4-21-2020

Production of DL-Methionine from Corn Syrup via Biosynthesis with Corynebacterium glutamicum

Dayoung Shin

Yvonne N. Szustakiewicz

Allison P. Walter

Production of DL-Methionine from Corn Syrup via Biosynthesis with Corynebacterium glutamicum

Abstract

DL-methionine is an important feed additive for poultry and swine diets. The amino acid is not produced by animals so it can only be obtained through diet. Methionine is important for protein growth and helps the health of livestock. Recent increase in meat consumption in Latin America and Asia, along with a growing consumer concern of animal welfare, has driven a growth in the methionine market. The methionine market is predicted to continue to grow for at least another five years (Methionine Market 2019). The proposed design is for a process that produces DL-methionine by fermentation with the bacteria, *Corynebacterium glutamicum*. The plant has a capacity of 250 kilotonnes/year and will be located in Cedar Rapids, IA. According to a 20 year profitability analysis, it has an estimated IRR of 16.43% and in 2042 it has an NPV of $5,650,200. In the third production year, the ROI will be 25.97%. The process begins with an aerobic fermentation, where the bacterial cells are grown up on a lab scale and then transferred into industrial scale pre-seed fermenters, seed fermenters, and then production fermenters. In order to produce feed grade DLM, the process after fermentation goes through heating, separation, triple effect evaporation, crystallization, and finally, drying. The final DLM product is 99% dry and 99% pure and will be sold for $3.60/kg.

Disciplines

Biochemical and Biomolecular Engineering | Chemical Engineering | Engineering

Department of Chemical and Biomolecular Engineering

220 South 33rd Street

Philadelphia, PA 19104

April 21, 2020

Dear Professor Bruce Vrana and Dr. Sue Ann Bidstrup Allen,

The enclosed report contains the solution to the design problem proposed by Stephen Tieri of DuPont. The proposed process design is for the biosynthetic production of DL-Methionine from corn syrup. *Corynebacterium glutamicum* is used in fed-batch fermentation. The proposed plant will be located in the U.S. Midwest and have the capacity to produce 250 kilotonnes/year of DL-Methionine.

This report contains detailed process design, economic analysis, and conclusions and recommendations for the implementation of the plant. The proposed plant is found to be economically feasible. It has an estimated IRR of 16.43% and in 20 years it has a total NPV of $5,650,200. Some of the continuous operations in this process were modeled using Aspen Plus v.11. We recommend that the design move forward with the specifications detailed within this report. Further research should be done to implement the design and guarantee financial success, as well as to prepare the product for a wider range of market applications.

Thank you for your help throughout the course of this project and thank you in advance for your feedback as to the efficacy of our design.

Sincerely,

Dayoung Shin _____

Yvonne Szustakiewicz _____

Allison Walter _____

Production of DL-Methionine from Corn Syrup via Biosynthesis with *Corynebacterium glutamicum*

Dayoung Shin | Yvonne Szustakiewicz | Allison Walter

Advised By:
Dr. Sue Ann Bidstrup Allen and Prof. Bruce Vrana

Project Proposed By:
Mr. Stephen Tieri

University of Pennsylvania
School of Engineering and Applied Sciences
Department of Chemical and Biomolecular Engineering
April 21, 2020

Table of Contents

Tables

Figures

Glossary

Word	**Abbreviation**
DL-Methionine	DLM
Corn Syrup	CS
Corynebacterium glutamicum	*C. glutamicum*
Million	MM

1. Abstract

DL-methionine is an important feed additive for poultry and swine diets. The amino acid is not produced by animals so it can only be obtained through diet. Methionine is important for protein growth and helps the health of livestock. Recent increase in meat consumption in Latin America and Asia, along with a growing consumer concern of animal welfare, has driven a growth in the methionine market. The methionine market is predicted to continue to grow for at least another five years (Methionine Market 2019). The proposed design is for a process that produces DL-methionine by fermentation with the bacteria, *Corynebacterium glutamicum*. The plant has a capacity of 250 kilotonnes/year and will be located in Cedar Rapids, IA. According to a 20 year profitability analysis, it has an estimated IRR of 16.43% and in 2042 it has an NPV of $5,650,200. In the third production year, the ROI will be 25.97%. The process begins with an aerobic fermentation, where the bacterial cells are grown up on a lab scale and then transferred into industrial scale pre-seed fermenters, seed fermenters, and then production fermenters. In order to produce feed grade DLM, the process after fermentation goes through heating, separation, triple effect evaporation, crystallization, and finally, drying. The final DLM product is 99% dry and 99% pure and will be sold for $3.60/kg.

2. Introduction

2.1 Project Origin

Methionine is an amino acid that is essential to both livestock and the human metabolism. It is essential for the animal feed market, which constitutes 98% of the total methionine market ("Methionine Market"). Because mammals cannot produce it, it must be obtained through their diet, either directly or through methionine-containing proteins.The amount of methionine in raw plants is insufficient so a supplementary source is needed. As a feed additive, it contributes to the efficient, healthy, and environmentally friendly nutrition of livestock, which makes it important for sustaining the world's animal protein supply ("Methionine Market Size"). Synthetic methionine is available in two forms: DL-Methionine (DLM), a racemic mixture of the two stereoisomers, and methionine hydroxy analog (MHA). For animal nutrition, the two are equivalent ("MetAMINO"). It is typically produced via a petrochemical route which involves hydrogen cyanide, a dangerous and hard-to-handle raw material. In recent years, biochemical routes to produce methionine have been developed. In 2015, the first bio-methionine plant was opened in Malaysia and was built for South Korea's biggest food company and a French specialty chemicals and advanced materials company ("Evonik").

Amino acids such as lysine, threonine, isoleucine, and histidine have been produced successfully by fermentation for decades. Only recently, as late as 2013, was a fermentation process developed to produce methionine. The process begins by heating a methionine-containing fermentation broth and then evaporating the broth in order to concentrate such broth. Next, the biomass is separated under temperatures ranging 70-100^{o}C to prevent

premature crystallization, and ultimately washed and dried to obtain crystalline, dry methionine (Boy, Klein, Schröder). While this fermentation process has successfully been developed, it only has been successfully developed on an industrial scale by one company. The main limitation in the process is the lack of solubility of methionine in the aqueous fermentation medium. In addition, considerable waste streams are produced, which is associated with high disposal costs.

2.2 Project Goals and Scope

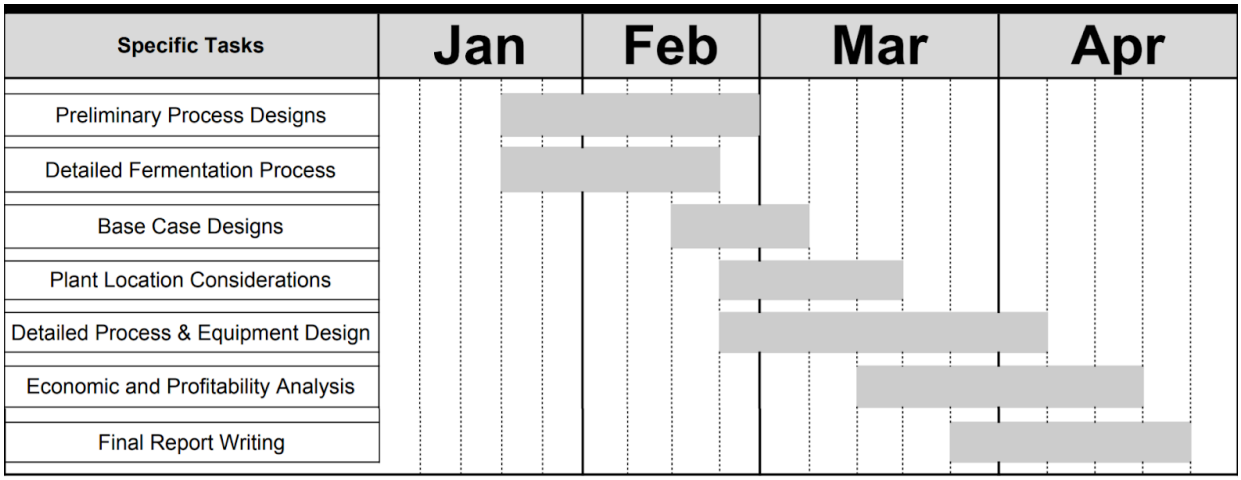
The goal of this project was to design a process to generate 150MM kg/yr of methionine. After a last minute calculation check found a discrepancy in the material balance, the production rate became 250MM kg/yr of methionine. Although this is a large amount of DLM to produce, it is not a market concern because the methionine market is expected to reach $5 billion by 2024 at a CAGR of 6% ("Methionine Market 2019"). See Section 4 for more in depth market analysis. Secondary goals included choosing which form of methionine to produce, plant location, and selection of process equipment.

The process detailed in this report begins with an aerobic fermentation section, consisting of six pre-seed fermenters, six seed fermenters, and 12 production fermenters. *Corynebacterium glutamicum*, a DLM producing microbe, has a high yield and selectivity. A complete cycle of fermentation takes about five days and is fed with media and corn syrup (CS).

In order to achieve the desired product purity, separation follows the fermentation. Separation operations include centrifugation, evaporation, and crystallization. The final product matches purity levels of DLM sold by Evonik and Novus, and is sold at a price competitive to Evonik and Novus. The proposed plant will be located in the U.S. Midwest and will produce

250 million kilograms of DLM per year. This location was chosen due to both its proximity to corn syrup plants, and to swine and poultry farms, which are large consumers of DLM.

2.3 Objective Time Chart



A graphic detailing the main tasks and timeline of the project is shown above. The major tasks in January included preliminary research into the petrochemical and biosynthetic methods of methionine production, as well as research on the market needs for the two choices of methionine form (DLM and MHA). Different choices of bacteria and raw materials were investigated for the fermentation process. Priorities in February included finalizing the fermentation operating conditions and beginning the base case design, complete with process flow diagrams. Consideration of the ideal plant location also began in February and was finalized in March based on cost, market, and accessibility of raw materials. Other work in March included designing the major equipment for both upstream and downstream parts of the process. More attention was given to economic considerations, including costing equipment and recycling process materials. As the project timeline entered April, sensitivity analyses were conducted for specific parts of the process, and the remainder of the equipment was designed.

Relevant process details and economic analyses were consolidated into the written report. The written report was submitted for review and then revised before re-submission for final evaluation.

2.4 Project Charter

Name of Project: Production of DL-Methionine from Corn Syrup via Biosynthesis with *Corynebacterium glutamicum*

Project Author: Stephen M. Tieri

Project Advisors: Dr. Sue Ann Bidstrup Allen and Professor Bruce Vrana

Project Leaders: Dayoung Shin, Yvonne Szustakiewicz, and Allison Walter

Specific Goals: Design a plant to produce 150 million kilograms of methionine per year

Project Scope:

In Scope

Produce an equivalent of 250 million kilograms of DLM per year from CS
Design process, including all equipment and process conditions
Determine optimal reactor conditions, separations to recycle unconverted feeds, and purification of product to meet quality specifications
Size and cost all equipment
Analyze profitability and economic of project by calculating cost of plant and required pricing premium of product
Safety considerations necessary for working with hazardous chemicals

Out of Scope

Kinetic data of reactions outside the published conditions
Testing of assumptions
Design of process control system
Considerations for start-up and shut down
Protocol and cost of crisis management (i.e. natural disasters)

Deliverables:

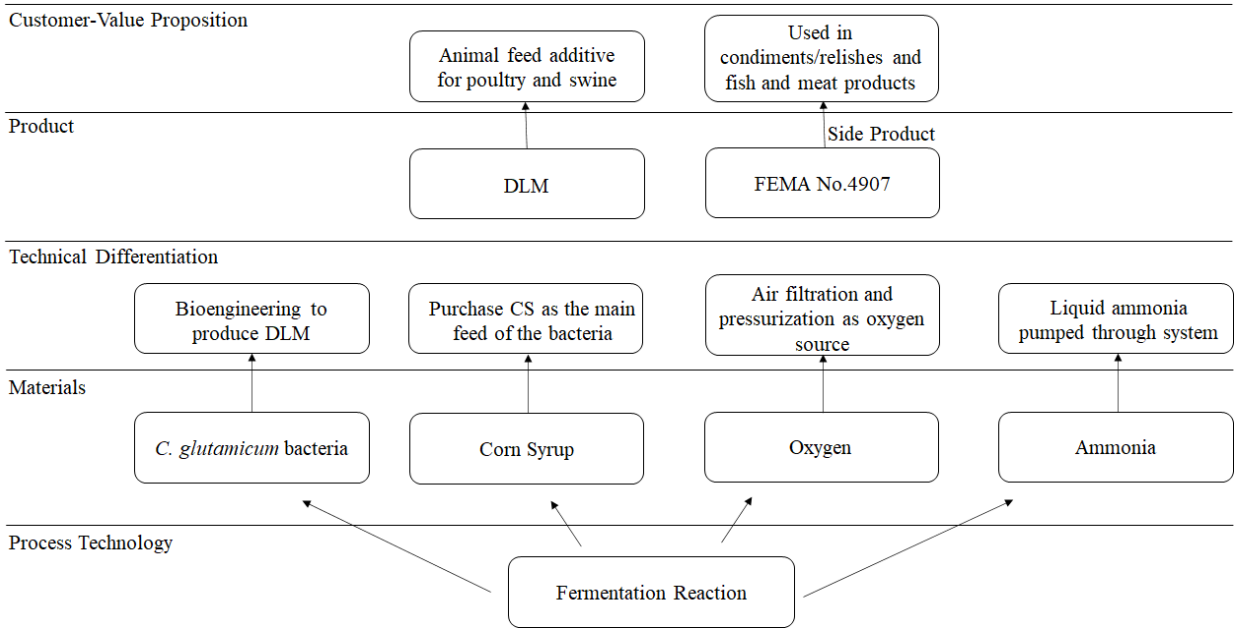
Flowsheet of process and simulation results
Mass and energy balances

Equipment design and operating parameters
Cost of plant
Economic and profitability analysis to understand plant feasibility
Sensitivity analyses of how changing parameters affect profitability of plant
Written report and presentation describing project

Timeline:

Initial presentation on November 18, 2019
Mid-semester presentation on March 3, 2020
Final report due on April 21, 2020
Final presentation on April 28, 2020

3. Innovation Map



4. Market and Competitive Analysis

This project involves the production of methionine as an animal feed additive. The global methionine market has been increasing since 2012 due to increased meat consumption in Asia Pacific and Latin America, recent animal disease outbreaks, and consumer awareness ("Methionine Market Size"). In 2015, global methionine production reached 1041 MM kilograms. The market for methionine is expected to grow at a CAGR of roughly 6% over the next five years, and will reach five billion dollars by 2024. ("Methionine Market 2019"). The improvement of energy, transportation costs, employee wages, and equipment depreciation will promote the cost of methionine in the next five years.

Animal feed additive accounts for 90% of world methionine consumption. Solid methionine, like DLM, can be used in other industries, while liquid methionine, like MHA, is only used in animal feed additives. DLM occupies 65% of the methionine additive market ("Methionine Market 2019"). The methionine market is not saturated by a lot of large companies and as stated above, the methionine market is expected to steadily grow for at least the next five years. Because of these two reasons, the fact that our plant produces ⅓ of the current DLM market does not raise high concerns. The company that builds this plant would become a top contender in the methionine market and compete with Evonik.

The methionine market concentration is low to medium. Major companies in the market include Evonik, Bluestar, NOVUS, and Sumitomo Chemical. Evonik accounts for 36.5% of global production and Bluestar accounts for 24.1% of global production. Evonik, Bluestar, and NOVUS occupy a large part of the market share in China, with local Chinese manufacturers

having small market share. The United States is the second largest producer of animal feed, following China ("Methionine Market 2019").

5. Customer Requirements

DLM produced by the plant will be sold as a feed additive for poultry and swine growth. High purity of the product was not a requirement by the customers since DLM is sold as animal feed additive. However, the current market provides very pure (>99%) DLM for feed additives. The final purity of our product will be 99% to compete efficiently in the existing market. Impurities, including salt and glucose, in the product do not violate the FEMA GRAS guideline and therefore can be present in the product.

Biomass, *Corynebacterium glutamicum*, resulting from the downstream process of fermentation will be sold as an animal feed additive with 10% moisture content. The side product does not violate the FEMA GRAS guideline and therefore can be sold. 10% moisture was selected based on that of the dried distiller's grain with solubles (DDGS) that is produced in ethanol production plants. The price of the side product was selected as 10% of the price of the DLM product since the side product is 10% DLM. Price of DDGS was also considered in determining the price of the side product but was rejected after profitability analysis.

6. Critical to Quality Variables - Product Requirements

N/A

7. Product Concepts

The product selected was DL-methionine (DLM) in crystalline form at 99% purity. The product will be sold only in the animal feed market. It will presumably be mixed with feed mixes made of corn, soya beans, cassava, and broken rice. This product was selected because it has higher purity than the liquid methionine-hydroxy analogue (MHA), is 100% bioavailable, and the manufacturing process is more efficient and cost effective. In addition, DLM is also easier to transport, store, and process than MHA. Lastly, DLM mixes more efficiently with other mainly dry feed components and does not stick to machinery.

8. Superior Product Concepts

N/A

9. Competitive Patent Analysis

The process detailed in this report was influenced by previous processes detailed in patent literature. Multiple patents exist for the production of methionine using bacteria. One such patent, U.S. patent 7,785,846 was used as a reference for much of the design of the fermentation techniques used in this report. The patent, titled *Method For The Production Of Methionine*, was published in 2005 and describes the fermentation conditions needed to successfully produce methionine from a *Corynebacterium glutamicum* bacteria strain. The authors detail the temperature, fermentation broth composition, and general processes to yield 1.5 kg methionine for every 20 kg of fermentation broth (Boy, Klein, Schröder). Another patent, international patent WO 2015/028674 A1 was used as a reference for much of the design of the cell culture techniques used in this report. The patent, titled *Microorganism for methionine production with improved methionine synthase activity and methionine efflux*, describes the batch fermentation process using *E. coli* in 2.5L reactors (Dischert, Figge, Vasseur). The relevant patents for this report can be found in full in Appendix F.

Evonik has developed a process to produce DL-methionine through fermentation. The plant was opened in Singapore in June, 2019 and will double the company's annual capacity of methionine. Very little detail on how the plant functions has been released.

The technology needed to produce DL-methionine through fermentation is well known, but has been hard to scale to an industrial size. Research has been done and continues to be done on genetically modifying the bacterial strain used for fermentation in order to increase the process yield.

10. Preliminary Process Synthesis

10.1 Fermentation Chemistry

The growth and fermentation of *Corynebacterium glutamicum* requires an aerobic process with glucose as the fuel source and air as the oxygen source. Bacterial cells are made up of about 50% carbon, 20% hydrogen, 14% oxygen, 8% nitrogen, and 3% or less of other minerals such as phosphorus, sulfur, potassium, magnesium, calcium, and iron (Huang). All of these elements must be introduced in the reactor feed through solutions or fuel sources in order for the bacteria to grow and multiply. *Corynebacterium glutamicum* uses glucose as its fuel source and ammonium chloride was chosen as the nitrogen source because it was present in a premade broth from Teknova. The chemistry for bacteria using glucose as fuel and producing methionine can be seen in Figure 10.1 (Bolten). In this aerobic process it can be seen that glucose goes through glycolysis to produce acetyl coenzyme A, which then goes through the TCA cycle to produce oxaloacetic acid, which then is converted to acetic acid, which is ultimately converted to methionine.

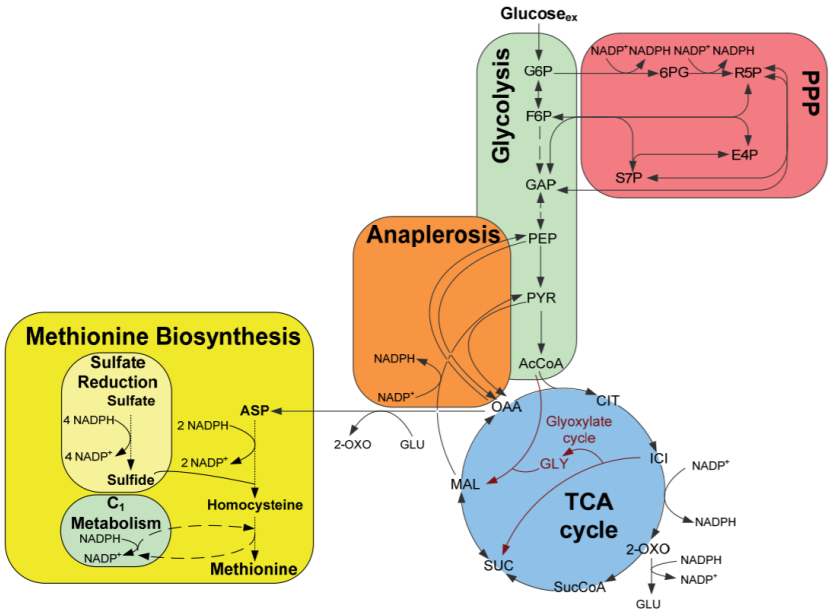


Figure 10.1. Chemical pathway for aerobic use of glucose as a carbon fuel source for methionine production.

10.2 Alternative Process Synthesis

There are three known methods of producing DLM: petrochemical synthesis, enzymatic synthesis, and biosynthesis involving fermentation. In choosing the synthesis route for this project, petrochemical and fermentation methods were considered as the starting routes because enzymatic synthesis requires homocysteine.

DLM produced via chemical synthesis is the major source of methionine in the market, representing around 60 % of global DLM capacity. The starting materials include methyl mercaptan (MMP), acrolein, ammonium carbonate, and inorganic cyanide. The reactions involved are hydantoin production, hydantoin hydrolysis, and DLM production. First, MMP and acrolein are heated together in presence of ammonium carbonate and inorganic cyanide to produce hydantoin. During the process, strong acid, such as HCl or HBr, is added to lower the

pH to less than 4 to increase the yield of hydantoin. However, this process releases toxic HCN and thus requires great care in handling. Hydantoin is subsequently hydrolyzed in basic condition, preferably by addition of BaOH, to produce DLM.

One major drawback of this process is the release of HCN in hydantoin production. Another drawback is the immense amount of energy duty that is needed during the process to control the reaction rate. Lastly, purification of methionine requires additional work-up to remove barium salts, which are toxic.

After carefully reviewing the petrochemical synthesis route, it was decided that fermentation will be used for DLM production due to the inherent toxicity of the traditional synthesis route. In addition, there is a growing opposition towards chemically produced feed additives in organic farming. Despite the fact that DLM synthesis via petrochemical route is reported to be more profitable than synthesis via fermentation, the trend will be reversed in the future due to the stricter regulations on chemically produced feed additives in organic farming. In fact, Evonik recently finished building a plant in Singapore capable of producing 150 kilo-tonnes of DLM using fermentation, indicating that large scale of DLM via fermentation is profitable.

10.3 Alternative Media Source

The media for this fermentation process requires glucose, a nitrogen source, a sulfur source, a phosphorous source, inorganic salts, and vitamins. The process of purchasing each of those individual components separately and then mixing them together was initially explored because of its cost benefits. Working with Teknova to create and purchase a premade concentrated Inoculum Broth (Soytone 25%, Yeast Extract 48%, Glycerol 10%, Na_2HPO_4 6%,

KH_2PO_4 3%, NH_4Cl 1%, $MgSO_4$ 0.25%, $CaCl_2$ 130 μM), with those needed sources was ultimately chosen to simplify the process. Although the Teknova broth is expensive, only corn syrup needs to be added as the glucose source. In addition, the Teknova Inoculum Broth only requires storage tanks for the broth and the corn syrup, while making a media from individual components would require storage tanks for each individual chemical. The total cost of raw materials is $0.383 per pound of DLM when using the Teknova broth. The total cost of raw materials is $0.108 per pound of DLM when making our own broth. This 27 cents difference saves $157MM per year. This figure does not factor into consideration that at least five more storage tanks would have to be purchased along with another mixer, but these extra capital costs would not outweigh the money saved on the cost of creating our own broth. Given the time constraints of the project and the goal to streamline the fermentation process, the Teknova broth was chosen. If this process was to be built, the plant process should involve purchasing individual, solid chemicals and mixing them with process water to create the broth.

10.4 Alternative Glucose Source

The carbon source for this process needed to be glucose so two different glucose sources were considered. High fructose corn syrup was initially suggested by the project author and costs 77.6 cents/kg, but only consists of 53% glucose. Corn syrup costs between 55 and 99 cents/kg and is 75% glucose. Because both high fructose corn syrup and corn syrup are produced in abundance in the U.S. and glucose is the second most massive component of the fermentation broth, corn syrup was chosen as the glucose source. While high fructose corn syrup can be less expensive than corn syrup, less mass of corn syrup is needed for the media due to its

higher glucose content. For this reason, corn syrup is more economically feasible than high fructose corn syrup.

10.5 Alternative Plant Locations

A variety of different plant locations were considered when designing this process before deciding to operate the plant in the US Midwest. Singapore, while convenient because of its proximity to China, which holds a large share of the methionine market, was ultimately not chosen because of the amount of wet feed (CS) required for the process and the country's carbon tax. China does produce a large amount of CS that could be shipped to Singapore, but the large volume of CS needed compared to the methionine yield made the US a more feasible location for the plant. In addition, Singapore has a carbon tax of $5 per tonne of greenhouse gas emissions, while the US has no carbon tax ("Carbon Tax"). Our plant design to produce DLM will emit 79,832 tonnes of CO_2 per year, so building and operating the plant in the US is more economically feasible than in Singapore.

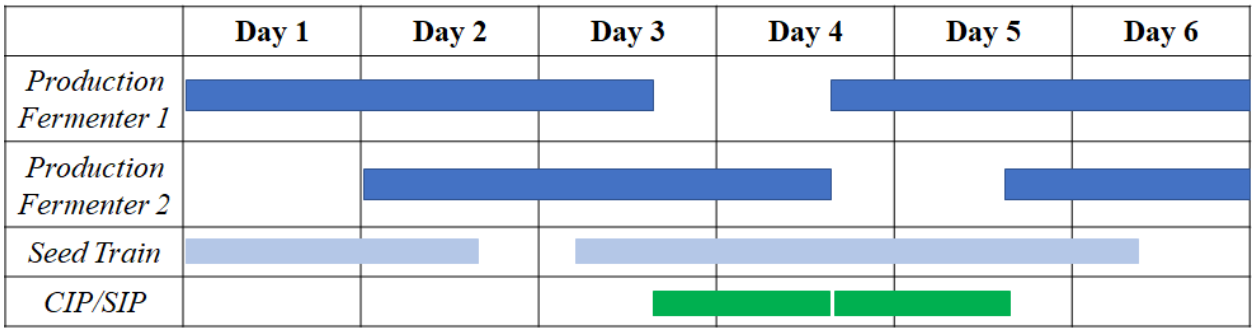
Cost of labor was also considered in comparing the viability of a US versus Singapore location. Construction workers are paid 30% more in the US than in Singapore, but engineers who would operate the plant are paid very similarly in the two countries. Because the cost of construction workers would be a one time payment and the cost of operators would be a continuous payment, the fact that the construction of the plant in Singapore would probably cost less than in the US was not considered very strongly.

Most of the CS produced in the US is made in the US Midwest. CS is expensive to ship because of its large water content and therefore the plant should be chosen so that a CS plant is nearby. Iowa and Illinois are the two of the largest state producers of corn. In addition, they

both border the Mississippi River, which is convenient for shipping purposes. Iowa has lower property taxes and income taxes than Illinois, so Iowa is the better economic choice for plant location.

The US Gulf Coast was not strongly considered because if the plant was to be in the US, it should be located as close as possible to where the CS is produced in order to limit costs of transport. While the US Gulf Coast borders the Gulf of Mexico, which is convenient for shipping product by sea, the costs of having to transport the CS from the US Midwest to the US Gulf Coast outweighs the cost of transporting the Methionine product from the US Midwest to the closest body of water for transport to China.

10.6 Batch Operation Schedule



The graphic above shows the proposed schedule for the operation of all major processes involved in this project. The plant will operate on a 7,500 hour per year schedule. There are a total of six pre-seed fermenters, six seed fermenters, and twelve production fermenters, creating six of the proposed schedules above that are running in parallel. The production fermenters will run continuously in 64 hour reactor campaigns before operations must be suspended, and there will be approximately 109 such reactor campaigns per production fermenter per year. The

operating schedule of the two production fermenters that are connected to the same seed fermenter will be staggered such that a new campaign will begin 24 hours after the first production fermenter's campaign. When a production fermenter campaign ends, the Clean-in-Place (CIP) system and Sterilization-in-Place system (SIP) will begin to sterilize the reactors, and this process will take approximately 24 hours. The reactor seed train process with one pre-seed fermenter and one seed fermenter will be initiated 24 hours before the end of a production fermenter. Technicians will be able to lengthen or shorten the seed train process in case of unexpected events that cause this time period to change,

In order to grow new batches of cells in a timely manner with appropriate quality checks in between each growth stage, a seed train is used in this process . The reactors from pre-seed to seed to production are sized up by a volumetric order of magnitude of 10. The estimated batch times for each of the units in the fermentation train are summarized in Table 10.6. These batch times were calculated based on the cell doubling times in each of the respective units. The seed train process will take a total of 77 hours, including turnaround. This can be increased and decreased according to process needs by adjusting the percentage of mass transferred from one seed reactor to the next. Additionally, each of the batch times was calculated based on the growth phase and the turnaround time, which was assumed to be 8 hours. The stationary phase of cell growth is not included in these batch times since the cells will be inoculated at optimal growth conditions.

Table 10.6. Batch growth times for each of the fermentation train units, adding to a total of 61 hours.

Unit	Unit Size (L)	Doubling Time (hr)	Final DCW (g/L)	Growth Time (hr)
Flask	2	2.68	0.67	24
Pre-seed	5,000	1.88	1	22
Seed	50,000	2.58	6	15
Production	500,000	3.89	20	20

11. Assembly of Database

11.1 Cost of Chemicals

The main raw materials in this process are corn syrup and Teknova Inoculum Broth. The prices for buying these materials in bulk were obtained from the websites of various vendors. Corn syrup will be purchased from ADM and transported via piping from their plant location in Cedar Rapids, IA at a bulk price of $0.88/kg. Teknova Inoculum Broth will be purchased from Teknova and transported via rail in tank wagons at a bulk price of $0.51/kg. We are making the assumption that we will work with Teknova to create a broth that is more concentrated than the broth that is sold on their website, which is 90.8% water. We are also making the assumption that we will work with Teknova so that they can sell the concentrated broth to us in bulk amounts. Because of these two assumptions, we made the estimation that we will be able to purchase their broth at $0.51/kg. The costs of all reactants and products are summarized in Table 11.1.

Table 11.1. Bulk price of main reactants and products.

Chemical Name	**Cost ($USD/kg)**
Teknova Inoculum Broth	$0.51
Corn syrup	$0.88
C. glutamicum	$402/0.4 mL aliquot
Methionine product	$3.6
FEMA No. 4907 side product	$0.36

11.2 Chemical Components and Thermophysical Properties

The Safety Data Sheets (SDS) for all chemical components can be found in Appendix E. Considerations based on safety of the chemicals were determined using the information from these SDS. Aspen simulations were used to model the media sterilization, triple effect evaporation, the heat exchanger of the crystallizer, and the combustion of natural gas for the rotary dryer. For calculations in Aspen, ammonium sulfate was used to model all of the salts that are involved in the process and dextrose was used to model glucose. The choice to use ammonium sulfate to model all salts was done to simplify calculations and was approved by project author, Stephen Tieri. For the stoichiometry of the formation of DLM, ammonium sulfate was also used to model all the salts that are involved in the process. This decision was proposed by consultant Rick Bockrath and was approved by project author, Stephen Tieri. The amount of air and ammonia needed for each reactor was based on this stoichiometry.

11.3 Cell Growth Kinetics and Bioreactor Rates

Information on cell growth kinetics was found in patents and literature. The Venkata paper shows a range of doubling times between 1.25-3 hours for *C.glutamicum* (Venkata, Vamsi, Venkata). For our calculations, a doubling time of 1.88 hours was calculated for the pre-seed reactor, 2.58 hours was calculated for the seed reactor, and 3.89 hours was calculated for the production fermenter. These doubling time calculations were based on growth rates which were based on initial and final dry cell weight and time allowed for growth. International patent WO 2015/028674 A1 had an example where the final concentration of methionine in solution reached was 20 g/L. It was assumed that during fermentation, the final concentration of methionine in

solution reached was 80 g/L in order to decrease the number of fermenters needed to produce 250MM kg/year of DLM per year. If 20 g/L was used as the final titer, 48 production fermenters would have been required; when 80 g/L is the final titer, only 12 production fermenters are required. It was concluded from US Patent 7,785,846 that for every 20 kg of fermentation broth put into the reactors, 1.5 kg of DLM product is produced. This ratio was used for the basis of our preliminary material balances. From US Patent 8,338,141, it was determined that the pH should be maintained at 7.5 during fermentation. The bulk density of the dried DLM is 1.34 kg/L. The production reactors can be run for 64 hours in continuous, sterile operation before needing to be stopped and cleaned with 95% process uptime. A seed train was created for cell growth as a fed-batch process, with reactors increasing in size until the target titer was reached in the largest reactor and the continuous process can begin.

11.4 Aspen Simulation Specifications

Aspen Plus V11 was used in the simulation of many of our processes. These processes include media sterilization, triple effect evaporation, the heat exchanger of the crystallizer, and the combustion of natural gas for the rotary dryer. The NRTL property method was used because most of the solutions involved in the process consist mainly of water and water is a polar molecule. It was also chosen because it is useful to calculate phase equilibria and several parts of the process involve liquid, vapor separation. All of the heat exchangers were modeled using HEATX. For all processes modeled in Aspen, dextrose was used to model glucose, and ammonium sulfate was used to model all of the salts and other minor components present in the Teknova broth (including yeast extract, soytone, glycerol, monosodium phosphate, monopotassium phosphate, ammonium chloride, and magnesium sulfate). Ammonium sulfate

was chosen because it is a source of nitrogen and sulfur, which are key elements necessary for growth in fermentation. This decision was approved by project author, Stephen Tieri. Other fermentation byproducts and minerals were ignored in modeling because their small weight percent was deemed insignificant to calculations. This decision was approved by project author, Stephen Tieri. DLM was present in Aspen's database, but many parameters were missing so values for parameters were added manually from doing outside research on DLM.

The media sterilization was modeled as three heat exchangers. The cold stream coming out of the second heat exchanger is used as the hot stream into the first heat exchanger to maximize energy efficiency. The hot stream out of the first heat exchanger is mixed with the cold stream out of the second heat exchanger to become the hot stream into the third heat exchanger to maximize energy efficiency.

The triple effect evaporation unit was modeled as three heat exchangers and three flash vessels. Operation at a maximum approach of 10^{o}C was set to maximize the heat transfer per unit area. Smaller temperature approaches were avoided as this would increase the heat exchanger area. Since there is no actual model for evaporators in Aspen, the exit cold stream from the heat exchangers were fed into FLASH2 separators. The duty of the triple effect evaporation was calculated by summing the duties of the heat exchangers in each effect.

The heat exchanger part of the crystallizer was modeled in Aspen to calculate the duty of cooling the liquid stream coming in from 112 to 5^{o}C. Water from triple effect evaporation comes in at 6^{o}C to cool the liquid input stream. The total duty of the crystallizer was calculated by summing the duty of the heat exchanger with the heat of fusion times the flow rate of DLM

going into the crystallizer. The latter calculation was completed by hand and can be seen in Appendix A.

The combustion of natural gas was modeled in Aspen to calculate how much natural gas was needed to heat the air required by the rotary dryer. Air and methane were inputs to an RSTOIC reactor, which modeled a furnace, and carbon dioxide and water were outputs. The flow rate of natural gas was increased until the exhaust from the furnace was at 170°C, as advised by consultant Rick Bockrath. The amount of natural gas needed was incorporated into utilities calculations.

12. Process Flow Diagrams and Material Balance

Process flow diagrams for different parts of the process are presented in sections 12.1-12.3. Section 12.1 and Figure 12.1 shows the media sterilization process. Section 12.2 and Figure 12.2.1 shows a single fermentation train in greater detail, including all equipment required for a single pre-seed and seed fermenter, and a pair of production fermenters. The full fermentation design and all batch operations are shown in Figure 12.2.2. Intermediate pumps for fermentation are not shown in the overall diagram, but can be referenced in detail in Figure 12.2.1. All downstream continuous processes are presented in Section 12.3 and Figure 12.3. Some streams appear in multiple figures to show the path of heat integration across the batch and continuous processes. Streams that contain significant amounts of DLM are bolded throughout the process flow diagrams. In Section 12.4, a portion of Figure 12.3 is depicted in the schematic labeled Figure 12.4 to indicate elevation of various units and to indicate where gravity and static head are used to assist with material transportation in the process.

12.1 Media Sterilization

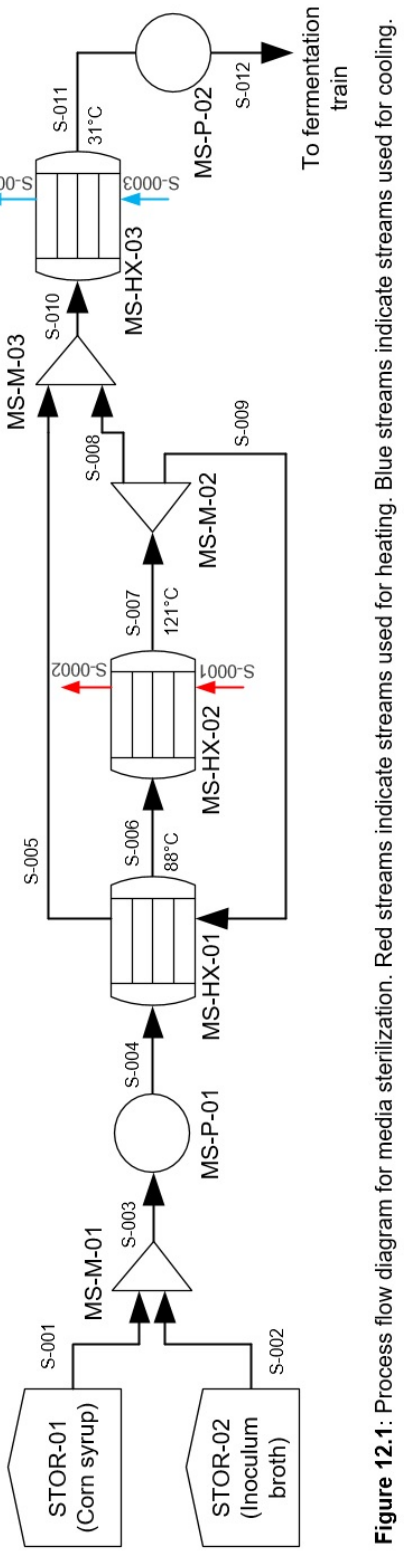


Figure 12.1: Process flow diagram for media sterilization. Red streams indicate streams used for heating. Blue streams indicate streams used for cooling.

Description	Media in	Media out	Media out	Media in	Media out
Stream #	**S-004**	**S-006**	**S-007**	**S-010**	**S-011**
Temperature (°C)	20	88	121	104	31
Pressure (bar)	1.01	1.01	1.01	1.01	1.01
Vapor Fraction	0	0	0	0	0
Total Flow (kg/hr)	52275	52275	52275	52275	52275
Component Flow (kg/hr)					
Water	42834.1	42834.1	42834.1	42834.1	42834.1
Glucose	5222.3	5222.3	5222.3	5222.3	5222.3
Minerals/Salts	42185.9	42185.9	42185.9	42185.9	42185.9
DLM	0	0	0	0	0

Description	Recycled media in	Recycled media out	Steam in	Steam out	CW in	CW out
Stream #	**S-009**	**S-005**	**S-0001**	**S-0002**	**S-0003**	**S-0004**
Temperature (°C)	121	104	175	132	20	44
Pressure (bar)	1.01	1.01	8.9	8.9	1.01	1.01
Vapor Fraction	0	0	1	1	0	0
Total Flow (kg/hr)	40641	40641	450382	450382	990840	990840
Component Flow (kg/hr)						
Water	33301.2354	33301.2354	450382	450382	990840	990840
Glucose	4060.0359	4060.0359	0	0	0	0
Minerals/Salts	32797.287	32797.287	0	0	0	0
DLM	0	0	0	0	0	0

12.2 Seed Train Design

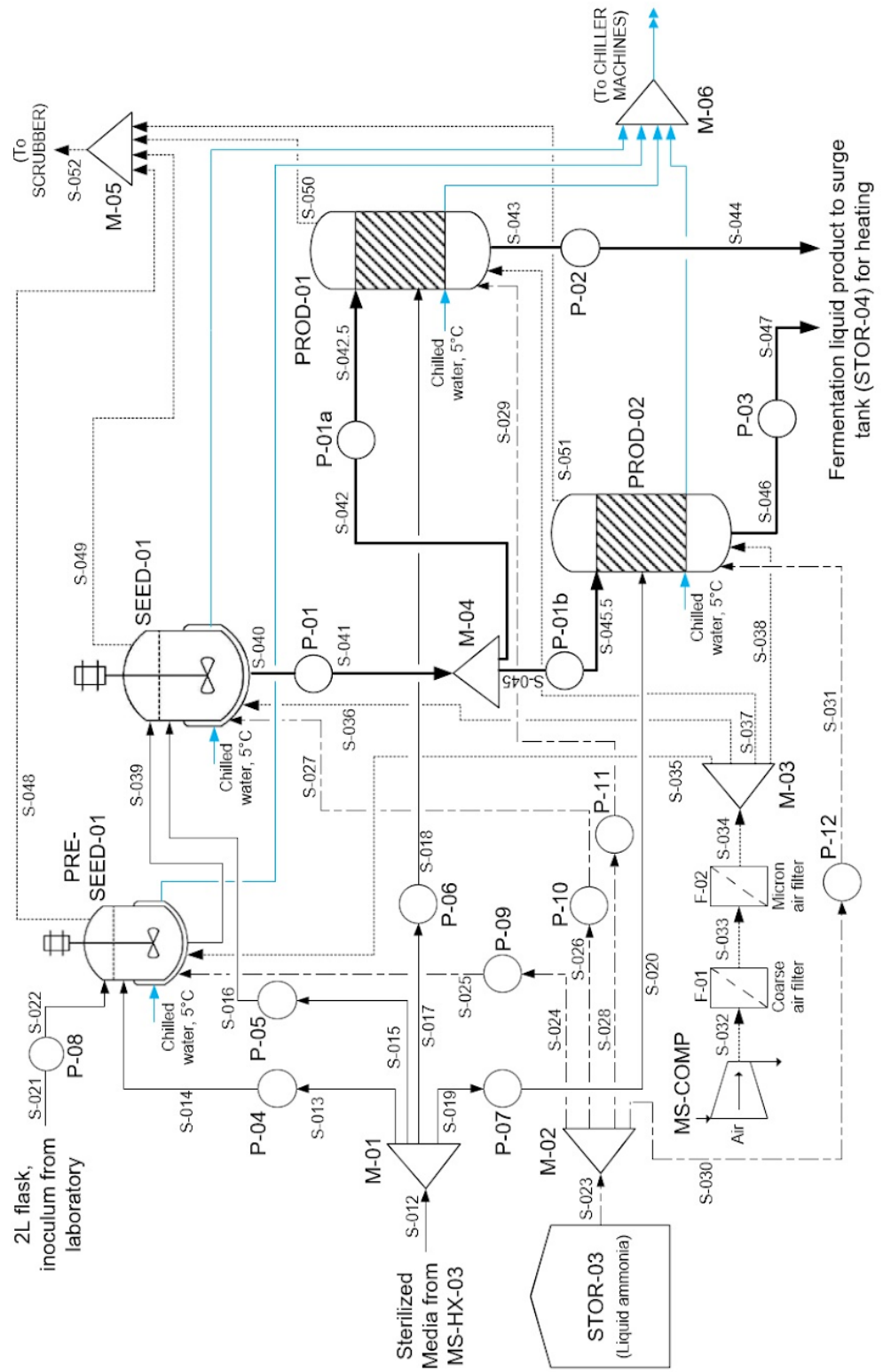


Figure 12.2.1: Process flow diagram for a single fermentation train (PRE-SEED-01, SEED-01, PROD-01, PROD-02). Includes all equipment required, including that shared among the six trains. Bold streams contain Met product. Blue indicates streams used for cooling and exist as a closed loop of chiller machines.

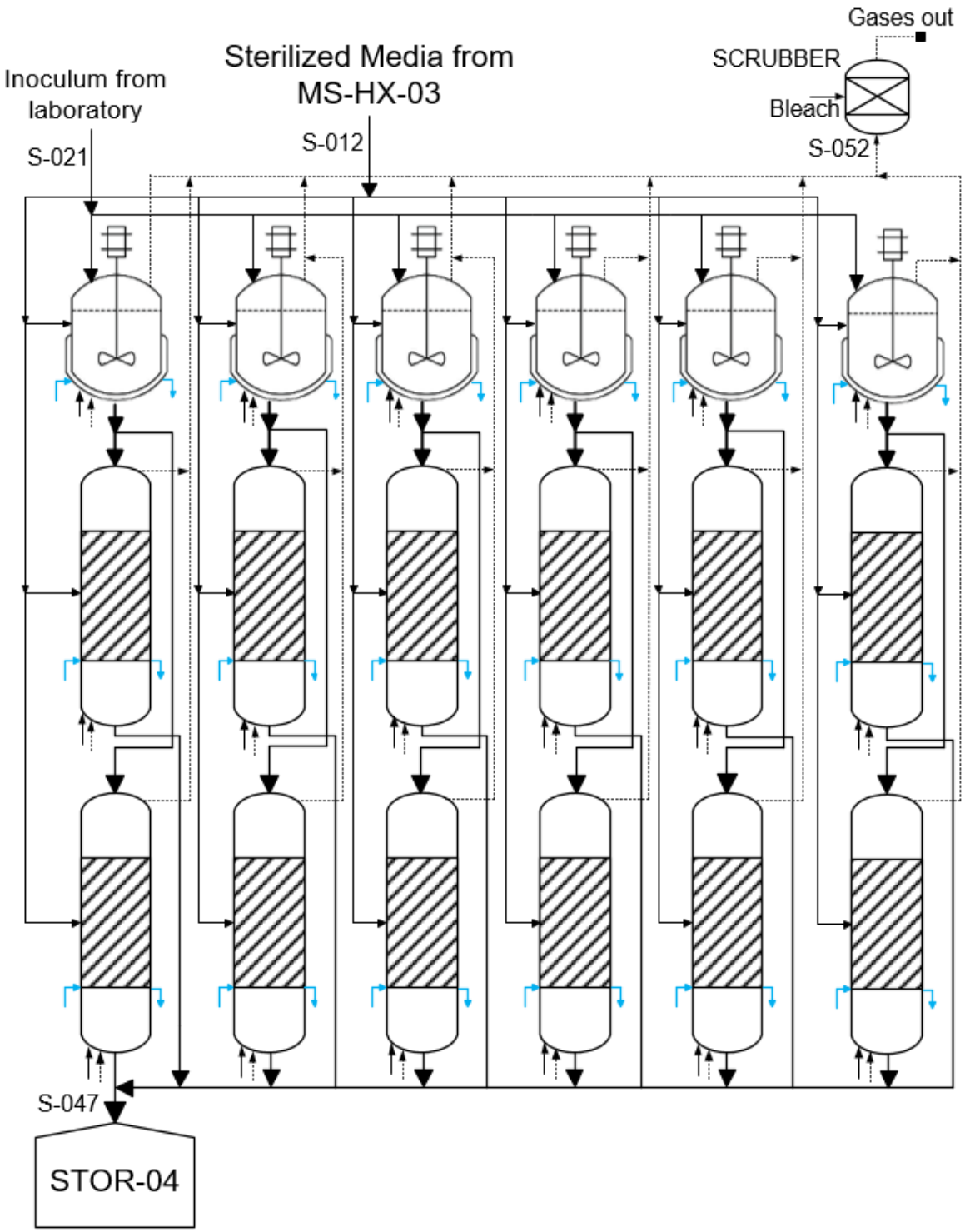


Figure 12.2.2: Process flow diagram for all batch operations, including fermentation and all associated pumps, heat exchangers, and storage tanks. Intermediate fermentation equipment shown in Figure 2 is not shown here for each fermentation train. Bold streams contain Met product. Blue indicates streams used for cooling. Square terminals indicate streams for disposal.

2 L Flask

Description	From Aliquot	Media in	Product to next reactor
Stream #	**N/A**	**N/A**	**S-021**
Twin Stream #'s			
Temperature (°C)	31.0	31.0	31.0
Pressure (bar)	1.0	1.0	1.2
Total Mass (g/batch)	8.1	1650.0	1658.1
Component Mass (g/batch)			
Cell Mass	0.1	0.0	77.3
Water	8.0	1348.1	1487.3
Glucose	0.0	164.8	0.2
Teknova Mix	0.0	137.4	10.8
Ammonia	0.0	0.0	0.0
DLM	0.0	0.0	40.0
Other Fermentation Byproducts	0.0	0.0	41.5
Carbon Dioxide	0.0	0.0	0.0
Air	0.0	0.0	0.0
Oxygen	0.0	0.0	0.0
Nitrogen	0.0	0.0	0.0
Side Biomass	0.0	0.0	1.1

5,000 L Reactor

Description	From Flasks	Media in	Compressed Air in	Liquid Ammonia in	Product to Next Reactor	Gas Vent
Stream #	**S-022**	**S-014**	**S-035**	**S-025**	**S-039**	**S-048**
Twin Stream #'s						
Temperature (°C)	31.0	31.0	20.0	20.0	31.0	31.0
Pressure (bar)	1.2	1.0	2.9	1.0	1.2	1.2
Total Mass (kg/batch)	6.6	4846.3	6700.0	0.2	5270.8	6282.4
Component Mass (kg/batch)						
Cell Mass	0.31	0.0	0.0	0.0	0.91	0.0
Water	5.9	3959.4	0.0	0.17	4727.9	274.3
Glucose	0.00066	484.1	0.0	0.0	0.48	0.0

Teknova Mix	0.043	403.7	0.0	0.0	34.3	0.0
Ammonia	0.0	0.0	0.0	0.07	0.0	0.0
DLM	0.16	0.0	0.0	0.0	360.0	0.0
Other Fermentation Byproducts	0.17	0.0	0.0	0.0	131.8	0.0
Carbon Dioxide	0.0	0.0	0.0	0.0	5.9	580.6
Air	0.0	0.0	6699.8	0.0	0.0	N/A
Oxygen	0.0	0.0	1406.9	0.0	0.0	134.7
Nitrogen	0.0	0.0	5292.8	0.0	0.0	5292.8
Side Biomass	0.0042	0.0	0.0	0.0	9.6	0.0

50,000 L Reactor

Description	From Last Reactor	Media in	Compressed Air in	Liquid Ammonia in	Product to Next Reactor	Gas Vent
Stream #	**S-039**	**S-016**	**S-036**	**S-027**	**S-040**	**S-049**
Twin Stream #'s						
Temperature (°C)	31.0	31.0	20.0	20.0	31.0	31.0
Pressure (bar)	1.2	1.0	3.1	1.0	1.2	1.2
Total Mass (kg/batch)	5270.8	45770.6	67000.0	2.3	55653.6	62390.2
Component Mass (kg/batch)						
Cell Mass	0.91	0.0	0.0	0.0	422.0	0.0
Water	4727.9	37394.6	0.0	1.6	49921.2	2631.5
Glucose	0.48	4572.5	0.0	0.0	4.6	0.0
Teknova Mix	34.3	3812.7	0.0	0.0	361.7	0.0
Ammonia	0.0	0.0	0.0	0.64	0.0	0.0
DLM	360.0	0.0	0.0	0.0	3400.0	0.0
Other Fermentation Byproducts	131.8	0.0	0.0	0.0	1391.3	0.0
Carbon Dioxide	5.9	0.0	0.0	0.0	62.4	5483.2
Air	0.0	0.0	66997.7	0.0	0.0	N/A
Oxygen	0.0	0.0	14069.5	0.0	0.0	1347.2
Nitrogen	0.0	0.0	52928.2	0.0	0.0	52928.2
Side Biomass	9.6	0.0	0.0	0.0	90.3	0.0

500,000 L Reactor

Description	From Last Reactor	Media in	Compressed Air in	Liquid Ammonia in	Product to STOR-04	Gas Vent
Stream #	**S-042**	**S-018**	**S-037**	**S-029**	**S-044**	**S-050**
Twin Stream #'s	**S-045**	**S-020**	**S-038**	**S-031**	**S-047**	**S-051**
Temperature (°C)	31.0	31.0	20.0	20.0	31.0	31.0
Pressure (bar)	1.2	1.0	5.1	1.0	1.2	1.2
Total Mass (kg/batch)	27826.8	484630.4	670000.0	24.2	553807.5	628673.9
Component Mass (kg/batch)						
Cell Mass	211.0	0.0	0.0	0.0	1972.2	0.0
Water	24960.6	395943.0	0.0	17.4	496765.3	27862.6
Glucose	2.3	48414.6	0.0	0.0	48.4	0.0
Teknova Mix	180.9	40369.7	0.0	0.0	3599.7	0.0
Ammonia	0.0	0.0	0.0	6.8	0.0	0.0
DLM	1700.0	0.0	0.0	0.0	36000.0	0.0
Other Fermentation Byproducts	695.7	0.0	0.0	0.0	13845.2	0.0
Carbon Dioxide	31.2	0.0	0.0	0.0	621.0	58057.9
Air	0.0	0.0	669975.8	0.0	0.0	N/A
Oxygen	0.0	0.0	140694.9	0.0	0.0	13472.5
Nitrogen	0.0	0.0	529280.9	0.0	0.0	529280.9
Side Biomass	45.1	0.0	0.0	0.0	955.7	0.0

12.3 Downstream Continuous Design

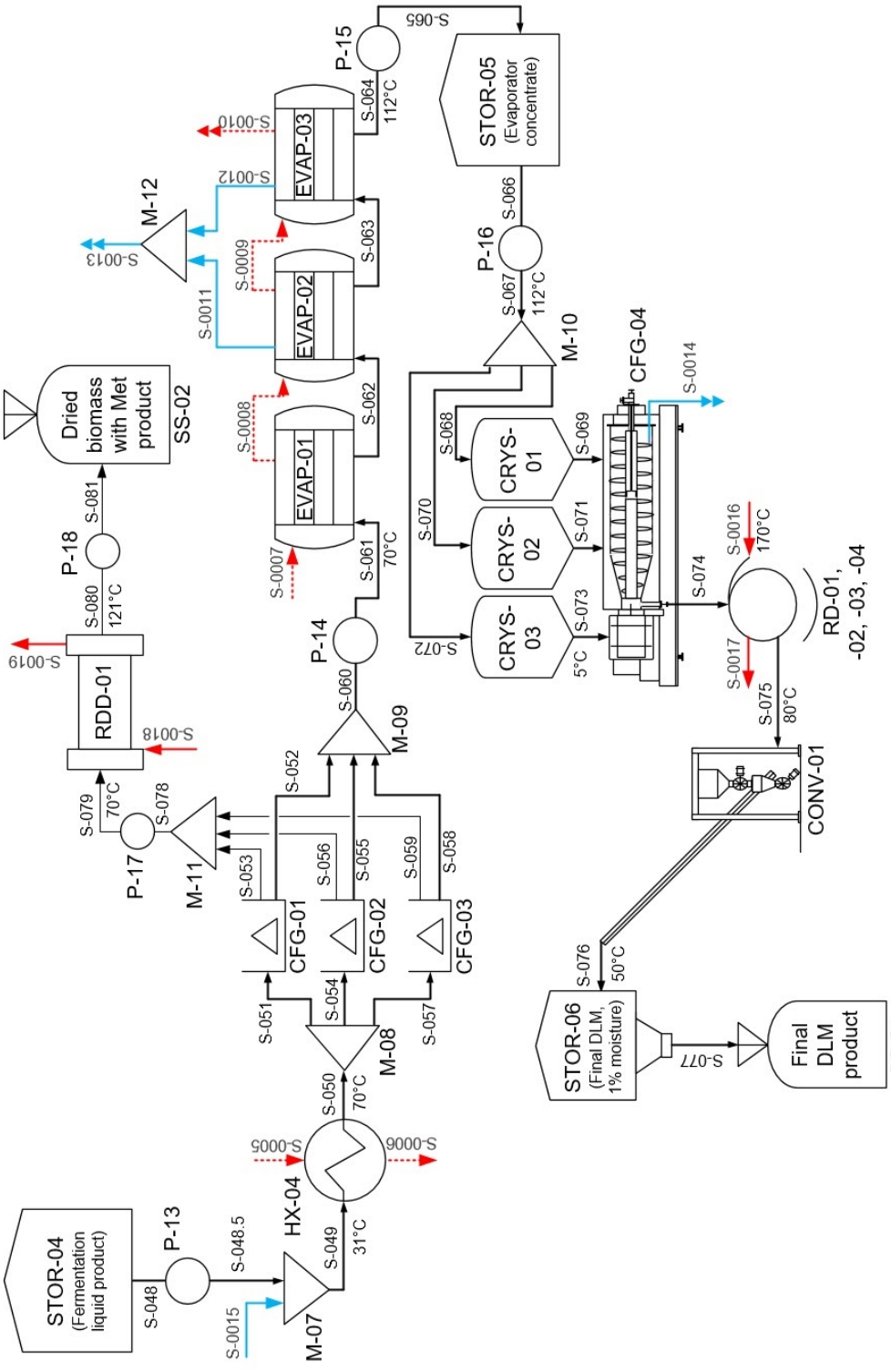


Figure 12.3 Process flow diagram for all downstream continuous operations and all associated pumps, heat exchangers, evaporators, crystallizers, centrifuges, rotary dryers, rotary drum dryers, bucket conveyors, and storage tanks. Bold streams contain majority DLM product. Light streams contain majority biomass product with some Met. Blue stream S-0015 is process water to be mixed with S-048.5 to add moisture to liquid fermentation product. Double-headed blue arrows indicate water streams to be cooled, sterilized, and recycled as process water. Red indicates streams used for heating. S-0016 and S-0017 are hot air streams. S-0018 represents a natural gas stream. Dotted red lines represent steam. S-0010 represents steam to be recycled after the triple-effect evaporation. Rotary dryer symbol represents four distinct rotary dryers (-01 to -04), each with its own hot air inlet and outlet streams. A comprehensive depiction of rotary dryer units RD-02, RD-03, RD-04 has been omitted from the diagram to maintain simplicity and conciseness of figure.

Description		Out of STOR-04, Into M-07	Process Water	Stream Into HX-04	Steam into HX-04	Steam out of HX-04	Out of HX-04	Split Out of Centrifuge	Out of Centrifuge	Side Biomass Stream
Stream #		**S-048**	**S-0015**	**S-049**	**S-0005**	**S-0006**	**S-050**	**S-052**	**S-060**	**S-078**
Twin Stream #								**S-055, S-058**		
Temperature	(°C)	31	25	30	180	150	70	70	70	70
Pressure	bar	1.2	1.0	1.2	1.0	1.0	1.0	1.0	1.0	1.0
Total Mass Flow	**(kg/hr)**	**648,888**	**53,533**	**683,788**	**1,995,700**	**1,995,700**	**683,788**	**226,819**	**680,456**	**3,332**
Liquid/Vapor Flow	**(kg/hr)**	**610,442**	**53,533**	**663,975**	**1,995,700**	**1,995,700**	**682,955**	**226,819**	**680,456**	**2,499**
Water	(kg/hr)	570,373	53,533	623,906	1,995,700	1,995,700	623,906	205,540	616,620	2,042
Salts	(kg/hr)	4,218	0	4,218	0	0	4,218	1,520	4,560	33
DLM (liquid)	(kg/hr)	18,980	0	18,980	0	0	37,960	13,679	41,037	294
Glucose	(kg/hr)	649	0	649	0	0	649	234	702	5
Other Fermentation Byproducts	(kg/hr)	16,222	0	16,222	0	0	16,222	5,846	17,538	125
Air	(kg/hr)	0	0	0	0	0	0	0	0	0
Solids Flow	**(kg/hr)**	**19,813**	**0**	**19,813**	**0**	**0**	**833**	**0**	**0**	**833**
Biomass	(kg/hr)	833	0	833	0	0	833	0	0	833
DLM (solid)	(kg/hr)	18,980	0	18,980	0	0	0	0	0	0

Description		Out of HX-04	Split Side Biomass Stream	Side Biomass Stream	Into Rotary Drum Dryer	Out of Rotary Drum Dryer
Stream #		**S-050**	**S-053**	**S-078**	**S-079**	**S-080**
Twin Stream #'s			**S-056, S059**			
Temperature	(°C)	70	70	70	70	121
Pressure	(bar)	1	1	1	1	1
Total Mass Flow	**(kg/hr)**	**683,788**	**1,111**	**3,332**	**3,332**	**1,398**
Liquid/Vapor Flow	**(kg/hr)**	**682,955**	**833**	**2,499**	**2,499**	**146**
Water	(kg/hr)	623,906	681	2,042	2,042	108
Salts	(kg/hr)	4,218	11	33	33	33
DLM (liquid)	(kg/hr)	37,960	98	294	294	0
Glucose	(kg/hr)	649	2	5	5	5
Other Fermentation Byproducts	(kg/hr)	16,222	42	125	125	0
Natural Gas	(kg/hr)	0	0	0	0	0
Solids Flow	**(kg/hr)**	**833**	**278**	**833**	**833**	**1,252**
Biomass	(kg/hr)	833	278	833	833	833
DLM (solid)	(kg/hr)	0	0	0	0	0
Other solids (salts, glucose, etc.)	(kg/hr)	0	0	0	0	419

Description		Feed to Evaporator	Steam into Effect 1	Liquid Out of Effect 1	Vapor Out of Effect 1	Liquid Out of Effect 2	Vapor Out of Effect 2	Liquid Out of Effect 3	Vapor Out of Effect 3	CW Out of Effect 2	CW Out of Effect 3	CW to be Recycled
Stream #		**S-061**	**S-0007**	**S-062**	**S-0008**	**S-063**	**S-0009**	**S-064**	**S-0010**	**S-0011**	**S-0012**	**S-0013**
Twin Stream #												
Temperature	(°C)	70	175	159	159	144	144	112	112	159	144	151
Pressure	bar	6.0	8.9	6.0	6.0	4	4	1.5	1.5	6	4	4.9
Total Mass Flow	**(kg/hr)**	**680,456**	**260,000**	**488,065**	**133,003**	**341,201**	**146,865**	**178,430**	**162,770**	**133,003**	**146,865**	**279,868**
Liquid/Vapor Flow	**(kg/hr)**	**680,456**	**260,000**	**488,065**	**133,003**	**341,201**	**146,865**	**178,430**	**162,770**	**133,003**	**146,865**	**279,868**
Water	(kg/hr)	616,620	260,000	445,833	133,003	298,968	146,865	136,198	162,770	133,003	146,865	279,868
Salts	(kg/hr)	4,560	0	2,174	0	2,174	0	2,174	0	0	0	0
DLM (liquid)	(kg/hr)	41,037	0	37,264	0	37,264	0	37,264	0	0	0	0
Glucose	(kg/hr)	702	0	621	0	621	0	621	0	0	0	0
Other Fermentation Byproducts	(kg/hr)	17,538	0	2,174	0	2,174	0	2,174	0	0	0	0
Air	(kg/hr)	0	0	0	0	0	0	0	0	0	0	0
Solids Flow	**(kg/hr)**	**0**	**0**	**0**	**0**	**0**	**0**	**0**	**0**	**0**	**0**	**0**
Biomass	(kg/hr)	0	0	0	0	0	0	0	0	0	0	0
DLM (solid)	(kg/hr)	0	0	0	0	0	0	0	0	0	0	0

Description		Feed to STOR-05	Feed to Crystallizer	Split Out of Crystallizer	*Slurry Out of Crystallizer to Centrifuge	Liquid out of Centrifuge	**Wet Cake out of Centrifuge to Rotary Dryer	***Cake Out of Rotary Dryer	****Air In	Air Out
Stream #		**S-065**	**S-066**	**S-069**	**N/A**	**S-0014**	**S-074**	**S-075**	**S-0016**	**S-0017**
Twin Stream #			**S-067**	**S-071, S-073**	**via gravity**					
					to centrifuge					
Temperature	(°C)	112	112	5	5	5	5	20	170	130
Pressure	bar	1.0	1.0	1.0	1.0	1.0	1.0	1.0	1.0	1.0
Total Mass Flow	**(kg/hr)**	**178,430**	**178,430**	**59,477**	**178,430**	**135,878**	**42,381**	**35,080**	**367,048**	**367,048**
Liquid/Vapor Flow	**(kg/hr)**	**178,430**	**178,430**	**47,988**	**143,963**	**135,878**	**8,085**	**352**	**367,048**	**367,048**
Water	(kg/hr)	136,198	136,198	45,399	136,198	128,540	7,648	352	0	0
Salts	(kg/hr)	2,174	2,174	725	2,174	2,052	122	0	0	0
DLM (liquid)	(kg/hr)	37,264	37,264	932	2,796	2,582	154	0	0	0
Glucose	(kg/hr)	621	621	207	621	586	35	0	0	0
Other Fermentation Byproducts	(kg/hr)	2,174	2,174	725	2,174	2,052	122	0	0	0
Air	(kg/hr)	0	0	0	0	0	0	0	367,048	367,048
Solids Flow	**(kg/hr)**	**0**	**0**	**11,489**	**34,468**	**0**	**34,296**	**34,728**	**0**	**0**
Biomass	(kg/hr)	0	0	0	0	0	0	0	0	0
DLM (solid)	(kg/hr)	0	0	11,489	34,468	0	34,296	34,449	0	0

*:Total flow and composition of the stream into the centrifuge

**: Total flow to all 4 rotary dryers

***: Total flow out of all 4 rotary dryers

****: Total air needed for all 4 rotary dryers

12.4 Elevation Considerations in Design

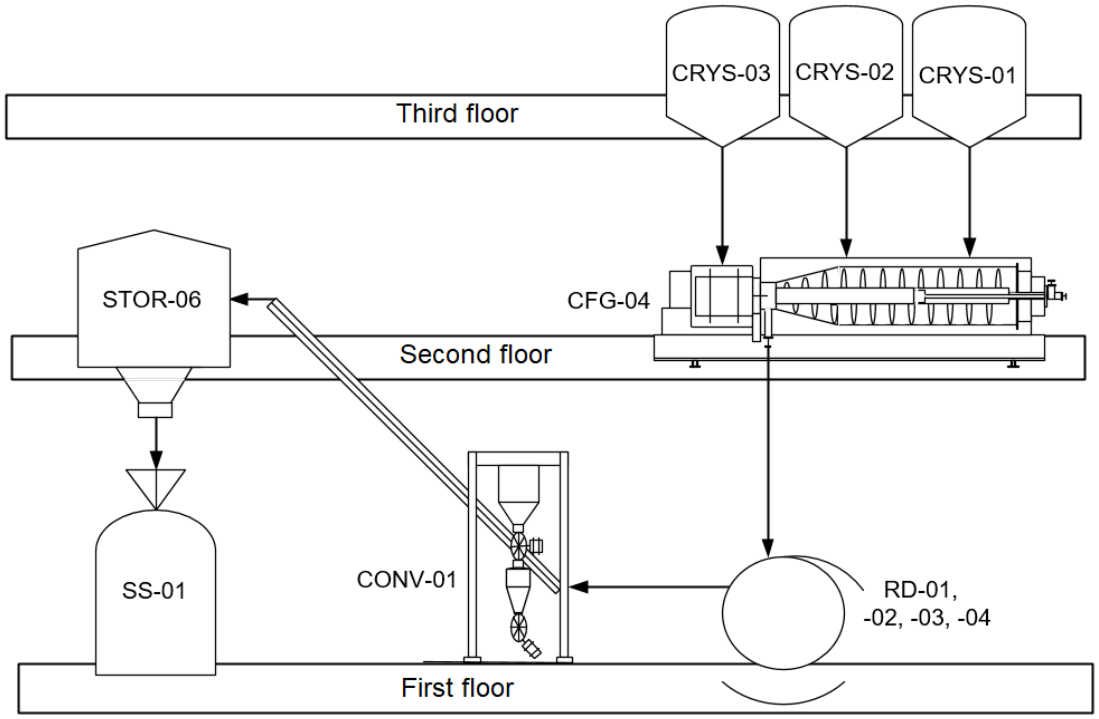


Figure 12.4. Schematic diagram showing elevation considerations to minimize pump and conveyor requirements. Crystallizers will be located on the third floor and feed their slurry product into the centrifuge via gravity and static head. The centrifuge will be located on the second floor and feed solid product into the rotary dryers located on the ground floor of the plant. A conveyor will cool the dried product as it is carried to the second floor storage bin. The bin contains a live bottom unit to be able to drop cooled product into the super sacks packing station located beneath the bin, on the ground floor. This set-up maximizes the use of gravity and static head to transport solid streams.

13. Process Descriptions

The first section of this process is the aerobic fermentation of *Corynebacterium glutamicum*. A 0.4 mL aliquot of the bacteria will be purchased from ATCC and grown up in a lab. 0.112 grams of the cell mass will be transferred into 2L flasks to grow for 24 hours. The contents of the flasks will then be transferred into pre-seed fermenters. The pre-seed stage occurs over 30 hours, which includes 8 hours of turnaround, in six 5,000 L fermenters (PRE-SEED-01 to -06). The cells are then transferred into six 50,000 L seed fermenters (SEED-01 to -06) where the fermentation reaction proceeds for 23 hours (including 8 hours of turnaround). Air is added to the pre-seed reactors at 0.29 vvm and to the seed reactors at 0.31 vvm. Chilled water at 6˚C is used to maintain the temperature of the fermenters at 31˚C and ammonia is added to maintain the pH at 7.5. Final DLM concentration in each fermenter is 80 g/L at the end of the fermentation period.

Each pre-seed fermenter feeds into one seed fermenter, each with a working volume of 42,500 L. Each seed fermenter feeds into two production fermenters, each with a working volume of 450,000 L (PROD-01 to -12). Air is added to the production fermenters at 0.29 vvm. Chilled water at 6˚C is used to maintain the temperature of the fermenters at 31˚C and ammonia is added to maintain the pH at 7.5. Final DLM concentration in each of the 12 fermenters is 80 g/L. The entire fermentation process is exothermic and aerobic.

The batch time for the total fermentation is 117 hours, with a cycle time of 69 hours, allowing for 109 batches per year. Each set of two production fermenters is staggered by 11.5 hours. See Appendix A for stagger time calculations. The stagger time is the only unique aspect

of scheduling this process. There are no bottlenecks because the fermentation time is similar in the seed and production fermenters. After fermentation, the production fermenters are emptied into a storage tank (STOR-04). Due to the staggered start times, only two production fermenters are emptied at the same time.

13.1 Feed Material Storage and Preparation

Several raw materials must be introduced into the fermentation system in order to enable aerobic cell growth, pH control, and DLM production. These raw materials must be prepared and stored in appropriate conditions to ensure safety, maximum growth, and no operation delays. There will be storage tanks for the corn syrup, Teknova Inoculum Broth, and liquid ammonia solution, the design specifications of which are described in Section 15.1.

13.1.1 Corn Syrup Storage

Liquid corn syrup will be delivered to our production plant from the ADM Corn Processing plant in Cedar Rapids, IA via pipelines at a price of 55 cents/kg. Corn syrup will be mixed with the Teknova Inoculum Broth and then sterilized to be fed to the fermenters. Because corn syrup is the only glucose source to enable cell growth, a shortage of corn syrup would result in needing to shut down production and restart the 64-hour campaign period. A 3 day supply of corn syrup will be stored in the tank at 20^{o}C and atmospheric pressure.

13.1.2 Teknova Inoculum Broth Storage

The Teknova Inoculum Broth is necessary to provide nutrients for the cells to grow. Our team made the assumption that we worked with Teknova to develop a more concentrated broth than the one listed on its site. The current Teknova Inoculum Broth for sale is 90.8% water and

we worked with Teknova to make a broth that is 6.75% water. Because this new concentrated broth consists of much less water and because we are buying in bulk, we estimated a discount on the price to buy the broth at 51 cents per kg. The broth will be delivered in 30,000 L tank wagons so 39 tank wagons will need to come into the plant each week. A seven day supply of the concentrated Teknova broth will therefore cost around $818,000. The broth is normally only sold in 1 L quantities, so we made the assumption that we worked with Teknova to sell the concentrated broth in 10,000 L quantities to fulfill our supply. The broth includes water, yeast extract, soytone, glycerol, monosodium phosphate, monopotassium phosphate, ammonium chloride, and magnesium sulfate heptahydrate. The broth will be diluted with process water so it becomes 90.8% water and then mixed with corn syrup to become the fermentation media. The media is then sterilized to be fed to the fermenters. A summary of the concentrations of the nutrients in the storage tank can be found in Table 15.1.2 A seven day supply of the broth will be held in two storage tanks at 20^{o}C and atmospheric pressure.

13.1.3 Ammonia Storage

28 wt% ammonia solution will be delivered to our production plant from American Elements via 15,000 L truck shipments at a price of $800 per 100 metric tons. The ammonia solution will be easier for storage and transport than gaseous ammonia because of its liquid state. A year's supply of ammonia solution will be stored in a tank with 20% head space to allow for some vaporization. Ammonia is very stable so a year's supply can be stored in one storage tank without concerns about degradation. The ammonia will be stored at 20^{o}C and atmospheric pressure, and will be used for pH control in the fermenters.

13.1.4 Media Sterilization Heat Exchangers

The media made of corn syrup and Teknova broth is at 20°C and needs to be heated for sterilization and then cooled back down to 31°C for entrance into the fermentation train. This sterilization will be achieved through a series of three shell-and-tube heat exchangers. Unsterilized media will flow into the first heat exchanger at 52,275 kg/hr. The first heat exchanger heats from 20°C to 88°C, has an area of 54.4 m^2, and requires 40,641 kg/hr of recycled heated media at 121°C. A fraction of the heated media that comes out of the second heat exchanger is recycled as the heating liquid for the first exchanger in order to decrease the amount of steam needed to be purchased. The second heat exchanger heats from 88°C to 121°C, has an area of 766 m^2, and requires 450,382 kg/hr of medium pressure steam. The third heat exchanger cools from 121°C to 31°C, has an area of 532 m^2, and requires 990,840 kg/hr of cooling water. All of the heat exchangers will be made from stainless steel to minimize corrosion and contamination.

13.2 Air Compression and Filtration

The oxygen source for fermentation will be air. The air will require a compression and filtration system to avoid contamination and prepare the gas for introduction to the fermenters. Because air is used instead of pure oxygen, a significant amount of nitrogen gas will be introduced into the system which will be inert in the process and must be vented for release to the atmosphere.

13.2.1 Air Compression

Because ambient air is the source of oxygen for the fermentation process, an air compressor is needed to extract air and properly pressurize it. The air will be sparged into the cell culture from the bottom of the reactor, so the gas must be pressurized to the correct amount. The pre-seed fermenter will have a height of approximately 5.7 m, so the compressor will pressurize air to 2.9 bar. The seed fermenter will have a height of approximately 7.8 m, so the compressor will pressurize the air to 3.1 bar. The production fermenter will have a height of approximately 25.6 m, so the compressor will pressurize the air to 5.1 bar. The compressor will be an oil-free screw compressor.

13.2.2 Air Filtration

Because ambient air will be used for the process, several air filters will be needed to ensure the quality and sterility of the air before it is introduced to the fermenter. Two different filters will be placed on either side of the air compressor to purify the gas. The first filter will be an M6 class ‘course’ filter to remove any large debris or contaminants before the gas is pressurized. The second filter will be an H13 class ‘fine’ filter, a high-performance particle filter with a sub-micron pore size that will purify the air before entering the fermenter (Jahnke, Pillarella, Weiner). The submicron pores are small enough that any microorganisms in the air that could contaminate the reactor would be removed. The small filter size will introduce a pressure drop across the unit and may require additional gas compression (Jahnke, Pillarella, Weiner).

13.3 Seed Train Growth

In order for the cell mass to reach the amount needed for the production fermenter, the cells must be grown from lab scale to manufacturing scale. Beginning with 2 mL aliquots at 0.0013 g/L, the cell solution is grown to 500,000 L at 20 g/L. The seed train consists of 2 L flasks, pre-seed fermenters, seed fermenters and a batch process at the final production volume.

Seed train growth is important for the growth of cells in an efficient manner and for quality control of the cells. The lab staff will ensure the quality of the cells at each step of the seed train before moving the cell mass to the next reactor. If the cells have a quality issue, the staff can address it before the cell volume is at a manufacturing scale.

13.3.1 Cell Bank Storage

In order to ensure reproducibility of batches in the DLM production process, a cell bank is used to store and freeze cell samples. The bank also ensures fresh samples are available in the case of contamination or failed batches. To generate the cell bank, an initial culture undergoes rigorous quality testing to ensure no bacteria or fungi has infiltrated the sample. The culture is then fractionated into 2 mL aliquots to form the master cell bank. The aliquots are then frozen to preserve the cells until they are needed to inoculate new reactor batches. If the cell banks are near depletion, one of the aliquots will be used to create a new master cell bank (Jahnke, Pillarella, Weiner). A diagram of this process is shown in Figure 13.3.1.

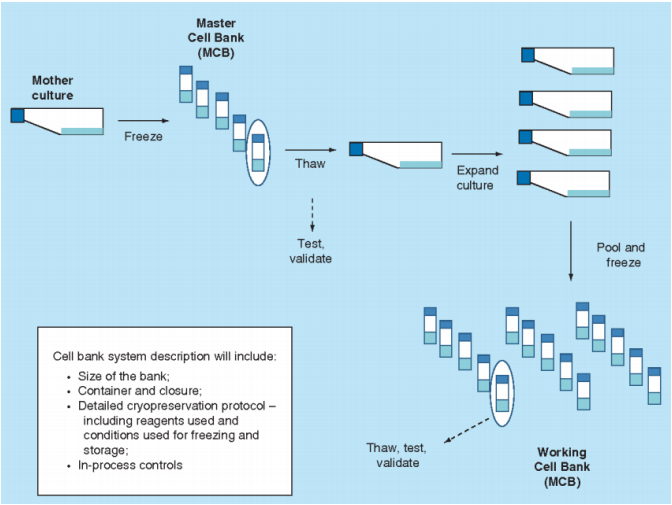


Figure 13.1.1. Process of creating a cell bank of 2 mL aliquots (Gargi).

The cell bank will store 300 2 mL aliquots of cells at a concentration of 0.0013 g/L. According to consultant Rick Bockrath, the freezer operates at -90ºC to ensure the cryopreservation of the cells. Dimethyl sulfoxide (DMSO) at 10% is used as a cryopreservation agent. Serum-free Freezing Media is used as the medium for cryopreservation. The cells are cooled at a rate of -1ºC/min (Jahnke, Pillarella, Weiner).

The low temperature seizes all molecular processes and prevents the generation of free radicals that would disrupt the efficacy of the cells after preservation. When the cells are thawed, the viability and purity of the cells are tested to ensure the cells are usable for the seed train. Personal protective equipment and biosafety precautions should be taken in order to guarantee the safety of lab personnel and the efficacy of the cell line (Jahnke, Pillarella, Weiner). Two cell banks will exist, one on site and one off site, in case of contamination or destruction of one of the cell banks.

13.3.2 2 L Flasks

24 2 L Erlenmeyer flasks are used as the first units to scale up the cells to manufacturing scale. Sterilized media will be fed into each flask at the beginning of the batch. Each flask will have a fill fraction of 25% and therefore a working volume of 0.5 L. Four 2 mL aliquot will be used to initiate cell growth in each 2 L flask. The final concentration in each flask will be 0.67 g/L, giving a final dry cell mass of 1.34 g. The efficacy, viability and purity of the cells are tested and 100% of the final batch product is fed to the next seed reactor to begin further cell growth. Four 2 L flasks are needed for each pre-seed reactor, which is the next unit in the seed train.

13.3.3 5,000 L Fermenters

Six 5,000 L batch fermenters are used as the second unit to scale up the cells to manufacturing scale. Sterilized media, compressed air, and liquid ammonia will be fed into each fermenter at the beginning of the batch. The reactor will have a fill fraction of 90% and therefore a working volume of 4,500 L. Cooling water through internal coils and external jackets will deliver cooling at the rate necessary to maintain a temperature of 31°C in the fermenter. The product from four 2 L flasks will be used to initiate cell growth in each individual reactor. The final concentration in each bioreactor will be 1 g/L, giving a final dry cell mass of 4 kg. A gas vent steam will be used to prevent pressure build up. The efficacy, viability and purity of the cells are tested and 100% of the final batch product is fed to the next seed reactor to begin further cell growth.

13.3.4 50,000 L Fermenters

Six 50,000 L batch fermenters are used as the third unit to scale up the cells to manufacturing scale. Sterilized media, compressed air, and liquid ammonia will be fed into each fermenter at the beginning of the batch. The reactor will have a fill fraction of 85% and therefore a working volume of 42,500 L. Cooling water through internal coils and external jackets will deliver cooling at the rate necessary to maintain a temperature of 31°C in the fermenter. The product from the 5,000 L reactors will be used to initiate cell growth. The final concentration in each bioreactor will be 6 g/L, giving a final dry cell mass of 255 kg. A gas vent steam will be used to prevent pressure build up. The efficacy, viability and purity of the cells are tested and 100% of the final batch product is fed to the continuous reactor to begin start up batch cell growth.

13.3.5 Chilling Machines

All of the pre-seed, seed, and production fermenters need cooling water to maintain a temperature of 31°C in the fermenter. The cooling water will be provided by chilling machines that pump out water at 6°C, which enter the fermenters through internal coils and external jackets. The water is then heated in the fermenters and pumped back out to the chilling machines to be cooled and go out to the fermenter again. The net requirement of cooling water for all the fermenters is 32MM liters. Assuming chilling machines that hold 3.6MM liters of cooling water, which was suggested by consultant Rick Bockrath, the process will need nine chilling machines.

13.4 Batch Growth Period

After the last seed reactor, the product from the 50,000 L reactors is the appropriate size to initiate cell growth in the continuous scale bioreactors of 500,000 L. Sterilized media, compressed air, and liquid ammonia will be fed into the fermenter at the beginning of the batch. The reactor will have a fill fraction of 90% and therefore a working volume of 450,000 L. Cooling water through internal coils and external jackets will deliver cooling at the rate necessary to maintain a temperature of 31°C in the fermenter. The final concentration in the bioreactor will be 20 g/L, giving a final dry cell mass of 9000 kg. A gas vent steam will be used to prevent pressure build up. The efficacy, viability, and purity of the cells are tested, and then the continuous fermenter growth will begin.

13.5 Continuous Bioreactor Growth

After the cells are grown in the 500,000 L fermenters to the target concentration of 20 g/L, the continuous phase of operation will begin. The continuous process consists of constant flow rates of products, reactants, and recycle streams flowing in and out of the fermenter system. Inlet streams to the bioreactor include sterilized media, compressed air, and liquid ammonia. Outlet streams from the bioreactor include a vented gas stream of nitrogen, carbon dioxide and excess oxygen and a liquid product stream which will then be heated. Each of the 500,000 L bioreactors has a campaign period of 64 hours to operate continuously before the operation must cease for the bioreactor to be sterilized and restarted with a new batch growth period. The 12 production fermenters will run on staggered schedules so that at least 6 fermenters are running at all times. One of the production fermenters is cleaned and restarted every 64 hours.

13.5.1 500,000 L Fermenters

12 500,000 L bioreactors will operate in coupled systems for bacteria to grow and DLM and FEMA No. 4907 byproduct to be formed. The reactor will have a fill fraction of 90% and therefore a working volume of 450,000 L. Cooling water will deliver cooling at the rate necessary to maintain a temperature of 31°C in the fermenter. The bioreactor will be maintained at a pressure of 1.2 bar, slightly above atmospheric pressure, to ensure that in the case of a leak, external contaminants will not immediately enter the bioreactor (Jahnke, Pillarella, Weiner). A cell density of 20 g/L will be maintained in the bioreactor, and reactant and product streams will flow in and out of the bioreactor at the flow rates specified in Section 12.2. The bioreactor will include agitation with power of 1118 kW.

13.5.2 Fermentation Product Storage

Each batch of solution coming out of two production fermenters will empty into a storage tank. This storage tank is for the transition from batch to continuous process. Once two fermentation batches have been completed and emptied into the storage tank, the contents of the tank will be pumped into a heating unit to solubilize DLM in solution. A 6 day's supply of fermentation product will be stored in the tank at 31°C and atmospheric pressure.

13.5.3 Cooling Water

Due to the highly exothermic nature of the bacterial growth process, large amounts of heat will be created in each bioreactor. 4.8 kW of heat is created in each pre-seed reactor, 273 kW of heat is created in each seed reactor, and 9,648 kW of heat is created in each production fermenter so extensive cooling is needed to maintain an operation temperature of 31°C in the

fermenter. The bioreactor will be made of smooth stainless steel to prevent corrosion and allow for easy sterilization between the continuous operation periods.

13.6 Fermentation Product Heat Exchanger

The first step in the downstream process after fermentation is the heating of the resulting fermentation broth to completely solubilize DLM produced. Initially, approximately 50% of DLM is in solid phase due to the low solubility of DLM in the fermentation broth at 31°C. Thus, the broth is heated from 31°C to 70°C using a floating-head, counter-current shell-and-tube heat exchanger; Steam at 180°C and 1 bar will be passed through the shell side while passing the broth through the tube to accomplish the heating. Required heat duty is 32 MW.

13.7 Disc Stack Centrifugation

The outlet of the fermentation product heat exchanger is flowed through a separator in order to separate the biomass pellets from the DLM containing supernatant. A total of three continuous nozzle centrifuges operate at 70°C and at 1 bar. The separators are assumed to completely separate biomass from the fermentation product broth such that the resulting supernatant stream does not contain any biomass. The pellet is assumed to contain 75% moisture content. The electricity needed to operate one separator unit is 170kW and the process water needed to ensure safety and to flush is 45 kilo-tonnes per year per unit. In total, 510kW of electricity and 134 kilo-tonnes of process water.

13.8 Rotary Drum Dryer

After the biomass is separated from the DLM containing liquid stream, the pellet is dried in a rotary drum dryer to decrease the moisture level to 10% and to kill the biomass at a high

temperature so that it can be sold as animal feed additive. Direct fired rotary drum dryer that utilizes heated natural gas is used to heat the side product stream from 70°C to 121°C. The outlet stream of the rotary drum dryer is assumed to contain all of the inlet biomass with decreased water content. 777MM kilograms of natural gas and heating duty of 26,000 MW is used per year.

13.8.1 FEMA 4907 Packaging

Once the biomass has been separated and dried, the FEMA 4907 pellets are funneled into super sacks to be sold.

13.9 Triple Effect Evaporation

After the FEMA 4907 side product is separated from the main product stream, the downstream flow goes into triple effect evaporation. The evaporation removes water and concentrates the amount of DLM in the stream from 6 wt% to 21 wt%. Triple effect evaporation consists of three stages of heat exchangers and flash vessels in series, with each stage operating at a lower temperature and pressure than the previous. The first stage operates at 159^{o}C and 6 bar, the second stage operates at 144^{o}C and 4 bar, and the third stage operates at 112^{o}C and 1.5 bar. Each of the three heat exchangers will have condensate streams which will be cooled and recycled to be used as process water diluting the Teknova broth and cooling water in the crystallizer. The steam from the third effect will also be recycled to be used in the first effect.

13.10 Crystallization

After triple effect evaporation, the liquid product stream from the flash vessel of the third effect flows into three crystallizers. Each crystallizer will precipitate out solid DLM that will be

part of a slurry that is 76 wt% water. 92% of the liquid methionine flowing into the crystallizer will precipitate out of solution due to cooling. The feed inlet will flow through a low-shear axial pump into a heat exchanger that cools the stream from 112ºC to 5ºC. 5ºC was chosen as the end temperature as recommended by Patent US7785846. The solubility of DLM at this temperature is 20.53g/L. A low-shear axial pump is used to reduce secondary nucleation. The velocity of the feed will be kept at a minimum of 2.44 m/s to prevent material from salting out on the tubes. The crystallization will take place at atmospheric pressure. Approximately a total of 34,468 kg/hr of DLM will be crystallized out of solution.

13.11 Decanter Centrifugation

The slurry out of the three crystallizers will feed into a cylindrical-conical screen bowl centrifuge. This centrifuge contains a rotating bowl connected to a conveyor and has continuous feed and discharge. The total inlet flow rate to the unit is 178,430 kg/hr. As stated in Section 13.10, this flow rate includes DLM solids which flow at 34,468 kg/hr. The purpose of this centrifugation step is to isolate the wet DLM cake from the remaining liquid stream, as opposed to placing a large duty on heating units by trying to evaporate such large amounts of water. Solids are discharged over the conical section and are further dewatered by means of a cylindrical screen. Because the outlet liquid stream is 95% water, it is sufficiently low in other components (i.e., salts, glucose, DLM), such that it can be cooled, sterilized, and recycled as process water for other units. These centrifugation units are considered to be high separation machines, so a recovery of 99.5% of solids was assumed. The total flow out of the centrifuge is 42,381 kg/hr, which includes 19% liquid moisture in the DLM cake.

13.12 Rotary Dryer

After centrifugation, the solid stream containing the DLM product will be a wet crystalline cake with 19% moisture. The final DLM to be packaged in Super Sacks is desired to have 1-2% moisture to minimize mold and bacterial growth, so a rotary dryer system will be used to dry the crystalline methionine to 1% moisture. The dryer system includes an induced draft (ID) fan to feed the gases, a cyclone, and a baghouse. The ID fan uses pressure to pull exhaust gases and particulates through the system and facilitates their removal. The cyclone uses centrifugal force to recover the bulk of any large particulates. The baghouse filters particulates out of the process air, assisting in pollution control and product recovery. These components support proper handling of exhaust gases in compliance with emissions requirements.

To keep dryer design specifications within reasonable limits, there will be a total of 4 rotary dryer units to accommodate and handle the large amount of solid flow. Each dryer will have a wet cake input stream of 19% moisture at 5^{o}C flowing in at 10,595 kg/hr. Hot air at 170^{o}C with 0.7% humidity will enter each dryer at a rate of 91,762 kg/hr. Inlet air velocity will be kept at 1.5 m/s to minimize dusting of the solid. Water will be evaporated at a rate of 1,950 kg/hr from each unit. The exit flow rate of the solid DLM stream (1% moisture) will be 8,770 kg/hr, for a total of 35,080 kg/hr DLM of 99% purity. The solid stream will be leaving the dryer at 80^{o}C. Assuming 85% uptime, this produces 250 kilotonnes of the DLM product per year.

13.12.1 Conveyor

The dried DLM product stream which exits the rotary dryer will be transported via a feed bucket conveyor to a storage tank one floor above. The feed bucket conveyor was chosen on the basis of trying to minimize crystal breakage. Other conveyor methods could potentially work as

well, but would require more thorough knowledge of the behavior of crystalline DLM. This conveyor will be approximately 30 feet long. It is important to note that the product leaves the dryers at 80ºC. As a burn protection measure for workers, the conveyor will be equipped with a cooling system to lower the product temperature to 50ºC. This will allow for safer handling of the product material.

13.12.2 DLM Product Storage

A storage bin is to be placed above the packing station in order to receive product from the conveyor unit and will store eight hours of material, so that several super sacks can be filled consecutively without emptying the bin.

13.13 DLM Final Packaging

From the storage unit, the DLM will be ready to be packaged for final distribution. The DLM will be dropped into large plastic super sacks via gravity from the storage unit located one floor above the packing station. These super sacks will roughly hold two tons of product. Once the package is filled, it will be ready for distribution.

14. Energy Balance and Utility Requirements

14.1 Utility Requirements

Table 14.1. Yearly amounts and costs for utilities needed in the process.

Utility	Yearly Amount (kg/yr)	Yearly Cost ($)
Medium Pressure Steam	4,107,090,000	$63,249,186
Low Pressure Steam	14,967,750,000	$198,023,333
Cooling Water	7,448,435,001	$196,529
Process Water	1,080,088,115	$227,987
Natural Gas	915,802,500	$201,934,451
Total:	28,519,165,620	$463,631,486

The utilities needed for this project are displayed in Table 14.1. The largest expense in utilities is due to the steam that is needed for media sterilization, the heat exchanger to heat the liquid product out of the fermentation, and triple effect evaporation. Chilling machines will supply the cooling water for the heat exchangers and the operation of these machines is reflected in the price above. Process water will be obtained from suppliers in the Midwest at ambient conditions.

The main utility needs for this process include chilled water for the fermentation vessels, cooling water for heat exchangers, electricity for operation of pumps and compressors, medium pressure steam for the media sterilization, and natural gas for the rotary dryers.

It was estimated that the pre-seed fermenters, seed fermenters, and production fermenters consume 1,407, 14,070, and 140,695 kg O_2 per batch, respectively. The amount of chilled water needed for each fermenter was calculated from the OUR and working volume of each respective reactor. Defining an overall heat transfer efficiency of 70% between submerged coils and the fermentation liquid and using chilled water at 6°C, we conservatively estimated a chilled water cooling requirement for each of the fermenters. Details of the calculations can be found in Appendix A.

14.2 Electricity Requirements

Table 14.2. Yearly energy requirements for process units.

Source	Duty (kW)	Yearly Energy Consumption (kW-hr)	Yearly Cost ($)
Pumps	157	1,175,600	82,292
Air Compressor	133	997,500	69,825
Pre-seed reactors (x6)	6.71	50,341	3,524
Seed reactors (x6)	381	2,854,422	199,810
Production reactors (x12)	14,794	110,956,230	7,766,936
Centrifuges	510	3,825,000	267,750
Decanter Centrifuge	300	2,250,000	157,500
Rotary Dryer	38	285,000	19,950
Conveyor	37	280,000	19,575
CIP System	17	127,500	8,925
SIP System	17	127,500	8,925
Total:	16,391	122,929,093	$8,605,012

The yearly energy requirements for the units involved in this process are detailed in Table 14.2. The yearly energy consumption of each unit was calculated from its kilowatt operating power for a 340 day work cycle. For units that are not part of the continuous process, like the reactors, the periods of down-time between use were considered when calculating the yearly energy consumption of those units. The majority of the energy costs are associated with the production fermenters. This is to be expected as they are 500,000 L reactors with 1,118 kW agitators and the process requires twelve of them.

15. Equipment List and Unit Descriptions

15.1 Feed Material Process Units

15.1.1 Corn Syrup Storage Tank

One 576,000 L stainless steel storage tank with a cone roof will be used to store a three day supply of corn syrup at room temperature of approximately 20°C and 1 bar. The corn syrup will be mixed with the Teknova broth to create the media for fermentation. The bare module cost of this storage tank is estimated to be $510,974 according to the Equipment Costing Spreadsheet.

15.1.2 Teknova Inoculum Broth Storage Tank

Two 640,000 L stainless steel storage tanks with cone roofs will be used to store a week's supply of inoculum broth at room temperature of approximately 20°C and 1 bar. The Teknova broth will be diluted with process water until it is 90.8% water. 553,000,000 L of process water per year will be needed to dilute the Teknova broth. The diluted Teknova broth will be mixed with the corn syrup to create the media for fermentation. The bare module cost of an individual storage tank is estimated to be $539,306 so the total bare module cost for both tanks is $1,078,612. according to the Equipment Costing Spreadsheet. Table 15.1.2 shows the makeup of the diluted Teknova Broth.

Table 15.1.2. Composition of the diluted Teknova Inoculum Broth to be entered into the mixer for combination with corn syrup.

Component Name	% of Broth
Water	6.75
Yeast Extract	48
Soytone	25
Glycerol	10
Monosodium Phosphate	6
Monopotassium Phosphate	3
Ammonium Chloride	1
Magnesium Sulfate Heptahydrate	0.25

15.1.3 Ammonia Storage Tank

A 37,000 L stainless steel storage tank with a cone roof will be used to store a year's supply of ammonia solution. The ammonia will be stored at 20°C and atmospheric pressure. The flow rate of ammonia into each of the production bioreactors is approximately 38.3 kg/hr, the flow rate into each of the seed bioreactors is approximately 3.62 kg/hr, and the flow rate into each of the pre-seed bioreactors is approximately 0.383 kg/hr. Therefore, the size tank should be sufficient in supplying all of the reactors for a year. Ammonia will enter at the bottom of all the fermenters as a pH controller. This storage tank is estimated to cost $126,437 according to the Equipment Costing Spreadsheet. Since the liquid ammonia will expand 850 times when evaporating, it is important that the tank has enough head space and can withstand a high enough pressure to allow for the variations in the ambient temperature of the tank due to climate

seasonality (Jahnke, Pillarella, Weiner). Gaseous anhydrous ammonia is not chosen as the form of ammonia because ammonia gas is highly toxic.

15.1.4 Media Sterilization Heat Exchangers

The media made from corn syrup and the Teknova broth needs to be sterilized to prevent contamination. Table 15.1.4 shows the composition of the media.

Table 15.1.4. Composition of the media for entry into the media sterilization heat exchangers.

Component Name	% of Broth
Water	81.94
Glucose	9.99
Yeast Extract	4.16
Soytone	2.16
Glycerol	0.87
Monosodium Phosphate	0.52
Monopotassium Phosphate	0.26
Ammonium Chloride	0.09
Magnesium Sulfate Heptahydrate	0.02

Three heat exchangers in series are used to heat the premade media from 20°C to 121°C and then cool it back down to 31°C so that it can be pumped into the fermentation process. The first heat exchanger heats from 20°C to 88°C, the second heat exchanger heats from 88°C to 121°C, and the third heat exchanger cools from 121°C to 31°C. All three heat exchangers are shell-and-tube floating head and are made of stainless steel. The areas of the heat exchangers are

54.4 m^2, 766 m^2, and 532 m^2 respectively. The bare module cost of the heat exchangers are $419,422, $1,505,748, and $1,148,453 respectively. The total bare module cost for the three heat exchangers is therefore $3.1MM.

15.1.5 Air Compressor

The air compressor is required to feed air to the pre-seed fermenters at 2.9 bar, the seed fermenters at 3.1 bar, and the production fermenters at 5.1 bar. The compressor will have a three-phase 178 horsepower engine that will operate at 3,250 rpm. All of the air will come out of the compressor at 5.1 bar. The size of the inlet control valve for the pre-seed and seed reactors will be adjusted so that the pressure drop of the valve equates to air at 3.1 bar going into the seed reactors and air at 2.9 bar going into the pre-seed reactors. The purchase cost of the compressor is $327,596.

15.1.6 Coarse Air Filters

Opakfil 2V air filters will be purchased from Camfil to serve as the first stage of air filtration. The M6 class filters will provide a 'coarse' filtration of incoming air to remove large contaminants. The filter media will be glass fiber with a media area of 8 m^2. The filter will be a V-bank filter with an ABS frame. The filter will add a negligible pressure drop that should not affect the amount of compression required. If the compressor needs to be replaced, the unit is fully incinerable. The dimensions of the filters will be 0.6 x 0.6 x 0.2 m. Each air filter will cost $4,000 (Jahnke, Pillarella, Weiner).

15.1.7 Submicron Air Filters

Absolute VG air filters will be purchased from Camfil to further purify air to be used in the process. The H13 class filters will have submicron pores to sterilize incoming air before it is introduced to the process. The filter media will be glass fiber with a media area of 46 m^2. The filter will be a V-bank box filter with an ABS frame. The filter will add a negligible pressure drop that should not affect the amount of compression required. The filters will be halogen-free. The dimensions of the filters will be 0.75 x 0.6 x 0.3 m. Each air filter will cost $4,000 (Jahnke, Pillarella, Weiner).

15.2 Seed Train Process Units

15.2.1 12 mL Test Tubes

Thermo Scientific Matrix 12.0 mL ScrewTop Tubes in Barcoded Latch Racks 3775BR will be used to store the genetically modified cells in the onsite cell bank. Each tube will be filled with 2 mL aliquots and there will be at least 192 tubes. These tubes are capable of storing cells at lower temperatures and will be stored at -90^{o}C in a freezer in the lab. The material is medical grade polypropylene, and the tubes come with certified sterility. Each package has 4 racks of 24 tubes per case. Two 96 tube packages will be purchased at $222 per package.

15.2.2 2 L Erlenmeyer Flasks

Thermo Scientific 2 L solid, non-vented Nalgene Erlenmeyer Flasks with Plain Bottom will be used to scale up the seed train. The material is polyethylene terephthalate, and the flasks come with certified sterility. Each flask will be loaded with four of the 2 mL aliquots and media to obtain a net 0.5 L of liquid volume. The flasks will be shaken on a table shaker for

approximately 24 hours. This process is operated in the lab to account for any growth problem. Four flasks are needed per pre-seed fermenter so six packages of four flasks will be purchased at $95 each for a total of $570.

15.2.3 5,000 L Pre-Seed Fermenters

Each of the six pre-seed fermenters is 5,000 L with a working volume of 4,500 L. The fermentation media of Teknova inoculum broth, corn syrup, and *C.glutamicum* are initially charged to the fermenter. The cycle time is 30 hours, which includes 22 hours of cell doubling, and 8 hours of turnaround. The fermenter is aerated at 0.29 vvm and non-dissolved gases exit through a vent. Ammonia is added to maintain the pH at 7.5 and the temperature is maintained at 31 ºC with 485 kg/hr chilled water at 6ºC. The molar yield of DLM is 0.217 mol per mol of glucose consumed and the productivity is 0.055 g/L-h. The concentration of DLM in the broth after fermentation is 80 g/L. The seed fermenter is 5.7 meters tall and has a diameter of 1.1 meters. It is constructed from stainless steel to prevent rust and corrosion. The total bare module cost of the fermenter, including the chilled water coils, is $233,000. The combined total bare module cost for all seed fermenters is $1.4MM.

15.2.4 50,000 L Seed Fermenter

Each of the six seed fermenters is 50,000 L with a working volume of 42,500 L. The fermentation media of Teknova inoculum broth, corn syrup, and *C.glutamicum* are initially charged to the fermenters. The cycle time is 23 hours, which includes 15 hours of cell doubling and 8 hours of turnaround. The fermenters are aerated at 0.31 vvm and non-dissolved gases exit through a vent. Ammonia is added to maintain the pH at 7.5 and the temperature is maintained at 31 ºC with 27,500 kg/hr chilled water at 6ºC. The molar yield of DLM is 0.217 mole per mole

of glucose consumed and the productivity is 0.33 g/L-h. The concentration of DLM in the broth after fermentation is 80 g/L. Each seed fermenter is 7.7 meters tall and has a diameter of 2.87 meters. It is constructed from stainless steel to prevent rust and corrosion. The total bare module cost of each fermenter, including the chilled water coils, is $730,000. The combined total bare module cost for the six seed fermenters is $4.4MM.

15.2.5 Chilling Machines

All of the fermenters need cooling water. Nine chilling machines will deliver cooling water at 6°C for all pre-seed, seed, and production fermenters. The cost of the chilling machines is based solely off the cost of the cooling water. Table 17.1 in Product and Process Design Principles 4th Edition is used to cost the machines. The fermenters need 32MM kg of cooling water, so the cost of the chilling machines will be $19.2MM.

15.3 Continuous Process Units

15.3.1 500,000 L Production Fermenters

Each seed fermenter feeds into two 500,000 L production fermenters with a working volume of 450,000 L. The cycle time is 64 hours, which includes 20 hours of biomass growth, 32 hours of methionine production, and 12 hours for turnaround. The fermenter is aerated at 0.31 vvm and non-dissolved gases exit through a vent. Ammonia is added to maintain the pH at 7.5 and the temperature is maintained at 31 °C with 970,500 kg/hr chilled water at 6 °C. The molar yield of DLM is 0.217 mole per mole of glucose consumed and the productivity is 1.1 g/L-h. The concentration of DLM in the broth after fermentation is 80 g/L. The production fermenter is 25.6 meters tall and has a diameter of 5 meters. It is constructed from stainless steel

to prevent rust and corrosion. The total bare module cost of the fermenter, including the agitator and chilled water coils, is $3.1MM. The combined total bare module cost for all production fermenters is $37.2MM.

15.3.2 Fermentation Product Storage Tank

A 1,220,000 L stainless steel storage tank with a cone roof will be used to store two batches worth's supply of the liquid product from fermentation. Sizing of the storage tank between batch and continuous processes was based on the mean residence time in the vessel. Since fermentation times are staggered, it was especially important to anticipate the maximum working volume and purchase storage tanks large enough to handle the volume input. The liquid will be stored at 31^{o}C and atmospheric pressure. The flow rate of the solution into the fermentation product heating unit is around 660,000 kg/hr. This storage tank was estimated to cost $750,000 according to the Equipment Costing Spreadsheet.

15.3.3 Gas Scrubber

Three model CS-17 chemical scrubbers will be purchased from Pollution Systems to remove contaminants from the vent gases of any cell culture before they are released to the atmosphere. Each scrubber will process vent gas at a flow rate of roughly 115,630 kg/hr and the scrubbing liquid will be water. The unit will be a packed bed scrubber made out of stainless steel. Each scrubber stack will have a height of 11 m and a diameter of 0.71 m. The unit will come equipped with a 30 horsepower process fan and 34,000 L/hr recycle pump (Jahnke, Pillarella, Weiner). The three gas scrubbers will cost $56,000.

15.3.4 Fermentation Product Heat Exchanger

One unit of counter-current, shell-and-tube heat exchanger will be used to heat the product liquid stream from 31°C to 70°C. 1,996,000 kg/hr of steam at 1 bar and 180°C will pass through the shell side to heat the liquid stream that passes through the tube side at a flow rate of 684,000 kg / hr. The heat duty for the heat exchanger is 32MW and the necessary surface area is 930 ft^2. The material of the heat exchanger is stainless steel and the length is 20 ft. Floating-head will be used due to the large flow rates. The purchase cost of this heat exchanger will be $112,000.

15.3.5 Disc Stack Centrifuges

Three units of stainless-steel Alfa Laval FEQX 520S-31CG large capacity disc stack nozzle centrifuges will be purchased to separate the biomass containing pellets from the DLM containing supernatant exiting the fermentation product heat exchanger. The units are continuously operated using 18 nozzles to remove the biomass-containing stream. The dimension of each centrifuge is 3 meters in height and 1.45 meters in diameter. The process occurs at 70°C and 1 bar. Each centrifuge will process a flow rate of 228,000 kg/ hr. Each unit consumes 170kW of power and 45 kilo-tonnes of process water, which is needed for purging and for safety. The resulting product streams are DLM containing liquid stream and 75% moisture biomass side stream. Each separator will cost $400,000.

15.3.6 Rotary Drum Dryer

1 unit of FEECO direct fired rotary drum dryer will be purchased. The biomass containing stream with moisture content of 75% exiting the centrifuge will flow through the stainless-steel rotary drum dryer to decrease the moisture content to 10%. Heated natural gas at

180°C will pass through the drum dryer at a flow rate of 103,600 kg / hr in counter-current direction. The unit will process 3,300 kg / hr of incoming stream continuously. The dimension of the unit is 30.5 meters in height and 4.6 meters in diameter. The heat duty of the drum dryer is 3.5 MW and the price of the natural gas is $3,426,000 per year. The purchase cost of the rotary drum dryer is $750,000.

15.3.7 Triple Effect Evaporation

The product from the centrifuges contains 6 wt% DLM. To obtain the desired amount of DLM product, it was necessary to concentrate the product to 21 wt% DLM. This was done with a triple effect falling film evaporator with forward feed arrangement. The feed to the evaporators unit is at 70 ºC and 6 bar. Low pressure steam is used to evaporate 133,003 kg/hr of water from the first effect operating at 6 bar. Vapor from the first effect is used to evaporate 146,865 kg/hr of water from the second effect. For this vapor to achieve evaporation in the second effect, it was necessary to reduce the pressure of the liquid feed to the second effect from 6 bar to 4 bar. The vapor from the second effect is used to vaporize 162,770 kg/hr of water from the third effect. To achieve this operation, it was necessary to lower the pressure of liquid feed to the third effect from 4 bar to 1.5 bar. The temperatures of the for the first, second, and third effects are 159ºC, 144ºC, 112ºC respectively.

The liquid condensate from the heat exchangers of the second and third effects is cooled and then recycled to be used as process water for the fermenters, and is pumped to the crystallizers for use as cooling water. The vapor from the third effect is recycled as steam for the first effect. Once the first batch is complete, the water from the triple effect evaporation can start

supplying 676 kg/hr per batch of process water to the fermenters and 394 kg/hr per batch of cooling water to the crystallizers.

Use of falling film evaporators was suggested to avoid side reactions. The 99% purity required for the product necessitated use of the expensive falling film evaporators network. The total bare module cost of the triple-effect evaporator unit is $8.3MM.

15.3.8 Crystallizer

The three crystallizers were sized using a residence time of 60 minutes, as recommended by consultant Daniel Green, to meet hourly production requirements and form solid crystals of appropriate size. Based on an inlet mass flow of 59,477 kg/hr into each crystallizer, the dimensions of the vessel were calculated to be 3.8 m in diameter and 5.75 m in height. A height to diameter ratio of 1.5 was recommended by industrial consultants in order to give the dimensions highlighted above. A particle size of 50 μm was assumed based on recommendations from consultant Art Etchells. Literature was found that stated the particle size of L-methionine to be 9.5 μm, but this small of a particle size would require upwards of 50 crystallizers of the same current size. The three crystallizers produce approximately 34,468 kg/hr of DLM crystals. The net heat duty requirement for all three crystallizers is 76,625 kW, which is satisfied by supplying each vessel with approximately 14,333 kg/hr of cooling water at 6^{o}C. The total heat duty is the sum of the duty of each heat exchanger and the heat of fusion of DLM. The bare module cost for an individual crystallizer is $1,357,000 so the net bare module cost for all three crystallizers is $4.1MM.

15.3.9 Decanter Centrifuge

The slurry out of the three crystallizers will feed into a cylindrical-conical screen bowl centrifuge to significantly dewater the wet DLM cake down to a moisture content of 19%. There will be a continuous feed of 178,430 kg/hr and discharge DLM solids with 19% moisture at a rate of 42,381 kg/hr to continue on to the subsequent drying step of the process. Up to 135,878 kg/hr of liquid (95% water, 5% salts, glucose, DLM, organic byproducts) can be cooled, sterilized, and recycled as process water to other units. Consultant Richard Bockrath provided specifications for a centrifuge which meets this capacity. A machine with a 44- inch bowl diameter and a 400Hp motor with 1600 RPM can handle up to 700 gpm of liquids and up to 10 tons/hr of solids. These centrifugation units are considered high separation machines, so a recovery of 99.5% of solids was assumed, per Rick Bockrath's recommendation. The centrifuge purchase cost for stainless steel construction in 1981 was $150,000 (Perry, Green). The total bare module cost for the decanter centrifuge is $437,500.

15.3.10 Rotary Dryer

The product from the crystallizers will be a wet cake of 19% moisture. The final step in the downstream process is drying the DLM product to reach a final moisture content of just 1%. Sizing calculations for the rotary dryer unit were performed with assistance from consultant Richard Bockrath. The rotary dryer system includes an induced draft (ID) fan to feed air, a cyclone, and a baghouse. This support equipment ensures appropriate handling of the exhaust gas to comply with local, state, and federal regulations. This type of dryer system typically features very high recovery rates because the solid does not leave the system, so 100% recovery was assumed for the baghouse.

To achieve realistic equipment dimensions, 4 rotary dryer units were designed. Based on an inlet mass flow rate of 10,595 kg/hr of wet crystalline product at 5°C to each dryer, and an inlet mass flow rate of air at 170°C with 0.7% humidity of 91,762 kg/hr, each dryer will have a diameter of 6 m and a length of 20 m. The inlet air velocity will be 1.5 m/s to avoid dusting of the solid. The outlet solid stream flowing at 35,080 kg/hr will contain 1% moisture and be of 99% purity. Electricity requirements for the 38 kW motor total to $ 19,950 per year. Natural gas requirements for heating the air to 170°C total to $ 7,274,020 per year per dryer. The bare module cost of a single rotary dryer unit constructed from stainless steel is $1.49MM, so the net bare module cost for 4 rotary dryers is $5.96MM.

15.3.11 Conveyor

A feed bucket conveyor system is used to transport the dried DLM product from the dryers up to a storage tank. This conveyor configuration was recommended by consultant Rick Bockrath to minimize crystal breakage during transport. Rick provided an estimate for a conveyor with a length of 30 feet for $50,000 bare equipment. A motor load of 50Hp for the conveyor was provided by Prof. Bruce Vrana. Prof. Vrana and Rick Bockrath also suggested supplementing the conveyor with a cooling system priced at $10M which cools the DLM product from 80°C to 50°C as a burn protection measure for plant workers. Full, complete design of the conveyor remains part of the future work for this process. The bare module cost for the conveyor is approximately $109M.

15.3.12 DLM Storage Tank

A storage tank placed above the packing station will receive product from the conveyor unit. The storage unit will include a live bottom which will allow solid product to fall via gravity into the super sacks packing station below it. The maximum working volume of the tank will be 281M liters to be able to store and fill several hours of material. This large volume permits the filling of several super sacks consecutively without emptying the tank. The storage unit was priced with assistance from Prof. Vrana for a total bare module cost of $393MM.

15.4 Additional Units

15.4.1 Pumps and Piping

The process will require several rudimentary pumps to provide the driving force necessary to transport the materials at the flow rates detailed in the stream reports and mass balances in Section 12. Pipe diameter considerations and pump design were considered out of scope for this project due to time constraints. All 22 pumps which have been costed are centrifugal pumps with 100 feet of head based on recommendations from Professor Bruce Vrana and consultant Art Etchells. The pressure drops associated with the pumps range from 0.37 bar to 5.7 bar, and the resulting power requirements range from 2 to 64 kW. There are six larger pumps which are responsible for transporting the inputs and outputs to and from the production fermenters. These pumps have an average bare module cost of $144,430. The other 16 pumps transport contents at a smaller flow rate because they either follow a splitter, or their contents are the biomass side stream, which is small in comparison to the main product streams. These have an average cost of $36,480. The pumps and piping will all be constructed from stainless steel.

Motors use totally enclosed fan cooled motor enclosures for protective purposes, since none of the materials are explosive.

15.4.2 Mixers and Splitters

Mixers and splitters of two or more fluid streams are needed at various points through the process, including in the creation of the premade media, and the feeding of compressed air and sterilized media to the pre-seed, seed, and production fermenters. In all of these cases, the streams being mixed or split consist of fairly homogeneous fluids; therefore, the mixing and splitting can be accomplished by a tee in the pipeline and appropriate flow measurements, controllers, and valves. All of these considerations are assumed to be covered by the bare module costs of the other process equipment, so the mixers and splitters in this process were not designed nor costed specifically.

15.4.3 Bank of Chillers

A bank of chilling machines is necessary to provide the cooling water needed for the fermenters in the process. The pre-seed, seed, and production fermenters will require 998,485 kg/hr of cooling water, with the largest need for cooling water coming from the production fermenters. The cooling water for all processes will need to be chilled to 6ºC. Due to the warm weather for half of the year in Cedar Rapids, Iowa, this chilled temperature can only be achieved with refrigeration. During the five cold months, this chilled temperature can be achieved without refrigeration. The yearly cost of the operation and maintenance of the cooling tower is reflected in the cost factors used when calculating the cost of utilities in Sections 14.1 and 19.2.

15.4.4 Steam Generator

A steam generator will be installed on site to provide the heated steam necessary for the project. The process will require 4,107,090,000 kg of medium pressure steam per year and 14,967,750,000 kg of low pressure steam per year. The majority of the steam will go to the heat exchanger that heats the liquid product out of the fermentation process. Steam is also needed in the media sterilization process and in the first effect of the triple effect evaporation. For the media sterilization and triple effect evaporation, the steam will be produced at 175°C and 8.9 bar. For the fermentation product heat exchanger, the steam will be produced at 180°C and 1 bar. The yearly cost of the operation and maintenance of the steam generator is reflected in the cost factors used when calculating the costs of utilities in Sections 14.1 and 19.2.

15.4.5 Clean-In-Place System

The Clean-In-Place (CIP) system is an automated cleaning system to clean the inside of the 500,000 L fermenters, pipes, and mixers between continuous batches. This will occur every 64 hours when a fermenter is emptied and refilled with new cell mass from the seed process. A single tank CIP system by Sani-Matic will be used for this process. A customized Sani-Matic Ultra Flow 110 will be used. This system is portable, so it can be easily transported in case the fermenters are far apart. The unit has a small footprint and also has the ability to self-clean. High turbulent flow rates and low water requirements make this unit very effective in cleaning process equipment. Diaphragm control valves set the cleaning circuit flow rates and control the rate of discharge to drain. Chemical conductivity is used to provide proof of rinse (Jahnke, Pillarella, Weiner). The tank schematic is shown below in Figure 15.4.5 (Sani-Matic).

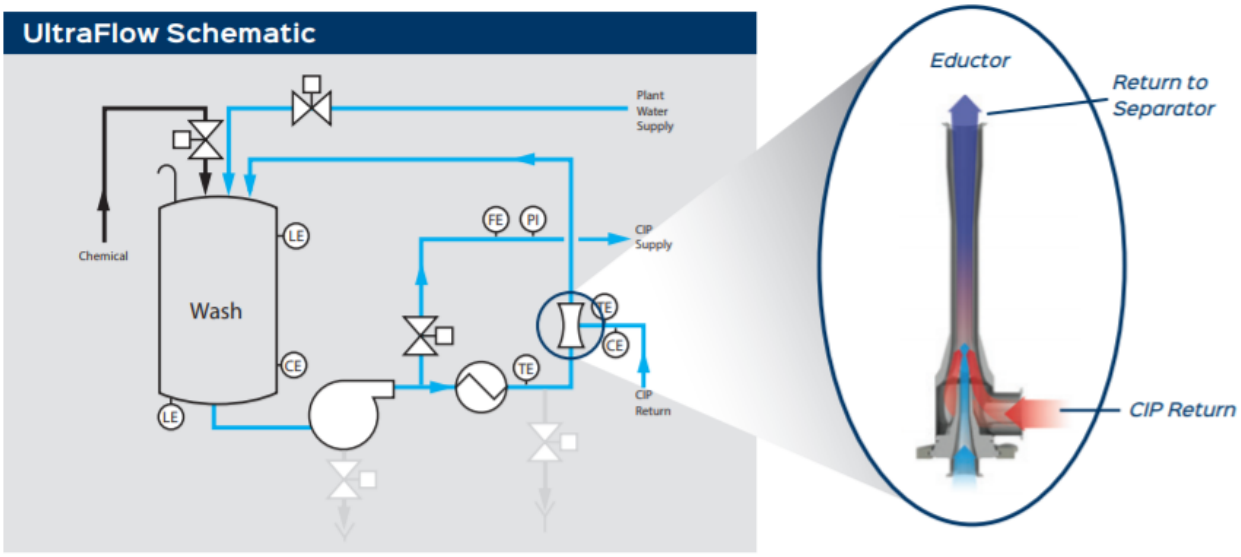


Figure.15.4.5. Schematic diagram of the Clean-In-Place system (Sani-Matic).

15.4.6 Sterilization-In-Place System

The Sterilization-in-Place (SIP) system is combined with the CIP system to ensure the final and automatic sterilization of units before the next batch is started. The SIP system ensures safety and efficiency, prevents toxic contamination of the product, and minimizes recontamination of the process. The SIP system also monitors and records critical process parameters. Saturated pure steam at a temperature higher than 121°C will be used to clean all fermenters, pipes, and mixers between continuous batches. A single-tank SIP system by Solida Biotech will be used for this process ("Clean-in-Place").

15.4.7 Final Packaging

The final product will be packaged in super sacks, each holding a mass of 1,800 kilograms of product. To accomodate for the production volumes of the plant, approximately 390 super sacks will be filled daily for shipment. Each super sack costs $29 and can be

purchased from ULINE, for a total of $4,128,472 per year. The super sacks are made of a woven polypropylene material and feature a duffel top with a tie closure as well as four lift loops on the corners for easy transport. It is assumed that buyers would be purchasing our product in units of super sacks for bulk animal feed usage.

16. Specification Sheets

CORN SYRUP STORAGE TANK			
Identfication:	Item:		*Storage tank*
	Item No.		STOR-01
	No. Required		1
Function:	Hold corn syrup before being mixed with inoculum broth		
Operation:	Batch		
Type:	Cone Roof		
	Stream In	**Stream Out**	
Stream ID	**N/A**	**S-001**	
Temperature (°C)	20		
Mass Fraction			
Methionine (solid)	0		
Methionine (liquid)	0		
Water	0.25		
Glucose	0.75		
Salts	0		
Biomass	0		
Yeast Extract	0		
Fermentation byproducts	0		
Design Data:	Material of Construction:	Stainless Steel	
	Max working vol (L):	518,000	
	Days storage:	3	
Purchase Cost:			$128,000
Bare Module Cost:			$511,000

INOCULUM BROTH STORAGE TANK			
Identfication:	Item:		*Storage tank*
	Item No.		STOR-02
	No. Required		2
Function:	Hold Teknova Inoculum Broth before being mixed with corn syrup		
Operation:	Batch		
Type:	Cone Roof		
	Stream In	**Stream Out**	
Stream ID	**N/A**	**S-002**	
Temperature (°C)	20		
Mass Fraction			
Water	0.0675		
Yeast Extract	0.48		
Soytone	0.25		
Glycerol	0.1		
Na2HPO4	0.06		
KH2PO4	0.03		
NH4Cl	0.01		
MgSO4	0.0025		
Design Data:	Material of Construction:	Stainless Steel	
	Max working vol (L):	582,000	
	Days of storage:	7	
Purchase Cost:			$135,000
Bare Module Cost:			$539,000

MEDIA STERILIZATION PUMP			
Identification:	Item:		*Pump*
	Item No.		MS-P-01 (02)
	No. Required		2
Function(s):	Pump contents into heat exchanger and splitter		
Operation:	Continuous		
Type:	Centrifugal, 3600 RPM, VSC, 75 Hp		
Stream ID	**Input**	**Output**	
	S-003	**S-004**	
Pressure (bar)	1.01	4	
Design Data:	Flow rate (gpm)	230	
	Construction Material	Stainless Steel	
	Pump Head (ft)	100	
	Brake Horsepower (Hp)	9	
Cost of utilites/year:	15 x 10^3 kWh of electricity	**$**	**3,600**
Purchase Cost:		**$**	**10,000**
Bare Module Cost:		**$**	**33,000**
Associated Cost:	Motor	**$**	**3,100**
Total Bare Module Cost:		**$**	**36,100**
Comments:	Totally enclosed, fan-cooled motor enclosure used		
	MS-P-01 and MS-P-02 are identical		

HEAT EXCHANGER			
Identification:	Item:		*Heat exchanger*
	Item No.		MS-HX-01
	No. Required		1
Function:	Preheat unsterilized media to 88°C		
Operation:	Continuous		
Type:	Shell-and-tube floating head		
Stream ID	**Tube Side**	**Shell Side**	
Stream In	**S-009**	**S-004**	
Stream Out	**S-005**	**S-006**	
Flow Rate (kg/hr)	40,641	52,275	
Inlet Temperature (°C)	121	20	
Outlet Temperature (°C)	104	88	
Design Data:	Surface area (m²)	83	
	LMTD (°C)	54.4	
	Heat Duty (kW)	3,838	
	Construction Materials	Stainless Steel/Stainless Steel	
Purchase Cost:		$	132,000
Bare Module Cost:		$	419,000

HEAT EXCHANGER			
Identification:	Item:		*Heat exchanger*
	Item No.		MS-HX-02
	No. Required		1
Function:	Heat unsterilized media from 88 to 121°C		
Operation:	Continuous		
Type:	Shell-and-tube floating head		
Stream ID	**Tube Side**	**Shell Side**	
Stream In	**S-0001**	**S-006**	
Stream Out	**S-0002**	**S-007**	
Flow Rate (kg/hr)	450,382	52,275	
Inlet Temperature (°C)	175	88	
Outlet Temperature (°C)	132	121	
Design Data:	Surface area (m²)	766	
	LMTD (°C)	42.3	
	Heat Duty (kW)	26,923	
	Construction Materials	Stainless Steel/Stainless Steel	
Purchase Cost:		$	475,000
Bare Module Cost:		$	1,506,000

HEAT EXCHANGER			
Identification:	Item:		*Heat exchanger*
	Item No.		MS-HX-03
	No. Required		1
Function:	Cool sterilized media to 31°C		
Operation:	Continuous		
Type:	Shell-and-tube floating head		
Stream ID	**Tube Side**	**Shell Side**	
Stream In	**S-010**	**S-0003**	
Stream Out	**S-011**	**S-0004**	
Flow Rate (kg/hr)	52,275	990,840	
Inlet Temperature (°C)	104	20	
Outlet Temperature (°C)	31	44	
Design Data:	Surface area (m²)	532	
	LMTD (°C)	59.6	
	Heat Duty (kW)	27,524	
	Construction Materials	Stainless Steel/Stainless Steel	
Purchase Cost:		$	362,000
Bare Module Cost:		$	1,148,000

AMMONIA STORAGE TANK			
Identfication:	Item:		*Storage tank*
	Item No.		STOR-03
	No. Required		1
Function:	Hold ammonia solution before being pumped into reactors		
Operation:	Batch		
Type:	Cone Roof		
	Stream In	**Stream Out**	
Stream ID	**N/A**	**S-023**	
Temperature (°C)	20		
Mass Fraction			
Ammonia	0.28		
Water	0.72		
Design Data:	Material of Construction:	Stainless Steel	
	Max working vol (L):	37,000	
	Days of storage:	365	
Purchase Cost:			$32,000
Bare Module Cost:			$126,000

AIR COMPRESSOR			
Identification:	Item:		*Compressor*
	Item No.		MS-COMP
	No. Required		1
Function:	Extracts ambient air and pressurizes it to be used by all reactors		
Operation:	Continuous		
	Input		**Output**
Stream ID	**N/A**		**S-032**
Flow (kg/hr)	Variable		Variable
Design Data:	Temperature	20°C	
	Pressure	1-1.7 bar	
	Weight (kg)	1,500	
	Motor speed (rpm)	1,750	
	Construction Materials	Stainless Steel	
	Compressor Style	Rotary Screw	
	Power (kW)	75	
Purchase Cost:		$540,000	
Bare Module Cost:		$1,160,000	

COARSE AIR FILTER			
Identification:	Item:		*Filter*
	Item No.		N/A
	No. Required		1
Function:	Preliminary coarse filtration of ambient air		
Operation:	Continuous		
	Input	**Output**	
Stream ID	**S-032**	**S-033**	
Flow (kg/hr)	5000	5000	
Design Data:	Temperature	20°C	
	Pressure	1 bar	
	Filter Media Material	Glass Fiber	
	Filter Class	M6	
	Media Area (m^2)	8	
	Dimensions	0.6 x 0.6 x 0.2 m	
	Weight (kg)	3	
Purchase Cost:		$4,000	
Bare Module Cost:		$12,800	

MICRON AIR FILTER			
Identification:	Item:		*Filter*
	Item No.		N/A
	No. Required		1
Function:	Fully purify and sterilize air leaving the compressor		
Operation:	Continuous		
	Input	**Output**	
Stream ID	**S-033**	**S-034**	
Flow (kg/hr)	5000	5000	
Design Data:	Temperature	20°C	
	Pressure	1 bar	
	Filter Media Material	Glass Fiber	
	Filter Class	H13	
	Media Area (m^2)	46	
	Dimensions	0.75 x 0.6 x 0.3 m	
	Weight (kg)	14	
Purchase Cost:		$4,000	
Bare Module Cost:		$12,800	

TEST TUBES			
Identification:	Item:		*Test Tubes*
	Item No.		N/A
	No. Required		96
Function:	Store aliqouts of *Corynebacterium glutamicum* cells		
Operation:	Batch		
Type:	N/A		
Temperature (°C)		-90	
Design Data:	Final working volume (mL):	2	
	Construction Materials:	Polypropylene	
	Sterility:	Sterile	
	Color:	Clear	
	Vendor:	Fisher-Scientific	
Purchase Cost:		$	444
Comments:	All tubes are identical		

ERLENMEYER FLASKS			
Identification:	Item:		*Erlenmeyer Flask*
	Item No.		N/A
	No. Required		24
Function:	Production of DLM from *Corynebacterium glutamicum* cells		
Operation:	Batch		
Type:	N/A		
	Inlet	**Outlet**	
Stream ID	**N/A**	**S-021**	
Temperature (°C)	31	31	
Total Mass (g/batch)	8.112	1,658.10	
Composition (g/batch)			
Cell Mass	0.112	77.3	
DLM	0	40	
Water	8	1,487.30	
Glucose	0	0.16	
Salts	0	10.8	
Carbon Dioxide	0	0	
Ammonia	0	0	
Oxygen	0	0	
Nitrogen	0	0	
Other Fermentation Byproducts	0	41.5	
Biomass	0	1.1	
Design Data:	Final working volume (L):	0.5	
	Construction Materials:	Polyethylene terephthalate	
Purchase Cost:		$	570
Comments:	FLASK-01 to 24 are identical		

PUMP			
Identification:	Item:		*Pump*
	Item No.		P-04 (08-12)
	No. Required		6
Function(s):	Pump contents into pre-seed, seed, and production fermenters		
Operation:	Continuous		
Type:	Centrifugal, 3600 RPM, VSC, 75 Hp		
Stream ID	**Input**	**Output**	
	S-013	**S-014**	
Pressure (bar)	1.01	3.7	
Design Data:	Flow rate (gpm)	40	
	Construction Material	Stainless Steel	
	Pump Head (ft)	100	
	Brake Horsepower (Hp)	2.5	
Cost of utilites/year:	26 x 10^2 kWh of electricity	**$**	**1000**
Purchase Cost:		**$**	**9,400**
Bare Module Cost:		**$**	**31,020**
Associated Cost:	Motor	**$**	**2,100**
Total Bare Module Cost:		**$**	**33,120**
Comments:	Totally enclosed, fan-cooled motor enclosure used		
	P-04 and P-08 through P-12 are identical		

PRE-SEED FERMENTER			
Identification:	Item:		*Vertical vessel*
	Item No.		PRE-SEED-01 (02-06)
	No. Required		6
Function:	Production of DLM from *Corynebacterium glutamicum* cells		
Operation:	Batch		
Type:	N/A		
	Inlet	**Outlet**	
Stream ID	**S-022**	**S-039**	
Temperature (°C)	31	31	
Total Mass (kg/batch)	6.6	5,271	
Composition (kg/batch)			
Cell Mass	0.3	0.9	
DLM	0.16	360	
Water	5.9	4,727.90	
Glucose	0.00066	0.48	
Salts	0.043	34.3	
Carbon Dioxide	0	5.9	
Ammonia	0	0	
Oxygen	0	0	
Nitrogen	0	0	
Other Fermentation Byproducts	0.17	131.8	
Biomass	0.0042	9.6	
Design Data:	Vessel Height (m):	5.7	
	Vessel Diameter (m):	1.1	
	Final working volume (L):	4,500	
	Pressure (bar):	1.2	
	Construction Materials:	Stainless Steel	
Cost of utilities/year:	7,800 kg chilled water	$	4,700
Purchase Cost:		$	9,900
Bare Module Cost:		$	232,000
Associated Costs:	Chilled Water Coils:	$	930
Total Bare Module Cost:		$	233,000
Comments:	PRE-SEED-01 to 06 are identical		

PUMP			
Identification:	Item:		*Pump*
	Item No.		P-05
	No. Required		1
Function(s):	Pump sterilized media into seed reactor		
Operation:	Continuous		
Type:	Centrifugal, 3600 RPM, VSC, 75 Hp		
Stream ID	**Input**	**Output**	
	S-015	**S-016**	
Pressure (bar)	1.01	3.7	
Design Data:	Flow rate (gpm)	201	
	Construction Material	Stainless Steel	
	Pump Head (ft)	100	
	Brake Horsepower (Hp)	8	
Cost of utilites/year:	13 x 10^3 kWh of electricity	**$**	**3200**
Purchase Cost:		**$**	**9,900**
Bare Module Cost:		**$**	**32,670**
Associated Cost:	Motor	**$**	**3,000**
Total Bare Module Cost:		**$**	**35,670**
Comments:	Totally enclosed, fan-cooled motor enclosure used		

SEED FERMENTER			
Identification:	Item:		*Vertical vessel*
	Item No.		SEED-01 (02-06)
	No. Required		6
Function:	Production of DLM from *Corynebacterium glutamicum* cells		
Operation:	Batch		
Type:	N/A		
	Inlet	**Outlet**	
Stream ID	**S-039**	**S-040**	
Temperature (°C)	31	31	
Total Mass (kg/batch)	5,270.80	55,653.60	
Composition (kg/batch)			
Cell Mass	0.9	422	
DLM	360	3,400	
Water	4,727.9	49,921.2	
Glucose	0.48	4.6	
Salts	34.3	361.7	
Carbon Dioxide	5.9	62.4	
Ammonia	0	0	
Oxygen	0	0	
Nitrogen	0	0	
Other Fermentation Byproducts	131.8	1,391.3	
Biomass	9.6	90.3	
Design Data:	Vessel Height (m):	7.77	
	Vessel Diameter (m):	2.87	
	Final working volume (L):	42,500	
	Pressure (bar):	1.2	
	Construction Materials:	Stainless Steel	
Cost of utilities/year:	444,000 kg chilled water	$	268,000
Purchase Cost:		$	93,500
Bare Module Cost:		$	721,000
Associated Costs:	Chilled Water Coils:	$	9,000
Total Bare Module Cost:		$	730,000
Comments:	SEED-01 to 06 are identical		

PUMP			
Identification:	Item:		*Pump*
	Item No.		P-06 (07)
	No. Required		2
Function(s):	Pump sterilized media into production fermenters		
Operation:	Continuous		
Type:	Centrifugal, 1800 RPM, VSC, 200 Hp		
Stream ID	**Input**	**Output**	
	S-017	**S-018**	
Pressure (bar)	1.01	3.7	
Design Data:	Flow rate (gpm)	2132	
	Construction Material	Stainless Steel	
	Pump Head (ft)	100	
	Brake Horsepower (Hp)	66	
Cost of utilites/year:	14 x 10^4 kWh of electricity	**$**	**25,700**
Purchase Cost:		**$**	**35,400**
Bare Module Cost:		**$**	**116,820**
Associated Cost:	Motor	**$**	**15,000**
Total Bare Module Cost:		**$**	**131,820**
Comments:	Totally enclosed, fan-cooled motor enclosure used		
	P-06 and P-07 are identical		

PUMP			
Identification:	Item:		*Pump*
	Item No.		P-01
	No. Required		1
Function(s):	Pump contents into splitter		
Operation:	Continuous		
Type:	Centrifugal, 3600 RPM, VSC, 75 Hp		
Stream ID	**Input**	**Output**	
	S-040	**S-041**	
Pressure (bar)	1.01	1.7	
Design Data:	Flow rate (gpm)	245	
	Construction Material	Stainless Steel	
	Pump Head (ft)	100	
	Brake Horsepower (Hp)	10	
Cost of utilites/year:	16 x 10^3 kWh of electricity	**$**	**3,800**
Purchase Cost:		**$**	**10,200**
Bare Module Cost:		**$**	**33,660**
Associated Cost:	Motor	**$**	**3,200**
Total Bare Module Cost:		**$**	**36,860**
Comments:	Totally enclosed, fan-cooled motor enclosure used		

PUMP			
Identification:	Item:		*Pump*
	Item No.		P-01a, (01b)
	No. Required		2
Function(s):	Pump contents into production fermenter		
Operation:	Continuous		
Type:	Centrifugal, 3600 RPM, VSC, 75 Hp		
Stream ID	**Input**	**Output**	
	S-042	**S-042.5**	
Pressure (bar)	1.01	3.7	
Design Data:	Flow rate (gpm)	122	
	Construction Material	Stainless Steel	
	Pump Head (ft)	100	
	Brake Horsepower (Hp)	5.5	
Cost of utilites/year:	8 x 10^3 kWh of electricity	**$**	**2,200**
Purchase Cost:		**$**	**9,300**
Bare Module Cost:		**$**	**30,690**
Associated Cost:	Motor	**$**	**2,500**
Total Bare Module Cost:		**$**	**33,190**
Comments:	Totally enclosed, fan-cooled motor enclosure used		
	P-01a and P-01b are identical		

PRODUCTION FERMENTER				
Identification:	Item:			*Vertical vessel*
	Item No.			PROD-01 (02-12)
	No. Required			12
Function:	Production of DLM from *Corynebacterium glutamicum* cells			
Operation:	Batch			
Type:	N/A			
	Inlet		**Outlet**	
Stream ID	**S-042**		**S-043**	
	S-045		**S-046**	
Temperature (°C)	31		31	
Total Mass (kg/batch)	27,826.8		553,807.5	
Composition (kg/batch)				
Cell Mass	211		1,972.2	
DLM	1,700		36,000	
Water	24,960.6		496,765.3	
Glucose	2.3		48.4	
Salts	180.9		3599.7	
Carbon Dioxide	31.2		621	
Ammonia	0		0	
Oxygen	0		0	
Nitrogen	0		0	
Other Fermentation Byproducts	695.7		13,845.20	
Biomass	45.1		955.7	
Design Data:	Vessel Height (m):	25.6		
	Vessel Diameter (m):	5		
	Final working volume (L):	450,000		
	Pressure (bar):	1.2		
	Construction Materials:	Stainless Steel		
Cost of utilities/year:	31,359,000 kg chilled water	$		18,925,000
Purchase Cost:		$		990,000
Bare Module Cost:		$		2,749,000
Associated Costs:	Agitator:	$		265,000
	Chilled Water Coils:	$		87,000
Total Bare Module Cost:		$		3,101,000
Comments:	PROD-01 to 12 are identical			

PUMP			
Identification:	Item:		*Pump*
	Item No.		P-02 (03)
	No. Required		2
Function(s):	Pump contents of production fermenter into storage tank		
Operation:	Continuous		
Type:	Centrifugal, 1800 RPM, VSC, 200 Hp		
Stream ID	**Input**	**Output**	
	S-043	**S-044**	
Pressure (bar)	1.01	3.7	
Design Data:	Flow rate (gpm)	2437	
	Construction Material	Stainless Steel	
	Pump Head (ft)	100	
	Brake Horsepower (Hp)	75	
Cost of utilites/year:	16 x 10^4 kWh of electricity	**$**	**29,000**
Purchase Cost:		**$**	**38,300**
Bare Module Cost:		**$**	**126,390**
Associated Cost:	Motor	**$**	**17,300**
Total Bare Module Cost:		**$**	**143,690**
Comments:	Totally enclosed, fan-cooled motor enclosure used		
	P-02 and P-03 are identical		

FERMENTATION PRODUCT STORAGE TANK			
Identfication:	Item:		*Storage tank*
	Item No.		STOR-04
	No. Required		1
Function:	Hold contents from fermenter and transfer to broth heating		
Operation:	Batch		
Type:	Cone Roof		
	Stream In		**Stream Out**
Stream ID	**S-044, S-047**		**S-048**
Temperature (°C)	31		
Composition (kg/batch)			
Methionine (solid)	18,000		
Methionine (liquid)	18,000		
Water	539,384		
Glucose	587		
Salts	3,600		
Biomass	956		
Fermentation byproducts	13,845		
Design Data:	Material of Construction:	Stainless Steel	
	Max working vol (L):	1,108,000	
	Days of storage:	6	
Purchase Cost:			$188,000
Bare Module Cost:			$750,000

GAS SCRUBBER			
Identfication:	Item:		*Gas scrubber*
	Item No.		N/A
	No. Required		3
Function:	Remove contaminants from all vent gas before it is vented out		
Operation:	Continuous		
Temperature (°C)	20		
Pressure (bar)	1		
	In	**Out**	
Stream ID	**S-052**	**N/A**	
Flow (kg/hr)	38,543	38,543	
Composition (wt%)			
Water	4.4		
CO2	9.2		
O2	2.1		
N2	84.3		
Design Data:	Material of Construction:	Stainless Steel	
	Removal Efficiency (%):	95	
	Process Fan (kW):	22.4 kW	
	Recycle Pump (L/hr):	34,000	
	Power Requirements:	40 V	
Purchase Cost:			$18,700
Bare Module Cost:			$77,800

FERMENTATION PRODUCT HEAT EXCHANGER			
Identification:	Item:		*Heat exchanger*
	Item No.		HX-04
	No. Required		1
Function:	To completely solubilize methionine		
Operation:	Continuous		
Type:	Shell-and-tube floating head		
Stream ID	**Tube Side**	**Shell Side**	
Stream In	**S-049**	**S-0005**	
Stream Out	**S-050**	**S-0006**	
Flow Rate (kg/hr)	683,788	1,955,700	
Inlet Temperature (°C)	31	180	
Outlet Temperature (°C)	70	150	
Design Data:	Surface area (m^2)	86.4	
	LMTD (°C)	114.4	
	Heat Duty (kW)	31,823.1	
	Construction Materials	Stainless Steel/Stainless Steel	
Purchase Cost:		$	119,944
Bare Module Cost:		$	380,221

BIOMASS SEPARATION			
Identification:	Item:		*Centrifuge*
	Item No.		CFG-01 (02, 03)
	No. Required		3
Function(s):	Separate biomass containing pellet from DLM containing supernatant		
Operation:	Continuous		
Type:	Large capacity nozzle centrifuge		
Stream ID	**Input** **S-051** **S-054** **S-057**		**Output** **S-052, S-053** **S-055, S-056** **S-058, S-059**
Design Data:	Flow Rate (kg/hr)		228,000
	Construction Material		Stainless Steel
	Power (kW)		170
Cost of utilites/year (per unit):	15 x 10^3 kWh of electricity	**$**	**1,050**
Cost of process water/year (per unit):	45 kilo-tonnes of process water	**$**	**12,100**
Purchase Cost (per unit)		**$**	**400,000**
Total Bare Module Cost (per unit)		**$**	**1,237,350**
Comments:	Pellet exists at 75% moisture level		
	CFG-01, CFG-02, and CFG-03 are identical		

PUMP			
Identification:	Item:		*Pump*
	Item No.		P-17
	No. Required		1
Function(s):	Pump contents into rotary drum dryer		
Operation:	Continuous		
Type:	Centrifugal, 3600 RPM, VSC, 75 Hp		
Stream ID	**Input**	**Output**	
	S-078	**S-079**	
Pressure (bar)	1.01	4	
Design Data:	Flow rate (gpm)	15	
	Construction Material	Stainless Steel	
	Pump Head (ft)	100	
	Brake Horsepower (Hp)	5	
Cost of utilites/year:	7 x 10^3 kWh of electricity	**$**	**2,000**
Purchase Cost:		**$**	**9,200**
Bare Module Cost:		**$**	**30,360**
Associated Cost:	Motor	**$**	**2,500**
Total Bare Module Cost:		**$**	**32,860**
Comments:	Totally enclosed, fan-cooled motor enclosure used		

ROTARY DRUM DRYER			
Identification:	Item:		*Rotary Drum Dryer*
	Item No.		RDD-01
	No. Required		1
Function(s):	Heats biomass pellet from 70°C to 121°C and lower moisture level to 10%		
Operation:	Continuous		
Type:	Direct fired with natural gas, continuous		
Stream ID	**Input**	**Output**	
	S-078	**S-079**	
Design Data:	Flow rate (kg/hr)	3,332	
	Construction Material	Stainless Steel	
	Diameter (m)	4.6	
	Height (m)	30.5	
	Power (kW)	3,460	
Purchase Cost:		**$**	**750,000**
Bare Module Factor:			**2**
Total Bare Module Cost:		**$**	**1,500,000**
Comments:	Natural gas is directly heated and flows in counter-current direction.		

PUMP			
Identification:	Item:		*Pump*
	Item No.		P-18
	No. Required		1
Function(s):	Pump contents into Super Sacks		
Operation:	Continuous		
Type:	Centrifugal, 3600 RPM, VSC, 75 Hp		
Stream ID	**Input**	**Output**	
	S-080	**S-081**	
Pressure (bar)	1.01	3.7	
Design Data:	Flow rate (gpm)	6	
	Construction Material	Stainless Steel	
	Pump Head (ft)	100	
	Brake Horsepower (Hp)	4	
Cost of utilites/year:	6 x 10^3 kWh of electricity	**$**	**1,800**
Purchase Cost:		**$**	**9,100**
Bare Module Cost:		**$**	**30,030**
Associated Cost:	Motor	**$**	**2,400**
Total Bare Module Cost:		**$**	**32,430**
Comments:	Totally enclosed, fan-cooled motor enclosure used		

PUMP			
Identification:	Item:		*Pump*
	Item No.		P-14
	No. Required		1
Function(s):	Pump contents into first evaporator		
Operation:	Continuous		
Type:	Centrifugal, 1800 RPM, VSC, 200 Hp		
Stream ID	**Input**	**Output**	
	S-060	**S-061**	
Pressure (bar)	1.01	6.7	
Design Data:	Flow rate (gpm)	2992	
	Construction Material	Stainless Steel	
	Pump Head (ft)	100	
	Brake Horsepower (Hp)	83	
Cost of utilites/year:	18 x 10^4 kWh of electricity	**$**	**33,000**
Purchase Cost:		**$**	**41,100**
Bare Module Cost:		**$**	**135,630**
Associated Cost:	Motor	**$**	**20,000**
Total Bare Module Cost:		**$**	**155,630**
Comments:	Totally enclosed, fan-cooled motor enclosure used		

TRIPLE EFFECT EVAPORATOR			
Identfication:	Item:		*Falling Film Evaporator*
	Item No.		EVAP-01, 02, 03
	No. Required		1
Function:	Concentrate filtered fermentation broth to 21 wt% water		
Operation:	Continuous		
Type:	Shell-and-tube floating head		
Stream ID	**In** **S-061**		**Out** **S-064**
Duty per Effect (kW)	91,500		
Flow rate into Effect 1 (kg/hr)	680,000		
Inlet Temperature (°C)	70		
Outlet Temperature (°C)	112		
Mass Evaporated Effect 1 (kg/hr)	133,003		
Mass Evaporated Effect 2 (kg/hr)	146,865		
Mass Evaporated Effect 3 (kg/hr)	162,770		
Design Data:			
Effect 1	Temperature (°C)	159	
	Pressure (bar)	6	
	Area (m²)	256	
Effect 2	Temperature (°C)	144	
	Pressure (bar)	4	
	Area (m²)	193	
Effect 3	Temperature (°C)	112	
	Pressure (bar)	1.5	
	Area (m²)	117	
	Construction Materials	Stainless Steel/Stainless Steel	
Cost of utilities/year:	MP Steam		**$ 274,000**
Purchase Cost:			**$ 2,262,000**
Bare Module Cost:			**$ 8,284,000**

PUMP			
Identification:	Item:		*Pump*
	Item No.		P-15 (16)
	No. Required		2
Function(s):	Pump contents into storage tank		
Operation:	Continuous		
Type:	Centrifugal, 3600 RPM, VSC, 75 Hp		
Stream ID	**Input**	**Output**	
	S-064	**S-065**	
Pressure (bar)	1.01	3.7	
Design Data:	Flow rate (gpm)	785	
	Construction Material	Stainless Steel	
	Pump Head (ft)	100	
	Brake Horsepower (Hp)	26	
Cost of utilites/year:	51 x 10^3 kWh of electricity	**$**	**10,300**
Purchase Cost:		**$**	**14,500**
Bare Module Cost:		**$**	**47,850**
Associated Cost:	Motor	**$**	**6,400**
Total Bare Module Cost:		**$**	**54,250**
Comments:	Totally enclosed, fan-cooled motor enclosure used		
	P-15, and P-16 are identical		

EVAPORATOR CONCENTRATE STORAGE TANK			
Identfication:	Item:		*Storage tank*
	Item No.		STOR-05
	No. Required		2
Function:	Hold concentrate from evaporation before crystallization		
Operation:	Batch		
Type:	Cone Roof		
	Stream In	**Stream Out**	
Stream ID	**S-065**	**S-066**	
Temperature (°C)	112		
Mass Fraction			
Methionine (solid)	0		
Methionine (liquid)	0.209		
Water	0.763		
Glucose	0.0035		
Salts	0.024		
Biomass	0		
Yeast Extract	0		
Fermentation byproducts	0		
Design Data:	Material of Construction:	Stainless Steel	
	Max working vol (L):	518,000	
	Hours of storage:	12	
Purchase Cost:			$193,000
Bare Module Cost:			$770,000

CRYSTALLIZER			
Identification:	Item:		*Crystallizer*
	Item No.		CRYS-01 (02-03)
	No. Required		3
Function:	Cool feed in so DLM precipiates out of solution		
Operation:	Continuous		
Type:	N/A		
	Inlet	**Outlet**	
Stream ID	**S-068, S-070, S-072**	**S-069, S-071, S-073**	
Temperature (°C)	112	5	
Flow (kg/hr)	59,477	59,477	
Composition (kg/hr)			
Water	45,399	45,399	
DLM (solid)	0	11,489	
DLM (liquid)	12,421	932	
Glucose	207	207	
Salts	725	725	
Fermentation Byproducts	725	725	
Design Data:	Vessel Height (m):	5.75	
	Vessel Diameter (m):	3.83	
	Final volume (L):	210,000	
	Residence time (hr):	1	
	Construction Materials:	Stainless Steel	
Cost of utilities/year:			
Purchase Cost:		$	364,000
Bare Module Cost:		$	1,357,000
Associated Costs:	Cooling water:	$	770
Total Bare Module Cost:		$	4,136,000

DECANTER CENTRIFUGE			
Identification:	Item:		*Centrifuge*
	Item No.		CFG-04
	No. Required		1
Function(s):	To separate out wet DLM cake from liquid stream after crystallizers		
Operation:	Continuous		
Type:	Conical-cylindrical solid bowl centrifuge, 1600 RPM, 400 Hp motor		
Stream ID	**Input** **S-69, S-71, S-73**	**Output** **S-74**	
Design Data:	Bowl diameter (in)	44	
	Flow rate (gpm)	723	
	Construction Material	Stainless Steel	
	Power (kW)	300	
Cost of utilites/year:	2 x 10^8 kWh of electricity	**$**	**157,500**
Purchase Cost		**$**	**150,000**
Bare Module Cost:		**$**	**304,500**
Associated Costs:	Motor	**$**	**133,000**
Total Bare Module Cost		**$**	**437,500**
Comments:	Also called helical conveyor or scroll conveyor centrifuges		

ROTARY DRYER			
Identification:	Item:		*Rotary Dryer*
	Item No.		RD-01 (02-04)
	No. Required		4
Function(s):	Concentrate wet cake crystalline product from 19% moisture to 1% moisture		
Operation:	Continuous		
Type:	System includes an ID fan, a cyclone and baghouse. 50 Hp motor		
Stream ID	**Input** **S-074** **S-0016**	**Output** **S-075** **S-0017**	
Design Data:	Flow rate of wet solids (kg/hr)	10,595	
	Air flow rate (kg/hr)	91,762	
	Natural gas flow rate (kg/hr)	4,630	
	Water evaporation rate (kg/hr)	1,950	
	Construction Material	Stainless Steel	
	Diameter (m)	6	
	Length (m)	20	
	Power (kW)	38	
Cost of utilities/yr	3 x 10^5 kWh of electricity	**$**	**20,000**
Cost of natural gas/yr		**$**	**7,274,000**
Purchase Cost:		**$**	**715,000**
Bare Module Cost:		**$**	**1,472,900**
Associated Costs:	Motor	**$**	**12,000**
Total Bare Module Cost:		**$**	**1,484,900**
Comments:	Air enters with humidity of 0.007 and exits with humidity of 0.0284. This air stream is heated by natural gas to 170°C. RD-01 and RD-02 through RD-04 are identical.		

FEED BUCKET CONVEYOR			
Identification:	Item:		*Conveyor*
	Item No.		CONV-01
	No. Required		1
Function(s):	To transport dried DLM product up to storage tank		
Operation:	Continuous		
Type:	Feed bucket conveyor, 50 Hp motor		
Stream ID	**Input S-075**	**Output S-076**	
Design Data:	Length (ft)	30	
	Flow rate (gpm)	116	
	Construction Material	Stainless Steel	
	Power (kW)	37	
Cost of utilites/year:	3 x 10^5 kWh of electricity	**$**	**19,600**
Purchase Cost		**$**	**50,000**
Bare Module Cost:		**$**	**87,000**
Associated Costs:	Motor	**$**	**12,000**
	Cooling jacket	**$**	**10,000**
Total Bare Module Cost		**$**	**109,000**
Comments:	Cooling jacket is included as a burn protection measure for workers. It cools the DLM product from 80°C to 50°C.		

DLM STORAGE TANK			
Identfication:	Item:		*Storage tank*
	Item No.		STOR-06
	No. Required		1
Function:	Hold DLM product from conveyor and feed via live bottom into Super Sacks stationed beneath unit		
Operation:	Batch		
Type:	Cone Roof with a live bottom		
Stream ID	**Stream In S-076**	**Stream Out S-077**	
Temperature (°C)	30		
Mass Fraction			
DLM	0.99		
Water	0.01		
Design Data:	Material of Construction:	Stainless Steel	
	Max working vol (L):	281,000	
	Hours of storage:	8	
Purchase Cost:			$ 98,100
Bare Module Cost:			$ 393,000

SUPER SACKS			
Identification:	Item:		*Super sack*
	Item No.		SS-01, SS-02
	No. Required		142,361
Function:	Used for final packaging of DLM and FEMA No. 4907		
Operation:	Batch		
		Input	**Output**
Stream ID		**S-077**	**N/A**
DLM Mass (kg/bag)		1800	1800
FEMA No. 4907 (kg/bag)		1800	1800
Design Data:	Temperature (°C):		20
	Pressure (bar):		1
	Volume (L):		1400
	Dimensions:		0.89 x 0.89 x 1.4 m
	Construction Materials:		Woven Polypropylene
	Additional Features:		Duffel top with tie closure, flat bottom, four lift loops
Purchase Cost:			$29 per bag
Bare Module Cost:			$121 per bag

CLEAN-IN-PLACE SYSTEM		
Identification:	Item:	*CIP System*
	Item No.	N/A
	No. Required	1
Function:	Used for cleaning fermenters, pipes, pumps, and heat exchangers	
Operation:	Continuous, once every 64 hours	
Temperature (ºC)	20-80	
Pressure (bar)	4.14	
Design Data:	Model: UltraFlow 110	
	Material: Wetted Surface - 316 stainlees steel	
	Non-wetted Surface - 304 stainlees steel	
	Size: 1.87 x 0.84 x 2.03 m	
	Pipe Diameter Cleaning Ability: 0.152 - 0.203 m	
	Tank Diameter Cleaning Ability: 4.5 m	
Utilities	Power: 15 kW	
Purchase Cost:	$175,000 per unit	
Bare Module Cost:	$728,000	
Comments:	A customized version of the Sani-Matic UltraFlow 110 will be used to accomodate the pipe diameter and tank diamater needed to clean the process. See Appendix D.	

STERILIZATION-IN-PLACE SYSTEM		
Identification:	Item:	*SIP System*
	Item No.	N/A
	No. Required	1
Function:	Used for cleaning fermenters, pipes, pumps, and heat exchangers	
Operation:	Continuous, once every 64 hours	
Temperature (ºC)	120	
Pressure (bar)	2	
Design Data:	Supplier: Solida Biotech	
	Material: Stainlees steel	
Utilities	Power: 15 kW	
Purchase Cost:	$100,000 per unit	
Bare Module Cost:	$416,000	
Comments:	A customized version of the Solida Biotech's SIP system will be used to accomodate the pipe diameter and tank diamater needed to clean the process. See Appendix D.	

17. Equipment Cost Summary

17.1 Equipment Cost Summary Table

Table 17.1.1 details the cost of equipment needed for the seed, batch, and continuous processes. For readability, figures are presented as rounded values, but calculations of total bare module costs and the overall equipment cost were computed with exact values. A CE index value of 600 was used in the calculations.

Table 17.1.1. Equipment costs for all units used in the process.

Equipment	Flowsheet Label	Amount per Order	Vendor	Purchase Cost ($USD)	Number Purchased	Bare Module Factor	Total Bare Module Cost ($USD)
Cell Preparation							
Cell Bank	N/A	1	ATCC	7,900	1	3.21	25,000
12 mL Test Tubes	N/A	96	Fisher Scientific	222	2	3.21	1,400
Storage							
Corn Syrup Storage Tank	STOR-01	1	N/A	128,000	1	4	511,000
Teknova Broth Storage Tank	STOR-02	1	N/A	135,000	2	4	1,079,000
Ammonia Storage Tank	STOR-03	1	N/A	31,600	1	4	126,000
Fermentation Product Storage Tank	STOR-04	1	N/A	188,000	1	4	750,000
Evaporator Concentrate Storage Tank	STOR-05	1	N/A	193,000	2	4	1,541,000
DLM Storage Tank	STOR-06	1	N/A	98,100	1	4	393,000
Seed Train Process							

Air Compressor	MS-COMP	1	Eaton Compressor	328,000	1	2.15	705,200
Coarse Air Filter	Course air filter	1	Camfil	4,000	1	2.32	9,280
Submicron Air Filter	Submicron air filter	1	Camfil	4,000	1	2.32	9,280
Pump	P-04, 08-12	1	Goulds Pumps	9,420	6	3.3	186,500
Pump	P-05	1	Goulds Pumps	9,900	1	3.3	32,700
Pump	P-06, 07	1	Goulds Pumps	35,400	2	3.3	233,600
2 L Flask	N/A	4	Fisher Scientific	95	6	3.21	1,800
5,000 L Reactor	PRE-SEED -01 to -06	1	Paul Mueller Company	56,000	6	4.16	1,394,000
Pump	P-01	1	Goulds Pumps	10,200	1	3.3	33,700
Pump	P-01a,01b	1	Goulds Pumps	9,300	2	3.3	61,200
50,000 L Reactor	SEED-01 to -06	1	Paul Mueller Company	173,000	6	4.16	4,327,000
500,000 L Reactor	PROD-01 to -12	1	Paul Mueller Company	661,000	12	4.16	32,990,000
Pump	P-02,03	1	Goulds Pumps	38,300	2	3.3	253,000
Scrubber	N/A	1	Pollution Systems	18,700	3	4.16	233,000
Batch Process/Continuous Process							
Pump	MS-PUMP -01	1	Goulds Pumps	10,000	1	3.3	33,000
Heat Exchanger	MS-HX-01	1	N/A	132,000	1	3.17	419,000
Heat Exchanger	MS-HX-02	1	N/A	475,000	1	3.17	1,506,000
Heat Exchanger	MS-HX-03	1	N/A	362,000	1	3.17	1,148,000
Pump	MS-PUMP	1	Goulds	10,000	1	3.3	33,000

	-02		Pumps				
Pump	P-13	1	Goulds Pumps	42,100	1	3.3	138,900
Heat Exchanger	HX-04	1	N/A	120,000	1	3.17	380,400
Centrifuge	CFG-01, 02, 03	1	Alfa Laval	400,000	3	3	3,600,000
Pump	P-17	1	Goulds Pumps	9,200	1	3.3	30,400
Pump	P-18	1	Goulds Pumps	9,100	1	3.3	30,000
Rotary Drum Dryer	RDD-01	1	FEECO	750,000	1	2	1,500,000
Pump	P-14	1	Goulds Pumps	41,100	1	3.3	135,600
Heat Exchanger	EVAP-01	1	N/A	593,000	1	3.17	1,879,000
Flash Vessel	N/A	1	N/A	563,000	1	4.16	2,344,000
Heat Exchanger	EVAP-02	1	N/A	322,000	1	3.17	1,020,000
Flash Vessel	N/A	1	N/A	328,000	1	4.16	1,363,000
Heat Exchanger	EVAP-03	1	N/A	224,000	1	3.17	709,000
Flash Vessel	N/A	1	N/A	233,000	1	4.16	969,000
Pump	P-15, 16	1	Goulds Pumps	14,500	2	3.3	95,700
Crystallizer	CRYS-01 to -03	1	Paul Mueller Company	364,000	3	4.16	2,608,000
Centrifuge	CFG-04	1	Alfa Level	150,000	1	3.0	450,000
Rotary Dryer	RD-01 to -04	1	N/A	715,000	4	2.06	5,892,000
Conveyor	CONV-01	1	N/A	50,000	1	1.74	87,000
Spares							
Pumps	N/A	1	Goulds Pumps	20,000	3	3.3	198,000
Filters	N/A	1	Camfil	4,000	1	2.32	9,300
Scrubber	N/A	1	Pollution	18,700	1	4.16	77,800

			Systems				
Other	N/A	1	Other	181,100	10	3.21	5,814,000
Product Purification							
Super Sacks	SS-01, -02	1	ULINE	29	143,000	4.16	17,252,000
Cleaning							
CIP System	N/A	1	Sani-Matic	175,000	1	4.16	728,000
SIP System	N/A	1	Solida Biotech	100,000	1	4.16	416,000
TOTAL							$97,389,410

Equipment purchase costs were calculated either by using the Equipment Costing Spreadsheet provided by Professors Bruce Vrana and Warren Seider or via a quote request from a vendor. The sources for each equipment cost can be found in Appendix C. The standard cost equations can be found in Product and Process Design Principles Fourth Edition. The total cost of the equipment is $97,389,410. Figure 17.1 shows a breakdown of the costs for each type of equipment.

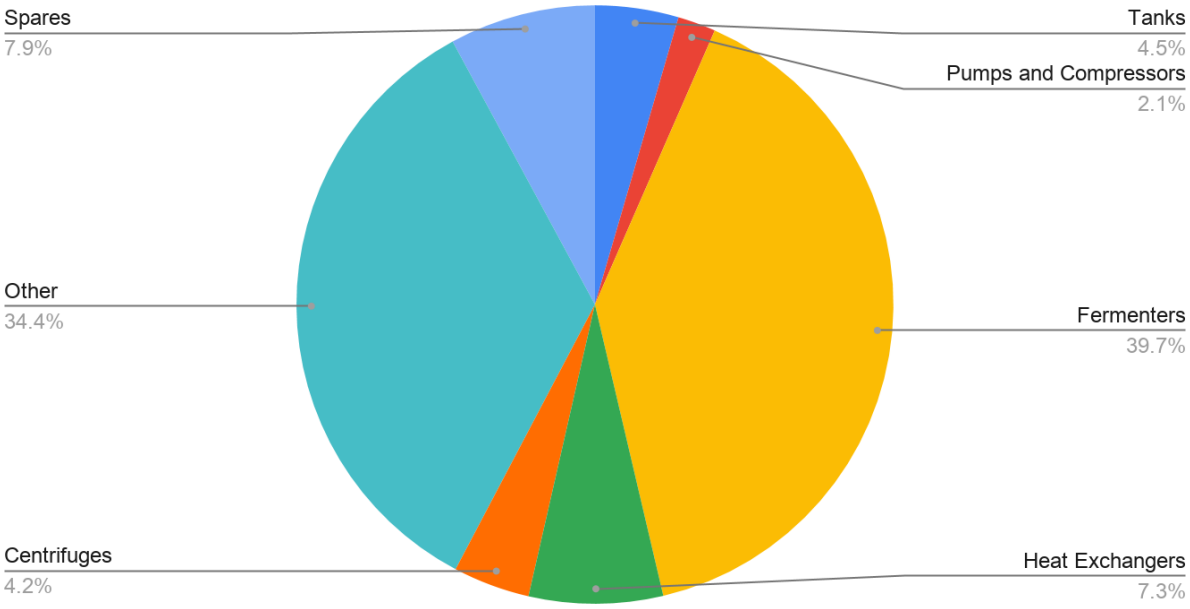


Figure 17.1. Cost breakdown of total equipment costs by equipment type.

As shown in the chart, the fermenters for the largest cost of the overall equipment. This was to be predicted as there are 24 fermenters in total, 12 of which are 500,000L. Fermenters are expensive units, and the process requires a considerable number of sizable reactors. The second most costly group of equipment is the Other section, which includes the cell bank, test tubes, flasks, air filters, scrubbers, flash vessels, crystallizers, rotary dryers, conveyor, super sacks, CIP, and SIP. The heat exchangers are the third most costly group and the least costly group is the pumps and compressors. The full, detailed list of all spare equipment is not represented in Table 17.1, but was calculated to be $7,704,000 in bare module cost based on the suggestion from author Steve Tieri that one should account for spare equipment to cost 7-10% of total bare module cost for all equipment.

The most expensive piece of equipment listed are the production fermenters. This is also to be predicted because reactors are expensive units and the reactors in this process are very large units. The next most expensive piece of equipment are the rotary dryers when excluding the super sacks. These are a significant part of the downstream process that have to do a considerable amount of drying so it is expected that they contribute a substantial amount to the cost of equipment. The bare module cost of the super sacks is so expensive due to the amount of product being packaged.

17.2 Unit Costing Consideration

17.2.1 Fermenters

The pre-seed, seed, and production fermenters were priced using the Pressure Vessel tab of the Equipment Costing Spreadsheet as recommended by Bruce Vrana. Fermenters are complex units to price, so Vrana suggested modeling the reactors as pressure vessels to estimate the cost.

17.2.2 Biomass Separation

The centrifuge was priced following the guidance of Professor Bruce Vrana.

17.2.3 Rotary Drum Dryer

The rotary drum dryer was priced following the guidance of Professor Bruce Vrana.

17.2.4 Triple Effect Evaporation

The triple effect evaporation was priced by combining the costs of heat exchangers and pressure vessels. The triple effect evaporation was modeled in Aspen Plus with three heat exchangers and three flash vessels. The costs were then estimated by combining the prices of the

three heat exchangers from the Equipment Costing Spreadsheet with the prices of the three flash vessels from the Equipment Costing Spreadsheet. The Pressure Vessel tab of the spreadsheet was used to model the flash vessels.

17.2.5 Crystallizer

The crystallizer was priced by combining the costs of a heat exchanger, a pressure vessel, and a pump. This calculation was performed using equations in the Equipment Costing Spreadsheet provided by Professor Vrana and Professor Seider. Pilot scale testing of the crystallizer is outside the scope of this report, so the design cannot be rigorously modeled. Thus the cost for the flash drying unit was based on assumptions of the residence time and the diameter-to-height ratio for the vessel, which was based on the assumption of the particle diameter.

17.2.6 Heat Exchangers

The heat exchangers were priced using the Equipment Costing Spreadsheet. Shell-and-tube heat exchangers were constructed from stainless steel. Preliminary calculations were performed to obtain the heat duty and the necessary surface area of each heat exchanger. The spreadsheet was then used to obtain the purchase cost and the total bare module cost of each heat exchanger.

17.2.7 Rotary Dryer

The rotary dryers were priced following the guidance of Professor Bruce Vrana.

17.2.8 DLM Storage Tank

The DLM storage tank was priced with guidance from Professor Bruce Vrana because the unit required a live bottom discharger.

18. Fixed Capital Investment Summary

The Profitability Analysis Spreadsheet 4.0 was used to model all financials involving the construction and operation of the plant. Financial models were estimated for 20 years of plant operation. The capital investment summary includes all capital costs that the plant will incur in the first year including purchase costs, initial construction, and equipment costs.

18.1 Equipment Costs

Individual equipment costs were presented in detail with bare module costs and vendors included in Section 17. The total bare module cost of equipment was calculated to be $100,444,079 by adding all the individual equipment costs and spare equipment costs for the process. A summary of the total bare module costs by equipment category can be seen in Figure 18.1.

Total Bare Module Costs:			
Fabricated Equipment	$	65,963,265	
Process Machinery	$	3,752,473	
Spares	$	7,704,000	
Storage	$	4,399,680	
Other Equipment	$	18,624,661	
Catalysts	$	-	
Computers, Software, Etc.	$	-	
Total Bare Module Costs:			$ 100,444,079

Figure 18.1. Total Bare Module Costs of equipment from Profitability Analysis Spreadsheet.

18.2 Total Permanent Investment

Additional fees associated with construction of the plant and royalties for outside research can be summarized in the total permanent investment of the plant. These fees were set to default values for general recommendations. A summary of the total permanent investment costs can be seen in Figure 18.2.

Total Permanent Investment

		% of Total Permanent Investment		
Year:	**2022**	100%	(default is first year of Construction, otherwise over-ride this year)	
	2023	0%		
	2024	0%		
	2025	0%		
		Cost of Site Preparations:	5.00%	of Total Bare Module Costs
		Cost of Service Facilities:	5.00%	of Total Bare Module Costs
		Allocated Costs for utility plants and related facilities:	$0	
		Cost of Contingencies and Contractor Fees:	18.00%	of Direct Permanent Investment
		Cost of Land:	2.00%	of Total Depreciable Capital
		Cost of Royalties:	$0	
		Cost of Plant Start-Up:	10.00%	of Total Depreciable Capital

Figure 18.2. Total Permanent Investment inputs from the Profitability Analysis Spreadsheet.

18.3 Investment Cost Summary

The output of total permanent investment from the Profitability Spreadsheet can be seen in Figure 18.3. The total permanent investment is $167,924,823 the majority of which is due to the bare module costs of the equipment.

Investment Summary				
Total Bare Module Costs:				
Fabricated Equipment	$	65,963,265		
Process Machinery	$	3,752,473		
Spares	$	7,704,000		
Storage	$	4,399,680		
Other Equipment	$	18,624,661		
Catalysts	$	-		
Computers, Software, Etc.	$	-		
Total Bare Module Costs:			**$**	**100,444,079**
Direct Permanent Investment				
Cost of Site Preparations:	$	5,022,204		
Cost of Service Facilities:	$	5,022,204		
Allocated Costs for utility plants and related facilities:	$	-		
Direct Permanent Investment			**$**	**110,488,487**
Total Depreciable Capital				
Cost of Contingencies & Contractor Fees	$	19,887,928		
Total Depreciable Capital			**$**	**130,376,415**
Total Permanent Investment				
Cost of Land:	$	2,607,528		
Cost of Royalties:	$	-		
Cost of Plant Start-Up:	$	13,037,642		
Total Permanent Investment - Unadjusted			$	146,021,585
Site Factor				1.15
Total Permanent Investment			**$**	**167,924,823**

Figure 18.3. Investment Cost Summary from outputs of the Profitability Analysis Spreadsheet.

19. Operating Cost - Cost of Manufacture

19.1 Raw Materials

The raw materials for this process include concentrated Teknova Inoculum Broth and corn syrup. The procurement of these raw materials are presented in Section 11. The costs of the raw materials and their ratio to kilograms of product are presented in Table 19.1. The costs for air, water, and natural gas are not included because they are accounted for in the cost of utilities. Lastly, the cost of the *Corynebacterium glutamicum* is not included because it is negligible compared to the costs of other raw materials.

Table 19.1. Annual utility requirements and costs.

Raw Material	Ratio (kg per kg product)	Cost ($USD/kg)
Teknova Broth	0.466	$0.51
Corn Syrup	0.687	$0.55

19.2 Utilities

The yearly requirements and costs for utilities including medium pressure steam, low pressure steam, natural gas, process water, cooling water, and electricity were presented in detail in Section 14. The standard prices for these utilities were found in Table 17.1 of Product and Process Design Principles 4th edition. The largest utilities costs include the electricity requirements for the production fermenters and the cooling water needed for heat exchangers throughout the process. The costs for utilities are presented in Table 19.2.

Table 19.2. Annual utility requirements and costs.

Utility	Ratio		Cost ($USD)		Yearly Requirement		Annual Cost ($USD)
Medium Pressure Steam (kg)	15.820	kg per kg product	$0.01540	per kg	4,107,090,000	kg	$63,249,186
Natural Gas (kg)	3.528	kg per kg product	$0.22050	per kg	915,802,500	kg	$201,934,451
Process Water (gal)	0.498	gal per kg product	$0.00080	per gal	284,983,672	gal	$227,987
Cooling Water (gal)	3.433	kg per kg product	$0.00010	per gal	1,965,286,280	gal	$196,529
Electricity (kWh)	0.215	kWh per kg product	$0.07000	per kWh	122,926,594	kWh	$8,604,862
Low Pressure Steam (kg)	57.653	kg per kg product	$0.01323	per kg	14,967,750,000	kg	$198,023,333

19.3 Variable Costs and Working Capital

The variable costs for the plant include general expenses and working capital. General expenses include selling/transfer expenses, direct and allocated research, administrative expenses, and management incentive compensation. Working capital includes accounts receivable, cash reserves, accounts payable, DLM inventory, and raw materials. The general expenses were all set to follow the general recommendations set in the Profitability Analysis Spreadsheet. A summary of the variable costs and working capital are presented in Figure 19.3.

Variable Costs			
General Expenses:			
Selling / Transfer Expenses:		3.00%	of Sales
Direct Research:		4.80%	of Sales
Allocated Research:		0.50%	of Sales
Administrative Expense:		2.00%	of Sales
Management Incentive Compensation:		1.25%	of Sales
Working Capital			
Accounts Receivable	a	30	Days
Cash Reserves (excluding Raw Materials)	a	30	Days
Accounts Payable	a	30	Days
DLM Inventory	a	4	Days
Raw Materials	a	2	Days

Figure 19.3. Variable Costs and Working Capital inputs from Profitability Analysis Spreadsheet.

19.4 Total Variable Cost and Working Capital Summary

The variable costs for the process, including general expenses, raw materials, byproducts, and utilities, can be evaluated together to calculate the total variable costs for the plant for one year. The total variable costs is $786,920,651 per year at 100% operating capacity.

The output of total variable costs and working capital from the Profitability Analysis Spreadsheet is presented in Figure 19.4.

Variable Cost Summary

Variable Costs at 100% Capacity:

General Expenses

Selling / Transfer Expenses:		$	27,993,029
Direct Research:		$	44,788,847
Allocated Research:		$	4,665,505
Administrative Expense:		$	18,662,020
Management Incentive Compensation:		$	11,663,762
Total General Expenses		$	107,773,163
Raw Materials	$0.382832 per lb of DLM		$219,153,934
Byproducts	$0.004000 per lb of DLM		($2,289,818)
Utilities	$0.807546 per lb of DLM		$462,283,372
Total Variable Costs		**$**	**786,920,651**

Working Capital

	2022	2023	2024
Accounts Receivable	$ 34,511,954	$ 17,255,977	$ 17,255,977
Cash Reserves	$ 17,931,386	$ 8,965,693	$ 8,965,693
Accounts Payable	$ (25,203,846)	$ (12,601,923)	$ (12,601,923)
DLM Inventory	$ 4,601,594	$ 2,300,797	$ 2,300,797
Raw Materials	$ 540,380	$ 270,190	$ 270,190
Total	**$ 32,381,468**	**$ 16,190,734**	**$ 16,190,734**
Present Value at 15%	*$ 28,157,798*	*$ 12,242,521*	*$ 10,645,670*

Total Capital Investment **$ 218,970,812**

Figure 19.4. Variable Cost Summary and Working Capital outputs from Profitability Analysis Spreadsheet.

19.5 Operations, Maintenance, Overhead

Plant operations, maintenance, and overhead also need to be incorporated into the fixed cost of the plant. It was determined that 6 operators would be needed per shift assuming 5 shifts. The operators would run the laboratories, ensure sake upkeep of the control systems used to regulate ammonia flow into the process, and closely monitor the batch seed train process. In addition, the operators would manage the super sacks for the transport of the DLM product. The

remaining values of maintenance, operating overhead, direct salaries and benefits, and direct wages and benefits were left as the default values from the Profitability Analysis Spreadsheet. Our plant should not deviate from these standards. A summary of operations, maintenance, and operating overhead is presented in Figure 19.5.

Fixed Costs		
Operations		
Operators per Shift:	6	(assuming 5 shifts)
Direct Wages and Benefits:	$40	/operator hour
Direct Salaries and Benefits:	15%	of Direct Wages and Benefits
Operating Supplies and Services:	6%	of Direct Wages and Benefits
Technical Assistance to Manufacturing:	$16,000.00	per year, for each Operator per Shift
Control Laboratory:	$20,000.00	per year, for each Operator per Shift
Maintenance		
Wages and Benefits:	4.50%	of Total Depreciable Capital
Salaries and Benefits:	25%	of Maintenance Wages and Benefits
Materials and Services:	100%	of Maintenance Wages and Benefits
Maintenance Overhead:	5%	of Maintenance Wages and Benefits
Operating Overhead		
General Plant Overhead:	7.10%	of Maintenance and Operations Wages and Benefits
Mechanical Department Services:	2.40%	of Maintenance and Operations Wages and Benefits
Employee Relations Department:	5.90%	of Maintenance and Operations Wages and Benefits
Business Services:	7.40%	of Maintenance and Operations Wages and Benefits

Figure 19.5. Operation, maintenance, and overhead inputs from Profitability Analysis Spreadsheet.

19.6 Other Fixed Costs

Other fixed costs for this process include property taxes and insurance, and straight line depreciation. No rental fees or licensing fees were accounted for. These values were left as the default from the Profitability Analysis Spreadsheet. A summary of inputs for these other fixed costs is presented in Figure 19.6.

Property Taxes and Insurance

Property Taxes and Insurance:	**2%**	**of Total Depreciable Capital**

Straight Line Depreciation

Direct Plant:	8.00%	**of Total Depreciable Capital, less 1.18 times the Allocated Costs for Utility Plants and Related Facilities**
Allocated Plant:	6.00%	**of 1.18 times the Allocated Costs for Utility Plants and Related Facilities**

Other Annual Expenses

Rental Fees (Office and Laboratory Space):	**$0**
Licensing Fees:	**$0**
Miscellaneous:	**$0**

Depletion Allowance

Annual Depletion Allowance:	**$0**

Figure 19.6. Other fixed costs inputs from Profitability Analysis Spreadsheet.

19.7 Total Fixed Costs

The output of total fixed costs from the Profitability Analysis Spreadsheet is presented in Figure 19.7, which includes operations, maintenance, operating overhead, and property taxes and insurances. The total fixed cost is $22,528,176 per year.

Fixed Cost Summary

Operations		
Direct Wages and Benefits	$	2,496,000
Direct Salaries and Benefits	$	374,400
Operating Supplies and Services	$	149,760
Technical Assistance to Manufacturing	$	480,000
Control Laboratory	$	600,000
Total Operations	**$**	**4,100,160**
Maintenance		
Wages and Benefits	$	5,866,939
Salaries and Benefits	$	1,466,735
Materials and Services	$	5,866,939
Maintenance Overhead	$	293,347
Total Maintenance	**$**	**13,493,959**
Operating Overhead		
General Plant Overhead:	$	724,489
Mechanical Department Services:	$	244,898
Employee Relations Department:	$	602,040
Business Services:	$	755,101
Total Operating Overhead	**$**	**2,326,529**
Property Taxes and Insurance		
Property Taxes and Insurance:	$	2,607,528
Other Annual Expenses		
Rental Fees (Office and Laboratory Space):	$	-
Licensing Fees:	$	-
Miscellaneous:	$	-
Total Other Annual Expenses	**$**	**-**
Total Fixed Costs	**$**	**22,528,176**

Figure 19.7. Total Fixed Cost Summary from Profitability Analysis Spreadsheet.

20. Profitability Analysis

20.1 Baseline Cash Flow Summary and Profitability

Profitability of the designed plant over the span of 20 years is assessed using the Profitability Analysis 4.0 spreadsheet provided by consultant Bruce Vrana. For the baseline process, product selling price of $3.60/ kg in the first year and side product selling price of $0.36/kg are assumed. The overall process is reasonably profitable, with IRR of 16.43 % and NPV of $ 5,650,200. In addition, the ROI of the third year of production is 26 %. The positive outlook accounts for any possible fermentation batch contamination and any other accidents that can occur during the process.

Cash Flow Summary

Year	Percentage of Design Capacity	Product Unit Price	Sales	Capital Costs	Working Capital	Var Costs	Fixed Costs	Depreciation	Depletion Allowance	Taxible Income	Taxes	Net Earnings	Cash Flow	Cumulative Net Present Value at 15%
2021	0%		-			-	-	-	-	-	-	-	-	-
2022	0%		-	(167,924,800)	(32,381,500)	-	-	-	-	-	-	-	(200,306,300)	(174,179,400)
2023	45%	$1.63	419,895,400	-	(16,190,700)	(354,114,300)	(22,528,200)	(26,075,300)	-	17,177,700	(3,950,900)	13,226,800	23,111,400	(156,703,900)
2024	68%	$1.65	636,141,600	-	(16,190,700)	(543,388,400)	(23,046,300)	(41,720,500)	-	27,986,400	(6,436,900)	21,549,600	47,079,300	(125,748,500)
2025	90%	$1.66	856,670,700	-	-	(741,181,800)	(23,576,400)	(25,032,300)	-	66,880,300	(15,382,500)	51,497,800	76,530,100	(81,992,200)
2026	90%	$1.68	865,237,400	-	-	(758,228,900)	(24,118,600)	(15,019,400)	-	67,870,400	(15,610,200)	52,260,200	67,279,600	(48,542,300)
2027	90%	$1.70	873,889,800	-	-	(775,668,200)	(24,673,400)	(15,019,400)	-	58,528,800	(13,461,600)	45,067,200	60,086,600	(22,565,200)
2028	90%	$1.71	882,628,700	-	-	(793,508,600)	(25,240,900)	(7,509,700)	-	56,369,500	(12,965,000)	43,404,500	50,914,200	(3,424,700)
2029	90%	$1.73	891,454,900	-	-	(811,759,300)	(25,821,400)	-	-	53,874,300	(12,391,100)	41,483,200	41,483,200	10,136,200
2030	90%	$1.75	900,369,500	-	-	(830,429,700)	(26,415,300)	-	-	43,524,500	(10,010,600)	33,513,800	33,513,800	19,663,000
2031	90%	$1.77	909,373,200	-	-	(849,529,600)	(27,022,800)	-	-	32,820,700	(7,548,800)	25,272,000	25,272,000	25,909,800
2032	90%	$1.78	918,466,900	-	-	(869,068,800)	(27,644,400)	-	-	21,753,800	(5,003,400)	16,750,400	16,750,400	29,510,200
2033	90%	$1.80	927,651,600	-	-	(889,057,400)	(28,280,200)	-	-	10,314,000	(2,372,200)	7,941,800	7,941,800	30,994,600
2034	90%	$1.82	936,928,100	-	-	(909,505,700)	(28,930,600)	-	-	(1,508,200)	346,900	(1,161,300)	(1,161,300)	30,805,800
2035	90%	$1.84	946,297,400	-	-	(930,424,300)	(29,596,000)	-	-	(13,723,000)	3,156,300	(10,566,700)	(10,566,700)	29,312,400
2036	90%	$1.86	955,760,400	-	-	(951,824,100)	(30,276,800)	-	-	(26,340,500)	6,058,300	(20,282,200)	(20,282,200)	26,819,900
2037	90%	$1.87	965,318,000	-	-	(973,716,000)	(30,973,100)	-	-	(39,371,200)	9,055,400	(30,315,800)	(30,315,800)	23,580,200
2038	90%	$1.89	974,971,100	-	-	(996,111,500)	(31,685,500)	-	-	(52,825,900)	12,149,900	(40,675,900)	(40,675,900)	19,800,300
2039	90%	$1.91	984,720,900	-	-	(1,019,022,100)	(32,414,300)	-	-	(66,715,500)	15,344,600	(51,370,900)	(51,370,900)	15,649,300
2040	90%	$1.93	994,568,100	-	-	(1,042,459,800)	(33,159,800)	-	-	(81,051,300)	18,641,800	(62,409,500)	(62,409,500)	11,264,100
2041	90%	$1.95	1,004,513,700	-	-	(1,066,436,200)	(33,922,500)	-	-	(95,844,900)	22,044,300	(73,800,600)	(73,800,600)	6,754,800
2042	90%	$1.97	1,014,558,900	-	64,762,900	(1,090,964,200)	(34,702,700)	-	-	(111,108,000)	25,554,800	(85,553,200)	(20,790,200)	5,650,200

Figure 20.1. The cash flow summary of the designed plant with a 20 year lifespan.

20.2 DLM Selling Price Sensitivity Analysis

A product selling price of $3.61/kg is chosen to compete effectively in the existing market. Different prices of DLM were used to assess the sensitivity of IRR on the product pricing. As Figure 20.2 shows, the IRR of the plant increases significantly as the product selling price is raised, suggesting that the profit of the plant is greatly affected by the DLM price. Price

with IRR of 16 % was chosen following guidance of consultant Michael Grady and consideration of the current market price of feed-grade DLM.

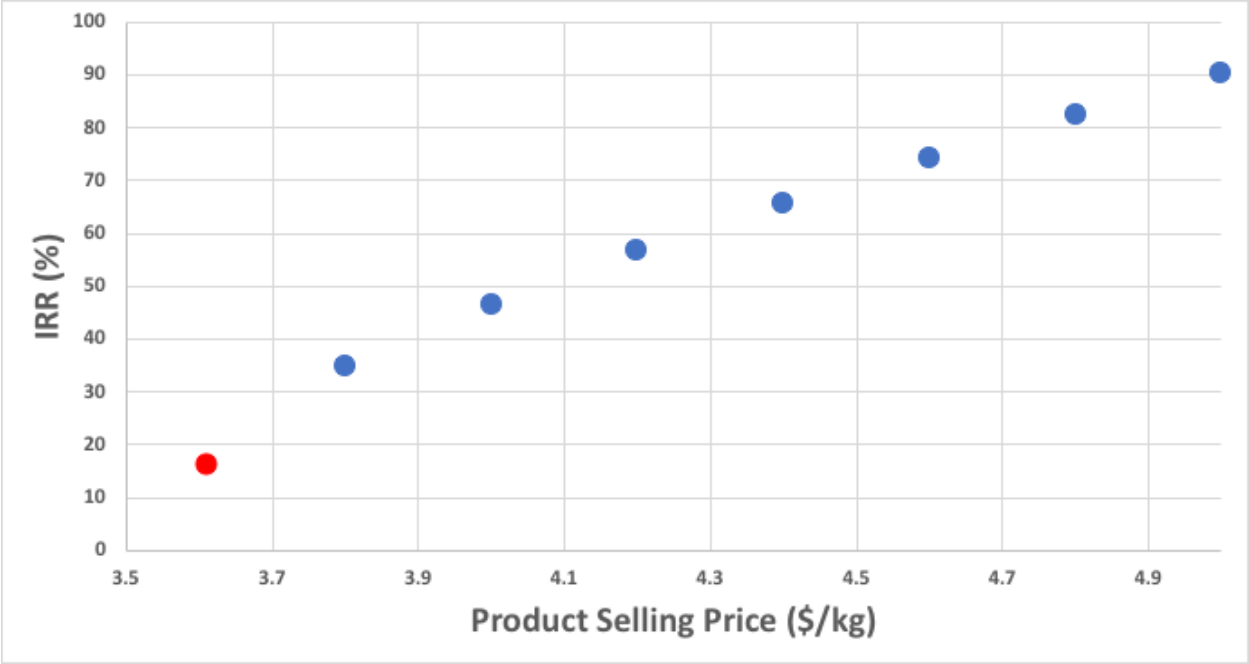


Figure 20.2. Relationship between IRR and product selling price. The red data point is the selected product price.

20.3 Corn Syrup Selling Price Sensitivity Analysis

Corn syrup is one of the main raw materials used in the plant. Different prices of corn syrup are used to determine the appropriate price and the sensitivity of the profit on the corn syrup selling price. As Figure 20.3 demonstrates, IRR of the plant is not as sensitive to the price of the corn syrup in the reasonably considered range as to the product selling price; data points are very closely clustered in the region between $0.8 - $0.9 (per kg of corn syrup). Overall, the price of the corn syrup was chosen according to the price provided from a manufacturer.

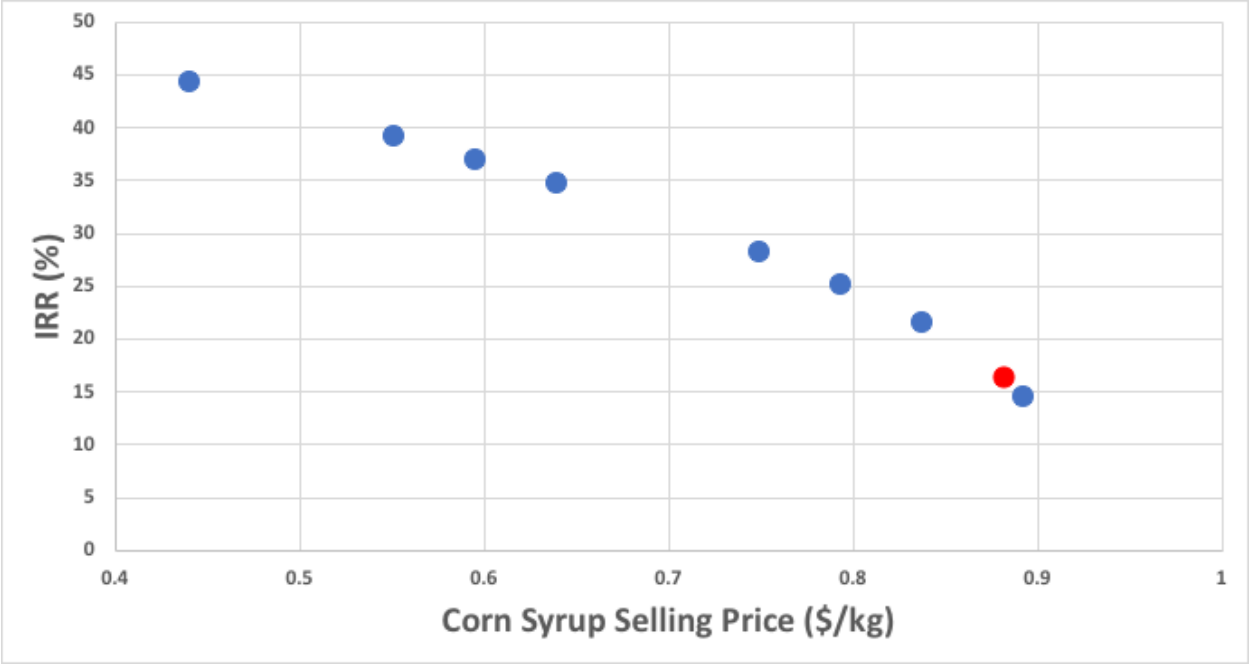


Figure 20.3. Relationship between IRR and the corn syrup selling price. The red data point represents the selected corn syrup selling price.

20.4 Teknova Broth Selling Price Sensitivity Analysis

Teknova pre-made broth will be purchased as the main fermentation broth in the plant. Initially, it was assumed that the pre-made broth would cost $50.7/kg following the guideline of the manufacturer. However, this led to a negative IRR, with NPV of $ -114 billion. Therefore, additional assumptions were made to decrease the price of the pre-made broth. First, it was assumed that Teknova can sell a much more concentrated broth with significantly decreased water content. Water would be purchased and added separately to the pre-made broth. Secondly, the price of the customized broth was assumed to be $0.232/kg. as this leads to a reasonable IRR of 16.43 %. Figure 20.4 illustrates the sensitivity of the plant profit based on the Teknova broth

selling price. As the figure depicts, pre-made broth that costs more than $0.55/kg leads to negative IRR.

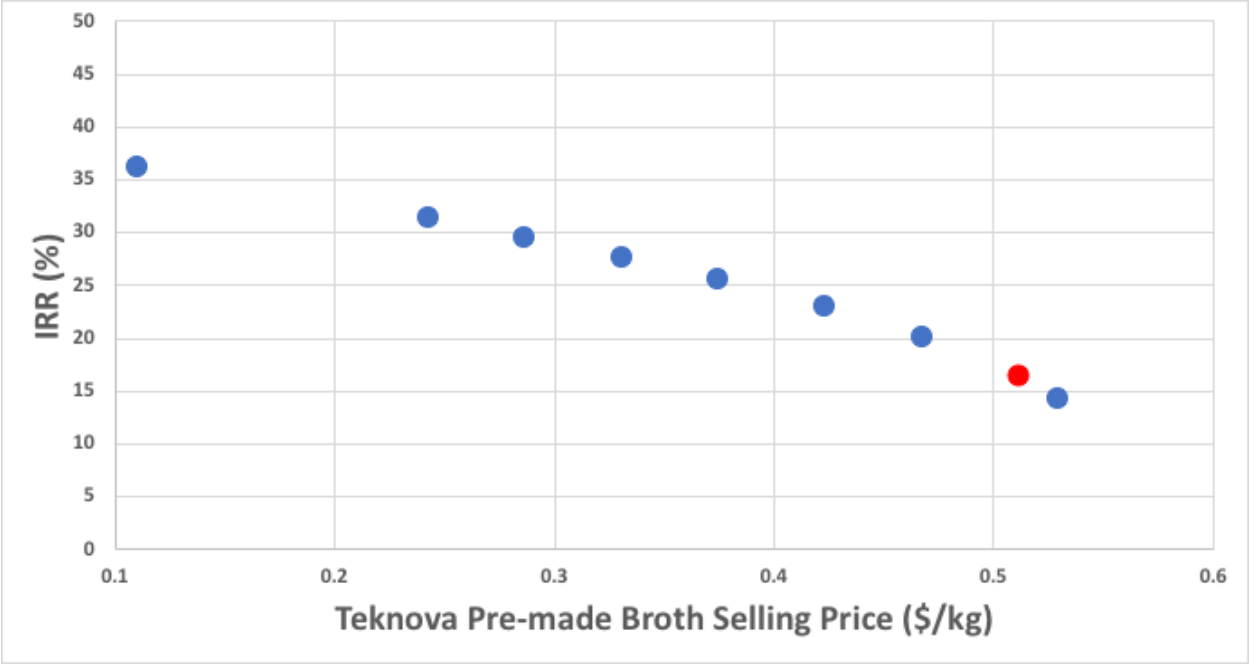


Figure 20.4. Relationship between IRR and Teknova pre-made broth selling price. The red data point represents the selected price of the pre-made broth.

21. Additional Considerations

21.1 Plant Location

Three locations were provided in the original project statement: the U.S. Midwest, U.S. Gulf Coast, and Singapore. While the majority of the DLM market is in Asia (33%), there is still substantial demand in the U.S. (23%). Plant production capacity for this project is 5% of the global DLM market ("Methionine Market 2019"). Since the final product is intended for use as poultry and swine feed, it is logical to locate near such farms, as they will be the main consumers.

Cost of labor was also considered in comparing the viability of a US versus Singapore location. Construction workers are paid 30% more in the US than in Singapore, but engineers who would operate the plant are paid very similarly in the two countries. Because the cost of construction workers would be a one time payment and the cost of operators would be a continuous payment, the fact that the construction of the plant in Singapore would probably cost less than in the US was not considered very strongly.

Most of the CS produced in the US is made in the US Midwest. CS is expensive to ship because of its large water content and therefore the plant should be chosen so that a CS plant is nearby. Iowa and Illinois are the two of the largest state producers of corn. In addition, they both border the Mississippi River, which is convenient for shipping purposes. Corn sweetener manufacturer Archer Daniels Midland Co. (ADM) has the world's largest corn mill located in Decatur, IL and Cargill Inc. has corn processing facilities in Cedar Rapids, IA. Iowa has lower

property taxes and corporate taxes than Illinois, so Iowa is the better economic choice for plant location.

21.2 Shipping

The main inputs and outputs of the process are CS, water, and DLM. As CS is a liquid raw material, it would be very costly to ship overseas from a U.S. producer to Asia or from Asia to the U.S. Therefore, it is most economically beneficial to locate the plant close to this critical raw material. DLM would be shipped as a dry product, implying a cheaper shipping rate; however, shipping overseas still adds significant economic burden. For example, shipping DLM from Iowa to China costs $15,134,361, while shipping from Singapore to China costs $9,614,697.

The US Gulf Coast was not strongly considered because if the plant was to be in the US, it should be located as close as possible to where the CS is produced in order to limit costs of transport. While the US Gulf Coast borders the Gulf of Mexico, which is convenient for shipping product by sea, the costs of having to transport the CS from the US Midwest to the US Gulf Coast outweighs the cost of transporting the Methionine product from the US Midwest to the closest body of water for transport to China.

This realization results in two economically viable options: locating the plant in the U.S. and serving as a DLM producer for U.S. or North American consumers, or locating in Singapore and meeting the DLM market demands in Asia.

21.3 Environmental Factors

There are two main routes to synthesize methionine, namely, the petrochemical and microbial production methods. Methionine is one of the more challenging amino acids to produce due to the fact that it contains sulfur. While the petrochemical route is more thermodynamically favorable, this route requires hydrogen cyanide (HCN), a highly toxic gas. The biosynthetic route does not require HCN, thus bypassing the most severe safety hazards for plant operators and communities near the plant.

In order to eliminate the biological hazards that could leave through the gas outlet stream from each pre-seed, seed, and production reactor as well as the continuous phase, a scrubber will be installed. The efficacy of this scrubber will be tested frequently in order to maintain safety of the air surrounding the plant. Additionally, safety precautions will be taken to educate manufacturing and lab staff on biological hazards to make sure potential contamination is limited (Jahnke, Pillarella, Weiner). Quantitative HAZOP studies will be used to identify and minimize risk of deviation from normal operations as well as to develop safeguards, to be ready in case of an emergency.

Another factor which was considered in determining the plant location was the existence of Singapore's carbon tax. The tax rate is $5 per metric ton of greenhouse gases, and by 2023, it will increase to between $10 and $15 per metric ton. The process outlined in this project produces roughly 80,000 metric tons, which would have an associated tax of US$282,863. The U.S. does not have any carbon tax.

The biomass side product from the fermentation process is "generally recognized as safe" (GRAS-approved) by FEMA. It is classified as "*Corynebacterium glutamicum* corn syrup

fermentation product FEMA No. 4907". With respect to the potential for some remaining organisms in this side stream, Nestec states that *C. glutamicum* is a non-pathogenic, non-toxigenic organism commonly used in food production. This means the side product can be sold for further use, specifically in condiments, fish products, seasonings, soups, and more.

21.4 Public and Employee Safety, Health, and Welfare

The chemicals, biocatalysts, and bacterium used in this project all have low hazard levels. Exposure to HCN can cause a wide range of dangerous effects: headaches, nausea, seizures, and even death. As described in section 21.3, the biosynthetic route avoids the traditional production risks associated with HCN. The process should be odorless under standard operating conditions, so odor from the plant will not be a concern for surrounding communities. The plant will operate in accordance with OSHA safety standards to minimize risk to employees. One specific example of reducing risk to employees is the inclusion of a cooling jacket on the feed bucket conveyor in Figure 12.3, to cool the dryer output stream from 80°C to 50°C. Handling materials at temperatures higher than 50°C presents burn potential for the worker which would require extra safety precautions.

21.5 Global, Cultural, Social, and Ethical Factors

There were several options available when selecting a bacterium for the fermentation process, including several genetically modified organisms (GMOs) such as genetically engineered *C. glutamicum*. While the U.S. and Asia are generally friendly towards GMOs, the non-GMO strain of *C. glutamicum* was used for this process to be able to sell DLM product to both organic and non-organic farms.

One of the units in the process, the rotary dryers in Figure 12.3 (RD-01 through RD-04), is part of a larger system, sometimes called a “dryer island”, which comes equipped with support equipment which ensures proper exhaust gas handling in compliance with local, state, and federal regulations. These specific components serve the purpose of removing particulates from the process gas to minimize and prevent their release into the atmosphere.

With regards to plant location, Singapore’s Jurong Island, a petrochemical hub, is already dense with refineries and chemical plants. Land for industry is already so limited that developers began preparing storage space within the island’s underground caves. Floating platforms will likely become the new source of additional space for industry needs. On the other hand, the U.S. Midwest consists of spacious plains with plentiful access to corn and waterways. Iowa and central/southern Illinois are very rural areas; construction of a methionine plant will promote additional employment of residents from communities nearby, and it will not drastically threaten the amount of available land.

22. Conclusions and Recommendations

Based on the design specifications and economic analysis of this process, the production of DLM was found to be profitable. The recycling of process water and steam throughout the process proved to be essential to this profitability. Additionally, the use of corn syrup as the glucose source was vital for economic success. High fructose corn syrup as the source of glucose was originally explored, but it is only 45% glucose and is more expensive than corn syrup. The process was found to have an IRR around 49% when the price of DLM was $4.04/kg or the price of corn syrup was $0.44/kg. Although both of those prices increase profitability, they are likely not very feasible as the higher DLM price would drive customers to other suppliers and that low of a corn syrup price may be difficult to find from a supplier. The profitability could have been further improved if the fermentation media was made from individual bulk chemicals rather than purchasing a premade Teknova broth. Creating media from the bulk chemicals would save 27.5 cents per pound of DLM and therefore save the entire process $157MM per year. This route of making the media would have higher capital costs because more storage tanks and mixers would be needed, but this cost would be almost negligible compared to the money saved on the price of raw materials. Capital costs could also be cut significantly if a corrosion study was conducted. Stainless steel 316 was used to construct all process units to err on the side of caution. Stainless steel 316 is more corrosion resistant than stainless steel 304, but also more expensive. There is potential for using stainless steel 304 instead.

The separation by centrifugation should be examined more thoroughly. Although the FEMA 4907 side product from the centrifugation can be sold, it is sold at one tenth of the price of DLM. Some DLM is lost in the side product so exploring a recycling separation process so that more of the DLM can continue downstream would increase profits. In addition, adding a recycle component to the crystallization would be beneficial to yield more product. Currently, 2% of the DLM is lost during crystallization. If the crystallization process is designed so that the streams out of the crystallizers are recycled back through the crystallizers, a yield of up to 99.9% DLM could be achieved. Another factor to consider is genetically modified bacteria. Although a GMO was not originally selected due to ethical concerns and the lack of complete properties and characteristics information, certain GMO strains could improve the productivity of the fermentation process and therefore could potentially significantly increase the amount of DLM produced. Lastly, scaling down the process should be considered. After the plant is constructed in three years, the DLM market should be assessed and an economic analysis should be completed to determine if it is more profitable to decrease production to 200 ktonnes or 150 ktonnes per year rather than 250 ktonnes per year.

23. Acknowledgements

The successful completion of this investigation could not have been completed without the guidance of many knowledgeable professionals whom we met throughout the year. We would like to sincerely thank Dr. Sue Ann Bidstrup Allen and Prof. Bruce Vrana for taking a personal interest in our project and ensuring we continued to move forward in the right direction. Our team would also like to thank Mr. Stephen Tieri for proposing this project and supporting us as we progressed through key decisions while challenging our problem solving skills. Additionally, we would like to thank the many industrial consultants who met with us on a weekly basis to offer advice that improved our chemical engineering knowledge, especially Rick Bockrath and Michael Grady. Lastly, we are very grateful for all the time Professor Fabiano put into helping us with our Aspen simulations.

24. Bibliography

Biological Industries Israel Beit Haemek. (2017, June). Cryopreservation Guide, https://www.bioind.com/media/wysiwyg/product/cryostem/CryopreservationGuide.pdf. Accessed 22 March 2020.

Bolten, Christopher J. "Bio-based Production of L-Methionine in Corynebacterium glutamicum." *Cuvillier-Verlag*, Accessed 3 Feb 2020.

Boy, Matthias, Klein, Daniela, and Schröder, Hartwig. Method for the Production of Methionine. US7,785,846 B2, United States Patent and Trademark Office, 31 Aug 2010.

"Carbon Tax." *National Environment Agency*, https://www.nea.gov.sg/our-services/climate-change-energy-efficiency/climate-change/carbon-tax. Accessed 1 March 2020.

"Clean-in-Place (CIP) and Sterilize-in-Place (SIP) systems." *Solida Biotech*, https://solidabiotech.com/product/clean-place-cip-sterilize-place-sip-systems/. Accessed 3 April 2020.

Dischert, Wanda, Figge, Rainer, and Vasseur, Perrine. Microorganism for methionine production with improved methionine synthase activity and methionine efflux. WO 2015/028674 A1, World Intellectual Property Organization, 5 March 2015.

"Evonik commissions second complex for MetAMINO production in Singapore." *Evonik*, 18 June 2019, https://corporate.evonik.com/en/evonik-commissions-second-complex-for-metamino-production-in-singapore-113765.html. Accessed 1 Feb 2020.

Gargi, Seth. "Recent advances in optimal cell banking of mammalian cells for biopharmaceutical production." *Biology*, 2015, Semantic Scholar, Accessed 15 March 2020.

Huang, C. "Microbial nutrients, growth, and cultivation." *National Taiwan University*, http://cthuang.bst.ntu.edu.tw/microbialmetabolism/mm2.pdf. Accessed 18 March 2020.

Jahnke, Craig, Pillarella, Corinne, and Weiner, Brielle. "Production of Single Cell Protein and Astaxanthin Using Methanol as Carbon Source." *University of Pennsylvania*, 20 May 2019, https://repository.upenn.edu/cgi/viewcontent.cgi?article=1113&context=cbe_sDr. Accessed 15 March 2020.

"MetAMINO Beset Handling." *Evonik*, https://animal-nutrition.evonik.com/product/feed-additives/en/products/methionine-and-derivatives/dl-methionine/metamino-hub/best-handling/. Accessed 1 Feb 2020.

"Methionine Market Highlights The Competitive Scenario Of The Market, Major Competitors and Benchmarking." *P&S Intelligence*, 1 Oct 2019, https://spacemarketnow.com/2019/10/01/methionine-market-highlights-the-competitive-scenario-of-the-market-major-competitors-and-benchmarking/. Accessed 26 Jan 2020.

"Methionine Market Size, Share & Trends Analysis Report by Raw Material, By Application, and Segment Forecasts, 2015-2022." *Grand View Research*, July 2016, https://www.grandviewresearch.com/industry-analysis/methionine-market. Accessed 28 Jan 2020.

"Methionine Market 2019 Global Industry Size, Share, Business Growth, Revenue, Trends, Global Market Demand Penetration and Forecast to 2024| 360 Market Updates." *MarketWatch*, 22 Oct 2019, https://www.marketwatch.com/press-release/methionine-market-2019-global-industry-size-share-business-growth-revenue-trends-global-market-demand-penetration-and-forecast-to-2024-360-market-updates-2019-10-22. Accessed 19 Feb 2020.

Perry, R. H., and D. W. Green, Eds., Chapter 19, in "Perry's Chemical Engineers' Handbook," 6th ed., McGraw-Hill, New York (1984).

Shuler, Michael L., and Fikret Kargi. Bioprocess engineering. New York: Prentice Hall, 2002. http://elliotswartz.com/cellbasedmeat/cleanmeat301 (Figure 13.1.1)

"UltraFlow: Powerful CIP in a Compact, Portable Design." *SaniMatic*, https://sanimatic.com/wp-content/uploads/2019/04/BPB-0006.2_UltraFlow_Brochure_09.2019.pdf. Accessed 25 March 2020.

Venkata, Narayana A., Vamsi, Priya A., Venkata, Nadh R., etc. "Methionine Production by Coryneform Bacteria through Fermentation." *RJPBCS*, Volume 4, Issue 2, June 2013, Accessed 19 Jan 2020.

Appendix A: Sample Calculations

A.1 Fermenters

Doubling Time for Pre-Seed Reactor

$$Initial\ DCW\ (\tfrac{g}{L}) = \frac{Working\ V\ of\ previous\ seed\ unit \times Final\ DCW\ of\ previous\ seed\ unit}{Working\ V\ of\ current\ unit} = \frac{2L \times 0.67\frac{g}{L}}{4500L} = 0.0003$$

$$Growth\ rate\ (\tfrac{m}{hr}) = \frac{ln(Final\ DCW/Initial\ DCW)}{Time} = \frac{ln(1/0.0003)}{22} = 0.37$$

$$Doubling\ Time\ (hr) = \frac{ln(2)}{Growth\ rate} = \frac{ln(2)}{0.37} = 1.88$$

Stagger Time

Stagger time is defined by the cycle time (sum of fermentation time, clean-in-place, steam-in-place, charge, etc.) and number of fermentation trains:

$$\frac{69\ hours}{6\ fermentation\ trains} = 11.5\ hours$$

OUR

Reaction stoichiometry, global productivity and molecular weight gives the oxygen uptake rate for each respective reactor. The below is the example calculation for the seed reactor.

$$Global\ Productivity = Specific\ productivity \times DCW = 0.055\frac{gr\ DLM}{hr \times gr\ DCW} \times 6\frac{gr}{L} = 0.33\frac{gr\ DLM}{hr \times L}$$

$$OUR = Molar\ ratio\ of\ O_2\ to\ DLM \times \frac{Global\ Productivity}{MW\ of\ DLM} \times \frac{1000\ millimoles}{1\ mole} = 20.86 \times \frac{0.33\frac{gr\ DLM}{hr \times L}}{149.21\frac{gr}{L}} \times 1000 = 46.13\frac{mmole\ O_2}{L \times hr}$$

Heat Generated in Reaction

Reaction stoichiometry gives the amount of Oxygen consumed during the reaction. This can be used to calculate the heat generated in the reaction (Shuler). 10^5

$$0.12 * 46.13\ mmol\ O_2\ uptake\ per\ L\ per\ hr * 42.5 \times 10^3\ L\ in\ seed\ fermenter = 2.35 \times 10^5\ \frac{kcal}{hr}$$

Chilled Water

Assuming 70% heat transfer efficiency and that the chilled water is cooled 6 degrees, the chilled water requirement can be calculated.

$$\frac{2.35 \times 10^5\ \frac{kcal}{hr} * 0.7}{0.9981\frac{kcal}{kg\ ^oC} * 6\ ^oC} = 27,500\frac{kg}{hr}\ chilled\ water\ for\ the\ seed\ fermenter$$

Ammonia

The pre-seed, seed, and production fermenters needed ammonia for pH control. Based off a 0.05 kg per m^3 of fermentation broth per hour rate given by consultant Rick Bockrath, the ammonia requirement can be calculated. The calculations for the pre-seed fermenter is shown below.

$$0.05 \times \frac{1\,m^3}{1000L} \times 4846\ \frac{kg\ fermentation\ broth}{batch} = 0.242\ kg\ per\ batch$$

A.2 Air Compressor

Compressor requirements were evaluated using the static head of liquid in the fermenter plus several different pressure drops.

Static head of liquid in fermenter + *P drop through inlet piping and inlet air control valve* + *P drop through inlet HEPA filters* + *P drop through exit piping and exit air control valve* + *P drop through exit gas scrubber* + *P to ensure positive pressure when discharging to atmosphere* = *Pressure at sparger*

$(84\,ft/30\,ft) + 0.5\,atm + 0.5\,atm + 0.6\,atm + 0.3\,atm + 0.3\,atm = 5\,atm = 5.1\,bar$ for the production fermenter

A.3 Broth Heating Unit

Broth heating was accomplished using a shell-and-tube heat exchanger.

Heat Duty Q (kJ/hr) = $(702421\ \frac{kg}{hr})\,(39°C)(4.182\ \frac{J}{g°C})(1000\ \frac{g}{kg}) = 1.14563 \times 10^8$

Q (BTU/hr) = $108584772.754\ \frac{BTU}{hr}$

Flow rate of steam (kg/hr) = $(1.14563 \times 10^8\ \frac{kJ}{hr})/(30°C \times 1.9135\ \frac{kJ}{kgK}) = 1.9957 \times 10^6\ \frac{kg}{hr}$

ΔT_{LM} (°F) = $\frac{(302-87.8)-(356-158)}{ln\frac{(302-87.8)}{(356-158)}} = 206°F$

F_T = 1, Overall Transfer Coefficient ($\frac{BTU}{Fft^2hr}$) = 567

Surface Area (ft^2) = $(108584772.754\ \frac{BTU}{hr})/(206°F \times 1 \times 567\ \frac{BTU}{Fft^2hr}) = 929.692\,ft^2$

A.4 Rotary Drum Dryer

Heat Duty Q (kJ/hr) = $(58400\ \frac{kg}{hr})(4.182\ \frac{J}{g°C})(51°C)(1000\ \frac{g}{kg}) = 1.24557 \times 10^7$

Flow rate of natural gas (kg/hr) = $(1.24557 \times 10^7\ \frac{kJ}{hr})/(2.614\ \frac{kJ}{kgK} \times 46°C) = 103587$

A.5 Triple Effect Evaporation

Sizing Flash Vessels

$$Velocity\ (ft/s) = 0.27\sqrt{(\rho_l - \rho_v)/\rho_v} = 0.27\sqrt{(54.43\frac{lb}{ft^3} - 0.134\frac{lb}{ft^3})/0.134\frac{lb}{ft^3}} = 5.44$$

ρ_v = *density of vapor stream* $(\frac{lb}{ft^3})$

ρ_l = *density of liquid stream* $(\frac{lb}{ft^3})$

$$Area\ (ft^2) = \frac{Vapor\ flow}{\rho_v \times Velocity} = \frac{514991\ \frac{lb}{hr}}{0.134\ \frac{lb}{ft^3} \times 5.44\ \frac{ft}{s}} \times \frac{1\ hr}{3600\ s} = 196.66$$

$$Diameter\ (ft) = \sqrt{\frac{Area \times 4}{\pi}} = \sqrt{\frac{196.66\ ft^2 \times 4}{\pi}} = 15.8$$

$$Length\ (ft) = Diameter \times 3 = 15.8 \times 3 = 47.4\ m$$

A.6 Crystallizer

Solid DLM Out of Crystallizer

$$Soluble\ DLM\ at\ 5\ ^oC\ (\frac{kg}{hr}) = \frac{Total\ Flow\ In}{\rho} \times Solubility\ at\ 5^oC \times 1000 = \frac{178430\ \frac{kg}{hr}}{924594\ \frac{kg}{L}} \times 20.53\frac{g}{L} \times \frac{1\ kg}{1000\ g} = 0.0040$$

$$Solid\ DLM\ Flow\ (\frac{kg}{hr}) = DLM\ Flow\ In - Soluble\ DLM\ at\ 5\ ^oC = 37264 - 0.0040 = 37263.996$$

Sizing Crystallizer Conical Vessel

$$Excess\ Force,\ F_g\ (\frac{kg \times m}{s^2}) = (\rho_s - \rho_l) \times g \times \frac{4}{3} \times \pi \times R_p^3 = (1340 - 997) \times 9.81 \times \frac{4}{3} \times \pi \times 0.00005^3 = 1.76 \times 10^{-9}$$

ρ_s = *density of particle* $(\frac{kg}{m^3})$

ρ_l = *density of solvent* $(\frac{kg}{m^3})$

g = *gravitational acceleration* $(\frac{m}{s^2})$

R_p = *radius of particle* (m)

$$Velocity\ (m/s) = \frac{F_g}{6 \times \pi \times \mu \times R} = \frac{1.76 \times 10^{-9}}{6 \times \pi \times 0.0017 \times 0.00005} = 0.0011\ \frac{m}{s}$$

μ = *viscosity of water* $(Pa \times s)$

$$Crossflow\ Area\ (m^2) = \frac{(Water\ flow)/3}{\rho_w \times Velocity} \times \frac{1\ hr}{3600\ s} = \frac{(136198\ \frac{kg}{hr})/3}{997\ \frac{kg}{m^3} \times 0.0011\ \frac{m}{s}} \times \frac{1\ hr}{3600\ s} = 11.50$$

ρ_w = *density of water* $(\frac{kg}{m^3})$

$$Diameter\ (m) = \sqrt{\frac{Area \times 4}{\pi}} = \sqrt{\frac{11.50\ m^2 \times 4}{\pi}} = 3.83$$

$$Length\ (m) = Diameter \times 1.5 = 3.83 \times 1.5 = 5.75$$

Duty of Crystallizer

$$Heat\ of\ Fusion\ of\ DLM:\ \Delta H_{fus} = 20.2\ \frac{kJ}{mol} \Rightarrow 20.2 \times 8168.92\ \frac{kmol}{hr} \times \frac{1000\ mol}{1\ kmol} = 165012184\ \frac{kJ}{hr}$$

Add Heat of Fusion to Duty From Crystallizer (*calculated in Aspen*)

$$\textit{Duty of crystallizer} = 10954976 \frac{cal}{sec} + 7358930 \frac{cal}{sec} = 18.3 \times 10^6 \frac{cal}{sec}$$

A.7 Rotary Dryer

Solid comes from centrifuge at 19% moisture. Assume hot air comes in at 170oC and 0.7% humidity.
The exit solid temperature must not exceed 80°C and the air velocity must not exceed 1.5 m/s in order to avoid dusting of the solid. Specific heat of the dry solid is cps = 1.94 kJ/kg-K.

$$\textit{Mass of dry solid desired, } Ls = 34,728\ kg/hr$$

(above value is taken from material balance to satisfy 250 kiloton demand)
This flow rate will require 4 dryer units. All subsequent calculations are for one unit.

$$\textit{Mass of dry solid desired, } Ls = 34,728/4\ kg/hr = 8,682\ kg/hr$$
$$\textit{Moisture in the wet solid, } x_1 = 0.19/0.81 = 0.2345$$
$$\textit{Moisture in the dry solid, } x_2 = 0.01/0.99 = 0.0101$$
$$\textit{Rate of water evaporation} = 8,682(0.2345 - 0.0101) = 1,948\ kg/hr$$

Calculate enthalpy of different streams:

$$H'S1 = [cps + (4.187) * X1](TS1 - 0) = (1.94 + (4.187 * .19) * (5 - 0)$$
$$= 13.67\ kJ/kg\ of\ dry\ solids$$

$$H'G2 = [1.005 + 1.88 * Y2](TG2 - 0) + Y2 * \lambda 0$$
$$= (1.005 + 1.88 * 0.007) * (170 - 0) + (0.007 * 2500)$$
$$= 190.6\ kJ/kg$$

$$H'G1 = [1.005 + 1.88 * Y1](TG1 - 0) + Y1 * \lambda 0$$
$$= [1.005 + 1.88 * Y1] * (60 - 0) + Y1 * 2500$$
$$= [1.005 + 1.88 * Y1] * (100 - 0) + (Y1 * 2500)$$
$$= (100.5 + 2688 * Y1)\ kJ/kg$$

Overall mass balance:

$$GS(Y1 - Y2) = LS(X1 - X2) = G2(Y1 - 0.007)$$
$$1,948 = G2(Y1 - 0.007)$$

$$LS(H'S2 - H'S1) = GS(H'G2 - H'G1)$$

$$8,682 * (158.58 - 13.67) = 1,948/(Y1 - 0.007) * (190.6 - 100.5 - 2688 * Y1)$$

$Y1 = 0.02838$ *and* $Gs = 1,948.24/((0.02838 - 0.007) = 91,124\ kg/hr$

Calculation of the shell diameter:

Humid volume, $VH = [(1/28.97) + (Y/18.02)]\ x\ 22.4\ x\ [(TG + 273)/273]$

Humid volume of the inlet gas, $(T = 170C,\ Y2 = 0.007)\ VH2 = 1.269\ m/kg\ dry\ air$

Humid volume of the exit gas $(T = 100C,\ Y1 = 0.0284)$ *or* $VH1 = 1.1047\ m^3/kg\ dry\ air$

The maximum volumetric gas flow rate $= GS\ x\ VH2$

$= 91,124 * 1.269 = 115,637\ m^3/hr = 32\ m^3/sec$

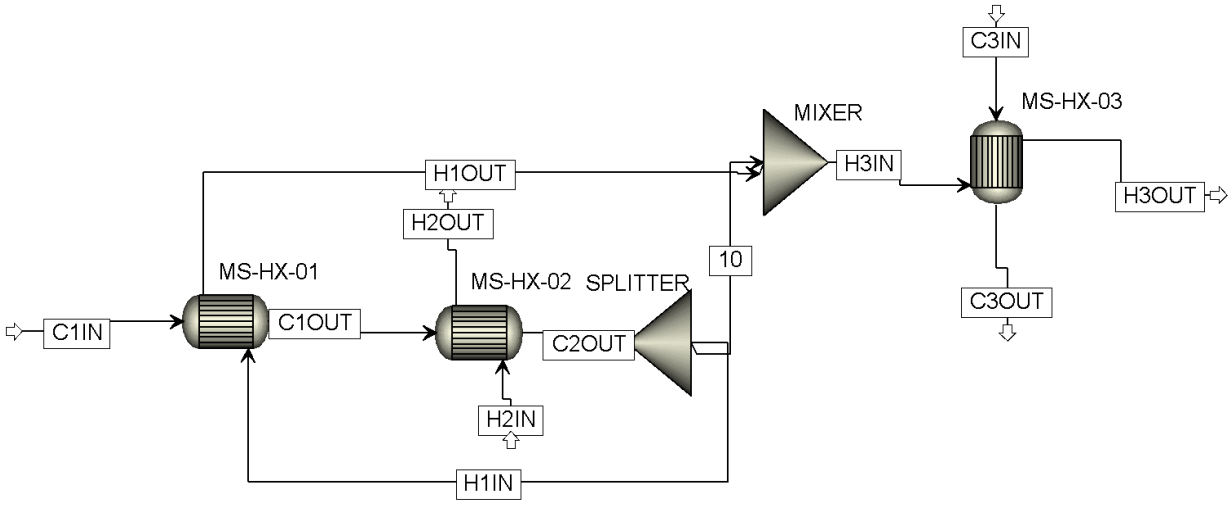
Take the maximum superficial air velocity to be 1.2 m/s (this is 20% less than the maximum allowable velocity since part of the dryer is filled with the moving solid, and the entire cross-section is not available for gas flow). If *d* is the diameter,

$(\pi d^2/4)\ x\ (1.2) = 32\ m^3/sec \rightarrow d = 5.84\ meters = 19.2\ feet$

Conservatively purchase a 6-meter, (or 20-foot) diameter unit for each of the 4 dryers.

Appendix B: Aspen Calculations

Media Sterilization Process Flow Diagram:



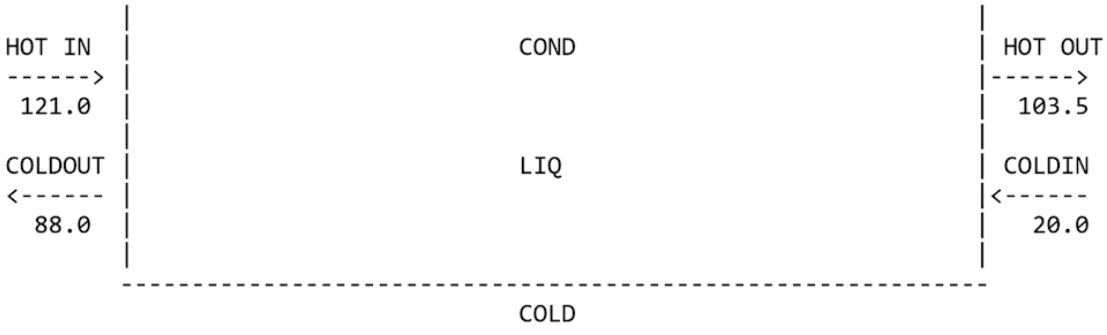
Media Sterilization Input:

```
;
;Input Summary created by Aspen Plus Rel. 37.0 at 18:31:09 Tue Apr 7, 2020
;Directory \\nestor\alliwa\  Filename C:\Users\alliwa\AppData\Local\Temp\~apeee7.txt
;


DYNAMICS
    DYNAMICS RESULTS=ON

IN-UNITS MET PRESSURE=bar TEMPERATURE=C DELTA-T=C PDROP=bar  &
        INVERSE-PRES='1/bar' SHORT-LENGTH=mm

DEF-STREAMS CONVEN ALL

MODEL-OPTION

DATABANKS 'APV110 PURE37' / 'APV110 AQUEOUS' / 'APV110 SOLIDS' &
         / 'APV110 INORGANIC' / 'APESV110 AP-EOS' /  &
        'NISTV110 NIST-TRC' / NOASPENPCD

PROP-SOURCES 'APV110 PURE37' / 'APV110 AQUEOUS' /  &
        'APV110 SOLIDS' / 'APV110 INORGANIC' / 'APESV110 AP-EOS' &
         / 'NISTV110 NIST-TRC'

COMPONENTS
    WATER H2O /
    DEXTROSE C6H12O6 /
    AMMON-01 "(NH4)2SO4"

SOLVE
    RUN-MODE MODE=SIM

FLOWSHEET
    BLOCK MS-HX-01 IN=H1IN C1IN OUT=H1OUT C1OUT
    BLOCK MS-HX-02 IN=H2IN C1OUT OUT=H2OUT C2OUT
    BLOCK SPLITTER IN=C2OUT OUT=H1IN 10
    BLOCK MIXER IN=H1OUT 10 OUT=H3IN
    BLOCK MS-HX-03 IN=H3IN C3IN OUT=H3OUT C3OUT

PROPERTIES NRTL

STREAM C1IN
    SUBSTREAM MIXED TEMP=20. PRES=1.01 MASS-FLOW=52275.
    MASS-FRAC WATER 0.8194 / DEXTROSE 0.0999 / AMMON-01  &
        0.0807

STREAM C3IN
    SUBSTREAM MIXED TEMP=20. PRES=1.01325 MOLE-FLOW=55000.
    MOLE-FLOW WATER 1. / DEXTROSE 0. / AMMON-01 0.

STREAM H2IN
```

Media Sterilization Report:

```
BLOCK:  MS-HX-01 MODEL: HEATX
------------------------------
  HOT SIDE:
  ---------
  INLET STREAM:             H1IN
  OUTLET STREAM:            H1OUT
  PROPERTY OPTION SET:   NRTL      RENON (NRTL) / IDEAL GAS
  COLD SIDE:
  ----------
  INLET STREAM:             C1IN
  OUTLET STREAM:            C1OUT
  PROPERTY OPTION SET:   NRTL      RENON (NRTL) / IDEAL GAS

                    ***  MASS AND ENERGY BALANCE  ***
                                    IN              OUT        RELATIVE DIFF.
   TOTAL BALANCE
      MOLE(KMOL/HR )            4334.43         4334.43          0.00000
      MASS(KG/HR   )            92916.2         92916.2        -0.156613E-15
      ENTHALPY(CAL/SEC )      -0.788942E+08   -0.788942E+08     0.188875E-15

                    ***  CO2 EQUIVALENT SUMMARY ***
   FEED STREAMS CO2E             0.00000      KG/HR
   PRODUCT STREAMS CO2E          0.00000      KG/HR
   NET STREAMS CO2E PRODUCTION   0.00000      KG/HR
   UTILITIES CO2E PRODUCTION     0.00000      KG/HR
   TOTAL CO2E PRODUCTION         0.00000      KG/HR

                       ***  INPUT DATA  ***

  FLASH SPECS FOR HOT SIDE:
  TWO    PHASE      FLASH
  MAXIMUM NO. ITERATIONS                                  30
  CONVERGENCE TOLERANCE                                    0.000100000

  FLASH SPECS FOR COLD SIDE:
  TWO    PHASE      FLASH
  MAXIMUM NO. ITERATIONS                                  30
  CONVERGENCE TOLERANCE                                    0.000100000

  FLOW DIRECTION AND SPECIFICATION:
    COUNTERCURRENT   HEAT EXCHANGER
    SPECIFIED COLD OUTLET TEMP
    SPECIFIED VALUE               C                      88.0000
    LMTD CORRECTION FACTOR                                1.00000

  PRESSURE SPECIFICATION:
    HOT  SIDE PRESSURE DROP       BAR                     0.0000
    COLD SIDE PRESSURE DROP       BAR                     0.0000

  HEAT TRANSFER COEFFICIENT SPECIFICATION:
```

```
  HOT LIQUID    COLD LIQUID      CAL/SEC-SQCM-K          0.0203
  HOT 2-PHASE   COLD LIQUID      CAL/SEC-SQCM-K          0.0203
  HOT VAPOR     COLD LIQUID      CAL/SEC-SQCM-K          0.0203
  HOT LIQUID    COLD 2-PHASE     CAL/SEC-SQCM-K          0.0203
  HOT 2-PHASE   COLD 2-PHASE     CAL/SEC-SQCM-K          0.0203
  HOT VAPOR     COLD 2-PHASE     CAL/SEC-SQCM-K          0.0203
  HOT LIQUID    COLD VAPOR       CAL/SEC-SQCM-K          0.0203
  HOT 2-PHASE   COLD VAPOR       CAL/SEC-SQCM-K          0.0203
  HOT VAPOR     COLD VAPOR       CAL/SEC-SQCM-K          0.0203

                    ***  OVERALL RESULTS  ***

STREAMS:
                 ------------------------------------
                 |                                  |
H1IN      ----->|               HOT                |-----> H1OUT
T=  1.2100D+02   |                                  |       T=  1.0351D+02
P=  1.0100D+00   |                                  |       P=  1.0100D+00
V=  9.5068D-01   |                                  |       V=  7.8956D-01
                 |                                  |
C1OUT     <-----|               COLD               |<----- C1IN
T=  8.8000D+01   |                                  |       T=  2.0000D+01
P=  1.0100D+00   |                                  |       P=  1.0100D+00
V=  0.0000D+00   |                                  |       V=  0.0000D+00
                 ------------------------------------

DUTY AND AREA:
  CALCULATED HEAT DUTY           CAL/SEC           917307.3981
  CALCULATED (REQUIRED) AREA     SQM                   83.0557
  ACTUAL EXCHANGER AREA          SQM                   83.0557
  PER CENT OVER-DESIGN                                  0.0000

HEAT TRANSFER COEFFICIENT:
  AVERAGE COEFFICIENT (DIRTY)    CAL/SEC-SQCM-K         0.0203
  UA (DIRTY)                     CAL/SEC-K          16861.8801

LOG-MEAN TEMPERATURE DIFFERENCE:
  LMTD CORRECTION FACTOR                                1.0000
  LMTD (CORRECTED)               C                     54.4013
  NUMBER OF SHELLS IN SERIES                             1

PRESSURE DROP:
  HOTSIDE, TOTAL                 BAR                    0.0000
  COLDSIDE, TOTAL                BAR                    0.0000

                    ***  ZONE RESULTS  ***

TEMPERATURE LEAVING EACH ZONE:

                                HOT
     ------------------------------------------------------------
```



ZONE HEAT TRANSFER AND AREA:

ZONE	HEAT DUTY CAL/SEC	AREA SQM	LMTD C	AVERAGE U CAL/SEC-SQCM-K	UA CAL/SEC-K
1	917307.398	83.0557	54.4013	0.0203	16861.8801

HEATX COLD-TQCU MS-HX-01 TQCURV INLET

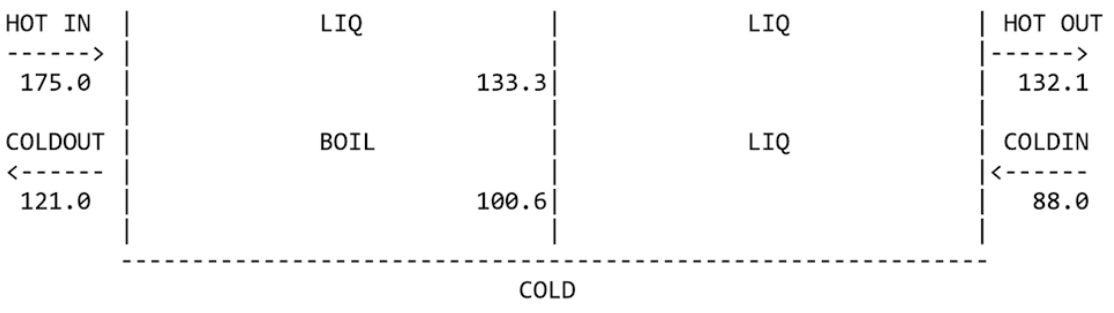
PRESSURE PROFILE: CONSTANT2
PRESSURE DROP: 0.0 BAR
PROPERTY OPTION SET: NRTL RENON (NRTL) / IDEAL GAS

DUTY CAL/SEC	PRES BAR	TEMP C	VFRAC
0.0	1.0100	88.0000	0.0
4.3681+04	1.0100	84.8992	0.0
8.7363+04	1.0100	81.7831	0.0
1.3104+05	1.0100	78.6520	0.0
1.7473+05	1.0100	75.5061	0.0
2.1841+05	1.0100	72.3456	0.0
2.6209+05	1.0100	69.1707	0.0
3.0577+05	1.0100	65.9816	0.0
3.4945+05	1.0100	62.7787	0.0
3.9313+05	1.0100	59.5621	0.0
4.3681+05	1.0100	56.3321	0.0
4.8049+05	1.0100	53.0890	0.0
5.2418+05	1.0100	49.8330	0.0
5.6786+05	1.0100	46.5645	0.0
6.1154+05	1.0100	43.2836	0.0
6.5522+05	1.0100	39.9908	0.0

```
!  6.9890+05 !     1.0100 !    36.6863 !     0.0    !
!  7.4258+05 !     1.0100 !    33.3705 !     0.0    !
!  7.8626+05 !     1.0100 !    30.0436 !     0.0    !
!  8.2994+05 !     1.0100 !    26.7060 !     0.0    !
!------------+------------+------------+------------!
!  8.7363+05 !     1.0100 !    23.3580 !     0.0    !
!  9.1731+05 !     1.0100 !    20.0000 !     0.0    !
-----------------------------------------------------

HEATX HOT-TQCUR MS-HX-01 TQCURV INLET
-------------------------------------
  PRESSURE PROFILE:      CONSTANT2
  PRESSURE DROP:         0.0         BAR
  PROPERTY OPTION SET:   NRTL      RENON (NRTL) / IDEAL GAS

-----------------------------------------------------
!  DUTY      !  PRES      !  TEMP      !  VFRAC     !
!            !            !            !            !
!            !            !            !            !
!            !            !            !            !
!  CAL/SEC   !  BAR       !  C         !            !
!            !            !            !            !
!============!============!============!============!
!     0.0    !     1.0100 !   121.0000 !     0.9507 !
!  4.3681+04 !     1.0100 !   117.9678 !     0.9453 !
!  8.7363+04 !     1.0100 !   115.5025 !     0.9393 !
!  1.3104+05 !     1.0100 !   113.5075 !     0.9328 !
!  1.7473+05 !     1.0100 !   111.8899 !     0.9260 !
!------------+------------+------------+------------!
!  2.1841+05 !     1.0100 !   110.5696 !     0.9188 !
!  2.6209+05 !     1.0100 !   109.4823 !     0.9114 !
!  3.0577+05 !     1.0100 !   108.5777 !     0.9038 !
!  3.4945+05 !     1.0100 !   107.8171 !     0.8961 !
!  3.9313+05 !     1.0100 !   107.1712 !     0.8882 !
!------------+------------+------------+------------!
!  4.3681+05 !     1.0100 !   106.6174 !     0.8802 !
!  4.8049+05 !     1.0100 !   106.1382 !     0.8722 !
!  5.2418+05 !     1.0100 !   105.7203 !     0.8641 !
!  5.6786+05 !     1.0100 !   105.3531 !     0.8559 !
!  6.1154+05 !     1.0100 !   105.0281 !     0.8477 !
!------------+------------+------------+------------!
!  6.5522+05 !     1.0100 !   104.7388 !     0.8395 !
!  6.9890+05 !     1.0100 !   104.4796 !     0.8312 !
!  7.4258+05 !     1.0100 !   104.2463 !     0.8229 !
!  7.8626+05 !     1.0100 !   104.0352 !     0.8146 !
!  8.2994+05 !     1.0100 !   103.8434 !     0.8063 !
!------------+------------+------------+------------!
!  8.7363+05 !     1.0100 !   103.6684 !     0.7979 !
!  9.1731+05 !     1.0100 !   103.5081 !     0.7896 !
-----------------------------------------------------
```

```
BLOCK:  MS-HX-02 MODEL: HEATX
------------------------------
  HOT SIDE:
  ---------
  INLET STREAM:             H2IN
  OUTLET STREAM:            H2OUT
  PROPERTY OPTION SET:   NRTL      RENON (NRTL) / IDEAL GAS
  COLD SIDE:
  ----------
  INLET STREAM:             C1OUT
  OUTLET STREAM:            C2OUT
  PROPERTY OPTION SET:   NRTL      RENON (NRTL) / IDEAL GAS

                    ***  MASS AND ENERGY BALANCE  ***
                                    IN              OUT        RELATIVE DIFF.
   TOTAL BALANCE
      MOLE(KMOL/HR )            27438.6         27438.6          0.00000
      MASS(KG/HR   )            502657.         502657.          0.00000
      ENTHALPY(CAL/SEC )     -0.500045E+09   -0.500045E+09    0.119199E-15

                    ***  CO2 EQUIVALENT SUMMARY ***
   FEED STREAMS CO2E             0.00000      KG/HR
   PRODUCT STREAMS CO2E          0.00000      KG/HR
   NET STREAMS CO2E PRODUCTION   0.00000      KG/HR
   UTILITIES CO2E PRODUCTION     0.00000      KG/HR
   TOTAL CO2E PRODUCTION         0.00000      KG/HR

                       ***  INPUT DATA  ***

  FLASH SPECS FOR HOT SIDE:
  TWO    PHASE      FLASH
  MAXIMUM NO. ITERATIONS                                  30
  CONVERGENCE TOLERANCE                                    0.000100000

  FLASH SPECS FOR COLD SIDE:
  TWO    PHASE      FLASH
  MAXIMUM NO. ITERATIONS                                  30
  CONVERGENCE TOLERANCE                                    0.000100000

  FLOW DIRECTION AND SPECIFICATION:
    COUNTERCURRENT   HEAT EXCHANGER
    SPECIFIED COLD OUTLET TEMP
    SPECIFIED VALUE               C                     121.0000
    LMTD CORRECTION FACTOR                                 1.00000

  PRESSURE SPECIFICATION:
    HOT  SIDE PRESSURE DROP       BAR                      0.0000
    COLD SIDE PRESSURE DROP       BAR                      0.0000

  HEAT TRANSFER COEFFICIENT SPECIFICATION:
    HOT LIQUID    COLD LIQUID     CAL/SEC-SQCM-K           0.0203
```

```
  HOT 2-PHASE   COLD LIQUID      CAL/SEC-SQCM-K           0.0203
  HOT VAPOR     COLD LIQUID      CAL/SEC-SQCM-K           0.0203
  HOT LIQUID    COLD 2-PHASE     CAL/SEC-SQCM-K           0.0203
  HOT 2-PHASE   COLD 2-PHASE     CAL/SEC-SQCM-K           0.0203
  HOT VAPOR     COLD 2-PHASE     CAL/SEC-SQCM-K           0.0203
  HOT LIQUID    COLD VAPOR       CAL/SEC-SQCM-K           0.0203
  HOT 2-PHASE   COLD VAPOR       CAL/SEC-SQCM-K           0.0203
  HOT VAPOR     COLD VAPOR       CAL/SEC-SQCM-K           0.0203

                     ***  OVERALL RESULTS  ***

STREAMS:
                 -------------------------------------
                 |                                   |
H2IN      ----->|               HOT                 |-----> H2OUT
T=  1.7500D+02   |                                   |       T=  1.3209D+02
P=  8.9253D+00   |                                   |       P=  8.9253D+00
V=  0.0000D+00   |                                   |       V=  0.0000D+00
                 |                                   |
C2OUT     <-----|               COLD                |<----- C1OUT
T=  1.2100D+02   |                                   |       T=  8.8000D+01
P=  1.0100D+00   |                                   |       P=  1.0100D+00
V=  9.5068D-01   |                                   |       V=  0.0000D+00
                 -------------------------------------

DUTY AND AREA:
  CALCULATED HEAT DUTY           CAL/SEC          6578391.9744
  CALCULATED (REQUIRED) AREA     SQM                 765.5467
  ACTUAL EXCHANGER AREA          SQM                 765.5467
  PER CENT OVER-DESIGN                                 0.0000

HEAT TRANSFER COEFFICIENT:
  AVERAGE COEFFICIENT (DIRTY)    CAL/SEC-SQCM-K           0.0203
  UA (DIRTY)                     CAL/SEC-K           155420.5385

LOG-MEAN TEMPERATURE DIFFERENCE:
  LMTD CORRECTION FACTOR                               1.0000
  LMTD (CORRECTED)               C                    42.3264
  NUMBER OF SHELLS IN SERIES                            1

PRESSURE DROP:
  HOTSIDE, TOTAL                 BAR                   0.0000
  COLDSIDE, TOTAL                BAR                   0.0000

                   ***  ZONE RESULTS  ***

TEMPERATURE LEAVING EACH ZONE:

                                HOT
      ------------------------------------------------------------
      |                           |                              |
```



ZONE HEAT TRANSFER AND AREA:

ZONE	HEAT DUTY CAL/SEC	AREA SQM	LMTD C	AVERAGE U CAL/SEC-SQCM-K	UA CAL/SEC-K
1	6398030.677	742.2344	42.4589	0.0203	150687.6887
2	180361.297	23.3123	38.1084	0.0203	4732.8498

HEATX COLD-TQCU MS-HX-02 TQCURV INLET

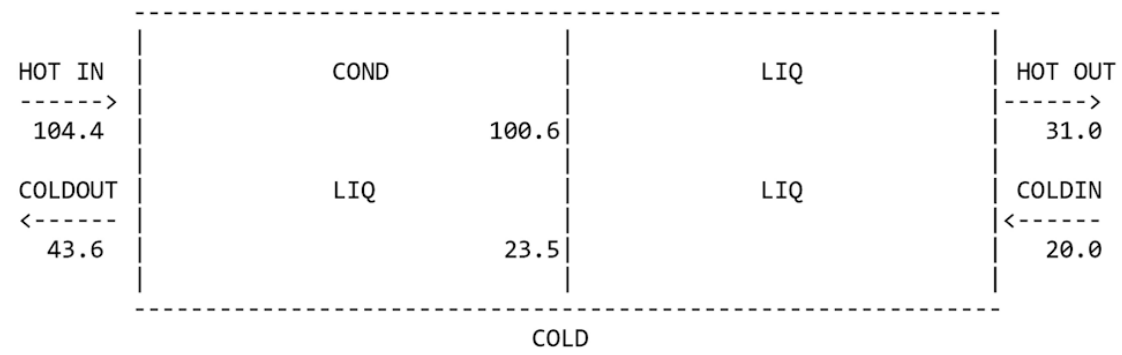
PRESSURE PROFILE: CONSTANT2
PRESSURE DROP: 0.0 BAR
PROPERTY OPTION SET: NRTL RENON (NRTL) / IDEAL GAS

DUTY CAL/SEC	PRES BAR	TEMP C	VFRAC
0.0	1.0100	121.0000	0.9507
3.1326+05	1.0100	109.9190	0.9146
6.2651+05	1.0100	106.0716	0.8710
9.3977+05	1.0100	104.3078	0.8252
1.2530+06	1.0100	103.3186	0.7786
1.5663+06	1.0100	102.6901	0.7317
1.8795+06	1.0100	102.2567	0.6846
2.1928+06	1.0100	101.9403	0.6373
2.5061+06	1.0100	101.6992	0.5900
2.8193+06	1.0100	101.5095	0.5426
3.1326+06	1.0100	101.3564	0.4952
3.4458+06	1.0100	101.2303	0.4478
3.7591+06	1.0100	101.1246	0.4003
4.0723+06	1.0100	101.0347	0.3528
4.3856+06	1.0100	100.9574	0.3053
4.6989+06	1.0100	100.8901	0.2578

```
!  5.0121+06 !     1.0100 !   100.8311 !     0.2103 !
!  5.3254+06 !     1.0100 !   100.7789 !     0.1628 !
!  5.6386+06 !     1.0100 !   100.7324 !     0.1152 !
!  5.9519+06 !     1.0100 !   100.6907 !  6.7705-02 !
!------------+------------+------------+------------!
!  6.2651+06 !     1.0100 !   100.6532 !  2.0168-02 !
!  6.3980+06 !     1.0100 !   100.6383 ! BUB>0.0    !
!  6.5784+06 !     1.0100 !    88.0000 !     0.0    !
-----------------------------------------------------

HEATX HOT-TQCUR MS-HX-02 TQCURV INLET
-------------------------------------
  PRESSURE PROFILE:      CONSTANT2
  PRESSURE DROP:         0.0         BAR
  PROPERTY OPTION SET:   NRTL      RENON (NRTL) / IDEAL GAS

-----------------------------------------------------
!  DUTY      !  PRES      !  TEMP      !  VFRAC     !
!            !            !            !            !
!            !            !            !            !
!            !            !            !            !
!  CAL/SEC   !  BAR       !  C         !            !
!            !            !            !            !
!============!============!============!============!
!     0.0    !     8.9253 !   175.0000 !     0.0    !
!  3.1326+05 !     8.9253 !   173.0643 !     0.0    !
!  6.2651+05 !     8.9253 !   171.1179 !     0.0    !
!  9.3977+05 !     8.9253 !   169.1606 !     0.0    !
!  1.2530+06 !     8.9253 !   167.1925 !     0.0    !
!------------+------------+------------+------------!
!  1.5663+06 !     8.9253 !   165.2137 !     0.0    !
!  1.8795+06 !     8.9253 !   163.2240 !     0.0    !
!  2.1928+06 !     8.9253 !   161.2236 !     0.0    !
!  2.5061+06 !     8.9253 !   159.2124 !     0.0    !
!  2.8193+06 !     8.9253 !   157.1904 !     0.0    !
!------------+------------+------------+------------!
!  3.1326+06 !     8.9253 !   155.1576 !     0.0    !
!  3.4458+06 !     8.9253 !   153.1141 !     0.0    !
!  3.7591+06 !     8.9253 !   151.0599 !     0.0    !
!  4.0723+06 !     8.9253 !   148.9949 !     0.0    !
!  4.3856+06 !     8.9253 !   146.9192 !     0.0    !
!------------+------------+------------+------------!
!  4.6989+06 !     8.9253 !   144.8328 !     0.0    !
!  5.0121+06 !     8.9253 !   142.7358 !     0.0    !
!  5.3254+06 !     8.9253 !   140.6281 !     0.0    !
!  5.6386+06 !     8.9253 !   138.5099 !     0.0    !
!  5.9519+06 !     8.9253 !   136.3810 !     0.0    !
!------------+------------+------------+------------!
!  6.2651+06 !     8.9253 !   134.2416 !     0.0    !
!  6.3980+06 !     8.9253 !   133.3308 !     0.0    !
!  6.5784+06 !     8.9253 !   132.0917 !     0.0    !
```

```
------------------------------------------------------

BLOCK:  MS-HX-03 MODEL: HEATX
------------------------------
  HOT SIDE:
  ---------
  INLET STREAM:             H3IN
  OUTLET STREAM:            H3OUT
  PROPERTY OPTION SET:   NRTL      RENON (NRTL) / IDEAL GAS
  COLD SIDE:
  ----------
  INLET STREAM:             C3IN
  OUTLET STREAM:            C3OUT
  PROPERTY OPTION SET:   NRTL      RENON (NRTL) / IDEAL GAS

                     ***  MASS AND ENERGY BALANCE  ***
                                  IN              OUT        RELATIVE DIFF.
   TOTAL BALANCE
      MOLE(KMOL/HR )            57438.6         57438.6         0.00000
      MASS(KG/HR   )          0.104312E+07    0.104312E+07      0.00000
      ENTHALPY(CAL/SEC )     -0.108533E+10   -0.108533E+10      0.00000

                     ***  CO2 EQUIVALENT SUMMARY ***
   FEED STREAMS CO2E             0.00000      KG/HR
   PRODUCT STREAMS CO2E          0.00000      KG/HR
   NET STREAMS CO2E PRODUCTION   0.00000      KG/HR
   UTILITIES CO2E PRODUCTION     0.00000      KG/HR
   TOTAL CO2E PRODUCTION         0.00000      KG/HR

                        ***  INPUT DATA  ***

  FLASH SPECS FOR HOT SIDE:
  TWO    PHASE      FLASH
  MAXIMUM NO. ITERATIONS                                  30
  CONVERGENCE TOLERANCE                                    0.000100000

  FLASH SPECS FOR COLD SIDE:
  TWO    PHASE      FLASH
  MAXIMUM NO. ITERATIONS                                  30
  CONVERGENCE TOLERANCE                                    0.000100000

  FLOW DIRECTION AND SPECIFICATION:
    COUNTERCURRENT   HEAT EXCHANGER
    SPECIFIED HOT OUTLET TEMP
    SPECIFIED VALUE               C                      31.0000
    LMTD CORRECTION FACTOR                                1.00000

  PRESSURE SPECIFICATION:
    HOT  SIDE PRESSURE DROP       BAR                     0.0000
    COLD SIDE PRESSURE DROP       BAR                     0.0000
```

```
HEAT TRANSFER COEFFICIENT SPECIFICATION:
  HOT LIQUID    COLD LIQUID      CAL/SEC-SQCM-K          0.0203
  HOT 2-PHASE   COLD LIQUID      CAL/SEC-SQCM-K          0.0203
  HOT VAPOR     COLD LIQUID      CAL/SEC-SQCM-K          0.0203
  HOT LIQUID    COLD 2-PHASE     CAL/SEC-SQCM-K          0.0203
  HOT 2-PHASE   COLD 2-PHASE     CAL/SEC-SQCM-K          0.0203
  HOT VAPOR     COLD 2-PHASE     CAL/SEC-SQCM-K          0.0203
  HOT LIQUID    COLD VAPOR       CAL/SEC-SQCM-K          0.0203
  HOT 2-PHASE   COLD VAPOR       CAL/SEC-SQCM-K          0.0203
  HOT VAPOR     COLD VAPOR       CAL/SEC-SQCM-K          0.0203

                    ***  OVERALL RESULTS  ***

STREAMS:
                 ------------------------------------
                 |                                  |
H3IN      ----->|                HOT               |-----> H3OUT
T=  1.0440D+02   |                                  |       T=  3.1000D+01
P=  1.0100D+00   |                                  |       P=  1.0100D+00
V=  8.2852D-01   |                                  |       V=  0.0000D+00
                 |                                  |
C3OUT     <-----|                COLD              |<----- C3IN
T=  4.3605D+01   |                                  |       T=  2.0000D+01
P=  1.0132D+00   |                                  |       P=  1.0132D+00
V=  0.0000D+00   |                                  |       V=  0.0000D+00
                 ------------------------------------

DUTY AND AREA:
  CALCULATED HEAT DUTY           CAL/SEC          6434805.0597
  CALCULATED (REQUIRED) AREA     SQM                 531.8016
  ACTUAL EXCHANGER AREA          SQM                 531.8016
  PER CENT OVER-DESIGN                                 0.0000

HEAT TRANSFER COEFFICIENT:
  AVERAGE COEFFICIENT (DIRTY)    CAL/SEC-SQCM-K       0.0203
  UA (DIRTY)                     CAL/SEC-K       107965.8335

LOG-MEAN TEMPERATURE DIFFERENCE:
  LMTD CORRECTION FACTOR                               1.0000
  LMTD (CORRECTED)               C                    59.6004
  NUMBER OF SHELLS IN SERIES                            1

PRESSURE DROP:
  HOTSIDE, TOTAL                 BAR                   0.0000
  COLDSIDE, TOTAL                BAR                   0.0000

                    ***  ZONE RESULTS  ***

TEMPERATURE LEAVING EACH ZONE:

                                  HOT
```



ZONE HEAT TRANSFER AND AREA:

ZONE	HEAT DUTY CAL/SEC	AREA SQM	LMTD C	AVERAGE U CAL/SEC-SQCM-K	UA CAL/SEC-K
1	5480723.279	393.3687	68.6280	0.0203	79861.3182
2	954081.781	138.4329	33.9476	0.0203	28104.5153

HEATX COLD-TQCU MS-HX-03 TQCURV INLET

PRESSURE PROFILE: CONSTANT2
PRESSURE DROP: 0.0 BAR
PROPERTY OPTION SET: NRTL RENON (NRTL) / IDEAL GAS

DUTY CAL/SEC	PRES BAR	TEMP C	VFRAC
0.0	1.0133	43.6049	0.0
3.0642+05	1.0133	42.4935	0.0
6.1284+05	1.0133	41.3807	0.0
9.1926+05	1.0133	40.2666	0.0
1.2257+06	1.0133	39.1512	0.0
1.5321+06	1.0133	38.0344	0.0
1.8385+06	1.0133	36.9164	0.0
2.1449+06	1.0133	35.7970	0.0
2.4514+06	1.0133	34.6764	0.0
2.7578+06	1.0133	33.5546	0.0
3.0642+06	1.0133	32.4315	0.0
3.3706+06	1.0133	31.3072	0.0
3.6770+06	1.0133	30.1817	0.0
3.9835+06	1.0133	29.0550	0.0
4.2899+06	1.0133	27.9271	0.0

4.5963+06	1.0133	26.7980	0.0
4.9027+06	1.0133	25.6678	0.0
5.2091+06	1.0133	24.5365	0.0
5.4807+06	1.0133	23.5327	0.0
5.5155+06	1.0133	23.4040	0.0
5.8220+06	1.0133	22.2704	0.0
6.1284+06	1.0133	21.1357	0.0
6.4348+06	1.0133	20.0000	0.0

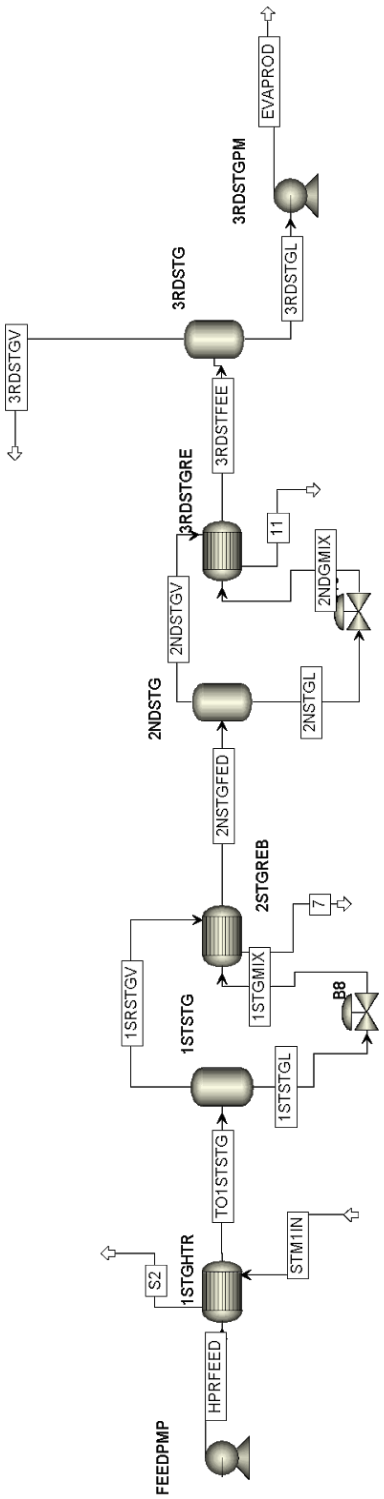
HEATX HOT-TQCUR MS-HX-03 TQCURV INLET

PRESSURE PROFILE: CONSTANT2
PRESSURE DROP: 0.0 BAR
PROPERTY OPTION SET: NRTL RENON (NRTL) / IDEAL GAS

DUTY	PRES	TEMP	VFRAC
CAL/SEC	BAR	C	
0.0	1.0100	104.4008	0.8285
3.0642+05	1.0100	103.3921	0.7830
6.1284+05	1.0100	102.7506	0.7371
9.1926+05	1.0100	102.3080	0.6911
1.2257+06	1.0100	101.9847	0.6449
1.5321+06	1.0100	101.7384	0.5986
1.8385+06	1.0100	101.5446	0.5522
2.1449+06	1.0100	101.3881	0.5059
2.4514+06	1.0100	101.2592	0.4594
2.7578+06	1.0100	101.1512	0.4130
3.0642+06	1.0100	101.0593	0.3666
3.3706+06	1.0100	100.9803	0.3201
3.6770+06	1.0100	100.9116	0.2737
3.9835+06	1.0100	100.8513	0.2272
4.2899+06	1.0100	100.7979	0.1807
4.5963+06	1.0100	100.7504	0.1342
4.9027+06	1.0100	100.7078	8.7715-02
5.2091+06	1.0100	100.6693	4.1217-02
5.4807+06	1.0100	100.6383	BUB>0.0
5.5155+06	1.0100	98.2192	0.0
5.8220+06	1.0100	76.5047	0.0

```
!  6.1284+06 !     1.0100 !    54.0699 !     0.0    !
!  6.4348+06 !     1.0100 !    31.0000 !     0.0    !
-----------------------------------------------------
```

Triple Effect Evaporation Input:



Triple Effect Evaporation Process Flow Diagram:

```
;
;Input Summary created by Aspen Plus Rel. 37.0 at 18:26:47 Tue Apr 7, 2020
;Directory \\nestor\alliwa\  Filename C:\Users\alliwa\AppData\Local\Temp\~apf19c.txt
;


DYNAMICS
    DYNAMICS RESULTS=ON

IN-UNITS MET PRESSURE=bar TEMPERATURE=C DELTA-T=C PDROP=bar  &
        INVERSE-PRES='1/bar' SHORT-LENGTH=mm

DEF-STREAMS CONVEN ALL

MODEL-OPTION

DATABANKS 'APV110 PURE37' / 'APV110 AQUEOUS' / 'APV110 SOLIDS' &
         / 'APV110 INORGANIC' / 'APESV110 AP-EOS' /  &
        'NISTV110 NIST-TRC' / NOASPENPCD

PROP-SOURCES 'APV110 PURE37' / 'APV110 AQUEOUS' /  &
        'APV110 SOLIDS' / 'APV110 INORGANIC' / 'APESV110 AP-EOS' &
         / 'NISTV110 NIST-TRC'

COMPONENTS
    WATER H2O /
    AMMON-01 "(NH4)2SO4" /
    DL-ME-01 C5H11NO2S /
    DEXTR-01 C6H12O6

SOLVE
    RUN-MODE MODE=SIM

FLOWSHEET
    BLOCK 1STGHTR IN=STM1IN HPRFEED OUT=S2 TO1STSTG
    BLOCK 1STSTG IN=TO1STSTG OUT=1SRSTGV 1STSTGL
    BLOCK 2STGREB IN=1SRSTGV 1STGMIX OUT=7 2NSTGFED
    BLOCK 2NDSTG IN=2NSTGFED OUT=2NDSTGV 2NSTGL
    BLOCK 3RDSTGRE IN=2NDSTGV 2NDGMIX OUT=11 3RDSTFEE
    BLOCK 3RDSTG IN=3RDSTFEE OUT=3RDSTGV 3RDSTGL
    BLOCK B8 IN=1STSTGL OUT=1STGMIX
    BLOCK B10 IN=2NSTGL OUT=2NDGMIX
    BLOCK 3RDSTGPM IN=3RDSTGL OUT=EVAPROD
    BLOCK FEEDPMP IN=FEED OUT=HPRFEED

PROPERTIES NRTL FREE-WATER=STEAMNBS
    PROPERTIES PENG-ROB / SRK

PROP-DATA PLXANT-1
    IN-UNITS MET PRESSURE=bar TEMPERATURE=C DELTA-T=C PDROP=bar  &
        INVERSE-PRES='1/bar' SHORT-LENGTH=mm
```

```
+ + + + + + + + + + + + + + + + + + + + + + + + + + + + + + + + + + + + + + + +
+ + + + + + + + + + + + + + + + + + + + + + + + + + + + + + + + + + + + + + + +
+ +                                                                         + +
+ +                    ASPEN PLUS CALCULATION REPORT                        + +
+ +                                                                         + +
+ + + + + + + + + + + + + + + + + + + + + + + + + + + + + + + + + + + + + + + +
+ + + + + + + + + + + + + + + + + + + + + + + + + + + + + + + + + + + + + + + +
```

ASPEN PLUS IS A TRADEMARK OF ASPEN TECHNOLOGY, INC. 781/221-6400

HOTLINE: U.S.A. 888/996-7100 EUROPE (44) 1189-226555

PLATFORM: WIN-X64
VERSION: 37.0 Build 395
INSTALLATION:

APRIL 7, 2020
TUESDAY
6:21:26 P.M.

TABLE OF CONTENTS

TABLE OF CONTENTS

RUN CONTROL SECTION

RUN CONTROL INFORMATION

THIS COPY OF ASPEN PLUS LICENSED TO UNIVERSITY OF PENNSYLVAN

TYPE OF RUN: NEW

INPUT FILE NAME: _0948rer.inm

OUTPUT PROBLEM DATA FILE NAME: _1922wrr
LOCATED IN:

PDF SIZE USED FOR INPUT TRANSLATION:
  NUMBER OF FILE RECORDS (PSIZE) =   0
  NUMBER OF IN-CORE RECORDS   =   256
PSIZE NEEDED FOR SIMULATION   =   1

CALLING PROGRAM NAME: apmain

FLOWSHEET SECTION

FLOWSHEET CONNECTIVITY BY STREAMS

STREAM	SOURCE	DEST	STREAM	SOURCE	DEST
STM1IN	----	1STGHTR	FEED	----	FEEDPMP
S2	1STGHTR	----	TO1STSTG	1STGHTR	1STSTG
1SRSTGV	1STSTG	2STGREB	1STSTGL	1STSTG	B8
7	2STGREB	----	2NSTGFED	2STGREB	2NDSTG
2NDSTGV	2NDSTG	3RDSTGRE	2NSTGL	2NDSTG	B10
11	3RDSTGRE	----	3RDSTFEE	3RDSTGRE	3RDSTG
3RDSTGV	3RDSTG	----	3RDSTGL	3RDSTG	3RDSTGPM
1STGMIX	B8	2STGREB	2NDGMIX	B10	3RDSTGRE
EVAPROD	3RDSTGPM	----	HPRFEED	FEEDPMP	1STGHTR

FLOWSHEET CONNECTIVITY BY BLOCKS

BLOCK	INLETS	OUTLETS
1STGHTR	STM1IN HPRFEED	S2 TO1STSTG
1STSTG	TO1STSTG	1SRSTGV 1STSTGL
2STGREB	1SRSTGV 1STGMIX	7 2NSTGFED
2NDSTG	2NSTGFED	2NDSTGV 2NSTGL
3RDSTGRE	2NDSTGV 2NDGMIX	11 3RDSTFEE
3RDSTG	3RDSTFEE	3RDSTGV 3RDSTGL
B8	1STSTGL	1STGMIX
B10	2NSTGL	2NDGMIX
3RDSTGPM	3RDSTGL	EVAPROD
FEEDPMP	FEED	HPRFEED

COMPUTATIONAL SEQUENCE

SEQUENCE USED WAS:
FEEDPMP 1STGHTR 1STSTG B8 2STGREB 2NDSTG B10 3RDSTGRE 3RDSTG 3RDSTGPM

OVERALL FLOWSHEET BALANCE

*** MASS AND ENERGY BALANCE ***

	IN	OUT	RELATIVE DIFF.
CONVENTIONAL COMPONENTS (KMOL/HR)			
WATER	46562.4	46562.4	-0.156262E-15
AMMON-01	32.9004	32.9004	0.00000

```
      DL-ME-01                  249.736         249.736         0.00000
      DEXTR-01                  3.44736         3.44736         0.00000
   TOTAL BALANCE
      MOLE(KMOL/HR )            46848.5         46848.5         0.00000
      MASS(KG/HR   )            881068.         881068.        -0.132130E-15
      ENTHALPY(CAL/SEC )      -0.829274E+09   -0.829248E+09   -0.311027E-04
 ASPEN PLUS   PLAT: WIN-X64   VER: 37.0                   04/07/2020  PAGE 3


                              FLOWSHEET SECTION

 OVERALL FLOWSHEET BALANCE (CONTINUED)

                     ***  CO2 EQUIVALENT SUMMARY ***
   FEED STREAMS CO2E             0.00000      KG/HR
   PRODUCT STREAMS CO2E          0.00000      KG/HR
   NET STREAMS CO2E PRODUCTION   0.00000      KG/HR
   UTILITIES CO2E PRODUCTION     0.00000      KG/HR
   TOTAL CO2E PRODUCTION         0.00000      KG/HR
 ASPEN PLUS   PLAT: WIN-X64   VER: 37.0                   04/07/2020  PAGE 4


                         PHYSICAL PROPERTIES SECTION

 COMPONENTS
 ----------

  ID       TYPE  ALIAS          NAME
   WATER    C     H2O            WATER
   AMMON-01 C     (NH4)2SO4      AMMONIUM-SULFATE
   DL-ME-01 C     C5H11NO2S      METHIONINE
   DEXTR-01 C     C6H12O6        DEXTROSE
 ASPEN PLUS   PLAT: WIN-X64   VER: 37.0                   04/07/2020  PAGE 5


                             U-O-S BLOCK SECTION

 BLOCK:  1STGHTR  MODEL: HEATX
 ------------------------------
   HOT SIDE:
   ---------
   INLET STREAM:             STM1IN
   OUTLET STREAM:            S2
   PROPERTY OPTION SET:   NRTL      RENON (NRTL) / IDEAL GAS
   COLD SIDE:
   ----------
   INLET STREAM:             HPRFEED
   OUTLET STREAM:            TO1STSTG
   PROPERTY OPTION SET:   NRTL      RENON (NRTL) / IDEAL GAS

                     ***  MASS AND ENERGY BALANCE  ***
```

```
                                    IN              OUT        RELATIVE DIFF.
 TOTAL BALANCE
    MOLE(KMOL/HR )            46848.5         46848.5          0.00000
    MASS(KG/HR   )            881068.         881068.          0.00000
    ENTHALPY(CAL/SEC )     -0.829249E+09   -0.829249E+09       0.00000

                      ***  CO2 EQUIVALENT SUMMARY ***
 FEED STREAMS CO2E             0.00000      KG/HR
 PRODUCT STREAMS CO2E          0.00000      KG/HR
 NET STREAMS CO2E PRODUCTION   0.00000      KG/HR
 UTILITIES CO2E PRODUCTION     0.00000      KG/HR
 TOTAL CO2E PRODUCTION         0.00000      KG/HR

                         ***  INPUT DATA  ***

 FLASH SPECS FOR HOT SIDE:
 TWO    PHASE      FLASH
 MAXIMUM NO. ITERATIONS                                  30
 CONVERGENCE TOLERANCE                                    0.000100000

 FLASH SPECS FOR COLD SIDE:
 TWO    PHASE      FLASH
 MAXIMUM NO. ITERATIONS                                  30
 CONVERGENCE TOLERANCE                                    0.000100000

 FLOW DIRECTION AND SPECIFICATION:
   COUNTERCURRENT   HEAT EXCHANGER
   SPECIFIED HOT VAPOR FRACTION
   SPECIFIED VALUE                                        0.0000
   LMTD CORRECTION FACTOR                                 1.00000
 ASPEN PLUS   PLAT: WIN-X64   VER: 37.0                 04/07/2020  PAGE 6


                             U-O-S BLOCK SECTION

 BLOCK:  1STGHTR  MODEL: HEATX (CONTINUED)

 PRESSURE SPECIFICATION:
   HOT  SIDE PRESSURE DROP        BAR                    0.0000
   COLD SIDE PRESSURE DROP        BAR                    0.0000

 HEAT TRANSFER COEFFICIENT SPECIFICATION:
   HOT LIQUID    COLD LIQUID      CAL/SEC-SQCM-K         0.0203
   HOT 2-PHASE   COLD LIQUID      CAL/SEC-SQCM-K         0.0203
   HOT VAPOR     COLD LIQUID      CAL/SEC-SQCM-K         0.0203
   HOT LIQUID    COLD 2-PHASE     CAL/SEC-SQCM-K         0.0203
   HOT 2-PHASE   COLD 2-PHASE     CAL/SEC-SQCM-K         0.0203
   HOT VAPOR     COLD 2-PHASE     CAL/SEC-SQCM-K         0.0203
   HOT LIQUID    COLD VAPOR       CAL/SEC-SQCM-K         0.0203
   HOT 2-PHASE   COLD VAPOR       CAL/SEC-SQCM-K         0.0203
   HOT VAPOR     COLD VAPOR       CAL/SEC-SQCM-K         0.0203
```

*** OVERALL RESULTS ***

STREAMS:

```
                 ------------------------------------
                 |                                  |
STM1IN    ----->|               HOT                |-----> S2
T=  1.7500D+02   |                                  |       T=  1.7497D+02
P=  8.9000D+00   |                                  |       P=  8.9000D+00
V=  1.0000D+00   |                                  |       V=  0.0000D+00
                 |                                  |
TO1STSTG  <-----|               COLD               |<----- HPRFEED
T=  1.5937D+02   |                                  |       T=  7.0145D+01
P=  6.0000D+00   |                                  |       P=  6.0000D+00
V=  2.2775D-01   |                                  |       V=  0.0000D+00
                 ------------------------------------
```

DUTY AND AREA:

CALCULATED HEAT DUTY	CAL/SEC	35032915.8751
CALCULATED (REQUIRED) AREA	SQM	7529.7843
ACTUAL EXCHANGER AREA	SQM	7529.7843
PER CENT OVER-DESIGN		0.0000

HEAT TRANSFER COEFFICIENT:

AVERAGE COEFFICIENT (DIRTY)	CAL/SEC-SQCM-K	0.0203
UA (DIRTY)	CAL/SEC-K	1528689.3687

LOG-MEAN TEMPERATURE DIFFERENCE:

LMTD CORRECTION FACTOR		1.0000
LMTD (CORRECTED)	C	22.9170
NUMBER OF SHELLS IN SERIES		1

PRESSURE DROP:

HOTSIDE, TOTAL	BAR	0.0000
COLDSIDE, TOTAL	BAR	0.0000

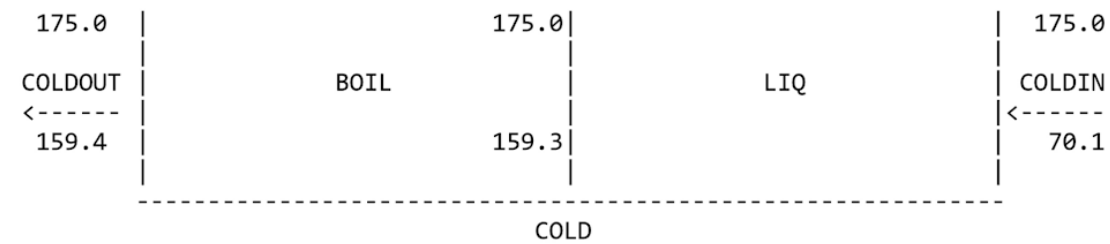
U-O-S BLOCK SECTION

BLOCK: 1STGHTR MODEL: HEATX (CONTINUED)

*** ZONE RESULTS ***

TEMPERATURE LEAVING EACH ZONE:

```
                                HOT
        ------------------------------------------------------------
        |                           |                              |
HOT IN  |            COND           |            COND              | HOT OUT
------> |                           |                              |------>
```



ZONE HEAT TRANSFER AND AREA:

ZONE	HEAT DUTY CAL/SEC	AREA SQM	LMTD C	AVERAGE U CAL/SEC-SQCM-K	UA CAL/SEC-K
1	18408905.626	5785.8291	15.6720	0.0203	1174633.3148
2	16624010.249	1743.9552	46.9530	0.0203	354056.0538

U-O-S BLOCK SECTION

HEATX COLD-TQCU 1STGHTR TQCURV INLET

PRESSURE PROFILE: CONSTANT2
PRESSURE DROP: 0.0 BAR
PROPERTY OPTION SET: NRTL RENON (NRTL) / IDEAL GAS

DUTY CAL/SEC	PRES BAR	TEMP C	VFRAC
0.0	6.0000	159.3660	0.2277
895.6069	6.0000	159.3659	0.2277
1.6682+06	6.0000	159.3542	0.2071
3.3365+06	6.0000	159.3430	0.1865
5.0047+06	6.0000	159.3323	0.1658
6.6729+06	6.0000	159.3222	0.1452
8.3412+06	6.0000	159.3126	0.1246
1.0009+07	6.0000	159.3034	0.1039
1.1678+07	6.0000	159.2946	8.3283-02
1.3346+07	6.0000	159.2863	6.2643-02
1.5014+07	6.0000	159.2782	4.2003-02
1.6682+07	6.0000	159.2705	2.1362-02
1.8351+07	6.0000	159.2632	7.2172-04
1.8409+07	6.0000	159.2629	BUB>0.0
2.0019+07	6.0000	151.3615	0.0

2.1687+07	6.0000	143.0023	0.0
2.3355+07	6.0000	134.4701	0.0
2.5024+07	6.0000	125.7668	0.0
2.6692+07	6.0000	116.8950	0.0
2.8360+07	6.0000	107.8578	0.0
3.0028+07	6.0000	98.6592	0.0
3.1696+07	6.0000	89.3039	0.0
3.3365+07	6.0000	79.7972	0.0
3.5033+07	6.0000	70.1454	0.0

U-O-S BLOCK SECTION

HEATX HOT-TQCUR 1STGHTR TQCURV INLET

PRESSURE PROFILE: CONSTANT2
PRESSURE DROP: 0.0 BAR
PROPERTY OPTION SET: NRTL RENON (NRTL) / IDEAL GAS

DUTY	PRES	TEMP	VFRAC
CAL/SEC	BAR	C	
0.0	8.9000	175.0000	1.0000
895.6069	8.9000	174.9730	DEW>1.0000
1.6682+06	8.9000	174.9730	0.9524
3.3365+06	8.9000	174.9730	0.9048
5.0047+06	8.9000	174.9730	0.8572
6.6729+06	8.9000	174.9730	0.8095
8.3412+06	8.9000	174.9730	0.7619
1.0009+07	8.9000	174.9730	0.7143
1.1678+07	8.9000	174.9730	0.6667
1.3346+07	8.9000	174.9730	0.6191
1.5014+07	8.9000	174.9730	0.5714
1.6682+07	8.9000	174.9730	0.5238
1.8351+07	8.9000	174.9730	0.4762
1.8409+07	8.9000	174.9730	0.4745
2.0019+07	8.9000	174.9730	0.4286
2.1687+07	8.9000	174.9730	0.3810
2.3355+07	8.9000	174.9730	0.3333

```
!  2.5024+07 !     8.9000 !   174.9730 !     0.2857 !
!  2.6692+07 !     8.9000 !   174.9730 !     0.2381 !
!  2.8360+07 !     8.9000 !   174.9730 !     0.1905 !
!------------+------------+------------+------------!
!  3.0028+07 !     8.9000 !   174.9730 !     0.1429 !
!  3.1696+07 !     8.9000 !   174.9730 !  9.5241-02 !
!  3.3365+07 !     8.9000 !   174.9730 !  4.7620-02 !
!  3.5033+07 !     8.9000 !   174.9730 !  6.3373-09 !
-----------------------------------------------------

BLOCK:  1STSTG   MODEL: FLASH2
------------------------------
  INLET STREAM:          TO1STSTG
  OUTLET VAPOR STREAM:   1SRSTGV
  OUTLET LIQUID STREAM:  1STSTGL
  PROPERTY OPTION SET:   NRTL      RENON (NRTL) / IDEAL GAS
 ASPEN PLUS   PLAT: WIN-X64   VER: 37.0                  04/07/2020  PAGE 10


                            U-O-S BLOCK SECTION

BLOCK:  1STSTG   MODEL: FLASH2 (CONTINUED)

                     ***  MASS AND ENERGY BALANCE  ***
                                  IN              OUT        RELATIVE DIFF.
   TOTAL BALANCE
      MOLE(KMOL/HR )            32416.3         32416.3          0.00000
      MASS(KG/HR   )            621068.         621068.       -0.187444E-15
      ENTHALPY(CAL/SEC )     -0.567563E+09   -0.567563E+09    0.594405E-13

                     ***  CO2 EQUIVALENT SUMMARY ***
   FEED STREAMS CO2E             0.00000      KG/HR
   PRODUCT STREAMS CO2E          0.00000      KG/HR
   NET STREAMS CO2E PRODUCTION   0.00000      KG/HR
   UTILITIES CO2E PRODUCTION     0.00000      KG/HR
   TOTAL CO2E PRODUCTION         0.00000      KG/HR

                         ***  INPUT DATA  ***
  TWO    PHASE  PQ  FLASH
  PRESSURE DROP         BAR                                  0.0
  SPECIFIED HEAT DUTY   CAL/SEC                              0.0
  MAXIMUM NO. ITERATIONS                                    30
  CONVERGENCE TOLERANCE                                      0.000100000

                          ***  RESULTS  ***
  OUTLET TEMPERATURE    C                                    159.37
  OUTLET PRESSURE       BAR                                  6.0000
  VAPOR FRACTION                                            0.22775
```

V-L PHASE EQUILIBRIUM :

COMP	F(I)	X(I)	Y(I)	K(I)
WATER	0.99117	0.98857	1.0000	1.0116
AMMON-01	0.10149E-02	0.13143E-02	0.32394E-82	0.24648E-79
DL-ME-01	0.77040E-02	0.99761E-02	0.24589E-81	0.24648E-79
DEXTR-01	0.10635E-03	0.13771E-03	0.38397E-09	0.27883E-05

BLOCK: 2NDSTG MODEL: FLASH2

INLET STREAM: 2NSTGFED
OUTLET VAPOR STREAM: 2NDSTGV
OUTLET LIQUID STREAM: 2NSTGL
PROPERTY OPTION SET: NRTL RENON (NRTL) / IDEAL GAS

U-O-S BLOCK SECTION

BLOCK: 2NDSTG MODEL: FLASH2 (CONTINUED)

*** MASS AND ENERGY BALANCE ***

	IN	OUT	RELATIVE DIFF.
TOTAL BALANCE			
MOLE(KMOL/HR)	25033.6	25033.6	0.00000
MASS(KG/HR)	488065.	488065.	-0.596310E-15
ENTHALPY(CAL/SEC)	-0.432946E+09	-0.432946E+09	-0.229641E-10

*** CO2 EQUIVALENT SUMMARY ***

FEED STREAMS CO2E	0.00000	KG/HR
PRODUCT STREAMS CO2E	0.00000	KG/HR
NET STREAMS CO2E PRODUCTION	0.00000	KG/HR
UTILITIES CO2E PRODUCTION	0.00000	KG/HR
TOTAL CO2E PRODUCTION	0.00000	KG/HR

*** INPUT DATA ***

TWO PHASE PQ FLASH		
PRESSURE DROP	BAR	0.0
SPECIFIED HEAT DUTY	CAL/SEC	0.0
MAXIMUM NO. ITERATIONS		30
CONVERGENCE TOLERANCE		0.000100000

*** RESULTS ***

OUTLET TEMPERATURE	C	144.31
OUTLET PRESSURE	BAR	4.0000
VAPOR FRACTION		0.32565

V-L PHASE EQUILIBRIUM :

COMP	F(I)	X(I)	Y(I)	K(I)
WATER	0.98857	0.98305	1.0000	1.0172
AMMON-01	0.13143E-02	0.19489E-02	0.72056E-82	0.36972E-79
DL-ME-01	0.99761E-02	0.14794E-01	0.54696E-81	0.36972E-79
DEXTR-01	0.13771E-03	0.20421E-03	0.18500E-09	0.90594E-06

U-O-S BLOCK SECTION

BLOCK: 2STGREB MODEL: HEATX

HOT SIDE:

INLET STREAM: 1SRSTGV
OUTLET STREAM: 7
PROPERTY OPTION SET: NRTL RENON (NRTL) / IDEAL GAS

COLD SIDE:

INLET STREAM: 1STGMIX
OUTLET STREAM: 2NSTGFED
PROPERTY OPTION SET: NRTL RENON (NRTL) / IDEAL GAS

*** MASS AND ENERGY BALANCE ***

	IN	OUT	RELATIVE DIFF.
TOTAL BALANCE			
MOLE(KMOL/HR)	32416.3	32416.3	0.00000
MASS(KG/HR)	621068.	621068.	0.187444E-15
ENTHALPY(CAL/SEC)	-0.567563E+09	-0.567563E+09	-0.210037E-15

*** CO2 EQUIVALENT SUMMARY ***

FEED STREAMS CO2E	0.00000	KG/HR
PRODUCT STREAMS CO2E	0.00000	KG/HR
NET STREAMS CO2E PRODUCTION	0.00000	KG/HR
UTILITIES CO2E PRODUCTION	0.00000	KG/HR
TOTAL CO2E PRODUCTION	0.00000	KG/HR

*** INPUT DATA ***

FLASH SPECS FOR HOT SIDE:
TWO PHASE FLASH
MAXIMUM NO. ITERATIONS 30
CONVERGENCE TOLERANCE 0.000100000

FLASH SPECS FOR COLD SIDE:
TWO PHASE FLASH
MAXIMUM NO. ITERATIONS 30
CONVERGENCE TOLERANCE 0.000100000

FLOW DIRECTION AND SPECIFICATION:
COUNTERCURRENT HEAT EXCHANGER

U-O-S BLOCK SECTION

BLOCK: 2STGREB MODEL: HEATX (CONTINUED)

PRESSURE SPECIFICATION:

HOT SIDE PRESSURE DROP	BAR	0.0000
COLD SIDE PRESSURE DROP	BAR	0.0000

HEAT TRANSFER COEFFICIENT SPECIFICATION:

HOT LIQUID	COLD LIQUID	CAL/SEC-SQCM-K	0.0203
HOT 2-PHASE	COLD LIQUID	CAL/SEC-SQCM-K	0.0203
HOT VAPOR	COLD LIQUID	CAL/SEC-SQCM-K	0.0203
HOT LIQUID	COLD 2-PHASE	CAL/SEC-SQCM-K	0.0203
HOT 2-PHASE	COLD 2-PHASE	CAL/SEC-SQCM-K	0.0203
HOT VAPOR	COLD 2-PHASE	CAL/SEC-SQCM-K	0.0203
HOT LIQUID	COLD VAPOR	CAL/SEC-SQCM-K	0.0203
HOT 2-PHASE	COLD VAPOR	CAL/SEC-SQCM-K	0.0203
HOT VAPOR	COLD VAPOR	CAL/SEC-SQCM-K	0.0203

*** OVERALL RESULTS ***

STREAMS:

1SRSTGV	----->	HOT	----->	7
T= 1.5937D+02				T= 1.5892D+02
P= 6.0000D+00				P= 6.0000D+00
V= 1.0000D+00				V= 0.0000D+00
2NSTGFED	<-----	COLD	<-----	1STGMIX
T= 1.4431D+02				T= 1.4412D+02
P= 4.0000D+00				P= 4.0000D+00
V= 3.2565D-01				V= 3.7494D-02

DUTY AND AREA:

CALCULATED HEAT DUTY	CAL/SEC	18408329.4667
CALCULATED (REQUIRED) AREA	SQM	6165.8267
ACTUAL EXCHANGER AREA	SQM	6165.8267
PER CENT OVER-DESIGN		0.0000

HEAT TRANSFER COEFFICIENT:

AVERAGE COEFFICIENT (DIRTY)	CAL/SEC-SQCM-K	0.0203
UA (DIRTY)	CAL/SEC-K	1251780.0436

```
  LOG-MEAN TEMPERATURE DIFFERENCE:
    LMTD CORRECTION FACTOR                                  1.0000
    LMTD (CORRECTED)                C                      14.7057
    NUMBER OF SHELLS IN SERIES                              1

  PRESSURE DROP:
    HOTSIDE, TOTAL                  BAR                     0.0000
    COLDSIDE, TOTAL                 BAR                     0.0000
 ASPEN PLUS   PLAT: WIN-X64   VER: 37.0                  04/07/2020  PAGE 14


                              U-O-S BLOCK SECTION

 BLOCK:  2STGREB  MODEL: HEATX (CONTINUED)

                         ***  ZONE RESULTS  ***

   TEMPERATURE LEAVING EACH ZONE:

                                    HOT
          ------------------------------------------------------------
          |                              |                             |
 HOT IN   |              VAP             |             COND            | HOT OUT
 ------>  |                              |                             |------>
  159.4   |                         158.9|                             |  158.9
          |                              |                             |
 COLDOUT  |              BOIL            |             BOIL            | COLDIN
 <------  |                              |                             |<------
  144.3   |                         144.3|                             |  144.1
          |                              |                             |
          ------------------------------------------------------------
                                    COLD

   ZONE HEAT TRANSFER AND AREA:

   ZONE       HEAT DUTY       AREA       LMTD       AVERAGE U       UA
              CAL/SEC         SQM         C         CAL/SEC-SQCM-K  CAL/SEC-K
     1       7517.050         2.4956      14.8366     0.0203          506.6556
     2   18400812.417      6163.3311      14.7057     0.0203      1251273.3880
 ASPEN PLUS   PLAT: WIN-X64   VER: 37.0                  04/07/2020  PAGE 15


                              U-O-S BLOCK SECTION

 HEATX COLD-TQCU 2STGREB  TQCURV INLET
 -------------------------------------
   PRESSURE PROFILE:      CONSTANT2
   PRESSURE DROP:         0.0         BAR
   PROPERTY OPTION SET:   NRTL      RENON (NRTL) / IDEAL GAS

 ------------------------------------------------------
```

DUTY	PRES	TEMP	VFRAC
CAL/SEC	BAR	C	
0.0	4.0000	144.3061	0.3257
7517.0497	4.0000	144.3060	0.3255
8.7659+05	4.0000	144.2937	0.3119
1.7532+06	4.0000	144.2818	0.2982
2.6298+06	4.0000	144.2703	0.2845
3.5063+06	4.0000	144.2592	0.2708
4.3829+06	4.0000	144.2486	0.2571
5.2595+06	4.0000	144.2383	0.2433
6.1361+06	4.0000	144.2285	0.2296
7.0127+06	4.0000	144.2189	0.2159
7.8893+06	4.0000	144.2097	0.2022
8.7659+06	4.0000	144.2008	0.1884
9.6425+06	4.0000	144.1923	0.1747
1.0519+07	4.0000	144.1840	0.1610
1.1396+07	4.0000	144.1759	0.1473
1.2272+07	4.0000	144.1681	0.1336
1.3149+07	4.0000	144.1606	0.1198
1.4025+07	4.0000	144.1533	0.1061
1.4902+07	4.0000	144.1462	9.2390-02
1.5779+07	4.0000	144.1393	7.8666-02
1.6655+07	4.0000	144.1327	6.4942-02
1.7532+07	4.0000	144.1262	5.1218-02
1.8408+07	4.0000	144.1199	3.7494-02

U-O-S BLOCK SECTION

HEATX HOT-TQCUR 2STGREB TQCURV INLET

PRESSURE PROFILE: CONSTANT2
PRESSURE DROP: 0.0 BAR
PROPERTY OPTION SET: NRTL RENON (NRTL) / IDEAL GAS

DUTY	PRES	TEMP	VFRAC

CAL/SEC	BAR	C	
0.0	6.0000	159.3660	1.0000
7517.0497	6.0000	158.9216	DEW>1.0000
8.7659+05	6.0000	158.9160	0.9528
1.7532+06	6.0000	158.9160	0.9051
2.6298+06	6.0000	158.9160	0.8575
3.5063+06	6.0000	158.9160	0.8099
4.3829+06	6.0000	158.9160	0.7622
5.2595+06	6.0000	158.9160	0.7146
6.1361+06	6.0000	158.9160	0.6669
7.0127+06	6.0000	158.9160	0.6193
7.8893+06	6.0000	158.9160	0.5717
8.7659+06	6.0000	158.9160	0.5240
9.6425+06	6.0000	158.9160	0.4764
1.0519+07	6.0000	158.9160	0.4287
1.1396+07	6.0000	158.9160	0.3811
1.2272+07	6.0000	158.9160	0.3335
1.3149+07	6.0000	158.9160	0.2858
1.4025+07	6.0000	158.9160	0.2382
1.4902+07	6.0000	158.9160	0.1906
1.5779+07	6.0000	158.9160	0.1429
1.6655+07	6.0000	158.9160	9.5277-02
1.7532+07	6.0000	158.9160	4.7639-02
1.8408+07	6.0000	158.9160	0.0

BLOCK: 3RDSTG MODEL: FLASH2

INLET STREAM: 3RDSTFEE
OUTLET VAPOR STREAM: 3RDSTGV
OUTLET LIQUID STREAM: 3RDSTGL
PROPERTY OPTION SET: NRTL RENON (NRTL) / IDEAL GAS

U-O-S BLOCK SECTION

BLOCK: 3RDSTG MODEL: FLASH2 (CONTINUED)

*** MASS AND ENERGY BALANCE ***

	IN	OUT	RELATIVE DIFF.
TOTAL BALANCE			
MOLE(KMOL/HR)	16881.3	16881.3	0.00000
MASS(KG/HR)	341201.	341201.	-0.170597E-15
ENTHALPY(CAL/SEC)	-0.283542E+09	-0.283542E+09	0.102875E-09

```
                    ***  CO2 EQUIVALENT SUMMARY ***
  FEED STREAMS CO2E             0.00000      KG/HR
  PRODUCT STREAMS CO2E          0.00000      KG/HR
  NET STREAMS CO2E PRODUCTION   0.00000      KG/HR
  UTILITIES CO2E PRODUCTION     0.00000      KG/HR
  TOTAL CO2E PRODUCTION         0.00000      KG/HR

                         ***  INPUT DATA  ***
  TWO    PHASE  PQ  FLASH
  PRESSURE DROP         BAR                                  0.0
  SPECIFIED HEAT DUTY   CAL/SEC                              0.0
  MAXIMUM NO. ITERATIONS                                    30
  CONVERGENCE TOLERANCE                                      0.000100000

                          ***  RESULTS  ***
  OUTLET TEMPERATURE    C                                    112.52
  OUTLET PRESSURE       BAR                                  1.5000
  VAPOR FRACTION                                             0.53521


  V-L PHASE EQUILIBRIUM :

     COMP              F(I)           X(I)           Y(I)           K(I)
     WATER            0.98305        0.96354         1.0000         1.0378
     AMMON-01         0.19489E-02    0.41932E-02    0.41342E-81    0.98593E-79
     DL-ME-01         0.14794E-01    0.31829E-01    0.31381E-80    0.98593E-79
     DEXTR-01         0.20421E-03    0.43937E-03    0.27466E-10    0.62514E-07

BLOCK:  3RDSTGPM MODEL: PUMP
------------------------------
  INLET STREAM:          3RDSTGL
  OUTLET STREAM:         EVAPROD
  PROPERTY OPTION SET:   NRTL      RENON (NRTL) / IDEAL GAS
 ASPEN PLUS   PLAT: WIN-X64   VER: 37.0                   04/07/2020  PAGE 18


                            U-O-S BLOCK SECTION

BLOCK:  3RDSTGPM MODEL: PUMP (CONTINUED)

                    ***  MASS AND ENERGY BALANCE  ***
                                 IN              OUT        RELATIVE DIFF.
   TOTAL BALANCE
      MOLE(KMOL/HR )           7846.21         7846.21          0.00000
      MASS(KG/HR   )           178430.         178430.        0.163110E-15
      ENTHALPY(CAL/SEC )    -0.140360E+09   -0.140360E+09   -0.605360E-05

                    ***  CO2 EQUIVALENT SUMMARY ***
   FEED STREAMS CO2E             0.00000      KG/HR
```

```
   PRODUCT STREAMS CO2E          0.00000      KG/HR
   NET STREAMS CO2E PRODUCTION   0.00000      KG/HR
   UTILITIES CO2E PRODUCTION     0.00000      KG/HR
   TOTAL CO2E PRODUCTION         0.00000      KG/HR

                        ***  INPUT DATA  ***
   OUTLET PRESSURE  BAR                                  2.00000
   DRIVER EFFICIENCY                                     1.00000

   FLASH SPECIFICATIONS:
   LIQUID PHASE CALCULATION
   NO FLASH PERFORMED
   MAXIMUM NUMBER OF ITERATIONS                          30
   TOLERANCE                                             0.000100000

                         ***  RESULTS  ***
   VOLUMETRIC FLOW RATE  L/MIN                       3,238.55
   PRESSURE CHANGE  BAR                                  0.50000
   NPSH AVAILABLE   M-KGF/KG                             0.0
   FLUID POWER  KW                                       2.69879
   BRAKE POWER  KW                                       3.55746
   ELECTRICITY  KW                                       3.55746
   PUMP EFFICIENCY USED                                  0.75863
   NET WORK REQUIRED  KW                                 3.55746
   HEAD DEVELOPED M-KGF/KG                               5.55242
 ASPEN PLUS   PLAT: WIN-X64   VER: 37.0                  04/07/2020  PAGE 19


                            U-O-S BLOCK SECTION

 BLOCK:  3RDSTGRE MODEL: HEATX
 ------------------------------
   HOT SIDE:
   ---------
   INLET STREAM:             2NDSTGV
   OUTLET STREAM:            11
   PROPERTY OPTION SET:   NRTL      RENON (NRTL) / IDEAL GAS
   COLD SIDE:
   ----------
   INLET STREAM:             2NDGMIX
   OUTLET STREAM:            3RDSTFEE
   PROPERTY OPTION SET:   NRTL      RENON (NRTL) / IDEAL GAS

                     ***  MASS AND ENERGY BALANCE  ***
                                  IN              OUT        RELATIVE DIFF.
    TOTAL BALANCE
       MOLE(KMOL/HR )            25033.6         25033.6         0.00000
       MASS(KG/HR   )            488065.         488065.        0.238524E-15
       ENTHALPY(CAL/SEC )      -0.432946E+09   -0.432946E+09     0.00000

                     ***  CO2 EQUIVALENT SUMMARY ***
```

```
 FEED STREAMS CO2E             0.00000      KG/HR
 PRODUCT STREAMS CO2E          0.00000      KG/HR
 NET STREAMS CO2E PRODUCTION   0.00000      KG/HR
 UTILITIES CO2E PRODUCTION     0.00000      KG/HR
 TOTAL CO2E PRODUCTION         0.00000      KG/HR

                        ***  INPUT DATA  ***

 FLASH SPECS FOR HOT SIDE:
 TWO    PHASE      FLASH
 MAXIMUM NO. ITERATIONS                                  30
 CONVERGENCE TOLERANCE                                    0.000100000

 FLASH SPECS FOR COLD SIDE:
 TWO    PHASE      FLASH
 MAXIMUM NO. ITERATIONS                                  30
 CONVERGENCE TOLERANCE                                    0.000100000

 FLOW DIRECTION AND SPECIFICATION:
   COUNTERCURRENT   HEAT EXCHANGER
   SPECIFIED HOT VAPOR FRACTION
   SPECIFIED VALUE                                       0.0000
   LMTD CORRECTION FACTOR                                1.00000
 ASPEN PLUS   PLAT: WIN-X64   VER: 37.0                 04/07/2020  PAGE 20


                           U-O-S BLOCK SECTION

 BLOCK:  3RDSTGRE MODEL: HEATX (CONTINUED)

 PRESSURE SPECIFICATION:
   HOT  SIDE PRESSURE DROP        BAR                    0.0000
   COLD SIDE PRESSURE DROP        BAR                    0.0000

 HEAT TRANSFER COEFFICIENT SPECIFICATION:
   HOT LIQUID    COLD LIQUID      CAL/SEC-SQCM-K         0.0203
   HOT 2-PHASE   COLD LIQUID      CAL/SEC-SQCM-K         0.0203
   HOT VAPOR     COLD LIQUID      CAL/SEC-SQCM-K         0.0203
   HOT LIQUID    COLD 2-PHASE     CAL/SEC-SQCM-K         0.0203
   HOT 2-PHASE   COLD 2-PHASE     CAL/SEC-SQCM-K         0.0203
   HOT VAPOR     COLD 2-PHASE     CAL/SEC-SQCM-K         0.0203
   HOT LIQUID    COLD VAPOR       CAL/SEC-SQCM-K         0.0203
   HOT 2-PHASE   COLD VAPOR       CAL/SEC-SQCM-K         0.0203
   HOT VAPOR     COLD VAPOR       CAL/SEC-SQCM-K         0.0203

                        ***  OVERALL RESULTS  ***

 STREAMS:
                 ---------------------------------------
                 |                                     |
 2NDSTGV   ----->|                HOT                  |-----> 11
```

```
  T=  1.4431D+02  |                                    |       T=  1.4369D+02
  P=  4.0000D+00  |                                    |       P=  4.0000D+00
  V=  1.0000D+00  |                                    |       V=  0.0000D+00
                  |                                    |
  3RDSTFEE  <-----|                COLD                |<----- 2NDGMIX
  T=  1.1252D+02  |                                    |       T=  1.1196D+02
  P=  1.5000D+00  |                                    |       P=  1.5000D+00
  V=  5.3521D-01  |                                    |       V=  7.2999D-02
                  ------------------------------------
```

DUTY AND AREA:

CALCULATED HEAT DUTY	CAL/SEC	20801958.1706
CALCULATED (REQUIRED) AREA	SQM	3257.8505
ACTUAL EXCHANGER AREA	SQM	3257.8505
PER CENT OVER-DESIGN		0.0000

HEAT TRANSFER COEFFICIENT:

AVERAGE COEFFICIENT (DIRTY)	CAL/SEC-SQCM-K	0.0203
UA (DIRTY)	CAL/SEC-K	661405.5997

LOG-MEAN TEMPERATURE DIFFERENCE:

LMTD CORRECTION FACTOR		1.0000
LMTD (CORRECTED)	C	31.4511
NUMBER OF SHELLS IN SERIES		1

PRESSURE DROP:

HOTSIDE, TOTAL	BAR	0.0000
COLDSIDE, TOTAL	BAR	0.0000

U-O-S BLOCK SECTION

BLOCK: 3RDSTGRE MODEL: HEATX (CONTINUED)

*** ZONE RESULTS ***

TEMPERATURE LEAVING EACH ZONE:

```
                                HOT
        ------------------------------------------------------------
        |                           |                           |
HOT IN  |            VAP            |           COND            | HOT OUT
------> |                           |                           |------>
 144.3  |                     143.7 |                           |  143.7
        |                           |                           |
COLDOUT |            BOIL           |           BOIL            | COLDIN
<------ |                           |                           |<------
 112.5  |                     112.5 |                           |  112.0
        |                           |                           |
        ------------------------------------------------------------
```

ZONE HEAT TRANSFER AND AREA:

ZONE	HEAT DUTY CAL/SEC	AREA SQM	LMTD C	AVERAGE U CAL/SEC-SQCM-K	UA CAL/SEC-K
1	11343.505	1.7750	31.4776	0.0203	360.3675
2	20790614.665	3256.0755	31.4511	0.0203	661045.2322

U-O-S BLOCK SECTION

HEATX COLD-TQCU 3RDSTGRE TQCURV INLET

PRESSURE PROFILE: CONSTANT2
PRESSURE DROP: 0.0 BAR
PROPERTY OPTION SET: NRTL RENON (NRTL) / IDEAL GAS

DUTY CAL/SEC	PRES BAR	TEMP C	VFRAC
0.0	1.5000	112.5230	0.5352
1.1344+04	1.5000	112.5224	0.5350
9.9057+05	1.5000	112.4714	0.5132
1.9811+06	1.5000	112.4243	0.4912
2.9717+06	1.5000	112.3812	0.4692
3.9623+06	1.5000	112.3416	0.4472
4.9528+06	1.5000	112.3050	0.4252
5.9434+06	1.5000	112.2712	0.4032
6.9340+06	1.5000	112.2399	0.3812
7.9246+06	1.5000	112.2107	0.3592
8.9151+06	1.5000	112.1835	0.3372
9.9057+06	1.5000	112.1580	0.3152
1.0896+07	1.5000	112.1342	0.2932
1.1887+07	1.5000	112.1118	0.2712
1.2877+07	1.5000	112.0908	0.2492
1.3868+07	1.5000	112.0709	0.2271
1.4859+07	1.5000	112.0522	0.2051
1.5849+07	1.5000	112.0345	0.1831
1.6840+07	1.5000	112.0178	0.1611
1.7830+07	1.5000	112.0019	0.1391

1.8821+07	1.5000	111.9868	0.1170
1.9811+07	1.5000	111.9724	9.5021-02
2.0802+07	1.5000	111.9588	7.2999-02

U-O-S BLOCK SECTION

HEATX HOT-TQCUR 3RDSTGRE TQCURV INLET

PRESSURE PROFILE: CONSTANT2
PRESSURE DROP: 0.0 BAR
PROPERTY OPTION SET: NRTL RENON (NRTL) / IDEAL GAS

DUTY	PRES	TEMP	VFRAC
CAL/SEC	BAR	C	
0.0	4.0000	144.3061	1.0000
1.1344+04	4.0000	143.6965	DEW>1.0000
9.9057+05	4.0000	143.6886	0.9529
1.9811+06	4.0000	143.6886	0.9053
2.9717+06	4.0000	143.6886	0.8576
3.9623+06	4.0000	143.6886	0.8100
4.9528+06	4.0000	143.6886	0.7623
5.9434+06	4.0000	143.6886	0.7147
6.9340+06	4.0000	143.6886	0.6670
7.9246+06	4.0000	143.6886	0.6194
8.9151+06	4.0000	143.6886	0.5717
9.9057+06	4.0000	143.6886	0.5241
1.0896+07	4.0000	143.6886	0.4765
1.1887+07	4.0000	143.6886	0.4288
1.2877+07	4.0000	143.6886	0.3812
1.3868+07	4.0000	143.6886	0.3335
1.4859+07	4.0000	143.6886	0.2859
1.5849+07	4.0000	143.6886	0.2382
1.6840+07	4.0000	143.6886	0.1906
1.7830+07	4.0000	143.6886	0.1429
1.8821+07	4.0000	143.6886	9.5291-02
1.9811+07	4.0000	143.6886	4.7645-02
2.0802+07	4.0000	143.6886	0.0

```
BLOCK:  B10      MODEL: VALVE
------------------------------
  INLET STREAM:          2NSTGL
  OUTLET STREAM:         2NDGMIX
  PROPERTY OPTION SET:   NRTL      RENON (NRTL) / IDEAL GAS

                     ***  MASS AND ENERGY BALANCE  ***
                                  IN              OUT        RELATIVE DIFF.
 ASPEN PLUS   PLAT: WIN-X64   VER: 37.0                   04/07/2020  PAGE 24


                             U-O-S BLOCK SECTION

BLOCK:  B10      MODEL: VALVE (CONTINUED)
   TOTAL BALANCE
      MOLE(KMOL/HR )            16881.3         16881.3         0.00000
      MASS(KG/HR   )            341201.         341201.        0.170597E-15
      ENTHALPY(CAL/SEC )      -0.304344E+09   -0.304344E+09   -0.195846E-15

                     ***  CO2 EQUIVALENT SUMMARY ***
   FEED STREAMS CO2E             0.00000      KG/HR
   PRODUCT STREAMS CO2E          0.00000      KG/HR
   NET STREAMS CO2E PRODUCTION   0.00000      KG/HR
   UTILITIES CO2E PRODUCTION     0.00000      KG/HR
   TOTAL CO2E PRODUCTION         0.00000      KG/HR

                         ***  INPUT DATA  ***

   VALVE OUTLET PRESSURE     BAR                           1.50000
   VALVE FLOW COEF CALC.                                  NO

                         FLASH SPECIFICATIONS:
   NPHASE                                                  2
   MAX NUMBER OF ITERATIONS                               30
   CONVERGENCE TOLERANCE                                   0.000100000

                         ***  RESULTS  ***

   VALVE PRESSURE DROP       BAR                           2.50000

BLOCK:  B8       MODEL: VALVE
------------------------------
  INLET STREAM:          1STSTGL
  OUTLET STREAM:         1STGMIX
  PROPERTY OPTION SET:   NRTL      RENON (NRTL) / IDEAL GAS

                     ***  MASS AND ENERGY BALANCE  ***
                                  IN              OUT        RELATIVE DIFF.
   TOTAL BALANCE
      MOLE(KMOL/HR )            25033.6         25033.6         0.00000
```

```
      MASS(KG/HR    )            488065.         488065.        0.238524E-15
      ENTHALPY(CAL/SEC )      -0.451354E+09   -0.451354E+09   -0.264115E-15

                     ***  CO2 EQUIVALENT SUMMARY ***
   FEED STREAMS CO2E             0.00000      KG/HR
   PRODUCT STREAMS CO2E          0.00000      KG/HR
   NET STREAMS CO2E PRODUCTION   0.00000      KG/HR
   UTILITIES CO2E PRODUCTION     0.00000      KG/HR
   TOTAL CO2E PRODUCTION         0.00000      KG/HR

                          ***  INPUT DATA  ***

 ASPEN PLUS   PLAT: WIN-X64   VER: 37.0                   04/07/2020  PAGE 25


                             U-O-S BLOCK SECTION

 BLOCK:  B8       MODEL: VALVE (CONTINUED)
   VALVE OUTLET PRESSURE     BAR                          4.00000
   VALVE FLOW COEF CALC.                                 NO

                          FLASH SPECIFICATIONS:
   NPHASE                                                 2
   MAX NUMBER OF ITERATIONS                              30
   CONVERGENCE TOLERANCE                                  0.000100000

                          ***  RESULTS  ***

   VALVE PRESSURE DROP       BAR                          2.00000

 BLOCK:  FEEDPMP  MODEL: PUMP
 ------------------------------
   INLET STREAM:          FEED
   OUTLET STREAM:         HPRFEED
   PROPERTY OPTION SET:   NRTL      RENON (NRTL) / IDEAL GAS

                     ***  MASS AND ENERGY BALANCE  ***
                                     IN              OUT        RELATIVE DIFF.
   TOTAL BALANCE
      MOLE(KMOL/HR )            32416.3         32416.3          0.00000
      MASS(KG/HR   )            621068.         621068.          0.00000
      ENTHALPY(CAL/SEC )      -0.602621E+09   -0.602596E+09   -0.413908E-04

                     ***  CO2 EQUIVALENT SUMMARY ***
   FEED STREAMS CO2E             0.00000      KG/HR
   PRODUCT STREAMS CO2E          0.00000      KG/HR
   NET STREAMS CO2E PRODUCTION   0.00000      KG/HR
   UTILITIES CO2E PRODUCTION     0.00000      KG/HR
   TOTAL CO2E PRODUCTION         0.00000      KG/HR

                          ***  INPUT DATA  ***
```

OUTLET PRESSURE BAR	6.00000
DRIVER EFFICIENCY	1.00000

FLASH SPECIFICATIONS:
LIQUID PHASE CALCULATION
NO FLASH PERFORMED

MAXIMUM NUMBER OF ITERATIONS	30
TOLERANCE	0.000100000

U-O-S BLOCK SECTION

BLOCK: FEEDPMP MODEL: PUMP (CONTINUED)

*** RESULTS ***

VOLUMETRIC FLOW RATE	L/MIN	10,845.7
PRESSURE CHANGE	BAR	4.82789
NPSH AVAILABLE	M-KGF/KG	9.22119
FLUID POWER	KW	87.2701
BRAKE POWER	KW	104.431
ELECTRICITY	KW	104.431
PUMP EFFICIENCY USED		0.83567
NET WORK REQUIRED	KW	104.431
HEAD DEVELOPED	M-KGF/KG	51.5832

STREAM SECTION

11 15 1SRSTGV 1STGMIX 1STSTGL

STREAM ID	11	15	1SRSTGV	1STGMIX	1STSTGL
FROM :	3RDSTGRE	----	1STSTG	B8	1STSTG
TO :	----	----	2STGREB	2STGREB	B8
SUBSTREAM: MIXED					
PHASE:	LIQUID	LIQUID	VAPOR	MIXED	LIQUID
COMPONENTS: KMOL/HR					
WATER	8152.2329	1.9819+05	7382.7781	2.4747+04	2.4747+04
AMMON-01	0.0	0.0	0.0	32.9004	32.9004
DL-ME-01	0.0	0.0	0.0	249.7361	249.7361
DEXTR-01	1.5082-06	0.0	2.8348-06	3.4474	3.4474
TOTAL FLOW:					
KMOL/HR	8152.2329	1.9819+05	7382.7781	2.5034+04	2.5034+04
KG/HR	1.4686+05	3.5704+06	1.3300+05	4.8807+05	4.8807+05
L/MIN	2812.2113	5.9127+04	7.3747+05	1.4468+05	9523.1702
STATE VARIABLES:					
TEMP C	143.6886	12.0000	159.3660	144.1199	159.3660
PRES BAR	4.0000	1.0135	6.0000	4.0000	6.0000

VFRAC	0.0	0.0	1.0000	3.7494-02	0.0
LFRAC	1.0000	1.0000	0.0	0.9625	1.0000
SFRAC	0.0	0.0	0.0	0.0	0.0
ENTHALPY:					
CAL/MOL	-6.5976+04	-6.8491+04	-5.6666+04	-6.4908+04	-6.4908+04
CAL/GM	-3662.2327	-3801.8505	-3145.4451	-3329.2163	-3329.2163
CAL/SEC	-1.4940+08	-3.7706+09	-1.1621+08	-4.5135+08	-4.5135+08
ENTROPY:					
CAL/MOL-K	-32.6489	-39.7471	-11.1248	-27.2297	-27.2767
CAL/GM-K	-1.8123	-2.2063	-0.6175	-1.3966	-1.3991
DENSITY:					
MOL/CC	4.8314-02	5.5864-02	1.6685-04	2.8838-03	4.3812-02
GM/CC	0.8704	1.0064	3.0058-03	5.6225-02	0.8542
AVG MW	18.0153	18.0153	18.0153	19.4964	19.4964

STREAM SECTION

2NDGMIX 2NDSTGV 2NSTGFED 2NSTGL 3RDSTFEE

STREAM ID	2NDGMIX	2NDSTGV	2NSTGFED	2NSTGL	3RDSTFEE
FROM :	B10	2NDSTG	2STGREB	2NDSTG	3RDSTGRE
TO :	3RDSTGRE	3RDSTGRE	2NDSTG	B10	3RDSTG
SUBSTREAM: MIXED					
PHASE:	MIXED	VAPOR	MIXED	LIQUID	MIXED
COMPONENTS: KMOL/HR					
WATER	1.6595+04	8152.2329	2.4747+04	1.6595+04	1.6595+04
AMMON-01	32.9004	0.0	32.9004	32.9004	32.9004
DL-ME-01	249.7361	0.0	249.7361	249.7361	249.7361
DEXTR-01	3.4474	1.5082-06	3.4474	3.4474	3.4474
TOTAL FLOW:					
KMOL/HR	1.6881+04	8152.2329	2.5034+04	1.6881+04	1.6881+04
KG/HR	3.4120+05	1.4686+05	4.8807+05	3.4120+05	3.4120+05
L/MIN	4.4425+05	1.1790+06	1.1855+06	6508.3606	3.2224+06
STATE VARIABLES:					
TEMP C	111.9588	144.3061	144.3061	144.3061	112.5230
PRES BAR	1.5000	4.0000	4.0000	4.0000	1.5000
VFRAC	7.2999-02	1.0000	0.3257	0.0	0.5352
LFRAC	0.9270	0.0	0.6743	1.0000	0.4648
SFRAC	0.0	0.0	0.0	0.0	0.0
ENTHALPY:					
CAL/MOL	-6.4902+04	-5.6790+04	-6.2261+04	-6.4902+04	-6.0466+04
CAL/GM	-3211.1294	-3152.3279	-3193.4353	-3211.1294	-2991.6484
CAL/SEC	-3.0434+08	-1.2860+08	-4.3295+08	-3.0434+08	-2.8354+08
ENTROPY:					
CAL/MOL-K	-25.7404	-10.6114	-20.8870	-25.8492	-14.2279
CAL/GM-K	-1.2735	-0.5890	-1.0713	-1.2789	-0.7039
DENSITY:					

MOL/CC	6.3333-04	1.1524-04	3.5195-04	4.3230-02	8.7313-05
GM/CC	1.2801-02	2.0762-03	6.8617-03	0.8737	1.7648-03
AVG MW	20.2117	18.0153	19.4964	20.2117	20.2117

STREAM SECTION

3RDSTGL 3RDSTGV 7 EVAPROD FEED

STREAM ID	3RDSTGL	3RDSTGV	7	EVAPROD	FEED
FROM :	3RDSTG	3RDSTG	2STGREB	3RDSTGPM	----
TO :	3RDSTGPM	----	----	----	FEEDPMP
SUBSTREAM: MIXED					
PHASE:	LIQUID	VAPOR	LIQUID	LIQUID	LIQUID
COMPONENTS: KMOL/HR					
WATER	7560.1277	9035.1174	7382.7781	7560.1277	3.2130+04
AMMON-01	32.9004	0.0	0.0	32.9004	32.9004
DL-ME-01	249.7361	0.0	0.0	249.7361	249.7361
DEXTR-01	3.4474	2.4816-07	2.8348-06	3.4474	3.4474
TOTAL FLOW:					
KMOL/HR	7846.2115	9035.1174	7382.7781	7846.2115	3.2416+04
KG/HR	1.7843+05	1.6277+05	1.3300+05	1.7843+05	6.2107+05
L/MIN	3238.5534	3.2191+06	2599.2028	3238.6297	1.0846+04
STATE VARIABLES:					
TEMP C	112.5230	112.5230	158.9160	112.5409	70.0000
PRES BAR	1.5000	1.5000	6.0000	2.0000	1.1721
VFRAC	0.0	1.0000	0.0	0.0	0.0
LFRAC	1.0000	0.0	1.0000	1.0000	1.0000
SFRAC	0.0	0.0	0.0	0.0	0.0
ENTHALPY:					
CAL/MOL	-6.4400+04	-5.7050+04	-6.5642+04	-6.4400+04	-6.6924+04
CAL/GM	-2831.9011	-3166.7653	-3643.7052	-2831.8839	-3493.0730
CAL/SEC	-1.4036+08	-1.4318+08	-1.3462+08	-1.4036+08	-6.0262+08
ENTROPY:					
CAL/MOL-K	-19.8892	-9.3116	-31.9001	-19.8882	-33.0025
CAL/GM-K	-0.8746	-0.5169	-1.7707	-0.8746	-1.7225
DENSITY:					
MOL/CC	4.0379-02	4.6778-05	4.7340-02	4.0378-02	4.9814-02
GM/CC	0.9183	8.4272-04	0.8528	0.9182	0.9544
AVG MW	22.7410	18.0153	18.0153	22.7410	19.1591

STREAM SECTION

HPRFEED S2 STM1IN TO1STSTG

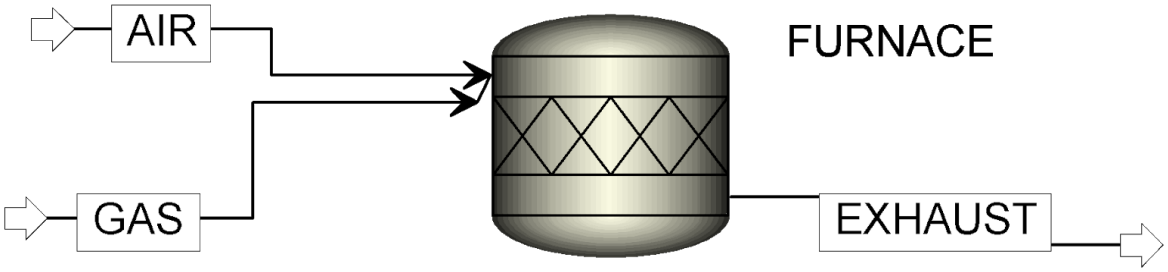
STREAM ID	HPRFEED	S2	STM1IN	TO1STSTG
FROM :	FEEDPMP	1STGHTR	----	1STGHTR
TO :	1STGHTR	----	1STGHTR	1STSTG
SUBSTREAM: MIXED				
PHASE:	LIQUID	LIQUID	VAPOR	MIXED
COMPONENTS: KMOL/HR				
WATER	3.2130+04	1.4432+04	1.4432+04	3.2130+04
AMMON-01	32.9004	0.0	0.0	32.9004
DL-ME-01	249.7361	0.0	0.0	249.7361
DEXTR-01	3.4474	0.0	0.0	3.4474
TOTAL FLOW:				
KMOL/HR	3.2416+04	1.4432+04	1.4432+04	3.2416+04
KG/HR	6.2107+05	2.6000+05	2.6000+05	6.2107+05
L/MIN	1.0847+04	5197.3601	1.0070+06	7.4700+05
STATE VARIABLES:				
TEMP C	70.1454	174.9730	175.0000	159.3660
PRES BAR	6.0000	8.9000	8.9000	6.0000
VFRAC	0.0	0.0	1.0000	0.2277
LFRAC	1.0000	1.0000	0.0	0.7723
SFRAC	0.0	0.0	0.0	0.0
ENTHALPY:				
CAL/MOL	-6.6921+04	-6.5276+04	-5.6537+04	-6.3031+04
CAL/GM	-3492.9284	-3623.3409	-3138.2698	-3289.8614
CAL/SEC	-6.0260+08	-2.6169+08	-2.2665+08	-5.6756+08
ENTROPY:				
CAL/MOL-K	-32.9944	-31.1149	-11.6142	-23.5981
CAL/GM-K	-1.7221	-1.7271	-0.6447	-1.2317
DENSITY:				
MOL/CC	4.9806-02	4.6281-02	2.3886-04	7.2326-04
GM/CC	0.9542	0.8338	4.3031-03	1.3857-02
AVG MW	19.1591	18.0153	18.0153	19.1591

PROBLEM STATUS SECTION

BLOCK STATUS

```
**************************************************************************
*                                                                        *
* Calculations were completed normally                                   *
*                                                                        *
* All Unit Operation blocks were completed normally                      *
*                                                                        *
* All streams were flashed normally                                      *
*                                                                        *
**************************************************************************
```

Combustion of Natural Gas for Rotary Dryer PFD:



Combustion of Natural Gas for Rotary Dryer Input:

```
;
;Input Summary created by Aspen Plus Rel.  37.0 at 21:25:55 Sun Apr 19, 2020
;Directory \nestor\alliwa\  Filename C:\Users\alliwa\AppData\Local\Temp\~ape4c8.txt
;


DYNAMICS
        DYNAMICS RESULTS=ON

IN-UNITS MET PRESSURE=bar TEMPERATURE=C DELTA-T=C PDROP=bar  &
        INVERSE-PRES='1/bar' SHORT-LENGTH=mm

DEF-STREAMS CONVEN ALL

MODEL-OPTION

DATABANKS 'APV110 PURE37' / 'APV110 AQUEOUS' / 'APV110 SOLIDS' &
        / 'APV110 INORGANIC' / 'APESV110 AP-EOS' /  &
        'NISTV110 NIST-TRC' / NOASPENPCD

PROP-SOURCES 'APV110 PURE37' / 'APV110 AQUEOUS' /  &
        'APV110 SOLIDS' / 'APV110 INORGANIC' / 'APESV110 AP-EOS' &
        / 'NISTV110 NIST-TRC'

COMPONENTS
        AIR AIR /
        METHA-01 CH4 /
        CARBO-01 CO2 /
        WATER H2O /
        OXYGE-01 O2 /
        NITRO-01 N2

SOLVE
        RUN-MODE MODE=SIM

FLOWSHEET
        BLOCK B2 IN=AIR GAS OUT=EXHAUST

PROPERTIES NRTL

STREAM AIR
```

```
        SUBSTREAM MIXED TEMP=25.  PRES=1.01 MASS-FLOW=87606.
        MASS-FRAC AIR 0.  / METHA-01 0.  / CARBO-01 0.  / WATER  &
        0.  / OXYGE-01 0.21 / NITRO-01 0.79

STREAM GAS
        SUBSTREAM MIXED TEMP=25.  PRES=1.  MASS-FLOW=4630.
        MASS-FRAC AIR 0.  / METHA-01 1.  / CARBO-01 0.  / WATER  &
        0.  / OXYGE-01 0.  / NITRO-01 0.

BLOCK B2 RSTOIC
        PARAM TEMP=170.  PRES=1.01
        STOIC 1 MIXED OXYGE-01 -2.  / METHA-01 -1.  / CARBO-01 1.  / &
        WATER 2.
        CONV 1 MIXED METHA-01 1.

EO-CONV-OPTI

STREAM-REPOR MOLEFLOW

PROPERTY-REP PCES
;
;
;
;
;
```

Combustion of Natural Gas for Rotary Dryer Report:

```
 ASPEN PLUS CALCULATION REPORT

 ASPEN PLUS IS A TRADEMARK OF          HOTLINE:
 ASPEN TECHNOLOGY, INC.                U.S.A. 888/996-7100
 781/221-6400                          EUROPE (44) 1189-226555

 PLATFORM: WIN-X64                                  APRIL 20, 2020
 VERSION: 37.0   Build 395                          MONDAY
 INSTALLATION:                                      6:43:05 P.M.

 ASPEN PLUS   PLAT: WIN-X64   VER: 37.0              04/20/2020  PAGE    I


      ASPEN PLUS (R) IS A PROPRIETARY PRODUCT OF ASPEN TECHNOLOGY, INC.
      (ASPENTECH), AND MAY BE USED ONLY UNDER AGREEMENT WITH ASPENTECH.
      RESTRICTED RIGHTS LEGEND:  USE, REPRODUCTION, OR DISCLOSURE BY THE
      U.S. GOVERNMENT IS SUBJECT TO RESTRICTIONS SET FORTH IN
      (i) FAR 52.227-14, Alt. III, (ii) FAR 52.227-19, (iii) DFARS
      252.227-7013(c)(1)(ii), or (iv) THE ACCOMPANYING LICENSE AGREEMENT,
      AS APPLICABLE. FOR PURPOSES OF THE FAR, THIS SOFTWARE SHALL BE DEEMED
      TO BE "UNPUBLISHED" AND LICENSED WITH DISCLOSURE PROHIBITIONS.
      CONTRACTOR/SUBCONTRACTOR: ASPEN TECHNOLOGY, INC. 20 CROSBY DRIVE,
      BEDFORD, MA 01730.
                              TABLE OF CONTENTS


            RUN CONTROL SECTION.....................................  1
                 RUN CONTROL INFORMATION............................  1

            FLOWSHEET SECTION.......................................  2
                 FLOWSHEET CONNECTIVITY BY STREAMS..................  2
                 FLOWSHEET CONNECTIVITY BY BLOCKS...................  2
                 COMPUTATIONAL SEQUENCE.............................  2
                 OVERALL FLOWSHEET BALANCE..........................  2

            PHYSICAL PROPERTIES SECTION.............................  3
                 COMPONENTS.........................................  3

            U-O-S BLOCK SECTION.....................................  4
                 BLOCK:  B2       MODEL: RSTOIC.....................  4
```

RUN CONTROL SECTION

RUN CONTROL INFORMATION

THIS COPY OF ASPEN PLUS LICENSED TO UNIVERSITY OF PENNSYLVAN

TYPE OF RUN: EDIT

INPUT FILE NAME: _5742ewl.inm

INPUT PROBLEM DATA FILE NAME : _5742ewl
OUTPUT PROBLEM DATA FILE NAME: _3114xma
LOCATED IN:

PDF SIZE USED FOR INPUT TRANSLATION:
NUMBER OF FILE RECORDS (PSIZE) = 0
NUMBER OF IN-CORE RECORDS = 256
PSIZE NEEDED FOR SIMULATION = 1

CALLING PROGRAM NAME: apmain
LOCATED IN: C:\Program Files\AspenTech\Aspen Plus V11.0\Engine\\xeq

SIMULATION REQUESTED FOR ENTIRE FLOWSHEET

FLOWSHEET SECTION

FLOWSHEET CONNECTIVITY BY STREAMS

STREAM	SOURCE	DEST	STREAM	SOURCE	DEST
GAS	----	B2	AIR	----	B2
EXHAUST	B2	----			

FLOWSHEET CONNECTIVITY BY BLOCKS

BLOCK	INLETS	OUTLETS
B2	AIR GAS	EXHAUST

COMPUTATIONAL SEQUENCE

SEQUENCE USED WAS:
B2

OVERALL FLOWSHEET BALANCE

*** MASS AND ENERGY BALANCE ***

	IN	OUT	GENERATION	RELATIVE DIFF.
CONVENTIONAL COMPONENTS (KMOL/HR)				
AIR	0.00000	0.00000	0.00000	0.00000
METHA-01	288.604	0.00000	-288.604	0.00000
CARBO-01	0.00000	288.604	288.604	0.00000
WATER	0.00000	577.207	577.207	0.00000
OXYGE-01	602.211	25.0033	-577.207	-0.530951E-16
NITRO-01	2587.75	2587.75	0.00000	0.00000
TOTAL BALANCE				
MOLE(KMOL/HR)	3478.57	3478.57	0.00000	0.130728E-15
MASS(KG/HR)	96392.0	96392.0		0.00000
ENTHALPY(CAL/SEC)	-0.142689E+07	-0.157596E+08		0.909459

*** CO2 EQUIVALENT SUMMARY ***

FEED STREAMS CO2E	115750.	KG/HR
PRODUCT STREAMS CO2E	12701.4	KG/HR
NET STREAMS CO2E PRODUCTION	-103049.	KG/HR
UTILITIES CO2E PRODUCTION	0.00000	KG/HR
TOTAL CO2E PRODUCTION	-103049.	KG/HR

PHYSICAL PROPERTIES SECTION

COMPONENTS

ID	TYPE	ALIAS	NAME
AIR	C	AIR	AIR
METHA-01	C	CH4	METHANE
CARBO-01	C	CO2	CARBON-DIOXIDE
WATER	C	H2O	WATER
OXYGE-01	C	O2	OXYGEN
NITRO-01	C	N2	NITROGEN

U-O-S BLOCK SECTION

BLOCK: B2 MODEL: RSTOIC

```
------------------------------
  INLET STREAMS:         AIR         GAS
  OUTLET STREAM:         EXHAUST
  PROPERTY OPTION SET:   NRTL      RENON (NRTL) / IDEAL GAS

                      ***  MASS AND ENERGY BALANCE  ***
                              IN          OUT       GENERATION   RELATIVE DIFF.
  TOTAL BALANCE
  MOLE(KMOL/HR )         3478.57       3478.57       0.00000      0.130728E-15
  MASS(KG/HR   )         96392.0       96392.0                     0.00000
  ENTHALPY(CAL/SEC )   -0.142689E+07 -0.157596E+08                 0.909459

                      ***  CO2 EQUIVALENT SUMMARY ***
   FEED STREAMS CO2E             115750.      KG/HR
   PRODUCT STREAMS CO2E          12701.4      KG/HR
   NET STREAMS CO2E PRODUCTION  -103049.      KG/HR
   UTILITIES CO2E PRODUCTION     0.00000      KG/HR
   TOTAL CO2E PRODUCTION        -103049.      KG/HR

                         ***  INPUT DATA  ***
  STOICHIOMETRY MATRIX:

   REACTION #   1:
    SUBSTREAM MIXED   :
    METHA-01  -1.00    CARBO-01   1.00    WATER      2.00    OXYGE-01  -2.00


  REACTION CONVERSION SPECS: NUMBER=    1
    REACTION #   1:
    SUBSTREAM:MIXED    KEY COMP:METHA-01 CONV FRAC:  1.000



  TWO    PHASE  TP  FLASH
  SPECIFIED TEMPERATURE C                                  170.000
  SPECIFIED PRESSURE    BAR                                  1.01000
  MAXIMUM NO. ITERATIONS                                    30
  CONVERGENCE TOLERANCE                                      0.000100000
  SIMULTANEOUS REACTIONS
  GENERATE COMBUSTION REACTIONS FOR FEED SPECIES           NO
 ASPEN PLUS   PLAT: WIN-X64   VER: 37.0                    04/20/2020  PAGE 5


                            U-O-S BLOCK SECTION

 BLOCK:  B2       MODEL: RSTOIC (CONTINUED)

                           ***  RESULTS  ***
  OUTLET TEMPERATURE    C                                    170.00
  OUTLET PRESSURE       BAR                                  1.0100
```

HEAT DUTY	CAL/SEC	-0.14333E+08
VAPOR FRACTION		1.0000

REACTION EXTENTS:

REACTION NUMBER	REACTION EXTENT KMOL/HR
1	288.60

V-L PHASE EQUILIBRIUM :

COMP	F(I)	X(I)	Y(I)	K(I)
CARBO-01	0.82966E-01	0.61980E-02	0.82966E-01	617.41
WATER	0.16593	0.97801	0.16593	7.8255
OXYGE-01	0.71878E-02	0.16096E-03	0.71878E-02	2059.6
NITRO-01	0.74391	0.15632E-01	0.74391	2195.0

STREAM SECTION

AIR EXHAUST GAS

STREAM ID	AIR	EXHAUST	GAS
FROM :	----	B2	----
TO :	B2	----	B2
SUBSTREAM: MIXED			
PHASE:	VAPOR	VAPOR	VAPOR
COMPONENTS: KMOL/HR			
AIR	0.0	0.0	0.0
METHA-01	0.0	0.0	288.6037
CARBO-01	0.0	288.6037	0.0
WATER	0.0	577.2074	0.0
OXYGE-01	602.2107	25.0033	0.0
NITRO-01	2587.7535	2587.7535	0.0
TOTAL FLOW:			
KMOL/HR	3189.9642	3478.5679	288.6037
KG/HR	9.1762+04	9.6392+04	4630.0000
L/MIN	1.3049+06	2.1150+06	1.1924+05
STATE VARIABLES:			
TEMP C	25.0000	170.0000	25.0000
PRES BAR	1.0100	1.0100	1.0000
VFRAC	1.0000	1.0000	1.0000
LFRAC	0.0	0.0	0.0
SFRAC	0.0	0.0	0.0
ENTHALPY:			

```
  CAL/MOL            -1.6797-13 -1.6310+04 -1.7799+04
  CAL/GM             -5.8393-15  -588.5827 -1.1094597
  CAL/SEC            -1.4884-10 -1.5760+07 -1.4269+06
ENTROPY:
  CAL/MOL-K              0.9684     2.7393   -19.2241
  CAL/GM-K            3.3666-02  9.8854-02    -1.1983
DENSITY:
  MOL/CC              4.0744-05  2.7412-05  4.0340-05
  GM/CC               1.1720-03  7.5960-04  6.4717-04
AVG MW                  28.7658    27.7103    16.0428
```


PROBLEM STATUS SECTION

BLOCK STATUS

```
**************************************************************************
*                                                                        *
* Calculations were completed normally                                   *
*                                                                        *
* All Unit Operation blocks were completed normally                      *
*                                                                        *
* All streams were flashed normally                                      *
*                                                                        *
**************************************************************************
```

Appendix C: Equipment Costing Sources

Equipment	Flowsheet Label	Cost Source
Cell Preparation		
Cell Bank	N/A	ATCC Site Listing
12 mL Test Tubes	N/A	Fisher Scientific Site Listing
Storage		
Corn Syrup Storage Tank	STOR-01	Equipment Costing Spreadsheet
Teknova Broth Storage Tank	STOR-02	Equipment Costing Spreadsheet
Ammonia Storage Tank	STOR-03	Equipment Costing Spreadsheet
Fermentation Product Storage Tank	STOR-04	Equipment Costing Spreadsheet
Evaporator Concentrate Storage Tank	STOR-05	Equipment Costing Spreadsheet
DLM Storage Tank	STOR-06	Bruce Vrana
Seed Train Process		
Air Compressor	MS-COMP	Equipment Costing Spreadsheet
Coarse Air Filter	Coarse air filter	Jahnke, Pillarella, Weiner Paper
Submicron Air Filter	Submicron air filter	Jahnke, Pillarella, Weiner Paper
Pump	P-04, 08-12	Equipment Costing Spreadsheet
Pump	P-05	Equipment Costing Spreadsheet
Pump	P-06, 07	Equipment Costing Spreadsheet
2 L Flask	N/A	Fisher Scientific Site Listing
5,000 L Reactor*	PRE-SEED-01 to -06	Equipment Costing Spreadsheet
Pump	P-01	Equipment Costing Spreadsheet
Pump	P-01a,01b	Equipment Costing Spreadsheet
50,000 L Reactor*	SEED-01 to -06	Equipment Costing Spreadsheet

500,000 L Reactor*	PROD-01 to -12	Equipment Costing Spreadsheet
Pump	P-02,03	Equipment Costing Spreadsheet
Scrubber	N/A	Jahnke, Pillarella, Weiner Paper
Batch Process/Continuous Process		
Pump	MS-PUMP-01	Equipment Costing Spreadsheet
Heat Exchanger	MS-HX-01 MS-HX-02 MS-HX-03	Equipment Costing Spreadsheet
Pump	MS-PUMP-02	Equipment Costing Spreadsheet
Pump	P-13	Equipment Costing Spreadsheet
Heat Exchanger	HX-04	Equipment Costing Spreadsheet
Centrifuge	CFG-01, 02, 03	Bruce Vrana
Pump	P-17	Equipment Costing Spreadsheet
Pump	P-18	Equipment Costing Spreadsheet
Rotary Drum Dryer	RDD-01	Bruce Vrana
Pump	P-14	Equipment Costing Spreadsheet
Heat Exchanger	EVAP-01	Equipment Costing Spreadsheet
Flash Vessel	N/A	Equipment Costing Spreadsheet
Heat Exchanger	EVAP-02	Equipment Costing Spreadsheet
Flash Vessel	N/A	Equipment Costing Spreadsheet
Heat Exchanger	EVAP-03	Equipment Costing Spreadsheet
Flash Vessel	N/A	Equipment Costing Spreadsheet
Pump	P-15, 16	Equipment Costing Spreadsheet
Crystallizer	CRYS-01 to -03	Equipment Costing Spreadsheet
Centrifuge	CFG-04	Consultant
Rotary Dryer	RD-01	Bruce Vrana
Conveyor	CONV-01	Consultant
Spares		

Pumps	N/A	Equipment Costing Spreadsheet
Filters	N/A	Jahnke, Pillarella, Weiner Paper
Scrubber	N/A	Jahnke, Pillarella, Weiner Paper
Other	N/A	Profitability Analysis Spreadsheet
Product Purification		
Super Sacks	SS-01, -02	ULINE Site Listing
Cleaning		
CIP System	N/A	Jahnke, Pillarella, Weiner Paper
SIP System	N/A	Solida Biotech Quote

*All reactors were priced using the Pressure Vessel tab of the Equipment Costing Spreadsheet, as recommended by Bruce Vrana.

Appendix D: Vendor Specification Sheets


SOLIDA BIOTECH
NEXT-GENERATION BIOREACTORS
IN SITU
STERILIZABLE
BIOREACTORS

BioSip

In situ Sterilizable Bioreactors

Solida Biotechnology team with over 20 years experience in sterile process engineering and bioprocess technologies has realised a complete range of laboratory and pilot SIP in situ sterilizable bioreactors and fermenters. Solida Biotechnology offer pre-assembled SIP bioreactors packages or custom made solutions based on detailed requirements.

Culture vessels are available in bacteriological or cell cultivation configurations in the standard volumes from 3 to 5000 litres , or customized volumes up to 50 cubic meters and more on request.

- **High Flexibility and Reliability** via PLC automation andBIOFLEX SCADA software
- **Modularity and upgrades** at any time thanks to our new Modular concept design
- **Quality without compromise** only certified materials are selected
- **Complete documentation.** IOQ, DQ and components traceability for GLP and cGMP
- **Service and Maintenance** with a worldwide network

SOLIDA BIOTECH SOLUTION
FULL CONTROL & FLEXIBILITY

EXPERIENCE IN BIOREACTORS DESIGN AND STERILE PROCESSING

Our laboratory and pilot SIP bioreactors are designed to guarantee better performances and improve process conditions like scaling up or scaling down, mixing and oxygen transfer, heating/cooling thermal transfer, sterility and cleaning procedures to optimize manufacturing time and costs saving.

BioSip automation & software Are based on leading supplier PLC's that runs under an advanced intuitive operating system. Software comprises a PLC with local visualization platform, HMI human interface touch screen and custom made configuration.

BioSip automated platforms guarantee the best performances, reliability, long term service and spare parts availability unless proprietary systems. The selection of trusted hardware components united with our background in fermentation and cell culture implemented into the BIO-SIP Controller Software ends up into a unique advanced SIP bioreactors solution.

BioSip Controllers series are powered through a UPS device protecting it from interference, overvoltage and power cuts. All units are provided with an automated re-start sequence in case of power supply failures. Hardware components are located into a classified waterproof cabinet IP55/65 certified.

BioSip Controller architecture can hold and simultaneously manage up to 2 or more Lab and Pilot bioreactors. Automation design and functionality allow to interchange vessel's size without modifying PLC or Software configuration.
Advanced technology reflect also the use of field-bus based I/O modules for accurate and fast data management and to allow easy maintenance and to be ready for any expansion later on. Each system can be upgraded and replaced at any time without any limitation.

Reduce lab space and energy consumption.

Easy to use with a simpler User Interface.

Full material traceability and certifications.

Various Accessories available.

Maximum flexibility with wide choose of Hardware solutions.

UNIQUE CONTROLLER FLEXIBILITY IN ONE BIOREACTOR

Simultaneous control and regulation:

- 2 x pH
- 2 x pO2
- 10 or more temperature
- 2 x level and foam
- 2 x stirrer speed
- 2 x pressure
- up to 8 x variable speed or fix speed peristaltic pumps
- up to 8 or more MFC's or rotameters
- up to 4 load cells
- 8 or more balances

Extra inputs:

- biomass monitors
- optical density
- gas analyzer
- pCO2
- methanol analyzer
- automated samplers and others.

Chosen of leading PLC:
Siemens, National Instruments, Allen Bradley, Delta V.

Chosen of Communication device:
Canopen, Interbus, Profibus, DeviceNet, ControlNet, ModBus, RS232/485, Ethernet, USB

BioSip HUMAN INTERFACE TOUCHSCREEN

The **Bio**Sip HMI-PC is a unique interface that allow full local control of the bioreactor. Large Touch-screens available in 15", 19", 21".

Functionalities:

- Full or empty automated sterilization cycles in one touch
- Preparation phases
- Pre-Inoculation set-up
- Inoculation assistance procedure
- Fermentation or Cell cultivation process start-up guided procedures
- Easy configuration of process parameters,
- P.I.D. settings,
- Probes and pumps calibration
- Dose monitoring for pumps and MFC's
- Up to 4 level of alarms
- Up to 4 password access
- Sequences programming
- Batches and feeding profiles formulations
- Cascade controls and exponential equations
- Online data recording with memory card
- Data download via USB/ Ethernet output
- Real-time data Visualization with graphic, curves and profiles displays.

Each unit is equipped with Batch, Feed-batch, Continuous modes of operation.

STANDARD **JACKETED VESSELS SPECIFICATIONS:**

Total volume	Working volume (L)	min.Working volume (L)	Aspect ratio Microbial culture	Aspect ratio h/d Cell cultivation
7L	5	1,5	2.7 / 3.1	2.1 / 1.5
10L	7,5	2,5	2.7 / 3.1	2.1 / 1.5
15L	11	3,5	2.7 / 3.1	2.1 / 1.5
20L	15	5	2.7 / 3.1	2.1 / 1.5
30L	22	7,5	2.7 / 3.1	2.1 / 1.5
40L	30	10	2.7 / 3.1	2.1 / 1.5
50L	37,5	12,5	2.7 / 3.1	2.1 / 1.5
70L	52,5	17,5	2.7 / 3.1	2.1 / 1.5
100L	75	25	2.7 / 3.1	2.1 / 1.5
150L	110	37,5	2.7 / 3.1	2.1 / 1.5
200L	150	50	2.7 / 3.1	2.1 / 1.5
300L	225	75	2.7 / 3.1	2.1 / 1.5
400L	300	100	2.7 / 3.1	2.1 / 1.5
500L	375	125	2.7 / 3.1	2.1 / 1.5

FEATURES & SPECIFICATIONS

FEATURES

- Smart pH and D.O. probes allow monitoring of all sensor functions making substantial advantages in bioprocess monitoring and control
- pH sensor empower fully integrated accuracy monitoring
- Monitoring of sensor quality (glass resistance, reference resistance, Checkref potential).
- D.O. optical sensors demonstrate a number of substantial advantages because of a symbiosis of sensor and measurement amplifier- an smart sensor.
- Variable or fix speed peristaltic pumps, autoclavable type.

The pump heads parts are assembled together and mounted to the front end of the metering pump.
Even if the separate parts are individually sterilized, handling is required for assembly which renders the product contact surfaces non-sterile.
As a result, Solida Biotech introduce onto his Bioreactors liquid metering pumps to avoid contamination problems caused by manual handling.

SPECIFICATIONS

Agitation system	Direct drive, single and double mechanical or magnetically coupled drive
Stirrer speed (rpm)	Standard range is 1 – 2000 rpm adjustable according to required configuration either bacterial, cell culture or both
Impellers	Rushton, Marine, Pitched Blade, adjustable and removable type impellers. Special impellers are also available.
Gas sparger	Porous sparger, L-type sparger, Sinterized sparger, fixed or removable type
Gas overlay	Included as standard feature
Gas mixing	Standard set-up include Air, O2, CO2 and N2 gas mixing station, our unit can hold up to 8 gasses. Standard set-up include Flowmeters with on/off automatic solenoid valve for gas flow regulation or Massflow controllers for automated gas flow control and data recording
Exhaust gas	Water cooled exhaust gas Condenser
Sampling	Sanitary sampling system with contained sampling pipe including sampling bottles available with various volumes. DN Ingold sampling port or Retractable-fit type are available
Harvesting	Sanitary contained Drain pipe or Dip tube Fixed height or Height adjustable
Liquid additions	Sanitary inlet ports for chemicals additions or contained resterilizable liquid addition pipe
pH	Optical or classic pH sensor, 12mm, Ingold connectors. PLC and SCADA Software Control: via acid pump or CO2 gas (Flowmeter or MFC) in combination with alkali pump and/or other actuators.
DO2	Optical or classic DO sensor, 12mm, Ingold connectors. PLC and SCADA Software Control: via or in combination with N2, Air, O2 (Flowmeter or automation MFC) and agitation or nutrient addition pump or other actuators
Temperature	Pt-100 sensor in thermo well plate. PLC and SCADA Software Control: cooling and/or heating jacket via bioreactor wall or via internal heat exchanger, cooling via tap water or chilled water
Foam	Height adjustable conductivity based foam and level sensor, High/Low foam sensors are also available. PLC and SCADA Software Control: Anti foam addition pump or other actuators.
Level	Height adjustable capacitative based level sensor. PLC and SCADA Software Control: pump for liquid addition or removal
Pressure	Pressure sensor top plate mounted. PLC and SCADA Software Control: modulated pressure valve, combined with air inlet, Flowmeters/MFC, agitation and other actuators
Weight	Load cells and balances are available. PLC and SCADA Software Control: pumps for liquid addition or removal, chemostat or continuous mode.
Probes and sensors available	Online Biomass probes, optical density sensors, CO2/O2/NH4/SO2 gas analyser, pCO2 sensor, conductivity, methanol/ethanol analyzers, Automated samplers PLC and SCADA Software Control integrations, OPC compliance.
Certifications	IQ, OQ, PQ protocols available Including full material traceability GLP and cGMP compliance. User and maintenance manuals are available in English, French, German, Spanish or Italian. Chinese, Indian, Russian and Japanese languages, on request.
After-sales support	Worldwide after-sales net-work with skilled engineers support. Remote diagnostic control and Online assistance 24/24h available.

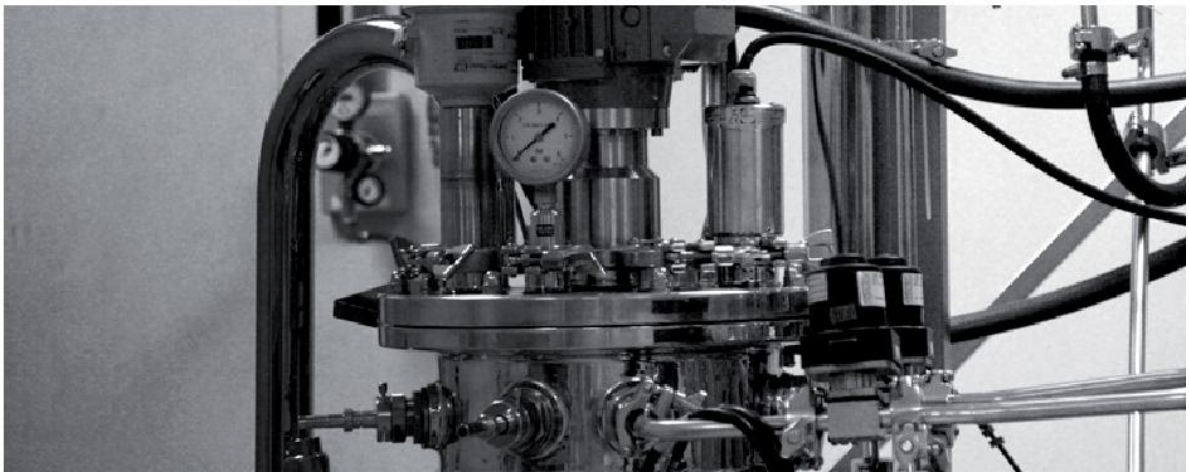
INDUSTRIAL PLANT PROJECTS

TURN-KEY SOLUTIONS BY SOLIDA BIOTECHNOLOGY

The concept of modularity using standard modules to customized lay out of the bioreactors has been extended to the stainless steel Industrial bioreactors and fermenters. The vessels are cGMP and comply with different pressure code throughout the world. The systems are fully documented and delivered with all necessary documentation for mechanical and electronical components. The Industrial Systems are ranging in sizes of 500L up to 50cm^3 or more. Our modular, pre-designed and configured turnkey system incorporating the most commonly requested functions and features.

COMPLEMENTARY PRODUCTS

- Complete, turnkey production-scale equipments
- Fully automatic in-situ sterilization and integrated steam generators
- Industrial PLC Automation controllers and SCADA Software in an IP55/IP65 stainless steel cabinet
- Integration of Online Analyzer's for complete process control
- Integration of Down-stream equipments
- Tangential flow and Dia-Filtration units
- Online Centrifuges
- Integration of Isolators and Laminar Flow cabinets
- Supply of Fill/Finishing and Capping automated or semi-automated machineries.




1 BioSIP HMI-PC
Full local controlwith large
touch-screenPC interface
2 High Quality
SS 316L bioreactors
Fully cGMP conform
3 Sanitary piping
Sterility and cleanability
concept design
4 BioSteam
Automated steam
generator unit
5 BioUPS
Back-up safety device
for continuous operations
6 BioSIP
Advanced Controller
Guaranteed flexibility
and upgrades at any time
BioSip
In situ
Sterilizable
Bioreactors

Solida Biotechnology
and his partners design and realise
machineries for the pharmaceutical industry.
Our firm's develop guideline is based
on researchand projectof new solutions
in full compliance with the quality and safety rules.

With a worldwide distribution network
Solida Biotechnology guarantees
full local support and after-sales services.

The production catalogue
includesover 100 machinemodels
and a line of accessories and complements
according to curent GMP and FDA rules.

Our production line includes:

Bioreactors & Fermenters
Filtration Units
Isolators & Glowe Testers
Sanitary Tanks
Sterilizing & Depirogenizing Units
Washing Machines
Confectioning & Final Confectioning Machines
Cryoplants
Automation & Software
Fornitures & Accessories
Turnkey Projects

SOLIDA BIOTECH

Street: Wagmullerstrasse, 23 - 80538 Munchen - Germany
T/+49 (0) 89 242 9090 20 - F/+49 (0) 89 242 9090 30

E-mail: sales@solidabiotech.com
www.solidabiotech.com

OPAKFIL 2V

V-Bank Filter

ADVANTAGES

- Light and robust

Application	Air conditioning applications and preparatory filtration in clean rooms
Type	V-Bank Filter
Frame	ABS
Media	Glass fiber
Separator	Hot-melt
Sealant	Polyurethane
Dimensions	Filter front dimensions according EN 15805
Rec. final pressure drop acc. EN 13053	Initial pressure drop + 100 Pa or initial pressure drop x3 (whichever is lower)
Max airflow	1,25 x nominal flow
Temperature max	70°C
RH. max	100%
Installation Options	Front and side access housings and frames are available.

Model Name	EN779	ISO 16890	Dimensions WxHxD (mm)	Air Flow/pressure drop (m³/h/Pa)	Media area (m²)	Weight (kg)	Energy (kWh/year)	Energy class	ePM1	ePM1min	ePM2,5	ePM2,5min	ePM10
2V6	M6	ePM10 70%	592x592x268	3400/85	8	3		E	28	28	40	40	74
2V6	M6	ePM10 70%	592x490x268	2800/85	7	2,5		E					
2V6	M6	ePM10 70%	592x287x268	1700/85	4	2		E					
2V7	F7	ePM1 55%	592x490x268	2800/100	7	2,5		C					
2V7	F7	ePM1 55%	592x287x268	1700/100	4	2		C					
2V8	F8	ePM1 70%	592x592x268	3400/120	8	3		E	72	72	80	80	92
2V8	F8	ePM1 70%	592x490x268	2800/120	7	2,5		E					
2V8	F8	ePM1 70%	592x287x268	1700/120	4	2		E					
2V9	F9	ePM1 80%	592x592x268	3400/180	8	3		E	83	83	87	87	95
2V9	F9	ePM1 80%	592x490x268	2800/180	7	2,5		E					
2V9	F9	ePM1 80%	592x287x268	1700/180	4	2		E					
2V7	F7	ePM1 55%	592x592x268	3400/100	8	3	1359	C	56	56	66	66	87

Energy Consumption, kWh/year: Calculated according to Eurovent Guideline 4/21-2019
Energy class: according to Eurovent RS 4/C/001-2019

ABSOLUTE VG XL, XXL

V-Bank Box Filter

ADVANTAGES

- High efficiency
- Halogen free
- VDI 6022
- Applicable up to 6000 m³/h air flow
- High air flow
- Low pressure drop
- Optimized, compact construction

Application	Efficiency final filtration in air conditioning systems, housings and diffusers
Type	V-Bank Box Filter
Frame	ABS
Gasket	EPDM
Media	Glass fiber
Separator	Hot-melt
Sealant	Polyurethane
Max. final pressure drop	600 Pa
Max airflow	Nominal flow rate (if not, efficiency drops)
Temperature max	70°C
RH. max	100%
Installation Options	FKB, 4N, CamSafe2

Art. No.	Type	EN1822	Dimensions WxHxD (mm)	Air Flow/pressure drop (m³/h/Pa)	Weight (kg)
1705008	VGXL11 610x610x292-PR	E11	610x610x290	4000/250	10
1705009	VGXXL11-305x610x292-PR	E11	305x610x292	2000/250	5
1705007	VGXXL11-610x610x292-PR	E11	610x610x292	5000/250	11
1705002	VGL13-610x610x292-PR-S	H13	610x610x292	3400/250	11
1705003	VGXL13-305x610x292-PR-S	H13	305x610x292	1700/250	5
1705001	VGXL13-610X610X292-PR-S	H13	610x610x292	4000/250	11
1705006	VGXL13-762x610x292-PR-S	H13	762x610x292	6000/380	14
1705014	VGXL14-305x610x292-PR-S	H14	305x610x292	1500/250	5
1705013	VGXL14-610x610x292-PR-S	H14	610x610x292	3000/250	11
1705015	VGXL14 305x610x292-PR-S	H14	305x610x292	3400/250	11
1705016	VGXL14 305x610x292 PR S	H14	305x610x292	1700/250	5

Type M = Gasket on one side

Other sizes,stainless steel or aluminium frames are available on request

FEQX 520S - Large capacity nozzle centrifuge

For yeast and distillery applications with solids recirculation system

Alfa Laval separator centrifuges for the yeast and distillery industries are available in many different sizes and configurations, each one designed and adapted for dealing with the widely varying separation tasks required. The FEQX 520S-31CG is the largest centrifuge with peripheral nozzles for these industries.

Applications

The FEQX 520S is specifically designed for separation of distiller's, and fodder yeast as well as other fermentation broths. Separation of particles with sizes down to 0.5 µm is possible.

Typical proceecing capacitys

Conventional fermentation of molasses/sugar w. recycle	180 m^3/h.
High conc. fermentation of molasses/sugar w. recycle	120 m^3/h
Spent sulphite liquor fermentation	130 m^3/h
Fodder yeast at 5% DM in the feed	90 m^3/h

Standard design

Separation takes place in the bowl, which is placed on a vertical spindle. An electric motor mounted vertically drives the spindle near the bottom via a flat belt. Two motors are available: a standard motor for variable frequency drive or a control-torque motor. All metallic parts that come in contact with the process liquid are made of high-grade stainless steel. The 18 nozzles can be reached from the outside via a hatch in the frame hood, which enables technicians to replace them easily and rapidly without dismantling the frame. The nozzles are made of tungsten carbide, suitable for abrasive solids. The inlet and outlet device is equipped with flanges. The bowl casing has connections for flushing above and below the bowl.

Special features

The FEQX 520S is equipped with a solids recirculation system, which constitutes a unique means of controlling the separation process. Recirculation makes it possible to use a larger nozzle size and to meet fluctuations in the solids flow. Circulating oil ensures that the bearings are lubricated. An external pump maintains the necessary pressure.

Basic equipment

Centrifuge with motor, set of tools, speed and vibration sensors, oil pressure switch, temperature sensors for the main spindle bearing and the motor winding, vibration dampening feet, foundation plate and standard set of spares.

Fig. 1 FEQX 520S complete with motor

Options

Control-torque motors of four different power ratings are available. Frequency drive is also possible. The bowl is available in two versions, with or without erosion protection. Liquid-wetted gaskets are made of nitrile rubber or food grade EPDM rubber. The centrifuge bowl is available with four different disc spacings. The connections are designed with either DIN or ANSI flanges.

Optional extras

The FEQX 520S can be fitted with a nozzle monitor. This consists of a microphone, which is hit by the jet from each individual nozzle. The signal is then transmitted to a box, which displays the result. The monitor shows whether the nozzles are clogged or worn out, thus enabling safe operation and high availability. A cover interlocking kit makes it impossible to start the centrifuge unless it is properly assembled. The FEQX 520S can be delivered as a complete system, including valve modules for process liquid and wash water, starter and control system. A conversion kit for rebuilding into FESX 520S is available.

Operating principles

The feed containing the liquid and the solids is introduced to the rotating centrifuge bowl (fig. 2) from the top via a stationary inlet pipe (1), and is accelerated in a distributor (2) before entering the disc stack (3). Separation takes place between the discs. The light phase moves through the disc stack towards the centre of the bowl, and is pumped out under pressure by means of a built-in paring disc (4). The yeast is collected at the bowl periphery and continuously discharged through the nozzles (5). Filler pieces (6) prevent build-up of the solids between the nozzles. The nozzle flow is collected in a cover around the bowl and further discharged into a pump. Part of the effluent from the pump can then be recirculated back to the nozzles through a pipe (7), a separate recirculation chamber (8), and recirculation tubes (9).

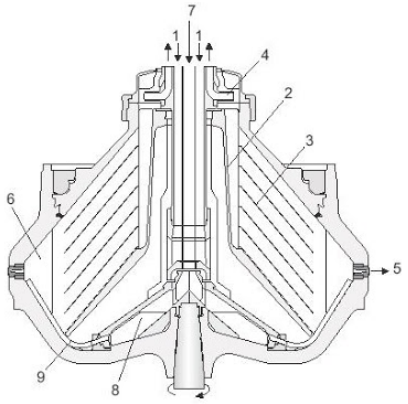


Fig. 2 Typical bowl drawing for a nozzle centrifuge with recirculation of solids. Details illustrated do not necessarily correspond to the centrifuge described.

Utilities consumption

Electric power	max. 170 kW [1]
Safety water	23-55 m^3/h [2]
Flushing water	60/460 l/h [3]

[1] At max process flow rate 120 m^3/h, nozzle flow rate 40 m^3/h, and recirculation rate 20 m^3/h. Power consumption increases with the flow rate

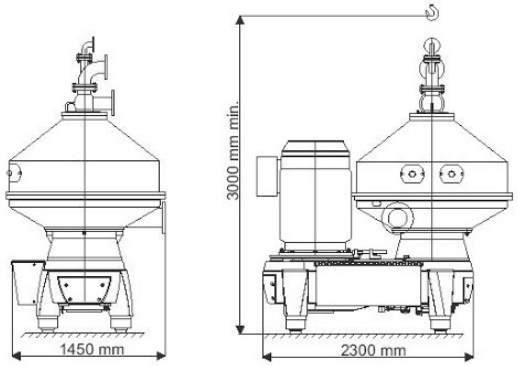
[2] The bowl should be filled at start, stop and normal operation. In case process liquid is not available, safety water should be used. The above figures refer to nozzle sizes from 1.6 to 2.5 mm and max, bowl speed. The safety water feed to separator should always exceed the nozzle flow by 10%.

[3] Above/below bowl. Intermittent flow.

Shipping data (approximate)

Centrifuge incl. bowl and motor	4570 kg
Bowl weight	1050 kg
Gross weight	4900 kg
Volume	8 m^3

Dimensions



Technical specification

Throughput capacity	max. 250 m^3/h [1]
Light liquid flow	max. 200 m^3/h
Nozzle flow	max. 100 m^3/h
Bowl volume	120 l
Bowl speed	3750 rpm
Motor speed, synchr. 50/60	1500/1800 rpm
Motor power installed	135/160/200 kW
Centrifugal force inside bowl	max. 6480 g
Starting time	5-8 mins
Stopping time without brake	80 mins
Feed temperature range	0 - 100 °C
Feed inlet pressure at inlet flange	100 kPa [2]
Outlet pressure at outlet flange	max. 500 kPa [3]
Sound pressure	89 dB(A) [4]

[1] Actual capacity depends on particle sizes, densities, viscosity and require degree of separation.

[2] At max. process flow rate 180 m^3/h. Inlet pressure increases with the flow rate.

[3] At outlet flow rate 80 m^3/h. Max. pressure decreases with flow rate.

[4] According to ISO 3744.

Material data

Bowl body	s.s. 1.4501 UNS S32760
Bowl hood, lock ring and distributor	s.s. 1.4462 UNS S31803
Solids cover and frame hood	s.s. 1.4401 UNS 316 00
In and outlet parts	s.s. 1.4401 UNS 316 00
Frame bottom part	Cast grey iron
Gaskets and O-rings	Nitrile rubber or food grade EPDM [1]

[1] In accordance with FDA 21 CFR 177.260

How to contact Alfa Laval
Contact details for all countries
are continually updated on our website.
Please visit www.alfalaval.com to
access the information direct.

SANI-MATIC

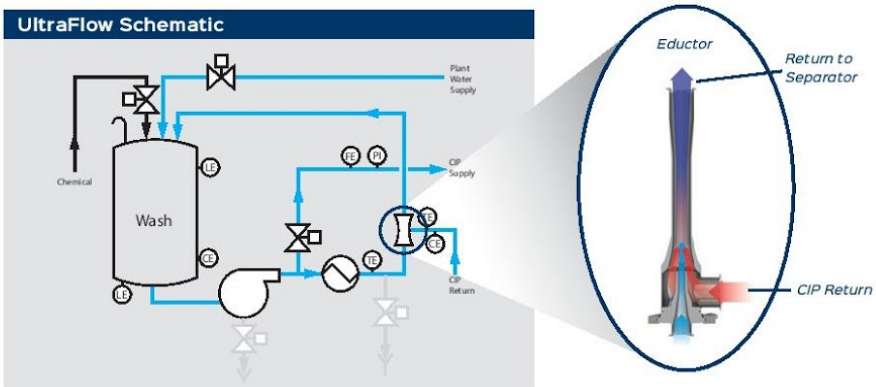
UltraFlow®: Powerful CIP in a Compact, Portable Design.

The Sani-Matic UltraFlow can operate with as low as 6 gallons of water vs. conventional CIP Systems, which must maintain a significant quantity of water in the supply tank to prevent pump cavitation.

Advantages

- **Small Footprint.** Space-saving design for installations with limited floor space. Fits through standard doorways with ease.
- **Wide Operating Range.** The systems range from 2–45 gpm and 5–110 gpm and are able to clean small and large applications.
- **Self-Cleaning.** Self-cleans without extra steps, and eliminates cross-contamination.
- **Portable.** Positioned on low-friction casters for easy movement between process suites. No expensive supply and return line installation required.
- **Water & Chemical Savings.** The high turbulent flow rate and low water requirements for operation reduce the amount of water and chemicals needed for a complete clean.
- **Low Outlets? No Problem.** Returns solutions with entrained air to accommodate vessels with low and restricted outlets.

UltraFlow Schematic



Documentation

Standard	Optional
Operation and maintenance manuals	Functional Specifications (FS)
Recommended spare parts (RSP) list	Configuration Specification (CS)
Mechanical Bill of Materials (BOM)	Factory Acceptance Test (FAT)
Instrumentation calibration procedures	Site Acceptance Test (SAT)
Material Test Reports (MTRs)	Installation and Operation Qualification (IQ/OQ)
Weld maps and weld logs (including qualification and inspection records)	Traceability matrix
Inspection test results, reports and certificates	Instrument data sheets
Component vendor documentation	Cleaning and passivation certificate
As-built General Assembly (GA) drawings	Borescope Inspection Video
As-built Process and Instrumentation Diagrams (P&ID)	Hydrostatic test certificate
As-built electrical drawings	Riboflavin spray coverage test

Features

UltraFlow 45

- 68" L x 24" W x 74" H (height may vary with options)
- Operating range of 2–45 gpm @ 50 psi
- Electric
- For process tank diameters up to 4.5'
- For process line diameters up to 2"
- Turbine flow meter

UltraFlow 110

- 74" L x 33" W x 80" H (height may vary with options)
- Operating range of 5–110 gpm @ 60 psi
- Electric
- For process tank diameters up to 10'
- For process line diameters up to 3"
- Turbine flow meter

Standard Features for Both Models

- A single centrifugal CIP supply pump
- Modulating diaphragm control valves to set cleaning circuit flow rates and to control the rate of discharge to drain
- Two chemical delivery systems comprised of pneumatic diaphragm pumps, removable chemical reservoirs
- Chemical conductivity, proof of rinse conductivity
- Supply and return temperature sensors
- Electric flow-through heater
- Discharge pressure gauge
- Low friction, non-marking casters
- Wetted surface: 316L stainless steel, 25 µin Ra
  Non-wetted surface: 304 stainless steel, 32 µin Ra
- UL listed, 304 stainless steel, NEMA 4X enclosure
- Allen-Bradley CompactLogix PLC
- Allen-Bradley PanelView Plus HMI
- Ethernet communication
- 40 customizable cleaning cycle programs
- Eductor return system

Optional Features for Both Models

- Vent filter assembly
- Pressure transmitter
- Mass flow meter
- Fixed position leveling feet
- Frame weld finish upgrade
- Sanitary flex hose package
- Piping insulation
- Fixed position seismic zone calculations
- Passivation
- Spare parts budget
- Larger electric heater
- Sani-Matic Start-up and Preventive Maintenance (PM) Services
- Wetted Surface: 15 µin Ra Electropolish (EP) finish
- Allen-Bradley PanelView Plus 1000
- Report ticket printer
- Stainless steel motor
- Steam Heat (shell and tube heat exchanger)
- Air blow manifold
- Chemical reservoir low level switches
- CIP supply routing valves
- Water connection bleed valves
- Sample valve

Operating Requirements

	UltraFlow 45	UltraFlow 110
Instrument Air	½" NPT, 10 scfm @ 90 psi	½" NPT, 10 scfm @ 90 psi
Water Supply	Two 1" tri-clamps, WFI, DI, potable ≤ 2 gpm @ 25 psi, 20°–80 °C	Two 1" tri-clamps, WFI, DI, potable ≤ 2 gpm @ 25 psi, 20°–80 °C
Drain	2" tri-clamp (controllable drain rate)	3" tri-clamp (controllable drain rate)
Dry Weight	900 lbs (approximate)	1,400 lbs (approximate)
Electrical Power (with electric heat)	12 kW, 27 amps (standard) or 24 kW, 43 amps (optional) @ 460V AC, 3PH	15 kW, 50 amps (standard) or 30 kW, 68 amps (optional) @ 460V AC, 3PH
Electrical Power (with optional steam heat)	11 amps @ 460V AC, 3PH	27 amps @ 460V AC, 3PH
- Plant Steam	¾" flange, 195 lbs/hr @ 50 psi	1 ½" flange, 540 lbs/hr @ 50 psi
- Plant Condensate	½" flange	1" flange
CIP Supply	1 ½" tri-clamp, 2–45 gpm @ 50 psi	2" tri-clamp, 5–110 gpm @ 60 psi
CIP Return	2" tri-clamp, 2–45 gpm @ 8.5' of head @ 80 °C	3" tri-clamp, 5–110 gpm @ 11' of head @ 80 °C
Vent/Overflow	2" tri-clamp	2" tri-clamp

Separator Chamber: Small Size, Big Performance

The combination of air and CIP return solution enters the sidewall port of the separator where centrifugal action separates air (upwards) and solution (downwards) to maintain adequate supply conditions for the CIP supply centrifugal pump.

Industry Standard Compliance

- FDA Current Good Manufacturing Practices (cGMP), CFR Title 21, Part 820
- Underwriters Laboratory (UL): Controls, Standard 508
- ANSI/ISA-88 (S88) Batch Control
- Authorized to Provide Canadian Registration Number (CRN)
- ASME BPE Standards

Air Control Systems

Packed Bed Chemical Scrubber, Model CS-17

The Model CS-17 is a Packed Bed Chemical Scrubber designed to efficiently remove the gas contaminant from a continuous process stream through a chemical reaction. This system includes fully automated controls to minimize operator interaction. Exhaust gas enters the scrubber and passes through a bed of packed media where it contacts a scrubbing solution to capture the pollutant. The scrubbing solution is introduced in counter-current flow by a liquid distribution spray nozzle.

This model, specifically designed for acid removal, uses dilute caustic as a neutralizing reagent to react with the acid and produce non-volatile, soluble salts and water. A chemical reagent pump adds caustic to fresh water to create a scrubbing solution. This water is then conveyed by the recirculation pump to the spray header to flood the packing where it will interface with the process stream.

The buildup of salts in the scrubbing solution is limited using fresh makeup water and blowdown. The cleaned exhaust stream then passes through a mist eliminator where water droplets are removed. Finally, the cleaned air stream is discharged to the atmosphere.

Base System Components

Stainless Steel Construction
Engineered Internal Packing
Recirculation Pump
Carbon Steel Interconnecting Ductwork
Carbon Steel Process Blower
Carbon Steel Exhaust Stack
NEMA 4 Control Panel
Touchscreen Operator Interface
Liquid Level Controls
Pressure Gauges and Transmitters
Chemical Metering Pump
pH Probe and Analyzer
Immersion Heater (as needed)

Specifications

Removal Efficiency:	95%
Air Flow Capacity:	17,500 ACFM
Pollutant Loading:	32 lbs/hr
Inlet Connection:	42" x 36"
Stack Height:	36'
Stack Diameter:	28"
Scrubber Process Fan:	30 HP, TEFC Motor
Recycle Pump:	150 GPM
Power Requirements:	480 V/ 3 ph / 60 Hz, 53 FLA

Pollution Systems
2170 Buckthorne Place
Suite 160
The Woodlands, Texas 77380

Phone (713) 574-6661
Fax (713) 456-2666
Email: Sales@PollutionSystems.com
Web: www.PollutionSystems.com

GOULDS PUMPS

RHEINHÜTTE PUMPEN

PRO services

ITT Brands

Centrifugal Pump Selection Guide

Company Overviews

Goulds Pumps

ITT Goulds Pumps is among the most widely recognized and respected brands in the global pump industry, serving customers in the oil and gas, mining, power generation, chemical, pulp and paper, and general industrial markets. As the only manufacturer to make digital monitoring standard on every process pump, ITT Goulds Pumps continues to lead the industry in both mechanical pump design and the adoption of smart technologies.

Rheinhütte Pumpen

As a specialist in corrosion and wear resistant materials the Rheinhütte Pumpen leads the field in know-how in many specific areas. The basis for our comprehensive pump range are the three material groups Metal, Plastics and Ceramics. This wide variety of materials and more than 20 different pump types offer the right solution for your specific project. In close communication with you our experienced project teams develop individual concepts for your sophisticated application. And if you need a standardized application just profit from our extensive pump range.

PRO Services

ITT PRO Services provides replacement parts, repair and upgrade services, reliability and maintenance programs, and asset management assistance to customers with the goals of extending equipment life, reducing Total Cost of Ownership (TCO), and increasing plant output. PRO Services includes, PumpSmart variable speed drive systems, and i-ALERT2 equipment health sensors.

Centrifugal Pump Selection Guide

Goulds Pumps and Rheinhütte pumpen... Serving the World's Industries

Goulds Pumps and Rheinhütte Pumpen presents this Centrifugal Pump Selection Guide to assist users in making an easy initial selection of the best pump for a particular service. To do this, simply refer to the selection chart on page 4 & 5 where the full line of Goulds Pumps, Rheinhütte Pumpen and PRO Services products are listed by category. For more details about your selection, refer to the page indicated. Contact your nearest Goulds Pumps or Rheinhütte Pumpen sales office or representative for a complete data package on any pump(s) in which you are interested. You will be furnished with any information you require to ensure proper pump selection for optimum reliability and performance.

Chemical

The family of chemical process pumps includes both ANSI and ISO models. Goulds Pumps and Rheinhütte Pumpen specialize in high alloys for our chemical pumps ranging from 316SS to Zirconium and other special alloys as requested. As well as a wide range of plastic materials. Unique non-metallic pumps offer distinct advantages when handling severe corrosives.

Magnetic drive pumps are designed for services where leakage cannot be tolerated. Our complete understanding of chemical processing and related industries gives us a clear advantage in finding solutions to these particular pumping problems.

Pulp and Paper

Goulds Pumps' leadership in the pulp & paper industry has been largely due to the success of our comprehensive range of pumps that stand up to the harsh operating requirements of this industry. The Model 3175 has been prized for performance since its introduction in 1968. Our latest 3180/3185 paper stock/process pump line extends the offering with better efficiencies, multiple impellers, metric flange option and greater hydraulic coverage. Other superior pumps include our 3420 & 3498 large double suction pumps for lo-pulse fan pumps & dilution pump applications along with our 3409 & 3410 models for black liquor transfer applications.

Mining and Minerals

Goulds Pumps' and Rheinhütte Pumpen' presence in the mining industry dates back to the late 1800s. Designed for the most severe applications, our pumps can be found in coal, aluminum, copper, iron, clay, phosphate, H_2SO_4, potash, soda ash, salt, gold and aggregate industries throughout the world.

Goulds Pumps and Rheinhütte Pumpen offers the widest range of rubber-lined and metal corrosion/abrasion-resistant slurry pumps in the industry, including vertical, horizontal and submersible designs for cyclone feed, tailings disposal, minerals processing, mine dewatering, clarifier underflow, oil sands, and sump services.

Power Generation

We offer a wide variety of pumps designed specifically for uses within this industry. The Model 3600, the most modern axially split multistage pump in the world, is ideally suited for boiler feed service.

Vertical turbine and double suction pumps can handle the most demanding condensate or circulating water needs. Sumps can be cleared with Goulds Pumps' line of vertical or submersible sump pumps. Heavy duty slurry pumps like the XHD, SRL and 5500 are specially designed for flue gas scrubbers and ash handling services. The Rheinhütte pump model GVSO is ideally suited for solar power systems, with an immersion depth of up to 17.5 metres and for temperatures up to 600°C. Plastic pumps are widely used for waste plant incinerations

Oil Refining and Gas Processing

We offer a full range of API 610 pumps to meet your demanding applications: BB1 axially-split, between-bearing pumps, BB2 between bearing radially split pumps, BB3 multistage axially split pumps, BB5 barrel multistage radially split pumps and overhung OH2/OH3 process pumps.

Vertical turbine pumps are available in any configuration including can pumps for low NPSH, fire pumps and submersibles. Design and manufacturing capabilities include standard commercial grades, ASME Section VIII and API-610 for total line capability.

Pipeline

Whether you are talking short distances between storage tank and truck, or long barren stretches between pumping stations, ITT has the right solution.

Transporting crude, refined product or water demands absolute care. ITT has dependable, efficient products that are crucial to managing your pipelines and transport requirements.

Our offerings include pumps for terminals and tank farms, booster pumps, mainline pumps. We also do hydraulic rerates of existing pumps to improve efficiency and TCO. Our PumpSmart® Smart Control delivers real-time control and protection of your pumps while providing valuable process insight.

Primary Metals

The wide range of products makes Goulds Pumps the ideal choice for the demanding services of this industry. We provide pumps for vertical and submersible abrasives handling, slurry pumps for scale pits, chemical pumps for pickle liquor and leaching solutions, vertical turbines, double suction pumps for cooling tower and dewatering applications, and pumps for waste acid, scrubber service, and quench. Rheinhütte Pumpen offers an extensive range of plastic pumps for the steel industry.

Water and Wastewater

We offer the most comprehensive line of double suction, end suction, multistage and vertical turbine pumps for chemical feed, water supply, booster, low lift, and high lift.

For non-clog solids handling, a range of horizontal, vertical sump, and submersible pumps have helped professional engineers solve pollution problems around the world.

Rheinhütte Pumpen offers an extensive range of plastic pumps for this industry.

Food and Beverage

Adhering to strict process requirements is only one of the reasons for Goulds Pumps' entry into the forefront of these industries. Goulds Pumps handle a wide variety of grain processing, water, wastes, biofuels, corrosives and erosives.

Breweries, bottling companies, canneries, and a multitude of food and liquid industries rely on Goulds Pumps for successful operations.

Fertilizer

Gould pumps and Rheinhütte Pumpen are the only Pump company in the world, that can offer a complete plant solution from one hand. We are a specialist in the field of pumping fluids in the Nitrogin and Phosphate Proccess for many decades. Optimal customer and engineering solutions are provided in a large selection of speciall alloys in metal, plastic and ceramic materials combined with special shaft seals to ensure realibity and safety for plants operators.

Market Segments - Selection Chart

ITT Goulds Pumps and Rheinhütte Pumpen makes the widest range of pumps in the industry — pumps to handle virtually any service. This selection chart is designed to help you find and specify the best pump for your service.

Product Category	Model	Pump Type	Chemical	Pulp & Paper	Mining & Minerals	Power Generation	Oil & Gas	Pipeline	Primary Metals	Water & Wastewater	Food & Beverage	Nature of Pumpage					Refer to Page
												Corrosive	High Temperature 260°C(500°F)	Abrasive	Solids		
															Non-Abrasive	Fibrous/ Stringy	
PRO Services	PRO Services	Rotating Equipment Services															34
Paper Stock/ Process	3175[1,2]	Paper Stock/Process															6
	3180/3185[1,2]	Paper Stock/Process															6
	3181/3186	High Temperature															6
Vertical Sump & Process	3171	Vertical Sump and Process															7
	NM3171	FRP Vert. Sump/Process															7
	CV3171	Non-Clog Vertical Sump Process															7
	LF3171	Low Flow, High Head Vertical Sump Process															7
	GVSO	Vertical Chemical Centrifugal pump															8
	GVRN	Vertical Acid Chemical Centrifugal pump															8
	RK	Vertical Chemical Pump															8
	RVKu	Vertical Plastic Pump															9
	RKuV	Vertical Plastic Pump, Cantilever Design															9
ANSI Process Pumps	3196[1,2]	ANSI Chemical Process															10
	HT3196[1]	ANSI High-Temperature Process															10
	LF3196[1,2]	Low Flow ANSI Process															10
	CV3196[1]	Non-Clog Process															10
	3796[1]	Self-Priming Process															11
	3996	ANSI In-Line Process															11
Sealless Process Pumps	3299	ANSI PFA PTFE Lined Sealless															12
	FNPM	Magnetic Drive Plastic Pump															12
	3296 EZMAG	ANSI Metallic Sealless Process															12
	3298	ANSI ETFE Lined Sealless															13
	SP3298	ANSI ETFE Lined Sealless															13
	V3298	ETFE Lined Sealless															13
Sealed Lined & Non-Metallic	3198[1]	ANSI PFA ETFE Lined Process															14
	NM3196[1]	ANSI FRP Process															14
	CPDR	Horizontal Standardized Chemical, Plastic															14
	RCNKu	Horizontal Standardized Chemical, Plastic															14
	RCNKu+	Horizontal Standardised Chemical, Plastic															15
	RCKu	Horizontal Chemical Pump, Plastic															15
	FNP	Standardized Chemical Pump, PFA-Lining															15
	FNC	Standardized Chemical Pump, Ceramic															15
	FGP	Horizontal Liquid Ring Pump, Ceramic															16
ISO Process Pumps	IC[1]	ISO Chemical Process															17
	RN	Standardized Chemical Pump															17
	RNSi	Acid Standardized Chemical Pump															17
	ICM	ISO Metallic Magnetic Drive															18
	RMKN	Magnetic Drive Metal Pump															18
	ICB	Close-Coupled ISO Process															18
	ICMB	Close-Coupled ISO Sealless															19
	ICP[1]	High-Temperature ISO Magnetic Drive															19
	ICMP	High-Temperature ISO Magnetic Drive															19
	ICO[1]	Open Impeller ISO Chemical Process															19
API 610 Process Pumps	3610[1]	Axially Split, 1-Stage (BB1)															20
	3620[1]	Radially Split, 1-Stage (BB2)															20
	3640[1]	Radially Split, 2-Stage (BB2)															20
	3600[1]	Axially Split, Multistage (BB3)															20
	7200CB	Barrel Multistage (BB5)															20
	7200SB	Barrel Multistage, In-Line Diffuser (BB5)															21
	3910	Vertical In-Line (OH3)															21
	API 3171	Industrial Duty Vertical Sump (VS4)															21
	3700[1]	1-Stage, Overhung (OH2)															22
	RCE	Heavy Duty Centrifugal Pump															6
	3700LFI	1-stage, Overhung, Radially Split (OH2)															22

[1]i-ALERT®2 standard | [2]NSF Certified — Ideally Suited for Service Indicated

Market Segments - Selection Chart

ITT Goulds Pumps and Rheinhütte Pumpen makes the widest range of pumps in the industry — pumps to handle virtually any service. This selection chart is designed to help you find and specify the best pump for your service.

Product Category	Model	Pump Type	Chemical	Pulp & Paper	Mining & Minerals	Power Generation	Oil & Gas	Pipeline	Primary Metals	Water & Wastewater	Food & Beverage	Nature of Pumpage: Corrosive	Nature of Pumpage: High Temperature 260°C(500°F)	Nature of Pumpage: Abrasive	Nature of Pumpage: Solids: Non-Abrasive	Nature of Pumpage: Solids: Fibrous/ Stringy	Refer to Page
Sump/ Abrasives/ Solids Handling	HSU	Submersible	■	■	■	■	■		■	■	■	■		■	■	■	23
	HSUL		■	■	■	■	■		■	■	■	■		■	■	■	23
	JCU		■	■	■	■	■		■	■	■	■		■	■	■	23
	VRS	Abrasive Slurry R.L. Cantilever	■	■	■	■				■	■			■	■		23
	VHS	Vertical Cantilever	■	■	■	■	■		■	■	■	■		■	■	■	23
	VJC		■	■	■	■	■		■	■	■			■	■		23
	RCEV	Vertical Cantilever	■		■	■	■		■	■		■		■	■	■	23
Abrasives Slurry/Solids Handling	XHD[1]	Severe Duty Slurry		■	■	■			■	■	■	■		■	■		24
	JC	Medium-Duty Abrasive Slurry	■	■	■	■			■	■		■		■			24
	SRL	Rubber-Lined Abrasive Slurry	■	■	■	■			■	■		■		■	■		24
	SRL-C	Rubber-Lined Abrasive Slurry	■	■	■	■			■	■		■		■	■		24
	SRL-S	Rubber-Lined Abrasive Slurry	■	■	■	■			■	■		■		■	■		24
	SRL-XT	Rubber-Lined Abrasive Slurry	■	■	■	■			■	■		■		■	■		24
	5500	Severe Duty Abrasive Slurry	■	■	■	■			■	■		■		■	■		24
	HS	Non-Clog Solids Handling	■	■	■	■	■		■	■	■	■		■	■	■	25
Multistage/ Axial Flow/ Double Suction	3393[1]	High-Pressure Multistage	■	■	■	■	■		■	■		■					26
	3316	Two-Stage	■	■	■	■	■			■					■		26
	3935	Diffuser-Type Multistage	■	■	■	■	■			■	■				■		26
	3355[1]	Multistage	■	■	■	■	■		■	■	■	■					26
	3400 Series[2]	Single Stage, Double Suction	■	■	■	■	■			■	■				■		27
	AF	Axial Flow	■	■	■	■				■		■			■		28
	RSU	Acid Axial Flow	■		■							■			■	■	28
	RPROP	Axial Flow	■	■	■	■			■	■		■		■	■	■	28
Vertical Mixed and Axial Flow	VIC[2]	Vertical Turbine/Can Type (VS6)	■	■	■	■	■	■	■	■	■	■	■	■	■		29
	VIT[2]	Vertical Industrial Turbine (VS1)	■	■	■	■	■		■	■	■	■		■	■	■	29
	VIDS	Double Suction Vertical (VS2 / VS7)	■	■	■	■	■			■	■			■			29
	VICR	Vertical Multistage Low Flow, High Head	■			■	■			■		■		■	■		29
	VCW[2]	Wet Pit Pumps (VS1 / VS3)	■	■			■			■							30
	VIS	Vertical Submersible			■		■			■		■					31
	VMP	Vertical Marine								■		■					31

[1]i-ALERT®2 standard | [2]NSF Certified

■ Ideally Suited for Service Indicated

See page 4-5 table for list of eight color-coded market designations.

Process Pumps

Goulds 3180 / 3185*
Paper Stock / Process
All customer requirements were considered in this line of paper stock / process pumps: excellent hydraulic coverage, high efficiency, extreme ease of maintenance, and mechanical reliability. The Model 3185 pump furnished with ISO or JIS flange drilling, metric fasteners, dimensions. Open, enclosed or Shearpeller™ impellers available. Features i-ALERT® 2 condition monitoring as standard. Model 3180 standard with ANSI flanges.

3180
- Capacities to 9,000 m³/h | 40,000 GPM
- Heads to 125 m | 410 ft
- Temperatures to 230° C | 446° F
- Pressures to 16 bar | 232 PSIG

3185 with Metric standards
- Capacities to 6,000 m³/h | 26,000 GPM
- Heads to 125 m | 410 ft
- Temperatures to 230°C | 446°F
- Pressures to 16 bar | 232 PSIG

Applications:
- Paper Stock
- Black Liquor
- Chemical Process
- Wastewater

NSF

Materials: Al/CD4MCuN, CD4MCuN, 316SS, 317SS, Hast-C, Alloy 20, Super Duplex. Other materials available upon request.

*i-ALERT®2 sensor installed (see pg 38 for details)

Goulds 3181 / 3186*
High Temperature Paper Stock / Process
End suction, top center line discharge, self-venting. Center line mounted for high temperature services. High efficiency enclosed impeller. TaperBore™ seal chamber standard with mechanical seal arrangement. Features i-ALERT® 2 condition monitoring as standard.

3181 with ANSI flanges
- Capacities to 3000 m³/h | 13,000 GPM
- Heads to 125 m | 410 ft
- Temperatures to 300°C | 508°F
- Pressures to 25 bar | 360 PSIG

3186 with ISO or JIS flanges
- Capacities to 3,000 m³/h | 13,000 GPM
- Heads to 125m | 410 ft
- Temperatures to 300°C | 508°F
- Pressures to 25 bar | 360 PSIG

Applications:
- Digester Recirculation
- Make-Up Liquor
- White Liquor
- Black Liquor
- High Pressure/High Temperature Pulp Mill Services
- Hot Oil

Materials: Duplex SS. Other materials available upon request.

*i-ALERT®2 sensor installed (see pg 38 for details)

Goulds 3175*
Paper Stock / Process
For the toughest services. Thousands of installations handle stock, solids, fibrous / stringy materials, abrasive slurries, and corrosives. Dynamic seal option eliminates mechanical seal problems. Features i-ALERT® 2 condition monitoring as standard.

- Capacities to 6,360 m³/h | 28,000 GPM
- Heads to 107 m | 350 ft
- Temperatures to 232° C | 450°F
- Pressures to 20 bar | 285 PSIG

NSF

Applications:
- Pulp & Paper Paper stock through 6% Consistency, Black Liquor, Hydropulper and Broke Service, Low NPSH Digester Circulation, Blow tank to Screens, Primary Screens Rejects, High Density Chlorine Tower to Washer, Flotation Cell Circulation
- Chemical Evaporator and Reboiler Circulation, Slurry Services
- Petroleum Corrosive/Abrasive Crude, Catalyst Slurry, Coke fines
- Steel Mill Descaling, Waste Treatment, Venturi Scrubber, Electro-Galvanizing Recirculation
- Food Fruit Pulps, Grain mash and Spent Grains, Evaporator Recirculation, Beet and Cane Sugar, Corn Products
- General Waste Treatment, Air Pollution Abatement, Acid Mine Water, Textile Slurries

Materials: Al/CD4MCuN, CD4MCuN, 316SS, 317SS, Hast-C, Alloy 20, Super Duplex. Other materials available upon request.

*i-ALERT®2 sensor installed (see pg 38 for details)

Rheinhütte RCE
Heavy Duty Chemical Centrifugal Pump in Metal
The RCE is a horizontal single-stage, end-suction, top-discharge, centrifugal pump with heavy duty bolted-down bearing pedestal. The sturdy design with front and back vanes on the impeller is primarily intended to fulfill very specific requirements as a standard chemical pump.

As a product-related special version, the RCE is the first choice in the chemical industry, the basic industry - especially in fertilizer production - environmental technology and many other industrial sectors.

It pumps organic and inorganic as well as aggressive liquids with high solid contents and is particularly suitable for hot media up to 450 degrees. Examples of applications include ammonium nitrate, molten sulphur, phosphoric acid, tar, pitch, urea melt, caustic soda, water glass, mash and cataphoretic paints. A special option of this series is the hydrodynamic shaft seal.

- Capacities to 1200 m³/h | 5283 GPM
- Heads to 180 m | 591 ft
- Temperature ranges from -40 °C to 450 °C | -40 °F to 842 °F
- Pressures to 16 bar | 232 PSIG

Applications:
- Phosphate fertilizer
- Ammonium nitrate melt
- Pitch and Tar
- Urea melt
- Molten Suphur
- Aggressive Slurries

Materials:
- 12 different cast irons, cast steels, Nickel based materials and high alloy cast steels

See page 4-5 table for list of eight color-coded market designations.

Vertical Sump & Process

Goulds 3171
Vertical Sump and Process

The "Veteran" vertical sump and process pump. Thousands of installations – industrial process, sump drainage, corrosive liquids, pollution control, molten sulfur. Rugged, heavy construction. Simple mounting.

- Capacities to 722 m³/h | 3,180 GPM
- Heads to 95 m | 344 ft
- Temperatures to 232° C | 450° F
- Pit Depths to 6 m | 20 ft

Applications:

- Industrial Process
- Industrial Sump Wastes
- Molten Sulfur
- Tank Unloading
- Corrosive and Non-CorrosiveLiquids

Materials: Cast Iron, Bronze-fitted, Carbon Steel, 316SS, Alloy 20, Hastelloy B and C, Duplex SS

Goulds NM3171
FRP Vertical Sump and Process

Designed for tough chemical sump pump applications. The fiberglass reinforced Vinyl Ester construction provides excellent corrosion resistance in aggressive acidic and caustic services. The true volute design provides the highest efficiencies in the industry for FRP pumps.

- Capacities to 284 m³/h |1,250 GPM
- Heads to 92 m | 300 feet
- Temperatures to 93° C | 200° F
- Pit Depts to 5 m | 16 Ft

Applications:

- Chemical/Petrochemical-Waste Acid, Sodium Hydroxide; Ferric Chloride, Sulfuric Acid, Spinfinish Wastes
- Utility-Coal pile runoff, Sea water, Demineralized water
- Metal Finishing-Spent pickling solutions, Electroplating rinses, Nickel plating bath
- General-Industrial process, Deionized water, Pollution control, Sump services

Materials: Glass reinforced Vinyl Ester. Other resins available upon request.

Goulds CV 3171
Vertical Sump and Process

The CV 3171 is a recessed impeller, circular volute type sump pump. Ideal for large solids and shear sensitive fluids. Circular volute minimizes radial loads making this the ideal pump for low flow process applications.

- Capacities to 295 m³/h | 1,300 GPM
- Heads to 126 m | 230 ft
- Temperatures to 232° C | 450° F
- Pit Depths to 6 m | 20 ft

Applications:

- Fiberous Wastewater
- Industrial Process
- Industrial Sump Wastes
- Tank Unloading
- Corrosive and Non-Corrosive Liquids
- Food Processing
- Chemical Slurries

Materials: Cast Iron, Duplex SS, 316SS, Alloy 20, Hastelloy B and C

Goulds LF 3171
Low Flow, High Head Vertical Sump Pump

The LF3171 is specifically designed to provide superior performance for low flow flow high head sump applications. Its concentric (circular volute) casing and open radial vane impeller are designed to eliminate hydraulic and mechanical problems at throttled low flows. Radial loads are reduced as much as 85% versus standard volutes at low flows.

- Capacities to 50 m³/h | 220 GPM
- Heads to 290 m | 950 ft.
- Temperatures to 232°C | 450°F
- Pit Depths to 6 m | 20 ft.

Applications:

- General Sump
- Lift Pump
- Tank Unloading
- Condensate
- Drum Pump
- Drain Pump
- Hydrocarbons / Oily Water
- Molten Sulfur
- Batch & Specialty Chemicals Sumps

Materials: Cast Iron, Duplex SS, 316SS, Alloy 20, Hastelloy B and C

See page 4-5 table for list of eight color-coded market designations.

Vertical Process Pumps

Rheinhütte GVSO
Vertical chemical centrifugal pump in metal

28 basic sizes in a single-stage or multi-stage design allow maximum flexibility even during the planning stage. This means that the GVSO covers volume flows of up to 4.000 m³/h and pumping heights of up to 150 m. Individual immersion depth adjustment from 0.5 to 17,5 m through the use of one or more intermediate bearings completes the maximum level of flexibility. At the customer's request, the connecting dimensions and the shape of the sole plate can be individually adapted to the container and pressure flange. If constant temperatures and viscosities are required, e.g. in the case of molten sulfur, the GVSO is also available as a heated version. Here we offer a customized heating jacket system for steam and other heat transfer media. Only two additional connections for feed and discharge are required here.

- Capacities to 4000 m³/h | 17612 GPM
- Heads to 150 m | 492 ft
- Temperatures to from -40 °C to 600 °C | -40 °F to 1112 °F
- Pressures to 40 bar | 580 PSIG

Applications:
Aggressive, also contaminated fluids, liquefied gases and melts like e.g. sulphuric acid at all concentrations, oleum, molten sulfur, Phthalic acid, tars and molten salts

Materials:
- 1.0619 (A 216 Grade WCA / WCB)
- 1.4136S (Rheinhütte material)
- 1.4306S (A 743 CF-3 (Type 304L))
- 1.4408 (A 743 CF-8M (Type 316))
- 1.4517 (A890 Grade 1B / A743 Gr. CD4MCuN)
- 1.4529S (A 743 CN3MN (AL-6 XN))
- 1.4581 (A 743 CF-8M (+Nb) / A 351 CF10MC (Type 316Cb)
- 1.7357 (A 217 Grade WC6)
- R30.20 (A 743 CN7M (Alloy 20))
- RH-RS (Rheinhütte material)
- RH-SX (alloy SX)

Rheinhütte GVRN
Acid Vertical chemical centrifugal pump.

The GVRN vertical centrifugal pump has been specially designed for use in modern sulphuric acid plants covers most demanding applications in this area. The high-alloyed material is particularly suitable for hot and highly concentrated acids. The thick-walled cast material ensures long pump service life. The compact design allows easy adaptation to existing system dimensions.

Special designs: wet design as well dry installation design.

- Capacities to 4000 m³/h | 17612 GPM
- Heads to 85 m | 279 ft
- Temperature ranges from -40 °C to 250 °C | -40 °F to 482 °F
- Pressures to 10 bar | 145 PSIG

Applications:
- HRS (Heat recovery system) Hot Sulfuric Acid
- Concentrated Sulphuric Acid

Materials:
- 1.4136S (Rheinhütte material)
- RH-RS (Rheinhütte material)
- RH-SX (alloy SX)

Rheinhütte RK
Vertical chemical centrifugal pump in metal

Vertical centrifugal pumps type RK are normally designed for open vessels/pits, to drain these objects rotational. This type of pump is not suitable for continuous operation or process operation. RK pumps are used for handling chemically aggressive media, contaminated liquids with solids up to a maximum grain size of 8 mm.

- Capacities to 56 m³/h | 247 GPM
- Heads to 55 m | 180 ft
- Temperature ranges from -40 °C to 100 °C | -40 °F to 212 °F
- Pressures to 10 bar | 145 PSIG

Applications:
Chemically aggressive media, contaminated liquids with solids up to a maximum grain size of 8 mm.

Materials:
- 1.4408 (A 743 CF-8M (Type 316))

Vertical Process Pumps

Rheinhütte RVKu
Chemical centrifugal pump in plastic

The vertical centrifugal pump RVKu is specially designed for pumping aggressive, slightly contaminated media such as acids, alkalis and chemical waste water. The closed impeller design with long axial throttle gaps allows immersion depths of up to 3m at operating temperatures of 80°C. Smooth running of the shaft is ensured by the use of a ball joint bearing located outside the aggressive zone as well as an immersion plain bearing made of SIC, carbon or ceramic.

- Capacities to 1000 m³/h | 4403 GPM
- Heads to 70 m | 230 ft
- Temperature ranges from -40 °C to 90 °C | -40 °F to 194 °F
- Pressures to 10 bar | 145 PSIG

Applications:

- Pickling
- Chemical wastewater
- Sulphuric acid (H2SO4)
- Surface treatment
- Hydrochloric acid (HCl)
- Fertilizer
- Plastic Production
- Functional media
- Dyes and Pigments
- Salts
- Metal Production
- Organics
- Nitric acid (HNO3)
- Flue gas scrubber
- Steel Industry
- Wasteplants Incineration

Materials:

- PP
- PE 1000
- PVDF

Rheinhütte RKuV
Chemical centrifugal pump in plastic

The vertical centrifugal pump RKuV has been specially developed for pumping aggressive media that are contaminated with solids or that crystallize out. The series is insensitive to dry running and operation against closed slide valves, as there is no plain bearing in the pumped medium. The series is also available as lightweight design RKuVL.

- Capacities to 120 m³/h | 528 GPM
- Heads to 60 m | 197 ft
- Temperature ranges from -40 °C to 100 °C | -40 °F to 212 °F
- Pressures to 10 bar | 145 PSIG

Applications:

- Pickling
- Chemical wastewater
- Sulphuric acid (H2SO4)
- Surface treatment
- Hydrochloric acid (HCl)
- Fertilizer
- Plastic Production
- Functional media
- Dyes and Pigments
- Salts
- Metal Production
- Organics
- Nitric acid (HNO3)
- Flue gas scrubber
- Steel Industry
- Wasteplants Incineration

Materials:

- PP
- PE 1000
- PVDF

See page 4-5 table for list of eight color-coded market designations.

ANSI Process Pumps

Goulds 3196 i-FRAME®*
ANSI Process
This is the original ANSI pump that has become the standard of the industry. Over 1,000,000 installations attest to the remarkable performance of the 3196. Available with a wide range of features for handling difficult applications. i-FRAME® power ends maximize reliability and MTBF (Mean Time Between Failure).

- Capacities to 1,364 m³/h | 7,000 GPM
- Heads to 223 m | 730 ft
- Temperatures to 371° C | 700° F
- Pressures to 26 bar | 375 PSIG

Applications:
- Chemical
- Petrochemical
- Pulp & Paper
- Primary Metals
- Food & Beverage
- General Industries

Materials: Ductile Iron, 316SS, CD4MCu, Alloy 20, Monel, Nickel, Hastelloy B and C, Titanium

NSF

*i-ALERT®2 sensor installed (see pg 38 for details)

Goulds HT 3196 i-FRAME®*
ANSI High Temperature Process Pump
Center line mounted in a heavy duty fabricated steel casing support, the Model HT 3196 minimizes shaft misalignment and piping strain associated with elevated temperatures up to 700° F. As a member of the ANSI pump family the HT3196 features Goulds Pumps' premier i-FRAME® power end, multiple seal chamber options including the TaperBore PLUS, and a wide variety of rigid and rugged mounting systems.

- Capacities to 1,023 m³/h | 4,500 GPM
- Heads to 282 m | 925 ft
- Temperatures to 371° C | 700° F
- Pressures to 31 bar | 450 PSIG

Applications:
- Hot Water
- Thermal Oils
- Heat Transfer Fluids
- Die/Mold Pre-Heating Systems
- Pilot Plants
- Electronic Heating and Cooling
- Reactor Heating
- Urea

Materials: Carbon Steel, 316SS, CD4MCu, Alloy 20, Hastelloy C

*i-ALERT®2 sensor installed (see pg 38 for details)

Goulds LF 3196 i-FRAME®*
Low Flow ANSI Process
Designed specifically to provide superior performance for low flow services. Features a concentric (circular volute) casing and open radial vane impeller to eliminate hydraulic and mechanical problems at low flows. Includes i-FRAME® power ends.

- Capacities to 50 m³/h | 220 GPM
- Heads to 282 m | 925 ft
- Temperatures to 371° C | 700° F
- Pressures to 31 bar | 450 PSIG

Applications:
- Specialty Chemicals
- Batch Chemical Process
- Reactor Feed
- Seal Water
- Shower Service
- Boiler Feed
- Condensate
- High Pressure Process
- Column Bottoms
- Hot Oil
- Column Reflux

Materials: Ductile Iron, 316SS, CD4MCu, Alloy 20, Hastelloy B and C

NSF

*i-ALERT®2 sensor installed (see pg 38 for details)

Goulds CV 3196 i-FRAME®*
Non-Clog ANSI Process
Perfect solution for handling bulky, fibrous, or shear-sensitive liquids. Recessed impeller design provides non-clog pumping with minimum solids degradation. Capability to handle liquids containing 10 to 20 percent air/gas. i-FRAME® power ends.

- Capacities to 610 m³/h | 2,700 GPM
- Heads to 134 m | 440 ft
- Temperatures to 260° C | 500° F
- Pressures to 20 bar | 285 PSIG

Applications:
- Filter Slurries
- Latex
- Polystyrene Beads
- Crystal Suspensions
- Screen Rejects
- Hydropulper pump
- Sodium Chlorate Slurry
- Fruit and Vegetable Suspensions
- Dye Liquor
- Fibrous Wastewater
- Long Fibre White Water
- Long Fibre White Water
- Primary Cleaner Pump

Materials: Ductile Iron, CD4MCu, Hastelloy B and C, Alloy 20

*i-ALERT®2 sensor installed (see pg 38 for details)

ANSI Process Pumps

Goulds 3796*
Self-Priming ANSI Process
One-piece casing eliminates need for separate priming chamber, air separator, valves or by-pass line. Fully open impeller can be trimmed to meet specific hydraulic requirements. Includes i-FRAME™ power ends.

- Capacities to 284 m³/h | 1,250 GPM
- Heads to 131 m | 430 ft
- Temperatures to 260° C | 500° F
- Suction Lifts to 6 m | 20 ft

Applications:
- Industrial Sump
- Mine Dewatering
- Chemical Transfer
- Bilge Water Removal
- Coal Pile Drainage
- Tank Car Unloading
- Filter Systems v Petroleum Transfer
- Column Bottoms and Reflux

Materials: Ductile Iron, 316SS,CD4MCu, Alloy 20, Hastelloy B and C, Titanium

*i-ALERT®2 sensor installed (see pg 38 for details)

Goulds 3996
In-Line ANSI Process
For corrosives, abrasives and high temperature. Fully open impeller, back pull-out design, heavy duty construction. Field alignment not required.

- Capacities to 318 m³/h | 1,400 GPM
- Heads to 213 m | 700 ft
- Temperatures to 260° C | 500° F
- Pressures to 26 bar | 375 PSIG

Applications:
- Caustic Transfer
- Acid Unloading
- Monomer/Polymer Transfer
- Liquid Nitrogen
- Liquid Ammonia
- Reflux and Light Tower Bottoms
- Waste Acid Recovery
- Pickle Liquor Circulation
- Chilled Water
- Filter Feed
- Condensate Return

Materials: Ductile Iron, 316SS, Monel, Alloy 20, Nickel, Hastelloy B and C, CD4MCu, Titanium

See page 4-5 table for list of eight color-coded market designations.

Sealless Process Pumps

Goulds 3299
Magnetic Drive ANSI Lined

Designed to handle moderate to severe corrosives with or without solids. Sealless design provides effective alternative to pumps with mechanical seal problems. Thick linings for extended pump life.

- Capacities to 95 m³/h | 425 GPM
- Heads to 149 m | 490 ft
- Temperatures to 180° C | 360° F
- Pressures to 19 bar | 275 PSIG

Applications:

- Hot Acids
- Acetic Acid
- Chlorinated Solvents
- Chloroform
- Freon 113
- Acetone
- Hydrofluoric Acid
- Sodium Hypochlorite
- Nitric Acid
- Amines
- Carbon Tetrachloride
- Dichloroethylene
- Ethers
- Bromine
- Chlorine Dioxide

Lining Material: PFA

Goulds 3296 EZMAG
Magnetic Drive ANSI Process

Robust, simple sealless design ideal for difficult liquids such as corrosives, pollutants, ultra-pure liquids and toxics. Meets ANSI dimensional specifications. Features a bearing cartridge for ease of maintenance and improved reliability.

- Capacities up to 159 m³/h | 700 GPM
- Heads to 213 m | 700 ft
- Temperatures to 280° C | 535° F
- Pressures to 19 bar | 275 PSIG

Applications:

- Batch Chemical Process
- Rail Car or Tank Unloading
- Specialty Chemicals

Materials: 316SS, others upon request

Rheinhütte FNPM
Magnetic drive pump in plastic

Pumps type FNPM are horizontal, single-stage, end-suction, top-discharge centrifugal pumps with magnetic coupling, dimensioned to comply with standards EN 22858, ISO 2858, NF 44-121 and BS 5257.

The scope of application covers the wide fields of chemical processing and environmental technology where chemically aggressive fluids of various concentrations at up to 190°C are to be handled. The constant standardization of the drive components and the unique magnetic cartridge reduce the variety of parts and enable cost-saving stocking concepts. The magnet cartridge is a pre-assembled,

ready-to-use unit containing all core components of the magnetic drive pump (e.g. plain bearings, impeller and containment shells). Replacement takes only a few minutes - then the old unit can be reconditioned.

The cost advantage over the usual quick-coupler unit: up to 25%.

- Capacities to 350 m³/h | 1541 GPM
- Heads to 100 m | 328 ft
- Temperature ranges from -40 °C to 190 °C | -40 °F to 374 °F
- Pressures to 16 bar | 232 PSIG

Applications:

- Hot Acids
- Acetic Acid
- Chlorinated Solvents
- Chloroform
- Steel Industry
- Acetone
- Hydrofluoric Acid
- Sodium Hypochlorite
- Nitric Acid
- Amines
- Chlorine Dioxide
- Flue gas scrubber
- Waste plants Incineration

Materials:

- PFA
- PTFE

See page 4-5 table for list of eight color-coded market designations.

Sealless Process Pumps

Goulds 3298
Magnetic Drive ANSI Lined

Designed to handle moderate to severe corrosives with or without solids. Sealless design provides effective alternative to pumps with mechanical seal problems. Thick linings for extended pump life.

- Capacities to 270 m³/h | 1,200 GPM
- Heads to 162 m | 500 ft
- Temperatures to 121°C | 250° F
- Pressures to 16 bar | 225 PSIG

Applications:
- Rail Car or Tank Unloading
- Batch Chemical Process
- Specialty Chemicals
- Column Reflux or Bottoms
- Reactor Feed

Lining Material: ETFE

Goulds V 3298
Vertical ANSI Lined Process

Ideal for moderate to severe corrosives. With or without solids, the 3298 can handle the tough chemical services. As a sealless design, it's an effective alternative to pumps with mechanical seal problems. Meets strictest EPA regulations.

- Capacities to 270 m³/h | 320 GPM
- Heads to 129 m | 425 ft
- Temperatures to 121°C | 250°F
- Pressures to 16 bar | 225 PSIG

Applications:
- Rail Car or Tank Unloading
- Batch Chemical Process
- Specialty Chemicals
- Column Reflux or Bottoms
- Reactor Feed

Materials: ETFE Construction

Goulds SP 3298
Self-Priming Lined

When suction pressure is negative and air or gases must be evacuated to accomplish pump priming, the SP 3298 has a self-priming dual volute that primes on demand with only an initial charge of liquid in the casing. Priming is accomplished within the casing, eliminating the need for auxiliary priming systems.

- Capacities to 70 m³/h | 310 GPM
- Heads to 42.5 m | 140 ft
- Temperatures to 121° C | 250° F
- Pressures to 12 bar | 175 PSIG
- Effective Static Lift to 6m | 20 ft

Applications:
- Rail Car or Tank Unloading
- Batch Chemical Process
- Specialty Chemicals
- Column Reflux or Bottoms
- Reactor Feed

Lining Material: ETFE

See page 4-5 table for list of eight color-coded market designations.

Sealed Lined & Non-Metallic

Goulds 3198*
PFA Process ANSI Lined
Virgin PFA Teflon® for handling a wide range of severe corrosive liquids, trace contaminants, and mixtures. The 3198 features ANSI B73.1 design, and i-ALERT® 2 power ends. Teflon® molded in place by high pressure technique and mechanically locked.

- Capacities to 182 m³/h | 800 GPM
- Heads to 137 m | 450 ft
- Temperatures to 149° C | 300° F
- Pressures to 16 bar | 225 PSIG

Applications:
- Hydrochloric Acid
- Hydrofluoric Acid
- Ferric Chloride
- Pickling Acid
- Plating Acid
- Plating Solutions
- Chlorinated Brine
- Chlorinated Hydrocarbons
- Sodium Hypochlorite
- Chlorine Dioxide

Material: PTFE

*i-ALERT®2 sensor installed (see pg 38 for details)

Rheinhütte CPDR
Standardized chemical pump in plastic
Pumps of the CPDR and CPRF type family are horizontal, single-stage, end-suction, top-discharge centrifugal pumps, standardized to EN 22858 (formerly DIN 24256; ISO 2858, NF 44-121, BS 5257 and ISO 5199). The installation length of type CPRF is simply longer about the channel width so that a free passage for the fluid is given. The pumps are used for handling chemically aggressive and/or inflammable liquids within the wide field of chemical processing and environmental technology. The CPDR with open impeller is foreseen for clean liquids and fluids with a small content of solids. The CPRF (Free Flow) is able to handle fluids with larger solids. Both types are possible in close-coupled design instead of bearing block.

- Capacities to 200 m³/h | 880 GPM
- Heads to 100 m | 328 ft
- Temperature ranges from -40 °C to 190 °C | -40 °F to 374 °F
- Pressures to 16 bar | 232 PSIG

Applications:
- Brine
- Chemical Wastewater
- Chloralkali
- Flue gas scrubbers
- Waste incineration plants
- Hydrochloric acid
- Sea water
- Steel industry
- Sulphuric acid

Materials:
- PP
- PE 1000
- PE 1000R
- PVDF
- PTFE

Goulds NM3196*
FRP ANSI Process
The Fiberglass reinforced Vinyl Ester construction provides excellent corrosion resistance in many aggressive acidic and caustic services. The random glass orientation and generous ribbing provides flange load ratings equal to a metal pump of the same size. The true volute design provides the highest efficiencies in the industry for FRP ANSI pumps.

- Capacities to 318 m³/h | 1,400 GPM
- Heads to 152 m | 500 ft
- Temperatures to 93° C | 200° F
- Pressures to 15 bar | 220 PSIG

Applications:
- Hydrochloric Acid Unloading
- Ferric Chloride
- Sulfuric Acid Transfer
- Sodium Sulphite
- Sulphate Liquors
- Plating Solutions
- Filter Feed
- Aquarium Water
- Sea Wter
- Chlorine Dioxide

Materials: Glass reinforced Vinyl Ester, other resins available upon request

*i-ALERT®2 sensor installed (see pg 38 for details)

Rheinhütte RCNKu
Standardized chemical pump in plastic
Pumps of the RCNKu and RCFKu type family are horizontal, single-stage, end-suction, top-discharge centrifugal pumps, standardized to EN 22858, ISO 2858, NF 44-121, BS 5257 and ISO 5199. With RCFKu (Free Flow), only the overall length is increased by the amount of the spiral width to ensure a free housing passage. The pumps are used for handling chemically aggressive and/or inflammable liquids within the wide field of chemical processing and environmental technology.

The RCNKu with closed impeller is foreseen for clean liquids and fluids with a small content of solids. The same applies for the RCKu with open impeller design. The RCFKu is able to handle fluids with larger solids

- Capacities to 2500 m³/h | 11007 GPM
- Heads to 100 m | 328 ft
- Temperature ranges from -40 °C to 190 °C | -40 °F to 374 °F
- Pressures to 16 bar | 232 PSIG

Applications:
- Brine
- Chemical Wastewater
- Chloralkali
- Flue gas scrubbers
- Waste incineration plants
- Hydrochloric acid
- Sea water
- Steel industry
- Sulphuric acid

Materials:
- PP
- PE 1000
- PE 1000R
- PVDF
- PTFE

See page 4-5 table for list of eight color-coded market designations.

Sealed Lined & Non-Metallic

Rheinhütte RCNKu+
Standardized chemical pump in plastic
Pumps of the RCNKu+ type family are horizontal, single-stage, end-suction, top-discharge centrifugal pumps, standardized to EN 22858 and ISO 5199. The pumps are used for handling chemically aggressive liquids within the wide field of chemical processing and environmental technology.

- Capacities to 400 m³/h | 1761 GPM
- Heads to 110 m | 361 ft
- Temperature ranges from -40 °C to 130 °C | -40 °F to 266 °F
- Pressures to 16 bar | 232 PSIG

Applications:
- Brine
- Chemical Wastewater
- Chloralkali
- Flue gas scrubbers
- Waste incineration plants
- Hydrochloric acid
- Sea water
- Steel industry
- Sulphuric acid

Materials:
- PP
- PE 1000
- PE 1000R
- PVDF

Rheinhütte RCKu
Standardized chemical pump in plastic
The RCKu is a cost-effective and place-saving bearing block pump for small power ratings.A variant of the RCKu is the RCKuF in close-coupled design instead of bearing block. The pumps are used for handling chemically aggressive and/or inflammable liquids within the wide field of chemical processing and environmental technology.

- Capacities to 20 m³/h | 88 GPM
- Heads to 50 m | 164 ft
- Temperature ranges from -40 °C to 130 °C | -40 °F to 266°F
- Pressures to 10 bar | 145 PSIG

Applications:
Chemically aggressive, even slightly contaminated media in all areas of the chemical industry and high-grade chemicals.

Materials:
- PP
- PE 1000
- PVDF

Rheinhütte FNP
Standardized chemical pump with PFA-Lining
The standard chemical pump FNP is universally applicable in the chemical and pharmaceutical industry, in petrochemistry and general process engineering for pumping chemically aggressive and corrosive media.

- Capacities to 70 m³/h | 308 GPM
- Heads to 95 m | 312 ft
- Temperature ranges from -30 °C to 190 °C | -22 °F to 374 °F
- Pressures to 16 bar | 232 PSIG

Applications:
- Hot Acids
- Acetic Acid
- Chlorinated Solvents
- Chloroform
- Acetone
- Hydrofluoric Acid
- Sodium Hypochlorite
- Nitric Acid
- Amines
- Chlorine Dioxide
- Flue gas scrubber
- Steel Industry
- Waste plants Incineration

Materials:
- PFA

Rheinhütte FNC
Standardized chemical pump in ceramic
Pumps of the FNC are horizontal, single-stage, end-suction, top-discharge centrifugal pumps, standardized to ISO 2858 and ISO 5199. The wear resistance and universal chemical resistance offers great advantages for use in corrosive and abrasive media. FNC pumps are used for handling chemically aggressive and/or abrasive liquids within the wide field of chemical processing and environmental technology.

- Capacities to 600 m³/h | 2642 GPM
- Heads to 90 m | 295 ft
- Temperature ranges from -40 °C to 120 °C | -40 °F to 248 °F
- Pressures to 10 bar | 145 PSIG

Applications:
- Chemical Industry
- Solids-containing fluids
- Titanium dioxide

Materials:
- FRIKORUND

Sealed Lined & Non-Metallic

Rheinhütte FGP
Liquid ring vacuum pump in ceramic

Our FGP liquid ring pump is the suitable partner for difficult evacuation and compression processes involving gases and vapours in all corrosion-critical areas of application in the chemical industry. Together with the freely selectable operating medium of the liquid ring, the FGP allows volume flows of up to 700 m³/h. In compressor mode gas pressures of up to p2 max = 2.5 barg are easily created. In vacuumode for aggressive media the pump produces suction pressures of p1 = 100 mbara up to p1 = 25 mbara with an additional gas ejector. And this all takes place at gas temperatures of up to 100 °C. The highly corrosion-resistant technical ceramic is perfectly suitable for all media containing chlorine and hydrogen chloride.

- Capacities to 700 m³/h | 3082 GPM
- Temperature ranges from -20 °C to 100 °C | -4 °F to 212 °F

Applications:
- Caustic gases
- Chemical industry
- Chlorine gas

Materials:
- FRIKORUND

ISO Process Pumps

Goulds IC i-FRAME®*
ISO Process

This series is designed in accordance with ISO 5199 and ISO 2858, making it ideal for worldwide chemical or industrial process applications. IC pumps are fitted with a patented seal chamber design called the Cyclone seal chamber, which has been proven to provide the optimum sealing environment for extended mechanical seal life. Optional inducer reduces NPSHr.

- Capacities to 450 m³/h | 1,980 GPM
- Heads to 160 m | 525 ft
- Temperature ranges from -40° C to 280°C | -40° F to 530° F
- Pressures to 25 bar | 360 PSIG

Applications:
- Chemical
- Petrochemical
- Pulp & Paper
- Primary Metals
- Food & Beverage
- General Industries

Materials: Ductile Iron, Carbon Steel, 316SS, Duplex SS, Alloy 20, Hastelloy C, Titanium

*i-ALERT®2 sensor installed (see pg 38 for details)

Rheinhütte RNSi
Standardized Acid Chemical Pump in SIGUSS

The RNSi (ferro-silicon cast iron "Si-Iron") pumps are used for handling chemically aggressive liquids within the wide field of chemical processing and environmental technology, especially for media based on sulphuric acid. Suitable for all Sulfuric acid concentration in all different Temperature ranges.

- Capacities to 1500 m³/h | 6604 GPM
- Heads to 100 m | 328 ft
- Temperature ranges from -40 °C to 300 °C | -40 °F to 572 °F
- Pressures to 10 bar | 145 PSIG

Applications:
- Sulphuric acid
- Titanium dioxide
- Spinning bath (Viscose fibre production)
- Electrolytes (Cu, Ni, Zn, etc)
- H2SO4 pickling
- H2SO4 regeneration

Materials:
- SIGUSS (A 518 Grade 3)

Rheinhütte RN
Standardized Chemical Pump in Metal

The pump RN is a horizontal, single-stage, endsuction, top-discharge centrifugal pumps, standardized to EN 22858 (formerly DIN 24256) ISO 2858, NF 44-121, BS 5257 and ISO 5199. RN (RNSI) pumps are used for handling chemically aggressive and/or inflammable liquids within the wide field of chemical processing and environmental technology.

- Capacities to 2700 m³/h | 11888 GPM
- Heads to 150 m | 492 ft
- Temperature ranges from -40 °C to 300 °C | -40 °F to 572 °F
- Pressures to 16 bar | 232 PSIG

Applications:
Chemically aggressive media in the whole area of chemical processing technology and other areas of industry.

Materials:
- 15 different pure metals (Nickel and Titan) , Nickel based materials and high alloy cast steels

See page 4-5 table for list of eight color-coded market designations.

ISO Process Pumps

Goulds ICM
ISO Metallic Magnetic Drive Process

The ICM pump is the optimum metallic sealless pump for process fluid services in the chemical, paper and general industries where ISO dimensions are preferred. The ICM is specifically designed to pump difficult fluids such as corrosives, high purity and toxic liquids. Its sealless, sturdy design combines with a wide variety of wet end materials. The bearings are chemical and abrasion resistant Silicon Carbide (SSiC). Optional Dryguard™ dry-run protection can be provided.

- Capacities to 400 m³/h | 1,760 GPM
- Heads to 210 m | 685 ft at 3,500 rpm
- Temperature ranges from -40° C to 180° C | -40° F to 360° F
- Pressures to 16 bar | 232 PSIG

Applications:
- Batch Chemical Process
- Rail Car or Tank Unloading
- Specialty Chemicals

Materials: Stainless Steel, Hastelloy, Ductile Iron, Alloy 20

Goulds ICB
Close-coupled ISO Process Pump

The ICB series is an extension to the IC series ISO 5199 frame mounted chemical pump series. These new pumps provide a compact and economical pumping solution ideal for OEM applications and confined spaces in industrial processes. No spacer coupling or alignment is required, reducing capital equipment costs and simplifying installation and maintenance. ICB pumps are fitted with our patented Cyclone seal chamber, proven to provide the optimum sealing environment for extended mechanical seal life.

- Capacities to 340 m³/h | 1,490 GPM
- Heads to 160 m | 525 ft
- Temperature ranges from -40° C to 140° C | -40° F to 280° F
- Pressures to 16 bar | 230 PSIG

Applications:
- Specialty Chemicals
- Batch Chemical Process
- Reactor Feed
- Seal Water
- Shower Service
- Boiler Feed
- Condensate
- High Pressure Process
- Column Bottoms
- Hot Oil
- Column Reflux

Materials: Ductile Iron, Carbon Steel, 316SS, Duplex SS

Rheinhütte RMKN
Magnetic drive pump in metal

The RMKN is a horizontal, single-stage, end-suction, top-discharge centrifugal pump with magnetic coupling, dimensioned to comply with standards ISO 2858. The pumps are designed to meet the technical requirements for magnetic drive pumps as determined by VDMA 24279 and DIN EN ISO 15783. The scope of application covers the wide fields of chemical processing and environmental technology where chemically aggressive fluids of various concentrations at up to 250°C are to be handled. For applications where the medium is kept at a constant temperature

the RMKN is available in a heated version. By using heating chamber systems, the RMKN is also very suitable for difficult cases, such as conveying molten sulfur, pitch and tar.

- Capacities to 500 m³/h | 2201 GPM
- Heads to 150 m | 492 ft
- Temperature ranges from -40 °C to 250 °C | -40 °F to 482 °F
- Pressures to 16 bar | 232 PSIG

Applications:
Aggressive, especially toxic, highly flammable, explosive or foul smelling media. In general all liquids that must not get into the atmosphere during the pumping process.

Materials:
- 12 different pure metals (Nickel and Titan) , Nickel based materials and high alloy cast steels

See page 4-5 table for list of eight color-coded market designations.

ISO Process Pumps

Goulds ICMB
Close-coupled ISO Magnetic Drive Process Pump

The ICMB is an extension of the ICM series frame mounted sealless process pump. This design provides a compact and economical solution ideal for OEM applications and confined spaces in industrial processes. No spacer coupling or alignment is required, reducing capital equipment costs and simplifying installation and maintenance. ICMB pumps are fitted with the same features as all other ICM pumps, including a patented bearing cartridge and a one piece high pressure containment shell.

- Capacities to 100 m³/h | 440 GPM
- Heads to 100 m | 330 ft at 3,500 rpm
- Temperature ranges from -40° C to 180° C | -40° F to 280° F
- Pressures to 16 bar | 232 PSIG

Applications:
- Batch Chemical Process
- Rail Car or Tank Unloading
- Specialty Chemicals

Materials: Stainless Steel, Hastelloy, Ductile Iron, Alloy 20

Goulds ICP*
High Temperature ISO Process Pump

The ICP is a heavy duty chemical process pump designed for extreme temperatures and pressures. The ICP complies with ISO standards and features the patented Cyclone Seal Chamber for extended seal service life. Center line casing design is self venting. Large capacity oil sump provides maximum bearing cooling.
Optional inducer reduces NPSHr.

- Capacities to 450 m³/h | 1,980 GPM
- Heads to 150 m | 492 ft
- Temperature ranges from -40° C to 280° C | -40° F to 535° F
- Pressures to 25 bar | 363 PSIG

Applications:
- Hot Water
- Thermal Oils
- Heat Transfer Fluids
- Die/Mold Pre-Heating Systems
- Pilot Plants
- Electronic Heating and Cooling
- Reactor Heating
- Urea

Materials: Carbon Steel, 316SS, Alloy 20, Duplex SS, Hastelloy C

*i-ALERT®2 sensor installed (see pg 38 for details)

Goulds ICMP
High Temperature ISO Metallic Magnetic Drive Process

The ICMP is a heavy-duty metallic sealless pump for applications with high temperature and pressure conditions. It is designed for aggressive, toxic and high purity media. The center line casing is optimal for the compensation of dimensional changes due to temperature fluctuations. SSiC Silicon Carbide plain bearings,with optional Dryguard™ dry run protection.

- Capacities to 400 m³/h | 1,760 gpm
- Heads to 210 m | 685 ft at 3500 rpm
- Temperature ranges from - 40° C to 280° C | - 40° F to 535° F
- Pressures to 25 bar | 365 PSIG

Applications:
- Batch Chemical Process
- Rail Car or Tank Unloading
- Specialty Chemicals

Materials: Stainless Steel, Hastelloy, Ductile Iron, Alloy 20

Goulds ICO i-FRAME® Series*
ISO process pump with i-ALERT®2 Intelligent Monitoring

Goulds Pumps IC family of ISO chemical process pumps is designed in accordance with ISO 5199 and ISO 2858, making it ideal for worldwide chemical or industrial process applications. The range includes the ICO pump which has the following features:

- Semi Open Impeller for improved solids and entrained gas handling
- 34 hydraulic sizes
- Flows up to 450 m³/h | 1980 GPM
- Heads up to 160m | 514 ft
- Temperatures from -40°C to 280°C | -40°F to 530°F
- Pressures up to 25 Bar | 360 PSI
- Available in a comprehensive range of materials for chemical and process applications that include Carbon Steel, 316SS, Duplex SS, Alloy 20, Hastelloy, Nickel and Titanium.

Features:
- Semi Open Impeller for improved solids handling
- ITT Goulds patented Cyclone Seal Chamber
- Suitable for mechanical seal or gland packing
- I-FRAME optimized Bearing Frame.
- Flanges drilled to DIN/ISO or ANSI
- Robust fabricated steel baseplate

*i-ALERT®2 sensor installed (see pg 38 for details)

See page 4-5 table for list of eight color-coded market designations.

API 610 Process Pumps

Goulds 3610 API 610 (BB1)
Horizontal Split Case, Double Suction
Designed for a wide range of industrial, municipal and marine services.

- Capacities to 11,355 m³/h | 50,000 GPM
- Heads to 215 m | 700 ft
- Temperatures to 205°C | 400°F
- Pressures to 42 bar | 600 PSIG

Applications:
- Petroleum refining, production, and distribution
- Petrochemical and demanding chemical processing
- High temperature applications including boiler circulation
- General industrial requiring high temperature or high pressures

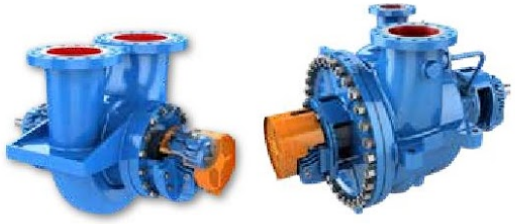
Materials: All API materials, custom materials available

Goulds 3620i* and 3640i®* API 610 (BB2)
Single and Two-Stage Between Bearings
Between bearings, radially split process pumps designed for smooth, reliable operation. Fully meets requirements of API 610.

- Capacities to 4,540 m³/h | 20,000 GPM
- Heads to 455 m | 1,500 ft
- Temperatures to 455° C | 850° F
- Pressures to 70 bar | 1,000 PSIG

Applications:
- Refinery – Tower bottoms, process feed, column reflux, circulation and pump around, process booster
- Power Plant – Boiler feed booster, boiler circulation, ash sluice

Materials: All API materials, custom materials available



*i-ALERT®2 sensor installed (see pg 38 for details)

Goulds 3600 i-FRAME®* API 610 (BB3)
Heavy Duty Multistage
Advanced design with proven operating history. Axially split, with many enhanced features that make it an extremely reliable, high performance pump well-suited to a wide range of services.

- Capacities to 1,930 m³/h | 8,500 GPM
- Heads to 2,740 m | 9,000 ft
- Temperatures to 205° C | 400° F
- Pressures to 275 bar | 4,000 PSIG

Applications:
- Refineries
- Injection offshore platforms
- Pipeline
- Boiler feed
- Descaling
- Mine dewatering
- Process transfer
- Desalination
- Water injection
- CO^2 injection

Materials: All API materials, custom materials available

*i-ALERT®2 sensor installed (see pg 38 for details)

7200CB (BB5)
Barrel Multistage Pumps
11th edition API compliant, severe service, barrel pumps, in-line diffuser style. For high temperatures, pressures and low specific gravities.

- Capacity: 910 m³/h | 4,000 GPM
- Head: 2,740 m | 9,000 ft
- Temperature: 425°C | 800°F
- Pressure: 275 bar | 4,000 PSIG

Applications:
- Petroleum refining, production, and distribution
- Petrochemical and demanding chemical processing
- High temperature applications including boiler circulation
- General industrial requiring high temperature or high pressures

Materials: All API materials, custom materials available

See page 4-5 table for list of eight color-coded market designations.

API 610 Process Pumps

Goulds 7200SB
API 610 11th Edition API BB5 Barrel Multistage, Radially Split In-Line Diffuser Type
High Temperature, High Pressure Low Specific Gravity BB5 Barrel Pumps for Critical Services.

- Capacity to 600 m³/h | 2,200 GPM
- Total Dynamic Head to 2430 m | 8,000 ft
- Temperature to 425° C | 800° F
- Pressure to 275 Bar | 4,000 PSIG
- Operating Speed to 3,600 RPM

Goulds API 3171 (VS4)
API 610 Vertical Sump and Process
For all refinery services requiring tank mount or sump duties. Fully compliant with 10th and 11th editions ISO 1370/API 610.

- Capacities to 722 m³/h | 3,180 GPM
- Heads to 160 m | 525 ft
- Temperatures to 232° C | 450° F
- Pit depths to 6 m | 20 ft

Applications:
- Industrial Process
- Industrial Sump Wastes
- Molten Sulfur
- Tank Unloading
- Corrosive & Non-Corrosive Liquids

Materials: Carbon Steel, 316SS, 12% Chrome Fitted, Duplex SS

Goulds 3910 API 610 (OH3)
Vertical In-Line with Bearing Frame
High pressure, high temperature services meets API 610 requirements. Back pull-out for ease of maintenance. Bearing frame carries pump loads.

- Capacities to 1,360 m³/h | 6,000 GPM
- Heads to 230 m | 750 ft
- Temperatures to 340° C | 650°F
- Pressures to 42 bar | 600 PSIG

Applications:
- Refinery Units – Distallation, Flasher, CCU, Hydrotreater, MTBE, Alkylation, Reformer, Gas Plant, Isomerization
- Petrochemical Plants – Olefins, BTX Recovery, Ethylene Glycol, Vinyl Chloride, Styrene, Phenol, Propylene Glycol, Alcohols, Ketones, Acids, Acrylonitrile, Anhydrides

Materials: All API materials, custom materials available

See page 4-5 table for list of eight color-coded market designations.

API 610 Process Pumps

Goulds 3700 API 610 (OH2)
Overhung Process
High temperature and high pressure process pumps designed to fully meet the requirements of API 610. Center line support for high temperature stability, maximum rigidity. Features tangential discharge for maximum hydraulic efficiency. Available in top suction design (Model 3710).

- Capacities to 1930 m³/h | 8,500 GPM
- Heads to 360 m | 1,200 ft
- Temperatures to 425° C | 800° F
- Pressures from full vacuum to 60 bar | 870 PSIG

Applications:

- Column Reflux
- Column Bottoms
- Reboiler
- Injection
- Fuel Blending
- Heat Transfer
- Slop Gas Oil Transfer
- Heavy Gas Oil
- Stripper Overhead
- Hot Oil
- Column Charge
- Reactor Feed
- Stabilizer Overhead
- Scrubber Circulation
- Tower Bottoms
- Offsite Hydrocarbon

Materials: All API materials, custom materials available

Goulds 3700LFI
API 610 11th Edition / ISO 13709 2nd Edition
API OH2 Overhung, Single Stage, Radially Split
High-Temperature and Pressure Process Pumps that meet or exceed ISO 13709 and API 610 11th edition. End-suction, centerline-mounted, overhung (OH2) API 610 process pump.

- Capacity to 88 m³/h | 390 GPM
- Total dynamic head to 503 m | 1650 ft
- Temperature to 425° C | 800° F
- Pressure to 75 bar | 1100 PSIG

Applications:

- Column Reflux
- Column Bottoms
- Reboiler
- Injection
- Fuel Blending
- Heat Transfer
- Slop Gas Oil
- Stripper Overhead
- Hot Oil
- Column Charge
- Reactor Feed
- Stabilizer Overhead
- Scrubber Circulation
- Tower Bottoms
- Offsite Hydrocarbon

Materials: Available in a wide range of materials including all API 610 constructions and custom application needs.

Sump/Abrasives/Solids Handling

Goulds HSU, HSUL & JCU Submersible

Three different models allow selection of the very best pump for the service conditions whether large, stringy, fibrous solids, or abrasive slurries.

- Capacities to 910 m³/h | 4,000 GPM
- Heads to 67 m | 220 ft
- Temperatures to 90° C | 194° F
- Solids to 152 mm | 6 inches

Applications:

- Waste Treatment Plants
- Sewage Wet Wells
- Reclaim Sumps
- Industrial Waste Sumps
- Sludge Pits
- Drainage Sumps
- Power Plants
- Collection Basins
- General Service Sumps

Materials: Cast Iron, High Chrome Iron, CD4MCuN, 316SS

Goulds VRS

Abrasive Slurry Handling

The VRS is designed using the proven reliability of the SRL and Goulds cantilever pumps. VRS offers higher efficiencies, with maximum reliability and interchangeability. Offered in standard lengths and a variety of elastomers.

- Capacities to 341 m³/h | 1,500 GPM
- Heads to 37 m | 120 ft
- Temperatures to 121° C | 250° F
- Pressures to 5 bar 75 PSIG
- Standard Lengths: 1.2 m | 4 ft and 1.8 m | 6 ft

Applications:

- Mineral Processing
- Non-metallic Mining
- Sand & Gravel
- Power Utility
- Pulp & Paper
- General Industry

Lining Materials: Natural Rubber, Neoprene, Nitrile, Polyurethane, Chlorobutyl, Hypalon, EPDM, and Metal / Alloy impeller available

Goulds VHS & VJC

Vertical Cantilever

Ideal for range of tough sump services: abrasive slurries – mine slurry, fly ash, foundry sand, clay, coal prep, power plants or large solids handling.

Model VHS

- Capacities to 1,590 m³/h | 7,000 GPM
- Heads to 42.6m | 140 ft
- Solids to 254 mm | 10 inches
- Lengths to 3.4 m | 11 feet

Materials: Cast Iron, High Chrome Iron, 316SS

Model VJC

- Capacities to 1,590 m³/h | 7,000 GPM
- Heads to 73 m | 240 ft
- Solids to 57 mm | 2 1/4 in
- Lengths to 3.4 m | 11 ft

Materials: Cast Iron, High Chrome Iron, 316SS

Applications: (Model VHS)

- Mill Scale
- Coal Slurry
- Coal Pile Runoff
- Sludge
- Clay Slurry
- Food Pulp
- Washdown Water
- Waste Paper Stock
- Black Liquor
- Plant Waste
- Sewage Treatment
- Ash Slurry

Applications: (Model VJC)

- Coal Prep Plant
- Iron Ore Slurry
- Steel Mills
- Power Plants
- Phosphoric Acid Plants
- Cement Mills
- Mine Slurry
- Foundries
- Alumina Refineries
- Phosphate Mines

Rheinhütte RCEV

Vertical chemical centrifugal pump in metal

Due to its non-pedestal design and impeller equipped with front and rear blades, the RCEV pump type is highly suitable for handling solids-laden liquids. It is possible to pump up to 30 percent solids with this type of pump, where the type and composition of the solids (hard, soft, light or heavy) plays a important role. The decision whether to use an open or closed impeller depends heavily on the type of the solids. The RCEV is used to convey mechanically contaminated, corrosive or neutral liquids such as phosphoric acid, highly contaminated sulphur, titanium dioxide slurries, copper dissolution acids as well as liquids that cannot tolerate localised heating by sliding bearings, e.g. concentrated ammonium nitrate solutions, DNT mixtures.

- Capacities to 900 m³/h | 3963 GPM
- Heads to 85 m | 180 ft
- Temperature ranges from -40 °C to 200 °C | -40 °F to 392 °F
- Pressures to 10 bar | 145 PSIG

Applications:

- Dirty sulphur
- Fertilizer
- Phosphate fertilizer
- Solids-containing fluids
- Sulphuric acid
- Ammonium Nitrate

Materials:

- 9 different cast irons, cast steels and high alloy cast steels

See page 4-5 table for list of eight color-coded market designations.

Abrasives/Solids Handling

Goulds XHD*
Extra Heavy Duty / Rubber and Metal Lined
The XHD lined slurry pump is designed for extremely tough slurry applications. Using advanced CFD technology for optimal hydraulics, it offers the lowest total cost of ownership features including adjustable suction liner and impeller plus double wall construction with extra wall thickness in high wear areas.

- Capacities to 2,950 m³/h | 13,000 GPM
- Heads to 85 m | 280 ft
- Pressures to 17 bar | 250 PSIG

Applications:
- Primary Metals – SAG/Ball Mill, Cyclone Feed, Tailings
- Mineral Processing – Slurry Transfer, Flotation Cells, Thickener Underflow
- Non-Metallic Mining – Heavy Media, Cyclone Feed, Raw Coal, Clay, Soda Ash and Phosphate Slurries, Slurry Heater, Slurry Digestion, Hydrate
- Power – Absorber Recycle, Gas Cooling, Filter Feed, Lime and Ash Slurries
- Sand & Aggregate – Sand Slurries, Tailings

Materials: HC 600, Endura Chrome

*i-ALERT®2 sensor installed (see pg 38 for details)

Goulds JC
Medium Duty Slurry
Ideal for most medium duty abrasive and/or corrosive slurry services. Extra thick wet end components extend wear life. Replaceable wear liner for low maintenance cost. Available with dynamic seal for elimination of seal problems, reduced maintenance. Variety of drive arrangements available for application flexibility

- Capacities to 1,600 m³/h | 7,000 GPM
- Heads to 73 m | 240 ft
- Temperatures to 121° C | 250° F
- Pressures to 10 bar | 127 PSIG
- Solids to 57 mm | 2.25 in

Applications:
- Wet scrubber systems
- Waste sludge
- Fracking slurries
- Paper mill wastes and liquors
- Clay and sand slurries
- Dirty water
- Kaolin water
- Carbon slurry
- Lime mud
- Precipitated $CaCO_3$

Materials: Cast Iron, High Chrome Iron, 316SS, CD4MCuN, Endura Chrome

Goulds SRL / SRL-C / SRL-S / SRL-XT
Abrasive and Corrosive Slurry Handling
The SRL pumps are designed to handle the toughest abrasive slurry. Features include wear-resistant rubber liners for maximum life and engineered for ease of maintenance. The SRL-S uses a Shearpeller® for froth applications.

- Capacities to 4,542 m³/h | 20,000 GPM
- Heads to 50 m | 164 ft
- Temperatures to 121° C | 250° F
- Pressures to 28 bar | 400 PSIG

Applications:
- Sag Mill
- Rod & Ball Mill
- Primary & Secondary Cyclone
- Thickener Feed
- Flotation Feed
- Tailings

Lining Materials: Natural Rubber, Neoprene, Nitrile, Polyurethane, Chlorobutyl, Hypalon, EPDM, Ceramic Composites and Metal Alloys

Goulds 5500
Severe Duty Slurry
The "Workhorse" of severe duty slurry pumps. It's not only built to stand up to the toughest services, but the Model 5500 is also designed for extreme ease of maintenance. A heavy duty power end, extra thick wall sections and easily replaceable wear parts add up to long, reliable operation.

- Capacities to 3,861 m³/h | 17,000 GPM
- Heads to 139 m | 425 ft
- Temperatures to 121°C | 250° F
- Pressures to 35 bar | 500 PSIG
- Solids to 127 mm | 5 in

Applications:
- Tailings
- Thickener Underflow
- Pipeline
- Potash
- Mud Disposal

Materials: High Chrome Iron, CD4MCuN, Endura Chrome

See page 4-5 table for list of eight color-coded market designations.

Abrasives/Solids Handling

Goulds HS
Hydro Solids
For handling sludges and slurries containing large solids, entrained air, fibrous materials, corrosives and abrasives. Features recessed, non-clog impeller.

- Capacities to 1,590 m³/h | 7,000 GPM
- Heads to 43 m | 140 ft
- Temperatures to 93° C | 200° F
- Pressures to 7 bar | 100 PSIG
- Solids to 254 mm | 10 in

Applications:

- Waste Treatment – Raw Sewage Sewage Sludge, Water, Resin, Fiber Water & Ashes, Textile Mill Effluent
- Pulp & Paper – Paper Stock, Plant Effluent, Black Liquor, Filtrate
- Food Processing – Beet Pulp, Dirty Water, Vegetable Refuse, Lemons, Tomato Wash Water
- Foundries & Steel Mills – Mill Scale Water & Slag, Grit
- Agriculture – Liquid Manure, Drainage Mulch, Seed, Water, Cane Wash, Sprigs Wood, Fiber
- Manufacturing – Paint Sludge, Plant Sewage & Sludge, Floor Wash, Clay Slip, Clarifier Sludge

Materials: Cast Iron, High Chrome, Iron, 316SS, CD4MCuN

See page 4-5 table for list of eight color-coded market designations.

Multistage/Double Suction

Goulds 3393*
High Pressure Multistage Ring Section Pump
Radially split, segmented casing, multistage pump designed with modular interstage components. Its multiple suction nozzle and discharge nozzle orientations allow adaptation to multiple piping installations. Multiple hydraulics for each pump size optimize efficiency across a vast range of applications. These pumps are particularly well suited for reverse osmosis, boiler feed, cogeneration, shower/spray service, pressure boosting and high pressure cleaning applications.

- Capacities to 750 m³/h | 3,300 GPM
- Heads to 1,000 m | 3,300 ft
- Temperatures to 177° C | 350° F
- Pressures to 114 bar | 1,650 PSIG

Applications:
- Reverse osmosis
- Boiler feed
- Cogeneration
- Shower / spray service
- Pressure boosting
- High Pressure Cleaning
- Snow making

Materials: 12% chrome, duplex and super duplex stainless steels, Other materials available upon request.

*i-ALERT®2 sensor installed (see pg 38 for details)

Goulds 3316
Two-Stage Splitcase
Horizontal split case pumps are ideally suited for boiler feed, mine dewatering and other services requiring moderately high heads with a wide range of operating conditions.

- Capacities up to 681 m³/h | 3,000 GPM
- Heads to 305 m | 1,000 ft
- Temperatures to 177° C | 350° F
- Pressures to 38 bar | 550 PSIG

Applications:
- Boiler Feed
- Mine Dewatering
- Booster
- High Pressure Process
- Condensate
- High Pressure Cleaning

Materials: Bronze-fitted, Cast Iron, 316SS, SS-Fitted. Other materials available upon request.

Goulds 3935
Centrifugal Diffuser Multistage
Centrifugal diffuser type multistage pumps well suited for boiler feed, reverse osmosis, petrochemical and hydrocarbon services.

- Capacities to 28 m³/h | 125 GPM
- Heads to 792 m | 2,600 ft
- Temperatures to 204° C | 400° F
- Pressures to 103 bar | 1,500 PSIG

Applications:
- Reverse Osmosis
- Boiler Feed
- Descaling
- High Pressure/High Temperature Cleaning
- Spraying Systems
- Hydraulic Systems
- Process Water
- Petrochemical & Hydrocarbon Service Transfer
- All Low Flow Applications – where efficiency is critical

Material: Carbon Steel. Other materials available upon request.

Goulds 3355
Multistage
Multistage ring section pump designed for high pressure services including: reverse osmosis, shower service, boiler feed and much more.

- Capacities to 340 m³/h | 1,500 USGPM
- Heads to 500 m | 1,640 ft
- Max speed to 3,600 min-1 | 3,600 rpm
- Discharge from 1½ in to 5 in
- Temperatures to 140° C | 280° F
- Pressures to 55 bar | 800 PSIG

Applications:
- Boiler Feed
- Condensate Return
- Deaerator
- Reverse Osmosis
- Shower/Spray Service
- Mine De-watering
- Cleaning Systems
- Seal Water Booster
- Product Transfer
- Reactor Feed
- Pressure Boosting

Materials: Stainless Steel, Stainless Fitted. Other materials available upon request.

See page 4-5 table for list of eight color-coded market designations.

Multistage/Double Suction

Goulds 3409
Medium Capacity

- Capacities to 2,725 m³/h | 12,000 GPM
- Heads to 259 m | 850 ft
- Temperatures to 120° C | 250° F
- Working Pressures to 2758 kPa | 400 PSIG

Applications:

- Process – Quench water, Stripper bottoms, Reboiler circulation, Cooling tower
- Pulp & Paper – Primary and secondary cleaner, filtrate, mill water supply Fan pump, Headbox supply, Shower
- Primary Metals – Cooling water, quench and leaching
- Municipal – High lift, low lift, wash water, waste water, raw water
- Power Generation – Cooling tower, Component cooling, Service water, Ash Sluicing, Heater drain
- Marine – Bilge and ballast, cargo, cooling water, fire pump
- General – River water, Brine, Sea water

Materials: Cast Iron / Bronze, All Iron, Cast Iron / Stainless Steel, Cast Iron / Ni-Al-Br, All Stainless Steel. Other materials available upon request.
(1724 kPa)

Goulds 3410
Small Capacity

- Capacities to 1,817 m³/h | 8,000 GPM
- Heads to 174 m | 570 ft
- Temperatures to 177° C | 350° F
- Pressures to 1,724 kPa | 250 PSIG

Applications:

- Process – Quench water, Stripper bottoms, Reboiler circulation, Cooling tower
- Pulp & Paper – Primary and secondary cleaner, filtrate, mill water supply shower, fan pump
- Primary Metals – Cooling water, quench and leaching
- Municipal – High lift, low lift, wash water, waste water, raw water
- Utilities – Cooling tower, component cooling, service water
- Marine – Bilge and ballast, cargo, cooling water, fire pump

Materials: Cast Iron / Bronze, All Iron, Cast Iron / Stainless Steel, Cast Iron / Ni-Al-Br, All Stainless Steel. Other materials available upon request.
(1724 kPa)

Goulds 3420
Large Capacity

- Capacities to 14,762 m³/h | 65,000 GPM
- Heads to 122 m | 400 ft
- Temperatures to 135°C | 275°F
- Working Pressures to 1379 kPa | 200 PSIG

Applications:

- Process – Quench water, Stripper bottoms, Reboiler circulation, Cooling tower
- Pulp & Paper – Primary and secondary cleaner, filtrate, mill water supply Fan pump, Headbox supply, Shower
- Primary Metals – Cooling water, quench and leaching
- Municipal – High lift, low lift, wash water, waste water, raw water
- Power Generation – Cooling tower, Component cooling, Service water, Ash Sluicing, Heater drain
- Marine – Bilge and ballast, cargo, cooling water, fire pump
- General – River water, Brine, Sea water

Materials: Cast Iron / Bronze, All Iron, Cast Iron / Stainless Steel, Cast Iron / Ni-Al-Br, All Stainless Steel. Other materials available upon request.
(1724 kPa)

Goulds 3498
Extra Large Capacity

- Capacities to 18,000 m³/h | 80,000 GPM
- Heads to 244 m | 800 ft
- Temperatures to 135°C | 275°F
- Working Pressures to 200 PSIG

Applications:

- Process – Quench water, Stripper bottoms, Reboiler circulation, Cooling tower
- Pulp & Paper – Primary and secondary cleaner, filtrate, mill water supply Fan pump, Headbox supply, Shower
- Primary Metals – Cooling water, quench and leaching
- Municipal – High lift, low lift, wash water, waste water, Raw water
- Power Generation – Cooling tower, Component cooling, Service water, Ash Sluicing, Heater drain
- Marine – Bilge and ballast, cargo, cooling water, fire pump
- General – River water, Brine, Sea water

Materials: Cast Iron / Bronze, All Iron, Cast Iron / Stainless Steel, Cast Iron / Ni-Al-Br, All Stainless Steel. Other materials available upon request.
(1724 kPa)

See page 4-5 table for list of eight color-coded market designations.

Axial Flow

Goulds Axial Flow®
Axial Flow
For continuous circulation of corrosive/abrasive solutions, slurries, and process wastes. Fabricated elbow or cast elbow designs available. Most suitable for low head, high capacity pumping.

- Capacities to 68,000 m³/h | 300,000 GPM
- Heads to 9.2 m | 30 ft
- Temperatures to 176° C | 350° F
- Available in cast iron, austenitic stainless steels, duplex alloys, nickel, nickel-chrome alloys, nickel-chrome-moly alloys, titanium and other alloys as required for the service
- Available in 6 - 66 inch sizes (larger sizes on application)

Materials: Cast Iron, 304SS, 316SS, CD4MCu, Nickel, Monel, Alloy 20, 904L, Titanium, Hastelloy, Sanicro 28. Other materials available upon request.

Applications:
- Chemical – Evaporator and Crystallizer Circulation
- Mining & Minerals – Phosphate, Soda Ash, Potash and Sodium Chloride Processing
- Petrochemical – Polymerization Reactors, Xylene
- Pulp & Paper – Black liquor evaporator, Chlorine dioxide generators
- Municipal – Sewage digesters
- General – Raw water pumping, Flood control, Marine ballast transfer

Rheinhütte RSU
Axial flow centrifugal pump in metal
Horizontal chemical circulating pump type RSU is designed for high flow rates at relatively low heads. By using specially designed screw impellers or propellers in Siguss is excellent suitability for the circulation of different acids, e.g. for the evaporation of spent and contaminated sulphuric acids. In order to achieve a sufficiently resistant - usually difficult to cast and machinable - materials, armoured versions are available.

- Capacities to 3400 m³/h | 14970 GPM
- Heads to 6 m | 20 ft
- Temperature ranges from -40 °C to 150 °C | -40 °F to 302 °F
- Pressures to 6bar | 87 PSIG

Applications:
- H2SO4 regeneration
- crystallizing lithium sulfate
- Titanium dioxide

Materials:
- SIGUSS (A 518 Grade 3)

Rheinhütte RPROP
Axial flow centrifugal pump in metal
Pumps of type RPROP are horizontal, axial flow circulation pumps, which are fitted in standard with propeller impeller and could be executed in special cases also with inducer impeller. Additionally, there is a close-coupled design (RPROPF) available, which can be installed suspended in the Pipeline. The RPROPF can also be executed with propeller or inducer impeller, but the flow direction is only one-sided. This pump type is installed in evaporation plants, crystallisation plants, pulp and paper industries, plastic production, regeneration plant for spend acid and reaction solutions and production of titanium dioxide.

- Capacities to 8500 m³/h | 37424 GPM
- Heads to 6,5 m | 21 ft
- Temperature ranges from -20 °C to 150 °C | -4 °F to 302 °F
- Pressures to 6 bar | 87 PSIG

Applications:
Crystal suspensions, brine, seawater, cellulose mash, abrasive media, aggressive acids and alkaline solutions. Crystal suspensions, brine, seawater, cellulose mash, abrasive media, aggressive acid, alkaline solutions, food and beverage.

Materials:
- 12 different Nickel based materials, high alloy cast steels and Titan

See page 4-5 table for list of eight color-coded market designations.

Vertical Mixed & Axial Flow

Goulds VIC (VS6)
Vertical Can-Type
A wide range of hydraulic conditions allows meeting requirements of virtually every pumping service. Designed to meet custom specifications of the user.
Model VIC can-type turbine meets API 610 specifications.

- Capacities to 15,900 m³/h | 70,000 GPM
- Heads to 1,067 m | 3,500 ft
- Pressures to 176 kg/cm² | 2,500 psi
- Bowl sizes from 152.4 mm to 1,400 mm | 6" to 55"
- Temperatures to 204°C | 400° F
- Horsepower to 3730 KW | 5,000 HP

Applications:
- Pipeline Booster
- Product Transfer, Refinery Blending
- Injection-Secondary Recovery
- Chemical Transfer
- Boiler Feed
- Condensate
- Cryogenics
- LNG Transfer
- Light Hydrobarbons
- Water Services

Materials: Any Machinable Alloy

*i-ALERT®2 sensor installed (see pg 38 for details)

Goulds VIT (VS1)
Vertical Turbine
A wide range of hydraulic conditions allows meeting requirements of virtually every pumping service. Designed to meet custom specifications of the user. Model VIT can-type turbine meets API-610 specifications.

- Capacities to 15,900 m³/h |70,000 GPM
- Heads to 1,067 m | 3,500 ft
- Pressures to 176 kg/cm² | 2,500 psi
- Bowl sizes from 152.4 mm to 1,400 mm| 6" to 55"
- Temperatures to 204°C | 400° F
- Horsepower to 3,730 KW | 5,000 HP

Applications:
- Cooling Water
- Seawater & River Water Intake
- Industrial Process Pumps
- Utility Circulating Water
- Condenser Circulating Water Pumps
- Fire Service
- Reclaimed Water

Materials: Any Machinable Alloy

*i-ALERT®2 sensor installed (see pg 38 for details)

Goulds VIDS (VS2/VS7)
Double Suction Vertical Pump
Unique specific designs that optimized results. Each model of the VIDS line is customized to conform to project specifications. They are available in open pit or can configurations.

- Capacities up to 15,900m³/h | 70,000 GPM
- Heads to 244 m | 800 ft on single stage configuration
  1,060 m | 3,500 ft on multi-stage configuration
- Temperatures to 204° C | 400° F
- Pressures to 76 kg/cm² | 2,500 psi
- Horsepower to 3,730 kw | 5,000 hp Maximum suspended solids concentration (1 stage): 10,000 PPM

Applications:
- Pipeline Booster
- Product Transfer, Refinery Blending
- Injection-Secondary Recovery
- Chemical Transfer
- Boiler Feed
- Condensate
- Cryogenics
- LNG Transfer
- Light Hydrocarbons
- Water Services
- Dirty water
- Sea water
- Reclaim & process water

Materials: Any Machinable Alloy

*i-ALERT®2 sensor installed (see pg 38 for details)

Goulds VICR (VS6)
Vertical Multistage Low Flow, High Head
A wide range of hydraulic conditions with a competitive advantage due to its compact design and reduced number of stages from the radial impeller configuration that can produce more head per stage.
Model VICR can vertical multistage low flow, high head meets API 610 specifications.

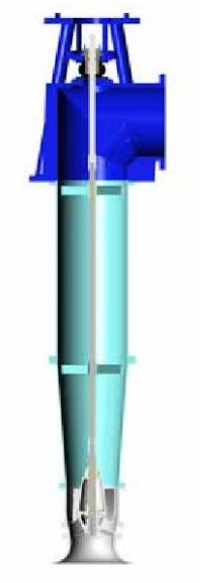
- Capacities to 636 m³/h | 2,800 GPM
- Heads to 1,372 m | 4,500 ft
- Temperatures to 204°C | 400° F
- Discharge flange sizes from 38 mm to 203 mm | 1.5" to 8"
- Powers to 3,000 KW | 4,000 hp

Applications:
- Pentane, Propane, LPG and other light hydrocarbons with specific gravities ranging from 0.2 to 1.0
- Hotwater applications such as Boiler feed water

*i-ALERT®2 sensor installed (see pg 38 for details)

See page 4-5 table for list of eight color-coded market designations.

Vertical Mixed & Axial Flow



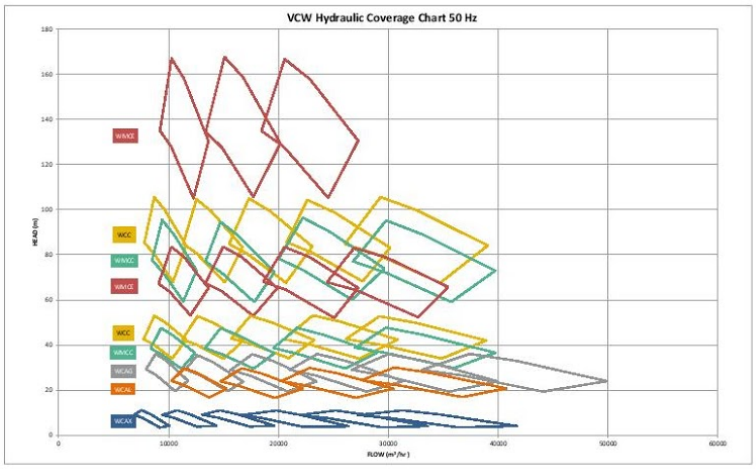
Goulds VCW (VS 1/VS 3)

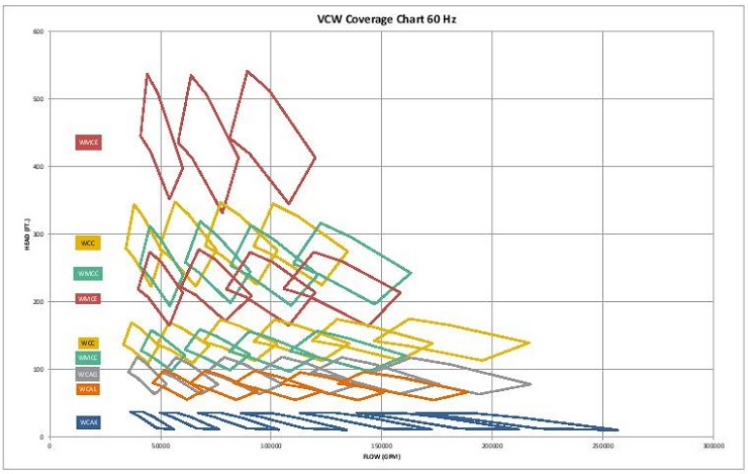
Vertical Mixed & Axial Flow

Custom designed for maximum reliability and high efficiency.

- Capacities to 91,000 m³/h | 400,000 GPM
- Heads to 180 m | 600 ft
- Powers to 7,500 KW | 10,000 hp

Materials: Bronze Fitted, All Bronze, SS Fitted, Ni Resist, All SS





*i-ALERT®2 sensor installed (see pg 38 for details)

NSF

See page 4-5 table for list of eight color-coded market designations.

Vertical Mixed & Axial Flow

Goulds VIS
Vertical Submersible

For deep settings or where use of lineshaft pumps is impractical. For irrigation, service water, deep well supply, offshore and mine dewatering.

- Capacities to 15,900 m³/h | 70,000 GPM
- Heads to 1,067m | 3,500 ft
- Pressures to 176 kg/cm² | 2,500 psi
- Bowl sizes from 152.4 mm to 1,400 mm | 6" to 55"

Applications:
- Irrigation
- Service Water
- Deep Well
- Sea Water Lift

Materials: Any Machinable Alloy

Goulds VMP
Vertical Marine

Goulds Model VMP pump is an automatically self-priming unit designed specially for efficient unloading and stripping of product tankers and barges.

- Capacities to 4,542 m³/h | 20,000 GPM
- Heads to 194m | 635 ft
- Temperatures to 120°C | 250° F

Applications:
- Product Stripping
- Ship Fire Pumps
- Ballast Pump
- Bilge
- Fuel Oil Transfer

Materials: Any Machinable Alloy

Bearings & Bearing Housings & Filters

To get superior MTBF you need two things: Optimum pump hydraulics and a robust pump structure. The new 360° i-FRAME housings deliver on the second point by providing a premium robust housing with unique features that raises the bar on what you can expect from your pump's long term performance. These 360° i-FRAME bearing housings include the new patented one piece design bearing housing for the Ball/Ball bearing arrangement, as well as the patent pending split bearing housing for the Sleeve/Ball and Sleeve/Tilt pad bearing arrangement.

Bearing housings constructed in ASTM A216 Grade WCB carbon steel. Three bearing arrangements available:

- Ball/Ball bearings
  - Duplex 40° Angular Contact Bearing Set on the Non-Drive End (NDE) to handle radial and axial loads. Bearing set is supplied with a light clearance
  - Deep Groove Ball Bearing on the Drive End (DE) to handle radial loads
- Sleeve/Ball bearings
  - Duplex 40° Angular contact Bearing Set on the Non-Drive End (NDE) handle axial loads. Bearing set is supplied with a light clearance.
  - Babbitt lined Sleeve Bearings handle radial loads on NDE and DE (Non Drive End and Drive End)
- Sleeve/Tilt pad bearings
  - Tilting pad Bearings are installed on NDE to handle axial load.
  - Babbitt lined Sleeve Bearings handle radial loads and are installed on NDE and DE (Non Drive End and Drive End).
  - This bearing configuration utilizes an external pressurized Lubrication Oil System (LOS) to lubricate and cool the bearings. Both API, standard and custom designed systems can be offered.

All bearing housings feature a 360° bearing saddle bolted to the casing positioned with precision dowels for accurate, repeatable alignment. The 360° bearing saddle is optimized for stiffness and rigidity of connection between the pump casing and housing along with increased bolt diameters. This provides significantly increased stiffness, resulting in reduced vibration.

The bearing housing exterior includes distinctive cooling fins optimized by CFD/FEA analysis to aid in heat dissipation.

The Ball/Ball and Sleeve/Ball 360° i-FRAME bearing housings have enhanced air cooling with axial fans and without the need for cooling water. The NDE side comes standard with a guarded extension to accept a fan for ease of field retrofit, so if your process needs to change, the fan can be fitted without the need for expensive pump disassembly and installation.

Bearing housing put through rigorous testing.

Bearings & Bearing Housings & Filters

Bearing oil contamination by wind-blown sand and dust together with atmospheric moisture are major contributors to bearings failing well before their design life. In an industry first, all Ball/Ball and Sleeve/Ball include a cartridge filter assembly that will help safeguard the bearing oil from debris contamination. The patent pending filter cartridge will also continuously work to scrub dissolved water from the bearing oil utilizing specifically engineered moisture absorbing materials built into the filter. The design allows for easy changeover of filter cartridges even while the pump is operating – no need to stop your process. All this additional reliability is achieved ***without*** the need for additional oil pumps or piping – no additional system complexity, monitoring, or control overhead.

Oil with Particulate**

Run time = 72 hrs*	Run time = 314 hrs*
Black Oil	Result: Clean Oil

Oil with Water

Run time = 0 hrs*	Run time = 72 hrs*
Cloudy Oil	Result: Clean Oil

*Continuous operation at 3100RPM
**Test dust used is ISO 12103-1, A3 Medium test dust

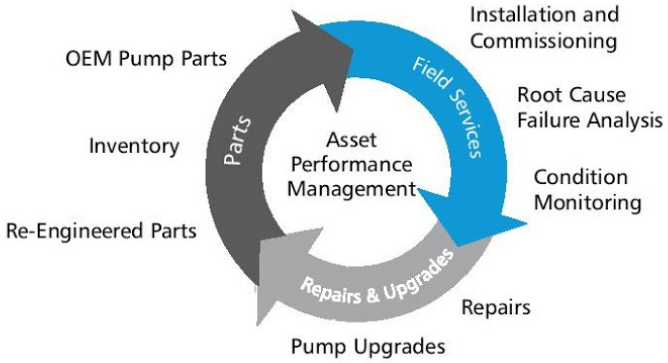
Another smart feature included as standard is the award winning, i-ALERT® 2. This provides class leading continuous machine monitoring with comprehensive wireless reporting including diagnostic quality vibration FFTs and operating history to the mobile phone or tablet of your choice. The bearing housings come equipped ***as standard*** with constant level oilers[1], sight window[1] and provisions for instrumentation including: RTD's, proximity probes[1], and accelerometers. If your monitoring needs change in the future, this comprehensive approach allows field retrofitting of almost any monitoring scheme without replacing your bearing housings or relying on ad-hoc instrument mounting.

[1]where appropriate, based on the bearing arrangement purchased.

Reliability has no quitting time.

PRO services

Building on centuries of pump design experience, **PRO Services** provides an array of services focused on reducing equipment total cost of ownership (TCO) and increasing plant output, including condition monitoring, predictive maintenance contracts, field service, engineered upgrades, inventory management, and overhauls for pumps and other rotating equipment.



Pump Upgrades

ITT PRO Services provide upgrades engineering services for a wide range of pumps.

Re-engineering hydraulics

is a the best way to address the root cause of many pump and system damaging mechanisms. ITT PRO Services is experienced at hydraulically re-rating any manufacturer's centrifugal pump for parameters such as Flow/Head, NPSH,Suction Recirculation, and Efficiency through dedicated aftermarket hydraulics engineering and laser scanning and casting technologies.

Custom drop-in replacement pumps

allow users to fit the hydraulic they need into the footprint available in order to save on piping, foundation, electrical, and civil work often required to completely change a pump train. This solution brings to bear the full capabilities of ITT PRO Services aftermarket mechanical and hydraulic expertise with the fullbacking of an industry leading OEM of centrifugal pumps.

PRX-OH2 Back Pull-out Upgrade

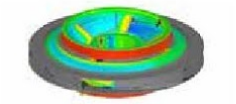
is a back pull-out assembly which provides a complete replacement to your existing equipment while keeping the existing impeller and casing.

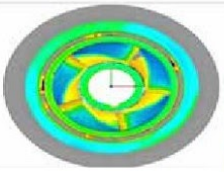
PRX-VSR Rebowl of Vertical Turbine Pumps

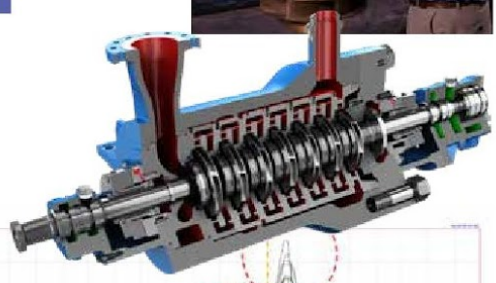
offers a more economical solution than a complete new pump, rebowling a pump could be the best option for many pump problems with no disturbance to the existing piping and re-using some of the original pump parts such as the discharge head,mounting plate and the driver.

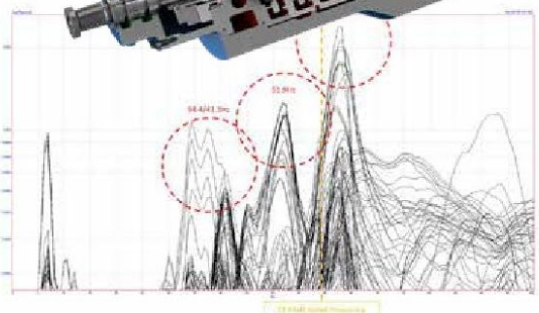
PRX-CBS

program exists to replace the internal bundle of a BB5 barrel pump to tailor hydraulics and provide quick sparing with minimized downtime.









ITT OEM and Re-engineered Parts

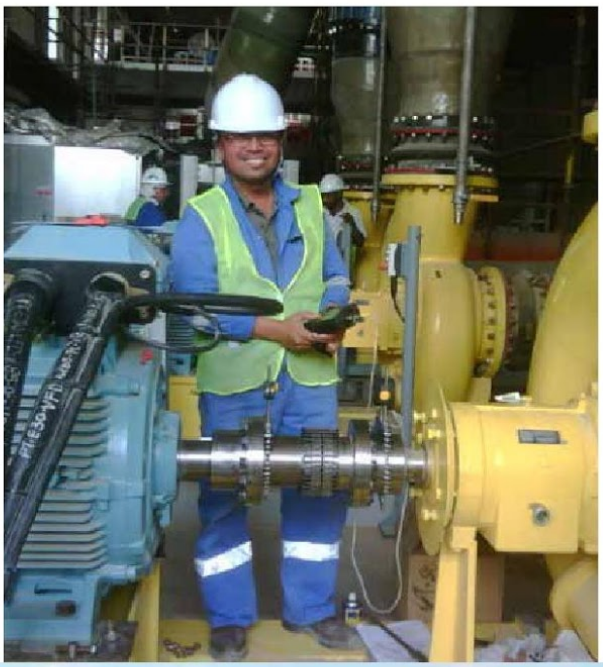
In addition to providing OEM parts for the full suite of ITT pump brands, we also supply replacement parts for all other pumps helping to solve pump performance or part supply issues for obsolete equipment.

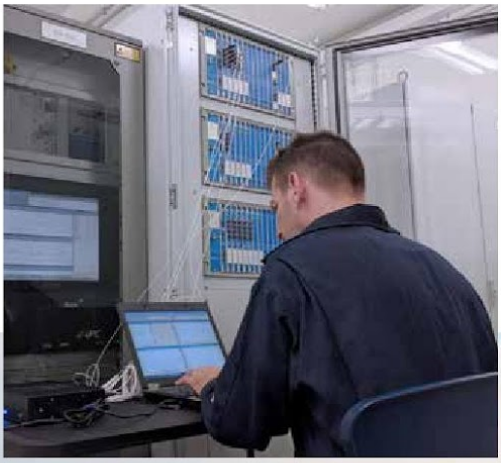
Utilizing global engineering center capability, regional manufacturing resources, combined with the latest available technology, PRO Services is able to re-engineer parts to improve hydraulic performance and equipment life cycle.

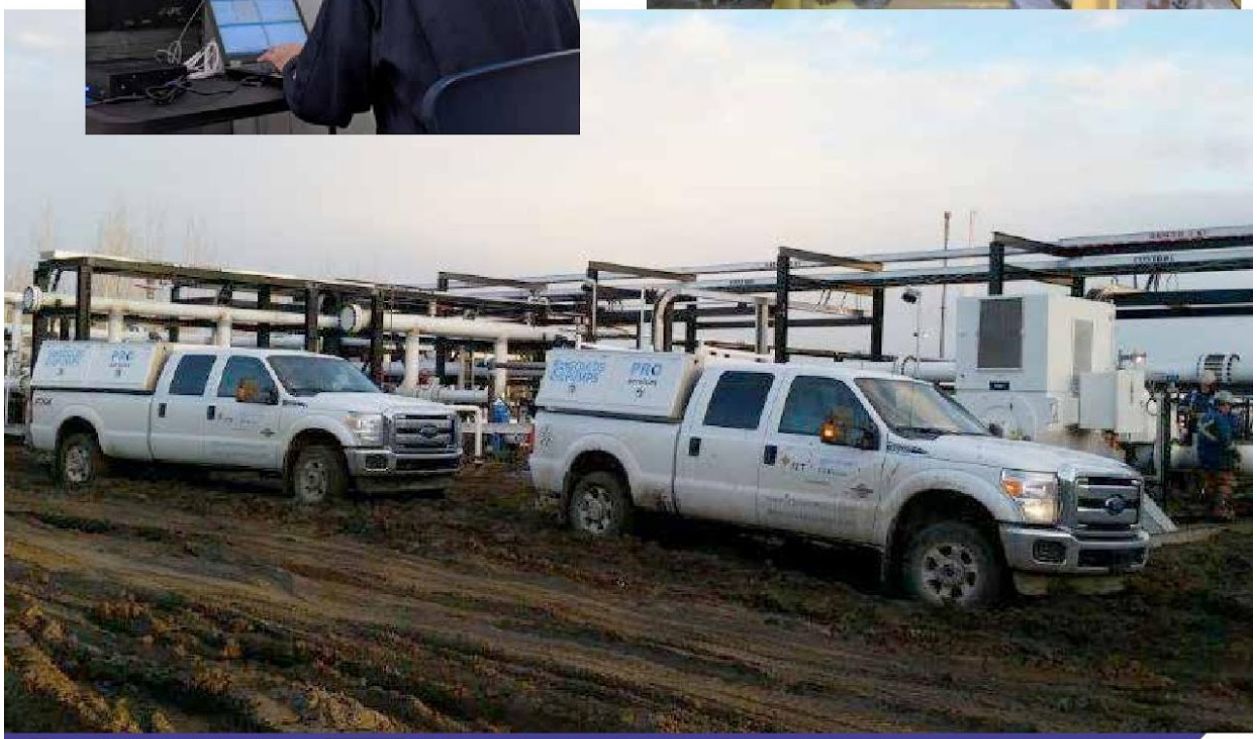
All parts meet or exceed OEM specifications at very competitive pricing and often with faster lead times. These parts deliver the advantages of ITT's advanced modeling capabilities and integrated pattern shop, and more than a century of pump design and manufacturing experience for a variety of applications worldwide.

Field Services

PRO Services brings its expertise and global coverage to support your equipment on site to ensure reliable, trouble free operation. Our Field Services team are experienced at supporting all types of pumps and rotating equipment. Using the latest industry equipment and capabilities we can provide a variety of services including installation & commissioning, machine analysis, removal and installation services and field repairs 24 hours a day, 7 days a week, 365 days a year.







i-ALERT®2 Monitoring Solution

Sensors | App | Ai Platform | Gateway www.i-alert.com

Bluetooth®

i-ALERT® Sensor

Monitor
Tracks vibration, temperature & run-time hours 24/7/365.

Alarm
Takes high resolution data when an alarm condition occurs and stores it for later analysis.

Trend
Captures data every 1-60 minutes and has up to 170 days of hourly on-board storage.

Analyze
Diagnose machine faults with vibration tools
Fast Fourier Transform (FFT) & Time Wave Form Analysis.

Environment
Rated for any industrial environment. water & dust resistant. Intrinsically Safe with a 3-year battery life (use dependent).
- ATEX Zone 0 AEx ia IIB Ga (Groups C & D)

Wireless
Sync data via Bluetooth Smart enabled smartphones and tablets.

Online Monitoring
Monitor and manage all of your i-ALERT® 2 enabled machines in one place - i-ALERT Ai Online Platform. This subscription service requires no software to download or dedicated hardware to run.

Spend less time collecting data and more time fixing problems. The i-ALERT mobile app has the ability to scan multiple i-ALERT® 2 sensors within range to quickly and safely inspect multiple machines.

Pressure Sensor

Process Monitoring
Directly measure and monitor the pressure and temperature of any process fluid. Build long term trends and capture transient events with the built in data logger.

Technical Specifications
Pressure: -14.7 to 10,000 psi
Temperature: -20°C to 85°C (-4°F to 185°F)
Wireless: Bluetooth V4, Range 30-100m (100-300ft)
Data Storage: 300 Days (based on hourly data)
Power: 2 Year Replaceable Lithium Battery (use dependent)
Enclosure: IP68 / NEMA4x
Wetted Material: 17-4 Stainless Steel

i-ALERT® Gateway

Secure Connection
The i-ALERT Gateway provides a secure connection between the i-ALERT sensors and the i-ALERT Ai portal. Apply power and let the gateway automatically connect to the cellular network and configure all the i-ALERT sensors in range.

Technical Specifications
Cellular WAN*: LTE, 3G
Bluetooth: Bluetooth 4.0 (Max 12.5 dBm)
Wireless Range: 30-100m (100-300ft)
Power: 120-240VAC or 6-90 VDC
Temperature: -30°C to 60°C (-22°F to 140°F)
Enclosure: IP68 / NEMA4x, Class 1 Division 2

i-ALERT® Ai Online Plateform

Monitor and manage all of your i-ALERT® 2 enabled machines and sensors in one place. This subscription service requires no software to download or dedicated hardware to run.

PumpSmart® Control Solutions

The industry award-winning and patented pump control logic delivers real-time control and protection of your pumps while also providing valuable process insight. By protecting against pump failure due to process upsets, PumpSmart keeps your operation running longer and reduces unplanned repair activities and expense. By right-sizing your pumps to your system, we can reduce not only your energy consumption, but also wear & tear on your process systems.

Features (Low Voltage)

- **Smart Flow**
  This patented feature allows PumpSmart to accurately control a process flow WITHOUT a flow meter.
- **Pump Protection**
  Provides the operator the ability to set protection for low flow, no flow, run-out and cavitation.
- **Flow Economy**
  Calculates process efficiency by flow of product versus energy consumption (gpm/kW).
- **Multi-Pump Control**
  Provides control for up to four pumps in a parallel for automatic lead/lag changeover, redundancy back-up and synchronized torque control while still communicating to a field bus or DCS system.
- **Options and Engineered Solutions**
  Available in a low-harmonic configuration guaranteed to meet IEEE519 harmonic specifications for industries requiring low-harmonic distortion on the utility line.

Features (Medium Voltage)

- **Pump Protection & Predictive Monitoring**
  Takes intelligent control of your pumping system to ensure it operates within the parameters needed for maximized output and it also prevents damage due to process upsets which cause critical "downtime".
- **Multi-Pump Control – Load Balancing**
  Ability to monitor or control multiple pumps while operating in parallel or series piping plans.
- **Upgrade and Improve your standard Medium Voltage VFD Pumping System!**
  Ability to analyze existing VFD controlled systems and give operators visibility to pump systems.

  Patented logic can improve overall system reliability and predictive monitoring capabilities.

Features (Engineered Solutions)

- Pre-Engineered or Custom Engineered Solutions for any pump project
- Dedicated Global Resources for design, drawings and site support
- Integrated Solutions for high energy centrifugal or PD type pumps
- ITT PumpSmart takes ownership of a fully integrated efficient pumping solution

Visit our website at
www.gouldspumps.com
www.rheinhuette.de
www.ittproservices.com

Pick Your Perfect Process Pump

Whether it's for severe corrosives, abrasive slurries, fibrous/stringy solids, high temperature liquids, hazardous fluids, low flow or high capacity services – Goulds Pumps and Rheinhütte pumps have a perfect, reliable solution. Our selection of fluid solutions includes horizontal and vertical configurations in a range of alloy and non-metallic constructions, sealed and sealless. Goulds Pumps' and Rheinhütte pumps have a wide range of products ensures that we have the right pump for virtually every application.

Pump Selection Checklist

The following Pump Selection Checklist is designed to assist users in reviewing most pump requirements for ultimate selection of the best pump. Your Goulds Pumps and Rheinhütte pumps representative has been specially trained in pump application and should be contacted to assist in final pump selection for optimum reliability and safety.

1A SYSTEM

Service: _____
Capacity: _____
Total Dynamic Head: _____
NPSH Available: _____
Suction Pressure: _____
Minimum Flow Rate: _____
Total Working Pressure: _____

2A LIQUID PROPERTIES

Liquid: _____
Vapor Pressure: _____
Specific Heat: _____
Viscosity: _____
Solids Size / Content: _____
Specific Gravity: _____
Temperature: _____
Characteristics: (flammable, explosive, carcinogenic, toxic, noxious, regulated, etc.):
_____

3A SAFETY / ENVIRONMENTAL

☐ UL label (explosion-proof enclosures)
☐ Regulations (government, local, plant)
☐ Temperature limits
☐ Fugitive emission limits
☐ Product purity
☐ Best Available Control Technology
☐ Reporting requirements

4A ECONOMY / RELIABILITY

☐ MTBF requirements
☐ Lubrication
☐ Cooling / Heating
☐ Operator experience
☐ Operator maintenance
☐ Extra product filtering
☐ Ease of installation

1B

Pump Size _____
Impeller diameter _____
HP, efficiency _____
NPSHR _____
Minimum Pump Flow _____
Speed (RPM) _____

2B

Materials of Construction _____
Bearing cooling _____
Sealing / flushing Requirements _____
Jacketing for Cooling / heating _____

3B

Explosion-proof enclosures _____
Safety protection options _____
Coupling guard options _____
Casing drain _____
Flange options _____
O-ring materials _____

4B

Type of lubrication _____
Start-up assistance _____
Operator training _____
Maintenance training _____
Baseplate options _____
Oil seal options _____

Member of Hydraulic Institute

GOULDS PUMPS | RHEINHÜTTE PUMPEN | PRO services

ITT Brands

240 Fall Street
Seneca Falls, NY 13148
Phone: 315.568.2811
Fax: 315.568.2418
www.gouldspumps.com

RHEINHÜTTE Pumpen GmbH
Rheingaustraße 96-98 – 65203 Wiesbaden – Germany
Tel: +49 (0)611 604-0 – Fax: +49 (0)611 604-328
info@rheinhuette.de
www.rheinhuette.de

B.PSG.en-US.2020-03

BIOPHARM TANKS

MUELLER
BIOPHARM SYSTEMS

Get to Know Mueller® BioPharm Systems

Since our inception in 1940, Paul Mueller Company has evolved from a small scale fabricator into a global process solution provider with one million square feet of manufacturing space. Mueller® offers a full range of tanks from shop-fabricated alloy vessels up through 20 feet in diameter to field-fabricated vessels up through 2,000,000 gallons; furthermore, we offer integrated systems, modular fabrication, field construction, plant maintenance and repair, and complete turnkey project execution. Our facility is uniquely qualified to handle large and complex fluid handling systems from project concept through installation. Mueller products are used in over 100 countries in a wide variety of applications. Paul Mueller Company delivers outstanding equipment and unique solutions to the process industries with our technical expertise, innovative engineering, and manufacturing resources.

We know that building a quality product starts from the ground up. Our unprecedented purpose is to make your system as valuable and efficient as it can be, and to guarantee that you receive the highest possible quality in our processes and products. With our skilled craftsmen, quality materials, and one of the best technologically advanced manufacturing facilities in the country we are able to build exceptional products at a reasonable price.

Mueller products are made by our highly skilled craftsmen, whose average experience exceeds 15 years. Our process is well defined and consistently developed. Each Mueller team member fully understands the importance that their individual roles play in producing a quality product. On any given day, their talent and pride of workmanship can be observed in any our production areas. Our central United States production facility lowers your transportation costs and speeds delivery of product to your location.

Mueller Transportation, Inc. lets us provide you with competitive delivery rates on standard products, as well as dedicated handling for large or critical delivery items. We offer a perfect package by working directly with you to resolve any transportation issues.

Mueller Field Operations, Inc. offers our customers more versatility and flexibility. Our field construction capabilities allow us to install Mueller advanced products at a low cost.

Factory technicians and field service available. Mueller offers rapid response to your service needs with trained factory personnel knowledgeable in all aspects of Paul Mueller Company equipment.

1

The Mueller Reputation

Every piece of Mueller BioPharm processing equipment is precision engineered for quality form and fit, close tolerances, and high quality finishes. You can depend on Mueller to deliver a product that will perform required functions and offer reliable product protection.

Mueller BioPharm Tanks

For decades, Mueller has been recognized as a trusted supplier of tanks and vessels to the pharmaceutical and biotech industry, and our cumulative experience in this field is unrivaled. From smaller portable tanks and "smart" tanks to larger processing vessels, we have the capabilities and engineering, manufacturing, and documentation resources to deliver your custom BioPharm tanks as required.

We provide you with a vast array of services, including a diverse engineering organization with specialists in the areas of heat transfer, agitation, and CIP, in addition to the most technologically advanced manufacturing capabilities.

Our extensive tank and vessel manufacturing capabilities, one million square foot facility, and hundreds of production workers and craftsmen allow us to provide the entire scope of these products in-house. Mueller manufactures 100% of the heads, shells, manways, and heat transfer surface within our facility. This, in conjunction with our electropolishing capability, lets us control the entire scope of supply for your tank or vessel. This means that you can expect tight control of quality and schedule throughout the manufacturing process, consistent documentation, and on-time delivery via Mueller Transportation, Inc.

In addition, we offer installation, full Factory Acceptance Testing (FAT), and extensive standard documentation packages, or we can supply a custom package to meet your project's specific needs. From projects requiring a single portable vessel to multiple quantity large vessel orders, let Mueller BioPharm Systems contribute to the success of your next project!

BioPharm Tanks

Portable Tanks

Mueller offers you a full range of portable vessels. Our engineering and manufacturing staff has decades of experience in the design and manufacture of portable tanks with heat transfer, agitation, top-head manways, and virtually any requirement that might exist.

"Smart" Tanks

A recent trend in the Biopharm industry, a "smart" tank is a vessel which has most or all of its required control hardware and capability integrated onto the vessel itself or an attached skid.

Our vessel capability, coupled with our highly skilled controls group, can meet all of your requirements for such a project. From initial consultation to software programming and FAT, we can meet your most complex project requirements.

Processing Tanks

Paul Mueller Company has the capability to fabricate the largest and most complex processing tanks the biopharm industry requires. We are experienced working with material from thin gauge up to one inch thick with any combination of fittings, manways, heat transfer, and mixing equipment. These capabilities mean there is almost no limit to the level of complexity and size of vessel we can manufacture.

We manufacture a wide range of heat transfer products, which means we can provide precise temperature control to meet your requirements, along with a variety of agitation devices for critical aseptic applications. Our capabilities also allow us to offer all surface finishes utilized in the biopharm industry.

Bioreactors and Fermenters

Paul Mueller Company custom bioreactor and fermentation systems are fully instrumented and integrated skid mounted systems built and designed to your custom specifications.

The complete systems are offered in sizes ranging from 20 liters to 25,000 liters to meet your unique needs.

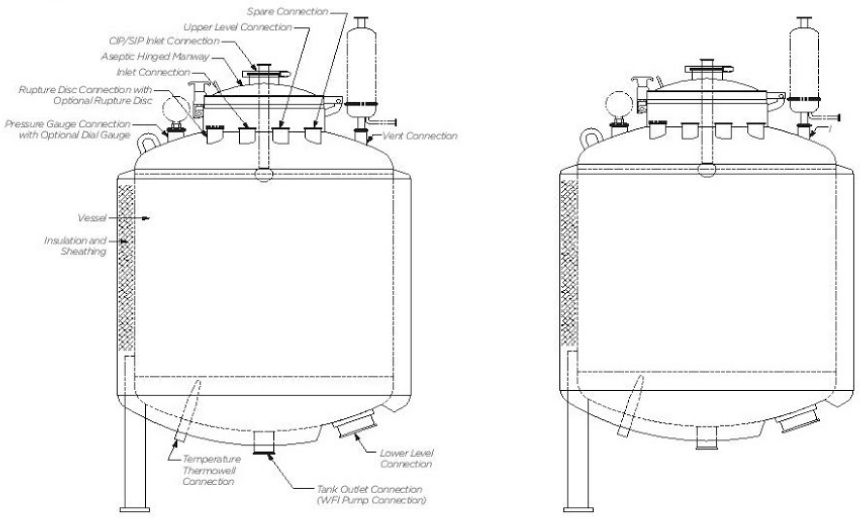
The equipment can be fully tested in our shop at elevation closely meeting the actual utility parameters at the installation site. We can even ship these systems using our own fleet of trucks.

Affordable High-Purity Water Storage

Water-for-Injection Tanks

Mueller WFI tanks are engineered specifically for the special needs of the pharmaceutical and biotech industry. They consist of a Type 316L stainless steel vessel and utilize sanitary clamp style connections, an aseptic manway, and a spray ball for interior sanitization. Chloride-free insulation surrounds the tank's sides and bottom, which is covered with Type 304 stainless steel outer sheathing. The vessel and all components are fabricated to the requirements of ASME Section VIII, Division 1. Standard tank sizes range from 250 to 15,000 gallons (945 to 56,780 liters) to satisfy a broad spectrum of capacity requirements. Custom fabrication is also available.

Specifications



Features and Benefits

- Seismic design means strength enough to withstand earthquakes up to and including Zone 4 conditions.
- With Paul Mueller Company's 75 years of experience in stainless steel fabrication and finishing, quality and reliability in design and construction are assured.
- Mueller manufactures a broad range of standard WFI tank sizes to meet your specific storage needs. In addition, we offer custom sizes and dimensions for special requirements.
- Insulated sides and bottom of vessel helps maintain WFI temperature.
- Mueller WFI tanks are integrated with our PyroPure® stills and pure steam generators and tested as a system prior to shipment, which speeds installation by pre-assembly and shop fit-up.
- CIP coverage and FAT testing is available. Each tank comes with a complete documentation package that speeds validation of your system.

Components

Vessel. The ASME Code stamped WFI vessel has a rating of 40 psig and full vacuum at 300°F. Interior surfaces are mechanically polished to 25 Ra maximum and then electropolished (BPE SF6) to maintain optimum sanitary conditions. Exterior surfaces are 2B or mill finish with welds buffed. Mechanically polished material and/or flush ground weld finishes are available options.

Aseptic Manway. The 18" hinged opening meets cGMPs standards for validation ease. It has an EPDM O-ring seal and is made of Type 316L stainless steel to match the vessel. It may be centered on top of the vessel or located off center, depending upon installation requirements. Silicone and Viton O-rings are available options.

Connections. Standard sanitary clamp-type connections include an outlet connection, inlet connection, CIP/SIP connection, vent connection, rupture disc connection, pressure gauge connection, upper and lower level connection, temperature thermowell connection, and a spare connection.

Insulation. A 2" chloride-free insulation surrounds the sides and bottom of each WFI vessel. A 12-gauge, Type 304 stainless steel sheathing is welded around the insulation to seal it from moisture.

Sanitary Spray Ball. When connected to a CIP or SIP system, the spray ball will rinse the vessel interior and top head with hot water to keep the tank environment sanitary. The spray ball and its components are removable for cleaning and inspection. The ball is constructed of electropolished Type 316L stainless steel. CIP coverage testing is available.

Optional Equipment

Heat Transfer Surface. Dimpled heat transfer surface can be included on the bottom of the vessel to keep WFI at a constant temperature using plant steam. The surface is ASME rated at 125 psig at 360°F and is constructed of 14- gauge Type 316L stainless steel. Additional heat transfer surface on the sidewall is also available.

Rupture Disc. Protects the vessel from excessive pressure buildup when combined with a sanitary port.

Pharmaceutical Vent Filter. Allows air in while protecting stored water from airborne contaminants with a 0.2 micron hydrophobic, steam sterilizable filter element. Both the filter and the element comply with requirements for LVP cGMPs.

Temperature Indicator. Provides a digital readout of WFI temperatures via an RTD probe.

Pressure/Vacuum Gauge. Stainless steel casing encloses a sanitary diaphragm pressure sensor. Gauge attaches to the vessel.

Sanitary Level Controller. Monitors tank level and can be set to activate a level alarm while starting/stopping the connected still.

Vortex Breaker. Prevents problems caused by high draw-off rates.

Material and Weld Finishes

Material Finishes

Mueller products can be fabricated with any of the following material finish options. These designations apply to stainless steel sheet, plate, pipe, and bar.

Types and Descriptions

Hot Rolled (HR). Rough, dull surface appearance. Most scale removed by pickling. Applies to all steel plate thicknesses above ¼". Also available in 7 gauge and ¼". Specify where surface finish is a low priority.

2B Mill Finish (2B). A smooth, bright, moderately reflective finish suitable for "as is" specifications or as a preliminary finish for further polishing. Available only in 10 gauge or thinner sheet material.

No. 3 Finish. A semi-polished surface achieved by finishing with the equivalent of an 80 grit abrasive. This finish has a pronounced grit line. Typically used with a No. 3 weld finish.

Hot-Rolled (HR)

2B Mill Finish (2B)

No. 3 Finish

No. 4 Finish

No. 6 Finish

No. 7 Finish

Industrial Electropolish (IND-EP)

Electropolish (EP)

No. 4 Finish. An aesthetic industrial finish with visible grain that prevents mirror-like reflectivity. Used where clean industrial surfaces are required. Typically used with a No. 4 weld finish (150 grit).

No. 6 Finish. This polished finish is achieved with the equivalent of 240 grit abrasive. Finer grit lines and higher reflectivity than No. 4 finish. Improved product release, cleanability, and appearance. Typically used with a No. 6 weld finish.

No. 7 Finish. Highly reflective surface obtained with the equivalent of 320 grit abrasive. Minimal grit lines. Used where product contact surfaces are critical. Typically used with a No. 7 weld finish.

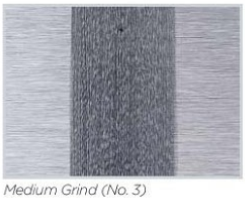
Industrial Electropolish (IND-EP). Reflective surface achieved by passing direct current through material that is suspended in electrolyte. Used where improved product release or cleanability is necessary.

Electropolish (EP). A highly reflective surface that provides the level of product release and cleanability required by the medical, chemical, pharmaceutical, and electronic industries. Process removes impurities and surface materials, but may not remove nonmetallic inclusions that may be present in parent material. Used to improve release on any of our product material finishes. Degree of improved performance depends on weld and material finishes specified prior to EP.

Weld Finishes

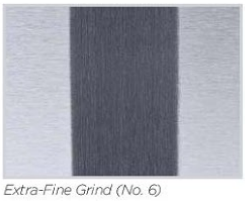
While it is possible to grind and polish every weld on a piece of equipment, in many cases it is not necessary or practical. The following describes the various weld finishes that are available from Mueller and, where applicable, the appropriate use of the finish.

Course Grind (No. 2) Industrial

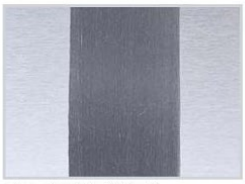


Medium Grind (No. 3)

Fine Grind (No. 4)



Extra-Fine Grind (No. 6)



Ultra-Fine Grind (No. 7)

Types and Descriptions

As-Is (AI). Characterized by fine spatter and smoke and weld discoloration. Tack welds, start-stop areas, and severe spatter are ground as required for nondestructive examination of the weld and weld area.

Sandblast (SB). Uniform, dull gray appearance to match cold- or hot-rolled material finish. Large spatter, slag, and burrs are first removed by grinding. Welds are then sandblasted to remove weld discoloration on material surfaces, leaving a clean, banded appearance.

Glass-Bead Blast (GB). Follows sandblasting to produce a satin, gray appearance closely matching a 2B finish.

Buff (BF). A process in which the weld is brightened. There is minimal removal of weld material. This finish is not flush and will contain crevices, ripples, silicone islands, and irregularities in the remaining weld material. Dark lines on either side of the weld and within the weld ripple may also remain. Generally used on exterior and interior plate surfaces where finish is not critical. Weld ripple size and appearance depends on the welding process used. Typically used with HR, CR, and 2B mill material finishes.

Coarse Grind (No. 2) Industrial. Welds are ground smooth but not flush. The upper surface of the weld bead is removed. Visual pits are not removed. This is not a 100% flush weld finish. Ra* is not applicable. Characterized by coarse grit lines which may run in any direction. Discoloration remains on both sides of weld. Used as a preparatory finish where a flush and uniform surface are required.

Medium Grind (No. 3). Weld is ground flush and all discoloration is removed. A near sanitary finish generally used where a flush and uniform surface is required. Moderate grit lines remain. Target Ra is 75.

Fine Grind (No. 4) 150 Grit. Results in an aesthetic industrial finish surface normally used with a No. 4 material finish for applications where clean industrial contact surfaces are required. Grain and grit lines are visible. Target Ra is 32.

Extra-Fine Grind (No. 6) 240 Grit. Finer grit lines and higher reflectivity than fine grind. Improves product release and cleanability. Target Ra is 25.

Ultra-Fine Grind (No. 7) 320 Grit. A highly reflective, sanitary surface with minimal grit lines. Normally used to provide excellent product release and cleanability. Use where sanitary product contact surfaces are most critical. Target Ra is 15.

**Ra: Roughness average is the most universally recognized parameter of roughness. Its arithmetical average definition is measured normal to the centerline (AA or CLA).*

Heat Transfer Solutions

Heat Transfer Surface

Paul Mueller Company offers a variety of heat transfer surfaces to meet your particular requirements. Mueller heat transfer surface is ideally suited for applications involving high pressure and temperature extremes. It can be routinely fabricated in an almost unlimited number of shapes, sizes, and materials to fit any vessel design. Styles are available for use with almost any type of refrigerant or heating media. We work closely with you on each project to select the right surface for your equipment.

Double-Embossed

Most commonly utilized in immersion applications, double-embossed Mueller Temp-Plate heat transfer surface helps maximize heating and cooling by using both sides of the heat transfer plate.

Half-Pipe Coil

Our half-pipe coil heat transfer surface can handle large volumes of flow and is suited for high pressure applications and low pressure drop requirements.

Dimpled

Dimpled Mueller Temp-Plate surface is machine punched and swaged prior to welding to increase the flow area in the passages.

Single-Embossed

Single-embossed Mueller Temp-Plate heat transfer surface is economical to use for interior tank walls, tank heads, and when a flat side is required.

Temp-Plate® Heat Transfer Surface

Mueller Temp-Plate heat transfer surface provides precise, consistent control capability with minimum pressure drop. Its design provides extremely efficient heat transfer performance that is more economical than other competitive types of heat transfer surface. Temp-Plate has spot-welded and inflated channels that induce the fluid turbulence necessary to attain high heat transfer coefficients. Lower flow rates are essential to achieve the high velocities of heating and cooling media.

Half-Pipe Coil Heat Transfer Surface

Mueller half-pipe coil heat transfer surface handles large volumes of flow and is suited for high pressure applications and low pressure drop requirements. It is ideal for cyclic heat transfer conditions where heating and cooling cycles occur several times a day, as it is very resistant to stress corrosion cracking. Available in a variety of materials, half-pipe coil may be used for heating or cooling using steam, hot oil, water, glycol, ammonia, and refrigerants. ASME Code stamping is available.

Dimpled Heat Transfer Surface

Mueller's dimpled Temp-Plate heat transfer surface is ideally suited for applications that involve high pressure and temperature extremes. It is routinely fabricated in an almost unlimited number of shapes, sizes, and materials to fit any vessel design. Styles are available for use with almost any type of heating or refrigerant media.

Documentation and Validation

Documentation

Material Traceability

The documentation for your system begins before the first drawing is generated or the first welding arc is struck. Material traceability is established with the purchase and receipt inspection of materials and is systematically maintained throughout the manufacturing and assembly processes.

Process Traceability

Many different processes take place during the fabrication of BioPharm equipment. Several methods are used to document that the equipment has been designed, fabricated, assembled, and tested appropriately. These include:

- Borescope inspection and video capabilities.
- Factory testing procedures.
- Inspection records.
- Software design specification (as required).
- Master inspection traveler.
- Weld records.

Submittals

After receipt of your order, Mueller will send you drawings for final approval. These documents define the mechanical scope of supply and allow procurement and fabrication of the key components to proceed so the schedule is minimized while ensuring that the proper equipment will be supplied. Subsequent submittals are provided for software and functional testing details as required. We encourage you to comment and provide feedback on these documents to ensure compliance with your project requirements.

Turnover Packages — Per BPE Requirements

The resulting turnover package provides a well organized and comprehensive validation reference that parallels customer protocols. In addition to the standard three-ring binders, packages are also provided in CD/DVD formats.

IQ/OQ Capabilities

Mueller offers installation qualification (IQ) and operational qualification (OQ) documents to support our products. Execution of these protocols can be performed by Paul Mueller Company service technicians at the time of start-up and commissioning.

Factory Acceptance Testing

Mueller factory acceptance testing starts prior to your arrival on site with your review and approval of the test documents. We also pre-test the equipment prior to your arrival. Any project specific requirements outlined in the functional specification and design specification documents will be checked and tested as needed.

Validation

As a world leader in water and processing systems for the finished pharmaceutical, bulk, API, biotechnology, medical device, and medical diagnostic industries, we have extensive industry experience preparing comprehensive turnover documentation and validation packages. The many projects that Mueller has completed have withstood scrutiny by the numerous customers, independent validation companies, as well as the Food and Drug Administration (FDA).

As the pharmaceutical industry has evolved, so has our approach to validation. We are qualified to provide documentation and validation compliance due to our extensive experience within the industry, our attention to regulatory changes, and our capability to adapt to each of our customers' specific needs. The optional completed installation qualification (IQ) and operational qualification (OQ) documentation and validation packages provide documented evidence that our systems are built and commissioned in accordance with user requirements specifications (URS), functional requirements specifications (FRS) and detail design specifications (DDS), as well as FDA and cGMP standards.

Paul Mueller Company maintains a staff of professionals with considerable experience within the pharmaceutical industries and broad educational backgrounds in quality, engineering, chemistry, and technical services. Since our validation and quality systems are integrated within the company structure, there are substantial benefits realized from shared databases as well as our detailed understanding of the equipment.

Industry Experience

Mueller has successfully provided documentation and validation assistance for large and small pharmaceutical and biotech projects including:

- Multiple-effect stills and pure steam generators.
- Seed train and production bioreactors, including controls and related process equipment.
- Process equipment for numerous buffer hold and preparation facilities consisting of as many as 40 vessels, as well as the associated controls, electrical equipment, structure, utility piping, and process piping.
- Vessels used in pharmaceutical and biotech service.

Complete Service from Start to Finish

Mueller Field Operations, Inc.

Mueller Field Operations, Inc., a wholly owned subsidiary of Paul Mueller Company, offers complete construction services with particular emphasis on expanded scope projects utilizing our construction management, engineering, procurement, and field integration capabilities. We provide specialized labor for on-site field erected tanks/vessels, equipment installation, vessel retrofit, vessel repair, and process piping that allows us to go beyond the capabilities of our manufacturing facility.

Mueller Field Operations, Inc. has extensive experience in providing on-site solutions in sanitary design for the food, juice, dairy, beer, wine, and pharmaceutical industries. Industrial applications, such as ASME and API code stamped equipment, are also available through our services.

In-house manufacturing of components in our state-of-the-art facility ensures that all parts such as tank heads, cylinders, manways, fittings, agitators, and heat transfer surface are fabricated correctly and coordinated to support our construction schedule in the field.

From project start to finish, we instill stringent quality control processes for design, component manufacturing, equipment transport, field installation, commissioning, final performance testing, to project completion. We also offer complete maintenance and start-up services to ensure our customer's needs are upheld.

Mueller Field Operations, Inc. is supported by Paul Mueller Company's nearly one million square foot manufacturing facilities, centrally located in Springfield, Missouri, and Osceola, Iowa. Manufactured components are delivered to the job site by Mueller's own fleet of trucks.

...We're with you from the ground up.

Mueller Product Support Team

Our Mission

The mission of the Mueller product support team is to meet and exceed our customers' expectations of value by setting the industry standard for exceptional service. In support of this mission, we maintain a technical staff of specialized technicians highly trained on our products, vendor software, controls, and the various trade disciplines. Our equipment is serving customers worldwide. Our factory-trained technicians are available to meet the needs of our customers and can normally be on-site within 72 hours of notification.

Paul Mueller Company makes some of the most reliable equipment in service today. However, no matter how well built a product is, continuous use without periodic inspection and maintenance may result in mechanical failure and costly downtime. When you buy Mueller equipment, you are not just buying machinery—you are investing in a partnership. We work together to assure that your equipment continues to perform at its best for years to come.

Our Services

Technical Support Via Phone, Fax, or Email

There is never a charge for technical support from the factory via telephone, fax, or email. Your experienced operators and our factory technicians are able to resolve most issues over the phone, which saves you time and money. Please call 888-281-5800, send a facsimile to 417-575-9662, or email us at biopharm@paulmueller.com.

Replacement Parts

Each documentation package includes a list of recommended replacement parts that will minimize downtime in the event of a failure. Mueller stocks the most critical replacement parts for your equipment. Our parts specialists literally provide replacement parts to you as quickly as possible when your machine is down.

"It has been our privilege to place the skills and techniques of Paul Mueller Company at the service of many of the nation's leading companies. It would be a further privilege to serve your company."

MUELLER

1600 West Phelps Street • Springfield, Missouri 65802, U.S.A.
Phone: 417-575-9000 • 1-800-MUELLER • Fax: 417-575-9662
www.paulmueller.com • Email: biopharm@paulmueller.com

PP-2167-1

Appendix E: SDS

ATCC

Product Sheet

Corynebacterium glutamicum (ATCC® 13032™)

Please read this FIRST

Storage Temp.
Frozen: -80°C or colder
Freeze-Dried: 2°C to 8°C
Live Culture: See Propagation Section

Biosafety Level
1

Intended Use

This product is intended for research use only. It is not intended for any animal or human therapeutic or diagnostic use.

Citation of Strain

If use of this culture results in a scientific publication, it should be cited in that manuscript in the following manner: *Corynebacterium glutamicum* (ATCC® 13032™)

American Type Culture Collection
PO Box 1549
Manassas, VA 20108 USA
www.atcc.org

800.638.6597 or 703.365.2700
Fax: 703.365.2750
Email: Tech@atcc.org

Or contact your local distributor

Description

Designation: 534 [NCIB 10025]
Deposited Name: *Micrococcus glutamicus* Kinoshita et al.
Product Description: Type strain. Genome sequenced strain.

Propagation

Medium
ATCC® Medium 3: Nutrient agar or nutrient broth

Growth Conditions
Temperature: 37°C
Atmosphere: Aerobic

Propagation Procedure

1. Open vial according to enclosed instructions.
2. Using a single tube of #3 broth (5 to 6 mL), withdraw approximately 0.5 to 1.0 mL with a Pasteur or 1.0 mL pipette. Rehydrate the entire pellet.
3. Aseptically transfer this aliquot back into the broth tube. Mix well.
4. Use several drops of the suspension to inoculate a #3 agar slant and/or plate.
5. Incubate the tubes and plate at 37°C for 24 hours.

Notes

Additional information on this culture is available on the ATCC® web site at www.atcc.org.

References

References and other information relating to this product are available online at www.atcc.org.

Biosafety Level: 1

Appropriate safety procedures should always be used with this material. Laboratory safety is discussed in the current publication of the *Biosafety in Microbiological and Biomedical Laboratories* from the U.S. Department of Health and Human Services Centers for Disease Control and Prevention and National Institutes for Health.

ATCC Warranty

ATCC® products are warranted for 30 days from the date of shipment, and this warranty is valid only if the product is stored and handled according to the information included on this product information sheet. If the ATCC® product is a living cell or microorganism, ATCC lists the media formulation that has been found to be effective for this product. While other, unspecified media may also produce satisfactory results, a change in media or the absence of an additive from the ATCC recommended media may affect recovery, growth and/or function of this product. If an alternative medium formulation is used, the ATCC warranty for viability is no longer valid.

Disclaimers

This product is intended for laboratory research purposes only. It is not intended for use in humans.
While ATCC uses reasonable efforts to include accurate and up-to-date information on this product sheet, ATCC makes no warranties or representations as to its accuracy. Citations from scientific literature and patents are provided for informational purposes only. ATCC does not warrant that such information has been confirmed to be accurate.
This product is sent with the condition that you are responsible for its safe storage, handling, and use. ATCC is not liable for any damages or injuries arising from receipt and/or use of this product. While reasonable effort is made to insure authenticity and reliability of materials on deposit, ATCC is not liable for damages arising from the misidentification or misrepresentation of such materials.

Please see the enclosed Material Transfer Agreement (MTA) for further details regarding the use of this product. The MTA is also available on our Web site at www.atcc.org

Additional information on this culture is available on the ATCC web site at www.atcc.org.

ATCC

Product Sheet

Corynebacterium glutamicum (ATCC® 13032™)

Please read this FIRST

Storage Temp.
Frozen: -80°C or colder
Freeze-Dried: 2°C to 8°C
Live Culture: See Propagation Section

Biosafety Level
1

Intended Use

This product is intended for research use only. It is not intended for any animal or human therapeutic or diagnostic use.

Citation of Strain

If use of this culture results in a scientific publication, it should be cited in that manuscript in the following manner: *Corynebacterium glutamicum* (ATCC® 13032™)

American Type Culture Collection
PO Box 1549
Manassas, VA 20108 USA
www.atcc.org

800.638.6597 or 703.365.2700
Fax: 703.365.2750
Email: Tech@atcc.org

Or contact your local distributor

Safety Data Sheet

according to 29CFR1910/1200 and GHS Rev. 3

Effective date : 12.27.2014

Corn Syrup

SECTION 1 : Identification of the substance/mixture and of the supplier

Product name : **Corn Syrup**

Manufacturer/Supplier Trade name:

Manufacturer/Supplier Article number: **S25339**

Recommended uses of the product and uses restrictions on use:

Manufacturer Details:

AquaPhoenix Scientific
9 Barnhart Drive, Hanover, PA 17331

Supplier Details:

Fisher Science Education
15 Jet View Drive, Rochester, NY 14624

Emergency telephone number:

Fisher Science Education Emergency Telephone No.: 800-535-5053

SECTION 2 : Hazards identification

Classification of the substance or mixture:

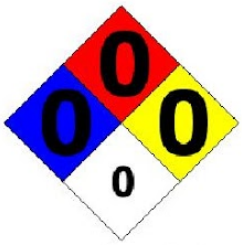
Not classified for physical or health hazards under GHS.

Hazard statements:
Precautionary statements:

Combustible Dust Hazard: :
May form combustible dust concentrations in air (during processing).
Combustible Dust Hazard: :
May form combustible dust concentrations in air (during processing).

Other Non-GHS Classification:

WHMIS
NFPA/HMIS



NFPA SCALE (0-4)

HMIS RATINGS (0-4)

SECTION 3 : Composition/information on ingredients

Ingredients:

Corn Syrup

CAS 50-99-7	D-Fructose	>75 %
CAS 7732-18-5	water, Purified	<25 %
		Percentages are by weight

SECTION 4 : First aid measures

Description of first aid measures

After inhalation: Loosen clothing as necessary and position individual in a comfortable position.Remove to fresh air. Give artificial respiration if necessary. If breathing is difficult give oxygen.Get medical assistance if cough or other symptoms appear.

After skin contact: Wash affected area with soap and water. Seek medical attention if irritation persists or if concerned.

After eye contact: Protect unexposed eye. Immediately flush eyes with water for at least 15 minutes. Remove contact lenses while rinsing.Immediately get medical assistance.

After swallowing: Dilute mouth with water or milk.Get medical assistance.

Most important symptoms and effects, both acute and delayed:

Nausea.Headache.Shortness of breath.Irritation.;

Indication of any immediate medical attention and special treatment needed:

If seeking medical attention provide SDS document to physician.

SECTION 5 : Firefighting measures

Extinguishing media

Suitable extinguishing agents: Use water, dry chemical, chemical foam, carbon dioxide, or alcohol-resistant foam.

For safety reasons unsuitable extinguishing agents:

Special hazards arising from the substance or mixture:

Dust can form an explosive mixture in air.

Advice for firefighters:

Protective equipment: Wear protective eyeware, gloves, and clothing.Refer to Section 8.

Additional information (precautions): Avoid contact with skin, eyes, and clothing.Avoid generating dust.

SECTION 6 : Accidental release measures

Personal precautions, protective equipment and emergency procedures:

Where risk assessment shows air-purifying respirators are appropriate use a full-face particle respirator type N100 (US) or type P3 (EN 143) respirator cartridges as a backup to engineering controls.When necessary use NIOSH approved breathing equipment.

Environmental precautions:

Methods and material for containment and cleaning up:

If necessary use trained response staff or contractor. Clean up spills immediately. Observe precautions for protective equipment.Absorb with suitable absorbent material such as sand or earth and containerize for disposal. Refer to Sections 5, 8, and 10.

Reference to other sections:

SECTION 7 : Handling and storage

Precautions for safe handling:

Wash hands before breaks and immediately after handling the product.Avoid contact with skin, eyes, and clothing.Minimize dust generation. Avoid ingestion and inhalation. Follow good hygiene procedures when handling chemical materials. Refer to Section 8.Do not eat, drink, smoke, or use personal products when handling chemical substances.

Conditions for safe storage, including any incompatibilities:

Keep container tightly closed in a cool, dry, and well-ventilated area. Store away from incompatible materials. Refer to Section 5.

SECTION 8 : Exposure controls/personal protection

Control Parameters:	, , OSHA PEL TWA (Total Dust) 15 mg/m3 (50 mppcf*) , , ACGIH TLV TWA (inhalable particles) 10 mg/m3
Appropriate Engineering controls:	Emergency eye wash fountains and safety showers should be available in the immediate vicinity of use or handling. Provide exhaust ventilation or other engineering controls to keep the airborne concentrations of vapor and mists below the applicable workplace exposure limits (Occupational Exposure Limits-OELs) indicated above.Normal ventilation is adequate.
Respiratory protection:	Not required under normal conditions of use. Normal ventilation is adequate.
Protection of skin:	Select glove material impermeable and resistant to the substance.Select glove material based on rates of diffusion and degradation.
Eye protection:	Safety glasses with side shields or goggles.
General hygienic measures:	Wash hands before breaks and at the end of work. Avoid contact with the eyes and skin.Perform routine housekeeping to prevent dust generation.Before wearing wash contaminated clothing.Dispose of contaminated gloves after use in accordance with applicable laws and good laboratory practices.

SECTION 9 : Physical and chemical properties

Appearance (physical state,color):	Clear viscous liquid	**Explosion limit lower**: **Explosion limit upper**:	Non Explosive Non Explosive
Odor:	Sweet odor	**Vapor pressure**:	Not Available
Odor threshold:	Not Available	**Vapor density**:	Not Available
pH-value:	5.9	**Relative density**:	1.54
Melting/Freezing point:	146.1°C	**Solubilities**:	Soluble in water
Boiling point/Boiling range:	Not Available	**Partition coefficient (n-octanol/water)**:	Not Available
Flash point (closed cup):	Not Applicable	**Auto/Self-ignition temperature**:	Not Applicable

Corn Syrup

Evaporation rate:	Not Available	**Decomposition temperature**:	Not Available
Flammability (solid,gaseous):	Not Applicable	**Viscosity**:	a. Kinematic:Not Available b. Dynamic: Not Available
Density: Not Available			

SECTION 10 : Stability and reactivity

Reactivity: None under normal processing.
Chemical stability: Stable under normal conditions.
Possible hazardous reactions:
Conditions to avoid: Excessive heat.Dust generation. Incompatible materials. Refer to Section 5.
Incompatible materials: Strong oxidizers.
Hazardous decomposition products: Carbon oxides.Irritating and highly toxic gases or fumes.

SECTION 11 : Toxicological information

Acute Toxicity:		
Oral:	25,800 mg/kg	LD50 Oral - rat
Chronic Toxicity: No additional information.		
Corrosion Irritation: No additional information.		
Sensitization:		No additional information.
Single Target Organ (STOT):		No additional information.
Numerical Measures:		No additional information.
Carcinogenicity:		No additional information.
Mutagenicity:		No additional information.
Reproductive Toxicity:		No additional information.

SECTION 12 : Ecological information

Ecotoxicity Persistence and degradability:
Bioaccumulative potential:
Mobility in soil:
Other adverse effects:

SECTION 13 : Disposal considerations

Waste disposal recommendations:

Chemical waste generators must determine whether a discarded chemical is classified as a hazardous waste. Chemical waste generators must also consult local, regional, and national hazardous waste regulations. Ensure complete and accurate classification.Dispose of empty containers as unused product.

SECTION 14 : Transport information

UN-Number

Not Dangerous Goods

UN proper shipping name

Not Dangerous Goods

Transport hazard class(es)
Packing group:Not Dangerous Goods
Environmental hazard:
Transport in bulk:
Special precautions for user:

SECTION 15 : Regulatory information

United States (USA)

SARA Section 311/312 (Specific toxic chemical listings):

None of the ingredients is listed

SARA Section 313 (Specific toxic chemical listings):

None of the ingredients is listed

RCRA (hazardous waste code):

None of the ingredients is listed

TSCA (Toxic Substances Control Act):

All ingredients are listed.

CERCLA (Comprehensive Environmental Response, Compensation, and Liability Act):

None of the ingredients is listed

Proposition 65 (California):

Chemicals known to cause cancer:

None of the ingredients is listed

Chemicals known to cause reproductive toxicity for females:

None of the ingredients is listed

Chemicals known to cause reproductive toxicity for males:

None of the ingredients is listed

Chemicals known to cause developmental toxicity:

None of the ingredients is listed

Canada

Canadian Domestic Substances List (DSL):

All ingredients are listed.

Canadian NPRI Ingredient Disclosure list (limit 0.1%):

None of the ingredients is listed

Canadian NPRI Ingredient Disclosure list (limit 1%):

None of the ingredients is listed

SECTION 16 : Other information

This product has been classified in accordance with hazard criteria of the Controlled Products Regulations and the

SDS contains all the information required by the Controlled Products Regulations.The responsibility to provide a safe workplace remains with the user.The user should consider the health hazards and safety information contained herein as a guide and should take those precautions required in an individual operation to instruct employees and develop work practice procedures for a safe work environment.The information contained herein is, to the best of our knowledge and belief, accurate.However, since the conditions of handling and use are beyond our control, we make no guarantee of results, and assume no liability for damages incurred by the use of this material.It is the responsibility of the user to comply with all applicable laws and regulations applicable to this material.Note:
GHS Full Text Phrases:

Abbreviations and acronyms:
IMDG: International Maritime Code for Dangerous Goods
PNEC: Predicted No-Effect Concentration (REACH)
CFR: Code of Federal Regulations (USA)
SARA: Superfund Amendments and Reauthorization Act (USA)
RCRA: Resource Conservation and Recovery Act (USA)
TSCA: Toxic Substances Control Act (USA)
NPRI: National Pollutant Release Inventory (Canada)
DOT: US Department of Transportation
IMDG: International Maritime Code for Dangerous Goods
IATA: International Air Transport Association
GHS: Globally Harmonized System of Classification and Labelling of Chemicals
IATA: International Air Transport Association
ACGIH: American Conference of Governmental Industrial Hygienists
CAS: Chemical Abstracts Service (division of the American Chemical Society)
NFPA: National Fire Protection Association (USA)
HMIS: Hazardous Materials Identification System (USA)
WHMIS: Workplace Hazardous Materials Information System (Canada)
DNEL: Derived No-Effect Level (REACH)
PNEC: Predicted No-Effect Concentration (REACH)
CFR: Code of Federal Regulations (USA)
SARA: Superfund Amendments and Reauthorization Act (USA)
RCRA: Resource Conservation and Recovery Act (USA)
GHS: Globally Harmonized System of Classification and Labelling of Chemicals
TSCA: Toxic Substances Control Act (USA)
NPRI: National Pollutant Release Inventory (Canada)
DOT: US Department of Transportation
ACGIH: American Conference of Governmental Industrial Hygienists
CAS: Chemical Abstracts Service (division of the American Chemical Society)
NFPA: National Fire Protection Association (USA)
HMIS: Hazardous Materials Identification System (USA)
WHMIS: Workplace Hazardous Materials Information System (Canada)
DNEL: Derived No-Effect Level (REACH)

Effective date : 12.27.2014
Last updated : 03.19.2015

SAFETY DATA SHEET

(in accordance with Regulation (EU) 2015/830)

108400-DL-Methionine

DC FINE CHEMICALS

SECTION 1: IDENTIFICATION OF THE SUBSTANCE AND OF THE COMPANY/UNDERTAKING.

1.1 Product identifier.

Product Name:	DL-Methionine
Product Code:	108400
Chemical Name:	DL-Methionine
CAS No:	59-51-8
EC No:	200-432-1
Registration No:	N/D

1.2 Relevant identified uses of the substance and uses advised against.

For manufacturing, processing, laboratory or repacking use only
Uses advised against:
Uses other than those recommended.

1.3 Details of the supplier of the safety data sheet.

Company:	**DC FINE CHEMICALS Ltd.**
Address:	Hill Top, 88
City:	NW11 6DY London (United Kingdom)
Telephone:	+44 (20) 7586 6800
Fax:	+44 (20) 7504 1701
E-mail:	info@dcfinechemicals.com
Web:	www.dcfinechemicals.com

1.4 Emergency telephone number: (Only available during office hours)

SECTION 2: HAZARDS IDENTIFICATION.

2.1 Classification of the substance.
The product is not classified as hazardous within the meaning of Regulation (EU) No 1272/2008.

2.2 Label elements.
Contains:
DL-Methionine

2.3 Other hazards.
In normal use conditions and in its original form, the product itself does not involve any other risk for health and the environment.

SECTION 3: COMPOSITION/INFORMATION ON INGREDIENTS.

3.1 Substances.

Chemical Name:	DL-Methionine
CAS No:	59-51-8
CE No:	200-432-1
Registration No:	N/D

3.2 Mixtures.
Not Applicable.

SECTION 4: FIRST AID MEASURES.

4.1 Description of first aid measures.
Due to the composition and type of the substances present in the product, no particular warnings are necessary.

Inhalation.

-Continued *on next page.*-

Take the victim into open air; keep them warm and calm. If breathing is irregular or stops, perform artificial respiration. Do not administer anything orally. If unconscious, place them in a suitable position and seek medical assistance.

Eye contact.
If wearing contact lenses, remove them. Wash eyes with plenty of clean and cool water for at least 10 minutes while pulling eyelids up, and seek medical assistance.

Skin contact.
Remove contaminated clothing. Wash skin vigorously with water and soap or a suitable skin cleaner. **NEVER** use solvents or thinners.

Ingestion.
If accidentally ingested, seek immediate medical attention. Keep calm. **NEVER** induce vomiting.

4.2 Most important symptoms and effects, both acute and delayed.
No known acute or delayed effects from exposure to the product.

4.3 Indication of any immediate medical attention and special treatment needed.
In case of doubt or when symptoms of feeling unwell persist, get medical attention. Never administer anything orally to persons who are unconscious.

SECTION 5: FIREFIGHTING MEASURES.

5.1 Extinguishing media.
Recommended extinguishing methods.
Extinguisher powder or CO_2. In case of more serious fires, also alcohol-resistant foam and water spray. Do not use a direct stream of water to extinguish.

5.2 Special hazards arising from the substance.
Special risks.
Fire can cause thick, black smoke. As a result of thermal decomposition, dangerous products can form: carbon monoxide, carbon dioxide. Exposure to combustion or decomposition products can be harmful to your health.

5.3 Advice for firefighters.
Use water to cool tanks, cisterns, or containers close to the heat source or fire. Take wind direction into account. Prevent the products used to fight the fire from going into drains, sewers, or waterways.
Fire protection equipment.
According to the size of the fire, it may be necessary to use protective suits against the heat, individual breathing equipment, gloves, protective goggles or facemasks, and gloves.

SECTION 6: ACCIDENTAL RELEASE MEASURES.

6.1 Personal precautions, protective equipment and emergency procedures.
For exposure control and individual protection measures, see section 8.

6.2 Environmental precautions.
Product not classified as hazardous for the environment, avoid spillage as much as possible.

6.3 Methods and material for containment and cleaning up.
The contaminated area should be immediately cleaned with an appropriate de-contaminator. Pour the decontaminator on the remains in an opened container and let it act various days until no further reaction is produced.

6.4 Reference to other sections.
For exposure control and individual protection measures, see section 8.
For later elimination of waste, follow the recommendations under section 13.

SECTION 7: HANDLING AND STORAGE.

-Continued *on next page.*-

7.1 Precautions for safe handling.
The product does not require special handling measures, the following general measures are recommended:
For personal protection, see section 8. Never use pressure to empty the containers. They are not pressure-resistant containers.
In the application area, smoking, eating, and drinking must be prohibited.
Follow legislation on occupational health and safety.
Keep the product in containers made of a material identical to the original.

7.2 Conditions for safe storage, including any incompatibilities.
The product does not require special storage measures.
As general storage measures, sources of heat, radiation, electricity and contact with food should be avoided.
Keep away from oxidising agents and from highly acidic or alkaline materials.
Store according to local legislation. Observe indications on the label.
The product is not affected by Directive 2012/18/EU (SEVESO III).
7.3 Specific end use(s).

SECTION 8: EXPOSURE CONTROLS/PERSONAL PROTECTION.

8.1 Control parameters.

The product does NOT contain substances with Professional Exposure Environmental Limit Values.The product does NOT contain substances with Biological Limit Values.
8.2 Exposure controls.

Measures of a technical nature:
Provide adequate ventilation, which can be achieved by using good local exhaust-ventilation and a good general exhaust system.

Concentration:	**100 %**		
Uses:	**For manufacturing, processing, laboratory or repacking use only**		
Breathing protection:			
If the recommended technical measures are observed, no individual protection equipment is necessary.			
Hand protection:			
PPE:	Protective gloves.		
Characteristics:	«CE» marking, category II.		
CEN standards:	EN 374-1, En 374-2, EN 374-3, EN 420		
Maintenance:	Keep in a dry place, away from any sources of heat, and avoid exposure to sunlight as much as possible. Do not make any changes to the gloves that may alter their resistance, or apply paints, solvents or adhesives.		
Observations:	Gloves should be of the appropriate size and fit the user's hand well, not being too loose or too tight. Always use with clean, dry hands.		
Material:	PVC (polyvinyl chloride)	Breakthrough time (min.): > 480	Material thickness (mm): 0,35
Eye protection:			
PPE:	Face shield.		
Characteristics:	«CE» marking, category II. Face and eye protector against splashing liquid.		
CEN standards:	EN 165, EN 166, EN 167, EN 168		
Maintenance:	Visibility through lenses should be ideal. Therefore, these parts should be cleaned daily. Protectors should be disinfected periodically following the manufacturer's instructions. Make sure that mobile parts move smoothly.		
Observations:	Face shields should offer a field of vision with a dimension in the central line of, at least, 150 mm vertically once attached to the frame.		
Skin protection:			
PPE:	Protective clothing.		
Characteristics:	«CE» marking, category II. Protective clothing should not be too tight or loose in order not to obstruct the user's movements.		
CEN standards:	EN 340		
Maintenance:	In order to guarantee uniform protection, follow the washing and maintenance instructions provided by the manufacturer.		
Observations:	The protective clothing should offer a level of comfort in line with the level of protection provided in terms of the hazard against which it protects, bearing in mind environmental conditions, the user's level of activity and the expected time of use.		

-Continued *on next page.*-

PPE:	Work footwear.
Characteristics:	«CE» marking, category II.
CEN standards:	EN ISO 13287, EN 20347
Maintenance:	This product adapts to the first user's foot shape. That is why, as well as for hygienic reasons, it should not be used by other people.
Observations:	Work footwear for professional use includes protection elements aimed at protecting users against any injury resulting from an accident

SECTION 9: PHYSICAL AND CHEMICAL PROPERTIES.

9.1 Information on basic physical and chemical properties.
Appearance:Solid
Colour: N.A./N.A.
Odour:N.A./N.A.
Odour threshold:N.A./N.A.
pH:5,6-6,1 (2%, 20ºC)
Melting point:275 ºC
Boiling Point: N.A./N.A.
Flash point: N.A./N.A.
Evaporation rate: N.A./N.A.
Inflammability (solid, gas): N.A./N.A.
Lower Explosive Limit: N.A./N.A.
Upper Explosive Limit: N.A./N.A.
Vapour pressure: N.A./N.A.
Vapour density:N.A./N.A.
Relative density:1,34 g/cm^3
Solubility:34 g/l (25ºC)
Liposolubility: N.A./N.A.
Hydrosolubility: N.A./N.A.
Partition coefficient (n-octanol/water): -2,41 (log Pow)
Auto-ignition temperature: 390ºC
Decomposition temperature: 280ºC
Viscosity: N.A./N.A.
Explosive properties: N.A./N.A.
Oxidizing properties: N.A./N.A.
N.A./N.A.= Not Available/Not Applicable due to the nature of the product

9.2. Other information.

SECTION 10: STABILITY AND REACTIVITY.

10.1 Reactivity.
The product does not present hazards by their reactivity.

10.2 Chemical stability.
Unstable in contact with:
- Bases.

10.3 Possibility of hazardous reactions.
Neutralization can occur on contact with bases.

10.4 Conditions to avoid.
- Avoid contact with bases.

10.5 Incompatible materials.
Avoid the following materials:
- Bases.

10.6 Hazardous decomposition products.
Depending on conditions of use, can be generated the following products:
- Corrosive vapors or gases.

-Continued *on next page.*-

SECTION 11: TOXICOLOGICAL INFORMATION.

11.1 Information on toxicological effects.
Repeated or prolonged contact with the product can cause the elimination of oil from the skin, giving rise to non-allergic contact dermatitis and absorption of the product through the skin.
Splatters in the eyes can cause irritation and reversible damage.

Toxicological information.

Name	Acute toxicity			
	Type	Test	Kind	Value
DL-Methionine CAS No: 59-51-8 EC No: 200-432-1	Oral	DL50	Rat	5 g/kg
	Dermal			
	Inhalation			

a) acute toxicity;
Not conclusive data for classification.

b) skin corrosion/irritation;
Not conclusive data for classification.

c) serious eye damage/irritation;
Not conclusive data for classification.

d) respiratory or skin sensitisation;
Not conclusive data for classification.

e) germ cell mutagenicity;
Not conclusive data for classification.

f) carcinogenicity;
Not conclusive data for classification.

g) reproductive toxicity;
Not conclusive data for classification.

h) STOT-single exposure;
Not conclusive data for classification.

i) STOT-repeated exposure;
Not conclusive data for classification.

j) aspiration hazard;
Not conclusive data for classification.

SECTION 12: ECOLOGICAL INFORMATION.

12.1 Toxicity.
No information is available regarding the ecotoxicity.

12.2 Persistence and degradability.
No information is available about persistence and degradability of the product.

12.3 Bioaccumulative potencial.
Information about the bioaccumulation.

Name	Bioaccumulation			
	Log Pow	BCF	NOECs	Level

-Continued *on next page.*-

DL-Methionine N. CAS: 59-51-8 EC No: 200-432-1	-2,41	-	-	Very low

12.4 Mobility in soil.
No information is available about the mobility in soil.
The product must not be allowed to go into sewers or waterways.
Prevent penetration into the ground.

12.5 Results of PBT and vPvB assessment.
No information is available about the results of PBT and vPvB assessment of the product.

12.6 Other adverse effects.
No information is available about other adverse effects for the environment.

SECTION 13 DISPOSAL CONSIDERATIONS.

13.1 Waste treatment methods.
Do not dump into sewers or waterways. Waste and empty containers must be handled and eliminated according to current, local/national legislation.
Follow the provisions of Directive 2008/98/EC regarding waste management.

SECTION 14: TRANSPORT INFORMATION.

Transportation is not dangerous. In case of road accident causing the product's spillage, proceed in accordance with point 6.
14.1 UN number.
Transportation is not dangerous.

14.2 UN proper shipping name.
Transportation is not dangerous.

14.3 Transport hazard class(es).
Transportation is not dangerous.

14.4 Packing group.
Transportation is not dangerous.

14.5 Environmental hazards.
Transportation is not dangerous.

14.6 Special precautions for user.
Transportation is not dangerous.

14.7 Transport in bulk according to Annex II of MARPOL 73/78 and the IBC Code.
Transportation is not dangerous.

SECTION 15: REGULATORY INFORMATION.

15.1 Safety, health and environmental regulations/legislation specific for the substance.
The product is not affected by the Regulation (EC) No 1005/2009 of the European Parliament and of the Council of 16 September 2009 on substances that deplete the ozone layer.

The product is not affected by Directive 2012/18/EU (SEVESO III).
The product is not affected by Regulation (EU) No 528/2012 concerning the making available on the market and use of biocidal products.
The product is not affected by the procedure established Regulation (EU) No 649/2012, concerning the export and import of dangerous chemicals.

15.2 Chemical safety assessment.

-Continued *on next page.*-

There has been no evaluation a chemical safety assessment of the product.

SECTION 16: OTHER INFORMATION.

Sections changed compared with the previous version:

1,16

It is recommended that the product only be employed for the purposes advised.

Abbreviations and acronyms used:
BCF: Bioconcentration factor.
CEN: European Committee for Standardization.
EC50: Half maximal effective concentration.
PPE: Personal protection equipment.
LC50: Lethal concentration, 50%.
LD50: Lethal dose, 50%.
Log Pow: Logarithm of the partition octanol-water.
NOEC: No observed effect concentration.

Key literature references and sources for data:
http://eur-lex.europa.eu/homepage.html
http://echa.europa.eu/
Regulation (EU) 2015/830.
Regulation (EC) No 1907/2006.
Regulation (EU) No 1272/2008.

The information given in this Safety Data Sheet has been drafted in accordance with COMMISSION REGULATION (EU) 2015/830 of 28 May 2015 amending Regulation (EC) No 1907/2006 OF THE EUROPEAN PARLIAMENT AND OF THE COUNCIL of 18 December 2006 concerning the Registration, Evaluation, Authorisation and Restriction of Chemicals (REACH), establishing a European Chemicals Agency, amending Directive 1999/45/EC and repealing Council Regulation (EEC) No 793/93 and Commission Regulation (EC) No 1488/94 as well as Council Directive 76/769/EEC and Commission Directives 91/155/EEC, 93/67/EEC, 93/105/EC and 2000/21/EC.

The information in this Safety Data Sheet on the Preparation is based on current knowledge and on current EC and national laws, as far as the working conditions of the users is beyond our knowledge and control. The product must not be used for purposes other than those that are specified without first having written instructions on how to handle. It is always the responsibility of the user to take the appropriate measures in order to comply with the requirements established by current legislation. The information contained in this Safety Sheet only states a description of the safety requirements for the preparation, and it must not be considered as a guarantee of its properties.

-End *of safety data sheet..*-

1.6 Freedom of Information Act, 5 U.S.C. 552

It is Nestec's view that all data and information presented in Parts 2 through 7 of this Notice do not contain any trade secret, commercial, or financial information that is privileged or confidential, and therefore all data and information presented herein are not exempt from the Freedom of Information Act, 5 U.S.C. Section 552.

Part 2. §170.230 Identity, Method of Manufacture, Specifications, and Physical or Technical Effect

2.1 Identity

2.1.1 Common or Usual Name

FEMA Common Name: *Corynebacterium glutamicum* corn syrup fermentation product

FEMA No.: 4907

Commercial Name: Savory Base 100 "Corn Sauce" (Savory Base 100)

Historical/alternative denotations (used in supporting documentation):

- He Wei C. Essence I;
- Savory Seasoning Sauce 1 (SSS 1);
- Corn Seasoning Sauce 1; and
- Savory Corn Sauce 1 (SCS 1).

2.1.2 Chemical Name

Not applicable.

2.1.3 Chemical Abstract Service (CAS) Number

Not applicable.

2.1.4 Chemical and Physical Characteristics

Savory Base 100 is a pale brown to brownish paste with a savory taste. Some of the constituents that contribute to the characteristic savory flavor of Savory Base 100 include glutamic acid, L-alanine, succinic acid, formic acid, and an intrinsic mix of other free and bound amino acids, organic acids, Amadori and Maillard products, and minerals and their salts.

2.2 Method ofManufacturing

2.2.1 Raw Materials and Processing Aids

The raw materials (carbon and nitrogen source) and processing aids (*e.g.*, salts and minerals, anti-foaming aids and pH adjustment aids) and food contact materials used during the production of Savory Base 100 are food grade quality[1] and are used in accordance with an appropriate federal regulation, or have been determined to be GRAS for their respective uses[2]. Corn glucose syrup is used as a carbon source and liquid anhydrous ammonia is used as a nitrogen source to support microbial growth and metabolism during fermentation.

2.2.2 Manufacturing Process

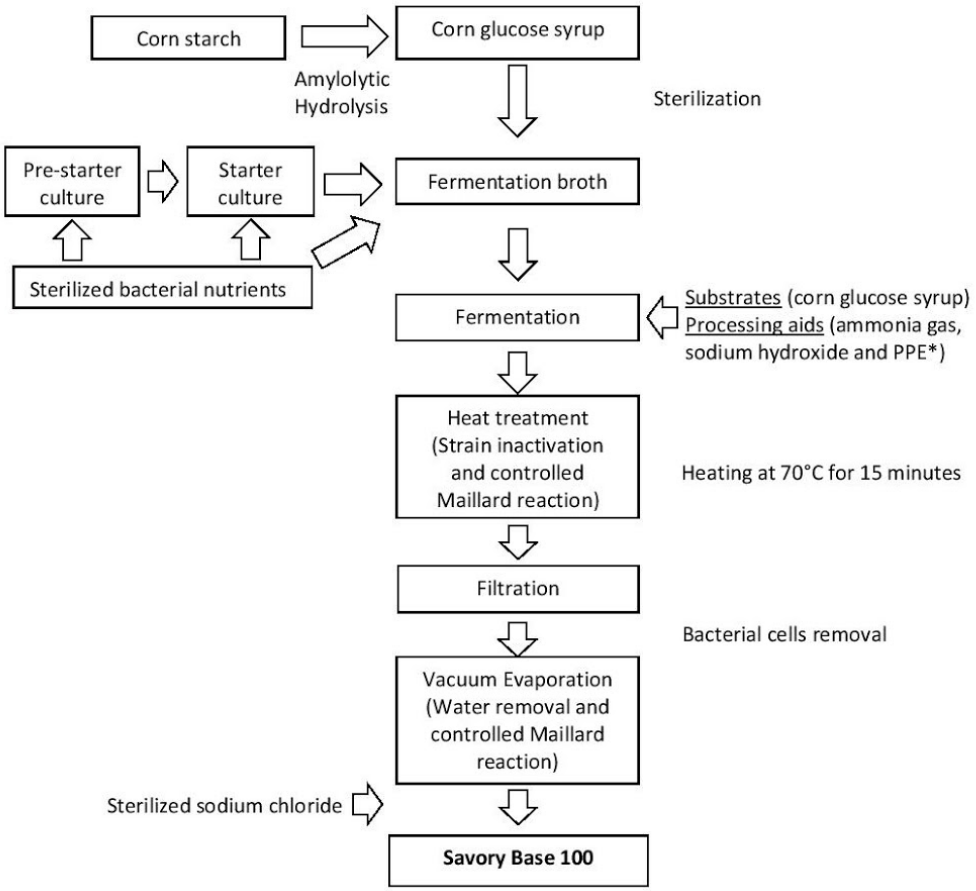
Savory Base 100 is manufactured by submerged fermentation of *C. glutamicum* in glucose-based media (enzymatically hydrolyzed corn starch) in compliance with requirements for risk-based preventive controls mandated by the FDA Food Safety Modernization Act (FSMA), current Good Manufacturing Practices (cGMPs) and the principles of Hazards Analysis and Critical Control Points (HACCP). Briefly, the process involves production of a fermentation broth, to which a *C. glutamicum* starter culture is added, followed by heating, filtration, and vacuum evaporation. A schematic overview of the production process is provided in Figure 2.2.2-1.

The submerged fermentation process is initiated by preparation of a fermentation broth (within a sterilized fermentation vessel), which contains sterilized nutrients for bacterial growth, substrates, and sterilized pH regulators. A small pre-starter culture is prepared separately with *C. glutamicum*, which is incubated in a medium containing the nutrients for optimum growth. This pre-starter culture is scaled up to produce the biomass, which is transferred to the primary fermentation vessel (containing the submerged fermentation broth) and then incubated. Processing aids are added during fermentation to regulate pH and reduce/prevent formation of foam. Substrates are also replenished during fermentation.

After fermentation is complete, the broth is heated to inactivate the bacteria, as well as to initiate a controlled Maillard reaction in order to achieve the desired color flavor and taste, before the broth is filtered to remove the bacterial cells (this process is monitored at Critical Control Point 3 of the HACCP plan); see Section 2.3.4 for information regarding the absence of the bacteria from the final product. The broth then undergoes vacuum evaporation, to remove water as well as initiate a second controlled Maillard reaction. At the same time sterilized sodium chloride is added to improve shelf life stability and microbial resistance against contaminants, producing the final Savory Base 100 "Corn Sauce".

[1] Specifications compliant with U.S Food Chemicals Codex, or equivalent international standard *E.g.,* US/EU Pharmacopoeia standards.
[2] *E.g.*, Antifoams or flocculants used in fermentation and recovery are used in accordance with the Enzyme Technical Association submissions to FDA.

Figure 2.2.2-1 Schematic Overview of the Manufacturing Process for Savory Base 100 "Corn Sauce" (Savory Base 100)



*Polyoxyethylene polyoxypropylene pentaerythritol ether

2.3 Product Specifications and Batch Analysis

2.3.1 Product Specifications

The product specifications for Savory Base 100 are presented in Table 2.3.1-1.

Table 2.3.1-1 Product Specifications and Analytical Methods for Savory Base 100 "Corn Sauce" (Savory Base 100)

Specification Parameter		Specification	Method
Appearance	As is	Uniform pale brown to brownish paste	Visual test
	After preparation	Clear solution and free from visible particles or insoluble matter	
Odor ('as is' and 'after preparation')		Characteristic of Savory Base 100 flavor, free from foreign and off odors	Organoleptic test
Taste (after preparation)		Characteristic of Savory Base 100 flavor, umami, slightly salty and not bitter or burned. Free from foreign and off flavors	Organoleptic test
pH (10% dry matter solution)		5.5 to 7	APHA 4500-H+
Compositional Parameters			
Moisture content (%)		27 to 34	IDF - FIL 26A
L-Glutamic acid (%) (free)		34 to 44	AOAC 982.30
L-Alanine (%)		0.8 to 2.3	AOAC 982.30
Succinic acid (%)		0.3 to 0.7	AOAC 986.13
Formic acid (%)		0.4 to 1.2	AOAC 986.13
Total nitrogen (%)		4 to 7	ISO/FDIS 16634
Ash (%)		10 to 18	AOAC 923.03
Sodium chloride (%)		5.5 to 8	AOAC 986.26
Heavy Metals			
Arsenic (mg/kg)		≤0.5	AOAC 984.27
Lead (mg/kg)		<0.02	AOAC 984.27
Cadmium (mg/kg)		<0.01	AOAC 984.27
Mercury (mg/kg)		<0.004	AOAC 984.27
Microbiological Parameters			
Aerobic plate count (CFU/g)		≤10,000	ISO 4833:2003 AOAC method 990.12
Yeasts and molds (CFU/g)		≤100	ISO-21527-2:2008
Enterobacteriaceae (CFU/g)		≤10	ISO 21528-2:2004
Salmonella		Negative/25g	-AFNOR TRA 02/08 – 03/01 alternative method according to ISO 16140 standard:2003 -AOAC 010602

AFNOR TRA = French National Organization for Standardization; AOAC = Association of Official Agricultural Chemists; APHA = American Public Health Association; CFU = colony forming units; FDIS = Final Draft International Standard; IDF – FIL = International Dairy Federation; ISO = International Standards Organization.

2.3.2 Batch Analyses

Data from the analysis of five non-consecutive lots of Savory Base 100 demonstrating the consistency of the manufacturing process and compliance with the ingredient specifications are presented in Table 2.3.2-1.

Table 2.3.2-1 Batch Analysis Data for 5 Representative Batches of Savory Base 100 "Corn Sauce" (Savory Base 100)

Specification Parameter		Specification	Manufacturing Lot				
			G151002[a]	G160302[b]	G160304[c]	G170213[d]	G170215[e]
Appearance	As is	Uniform pale brown to brownish paste	Conforms	Conforms	Conforms	Conforms	Conforms
	After preparation	Clear solution and free from visible particles or insoluble matter	Conforms	Conforms	Conforms	Conforms	Conforms
Odor ('as is' and 'after preparation')		Characteristic of Savory Base 100 flavor, free from foreign and off odors	Conforms	Conforms	Conforms	Conforms	Conforms
Taste (after preparation)		Characteristic of Savory Base 100 flavor, umami, slightly salty and not bitter or burned. Free from foreign and off flavors	Conforms	Conforms	Conforms	Conforms	Conforms
pH (10% dry matter solution)		5.5 to 7	5.6	5.6	5.5	5.5	6.3
Compositional Parameters							
Loss on drying (%)		27 to 34	33	32	32	31	29
L-Glutamic acid (%) (free)		34 to 44	37.00	37.20	39.70	35.2	34.1
L-Alanine (%) (free)		0.8 to 2.3	1.23	0.98	0.82	2.23	1.83
Succinic acid (%)		0.3 to 0.7	0.56	0.61	0.55	0.38	0.33
Formic acid (%)		0.4 to 1.2	1	0.73	0.42	0.68	1.18
Total nitrogen (%)		4 to 7	6.3	6.4	6.2	6.2	5.8
Ash (%)		10 to 18	11	13	12	14	15
Sodium chloride (%)		5.5 to 8	5.6	7.1	6.5	6.5	7.6
Heavy Metals							
Arsenic (mg/kg)		≤0.5	<0.05	<0.02	<0.05	<0.05	<0.05
Lead (mg/kg)		<0.02	<0.02	<0.007	<0.02	0.028	<0.02
Cadmium (mg/kg)		<0.01	<0.01	<0.005	<0.01	<0.01	<0.01
Mercury (mg/kg)		<0.004	<0.003	<0.003	<0.004	<0.003	<0.003
Microbiological Parameters							
Aerobic plate count (CFU/g)		≤10,000	450	10	<10	<100	<100
Yeasts and molds (CFU/g)		≤100	<10	<10	<10	<10	<10

Table 2.3.2-1 Batch Analysis Data for 5 Representative Batches of Savory Base 100 "Corn Sauce" (Savory Base 100)

Specification Parameter	Specification	Manufacturing Lot				
		G151002[a]	G160302[b]	G160304[c]	G170213[d]	G170215[e]
Enterobacteriaceae (CFU/g)	≤10	<10	<10	<10	<10	<10
Salmonella	Negative/ 25g	Negative/ 25 g	Negative/ 25 g	Negative/ 25 g	Negative/ 25 g	Negative/ 25 g

CFU = colony forming units.
[a] Manufacturing date: October 18, 2015.
[b] Manufacturing date: March 2, 2016.
[c] Manufacturing date: March 3, 2016.
[d] Manufacturing date: February 25, 2017.
[e] Manufacturing date: February 26, 2017.

2.3.3 Additional Chemical Characterization

The mineral profile of 5 non-consecutive industrial scale lots of Savory Base 100 are presented in Table 2.3.3-1.

Table 2.3.3-1 Mineral Profile for 5 Non-Consecutive Lots of Savory Base 100 "Corn Sauce"

Parameter (values given on a dry weight basis)	Manufacturing Lot				
	G151002[a]	G160302[b]	G160304[c]	G170213[d]	G170215[e]
Mineral profile					
Sodium (%)	4.03	4.79	4.57	6.02	7.65
Potassium (%)	0.94	1.00	0.89	0.71	0.75
Magnesium (%)	0.06	0.07	0.06	0.04	0.05
Calcium (%)	0.02	0.02	0.02	0.02	0.02
Chloride (%)	3.32	3.65	4.11	3.77	4.5
Phosphate (%)	0.49	0.54	0.45	0.56	0.61
Sulfate (%)	0.15	0.20	0.14	0.16	0.15

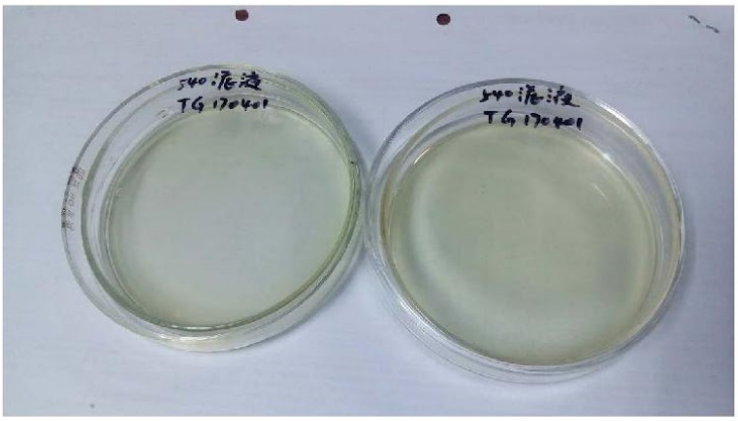
[a] Manufacturing date: October 18, 2015.
[b] Manufacturing date: March 2, 2016.
[c] Manufacturing date: March 3, 2016.
[d] Manufacturing date: February 25, 2017.
[e] Manufacturing date: February 26, 2017.

2.3.4 Other Impurities from Fermentation Media

2.3.4.1 Production Organism

The production organism (*C. glutamicum*) is excluded from the fermentate during production of Savory Base 100 using microfiltration (0.22 µm). The effectiveness of the microfiltration system was evaluated using 1 mL of Savory Base 100 filtrate, which was mixed with 15 to 20 mL of plate count agar (PCA), cooled at 46°C and then incubated at 36±1°C for approximately 48 hours. As shown in Figure 2.3.4.1-1, no microbial growth was detectable in the media.

Figure 2.3.4.1-1 Absence of the Production Organism Following Microfiltration



Absence of the fermentation strain is also corroborated by the low residual levels of protein in Savory Base 100. Three samples of Savory Base 100 were analyzed for protein content using sodium dodecyl sulfate polyacrylamide gel electrophoresis (SDS-PAGE) withCoom assie blue staining and the Bradford assay. As shown in Table 2.3.4.1-1, no appreciable protein levels could be detected in the ingredient. Small quantities of oligopeptides or other interfering substances likely account for the residual levels of protein that were detected.

Table 2.3.4.1-1 Protein Content of Savory Base 100 "Corn Sauce"

Sample	Bradford Assay	SDS-PAGE
	Protein Concentration (mg/mL)	Protein quantity (intact/theoretical protein content) (ppm)
1 (Lot L4K-00001)	0.005 ± 0.003	33
2 (Lot L4K-00002)	ND	92
3 (Lot L4K-00003)	0.193 ± 0.002	139

ND = not detected; SDS-PAGE = sodium dodecyl sulfate polyacrylamide gel electrophoresis.

2.3.4.2 Biogenic Amines

Biogenic amines are biologically active organic compounds present naturally inanimals and humans. The main source of exogenous amines is through consumption of foods such as fish, fish products and fermented foodstuffs (meat, dairy, vegetables, beers, and wines) (EFSA, 2011). As detailed in Table 2.3.4.2-1 below, results of analyses for biogenic amines did not identify detectable levels of phenethylamine, cadaverine, histamine, spermidine or spermine in Savory Base 100. Only minimal levels of putrescine (1.4 mg/kg), tyramine (5.4 mg/kg) and tryptamine (3.5 mg/kg) were detected, which are far below (or within, in the case of tryptamine) reported mean values of putrescine (87.3 to 222 mg/kg), tyramine (24.7 to 235 mg/kg) and tryptamine (2.4 to 7.2 mg/kg) detected in sauerkraut (Sahu *et al.*, 2015) and also lower than maximum levels found in other commercial ready-to-eat products (Table 2.3.4.2-2).

Table 2.3.4.2-1 Biogenic Amine Levels in Savory Base 100 "Corn Sauce"

Specification Parameter	Result (mg/kg)	Quantification Limit	Method of Analysis
Phenethylamine	<LQ	1	AM-BIOGE 2014 Rev.3 - HPLC-DAD
Cadaverine	<LQ	1	
Histamine	<LQ	1	
Putrescine	1.4 ± 0.4	1	
Spermidine	<LQ	1	
Spermine	<LQ	1	
Tyramine	5.4 ± 1.3	1	
Tryptamine	3.5 ± 0.9	0.5	
Biogenic Amine Index	1.4 ± 0.43	N/A	

HPLC-DAD = high performance liquid chromatography with diode array detection; LQ = quantification limit; N/A = not applicable.

Table 2.3.4.2-2 Biogenic Amine Levels in Commercial Ready-to-Eat Products

Specification Parameter	Result (mg/kg)					
	Soy Products	Miso Products	Ketchup	Finnish Dry Sausages	Washed-Rind	Parmesan
Phenylethylamine	NR	NR	NR	<1 to 48	NR	NR
Cadaverine	nd to 128	nd to 201	1.4 to 131	NR	NR	NR
Histamine	nd to 234	nd to 221	2 to 18	<1 to 200	Nd	1.4 ± 0.04
Putrescine	nd to 360	nd to 12	2.4 to 165	NR	NR	NR
Spermidine	NR	NR	NR	NR	NR	30.7 ± 1.9
Spermine	NR	NR	NR	NR	13.6 (nd to 70.5)	NR
Tyramine	nd to 237	nd to 434	4.5 to 149	82	NR	NR
Tryptamine	NR	NR	NR	<10 to 91	NR	NR
Biogenic Amine Index	nd to 959	nd to 868	10 to 463	NR	6.6	9.8

nd = not detected; NR = not reported.
Results presented as the range (soy products, miso products and ketchup) or the mean concentration (non-irradiated blue cheese, washed-rind, and parmesan).
Sources: Eerola *et al.* (1998); Prester (2016).

2.3.5 Other Internal Quality Control Analyses

2.3.5.1 Mycotoxins

As part of Nestec's internal quality control procedures, select lots of Savory Base 100 are routinely analyzed for mycotoxin contamination. The results of analysis of 5 non-sequential batches for Savory Base 100 are summarized in Table 2.3.5.1-1.

Table 2.3.5.1-1 Analysis of Mycotoxins in 5 Batches of Savory Base 100

Parameter	Specifications	Batch Number				
		G151002[a]	G160302[b]	G160304[c]	G170213[d]	G170215[e]
Aflatoxins (Sum of B and G) (µg/kg)	≤4	<4	<4	<4	<4	<4
Ochratoxin A (µg/kg)	≤0.5	<0.5	<0.5	<0.5	<0.5	<0.5
Fumonisins (Sum of B_1 and B_2) (µg/kg)	≤100	<100	<100	<100	<100	<100
Deoxynivalenol/Vomitoxin (µg/kg)	≤50	<50	<50	<50	<50	<50
Zearalenone (µg/kg)	≤20	<20	<20	<20	<20	<20

[a] Manufacturing date: October 18, 2015.
[b] Manufacturing date: March 2, 2016.
[c] Manufacturing date: March 3, 2016.
[d] Manufacturing date: February 25, 2017.
[e] Manufacturing date: February 26, 2017.

2.3.5.2 Heterocyclic Amines

As previously discussed in Section 2.1.4, Maillard reaction products, formed from the reaction between a reducing sugar and a food-grade nitrogen source (*e.g.*, amino acids), contribute to the distinct desirable flavor notes in Savory Base 100. However, Maillard-type reactions may also rise to undesirable substances such as heterocyclic amines (HCAs). These carcinogenic by-products are formed in the presence of creatine or creatinine (major components of muscle in meats and fish) and during heat processing of animal products at temperatures greater >130°C (Jägerstad *et al.*, 1991; Skog *et al.*, 1998), due to the reaction between creatine or creatinine with amino acids and sugars. Although the fermentation broth used in the manufacture of Savory Base 100 is enriched in amino acids and sugars, it does not contain creatine or creatinine, as it is not derived from animal sources. In addition, the temperature used during the manufacturing process of Savory Base 100 (*i.e.*, 70°C) does not reach a temperature at which formation of HCAs is favorable (*i.e.*, >130°C). Considering this, neither the composition nor the manufacturing process of Savory Base 100 is conducive to formation of such by-products.

2.4 Stability Data

The sensory and microbiological and chemical stability of Savory Base 100 was tested using a single lot of Savory Base 100 (lot number 363976). Each sample (100 g) was stored in a dual-layered, low-density polyethylene (LDPE) bag (enclosed within an aluminum pouch) and stored for up to 360 days (1 year). Sensory and chemical stability was evaluated at 30, 90, 150, 180, 240, 300, and 360 days, while microbiological stability was analyzed after 1 year only.

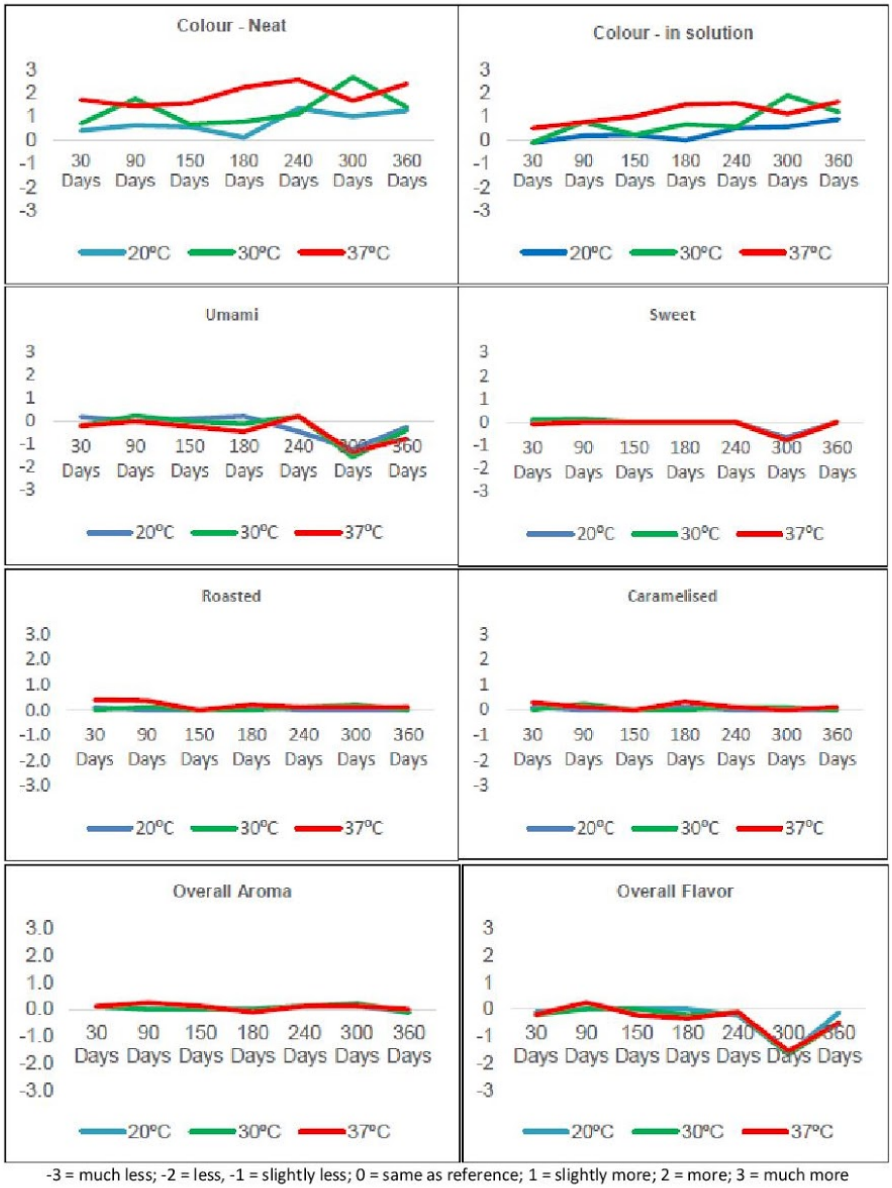
2.4.1 Sensory Stability

A panel of 8 trained internal sensory evaluators used a 7-point bipolar evaluation scale to score samples for taste (umami, sweet, roasted, caramelized and overall flavor), color [neat and in solution (as prepared for tasting)] and smell (overall aroma); the scoring scale is given as part of Figure 2.4.1-1. Tasting doses were prepared by dilution of 4 g Savory Base 100 paste in 1 liter of water (90°C) followed by stirring until visibly homogeneous; samples were served at 70°C (±5°C) for tasting. Test samples were stored (blinded and identifiable only by 3-digit code) at temperatures of 20, 30, or 37°C and at relative humidities of 50, 70, and

75%, respectively; samples stored at 4°C were assumed to be stable for the analysis period and were used as the reference (labeled as such).

As illustrated in Figure 2.4.1-1, the color of samples (whether neat or in solution) were darker with increasing temperature and humidity, and generally became darker over time. In terms of taste, there were minimal changes in roasted and caramelized flavors (regardless of temperature, humidity, or time); however, umami, sweet and overall flavor were all less detectable after 300 days (at all temperatures), then became slightly more similar to the reference after 1 year.

Figure 2.4.1-1 Sensory Stability Evaluation of Savory Base 100 "Corn Sauce"



-3 = much less; -2 = less, -1 = slightly less; 0 = same as reference; 1 = slightly more; 2 = more; 3 = much more

2.4.2 Chemical Stability

For evaluation of chemical stability, samples were homogenized before analysis of water activity, pH, and total acidity (as acetic or citric acid) when stored refrigerated (4°C) or at temperatures of 20, 30, or 37°C and at relative humidities of 50, 70, and 75%, respectively. As shown in Table 2.4.2-1 below there were no significant changes in any of the parameters measured (with all values remaining within specification, where applicable), regardless of temperature and relative humidity, when Savory Base 100 was stored for up to 1 year. Savory Base 100 is stable for at least 1 year under accelerated conditions.

Table 2.4.2-1 Accelerated Stability of Savory Base 100 "Corn Sauce" (Lot 363976)

Parameter	Specification	Analytical Data						
		Time (days)						
		30	90	150	180	240	300	360
Temperature = 4°C								
Water activity at 25°C	≤0.75	0.731	0.723	0.721	0.722	0.724	0.724	0.722
pH at 25°C	5.5-7.0	6.15	6.20	6.27	6.28	6.24	6.23	6.24
Total acidity – as acetic acid (g/100g)	N/A	2.17	2.19	2.24	2.51	2.32	2.20	2.04
Total acidity – as citric acid (g/100g)	N/A	2.53	2.55	2.61	2.93	2.57	2.50	2.38
Temperature = 20°C, RH = 50%								
Water activity at 25°C	≤0.75	0.728	0.726	0.726	0.723	0.739	0.720	0.723
pH at 25°C	5.5 to 7.0	6.16	6.27	6.19	6.29	6.22	6.22	6.24
Total acidity – as acetic acid (g/100g)	N/A	2.17	2.19	2.24	2.51	2.32	2.20	2.04
Total acidity – as citric acid (g/100g)	N/A	2.53	2.55	2.61	2.93	2.57	2.50	2.38
Temperature = 30°C, RH = 70%								
Water activity at 25°C	≤0.75	0.725	0.723	0.724	0.720	0.723	0.720	0.723
pH at 25°C	5.5 to 7.0	6.16	6.21	6.23	6.24	6.30	6.27	6.24
Total acidity – as acetic acid (g/100g)	N/A	2.19	2.20	2.17	2.47	2.22	2.11	2.06
Total acidity – as citric acid (g/100g)	N/A	2.55	2.57	2.53	2.88	2.46	2.47	2.40
Temperature = 37°C, RH = 75%								
Water activity at 25°C	≤0.75	0.723	0.720	0.720	0.717	0.716	0.725	0.725
pH at 25°C	5.5 to 7.0	6.17	6.32	6.22	6.23	6.29	6.25	6.21
Total acidity – as acetic acid (g/100g)	N/A	2.18	2.19	2.14	2.47	2.21	2.11	2.06
Total acidity – as citric acid (g/100g)	N/A	2.55	2.55	2.50	2.88	2.46	2.43	2.40

N/A = not applicable; RH = relative humidity.

2.4.3 Microbiological Stability

Savory Base 100 was also analyzed for the presence of microorganisms (Enterobacteriaceae and aerobic plate count) on Day 0 at room temperature and after 1 year when stored refrigerated (4°C) or at temperatures of 20, 30, or 37°C and at relative humidities of 50, 70, and 75%, respectively. These data are presented in Table 2.4.3-1 and show that the numbers of bacteria present in the sample after 1 year remained consistent with those on Day 0 (regardless of storage conditions), and within proposed specifications, demonstrating that Savory Base 100 is microbiologically stable for at least 1 year under accelerated conditions.

Table 2.4.3-1 Microbiological Stability of Savory Base 100 "Corn Sauce" (Lot 363976)

Time (days)	Storage Conditions	Enterobacteriaceae (CFU/g)		Aerobic Plate Count (CFU/g)	
		Specification	Analytical Data	Specification	Analytical Data
0	Room temperature	≤10	<10	≤10,000	<1,000
360	Temperature = 4°C		<10		270
	Temperature = 20°C, RH = 50%		<10		340
	Temperature = 30°C, RH = 70%		<10		200
	Temperature = 37°C, RH = 75%		<10		290

CFU = colony forming units; RH = relative humidity.

Part 3. §170.235 Dietary Exposure

3.1 Current Regulatory Status in the United States

Savory Base 100, under the substance name, "*Corynebacterium glutamicum* corn syrup fermentation product", was granted FEMA GRAS status for use as a flavoring agent in a variety of food and beverage products at use levels up to 5,100 ppm (FEMA No. 4907).

3.2 Estimated Dietary Consumption of Savory Base 100 from Intended Food Uses

3.2.1 Methodology

An assessment of the anticipated dietary exposure to Savory Base 100 as an ingredient under the intended conditions of use (see Table 3.1.2-1) was conducted using data available in the 2011-2012 cycles of the U.S. National Center for Health Statistics' (NCHS) National Health and Nutrition Examination Survey (NHANES) (CDC, 2015). A summary of the survey and methodology employed in the intake assessment of Savory Base 100 along with the pertinent results is presented herein.

The NHANES data are collected and released in 2-year cycles with the most recent cycle containing data collected in 2011-2012. Information on food consumption was collected from individuals *via* 24-hour dietary recalls administered on 2 non-consecutive days (Day 1 and Day 2). In addition to collecting information on the types and quantities of foods being consumed, NHANES contain socio-economic, physiological, and demographic information from individual participants in the survey, such as sex, age, height and weight, and other variables useful in characterizing consumption. The inclusion of this information allows for further assessment of food intake based on consumption by specific population groups of interest within the total population. Sample weights were incorporated with NHANES data to

compensate for the potential under-representation of intakes from specific populations and allow the data to be considered nationally representative (USDA, 2014; CDC, 2015). The NHANES data were employed to assess the mean and 90th percentile intake of Savory Base 100 for each of the following population groups:

- Infants and young children, ages 0 to 2 years;
- Children, ages 3 to 11;
- Female teenagers, ages 12 to 19;
- Male teenagers, ages 12 to 19;
- Female adults, ages 20 and up;
- Male adults, ages 20 and up; and
- Total population (all age and gender groups combined).

Consumption data from individual dietary records, detailing food items ingested by each survey participant, were collated by computer and used to generate estimates for the intake of Savory Base 100 by the U.S. population. Estimates for the daily intake of Savory Base 100 represent projected 2-day averages for each individual from Day 1 and Day 2 of NHANES 2011-2012 data, and these individual average amounts comprised the distribution from which mean and percentile intake estimates were generated. Mean and percentile estimates were generated incorporating survey weights in order to provide representative intakes for the entire U.S. population. "*Per capita*" intake refers to the estimated intake of Savory Base 100 averaged over all individuals surveyed, regardless of whether they potentially consumed food products containing Savory Base 100, and therefore includes individuals with "zero" intakes (*i.e.*, those who reported no intake of food products containing Savory Base 100 during the 2 survey days). "Consumer-only" intake refers to the estimated intake of Savory Base 100 by those individuals who reported consuming food products in which the use of Savory Base 100 is currently under consideration. Individuals were considered "consumers" if they consumed 1 or more food products in which Savory Base 100 is proposed for use on either Day 1 or Day 2 of the survey.

3.2.2 Estimated Intake of Savory Base 100 from Proposed Food-Uses

The estimates for the intake of Savory Base 100 was generated using the maximum use level indicated for each intended food-use, as presented in Table 1.3-1, together with food consumption data available from the 2011-2012 NHANES dataset. A summary of the estimated daily intake of Savory Base 100 from proposed food-uses is provided in Table 3.2.2-1 on an absolute basis (mg/person/day) and in Table 3.2.2-2 on a body weight basis (mg/kg body weight/day).

The percentage of consumers was high among all age groups evaluated in the current intake assessment; greater than 43.4% of the population groups consisted of users of those food products in which Savory Base 100 is currently proposed for use. Female adults had the greatest percentage of users at 82.3%; infants and young children had a notably lower percent consumers than all other age groups (43.4%). The consumer-only estimates are more relevant to risk assessments as they represent exposures in the target population; consequently, only the consumer-only intake results are discussed in detail herein.

Among the total population, the mean and 90th percentile consumer-only intakes of Savory Base 100 were determined to be 197 and 477 mg/person/day, respectively. Of the individual population groups, male adults were determined to have the greatest mean and 90th percentile consumer-only intakes of Savory Base 100 on an absolute basis, at 230 and 556 mg/person/day, respectively, while infants and young children had the lowest mean and 90th percentile consumer-only intakes of 105 and 290 mg/person/day, respectively (Table 3.2.2-1).

Table 3.2.2-1 Summary of the Estimated Daily Intake of Savory Base 100 from Proposed Food-Uses in the U.S. by Population Group (2011-2012 NHANES Data)

Population Group	Age Group (Years)	*Per capita* Intake (mg/day)		Consumer-Only Intake (mg/day)			
		Mean	90th Percentile	% Users	n	Mean	90th Percentile
Infants and Young Children	Up to 2	46	172	43.4	315	105	290
Children	3 to 11	105	291	71.7	1,138	147	359
Female Teenagers	12 to 19	138	400	76.0	391	182	443
Male Teenagers	12 to 19	170	455	75.5	384	226	537
Female Adults	20 and up	151	392	82.3	1,790	183	436
Male Adults	20 and up	186	492	80.8	1,685	230	556
Total Population	All ages	154	404	78.3	5,703	197	477

NHANES = National Health and Nutrition Examination Survey; Savory Base 100 = Savory Base 100 "Corn Sauce"; U.S. = United States.

On a body weight basis, infants and young children were identified as having the highest mean and 90th percentile consumer-only intakes of any population group, of 8.8 and 23.1 mg/kg body weight/day, respectively. Female adults had the lowest mean and 90th percentile consumer-only intakes of 2.7 and 6.3 mg/kg body weight/day, respectively (Table 3.1.2-2).

Table 3.2.2-2 Summary of the Estimated Daily Per Kilogram Body Weight Intake of Savory Base 100 from Proposed Food-Uses in the U.S. by Population Group (2011-2012 NHANES Data)

Population Group	Age Group (Years)	*Per Capita* Intake (mg/day)		Consumer-Only Intake (mg/day)			
		Mean	90th Percentile	% Users	n	Mean	90th Percentile
Infants and Young Children	Up to 2	3.8	14.5	43.4	314	8.8	23.1
Children	3 to 11	4.0	12.0	71.7	1,138	5.6	14.5
Female Teenagers	12 to 19	2.4	7.7	76.2	383	3.1	8.8
Male Teenagers	12 to 19	2.6	6.9	75.6	382	3.4	8.6
Female Adults	20 and up	2.2	5.6	82.3	1,774	2.7	6.3
Male Adults	20 and up	2.2	6.1	80.7	1,670	2.8	6.8
Total Population	All ages	2.5	6.7	78.3	5,661	3.2	7.8

bw = body weight; NHANES = National Health and Nutrition Examination Survey; Savory Base 100 = Savory Base 100 "Corn Sauce"; U.S. = United States.

3.2.3 Summary and Conclusions

Consumption data from the 2011-2012 NHANES dataset and information pertaining to the individual proposed food-uses of Savory Base 100 were used to estimate the "*per capita*" and consumer-only intakes for specific demographic groups and for the total U.S. population. Several conservative assumptions have been included in the present assessment, which means that resulting values may be considered 'worst case' estimates of exposure for the target population. For example, it was assumed that all food products within a food category contain the ingredients at the maximum specified level of use. In addition, it is well-established that the length of a dietary survey affects the estimated consumption of individual users. Short-term surveys, such as the typical 2- or 3-day dietary surveys, may overestimate the consumption of food products that are consumed relatively infrequently (Anderson, 1988). It should also be noted that the FEMA

GRAS uses are the same as those proposed herein, so consideration for additive exposure form FEMA GRAS uses was not deemed to be necessary.

In summary, on a consumer-only basis, the resulting mean and 90th percentile intakes of Savory Base 100 by the total U.S. population from all proposed food-uses in the U.S., were estimated to be 197 mg/person/day (3.2 mg/kg body weight/day) and 477 mg/person/day (7.8 mg/kg body weight/day), respectively. Among the individual population groups, the highest mean and 90th percentile intakes of Savory Base 100 were determined to be 230 mg/person/day (2.8 mg/kg body weight/day) and 556 mg/person/day (6.8 mg/kg body weight/day), respectively, as identified among male adults. When intakes of Savory Base 100 were expressed on a body weight basis, infants and young children had the highest mean and 90th percentile consumer-only intakes of 8.8 mg/kg body weight/day and 23.1 mg/kg body weight/day, respectively.

Part 4. §170.240 Self-Limiting Levels of Use

No known self-limiting levels of use are associated with Savory Base 100.

Part 5. §170.245 Experience Based on Common Use in Food Before 1958

Not applicable, as Savory Base 100 was not used in food before 1958.

Part 6. §170.250 Narrativeand Safety Information

The safety of Savory Base 100 is demonstrated based on the following pivotal information: 1) published toxicological studies (Tafazoli *et al*., 2017), including an acute oral toxicity study, a 90-day subchronic oral toxicity study, and a battery of *in vitro* genotoxicity and mutagenicity assay; 2) information on the compositional identity of Savory Base 100 demonstrating that they are common component of the diet with a history of safe use; 3) information establishing the safety of the fermentation organism. Each of the aforementioned points is discussed in detail in the following sections.

6.1 Metabolic Fate

The absorption, distribution, metabolism, and excretion (ADME) of Savory Base 100 has not been investigated; however, Savory Base 100 is mainly composed of amino acids, minerals, water, sugars, and organic acids that are normal components of human diet and as such, are expected to be digested and metabolized in a similar manner to other commonly consumed nutrients.

6.2 Toxicological Studies

6.2.1 Acute Toxicity

The acute oral toxicity of Savory Base 100 (identified as 'GA-NRC' in the study report) in rats has been evaluated in a study conducted in compliance with the Organisation for Economic Co-operation and Development (OECD) principles of Good Laboratory Practice (GLP) (OECD, 1998a) and according to Directive 86/609/EEC (EC, 1986), Directive 2001/83/EC (EC, 2001) and Commission Regulation (EC) No 440/2008 (EC, 2008) (Tafazoli *et al*., 2017).

Groups of 5 male and 5 female Wistar rats were administered a single dose of 0 (drinking water), 100, 500, or 2,000 mg/kg body weight Savory Base 100, by gavage, at a dose volume of 10 mL/kg body weight. Animals were observed shortly after dosing, at 6 hours after dosing and then once daily until the end of the study (14 days). Body weights were recorded on the day of dosing and 3 times a week thereafter. At the end of the observation period, animals were subjected to a macroscopic necropsy, where any abnormalities were fixed and subsequently examined microscopically.

There were no deaths and no test item-related clinical signs or effects on body weight (a statistically significant (5%) reduction in body weight for males given 500 mg/kg body weight on Day 14 was considered not toxicologically relevant, due to absence of a dose-response).

There were also no macroscopic or microscopic changes that were considered to be related to Savory Base 100. White deposits observed in the spleen of 2 females from each of the low and high-dose groups were confirmed microscopically to be slight capsular fibroses. However, these were isolated instances (only seen for 2 out of 5 females in each of the affected groups) and there was no evidence of a dose-related response. Isolated instances of unilateral pelvic dilatation (1 high-dose male and 1 control female) and red sports on the thymus (1 low dose male) were also considered to be unrelated to the test item. It was concluded, therefore, that 2,000 mg/kg body weight (the highest dose tested) was the no-observed-adverse-effect level (NOAEL).

6.2.2 Repeated-Dose Toxicity

A 90-day repeat dose oral toxicity study was conducted to investigate the subchronic toxicity of NRC Mix [a combination of Savory Base 100 and the related Savory Base 200 "Corn Sauce" (Savory Base 200) in a 2:1 ratio] in rats (Tafazoli *et al.*, 2017). NRC Mix contained 37.8±0.2% glutamic acid (primarily from Savory Base 100) and 14.5±0.4% IMP (primarily from Savory Base 200). Savory Base 200 is the subject of a concurrent GRAS Notice.

The study was performed in compliance with the OECD principles of GLP (OECD, 1998a) and according to Directive 2001/83/EC (EC, 2001), OECD Test Guideline 408 (OECD, 1998b) and Commission Regulation (EC) No 440/2008 (EC, 2008). Given that Savory Base 100 will often be used in combination with Savory Base 200, the test articles were used in combination.

Groups of 10 male and 10 female Wistar rats were given 0 (basal diet), 1, 2.5, or 7% NRC Mix (equivalent to approximately 500, 1,250, or 3,500 mg/kg body weight/day NRC Mix, which equates to approximately 333, 833, or 2,333 mg/kg body weight/day Savory Base 100), in the diet for 90 days; doses were selected based on data derived from an internal palatability study. An additional 5 males and 5 females were included in the control and high-dose groups and also fed for 90 days, after which time they were kept untreated for a further 4 weeks, to assess the reversibility of any effects seen during the treatment period.

Animals were observed daily for changes in behavior and appearance, with ophthalmoscopic examinations performed once before the start of dosing and once towards the end of the treatment period. Body weights were recorded 3 times each week, food intake was recorded once weekly, and water consumption was recorded every 4 days from Week 2 onwards. Blood samples were taken from the retro-orbital sinus for clinical pathology from main study animals before dosing and at the end of the treatment period, with recovery animals sampled towards the end of both the treatment and recovery periods; urine samples were collected once before dosing and at the end of the treatment and recovery periods (where applicable).

All animals were subjected to a macroscopic necropsy, where selected organs were weighed and, for animals in the control and high-dose groups only, the following tissues were examined microscopically: liver, kidneys, adrenals, spleen, pancreas, heart, lung, aorta, thymus, larynx, thyroid gland, parathyroid glands, salivary glands, tongue, trachea, bronchus, esophagus, stomach, small and large intestines, urinary bladder, prostate gland, seminal vesicles, testes, epididymides, ovaries, vagina, uterus, lymph nodes, brain, pituitary gland, skin, mammary gland, eyes, optic nerves, lacrimal glands, skeletal muscle, sciatic nerve, spinal cord, and bone marrow.

There were no test item-related deaths or clinical signs during the study. The death of 1 male in the mid-dose group on Day 90 was considered incidental as it was an isolated incident, but no reason for the death was identified at necropsy. There were also no ocular changes that were considered to be related to administration of the test item.

Mean body weights for test item-treated males were statistically significantly higher ($p<0.05$ to $p<0.005$) than those of the controls at the end of the treatment period; however, these increases were not dose-related (increases of 10, 14, and 6% at 1, 2.5, or 7.5% NRC Mix, respectively). Female groups given NRC Mix also gained slightly more weight than controls after 89 days (6 to 7%), but, as with the males, there was no dose-response relationship. All test item-treated male and female groups were heavier than controls on Day 1, despite mean body weights being similar on arrival; therefore, these animals were already gaining more weight than controls before NRC Mix was introduced into the diet. Body weight increases may in part be due to organoleptic properties of the savory base resulting in an apparent increase in food intake by the savory base groups during the early phase of the study. Nonetheless, the body weight changes were considered to be non-adverse.

Although there were statistically significant ($p<0.05$ to $p<0.005$) increases in mean food consumption in various weeks during the treatment period for both males and females (mostly for males given 1 or 2.5% NRC Mix, correlating with the increased body weights for these groups), food consumption in Week 13 was similar between test item-treated groups and controls.

High-dose males drank statistically significantly ($p<0.05$) more (18%) than controls after 90 days, with a dose-related increase in mean water consumption observed for females (increases of 13, 17, and 40% at 1, 2.5, or 7.5% NRC Mix, respectively), which was statistically significant ($p<0.005$) at the high dose; by the end of the recovery period, water consumption for high-dose groups dropped to either less than (males) or similar to (females) that of the controls. Increased water consumption was to be expected given the salt content of Savory Base ingredients. In the absence of biologically relevant changes in the kidney or in relevant clinical chemistry or urinary parameters, these findings were considered to be non-adverse.

Various statistically significant findings were reported among hematology parameters for test item-treated males and females at the end of the treatment period. Increases in hemoglobin count [4 and 7% ($p<0.005$) for high-dose males and females, respectively] and in hematocrit (for both sexes at the high-dose) were minor and there was only a dose-response relationship for females, hence these were considered to be physiological variations, unrelated to the test item. Differences in other hematological parameters were minor, inconsistent between the sexes, and/or did not show a relationship with dose and were likely also to be due to normal biological variation rather than any effect of the test item.

There were no test item-related differences in coagulation parameters at the end of the treatment period. Where statistically significant differences were reported [shortened mean activated partial thromboplastin time (APTT) for mid- (9%, $p<0.01$) and high-dose (8%, $p<0.05$) males and shorted mean prothrombin time (PT) for low dose females (4%, $p<0.05$)], there was no dose-response relationship and the changes were in the wrong direction for biological relevance (elongation of APTT and/or PT are considered to be biologically relevant changes). The statistically significantly ($p<0.01$) shortened PT (14%) for males at the end of the recovery period was also in the wrong direction for biological relevance and considered not test item-related.

There were numerous sporadic statistically significant differences in clinical chemistry parameters between test item-treated groups and controls; however, these differences were either of low magnitude, inconsistent between the sexes or did not show a dose-response relationship and were therefore considered to be toxicologically irrelevant. There were no test item-related differences in urinalyses parameters.

There were no differences in body weight-related organ weights between test item-treated groups and controls. Brain weight-relative organ weights can be notably affected by variations in terminal body weights (which were reported in this study), therefore the statistically significant differences in brain weight-related organ weights [increased thymus and spleen weights for males given 1 (thymus only), 2.5, or 7% NRC Mix, respectively, and reduced adrenal gland weight at the high dose] were considered not biologically relevant, in the absence of any changes in body weight-relative weights or of histological changes for any of these organs. Furthermore, these statistically significant differences weren't reported for females and the changes in thymus and adrenal weights were clearly not dose-related.

There were no test item-related macroscopic changes. Histopathological findings included hepatic steatosis (primarily in the periportal region), which was reported for 7 out of 20 controls and 13 out of 20 high-dose animals; this was also reported at the end of the recovery period in all 5 control males and 1 out of 5 control females and in 4 out of 5 males and 2 out of 5 females in the high-dose group. These effects were considered by the author as not test item-related, as they were not associated with any necrosis or increases in liver enzyme activities or liver weights (neither absolute nor relative), so the low and mid dose groups were not subject to histopathological examination. The histopathology report does not specify whether the changes were micro- or macrovesicular; however, as the droplets were described as "medium" this appears to indicate that these were macrovesicular fatty changes, which are the most common form of liver fatty changes that may be seen sporadically in control animals and are considered benign changes presumably as a result of nutritional, metabolic or hormonal derangement (Greaves and Faccini, 1992; Thoolen *et al.*, 2010; Greaves, 2012); therefore, these changes were considered not test item-related.

Kidney tubular mineralization (also known as nephrocalcinosis) was reported in 4 out of 10 high-dose females and 1 control female at the end of the treatment period and in 4 of the 5 high-dose females at the end of the recovery period. Nephrocalcinosis is a common spontaneous minor lesion that develops in young and adult rats, primarily females (Gad, 2016); this finding was not reported in males in this study. Increased susceptibility to nephrocalcinosis is known to occur from dietary manipulation and it has been reported that imbalances in the calcium and phosphorus content of diets, calcium:phosphorus ratio of diets, deficiency of magnesium and/or chloride and high urinary pH can all contribute to the development of nephrocalcinosis (Reeves *et al.*, 1993; Rao, 2002). Considering the high mineral content of Savory Base ingredients, the likely unbalanced provision of minerals in the test diet relative to the control diet could be responsible for the observed effects in the kidneys; however, no single mechanism that explains the association between the dietary factors contributing to the incidence of nephrocalcinosis has been identified. In general, these mineral deposits are of no pathological significance (Seely and Brix, 2014) and

in the absence of correlating markers of kidney impairment, were considered not to be toxicologically relevant.

At the end of the treatment period, non-specific and incidental findings included chronic focal myocarditis (4 out of 10 high-dose males and 1 of the 10 female controls) and hyperplasia of lymph follicles in both the small intestine (4 males and 2 females from the high-dose group, compared with 3 males and 1 female in the control group) and large intestine (2 and 1 high-dose males and females, respectively, compared with 4 male and 4 female controls) were reported. At the end of the recovery period, focal myocarditis was reported in only 1 high-dose male, hyperplasia of the lymph follicles in the small intestine was reported in 1 male and 2 females from the high-dose group, compared with 2 and 4 control males and females, respectively and hyperplasia of lymph follicles in the large intestine was reported in 2 males and 3 females from the high-dose group, in comparison to 3 male and 3 female controls.

The incidence of chronic focal myocarditis reported in high-dose males was considered to be toxicologically irrelevant, as these histological observations were similar to the spontaneous lesions commonly reported in test and control rats, with a higher occurrence in males (Gaunt *et al.*, 1967; Jokinen *et al.*, 2011). Instances of hyperplasia of lymph follicles in the small and large intestine were small in magnitude and occurred at a similar frequency in test item-treated and control groups, and were therefore also considered biologically irrelevant.

The NOAEL was reported to be 7% NRC Mix (the highest dose tested, equivalent to approximately 3,500 mg/kg body weight/day NRC Mix, which corresponds to a NOAEL of approximately 2,333 mg/kg body weight/day for Savory Base 100 (based on a the 2:1 ratio of Savory Base 100 and Savory Base 200).

6.2.3 Mutagenicity and Genotoxicity

6.2.3.1 Bacterial Reverse Mutation Test

The potential mutagenicity of Savory Base 100 (identified as 'GA-NRC' in the study report) was evaluated in a bacterial reverse mutation test (Ames test), which was performed in compliance with the OECD principles of GLP (OECD, 1998a) and according to OECD Test Guideline 471 (OECD, 1997), Commission Regulation (EC) No 2000/32/EC (EC, 2000), US EPA Health Effects Test Guidelines OPPTS 870.5100 (U.S. EPA, 1998), ICH Guidance S2A (ICH, 1995) and ICH Guidance S2B (ICH, 1997) (Tafazoli *et al.*, 2017).

An initial preliminary range-finding test was conducted using the plate incorporation method at Savory Base 100 concentrations of 5 to 5,000 µg/plate, using *Salmonella typhimurium* (*S. typhimurium*) strains TA98 and TA100, in the absence and presence of S9 metabolic activation. Since the results of this test were negative, 2 separate tests (plate incorporation assay and pre-incubation assay) were conducted using *S. typhimurium* strains TA98, TA100, TA1535, and TA1537 and *Escherichia coli* strain WP2 uvrA, which were treated with Savory Base 100 at concentrations of 51.2, 128, 320, 800, 2,000, and 5,000 µg/plate in the absence and presence of S9 mix.

Three negative control groups [untreated, vehicle (distilled water) and dimethyl sulfoxide] were used, and positive controls were also included in the absence (4-nitro-1,2-phenylene-diamine, sodium azide, 9-aminoacridine and methyl-methanesulfonate) and presence (2-aminoanthracene) of metabolic activation. A positive result for mutagenicity was defined as a dose-dependent, reproducible, and biologically relevant 2- (in *S. typhimurium* T100) or 3-fold (in the other tested strains) increase in the number of revertant colonies, compared to that of the vehicle control group.

Savory Base 100 showed no evidence of mutagenicity in any of the tests, in the absence or presence of metabolic activation. In contrast, the positive controls induced biologically relevant increases in revertant colony counts (with metabolic activation where required), which demonstrated the sensitivity of the assay and metabolic activity of the S9 preparations. It was concluded, therefore, that Savory Base 100 is non-mutagenic at concentrations up to 5,000 µg/plate, in the absence or presence of metabolic activation.

6.2.3.2 In Vitro Mammalian Cell Gene Mutation Test

The mutagenic potential of Savory Base 100 was investigated in an *in vitro* mammalian cell gene mutation test conducted in compliance with the OECD principles of GLP (OECD, 1998a) and according to OECD Test No. 476 (OECD, 2015) and Commission Directive (EC) No 2000/32/EC (EC, 2000) (Tafazoli *et al*., 2017).

A preliminary dose range-finding study (where Savory Base 100 was not cytotoxic at concentrations up to 5,000 µg/mL) was followed by 2 independent experiments (each conducted in duplicate) using V79 Chinese hamster lung (CHL) cells. For both of these experiments, the vehicle [Dulbecco's Modified Eagle's (DME) medium] and dimethyl sulfoxide (DMSO) served as the negative controls and positive controls were included in the absence (ethylmethane sulfonate) and presence (7,12-dimethyl benzanthracene) of S9 metabolic activation.

In the first experiment, CHL cells were exposed to Savory Base 100 for 3 hours at concentrations of 312.50, 625, 1,250, 2,500, or 5,000 µg/mL in the absence or presence of S9 metabolic activation. In the second, CHL cells were exposed to Savory Base 100 for 20 hours (in the absence of S9) or 3 hours (in the presence of S9) at concentrations of 156.25 (presence of S9 only), 312.50, 625, 1,250, 2,500, or 5,000 µg/mL.

After the incubation period, for both experiments, the cells were washed with DME, detached with trypsin-ethylenediaminetetraacetic acid (EDTA) solution, and cultured to determine survival and to allow for expression of the mutant phenotype. Once mutant colonies had been selected, they were fixed, stained with Giemsa, and counted for either mutant selection or cloning efficiency. Mutant frequency was calculated by division of the total number of mutant colonies by the number of cells selected, corrected for cloning efficient of cells before mutant selection. Positive mutagenic responses were defined as dose-related, reproducible, and statistically significant increases in mutant frequency.

For both experiments, in the absence or presence of S9, no statistically significant increases in mutation frequency were reported for Savory Base 100 treated cells, compared with that of the negative controls. Sensitivity of the assay and efficacy of the S9 preparations was confirmed by the significant increases in mutation frequency for the positive controls. It was concluded that Savory Base 100 is not mutagenic at concentrations up to 5,000 µg/mL, in the absence and presence of metabolic activation.

6.2.3.3 In Vitro Mammalian Cell Micronucleus Test

The clastogenic and aneugenic potential of Savory Base 100 (identified as He Wei C. Essence I in the study report) was evaluated in an unpublished corroborative *in vitro* mammalian cell micronucleus test, conducted using human lymphocytes, in compliance with the OECD principles of GLP (OECD, 1998a) and according to OECD Test No. 487 (OECD, 2014) (Chevallier, 2017). A copy of the full study report is provided in Appendix A.

An initial preliminary cytotoxicity test was conducted using Savory Base 100 at concentrations of 0 to 5,000 µg/mL, in the presence (3-hour treatment) and absence (3 and 24-hour treatments) of S9 metabolic activation; there was no evidence of cytotoxicity reported at any concentration. Cytotoxicity was assessed again in the main experiment. In the absence of S9 (at the same dose levels and under similar conditions to

those used in the preliminary test), there was no evidence of cytotoxicity after a 3-hour treatment, but slight to moderate cytotoxicity was reported at concentrations ≥2,500 µg/mL after 24 hours continuous treatment. However, there was no evidence of cytotoxicity in the presence of S9 after a 3-hour treatment under similar conditions to those described above.

In the main experiment for micronucleus analysis, 5,000 µg/mL was considered to produce extreme culture conditions, therefore, human lymphocytes were treated with Savory Base 100 at 312.5, 625, 1,250, 2,500, or 3,750 µg/mL with S9 (3 hours) and without S9 (3 and 24-hour treatments). The vehicle (water for injection) was used as a negative control and positive controls were included in the absence (colchicine and mitomycin C) and presence (cyclophosphamide) of metabolic activation. A positive result for clastogenicity/aneugenicity was defined as a dose-dependent, statistically significant increase in the frequency of micronucleated binucleated cells (MNBC), with the frequency of MNBC also being above the vehicle background range for at least 1 dose level.

Savory Base 100 showed no evidence of clastogenicity or aneugenicity in any of the tests, in the absence or presence of metabolic activation. In contrast, the positive controls induced biologically relevant increases in MNBC (with metabolic activation where required), which demonstrated the sensitivity of the assay and metabolic activity of the S9 preparations. It was concluded that Savory Base 100 is neither clastogenic nor aneugenic at concentrations up to 3,750 µg/mL, in the absence and presence of metabolic activation.

6.3 Additional Safety Information on Major Constituents of Savory Base 100

The constituents of Savory Base 100 have a long history of consumption as part of existing food stuffs and the characteristic savory taste of the ingredient results from a specific intrinsic mix of these compounds (including free and bound amino acids, organic acids, Amadori and Maillard products, minerals and their salts), all of which individually contribute to the overall taste. Dietary intakes of the flavoring compounds are consistent with levels commonly used in foods, and/or are well below acceptable daily intake (ADI) values that have been derived.

6.3.1 Glutamic Acid

A major constituent of Savory Base 100 is the amino acid glutamic acid. Glutamic acid is a non-essential amino acid and as a constituent of protein is consumed from a host of protein containing food sources, including meat, eggs, fish, milk, and vegetables. The safety of glutamic acid in particular has been well characterized and reported in safety evaluations of an extensive collection of animal and human studies, conducted firstly by the Joint FAO/WHO Expert Committee on Food Additives (JECFA) at the 14th and 17th JECFA meetings in 1970 and 1974, respectively (JECFA, 1970, 1974). A further evaluation of additional data at the 31st JECFA (1988) resulted in the allocation of a group ADI 'not specified' for glutamic acid and its ammonium, calcium, potassium, magnesium and sodium salts, which is applicable to substances with very low toxicity and indicates that the total dietary intake of glutamic acid, arising from its use at the levels necessary to achieve the desired effect and from its acceptable background levels in food, does not, in the opinion of JECFA, represent a hazard to health. This conclusion was reiterated by the Scientific Committee on Food (SCF) in 1991 (JECFA, 1988; SCF, 1991). Furthermore, glutamic acid is approved as a food additive (E 620) in the European Union (EU), under Commission Regulation (EU) No 1129/2011. Glutamic acid (E 620) is a Group I additive, authorized at levels up to 10 g/kg in numerous food categories; additionally, it is authorized for use in salt substitutes, seasonings, and condiments at *quantum satis* (European Union, 2011).

Recently, the European Food Safety Authority (EFSA) Panel on Food Additives and Nutrient Sources added to Food (ANS) re-evaluated the safety of glutamic acid and its salts for use as food additives (EFSA, 2017). Following its re-evaluation of the technical, safety, and exposure data available for glutamic acid and related glutamates, the Panel derived a group ADI of 30 mg/kg body weight/day, expressed as glutamic acid, for glutamic acid and glutamates. This ADI was based on the NOAEL of 3,200 mg monosodium glutamate/kg body weight/day from the neurodevelopmental toxicity study (Vorhees *et al.*, 1979), and applying the default uncertainty factor of 100.

Dietary intakes of glutamic acid from protein in the typical diet have been estimated to be *ca.* 15 g/person per day (Stamler *et al.,* 2009). Only free glutamic acid imparts flavor enhancing properties to foods, and free glutamic acid is present in a number of natural and fermented foods (Table 6.3.1-1).

Table 6.3.1-1 Foods Rich in Free Glutamic Acid

Food Product	Free Glutamic Acid (mg)	Serving Size
Human milk	300	1000 g
Cantaloupe	50	100 g
Grapes	40	100 g
Vegemite	143	10 g
Marmite	196	10 g
Tomato paste	62 to 64	10 g
Parmesan cheese	36 to 127	10 g
Soy sauce	5 to 126	10 g
Fish sauce	73 to 138	10 g
Oyster sauce	90	10 g
Condensed soups	0 to 480	100 g
Sauces, mixes, seasonings	2 to 190	10 g
Chinese restaurant meals	<10 to 1500	100 g
Italian restaurant meals	10 to 230	100 g
Western restaurant meals	<10 to 710	100 g

Sources: JECFA (1988); Yoshida (1988); Nichols and Jones (1991); Daniels *et al.* (1995).

In the U.S., L-glutamic acid and its glutamate salts are GRAS when used as a salt substitute when used in accordance with good manufacturing practice (§182.1045; §182.1047; §182.1500; §182.1516; §182.1). The GRAS use of L-glutamic acid and L-glutamates as flavoring enhancers was evaluated by the Select Committee on GRAS Substances (SCOGS) (FASEB, 1980). The committee commented on the reported cases of "Chinese Restaurant Syndrome" in certain individuals, and that the use of Monosodium Glutamate in restaurant and/or home prepared foods was not under the purview of the Select Committee since its evaluation was limited to processed foods. The committee concluded that

> "*There is no evidence in the available information on L-glutamic acid, L-glutamic acid hydrochloride, monosodium L-glutamate, monoammonium L-glutamate, and monopotassium L-glutamate that demonstrates, or suggests reasonable grounds to suspects, a hazard to the public when they are used at levels that are now current and in the manner now practices. However, it is not possible to determine, without additional data, whether a significant increase in consumption would constitute a dietary hazard*".

Another source of glutamic acid is from yeast extracts, which are commonly consumed ingredients that are GRAS under Title 21 Food and Drug of the Code of Federal Regulations (CFR) §184.1983 (U.S. FDA, 2017). Savory Base 100 is compositionally similar to yeast extracts and will be used as a replacement for them in foods. A comparison of yeast extracts [as defined in the Food Chemicals Codex (FCC, 2016)] with Savory Base 100 is presented in Table 6.3.1-2 below.

Table 6.3.1-2 Comparison of Savory Base 100 "Corn Sauce" (Savory Base 100) with Yeast Extracts (as Defined in the Food Chemicals Codex)

Parameter	Yeast Extract	Savory Base 100
Description	Yeast extract occurs as a liquid, paste, powder, or granular substance.	Savory Base 100 occurs as a pale brown to brownish paste.
	It comprises the water-soluble components of the yeast cell, the composition of which is primarily amino acids, peptides, carbohydrates, and salts.	Savory Base 100 is composed of glutamic acid (34 to 44%), water (27 to 34%), ash (10 to 18%), total nitrogen (4 to 7%), sodium chloride (5.5 to 8%) and other free amino acids (1 to 3%).
	Yeast extract is produced through the hydrolysis of peptide bonds by the naturally occurring enzymes present in edible yeasts or by the addition of food-grade enzymes.	Corn syrup serves as the substrate and *C. glutamicum* is the source of the enzymes.
	Food-grade salts may be added during processing.	Sodium chloride is added during manufacture.
Function	Flavoring agent, flavor enhancer.	Savory flavoring ingredient.
Assay		
Protein	≥42% protein	-
Total Nitrogen	-	4 to 7%.
α-Amino Nitrogen/ Total Nitrogen Percent Ratio	15 to 55%	N/A
Ammonia Nitrogen	≤2%	<2% (Analytical results)
Insoluble Matter	≤2%	Not provided
Lead	≤2 mg/kg	<0.02 mg/kg
Mercury	≤3 mg/kg	<0.003 mg/kg
Potassium	≤13%	0.94% (Analytical results)
Sodium Chloride	≤50%	5 to 7%
Microbial Limits		
Aerobic plate count	≤50,000 CFU/g	≤10,000 CFU/g
Coliforms	≤10 CFU/g	No specification
Salmonella	Negative in 25 g	Negative in 25 g
Yeast and Molds	≤50 CFU/g	≤100 CFU/g

CFU = colony forming units; N/A = not applicable.

Savory Base 100 is intended for use as an alternative to yeast extracts for general food use, and therefore, will not increase dietary intakes of glutamic acid above levels currently occurring by way of existing regulations for glutamic acid and its salts discussed above.

Based on the results of analysis of 3 batches of Savory Base 100, the glutamic acid content of the product averages about 38% (see Table 2.3.3-1). As previously indicated, EFSA has recently established an ADI of 30 mg/kg body weight/day. The 90th percentile intakes of Savory Base 100 were estimated to be 477 mg/person/day (see Table 3.1.2-1; the daily intakes of glutamic acid, as a major component of Savory Base 100, is calculated to be 174.77 mg/day (equivalent to 2.49 mg/kg body weight/day for a 70 kg individual). This intake is well below the ADI of 30 mg/kg body weight/day for glutamic acid as established by EFSA and is not expected to raise a safety concern.

6.3.2 L-Alanine

L-alanine is a non-essential amino acid, which is a natural constituent of proteins in plants and animals (Burdock, 2009). L-alanine is permitted for direct addition to foods for nutritive purposes at levels up to 6.1% by weight of total protein (21 CFR §172.320 - U.S. FDA, 2017). L-Alanine has been allocated an ADI of 'acceptable' by JECFA (2004). Based on the results of analysis of 3 batches of Savory Base 100, the L-alanine content of the product averages about 1.43% (see Table 2.3.3-1). Since the 90th percentile intakes of Savory Base 100 were estimated to be 477 mg/person/day (see Table 3.1.2-1), the daily intakes of L-alanine, as a component of Savory Base 100, was calculated to be 6.82 mg/day, and this is not expected to raise a safety concern.

6.3.3 Formic Acid

Formic acid is a natural constituent of many foods consumed by humans, such as apple, papaya, pear, raspberry, strawberry, cheeses, breads, yogurt, milk, cream, and fish (Burdock, 2009). It is also a metabolite in intermediary metabolism and a precursor in the biosynthesis of several body constituents (FASEB, 1976). Formic acid is permitted for direct addition to food intended for human consumption with no limitations other than good manufacturing practice (GMP) (21 CFR §186.1316 - U.S. FDA, 2017). Formic acid has been allocated an ADI of '0 to 3 mg/kg body weight/day' by JECFA (1997). Based on the results of the product averages about 0.80% (see Table 2.3.2-1). Since the 90th percentile consumer-only intakes of Savory Base 100 were estimated to be 477 mg/person/day (see Table 3.1.2-1), the daily intakes of formic acid, as a component of Savory Base 100, was calculated to be 3.82 mg/day (equivalent to 0.054 mg/kg body weight/day for a 70 kg individual). This intake is well below the ADI of 3 mg formic acid/kg body weight/day established by JECFA.

6.3.4 Succinic Acid

Succinic acid, an intermediate metabolite of the tricarboxylic acid cycle and an end-product of aerobic and anaerobic metabolism (Song and Lee, 2006), can be produced from yeast fermentation in the processing of sake and wine (Arikawa *et al.,* 1999; Song and Lee, 2006). In the U.S., succinic acid produced by chemical synthesis or fermentation is GRAS for use as a flavor enhancer, and pH control agent in food at levels consistent with 21 CFR §184.1091 and not to exceed cGMP (U.S. FDA, 2017). In a 13-week subchronic oral toxicity study by Maekawa *et al.* (1990), the toxicity of monosodium succinate was evaluated in groups of 10 male and 10 female F344 rats *via* the drinking water at concentrations of 0 (control), 0.3, 0.6, 1.25, 2.5, 5, or 10%. No dose-related adverse effects were reported in hematological, biochemical, or histopathological parameters at any dose. The authors concluded that the NOAEL was 1.25% (equivalent to 1,250 mg/kg body weight/day or 1,050 mg/kg body weight/day as succinic acid), based on decreased body weight gain noted at higher doses (Maekawa *et al.*, 1990). The food intakes were not measured in this study. In a follow-up 2-year carcinogenicity study, no statistically significant differences were reported between the control and treated animals in overall tumor incidence, or mean survival times in either sex, when groups of 50 male and 50 female F344 rats were administered monosodium succinate through the drinking water at

doses up to 2% for 104 weeks, corresponding to daily intakes of up to 1,093 mg/kg body weight/day for males and 773 mg/kg body weight/day for females (Maekawa *et al.*, 1990). The results of an *in vitro* reverse mutation assay and a chromosomal aberration test demonstrated that succinic acid was neither mutagenic nor clastogenic (Ishidate *et al.*, 1984). Based on the results of analysis of 3 batches of Savory Base 100, the succinic acid content of the product averages about 0.53%. Considering that the 90th percentile consumer-only intakes of Savory Base 100 was estimated to be 477 mg/person/day, and the daily intakes of succinic acid, as a component of Savory Base 100, was calculated to be 2.53 mg/day (equivalent to intakes of 0.036 mg succinic acid/kg body weight/day for a 70-kg individual), which provides a large margin of safety when compared to the NOAEL of 1,050 mg succinic acid/kg body weight/day, as determined in the 13-week oral toxicity study by Maekawa *et al.* (1990).

6.4 Safety of the Source Organism

6.4.1 Identity

The *C. glutamicum* strain used by Nestec in the production of Savory Base 100 is deposited in several international culture collections. Initially deposited as *Micrococcus glutamicus* strain 13032 by Kyowa Ferm. Ind. Co., Ltd., the production organism currently has the strain designation *C. glutamicum* 534 [ATCC 13032] and represents the type strain for the species (ATCC, 2016; Ikeda and Nakagawa, 2003).

The complete genome of *C. glutamicum* ATCC 13032 was sequenced in 1998, which was further characterized and annotated in 2001 and 2002 (reviewed in Ikeda and Nakagawa, 2003) and is also publicly available (NCBI, 2016). The central carbon pathway, physiology, and regulation of main and specific metabolic pathways for this strain have been well characterized, as it has significant industrial applications and much interest has been focused on optimizing production performance from this microorganism (Wieschalka *et al.*, 2013).

6.4.2 Pathogenicity and Toxicogenicity

There are no documented case-reports of *C. glutamicum* being pathogenic or toxic to humans or animals. *C. glutamicum* fulfils the requirements for Qualified Presumption of Safety (QPS) when it is used for amino acid production (EFSA, 2013); Savory Base 100 being enriched in amino acids. *C. glutamicum* ATCC 13032 is classified as a Biosafety Level 1 by the American Type Culture Collection (ATCC), meaning the microorganism is not known to consistently cause disease in healthy adult humans and is of minimal potential hazard to laboratory personnel and the environment.

C. glutamicum has a long history of use in the food production industry. First isolated in 1956, *C. glutamicum* was initially characterized by its unique natural ability to produce large amounts of glutamic acid (the predominant amino acid in Savory Base 100) from sugar and ammonia (Vertès *et al.*, 2013). Moreover, *C. glutamicum* has been used for the production of glutamic acid in the U.S. since 1961 (Kinoshita *et al.*, 1961a,b; Kalinowski *et al.*, 2003); in 2005 alone, 1.5 million tons of glutamate were produced using fermentation by *C. glutamicum*, in addition to several thousand tons of threonine, lysine, isoleucine and tryptophan (Smith *et al.*, 2010). *C. glutamicum* has also been identified as a surface microflora in cheese during ripening, indicating that this organism has a history of consumption as a species in cheese (Dolci *et al.*, 2009).

A number of *Corynebacterium* spp. (*C. ammoniagenes, C. casei, C. flavescens,* and *C. variabile*) have been listed in the International Dairy Federation (IDF) 2012 inventory of microbial species with technological beneficial role in fermented food products (IDF, 2012).

Corynebacterium spp. have also been used globally for number of years in the production of a variety of foods including cereals, bread, alcoholic beverages, and native dishes. *Corynebacterium* are responsible for the hydrolysis of starch to organic acids in the production of cassava and the West African maize porridge ogi (which can be cooked and then cooled to produce agidi, a weaning food or breakfast cereal) and are also involved in the fermentation of ugba (a Nigerian snack and condiment) from African oil bean seeds (Hahn, 1988; Haard *et al*., 1999; Osungbaro, 2009; Nwagu *et al*., 2011). A novel *Corynebacterium* species (termed by the authors as *C. nuruki* strain S6-4) was isolated from an alcohol fermentation starter (nuruk), which is used in the fermentation of rice to produce the Korean alcoholic beverage makgeolli (Shin *et al*., 2011); *Corynebacterium* spp. have also been detected in doenjang-meju (Korean fermented soybean paste), sufu (Chinese fermented bean curd) and sayur asin (Indonesian fermented mustard cabbage) (Puspito and Fleet, 1985; Cheng and Han, 2014; Jung *et al*., 2016).

6.5 Expert Panel Evaluation

Nestec has concluded that Savory Base 100 meeting appropriate food-grade specifications and manufactured consistent with cGMP is GRAS for use as an ingredient in various food products, as described in Part 1.3, on the basis of scientific procedures.

The GRAS determination is based on data generally available in the public domain pertaining to the safety of Savory Base 100 and based on a unanimous opinion among a panel of experts ("the Expert Panel"), who are qualified by scientific training and experience to evaluate the safety of food ingredients. The Expert Panel consisted of the following qualified scientific experts: Professor Emeritus Joseph F. Borzelleca (Virginia Commonwealth University School of Medicine), Professor Eric A. Johnson (University of Wisconsin-Madison), and Professor Emeritus John A. Thomas (Indiana University School of Medicine). The Expert Panel was selected and convened prior to issuance of the FDA's guidance for industry on *Best Practices for Convening a GRAS Panel* (U.S., FDA 2017), and therefore no formal written GRAS Panel policy was in place at the time of Expert Panel meeting. However, the notifier confirms that prior to convening the Panel all reasonable efforts were made to identify and select a balanced Expert Panel with expertise in food safety, toxicology, and microbiology, and efforts were placed on identifying conflicts of interests or relevant appearance issues that would potentially bias the outcome of the Expert Panel deliberations; no such conflicts of interests or appearance conflicts were identified. The Expert Panel received a reasonable honorarium as compensation for the Expert Panel's time, and honoraria provided to the Expert Panel were not contingent upon the outcome of the Expert Panel deliberations.

The Expert Panel, convened by Nestec, independently and critically evaluated all data and information presented herein, and concluded that Savory Base 100 is GRAS for use as an ingredient in various food products, as described in Section 1.3, based on scientific procedures. A summary of data and information reviewed by the Expert Panel and evaluation of such data as it pertains to the proposed GRAS uses of Savory Base 100, are presented in Appendix B.

6.6 Conclusions

Based on the data and information presented herein, Nestec has concluded that Savory Base 100, meeting appropriate food-grade specifications and manufactured according to cGMP, is safe for use in various food products as presented in Section 1.3. Nestec also has further concluded that pivotal data and information relevant to the safety of Savory Base 100 are publicly available and therefore the intended uses of Savory Base 100 can be determined to be Generally Recognized as Safe (GRAS) on the basis of scientific procedures.

Inoculum Broth with Calcium Chloride

Section 1. Identification

Product Identifier

Catalog Number: I4220

Recommended use of the chemical and restrictions on use
For research use only. Not intended for human or animal diagnostic or therapeutic uses

Manufacture/Supplier Details: Alpha Teknova, Inc.
2290 Bert Dr.
Hollister, CA 95023
Telephone Number: 831-637-1100

Emergency Telephone Numbers
CHEMTREC Emergency Phone Number: (800) 424-9300

Section 2. Hazard Identification

Classification of the substance/mixture: Not a hazardous substance or mixture.

GHS Label elements, including precautionary statements
The substance is classified and labeled according to the Globally Harmonized System (GHS).
Not a hazardous substance or mixture.

Hazard statements: None

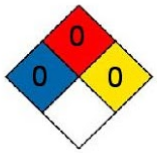
Signal word: None

Precautionary Statements: None

HMIS rating (Scale 0-4):

HEALTH	0
FIRE	0
REACTIVITY	0

NFPA Rating:



Hazards not otherwise classified: None

Section 3. Composition/Information On Ingredients

Chemical Name	Identifiers	Hazardous	Approximate Percentage %
Magnesium Sulfate	**CAS:** 7487-88-9	nonhazardous	0.10%
Soytone	**CAS:** N/A	nonhazardous	2.5%
Yeast extract	**CAS:** 8013-01-2	nonhazardous	4.80%
Glycerol	**CAS:** 56-81-5	nonhazardous	1%
Sodium Phosphate Dibasic	**CAS:** 7558-79-4	nonhazardous	12%
Potassium Phosphate Monobasic	**CAS:** 7778-77-0	nonhazardous	6%

Ammonium Chloride	**CAS:**12125-02-9	nonhazardous	2%
Calcium Chloride	**CAS:** 10043-52-4	nonhazardous	0.013%
Water	**CAS:** N/A	nonhazardous	-

Chemical Characterization: Mixture

Any concentration shown as a range is to protect confidentiality or is due to batch variation.

Section 4. First-Aid Measures

Description of necessary measures

Inhalation: Remove victim to fresh air and keep at rest in a position comfortable for breathing. If not breathing, if breathing is irregular or if respiratory arrest occurs, provide artificial respiration or oxygen by trained personnel. It may be dangerous to the person providing aid to give mouth-to-mouth resuscitation. Get medical attention if adverse health effects persist or are severe. If unconscious, place in recovery position and get medical attention immediately. Maintain an open airway.

Skin: Flush contaminated skin with plenty of water. Remove contaminated clothing and shoes. Get medical aid if irritation develops or persists

Eye: Immediately flush eyes with plenty of water, occasionally lifting the upper and lower eyelids. Check for and remove any contact lenses. Continue to rinse for at least 10 minutes. Get medical attention.

Ingestion: Do not induce vomiting. Get medical attention if adverse health effects develop or persist.

Most important symptoms/effects, acute and delayed
No further relevant information available

Indication of any immediate medical attention and special treatment needed
All treatment should be based on observed sign and symptoms of distress in the patient. Consideration should be given to the possibility that overexposure to materials other than this product may have occurred.

Section 5. Fire Fighting Measures

Extinguishing media:
Suitable extinguishing media: This is a nonflammable solution
Use an extinguishing agent suitable for the surrounding fire.

Unsuitable Extinguishing: None known

Special hazards arising from the substance or mixture
Unusual fire and explosion hazards: No specific fire or explosion hazard
Hazardous Combustions products: None known

Special protective equipment and precautions for the fire-fighters: No special measures required

Section 6. Accidental Release Measures

Personal precautions, protective equipment and emergency procedures
Wear appropriate protective equipment including respiratory protection as conditions warrant. Do not touch or walk through spilled material. Do not touch damaged containers or spilled materials unless wearing appropriate protective clothing.

Environmental precautions
Avoid run off to waterways and sewers.

Methods and materials for containment and cleaning up
Absorb with liquid-binding material.
Dispose contaminated material as waste according to item 13.

Section 7. Handling And Storage

Precautions for safe handling:	No special handling requirements for normal use of this material.
Conditions for safe storage, including and incompatibilities Storage:	Keep labeled container tightly closed and upright.
Incompatible materials or Ignition sources:	Keep away from strong oxidizers.

Section 8. Exposure Controls/Personal Protection

Control parameters

Occupational exposure Limit values:	Contains no substances with occupational exposure limit values.
Appropriate engineering controls:	General industrial hygiene practice.

Personal protective equipment

Eye protection:	Use equipment for eye protection tested and approved under appropriate government standards such as NIOSH (US) or EN 166(EU).
Hand Protection:	Protective gloves. The glove material has to be impermeable and resistant to the product/the substance/ the preparation.
Respiratory Protection:	None required under normal conditions of use

Section 9. Physical And Chemical Properties

Appearance	:	liquid
Odor	:	none
Odor Threshold	:	no data available
pH	:	no data available
Melting point/freezing point	:	no data available
Initial boiling point & boiling range	:	no data available
Flash point	:	no data available
Evaporation rate	:	no data available
Flammability (solid, gas)	:	no data available
Partition coefficient	:	no data available
Vapor pressure	:	no data available
Vapor density	:	no data available
Relative density (water)	:	no data available
Solubility	:	Soluble in water
Upper/lower flammability or explosive limits	:	no data available
Auto-ignition temperature	:	no data available
Decomposition temperature	:	no data available
Viscosity	:	no data available

Other information: None

Section 10. Stability And Reactivity

Reactivity:	No specific test data related to reactivity available for this product or its ingredients
Chemical stability	Stable under recommended storage conditions.
Possibility of hazardous reactions	Under normal conditions of storage and used, hazardous reactions should not occur.
Conditions to avoid	No specific data
Incompatible materials	No specific data
Hazardous decomposition products:	Under normal conditions of storage and used, hazardous decomposition products should not be produced.

Section 11. Toxicological Information

Information on toxicological effects
Acute toxicity

Product/Ingredient Name	CAS	Result	Species	Dose	Exposure
Inoculum Broth with Calcium Chloride	-	-	-	-	-

Irritation/Corrosion

Product/Ingredient Name	Result	Species	Score	Exposure/Observation
Inoculum Broth with Calcium Chloride	-	-	- -	-

Sensitization:	Not available
Mutagenicity:	Not available

Carcinogenicity

Product/Ingredient Name	OSHA	IARC	NTP
Inoculum Broth with Calcium Chloride	None of the ingredients listed	None of the ingredients listed	None of the ingredients listed

Reproductive toxicity:	Not available
Teratogenicity:	Not available
Specific target organ toxicity- single exposure	Not available
Specific target organ toxicity- repeated exposure	Not available
Aspiration Hazard	Not available

Information on the likely routes of exposure
Routes of entry anticipated: Oral

Symptoms related to the physical, chemical and toxicological characteristics

Eye contact:	No specific data
Inhalation:	No specific data
Skin contact:	No specific data
Ingestion:	No specific data
Aspiration Hazard	No specific data

Delayed and immediate effects and also chronic effects from short and long term exposure
No known significant effects or critical hazards.

Numerical measures of toxicity
Not available

Section 12. Ecological Information

Toxicity

Product/ingredient name	Result	Species	Exposure
Inoculum Broth with Calcium Chloride	-	-	-

Persistence and degradability:	Not available
Bioaccumulative potential	Not available
Mobility in soil	Not available
Other adverse effects	Not available
Aspiration Hazard	No known significant effects or critical hazards.

Section 13. Disposal Considerations

Waste treatment methods

Product waste: Dispose of content and/or container in accordance with local, regional, national, and/or international regulations

Packaging waste: Dispose of content and/or container in accordance with local, regional, national, and/or international regulations

Section 14. Transport Information

	UN Number	UN proper shipping name	Transport hazard class(es)	Packaging group	Environmental Hazards
DOT	Not regulated	-	-	-	none
IMDG	Not regulated	-	-	-	none
IATA	Not regulated	-	-	-	none

Transport in bulk according to Annex II of Marpol 73/78 and the IBC code
This product is provided only in non-bulk containers

Special Precautions for user
None specified

Section 15. Regulatory Information

Safety health and environmental regulations/ legislation specific for the substance or mixture

Sara	:	Not applicable
Section 355 (Extremely hazardous substances)	:	Substance is not listed
Section 313 (Specific toxic chemical listings)	:	Substance is not listed
SARA 311/312 Hazards No SARA Hazards TSCA (Toxic substances control act) Proposition 65:	:	Substance is not listed
Chemicals known to cause cancer	:	Substance is not listed
Chemicals known to cause reproductive toxicity or females	:	Substance is not listed
Chemicals known to cause reproductive toxicity for males	:	Substance is not listed
Chemicals known to cause developmental toxicity	:	Substance is not listed
Carcinogenic categories		
EPA (Environmental protection agency)	:	Substance is not listed
TLV (Threshold limit value established by ACGIH)	:	Substance is not listed
NIOSH-CA (National institute for occupational safety and health)	:	Substance is not listed
OSHA-CA (occupational safety & health administration)	:	Substance is not listed
GHS label elements	:	The product is classified and labeled according to the Globally Harmonized System (GHS).
Hazard pictograms	:	Not applicable
Decomposition temperature	:	Not applicable
Viscosity	:	Not applicable
Signal Word	:	None
Hazard-determining components of labeling	:	Not applicable
Hazard statements	:	None
Precautionary statements	:	Not applicable
National regulations	:	Substance is not listed
State right to know	:	Substance is not listed
Chemical safety assessment		A Chemical Safety Assessment has not been carried out.

Section 16. Other Information

Disclaimer/Statement of Liability: The information contained herein is believed to be accurate but is not warranted to be so. Data and calculation are based on information furnished by the manufacturers of the components of the product. Uses are advised to confirm in advance of need that information is current, applicable and suited to the circumstance of use. Vendor assumes no responsibility for injury to vendee or third persons proximately caused by the material if reasonable safety procedures are not adhered to as stipulated in the data sheet. Furthermore, vendor assumes no responsibility for injury caused by abnormal use of this material even if reasonable safety procedures are followed. Any questions regarding this product should be directed to the manufacturer of the product as described in section 1.

Warranty
The above information is believed to be correct but does not purport to
Be all inclusive and shall be used only as a guide. Teknova, inc. Shall not be held liable for any damage resulting from handling or from contact with the above product.
Teknova, inc.

Key to abbreviations: NDA= No data available

Appendix F: Patents

US007785846B2

(12) **United States Patent**
Boy et al.

(10) **Patent No.: US 7,785,846 B2**
(45) **Date of Patent: Aug. 31, 2010**

(54) **METHOD FOR THE PRODUCTION OF METHIONINE**

(75) Inventors: **Matthias Boy**, Langen (DE); **Daniela Klein**, Mannheim (DE); **Hartwig Schröder**, Nußloch (DE)

(73) Assignee: **Evonik Degussa GmbH**, Essen (DE)

(*) Notice: Subject to any disclaimer, the term of this patent is extended or adjusted under 35 U.S.C. 154(b) by 30 days.

(21) Appl. No.: **10/583,304**

(22) PCT Filed: **Dec. 17, 2004**

(86) PCT No.: **PCT/EP2004/014423**

§ 371 (c)(1),
(2), (4) Date: **Jun. 15, 2006**

(87) PCT Pub. No.: **WO2005/059155**

PCT Pub. Date: **Jun. 30, 2005**

(65) **Prior Publication Data**

US 2007/0122888 A1 May 31, 2007

(30) **Foreign Application Priority Data**

Dec. 18, 2003 (DE) 103 59 668

(51) **Int. Cl.**
C12P 13/12 (2006.01)

(52) **U.S. Cl.** **435/113**

(58) **Field of Classification Search** ..... None
See application file for complete search history.

(56) **References Cited**

U.S. PATENT DOCUMENTS

3,139,386	A *	6/1964	Takesue et al.	435/113
4,601,893	A	7/1986	Cardinal	
5,053,328	A	10/1991	Muetze et al.	
5,175,108	A	12/1992	Bachmann et al.	
5,840,358	A	11/1998	Höfler et al.	
6,017,555	A	1/2000	Stevens et al.	
2002/0197605	A1	12/2002	Nakagawa et al.	
2005/0260721	A1	11/2005	Kröger et al.	
2006/0003425	A1	1/2006	Kröger et al.	
2006/0068476	A1	3/2006	Kröger et al.	
2006/0223149	A1	10/2006	Kröger et al.	

FOREIGN PATENT DOCUMENTS

DE	3533198	3/1987
DE	10136986	3/2002
DE	10217058	11/2003
DE	10222858	12/2003
DE	10239073	3/2004
DE	10239082	3/2004
DE	10239308	3/2004
EP	0472869	7/1991
EP	0809940	4/1997
EP	1108790	6/2001
FR	1331847	7/1963
JP	10229891	9/1998
WO	WO-02/10209	2/2002
WO	WO-03/087386	10/2003
WO	WO-2005/007862	1/2005

OTHER PUBLICATIONS

Hsiao & Glatz, Biotechnology & Bioengineering, 1994, vol. 44, p. 1228-1234.*
Hermann, T., Journal of Biotechnology, Sep. 2003,vol. 104, p. 155-172.*
Kumar et al., Process Biochemistry, Mar. 2003, vol. 38, p. 1165-1171.*
Yuan et al., Journal of Chemical Technology and Biotechnology, 2002, vol. 77, p. 602-606.*
Kusumoto I., J. Nutr. 2001, vol. 131, p. 2552S-2555S.*
Hermann T., Journal of Biotechnology, Sep. 2003,vol. 104, p. 155-172.*
"Ionization Constants and pH Values at the Isoelectric Points of The Amino Acids in Water at 25°C." In Handbook of Chemistry and Physics. pp. C-741-C-743.
Hermann, T. "Industrial Production of Amino Acids by Coryneform Bacteria", Journal of Biotechnology, 2003, vol. 104, pp. 155-172.
Ullmann's Encyclopedia of Industrial Chemistry, 1985, vol. A2, pp. 71-72.
Motoyama, H., et al., "Overproduction of L-Lysine from Methanol by *Methylobacillus glycogenes* Derivatives Carrying a Plasmid with a Mutated *dapA* Gene", Applied and Environmental Microbiology, 2001, vol. 67, No. 7, pp. 3064-3070.
Eikmanns, B. J., et al., "Molecular Aspects of Lysine, Threonine, and Isoleucine Biosynthesis in Corynebacterium glutamicum", Antonie van Leeuwenhoek, 1993, vol. 64, pp. 145-163.
Eikmanns, B. J., "Identification, Sequence Analysis, and Expression of a *Corynebacterium glutamicum* Gene Cluster Encoding the Three Glycolytic Enzymes Glyceraldehyde-3-Phosphate Dehydrogenase, 3-Phosphoglycerate Kinase, and Triosephosphate Isomerase", Journal of Bacteriology, 1992, vol. 174, No. 19, pp. 6076-6086.
Martin, J. F., et al., "Cloning Systems in Amino Acid-Producing Corynebacteria", Bio/Technology, 1987, vol. 5, pp. 137-146.
Guerrero, C., et al., "Directed Mutagenesis of a Regulatory Palindromic Sequence Upstream from the *Brevibacterium lactofermentum* Tryptophan Operon", Gene, 1994, vol. 138, pp. 35-41.
Tsuchiya, M., et al., "Genetic Control Systems of *Escherichia coli* Can Confer Inducible Expression of Cloned Genes in Coryneform Bacteria", Bio/Technology, 1988, vol. 6, pp. 428-430.
Eikmanns, B. J., et al., "A Family of *Corynebacterium glutamicum/Escherichia coli* Shuttle Vectors for Cloning, Controlled Gene Expression, and Promoter Probing", Gene, 1991, vol. 102, pp. 93-98.
Schwarzer, A., et al., "Manipulation of *Corynebacterium glutamicum* by Gene Disruption and Replacement", Bio/Technology, 1991, vol. 9, pp. 84-87.

(Continued)

Primary Examiner L. Blaine Lankford
Assistant Examiner—Kade Ariani
(74) *Attorney, Agent, or Firm*—Smith, Gambrell & Russell, LLP

(57) **ABSTRACT**

The present invention relates to a process for producing methionine by fermentation, a process for isolating the methionine formed, the methionine-containing biomass produced in isolation, use thereof for producing a feedstuff or feed supplement, and also to the use of the isolated methionine for producing foodstuffs or feedstuffs or food supplements or feed supplements.

16 Claims, 4 Drawing Sheets

OTHER PUBLICATIONS

Reinscheid, D. J., et al., "Stable Expression of *hom-1-thrB* in *Corynebacterium glutamicum* and its Effect on the Carbon Flux to Threonine and Related Amino Acids", Applied and Environmental Microbiology, 1994, vol. 60, No. 1, pp. 126-132.

LaBarre, J., et al., "Gene Replacement, Integration, and Amplification at the *gdhA* Locus of *Corynebacterium glutamicum*", Journal of Bacteriology, 1993, vol. 175, No. 4, pp. 1001-1007.

Malumbres, M., et al., "Codon Preference in Corynebacteria", Gene, 1993, vol. 134, pp. 15-24.

Jensen, P. R., et al., "Artificial Promoters for Metabolic Optimization", Biotechnology and Bioengineering, 1998, vol. 58, Nos. 2 & 3, pp. 191-195.

Makrides, S.C., "Strategies for Achieving High-Level Expression of Genes in *Escherichia coli*" Microbiological Reviews, 1996, vol. 60, No. 3, pp. 512-538.

Pátek, M., et al., "Leucine Synthesis in *Corynebacterium glutamicum*: Enzyme Activities, Structure of *leuA*, and Effect of *leuA* Inactivation on Lysine Synthesis", Applied and Environmental Microbiology, 1994, vol. 60, No. 1, pp. 133-140.

Malakhova, I. I., et al., "Thin-Layer Chromatography of Free Amino Acids. Selection of Conditions for the Separation of L-Lysine, L-Homoserine, and L-Threonine", Russian Biotechnology, 1996, No. 11, pp. 26-31.

Schmidt, S., et al., "Near Infrared Spectroscopy in Fermentation and Quality Control for Amino Acid Production", Bioprocess Engineering, 1998, vol. 19, pp. 67-70.

Roy, S. K., et al., "Production and Purification of Methionine from a Multi-Analog Resistant Mutant B_2US-215 of *Bacillus megaterium* B71", J. Microbial Biotechnology, 1989, vol. 4, No. 1, pp. 35-41.

* cited by examiner

Fig. 1

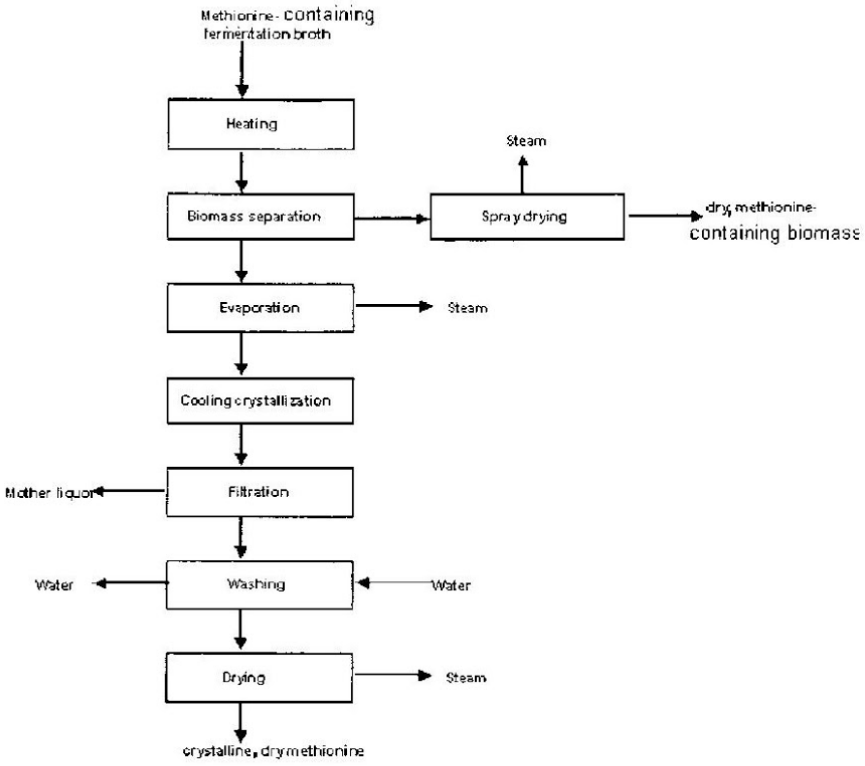

Methionine-containing fermentation broth
Heating
Steam
Biomass separation
Spray drying
dry, methionine-containing biomass
Evaporation
Steam
Cooling crystallization
Filtration
Mother liquor
Water
Washing
Water
Drying
Steam
crystalline, dry methionine

Fig. 2

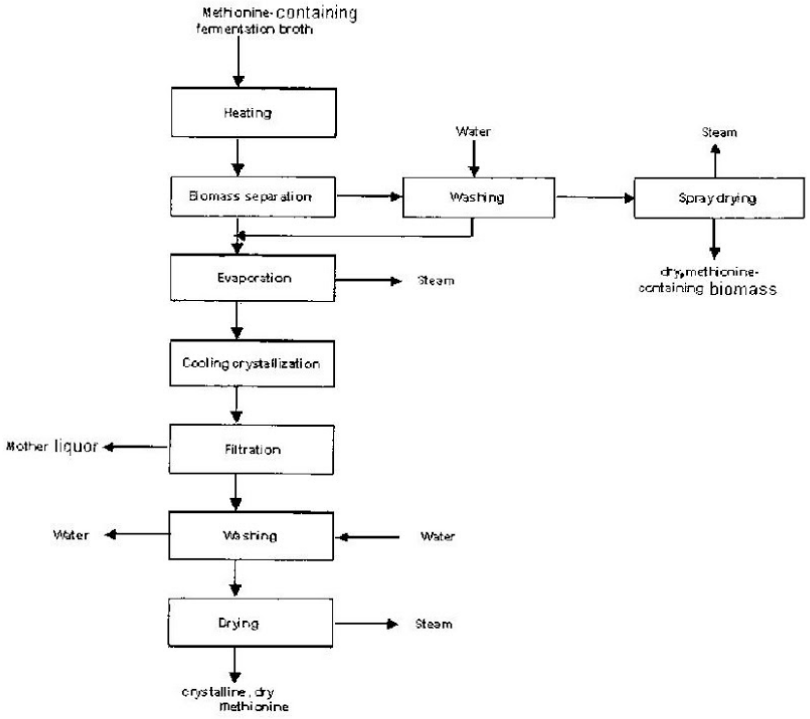

Methionine-containing fermentation broth
Heating
Water
Steam
Biomass separation
Washing
Spray drying
Evaporation
Steam
dry, methionine-containing biomass
Cooling crystallization
Mother liquor
Filtration
Water
Washing
Water
Drying
Steam
crystalline, dry methionine

Fig. 3

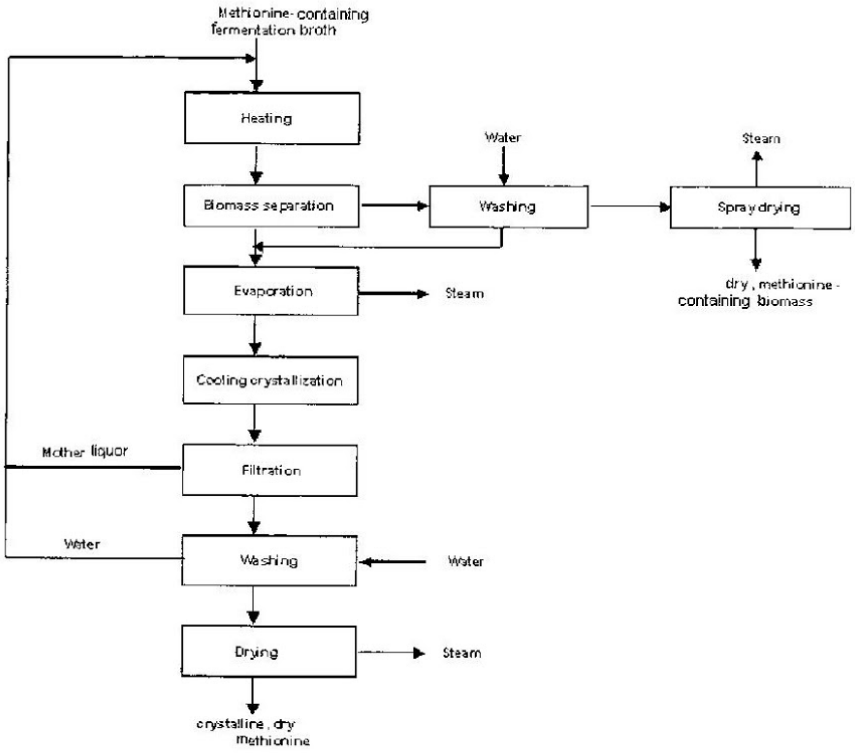
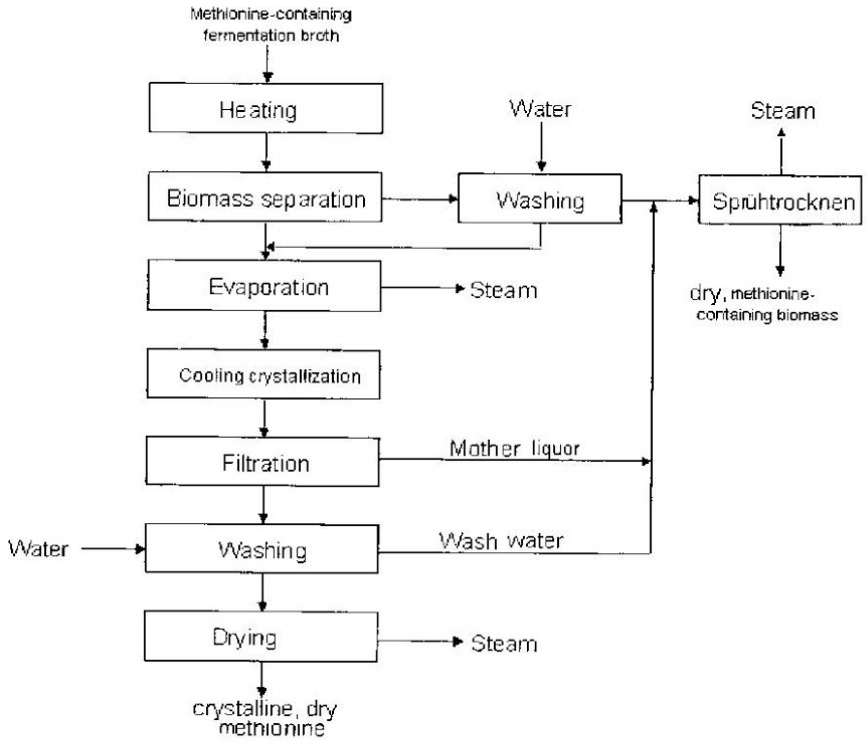

Methionine-containing fermentation broth
Heating
Water
Steam
Biomass separation
Washing
Spray drying
Evaporation
Steam
dry, methionine-containing biomass
Cooling crystallization
Mother liquor
Filtration
Water
Washing
Water
Drying
Steam
crystalline, dry methionine

Fig. 4


Methionine-containing fermentation broth
Heating
Water
Steam
Biomass separation
Washing
Sprühtrocknen
Evaporation
Steam
dry, methionine-containing biomass
Cooling crystallization
Filtration
Mother liquor
Water
Washing
Wash water
Drying
Steam
crystalline, dry methionine

METHOD FOR THE PRODUCTION OF METHIONINE

RELATED APPLICATIONS

This application is a national stage application (under 35 U.S.C. 371) of PCT/EP2004/014423 filed Dec. 17, 2004, which claims benefit of German application 103 59 668.2 filed Dec. 18, 2003.

The present invention relates to a process for producing methionine by fermentation, a process for isolating the methionine formed, the methionine-containing biomass obtained in the isolation, its use for producing a feedstuff or feed supplement, and also to the use of the isolated methionine for producing foods or feeds or food or feed supplements.

PRIOR ART

Methionine is used in the most varied sectors, including the food, feed, cosmetics and pharmaceutical industries.

Hitherto, only the chemical production processes for D,L-methionine have been of industrial importance. Starting materials for this synthesis are hydrogen sulfide, methylmercaptan, acrolein, Prussic acid or methylmercaptopropionaldehyde (see Ullmann's Encyclopedia of Industrial Chemistry (1985), Vol. A2, page 71).

Methionine is also produced by natural cellular metabolic processes. Its production on an industrial scale would most expediently be carried out by means of bacterial cultures which have been developed to produce and secrete large amounts of the desired substance. Organisms which are particularly suitable for this purpose are nonpathogenic coryneform bacteria.

It is known that methionine can be produced by fermenting strains of coryneform bacteria in particular *Corynebacterium glutamicum*. Because of the great importance, work is continuously being carried out on improving the production process. Improvements on the process can relate to, for example, fermentation measures, the composition of the nutrient media, or the intrinsic performance properties of the microorganism itself.

To improve the performance properties of these microorganisms with respect to producing a defined molecule, use can be made of methods of mutagenesis, selection and mutant selection or methods of recombinant DNA technology for the strain improvement of amino acid-producing strains, for example of *Corynebacterium*, by amplifying or turning off individual amino acid biosynthesis genes and thus inducing an improvement of the amino acid production.

For instance, WO-A-02/10209 and DE-A-101 36 986 describe a process for producing L-methionine by fermentation, using L-methionine-producing genetically modified coryneform bacteria. There, a description is given, inter alia, of a process for producing L-methionine comprising the fermentation of the bacteria, the enrichment of the amino acid in the medium or in the bacteria and the isolation of the amino acid. Furthermore, a process is described for producing L-methionine-containing animal feed additive from a fermentation broth which comprises the following steps: a) fermenting L-methionine-producing microorganisms; b) concentrating the fermentation broth, for example by evaporation; c) separating off the biomass (0-100%), for example by centrifugation; and d) drying, for example by freeze drying or spray drying, spray granulation.

Swapan et al. describe, in J. Microbial Biotechnology, 4 (1), 35-41 (1989), the microbial production of methionine by means of a *Bacillus megaterium* mutant by separating off the cells from the fermentation broth, adjusting the pH to 5, treatment with activated carbon and ion-exchange chromatography.

DE-A-35 33 198 discloses the production of L-leucine by fermentation using special thermophilic bacteria. The fermentation is performed at +60° C. continuously with retention of biomass, separation of product-containing, spent medium, cooling (down to +2° C.) in a crystallizer, production of the amino acid which has crystallized out and recirculation of the mother liquor to the reactor. The production of methionine by fermentation is not described therein.

The processes which have been described hitherto for the microbial production of methionine do not yet satisfy the requirements of production on an industrial scale. A reason for this is, firstly, the limited solubility of methionine in the aqueous fermentation medium, which has the effect that, at high biosynthesis output, methionine precipitates out in the fermentation broth and thus makes purification difficult. A further reason is that in the case of work according to the prior art, considerable waste streams are produced, the disposal of which is associated with high cost.

BRIEF DESCRIPTION OF THE INVENTION

It is an object of the present invention, therefore, to provide an improved process for isolating methionine produced by fermentation which is applicable, in particular, to those fermentation broths which comprise methionine in part in crystalline form. A further object is to provide a workup process for methionine-containing fermentation broths which produces virtually no waste streams and thus can be carried out particularly economically.

We have found that the above object is surprisingly achieved by providing a workup process which specifically exploits the solubility properties of methionine for separating off the biomass. The process utilizes crystallization as a purification method for L-methionine produced by fermentation. It gives two different products for use as feed additive (low-concentration and high-concentration product). In preferred variants, virtually no waste streams are produced and thus particularly economical methionine production on an industrial scale is permitted.

DETAILED DESCRIPTION OF THE INVENTION

A) General Definitions

"Methionine", for the purposes of the invention, in principle covers L- or D-methionine, mixtures of these isomers, for example racemates, but preferably L-methionine.

The solubility of methionine in water is about 30 g/l at 20° C., and at 70° C. it is greater than 90 g/l. In the fermentation broth under these conditions, solubilities of comparable order of magnitude are observed.

Process measures such as "concentrating", "separating", "washing", "drying", for the purposes of the present invention, cover all processes present in the field of specialist skill. For example, "concentrating" can be taken to mean evaporating the liquid phase under atmospheric pressure or with the application of a vacuum. "Concentrating" can be carried out, for example, using familiar techniques, such as reverse osmosis or nanofiltration or customary apparatuses, for example a falling-film evaporator, thin-film evaporator or rotary evaporator, or combinations thereof. "Separating" can cover, for example, centrifuging, filtering, decanting, or combinations of these processes. "Washing" can cover, for example, filtering off a solid and single or repeated washing, if appropriate

after suspending the filter residue. "Drying" can cover, for example, freeze drying, spray drying, spray granulation, fluidized-bed drying or combinations of these processes.

B) Preferred Embodiments of the Invention

The present invention firstly relates to a process for isolating methionine produced by fermentation, which comprises

a) heating a methionine-containing liquid fraction produced in the fermentation of a methionine-producing microorganism, which liquid fraction comprises, in particular, methionine in partially undissolved form, to a temperature which is sufficient to increase the solubility of methionine in the liquid phase, preferably to bring methionine essentially completely into solution,

b) obtaining therefrom a methionine-enriched liquid phase and

c) crystallizing out methionine, if appropriate after concentrating the enriched liquid phase.

Methionine is "essentially" completely in solution if it is, for example, more than 95% dissolved, preferably more than 98% dissolved, in particular 100% dissolved, based on the total methionine content in the liquid phase.

A methionine-containing "liquid fraction" is typically the broth which is obtained from the fermentation process and comprises, in particular, methionine in partially undissolved form and if appropriate can have other solid constituents which can be customarily present in fermentation broths; or a liquid derived therefrom, obtained for example by suitable pretreatment. A "pretreatment" could consist, for example, in concentration by evaporation, or in addition of substances. For example, methionine-containing fractions could be added to the broth from previous workup batches, or adjuncts (see below) which promote the following processing steps or which promote the use of the product (for example a feed additive) as directed.

The content of undissolved methionine in the, if appropriate, fortified fermentation broth is, based on the total weight of the fermentation broth, in the range of from about 1 to 10% by weight, preferably from about 3 to 8% by weight, or, based on the total solids content, in the range of from about 30 to 80% by weight, preferably from about 50 to 57% by weight.

For example, an inventive fermentation can give a methionine content of about 96 g/l, of which, at a typical fermentation temperature, about 46 g/l are in solution and about 50 g/l are undissolved.

The methionine content of the enriched liquid phase is, based on the dry residues thereof, in the range of from about 60 to 100% by weight or about 90 to 100% by weight, as for example from about 75 to 85% by weight, or about 95 to 100% by weight, each based on dry mass.

To bring methionine essentially into solution, in stage a), the liquid is heated to a temperature in the range of from about 60 to 120° C., preferably from about 70 to 100° C., depending on the amount of the product to be dissolved. If appropriate, it can be necessary to operate under a slightly elevated pressure, for example from 1 to 5 atm.

Preferably, the liquid fraction used in stage a) is the biomass-containing fermentation broth without further pretreatment.

The methionine-enriched liquid phase of stage b) is preferably obtained by separating off the biomass from the heated fermentation broth which is enriched with dissolved methionine. To prevent premature crystallization of the methionine, likewise elevated temperature is employed during biomass separation, preferably a temperature in the range specified above.

In a preferred embodiment of the invention

d) the crystallized methionine is separated off,

e) the solid, preferably crystalline, methionine which has been separated off is if appropriate washed and

f) if appropriate dried.

According to a further preferred process variant, the biomass separated off in stage b) is

g1) if appropriate washed, the liquid used for the washing being if appropriate heated, and

g3) dried.

It can become necessary to heat the wash liquid if, for example, solid methionine is to be present in the biomass fraction separated off, and it is desired to produce methionine from the biomass fraction as far as possible.

To avoid waste streams, preferably

g2) the wash liquid produced in stage g1) is combined with the methionine-enriched liquid phase from stage b).

The methionine-containing liquid phases of the stage b) obtained in accordance with the above procedures are then further concentrated, for example by evaporation with heating and if appropriate applying a vacuum. The methionine content in the resultant concentrate is in the range of from about 10 to 40% by weight, based on the total weight of the concentrate. The methionine is preferably separated off by cooling crystallization. For this the solution is cooled to temperatures in the range from 0 to 20° C. After crystallization is complete, the solid methionine is washed with cold wash liquid, for example water, and dried, if appropriate with gentle heating.

According to a further process variant, the mother liquor produced in stage d) is

d1) combined with the methionine-containing liquid fraction from another fermentation batch using a methionine-producing microorganism; or

d2) added to the biomass separated off from the same or another fermentation batch using a methionine-producing microorganism before the drying according to stage g3).

According to a further process variant, the wash liquid produced in stage e) is

e1) combined with the methionine-containing liquid fraction from another fermentation batch using a methionine-producing microorganism; or

e2) added to the biomass separated off from the same or another fermentation batch using a methionine-producing microorganism before the drying according to stage g3).

Recirculating mother liquor and wash liquid further prevents production of waste streams.

According to the invention, in addition, preferably, the drying according to stage g3) comprises a spray-drying step.

The present invention further relates to a process for producing methionine by fermentation, a natural or recombinant microorganism being fermented in a manner known per se and the methionine formed being isolated by a process according to the definition above.

In a preferred embodiment, the inventive processes are carried out using a methionine-producing microorganism selected from natural or recombinant bacteria of the genus *Corynebacterium*.

The invention further relates to the use of the dry material obtainable according to the above stage g3) for producing a feedstuff or a feed supplement (feed additive).

The present invention also relates to the use of the inventively isolated methionine for producing a foodstuff or feedstuff or food supplement or feed supplement.

The invention finally relates to methionine-containing dried biomasses obtainable by a process according to the definition above; feed additives, comprising a biomass of this

type; and also feed compositions comprising such a feed additive in addition to customary feedstuff constituents.

In the sections below, further developments of the invention are described.

C) Host Cells Used According to the Invention

For the inventive process, use is preferably made of coryneform bacteria. Preferably, these are bacteria of the genus *Corynebacterium*. Of the genus *Corynebacterium*, in particular the species *Corynebacterium glutamicum* is to be mentioned which is known in speciality for its ability to produce L-amino acids.

Examples of suitable strains which may be mentioned are:

of the genus *Corynebacterium:*

Corynebacterium glutamicum ATCC 13032, *Corynebacterium acetoglutanicum* ATCC 15806, *Corynebacterium acetoacidophilum* ATCC 13870, *Corynebacterium thermoaminogenes* FERM BP-1539, *Corynebacterium melassecola* ATCC 17965; *Corynebacterium glutamicum* KFCC10065; or *Corynebacterium glutamicum* ATCC21608

or of the genus *Brevibacterium:*

Brevibacterium flavum ATCC 14067; *Brevibacterium lactofermentum* ATCC 13869 and *Brevibacterium divaricatum* ATCC 14020 are to be mentioned;

(KFCC=Korean Federation of Culture Collection: ATCC=American Type Culture Collection; FERM BP=Collection of the National Institute of Bioscience and Human-Technology, Agency of Industrial Science and Technology, Japan)

The bacterial strains can be used unmodified or genetically modified in a suitable manner. For instance, microorganisms can be used, for example, in which genes of the methionine biosynthesis pathway are amplified, so that more methionine is present in the cell. Alternatively, or additionally, it is also possible to switch off or attenuate genes which are involved in methionine-degrading metabolic pathways. Suitable strategies for improving methionine production are known from the prior art and are described, for example, in WO-A-02/10209, DE-A-102 170 58, DE-A-102 393 08, DE-A-102 390 73, DE-A-102 390 82 and DE-A-102 228 58 which are expressly incorporated herein by reference.

In order to reduce the activity or amount of an enzyme which could lower the methionine content, those skilled in the art can carry out differing measures individually or in combinations. By reducing the transcription frequency of the gene which codes for the inventive protein, the concentration of the relevant protein can be lowered. This can be achieved by those skilled in the art by modifying or exchanging the promoter or regulation region and also the ribosome binding site of the coding gene. Downstream of the coding region, those skilled in the art can modify terminators or introduce sequences which lead to a reduced stability of the transcript. These measures reducing the life of the mRNA make it possible to lower the expression of the associated protein and thus its concentration.

At the level of the expressed enzyme, fusioned sequences can lead to an increased breakdown rate and thus likewise to a lowering of the concentration of the protein. In addition, those skilled in the art, by means of targeted or untargeted mutagenesis of the coding gene can change the activity, the substrate affinity and the substrate specificity. The activity of enzymes can be affected by mutations in the corresponding genes in such a manner that partial or complete reduction of the reaction velocity of the enzymatic reaction occurs. Examples of such mutations are known to those skilled in the art (Motoyama H. Yano H. Terasaki Y. Anazawa H. Applied & Environmental Microbiology. 67:3064-70, 2001, Eikmanns B J. Eggeling L. Sahm H. Antonie van Leeuwenhoek. 64:145-63, 1993-94). Mutants of the protein can also lead to reduced or inhibited homo- or heteromultimerization of enzyme complexes and thus likewise to an impairment of the enzymatic properties.

Genes modified in this manner can either be present in plasmids, or preferably integrated in the chromosome. In this case, the original gene which has not been modified in this manner can still additionally be present, but preferably can be exchanged for the modified gene.

To reduce the activity of an enzyme measured in a coryneform bacterium, it can be sufficient to express genes which code for functional equivalents, such as artificially manufactured mutants or natural homologes from other organisms. In this case, the original gene can still additionally be present, but preferably can be exchanged for the modified or homologous gene.

In addition, it can be advantageous for the bacterial production of methionine to amplify one or more enzymes of the methionine biosynthesis pathway, of the cysteine metabolic pathway, of aspartate semialdehyde synthesis, of glycolysis, of anaplerosis, of pentose phosphate metabolism, of the citric acid cycle or of amino acid export.

For instance, for the production of methionine, one or more of the following genes can be amplified:

- the gene lysC coding for an aspartate kinase (EP 1 108 790 A2; DNA-SEQ NO. 281),
- the gene asd coding for an aspartate semialdehyde (EP 1 108 790 A2; DNA-SEQ NO. 282),
- the gene gap coding for glyceraldehyde-3-phosphate dehydrogenase (Eikmanns (1992), Journal of Bacteriology 174: 6076-6086),
- the gene pgk coding for 3-phosphoglycerate kinase (Eikmanns (1992), Journal of Bacteriology 174: 6076-6086),
- the gene pyc coding for pyruvate carboxylase (Eikmanns (1992), Journal of Bacteriology 174: 6076-6086),
- the gene tpi coding for triose-phosphate isomerase (Eikmanns (1992), Journal of Bacteriology 174: 6076-6086),
- the gene metA coding for homoserine O-acetyltransferase (EP 1 108 790 A2; DNA-SEQ NO. 725),
- the gene metB coding for cystathioninegamma-synthase (EP 1 108 790 A2; DNA-SEQ NO. 3491),
- the gene metC coding for cystathioninegamma-lyase (EP 1 108 790 A2; DNA-SEQ NO. 3061),
- the gene metH coding for cystathioninesynthase (EP 1 108 790 A2; DNA-SEQ NO. 1663),
- the gene glyA coding for serinehydroxymethyltransferase (EP 1 108 790 A2; DNA-SEQ NO. 1110),
- the gene metY coding for O-acetylhomoserinesulfhydrylase (EP 1 108 790 A2;DNA-SEQ NO. 726),
- the gene metF coding for methylenetetrahydrofolatereductase (EP 1 108 790 A2; DNA-SEQ NO. 2379),
- the gene serC coding for phosphoserine aminotransferase (EP 1 108 790 A2; DNA-SEQ NO. 928)
- a gene serB coding for phosphoserine phosphatase (EP 1 108 790 A2; DNA-SEQ NO. 334, DNA-SEQ NO. 467, DNA-SEQ NO. 2767)
- the gene cysE coding for serine acetyl-transferase (EP 1 108 790 A2; DNA-SEQ NO. 2818)
- the gene cysK coding for cysteine synthase (EP 1 108 790 A2; DNA-SEQ NO. 2817),
- the gene hom coding for a homoserine dehydrogenase (EP 1 108 790 A2; DNA-SEQ NO. 1306)

In addition, it can be advantageous for the inventive production of methionine to mutate simultaneously at least one

of the following genes in such a manner that the activity of the corresponding proteins, compared with non-mutated proteins, is affected to a lesser extent, or is not affected, by a metabolite, or that their specific activity is increased:

- the gene lysC coding for an aspartate kinase (EP 1 108 790 A2; DNA-SEQ NO. 281),
- the gene pyc coding for pyruvate carboxylase (Eikmanns (1992). Journal of Bacteriology 174: 6076-6086).
- the gene metA coding for homoserine O-acetyltransferase (EP 1 108 790 A2; DNA-SEQ NO. 725),
- the gene metB coding for cystathionine gamma-synthase (EP 1 108 790 A2; DNA-SEQ NO. 3491),
- the gene metC coding for cystathionine gamma-lyase (EP 1 108 790 A2; DNA-SEQ NO. 3061),
- the gene metH coding for methionine synthase (EP 1 108 790 A2; DNA-SEQ NO. 1663),
- the gene glyA coding for serine hydroxymethyltransferase (EP 1 108 790 A2; DNA-SEQ NO. 1110),
- the gene metY coding for O-acetylhomoserine sulfhydrylase (EP 1 108 790 A2; DNA-SEQ NO. 726),
- the gene metF coding for methylenetetrahydrofolate reductase (EP 1 108 790 A2; DNA-SEQ NO. 2379),
- the gene serC coding for phosphoserine aminotransferase (EP 1 108 790 A2; DNA-SEQ NO. 928)
- a gene serB coding for phosphoserine phosphatase (EP 1 108 790 A2; DNA-SEQ NO. 334, DNA-SEQ NO. 467, DNA-SEQ NO. 2767)
- the gene cysE coding for serine acetyltransferase (EP 1 108 790 A2; DNA-SEQ NO. 2818)
- the gene cysK coding for cysteine synthase (EP 1 108 790 A2; DNA-SEQ NO. 2817),
- the gene hom coding for a homoserine dehydrogenase (EP 1 108 790 A2; DNA-SEQ NO. 1306)

In addition, it can be advantageous for the production of methionine to attenuate one or more of the following genes, in particular to reduce or switch off their expression:

- the gene metK coding for S-adenosylmethionine synthase (E.C.2.5. 1.6)
- the gene thrB coding for homoserine kinase (EP 1 108 790 A2; DNA-SEQ NO. 3453)
- the gene ilvA coding for threonine dehydratase (EP 1 108 790 A2; DNA-SEQ NO. 2328)
- the gene thrC coding for threonine synthase (EP 1 108 790 A2; DNA-SEQ NO. 3486)
- the gene ddh coding for meso-diaminopimelate D-dehydrogenase (EP 1 108 790 A2; DNA-SEQ NO. 3494)
- the gene pck coding for phosphoenolpyruvate carboxykinase (EP 1 108 790 A2; DNA-SEQ NO. 3157)
- the gene pgi coding for glucose-6-phosphate 6-isomerase (EP 1 108 790 A2; DNA-SEQ NO. 950)
- the gene poxB coding for pyruvate oxidase (EP 1 108 790 A2; DNA-SEQ NO. 2873)
- the gene dapA coding for dihydrodipicolinate synthase (EP 1 108 790 A2; DNA-SEQ NO. 3476)
- the gene dapB coding for dihydrodipicolinate reductase (EP 1 108 790 A2; DNA-SEQ NO. 3477)
- gene lysA coding for diaminopicolinate decarboxylase (EP 1 108 790 A2; DNA-SEQ NO. 3451)

In addition it can be advantageous for the production of methionine to mutate at least one of the abovementioned genes metK, thrB, ilvA, thrC, ddh, pck, pgi, poxB, dapA, dapB, lysA in such a manner that the enzymatic activity of the corresponding protein is partially or completely cut back.

In addition it can be advantageous for the production of methionine to eliminate further unwanted side reactions (Nakayama: "Breeding of Amino Acid Producing Microorganisms", in: Overproduction of Microbial Products, Krumphanzl, Sikyta, Vanek (eds.). Academic Press, London, UK, 1982).

To achieve overexpression, those skilled in the art can take differing measures individually or in combination. Thus, the number of copies of the corresponding genes can be increased, or the promoter and regulation region or the ribosome binding site which is upstream of the structural gene can be mutated. Expression cassettes act in the same manner which are incorporated upstream of the structural gene. By means of inducible promoters it is additionally possible to increase the expression in the course of production of L-methionine by fermentation. Measures to prolong the life of the mRNA likewise improve expression. Furthermore, by inhibiting the breakdown of the enzyme protein, the enzyme activity is likewise increased. The genes or gene constructs can be present in plasmids with differing numbers of copies or integrated in the chromosome and amplified. Alternatively, overexpression of the relevant genes can further be achieved by changing the media composition and culture conditions.

Those skilled in the art find instructions in this respect, inter alia, in Martin et al. (Biotechnology 5, 137-146 (1987)), in Guerrero et al. (Gene 138, 35-41 (1994)), Tsuchiya and Morinaga (Bio/Technology 6, 428-430 (1988)), in Eikmanns et al. (Gene 102, 93-98 (1991)), in EP 0472869, in U.S. Pat. No. 4,601,893, in Schwarzer and Pühler (Biotechnology 9, 84-87 (1991), in Remscheid et al. (Applied and Environmental Microbiology 60, 126-132 (1994), in LaBarre et al. (Journal of Bacteriology 175, 1001-1007 (1993)), in WO 96/15246, in Malumbres et al. (Gene 134, 15-24 (1993)), in JP-A-10-229891, in Jensen and Hammer (Biotechnology and Bioengineering 58, 191-195 (1998)), in Makrides (Microbiological Reviews 60:512-538 (1996) and in known textbooks of genetics and molecular biology.

D) Carrying Out the Inventive Fermentation

The microorganisms produced according to the invention can be cultured for the production of methionine continuously or batchwise in the batch process (batch culture) or in the fed batch process, or repeated fed batch process. A summary of known culture methods may be found in the textbook by Chmiel (Bioprozeßtechnik 1. Einführung in die Bioverfahrenstechnik [Process Biotechnology 1. Introduction to process biotechnology] (Gustav Fischer Verlag, Stuttgart, 1991)) or in the textbook by Storhas (Bioreaktoren und periphere Einrichtungen [Bioreactors and peripherals] (Vieweg Verlag, Brunswick/Wiesbaden, 1994)).

The culture medium to be used has to satisfy the requirements of the respective strains in a suitable manner. Descriptions of culture media of various microorganisms are given in the manual "Manual of Methods für General Bacteriology" of the American Society für Bacteriology (Washington D.C., USA, 1981).

The media which can be used according to the invention usually comprise one or more carbon sources, nitrogen sources, inorganic salts, vitamins and/or trace elements.

Preferred carbon sources are sugars, such as mono-, di- or polysaccharides. Very good carbon sources are, for example, glucose, fructose, mannose, galactose, ribose, sorbose, ribulose, lactose, maltose, sucrose, raffinose, starch or cellulose. Sugars can also be added to the media via complex compounds, such as molasses, or other by-products of sugar refining. It can also be advantageous to add mixtures of various carbon sources. Other possible carbon sources are oils and fats, for example soybean oil, sunflower oil, peanut oil and coconut fat; fatty acids, for example palmitic acid, stearic acid

or linoleic acid; alcohols, for example glycerol, methanol or ethanol: and organic acids, for example acetic acid or lactic acid.

Nitrogen sources are usually organic or inorganic nitrogen compounds or materials which contain these compounds. Examples of nitrogen sources comprise ammonia gas or ammonium salts such as ammonium sulfate, ammonium chloride. ammonium phosphate. ammonium carbonate or ammonium nitrate, nitrates, urea, amino acids or complex nitrogen sources such as corn steep liquor, soybean meal, soybean protein, yeast extract, meat extract and others. The nitrogen sources can be used individually or as a mixture.

Inorganic salt compounds which can be present in the media comprise the chloride, phosphorus or sulfate salts of calcium, magnesium, sodium, cobalt, molybdenum, potassium. manganese, zinc. copper and iron.

Sulfur sources which can be used for the production of methionine are inorganic sulfur compounds, for example sulfates, sulfites, dithionites, tetrathionates, thiosulfates, sulfides, but also organic sulfur compounds, such as mercaptans and thiols.

As phosphorus source. use can be made of phosphoric acid. potassium dihydrogen phosphate or dipotassium hydrogen phosphate or the corresponding sodium salts.

Chelating agents can be added to the medium to keep metal ions in solution. Particularly suitable chelating agents comprise dihydroxyphenols, such as catechol or protocatechuate. or organic acids, such as citric acid.

The fermentation media used according to the invention usually also comprise other growth factors, such as vitamins or growth promoters. which include, for example, biotin. riboflavin. thiamine, folic acid. nicotinic acid. panthothenate and pyridoxine. Growth factors and salts frequently originate from complex media components, such as yeast extract, molasses. corn steep liquor and the like. In addition, suitable precursors can be added to the culture medium. The exact composition of the media compounds depends greatly on the respective experiment and is decided individually for each specific case. Information on media optimization is obtainable from the textbook "Applied Microbiol. Physiology. A Practical Approach" (editors P. M. Rhodes. P. F. Stanbury. IRL Press (1997) pp. 53-73, ISBN 0 19 963577 3). Growth media may also be obtained from commercial suppliers, such as Standard 1 (Merck) or BHI (Brain heart infusion, DIFCO) and the like.

All media components are sterilized either by heat (20 min at 1.5 bar and 121° C.) or by sterile filtration. The components can either be sterilized together or if necessary separately. All media components can be present at the start of the culture or optionally can be added continuously or batchwise.

The temperature of the culture is usually from 15° C. to 45° C., preferably from 25° C. to 40° C., and can be kept constant or changed during the experiment. The pH of the medium should be in the range from 5 to 8.5, preferably around 7.0. The pH for the culture can be regulated during culture by adding basic compounds, such as sodium hydroxide, potassium hydroxide, ammonia or ammonia water, or acidic compounds, such as phosphoric acid or sulfuric acid. To control foam development, antifoamers can be used, for example fatty acid polyglycol esters. To maintain the stability of plasmids, suitable selective substances, for example antibiotics, can be added to the medium. To maintain aerobic conditions, oxygen or oxygen-containing gas mixtures, for example ambient air, are introduced into the culture. The culture is continued until a maximum of the desired product has formed. This goal is usually achieved within from 10 hours to 160 hours.

The resultant methionine-containing fermentation broths usually have a dry mass of from 7.5 to 25% by weight.

It is, furthermore, advantageous if the fermentation is run under sugar-limiting conditions at least at the end. but in particular over at least 30% of the fermentation time. That is to say that during this time the concentration of utilizable sugar in the fermentation medium is kept at from ≧0 to 3 g/l, or is reduced.

E) Purification of Methionine

If the methionine obtained according to the invention after crystallization should still not have the desired purity, it can be further purified. For this the product is subjected in dissolved form to chromatography using a suitable resin, the desired product or the impurities being wholly or partially retained on the chromatographic resin. These chromatographic steps can be repeated if necessary, the same or different chromatographic resins being used. Those skilled in the art are conversant in the selection of suitable chromatographic resins and their most effective application. The purified product can be concentrated by filtration or ultrafiltration and stored at a temperature at which the stability of the product is maximal.

The identity and purity of the isolated compound can be determined by known techniques. These include high-performance liquid chromatography (HPLC), spectroscopic methods, color methods, thin-layer chromatography, NIRS, enzyme testing or microbiological tests. These analytical methods are summarized in: Patek et al. (1994) Appl. Environ. Microbiol. 60:133-140; Malakhova et al. (1996) Biotekhnologiya 11 27-32; and Schmidt et al. (1998) Bioprocess Engineer. 19:67-70. Ullmann's Encyclopedia of Industrial Chemistry (1996) Vol. A27, VCH: Weinheim. pp. 89-90, pp. 521-540. pp. 540-547, pp. 559-566. 575-581 and pp. 581-587: Michal, G (1999) Biochemical Pathways: An Atlas of Biochemistry and Molecular Biology, John Wiley and Sons; Fallon, A. et al. (1987) Applications of HPLC in Biochemistry in: Laboratory Techniques in Biochemistry and Molecular Biology, Vol. 17.

F) Drying the Biomass

After the fermentation is completed, the methionine-containing fermentation broth can be processed directly to give the finished dry feedstuff additive. According to a preferred embodiment of the invention, however, first the biomass content is wholly or partially, preferably completely. removed, for example by centrifugation, from the fermentation broth and processed to form inventive feedstuff additive. The resultant biomass still contains a certain fraction of methionine, which if desired can be decreased by intermediate provision of a wash step.

The inventive biomass can be worked up to give a suitable dry product by various processes from the prior art which are known per se. In particular, suitable processes for the production are drying processes, such as spray drying, spray granulation, contact drying, fluidized-bed drying or freeze drying. Suitable processes are described, for example in:

O. Krischer, W. Kast, *Trocknungstechnik* [Drying technology] first volume, "Die wissenschaftlichen Grundlagen der Trocknungstechnik" [The scientific bases of drying technology]. Springer-Verlag 1978: Krischer/Kröll, *Trocknungstechnik* [Drying technology] second volume, "Trockner und Trocknungsverfahren" [Dryers and drying methods], Springer-Verlag 1959; K. Kröll. W. Kast. *Trocknungstechnik* third volume, "Trocknen und Trockner in der Produktion" [Drying and dryers in production], Springer-Verlag 1989; K. Masters, "Spray Drying Handbook", Longman Scientific & Technical 1991, 725 pages; H. Uhle-

mann, L. Mörl, "Wirbelschicht—Sprühgranulation" [Fluidized-bed spray granulation], Springer-Verlag 2000: Freeze drying: Georg-Wilhelm Oetjen, "Gefriertrocknen" [Freeze drying], VCH 1997; and also EP-A-0 809 940. The disclosure of the above-described publications is expressly incorporated herein by reference.

Particularly preferably, the inventive drying step is performed by spray drying, for example spray drying with an integrated fluidized bed, or by spray granulation.

If desired, the drying can be performed in the presence of a suitable support material which is suitable for feedstuff use, as a result of which, in particular, the free-flowing ability and thus the product quality can be improved.

Support materials which are suitable for feedstuff use and which can be used are customary inert supports. An "inert" support shall not exhibit any adverse interactions with the food adjuncts present in the additive and must be safe for use as aid in feedstuff additives. Examples of suitable support materials which may be mentioned are: inorganic or organic compounds of natural or synthetic origin. Examples of suitable low-molecular-weight inorganic supports are salts, such as sodium chloride, calcium carbonate, sodium sulfate and magnesium sulfate, or silicic acid. Examples of suitable organic supports are, in particular, sugars, for example glucose, fructose, sucrose and also dextrins and starch products. Examples of higher-molecular-weight organic supports which may be mentioned are: starch and cellulose preparations, such as, in particular, corn starch, cereal flours, for example wheat, rye, barley and oat flour, or mixtures thereof, or wheat semolina bran. The support material can be present in the preparation, based on dry basis, in a quantity of from about 5 to 85% by weight, for example from about 10 to 30% by weight, from 20 to 40% by weight or from 50 to 85% by weight.

Hereinafter, some preferred drying techniques are to be dealt with briefly in general form.

The spray drying can be carried out by first pumping the still-moist biomass to the atomizer in the spray tower. The atomization is performed, for example, by means of a pressure nozzle (single-component nozzle), a two-component nozzle or a centrifugal atomizer. The droplets are dried by a hot air stream passed into the spray dryer. When centrifugal atomizers are used, the drying is preferably performed in cocurrent flow. When nozzles are used, the drying can also be performed in countercurrent flow or crossflow. The dried powder can be discharged at the tower or it is carried along with the air stream and separated in a cyclone and/or filter. Depending on the product and procedure, a post-drying may be required, which can be performed in an internal fluidized bed flanged to the spray dryer or in an external fluidized bed.

In a variant of the inventive drying process, a continuous or batchwise fluidized-bed agglomeration is provided downstream of the drying step, in particular the spray drying. For this, a fluidized-bed dryer is charged, at the start of the process, with a pulverulent material, for example pulverulent additive obtained by spray drying. The material is fluidized, for example, by feeding preheated air. A liquid phase, for example further biomass or a binder-containing solution, is sprayed onto the fluidized bed, and as a result the powder which has been charged is wetted with this solution and, by its adhesive properties, increasingly agglomerated. At the same time, continuously or semicontinuously, in cycles at intervals, a subquantity of agglomerate is discharged from the fluidized bed. The discharge is classified, for example using a screen. Coarse material produced in this procedure can be ground and continuously recirculated to the fluidized bed. Fines, for example from the exhaust air filter system, can likewise be continuously recirculated.

A further preferred process variant comprises spray drying biomass to give a powder, coupled with the subsequent agglomeration of the spray-dried powder. This can be performed batchwise or continuously. Preference is given to the continuous procedure. Processes of this type can be carried out using conventional spray-drying plants. Advantageously, however, the procedure is carried out in apparatuses which are known as FSD (fluidized spray dryer), SBD (spray bed dryer) or MSD (multi stage dryer).

A fluidized spray dryer (FSD) drying plant for continuous production of an inventive dry product can be operated in particular according to the following pathline: wet biomass is introduced via a feedline into the top of the FSD dryer and atomized using an atomizer. The drying is performed by introducing air in cocurrent flow. The air is preheated via a heater. The spray-dried powder collects in the integrated fluidized bed in the bottom of the FSD dryer and is there sprayed using a spraying apparatus using compressed air, for example, with a binder solution and fluidized using introduced air. The air for this is preheated and fed via a feedline beneath the gas distributor of the integrated fluidized bed. The resultant preagglomerate then passes into a downstream external fluidized bed. Preheated air is introduced into this external fluidized bed from beneath via a further feedline. The preagglomerate charged in the fluidized bed is again sprayed using a further spraying apparatus using compressed air (for example with binder solution) and agglomerated to form the end product. The finished agglomerate is discharged from the fluidized bed and can be further worked up as described above.

The composition and amount of the liquids sprayed depend on the adhesive properties of the solution sprayed in, the agglomerate size to be achieved and the process conditions.

In the event that the adhesive properties of the sprayed biomass are not sufficient to ensure that the particles stick together stably after spraying, the use of a binder in addition is advantageous. This avoids that the agglomerates disintegrate again on drying. In such cases it is preferred to spray a binder which is soluble or dispersible in an aqueous medium into the fluidized bed. Examples of suitable binders which may be mentioned are solutions of carbohydrates, for example glucose, sucrose, dextrins etc., sugar alcohols, for example mannitol, or polymer solutions, for example solutions of hydroxypropylmethylcellulose (HPMC), polyvinylpyrrolidone (PVP), ethoxylated cellulose (EC), ethylcellulose or propylcellulose. As a result of suitable choice of amount and adhesive properties of the binder sprayed in, agglomerates of differing size and strength are formed.

If the binder is sprayed on as a separate solution, the binder content of the solution is in the range of from about 1 to 30% by weight, based on the total weight of the solution. The binder is likewise present in this case dissolved in an aqueous medium, preferably sterile demineralized water. Customary additives, for example buffer or solubilizer, can likewise be present.

The content of binder in the end product is according to the invention from 0 to about 20% by weight, for example from about 1 to 6% by weight. The optimum amount is also a function of the type of binder selected. It is necessary to ensure that adverse effects on the product are avoided.

G) Formulations

i) Feedstuff Additives and Feedstuff Compositions:

The inventive methionine-containing feedstuff additive is preferably in the form of a finely divided free-flowing powder, or in granulated form. Particles can be, for example in a size range of from 5 to 200 μm, for example from 10 to 150 μm, from 20 to 100 μm or from 30 to 80 μm, without being restricted thereto.

The bulk density of the inventive additives can be, for example, in the range of from about 100 to 600 g/l, for example from 150 to 400 g/l, or from 200 to 350 g/l, without being restricted thereto.

The methionine content of the inventive additive varies according to the manner of production.

Methionine crystals available according to the invention have a methionine content of greater than 60% by weight, for example of from about 70 to 98% by weight, preferably from about 80 to 95% by weight, particularly preferably from about 87 to 95% by weight. The content of salts (residues from the fermentation broth) can be in the range of from about 0 to 20% by weight, in particular of from about 5 to 15% by weight. Other fermentation minor constituents can be present in an amount of from about 0 to 20% by weight, in particular of from about 5 to 15% by weight.

Biomass methionine of the invention has a methionine content of more than 3% by weight, for example from about 5 to 40% by weight, or from about 10 to 35% by weight. The content of salts can be in the range of from about 0 to 30% by weight, such as from about 5 to 25% by weight. Other minor fermentation constituents can be present at a content of from about 0 to 20% by weight, such as from about 5 to 15% by weight.

The residual moisture content of the finished additive is preferably in the range of less than about 3-5% by weight, based on the total weight of the additive. The above percentages by weight are based on the total weight of the dry product (preferably without residual moisture).

In addition to the above-described constituents, the inventive formulations, as already mentioned above, can comprise further adjuncts, which can be added before, during or after workup of the biomass. Examples which can be mentioned are preservatives, antibiotics, antimicrobial additives, antioxidants, chelating agents, physiologically harmless salts, flavorings, colorings and the like. Nutritionally relevant adjuncts can also be present, for example vitamins (for example vitamins A, B_1, B_2, B_6, B_{12}, C, D_3, and/or E, K_3, folic acid, nicotinic acid, pantothenic acid); taurine, carboxylic acids and salts thereof, for example tricarboxylic acids, such as citrate, isocitrate, trans-/cisaconitate and/or homocitrate, enzymes, carotenoids, minerals, for example P, Ca, Mg and/or Fe, and trace elements, such as Se, Cr, Zn, Mn, proteins, carbohydrates, fats, amino acids. In addition pyruvic acid, L-carnitine, lipoic acid, coenzyme Q10, aminocarboxylic acids, for example creatine, orotic acid, myoinositol, flavonoids, betaine, p-aminobenzoic acid can be present.

The inventive methionine-containing feed additives can be incorporated into commercially conventional animal feed formulations, which can then be fed, for example, to cattle, pigs, sheep, poultry and the like. For this the inventive additive is mixed with customary animal feed constituents and if appropriate processed into final form, for example pelleted. Customary animal feed constituents are, for example, corn, barley, manioc, oats, soybean, fishmeal, wheat semolina bran, soybean oil, chalk, minerals, trace elements, amino acids and vitamins.

ii) Food and Feed Supplements

The inventively produced methionine is used as an adjunct in foodstuffs and feedstuffs or as an adjunct in food supplements and feed supplements, for example multivitamin preparations. The inventively produced product can be incorporated for this in the desired amount and in a manner known per se into conventional foodstuffs and feedstuffs or food supplements and feed supplements. The methionine can be present in this case, depending on use, in differing expedient amounts.

iii) Coated Formulations

The above-described inventive formulations can if appropriate additionally have a coating. They are furnished in this case with a coating composition which comprises at least one compound selected from:

- poly(alkylene glycol)s, in particular poly(ethylene glycol)s, for example having a number-average molecular weight of from about 400 to 15 000, for example from 400 to 10 000;
- poly(alkylene oxide) polymers or copolymers, for example having a number-average molecular weight of from about 4000 to 20 000, in particular block copolymers of polyoxyethylene and polyoxypropylene;
- substituted polystyrenes, maleic acid derivatives and styrene-maleic acid copolymers;
- vinyl polymers, in particular polyvinylpyrrolidones, for example having a number-average molecular weight of from about 7000 to 1 000 000; either alone or in combination with other compounds, such as cellulose ethers or starches;
- vinylpyrrolidone/vinyl acetate copolymers, for example having a number-average molecular weight of from about 30 000 to 100 000;
- poly(vinyl alcohol)s, for example having a number-average molecular weight of from about 10 000 to 200 000, and poly(phthalic acid vinyl ester)s;
- hydroxypropylmethylcelluloses, for example having a number-average molecular weight of from about 6000 to 80 000;
- alkyl(meth)acrylate polymers and copolymers, for example having a number-average molecular weight of from about 100 000 to 1 000 000, in particular ethyl acrylate/methyl methacrylate copolymers and methacrylate/ethyl acrylate copolymers;
- poly(vinyl acetate)s, for example having a number-average molecular weight of from about 250 000 to 700 000, if appropriate stabilized with poly-vinylpyrrolidone;
- polyalkylenes, in particular polyethylenes;
- aromatic polymers, for example lignins;
- poly(acrylic acid)s;
- polyacrylamides;
- polycyanoacrylates;
- phenoxyacetic acid-formaldehyde resins;
- cellulose derivatives, such as ethylcellulose, ethylmethylcellulose, methylcellulose, hydroxypropylcellulose, hydroxypropylmethylcellulose, carboxymethylcellulose, cellulose acetate phthalate;
- animal, vegetable or synthetic fats and modified fats, for example polyglycols, fatty alcohols, ethoxylated fatty alcohols, higher fatty acids; mono-, di- and triglycerides of higher fatty acids, for example glyceryl monostearate, alkylarylethoxylates and cocomonoethanolamides;
- animal and plant waxes or chemically modified animal and plant waxes, such as beeswax, candelilla wax, carnauba wax, montan ester wax and rice germ oil wax, spermaceti, lanolin, jojoba wax, sasol wax;

animal and vegetable proteins, for example gelatin, gelatin derivatives, gelatin substitutes, casein, whey, keratin, soybean protein; zein and wheat protein;

mono- and disaccharides, oligosaccharides, polysaccharides, for example hyaluronic acid, pullulan, elsinan, starches, modified starches, and also pectins, alginates, chitosan, carrageenan;

vegetable oils, for example sunflower, thistle, cottonseed, soybean, corn germ, olive, rapeseed, linseed, coconut, palm kernel oils; synthetic or semisynthetic oils, for example medium-chain triglycerides or mineral oils; animal oils, for example herring, sardine and whale oils;

hardened (hydrogenated or partially hydrogenated) oils/fats, for example of the abovementioned, in particular hydrogenated palm oil, hydrogenated cottonseed oil, hydrogenated soybean oil;

lacquer coatings, for example terpenes, in particular shellack, tolubalsam, perubalsam, sandarac, and silicone resins;

fatty acids, both saturated and also monounsaturated and polyunsaturated C_6 to C_{24}-carboxylic acids;

silicas;

and mixtures thereof.

Adding plasticizers or emulsifiers to fats or waxes before coating can if appropriate be advantageous to improve the flexibility of the film.

Coatings are applied in a manner known per se, if appropriate together with additives, generally via devices for making addition dropwise or by spraying onto the product of value which has been charged in a mixer. Examples of this are lances, sprinkler heads, single-fluid or multiple-fluid nozzles, or rotating dropping or atomizing devices. In the simplest case it is possible also to make the addition locally as a concentrated jet. Alternatively, the coating material can first be charged into the mixer, in order thereafter to add the product of value. Another possibility is the addition of initially solid coating material which, as a result of wall heating, or owing to mechanical energy input, melts and coats the product of value.

The invention will now be described in more detail with reference to the accompanying figures. FIGS. **1** to **4** show different developments of an inventive process for producing crystalline dry methionine and dry methionine-containing biomass ("biomass methionine").

EXAMPLE 1

a) Production of Methionine by Fermentation

To produce a representative fermentation broth for the purification of methionine, a laboratory fermentation was carried out. The *Corynebacterium glutamicum* strain ATCC13032 (American Type Culture Collection, Manassas, USA) was grown in a preliminary culture of 200 ml of BHI medium (Difco/Becton Dickinson Franklin Lakes, USA). In the Techfors fermenter, the preliminary culture was then inoculated into the culture medium (approximately 14 l).

The fermentation medium of the main culture had the following composition

2 g/l of KH_2PO_4
2 g/l of K_2HPO_4
10 g/l of ammonium sulfate
100 g/l of glucose
5 g/l of yeast extract
20 mg/l of kanamycin
1 g/l of KS911 ASM antifoam
pH 7.0

made up with demineralized water to the desired final volume

Trace salt solution 1 ml/l of medium

$FeSO_4 * 7\ H_2O$	10 g/l
$MnSO_4 * 4\text{–}6\ H_2O$	10 g/l
$ZnSO_4$	2 g/l
$MgSO_4 * 7\ H_2O$	250 g/l
Adjust to pH 1 using HCl	

1 ml/l of protocatechuate solution (stock solution 300 mg/10 ml)

biotin	1 mg/l
thiamine	1 mg/l
$CaCl_2$	5 mg/l

After the fermenter has been inoculated by the preliminary culture, the fermenter was kept at pH 7 by adding base (25% NH_4OH) and fermented until the sugar had been consumed. This was indicated by an increase in the pO_2 value or by a decrease in OTR and CTR.

b) Workup of the Fermentation Broth

The procedure for the workup is outlined diagrammatically in FIG. 1.

The fermentation broth produced according to section a) acts as starting material. At a fermentation temperature between 30 and 40° C., approximately 50% of the methionine present is in crystalline form. The starting product has a water content of approximately 86%, a fortified methionine content of approximately 9% and a biomass content of approximately 3%. Other fermentation by-products and minerals are present in the fermentation broth in traces (approximately 2.5% by weight).

20 kg of this fermentation broth are heated at 70° C. for 15 minutes. The methionine passes completely into solution as a result. At a constant temperature, the biomass is then centrifuged off. The supernatant (approximately 15 kg) is then concentrated to a methionine content of 20% at 100° C. and atmospheric pressure. The concentrate is then cooled to 5° C. at 5 K/h, as a result of which a majority of the methionine crystallizes out. The crystals are then separated off from the crystal magma on a vacuum filter, washed with 4 liters of water previously equilibrated at 5° C. and then blown dry with nitrogen at 40° C. By means of this procedure, 1.3 kg of dry methionine were isolated at a purity of approximately 90%.

The residue of the centrifugation (approximately 5 kg), in addition to dry biomass, also comprises approximately 6% methionine. By spray drying, this residue can be converted into approximately 0.7 kg of slightly yellowish and free-flowing dry powder having a residual moisture of 3%, which, in addition to the dry biomass and other fermentation by-products and mineral salts, also comprises methionine (approximately 30%).

The spray drying was performed in a laboratory spray dryer using the following instrument settings:

Inlet temperature: 200° C.,

Outlet temperature: 80-82° C.

The heating gas used was 60 m^3/h of nitrogen. The nozzle gas was sprayed at a pressure of 2 bar through a 1.2 mm nozzle.

EXAMPLE 2

Starting from the same starting material, the process is modified to the extent that the biomass is separated off by means of centrifugation and the biomass which is separated off is then washed with 5 l of water (FIG. **2**). After centrifugation, the resulting supernatant is added to the supernatant of the first biomass separation. The entire supernatant is concentrated to a methionine content of 16% at 100° C. and atmospheric pressure. By cooling the concentrate to 5° C. at 5 K/h, the methionine is crystallized out. The crystals are separated off on a vacuum filter, washed with 4.5 liters of water previously equilibrated at 5° C. and then dried with nitrogen at 40° C. By means of this procedure, the amount of dry methionine is increased to approximately 1.5 kg. The purity of the isolated crystals is again approximately 90%.

The residue of the biomass which is separated off and washed is converted into approximately 0.5 kg of dry product by spray drying.

The product thus produced which, in addition to dry biomass and other fermentation by-products and mineral salts, also comprises methionine (approximately 10%), was free flowing.

EXAMPLE 3

Starting from the same starting material, the process of Example 2 was additionally modified to the extent that the mother liquor and the wash water which are produced when the crystalline methionine is separated off are added in a next batch to the methionine-containing fermentation broth (FIG. **3**).

With an otherwise similar procedure to Example 2, in the fermentation approximately 1.5 kg of dry methionine are obtained from the crystallization at a purity of approximately 90% and approximately 0.5 kg of product is obtained from the spray-dried biomass having a methionine content of approximately 10%.

EXAMPLE 4

Starting from the same starting material, the process of Example 2 is additionally modified to the extent that the mother liquor and the wash water which are produced when the crystalline methionine is separated off are added to the biomass stream before the spray drying (FIG. **4**).

With an otherwise similar procedure to Example 2, approximately 1.1 kg of methionine-containing biomass are produced, which, in addition to dry biomass and other fermentation by-products and mineral salts, also comprises methionine (approximately 30%). The amount of dry methionine is 1.5 kg with a purity of approximately 90%.

EXAMPLE 5

A process variant is also conceivable in which a portion of the mother liquor and of the wash water, after the crystallization of the methionine, is added to the fermenter broth before biomass separation, and the other substream is added to the biomass stream before spray drying (combination of Examples 3 and 4).

We claim:

1. A process for preparing methionine comprising,

a) culturing a methionine-producing microorganism under conditions appropriate to produce methionine;

b) heating the culture at 60° C. to 120° C. for a period of time sufficient for solubilizing any methionine which has crystallized; and

c) while maintaining the temperature at 60° C. to 120° C. separating the fermentation broth from the microorganisms.

2. The process of claim **1**, wherein the heating is performed to a temperature of about 70 to 100° C. in stage b).

3. The process of claim **1**, further comprising

d) crystallizing methionine out of the fermentation broth.

4. The process of claim **1**, further comprising washing the microorganism to give a spent wash liquid and adding the spent wash liquid to the fermentation broth of step c).

5. The process of **3**, further comprising

e) separating off the crystallized methionine to give a mother liquid,

f) washing the crystallized methionine which has been separated off to give a wash liquid, and

g) drying the crystallized methionine.

6. The process of claim **5**, wherein the mother liquid produced in stage e) is combined with a methionine-containing liquid fraction from another fermentation batch.

7. The process of claim **5**, wherein the wash liquid produced in stage f) is combined with a methionine-containing liquid fraction from another fermentation batch.

8. The process of claim **1**, wherein the microorganism is a natural or recombinant microorganism.

9. The process of claim **8**, wherein the microorganism is a natural or recombinant bacterium of the genus Corynebacterium.

10. The process of claim **8**, wherein L-methionine is produced.

11. The process of claim **1**, further comprising concentrating the fermentation broth and then crystallizing methionine out of the concentrated fermentation, broth.

12. The process of claim **4**, further comprising drying the washed microorganism.

13. The process of claim **12**, wherein the drying comprises spray-drying.

14. The process of claim **1**, wherein the amount of methionine in the fraction containing the microorganism ranges from more than 3% to about 40% by weight.

15. The process of claim **5**, further comprising separating off the microorganism from the same or another fermentation batch and adding the mother liquid produced in stage e) to the separated off microorganism.

16. The process of claim **5**, further comprising separating off the microorganism from the same or another fermentation batch and adding the wash liquid produced in stage f) to the separated off microorganism.

* * * * *

(12) INTERNATIONAL APPLICATION PUBLISHED UNDER THE PATENT COOPERATION TREATY (PCT)

(19) World Intellectual Property Organization
International Bureau

(43) International Publication Date
5 March 2015 (05.03.2015)

WIPO | PCT

(10) International Publication Number
WO 2015/028674 Al

(51) International Patent Classification:
C12P 13/12 (2006.0 1) *C12N 9/1 0* (2006.0 1)

(21) International Application Number:
PCT/EP2014/068539

(22) International Filing Date:
1 September 2014 (01 .09.2014)

(25) Filing Language: English

(26) Publication Language: English

(30) Priority Data:
13306185.3 30 August 2013 (30.08.2013) EP

(71) Applicant: **METABOLIC EXPLORER** [FR/FR]; Biopole Clermont-limagne, F-63360 Saint-Beauzire (FR).

(72) Inventors: **DISCHERT, Wanda;** 67 rue de Coulogne, F-63270 Vic-le-Comte (FR). **VASSEUR, Perrine;** 8 route de Surat, F-63270 Martres-sur-Morges (FR). **FIGGE, Rainer;** 4 rue du Bosquet, F-63450 Le Crest (FR).

(74) Agent: **REGIMBEAU;** 139 rue Vendome, F-69477 Lyon Cedex 06 (FR).

(81) Designated States *(unless otherwise indicated, for every kind of national protection available):* AE, AG, AL, AM, AO, AT, AU, AZ, BA, BB, BG, BH, BN, BR, BW, BY, BZ, CA, CH, CL, CN, CO, CR, CU, CZ, DE, DK, DM, DO, DZ, EC, EE, EG, ES, FI, GB, GD, GE, GH, GM, GT, HN, HR, HU, ID, IL, IN, IR, IS, JP, KE, KG, KN, KP, KR, KZ, LA, LC, LK, LR, LS, LT, LU, LY, MA, MD, ME, MG, MK, MN, MW, MX, MY, MZ, NA, NG, NI, NO, NZ, OM, PA, PE, PG, PH, PL, PT, QA, RO, RS, RU, RW, SA, SC, SD, SE, SG, SK, SL, SM, ST, SV, SY, TH, TJ, TM, TN, TR, TT, TZ, UA, UG, US, UZ, VC, VN, ZA, ZM, ZW.

(84) Designated States *(unless otherwise indicated, for every kind of regional protection available):* ARIPO (BW, GH, GM, KE, LR, LS, MW, MZ, NA, RW, SD, SL, ST, SZ, TZ, UG, ZM, ZW), Eurasian (AM, AZ, BY, KG, KZ, RU, TJ, TM), European (AL, AT, BE, BG, CH, CY, CZ, DE, DK, EE, ES, FI, FR, GB, GR, HR, HU, IE, IS, IT, LT, LU, LV, MC, MK, MT, NL, NO, PL, PT, RO, RS, SE, SI, SK, SM, TR), OAPI (BF, BJ, CF, CG, CI, CM, GA, GN, GQ, GW, KM, ML, MR, NE, SN, TD, TG).

Declarations under Rule 4.17:

— *of inventorship (Rule 4.17(iv))*

Published:

— *with international search report (Art. 21(3))*

— *with sequence listing part of description (Rule 5.2(a))*

W° 2015/028674 A1

(54) Title: MICROORGANISM FOR METHIONINE PRODUCTION WITH IMPROVED METHIONINE SYNTHASE ACTIVITY AND METHIONINE EFFLUX

(57) Abstract: The present application is related to a recombinant microorganism optimised for the fermentative production of methionine and/or its derivatives, wherein in said recombinant microorganism, the cobalamin-dependent methionine synthase activity and the methionine efflux are enhanced. The application is also related to a method for optimizing the fermentative production of methionine and/or its derivatives comprising the steps of: c. culturing a recombinant microorgamsm wherein in said microorganism, the cobalamin-dependent methionine synthase activity and the methionine efflux are enhanced, in an appropriate culture medium comprising a fermentable source of carbon and a source of sulphur, and d. recovering methionine and/or its derivatives from the culture medium.

Microorganism for methionine production with improved methionine synthase activity and methionine efflux

FIELD OF THE INVENTION

The present invention relates to a recombinant microorganism useful for the production of L-methionine and/or its derivatives and process for the preparation of L-methionine. The microorganism of the invention is modified in a way that the L-methionine production is improved by enhancing its cobalamin-dependant methionine synthase activity as well as its L-methionine export. In particular, the genes *metH, fldA, fpr* or their homologous genes and the genes *ygaZ* and *ygaH* or their homologous genes are overexpressed in the microorganism.

PRIOR ART

Sulphur-containing compounds such as cysteine, homocysteine, methionine or S-adenosylmethionine are critical to cellular metabolism. In particular L-methionine, an essential amino acid, which cannot be synthesized by animals, plays an important role in many body functions. Most of the methionine produced industrially is widely used as an animal feed and food additive.

With the decreased use of animal-derived proteins as a result of BSE and chicken flu, the demand for pure methionine has increased. Commonly, D,L-methionine is produced chemically from acrolein, methyl mercaptan and hydrogen cyanide. However, the racemic mixture does not perform as well as pure L-methionine (Saunderson, 1985). Additionally, although pure L-methionine can be produced from racemic methionine, for example, through the acylase treatment of N-acetyl-D,L-methionine, this dramatically increases production costs. Accordingly, the increasing demand for pure L-methionine coupled with environmental concerns render microbial production of methionine an attractive prospect.

Other important amino acids, such as lysine, threonine and tryptophan are produced via fermentation for use in animal feed. Therefore, these amino acids can be made using glucose and other renewable resources as starting materials. The production of L-methionine via fermentation has not been successful yet, but the development of the technology is on going.

Different approaches for the optimisation of L-methionine production in microorganisms have been described previously (see, for example, Patents or patent applications US7,790,424, US7,61 1,873, WO2002/10209, WO2005/059093 and WO2006/008097); however, industrial production of L-methionine from microorganisms requires further improvements.

In *Escherichia coli,* two distinct enzymes catalyze the terminal step in *de novo* biosynthesis of methionine; the cobalamin-dependent methionine synthase (MetH, EC 2.1.1.13) which contains a prosthetic group that is required for activity and the cobalamin-independent methionine synthase (MetE, EC 2.1.1.14) (Foster *et al.,* 1961; Gonzalez *et al.,* 1992). The cobalamin-dependent methionine synthase, MetH, is a protein of ~136 kDa containing four domains: a domain containing the cobalamin cofactor (Cob domain), a domain binding the methyl-THF substrate (CH3-THF domain), a domain binding the homocysteine substrate (Hey domain), and a domain binding S-Adenosyl-Methionine (SAM) (Adomet domain) (Matthews, 2001). In the presence of oxygen, the enzyme is inactivated by oxidation (Banerjee *et al.,* 1990). In order to reactivate the enzyme, a reductive methylation occurs. The reaction involves a methyl group provided by SAM bound to the AdoMet domain of the enzyme, and two electrons transferred via an external transport chain. The two electrons are provided by NADPH and transferred via a downhill potential driven redox chain composed of a FAD-containing flavodoxine reductase, FldA and a FMN-containing flavodoxine reductase, Fpr (Fujii & Huennekens, 1974; Wan & Jarrett, 2002) in *Escherichia coli.* As disclosed in patent application WO2009/144270, in *Corynebacterium glutamicum,* functional homologues of FldA and Fpr have been identified. They are respectively FdxC, FdxD or FdxA and FprA1, FprA2, FprA3 or FldR1.

The protein complex YgaZ and YgaH is a member of the branched chain amino acid exporter (LIV-E) family responsible for export of L-valine. In the same manner, YgaZH is also involved in the export of methionine as it was shown by Trotschel and colleagues for BrnFE, the homolog of YgaZH from *Corynebacterium glutamicum* (Trotschel *et al,* 2005).

Numerous patents applications were filed on the improvement of the methionine synthase activity by different means in order to produce L-methionine:

- WO2007/012078 and WO2007/135188 from BASF claim among other modifications, genetic alteration leading to overexpression of at least *metH* and/or *metE,*
- WO2009/144270 from Evonik discloses a method of producing methionine with a microorganism that displays an increased amount and/or activity of a cob(I)alamin-dependent MetH reactivation system,
- WO2008/080900 from Evonik claims a $MetH^{FBR}$ form (FeedBack Resistant) which should be more resistant to high L-methionine concentrations.

In the same manner few patents disclose the overexpression of genes encoding the methionine excretion system in different micro organisms:

- Reduction of the L-methionine uptake in *Corynebacterium* is described in patent applications WO2002/097096 and WO2005/085463 (Degussa) or,

- Overexpression of a branched chain amino acid exporter (YgaZH) responsible for the export of L-valine and L-methionine is disclosed in patent applications EP1239041 (Ajinomoto) and WO2008/08221 1 (CJ Corporation).

Inventors have found surprisingly and unexpectedly that the increase of the L-methionine efflux together with the enhancement of the cobalamin-dependant L-methionine synthase activity in a recombinant L-methionine overproducer microorganism improve the methionine production.

SUMMARY OF THE INVENTION

The invention relates to recombinant microorganism and method for optimising the production of methionine and/or its derivatives, wherein the cobalamin-dependent methionine synthase activity and the methionine efflux are enhanced. In the recombinant microorganism, cobalamin-dependent methionine synthase activity is enhanced by overexpressing the expression of *metH,* and optionally the expression of the genes *fldA* and *fpr* from *E. coli* or their homologous genes from *C. glutamicum* whereas methionine efflux is enhanced by overexpressing the genes *ygaZH* from *E. coli* or *brnFE* from *C. glutamicum* or their homologous genes.

The recombinant microorganism may also comprise other genetic modifications such as:

- an increased expression of at least one of the following genes: *ptsG, pyc, pntAB, cysP, cysll, cysW, cysA, cysM, cysJ, cysl, cysH, gcvT, gcvH, gcvP, Ipd, serA, serB, serC, cysE, metF, metA, metA** allele encoding for an enzyme with reduced feed-back sensitivity to S-adenosylmethionine and/or methionine, *thrA,* or a *thrA** allele encoding for an enzyme with reduced feed-back inhibition to threonine and/or
- an attenuated expression of one of the following genes: *metJ, pykA, pykF, purU, ybdL, udhA, dgsA, metE, metN, metl, metQ or yncA.*

In a particular embodiment, the present invention is related to a recombinant microorganism wherein: a) the genes *metH,* and optionally the genes *fldA* and *fpr* from *E. coli* or their homologous genes from *C. glutamicum* are overexpressed, b) the genes *ygaZ* and *ygaH* from *E. coli* or the genes *brnF* and *brnE* from *C. glutamicum* or their homologous genes originating from *Citrobacter koseri, Shigella flexneri, Raoultella ornithinolytica, Enterobacter sp., Yersinia enterocolitica, Photorhabdus luminescens, Citrobacter youngae* or *Citrobacter freundii* are overexpressed, and c) the expression of the genes *metA*, cysPUWAM, cysJIH, gcvTHP, metF, serA, serB, serC, cysE, thrA*, ptsG* and *pyc* are enhanced; and d) the expression of the genes *metJ, pykA, pykF, purll, yncA, dgsA* and *metE* are attenuated.

Preferably, the recombinant microorganism is *Escherichia coli* or *Corynebacterium glutamicum.*

DETAILED DESCRIPTION OF THE INVENTION

Before describing the present invention in detail, it is to be understood that this invention is not limited to particularly exemplified methods and may, of course, vary. It is also to be understood that the terminology used herein is for the purpose of describing particular embodiments of the invention only, and is not intended to be limiting, which will be limited only by the appended claims.

All publications, patents and patent applications cited herein, whether *supra* or *infra,* are hereby incorporated by reference in their entirety.

Furthermore, the practice of the present invention employs, unless otherwise indicated, conventional microbiological and molecular biological techniques within the skill of the art. Such techniques are well known to the skilled worker, and are explained fully in the literature.

It must be noted that as used herein and in the appended claims, the singular forms "a", "an", and "the" include plural reference unless the context clearly dictates otherwise. Thus, for example, a reference to "a microorganism" includes a plurality of such microorganisms, and a reference to "an endogenous gene" is a reference to one or more endogenous genes, and so forth. Unless defined otherwise, all technical and scientific terms used herein have the same meanings as commonly understood by one of ordinary skill in the art to which this invention belongs. Although any materials and methods similar or equivalent to those described herein can be used to practice or test the present invention, the preferred materials and methods are now described.

In the claims that follow and in the consecutive description of the invention, except where the context requires otherwise due to express language or necessary implication, the word "comprise", "contain", "involve" or "include" or variations such as "comprises", "comprising", "containing", "involved", "includes", "including" are used in an inclusive sense, i.e. to specify the presence of the stated features but not to preclude the presence or addition of further features in various embodiments of the invention.

The term "methionine" and "L-methionine" designate the essential sulphur-containing amino-acid with chemical formula $H0_2CCH(NH_2)CH_2CH_2SCH_3$ and CAS number 59-51-8 or 63-68-3 for the specific L-isomer.

"Derivatives of methionine" refers to molecules analogs to methionine which present the same chemical backbone but differ from methionine with at least one chemical group. In this invention, preferred methionine derivatives are N-acetyl methionine (NAM), S-

adenosyl methionine (SAM) and hydroxy-methionine (or methionine hydroxy analogue or MHA).

The term "microorganism", as used herein, refers to a bacterium, yeast or fungus which is not modified artificially. Preferentially, the microorganism is selected among *Enterobacteriaceae, Bacillaceae, Streptomycetaceae* and *Corynebacteriaceae.* More preferentially the microorganism is a species of *Escherichia, Klebsiella, Pantoea, Salmonella, or Corynebacterium.* Even more preferentially the microorganism of the invention is either the species *Escherichia coli* or *Corynebacterium glutamicum.*

The term "recombinant microorganism" or "genetically modified microorganism", as used herein, refers to a bacterium, yeast or fungus that is not found in nature and is genetically different from its equivalent found in nature. It means, it is modified either by introduction or by deletion or by modification of genetic elements. It can also be transformed by forcing the development and evolution of new metabolic pathways by combining directed mutagenesis and evolution under specific selection pressure (see, for example, WO2004/076659 or WO2007/01 1939).

A microorganism may be modified to express exogenous genes if these genes are introduced into the microorganism with all the elements allowing their expression in the host microorganism. The modification or "transformation" of microorganisms with exogenous DNA is a routine task for those skilled in the art.

A microorganism may be modified to modulate the expression level of an endogenous gene.

The term "endogenous gene" means that the gene was present in the microorganism before any genetic modification. Endogenous genes may be overexpressed by introducing heterologous sequences in addition to, or to replace endogenous regulatory elements, or by introducing one or more supplementary copies of the gene into the chromosome or a plasmid. Endogenous genes may also be modified to modulate their expression and/or activity. For example, mutations may be introduced into the coding sequence to modify the gene product or heterologous sequences may be introduced in addition to or to replace endogenous regulatory elements. Modulation of an endogenous gene may result in the up-regulation and/or enhancement of the activity of the gene product, or alternatively, down regulate and/or lower the activity of the endogenous gene product.

Another way to modulate their expression is to exchange the endogenous promoter of a gene (e.g., wild type promoter) with a stronger or weaker promoter to up or down regulate expression of the endogenous gene. These promoters may be homologous or heterologous. It is well within the ability of the person skilled in the art to select appropriate promoters.

Contrariwise, "exogenous gene" means that the gene was introduced into a microorganism, by means well known by the man skilled in the art whereas this gene is not naturally occurring in the microorganism. Exogenous genes may be integrated into the host

chromosome, or be expressed extra-chromosomally by plasmids or vectors. A variety of plasmids, which differ with respect to their origin of replication and their copy number in the cell, are well known in the art. These genes may be homologous.

In the context of the invention, the term "homologous gene" is not limited to designate genes having a theoretical common genetic ancestor, but includes genes which may be genetically unrelated that have, none the less, evolved to encode protein which perform similar functions and/or have similar structure. Therefore the term 'functional homolog" for the purpose of the present invention relates to the fact that a certain enzymatic activity may not only be provided by a specific protein of defined amino acid sequence, but also by proteins of similar sequence from other (un)related microorganisms.

Using the references given in Genbank for known genes, those skilled in the art are able to determine the equivalent genes in other organisms, bacterial strains, yeast, fungi, mammals, plants, etc. This routine work is advantageously done using consensus sequences that can be determined by carrying out sequence alignments with genes derived from other microorganisms and designing degenerate probes to clone the corresponding gene in another organism. These routine methods of molecular biology are well known to those skilled in the art.

The terms "improved methionine production", "improve methionine production" and grammatical equivalents thereof, as used herein, refer to an increased methionine/carbon source yield (ratio of gram/mol methionine produced per gram/mol carbon source consumed that it can be expressed in percent) and/or an improved purity of produced methionine. In this invention, the purity of the produced methionine may be increased by decreasing the production of ketomethylvalerate and/or homolanthionine. Methods for determining the amount of carbon source consumed and of methionine produced are well known to those in the art. The yield and/or the purity of produced methionine are higher in the recombinant microorganism compared to the corresponding unmodified microorganism.

The terms "microorganism optimised for the fermentative production of methionine" refers to microorganisms evolved and/or genetically modified to present an improved methionine production in comparison with the endogenous production of the corresponding wild-type microorganisms. Such microorganisms "optimised" for methionine production are well known in the art, and have been disclosed in particular in patent applications WO2005/1 11202, WO2007/077041, WO2009/043803 and WO2012/098042.

According to the invention the terms "fermentative production", "culture" or "fermentation" are used to denote the growth of bacteria. This growth is generally conducted in fermenters with an appropriate culture medium adapted to the microorganism

being used and containing at least one simple carbon source, and if necessary co-substrates.

An "appropriate culture medium" designates a medium (e.g., a sterile, liquid media) comprising nutrients essential or beneficial to the maintenance and/or growth of the cell such as carbon sources or carbon substrates, nitrogen sources, for example, peptone, yeast extracts, meat extracts, malt extracts, urea, ammonium sulfate, ammonium chloride, ammonium nitrate and ammonium phosphate; phosphorus sources, for example, monopotassium phosphate or dipotassium phosphate; trace elements (e.g., metal salts), for example magnesium salts, cobalt salts and/or manganese salts; as well as growth factors such as amino acids and vitamins.

The term "carbon source" or "carbon substrate" or "source of carbon" according to the present invention denotes any source of carbon that can be used by those skilled in the art to support the normal growth of a microorganism, including monosaccharides (such as glucose, galactose, xylose, fructose or lactose), oligosaccharides, disaccharides (such as sucrose, cellobiose or maltose), molasses, starch or its derivatives, hemicelluloses and combinations thereof. An especially preferred simple carbon source is glucose. Another preferred simple carbon source is sucrose. The carbon source can be derived from renewable feed-stock. Renewable feed-stock is defined as raw material required for certain industrial processes that can be regenerated within a brief delay and in sufficient amount to permit its transformation into the desired product. Vegetal biomass treated or not, is an interesting renewable carbon source.

The term "source of sulphur" according to the invention refers to sulphate, thiosulfate, hydrogen sulphide, dithionate, dithionite, sulphite, methylmercaptan, dimethylsulfide and other methyl capped sulphides or a combination of the different sources. More preferentially, the sulphur source in the culture medium is sulphate or thiosulfate or a mixture thereof.

The terms "source of nitrogen" corresponds to either an ammonium salt or ammoniac gas. The nitrogen source is supplied in the form of ammonium or ammoniac.

The terms "attenuation" or "expression attenuated" mean in this context that the expression of a gene or the production of an enzyme is decreased or suppressed compared to the non modified microorganism leading to a decrease in the intracellular concentration of a ribonucleic acid, a protein or an enzyme compared to the non modified microorganism. The man skilled in the art knows different means and methods to measure ribonucleic acid concentrration or protein concentration in the cell including for instance use of Reverse Transcription Polymerase Chain Reaction (RT-PCR) and Real-time Polymerase Chain Reaction (qPCR) to determine ribonucleic acid concentration and use of specific antibody to determine concentration of specific protein.

Decrease or suppression of the production of an enzyme is obtained by the attenuation of the expression of gene encoding said enzyme.

Attenuation of genes may be achieved by means and methods known to the man skilled in the art. Generally, attenuation of gene expression may be achieved by:

- Mutating the coding region or the promoter region or,
- Deleting of all or a part of the promoter region necessary for the gene expression or,
- Deleting of all or a part of the coding region of the gene by homologous recombination or,
- Inserting an external element into coding region or into promoter region or,
- Expressing the gene under control of a weak promoter or an inducible promoter.

The man skilled in the art knows a variety of promoters which exhibit different strength and which promoter to use for a weak or an inducible genetic expression.

The term "activity" of an enzyme is used interchangeably with the term "function" and designates, in the context of the invention, the reaction that is catalyzed by the enzyme. The man skilled in the art knows how to measure the enzymatic activity of said enzyme.

The terms "attenuated activity" or "reduced activity" of an enzyme mean either a reduced specific catalytic activity of the protein obtained by mutation in the aminoacids sequence and/or decreased concentrations of the protein in the cell obtained by mutation of the nucleotidic sequence or by deletion of the coding region of the gene.

The terms "enhanced activity" or "increased activity'Of an enzyme designate either an increased specific catalytic activity of the enzyme, and/or an increased quantity/availability of the enzyme in the cell, obtained for example by overexpressing the gene encoding the enzyme.

The terms "increased expression", "enhanced expression" or "overexpression" and grammatical equivalents thereof, are used interchangeably in the text and have a similar meaning. These terms mean that the expression of a gene or the production of an enzyme is increased compared to the non modified microorganism leading to an increase in the intracellular concentration of a ribonucleic acid, a protein or an enzyme compared to the non modified microorganism. The man skilled in the art knows different means and methods to measure ribonucleic acid concentration or protein concentration in the cell including for instance use of Reverse Transcription Polymerase Chain Reaction (RT-PCR) and Real-time Polymerase Chain Reaction (qPCR) to determine ribonucleic acid concentration and use of specific antibody to determine concentration of specific protein.

Increase production of an enzyme is obtained by increasing expression of the gene encoding said enzyme.

To increase the expression of a gene, the man skilled in the art knows different techniques such as:

- Increasing the number of copies of the gene in the microorganism. The gene is encoded chromosomally or extrachromosomally. When the gene is located on the chromosome, several copies of the gene can be introduced on the chromosome by methods of recombination, known by the expert in the field (including gene replacement). When the gene is located extra-chromosomally, it may be carried by different types of plasmids that differ with respect to their origin of replication and thus their copy number in the cell. These plasmids are present in the microorganism in 1 to 5 copies, or about 20 copies, or up to 500 copies, depending on the nature of the plasmid: low copy number plasmids with tight replication (e.g for *E. coli* pSClOl, RK2), low copy number plasmids (e.g for *E. coli* pACYC, pRSFlOlO) or high copy number plasmids (e.g for *E. coli* pSK bluescript II).

- Using a promoter leading to a high level of expression of the gene. The man skilled in the art knows which promoters are the most convenient, for example promoters *Ftrc, Ftac, Viae,* or the lambda promoter *cl* are widely used. These promoters can be "inducible" by a particular compound or by specific external condition like temperature or light. These promoters may be homologous or heterologous.

- Attenuating the activity or the expression of a transcription repressor, specific or non-specific of the gene.

- Using elements stabilizing the corresponding messenger RNA (Carrier and Keasling, 1999) or elements stabilizing the protein (e.g., GST tags, GE Healthcare).

The terms "encoding" or "coding" refer to the process by which a polynucleotide, through the mechanisms of transcription and translation, produces an amino-acid sequence. The gene(s) encoding the enzyme(s) can be exogenous or endogenous.

The terms "feed-back sensitivity" or "feed-back inhibition" refer to a cellular mechanism control in which an or several enzymes that catalyse the production of a particular substance in the cell are inhibited or less active when that substance has accumulated to a certain level. So the terms "reduced feed-back sensitivity" or "reduced feed-back inhibition" mean that the activity of such a mechanism is decreased or suppressed compared to a non modified microorganism. The man skilled in the art knows how to modify the enzyme to obtain this result. Such modifications have been described in the patent application WO2005/1 11202 or in the patent US7,61 1,873.

In a first aspect of the invention, a recombinant microorganism is optimised for the fermentative production of methionine and/or its derivatives by enhancing the cobalamin-dependent methionine synthase activity and by enhancing the methionine efflux in said microorganism. Preferably, the recombinant microorganism is chosen among *Enterobacteriaceae* or *Corynebacteriaceae.* More preferably, the recombinant

microorganism of the invention is chosen among *Escherichia coli* or *Corynebacterium glutamicum*.

As described above, cobalamin-dependent methionine synthase activity is mediated by MetH enzyme. This enzyme needs a reactivation system for having a sustained activity. This system is encoded by two genes, *fldA* and *fpr* in *E. coli* and by respectively gene chosen among *fdxC, fdxD* or *fdxA* and among *fprAl, fprA2, fprA3* or *fldRl* in C. *glutamicum*. In this application, the terms "MetH and its reactivation system" or *"metH, fldA, fpr"* relate to the cobalamin-dependent methionine synthase and its reactivation system both in *E. coli* and in *C. glutamicum* or their encoding genes both from *E. coli* and from *C. glutamicum*. Thus, enhancement of cobalamin-dependent methionine synthase activity is preferably carried out by overexpression of *metH* gene and also of its reactivation system encoded by *fldA* and *fpr* genes.

In one embodiment of the invention, the cobalamin-dependent methionine synthase activity is enhanced by overexpressing (enhancing their expression) genes *metH, fldA, fpr* from *E. coli* or their homologous genes from *C. glutamicum*. Preferably, these genes are overexpressed under a promoter different from their wild-type promoter.

More preferably, the genes *metH, fldA* or *fpr* or their homologous genes from C. *glutamicum* are overexpressed chromosomally, i.e. these genes are overexpressed from the chromosome. One or several supplementary copies of each gene are introduced on the chromosome of the microorganism. They are integrated at different loci selected from the list disclosed in the patent application WO201 1/073122, and whose deletions do not have impact on methionine production. The wild-type copy of the coding sequence of each gene is conserved, but their promoter region may be replaced by articial promoter and/or Ribosome Binding Site (RBS).

In a specific embodiment of the invention:

- wild-type *metH* gene is conserved with replacement of its natural promoter and RBS, and two additional copies are introduced on the chromosome, and
- wild-type *fldA* and *fpr* genes and their promoter regions are conserved, and one additional copy of each gene is introduced on the chromosome.

Additional copies of the introduced genes are expressed under control of artificial promoter and RBS.

In amino-acid producer microorganisms, methionine is excreted by a specific efflux transporter. Notably, in *E. coli,* this transporter is called YgaZH and is encoded by the *ygaZ* and *ygaH* genes whereas in *C. glutamicum,* it is named BrnFE and is encoded by the *brnF* and *brnE* genes. Functional homologues of this methionine efflux system have been identified in several other microorganisms. In the invention, recombinant microorganism overexpresses *ygaZH* genes from *E. coli* or *brnFE* genes from *C. glutamicum*. Alternatively, the recombinant microorganism of the invention may overexpress functional

homologues of YgaZH or of BrnFE transporters. YgaZ and YgaH homologous protein are presented respectively in Table 1 and Table 2.

Table 1: YgaZ homologous proteins

Acession Number	Name	Organism
YP_001455539.1 NC_009792.1. ABV15103.1	hypothetical protein CKO_04031 [Citrobacter koseri ATCC BAA-895]	*Citrobacter koseri*
WP_005122932.1 EIQ78635.1	membrane protein [Shigella flexneri]	*Shigella flexneri*
YP_007877063.1 AGJ89511.1 WP_015585890.1	hypothetical protein RORB6_24155 [Raoultella ornithinolytica B6]	*Raoultella ornithinolytica*
YP_008107733.1 AGN85393.1 WP_020454909.1	membrane protein [Enterobacter sp. R4-368]	*Enterobacter sp.*
WP_004959353.1 EFE95945.1	membrane protein [Serratia odorifera]	*Serratia odorifera*
YP_003884334.1 ADM99777.1	amino acid transporter [Dickeya dadantii 3937] Erwinia chrysanthemi (strain 3937)	*Dickeya dadantii*
YP_006647984.1 AFR04731.1	amino acid transporter [Pectobacterium carotovorum subsp. carotovorum PCC21]	*Pectobacterium carotovorum subsp. Carotovorum*
YP_001007412.1 CAL13268.1	putative amino acid transporter [Yersinia enterocolitica subsp. enterocolitica 8081]	*Yersinia enterocolitica subsp. Enterocolitica*
NP_928590.1 CAE13573.1	hypothetical protein plu1279 [Photorhabdus luminescens subsp. laumondii TT01]	*Photorhabdus luminescens subsp. Laumondii*
WP_004847360.1 EHM42581.1	membrane protein [Hafnia alvei]	*Hafnia alvei*
WP_016157304.1 EOQ28426.1	inner membrane protein YgaZ [Citrobacter sp. KTE32]	*Citrobacter sp. KTE32*
WP_006687199.1 EFE06904.1	membrane protein [Citrobacter youngae] putative azaleucine resistance protein AzlC [Citrobacter youngae ATCC 29220]	*Citrobacter youngae*
YP_005198838.1 AEX50698.1	putative branched-chain amino acid permease (azaleucine resistance) [Rahnella aquatilis CIP 78.65 = ATCC 33071]	*Rahnella aquatilis*
WP_009111644.1 EHD20336.1.	membrane protein [Brenneria sp. EniD312]	*Brenneria sp.*
YP_003469114.1 CBJ82350.1	amino acid transporter [Xenorhabdus bovienii SS-2004]	*Xenorhabdus bovienii*
WP_000841919.1	membrane protein [Shigella flexneri]	*Shigella flexneri*
WP_000445647.1	membrane protein [Shigella dysenteriae]	*Shigella dysenteriae*
WP_000445645.1	membrane protein [Shigella flexneri]	*Shigella flexneri*
EFP71467.1	azlC family protein [Shigella dysenteriae 1617]	*Shigella dysenteriae*
WP_005063865.1	membrane protein [Shigella flexneri]	*Shigella flexneri*

WP_00 1428008. 1	membrane protein [Shigella dysenteriae]	*Shigella dysenteriae*
WP_005031 133. 1	membrane protein [Shigella dysenteriae]	*Shigella dysenteriae*
WP_004993748. 1	membrane protein [Shigella boydii]	*Shigella boydii*
WP_0050991 5 1.1	membrane protein [Shigella flexneri]	*Shigella flexneri*
NP_708495. 1	hypothetical protein SF2709 [Shigella flexneri 2a str. 301]	*Shigella flexneri*
YP_4091 84. 1. NC_00761 3.1. ABB67356	hypothetical protein SBO_2835 [Shigella boydii Sb227]	*Shigella boydii*
WP_0051 19769. 1	branched-chain amino acid permease [Shigella flexneri]	*Shigella flexneri*
WP_00382597 1.1	membrane protein [Citrobacter sp. 30_2]	*Citrobacter sp.*
WP_01 6 1541 56. 1	inner membrane protein YgaZ [Citrobacter sp. KTE1 5 1]	*Citrobacter sp.*
WP_003839672. 1	hypothetical protein [Citrobacter freundii]	*Citrobacter freundii*
WP_01 6 15087 1.1	inner membrane protein YgaZ [Citrobacter sp. KTE30]	*Citrobacter sp.*
WP_0 1907753 1.1	membrane protein [Citrobacter freundii]	*Citrobacter freundii*
WP_003037292. 1	membrane protein [Citrobacter sp. L 17]	*Citrobacter sp.*
WP_009652545. 1	membrane protein [Klebsiella sp. OBRC7]	*Klebsiella sp.*
WP_004853460. 1	membrane protein [Klebsiella oxytoca]	*Klebsiella oxytoca*
YP_0050 16079. 1	AzIC family protein [Klebsiella oxytoca KCTC 1686]	*Klebsiella oxytoca*
WP_004866792. 1	membrane protein [Klebsiella oxytoca]	*Klebsiella oxytoca*
WP_01 7459327. 1	membrane protein [Enterobacter cloacae]	*Enterobacter cloacae*
WP_004205700. 1	AzIC family protein [Klebsiella pneumoniae]	*Klebsiella pneumoniae*
CDA02044. 1	azIC family protein [Klebsiella variicola CAG:634]	*Klebsiella variicola*
WP_0041 23979. 1	membrane protein [Klebsiella oxytoca]	*Klebsiella oxytoca*
WP_0041 32932. 1	azIC family protein [Klebsiella oxytoca]	*Klebsiella oxytoca*
WP_01 790061 6.1	membrane protein [Klebsiella pneumoniae]	*Klebsiella pneumoniae*
YP_002236980. 1	AzIC family protein [Klebsiella pneumoniae 342]	*Klebsiella pneumoniae*
YP_005228384. 1	putative amino acid transport protein [Klebsiella pneumoniae subsp. pneumoniae HS 11286]	*Klebsiella pneumoniae subsp. Pneumoniae*
YP_00 1336647. 1	putative amino acid transport protein [Klebsiella pneumoniae subsp. pneumoniae MGH 78578]	*Klebsiella pneumoniae subsp. Pneumoniae*
WP_0 16947585. 1	membrane protein [Klebsiella pneumoniae]	*Klebsiella pneumoniae*
YP_005956056. 1	putative amino acid transport protein [Klebsiella pneumoniae KCTC 2242]	*Klebsiella pneumoniae*
WP_020803754. 1	inner membrane protein YgaZ [Klebsiella pneumoniae]	*Klebsiella pneumoniae*
WP_0 16 16 1678. 1	inner membrane protein YgaZ [Klebsiella sp.	*Klebsiella sp.*

	KTE92]	
WP_004174723.1	membrane protein [Klebsiella pneumoniae]	*Klebsiella pneumoniae*
WP_004114705.1	membrane protein [Klebsiella oxytoca]	*Klebsiella oxytoca*
YP_007990259.1	ygaZ [Klebsiella pneumoniae]	*Klebsiella pneumoniae*
WP_004104780.1	membrane protein [Klebsiella oxytoca]	*Klebsiella oxytoca*
WP_007370573.1	membrane protein [Kosakonia radicincitans]	*Kosakonia radicincitans*
WP_007370573.1	membrane protein [Kosakonia radicincitans]	*Kosakonia radicincitans*
NP_668256.1	hypothetical protein y0925 [Yersinia pestis KIM10+]	*Yersinia pestis*
WP_005119769.1	branched-chain amino acid permease [Shigella flexneri]	*Shigella flexneri*
YP_069400.1	LIV-E family branched chain amino acid exporter large subunit [Yersinia pseudotuberculosis IP 32953]	*Yersinia pseudotuberculosis*
WP_017893772.1	membrane protein [Serratia sp. S4]	*Serratia sp.*
YP_001479963.1	AzlC family protein [Serratia proteamaculans 568]	*Serratia proteamaculans*
WP_005189088.1	membrane protein [Yersinia intermedia]	*Yersinia intermedia*
YP_004297214.1	putative amino acid transporter [Yersinia enterocolitica subsp. palearctica 105.5R(r)]	*Yersinia enterocolitica subsp. Palearctica*
WP_019081387.1	membrane protein [Yersinia enterocolitica]	*Yersinia enterocolitica*
WP_004392936.1	membrane protein [Yersinia kristensenii]	*Yersinia kristensenii*
WP_016929851.1	membrane protein [Serratia marcescens]	*Serratia marcescens*
WP_019845222.1	membrane protein [Dickeya zeae]	*Dickeya zeae*
YP_003334823.1	AzlC family protein [Dickeya dadantii Ech586]	*Dickeya dadantii*
YP_003042011.1	conserved hypothetical protein [Photorhabdus asymbiotica]	*Photorhabdus asymbiotica*
WP_016941678.1	membrane protein [Dickeya zeae]	*Dickeya zeae*
WP_005274999.1	membrane protein [Yersinia bercovieri]	*Yersinia bercovieri*
CAC44347.1	YgaZ protein [Erwinia chrysanthemi]	*Erwinia chrysanthemi*
WP_004704053.1	membrane protein [Yersinia aldovae]	*Yersinia aldovae*
YP_003003219.1	AzlC family protein [Dickeya zeae Ech1591]	*Dickeya zeae*
WP_004707388.1	membrane protein [Yersinia frederiksenii]	*Yersinia frederiksenii*
WP_008812528.1	membrane protein [Enterobacteriaceae bacterium 9_2_54FAA]	*Enterobacteriaceae bacterium*
YP_008231812.1	membrane protein [Serratia liquefaciens ATCC 27592]	*Serratia liquefaciens*
YP_051597.1	amino acid transporter [Pectobacterium atrosepticum SCRI1043]	*Pectobacterium atrosepticum*
WP_019455591.1	membrane protein [Serratia marcescens]	*Serratia marcescens*
YP_007407667.1 AGE19648.1 NC_020211.1.	putative amino acid transporter YgaZ [Serratia marcescens WW4]	*Serratia marcescens*
WP_004716726.1	membrane protein [Yersinia rohdei]	*Yersinia rohdei*
YP_003018879.1	AzlC family protein [Pectobacterium carotovorum subsp. carotovorum PC1]	*Pectobacterium carotovorum subsp. Carotovorum*
WP_004873538.1	membrane protein [Yersinia mollaretii]	*Yersinia mollaretii*
WP_005975645.1	membrane protein [Pectobacterium wasabiae]	*Pectobacterium wasabiae*

YP_003260827.1	AzlC family protein [Pectobacterium wasabiae WPP163]	*Pectobacterium wasabiae*
YP_002986523.1	AzlC family protein [Dickeya dadantii Ech703]	*Dickeya dadantii*
YP_007345875.1 AGB83690.1	putative branched-chain amino acid permease (azaleucine resistance) [Serratia marcescens FGI94]	*Serratia marcescens*
YP_004211503.1	AzlC family protein [Rahnella sp. Y9602]	*Rahnella sp.*
YP_005400523.1	AzlC family protein [Rahnella aquatilis HX2]	*Rahnella aquatilis*
WP_010305354.1	membrane protein [Pectobacterium carotovorum]	*Pectobacterium carotovorum*
WP_010848732.1	conserved hypothetical protein [Xenorhabdus nematophila]	*Xenorhabdus nematophila*
YP_003711585.1 CBJ89380.1	hypothetical protein XNC1_1315 [Xenorhabdus nematophila ATCC 19061]	*Xenorhabdus nematophila*
YP_006500218.1 AFN33798.1	hypothetical protein A225_4537 [Klebsiella oxytoca E718]	*Klebsiella oxytoca*
EHT06520.1	inner membrane protein YgaZ [Klebsiella oxytoca 10-5246]	*Klebsiella oxytoca*
EKP29343.1	AzlC family protein [Klebsiella oxytoca M5al]	*Klebsiella oxytoca*
EJK15416.1	putative amino acid transport protein [Klebsiella pneumoniae subsp. pneumoniae KPNIH18]	*Klebsiella pneumoniae subsp. Pneumoniae*
YP_006500218.1	hypothetical protein A225_4537 [Klebsiella oxytoca E718]	*Klebsiella oxytoca*
YP_002920871.1	putative amino acid transport protein [Klebsiella pneumoniae subsp. pneumoniae NTUH-K2044]	*Klebsiella pneumoniae subsp. Pneumoniae*
YP_003437997.1	AzlC family protein [Klebsiella variicola At-22]	*Klebsiella variicola*
YP_003260827.1	AzlC family protein [Pectobacterium wasabiae WPP163]	*Pectobacterium wasabiae*
WP_010305354.1	membrane protein [Pectobacterium carotovorum]	*Pectobacterium carotovorum*
YP_404404.1 ABB62913.1	hypothetical protein SDY_2877 [Shigella dysenteriae Sd197]	*Shigella dysenteriae*
YP_311671.1. NC_007384.1. AAZ89436.1	hypothetical protein SSON_2826 [Shigella sonnei Ss046]	*Shigella sonnei*

Table 2: YgaH homologous proteins

Acession Number	Name	Organism
YP_001455540.1 ABV15104.1	hypothetical protein CKO_04032 [Citrobacter koseri ATCC BAA-895]	*Citrobacter koseri*
WP_005122930.1 EIQ78634.1	branched-chain amino acid ABC transporter permease [Shigella flexneri]	*Shigella flexneri*
YP_007877062.1 AGJ89510.1	L-valine exporter [Raoultella ornithinolytica B6]	*Raoultella ornithinolytica*
YP_008107734.1 WP_020454910.1 AGN85394.1	branched-chain amino acid ABC transporter permease [Enterobacter sp. R4-368]	*Enterobacter sp.*

WP_004959351.1 EFE95944.1	branched-chain amino acid ABC transporter permease [Serratia odorifera]	*Serratia odorifera*
YP_003884335.1 ADM99778.1	hypothetical protein Dda3937_00895 [Dickeya dadantii 3937]	*Dickeya dadantii*
YP_006647985.1 AFR04732.1	hypothetical protein PCC21_033290 [Pectobacterium carotovorum subsp. carotovorum PCC21]	*Pectobacterium carotovorum subsp. carotovorum*
YP_001007413.1 CAL13269.1	hypothetical protein YE3239 [Yersinia enterocolitica subsp. enterocolitica 8081]	*Yersinia enterocolitica subsp. enterocolitica*
NP_928589.1 CAE13572.1	hypothetical protein plu1278 [Photorhabdus luminescens subsp. laumondii TTO1]	*Photorhabdus luminescens subsp. laumondii*
WP_004847362.1 EHM42582.1	branched-chain amino acid ABC transporter permease [Hafnia alvei]	*Hafnia alvei*
WP_016154157.1 EOQ28427.1 EOQ47452.1	L-valine exporter [Citrobacter sp. KTE32]	*Citrobacter sp.*
WP_006687198.1 EFE06903.1	branched-chain amino acid ABC transporter permease [Citrobacter youngae]	*Citrobacter youngae*
YP_005198837.1 AEX50697.1	Branched-chain amino acid transport protein AzlD [Rahnella aquatilis CIP 78.65 = ATCC 33071]	*Rahnella aquatilis*
WP_009111643.1 EHD20335.1.	branched-chain amino acid ABC transporter permease [Brenneria sp. EniD312]	*Brenneria sp. EniD312*
YP_003469115.1 CBJ82351.1	transporter [Xenorhabdus bovienii SS-2004]	*Xenorhabdus bovienii*
NP_708496.1	L-valine exporter [Shigella flexneri 2a str. 301]	*Shigella flexneri*
YP_409183.1. NC_007613.1. ABB67355.1.	conserved hypothetical protein [Shigella boydii Sb227]	*Shigella boydii*
WP_000119765.1	branched-chain amino acid ABC transporter permease [Shigella flexneri]	*Shigella flexneri*
WP_003825969.1	branched-chain amino acid ABC transporter permease [Citrobacter sp. 30_2]	*Citrobacter sp.*
WP_003037297.1	branched-chain amino acid ABC transporter permease [Citrobacter freundii]	*Citrobacter freundii*
WP_003037297.1	branched-chain amino acid ABC transporter permease [Citrobacter freundii]	*Citrobacter freundii*
EKU35015	liv-e family branched chain amino acid small subunit [Citrobacter sp. L17]	*Citrobacter sp.*
WP_009652550.1	branched-chain amino acid ABC transporter permease [Klebsiella sp. OBRC7]	*Klebsiella sp.*
WP_004853462.1	branched-chain amino acid ABC transporter permease [Klebsiella oxytoca]	*Klebsiella oxytoca*
YP_005016080.1	putative L-valine exporter [Klebsiella oxytoca KCTC 1686]	*Klebsiella oxytoca*
WP_017459326.1	branched-chain amino acid ABC transporter permease [Enterobacter cloacae]	*Enterobacter cloacae*
WP_004205699.1	L-valine exporter [Klebsiella pneumoniae]	*Klebsiella pneumoniae*
WP_004123982.1	branched-chain amino acid ABC transporter permease [Klebsiella oxytoca]	*Klebsiella oxytoca*
WP_004132928.1	L-valine exporter [Klebsiella oxytoca]	*Klebsiella oxytoca*
YP_002236979.1	hypothetical protein KPK_1115 [Klebsiella pneumoniae 342]	*Klebsiella pneumoniae*

YP_005228385.1	hypothetical protein KPHS_40850 [Klebsiella pneumoniae subsp. pneumoniae HS11286]	*Klebsiella pneumoniae subsp. Pneumoniae*
YP_001336648.1	hypothetical protein KPN_03012 [Klebsiella pneumoniae subsp. pneumoniae MGH 78578]	*Klebsiella pneumoniae subsp. Pneumoniae*
YP_005956057.1. NC_017540.1.	putative L-valine exporter [Klebsiella pneumoniae KCTC 2242]	*Klebsiella pneumoniae*
WP_020803764.1	hypothetical protein [Klebsiella pneumoniae]	*Klebsiella pneumoniae*
WP_004114708.1	branched-chain amino acid ABC transporter permease [Klebsiella oxytoca]	*Klebsiella oxytoca*
WP_004104783.1	branched-chain amino acid ABC transporter permease [Klebsiella oxytoca]	*Klebsiella oxytoca*
WP_007370572.1 EJI92176.1	branched-chain amino acid transport family protein [Kosakonia radicincitans]	*Kosakonia radicincitans*
EJI93105.1	branched-chain amino acid transport family protein [Enterobacter radicincitans DSM 16656]	*Enterobacter radicincitans*
NP_668255.1	hypothetical protein y0924 [Yersinia pestis KIM10+]	*Yersinia pestis*
YP_069399.1	hypothetical protein YPTB0858 [Yersinia pseudotuberculosis IP 32953]	*Yersinia pseudotuberculosis*
YP_001479964.1	hypothetical protein Spro_3740 [Serratia proteamaculans 568]	*Serratia proteamaculans*
WP_005189085.1	branched-chain amino acid ABC transporter permease [Yersinia intermedia]	*Yersinia intermedia*
YP_004297213.1	hypothetical protein YE105_C1014 [Yersinia enterocolitica subsp. palearctica 105.5R(r)]	*Yersinia enterocolitica subsp. Palearctica*
WP_019081388.1	branched-chain amino acid ABC transporter permease [Yersinia enterocolitica]	*Yersinia enterocolitica*
WP_004392937.1	branched-chain amino acid ABC transporter permease [Yersinia kristensenii]	*Yersinia kristensenii*
WP_016929852.1	branched-chain amino acid ABC transporter permease [Serratia marcescens]	*Serratia marcescens*
WP_019845221.1	branched-chain amino acid ABC transporter permease [Dickeya zeae]	*Dickeya zeae*
YP_003334824.1	hypothetical protein Dd586_3285 [Dickeya dadantii Ech586]	*Dickeya dadantii*
YP_003042012.1. NC_012962.1.	conserved hypothetical protein [Photorhabdus asymbiotica]	*Photorhabdus asymbiotica*
WP_016941677.|	branched-chain amino acid ABC transporter permease [Dickeya zeae]	*Dickeya zeae*
WP_005275000.1	branched-chain amino acid ABC transporter permease [Yersinia bercovieri]	*Yersinia bercovieri*
CAC44348.1	YgaH protein [Erwinia chrysanthemi]	*Erwinia chrysanthemi*
WP_004704054.1	branched-chain amino acid ABC transporter permease [Yersinia aldovae]	*Yersinia aldovae*
YP_003003218.1	hypothetical protein Dd1591_0860 [Dickeya zeae Ech1591]	*Dickeya zeae Ech1591*
WP_004707387.1	branched-chain amino acid ABC transporter permease [Yersinia frederiksenii]	*Yersinia frederiksenii*
WP_008812527.1	branched-chain amino acid ABC transporter permease [Enterobacteriaceae bacterium 9_2_54FAA]	*Enterobacteriaceae bacterium*
YP_008231813.1	branched-chain amino acid ABC transporter permease [Serratia liquefaciens ATCC 27592]	*Serratia liquefaciens*

YP_051598.1	hypothetical protein ECA3510 [Pectobacterium atrosepticum SCRI1043]	*Pectobacterium atrosepticum*
WP_019455592.1	branched-chain amino acid ABC transporter permease [Serratia marcescens]	*Serratia marcescens*
YP_007407668.1	putative amino acid transporter YgaH [Serratia marcescens WW4]	*Serratia marcescens*
WP_004716724.1	branched-chain amino acid ABC transporter permease [Yersinia rohdei]	*Yersinia rohdei*
YP_003018880.1. NC_012917.1.	hypothetical protein PC1_3328 [Pectobacterium carotovorum subsp. carotovorum PC1]	*Pectobacterium carotovorum subsp. Carotovorum*
WP_004873539.1	branched-chain amino acid ABC transporter permease [Yersinia mollaretii]	*Yersinia mollaretii*
WP_005975643.1	branched-chain amino acid ABC transporter permease [Pectobacterium wasabiae]	*Pectobacterium wasabiae*
YP_003260828.1	hypothetical protein Pecwa_3484 [Pectobacterium wasabiae WPP163]	*Pectobacterium wasabiae*
YP_002986522.1	hypothetical protein Dd703_0892 [Dickeya dadantii Ech703]	*Dickeya dadantii*
YP_007345876.1	Branched-chain amino acid transport protein (AzlD) [Serratia marcescens FGI94]	*Serratia marcescens*
YP_004211502.1	branched-chain amino acid transport [Rahnella sp. Y9602]	*Rahnella sp.*
YP_005400522.1 NC_017047.1.	putative L-valine exporter [Rahnella aquatilis HX2]	*Rahnella aquatilis*
WP_010305358.1	branched-chain amino acid ABC transporter permease [Pectobacterium carotovorum]	*Pectobacterium carotovorum*
YP_003711584.1. NC_014228.1.	hypothetical protein XNC1_1314 [Xenorhabdus nematophila ATCC 19061]	*Xenorhabdus nematophila*
YP_006500219.1 AFN29790.1	branched-chain amino acid transport [Klebsiella oxytoca E718]	*Klebsiella oxytoca*
EHT06521.1	hypothetical protein HMPREF9690_03780 [Klebsiella oxytoca 10-5246]	*Klebsiella oxytoca*
EKP29342.1.	L-valine exporter [Klebsiella oxytoca M5al]	*Klebsiella oxytoca*
EJK15417.1.	putative L-valine exporter [Klebsiella pneumoniae subsp. pneumoniae KPNIH18]	*Klebsiella pneumoniae subsp. Pneumoniae*
YP_006500219.1	branched-chain amino acid transport [Klebsiella oxytoca E718]	*Klebsiella oxytoca*
BAH64805.1.	hypothetical protein KP1_4275 [Klebsiella pneumoniae subsp. pneumoniae NTUH-K2044]-ygaH	*Klebsiella pneumoniae subsp. Pneumoniae*
YP_003437996.1	hypothetical protein Kvar_1056 [Klebsiella variicola At-22]	*Klebsiella variicola*
YP_003260828.1	hypothetical protein Pecwa_3484 [Pectobacterium wasabiae WPP163]	*Pectobacterium wasabiae*
WP_010282658.1	branched-chain amino acid ABC transporter permease [Pectobacterium carotovorum]	*Pectobacterium carotovorum*
YP_404405.1. NC_007606.1. ABB62914.1.	hypothetical protein SDY_2878 [Shigella dysenteriae Sd197]	*Shigella dysenteriae*
WP_000119748.1	branched-chain amino acid ABC transporter permease [Shigella dysenteriae]	*Shigella dysenteriae*
YP_311672.1 AAZ89437.1	hypothetical protein SSON_2827 [Shigella sonnei Ss046]	*Shigella sonnei*

WP_0051 50562. 1	putative membrane protein [Shigella sonnei]	*Shigella sonnei*
WP_0001 19744. 1	branched-chain amino acid ABC transporter permease [Shigella boydii]	*Shigella boydii*
WP_002427075. 1	branched-chain amino acid ABC transporter permease [Yersinia pestis]	*Yersinia pestis*
WP_0 1749 1438. 1	branched-chain amino acid ABC transporter permease [gamma proteobacterium WG36]	*gamma proteobacterium*
WP_0023661 38. 1	branched-chain amino acid transport family protein, partial [Yersinia pestis]	*Yersinia pestis*

With accession number disclosed in the tables for each homolog the man skilled in the art is able to obtain the amino acid sequence and its nuceotidic coding sequence on NCBI databases for instance.

From the amino acid sequence or nucleotidic sequence, it is a routine task for the man skilled in the art to obtain genes encoding these homologues. It can be done either by artificial synthesis of the gene coding the protein of interest from its amino acid sequence or by PCR amplification of the coding region of interest from the corresponding genomic DNA. In the context of the invention, these genes are called "*ygaZ* or *ygaH* homologous genes". The sequences of these *ygaZH* homologous genes may be adjusted to the codon bias of the host microorganism.

In a specific embodiment of the invention, the recombinant microorganism overexpresses the genes *ygaZ* and *ygaH* from *E. coli* coding the proteins whose sequences are respectively disclosed in SEQ ID NO: 1 and SEQ ID NO: 2 or *brnF* and *brnE* from *C. glutamicum* or their homologous genes. Preferably, *ygaZ* and *ygaH* homologous genes are composed by the gene pair originating from the same organism and composed by the homologous gene of *ygaZ* and the homologous gene of *ygaH*. However mismatch pair of an *ygaZ* homologous gene from a first organism and an *ygaH* homologous gene from a second organism could be used. Preferably, the genes *ygaZH, brnFE* or their homologous genes are overexpressed.

YgaZH homologous genes are chosen among genes encoding the YgaZ and YgaH homologues disclosed respectively in table 1 and in table 2. Preferably, *ygaZH* homologous genes are chosen among genes encoding YgaZH homologues from *Citrobacter* species, *Shigella* species, *Raoultella* species, *Enterobacter* species, *Yersinia* species and *Photorhabdus* species. More preferably *ygaZH* homologous genes originate from *Citrobacter koseri, Shigella flexneri, Raoultella ornithinolytica, Enterobacter sp., Yersinia enterocolitica, Photorhabdus luminescens, Citrobacter youngae* or *Citrobacter freundii.* Most preferably, *ygaZH* homologous genes originate from *Citrobacter koseri, Citrobacter youngae, Citrobacterfreundii* or *Enterobacter sp.*.

Therefore, *ygaZH* homologous genes are preferably chosen among genes coding the pair of YgaZ homolog and YgaH homolog defined respectively by: SEQ ID NO: 3 and SEQ ID

NO: 4 from *Citrobacter koseri,* SEQ ID NO: 5 and SEQ ID NO: 6 from *Shigella flexneri,* SEQ ID NO: 7 and SEQ ID NO: 8 from *Raoultella ornithinolytica,* SEQ ID NO: 9 and SEQ ID NO: 10 from *Enterobacter sp.* (R4-368), SEQ ID NO: 11 or 12 and SEQ ID NO: 13 or 14 from *Yersinia enterocolitica subsp. enterocolitica,* SEQ ID NO: 15 and SEQ ID NO: 16 from *Photorhabdus luminescens subsp. laumondii,* SEQ ID NO: 17 and SEQ ID NO: 18 from *Citrobacter youngae,* SEQ ID NO: 19 and SEQ ID NO: 20 from *Citrobacter freundii.*

In a preferred embodiment of the invention, these genes *ygaZH* or *brnFE* or homologous genes originating from *Citrobacter koseri, Shigella flexneri, Raoultella ornithinolytica, Enterobacter sp., Yersinia enterocolitica, Photorhabdus luminescens, Citrobacter youngae* or *Citrobacter freundii* are overexpressed under the control of an inducible promoter. The man skilled in the art knows such inducible promoters. For instance, promoters like λP_R or λP_L may be used to overexpress *ygaZH* genes or *brnFE* genes or *ygaZH* homologous genes originating from *Citrobacter koseri, Shigella flexneri, Raoultella ornithinolytica, Enterobacter sp., Yersinia enterocolitica, Photorhabdus luminescens, Citrobacter youngae* or *Citrobacter freundii* in the recombinant microorganism of the invention.

It is another object of the invention to identify *ygaZH* homologous genes and to overexpress said genes in amino-acid producer microorganism, alone or in combination with other genetic modifications as disclosed below.

Optimisation of methionine biosynthesis pathway

The recombinant microorganism according to the invention is modified for improving the production of methionine. Genes involved in methionine production are well known in the art, and comprise genes involved in the methionine specific biosynthesis pathway as well as genes involved in precursor-providing pathways and genes involved in methionine consuming pathways.

Efficient production of methionine requires the optimisation of the methionine specific pathway and several precursor-providing pathways. Methionine producing strains have already been described, in particular in patent applications WO2005/1 11202, WO2007/077041 and WO2009/043803. These applications are incorporated as reference into this application.

Except otherwise stated, all the genes mentioned below concerning optimisation of methionine biosynthesis pathway are referring to those from *E. coli.*

In a specific embodiment of the invention, the recombinant microorganism is modified as described below: the expression of at least one gene chosen among *ptsG, pyc, pntAB, cysP, cysll, cysW, cysA, cysM, cysJ, cysl, cysH, gcvT, gcvH, gcvP, Ipd, serA, serB, serC, cysE, metF, metA, metA* *allele encoding for an enzyme with reduced feed-back sensitivity

to S-adenosylmethionine and/or methionine, *thrA,* and *thrA* * allele encoding for an enzyme with reduced feed-back inhibition to threonine is increased.

• *ptsG* encodes the PTS enzyme $IICB^{Glc}$ as described in patent application WO2013/001055,

· *pyc* encodes a pyruvate carboxylase as described in patent application WO20 13/00 1055. In a preferred embodiment, the *pyc* gene is heterologous and is chosen from *pyc* genes from *Rhizobium etli, Bacillus subtilis, Lactococcus lactis, Pseudomonas fluorescens* or *Corymb acterium* species,

• *pntAB* encode subunits of a membrane-bound transhydrogenase, such as described in patent application WO2012/055798,

• *cysP* encodes a periplasmic sulphate binding protein, as described in WO2007/077041 and in WO2009/043803,

• *cysU* encodes a component of sulphate ABC transporter, as described in WO2007/077041 and in WO2009/043803,

· *cysW* encodes a membrane bound sulphate transport protein, as described in WO2007/077041 and in WO2009/043803,

• *cysA* encodes a sulphate permease, as described in WO2007/077041 and in WO2009/043803,

• *cysM* encodes an O-acetyl serine sulfhydralase, as described in WO2007/077041 and in WO2009/043803,

• *cysI* and *cysJ* encode respectively the alpha and beta subunits of a sulfite reductase as described in WO2007/077041 and in WO2009/043803. Preferably *cysI* and *cysJ* are overexpressed together,

• *cysH* encodes an adenylylsulfate reductase, as described in WO2007/077041 and in WO2009/043803.

Increasing CI metabolism is also a modification that leads to improved methionine production. It relates to the increase of the activity of at least one enzyme involved in the CI metabolism chosen among GcvTHP, Lpd, MetF or MetH. In a preferred embodiment of the invention, the one carbon metabolism is increased by enhancing the expression and/or the activity of at least one of the following:

• *gcvT, gcvH, gcvP,* and *Ipd,* coding for the glycine cleavage complex, as described in patent application WO 2007/077041. The glycine-cleavage complex (GCV) is a multienzyme complex that catalyzes the oxidation of glycine, yielding carbon dioxide, ammonia, methylene-THF and a reduced pyridine nucleotide. The GCV complex consists of four protein components, the glycine dehydrogenase said P-protein (GcvP), the lipoyl-GcvH-protein said H-protein (GcvH), the aminomethyltransferase said T-protein (GcvT), and the dihydrolipoamide dehydrogenase said L-protein (GcvL or Lpd). P-protein catalyzes the pyridoxal phosphate-dependent liberation of C02 from glycine, leaving a

methylamine moiety. The methylamine moiety is transferred to the lipoic acid group of the H-protein, which is bound to the P-protein prior to decarboxylation of glycine. The T-protein catalyzes the release of NH3 from the methylamine group and transfers the remaining CI unit to THF, forming methylene-THF. The L protein then oxidizes the lipoic acid component of the H-protein and transfers the electrons to NAD^+, forming NADH;

• *MetF* encoding a methylenetetrahydro folate reductase, as described in patent application WO2007/07704.

The overexpression of at least one of the following genes involved in serine biosynthesis also reduces the production of the by-product isoleucine:

· *serA* which encodes a phosphoglycerate dehydrogenase, as described in WO2007/077041 and in WO2009/043803,

• *serB* which encodes a phosphoserine phosphatase, as described in WO2007/077041 and in WO2009/043803,

• *serC* which encodes a phosphoserine aminotransferase, as described in WO2007/077041 and in WO2009/043803.

The overexpression of the following genes has already been shown to improve the production of methionine:

• *cysE* encodes a serine acyltransferase; its overexpression allows an increase in methionine production, as described in WO2007/077041;

· *metA* encodes a homoserine succinyltransferase. The allele *metA** codes for an enzyme with reduced feed-back sensitivity to S-adenosylmethionine and/or methionine. Preferentially, the allele *metA** described in the patent application WO2005/1 11202 is used;

• *thrA* encodes an aspartokinase /homoserine dehydrogenase; the *thrA* * allele codes for an enzyme with reduced feed-back inhibition to threonine, as described in WO2005/1 11202.

In a specific embodiment of the invention, at least one of said genes is under control of an inducible promoter. In a preferred embodiment of the invention, at least one of these genes is under the control of a temperature inducible promoter. Preferably, the expression of at least one of the genes: *thrA, cysE, metA,* is under the control of an inducible promoter, directly or indirectly. More preferably, the genes *thrA, cysE* and *metA* are under control of an inducible promoter, directly or indirectly. In a preferred embodiment of the invention, expression of *thrA* gene is under direct control of an inducible promoter and expression of *cysE* gene is under polar effect of inducible expression of *thrA* gene. In another preferred embodiment of the invention, expression of *thrA* gene is under direct control of an inducible promoter and expressions of *cysE* and *metA* genes are under polar effect of inducible expression of *thrA* gene.

In a most preferred embodiment, the temperature inducible promoter belongs to the family of P_R promoters. A methionine producing strain having genes under control of inducible promoters is described in patent application WO201 1/073122.

In another specific embodiment of the invention, the microorganism has been further modified, and the expression of at least one of the following genes is attenuated: *metJ, pykA, pykF, purU, ybdL, yncA, metE, dgsA, metN, metl, metQ* or *udhA*.

• The gene *metJ* codes for the repressor protein MetJ (GenBank 1790373), responsible for the down-regulation of the methionine regulon as was suggested in patent application JP2000/1 57267,

· The genes *pykA* and *pykF* code for the enzymes 'pyruvate kinase'. The attenuation of the expression of at least one or both of the pyruvate kinases decreases the consumption of phosphoenol pyruvate (PEP). Increased availability of PEP can increase the production of oxaloacetate, an important precursor of aspartate, which in turn is a precursor of methionine, as described in WO2007/077041 and in WO2009/043803,

· *purU* codes for a formyltetrahydrofolate deformylase, an enzyme that catalyzes the formyl-THF deformylase reaction. The attenuation of the deformylase activity increases the production of methyl-THF that is required for methylation of homocysteine. Loss of C I metabolites by deformylation leads to an increased production of homocysteine that cannot be transformed into methionine. Homocysteine can then be a substrate for the enzyme cystathionine gamma synthase (MetB) that can catalyze the reaction between O-succinylhomoserine and homocysteine resulting in the production of homolanthionine, as described in WO2007/077041 and in WO2009/043803,

• *ybdL* encodes an aminotransferase as described in patent application WO20 12/090021,

· *yncA* encodes a N-acyltransferase, as described in patent application WO20 10/020681,

• *metE* encodes a cobalamin-independent methionine synthase, as described in patent application PCT/IB2012/001336,

• *dgsA,* better known as Mlc, encodes a transcriptional dual regulator that controls the expression of genes encoding enzymes of the phosphotransferase (PTS) and phosphoenolpyruvate (PEP) systems as described in patent application WO2013/001055,

• *metN, metl, metQ,* encode a methionine uptake system,

• *udhA* encodes soluble pyridine nucleotide transhydrogenase, as described in patent application WO2012/055798.

In a more preferred embodiment of the invention, the fermentative production of methionine and/or its derivatives by a recombinant microorganism, wherein the methionine import is attenuated and the methionine efflux is enhanced, from glucose as a main carbon

source, may be achieved through a combination of the above discussed modifications in said microorganism, for example:

- the expression of the gene *metJ* is attenuated and the expression of a *metA** allele encoding for an enzyme with reduced feed-back sensitivity to S-adenosylmethionine and/or methionine *(MetA *)* is enhanced;
- the expression of the gene *metJ* is attenuated; the expression of a *metA** allele encoding for an enzyme with reduced feed-back sensitivity to S-adenosylmethionine and/or methionine *(MetA *)* is enhanced; and the expression of a *thrA ** allele encoding for an enzyme with reduced feed-back inhibition to threonine *(thrA *)* is enhanced;
- the expression of the gene *metJ* is attenuated; the expression of a *metA** allele encoding for an enzyme with reduced feed-back sensitivity to S-adenosylmethionine and/or methionine *(MetA*)* is enhanced; the expression of a *thrA** allele encoding for an enzyme with reduced feed-back inhibition to threonine *(thrA *)* is enhanced; and the expression of the gene *cysE* is enhanced;
- the expression of the gene *metJ* is attenuated; the expression of a *metA** allele encoding for an enzyme with reduced feed-back sensitivity to S-adenosylmethionine and/or methionine *(MetA*)* is enhanced; the expression of a *thrA** allele encoding for an enzyme with reduced feed-back inhibition to threonine *(thrA *)* is enhanced; the expression of the gene *cysE* is enhanced; and the expression of the genes *metF* is enhanced.

In a particular aspect of the invention, the recombinant microorganism comprises the following genetic modifications:

- the genes *metH,* and *fldA* and *fpr* from *E. coli* or their homologous genes from *C glutamicum* are overexpressed,
- the genes *ygaZ* and *ygaH* from *E. coli* or the genes *brnF* and *brnE* from *C. glutamicum* or their homologous genes originating from *Citrobacter koseri, Shigella flexneri, Raoultella ornithinolytica, Enterobacter sp., Yersinia enterocolitica, Photorhabdus luminescens, Citrobacter youngae* or *Citrobacterfreundii* are overexpressed,
- the expression of the genes *metA*, cysPUWAM, cysJIH, gcvTHP, metF, serA, serB, serC, cysE, thrA *, ptsG* and *pyc* are enhanced, and
- the expression of genes *metJ, pykA, pykF, purU, metE, dgsA and yncA* are attenuated.

In a particular embodiment of the invention, the microorganism to be modified is from the bacterial family *Enterobacteriaceae* or *Corynebacteriaceae.*

Preferentially, the microorganism to be modified is *Escherichia coli* or *Corynebacterium glutamicum.*

Culture conditions

In a second aspect of the invention, a method is optimised for the fermentative production of methionine and/or its derivatives. It comprises the followings steps:

- Culturing a recombinant microorganism wherein the cobalamin-dependent methionine synthase activity and the methionine efflux are enhanced by overexpressing respectively the genes *metH,* and optionally the genes *fldA* and *fpr* genes from *E. coli* or their homologous genes from *C. glutamicum* and the genes *ygaZH* from *E. coli* or the genes *brnFE* from *C. glutamicum* or their homologous genes in an appropriate culture medium comprising a fermentable source of carbon and a source of sulphur, and,
- Recovering methionine and/or its derivatives from the culture medium.

Those skilled in the art are able to define the culture conditions for the microorganisms according to the invention. In particular the bacteria are fermented at a temperature between 20°C and 55°C, preferentially between 25°C and 40°C, and more specifically about 30°C for *C. glutamicum* and about 37°C for *E. coli.*

For *E. coli,* the culture medium can be of identical or similar composition to an M9 medium (Anderson, 1946), an M63 medium (Miller, 1992); or a medium such as defined by Schaefer *et al,* (1999).

For *C. glutamicum,* the culture medium can be of identical or similar composition to BMCG medium (Liebl *et al,* 1989) or to a medium such as described by Riedel *et al.,* (2001).

In the method of the invention, the *ygaZH* homologous genes which are overexpressed in the recombinant microorganism are preferably chosen among the group consisting in homologous genes from *Citrobacter* species, *Shigella* species, *Raoultella* species, *Enterobacter* species, *Yersinia* species and *Photorhabdus* species, and more preferably originate from *Citrobacter koseri, Shigella flexneri, Raoultella ornithinolytica, Enterobacter sp., Yersinia enterocolitica, Photorhabdus luminescens, Citrobacter youngae* or *Citrobacter freundii.*

In a specific embodiment of the method, the recombinant microorganism comprises the following genetic modifications:

a. overexpression of the genes *metH,* and *fldA* and *fpr* from *E. coli,* or their homologous genes from *C. glutamicum* and
b. overexpression of the genes *ygaZH* from *E. coli,* or *brnFE* from C. *glutamicum* or their homologous genes.

In this specific embodiment of the invention, said *ygaZH* homologous genes are preferably chosen among the group consisting in homologous genes from *Citrobacter* species, *Shigella* species, *Raoultella* species, *Enterobacter* species, *Yersinia* species and *Photorhabdus* species, and more preferably chosen among the groups consisting in homologous genes from *Citrobacter koseri, Shigella flexneri, Raoultella ornithinolytica, Enterobacter sp., Yersinia enterocolitica, Photorhabdus luminescens, Citrobacter youngae* or *Citrobacterfreundii.*

In the method of the invention, the *ygaZH* homologous genes which are overexpressed in the recombinant microorganism are most preferably originating from *Citrobacter koseri, Citrobacter youngae, Citrobacterfreundii* or *Enterobacter sp..*

In some embodiment of the invention, the growth of the recombinant microorganism is subjected to a limitation or starvation / deficiency for one or several inorganic substrate, in particular phosphate and/or potassium, in the culture medium. It refers to condition under which growth of the microorganisms is governed by the quantity of an inorganic chemical supplied that still permits weak growth. Such limitation in microorganism growth has been described in the patent application WO2009/043372. In a preferred embodiment of the invention, the culture is subjected to phosphate limitation.

The action of "recovering methionine and/or its derivatives from the culture medium" designates the action of recovering L-methionine and/or one of its derivatives, in particular N-acetyl methionine (NAM) and S-adenosyl methionine (SAM) and all other derivatives that may be useful such as hydroxy-methionine (or methionine hydroxy analogue or MHA). The methods for the recovery and purification of the produced compounds are well known to those skilled in the art (see in particular WO2005/007862, WO2005/059155). Preferably, the step of recovering methionine and/or its derivatives comprises a step of concentration of methionine and/or its derivatives in the fermentation broth.

The amount of product in the fermentation medium can be determined using a number of methods known in the art, for example, high performance liquid chromatography (HPLC) or gas chromatography (GC). For example the quantity of methionine obtained in the medium is measured by HPLC after OPA/Fmoc derivatization using L-methionine (Fluka, Ref 64319) as a standard. The amount of NAM is determinated using refractometric HPLC using NAM (Sigma, Ref 01310) as a standard.

EXAMPLES

The following experiments demonstrate how overexpression of genes encoding for the L-methionine excretion system together with the overexpression of genes encoding for the B12-dependent methionine synthase and its reactivation system in microorganisms such as *E. coli* and *C. glutamicum* improved methionine production.

In the examples given below, methods well known in the art were used to construct *E. coli* and *C. glutamicum* strains containing replicating vectors and/or various chromosomal insertions, deletions, and substitutions using homologous recombination well described by Datsenko & Wanner, (2000) for *E.coli* and in patent WO2007012078 for *C. glutamicum.*

In the same manner, the use of plasmids or vectors to express or overexpress one or several genes in a recombinant microorganisms are well known by the man skilled in the art.

Examples of suitable *E.coli* expression vectors include pTrc, pACYC184n pBR322, pUC18, pUC19, pKC30, pRep4, pHSl, pHS2, pPLc236 etc...

Examples of suitable *C. glutamicum* and *E.coli* shuttle vectors are e. g. pClik5aMCS (WO2005059093) or can be found in Eikmanns *et al,* (1991).

Examples for suitable vectors to manipulate Corynebacteria can be found in the handbook of Corynebacteria edited by Eggeling and Bott in 2005.

PROTOCOLS

Several protocols have been used to construct methionine producing strains described in the following examples.

Protocol 1 (Chromosomal modifications by homologous recombination, selection of recombinants and antibiotic cassette excision) and protocol 2 (Transduction of phage PI) used in this invention have been fully described in patent application WO20 13/00 1055.

Protocol 3: Construction of recombinant plasmids

Recombinant DNA technology is well described and known by the man skilled in the art. Briefly, the DNA fragments are PCR amplified using oligonucleotides (the person skilled in the art will is able to design) and MG1655 genomic DNA as matrix. The DNA fragments and selected plasmid are digested with compatible restriction enzymes, ligated and then transformed in competent cells. Transformants are analysed and recombinant plasmids of interest are verified by DNA sequencing.

Table 3: Sequences cited in the following examples

SEQ ID N°	Sequence 5' → 3'
21	AACACTGCAAAATCCTGCTATTTGATTTGTATGAGTGATA AGTGTAACGCCGAATAATCGTCGTTGGCGAATTTTACGAC TCTGACAGGAGGTGGCAATG
22	GAGAAAGTAAACGTAACATGATGACGACAATTCTGACGA TTCATGTTCCTTCAACGCCGGGGCGCGCATGGAATATGCT GGTGGCACTTCAGGCAGGAAA
23	TGAGGAATAGACAATGTTAGTTAGTAAAAGCAACGGATT TAACGCTAGCGCAGTTTTGGGTAGTGGAAGTTATAATGAA AATAAATCTTCTAAACACATG
24	TGCGCTAAAAGAAATGAATAGAACCTTTTCGATAATATAA GAAAAAGTGATTTTCATGTTGGTTTACTTAAGCCAAGTAG

	TACGCGTAGTGTTATTTTAG
25	AAATTATTCTTGTATCTTTGTTATAATATGGGAAAGTGCA ACCAT
26	CGTTAATCAGCAGGTTAGCCAGCCACAAAAAGCCATTGA GAAAATTATTGATTTTACATGGGATTATTATATTGCTAAT CCTTGGTTTTTAAAAATTGTG
27	TCATCTACCGCGCACGAATAAAACTGCCATCCGGCTGGCG GGTGAACAGGACCTGTTGATTATTCCCCGTATCAATGGTT AAGCCCGTCACCACGCCGCT

EXAMPLE 1: Overproduction of the cobalamin-dependent methionine synthase or Overproduction of a L-methionine secretion system in a L-methionine overproducer *E.coli* recombinant strain - Strain 1 and Construction of strains 2, 3, 4, 5 and 6

Strain 1 - Reference strain

Methionine producing strain 17 described in patent application WO20 13/00 1055 (which is incorporated as reference into this application) was renamed strain 1 in this present application. For reminder this strain overexpressed *metH* owing artificial promoter and ribosome binding site integrated in front of *metH* gene at its endogenous locus (for details see as patent application WO2007/077041). This strain contains also the mutation in *metE* gene disclosed in patent application WO2013/190343.

Construction of strain 5 - Overproduction of the cobalamin-dependent methionine synthase, overexpression of *metH, fldA* and *fpr*

The *E.coli* gene encoding the cobalamin-dependent methionine synthase, *metH* and genes *fldA* and *fpr* encoding for the reactivation system of MetH, were all overexpressed in genetic background of strain 1.

Before using strain 1, the antibiotic cassette was removed from *AdgsA* modification using the Flp recombinase as described by Datsenko & Wanner, 2000 (according to Protocol 1). The kanamycin sensible transformants were selected and the absence of antibiotic cassette at *AdgsA* locus was verified by a PCR analysis with appropriate oligonucleotides. The strain retained was designated strain 2.

To overexpress *metH,* this gene, operatively linked to the same promoter and ribosome binding site as described in patent application WO2007/077041 was integrated on the chromosome at two different loci *ybeM* and *ypjC* (selected from the list disclosed in the patent application WO201 1/073122 and whose deletion do not have impact on methionine production).

To strongly overexpress *metH,* the homologous recombination strategy described by Datsenko & Wanner, 2000 (according to Protocol 1) was used. For both chromosomal integrations, a fragment carrying *metH* gene linked to its artificial promoter and a resistance marker both flanked by DNA sequences homologous to the targeted integration

locus *ybeM* or *ypjC* was PCR amplified by the overlapping PCR technique (overlapping oligonucleotides). The sequences for recombination into *ybeM* and *ypjC* are referred as SEQ ID N° 21 and 22, and SEQ ID N° 23 and 24 (listed in table 3), for *ybeM* and *ypjC* respectively. The PCR products *"AybeM::metH::Km"* and *"AypjC::metH::Cm"* obtained were then introduced by electroporation into the strain MG1655 *metA** (pKD46), separately. The antibiotic resistant transformants were selected and the insertion of the *metH* gene with the resistance cassette at the targeted locus was verified by a PCR analysis with appropriate oligonucleotides. The strains retained were designated MG1655 *metA*\\ AybeM::metH::Km* and MG1655 *metA*\\ AypjC::metH::Cm.* Finally, the *AybeM::metH::Km* and *AypjC::metH::Cm* chromosomal integrations were transferred by PI phage transduction successively (according to Protocol 2) from the MG1655 *metA*\\ AybeM::metH::Km* and MG1655 *metA*\\ AypjC::metH* to strain 2. Chloramphenicol or kanamycin resistant transductants were selected and the presence of *AybeM::metH::Km* and *AypjC::metH::Cm* chromosomal integrations were verified by a PCR analysis with appropriate oligonucleotides. The strain retained was called strain 3.

The antibiotic cassettes were removed from chromosomal integrations made at *ybeM* and *ypjC* loci into strain 3 using the Flp recombinase as described by Datsenko & Wanner, 2000 (according to Protocol 1). The kanamycin and chloramphenicol sensible transformants were selected and the absence of antibiotic cassette at both loci was verified by a PCR analysis with appropriate oligonucleotides. The strain retained was designated strain 4.

To overexpress *fldA* and *fpr,* these genes, were operatively linked to artificial promoters and to artificial ribosome binding site and were integrated onto the chromosome at the ytfA locus (same selection criteria as ybeM and ypjC loci, see above). The artificial promoters were constructed with SED ID N° 25 for *fldA* and as described for the overexpression of *cysPUWAM* operon in patent application WO2009/043803 for *fpr.* The artificial ribosome binding sites are the same as described to overexpress *ptsG* gene in strain 17 disclosed in patent application WO2013/001055.

To add copies of *fldA* and *fpr* overexpression onto the chromosome, the homologous recombination strategy described by Datsenko & Wanner, 2000 (according to Protocol 1) was used. A fragment carrying *fldA* and *fpr* genes, with their respective promoters, and a resistance marker, both flanked by DNA sequence homologous to the integration locus *ytfA* was PCR amplified by overlapping PCR technique (overlapping oligonucleotides). The sequences for recombination into the *ytfA* locus are referred as SEQ ID N° 26 and 27 (listed in table 3). The PCR product *"AytfA::fldA-fpr::Km"* obtained was then introduced by electroporation into the MG1655 *metA** (pKD46) strain. The antibiotic resistant transformants were then selected and the insertion of the *fldA-fpr* genes with the resistance cassette at the *ytfA* locus was verified by a PCR analysis with appropriate oligonucleotides.

The strain retained was designated MG1655 *metA*\ \ AytfA::fldA-fpr::Km.* Finally, the *AytfA: :fldA-fpr: :Km* chromosomal integration was transferred by PI phage transduction (according to Protocol 2) from the MG1655 *metA*\ \ AytfA: :fldA-fpr::Km* to strain 4. Kanamycin resistant transductants were selected and the presence of *AytfA: :fldA-fpr: :Km* chromosomal integration was verified by a PCR analysis with appropriate oligonucleotides. The strain retained was called strain 5.

Construction of strain 6 - Overproduction of a L-methionine secretion system, overexpression *ofygaZH*

The *E.coli* genes *ygaZH* encoding the exporter of methionine were overexpressed in strain 1. They were cloned on the moderate plasmid copy number pCL1920 (Lerner & Inouye, 1990) with the use of the natural promoter of *ygaZ.* This plasmid was named pME1247. Finally, the plasmid pME1247 was transformed into strain 1, giving the strain 6.

EXAMPLE 2: Overproduction of the cobalamin-dependent methionine synthase and overproduction of a L-methionine secretion system in a L-methionine overproducer *E.coli* strain - Construction of strain 7

The *E.coli* genes *ygaZH* encoding the exporter of methionine, were overexpressed in strain 5. The plasmid pME1247 was transformed into strain 5, giving rise to strain 7.

EXAMPLE 3: Overproduction of the cobalamin-dependent methionine synthase or its reactivation system or overproduction of a L-methionine secretion system in a L-methionine overproducer *C. glutamicum* recombinant strain - Construction of strains A to F

The *C. glutamicum* strain ATCC 13032 *horn* ask* metH* (designated strain A in the following) is described in patent WO2007/0 12078.

In that strain A, *horn** and *ask** correspond to feedback resistant alleles of homoserine dehydrogenase encoding the protein Hsdh S393F and of aspartate kinase encoding the protein Ask T31II also called LysC T31II, respectively.

This strain A is subsequently mutagenized with N-Methyl-N'-nitroguanidine as described in patent WO2007/012078. Clones that show a methionine titer that is at least twice that in strain A are isolated. One such clone, used in further experiments, is named strain B. This strain B is a *C. glutamicum* L-methionine producer.

Then, the *C. glutamicum* strain B is modified as described in patents WO2007/0 12078 and WO2004/050694 to obtain the strain C including *hsk* metYmetA metF DmcbR.*

The mutated allele *hsk** encoding the homoserine kinase Hsk T190A is overexpressed as well as *metY* encoding the O-acetylhomoserine sulfhydrylase, *metA* encoding the

homoserine acetyl-transferase, *metF* encoding the homocysteine methylase and *mcbR* gene is deleted.

In order to increase the cobalamin-dependent methionine synthase activity in *C. glutamicum* L-methionine producer strain C, *metHcg (metH* gene from *C. glutamicum)* is overexpressed together with *fprAl* gene encoding a ferredoxin reductase working as MetH reoxidation protein. These modifications are performed according the description of patent WO2009/ 144270. The resulting strain is called strain D.

Another way to increase the cobalamin-dependent methionine synthase activity in *C. glutamicum* L-methionine producer strain C, is to overexpress *metHEc (metH* gene from *E. coli)* together with *fldA* and *fpr* genes from *E. coli* encoding the flavodoxins involved into the reactivation of MetH enzyme. This is achieved according to the description of patent WO2009/ 144270. The resulting strain is called strain E.

In order to increase the L-methionine excretion system specific of *C. glutamicum* in strain C, the *brnFE* operon is overexpressed from the *E. coli-C. glutamicum* shuttle expression vector pEC-XT99A (EP 1085094). The plasmid was constructed in *E. coli* from PCR-generated fragments by using *C. glutamicum* ATCC 13032 DNA as a template. The plasmid was constructed as described by Trotschel *et al.,* (2005) in pEC-XT99A, and the resulting plasmid pCBl is subsequently transformed into strain C giving rise to strain F.

EXAMPLE 4: Combined overproduction of the cobalamin-dependent methionine synthase with the overproduction of a L-methionine secretion system in a *C. glutamicum* L-methionine overproducer strain - Construction of strains G and H

In order to combine the overproduction of MetHco, FprAl or $MetH_Ec$, FldA, Fpr in *C. glutamicum* with the overproduction of the specific L-methionine excretion system BrnFE, the plasmid pCBl described above is introduced by electroporation into strains D and E giving rise to strains G and H respectively.

Strain G carries only genes belonging to *C. glutamicum* whereas strain H carries the cobalamin-dependent methionine synthase and its reactivation system from *E. coli.*

The exporter is in all cases BrnFE.

EXAMPLE 5: Production of L-methionine by fermentation in bio-reactor with *E.coli* strains

Strains described in previous examples were tested under production conditions in 2.5 L reactors (Pierre Guerin) using a fedbatch strategy.

Briefly, an 24 hours culture grown in 10 mL LB medium with 2.5 $g.L^{-1}$ glucose was used to inoculate a 24 hours preculture in minimal medium (Bla). These incubations were carried out in 500 mL baffled flasks containing 50 mL of minimal medium (Bla) in a

rotary shaker (200 RPM). The first preculture was realized at a temperature of 30°C, the second one at a temperature of 34°C.

A third preculture step was carried out in bio-reactors (Sixfors) filled with 200 mL of minimal medium (Bib) inoculated to a biomass concentration of 1.2 $g.L^{-1}$ with 5 mL concentrated preculture. The preculture temperature was maintained constant at 34°C and the pH was automatically adjusted to a value of 6.8 using a 10 % NH_4OH solution. The dissolved oxygen concentration was continuously adjusted to a value of 30 % of the partial air pressure saturation with air supply and /or agitation. After glucose exhaustion from the batch medium, the fedbatch was started with an initial flow rate of 0.7 $mL.h^{-1}$, before increasing exponentially for 26 hours with a growth rate of 0.13 h^{-1} in order to obtain a final cellular concentration of about 20 $g.L^{-1}$.

Table 4: Preculture batch mineral medium composition (B1a and Bib)

Compound	B1a Concentration ($g.L^{-1}$)	B1b Concentration ($g.L^{-1}$)
$Zn(CH_3COO)_2.2H_2O$	0.0130	0.0130
$CuCl_2.2H_2O$	0.0015	0.0015
$MnCl_2.4H_2O$	0.0150	0.0150
$CoCl_2.6H_2O$	0.0025	0.0025
H_3BO_3	0.0030	0.0030
$Na_2MoO_4.2H_2O$	0.0025	0.0025
Fe(III) citrate H_2O	0.1064	0.1064
EDTA	0.0084	0.0084
$MgSO_4.7H_2O$	1.00	1.00
$CaCl_2.2H_2O$	0.08	0.08
Citric acid	1.70	1.70
KH_2PO_4	4.56	4.56
$K_2HPO_4.3H_2O$	2.53	2.53
$(NH_4)_2HPO_4$	1.11	1.11
$(NH_4)_2SO_4$	4.90	4.90
$(NH_4)_2S_2O_5$	1.00	1.00
Thiamine	0.01	0.01
Vitamin B12	0.01	0.01
Glucose	30.00	5.00
MOPS	30.00	0.00
NH_4OH 28%	Adjusted to pH 6.8	Adjusted to pH 6.8

Table 5: Preculture fedbatch mineral medium composition (Fl)

Compound	Concentration ($g.L^{-1}$)
$Zn(CH_3COO)_2.H_2O$	0.0104
$CuCl_2.2H_2O$	0.0012
$MnCl_2.4H_2O$	0.0120
$CoCl_2.6H_2O$	0.0020
H_3BO_3	0.0024
$Na_2MoO_4.2H_2O$	0.0020
Fe(III) citrate H_2O	0.0524
EDTA	0.0067
$MgSO_4$	5.00
$(NH_4)_2SO_4$	8.32
Na_2SO_4	8.95
$(NH_4)_2S_2O_3$	24.80
Thiamine	0.01
Glucose	500.00
Vitamin B12	0.01
NH_4OH 28%	Adjusted to pH 6.8

Subsequently, 2.5 L fermentors (Pierre Guerin) were filled with 600 or 620 mL of minimal medium (B2) and were inoculated to a biomass concentration of 3,2 $g.L^{-1}$ with a preculture volume ranging between 80 to 100 mL.

Cell growth is controlled by phosphate, that is why the final phosphate concentration in batch medium B2 was adjusted to a value comprised between 0 to 20 mM, by addition of different concentrations of KH_2PO_4, K_2HPO_4 and $(NH_4)_2HPO_4$. In the same manner, the final phosphate concentration of F2 medium was adjusted to a value comprise between 5 to 30 mM, by addition of different concentrations of KH_2PO_4, K_2HPO_4 and $(NH_4)_2HPO_4$. Thiosulfate concentration in fedbatch medium can be adjusted in order to prevent a starvation of this compound during the culture.

Table 6: Culture batch mineral medium composition (B2)

Compound	Concentration ($g.L^{-1}$)
$Zn(CH_3COO)_2.2H_2O$	0.0130
$CuCl_2.2H_2O$	0.0015
$MnCl_2.4H_2O$	0.0150
$CoCl_2.6H_2O$	0.0025
H3BO3	0.0030

$Na_2MoO_4.2H_2O$	0,0025
Fe(III) citrate H_2O	0.1064
EDTA	0,0084
$MgSO_4.7H_2O$	1.00
$CaCl_2.2H_2O$	0.08
Citric acid	1.70
$(NH_4)_2S_2O_3$	7.74
Thiamine	0.01
Vitamin B12	0.01
Biotin	0.10
Glucose	10
NH_4OH 28%	Adjusted to pH 6.8
IPTG	0,0047

Table 7: Culture fedbatch medium composition (F2)

Compound	**Concentration ($g.L^{-1}$)**
$Zn(CH_3COO)_2.2H_2O$	0,0104
$CuCl_2.2H_2O$	0.0012
$MnCl_2.4H_2O$	0,0120
$CoCl_2.6H_2O$	0.0020
H_3BO_3	0,0024
$Na_2MoO_4.2H_2O$	0,0020
Fe(III) citrate H_2O	0,0524
EDTA	0.0067
$MgSO_4$	5.00
$(NH_4)_2S_2O_3$	60.00
Thiamine	0.01
Vitamin B12	0.01
Biotin	0.10
Glucose	500
IPTG	0,0047

The culture temperature was maintained constant at 37 °C and pH was maintained to the working value (6.8) by automatic addition of NH_4OH solutions (10 % and 28 %). The initial agitation rate was set at 200 RPM during the batch phase and was increased up to 1000 RPM during the fedbatch phase. The initial airflow rate was set at 40 $NL.h^{-1}$ during

the batch phase and was augmented to 100 $NL.h^{-1}$ at the beginning of the fedbatch phase. The dissolved oxygen concentration was maintained at values between 20 and 40%, preferentially 30% saturation by increasing the agitation.

IPTG was added in batch and fedbatch media when it was necessary at a final concentration of 20 µM. When it was needed, antibiotics were added at a concentration of 50 $mg.L^{-1}$ for spectinomycin, 30 $mg.L^{-1}$ for chloramphenicol, 50 $mg.mL^{-1}$ for kanamycin and 100 $mg.L^{-1}$ for ampicillin.

When the cell mass reached a concentration close to 5 $g.L^{-1}$, the fedbatch was started with an initial flow rate of 5 $mL.h^{-1}$. Feeding solution was injected with a sigmoid profile with an increasing flow rate that reached 24 $mL.h^{-1}$ after 25 hours. The precise feeding conditions were calculated by the equation: $Q(t) = p1 + \frac{p2}{1+e^{-p3(t-p4)}}$.

where Q(t) is the feeding flow rate in $mL.h^{-1}$ with pi = 1.80, p2 = 22.4, p3 = 0.27, p4 = 6.50. This flow rate was increased from 10 to 50 %, preferentially between 20 and 30 % throughout the entire culture.

After 25 hours fedbatch, feeding solution pump was stopped and culture was finalized after glucose exhaustion.

Extracellular amino acids were quantified by HPLC after OPA/Fmoc derivatization and other relevant metabolites were analyzed using HPLC with refractometric detection (organic acids and glucose) and GC-MS after silylation.

Impact of the combination of *metH, fldA, fpr* overexpression and *ygaZH* overexpression in *E. coli* was tested. The results are presented in Table 8.

Table 8: Maximal and final methionine yields and homolanthionine concentrations produced in fedbatch cultures by the different strains. The performances of the strains of interest, strains 5, 6 and 7 are compared to the reference strain 1 and were cultivated in same conditions. The symbol ~ indicates that there is no difference between the strains, the symbol + indicates an increase between 1 to 5 %, the symbol ++ indicates an increase between 5 to 10 % and the symbol +++ indicates an increase greater than 10%. For the definition of methionine/glucose yield see below.

Strain	**Strain 1**	**Strain 6**	**Strain 5**	**Strain 7**
Number of repetitions	n = 4	n = 1	n = 1	n = 2
Max methionine yield	reference	~	++	+++
Final methionine yield	reference	~	~	++
Homolanthionine (mM) Concentration at the final point	14.8	ND	3.6	2.5
MetH Specific activity (mUI/mg of protein)	230	230	1500	ND

These results show that in *E. coli,* the overexpression *oiygaZH* genes only is of no benefit to the production of methionine (strain 6). The overexexpression of the cobalamin-dependent methionine synthase system in *E. coli* (strain 5) leads to an improved production of methionine. Surprisingly, we observe that the combination of overexpression of the genes *ygaZH* and the cobalamin-dependent methionine synthase system has a synergistic effect on the methionine production leading to an unexpected increased production of methionine. Moreover this combination has also a favourable impact on the homolanthionine production leading to a methionine with better purity.

Determination of methionine/glucose yield (Y_{met})

The reactor volume was calculated by adding to the initial volume the amount of solutions added to regulate the pH and to feed the culture and by subtracting the volume used for sampling and lost by evaporation.

The fedbatch volume was followed continuously by weighing the feeding stock. The amount of injected glucose was then calculated on the basis of the injected weight, the density of the solution and the glucose concentration determined by the method of Brix ([Glucose]). The methionine yield was expressed as followed:

$$Y_{met} = \frac{Methionine_t * V_T - Methionine^{\wedge} * v_Q \times 100}{Consumed\ glu\cos e_t}$$

With Methionineo and $Methionine_t$ respectively the initial and final methionine concentrations and Vo and V_t the initial and the instant t volumes.

The consumed glucose was calculated as follows:

$$fed\ volume_t = \frac{fed\ weight_0}{density\ fed} - \frac{fed\ weight_t}{solution}$$

Injected $Glucose_t$ = fed $volume_t$ * [Glucose]

Consumed $glucose_t$ = [Glucose]o * Vo + Injected Glucose - $[Glucose]_{residuai}$ * V_t With [Glucose]o, [Glucose], $[Glucose]_{residuai}$ respectively the initial, the fed and the residual glucose concentrations.

Cobalamin-dependent methionine synthase activity assay

The cobalamin-dependent methionine activity assay is an adaptation of the assay described by Drummond *et al.,* in 1995.

The Cobalamin-dependent methionine synthase activity was assayed by measuring the product tetrahydrofolate (H4folate) concentration with a spectrophotometer at a wavelength of 350 nm and at a constant temperature of 37°C.

The reaction mixture was carried out in 80 mM potassium phosphate pH7.2, 20 mM DTT, 15 µM S-adenosylmethionine (SAM), 0.6 mM (6R,S)-5-Methyl-5,6,7,8-tetrahydrofolic acid, 40µM Hydroxocobalamin, O.lmM Zinc chloride and 8µg of crude extract in a final volume of 800µ1. The reaction mixture was incubated for 10 min at 37°C before to start the reaction by the addition of the substrate homocysteine at a final concentration of 0.8 mM. After 5 min at 37°C, 200 µï of acidic derivatization solution (4M HC1 in 60% formic acid) was added to quench the turnover bringing the volume to 1ml, and the tubes are heated at 80°C for 10 min. This step is necessary to stabilize the enzymatic product of the reaction, the tetrahydro folate which is not stable in acid. The heat leads to the formation of the methenyltetrahydro folate which absorbs light at 350 nm, while residual substrate 5-methyltetrahydro folate does not contribute to the absorbance at 350 nm. The reaction blank contained all components of the reaction mixture except the substrate homocysteine.

Quantification of the FldA and Fpr proteins levels

In order to quantify the two proteins, antibodies were generated against the flavodoxin-1 (fldA) and the flavodoxin reductase (fpr) (Antibodies from rabbit, Eurogentec) and used in Western blot experiments. Western blot detection was carried out using goat anti-rabbit AP. The proteins levels of FldA and Fpr on stained blots were quantified with a commercially available imaging system (Epson Expression 1680 professional) and compared in the different strains described in this patent.

EXAMPLE 6: Production of L-methionine by fermentation with *C. glutamicum* strains

Strains are cultivated in flask in the same conditions as described in patent application WO2009/ 144270.

Table 9: Methionine titers produced by *C. glutamicum* strains D, E, F, G and H compared to reference strain C. The symbol ~ indicates that there is no difference between the strains, the symbol + indicates an increase between 1 to 3 %, the symbol ++ indicates an increase greater than 3%.

Strain	Strain C	Strain F	Strain D	Strain E	Strain G	Strain H
Number of repetitions	n = 10	n = 2	n = 2	n = 2	n = 2	n = 2
Methionine Titer % compared to the strain	reference	~	+	~	++	+

Similarly to *E. coli,* in *C. glutamicum,* the combination of overexpression of the genes *brnFE* and the cobalamin-dependent methionine synthase system (from *E. coli* - strain H and from *C. glutamicum* - strain G) has a synergistic effect on the methionine production leading to an unexpected increased production of methionine.

EXAMPLE 7: Overproduction of the cobalamin-dependent methionine synthase and overproduction of homologous L-methionine secretion systems in an *E.coli* strain overproducer of L-methionine - Construction of strains 8 to 17

The *ygaZH* homologous genes from *Citrobacter* species, *Raoultella* species, *Shigella* species, *Enterobacter* species, *Yersinia* species and *Photorhabdus* species were overexpressed in genetic background of strain 5.

Before using strain 5, the antibiotic cassette of the chromosomal integration made at *ytfA* locus was removed using the Flp recombinase as described by Datsenko & Wanner, 2000 (according to Protocol 1). The kanamycin sensible transformants were selected and the absence of antibiotic cassette at *ytfA* locus was verified by a PCR analysis with appropriate oligonucleotides. The resulting strain was named strain 8.

Construction of strain 9 - Overproduction of the endogenous L-methionine secretion system, overexpression *ofygaZH* from *E. coli*

To compare the effect of the overexpression *ofygaZH* from *E. coli* and overexpression of *ygaZH* homologues in the same genetic background, the plasmid pME1247 carrying *ygaZH* from *E. coli* was transformed into strain 8, giving rise to strain 9.

Construction of strains 10 to 17 - Overproduction of homologous L-methionine secretion systems, overexpression *ofygaZH* from genus and species listed in table 10

To overexpress the *ygaZH* homologous genes listed in table 10, each couple of genes was cloned on the moderate copy number plasmid pCL1920 (Lerner & Inouye, 1990) with the use of the natural promoter and natural ribosome binding site of *E. coli ygaZ* gene as previously described for *E. coli ygaZH* genes, As specified in table 11, the *ygaZH* homologue genes were either amplified from genomic DNA of the corresponding strain or chemically synthesized, with or without optimizing the codon usage to *E. coli* (as proposed by GeneArt® Gene Synthesis service with GeneOptimizer® software - Lifetechnologies). The amplified DNA fragments comprising the *ygaZH* homologous genes are disclosed in SEQ ID indicated in the Table 11. The resulting plasmids were named as mentioned in table 11. Finally each plasmid was transformed into strain 8, giving the strains 10 to 17, as mentioned in table 11.

Table 10: YgaZH homologue proteins

Organism	ygaZ		ygaH	
	Acession Number	Name	Acession Number	Name
Citrobacter koseri	YP_001455539.1 NC_009792.1. ABV15103.1	hypothetical protein CKO_04031 [Citrobacter koseri ATCC BAA-895]	YP_001455540.1 ABV15104.1	hypothetical protein CKO_04032 [Citrobacter koseri ATCC BAA-895]
Shigella flexneri	WP_005122932.1 EIQ78635.1	membrane protein [Shigella flexneri]	WP_005122930.1 EIQ78634.1	branched-chain amino acid ABC transporter permease [Shigella flexneri]
Raoultella ornithinolytica	YP_007877063.1 AGJ89511.1 WP_015585890.1	hypothetical protein RORB6_24155 [Raoultella ornithinolytica B6]	YP_007877062.1 AGJ89510.1	L-valine exporter [Raoultella ornithinolytica B6]
Enterobacter sp.	YP_008107733.1 AGN85393.1 WP_020454909.1	membrane protein [Enterobacter sp. R4-368]	YP_008107734.1 WP_020454910.1 AGN85394.1	branched-chain amino acid ABC transporter permease [Enterobacter sp. R4-368]
Yersinia enterocolitica subsp. Enterocolitica	EKA28834.1 YWA314-01718	putative amino acid transporter [Yersinia enterocolitica subsp. enterocolitica WA-314]	EKA28833.1 ou YWA314-01713	hypothetical protein YE3239 [Yersinia enterocolitica subsp. Enterocolitica WA-314]
Photorhabdus luminescens subsp. Laumondii	NP_928590.1 CAE13573.1	hypothetical protein plu1279 [Photorhabdus luminescens subsp. laumondii TTO1]	NP_928589.1 CAE13572.1	hypothetical protein plu1278 [Photorhabdus luminescens subsp. laumondii TTO1]
Citrobacter youngae	WP_006687199.1 EFE06904.1	membrane protein [Citrobacter youngae] putative azaleucine resistance protein AzlC [Citrobacter youngae ATCC 29220]	WP_006687198.1 EFE06903.1	branched-chain amino acid ABC transporter permease [Citrobacter youngae]
Citrobacter freundii	WP_003839672.1	hypothetical protein [Citrobacter freundii]	WP_003037297.1	branched-chain amino acid ABC transporter permease [Citrobacter freundii]

Table 11: Plasmids and strains carrying *ygaZH* homologue genes

Microorganism	Chemical synthesis	Codon usage optimisation	SEQ ID N°	Plasmid name	Strain name
Citrobacter koseri	no	no	28	pME1277	Strain 10
Shigella flexneri	yes	no	29	pME1274	Strain 11
Raoultella ornithinolytica	yes	yes	30	pME1275	Strain 12
Enterobacter sp.	yes	yes	31	pME1283	Strain 13
Yersinia enterocolitica subsp. Enterocolitica	no	no	32	pME1287	Strain 14
Photorhabdus luminescens subsp. Laumondii	no	no	33	pME1281	Strain 15
Citrobacter youngae	yes	yes	34	pME1311	Strain 16
Citrobacter freundii	yes	yes	35	pME1307	Strain 17

EXAMPLE 8: Production of L-methionine by fermentation in flask experiments

Recombinant L-methionine producers overeproducing the cobalamin dependant methionine synthase MetH as well as different L-methionine secretion systems from various microorganisms (homologous to YgaZH from *E.coli)* were evaluated in small Erlenmeyer flasks.

Production strains were evaluated in small Erlenmeyer flasks. A 5.5 mL preculture was grown at 30°C for 21 hours in a mixed medium (10 % LB medium (Sigma 25 %) with 2.5 $g.L^{-1}$ glucose and 90 % minimal medium PCI). It was used to inoculate a 50 mL culture to an OD_{600} of 0.2 in medium PCI. Spectinomycin was added at a concentration of 50 $mg.L^{-1}$ and gentamycin at 10 $mg.L^{-1}$ when it was necessary. The temperature of the cultures was 37°C. When the culture had reached an OD_{600} of 5 to 7, extracellular amino acids were quantified by HPLC after OPA/Fmoc derivatization and other relevant metabolites were analyzed using HPLC with refractometric detection (organic acids and glucose) and GC-MS after silylation.

Table 12: Minimal medium composition (PCI)

Compound	Concentration ($g.L^{-1}$)
$ZnSO_4.7H_2O$	0.0040
$CuCl_2.2H_2O$	0.0020
$MnSO_4.H_2O$	0.0200
$CoCl_2.6H_2O$	0.0080
H_3BO_3	0.0010
$Na_2MoO_4.2H_2O$	0.0004
$MgSO_4.7H_2O$	1.00
Citric acid	6.00
$CaCl_2.2H_2O$	0.04
K_2HPO_4	8.00
Na_2HPO_4	2.00
$(NH_4)_2HPO_4$	8.00
NH_4Cl	0.13
NaOH 4M	Adjusted to pH 6.8
$FeSO_4.7H_2O$	0.04
Thiamine	0.01
Glucose	20.00
Ammonium thiosulfate	5.61
Vitamin B12	0.01
MOPS	20.00
IPTG	0.0048

Table 13: Methionine yield (Ymet) in g methionine / % g of glucose produced in flask culture by the strains of interest carrying overexpressions *ofygaZH* homologues genes as well as *metH*,*fldA* and*fpr* genes. For the precise definition of methionine/glucose yield see below. "n" indicates the number of repeats.

Strain	Y_{met}
Strain 8 (n= 2)	**16.0**
Strain 9 (*E.coli*) (n= 10)	**16.2**
Strain 10 *(C. koseri)* (n=4)	**18.4**
Strain 11 (*S.flexneri)* (n=1)	**16.6**
Strain 12 (*R. ornithinolytica)* (n=2)	**16.2**

Strain 13 (*Enterobacter sp.)* (n=2)	**18.8**
Strain 14 (*Y. enterocolitica subsp. Enterocolitica)* (n=2)	**16.3**
Strain 15 (*P. luminescens subsp. Laumondii)* (n=2)	**16.1**
Strain 16 (*C. youngae)* (n=2)	**18.1**
Strain 17 (*C. freundii)* (n=2)	**18.4**

As can be seen in table 13, overexpression of *ygaZH* homologous genes from various microorganisms in the L-methionine producer overexpressing *metH, fldA, fpr* genes, leads to equivalent or better performances than those obtained with strain 9 which overexpresses *ygaZH* from *E.coli.* The homologous L-methionine secretion systems from other microorganisms than *E. coli* can replace the endogenous proteins of the bacterium.
The homologous proteins YgaZH from *Citrobacter Koseri* (strain 10, Ymet=19,6g/g), *Citrobacter youngae* (strain 16, Ymet=19,6g/g), *Citrobacter freundii* (strain 17, Ymet=19,6g/g) and *Enterobacter sp.* (Strain 13, Ymet=19,4g/g) showed the best L-methionine yields of production compared to strain 9 (Ymet=18.7g/g).

The methionine yield was expressed as followed:

$$Y_{met} = \frac{methionine\ (g)}{consummed\ glucose\ (g)} * 100$$

REFERENCES

- **Anderson,** 1946, *Proc. Natl. Acad. Sci.* USA **32**:120-128.
- **Banerjee R.V., Harder S.R., Ragsdale S.W., Matthews R.G.,** 1990, *Biochemistry,* **29:** 1129-1 135
- **Carrier T., Keasling J.D.,** 1999, *Biotechnology Progress,* **15**:58-64
- **Datsenko K.A., Wanner B.L.,** 2000, *Proceedings of the National Academy of Sciences of the USA,* **97**:6640-6645
- **Drummond J.T., Jarrett J., Gonzalez J.C., Huang S., Matthews R.G.,** 1995, *Analytical Biochemistry,* **228(2)**:323-329.
- **Eikmanns B.J., Kleinertz E., Liebl W., Sahm H.,** 1991, *Gene,* **102**:93-98
- **Foster M.A., Jones K.M., Woods D.D.,** 1961, *BiochemicalJournal,* **80**:519-531
- **Fujii K. and Huennekens F.M.,** 1974, *Journal of Biological Chemistry,* **249 (21)**:6745-6753
- **Gonzalez J.C., Banerjee R.V., Huang S., Summer J.S., Matthews R.G.,** 1992, *Biochemistry,* **31**:6045-6056
- **Lerner C.G. and Inouye M.,** 1990, *Nucleic Acids Research,* **18(15)**:463 1
- **Liebl *et al.,*** 1989, *Appl. Microbiol. Biotechnol.* **32:** 205-210.
- **Matthews R.G.,** 2001, *Accounts of Chemical Research,* **34**:681-689
- **Miller,** *1992; "A Short Course in Bacterial Genetics: A Laboratory Manual and Handbook for Escherichia coli and Related Bacteria", Cold Spring Harbor Laboratory Press, Cold Spring Harbor, New York.*
- **Riedel *et al,*** *2001, J. Mol. Microbiol. Biotechnol. 3: 573-583.*
- **Saunderson C.L.,** 1985, *British Journal of Nutrition,* **54**:621-633
- **Schaefer *et al.*** 1999, *Anal. Biochem.* **270:** 88-96.
- **Trotschel C , Deutenberg D., Bathe B., Burkovski A., Kramer R.,** 2005, *Journal of Bacteriology.* **187(ll)**:3786-3794
- **Wan J.T. and Jarrett J.T.,** 2002, *Archives of Biochemistry and Biophysics,* **406**: 116-126

CLAIMS

1. A recombinant microorganism optimised for the fermentative production of methionine and/or its derivatives, wherein in said recombinant microorganism, the expression of *metH* from *E. coli,* and optionally the expression of the genes *fldA* and *fpr* from *E. coli* or their homologous genes from *C. glutamicum* are enhanced and the genes *ygaZH* from *E. coli,* or the genes *brnFE* from *C. glutamicum* or their homologous genes are overexpressed.

2. The recombinant microorganism of claim 1, wherein said genes *metH, fldA* and *fpr* or their homologous genes from *C. glutamicum* are overexpressed chromosomally.

3. The recombinant microorganism of anyone of claims 1 or 2, wherein said *ygaZH* homologous genes are chosen among the group consisting in homologous genes from *Citrobacter* species, *Shigella* species, *Raoultella* species, *Enterobacter* species, *Yersinia* species and *Photorhabdus* species.

4. The recombinant microorganism of claim 3, wherein *ygaZH* homologous genes originate from *Citrobacter koseri, Shigella flexneri, Raoultella ornithinolytica, Enterobacter sp., Yersinia enterocolitica, Photorhabdus luminescens, Citrobacter youngae* or *Citrobacterfreundii.*

5. The recombinant microorganism of claim 1 to 4, wherein said *ygaZH* or *brnFE* or homologous genes are expressed under control of inducible promoter.

6. The recombinant microorganism of anyone of claims 1 to 5, wherein the expression of at least one of the following genes is also increased: *ptsG, pyc, pntAB, cysP, cysll, cysW, cysA, cysM, cysJ, cysl, cysH, gcvT, gcvH, gcvP, Ipd, serA, serB, serC, cysE, metF, metA, metA* * allele encoding for an enzyme with reduced feed-back sensitivity to S-adenosylmethionine and/or methionine, *thrA,* or a *thrA** allele encoding for an enzyme with reduced feed-back inhibition to threonine.

7. The recombinant microorganism of claim 6, wherein at least one of said genes is under the control of an inducible promoter.

8. The recombinant microorganism of anyone of claims 1 to 7, wherein the expression of at least one of the following genes is also attenuated: *metJ, pykA, pykF, purU, ybdL, yncA, metE, dgsA, metN, metl, metQ* or *udhA.*

9. The recombinant microorganism of anyone of claims 1 to 8, wherein:

a. said genes *metH, and fldA* and *fpr* or their homologous genes from C. *glutamicum* are overexpressed,

b. said genes *ygaZ* and *ygaH* or the genes *brnF* and *brnE* or their homologous genes originate from *Citrobacter koseri, Shigella flexneri, Raoultella ornithinolytica, Enterobacter sp., Yersinia enterocolitica, Photorhabdus luminescens, Citrobacter youngae* or *Citrobacter freundii* are overexpressed,

c. the expression of the genes *metA*, cysPUWAM, cysJIH, gcvTHP, metF, serA, serB, serC, cysE, thrA *, ptsG* and *pyc* are enhanced; and

d. the expression of the genes *metJ, pykA, pykF, purll, dgsA, metE* and *yncA* are attenuated.

10. A method for optimizing the fermentative production of methionine and/or its derivatives comprising the steps of:

a. culturing a recombinant microorganism wherein in said microorganism, the expression of *metH* from *E.coli,* and optionally the expression of the genes *fldA* and*fpr* from *E. coli* or their homologous genes from *C. glutamicum* are enhanced and the genes *ygaZH* from *E. coli,* or *brnFE* from *C. glutamicum* or their homologous genes are overexpressed, in an appropriate culture medium comprising a fermentable source of carbon and a source of sulphur, and

b. recovering methionine and/or its derivatives from the culture medium.

11. The method of claim 10, wherein said *ygaZH* homologous genes are chosen among the group consisting in homologous genes from *Citrobacter* species, *Shigella* species, *Raoultella* species, *Enterobacter* species, *Yersinia* species and *Photorhabdus* species.

12. The method of claim 11, wherein said *ygaZH* homologous genes originate from *Citrobacter koseri, Shigella flexneri, Raoultella ornithinolytica, Enterobacter sp., Yersinia enterocolitica, Photorhabdus luminescens, Citrobacter youngae* or *Citrobacterfreundii.*

13. The method of anyone of claims 10 to 12 wherein growth of the recombinant microorganism is subjected to limitation or deficiency for one or several inorganic substrate(s), in particular phosphate and/or potassium, in the culture medium.

14. The method of anyone of claims 10 to 13, wherein the step of recovering methionine and/or its derivatives comprises a step of concentration of methionine and/or its derivatives in the fermentation broth.

15. The recombinant microorganism of anyone of claims 1 to 9 or the method of anyone of claims 10 to 14, wherein said recombinant microorganism is chosen among *Enterobacteriaceae* or *Corynebacteriaceae,* preferably among *Escherichia coli* or *Corymb acterium glutamicum.*

INTERNATIONAL SEARCH REPORT

International application No
PCT/EP2014/068539

A. CLASSIFICATION OF SUBJECT MATTER
INV. C12P13/12 C12N9/1007
ADD.

According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)
C12P C12N

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practicable, search terms used)

EPO-Internal , EMBASE, WPI Data

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
Y	WO 2008/127240 Al (CARGI LL INC [US] ; CJ CORP [KR] ; BRAZEAU BRIAN [US] ; CHANG JIN-S00K [KR) 23 October 2008 (2008-10-23) exampl e 4	1-15
Y	W0 2009/144270 Al (EV0NI K DEGUSSA GMBH [DE] ; ZELDER 0SKAR [DE] ; SCHROEDER HARTWIG [DE] ; K) 3 December 2009 (2009-12-03) page 14, l i n e 23 - page 19, l i n e 10	1-15
Y	US 2012/190084 Al (SCHNEIDER FRANK [DE] ET AL) 26 July 2012 (2012-07-26) paragraph [0107] - paragraph [0204] paragraphs [0217] , [0224]	1-15

☐ Further documents are listed in the continuation of Box C.

☒ See patent family annex.

* Special categories of cited documents :

"A" document defining the general state of the art which is not considered to be of particular relevance

"E" earlier application or patent but published on or after the international filing date

"L" documentwhich may throw doubts on priority claim(s) orwhich is cited to establish the publication date of another citation or other special reason (as specified)

"O" document referring to an oral disclosure, use, exhibition or other means

"P" document published prior to the international filing date but later than the priority date claimed

"T" later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention

"X" document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone

"Y" document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art

"&" document member of the same patent family

Date of the actual completion of the international search	Date of mailing of the international search report
19 November 2014	01/12/2014

Name and mailing address of the ISA/	Authorized officer
European Patent Office, P.B. 5818 Patentlaan 2 NL - 2280 HV Rijswijk Tel. (+31-70) 340-2040, Fax: (+31-70) 340-3016	Spri nks , Matthew

Form PCT/ISA/210 (second sheet) (April 2005)

INTERNATIONAL SEARCH REPORT

Information on patent family members

International application No
PCT/EP2014/068539

Patent document cited in search report	Publication date	Patent family member(s)	Publication date
WO 2008127240 A1	23-10-2008	CN 101688190 A	31-03-2010
		EP 2132306 A1	16-12-2009
		EP 2520645 A2	07-11-2012
		EP 2808381 A1	03-12-2014
		EP 2808382 A1	03-12-2014
		ES 2400261 T3	08-04-2013
		JP 5497628 B2	21-05-2014
		JP 2010523145 A	15-07-2010
		KR 20100019417 A	18-02-2010
		US 2011183383 A1	28-07-2011
		US 2013260424 A1	03-10-2013
		WO 2008127240 A1	23-10-2008
WO 2009144270 A1	03-12-2009	CN 102149823 A	10-08-2011
		EP 2281054 A1	09-02-2011
		US 2009311756 A1	17-12-2009
		WO 2009144270 A1	03-12-2009
US 2012190084 A1	26-07-2012	CN 103328644 A	25-09-2013
		EP 2479279 A1	25-07-2012
		EP 2665826 A1	27-11-2013
		JP 2014504875 A	27-02-2014
		US 2012190084 A1	26-07-2012
		WO 2012098042 A1	26-07-2012

Form PCT/ISA/210 (patent family annex) (April 2005)

US008338141B2

(12) **United States Patent**
Lorbert et al.

(10) **Patent No.: US 8,338,141 B2**
(45) **Date of Patent: Dec. 25, 2012**

(54) **METHIONINE RECOVERY PROCESSES**

(75) Inventors: **Steve Lorbert**, St. Louis, MO (US); **Jennifer Wu**, St. Charles, MO (US); **Farooq Uraizee**, Valley Park, MO (US); **Charles Steven Schasteen**, St. Louis, MO (US)

(73) Assignee: **Novus International, Inc.**, St. Charles, MO (US)

(*) Notice: Subject to any disclaimer, the term of this patent is extended or adjusted under 35 U.S.C. 154(b) by 1786 days.

(21) Appl. No.: **10/886,863**

(22) Filed: **Jul. 8, 2004**

(65) **Prior Publication Data**

US 2005/0089975 A1 Apr. 28, 2005

Related U.S. Application Data

(60) Provisional application No. 60/485,564, filed on Jul. 8, 2003, provisional application No. 60/485,565, filed on Jul. 8, 2003.

(51) **Int. Cl.**
C12P 13/12 (2006.01)
A61K 31/198 (2006.01)

(52) **U.S. Cl.** **435/113**; 514/562

(58) **Field of Classification Search** 435/113; 514/562

See application file for complete search history.

(56) **References Cited**

U.S. PATENT DOCUMENTS

2,471,053	A		5/1949	Almquist
3,139,386	A		6/1964	Shigeshi
3,205,261	A		9/1965	Asano et al.
3,219,543	A		11/1965	Douros
3,729,381	A		4/1973	Nakayama
4,148,688	A	*	4/1979	Yamada et al. 435/113
4,278,765	A		7/1981	Debabov
4,418,688	A		12/1983	Loeb
4,601,893	A		7/1986	Cardinal
4,827,029	A		5/1989	Kleemann
4,837,371	A		6/1989	Ogawa
5,215,897	A		6/1993	Sakashita
5,268,293	A		12/1993	Oh
5,431,933	A		7/1995	Binder
5,463,120	A		10/1995	Giraud
5,622,710	A		4/1997	Binder
5,714,355	A	*	2/1998	Wagner et al. 435/106
5,840,358	A		11/1998	Hofler
5,840,551	A		11/1998	Werning
6,040,160	A		3/2000	Kojima
6,379,934	B1		4/2002	Tilg
6,656,710	B2		12/2003	Bommarius
6,673,942	B1		1/2004	Kottenhahn
2002/0028490	A1		3/2002	Molenaar
2002/0048793	A1		4/2002	Bathe
2002/0049305	A1		4/2002	Bathe
2002/0102664	A1		8/2002	Bathe
2002/0110877	A1		8/2002	Bathe
2002/0110878	A1		8/2002	Moeckel
2002/0142405	A1		10/2002	Tilg
2003/0045753	A1		3/2003	Ponceblanc
2003/0049803	A1		3/2003	Rieping
2003/0054503	A1		3/2003	Rieping
2003/0059903	A1		3/2003	Rieping
2003/0092026	A1		5/2003	Rey

FOREIGN PATENT DOCUMENTS

EP	0472869	A3	7/1991
EP	0472869	B1	7/1991
WO	WO09615246	A1	11/1995

OTHER PUBLICATIONS

International Search Report of PCT/US04/21756.
CRC Handbook of Chemistry & Physics 51st Ed. 1970-1971. pp. C-741 and C-743.
http://www.formedium.com/USA/amino acids-and vitamins/htm#L-Methionine.
Examination Report dated Aug. 27, 2012 (received from Foreign associate on Sep. 6, 2012) from related EP Application No. 04 777 690.1-1521, 5 pages.

* cited by examiner

Primary Examiner Herbert J Lilling

(57) **ABSTRACT**

The present invention relates to a method of making a methionine preparation, for example for an animal feed additive. The invention also related to methods for increasing the solubility of a methionine preparation.

10 Claims, 1 Drawing Sheet

pK and pI Values at 25°C and Solubility of Amino Acids[a]

Amino acid	pK_1 (COOH)[b]	pK_2 (NH_3^+)[b]	pK_3 (NH_3^+)[b]	pK_4 (NH_3^+)	pI	Solubility, g in 100 g of water				
						0°C	25°C	50°C	75°C	100°C
Divalent										
glycine	2.34	9.60			5.97	14.18	24.99	39.10	54.39	67.17
alanine	2.34	9.69			6.00	12.11	16.72	23.00	31.89	44.04
L-valine	2.32	9.62			5.96	8.34	8.85	9.62	10.24	
L-leucine	2.36	9.60			5.98	2.27	2.33 (35°C)	2.66	3.82	5.64
L-isoleucine	2.26	9.62			5.94	3.79	4.12	4.82	6.08	8.26
serine	2.21	9.15			5.68	2.20	5.02	10.34	19.21	32.24
L-threonine	2.15	9.12			5.64		freely soluble in water			
L-proline	1.99	10.60			6.30	127.4	162.3	206.7	239.0 (65°C)	
L-hydroxyproline	1.82	9.65			5.74	28.86	36.11	45.18	56.67 (65°C)	
L-phenylalanine	1.83	9.13			5.48	1.98	2.97	4.43	6.62	9.90
L-tryptophan	2.38	9.39			5.89	0.82	1.14	1.71	2.80	4.99
DL-methionine	2.28	9.21			5.74	1.82	3.38	6.07	10.52	17.60
Trivalent										
L-aspartic acid	1.88	3.65 (COOH)	9.60		2.77	0.21	0.50	1.20	2.88	6.89
L-glutamic acid	2.19	4.25 (COOH)	9.67		3.22	0.34	0.84	2.19	5.53	14.00
L-tyrosine	2.20	9.11	10.07 (OH)		5.66	0.02	0.06	0.11	0.24	0.57
L-cysteine	1.71	8.33 (?)	10.78 (?)				freely soluble in water			
L-histidine	1.78	5.97 (Im)[c]	8.97		7.47		4.29			
L-arginine	2.18	9.09	13.2 (Guan)[d]		11.15	the said aq soln contains 15% (wt/wt), at 21°C				
L-lysine	2.20	8.90	10.28		9.59	very freely soluble in water				
Tetravalent										
L-cystine	<1	2.1 (COOH)	8.02	8.71	5.03	0.005	0.011	0.024	0.052	0.114

[a] Refs. 18–19.
[b] Unless otherwise stated.
[c] Im = imidazoyl group.
[d] Guan = guanidino group.

383

METHIONINE RECOVERY PROCESSES

RELATED APPLICATIONS

This application claims the benefit of U.S. Provisional Patent Application Ser. No. 60/485,564, filed Jul. 8, 2003, and U.S. Provisional Patent Application Ser. No. 60/485,565, filed Jul. 8. 2003.

FIELD OF THE INVENTION

The present invention relates to an improved method of producing a methionine preparation.

BACKGROUND OF THE INVENTION

Methionine is a sulfur-containing amino acid which is essential in the nutrition of animals, and is often used as a feed additive to animals including poultry, pigs, cows, fish, equine species and even companion animals like dogs and cats. Historically, the methionine used for animal nutrition has been the racemic mixture of D and L methionine. Methionine is unusual in that most animals can utilize both the D and L forms of the amino acid. For all other essential amino acids, only the L form of the amino acid has nutritive value. Some specific animal studies have been completed which show benefits for feeding L-methionine as opposed to the racemic mixture. Faster absorption and better utilization in the muscle has been shown for some species under some selected feeding conditions. There are some specific applications outside of animal nutrition where the use of L-methionine is preferred. For example. L-methionine has known uses in human medicine and in the pharmaceutical industry. It is useful as a lipotropic agent and for the treatment of liver disease in animals. L-methionine and L-methionine derivatives are required for manufacturing therapeutic peptides. which are synthesized from single amino acids. Unfortunately. the production of the single isomer, L-methionine, is much more difficult and expensive as compared to producing the racemic mixture. Therefore. it would be very beneficial to establish a relatively inexpensive, industrial process for the production of L-methionine for human healthcare which also could be used for animal nutrition.

Several methods have been available for the production of L-methionine. For example. there is a process for the production of L-methionine by optically resolving DL-methionine prepared by a synthetic method (Pokorny et al., 1970, Phytochemistry 9:2175). Commercial production of L-methionine using acylase catalyzed cleavage of N-acetyl-D,L methionine is well known (see. for example. U.S. Pat. No. 4.827,029 and U.S. Pat. No. 6.656,710). These processes are fairly complex and therefore add significant additional cost in separating L-methionine from the racemic mixture. Processes based on selective crystallization are also known (see U.S. Pat. No. 6,673.942). It is also known to produce L-methionine by hydrolyzing proteins. Additionally, it is known to produce L-methionine by a microbiological process (e.g., fermentation).

There has been much published regarding the development of bacterial and yeast strains for L-methionine production. It is well known that L-methionine synthesis is tightly regulated in microorganisms. Consequently, the productivity of these microorganisms has been low with respect to methionine production. Kase and Nakayama isolated *Coryneform* mutants capable of producing 2 g methionine/liter (Agr Biol. Chem., 39(1), 153-160, 1975). Gomes and coworkers have also used classical mutagenesis techniques to isolate methionine analog resistant mutants of *Corynebacterium lilium*. Production of methionine by their isolate was shown to be much improved over the wild type starting strain. but much below commercial titres typically seen for other amino acids like lysine which is produced by fermentation. Commercial lysine fermentations typically reach titres approaching 100 g lysine/liter (see U.S. Pat. No. 5,268.293).

More recently. it has been reported by Moeckel, et al. in U.S. patent application No. 2002/0110878 that L-methionine production can be improved dramatically through the amplification of key genes in the methionine pathway. The expression of native *Coryneform* metA and metY genes was improved through the use of a specially constructed plasmid system. Shake flask fermentations of this strain reached a final methionine concentration of 16.0 grams of methionine/liter (g/L). Modification of other genes in the pathway in combination with the improvements in metA and metY should result in strains with even higher productivities. Using larger commercial fermentation systems which can support higher cell densities, these highly productive methionine-producing strains should be capable of producing methionine at high titre. Development of high methionine producing strains of *E. coli* is also being investigated. (See JP2000-139471 and 157267).

Because of the low solubility of methionine under normal fermentation conditions. its separation from whole cells and other fermentation broth component is a major issue which needs to be solved to be able to produce L-methionine economically. Typically, a neutral pH is preferred for the production of L-amino acids. For example, U.S. Pat. No. 3,729,381 teaches that a neutral pH is preferred to obtain high yield of L-methionine by fermentation (e.g., claim 3, and column 3, lines 28-31). U.S. Pat. No. 5,840,551 also teaches a method of producing L-amino acids by fermentation using neutral pH (e.g., see Example 1). The preferred fermentation temperature for organisms like *Corynebacteria* and *E. coli* is in the range of 30-37° C. Because of L-methionine's low solubility, both soluble and insoluble methionine fractions would exist in the broth. An effective separations process is needed to produce purified L-methionine from fermentation broth.

SUMMARY OF THE INVENTION

The present invention provides methods for recovering purified L-methionine from fermentation broth. The purification strategies rely on methods for increasing the solubility of an L-methionine preparation, so that the it can subsequently be separated from whole cells and other fermentation broth solids. The L-methionine which has been solubilized can then be selectively crystallized to separate it from the more soluble components in the fermentation broth. Methionine solubility is manipulated to make a purified methionine end product which can be dried and granulated for use in the animal feed sector.

The methods of the present invention. which include methods comprising adjusting the pH of the methionine to an acidic or basic pH, and/or increasing the temperature of the methionine preparation to at least 40° C., are useful for increasing the recovery of L-methionine. L-methionine has limited aqueous solubility, resulting in loss of significant amounts as insoluble material from high methionine titre fermentation broths. The present invention provides simple, cost-effective methods for maximizing the recovery of L-methionine in making a methionine preparation from a fermentation broth.

In one aspect, the invention provides a method of increasing the solubility of a methionine preparation comprising

adding an acid or a base into the methionine preparation. If an acid is added. sulfuric acid, hydrochloric acid. phosphoric acid, nitric acid or 2-hydroxy-4-(methylthio)butanoic acid can be used. In one embodiment, adding the acid decreases the pH of the methionine preparation to between pH 1.5-3. Alternatively, ammonium hydroxide, sodium hydroxide or potassium hydroxide can be used as the base in the method. In one embodiment. adding the base increases the pH of the methionine preparation to a pH 8.5 or above.

The method according to this aspect can further comprise increasing the temperature to at least 40° C. The temperature can be further increased to at least 50° C., typically at least 60° C. or preferably at least 70° C.

In another aspect, the invention provides a method of increasing the solubility of a methionine preparation comprising increasing temperature of the methionine preparation to at least 40° C. The temperature can also be increased to at least 50° C., typically at least 60° C. In one embodiment, the temperature is increased to at least 70° C.

The method according to this aspect can further comprise adding an acid or base to the methionine preparation. The acid can be sulfuric acid, hydrochloric acid, phosphoric acid, nitric acid or 2-hydroxy-4-(methylthio)butanoic acid. In one embodiment, adding the acid decreases the pH of the methionine preparation to between pH 1.5-3. The base can be ammonium hydroxide. sodium hydroxide or potassium hydroxide. In one embodiment, adding the base increases the pH of the methionine preparation to a pH above pH 8.5.

In another aspect of this invention, a method of making a methionine preparation is provided. comprising the following steps:

(a) culturing a methionine-producing microorganism in a fermentation medium to yield a fermentation broth;
(b) solubilizing methionine in the fermentation broth by addition of an acid to lower the pH to 3.5 or below. or a base to raise the pH to 8.5 or above;
(c) removing insoluble material from the fermentation broth to yield a clarified broth;
(d) crystallizing methionine from the clarified broth; and,
(e) isolating the methionine crystals to produce a methionine preparation.

According to one embodiment, the temperature of the fermentation broth can be raised to further increase the solubility of methionine prior to removal of the insoluble material. In one embodiment. the temperature is raised to at least 40° C. In another embodiment, the temperature is raised to at least 50° C. In a preferred embodiment, the temperature is raised to at least 60° C. In a most preferred embodiment, the temperature is raised to at least 70° C.

If an acid is added. sulfuric acid, hydrochloric acid. phosphoric acid, nitric acid or 2-hydroxy-4-(methylthio)butanoic acid can be used. Alternatively, ammonium hydroxide, sodium hydroxide or potassium hydroxide can be used as the base in the method.

The insoluble material can be removed from the fermentation broth by filtration or centrifugation. Upon removal of insoluble material, the clarified broth can optionally be concentrated. Once removed of insoluble material. the methionine in the clarified broth can be crystallized by reducing the temperature to below 10° C., preferably at or below 4° C., and by adjusting the pH to between pH 5.5 and 6. The methionine preparation can be dried and optionally granulated for use.

In still another aspect. the invention provides for a method of making a methionine preparation comprising:

(a) culturing a methionine-producing microorganism in a fermentation medium to yield a fermentation broth;
(b) solubilizing methionine in the fermentation broth by raising the temperature of the broth to at least 40° C.;
(c) removing insoluble material from the fermentation broth to yield a methionine-enriched clarified broth;
(d) crystallizing methionine from the clarified broth; and,
(e) isolating the methionine crystals to produce a methionine preparation.

In one embodiment. the temperature of broth is raised to at least 50° C. In another embodiment, the temperature is raised to at least 60° C. In still another embodiment, the temperature is raised to at least 70° C.

To further solubilize methionine, the method can further comprise raising the pH of the broth to at least 8.5, or lowering the pH to 3.5 or below. The pH can be lowered by addition of an acid. In one embodiment, the acid can be selected from the group consisting of 2-hydroxy-4-(methylthio)butanoic acid, hydrochloric acid, sulfuric acid, phosphoric acid and nitric acid. Alternatively, the pH can be raised to at least 8.5 by addition of a base. The base can be selected from the group consisting of ammonium hydroxide, sodium hydroxide and potassium hydroxide.

The clarified broth produced in this aspect can be further dried to yield a dried methionine preparation.

In another aspect, the invention provides for a method of making a methionine preparation comprising:

(a) culturing a methionine-producing microorganism in a fermentation medium to produce a fermentation broth:
(b) separating methionine-enriched insoluble material from the fermentation broth;
(c) solubilizing methionine from the methionine-enriched insoluble material by addition of an acid to lower the pH to 3.5 or below, or a base to raise the pH to 8.5 or above to produce a methionine-enriched broth:
(d) removing insoluble material to yield a methionine-enriched clarified broth;
(e) optionally combining the methionine-enriched clarified broth from step d with the soluble methionine fraction from step b: and,
(e) crystallizing methionine from the methionine-enriched fractions; and,
(f) isolating the methionine crystals to produce a methionine preparation.

The insoluble material can be collected by centrifugation or filtration. Once collected. the insoluble material can be resuspended in solution before addition of an acid or base. According to one embodiment, the temperature can be raised to at least 40° C. in order to further increase the solubility of methionine. In another embodiment. the temperature is raised to at least 50° C. In a preferred embodiment. the temperature is raised to at least 60° C. In a most preferred embodiment, the temperature is raised to at least 70° C.

If an acid is added, sulfuric acid, hydrochloric acid. phosphoric acid, nitric acid or 2-hydroxy-4-(methylthio)butanoic acid can be used. Alternatively, ammonium hydroxide, sodium hydroxide or potassium hydroxide can be used as the base in the method.

Once the methionine has been solubilized. the remaining insoluble material can be removed by filtration or centrifugation. Upon removal of insoluble material, the clarified broth can be concentrated. Once removed of insoluble material, methionine can be purified from the clarified broth by crystallization. Crystallization can be performed by reducing the temperature to below 10° C.. preferably at or below 4° C.. and by adjusting the pH to between pH 5.5 and 6. The methionine preparation can be dried and optionally granulated for use.

In still another aspect, the invention provides a method of making a methionine preparation comprising the following steps:

a) culturing a methionine-producing microorganism in a fermentation medium, wherein the pH of the fermentation medium is adjusted to an acidic pH or basic pH;

b) obtaining a methionine-containing fermentation broth from the culturing; and

c) concentrating the methionine-containing fermentation broth to produce a methionine preparation.

The pH can be adjusted to between pH 1.5 and pH 3. In a particular embodiment, the pH is adjusted by adding sulfuric acid, hydrochloric acid, phosphoric acid, nitric acid or 2-hydroxy-4-(methylthio)butanoic acid. Alternatively, the pH can be adjusted to 8.5 or above. Ammonium hydroxide, sodium hydroxide or potassium hydroxide can be added to adjust the pH.

The method according to this aspect can further comprise adding 2-hydroxy-4-)methylthio)butanoic acid to the fermentation medium and/or to the fermentation broth.

The fermentation broth can be further dried to obtain an animal feed additive in the desired powder or granule form. The methionine preparation can also be further dried to obtain a dried methionine preparation.

In one embodiment, the pH of the methionine preparation is adjusted to between pH 7.5-12 before drying. Alternatively, the pH of the methionine preparation is adjusted to pH 9-11 before drying. Ammonium stripping and recrystallization can additionally be performed in this method.

The pH of the methionine preparation can additionally be adjusted to between pH 2.5-7 before drying. In another embodiment, the pH of the methionine preparation is adjusted to pH 4-7 before drying. In yet another embodiment, the pH of the methionine preparation is adjusted to between pH 5-7 before drying.

In another aspect, the invention provides a method of making a methionine preparation comprising the following steps:

a) culturing a methionine-producing microorganism in a fermentation medium, wherein the pH of the fermentation medium is adjusted:

b) obtaining a methionine-containing fermentation broth from the culturing; and

c) removing biomass from the methionine-containing fermentation broth to produce a methionine preparation.

The pH can be adjusted to between pH 1.5 and pH 3. In a particular embodiment, the pH is adjusted by adding sulfuric acid, hydrochloric acid, phosphoric acid, nitric acid or 2-hydroxy-4-(methylthio)butanoic acid. Alternatively, the pH can be adjusted to between pH 8 and pH 10. Ammonium hydroxide, sodium hydroxide or potassium hydroxide can be added to adjust the pH.

The method according to this aspect can further comprise adding 2-hydroxy-4-methylthiobutanoc acid to the fermentation medium and/or to the fermentation broth.

In yet another embodiment, the method further comprises drying the fermentation broth to obtain an animal feed additive in the desired powder or granule form. The methionine preparation can be further dried to obtain a dried methionine preparation.

The pH of the methionine preparation can be further adjusted to between pH 7.5-12 before drying. In one embodiment, the pH of the methionine preparation is adjusted to between pH 9-11 before drying. In another embodiment, the method further comprises ammonium stripping and crystallization before drying.

Alternatively, the pH of the methionine preparation can be adjusted to pH between 2.5-7 before drying. In one embodiment, the pH of the methionine preparation is adjusted to between pH 4-7 before drying. In another embodiment, the pH of the methionine preparation is adjusted to between pH 5-7 before drying.

In another aspect, the invention provides a method of making a methionine preparation comprising the following steps:

a) culturing a methionine-producing microorganism in a fermentation medium;

b) obtaining a methionine-containing fermentation broth from the culturing, wherein the pH of the fermentation broth is adjusted; and

c) concentrating the methionine-containing fermentation broth to produce a methionine preparation.

The pH can be adjusted to between pH 1.5 and pH 3. In a particular embodiment, the pH is adjusted by adding sulfuric acid, hydrochloric acid, phosphoric acid, nitric acid or 2-hydroxy-4-(methylthio)butanoic acid. Alternatively, the pH can be adjusted to between pH 8 and pH 10. Ammonium hydroxide, sodium hydroxide or potassium hydroxide can be added to adjust the pH.

2-hydroxy-4-(methylthio)butanoic acid can be added to the fermentation medium and/or to the fermentation broth.

In yet another embodiment, the method further comprises drying the fermentation broth to obtain an animal feed additive in the desired powder or granule form. The methionine preparation can be further dried to obtain a dried methionine preparation.

The pH of the methionine preparation can be adjusted to between pH 7.5-12 before drying. In one embodiment, the pH of the methionine preparation is adjusted to between pH 9-11 before drying.

In still another embodiment, the method further comprises ammonium stripping and crystallization before drying.

Alternatively, the pH of the methionine preparation can be adjusted to between pH 2.5-7 before drying. In one embodiment, the pH of the methionine preparation is adjusted to between pH 4-7 before drying. In another embodiment, the pH of the methionine preparation is adjusted to between pH 5-7 before drying.

In yet another aspect, the invention provides a method of making a methionine preparation comprising the following steps:

a) culturing a methionine-producing microorganism in a fermentation medium;

b) obtaining a methionine-containing fermentation broth from the culturing, wherein the pH of the fermentation broth is adjusted; and

c) removing from the methionine-containing fermentation broth to produce a methionine preparation.

In one embodiment, the method further comprises adding 2-hydroxy-4-(methylthio)butanoic acid to the fermentation medium. Alternatively, 2-hydroxy-4-(methylthio)butanoic acid can be added to the fermentation broth.

In one embodiment, the pH is adjusted to between pH 1.5 and pH 3. The pH can be adjusted by adding sulfuric acid, hydrochloric acid, phosphoric acid, nitric acid or 2-hydroxy-4-(methylthio)butanoic acid.

In another embodiment, the pH is adjusted to between pH 8 and pH 10. The pH can be adjusted by adding ammonium hydroxide, sodium hydroxide or potassium hydroxide.

In yet another aspect, the invention provides for a method of producing a feed additive, comprising:

(a) culturing a methionine-producing microorganism in a fermentation medium to produce a fermentation broth; and,

(b) drying the fermentation broth to obtain an animal feed additive in the desired powder or granule form.

The methionine preparation can be further dried to obtain a dried methionine preparation.

The pH of the methionine preparation can be adjusted to between pH 7.5-12 before drying. In one embodiment, the pH of the methionine preparation is adjusted to between pH 9-11 before drying. The method can further comprise ammonium stripping and crystallization before drying.

Alternatively, the pH of the methionine preparation can be adjusted to between pH 2.5-7 before drying. In one embodiment, the pH of the methionine preparation is adjusted to between pH 4-7 before drying. In another embodiment, the pH of the methionine preparation is adjusted to between pH 5-7 before drying.

In another aspect, the invention provides an acidified fermentation broth comprising methionine. The acidified fermentation broth has a pH of 1 to 5, typically a pH of between 1 to 4. In another embodiment, the acidified fermentation broth has a pH of 1 to 3. In a particular embodiment, the acidified fermentation broth has a pH of 1.5 to 3. The acidified fermentation broth can further comprise 2-hydroxy-4-(methylthio)butanoic acid.

BRIEF DESCRIPTION OF DRAWINGS

FIG. 1 is a table showing the solubility of various amino acids.

DETAILED DESCRIPTION OF THE INVENTION

DEFINITIONS

The term "amino acid preparation" refers to a preparation of L-amino acids including L-asparagine, L-threonine, L-serine, L-glutamate, L-glycine, L-alanine, L-cysteine, L-valine, L-methionine, L-isoleucine, L-leucine, L-tyrosine, L-phenylalanine, L-histidine, L-lysine, L-tryptophan and L-arginine, and the salts thereof (such as methionine hydrochloride or methionine sulfate). An "amino acid preparation", according to the present invention, can be made by any known methods in the art and as described herein, preferably by fermentation of an amino acid producing microorganism. An "amino acid preparation" of the present invention can be in any liquid or dry forms known in the art, and it can be a purified amino acid or the salt thereof (i.e., at least 95% by weight), or it can contain less than 95% by weight of amino acid or the salt thereof, but also contain other components (e.g., culture broth, and/or whole bacteria cells) in addition to the amino acid. An "amino acid preparation", according to the present invention, can also contain two or more amino acids and the salts thereof. Preferably, the "amino acid preparation" is in a form that can be used as an animal feed supplement.

The term "methionine preparation" refers to an amino acid preparation containing a methionine. A "methionine preparation" can be prepared by any known methods in the art and as described herein, preferably by fermentation of a methionine-producing microorganism. A "methionine preparation" of the present invention can be in any liquid or dry forms known in the art, and it can be a purified methionine or the salt thereof (i.e., at least 95% methionine by weight), or it can contain less than 95% by weight of methionine or the salt thereof, but also contain other components (e.g., culture broth, and/or whole bacteria cells) in addition to methionine. A "methionine preparation", according to the present invention, can also contain methionine and one or more other amino acids and their salts thereof. Preferably, the "methionine preparation" is in a form that can be used as an animal feed supplement.

The term "purified amino acid preparation", as used herein, refers to one form of the amino acid preparation as define herein above which has an amino acid content (% per weight) of at least 90%, for example, 92%, 94%, 96%, 98%, or 100%.

The term "dried amino acid preparation", as used herein, refers to one form of the amino acid preparation as defined herein above which has a water content (% per weight) of at most 10%, e.g., 8%, 6%, 4%, 2%, 1% or 0%.

The term "purified methionine preparation", as used herein, refers to one form of the methionine preparation as define herein above which has a methionine content (% per weight) of at least 90%, for example, 92%, 94%, 96%, 98%, or 100%.

The term "dried methionine preparation", as used herein, refers to one form of the methionine preparation as defined herein above which has a water content (% per weight) of less than 10%, e.g., 8%, 6%, 4%, 2%, 1% or 0%.

The term "solubility", as used herein, refers to the solid/liquid solubility, i.e., the ability or tendency of an amino acid to blend uniformly with a liquid, e.g., water. Solids vary from 0-100% in their degree of solubility in liquids, depending on the chemical nature of the substances: to the extent that they are soluble, they lose their crystalline form and become molecularly or ionically dispersed in the solvent to form a true solution. The "solubility of a methionine preparation" and "solubility of methionine in a methionine preparation", as used herein, refer to the aqueous solubility of methionine, e.g., in water, expressed as g/L. For example, DL-methionine has a water solubility of 33.81 g/L at 25° C. according to Merck Index, 12th Edition, 1996.

The term "increasing the solubility" refers to the increase of aqueous soluble amino acid concentration by pH adjustment as compared to the concentration of aqueous soluble methionine before adjusting the temperature and/or pH. There is an "increase" in solubility when the solubility of an amino acid is at least 20% greater (e.g., 21%, 25%, 30%, 40%, 50%, 60%, 70%, 80%, 90%, 100%, 200%, 300%, 400%, 500% greater, or more, than its solubility at pH 7.0 at the same temperature. For example, if a methionine preparation has a water soluble methionine concentration of 33.81 g/L at 25° C. at pH 7.0, it is said that there is an increase in the solubility of such methionine preparation if, by adjusting the pH and/or temperature, the methionine preparation has a water soluble methionine concentration of at least 40.57 g/L, e.g., at least 47.33 g/L, 50.72 g/L, 54.10 g/L, 57.48 g/L, 60.86 g/L, 64.24 g/L, 67.62 g/L, 101.43 g/L, 135.24 g/L, 169.05 g/L or more. The water soluble concentration of amino acid can be measured by any methods for solubility determination known in the art, e.g., as described in Daniels et al. 1970, Experimental Physical Chemistry, 7th ed., New York: McGraw-Hill; Halpern, A. M. and Reeves, H. L., 1988, Experimental Physical Chemistry, A laboratory Textbook, Scott Foreman and Company; Showmaker et al., 1981, Experiments in Physical Chemistry, 4th ed., New York: McGraw-Hill.

The term "base", as used herein, refers to any substance which can alter the pH of a solution from a neutral pH of 7.0 to a basic pH (i.e., 7.1 to 14). Typically, a base is a substance of a large class of compounds with one or more of the following properties: bitter taste, slippery feeling in solution, ability to turn litmus blue and to cause other indicators to take on characteristic colors, ability to react with (neutralize) acids to form salts. Included are both hydroxides and oxides of metals. Water-soluble hydroxides such as sodium, potassium, and ammonium hydroxide undergo ionization to produce hydroxyl ion (OH^-) in considerable concentration, and it is this ion that causes the previously mentioned properties common to bases. Such a base is strong or weak according to the

fraction of the molecules that breaks down (ionizes) into positive ion and hydroxyl ion in the solution. Base strength in solution is expressed by pH. Common strong bases (alkalis) are sodium and potassium hydroxides, ammonium hydroxide, etc. These are caustic and corrosive to skin, eyes, and mucous membranes. The pH range of basic solutions is from 7.1 to 14. Modern chemical terminology defines bases in a broader manner. A Lowry-Bronsted base in any molecular or ionic substance that can combine with a proton (hydrogen ion) to form a new compound. A Lewis base is any substance that provides a pair of electrons for a covalent bond with a Lewis acid. Examples of such bases are hydroxyl ion and most anions. metal oxides, and compounds of oxygen. nitrogen, and sulfur with non-bonded electron pairs (such as water, ammonia, and hydrogen sulfide).

The term "acid". as used herein. refers to any substance which can alter the pH of a solution from a neutral pH of 7.0 to an acidic pH (i.e.. 6.9 to 1). Typically, an acid is a substance of a large class of chemical substances whose water solutions have one or more of the following properties: sour taste, ability to make litmus dye turn red and to cause other indicator dyes to change to characteristic colors. ability to react with and dissolve certain metals to form salts, and ability to react with bases or alkalis to form salts. All acids contain hydrogen. In water, ionization or splitting of the molecule occurs so that some or most of this hydrogen forms H_3O^+ ions (hydronium ions). usually written more simply as H^+ (hydrogen ion). Acids are referred to as strong or weak according to the concentration of H^+ ion that results from ionization. Hydrochloric, nitric, and sulfuric are strong or highly ionized acids: acetic acid (CH_3COOH) and carbonic acid (H_2CO_3) are weak acids. Tenth normal hydrochloric acid is 100 times as acid (pH 1) as tenth normal acetic acid (pH 3). The pH range of acids is from 6.9 to 1. The hydroxy analog of methionine (e.g., 2-hydroxy-4-(methylthio)butanoic acid is an "acid" under the definition of the present invention.

The present invention is based on the unexpected discovery that the solubility of a methionine preparation can be increased by adjusting the pH during the preparation process to a basic or acidic pH. The present invention is also based on the discovery that solubility of methionine in a fermentation broth can be dramatically increased by increasing the temperature to at least 40° C.

The present invention can be used in combination with any known method of producing methionine preparation, e.g.. high purity methionine or fermentation broth containing methionine. The present invention can be used for methionine preparation by fermentation, for example, as described in U.S. Pat. Nos. 3.729.381, 5.840.551, 6.379.934: 5.431,933: 5.622,710: 5.840,358: or by optical resolution of DL-amino acids prepared in an organic synthetic-chemical method: or by chemico-enzymatic processes as described in U.S. Pat. No. 5,215,897; Japanese Patent Publication Nos. 22380/66, 2274/79. 18867/82, Japanese Patent Application Kokai (Laid-Open) No. 140890/84). the entirety of each is hereby incorporated by reference.

A preferred method of producing a methionine preparation, according to the present invention, is by fermentation of a methionine-producing microorganism. It should be understood. however, the pH adjustment as taught in the present invention can readily be applied to other methods of preparing a methionine preparation, e.g., by chemical synthesis and protein hydrolysis. so long as the pH as adjusted does not interfere with the production of methionine by such method. In addition, the pH adjustment as taught in the present invention can also be used to increase the solubility of other amino acid preparation, in particular, for other amino acid with low solubility problems, e.g., leucine, isoleucine, serine, glutamic acid. aspartic acid, some aromatic ring-containing amino acids such as tryptophan. tyrosine. phenylalanine. other sulfur-containing amino acids such as cysteine.

The present invention provides a method of making a methionine preparation by fermentation of a methionine-producing microorganism. The adjustment of pH can occur at any step of the process of making such a methionine preparation so long as it does not interfere with the fermentation process and the production of methionine by the methionine-producing microorganism. Likewise, the increase in temperature can occur at any stage of the process, so long as it does not interfere with the fermentation process and the production of methionine by the methionine-producing microorganism.

In one embodiment. the method of making such a methionine preparation comprises a) culturing a methionine-producing microorganism in a fermentation medium, where the pH of the fermentation medium is adjusted to an acidic or basic pH; b) obtaining a methionine-containing fermentation broth from said the culturing: and c) concentrating the methionine-containing fermentation broth to produce a methionine preparation.

In another embodiment, the method of making a methionine preparation comprises a) culturing a methionine-producing microorganism in a fermentation medium; b) obtaining a methionine-containing fermentation broth from said the culturing, where the pH of the fermentation broth is adjusted to an acidic or basic pH; and c) concentrating said the methionine-containing fermentation broth to produce a methionine preparation.

In addition to adjusting the pH to an acidic or basic pH in the aforementioned embodiments, the solubility of methionine can be further increased by increasing the temperature of the fermentation medium or fermentation broth to at least 40° C.

In yet another embodiment, the method of making such a methionine preparation comprises a) culturing a methionine-producing microorganism in a fermentation medium; b) increasing the temperature of the fermentation medium to at least 40° C.: c) obtaining a methionine-containing fermentation broth from the culturing; and d) concentrating the methionine-containing fermentation broth to produce a methionine preparation.

In addition to providing the primary means of separating the methionine from the whole cells, lowering or raising pH in combination with temperature will aid in inactivating the cells. Whole cell inactivation is usually performed in conjunction with removing the cells from the broth. The inactivated cells can be used as an animal feed supplement, or are disposed of in waste treatment operations.

Microorganism

Any microorganism can be effectively used in this invention on the sole condition that it should be able to produce an amino acid. e.g.. methionine. The microorganisms to which the present invention relates can prepare amino acids from glucose, sucrose, lactose, fructose, maltose, molasses, starch, cellulose or from glycerol, ethanol, and other carbohydrates.

Examples of useful methionine producing bacteria include. but are not limited to, those described in U.S. patent application Nos. 2003/0,092,026A1; 2003/0,059,903A1; 2003/0,054,503A1; 2003/0,049,803A1; 2002/0,142,405A1; 2002/0.110,878A1: 2002/0,110,877A1; 2002/0,102.664A1; 2002/0.049,305A1: 2002/0,048,793A1; 2002/0,028.490A1; and U.S. Pat. Nos. 6.379.934B1: 6,040,160; 3,219.543; 3,729,381; 3,756,916; 3,139,386, each of the patents and patent applications is hereby incorporated in its entirety.

Most of the useful bacteria for the present invention are classified as *Corynebacterium, Brevibacterium, Arthrobacter* or *Microbacterium*. All of the genera are found within the class Schizomycetes. *Brevibacterium* is a genus within the family Brevibacteriaceae, order Eubacteriales and is generally characterized by: short, unbranching rods; generally non-motile: type of motility of motile species is peritrichous or uncertain; sometimes chromogenic, with non-water soluble reddish, reddish orange, yellow or brown pigments; may or may not reduce nitrates; glucose broth usually becomes acid; lactose not fermented; proteolytic action varies with the species: aerobic and facultatively anaerobic: rarely microaerophilic. *Corynebacterium* is a genus within the family Corynebacteriaceae, order Eubacteriales, and is generally characterized by: straight to slightly curved rods with irregularly stained segments. sometimes granules; frequently show club-shaped swellings; snapping division produces angular and palisade (picket-fence) arrangements of cells: non-motile with exceptions among the plant pathogens; Gram-positive, but sometimes young cells and sometimes old cells losing the stain easily: granules invariably Gram-positive; generally quite aerobic, but microaerophilic or even anaerobic species occur; catalase-positive; may or may not liquefy gelatin; may or may not produce nitrites from nitrates; may or many not ferment sugars, but seldom, if ever, is a high acidity produced; many species oxidize glucose completely to CO_2 and H_2O without producing visible gas. *Arthrobacter* is a genus within the family Corynebacteriaceae, order Eubacteriales, and is generally characterized by: in young cultures the cells appear as rods which may vary in size and shape from straight to bent. curved. swollen or club-shaped forms; snapping division may show angular cell arrangement: short filament formation with rudimentary budding may occur, especially in richer liquid media; Gram-negative or Gram-variable, coccoid cells are characteristically observed in cultures and are Gram-negative to Gram-positive: larger coccoid cells which give rise to one or more rod-shaped cells on fresh transfer also occur; generally non-motile; growth on solid media soft or viscous; growth on liquid media generally not profuse; most species liquefy gelatin: little or no acid from carbohydrates: nitrites generally produced from nitrates; indole not produced; aerobic; most species show little or no growth at 37° C. *Microbacterium* is a genus within the family Corynebacteriaceae, order Eubacteriales and is characterized by: small rods with roundel ends: vary in length from 0.5 to 30 microns: non-motile: granulations demonstrable with methylene blue stain; Gram-positive; good surface growth on media supplemented with milk or yeast extract, acid production weak with principally L (+)-actic acid produced from fermented carbohydrates; catalase-positive optimum temperature. 32° C.

Specific non-limiting suitable strains of the genus *Corynebacterium*, in particular of the species *Corynebacterium glutamicum* (*C. glutamicum*), are in particular the known strains as follows:

Corynebacterium glutamicum ATCC21608
Corynebacterium glutamicum ATCC13032
Corynebacterium acetoglutamicum ATCC15806
Corynebacterium acetoacidophilum ATCC13870
Corynebacterium melassecola ATCC17965
Corynebacterium thermoaminogenes FERM BP-1539
Brevibacterium flavum ATCC14067
Brevibacterium lactofermentum ATCC13869 and
Brevibacterium divaricatum ATCC14020
or L-amino acid-producing mutants or strains prepared therefrom.

Microorganisms of the family Enterobacteriaceae selected from the genera *Escherichia, Erwinia, Providencia* and *Serratia* may also be used for the production of amino acids, e.g., methionine. according to the present invention.

Specific useful non-limiting suitable strains of the genus *Escherichia*. in particular those of the species *Escherichia coli* include for example:

Escherichia coli TF427
Escherichia coli H4578
Escherichia coli KY10935
Escherichia coli VNIIgenetika MG442
Escherichia coli VNIIgenetika M1
Escherichia coli VNIIgenetika 472T23
Escherichia coli BKIIM B-3996
Escherichia coli kat 13
Escherichia coli KCCM-10132

Specific non-limiting suitable strains of the genus *Serratia*, in particular of the species *Serratia marcescens* include for example:

Serratia marcescens HNr21
Serratia marcescens TLr156
Serratia marcescens T2000

The methods of mutagenesis, selection and mutant choice have been used to improve the microorganisms for the production of amino acids. For example, strains that are resistant to antimetabolites such as, for example, the lysine analogues of S-(2-aminoethyl)-cysteine or which are auxotrophic for significant regulatory amino acids. and produce L-amino acids, are obtained in this way.

Mutant strains of coryneform glutamic acid-producing bacteria represented by *Corynebacterium glutamicum* which exhibit resistance to analogues of methionine (for example; α-methylmethionine. ethionine. norleucine, N-acetylnorleucine. Trifluoromethylhomocysteine, 2-amino-5-heptenoic acids, 2-amino-4-hexenoic acid, seleno-methionine, methionine sulfoximine, methoxinine, 1-aminocyclopentane carboxylic acid. etc.), are also excellent producers of L-methionine (e.g., as described in U.S. Pat. No. 3.729,381, hereby incorporated by reference in its entirety). Resistance to analogues of methionine can be determined by checking if the mutant can grow in a medium containing 500 μg/ml of an analogue though the concentration varies depending upon the microorganisms and the analogues. It can be generally stated that the following L-glutamic acid-producing microorganisms are preferred in connection with the process of the present invention: *Brevibacterium glutamigenum, Brevibacterium lactoferinentum, Brevibacterium saccharolyficum, Brevibacterium thiogenitalls, Brevibacterium* sp.. *Corynebacterium* sp., *Corynebacterium callunae, Corynebacterium acetoacidophilum, Corynebacterium glutamicum, Corynebacterium melassecola, Microbacteriumflavum*, var. *glutamicum, Arthrobacter* sp. A particularly preferred mutant strain of *Corynebacterium glutamicum* has been deposited with the American Type Culture Collection, Rockville, Md., and has been accorded accession number ATCC 21608.

Recombinant DNA techniques can be used for strain-improvement for the production of amino acids (e.g., see U.S. Pat. No. 4,278,765, hereby incorporated by reference in its entirety). Reference is made to standard textbooks of molecular biology that contain definitions and methods and means for carrying out basic recombinant DNA techniques. encompassed by the present invention. See. for example. Maniatis et al., Molecular Cloning: A Laboratory Manual, Cold Spring Harbor Laboratory, New York (1982) and Sambrook et al., Molecular Cloning: A Laboratory Manual. Cold Spring Harbor Laboratory. New York (1989). hereby incorporated by reference in their entirety.

For example, L-amino acid producing strains of *Corynebacterium glutamicum* can be improved by transformation of

individual amino acid biosynthetic genes. Review articles about this topic can be found. inter alia. in Kinoshita ("Glutamic Acid Bacteria", in: Biology of Industrial Microorganisms. Demain and Solomon (Eds.), Benjamin Cummings, London, UK, 1985, 115-142), Hilliger (BioTec 2, 40-44 (1991)), Eggeling (Amino Acids 6, 261-272 (1994)), Jetten and Sinskey (Critical Reviews in Biotechnology 15, 73-103 (1995)) and Sham et al. (Annuals of the New York Academy of Science 782. 25-39 (1996)), incorporated by reference in their entirety.

For methionine production, genes encoding methionine biosynthetic pathway can be transformed into desired bacteria e.g., *Corynebacterium glutamicum*. Such genes are known in the art. for example. as described in U.S. patent applications 2003/0,092,026A1 (metD); 2002/0,110,878A1 (metY): 2002/0,110,877A1 (metE); 2002/0,102,664A1 (metR and metZ); 2002/0,049,305A1 (metF); 2002/0,048,793A1 (metH), each of the patent applications is hereby incorporated in its entirety.

To increase the production of methionine from recombinant bacteria, the copy number of the corresponding gene can be increased or the promoter and regulation region or the ribosome bonding site, which are located upstream of the coding sequence, can be mutated. Expression cassettes, which are incorporated upstream of the coding sequence. operate in the same way.

It is also possible to increase expression during the course of fermentative methionine production with inducible promoters. Expression is also improved by measures aimed at prolonging the lifetime of m-RNA. Furthermore, enzyme activity can also be amplified by inhibiting degradation of the enzyme protein. The genes or gene constructs can either be present in plasmids with different copy numbers or be integrated and amplified in the chromosome. Alternatively, overexpression of the genes concerned can also be achieved by modifying the composition of the media and management of the culture.

Instructions for these procedures can be found by a person skilled in the art in, inter alia, Martin et al. (Bio/Technology 5. 137-146 (1987)), in Guerrero et al. (Gene 138. 35-41 (1994)). Tsuchiya and Morinaga (Bio/Technology 6, 428-430 (1988), in Eikmanns et al. (Gene 102, 93-98 (1991)), in European Patent EP-B 0 472 869, in U.S. Pat. No. 4,601,893, in Schwarzer and Puhler (Bio/Technology 9, 84-87 (1991), in Reinscheid et al. (Applied and Environmental Microbiology 60, 126-132 (1994)), in LaBarre et al. (Journal of Bacteriology 175, 1001-1007 (1993)), in Patent Application WO 96/15246. in Malumbres et al. (Gene 134. 15-24 (1993)), in Japanese Patent JP-A-10-229891. in Jensen and Hammer (Biotechnology and Bioengineering 58, 191-195 (1998)), in Makrides (Microbiological Reviews 60:512-538 (1996)) and in well-known textbooks relating to genetics and molecular biology, each of which is hereby incorporated by reference in its entirety.

In an preferred embodiment, L-methionine is produced by culturing in a nutrient medium an L-methionine-producing certain type mutant strain of coryneform glutamic acid producing bacteria represented by *Corynebacterium glutamicum*. accumulating L-methionine in the culture liquor and recovering L-methionine therefrom.

Fermentation

Culturing and fermentation of the suitable amino acid producing bacteria can be performed according to any method known in the art, e.g., as described in U.S. patent application Nos. 2003/0,092,026A1; 2002/0,142,405A1; U.S. Pat. No. 3,546,071, hereby incorporated by reference in their entirety.

The culture medium to be used must meet the requirements of the particular strains in a suitable manner. Descriptions of culture media for various microorganisms are contained in the handbook "Manual of Methods for General Bacteriology" of the American Society for Bacteriology (Washington D.C., USA, 1981).

The culture medium employed in the present invention can be either synthetic or natural, so long as the medium properly contains a carbon source, a nitrogen source, inorganic compounds and small amounts of additional nutrients necessary for the specific microorganism used. Other than the above, there are no special restrictions attached to other essentials of the medium composition.

The following substances can be used individually, or as a mixture, as the source of carbon:

(a) sugars and carbohydrates, such as e.g. glucose, sucrose, lactose, fructose. maltose, molasses. starch and cellulose,

(b) oils and fats, such as, soy oil, sunflower oil, groundnut oil and coconut fat,

(c) fatty acids, such as palmitic acid, stearic acid and linoleic acid,

(d) alcohols, such as glycerol and ethanol, and

(e) organic acids, such as acetic acid, pyruvic acid, fumaric acid, lactic acid.

The following substances can be used individually. or as a mixture. as the source of nitrogen:

(a) Organic nitrogen-containing compounds, such as peptones, yeast extract, meat extract, malt extract, corn steep liquor, soya bean flour and urea, or

(b) inorganic compounds, such as ammonium sulfate, ammonium chloride, ammonium phosphate. ammonium carbonate and ammonium nitrate,

The following sources can be used individually, or as a mixture. as the source of phosphorus:

Phosphoric acid. potassium dihydrogen phosphate or dipotassium hydrogen phosphate or the corresponding sodium-containing salts.

The culture medium must furthermore comprise salts of metals. such as magnesium sulfate or iron sulfate, which are necessary for growth.

Essential growth substances, such as amino acids and vitamins, can be employed in addition to the above-mentioned substances. Suitable precursors can moreover be added to the culture medium. The starting substances mentioned can be added to the culture in the form of a single batch, or can be fed in during the culture in a suitable manner.

Acid compounds, such as an inorganic acid, e.g., hydrochloric acid. hydrobromic acid. sulfuric acid, phosphoric acid. hydrogen bromide, etc. or an organic acid, e.g., formic acid. acetic acid, propionic acid, trichloroacetic acid. trifluoroacetic acid, etc., can be added into the fermentation medium in a suitable manner, e.g., either manually or mechanically, to control the pH of the culture. and to increase solubility of methionine in the fermentation medium.

Antifoams, such as, for example, fatty acid polyglycol esters, can be employed to control the development of foam. Suitable substances having a selective action, such as, for example. antibiotics. can be added to the medium to maintain the stability of plasmids if recombinant bacteria stains are used. To maintain aerobic conditions, oxygen or oxygen-containing gas mixtures, such as air, are introduced into the culture. The temperature of the culture is usually between 20° C. to 45° C.. and preferably between 25° C. to 40° C. Culturing is continued until a maximum of the desired product has formed. This target is usually reached within 10 hours to 160 hours.

In one embodiment, the microorganism is grown in a seed medium prior to being used for inoculation of the culture medium. The seed medium is incubated under favorable growth conditions for a period of time sufficient to develop a suitable organism population, typically for about 24 hours. The seed medium is then used to inoculate the culture medium. Fermentation is then carried out until a considerable amount of L-methionine is produced and accumulated in the resultant medium, usually 1 to 5 days. After the completion of culturing, the L-methionine is readily recovered from the medium by separating the medium from the cells and subjecting the cell free medium to an ion exchange resin treatment or the like.

Post Fermentation

The fermentation broth prepared in this manner, in particular containing methionine, is then further processed. Depending on requirements, all or some of the biomass can be removed from the fermentation broth by separation methods. The removal of biomass may be particularly important to make a cell free preparation for animal feed when certain host (e.g., *E. coli*) is used. Examples of such separation methods are centrifugation, filtration, decanting or a combination thereof. Alternatively, the biomass can be left completely in the fermentation broth. This broth can optionally be thickened or concentrated by known methods. Examples of such thickening or concentrating methods include conventional methods such as evaporation, reverse osmosis, or by nanofiltration. Examples of instruments that can be used in evaporation processes include methods a rotary evaporator, thin film evaporator, and falling film evaporator. This thickened or concentrated fermentation broth can then be worked up. Examples of methods used to work up the thickened or concentrated fermentation broth include freeze drying, spray drying, spray granulation or by other processes. Optionally, the fermentation broth can be worked up to yield a preferably free-flowing, finely divided powder.

In an alternative embodiment, the starting point for making a methionine preparation can be the insoluble material collected from the fermentation broth. Because of the inherently low solubility of methionine in aqueous solutions, a great proportion of the methionine produced by the microorganism often exists in the insoluble state. Therefore, collection of insoluble material following fermentation by filtration or centrifugation can serve as the starting point for making the methionine preparation.

To increase recovery of methionine in the insoluble phase, the pH of the fermentation broth can be adjusted to further reduce its solubility. The temperature of the fermentation broth may additionally be reduced, for example, to less than 20° C., typically less than 10° C., less than 6° C. or less than 4° C. Using insoluble material as the starting point for making a methionine preparation is especially advantageous in situations where large volumes of fermentation broth is processed; due to the abundance of insoluble methionine, collecting the insoluble material serves as a significant enrichment step. The collected insoluble material may be resuspended in water or a solution. The choice of solution depends on the ideal conditions for ensuring that the microorganism remains relatively intact. Factors to consider include osmolarity, the presence of monovalent and divalent cations to maintain integrity of the cell wall, and the absence of compounds with detergent-like properties, which can disrupt the cell membrane and release the intracellular contents of the microorganism. In addition, it is preferable that the solution not possess strong pH buffering capacity that may interfere with altering the pH to increase the solubility of methionine.

To solubilize methionine, either from a fermentation broth or insoluble material resuspended in solution, the temperature of the solution can be increased to at least 40° C. to increase the solubility of methionine within the fermentation broth. In one embodiment, the temperature of the fermentation broth is raised to at least 50° C. In another embodiment, the temperature of the fermentation broth is raised to at least 60° C. In yet another embodiment, the temperature of the fermentation broth is raised to at least 70° C.

If it is desirable to remove the biomass, some or all of the biomass can be removed from the fermentation broth by separation methods such as filtration or centrifugation. The biomass is preferably removed while the temperature is increased (i.e., when the solubility of methionine is increased) to reduce the loss of insoluble methionine during this step.

Alternatively, or in addition to increasing the temperature of the fermentation broth, an acid compound can be added into the fermentation broth in a suitable manner, e.g., either manually or mechanically, at the end of the fermentation, but before the optional removal of the biomass, an acid compound can be added into the fermentation broth in a suitable manner, e.g., either manually or mechanically, to control the pH of the broth and to increase the solubility of methionine within the broth.

In one embodiment, a pH of 4.0 or below is achieved for the fermentation broth to increase the solubility of methionine. In another embodiment, a pH of 3.5 or below is achieved for the fermentation broth to increase the solubility of methionine. In another embodiment, a pH of 3.0 or below is achieved for the fermentation broth to increase the solubility of methionine.

As previously stated, the adjustment of pH to an acidic pH can be performed in combination with increasing the temperature of the fermentation broth to at least 40° C. For example, the pH of the fermentation broth can be adjusted to an acidic pH of 3.0, and the temperature of the fermentation broth increased to 50° C. pH can be measured with or without the removal of any biomass according to the present invention.

The solubility of methionine in an acidified such fermentation medium or fermentation broth (25° C.) is at least 40.57 g/L, for example, at least 47.33 g/L, 50.72 g/L, 54.10 g/L, 57.48 g/L, 60.86 g/L, 64.24 g/L, 67.62 g/L, 101.43 g/L, 135.24 g/L, 169.05 g/L or more.

Optionally, the acidified broth (i.e., the broth with an acidic pH) can be used for further purification of methionine by ion exchange methods known in the art, e.g., as described in Spackman et al. (1958, Analytical Chemistry, 30: 1190), U.S. patent applications 2002/0.110.877A1 and 2003/0,045,753A1, each of which is hereby incorporated by reference in its entirety.

When ion exchange is used, the acidified broth is passed over a cationic ion column to separate the methionine from the other broth constituent (e.g., the biomass). The bound methionine is then eluted from the column with ammonium hydroxide (or any other base). The ammonia can then be stripped from methionine to make methionine free base to make a purified methionine preparation.

Alternatively, the acid compound can be added after the removal of biomass, but before thickening or concentrating. This is useful if the methionine solubility at the end of the fermentation is below the solubility at a neutral pH.

In some embodiments of the invention, it is preferred to adjust the pH of the broth back to pH 2.5-7, preferably, pH 3.5-7, more preferably, 5-7, before drying to make the preparation more suitable for animal feeding.

As an alternative to adding an acid compound, basic compounds, such as sodium hydroxide, potassium hydroxide, ammonia or aqueous ammonia, ammonium hydrochloride, ammonium sulfate can be added into the fermentation broth in a suitable manner, e.g., either manually or mechanically, at the end of the fermentation, but before the optional removal of the biomass, to control the pH of the broth and to increase the solubility of methionine. In one embodiment, the pH of the broth is increased to at least 8.5. In another embodiment, the pH is increased to at least 9.0. In yet another embodiment, the pH is increased to at least 9.5. In still another embodiment, the pH is increased to at least 10.0. Similar to the embodiments involving acid addition, the adjustment of pH to a basic pH can be performed in combination with increasing the temperature of the fermentation broth to at least 40° C. For example, the pH of the fermentation broth can be adjusted to a basic pH of 8.5, and the temperature of the fermentation broth increased to 70° C.

Alternatively, the base can be added after the removal of biomass, but before thickening or concentrating. This is useful if the methionine solubility at the end of the fermentation is below the solubility at a neutral pH.

In one embodiment, a liquid sodium methionate or ammonium methionate product is obtained.

Once the insoluble material (containing whole cells) is removed from the methionine preparation, additional steps of methionine purification can be performed. The methionine preparation can be decolorized using absorbents such as activated carbon, for example the Darco KB and KB-B activated carbon (American Norit Co. Buford, Ga.). The absorbent can simply be mixed with the methionine preparation at a range of about 1-15 g/L of methionine preparation and stirred, with optional heating to between 40° C. and 70° C., for 1 to 24 hrs. The activated carbon can then be removed by simple filtration.

In another embodiment, a purified methionine preparation that is essentially cell free is obtained by taking the ammonium methionate salt solution which is at high pH to strip off the ammonia. The methionate solution can then be concentrated and crystallized to effectively produce a purified methionine preparation, for example, as described for a chemically-produced ammonium methionate solution in U.S. Pat. No. 6,417,395 (the entirety of which is hereby incorporated by reference). Prior to crystallization, the methionine preparation can be further concentrated, as described above, in order increase recovery. Crystallization of methionine can also be achieved by a number of means, for example by addition of aluminum salts of organic acids as is described in U.S. Pat. No. 5,463,120, by addition of alcohols, phenols or ketones as described in Japanese Patent published under JP 68-024890, or by addition of anionic or non-ionic surface active agents, as disclosed in Japanese Patent published under JP 71-019610. The three patents mentioned above are hereby incorporated in their entirety by reference. Crystallization of methionine can also be achieved by adjusting the pH of the methionine preparation to pH 5.74, the isoelectric point of methionine, and reducing the temperature to below 10° C., typically less than or equal to 4° C. and permitting the crystals to form for at least 3 hrs, typically overnight. Methionine crystals can be collected, for example, by filtration or centrifugation directly from the chilled solution, or after allowing the solution to warm to room temperature.

Both the collected crystals and the remaining solution can be analyzed for purity of methionine. Purity can be analyzed by any number of means to determine the methionine content of the crystal and the solution, including HPLC. If a significant amount of methionine remains in solution (in the mother liquor), then crystallization can be repeated. If needed, the crystals can be redissolved, and crystallization repeated in order to increase purity.

The free-flowing, finely divided powder can be converted by suitable compacting or granulating processes, e.g., as described in U.S. patent application Nos. 2003/0,092,026A1 and 2003/0,059,903A1, the entirety of each is hereby incorporated by reference. Preferably, the powder can be converted into a coarse-grained, readily free-flowing, storable and largely dust-free product. During granulation or compaction, it is advantageous to employ conventional organic or inorganic auxiliary substances or carriers. Examples of such organic or inorganic auxiliary substances or carriers include starch, gelatin, cellulose derivatives or similar substances. Further, these substances can be used as binders, gelling agents or thickeners in foodstuffs or feedstuffs processing. Further examples of these substances include silicas, silicates or stearates.

Alternatively, the product can be absorbed onto an organic or inorganic carrier substance that is known and conventional in feedstuffs processing. Examples of such organic or inorganic carrier substances include silicas, silicates, grits, bran, meals, starches, sugars or others. Further, the product simultaneously or subsequently mixed and/or stabilized with conventional thickeners and/or binders.

Finally, the product can be brought into a state in which it is stable to digestion by animal stomachs, in particular the stomach of ruminants, by coating processes, i.e. coating. Examples of such conventional coating processes include those that use film-forming agents. Examples of film-forming agents include metal carbonates, silicas, silicates, alginates, stearates, starches, gums and cellulose ethers.

If the biomass (i.e., insoluble material) is separated from methionine during the preparation, further inorganic solids which can be optionally added during the fermentation can be optionally removed. In addition, organic substances can be optionally formed and/or added and are optionally present in solution in the fermentation broth.

Examples of the above-mentioned organic substances include organic by-products. Organic by-products can be optionally produced, in addition to the L-methionine, and can be optionally discharged by the microorganisms employed in the fermentation. Examples of organic by-products include L-amino acids chosen from the group consisting of L-valine, L-threonine, L-alanine or L-tryptophan. Further examples of organic by-products include vitamins chosen from the group consisting of vitamin B1 (thiamine), vitamin B2 (riboflavin), vitamin B5 (pantothenic acid), vitamin B6 (pyridoxine), vitamin B12 (cyanocobalamin), nicotinic acid/nicotinamide and vitamin E (tocopherol). Even further examples of organic by-products include organic acids. Examples of organic acids are those that contain one to three carboxyl groups. Examples of organic acids containing one to three carboxyl groups include acetic acid, lactic acid, citric acid, malic acid and/or fumaric acid. Finally, Examples of organic by-products include sugars. Examples of sugars include such trehalose. These compounds are optionally desired if they improve the nutritional value of the product.

Purified organic substances, including methionine (e.g., L, D, or D/L methionine) or methionine esters or the hydroxy analog of methionine (e.g., 2-hydroxy-4-(methylthio)butanoic acid, provided by Novus International, St. Louis, Mo., USA) can also be added into the fermentation medium or broth, e.g., during a suitable process step (e.g., into the fermentation medium or the fermentation broth, or concentrated

fermentation broth, or the dry methionine preparation). The addition of such material increases the methionine content of the methionine preparation.

Such organic substances can be in many forms. Examples of such forms include concentrate and/or pure substance in solid and/or liquid form. These organic substances mentioned can optionally be added individually or as mixtures to the resulting or concentrated fermentation broth, or also optionally during the drying or granulation process. It is likewise possible to optionally add an organic substance or a mixture of several organic substances to the fermentation broth and also to add further organic substances or a mixture of several organic substances during a later process step. Examples of such as later step can include a granulation step.

In one aspect of the invention, the biomass can be separated from methionine to the extent of up to 70%, preferably up to 80%, preferably up to 90%, preferably up to 95%, and particularly preferably up to 100%.

In another aspect of the invention, less than 20% of the biomass, preferably less than 15%, preferably less than 10%, preferably less than 5%, particularly preferably no biomass is separated from methionine.

The methionine preparation made in the present invention can be used as a feed additive for animal nutrition. Alternatively, the fermentation broth obtained after culturing a methionine-producing microorganism can be used as a feed additive.

The L-methionine content of the animal feed additive is conventionally 1% per weight to 80% per weight, preferably 2% per weight to 80% per weight, particularly preferably 4% per weight to 80% per weight, and very particularly preferably 8% per weight to 80% per weight, based on the dry weight of the animal feed additive. Contents of 1% per weight to 60% per weight, 2% per weight to 60% per weight, 4% per weight to 60% per weight, 6% per weight to 60% per weight, 1% per weight to 40% per weight, 2% per weight to 40% per weight or 4% per weight to 40% per weight are likewise possible. The ranges for content of the animal feed additive include all specific values and subranges therebetween, such 2, 4, 6, 8, 10, 15, 20, 25, 30, 35, 40, 45, 50, 55, 60, 65, 70, and 75% per weight. The water content of the feed additive is conventionally up to 5% per weight, preferably up to 4% per weight, and particularly preferably less than 2% per weight.

An animal feed additive according to the present invention can comprise 1% per weight to 80% per weight L-methionine, D-methionine, D, L-methionine, or a mixture thereof with 1 to 40% per weight of a second amino acid, e.g., L-lysine, D-lysine or D,L-lysine, or several second amino acids, based on the dry weight of the animal feedstuffs additive. The ranges for content of L-methionine, D-methionine, D,L-methionine, or a mixture thereof with the second amino acid in the animal feedstuffs additive include all specific values and subranges therebetween, such 2, 4, 6, 8, 10, 15, 20, 25, 30, 35, 40, 45, 50, 55, 60, 65, 70, and 75% per weight. The ranges for content of the second amino acid, e.g., L-lysine, D-lysine or D, L-lysine in the mixture with L-methionine, D-methionine, D,L-methionine in the animal feedstuffs additive include all specific values and subranges therebetween, such 2, 4, 6, 8, 10, 10 15, 20, 25, 30, and 35% per weight.

Drying

It is one object of the present invention to provide a dried methionine preparation. The present method of making a methionine preparation thus can further comprise a drying step as described herein above.

Practice of certain specific embodiments of the invention is illustrated by the following representative examples.

EXAMPLES

Example 1

Culturing Methionine Producing Microorganisms in Fermentation Medium

Methionine producing bacteria *Corynebacterium glutamicum* ATCC21608, obtained from American Type Culture Collection, was aseptically added to 5 mL of nutrient broth (DIFCO) and allowed to grow for 24 hours at 30° C. under vigorous shaking. After the 24 hour incubation, the culture was used to seed 100 ml of nutrient broth (DIFCO) and allowed to grow for an additional 18 hours at 30° C. under vigorous shaking. Aliquots of the bacteria were then transferred to nutrient agar plates and the plates placed in an incubator for 24 hours. The plates were examined for growth and were refrigerated until further use.

Colonies from agar plates were aseptically transferred into 250 mL flasks containing 30 ml of nutrient broth (DIFCO) and allowed to grow at 30° C. After 24 hours of growth, these microorganisms were used as seed culture in a 3-L fermenter containing growth media consisting of 50 g/L glucose, 50 g/L soy protein hydrolyzate, 1 g/L K_2HPO_4, 45 g/L $(NH_4)_2SO_4$, 400 mg/L $MgSO_4.7H_2O$, 10 mg/L $MnSO_4.4H_2O$, 0.2 mg/L thiamin HCl and 0.05 mg/L biotin. Fermentation was carried out at 31° C. under vigorous agitation and good aeration. pH was maintained at 7.45 during fermentation by addition of ammonium hydroxide (NH_4OH). The organisms grew quite well under these conditions with a typical optical density (as measured as OD_{600}) in the range of 25 to 30 after only 24 hours. Fermentation batches were terminated once the initial glucose charge was consumed. The residual L-methionine produced during these fermentations was supplemented with additional L-methionine as described in the Examples below.

Example 2

Increased Recovery of L-Methionine at Basic pH

The fermentation broth produced by growing *Corynebacterium glutamicum* ATCC 21608, as discussed in Example 1, was supplemented with L-methionine to give a final methionine concentration of 75.2 g/L in the slurry. The resulting slurry consists of soluble and insoluble L-methionine, microbial cells and constituents of fermentation media. The pH of the slurry was measured to be 5.54. A well-mixed sample of this slurry was drawn, and solids removed from the sample by centrifugation followed by filtration. The concentration of methionine in the filtrate of the slurry was determined by HPLC to be 38 g/L, giving a concentration of soluble methionine in a typical fermentation broth.

To the remaining slurry, a 50% solution of sodium hydroxide (NaOH) was added at a level corresponding to 1 equivalent weight of L-methionine, raising its pH to 9.36. From a well-mixed sample of this slurry, solids were removed as before by centrifugation and filtration. Soluble methionine concentration in the filtrate was 78 g/L. The difference with the concentration in a typical fermentation broth represents an increase in solubility of methionine in the slurry due to the addition of NaOH. NaOH concentration was further increased in the slurry to 2 equivalent weight of L-methionine, which raised the pH to 10.22. At this stage the solubility of methionine in the solution was unchanged. The pH of the

slurry was raised to 12.53 by increasing the concentration of NaOH to 2.5 equivalent weight L-methionine. This did not further increase the solubility of L-methionine. At this point, the whole cells were separated from the methionine and fermentation solubles by centrifugation.

To the cell free broth containing the solubilized L-methionine, sulfuric acid (96% w/w) was added and its pH lowered to 5.95, the isoelectric point of L-methionine. At this pH, the methionine molecule does not have any charge, and results in the formation of methionine crystals. The slurry was then stored overnight at 4° C. to allow for precipitation of the crystals. The slurry was then centrifuged to collect the methionine crystals. The concentration of methionine remaining in the supernatant was found to be 33 g/L. The collected crystals were dried in a lyophilizer. The overall recovery of methionine using this of the process was 73%.

Example 3

Increased Recovery of L-Methionine at Basic pH

Methionine was added to the fermentation broth at a concentration of 56 g/L. In addition to soluble methionine, the slurry had insoluble methionine, microbial cells and components of fermentation media. 50% NaOH was added to the slurry at a concentration equal to 0.5 eq. weight of methionine. The pH of the slurry after addition of NaOH was 8.92 and the soluble methionine concentration in it was 58 g/L. Additional NaOH was added to the slurry to 1 eq weight of L-methionine, raising the pH of the slurry to 9.38. A sample of the slurry was removed and filtered as previously described. The concentration of methionine in the filtrate was found to be 56 g/L. No additional methionine was solubilized by increasing the concentration of NaOH to 1.5, 2 and 2.5 eq wt of methionine. The pH of the slurry was 9.82, 10.47 and 12.23 after addition of NaOH of 1.5, 2 and 2.5 eq wt of methionine, respectively.

Example 4

Increased Recovery of L-Methionine at Elevated Temperatures

Methionine was added to fermentation broth at a concentration of 77 g/L. The slurry was then heated to 70° C. without pH adjustment. This slurry was then filtered through a 0.45 micron filter in a heated filtration unit to remove the whole cells and other insoluble fermentation broth components. The filtrate was collected and analyzed for methionine. The concentration of methionine in the filtrate was 74.6 g/L. Thus, by raising the temperature of L-methionine containing fermentation broth from 22° C. to 70° C. increased the solubility of methionine from 38 g/L to 74.6 g/L. The filtrate was subsequently cooled to crystallize the free methionine. The methionine crystals were freeze-dried to remove residual moisture.

Example 5

Increased Recovery of L-Methionine at Acidic pH

The following examples demonstrate the use of an acid to increase the solubility of methionine.

A slurry containing 75 g/L of methionine was prepared by supplementing the fermentation broth produced with L-methionine as previously described.

Concentrated sulfuric acid (H_2SO_4, 96% w/w) was added in a concentration equal to 1 equivalent weight of methionine to the slurry, lowering the pH of the slurry to 2.49. Methionine concentration was measured from a sample after centrifugation and filtration as previously described. The concentration of soluble methionine increased to 75 g/L. Further addition of H_2SO_4 at 1.5 and 2 equivalents did not cause additional increase in the solubility of methionine in the fermentation broth. The pH of the slurry was 1.93 at 1.5 eq H_2SO_4 and 1.68 at 2 eq H_2SO_4.

At pH of 1.68, cells from the slurried fermenter broth were separated first by centrifugation followed by filtration through a 0.45 micron filter. The pH of the filtrate was then raised to 5.75, the isoelectric point of methionine by addition of 50% NaOH, resulting in formation of L-methionine crystals. The slurry was placed in a refrigerator at 4° C. for 18 hours to allow for additional crystallization of methionine. The crystals were then removed by centrifugation and filtration and further dried on a lyophilizer. The dried crystals were harvested from the lyophilizer. The concentration of methionine remaining in the supernatant (mother liquor) was 47.2 g/L. To recover additional methionine, the mother liquor was then placed in the refrigerator overnight. Crystals were collected by centrifugation at 4° C. by using a pre-chilled centrifuge. As a result of lower temperature, additional L-methionine was crystallized and recovered.

Example 6

Increased Recovery of L-Methionine at Acidic pH

L-methionine was added to fermentation broth at a final concentration of 55 g L-methionine per liter of the broth (55 g/L) as determined by HPLC. H_2SO_4 (96% w/w) at a concentration of 1 equivalent weight of methionine was added to this slurry, decreasing the pH of the broth to 2.39. The solubility of methionine in the broth at this pH was found to be 55 g/L, by measuring a sample which was centrifuged and filtered to remove insoluble material as previously described. Adding more H_2SO_4 to the broth at 1.5 and 2 times the equivalent weight to L-methionine further lowered the pH of the broth to 2.09 and 1.74 respectively, but did not result in further recovery of methionine.

For optimal recovery of methionine, solids from the acidified slurry above were removed by centrifugation and filtration and the pH of the recovered filtrate was raised to 5.6 using NaOH. Cooling the slurry to 4° C. for 18 hours resulted in formation of crystals in the solution. The concentration of methionine remaining in the supernatant (mother liquor) was 39.5 g/liter. This solution was placed again in the refrigerator at 4° C. for 18 hours to allow for formation of additional methionine crystals. Crystals formed after 18 hours at 4° C. were filtered and dried in a lyophilizer.

OTHER EMBODIMENTS

The foregoing examples demonstrate experiments performed and contemplated by the present inventors in making and carrying out the invention. It is believed that these examples include a disclosure of techniques which serve to both apprise the art of the practice of the invention and to demonstrate its usefulness. It will be appreciated by those of skill in the art that the techniques and embodiments disclosed herein are preferred embodiments only that in general numerous equivalent methods and techniques may be employed to achieve the same result.

All of the references identified hereinabove, are hereby expressly incorporated herein by reference to the extent that they describe, set forth, provide a basis for or enable compositions and/or methods which may be important to the practice of one or more embodiments of the present inventions.

The invention claimed is:

1. A method of making a methionine preparation comprising:
(a) culturing a methionine-producing microorganism in a fermentation medium to yield a fermentation broth;
(b) solubilizing methionine in said fermentation broth by raising the pH 8.5 or above, or lowering the pH to 3.5 or below;
(c) removing insoluble material from said fermentation broth to yield a clarified broth;
(d) crystallizing methionine from said clarified broth; and,
(e) isolating the methionine crystals to produce a methionine preparation.

2. The method of claim 1, wherein said base is selected from the group consisting of sodium hydroxide, potassium hydroxide and ammonium hydroxide.

3. The method of claim 1, wherein temperature of said fermentation broth is increased to at least 40° C. prior to removal of said insoluble material.

4. The method of claim 1, wherein temperature of said methionine-enriched broth to at least 40° C.

5. A method of making a methionine preparation comprising:
(a) culturing a methionine-producing microorganism in a fermentation medium to yield a fermentation broth;
(b) solubilizing methionine in said fermentation broth by raising the temperature of the broth to at least 40° C.;
(c) removing insoluble material from said fermentation broth to yield a methionine-enriched clarified broth;
(d) crystallizing methionine from said clarified broth; and,
(e) isolating the methionine crystals to produce a methionine preparation.

6. The method of claim 5, wherein said solubilizing further comprises raising the pH 8.5 or above, or lowering the pH to 3.5 or below.

7. The method of claim 5, wherein a methionine preparation is made by drying said clarified broth.

8. A method of making a methionine preparation comprising:
(a) culturing a methionine-producing microorganism in a fermentation medium to produce a fermentation broth;
(b) collecting methionine-enriched insoluble material from said fermentation broth;
(c) solubilizing methionine from said methionine-enriched insoluble material by addition of an acid to lower the pH to 3.5 or below, or a base to raise the pH to 8.5 or above to produce a methionine-enriched broth;
(d) crystallizing methionine from said methionine-enriched broth; and,
(e) isolating the methionine crystals to produce a methionine preparation.

9. A method of making a methionine preparation comprising:
(a) culturing a methionine-producing microorganism in a fermentation medium;
(b) obtaining a methionine-containing fermentation broth from said culturing, wherein the pH of said fermentation broth is adjusted to an acidic pH or basic pH; and
(c) concentrating said methionine-containing fermentation broth and optionally drying to produce a methionine preparation.

10. A method of making a methionine preparation comprising:
(a) culturing a methionine-producing microorganism in a fermentation medium;
(b) obtaining a methionine-containing fermentation broth from said culturing, wherein the pH of said fermentation broth is adjusted to an acidic pH or basic pH; and
(c) removing from said methionine-containing fermentation broth to produce a methionine preparation.

* * * * *

UNITED STATES PATENT AND TRADEMARK OFFICE

CERTIFICATE OF CORRECTION

PATENT NO.	: 8,338,141 B2
APPLICATION NO.	: 10/886863
DATED	: December 25, 2012
INVENTOR(S)	: Steve Lorbert et al.

It is certified that error appears in the above-identified patent and that said Letters Patent is hereby corrected as shown below:

In the Claims

Column 23, Claim 4, line 2, after the word "broth" and before "to", insert the following:
--is increased--

Column 24, Claim 6, line 2, after the word "pH" and before "8.5", insert the following: --to--

Signed and Sealed this
Eighth Day of April, 2014

Michelle K. Lee

Michelle K. Lee
Deputy Director of the United States Patent and Trademark Office

UNITED STATES PATENT AND TRADEMARK OFFICE

CERTIFICATE OF CORRECTION

PATENT NO.	: 8,338,141 B2
APPLICATION NO.	: 10/886863
DATED	: December 25, 2012
INVENTOR(S)	: Steve Lorbert et al.

It is certified that error appears in the above-identified patent and that said Letters Patent is hereby corrected as shown below:

In the Claims

Column 23, Claim 4, line 27, after the word "broth" and before "to", insert the following:
--is increased--

Column 24, Claim 6, line 2, after the word "pH" and before "8.5", insert the following: --to--

This certificate supersedes the Certificate of Correction issued April 8, 2014.

Signed and Sealed this
Sixth Day of May, 2014

Michelle K. Lee

Michelle K. Lee
Deputy Director of the United States Patent and Trademark Office