5-20-2019

Production of Single Cell Protein and Astaxanthin Using Methanol as Carbon Source

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Abstract
Singlecell protein (SCP) is the biomass of unicellular organisms, such as bacteria or yeast, which is used commonly as a food source for animals. With a high protein content, a broad amino acid profile, and the ability to produce essential organic compounds and vitamins, SCP is a promising alternative to other classical sources of animal feed. Several processes have been developed to manufacture SCP for use in feedstocks for the sustainable farming of fish and other aquatic life, or aquaculture, which is one of the fastest growing food markets in the world. Here, a process is presented for the production of 8,800 MT of SCP per year using methanotrophic bacteria with methanol as the carbon source. To increase process profitability, the cells will be genetically engineered to produce astaxanthin, a carotenoid pigment found naturally in aquatic algae. When used as a feed supplement for farmed salmon, these SCP will serve as a nutritional additive and ensure that the salmon possess the pink pigmentation consumers expect. The final product is SCP with 0.3% by weight astaxanthin sold for $16,500/MT. The high market price of astaxanthin significantly improves the profitability of the process. According to a 10year profitability analysis, the predicted IRR is 41.9%. In 2020, the Net Present Value of the project will be $129,000,000. In the third production year, the ROI will be 56.0%.

Disciplines
Biochemical and Biomolecular Engineering | Chemical Engineering | Engineering

Letter of Transmittal

University of Pennsylvania, School of Engineering and Applied Science
Department of Chemical and Biomolecular Engineering
220 South 33rd Street
Philadelphia, PA 19104

April 23, 2019

Dear Dr. Bomyi Lim and Professor Bruce Vrana,

The following report contains the design for a process that uses methanotrophic bacteria to produce single cell protein and astaxanthin. The process produces 8,800 MT of single cell protein and 26.4 MT of astaxanthin per year. By adding in the astaxanthin, the process has become more profitable than previous processes just producing single cell protein. The design includes seed reactor scale up, batch start up, continuous operation, purification, and packaging of the final product.

The economic analysis for the process synthesized shows very favorable results, especially compared to a process producing just single cell protein. The return on investment of the process is 56.0% during the third year of production. With just single cell protein production, the lower selling price gives a negative IRR and ROI. The IRR for the process is 41.9% and the net present value is $129,000,000. 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,

Corinne Pillarella Craig Jahnke Brielle Weiner

Production of Single Cell Protein and Astaxanthin Using Methanol as Carbon Source

Craig Jahnke
Corinne Pillarella
Brielle Weiner

Project Author: Dr. Jeffrey Cohen
Project Advisor: Dr. Bomyi Lim

University of Pennsylvania
School of Engineering and Applied Sciences
Department of Chemical and Biomolecular Engineering
April 23, 2019

Table of Contents

1. Abstract

Single-cell protein (SCP) is the biomass of unicellular organisms, such as bacteria or yeast, which is used commonly as a food source for animals. With a high protein content, a broad amino acid profile, and the ability to produce essential organic compounds and vitamins, SCP is a promising alternative to other classical sources of animal feed. Several processes have been developed to manufacture SCP for use in feedstocks for the sustainable farming of fish and other aquatic life, or aquaculture, which is one of the fastest growing food markets in the world. Here, a process is presented for the production of 8,800 MT of SCP per year using methanotrophic bacteria with methanol as the carbon source. To increase process profitability, the cells will be genetically engineered to produce astaxanthin, a carotenoid pigment found naturally in aquatic algae. When used as a feed supplement for farmed salmon, these SCP will serve as a nutritional additive and ensure that the salmon possess the pink pigmentation consumers expect. The final product is SCP with 0.3% by weight astaxanthin sold for $16,500/MT. The high market price of astaxanthin significantly improves the profitability of the process. According to a 10-year profitability analysis, the predicted IRR is 41.9%. In 2020, the Net Present Value of the project will be $129,000,000. In the third production year, the ROI will be 56.0%.

2. Introduction

2.1 Project Origin

Single cell protein (SCP) can be generated using a fermentation process of methanotrophic bacteria with methanol, ammonia, oxygen, and trace elements as raw materials. The product of this fermentation process is used as feed for various fish and crustaceans. However, this process has proven in some cases to be unprofitable because of low market prices for SCP. To increase revenue per unit of cell mass, the original cell lines can be genetically modified to produce astaxanthin along with SCP.

Astaxanthin is a 40-carbon carotenoid that enhances pigmentation and antioxidant activity in salmon. The chemical structure of astaxanthin is shown in Figure 2.1b (2). Astaxanthin is naturally present in the diet of wild salmon through eating astaxanthin-rich plankton. Astaxanthin is beneficial to the health of the fish as it is a powerful antioxidant that has anti-inflammatory qualities, improves blood flow, and protects and enhances mitochondria (1). When farmed salmon are fed a diet with astaxanthin, they appear more vibrant when on the shelf and more visually similar to wild caught salmon. Therefore, the fish are more visually appealing to consumers in the grocery store. A photo of regular farmed salmon (left) versus wild salmon with an astaxanthin-rich diet (right) is shown in Figure 2.1a to demonstrate the drastic effect that astaxanthin has on the appearance of the salmon.

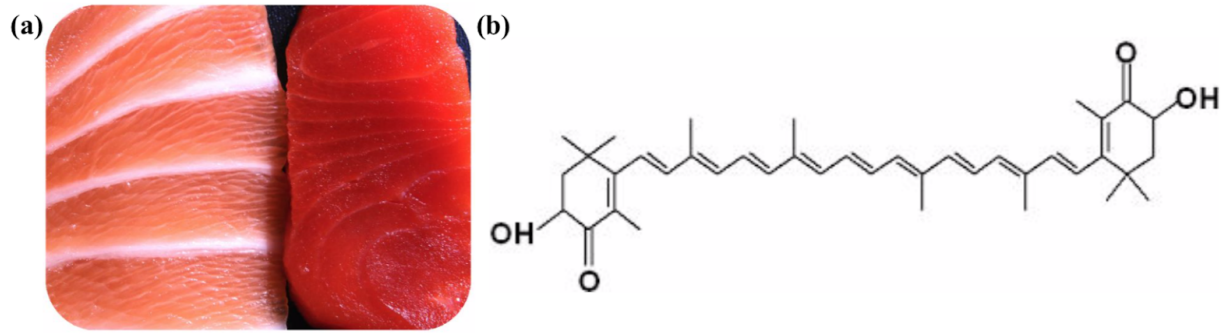


Figure 2.1. (a) Coloration of traditional farmed vs. wild, astaxanthin rich salmon (1). (b). Chemical structure of astaxanthin (2).

The SCP production process is loosely based on that developed by Norferm, a Norwegian subsidiary of DuPont and StatOil that operated from 1998 to 2005. This company used a bacterial fermentation process to produce SCP. However, the company was forced to liquidate in 2006 due to low fishmeal prices (3). Another company, Calysta, is currently producing SCP product successfully using the Norferm process and technology for fermentation (4). It is important to note that neither of these companies created astaxanthin along with SCP, which sets the process design developed in this report apart from current industry standards.

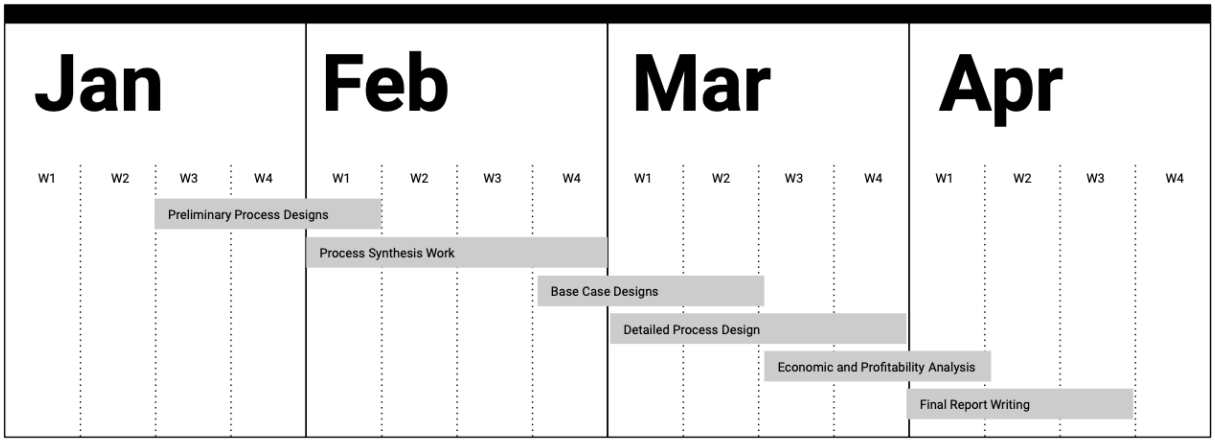
2.2 Project Goals and Scope

The goal of this project was to design a continuous process to generate a product consisting of SCP with 0.3% by weight astaxanthin using methanol as a carbon source. Ideally, the process would be more profitable than one simply creating SCP. Based solely on the market prices of SCP and astaxanthin, generating astaxanthin in the process should increase revenue approximately ten-fold. In order to run the process according to all of the process specifications, many important intermediate goals were met as well. These included but were not limited to

affordable raw material acquisition, selection of geographic location, and selection of process equipment.

This project was completed based on the assumption that methanotrophic bacteria could be genetically modified to create astaxanthin along with SCP. The scope of this design project began with the seed production of these cells which were assumed to be previously acquired by a third-party genetic engineering company. The raw material demands and process specifications for the seed batch processes as well as the continuous reactor process were determined. The project scope with respect to the process ends after the product is packaged. In addition to process specifications, economic and policy analysis were also completed to determine the validity and efficacy of the process created.

2.3 Objective Time Chart



Above is a graphic showing the proposed timeline of the major steps necessary to complete this report. Specifically, the tasks completed in January included preliminary research on the process and its products, an investigation into the scope of the market for SCP and astaxanthin, and an initial profitability analysis. In February, the raw materials that would serve

as the carbon and nitrogen sources were determined, a process flow diagram was created, and the ideal plant location was selected based on cost and access to resources. The work in March consisted of finalizing several important details for the design of the process such as the major equipment and the bioreactor operating conditions. With the process design completed, a comprehensive economic analysis of the project was begun. Finally, in April, the financial analysis was completed before beginning to synthesize all the relevant findings into the report. The report was then revised and edited before being submitted for final review.

2.4 Project Charter

Project Name: Production of Single Cell Protein and Astaxanthin Using Methanol as Carbon Source

Project Leaders: Corinne Pillarella, Brielle Weiner, Craig Jahnke

Specific Goals: The goal of this project is to develop a process to produce single cell protein and astaxanthin using genetically engineered methanotrophic bacteria. This process should be more profitable than a plant producing just single cell protein, as the market price for astaxanthin is comparatively high.

Project Scope:

In Scope:

- Design of seed train to grow cell mass from lab scale to continuous fermenter size
- Design process for two large scale fermenters
- Downstream purification of product including centrifugation and drying
- Financial analysis including evaluation of cost, profitability, and cash flow

Out of Scope:

- Genetic modification of cell line
- Safety approval needed from governments where the product is manufactured and sold
- Distribution of product to consumer

Deliverables

Market Assessment to Determine Potential for Profit

- Verification that market demands for single cell protein and astaxanthin support the profitability of the production process

Technical Process Design

- Verification that a process can be designed to produce 8,800 MT of cell mass annually

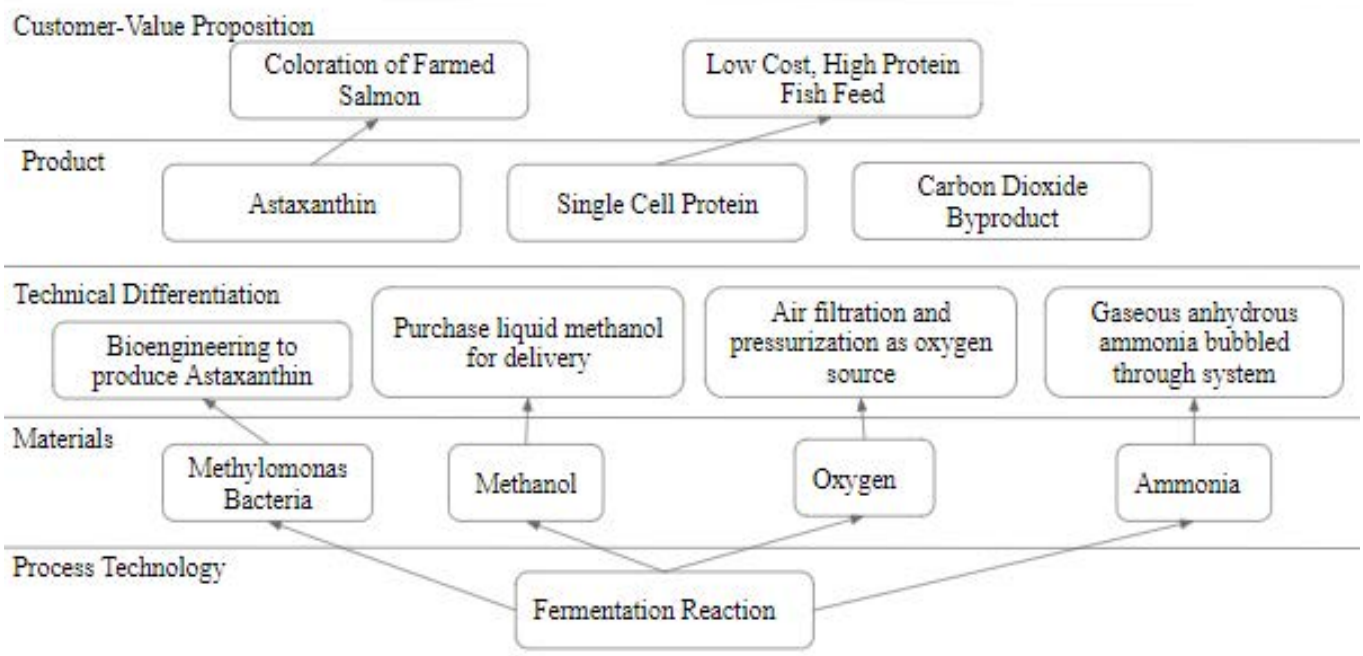
Manufacturing Feasibility Assessment

- Determination of best geographic plant location
- Verification that the process designed is in fact profitable and market-ready

Timeline

Time to design and build the plant along with 10 years of production and sales.

3. Innovation Map



4. Market and Competitive Assessment

4.1 Astaxanthin

The market for astaxanthin has been growing in recent years as its applications have been widened from aquaculture and animal feed to nutraceuticals and cosmetics. In 2017, the global astaxanthin market size was over $550 million and has continued to grow (5). Currently, aquaculture and animal feed remains the largest market segment for astaxanthin at around 45%, but the human markets are growing at a fast pace (6). Astaxanthin is necessary to include in aquaculture feed, particularly in salmon, since wild salmon get astaxanthin naturally through their diets of astaxanthin-rich plankton. Astaxanthin provides the pink pigmentation necessary for farmed salmon to be marketed appropriately and acts as a powerful antioxidant to increase salmon growth and health.

The astaxanthin market is currently dominated by synthetic production techniques involving chemical synthesis of three different astaxanthin isomers. This synthetic astaxanthin is commonly derived from petrochemicals and thus raises issues of concern for its biological functions and food safety (7). Some major synthetic producers of astaxanthin include DSM in the Netherlands, BASF in France, and NHU in China. Pure synthetic astaxanthin has an average selling price of around $2,000/kg and average production cost of $1,000/kg and is only approved for use in the aquaculture feed market.

For astaxanthin to be used for human consumption, it must be produced naturally through sources such as algae, yeast, and bacteria. This natural astaxanthin is viable for use in human

markets and has been found to provide better pigmentation for aquaculture (7). Natural astaxanthin is made only from two of the three isomers found in synthetic astaxanthin. Thus, natural astaxanthin is often preferred and has a higher selling price of up to $7,000/kg. Some of the major natural producers of astaxanthin include BGG in China and Algatech in Israel.

4.2 Single Cell Protein

Currently about 14 million MT of cereal, plant-based protein are consumed annually for aquaculture feed. Additionally, due to increasing fishing regulations, the ability to use by-catch as aquaculture feed is decreasing and alternative food sources are needed to support this large global market. SCP is a high protein content alternative to plant-based proteins for aquaculture and animal feed derived from bacteria, algae, or fungi. Given the high protein content of around 50-80%, SCP has the potential to replace up to 50% of fish meal. Additionally, feeding trials with salmon showed improved salmon growth rates of up to 0.2% per day with SCP in comparison to more traditional cereal proteins. Due to these factors, the market for SCP is growing globally to meet increasing demand. Some major producers of bacterial SCP from methane or methanol fuel sources include Imperial Chemical Industries, Unibio A/S and Calysta (8). These products sell for around $1,500/MT. Some SCP manufacturers, including Norferm, struggle economically due to the relatively high production and selling costs of SCP in comparison to the low cost of bulk cereal protein.

4.3 Our Product

Given that our product would consist of SCP derived from bacteria and 0.3% by weight of astaxanthin, the selling cost would be around $16.50/kg intended for the aquaculture market. Sensitivity analysis on the possible product prices will be presented in Section 20.2. Use in this market prevents the necessity of separating the two products after creation as they can instead by sold together and diluted with bulk cereal protein for aquaculture feed. Astaxanthin need only be in farmed salmons' diets at a concentration of 60 mg/kg feed, so salmon could be fed our astaxanthin product at a mass ratio of 1 to 50 with bulk cereal protein to be sufficient (9). With these regulations in mind, at the production rate of 8,800 MT/year, our product would be able to feed about 326,000 MT of salmon per year, representing about 14.5% of the total worldwide salmon market (10). This product with approximately 70% protein content would distinguish our business economically from those of solely SCP manufacturers. In order to have these two components produced together, the bacteria will need to be genetically modified, which raises the concern of introducing genetically modified organism (GMO) labelling and approval into our product and process. The GMO approval process will be discussed in Section 21.5.

5. Customer Requirements

N/A

6. CTQ Variables-Product Requirements

N/A

7. Product Concepts

Astaxanthin has applications in industries other than fish feed, including cosmetics, nutritional supplements for humans, and food coloring. For this reason, the benefits of separating the astaxanthin from the SCP were considered. By separating the products, the astaxanthin could potentially be used in the salmon feed market and in the human market. However, rigorous purification and health safety requirements would need to be met in order to enter the human market. These additional requirements could take many years, preventing any revenue long into the future. In addition to the added cost of purchasing and upkeep of separation equipment, gaining approval for products for human consumption can cost millions of dollars, potentially threatening the profitability of the process. Lastly, although the human consumption market for astaxanthin is growing, it is only 20% of the current fish feed market.

8. Superior Product Concepts

The product selected was a mixture of SCP and astaxanthin with astaxanthin at a concentration of 0.3% by weight. This product will be sold only in the fish feed market. It will presumably be mixed with other forms of fish food in order to dilute the astaxanthin to the concentration desired by the fish farmer. This product was selected because additional purity requirements needed for human consumption will not need to be met. In addition, the fish food market is much larger than the human consumption market for astaxanthin. This method will also avoid additional equipment cost for SCP and astaxanthin separation.

9. Competitive Patent Analysis

The process detailed in this report was influenced by previous processes detailed in patent literature. Several patents exist for the production of SCP using methanotrophic bacteria or yeast. One such patent, U.S. Patent 4,048,013, was used as a reference for much of the design of the cell culture techniques used in this report. The patent, titled *Process for producing single-cell protein from methanol using methylomonas sp. DSM 580*, was published in 1976 and describes the cell culturing conditions needed to successfully produce SCP from a methanotrophic bacteria strain, Methylomonas sp. DSM 580, fed with methanol (11). The authors detail the temperature, methanol and trace mineral concentrations, and growth time for bacterial cultures of 80 L and 340 L. The relevant patents for this report can be found in full in Appendix F.

As previously discussed, several other companies such as Norferm and Calysta have developed processes for producing high quality SCP from methanotrophic bacteria fed with methanol or methane. While the culturing techniques used and the nutritional value of the SCP produced may be quite similar, the distinguishing feature of this process is the ability of the bacteria to produce astaxanthin.

The technology needed to genetically modify the bacterial cells to produce this secondary product is well known. Furthermore, in the 1980 case *Diamond vs. Chakrabarty*, the Supreme Court of the U.S. ruled that genetically modified organisms such as this are eligible to be patented. Similar policies on biological patents exist in other countries, such as Canada and the European Union members.

10. Preliminary Process Synthesis

10.1 Fermentation Chemistry

The growth and fermentation of methanotrophic bacteria requires an aerobic process with methanol as the fuel source and ammonia as the nitrogen 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 (12). Thus, in order for bacteria to grow and multiply, all of these elements must be introduced in the reactor feed through mineral solutions or fuel sources. In the case of methanotrophic bacteria, methane or methanol must be used as the carbon source and ammonia must be used as the nitrogen source. The chemistry for methanotrophic bacteria using methane as fuel and converting methane to carbon dioxide can be seen in Figure 10.1 (13). In this process, it can be seen that in an aerobic process, methane is first converted to methanol, which is why methanol is also a viable carbon fuel source for this strain of bacteria. The methanol is then converted to formaldehyde and formic acid before ultimately being released as carbon dioxide.

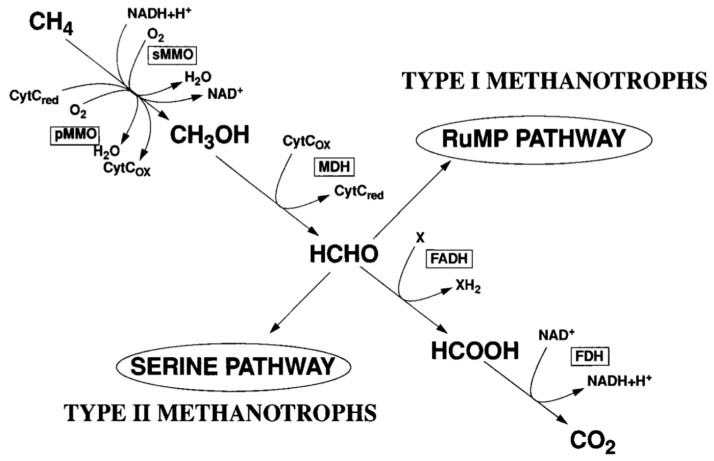


Figure 10.1. Chemical pathway for aerobic use of methane as a carbon fuel source for methanotrophic bacterial growth (13).

10.2 Alternative Carbon Fuel Source

Many decisions were made in the design of this process regarding the choice of raw materials and processing steps. Most importantly, the decision was made to use methanol as the fuel source rather than methane for a variety of reasons including ease of delivery and preparation, cooling requirements, oxygen requirements, safety considerations, and overall cost. In this process, when methane enters as fuel for the methanotrophic bacteria, the bacteria immediately converts the methane to methanol, which explains why methanol is also a viable fuel source for this strain of bacteria. This conversion process of methane to methanol is highly exothermic, contributing to 60% more heat created in the process and thus 60% more cooling required. Since only the enthalpy of fermentation of methane was given in the project statement, the change in enthalpy of fermentation what estimated by finding the ratio of the heat of combustions of methanol to methane. This ratio was then applied to the given enthalpy of fermentation to find that the enthalpy of fermentation decreases from -23.9 kJ/g dry mass to -9.76 kJ/g dry mass.

The use of methane would also require much greater amounts of oxygen fed to the process since oxygen is required in the conversion of methane to methanol. This oxygen would need to be introduced into the system in the form of nearly pure oxygen as opposed to air, which greatly increases reactant costs and introduces some safety concerns regarding the flammability and explosivity limits of methane-oxygen systems.

Finally, methanol can be directly delivered as a liquid from manufacturers with plants conveniently located within Canada for use in the fermentation process. Due to the large quantities of methanol required for this process, delivery and storage in a liquid form is most

convenient and economical. Methane, however, is a product of natural gas which would need to be separated either through the building of on an onsite natural gas separation plant or the purchase of nearly pure methane from an outside manufacturer. Since methane is in gas form, delivery of this material at the large quantities needed would prove much more difficult and costlier. As described in the project statement, higher alkanes in the fuel feed will be converted to organic acids by the bacteria which will slow or stop cell growth, thus separation of the natural gas would be essential. While the cost of purchasing pure methane at $290/MT is less than the cost of purchasing methanol at $432/MT, it was ultimately decided that this material cost does not make up for the additional risks and other costs associated with the use of methane, further justifying this choice in fuel.

10.3 Alternative Nitrogen Source

The nitrogen source for this process needed to be an ammonia product, either ammonium hydroxide or anhydrous ammonia. The decision was made to use anhydrous ammonia for a variety of reasons, including the higher concentration of ammonia and the prevalence of anhydrous ammonia suppliers due to its very common use in farming as a fertilizer. According to Nutrien's material data sheets, their anhydrous ammonia product is guaranteed to be at least 99.5% ammonia by weight whereas their ammonium hydroxide product is only guaranteed to be 29.5% ammonia by weight (14). Because of this, using anhydrous ammonia will be much more effective as a reactant in the fermentation process. Additionally, anhydrous ammonia is readily available in bulk quantities from suppliers all over the world including close to our chosen plant location in Canada due to its common use as a farm fertilizer. The price difference between the

two products is negligible and thus does not provide any additional reasoning to consider using ammonium hydroxide over anhydrous ammonia.

10.4 Alternative Cooling Methods

In order to achieve proper temperature regulation in this process, an extensive cooling system is needed to combat the highly exothermic bacterial fermentation process. It was found that with an enthalpy of fermentation of -9.76 kJ/g dry mass, the total cooling required for each 150,000 L bioreactor would be 1,708.3 kW to maintain a temperature of 33°C. Multiple methods of cooling were considered including a cooling jacket around the bioreactor, cooling the inlet material streams, cooling the recycled media after sterilization, and building a recycle cooling loop. After some initial calculations, it was found that the cooling jacket would only be able to provide 18% of the cooling necessary (15). Because of the relatively higher price of adding a cooling jacket in comparison to the low levels of cooling it would contribute, the decision was made to forgo adding a cooling jacket to the large bioreactors. It was found, however, that a cooling jacket would be sufficient for heat exchange in the smaller bioreactors of the seed train.

Similarly, it was decided that based on the flow rates of the sterilized media and methanol streams into the bioreactor, cooling these streams alone would not provide adequate cooling for the bioreactor. Resultantly, it was decided that a large heat exchanger would need to be added to cool the bioreactor by continuously recycling a portion of the bioreactor volume and cooling the stream with cooling water. It was found that this heat exchanger would need a batch turnover rate of about 1.7 batches per hour with the specifications of heat exchanger dimensions, stream temperatures, and flow rates given in Section 15.3.4.

10.5 Alternative Plant Locations

A variety of different plant locations were considered when designing this process before deciding to operate the plant in Alberta, Canada. The United States, while convenient because of personal familiarity and relaxed GMO regulations, was not strongly considered because of its fairly small salmon market—most of the salmon consumed in the United States is internationally imported. Instead, the locations most strongly considered were those of the largest salmon producers in the world due to ease of accessibility to customers, namely Norway, Chile, and Canada.

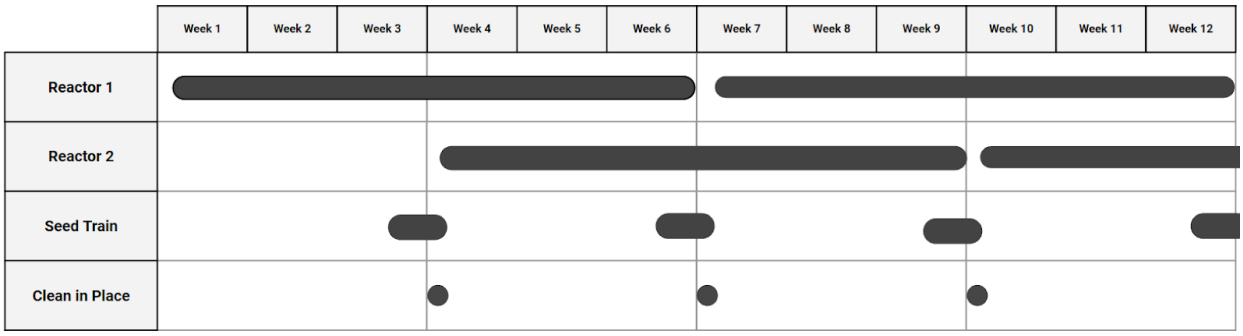
Additionally, while Chile is the 2nd largest producer of farmed salmon, Chile's aquaculture market currently has some concerns including a large algae boom due to natural disasters and rising seawater temperatures and disease outbreaks of deadly *P. salmonis* bacteria and resultantly very high amounts of antibiotic use (16). Furthermore, Chile's GMO regulations are fairly strict in that the country allows imports GMO crops and exports of GMO seeds but does not allow for the cultivation of GMO crops, so getting this product approved might be rather difficult.

Norway was strongly considered as the operation site for this plant because it is the largest producer of farmed salmon in the world, has a cold climate supportive of the necessary extensive cooling, and has methanol and anhydrous ammonia plants conveniently located within the country. Additionally, Norway has economic incentives for new businesses which would be generally beneficial for building this plant. However, Norway has extremely strict GMO regulations in that it does not allow for any GMO production within Norway for domestic use or for export according to the *Norwegian Gene Technology Act*. Even if the product were to be

allowed in Norway, the overwhelmingly negative public perception of GMOs would deter salmon farmers from purchasing the product (17). This limiting factor focused a different location to be chosen for plant operation.

Ultimately, Alberta, Canada was chosen as the ideal plant location for this process. Canada's cold climate can help assist in the cooling efforts needed in this process by keeping cooling water and raw materials at a low initial temperature. The convenient location of both anhydrous ammonia and methanol plants with rail delivery lines within Alberta was also a strong driving force for choosing this exact location. Canada is currently the 4th largest producer of farmed salmon in the world and is known to have very relaxed regulations on GMO products, making the product approval process fairly quick and easy for convenient use by customers located domestically. A more detailed description of this plant location can be found in Section 21.1.

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 340 day per year schedule. The two large 150,000 L fermenters will run continuously in 6-week reactor campaigns before operation must be suspended, and there will be approximately 8 such reactor campaigns per fermenter per year.

The operating schedule of the two reactors will be staggered such that a new campaign will begin at the start of the fourth week of the other reactor's campaign. When a large fermenter campaign ends, the Clean-in-Place (CIP) system will begin to sterilize the reactor, and this process will take approximately 24 hours. The reactor seed train process with 6 seed reactors will be initiated 24 hours before the end of a reactor. This gives 48 hours for the seed train to be completed before the reactor is ready to be inoculated with cells from the seed train to begin a new campaign. If unexpected events cause this time period to change, technicians will be able to lengthen or shorten the seed train process if necessary. Once inoculated, the large fermenter will have a 6-hour batch period before continuous operations resume.

A seed train is used in this process to grow new batches of cells in a timely manner with appropriate quality checks in between each growth stage. As a general rule of thumb, subsequent reactors in a seed train should be no more than 20 times larger than the previous reactors to ensure that time is not wasted on batch growth processes should a batch prove below quality standards. The estimated batch times for each of the 6 seed reactors to get to the peak cell concentration of 35 g/L are summarized in Table 10.6. These batch times were calculated based on the cell doubling time of 1.5 hours. Each subsequent reactor will be inoculated with 80% of the contents of the previous reactor to allow for some flexibility in the seed train process. To slow down the seed train process, the reactor can be inoculated with less than 80% and to speed up the process, the reactor can be inoculated with more than 80%. Assuming 80% transfer, the seed train process will take a total of 38.81 hours. This can be increased and decreased according to process needs by adjusting the percentage of mass transferred from one seed reactor to the next. The leftover bioreactor contents will be appropriately disposed of through the biowaste

inactivation system. Additionally, each of the batch times was calculated based only on the growth phase, also known as the log phase, of cell growth. The stationary phase of cell growth, also known as the lag phase, 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 seed train reactors, adding to a total of 38.81 hrs.

Reactor size (L)	**Reactor #**	**Startup time (hr)**	**Initial Cell Mass (g)**
0.01	1	6.48	0.014
0.1	2	5.47	0.224
2	3	6.97	2.24
25	4	5.95	44.8
500	5	6.97	560
10000	6	6.97	11200

11. Assembly of Database

11.1 Cost of Chemicals

The main raw materials in this process are methanol, ammonia, oxygen, and methylomonas bacteria. The prices for buying these materials in bulk were obtained from the websites of various vendors in Alberta, Canada within an hour driving distance of the planned plant location. Methanol will be purchased from Methanex for $432/MT and delivered on rail from their plant located in Medicine Hat, Alberta (18). Anhydrous ammonia will be purchased as a liquified compressed gas from Nutrien for $473/MT and delivered on rail from their plant located in Carseland, Alberta (19). The costs of water and utilities including tap water, cooling water, steam, and electricity were taken from standard guidelines and are summarized in Section 19. Air will be used as the oxygen source for the process, the cost of which is covered in utilities costs and unit costs for the air compressor and air filters. Costs of the trace minerals were estimated from Alibaba for bulk mineral purchases of bags 1 MT to 25 MT in size. The methylomonas bacteria will be genetically modified by a third party and purchased for the development of a cell bank onsite. The cost for this specialized strain of bacteria is estimated to be $438 for a 0.4 mL aliquot of cells, as found from a similar bacterial strain being sold by Cedarlane Laboratories (20). The cost of the genetic modification is included in the service fees in later economic analysis.

The product for sale is a mixture of SCP and astaxanthin at 0.3% by weight. Based on the market price of SCP of $1,500/MT and a conservative price of natural astaxanthin of $5,000/kg, this mixture product should sell for $16,500/kg. This value was obtained by multiplying the market price of the products separately by their mass fractions in the overall product. Sensitivity

analysis of the product selling price is presented in Section 20.2. The raw costs of all reactants and products are summarized in Table 11.1.

Table 11.1. Costs of all chemical reactants and products in USD per MT unless otherwise specified.

Chemical Name	Cost ($USD/MT)
Methanol	$432
Anhydrous ammonia	$473
Water	--
Air	--
Methylomonas bacteria	$438/ 0.4 mL aliquot
Disodium hydrogen phosphate	$800
Potassium dihydrogen phosphate	$1,000
Magnesium sulfate heptahydrate	$100
Calcium chloride	$200
Iron (II) sulfate heptahydrate	$100
SCP/Astaxanthin product	$16,500

11.2 Chemical Components and Thermophysical Properties

The Material Safety Data Sheet (MSDS) for all chemical components can be found in Appendix E. Considerations based on safety of the chemicals were determined using the information from these MSDS. No ASPEN simulations were used in the design of this process, so a complete database of chemical properties was not established. For calculations involving heat exchange and cooling, the system was assumed to have the same heat capacity and overall heat transfer coefficient as water. For the mass balance, the elemental makeup of the bacterial cells was assumed using general information about bacterial cells to include carbon, oxygen, nitrogen, hydrogen, phosphorus, sulfur, potassium, magnesium, calcium, and iron (12).

11.3 Cell Growth Kinetics and Bioreactor Rates

Information on cell growth kinetics and the bioreactor functionality was provided in the project statement. The project statement explained that the cell doubling time is 1.5 hours and that the peak cell density in the bioreactor on a bone-dry basis is 35 g/L. It was assumed that during the continuous phase of bioreactor operation, the concentration of cells was present at this peak cell density. It was concluded from U.S. Patent 4,048,013 that methanol should be introduced in the system at a weight ratio of 1 g methanol/0.44 g cell dry mass, which is the basis for the feed flow rate in this system (11). From U.S. Patent 4,166,004, it was found that the bioreactor should be maintained at a pH between 6.0 and 7.2, and the methanol concentration cannot exceed 1%, though the ideal concentration ranges from 5 to 100 ppm (21). The bulk density of the dried biomass is 0.7 kg/L. Additionally, the bioreactor can be run for 6 weeks 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 bioreactors increasing in size until the peak cell density in the largest bioreactor is obtained and the continuous process can be initiated.

12. Process Flow Diagrams and Material Balances

12.1 Overall Flow Sheet

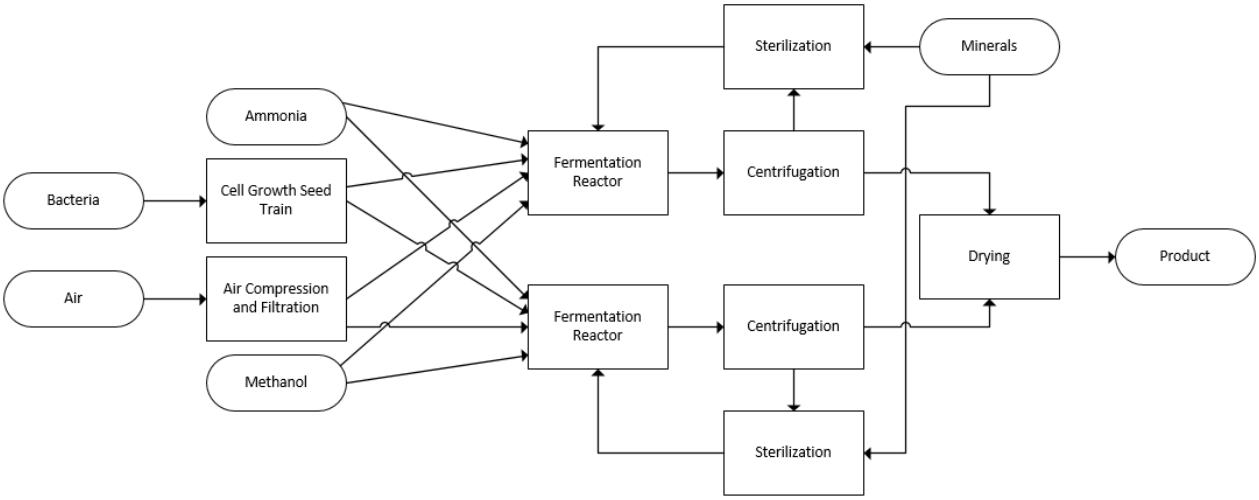


Figure 12.1. Overall flowsheet for the process including all major processes, reactants, and products.

12.2 Seed Reactor Design

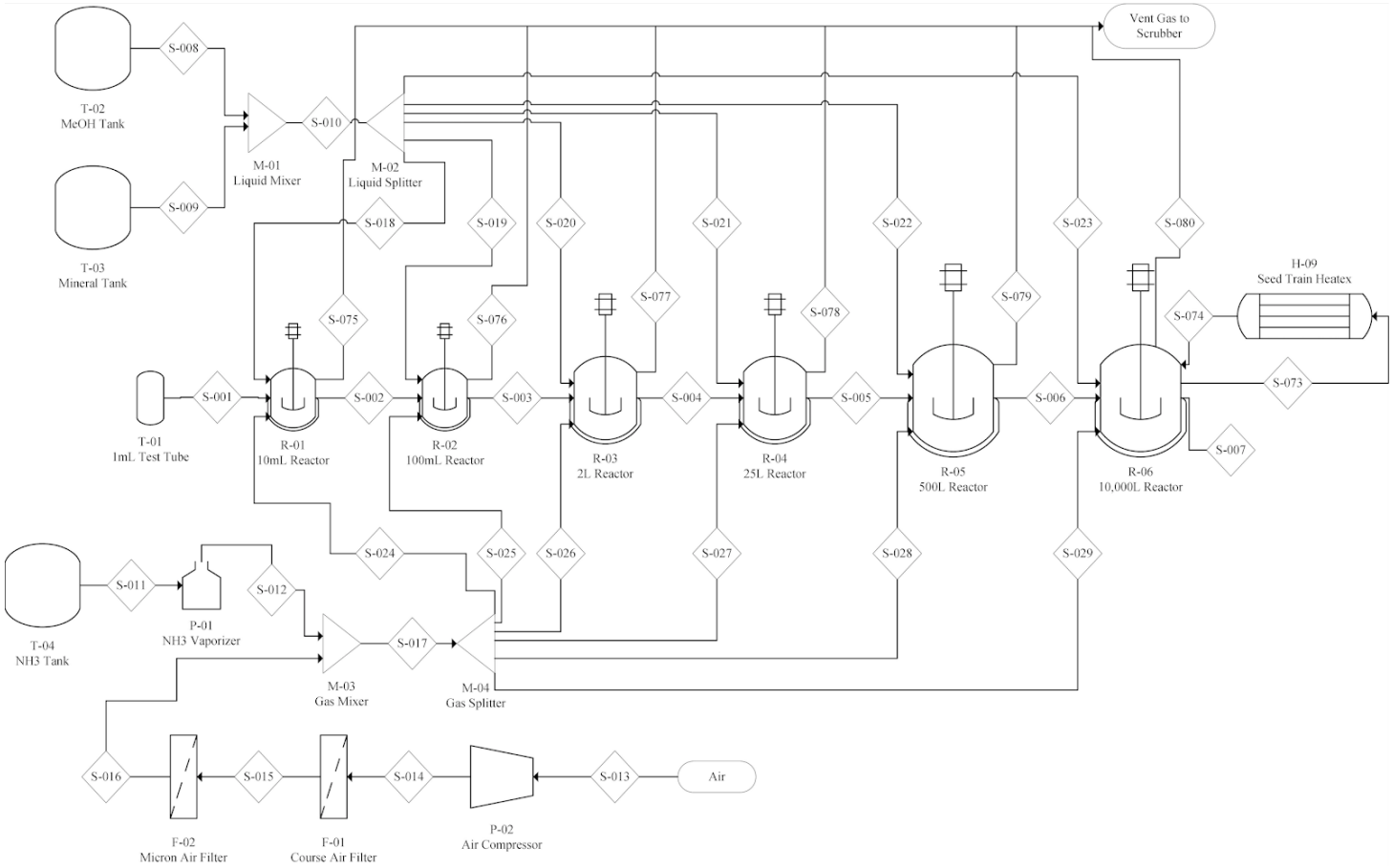


Figure 12.2. Flowsheet for the seed train reactors and reactant processing steps.

12.3 Seed Reactor Mass Balances

Table 12.3.1. Mass balances for the six batch seed reactors.

Seed Reactor 1							
Stream	**S-008**	**S-009**	**S-010**	**S-018**	**S-001**	**S-011**	**S-012**
Description	**Methanol in**	**Mineral/water in**	**Methanol/mineral /water mix**	**S-010 connect**	**From aliquot**	**Ammonia tank**	**Vaporized ammonia**
Temp (°C)	20.0	20.0	20.0	20.0	32.0	20.0	20.0
Pressure (bar)	1.0	1.0	1.0	1.0	1.0	1.0	1.5
Total Mass (mg/batch)	604.5	7,649.8	8,254.3	8,254.3	1,014.0	25.8	25.8
Component Mass (mg/batch)							
Cell Mass	0.0	0.0	0.0	0.0	14.0	0.0	0.0
Astaxanthin	0.0	0.0	0.0	0.0	0.0	0.0	0.0
Methanol	604.5	0.0	604.5	604.5	0.0	0.0	0.0
Ammonia	0.0	0.0	0.0	0.0	0.0	25.8	25.8
Water	0.0	7,600.0	7,600.0	7,600.0	1,000.0	0.0	0.0
Carbon Dioxide	0.0	0.0	0.0	0.0	0.0	0.0	0.0
Air	0.0	0.0	0.0	0.0	0.0	0.0	0.0
Oxygen	0.0	0.0	0.0	0.0	0.0	0.0	0.0
Nitrogen	0.0	0.0	0.0	0.0	0.0	0.0	0.0
Mineral Mix	0.0	49.8	49.8	49.8	0.0	0.0	0.0

Seed Reactor 1 continued								
Stream	**S-013**	**S-014**	**S-015**	**S-016**	**S-017**	**S-024**	**S-002**	**S-075**
Description	**Air in**	**Compressed air**	**Filter 1 air**	**Filter 2 air**	**Air/ammonia mix**	**S-017 connect**	**Product to next reactor**	**Gas Vent**
Temp (°C)	20.0	20.0	20.0	20.0	20.0	20.0	32.0	32.0
Pressure (bar)	1.0	1.5	1.5	1.5	1.5	1.5	1.2	1.2
Total Mass (mg/batch)	3,689.0	3,689.0	3,689.0	3,689.0	3,714.8	3,714.8	8,824.0	3,288.8
Component Mass (mg/batch)								
Cell Mass	0.0	0.0	0.0	0.0	0.0	0.0	224.0	0.0
Astaxanthin	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
Methanol	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
Ammonia	0.0	0.0	0.0	0.0	25.8	25.8	0.0	0.0
Water	0.0	0.0	0.0	0.0	0.0	0.0	8,600.0	81.6
Carbon Dioxide	0.0	0.0	0.0	0.0	0.0	0.0	0.0	399.0
Air	3,689.0	3,689.0	3,689.0	3,689.0	3,689.0	3,689.0	0.0	N/A
Oxygen	774.7	774.7	774.7	774.7	774.7	774.7	0.0	258.2
Nitrogen	2,914.3	2,914.3	2,914.3	2,914.3	2,914.3	2,914.3	0.0	2,550.0
Mineral Mix	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0

Seed Reactor 2							
Stream	**S-008**	**S-009**	**S-010**	**S-019**	**S-002**	**S-011**	**S-012**
Description	**Methanol in**	**Mineral/water in**	**Methanol/mineral /water mix**	**S-010 connect**	**From last reactor**	**Ammonia tank**	**Vaporized ammonia**
Temp (°C)	20.0	20.0	20.0	20.0	32.0	20.0	20.0
Pressure (bar)	1.0	1.0	1.0	1.0	1.2	1.0	1.5
Total Mass (g/batch)	5.9	76.5	82.4	82.4	8.8	0.3	0.3
Component Mass (g/batch)							
Cell Mass	0.0	0.0	0.0	0.0	0.2	0.0	0.0
Astaxanthin	0.0	0.0	0.0	0.0	0.0	0.0	0.0
Methanol	5.9	0.0	5.9	5.9	0.0	0.0	0.0
Ammonia	0.0	0.0	0.0	0.0	0.0	0.3	0.3
Water	0.0	76.0	76.0	76.0	8.6	0.0	0.0
Carbon Dioxide	0.0	0.0	0.0	0.0	0.0	0.0	0.0
Air	0.0	0.0	0.0	0.0	0.0	0.0	0.0
Oxygen	0.0	0.0	0.0	0.0	0.0	0.0	0.0
Nitrogen	0.0	0.0	0.0	0.0	0.0	0.0	0.0
Mineral Mix	0.0	0.5	0.5	0.5	0.0	0.0	0.0

Seed Reactor 2 continued								
Stream	**S-013**	**S-014**	**S-015**	**S-016**	**S-017**	**S-025**	**S-003**	**S-076**
Description	**Air in**	**Compressed air**	**Filter 1 air**	**Filter 2 air**	**Air/ammonia mix**	**S-017 connect**	**Product to next reactor**	**Gas vent**
Temp (°C)	20.0	20.0	20.0	20.0	20.0	20.0	32.0	32.0
Pressure (bar)	1.0	1.5	1.5	1.5	1.5	1.5	1.2	1.2
Total Mass (g/batch)	32.5	32.5	32.5	32.5	32.7	32.7	86.8	32.1
Component Mass (g/batch)								
Cell Mass	0.0	0.0	0.0	0.0	0.0	0.0	2.2	0.0
Astaxanthin	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
Methanol	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
Ammonia	0.0	0.0	0.0	0.0	0.3	0.3	0.0	0.0
Water	0.0	0.0	0.0	0.0	0.0	0.0	84.6	0.8
Carbon Dioxide	0.0	0.0	0.0	0.0	0.0	0.0	0.0	3.9
Air	32.5	32.5	32.5	32.5	32.5	32.5	0.0	N/A
Oxygen	7.6	7.6	7.6	7.6	7.6	7.6	0.0	2.5
Nitrogen	24.9	24.9	24.9	24.9	24.9	24.9	0.0	24.9
Mineral Mix	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0

Seed Reactor 3							
Stream	**S-008**	**S-009**	**S-010**	**S-020**	**S-003**	**S-011**	**S-012**
Description	**Methanol in**	**Mineral/water in**	**Methanol/mineral/ water mix**	**S-010 connect**	**From last reactor**	**Ammonia tank**	**Vaporized ammonia**
Temp (℃)	20.0	20.0	20.0	20.0	32.0	20.0	20.0
Pressure (bar)	1.0	1.0	1.0	1.0	1.2	1.0	1.5
Total Mass (g/batch)	122.5	1530.1	1,652.6	1,652.6	86.8	5.2	5.2
Component Mass (g/batch)							
Cell Mass	0.0	0.0	0.0	0.0	2.2	0.0	0.0
Astaxanthin	0.0	0.0	0.0	0.0	0.0	0.0	0.0
Methanol	122.5	0.0	122.5	122.5	0.0	0.0	0.0
Ammonia	0.0	0.0	0.0	0.0	0.0	5.2	5.2
Water	0.0	1,520.0	1,520.0	1,520.0	84.6	0.0	0.0
Carbon Dioxide	0.0	0.0	0.0	0.0	0.0	0.0	0.0
Air	0.0	0.0	0.0	0.0	0.0	0.0	0.0
Oxygen	0.0	0.0	0.0	0.0	0.0	0.0	0.0
Nitrogen	0.0	0.0	0.0	0.0	0.0	0.0	0.0
Mineral Mix	0.0	10.1	10.1	10.1	0.0	0.0	0.0

Seed Reactor 3 continued								
Stream	**S-013**	**S-014**	**S-015**	**S-016**	**S-017**	**S-026**	**S-004**	**S-077**
Description	**Air in**	**Compressed air**	**Filter 1 air**	**Filter 2 air**	**Air/ammonia mix**	**S-017 connect**	**Product to next reactor**	**Gas vent**
Temp (℃)	20.0	20.0	20.0	20.0	20.0	20.0	32.0	32.0
Pressure (bar)	1.0	1.5	1.5	1.5	1.5	1.5	1.2	1.2
Total Mass (g/batch)	678.2	678.2	678.2	678.2	683.4	683.4	1,649.4	670.7
Component Mass (g/batch)								
Cell Mass	0.0	0.0	0.0	0.0	0.0	0.0	44.8	0.0
Astaxanthin	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
Methanol	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
Ammonia	0.0	0.0	0.0	0.0	5.2	5.2	0.0	0.0
Water	0.0	0.0	0.0	0.0	0.0	0.0	1604.6	16.6
Carbon Dioxide	0.0	0.0	0.0	0.0	0.0	0.0	0.0	81.2
Air	678.2	678.2	678.2	678.2	678.2	678.2	0.0	N/A
Oxygen	158.0	158.0	158.0	158.0	158.0	158.0	0.0	52.7
Nitrogen	520.2	520.2	520.2	520.2	520.2	520.2	0.0	520.2
Mineral Mix	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0

Seed Reactor 4							
Stream	**S-008**	**S-009**	**S-010**	**S-021**	**S-004**	**S-011**	**S-012**
Description	**Methanol in**	**Mineral/water in**	**Methanol/mineral/ water mix**	**S-010 connect**	**From last reactor**	**Ammonia tank**	**Vaporized ammonia**
Temp (°C)	20.0	20.0	20.0	20.0	32.0	20.0	20.0
Pressure (bar)	1.0	1.0	1.0	1.0	1.2	1.0	1.5
Total Mass (g/batch)	1,493.6	19,122.5	20,616.1	20,616.1	1,649.4	63.6	63.6
Component Mass (g/batch)							
Cell Mass	0.0	0.0	0.0	0.0	44.8	0.0	0.0
Astaxanthin	0.0	0.0	0.0	0.0	0.0	0.0	0.0
Methanol	1,493.6	0.0	1,493.6	1,493.6	0.0	0.0	0.0
Ammonia	0.0	0.0	0.0	0.0	0.0	63.6	63.6
Water	0.0	19,000.0	19,000.0	19,000.0	1,604.6	0.0	0.0
Carbon Dioxide	0.0	0.0	0.0	0.0	0.0	0.0	0.0
Air	0.0	0.0	0.0	0.0	0.0	0.0	0.0
Oxygen	0.0	0.0	0.0	0.0	0.0	0.0	0.0
Nitrogen	0.0	0.0	0.0	0.0	0.0	0.0	0.0
Mineral Mix	0.0	122.5	122.5	122.5	0.0	0.0	0.0

Seed Reactor 4 continued								
Stream	**S-013**	**S-014**	**S-015**	**S-016**	**S-017**	**S-027**	**S-005**	**S-078**
Description	**Air in**	**Compressed air**	**Filter 1 air**	**Filter 2 air**	**Air/ammonia mix**	**S-017 connect**	**Product to next reactor**	**Gas vent**
Temp (°C)	20.0	20.0	20.0	20.0	20.0	20.0	32.0	32.0
Pressure (bar)	1.0	1.5	1.5	1.5	1.5	1.5	1.2	1.2
Total Mass (g/batch)	8,265.9	8,265.9	8,265.9	8,265.9	8,329.5	8,329.5	21,164.6	8,174.1
Component Mass (g/batch)								
Cell Mass	0.0	0.0	0.0	0.0	0.0	0.0	560.0	0.0
Astaxanthin	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
Methanol	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
Ammonia	0.0	0.0	0.0	0.0	63.6	63.6	0.0	0.0
Water	0.0	0.0	0.0	0.0	0.0	0.0	20,604.6	202.9
Carbon Dioxide	0.0	0.0	0.0	0.0	0.0	0.0	0.0	989.4
Air	8,265.9	8,265.9	8,265.9	8,265.9	8,265.9	8,265.9	0.0	N/A
Oxygen	1,926.0	1,926.0	1,926.0	1,926.0	1,926.0	1,926.0	0.0	642.0
Nitrogen	6,339.9	6,339.9	6,339.9	6,339.9	6,339.9	6,339.9	0.0	6,339.9
Mineral Mix	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0

Seed Reactor 5							
Stream	**S-008**	**S-009**	**S-010**	**S-022**	**S-005**	**S-011**	**S-012**
Description	**Methanol in**	**Mineral/water in**	**Methanol/mineral/ water mix**	**S-010 connect**	**From last reactor**	**Ammonia tank**	**Vaporized ammonia**
Temp (℃)	20.0	20.0	20.0	20.0	32.0	20.0	20.0
Pressure (bar)	1.0	1.0	1.0	1.0	1.2	1.0	1.5
Total Mass (kg/batch)	30.6	382.5	413.2	413.2	580.6	1.3	1.3
Component Mass (kg/batch)							
Cell Mass	0.0	0.0	0.0	0.0	560.0	0.0	0.0
Astaxanthin	0.0	0.0	0.0	0.0	0.0	0.0	0.0
Methanol	30.6	0.0	30.6	30.6	0.0	0.0	0.0
Ammonia	0.0	0.0	0.0	0.0	0.0	1.3	1.3
Water	0.0	380.0	380.0	380.0	20.6	0.0	0.0
Carbon Dioxide	0.0	0.0	0.0	0.0	0.0	0.0	0.0
Air	0.0	0.0	0.0	0.0	0.0	0.0	0.0
Oxygen	0.0	0.0	0.0	0.0	0.0	0.0	0.0
Nitrogen	0.0	0.0	0.0	0.0	0.0	0.0	0.0
Mineral Mix	0.0	2.5	2.5	2.5	0.0	0.0	0.0

Seed Reactor 5 continued								
Stream	**S-013**	**S-014**	**S-015**	**S-016**	**S-017**	**S-028**	**S-006**	**S-079**
Description	**Air in**	**Compressed air**	**Filter 1 air**	**Filter 2 air**	**Air/ammonia mix**	**S-017 connect**	**Product to next reactor**	**Gas vent**
Temp (℃)	20.0	20.0	20.0	20.0	20.0	20.0	32.0	32.0
Pressure (bar)	1.0	1.5	1.5	1.5	1.5	1.5	1.2	1.2
Total Mass (kg/batch)	169.6	169.6	169.6	169.6	170.9	170.9	411.8	167.7
Component Mass (kg/batch)								
Cell Mass	0.0	0.0	0.0	0.0	0.0	0.0	11.2	0.0
Astaxanthin	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
Methanol	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
Ammonia	0.0	0.0	0.0	0.0	1.3	1.3	0.0	0.0
Water	0.0	0.0	0.0	0.0	0.0	0.0	400.6	4.2
Carbon Dioxide	0.0	0.0	0.0	0.0	0.0	0.0	0.0	20.3
Air	169.6	169.6	169.6	169.6	169.6	169.6	0.0	N/A
Oxygen	39.5	39.5	39.5	39.5	39.5	39.5	0.0	13.2
Nitrogen	130.0	130.0	130.0	130.0	130.0	130.0	0.0	130.0
Mineral Mix	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0

Seed Reactor 6							
Stream	**S-008**	**S-009**	**S-010**	**S-029**	**S-006**	**S-011**	**S-012**
Description	**Methanol in**	**Mineral/water in**	**Methanol/mineral/ water mix**	**S-010 connect**	**From last reactor**	**Ammonia tank**	**Vaporized ammonia**
Temp (°C)	20.0	20.0	20.0	20.0	32.0	20.0	20.0
Pressure (bar)	1.0	1.0	1.0	1.0	1.2	1.0	1.5
Total Mass (kg/batch)	612.7	7,650.3	8,263.0	8,263.0	411.8	26.1	26.1
Component Mass (kg/batch)							
Cell Mass	0.0	0.0	0.0	0.0	11.2	0.0	0.0
Astaxanthin	0.0	0.0	0.0	0.0	0.0	0.0	0.0
Methanol	612.7	0.0	612.7	612.7	0.0	0.0	0.0
Ammonia	0.0	0.0	0.0	0.0	0.0	26.1	26.1
Water	0.0	7,600.0	7,600.0	7,600.0	400.6	0.0	0.0
Carbon Dioxide	0.0	0.0	0.0	0.0	0.0	0.0	0.0
Air	0.0	0.0	0.0	0.0	0.0	0.0	0.0
Oxygen	0.0	0.0	0.0	0.0	0.0	0.0	0.0
Nitrogen	0.0	0.0	0.0	0.0	0.0	0.0	0.0
Mineral Mix	0.0	50.3	50.3	50.3	0.0	0.0	0.0

Seed Reactor 6 continued										
Stream	**S-013**	**S-014**	**S-015**	**S-016**	**S-017**	**S-029**	**S-007**	**S-073**	**S-074**	**S-080**
Description	**Air in**	**Compressed air**	**Filter 1 air**	**Filter 2 air**	**Air/ammonia mix**	**S-017 connect**	**Product to next reactor**	**Heatex in**	**Heatex out**	**Gas vent**
Temp (°C)	20.0	20.0	20.0	20.0	20.0	20.0	32.0	32.0	32.0	32.0
Pressure (bar)	1.0	1.5	1.5	1.5	1.5	1.5	1.2	1.2	1.2	1.2
Total Mass (kg/batch)	3,391.1	3,391.1	3,391.1	3,391.1	3,417.2	3,417.2	8,280.6	Eqn 12.3.1	Eqn 12.3.1	3,353.5
Component Mass (kg/batch)										
Cell Mass	0.0	0.0	0.0	0.0	0.0	0.0	280.0	0.0	0.0	0.0
Astaxanthin	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
Methanol	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
Ammonia	0.0	0.0	0.0	0.0	26.1	26.1	0.0	0.0	0.0	0.0
Water	0.0	0.0	0.0	0.0	0.0	0.0	8,000.6	Eqn 12.3.1	Eqn 12.3.1	83.2
Carbon Dioxide	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	405.9
Air	3,391.1	3,391.1	3,391.1	3,391.1	3,391.1	3,391.1	0.0	0.0	0.0	N/A
Oxygen	790.2	790.2	790.2	790.2	790.2	790.2	0.0	0.0	0.0	263.4
Nitrogen	2,601.0	2,601.0	2,601.0	2,601.0	2,601.0	2,601.0	0.0	0.0	0.0	2,601.0
Mineral Mix	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0

To ensure cell viability in the seed reactors, the methanol concentration cannot exceed 1% by weight at any point inside the reactors. For this reason, the methanol is added to the reactor at a rate proportional to the rate of cell growth. This helps ensure that excess methanol will not exist at quantities higher than 1% inside the reactor. In addition to this control measure, the controllers that come as part of the bioreactors will verify that the methanol levels inside the reactors are not toxic, and control the methanol flow rate appropriately if the methanol concentration is too high. The amount of methanol listed in the mass balance table is the total methanol input to the reactor over the course of each batch reaction in the seed train. The

equations representing the methanol flow rate to each seed reactor is given in Table 12.3.2 below. The method through which these equations were developed can be found in Appendix A.

Table 12.3.2. Variable methanol flow into the batch seed train reactors.

Seed Reactor	Equation for Methanol Flow Rate (g/hr)
1	$M[g/hr]=0.0154[g/hr]e^{0.459[1/hr]t[hr]}$
2	$M=0.246e^{0.459t}$
3	$M=2.46e^{0.459t}$
4	$M=49.3e^{0.459t}$
5	$M=616e^{0.459t}$
6	$M=12{,}320e^{0.459t}$

In order to maintain a reactor temperature of 33°C, it is necessary to adjust the flow rate of reactor contents through the heat exchanger. As the log phase growth progresses, more heat is generated by the cell mass. This means that the heat exchanger conditions must adjust to account for the additional cooling that is needed throughout the batch reaction. Because the heat exchange area is constant throughout the process, the coolant flow rate, the flow rate of the reactor contents through the exchanger, and the log mean temperature difference must change. Only the adjustment of the flow rate of the reactor contents are shown here for mass balance purposes. This equation was derived in a similar fashion to the methanol flow rate adjustments. The derivation is shown in Appendix A. The recycle flow rate equation is:

$$\text{Recycle Flow Rate [kg/hr]}=2{,}224[kg/hr]e^{26.9[1/hr]t[hr]} \qquad \text{(Equation 12.3.1)}$$

12.4 Batch Process Design

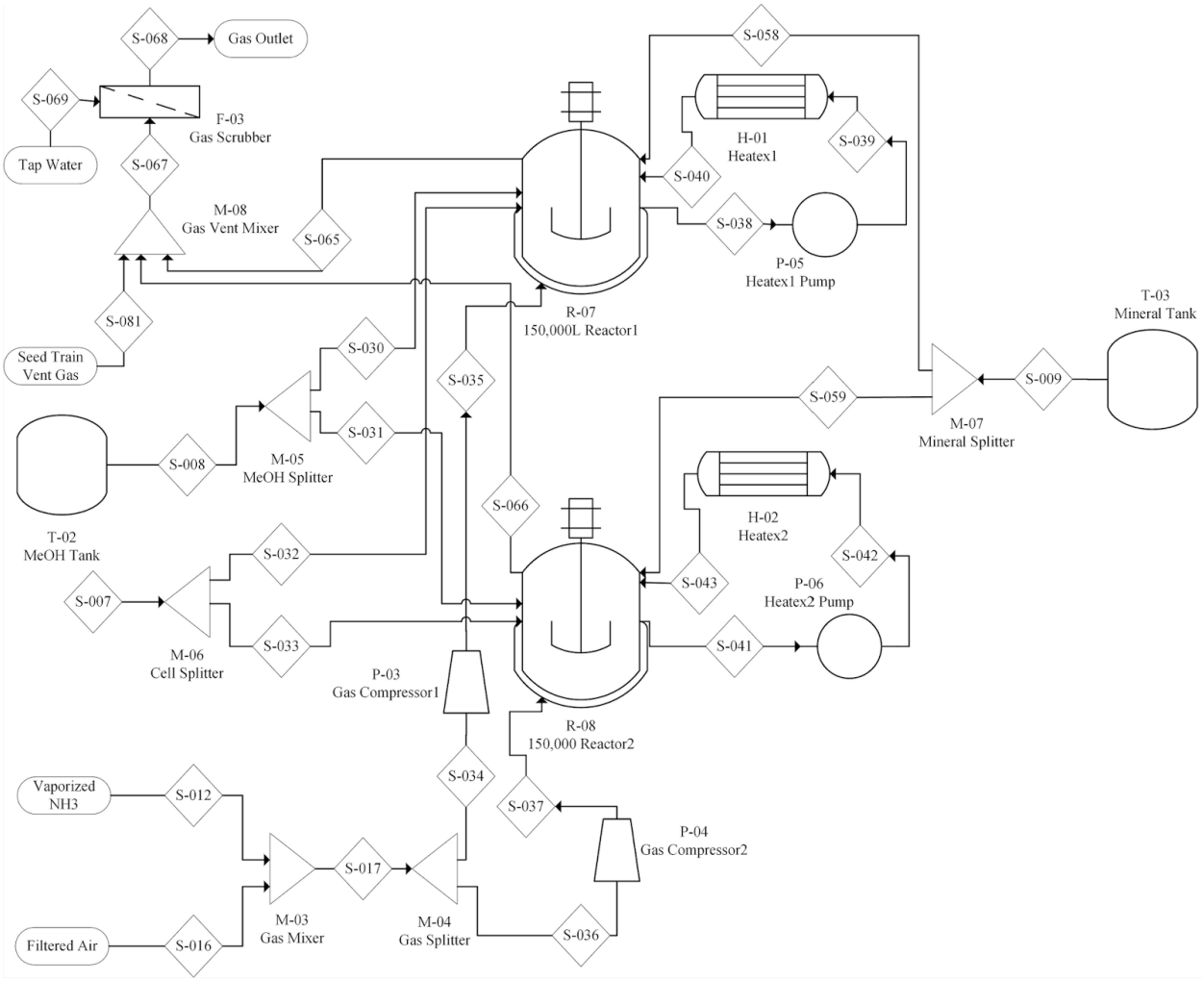


Figure 12.4. Flowsheet for the twin bioreactors operating under batch conditions.

12.5 Batch Process Mass Balances

Table 12.5.1. Mass balances for the large 150,000 L reactors in batch operation.

Batch Reactor (assuming one batch at a time)							
Stream	**S-008**	**S-030**	**S-007**	**S-032**	**S-012**	**S-016**	**S-017**
Twin Stream Number		**S-031**		**S-033**			
Description	**Methanol in**	**S-008 connect**	**Cells in**	**S-007 connect**	**Vaporized NH3**	**Filtered air**	**Mixed gas**
Temp	20.0	20.0	20.0	20.0	32.0	20.0	20.0
Pressure	1.0	1.0	1.0	1.0	1.2	1.0	1.5
Total Mass (MT/batch)	9.1	9.1	18.1	18.1	0.4	50.2	50.5
Component Mass (MT/batch)							
Cell Mass	0.0	0.0	0.3	0.3	0.0	0.0	0.0
Astaxanthin	0.0	0.0	0.0	0.0	0.0	0.0	0.0
Methanol	9.1	9.1	0.0	0.0	0.0	0.0	0.0
Ammonia	0.0	0.0	0.0	0.0	0.4	0.0	0.4
Water	0.0	0.0	8.0	8.0	0.0	0.0	0.0
Carbon Dioxide	0.0	0.0	0.0	0.0	0.0	0.0	0.0
Air	0.0	0.0	0.0	0.0	0.0	50.2	50.2
Oxygen	0.0	0.0	0.0	0.0	0.0	11.7	11.7
Nitrogen	0.0	0.0	0.0	0.0	0.0	38.5	38.5
Mineral Mix	0.0	0.0	0.0	0.0	0.0	0.0	0.0

Batch Reactor continued						
Stream	**S-034**	**S-035**	**S-009**	**S-058**	**S-038**	**S-039**
Twin Stream Number	**S-036**	**S-037**		**S-059**	**S-041**	**S-042**
Description	**S-017 connect**	**Compressed gas**	**Mineral/water in**	**S-009 connect**	**To heatex pump**	**To heatex**
Temp	20.0	20.0	20.0	20.0	32.0	32.0
Pressure	1.0	1.5	1.0	1.0	1.2	1.2
Total Mass (kg/batch)	50.5	50.5	107.0	107.0	Eqn 12.5.2	Eqn 12.5.2
Component Mass (kg/batch)						
Cell Mass	0.0	0.0	0.0	0.0	0.0	0.0
Astaxanthin	0.0	0.0	0.0	0.0	0.0	0.0
Methanol	0.0	0.0	0.0	0.0	0.0	0.0
Ammonia	0.4	0.0	0.0	0.0	0.0	0.0
Water	0.0	0.0	106.0	106.0	Eqn 12.5.2	Eqn 12.5.2
Carbon Dioxide	0.0	0.0	0.0	0.0	0.0	0.0
Air	50.2	50.2	0.0	0.0	0.0	0.0
Oxygen	11.7	11.7	0.0	0.0	0.0	0.0
Nitrogen	38.5	38.5	0.0	0.0	0.0	0.0
Mineral Mix	0.0	0.0	1.0	1.0	0.0	0.0

Batch Reactor continued						
Stream	**S-040**	**S-065**	**S-081**	**S-067**	**S-068**	**S-069**
Twin Stream Number	**S-043**	**S-066**				
Description	**From heatex**	**Gas vent**	**Gas vent from seed train**	**Mixed to gas vent**	**Gas outlet**	**Water to scrubber**
Temp	26.0	32.0	32.0	32.0	32.0	20.0
Pressure	1.2	1.2	1.2	1.2	1.2	1.0
Total Mass (kg/batch)	Eqn 12.5.2	171.1	3.5	174.6	174.6	1,272.4
Component Mass (kg/batch)						
Cell Mass	0.0	0.0	0.0	0.0	0.0	0.0
Astaxanthin	0.0	0.0	0.0	0.0	0.0	0.0
Methanol	0.0	0.0	0.0	0.0	0.0	0.0
Ammonia	0.0	0.0	0.0	0.0	0.0	0.0
Water	Eqn 12.5.2	3.9	0.1	4.0	4.0	1,272.4
Carbon Dioxide	0.0	6.0	0.4	6.4	6.4	0.0
Air	0.0	N/A	N/A	N/A	N/A	0.0
Oxygen	0.0	38.5	0.3	38.7	38.7	0.0
Nitrogen	0.0	122.7	2.7	125.4	125.4	0.0
Mineral Mix	0.0	0.0	0.0	0.0	0.0	0.0

Similar to the methanol flow rate adjustments described in Section 12.3, the methanol flow rates in the batch reactor also must change over time to prevent methanol build up in the reactor. The relationship is shown in Equation 12.5.1. below.

$$\text{Methanol Flow Rate [kg/hr]} = 245[\text{kg/hr}]e^{0.459[1/\text{hr}]t[\text{hr}]} \qquad \text{(Equation 12.5.1)}$$

Also similar to the seed train reactors, the flow rate of reactor medium that is recycled through the heat exchanger must be adjusted as the cell growth progresses. This relationship is shown in Equation 12.5.2.

$$\text{Recycle Flow Rate [kg/hr]} = 44{,}476[\text{kg/hr}]e^{27.0[1/\text{hr}]t[\text{hr}]} \qquad \text{(Equation 12.5.2)}$$

12.6 Continuous Process Design

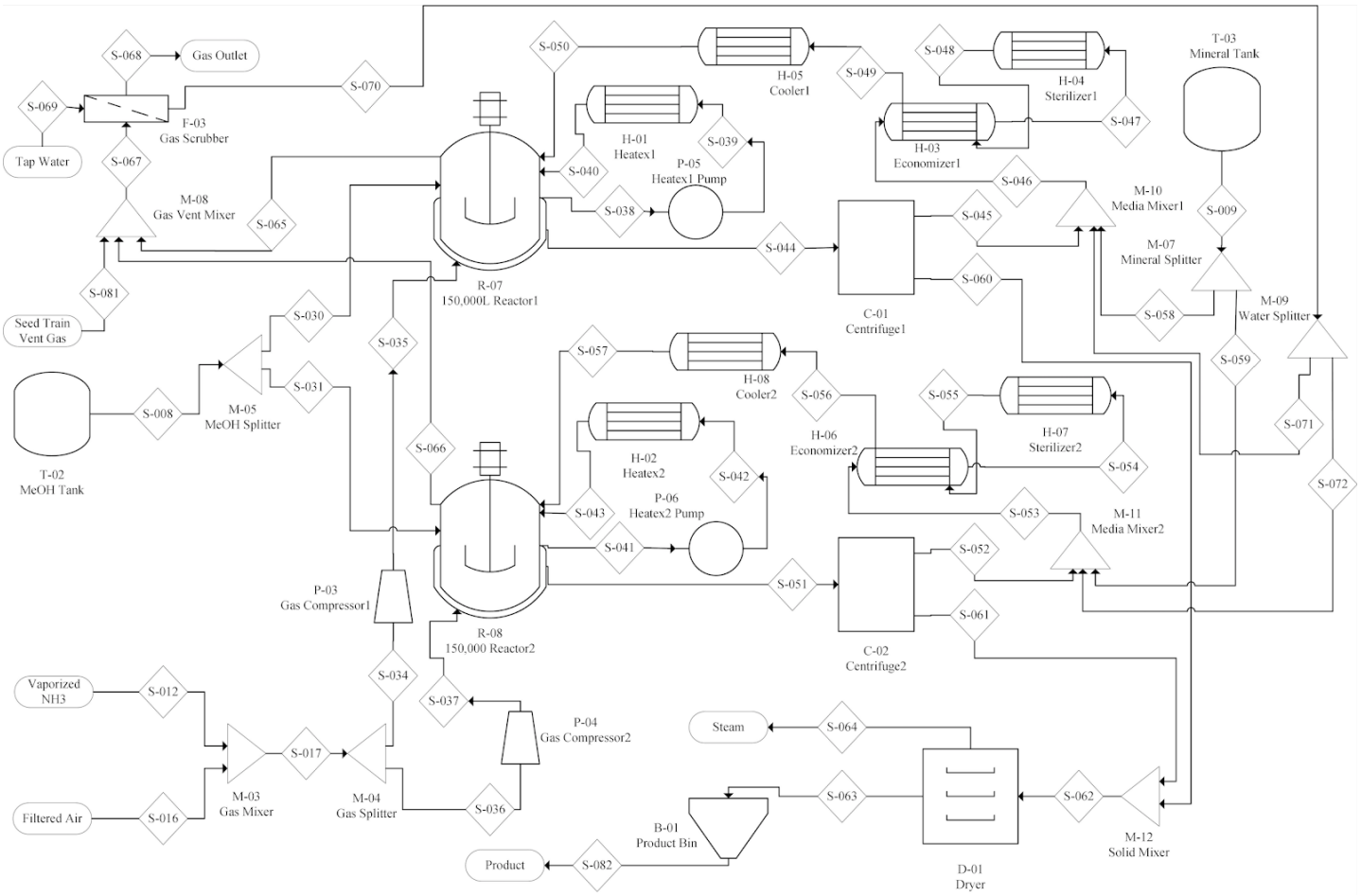


Figure 12.6. Flowsheet for the twin bioreactors operating continuously including downstream processing steps and heat exchange units.

12.7 Continuous Process Bioreactor Balance

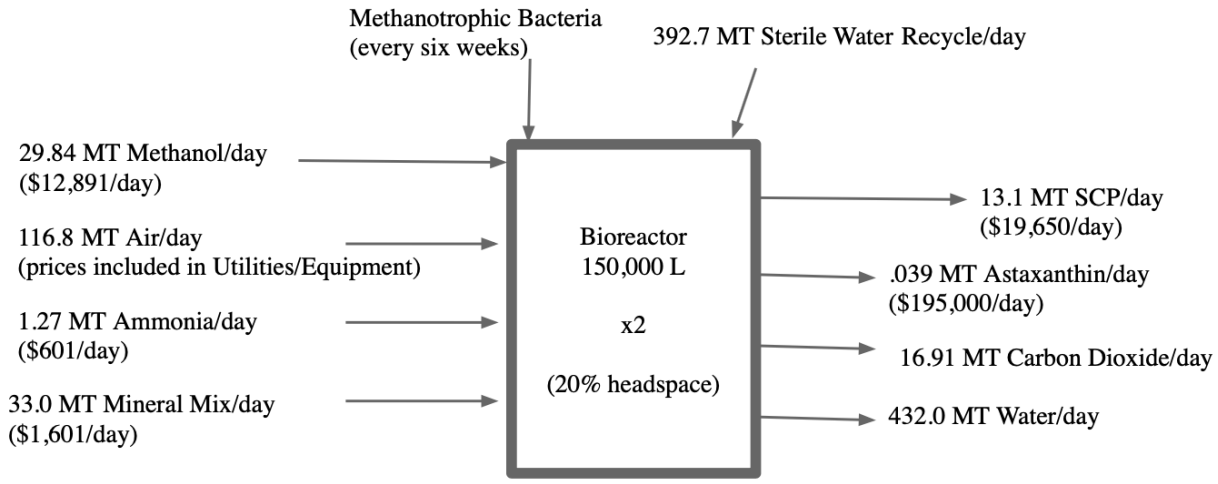


Figure 12.7. Overall daily input of raw materials and output of products with associated costs for continuous bioreactor operation.

12.8 Continuous Process Mass Balances

Table 12.8.1. Mass balances for the large 150,000 L reactors in continuous operation.

Inlet to Fermenter					
Stream Number	**S-008**	**S-030**	**S-012**	**S-016**	**S-017**
Twin Stream Number		**S-031**			
Description	**Methanol in**	**Methanol split**	**Ammonia in**	**Air in**	**Ammonia/air mix**
Temp	20.0	20.0	20.0	20.0	20.0
Pressure	1.0	1.0	1.0	1.0	1.0
Total Flow Rate (kg/hr)	2,487.1	1,243.6	106.0	9,735.2	9,841.2
Component Flow Rate (kg/hr)					
Cell Mass	0.0	0.0	0.0	0.0	0.0
Astaxanthin	0.0	0.0	0.0	0.0	0.0
Methanol	2,487.1	1,243.6	0.0	0.0	0.0
Ammonia	0.0	0.0	106.0	0.0	106.0
Water	0.0	0.0	0.0	0.0	0.0
Carbon Dioxide	0.0	0.0	0.0	0.0	0.0
Air	0.0	0.0	0.0	9,735.2	9,735.2
Oxygen	0.0	0.0	0.0	1,283.8	1,283.8
Nitrogen	0.0	0.0	0.0	8,451.4	8,451.4
Mineral Mix	0.0	0.0	0.0	0.0	0.0

Inlet to Fermenter continued			External Heat Exchanger		
Stream Number	**S-034**	**S-035**	**S-038**	**S-039**	**S-040**
Twin Stream Number	**S-036**	**S-037**	**S-041**	**S-042**	**S-043**
Description	**Ammonia/air mix split**	**Ammonia/air mix after compressor**	**Fermenter to heatex**	**From pump to heatex**	**Fermenter from heatex**
Temp	20.0	20.0	33.0	33.0	22.0
Pressure	1.0	2.35	1.2	1.2	1.2
Total Flow Rate (kg/hr)	4,920.6	4,920.6	280,899.8	262,352.5	262,352.5
Component Flow Rate (kg/hr)					
Cell Mass	0.0	0.0	8,263.7	7,718.1	7,718.1
Astaxanthin	0.0	0.0	24.6	22.9	22.9
Methanol	0.0	0.0	0.0	0.0	0.0
Ammonia	53.0	53.0	0.0	0.0	0.0
Water	0.0	0.0	272,611.5	254,611.5	254,611.5
Carbon Dioxide	0.0	0.0	0.0	0.0	0.0
Air	4,867.6	4,867.6	0.0	0.0	0.0
Oxygen	641.9	641.9	0.0	0.0	0.0
Nitrogen	4,225.7	4,225.7	0.0	0.0	0.0
Mineral Mix	0.0	0.0	0.0	0.0	0.0

Centrifuge				Mineral Tank to Sterilizer	
Stream Number	**S-044**	**S-045**	**S-060**	**S-009**	**S-058**
Twin Stream Number	**S-051**	**S-052**	**S-061**		**S-059**
Description	**Product to centrifuge**	**Centrifuge media recycle**	**Centrifuge concentrated product**	**Minerals/water in**	**Minerals/water in split**
Temp	32.0	32.0	32.0	20.0	20.0
Pressure	1.2	1.2	1.0	1.0	1.0
Total Flow Rate (kg/hr)	18,547.3	16,363.1	2,184.2	1,474.2	737.1
Component Flow Rate (kg/hr)					
Cell Mass	545.6	0.0	545.6	0.0	0.0
Astaxanthin	1.6	0.0	1.6	0.0	0.0
Methanol	0.0	0.0	0.0	0.0	0.0
Ammonia	0.0	0.0	0.0	0.0	0.0
Water	18,000.0	16,363.1	1,636.9	1,272.4	636.2
Carbon Dioxide	0.0	0.0	0.0	0.0	0.0
Air	0.0	0.0	0.0	0.0	0.0
Oxygen	0.0	0.0	0.0	0.0	0.0
Nitrogen	0.0	0.0	0.0	0.0	0.0
Mineral Mix	0.0	0.0	0.0	201.8	100.9

Gas Scrubber						
Stream Number	**S-069**	**S-065**	**S-067**	**S-068**	**S-070**	**S-071**
Twin Stream Number		**S-066**				**S-072**
Description	**Tap water to scrubber**	**Gas vent stream**	**Mixed gas vent streams**	**Gas outlet from scrubber**	**Scrubber water recycle**	**Split scrubber water recycle**
Temp	20.0	32.0	32.0	32.0	20.0	20.0
Pressure	1.0	1.2	1.2	1.2	1.0	1.0
Total Flow Rate (kg/hr)	1,272.4	5,279.7	10,559.3	10,559.3	1,272.4	636.2
Component Flow Rate (kg/hr)						
Cell Mass	0.0	0.0	0.0	0.0	0.0	0.0
Astaxanthin	0.0	0.0	0.0	0.0	0.0	0.0
Methanol	0.0	0.0	0.0	0.0	0.0	0.0
Ammonia	0.0	0.0	0.0	0.0	0.0	0.0
Water	1,272.4	135.2	270.4	270.4	1,272.4	636.2
Carbon Dioxide	0.0	704.8	1,409.6	1,409.6	0.0	0.0
Air	0.0	0.0	0.0	0.0	0.0	0.0
Oxygen	0.0	214.0	427.9	427.9	0.0	0.0
Nitrogen	0.0	4,225.7	8,451.4	8,451.4	0.0	0.0
Mineral Mix	0.0	0.0	0.0	0.0	0.0	0.0

Economizer/Sterilizer Loop					
Stream Number	**S-046**	**S-047**	**S-048**	**S-049**	**S-050**
Twin Stream Number	**S-053**	**S-054**	**S-055**	**S-056**	**S-057**
Description	**Mixed economizer/ sterilizer loop**	**Economizer to sterilizer**	**Sterilizer to economizer**	**Economizer to cooler**	**Cooler to fermenter**
Temp	32.0	52.0	121.0	102.0	32.0
Pressure	1.2	1.2	1.2	1.2	1.2
Total Flow Rate (kg/hr)	18,271.6	18,271.6	18,271.6	18,271.6	18,271.6
Component Flow Rate (kg/hr)					
Cell Mass	0.0	0.0	0.0	0.0	0.0
Astaxanthin	0.0	0.0	0.0	0.0	0.0
Methanol	0.0	0.0	0.0	0.0	0.0
Ammonia	0.0	0.0	0.0	0.0	0.0
Water	18,271.6	18,271.6	18,271.6	18,271.6	18,271.6
Carbon Dioxide	0.0	0.0	0.0	0.0	0.0
Air	0.0	0.0	0.0	0.0	0.0
Oxygen	0.0	0.0	0.0	0.0	0.0
Nitrogen	0.0	0.0	0.0	0.0	0.0
Mineral Mix	201.8	0.0	0.0	0.0	0.0

To Dryer			
Stream Number	**S-062**	**S-064**	**S-063**
Twin Stream Number			
Description	**Mixed centrifuge product discharge**	**Steam from dryer**	**Finished Product**
Temp	32.0	100.0	32.0
Pressure	1.0	1.5	1.0
Total Flow Rate (kg/hr)	4,368.3	3,273.8	1,094.5
Component Flow Rate (kg/hr)			
Cell Mass	1,091.3	0.0	1,091.3
Astaxanthin	3.2	0.0	3.2
Methanol	0.0	0.0	0.0
Ammonia	0.0	0.0	0.0
Water	3,273.8	3,273.8	0.0
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
Mineral Mix	0.0	0.0	0.0

13. Process Descriptions

13.1 Feed Material Storage and Preparation

Many raw materials must be introduced into the bioreactor systems in order to enable aerobic cell growth, astaxanthin and protein creation, pH control, and foam management. These raw materials must be carefully prepared and stored in appropriate conditions to ensure safety, maximum growth, and no operation delays. There will be storage tanks for the methanol, mineral growth media, and ammonia, the design specifications of which are described in Section 15.1.

13.1.1 Methanol Storage

Liquid methanol will be delivered to our production plant from the Methanex plant in Medicine Hat, Canada via railway in three day increments at a price of $432/MT. Methanol will be fed to each of the two largest bioreactors at a rate of 1,243.6 kg/hr, so it is important to have an extra supply of methanol in stock at the plant location in case there are any weather-related delays in delivery. Since methanol is the only carbon source to enable cell growth, a shortage of methanol would result in needing to shut down production and restart the 6-week campaign period. A weeks supply of methanol will be stored without refrigeration, due to the cold climate in Alberta, in a tank at around 20°C and atmospheric pressure.

13.1.2 Mineral Growth Media Preparation

Mineral growth media is necessary to provide cells with trace elements needed as nutrients to grow. The minerals will be delivered in large bags of 1 MT to 25 MT in size and range from about $100/MT to $1,000/MT for the different minerals needed. These minerals include disodium hydrogen phosphate, potassium dihydrogen phosphate, magnesium sulfate

heptahydrate, calcium chloride, and iron (II) sulfate heptahydrate. Excess minerals can be stored for long periods of time in the delivery bags in solid form, so it is reasonable to keep up to a month's supply of solid minerals onsite. The minerals will be mixed with water and dissolved with agitation at concentrations appropriate to supply enough minerals to the large bioreactors with a flow rate of 10.6 L/min. The minerals should all dissolve fairly easily due to their storage at concentrations well below the solubility limits of each mineral. The media will be sterilized before it is used in the seed and batch reactors. A summary of the concentrations of the minerals in the storage tank can be found in Table 15.1.2. A three-day supply of the dissolved mineral solution will be held in the storage tank at 20°C and atmospheric pressure.

13.1.3 Ammonia Storage

Anhydrous ammonia will be delivered to our production plant from the Nutrien plant in Carseland, Canada via railway in three day increments at a price of $473/MT. The anhydrous ammonia will be in the form of a liquified compressed gas for ease of storage and transport. A weeks supply of anhydrous ammonia will be stored in a tank with 20% head space to allow for some vaporization. The expected range of conditions for storage are temperatures between 0°C and 20°C with associated vapor pressures of 1.1 bar and 13.6 bar, respectively (22). The ammonia storage will be in compliance with Canada's Fertilizer Safety & Security Council Ammonia Code of Practice.

13.2 Air Compression and Filtration

Air will be used at the oxygen source for fermentation, which will require a careful compression and filtration system to avoid contamination and prepare the gas for inlet to the bioreactors. Additionally, with the use of air 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

With the decision to use ambient air as the source of oxygen for the fermentation process, an air compressor will be 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 reactor will be operated at a gauge pressure of 1.22 bar, and the height of the liquid in the reactor will be approximately 7.5 m, meaning the pressure at the bottom of the reactor will be the sum of these two pressures, 1.96 bar. The compressor will be an oil-free screw compressor, and it will pressurize the air in 20% excess of the minimum pressure to 2.35 bar.

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 ‘coarse’ 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. The submicron pores are small enough that any microorganisms in the air that could contaminate the reactor would be removed. This small filter size will introduce a pressure drop across the unit and may require additional gas compression.

13.3 Seed Train Growth

In order for the cell mass to reach the amount needed for the continuous fermenter, the cells must be grown from the lab scale to manufacturing scale. Beginning with 1 mL aliquots at 14 g/L concentration, the cell solution is grown to 150,000 L at 35 g/L concentration. The seed train consists of 6 fermenters of increasing size and a batch process at the final production volume.

Seed train growth is important not only for the growth of cells in a timely manner, but also 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. In the event that the cells had a quality issue, the issue is addressed before the cell volume is at manufacturing scale.

13.3.1 Cell Bank Storage

In order to ensure reproducibility of batches in the SCP and astaxanthin production process, a cell bank is used to store and freeze cell samples. This also functions to ensure 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. After this, the culture is fractionated into 1 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 bank nears depletion, one of the aliquots will be used to create a new master cell bank. A diagram of this process is shown in Figure 13.3.1 (23).

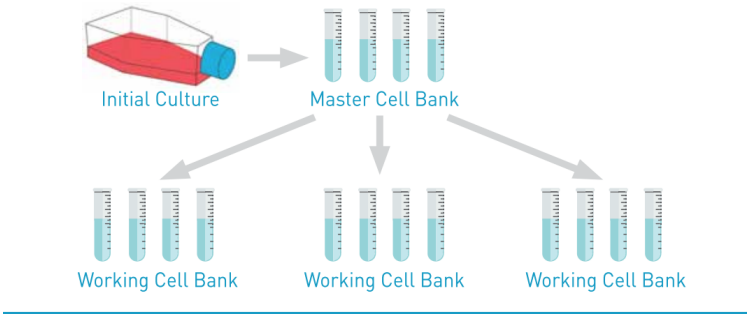


Figure 13.1.1. Process of creating a cell bank of 1 mL aliquots (23).

The cell bank will store 300-1 mL aliquots of cells at a concentration of 14 g/L. The freezer operates at -80°C to ensure the cryopreservation of the cells (23). 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 -1°C/min.

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.

Two cell banks will exist, one on site and one off site. This will act as an insurance policy in the event of contamination or destruction of one of the cell banks.

13.3.2 10 mL Bioreactor

A 10 mL batch fermenter is used as Seed Reactor 1. All raw materials will be fed into the fermenter at the beginning of the batch, except methanol will be fed at the rate of cell consumption in order to keep the methanol concentration in the reactor less than 1% by weight. A proportional integral controller will adjust the methanol flow rate into the reactor in the event

that the methanol exceeds 1% by weight. The reactor will have a fill fraction of 80% and therefore a usable volume of 8 mL. A 1 mL aliquot will be used to initiate cell growth in the 10 mL bioreactor. The final concentration in the bioreactor will be 35 g/L, giving a final dry cell mass of 0.28 g. A gas vent stream will be used to prevent pressure build up. The efficacy, viability, and purity of the cells are tested and 80% of the final batch product is fed to the next seed reactor to begin further cell growth.

13.3.3 100 mL Bioreactor

A 100 mL batch fermenter is used as Seed Reactor 2. All raw materials will be fed into the fermenter at the beginning of the batch, except methanol will be fed at the rate of cell consumption in order to keep the methanol concentration in the reactor less than 1% by weight. A proportional integral controller will adjust the methanol flow rate into the reactor in the event that the methanol exceeds 1% by weight. The reactor will have a fill fraction of 80% and therefore a usable volume of 80 mL. The product from Seed Reactor 1, the 10 mL fermenter, will be used to initiate cell growth. The final concentration in the bioreactor will be 35 g/L, giving a final dry cell mass of 2.8 g. A gas vent stream will be used to prevent pressure build up. The efficacy, viability, and purity of the cells are tested and 80% of the final batch product is fed to the next seed reactor to begin further cell growth.

13.3.4 2 L Bioreactor

A 2 L batch fermenter is used as Seed Reactor 3. All raw materials will be fed into the fermenter at the beginning of the batch, except methanol will be fed at the rate of cell consumption in order to keep the methanol concentration in the reactor less than 1% by weight. A proportional integral controller will adjust the methanol flow rate into the reactor in the event

that the methanol exceeds 1% by weight. The reactor will have a fill fraction of 80% and therefore a usable volume of 1.6 L. The product from Seed Reactor 2, the 100 mL fermenter, will be used to initiate cell growth. The final concentration in the bioreactor will be 35 g/L, giving a final dry cell mass of 56 g. A gas vent stream will be used to prevent pressure build up. The efficacy, viability, and purity of the cells are tested and 80% of the final batch product is fed to the next seed reactor to begin further cell growth.

13.3.5 25 L Bioreactor

A 25 L batch fermenter is used as Seed Reactor 4. All raw materials will be fed into the fermenter at the beginning of the batch, except methanol will be fed at the rate of cell consumption in order to keep the methanol concentration in the reactor less than 1% by weight. A proportional integral controller will adjust the methanol flow rate into the reactor in the event that the methanol exceeds 1% by weight. The reactor will have a fill fraction of 80% and therefore a usable volume of 20 L. The product from Seed Reactor 3, the 2 L fermenter, will be used to initiate cell growth. The final concentration in the bioreactor will be 35 g/L, giving a final dry cell mass of 700 g. A gas vent stream will be used to prevent pressure build up. The efficacy, viability, and purity of the cells are tested and 80% of the final batch product is fed to the next seed reactor to begin further cell growth.

13.3.6 500 L Bioreactor

A 500 L batch fermenter is used as Seed Reactor 5. All raw materials will be fed into the fermenter at the beginning of the batch, except methanol will be fed at the rate of cell consumption in order to keep the methanol concentration in the reactor less than 1% by weight. A proportional integral controller will adjust the methanol flow rate into the reactor in the event

that the methanol exceeds 1% by weight. The reactor will have a fill fraction of 80% and therefore a usable volume of 400 L. The product from Seed Reactor 4, the 25 L fermenter, will be used to initiate cell growth. The final concentration in the bioreactor will be 35 g/L, giving a final dry cell mass of 14 kg. A gas vent stream will be used to prevent pressure build up. The efficacy, viability, and purity of the cells are tested and 80% of the final batch product is fed to the next seed reactor to begin further cell growth.

13.3.7 10,000 L Bioreactor

A 10,000 L batch fermenter is used as Seed Reactor 6. All raw materials will be fed into the fermenter at the beginning of the batch, except methanol will be fed at the rate of cell consumption in order to keep the methanol concentration in the reactor less than 1% by weight. A proportional integral controller will adjust the methanol flow rate into the reactor in the event that the methanol exceeds 1% by weight. The reactor will have a fill fraction of 80% and therefore a usable volume of 80,000 L. An external heat exchanger will deliver cooling at the rate necessary to maintain a temperature of 33°C in the fermenter. The product from Seed Reactor 5, the 500 mL fermenter, will be used to initiate cell growth. The final concentration in the bioreactor will be 35 g/L, giving a final dry cell mass of 280 kg. A gas vent stream will be used to prevent pressure build up. The efficacy, viability, and purity of the cells are tested and 80% of the final batch product is fed to the continuous reactor to begin start up batch cell growth.

13.4 Batch Growth Period

After the last seed reactor, the product from Seed Reactor 6 is the appropriate size to initiate cell growth in the continuous scale bioreactor of 150,000 L. All raw materials will be fed into the fermenter at the beginning of the batch, except methanol will be fed at the rate of cell

consumption in order to keep the methanol concentration in the reactor less than 1% by weight. A proportional integral controller will adjust the methanol flow rate into the reactor in the event that the methanol exceeds 1% by weight. The reactor will have a fill fraction of 80% and therefore a usable volume of 120,000 L. An external heat exchanger will deliver cooling at the rate necessary to maintain a temperature of 33°C in the fermenter. The final concentration in the bioreactor will be 35 g/L, giving a final dry cell mass of 4.2 MT. A gas vent stream will be used to prevent pressure build up. The efficacy, viability, and purity of the cells are tested, then the continuous fermenter growth will begin.

13.5 Continuous Bioreactor Growth

After the cells are grown in the 150,000 L bioreactors to the peak concentration of 35 g/L, the continuous phase of operation will begin. This continuous process will consist of constant flow rates of products, reactants, and recycle streams flowing in and out of the bioreactor system. More specifically, inlet streams to the bioreactor include a liquid methanol stream, a gaseous ammonia and air stream, a cooled recycle stream, and a sterilized mineral mix, water, and recycled media stream. A proportional integral controller will be used to ensure that the methanol concentration in the bioreactor does not exceed 1% and adjust the flow rate accordingly if needed. Ideally the methanol concentration will stay around 50 ppm which the cells prefer for maximum growth. Outlet streams from the bioreactor include a vented gas stream of nitrogen, carbon dioxide and excess oxygen and a liquid product stream which the solids are then removed from through centrifugation and drying to create the final solid product for sale. Each of the 150,000 L bioreactors has campaign period of 6 weeks to operate continuously before the operation must cease for the bioreactor to be sterilized and restarted with a new batch

growth period. The two large bioreactors will run on opposite time schedules; therefore, one of the bioreactors is cleaned and restarted every three weeks.

13.5.1 150,000 L Bioreactors

Two large 150,000 L bioreactors will exist as identical twin systems for bacteria to grow and SCP and astaxanthin product to be formed. The reactor will have a fill fraction of 80% and therefore a usable volume of 120,000 L. An external heat exchanger will deliver cooling at the rate necessary to maintain a temperature of 33°C in the fermenter. The bioreactor will be maintained at a pressure of 1.22 bar, slightly above atmospheric pressure, to ensure that in the case of a leak, external contaminants will not immediately enter the bioreactor. A cell density of 35 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 12.6. The bioreactor will include agitation with power of 23.8 kW. The methanol inlet stream will flow directly into the liquid-gas interface near the agitator to prevent toxic hotspots of methanol in the bioreactor. A proportional integral controller will adjust the methanol flow rate into the reactor in the event that the methanol exceeds 1% by weight.

13.5.2 Cooling Recycle Heat Exchangers

Due to the highly exothermic nature of the bacterial growth process, 1,708.3 kW of heat will be created in each bioreactor, creating a need for extensive cooling to maintain the operation temperature of 33°C in the fermenter. This cooling will be achieved through a large shell and tube heat exchanger off of each bioreactor taking cooling a portion of the bioreactor contents and recycling it back into the bioreactor at a lower temperature of 22°C. This heat exchanger will have 440 cooling tubes each 3.8 cm in diameter and 10 m in length, with a 12°C cooling water

flow rate of 4,338 L/min. It is reasonable to assume a 12°C inlet temperature of cooling water due to the cold climate in Alberta, but a backup refrigeration system will be put on the cooling tower in case of unseasonably warm days. The cooling water will likely be at a colder temperature in the freezing winter months, in which case the flow rate of cooling water will be decreased to adjust for this temperature change. The flow rate of bioreactor recycle will be 3,944 L/min, resulting in 1.7 batch turnovers per hour through the heat exchanger. The bioreactor will be made of smooth stainless steel to prevent corrosion and allow for easy sterilization between the continuous operation periods.

The heat added via energy from the agitator was considered while determining the amount of cooling needed. However, a simple calculation determined that the energy added to the reactor through the agitator was approximately 1% of the heat produced in the reaction, therefore it was considered to be negligible.

13.6 Solid Bowl Centrifugation

A solid bowl centrifuge will be used to separate the bulk of the media used to grow the cell mass from the dry cell mass. The cell mass leaves the centrifuge diluted one to four in water; therefore, the centrifuge product is a slurry, not a final product.

The solid bowl centrifuge uses centrifugal force to separate two different substances with difference densities (24). A diagram of the solid bowl centrifuge is shown in Figure 13.6 (25). The Alfa Laval centrifuge can handle the flow rate of product diluted in media from one fermenter. Two centrifuges will be used, one for each fermenter. The liquid discharge from the centrifuge will be recycled back to the process. The water lost in the solid discharge will be

evaporated from the product in the dryer and replenished through the water and mineral feed to ensure no loss of water volume throughout the process.

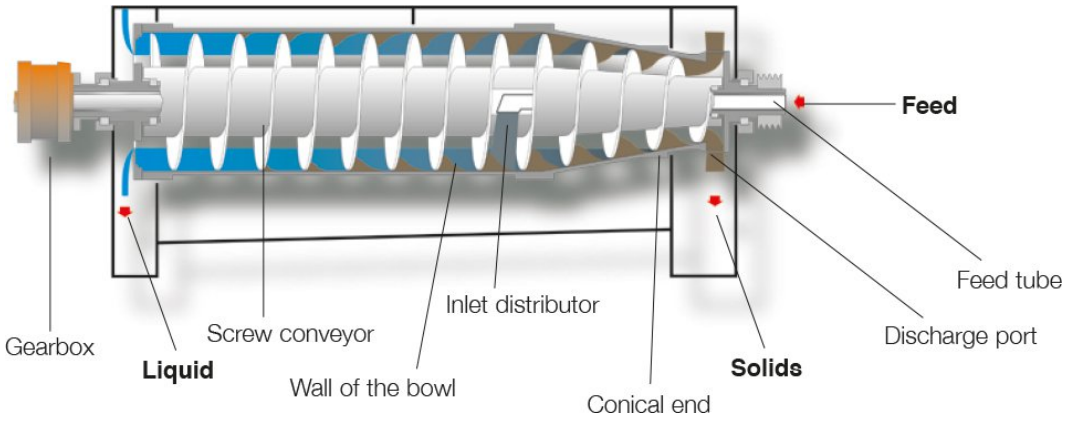


Figure 13.6. Diagram of a solid bowl centrifuge with major components labeled (25).

13.7 Recycle Sterilization

In order to maintain the sterile nature of the fermenter, the water entering the fermenter must be sterilized first. Before the sterilizer unit, the liquid media discharge from the centrifuge, the mineral mix, and the recycle water from the scrubber are mixed and sent to the sterilization unit. This unit involves an economizer, sterilizer, and cooler. A diagram of the sterilization loop is shown in Figure 13.7 (26).

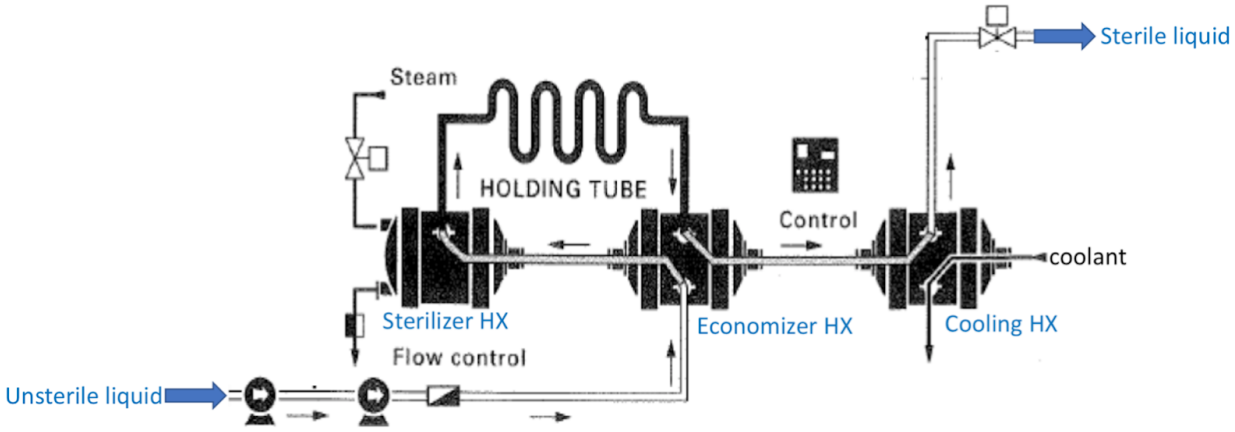


Figure 13.7. Diagram of sterilization loop (26).

13.7.1 Economizer

The economizer utilizes the incoming cool stream from the mixed stream and the sterilizer output to exchange heat to decrease the duty of the sterilizer and cooler. The unit will be a shell and tube heat exchanger with cooling tubes of 3.81 cm and length of 9.14 m. The exchanger has 85 tubes. The cold stream will be exiting the fermenter at 33°C and will exit the economizer at 52°C. This stream will continue onto the sterilizer. The sterilizer outlet stream will be at the sterilization temperature of 140°C. After the economizer, it will be at 102°C. This will proceed to the cooler to return to the reactor.

13.7.2 Sterilizer

The sterilization time needed at a temperature of 140°C is 2.5 minutes (27). A heat exchanger with high temperature steam will be used to heat the media stream from 52°C, the exit temperature of the economizer, to 140°C, the temperature needed for sterilization. Steam at 150°C will be used and will exit at 100°C. The unit will be a shell and tube heat exchanger with cooling tubes of 3.81 cm and length of 9.14 m. The exchanger has 112 tubes and a steam flow rate of 1,171 L/min. A 0.15 m diameter tube with a length of 10 m will be used to achieve the required residence time in the sterilizer unit at 140°C. After this tube length, the media is adequately sterilized.

13.7.3 Cooler

After the sterilizer and the economizer, more cooling is needed before the media can return to the fermenter. The hot stream enters the cooler at 102°C and leaves at 33°C, which is the operating temperature of the fermenter. The cooling water is chilled at 12°C and leaves at 33°C. The unit will be a shell and tube reactor with cooling tube diameters of 3.81 cm and

cooling tube lengths of 9.14 m. It has 54 tubes and a coolant flow rate of 955 L/min of cooling water.

13.8 Dryer

After centrifugation, the solid discharge of cell mass will still be quite wet, as it will be roughly 75% water by weight. To remove this water, a spray dryer will be used to fully dry the SCP. Spray drying consists of three main steps. First, the cell mass slurry will be atomized to produce small particles. Next, a drying gas will be sprayed over the atomized liquid stream. Finally, dry particles will be formed and separated from the exhaust gas. The spray dryer will use air as the drying gas will enter at an inlet temperature of 300°C and an exit temperature of 90°C. To protect the SCP from being damaged by the high heat of the drying gas, a co-current spray dryer will be used, so that the hotter inlet gas will only be in contact will the still wet particles. The spray dryer will also be open-loop, meaning the drying gas will be vented, not recycled. A diagram of this unit can be seen in Figure 13.8 (28).

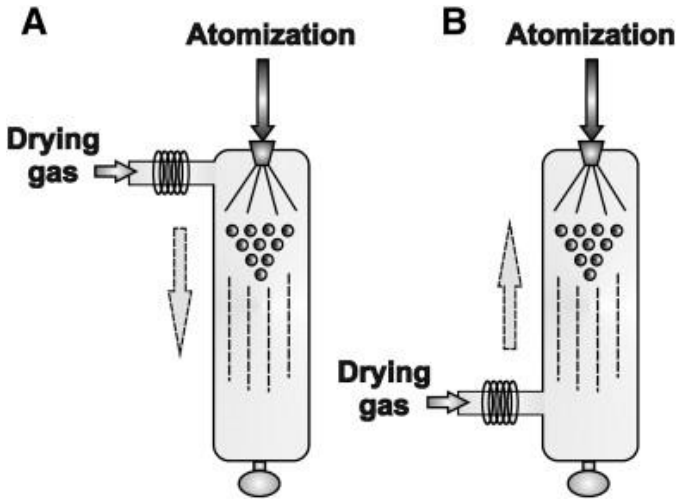


Figure 13.8. Diagram of a spray dryer in cocurrent and countercurrent flow (28).

13.9 Final Packaging

After drying, the SCP will be ready to be packaged for final distribution. From the spray dryer, the SCP will be dropped into a holding bin with a bin activator, more information about which can be found in Appendix D. Here, a motor will vibrate the product in the bin at a constant rate to allow for even distribution into large plastic packages known as super sacks. These super sack containers will be roughly one cubic meter in volume. 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 (MT/yr)	Yearly Cost ($)
High Pressure Steam	867,536.9	$15,268,650
Cooling Water	2,451,227.3	$66,183
Process Water	147,053.0	$39,704
Total:	3,857,699.1	$15,374,537

The utilities needed for this project and their projected cost are displayed in Table 14.1. The largest expense in utilities is due to the high pressure steam needed to sterilize new and recycled media in the sterilizer/economizer loop. Cooling towers will be used to supply the cooling water needed for the many heat exchangers, and the operation of those cooling towers is reflected in the price above. Process water will be procured from local suppliers at ambient conditions.

14.2 Electricity Requirements

Table 14.2. Yearly energy requirements for process units.

Source	Duty (kW)	Yearly Energy Consumption (kW-hr)	Yearly Cost ($)
Pumps	3.94	32,174.9	$2,252
Compressors	186.45	,1521,432.0	$106,500
150,000L bioreactor	23.80	194,208.0	$13,595
150,000L bioreactor	23.80	194,208.0	$13,595
Seed Train Reactors	19.12	14,684.2	$1,028
Bin Activator	0.10	791.5	$55
Mineral Storage Tank	17.70	144,432.0	$10,110
Centrifuges	168.00	1,37,0880.0	$95,962
CIP System	15.00	6,000.0	$420
Spray Dryer	4,418.55	36,055,368.0	$2,523,876
Total:	4,876.46	39,534,178.6	$2,767,392

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, such as units in the seed train, the periods of down-time between use were considered when calculating the yearly energy consumption of those units. From the table, it can be seen that the majority of the energy costs are associated with the operation of the spray dryer. Spray dryers are a notoriously large consumer of electricity, as a study of spray dryers in the U.K. found that the average industrial dryer required 1.35 kW-h of energy per kg of water evaporated (29).

15. Equipment List and Unit Descriptions

15.1 Feed Material Process Units

15.1.1 Methanol Storage Tank (T-02)

Two 250,000 L stainless steel storage tanks with cone roof will be used to store a six day supply of methanol at room temperature of approximately 20°C and 1 bar. The flow rate of methanol into each of the large bioreactors is around 29.84 MT/day, so this size tank should be sufficient in supplying both large bioreactors or one bioreactor and the seed train for three days each. This storage tank was estimated to cost $343,091 each according to the Equipment Costing Spreadsheet. Liquid methanol is highly flammable, and the vapor can be toxic, so this storage tank will comply with appropriate safety regulations to avoid these dangerous risks. Two tanks will be used to ensure that no delays are incurred in operation due to weather related delivery issues.

15.1.2 Mineral Growth Media Storage Tank (T-03)

A 90,000 L stainless steel storage tank with cone roof will be used to store a three day supply of mineral growth media at room temperature of approximately 20°C and 1 bar. The flow rate of mineral growth media into each of the large bioreactors is around 15,000 L/day, so this size tank should be sufficient in supplying both bioreactors or one bioreactor and the seed train. This storage tank was estimated to cost $198,117 according to the Equipment Costing Spreadsheet. This tank will require agitation with a power consumption of 17.7 kW in order to ensure all the minerals are fully and equally dissolved. The minerals should dissolve easily as their concentrations, listed in Table 15.1.2. Are well below the solubility limits for each of the

minerals in tap water. The mineral growth media will run through the sterilization process described in Section 15.3.8 before entrance into the bioreactors to ensure no contaminants are introduced into the system.

Table 15.1.2. Concentrations of minerals present in mineral growth media storage tank for entry into the sterilizer and bioreactors.

Mineral Name	Concentration (g/100 mL)
Disodium hydrogen phosphate	8.70
Potassium dihydrogen phosphate	2.99
Magnesium sulfate heptahydrate	2.12
Calcium chloride	1.19
Iron (II) sulfate heptahydrate	0.85

15.1.3 Ammonia Storage Tank (T-04)

Two 7,500 L stainless steel storage tanks with cone roof will be used to store a six day supply of anhydrous ammonia as a liquified compressed gas. The expected range of conditions for storage are temperatures between 0°C and 20°C with associated vapor pressures of 1.1 bar and 13.6 bar, respectively. The flow rate of ammonia into each of the large bioreactors is around 1.27 MT/day, so this size tank should be sufficient in supplying both large bioreactors or one bioreactor and the seed train for three days each. This storage tank was estimated to cost $55,374 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 variations in the ambient temperature of the tank due to climate seasonality. Furthermore, ammonia gas is highly toxic, so this tank will need to comply with all safety regulations to avoid the risk of leakage. Two of these tanks will be used to keep a backup supply of ammonia to avoid weather related delays. Additionally, due to the safety

concerns of ammonia, two smaller tanks are more appropriate for storage than one larger tank to minimize pressure buildup and other risks.

15.1.4 Ammonia Vaporizer (P-01)

An Azeovare Steam Powered Anhydrous Ammonia Vaporizer model A480SAA will be purchased to vaporize the anhydrous ammonia safely and efficiently before entering the bioreactors. This stainless steel vaporizer is designed for maximum safety and has 100% turndown capabilities if the plant is running at less than full capacity. Furthermore, while a flow rate of about 110 kg/hr will typically be needed, this unit has a capacity of 218 kg/hr which will provide some leniency for production capacity. The typical operating pressure for this unit is 17.2 bar, but has been tested at pressures of up to 25.8 bar to ensure safety. This vaporizer utilizes steam to power the vaporization process and would require about 59 kg/hr steam to be input into the heat exchanger under normal operating conditions. The purchasing cost of this unit is $40,000.

15.1.5 Air Compressor (P-02)

A 100 horsepower rotary screw air compressor will be purchased from Eaton Compression to pressurize the air needed for the large fermenters to 2.35 bar. The compressor will have a three phase 100 horsepower engine that will operate at 1,750 rpm. The dimensions of the compressor will be roughly 1.4 x 2.1 x 1.8 meters. The compressor will utilize 8,000-hour synthetic oil, a cast iron motor, and a gear driven direct drive. The purchasing cost of the air compressor unit is $324,348.

15.1.6 Coarse Air Filters (F-01)

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.

15.1.7 Submicron Air Filters (F-02)

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.

15.2 Seed Train Process Units

15.2.1 1 mL Test Tubes (T-01)

Thermo Scientific Matrix 1.0 mL ScrewTop Tubes in Barcoded Latch Racks 3741BR will be used to store the genetically modified cells in the on-site cell bank. These tube are capable of storing cells at low temperatures. They will be stored at -80°C in a freezer. The material is medical grade polypropylene, and the tubes come with certified sterility. Each package has 5 racks of 96 tubes per case. Two 480 tube cases will be purchased at $584 per case.

15.2.2 10 mL Bioreactor (R-01)

The 10 mL bioreactor is the first reactor in the seed train scale up. The Sartorius Stedim Biotech ambr 15 cell culture Single-Use Advanced Micro Bioreactor System is used. This is an automated microscale bioreactor with 24 to 48 parallel processing and control to ensure reproducibility and quality samples. The bioreactor comes with predictive pH and dissolved oxygen control. The unit also has temperature control. Each micro bioreactor has a gas sparge tube, sample port, and an impeller to make for easy and controlled use. Small pumps are also available to ensure controlled flow into the reactor. The 10 mL bioreactor system will cost $313.

15.2.3 100 mL Bioreactor (R-02)

The 100 mL bioreactor is the second reactor in the seed train scale up. The Sartorius Stedim Biotech ambr 250 cell culture Single-Use Advanced Micro Bioreactor System is used. This is an automated microscale bioreactor with 8 parallel processing and control to ensure reproducibility and quality samples. The bioreactor comes with predictive pH and dissolved oxygen control. The unit also has temperature control. Each micro bioreactor has a gas sparge tube, sample port, and an impeller to make for easy and controlled use. Pumps are also available to ensure controlled flow into the reactor. The 100 mL bioreactor will cost $1,250.

15.2.4 2 L Bioreactor (R-03)

The 2 L bioreactor is the third reactor in the seed train scale up. The Sartorius Stedim Biotech BIOSTAT A cell cultivation and microbial fermentation unit is used. This bioreactor is designed to be simple and easy to use. The BIOSTAT A is used with the UniVessel SU single use vessel. Automatic aeration control allows for the control of air and other gases entering the reactor, which will be particularly useful when adjusting the methanol flow rate over the course

of the experiment. The bioreactor comes with predictive pH and dissolved oxygen control. The unit also has temperature control, a gas sparge tube, a sample port, and an impeller to make for easy and controlled use. Pumps are also available to ensure controlled flow into the reactor. The 2 L bioreactor will cost $7,540.

15.2.5 25 L Bioreactor (R-04)

The 25 L bioreactor is the fourth reactor in the seed train scale up. The Sartorius Stedim Biotech BIOSTAT STR Bioreactor and Flexsafe STR Bags unit is used. The bioreactor will be custom made to accommodate the 25 L working volume needed. The single use bioreactor comes with replaceable bags, connectors, and tubes. Automatic aeration control allows for the control of air and other gases entering the reactor, which will be particularly useful when adjusting the methanol flow rate over the course of the experiment. The bioreactor comes with predictive pH and dissolved oxygen control. The unit also has temperature control, a gas sparge tube, a sample port, and two Rushton impellers to make for easy and controlled use. Pumps are also available to ensure controlled flow into the reactor. The 25 L bioreactor will cost $34,317.

15.2.6 500 L Bioreactor (R-05)

The 500 L bioreactor is the fourth reactor in the seed train scale up. The Sartorius Stedim Biotech BIOSTAT STR 500 Bioreactor and Flexsafe STR Bags unit is used. The single use bioreactor comes with replaceable bags, connectors, and tubes. Automatic aeration control allows for the control of air and other gases entering the reactor, which will be particularly useful when adjusting the methanol flow rate over the course of the experiment. The bioreactor comes with predictive pH and dissolved oxygen control. The unit also has temperature control, a gas sparge tube, a sample port, and two Rushton impellers to make for easy and controlled use.

Pumps are also available to ensure controlled flow into the reactor. The 500 L bioreactor will cost $207,072.

15.2.7 10,000L Bioreactor (R-06)

The 10,000 L bioreactor is the fourth reactor in the seed train scale up. The Sartorius Stedim Biotech BIOSTAT STR Bioreactor and Flexsafe STR Bags unit is used. The bioreactor will be custom made to accommodate the 10,000 L working volume needed. The single use bioreactor comes with replaceable bags, connectors, and tubes. Automatic aeration control allows for the control of air and other gases entering the reactor, which will be particularly useful when adjusting the methanol flow rate over the course of the experiment. The bioreactor comes with predictive pH and dissolved oxygen control. The unit also has temperature control, a gas sparge tube, a sample port, and two Rushton impellers to make for easy and controlled use. Pumps are also available to ensure controlled flow into the reactor. The 10,000 L bioreactor will cost an estimated $1,249,508.

15.2.8 Seed Train Heat Exchanger (H-09)

The sixth seed train reactor, 10,000 L, needs an external heat exchanger to provide adequate cooling to maintain a 33 °C reactor temperature. Because this process is batch, the coolant flow rate, the recycle flow rate, and the log mean temperature difference of reactor contents through the heat exchanger will change with time. These relationships can be found in the specification sheet for the 10,000 L seed train heat exchanger. The heat exchanger is a fixed head shell and tube heat exchanger with 53 tubes that are 9.14 m long and 3.61 cm in diameter. The heat exchanger is made of Stainless Steel 304. The heat exchanger will have a changing log mean temperature difference ranging from 0.45 °C when the least amount of cooling is needed at

the beginning of the batch to 10.49°C at the end of the batch. This is caused by a change in the coolant outlet temperature. The coolant always enters at 12°C, but exits at 33°C at the beginning of the batch and 22°C at the end of the batch. The change in the hot stream temperature is constant throughout the process, being cooled from 33°C to 26°C. The seed train heat exchanger will cost an estimated $182,000.

15.2.9 150,000 L Batch Process Heat Exchanger (H-01/H-02 during Batch)

The large scale 150,000 L reactors each need an external heat exchanger to provide adequate cooling to maintain a 33°C reactor temperature. Because this process is batch, the coolant flow rate, the recycle flow rate, and the log mean temperature difference of reactor contents through the heat exchanger will change with time. These relationships can be found in the specification sheet for the 150,000 L batch process heat exchanger. The heat exchanger is a fixed head shell and tube heat exchanger with 691 tubes that are 9.14 m long and 3.61 cm in diameter. The heat exchanger is made of Stainless Steel 304. The heat exchanger will have a changing log mean temperature difference ranging from 0.69°C when the least amount of cooling is needed at the beginning of the batch to 11.9°C at the end of the batch. This is caused by a change in the coolant outlet temperature. The coolant always enters at 12°C, but exits at 33°C at the beginning of the batch and 22°C at the end of the batch. The change in the hot stream temperature is constant throughout the process, being cooled from 33°C to 26°C. The batch start up heat exchanger will cost an estimated $905,000.

15.3 Continuous Process Units

15.3.1 Gas Compressors (P-03, P-04)

Two 150 horsepower rotary screw air compressors will be purchased from Eaton Compressor to pressurize the air needed for the large fermenters to 2.35 bar. Each compressor will have a three phase 150 horsepower engine that will operate at 1,750 rpm. The dimensions of the compressors will be roughly 1.6 x 2.6 x 1.9 m. The compressors will utilize 8,000-hour synthetic oil, a cast iron motor, and a gear driven direct drive. Each gas compressor will cost $324,348.

15.3.2 150,000 L Reactors (R-07, R-08)

Two large 150,000 L reactors made of Stainless Steel 304 are needed for the batch start up period and the continuous production of SCP and astaxanthin. As shown in the mass balance, the reactors will have a gas inlet at the bottom of the reactor that allows the gas to bubble through the reactor to be consumed. The top of the reactor has a gas vent stream that goes to a gas scrubber. Methanol and mineral solution enter near the agitator to eliminate methanol hotspots that could be toxic to the cells. The agitator will need 23.8 kW power to sufficiently mix the components of the bioreactor for maximum cell growth. The reactor has an external cooler to maintain the 33°C temperature inside the reactor that is ideal for cell growth. The product of the reactor is fed to the centrifuge and the liquid effluent from the centrifuge is sterilized and recycled back to the reactor. The reactor has an 80% fill fraction and thus a usable volume of 120,000 L and is maintained at a pressure of 1.22 bar to prevent contamination in the case of possible leakage. Each of these bioreactors will cost $1,312,331 and will be custom designed by Paul Mueller Company.

15.3.3 Heat Exchanger Pumps (P-05, P-06)

Two large pumps will be needed to drive the reactor contents through the two external heat exchangers used to maintain the temperature of the reactor at 33°C. The units will need to need to pump 3,943.4 L/min of the reactor culture through roughly 10 m of tubing, and the resulting pressure drop of the material will be 0.223 bar. A model CV 3196 Non-clog pump will be purchased from Goulds Pumps. This pump is design for handling bulky materials that are sensitive to shear force. The pump has a flow capacity of 610 m^3/hr and a maximum head of 134 m. These two heat exchanger pumps will cost $7,290 each.

15.3.5 Heat Exchangers (H-01, H-02)

The continuous 150,000 L bioreactors need external heat exchangers to maintain an adequate fermentation temperature of 33 °C inside of the reactor. The heat exchanger is a fixed head shell and tube heat exchanger with 455 tubes that are 9.14 m long and 3.61 cm in diameter. The heat exchanger is made of Stainless Steel 304. The cool stream is tube side and the hot stream is shell side. The heat exchanger will require a log mean temperature difference of 10.5°C, resulting from a change in the hot stream of 33°C to 22°C and a coolant of chilled water flow rate temperature increase from 12°C to 22°C. The coolant flow rate required is 4,487.9L/min. The reactor contents will recycle through the heat exchanger at 4,079.9 L/min. Each of these large heat exchangers will cost $619,834.

15.3.5 Gas Scrubber (F-03)

A Model CS-17 chemical scrubber will be purchased from Pollution Systems to remove contaminants from the vent gases of any cell culture before they are released to the atmosphere. The scrubber will process vent gas at a flow rate of roughly 10,559 kg/hr, and the scrubbing

liquid will be water. The unit will be a packed bed scrubber with a stainless steel construction. The scrubber has a removal efficiency of 95% and a maximum air flow capacity of 29,733 m^3/hr. The scrubber stack will have a height of 11 m and a diameter of 0.7 m. The unit will come equipped with a 30 horsepower process fan 34,069 L/hr recycle pump. The gas scrubber will cost $18,687.

15.3.6 Solid Bowl Centrifuges (C-01, C-02)

The centrifuge is used as the first step in the separation of the media recycle and the finished dry mass product. A solid bowl centrifuge is used to create a liquid recycle stream that is fed to the sterilizer loop and back into the reactor and a cell slurry at a 25% by weight cell concentration. A horizontal cylindrical bowl has a screw conveyor that separates the solids and liquid via centrifugal force. A distributor accelerates the liquid to produce this effect. A conveyor inside the bowl takes the solids on the wall of the centrifuge and brings them to the end of the centrifuge, which is conical. The Alfa Laval Foodec centrifuge will be used to separate the liquid recycle and the cell slurry. A processing rate of 15,000 to 25,000 L/hr is needed. The bowl size is 0.762 m. The utilities required are 149-186 kW and the unit voltage is 480-4,160 V. Two centrifuges will be purchased, one to process the fermenter effluent from each reactor. Additionally, this will ensure that in the event of a technical issue with the centrifuge, the process can still operate at half capacity. Each of the solid bowl centrifuges will cost $96,224.

15.3.7 Economizers (H-03, H-06)

After the liquid recycle stream is separated in the centrifuge, it must be sterilized before going back into the reactor. The economizer is meant to optimize heat exchange between the stream entering the sterilizer and the stream leaving the sterilizer. This will decrease the duty of

the sterilizer and the cooler that cools the stream back to a suitable temperature for the reactor. The amount of heat exchanged is 3,804 kW. The economizer is a fixed head shell and tube heat exchanger with 86 tubes that are 9.14 m long and 3.61 cm in diameter. The cool stream is tube side and the hot stream is shell side. The economizer is made of Stainless Steel 304. The economizer will require a log mean temperature difference of 78.7°C, resulting from a change in the hot stream coming from the sterilizer of 140°C to 102°C and a stream of liquid recycle flow increasing from 33°C to 52°C. The flow rate of the hot and cold stream is the same because they are connected streams with a flow rate of 272.7 L/min. Each of the economizers will cost $70,314.

15.3.8 Sterilizers (H-04, H-07)

A sterilizer is needed to make sure all unwanted bacteria is eliminated from the recycle media. In order to achieve sterilization, the media must be at 140°C for 2.5 minutes (27). The sterilizer has two parts, one to achieve 140°C in the mixture, and another holding tube to maintain this temperature for the appropriate amount of time. The heat exchange portion of the sterilizer is a shell in tube heat exchanger made of Stainless Steel 304. The amount of heat needed to raise the media to this temperature is 1,673 kW. For heat exchange, 112 tubes are needed. Each tube has a diameter of 3.81 cm and a length of 9.14 m. The cool stream is tube side and the hot stream is shell side. The inlet stream will enter at 52°C from the economizer. It will leave at 140°C and enter the holding tube. The holding tube is a 0.15 m diameter tube of length 38.6 m. It is insulated with polyurethane. Steam will enter the reactor at 150°C and leave at 100°C. The flow rate of steam is 1,771 L/min. After being adequately sterilized, the stream will

enter the economizer then the cooler to be cooled to the temperature of the fermenter. Each of the sterilizers will cost $80,189.

15.3.9 Coolers (H-05, H-08)

After the sterilizer and economizer, a shell in tube heat exchanger is used to cool the media back to the temperature of the reactor, 33°C. The stream leaves the economizer at 102°C and is cooled to 33°C. In order to accomplish this cooling, 1,331 kW of energy is exchanged. The coolant is chilled water entering at 12°C and leaving at 33°C. The log mean temperature difference is therefore 39.9°C. The tubes are 3.81 cm in diameter and 9.14 m in length. The cool stream is tube side and the hot stream is shell side. The number of tubes needed to accomplish cooling is 54. The coolant flow rate is 954.5 L/min. The effluent from the heat exchanger is returned to the fermenter. Each of the coolers will cost $58,133.

15.3.10 Dryer (D-01)

A spray dryer will be purchased from GEA to remove any excess moisture from the solid discharge stream exiting the centrifuge. The dryer will have to have to remove water at a rate of 3,273.8 kg/hr from a solid slurry flow rate of 4,368.3 kg/hr. Because of this high water evaporation rate requirement, a special order will likely have to be placed with a manufacturer to produce a unit capable of processing the amount of product required for this project. The dryer design will be based on the Versatile-SD dryer from GEA. The drying gas will be air, and the inlet temperature of the gas will be approximately 300°C. The air-flow will run co-currently will the atomized cell particles, and the gas will exit at a temperature of 90°C. The unit will be quite large, with dimensions exceeding 20 x 10 x 20 m, so this large footprint will have to be accounted for on the plant floor. The dryer will cost $374,615.

15.3.11 Live Bottom Bin (B-01)

A 10,000 L stainless steel holding bin with a Wamgroup bin activator model BA-040 will be utilized to ensure even distribution of product into super sacks for final packaging. The bin activator consists of a 41 cm diameter vibrating cone and convex baffle plate to ensure even discharge from the holding bin supportive of the product flow rate of 1,094 kg/hr. The purchase cost of the bin activator is $1,330 in addition to the holding bin cost of $63,862. The bin activator will require a single motor with 230V and 1 A current for a power consumption of 96.9 W. The product from this live bottom bin will drop directly into the super sacks for final packaging and distribution.

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. These pumps were designed using the dP Pump Designer software. For most of the streams, a pipe inner diameter of 131.7 mm and a length of 10 m was assumed. For streams with large flow rates, such as the streams leading to the external heat exchangers of the 150,000 L fermenters, the pipe diameter was increased to 206.5 mm. Other factors such as the elevation change were also taken into account. It was also assumed that there would be two 90-degree bends in the piping for all pumps calculated. The pressure drops associated with the pumps range from 0.2 bar to 0.5 bar, and the resulting power requirements range from 3.5 to 21.8 W. The piping will all be constructed with stainless steel.

15.4.2 Mixers and Splitters

Mixers and splitters of two or more fluid streams are needed at various points throughout the process, including at the exits to the material holding tanks, the entrances to the economizer and sterilizer loops, and the entrance to the drier. In each of these cases, the streams being mixed or split consist of fairly homogeneous fluids and thus the mixing and splitting can be accomplished most simply 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 Water Cooling Tower

A water cooling tower will have to be installed on site to provide the cooling water needed for the heat exchangers and coolers in the process. The overall process will require 2,843,109 MT of cooling water per year, with the largest need for cooling water coming from the two external heat exchangers used to regulate the temperature of the 150,000 L bioreactors. The cooling water for all processes will need to be chilled to 12°C. Due to the cold climate in Alberta, Canada, for much of the year this chilled temperature can be achieved without refrigeration, though during the warmer months some refrigeration might be necessary. 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 867,536.9 MT of high pressure steam per year. The majority of

this steam will go to the sterilizer to heat recycled and fresh media to 140°C for 2.5 minutes, and a smaller quantity of steam will be needed for the ammonia vaporizer. The steam will be produced with a temperature of 150°C for these processes. The yearly cost of the operation and maintenance of the steam generator is reflected in the cost factors used when calculating the cost 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 150,000 L fermenters, pipes, mixers, and heat exchangers between continuous batches. This will occur every 6 weeks 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 the process. A customized Sani-Matic Ultra Flow 110 will be used. This system is portable, so it can be easily transported in the event that 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 incredibly 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. The tank schematic is shown below in Figure 15.4.5 (30).

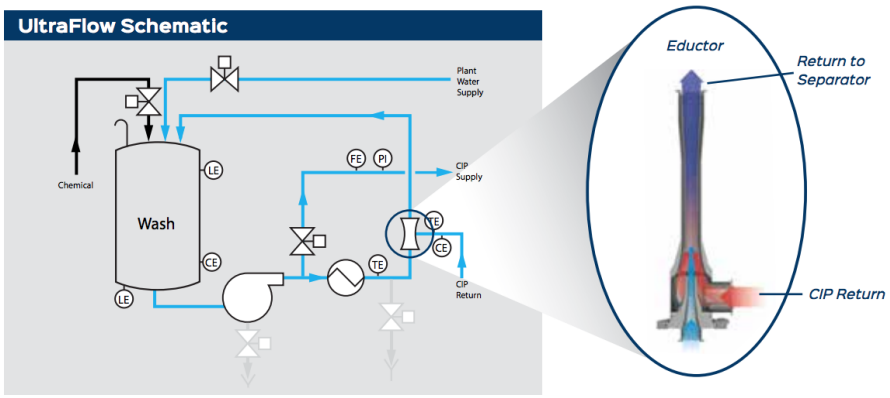


Figure 15.4.5. Schematic diagram of the Clean-In-Place system (30).

15.4.6 Bacterial Genetic Modification Lab

The methylomonas bacteria will need to be genetically modified by researchers and lab staff to create astaxanthin as a co-product. Genetic modification techniques of this sort are very well established in the field so hired researchers should be able to readily complete the desired modification. This process will be included in the services and royalties fees of the plant. A more detailed sensitivity analysis is explored in Section 20.3 to determine the appropriate royalties considering upfront payments or an annual share of the profits.

15.4.7 Biowaste Inactivation System

A biowaste inactivation system is needed to kill any live cells remaining throughout the startup seed train, given the design that only 80% of each batch is transferred to the following bioreactor unit. This includes liquid volumes from all CIP washes from the bioreactors. The inactivation process requires mixing bleach with the liquid solution containing cells to inactivate the cells before disposal. This system will be included in the annual expenses of the plant.

15.4.8 Quality Control Lab

A quality control lab will be utilized to ensure the manufactured product meets the quality specifications, as well as GMO and Canadian Food Inspection Agency (CFIA) regulations. Various samples will be taken throughout the process with a specific focus on proper bacterial function and appropriate astaxanthin concentration. This lab will be included in the annual expenses of the plant.

15.4.9 Final Packaging

The final product will be packaged in super sacks, each holding a volume of about 700 L or 495 kg product. To accommodate for the production volumes of the plant, about 53 super

sacks will be filled daily for shipment. Each super sack costs $15 and can be purchases from Bag Corp, for a total of $270,300 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 expected that customers would be purchasing our product in units of super sacks for bulk animal feed usage.

16. Specification Sheets

Methanol Storage Tank (T-02)

Description and Function	A 250,000 L storage tank will be used to hold liquid methanol delivered via railway before input as fuel for the bioreactors.		
Vendor	N/A		
Operation	Batch input, continuous output		
Materials Handled		Input (MT/day)	Output (MT/day)
	Methanol	59.68	59.68
Characteristics	Material: Stainless Steel 304 Volume: 250,000 L Working Capacity: 80% Height/Diameter Ratio: 2/1 Flow Capabilities: 100,000-250,000 L/day		
Operating Conditions/ Design Data	Temperature: 20°C Pressure: 1 bar Duration: 3 days		
Purchase Cost	$343,091		
Utilities	N/A		
Comments and Drawings	N/A		

Mineral Growth Media Storage Tank (T-03)

Description and Function	A 90,000 L storage tank with agitation will be used to mix and hold a three day supply of mineral growth media.
Vendor	N/A
Operation	Batch input, continuous output

Materials Handled

	Input (MT/day)	Output (MT/day)
Water	30.536	30.536
Disodium Phosphate	2.656	2.656
Potassium Phosphate	0.914	0.914
Magnesium Sulfate	0.649	0.649
Calcium Chloride	0.363	0.363
Iron Sulfate	0.261	0.261

Characteristics	Material: Stainless Steel 304 Volume: 90,000 L Working Capacity: 80% Height/Diameter Ratio: 2/1 Flow Capabilities: 15,000-40,000 L/day
Operating Conditions/ Design Data	Temperature: 20°C Pressure: 1 bar Duration: 3 days
Purchase Cost	$198,177
Utilities	Power: 17.7 kW
Comments and Drawings	N/A

Ammonia Storage Tank (T-04)

Description and Function	A 7,500 L storage tank will be used to hold anhydrous ammonia as a liquified compressed gas delivered via railway.		
Vendor	N/A		
Operation	Batch input, continuous output		
Materials Handled		Input (MT/day)	Output (MT/day)
	Ammonia	2.54	2.54
Characteristics	Material: Stainless Steel 304 Volume: 7,500 L Working Capacity: 80% Height/Diameter Ratio: 2/1 Flow Capabilities: 1,000-3,000 L/day		
Operating Conditions/ Design Data	Temperature: 0-20°C Pressure: 1-14 bar Duration: 3 days		
Purchase Cost	$55,374		
Utilities	N/A		
Comments and Drawings	N/A		

Ammonia Vaporizer (P-01)

Description and Function	The ammonia vaporizer utilizes steam to vaporize the anhydrous ammonia stored as a liquified gas for preparation for entry into the bioreactors.		
Vendor	Algas SDI		
Operation	Continuous		
Materials Handled		Input (MT/day)	Output (MT/day)
	Ammonia	2.54	2.54
Characteristics	Model: A480SAA Material: Stainless steel Weight: 318 kg Vaporization Capacity: 218 kg/hr Heat Exchanger Design Capacity: 17.2 bar Heat Exchanger Test Capacity: 25.8 bar		
Operating Conditions/ Design Data	Temperature: 20°C Pressure: 8.57 bar		
Purchase Cost	$40,000		
Utilities	Steam: 59 kg/hr		
Comments and Drawings	See Appendix D		

Air Compressor (P-02)

Description and Function	The compressor extracts ambient air and adequately pressurizes it to be used for all the reactors in the reactor seed train.
Vendor	Eaton Compressor
Operation	Continuous during Seed Train operation
Materials Handled	Air — Input (kg/hr): variable — Output (kg/hr): variable
Characteristics	Material: Stainless steel Weight: 1.5 MT Compressor Style: Rotary Screw Oil-Type: 8,000-hour synthetic Maximum Flow Rate: 718.7 m^3/hr at 6.89 bar Motor Speed: 1,750 rpm Control System: graphic user interface with user-friendly Logika Programmable Logic Computer.
Operating Conditions/ Design Data	Temperature: 20°C Pressure: 1.01-1.70 bar
Purchase Cost	$324,000 per unit
Utilities	Power: 75 kW Unit Voltage: 460 V
Comments and Drawings	N/A

Coarse Air Filter (F-01)

Description and Function	M6 class filters provide a preliminary coarse filtration of ambient air to be used in the process before compression.		
Vendor	Camfil		
Operation	Continuous and Seed Train operations		
Materials Handled		Input (kg/hr)	Output (kg/hr)
	Air	(up to) 4,867.6	(up to) 4,867.6
Characteristics	Filter Media Material: Glass Fiber Filter Class: M6 Media area: 8 m^2 Filter Type: V-bank filter Dimensions: 0.6 x 0.6 x 0.2 m Air flow: 3,400 m^3/hr Frame: ABS Weight: 3 kg		
Operating Conditions/ Design Data	Temperature: 20°C Pressure drop: <0.01 bar		
Purchase Cost	$4,000		
Utilities	N/A		
Comments and Drawings	See Appendix D		

Submicron Air Filter (F-02)

Description and Function	H-13 class filters with submicron pore-size to fully purify and sterilize air leaving the compressor before it is introduced to the active cell cultures in the process.		
Vendor	Camfil		
Operation	Continuous and Seed Train operations		
Materials Handled		Input (kg/hr)	Output (kg/hr)
	Air	(up to) 4,867.6	(up to) 4,867.6
Characteristics	Filter Media Material: Glass Fiber Filter Class: H13 Media area: 46 m^2 Filter Type: V-bank box filter Dimensions: 0.75 x 0.6 x 0.3 m Air flow: 6,000 m^3/hr Frame: ABS Weight: 14 kg		
Operating Conditions/ Design Data	Temperature: 20°C Pressure drop: <0.01 bar		
Purchase Cost	$4,000		
Utilities	N/A		
Comments and Drawings	See Appendix D		

1 mL Test Tubes (T-01)

Description and Function	The 1 mL tubes are used to store aliquots of genetically modified bacteria at 14 g/L for the purpose of a cell bank. The tubes will be placed in a -80°C freezer to ensure the cell metabolism is halted.
Vendor	Fisher Scientific
Operation	Storage
Materials Handled	Genetically Modified Methylomonas Clara Bacteria
Characteristics	Model: Matrix 1.0 mL ScrewTop Tubes in barcoded latch racks Material: Virgin Class VI Medical Grade Polypropylene with Virgin Class VI Medical Grade Silicone O-Ring Capacity: 1.0 mL Sterility: Sterile Color: Clear Shape: V-Bottom ScrewTop Tubes Package Configuration: 5 racks of 96/case totaling 480 tubes
Operating Conditions/ Design Data	Temperature: -80°C Pressure: 1 bar Duration: up to 10 years
Purchase Cost	$584 per 480 tubes
Utilities	N/A
Comments and Drawings	N/A

10 mL Bioreactor (R-01)

Description and Function	The 10 mL Micro Bioreactor system will be used as the first step in the seed train scale up process.
Vendor	Sartorius Stedim Biotech
Operation	Batch

Materials Handled

	Input (mg/batch)	Output(mg/batch)
Cell Mass	0.0	266.0
Astaxanthin	0.0	0.798
Carbon Dioxide	0.0	399.0
Methanol	604.5	0.0
Ammonia	25.8	0.0
Oxygen	774.7	258.2
Nitrogen	2,914.3	2,545.0
Water	7,600	8175
Disodium Phosphate	27.0	0.0
Potassium Phosphate	9.3	0.0
Magnesium Sulfate	6.6	0.0
Calcium Nitrate	5.5	0.0
Iron Sulfate	1.4	0.0

Characteristics

Model: ambr 15 cell culture Industry Standard Single-Use Advanced Micro Bioreactor System
Usable volume: 10-15 mL
Number of Samples in Parallel: up to 48
Available Control: pH, DO, feed flow, reagent flow, carbon dioxide flow, nitrogen flow, oxygen flow
Individual Bioreactor Vessel: integral impeller, sparge gas tube, sample port
Liquid Handling Unit: dual pipette heads, automated vessel decapper, plate delidder
Software: online experiment monitoring

Operating Conditions/ Design Data

Temperature: 33°C
Pressure: 1.15 bar
Duration: 6.5 hours

Purchase Cost	$314
Utilities	N/A
Comments and Drawings	See Appendix D

100 mL Bioreactor (R-02)

Description and Function	The 100 mL Micro Bioreactor system will be used as the second step in the seed train scale up process.
Vendor	Sartorius Stedim Biotech
Operation	Batch

Materials Handled

	Input (g/batch)	Output (g/batch)
Cell Mass	0.0	2.6
Astaxanthin	0.0	0.0077
Carbon Dioxide	0.0	3.9
Methanol	5.9	0.0
Ammonia	0.25	0.0
Oxygen	7.6	2.5
Nitrogen	24.9	24.9
Water	76.0	81.6
Disodium Phosphate	0.26	0.0
Potassium Phosphate	0.089	0.0
Magnesium Sulfate	0.064	0.0
Calcium Nitrate	0.053	0.0
Iron Sulfate	0.014	0.0

Characteristics	Model: ambr 250 modular Single-Use Benchtop Bioreactor with Simplified Operation for Increased Productivity Usable Volume: 100-250 mL Number of Samples in Parallel: up to 8 Pumps: peristaltic pump, 5 integrated syringe pumps Available Control: pH, temperature, DO, foam, carbon dioxide, oxygen, nitrogen Individual Bioreactor Vessel: Rushton impellers, sparge tube, exhaust gas condenser, gas tube Software: capable of process definition, runtime, experiment viewer, results viewer
Operating Conditions/ Design Data	Temperature: 33°C Pressure: 1.15 bar Duration: 5.5 hours
Purchase Cost	$1,250
Utilities	N/A
Comments and Drawings	See Appendix D

2 L Bioreactor (R-03)

Description and Function	The 2 L Bioreactor system will be used as the third step in the seed train scale up process.
Vendor	Sartorius Stedim Biotech
Operation	Batch

Materials Handled

	Input (g/batch)	Output (g/batch)
Cell Mass	0.0	53.8
Astaxanthin	0.0	0.16
Carbon Dioxide	0.0	81.2
Methanol	122.5	0.0
Ammonia	5.2	0.0
Oxygen	158.0	52.7
Nitrogen	520.2	520.2
Water	1,520	1,637.2
Disodium Phosphate	5.45	0.0
Potassium Phosphate	1.88	0.0
Magnesium Sulfate	1.33	0.0
Calcium Nitrate	1.10	0.0
Iron Sulfate	0.29	0.0

Characteristics

Model: BIOSTAT A cell cultivation and microbial fermenter
Vessel Model: UniVessel SU (Single Use)
Usable Volume: 2 L
Pumps: 3 internal pumps, 1 external pump
Pump Speed: internal pumps 43 rpm; external pumps 200 rpm
Motor rpm: 30-1,100 rpm
Available Control: pH, temperature, DO, foam, carbon dioxide, oxygen, nitrogen
Individual Bioreactor Vessel: Rushton impellers, sparge tube, exhaust gas condenser, gas tube
Software: Chemometrics Toolbox, BioPAT MODDE, BioPAT

Operating Conditions/ Design Data

Temperature: 33°C
Maximum Relative Humidity: 80%
Pressure: 1.15 bar
Duration: 7.0 hours

Purchase Cost	$7,539
Utilities	Power: 4.78 kW
Comments and Drawings	See Appendix D

25 L Bioreactor (R-04)

Description and Function	The 25 L Bioreactor system will be used as the fourth step in the seed train scale up process.
Vendor	Sartorius Stedim Biotech
Operation	Batch

Materials Handled

	Input (g/batch)	Output (g/batch)
Cell Mass	0.0	655.2
Astaxanthin	0.0	1.97
Carbon Dioxide	0.0	989.4
Methanol	1,493.6	0.0
Ammonia	63.66	0.0
Oxygen	1,926.0	642.0
Nitrogen	6,339.9	6,339.9
Water	19,000.0	20,428.0
Disodium Phosphate	66.34	0.0
Potassium Phosphate	22.9	0.0
Magnesium Sulfate	16.2	0.0
Calcium Nitrate	13.4	0.0
Iron Sulfate	3.57	0.0

Characteristics

Model: BIOSTAT STR Bioreactor and Flexsafe STR Bags
Usable Volume: 25 L
Sensor Window: reusable sensors that connect to external devices
Aeration Control: 0.8 mm ring sparger and a 150 μm micro-sparger
Carbon Dioxide Control: using stripping gas flow
Stirrer Design: two Rushton impellers on a magnetically coupled center-line shaft
Cooling: double wall for fast cooling
Software: BioPAT MFCS- Turnkey SCADA Solution
Flexsafe STR Bag Design:
1:4 turndown ratio
Tubing: C-Flex 374
Connections: Luer sterile connectors and tube welding
Sensors: single use pH and DO probes

Operating Conditions/ Design Data

Temperature: 33°C
Pressure: 1.15 bar
Relative Humidity Range: less than 85%
Duration: 5.95 hours

Purchase Cost	$34,317
Utilities	Power: 4.78 kW
Comments and Drawings	The BIOSTAT STR will be custom made to accommodate the usable volume needed for this reactor. See Appendix D

500 L Bioreactor (R-05)

Description and Function	The 500 L Bioreactor system will be used as the fifth step in the seed train scale up process.
Vendor	Sartorius Stedim Biotech
Operation	Batch

Materials Handled

	Input (kg/batch)	Output (kg/batch)
Cell Mass	0.0	13.4
Astaxanthin	0.0	0.040
Carbon Dioxide	0.0	20.3
Methanol	30.6	0.0
Ammonia	1.31	0.0
Oxygen	39.5	13.2
Nitrogen	130.0	130.0
Water	380.0	409.3
Disodium Phosphate	1.36	0.0
Potassium Phosphate	0.47	0.0
Magnesium Sulfate	0.33	0.0
Calcium Nitrate	0.28	0.0
Iron Sulfate	0.073	0.0

Characteristics

Model: BIOSTAT STR 500 Bioreactor and Flexsafe STR Bags
Usable Volume: 500 L
Sensor Window: reusable sensors that connect to external devices
Aeration Control: 0.8 mm ring sparger and a 150 μm micro-sparger
Carbon Dioxide Control: using stripping gas flow
Stirrer Design: two Rushton impellers on a magnetically coupled center-line shaft
Cooling: double wall for fast cooling
Software: BioPAT MFCS- Turnkey SCADA Solution
Flexsafe STR Bag Design
1:4 turndown ratio
Tubing: C-Flex 374
Connections: Luer sterile connectors and tube welding
Sensors: single use pH and DO probes

Operating Conditions/ Design Data

Temperature: 33°C
Pressure: 1.15 bar
Relative Humidity Range: less than 85%
Duration: 6.97 hours

Purchase Cost	$207,072
Utilities	Power: 4.78 kW
Comments and Drawings	See Appendix D

10,000 L Bioreactor (R-06)

Description and Function	The 10,000 L Bioreactor system will be used as the sixth and final step in the seed train scale up process.
Vendor	Sartorius Stedim Biotech
Operation	Batch

Materials Handled

	Input (kg/batch)	Output (kg/batch)
Cell Mass	0.0	268.8
Astaxanthin	0.0	0.806
Carbon Dioxide	0.0	405.9
Methanol	612.7	0.0
Ammonia	26.1	0.0
Oxygen	790.2	263.3
Nitrogen	2,601.0	2,601.0
Water	7,600	8,185.0
Disodium Phosphate	27.3	0.0
Potassium Phosphate	9.38	0.0
Magnesium Sulfate	6.66	0.0
Calcium Nitrate	5.51	0.0
Iron Sulfate	1.46	0.0

Characteristics

Model: BIOSTAT STR 500 Bioreactor and Flexsafe STR Bags
Usable Volume: 10,000 L
Sensor Window: reusable sensors that connect to external devices
Aeration Control: 0.8 mm ring sparger and a 150 μm micro-sparger
Carbon Dioxide Control: using stripping gas flow
Stirrer Design: two Rushton impellers on a magnetically coupled center-line shaft
Cooling: double wall for fast cooling
Software: BioPAT MFCS- Turnkey SCADA Solution
Flexsafe STR Bag Design:
1:4 turndown ratio
Tubing: C-Flex 374
Connections: Luer sterile connectors and tube welding
Sensors: single use pH and DO probes

Operating Conditions/ Design Data

Temperature: 33°C
Pressure: 1.15 bar
Relative Humidity Range: less than 85%
Duration: 6.97 hours

Purchase Cost	$1,249,508
Utilities	Power: 4.78 kW
Comments and Drawings	See Appendix D

10,000 L Seed Train Heat Exchanger (H-09)

Description and Function	The batch 10,000 L bioreactors need external cooling in order to maintain a temperature of 33°C. A shell in tube heat exchanger with chilled cooling water is needed for this purpose.
Vendor	N/A
Operation	Batch
Materials Handled	10,000 L batch bioreactor contents
Characteristics	Type: Shell in tube, fixed head Effective Surface Area: 57.15 m^2 LMTD: 0.45 °C /10.49 °C Heat Exchanged: 14.7 kW - 340.3 kW Heat Transfer Coefficient: 567 W/m^2C Tube Side Material: Stainless Steel 304 Shell Side Material: Stainless Steel 304 Number of Tubes Required: 53 Tube Length: 9.14 meters Tube Diameter: 3.81 centimeters Coolant Flow Rate Control Equation: Coolant FR[MT/hr]=0.602[MT/hr]$e^{36.7[1/hr]t[hr]}$ Recycle Flow Rate Control Equation: Recycle FR[MT/hr]=1.81[MT/hr]$e^{29.7[1/hr]t[hr]}$
Operating Conditions/ Design Data	Temperature: 33°C Pressure: 1.15 bar
Purchase Cost	$182,000 per unit
Utilities	Cooling Water: 200 L/min
Comments and Drawings	N/A

150,000 L Batch Process Heat Exchanger (H-01, H-02 during Batch Process)

Description and Function	The batch 150,000 L bioreactors need external cooling in order to maintain a temperature of 33°C. A shell in tube heat exchanger with chilled cooling water is needed for this purpose. Because the heat exchangers are on opposite schedules, only one heat exchanger of this nature is necessary.
Vendor	N/A
Operation	Batch
Materials Handled	150,000 L batch bioreactor contents
Characteristics	Type: Shell in tube, fixed head Effective Surface Area: 755.7 m^2 LMTD: 0.69 °C /11.89 °C Heat Exchanged: 294.1 kW - 5,097.8 kW Heat Transfer Coefficient: 567 W/m^2C Tube Side Material: Stainless Steel 304 Shell Side Material: Stainless Steel 304 Number of Tubes Required: 691 Tube Length: 9.14 meters Tube Diameter: 3.81 centimeters Coolant Flow Rate Control Equation: Coolant FR[MT/hr]=13.3[MT/hr]e^$^{33.5[1/hr]t[hr]}$ Recycle Flow Rate Control Equation: Recycle FR[MT/hr]=44.5[MT/hr]e^$^{27.0[1/hr]t[hr]}$
Operating Conditions/ Design Data	Temperature: 33°C Pressure: 1.15 bar
Purchase Cost	$905,000 per unit
Utilities	Cooling Water: 4,225.4 L/min
Comments and Drawings	N/A

Gas Compressors (P-03, P-04)

Description and Function	The compressors extract ambient air and pressurize it to an adequate pressure to be sparged into the bottom of the large bioreactors.
Vendor	Eaton Compressor
Operation	Continuous
Materials Handled	Air — Input (kg/hr): 4,867.6 — Output (kg/hr): 4,867.6
Characteristics	Material: Stainless steel Weight: 1.58 MT Compressor Style: Rotary Screw Oil-Type: 8,000-hour synthetic CFM: 706 at 100 psi Motor Speed: 1,750 rpm Control System: graphic user interface with user-friendly Logika Programmable Logic Computer.
Operating Conditions/ Design Data	Temperature: 20°C Pressure: 2.67 bar
Purchase Cost	$324,000 per unit
Utilities	Power: 111 kW Unit Voltage: 650 V
Comments and Drawings	N/A

150,000 L Bioreactors (R-07, R-08)

Description and Function: The 150,000 L Bioreactors are used to generate SCP mass with astaxanthin in the batch and continuous process.

Vendor: Paul Mueller Company

Operation: Batch/ Continuous

Materials Handled

Batch Process	Input (MT/batch)	Output (MT/batch)
Cell Mass	0.0	4.0
Astaxanthin	0.0	0.012
Carbon Dioxide	0.0	6.0
Methanol	9.1	0.0
Ammonia	0.39	0.0
Oxygen	11.7	38.5
Nitrogen	38.5	122.7
Water	114	126.5
Disodium Phosphate	0.40	0.0
Potassium Phosphate	0.033	0.0
Magnesium Sulfate	0.11	0.0
Calcium Nitrate	0.060	0.0
Iron Sulfate	0.023	0.0

Continuous Process	Input (kg/hr)	Output (kg/hr)
Cell Mass	0.0	545.8
Astaxanthin	0.0	1.63
Carbon Dioxide	0.0	704.2
Methanol	1,241.7	0.0
Ammonia	52.9	0.0
Oxygen	641.7	214.2
Nitrogen	4,225.0	4,225.0
Water	17,635.5	18,133.3
Disodium Phosphate	55.4	0.0
Potassium Phosphate	19.2	0.0
Magnesium Sulfate	13.3	0.0
Calcium Nitrate	7.50	0.0
Iron Sulfate	5.42	0.0

Characteristics:
Model: WFI Process Tank
Usable Volume: 150,000 L
Material: Stainless Steel 304 with 2” chloride free insulation
Height: 9.43 m
Diameter: 4.5 m

Operating Conditions/ Design Data	Temperature: 33°C Pressure: 1.15 bar Relative Humidity Range: less than 85% Duration: 6 weeks
Purchase Cost	$1,312,331
Utilities	Power: 23.8 kW
Comments and Drawings	See Appendix D

Heat Exchanger Pumps (P-05, P-06)

Description and Function	Two large pumps will be needed to provide the driving force necessary to move a constant flow rate of reactor culture through the external heat exchangers of the 150,000 L bioreactors.
Vendor	Goulds Pumps
Operation	Continuous
Materials Handled	150,000 L continuous bioreactor contents
Characteristics	Material: Cast Iron Maximum Flow Rate: 610 m^3/hr Maximum Temperature: 260°C
Operating Conditions/ Design Data	Reactor Contents Flow rate: 3,943 L/min Pressure drop: 0.223 bar
Purchase Cost	$7,200 per unit
Utilities	Power: 1624 W
Comments and Drawings	See Appendix D

Pumps and Piping

Description and Function	Pumps and piping have been designed to transport materials to and from all units in the process. The pumps all have unique pressure drops and power requirements based on the material and flow rate they are responsible for transporting.
Vendor	Goulds Pumps
Operation	Continuous
Materials Handled	Various
Characteristics	Material: Cast Iron Max Flow Rates: 610 m^3/hr Maximum Temperature: 260°C Pipe Diameter: 131.3-206.5 mm Pipe Material: Stainless Steel
Operating Conditions/ Design Data	Flow rates: 636.2-18547.3 kg/hr Pressure drop: 0.2-0.5 bar
Purchase Cost	$7,200 per unit
Utilities	Power: 3.5-99.7 W
Comments and Drawings	See Appendix C for a more detailed breakdown of each pump. See Appendix D for a vendor specification sheet with images.

Heat Exchangers (H-01, H-02)

Description and Function	The continuous 150,000 L bioreactors need external cooling in order to maintain a temperature of 33°C. A shell in tube heat exchanger with chilled cooling water is needed for this purpose.
Vendor	N/A
Operation	Continuous
Materials Handled	150,000 L continuous bioreactor contents
Characteristics	Type: Shell in tube, fixed head Effective Surface Area: 498.42 m^2 LMTD: 10.5 °C Heat Exchanged: 2967.5 kW Heat Transfer Coefficient: 567 W/m^2C Tube Side Material: Stainless Steel 304 Shell Side Material: Stainless Steel 304 Number of Tubes Required: 455 Tube Length: 9.14 m Tube Diameter: 3.81 cm
Operating Conditions/ Design Data	Pressure: 1.15 bar
Purchase Cost	$619,834 per unit
Utilities	Cooling Water: 4,487.9 L/min
Comments and Drawings	N/A

Gas Scrubber (F-03)

Description and Function	The vent gas from all cell cultures will need to be process by the gas scrubber to remove contaminants such as methane and organic molecules before it can be vented.
Vendor	Pollution Systems
Operation	Continuous

Materials Handled

	Input (kg/hr)	Output (kg/hr)
Vent Gas	10,559.3	10,559.3
Water	1,272.4	1,272.4

Characteristics	Material: Stainless steel Pollutant Removal Efficiency: 95% Maximum Air Flow: 29,732.7 m^3/hr Process Fan: 22.4 kW Recycle Pump: 34,069 L/hr Power Requirements: 40 V Pollutant Loading: 14.5 kg/hr Control System: Touch screen operated interface
Operating Conditions/ Design Data	Temperature: 20°C Pressure: 1 bar
Purchase Cost	$18,687
Utilities	Process Water: 21.2 L/min
Comments and Drawings	See Appendix D

Solid Bowl Centrifuge (C-01, C-02)

Description and Function	The solid bowl centrifuge separates the fermenter discharge into a cell slurry at 25 weight % concentration and the liquid media recycle stream. The centrifuge uses flow over a screw to separate the mixture via centrifugal force.
Vendor	Alfa Laval
Operation	Continuous

Materials Handled

	Input (kg/hr)	Output (kg/hr)	
		Media Recycle	Product Stream
Cell Mass	545.6		545.6
Astaxanthin	1.6		1.6
Water	18,000.0	16,363.1	1,636.1

Characteristics	Material: Stainless steel Weight: 20.9 MT Processing Rate: 15,000-25,000 L/hr Bowl Size: 762 mm Solid Feed Rate Maximum: 28% Control System: graphic user interface with many communication options
Operating Conditions/ Design Data	Temperature: 33°C Pressure: 1.15 bar
Purchase Cost	$175,000 per unit
Utilities	Power: 149-186 kW Unit Voltage: 480 - 4,160 V
Comments and Drawings	See Appendix D

Economizers (H-03, H-06)

Description and Function	The continuous 150,000 L bioreactors need sterilization of the liquid recycle stream before the media can be returned to the reactor. This requires a series of heat exchangers, including an economizer, sterilizer, and cooler. The inlet and outlet streams from the sterilizer are run through an economizer to decrease the duty of the sterilizer and the cooler. A shell in tube heat exchanger is used for this purpose.
Vendor	N/A
Operation	Continuous

Materials Handled

Continuous Process	Input (kg/hr)	Output (kg/hr)
Water	18,271.6	18,271.6
Disodium Phosphate	55.4	55.4
Potassium Phosphate	19.2	19.2
Magnesium Sulfate	13.3	13.3
Calcium Nitrate	7.50	7.50
Iron Sulfate	5.42	5.42

Characteristics

Type: Shell in tube, fixed head
Effective Surface Area: 93.0 m^2
LMTD: 78.7 °C
Heat Exchanged: 380.4 kW
Heat Transfer Coefficient: 567 W/m^2C
Tube Side Material: Stainless Steel 304
Shell Side Material: Stainless Steel 304
Number of Tubes Required: 86
Tube Length: 9.14 m
Tube Diameter: 3.81 cm

Operating Conditions/ Design Data	Pressure: 1.15 bar
Purchase Cost	$222,895 per unit
Utilities	N/A
Comments and Drawings	N/A

Sterilizers (H-04, H-07)

Description and Function	The continuous 150,000 L bioreactors need sterilization of the liquid recycle stream before the media can be returned to the reactor. This requires a series of heat exchangers, including an economizer, sterilizer, and cooler. The stream that was heated in the economizer enters the sterilizer to reach adequate temperature for sterilization.
Vendor	N/A
Operation	Continuous

Materials Handled

Continuous Process	Input (kg/hr)	Output (kg/hr)
Water	18,271.6	18,271.6
Disodium Phosphate	55.4	55.4
Potassium Phosphate	19.2	19.2
Magnesium Sulfate	13.3	13.3
Calcium Nitrate	7.50	7.50
Iron Sulfate	5.42	5.42

Characteristics

Type: Shell in tube, fixed head
Effective Surface Area: 121.8 m^2
LMTD: 24.2 °C
Heat Exchanged: 464.9 kW
Heat Transfer Coefficient: 567 W/m^2C
Tube Side Material: Stainless Steel 304
Shell Side Material: Stainless Steel 304
Number of Tubes Required: 112
Tube Length: 9.14 m
Tube Diameter: 3.81 cm
Holding Tube Diameter: 0.15 m
Holding Tube Length: 38.6 m
Holding Tube Flow: Turbulent

Operating Conditions/ Design Data	Pressure: 1.15 bar
Purchase Cost	$254,200 per unit
Utilities	High Pressure Steam: 1770.9 L/min
Comments and Drawings	N/A

Coolers (H-05, H-08)

Description and Function	The continuous 150,000 L bioreactors need sterilization of the liquid recycle stream before the media can be returned to the reactor. This requires a series of heat exchangers, including an economizer, sterilizer, and cooler. The stream that was heated in the sterilizer enters the cooler to reach adequate temperature for return to the fermenter.
Vendor	N/A
Operation	Continuous

Materials Handled

Continuous Process	Input (kg/hr)	Output (kg/hr)
Water	18,271.6	18,271.6
Disodium Phosphate	55.4	55.4
Potassium Phosphate	19.2	19.2
Magnesium Sulfate	13.3	13.3
Calcium Nitrate	7.50	7.50
Iron Sulfate	5.42	5.42

Characteristics	Type: Shell in tube, fixed head Effective Surface Area: 58.8 m^2 LMTD: 39.9 °C Heat Exchanged: 369.8 kW Heat Transfer Coefficient: 567 W/m^2C Tube Side Material: Stainless Steel 304 Shell Side Material: Stainless Steel 304 Number of Tubes Required: 54 Tube Length: 9.14 m Tube Diameter: 3.81 cm
Operating Conditions/ Design Data	Pressure: 1.15 bar
Purchase Cost	$184,283 per unit
Utilities	Cooling Water: 954.5 L/min
Comments and Drawings	N/A

Dryer (D-01)

Description and Function	The spray dryer will dry solid discharge from the centrifuge to remove excess water still present after centrifugation. The spray dryer will atomize the slurry into particles and dry them with a stream of heated air.
Vendor	GEA Process Engineering
Operation	Continuous

Materials Handled

	Input (kg/hr)	Output (kg/hr)
Cell Slurry	4368.3	0
Water	0	3273.8
Dry Cells	0	1091.3

Characteristics	Material: Stainless steel Drying Medium: Air Gas Inlet Temperature: 300°C Gas Outlet Temperature: 90°C Mean Particle Size: 20 μm
Operating Conditions/ Design Data	Temperature: 90-300°C Pressure: 1 bar
Purchase Cost	$375,000
Utilities	Power: 4418.55 kW
Comments and Drawings	See Appendix D

Live Bottom Bin (B-01)

Description and Function	A 5,000 L holding tank with a bin activator will be used to collect final product from the dryer and distribute it evenly into the super sacks through vibration of the solid product.		
Vendor	Wamgroup		
Operation	Continuous		
Materials Handled		Input (kg/hr)	Output (kg/hr)
	SCP	1,091.7	1,091.7
	Astaxanthin	3.24	3.24
Characteristics	Material: Stainless Steel 304 Bin Volume: 10,000 L Bin Height/Diameter Ratio: 2/1 Activator Model: BA-040 Activator Entry Diameter: 41 cm Activator Exit Diameter: 10.2 cm Activator Weight: 59 kg		
Operating Conditions/ Design Data	Temperature: 33°C Pressure: 1 bar		
Purchase Cost	Holding Bin: $63,862 Bin Activator: $1,330		
Utilities	Power: 96.9 W		
Comments and Drawings	See Appendix D		

Super Sacks

Description and Function	Super sacks with a volume of 1,000 L each are used for final packaging of the SCP/astaxanthin product before distribution.		
Vendor	Bag Corp		
Operation	Batch		
Materials Handled		Input (kg/bag)	Output (kg/bag)
	SCP	689.5	689.5
	Astaxanthin	2.1	2.1
Characteristics	Material: Woven Polypropylene Volume: 705 L Dimensions: 89 cm x 89 cm x 89 cm Additional Features: duffel top with tie closure, flat bottom, four lift loops		
Operating Conditions/ Design Data	Temperature: 20°C Pressure: 1 bar		
Purchase Cost	$15 per bag		
Utilities	N/A		
Comments and Drawings	N/A		

Clean-In-Place System

Description and Function	The Clean-In-Place system is used to clean the fermenters, pipes, pumps, and heat exchangers involved in the batch and continuous process. Water with cleaning chemicals will flow through the process units and CIP system through an automated system preset by the vendor customized for this plant. The CIP system will be used every 6 weeks per fermenter.
Vendor	Sani-Matic
Operation	Continuous, once every 6 weeks
Materials Handled	Process Residue, water, cleaning fluid
Characteristics	Model: UltraFlow 110 Material: Wetted Surface-316L stainless steel 25 µin Ra; Non-wetted Surface- 304 stainless steel 32 µin Ra Flow Rate: 18.9-416.4 L/min Size: 1.87 m L x 0.84 m W x 2.03 m H Pipe Diameter Cleaning Ability: 0.152-0.203 m Tank Diameter Cleaning Ability: 4.5 m Software: Allen-Bradley CompactLogix PLC; Allen-Bradley PanelView Plus HMI
Operating Conditions/ Design Data	Temperature: 20-80°C Pressure: 4.14 bar
Purchase Cost	$175,000 per unit
Utilities	Power: 15 kW
Comments and Drawings	A customized version of the Sani-Matic UltraFlow 110 will be used in order to accommodate the pipe diameter and tank diameter needed to clean the process. See Appendix D.

17. Equipment Cost Summary

Table 17.1 details the cost of equipment needed for the seed process, batch process, and continuous process. Although rounded numbers are shown for simplicity, unrounded values were used to calculate total bare module costs and the overall equipment cost. A CE index value of 600 was used in the calculations.

Table 17.1. 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	K2 Scientific	8,300	1	3.17	26,300
1 mL Test Tubes	N/A	480	Fisher Scientific	583	2	3.17	3,700
Storage							
Methanol Storage Tank	T-02	1	N/A	343,000	2	3.21	2,200,000
Mineral Growth Media Storage Tank	T-03	1	N/A	198,000	1	3.21	636,000
Ammonia Storage Tank	T-04	1	N/A	55,400	2	3.21	35,600
Seed Train Process							
Pump	S-008	1	Goulds Pumps	7,210	1	2.15	15,500
Pump	S-009	1	Goulds Pumps	7,210	1	2.15	15,500
Pump	S-011	1	Goulds Pumps	7,210	1	2.15	15,500
Ammonia Vaporizer	P-01	1	Algas SDI	40,000	1	2.15	68,000
Compressor	S-012	1	Eaton Compressor	324,000	1	2.15	697,000
Compressor	S-013	1	Eaton Compressor	324,000	1	2.15	697,000
Air Compressor	P-02	1	Eaton Compressor	324,000	1	2.15	697,000
Compressor	S-014	1	Eaton Compressor	324,000	1	2.15	697,000
Coarse Air Filter	F-01	1	Camfil	4,000	1	2.32	9,280

Compressor	S-015	1	Eaton Compressor	324,000	1	2.15	697,000
Submicron Air Filter	F-02	1	Camfil	4,000	1	2.32	9,280
Compressor	S-016	1	Eaton Compressor	324,000	1	2.15	697,000
10 mL Reactor	R-01	1	Sartorius Stedim Biotech	312	1	4.16	1,310
100 mL Reactor	R-02	1	Sartorius Stedim Biotech	1,250	1	4.16	5,200
2 L Reactor	R-03	1	Sartorius Stedim Biotech	7,540	1	4.16	31,400
25 L Reactor	R-04	1	Sartorius Stedim Biotech	34,300	1	4.16	143,000
500 L Reactor	R-05	1	Sartorius Stedim Biotech	207,000	1	4.16	861,000
10,000 L Reactor	R-06	1	Sartorius Stedim Biotech	1,250,000	1	4.16	5,200,000
Pump	S-073	1	Goulds Pumps	7,210	1	2.15	15,500
Cooler	H-09	1	N/A	57,000	1	3.17	182,000
Pump	S-074	1	Goulds Pumps	7,210	1	2.15	15,500
Batch Process/Continuous Process							
Gas Compressor	P-03, P-04	1	Eaton Compressor	324,000	2	2.15	1,390,000
Compressor	S-035, S-037	1	Eaton Compressor	324,000	2	2.15	1,390,000
Pump	S-030, S-031	1	Goulds Pumps	7,210	2	2.15	31,000
150,000 L Reactor	R-07, R-08	1	Paul Mueller Company	1,310,000	2	4.16	10,900,000
Pump	S-038/P-05, S-041/P-06	1	Goulds Pumps	7,210	2	2.15	31,000
Batch Heat Exchanger	H-01/H-02	1	N/A	285,000	1	3.17	905,000
Heat Exchanger	H-01, H-02	1	N/A	619,000	2	3.17	3,930,000
Pump	S-044, S-051	1	Goulds Pumps	7,210	2	2.15	31,000
Centrifuge	C-01, C-02	1	Alfa Laval	96,200	2	2.03	390,000
Pump	S-045, S-052	1	Goulds Pumps	7,210	2	2.15	31,000

Pump	S-060, S-061	1	Goulds Pumps	7,210	2	2.15	31,000
Pump	S-058, S-059	1	Goulds Pumps	7,210	2	2.15	31,000
Pump	S-070	1	Goulds Pumps	7,210	1	2.15	15,500
Pump	S-071, S-072	1	Goulds Pumps	7,210	2	2.15	31,000
Pump	S-046, S-053	1	Goulds Pumps	7,210	2	2.15	31,000
Economizer	H-03, H-06	1	N/A	70,300	2	3.17	446,000
Sterilizer	H-04, H-07	1	N/A	80,200	2	3.17	508,000
Cooler	H-05, H-08	1	N/A	58,100	2	3.17	369,000
Scrubber	F-03	1	Pollution Systems	18,700	1	4.16	77,700
Pump	S-069	1	Goulds Pumps	7,210	1	2.15	15,500
Spares							
Pumps	N/A	1	Goulds Pumps	7,210	5	2.15	77,500
Compressors	N/A	1	Eaton Compressor	324,000	2	2.15	1,390,000
Heat Exchanger	N/A	1	N/A	619,000	1	3.17	1,960,000
Scrubber	N/A	1	Pollution Systems	18,700	1	4.16	77,700
Product Purification							
Dryer	D-01	1	GEA Process Engineering	375,000	1	2.06	772,000
Product Bin	B-01	1	Wamgroup	1,330	1	4.16	5,560
Product Bags	N/A	1	Bag Corp	15	180,000	4.16	11,200,000
Cleaning							
CIP System		1	Sani-Matic	175,000	1	4.16	728,000
						TOTAL	**50,900,000**

Equipment purchase costs were calculated either by using the Equipment Costing Spreadsheet provided by Bruce Vrana and Warren Seider or via quote request from a vendor. The sources for each equipment cost can be found in Appendix B. The standard cost equations can be

found in Product and Process Design Principles Fourth Edition. The total cost of the equipment is $50,900,000 USD. Figure 17.1 shows a breakdown of the costs for each type of equipment.

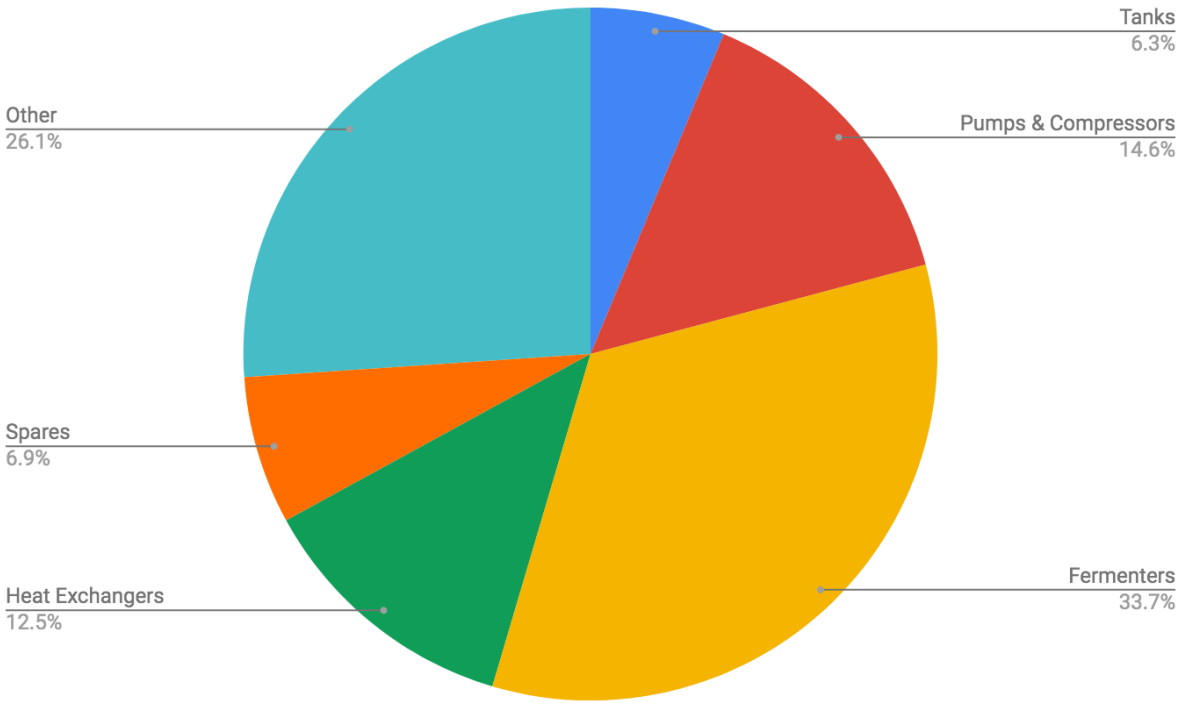


Figure 17.1. Cost breakdown of total equipment costs by equipment type.

As shown in the chart, the fermenters pose the largest cost for the overall equipment. This is most likely due to the large size of the 150,000 L fermenters for the continuous phase. The "other" section, representing cell preparation steps, filters, centrifuge, scrubber, product purification, and clean in place, is the second most costly group. Following the "other" section are pumps and compressors, heat exchangers, spares, and tanks, in that order.

The most expensive piece of equipment listed is the product bags for the finished cell mass before shipment to customers. The total bare module cost of the bags is $11,200,000. However, this is because the number of bags accounted for in the cost was enough for 10 years

of product production. The next most expensive piece of equipment are the 150,000 L reactors. The two reactors cost a total of $10,900,000. These are the main process component, so it is expected that they contribute a significant amount to the cost of equipment.

18. Fixed Capital Investment Summary

The Profitability Analysis Spreadsheet 4.0 was used to model all financials involving the construction and operation of this plant. Financial models were estimated for ten 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 in Section 17, including vendors, and bare module costs factored in. The total bare module cost of equipment was calculated to be $50,851,719 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	$	24,505,868	
Process Machinery	$	19,346,167	
Spares	$	3,514,807	
Storage	$	2,728,734	
Other Equipment	$	756,143	
Catalysts	$	-	
Computers, Software, Etc.	$	-	
Total Bare Module Costs:			$ 50,851,719

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 use can be summarized in the total permanent investment of the plant. These fees were set to the default values for general recommendations except for the royalties, which was

estimated at a baseline cost of $5,000,000 for the genetic modification of the bacteria. A detailed sensitivity analysis on these royalties will be presented in Section 20.3. A summary of the total permanent investment costs can be seen in Figure 18.2.

Total Permanent Investment		
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:	$5,000,000	
Cost of Plant Start-Up:	10.00%	of Total Depreciable Capital

Figure 18.2. Total Permanent Investment inputs from 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. It can be seen that the total permanent investment is $78,926,195, the majority of which is due to the bare module costs of equipment.

Investment Summary			
Total Bare Module Costs:			
Fabricated Equipment	$	24,505,868	
Process Machinery	$	19,346,167	
Spares	$	3,514,807	
Storage	$	2,728,734	
Other Equipment	$	756,143	
Catalysts	$	-	
Computers, Software, Etc.	$	-	
Total Bare Module Costs:			**$ 50,851,719**
Direct Permanent Investment			
Cost of Site Preparations:	$	2,542,586	
Cost of Service Facilities:	$	2,542,586	
Allocated Costs for utility plants and related facilities:	$	-	
Direct Permanent Investment			**$ 55,936,891**
Total Depreciable Capital			
Cost of Contingencies & Contractor Fees	$	10,068,640	
Total Depreciable Capital			**$ 66,005,532**
Total Permanent Investment			
Cost of Land:	$	1,320,111	
Cost of Royalties:	$	5,000,000	
Cost of Plant Start-Up:	$	6,600,553	
Total Permanent Investment - Unadjusted			$ 78,926,195
Site Factor			1.00
Total Permanent Investment			**$ 78,926,195**

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

19. Operating Cost-Cost of Manufacture

19.1 Raw Materials

The raw materials for this process include methylomonas bacteria, methanol, ammonia, oxygen, and various minerals. The procurement of these raw products as well as their general costs per metric ton were presented in Section 11.1. A more detailed analysis of annual costs and requirements are presented in Table 19.1. The annual totals assume each bioreactor completes 8 full cycles per year, which corresponds to 16 total batch processes annually. Additionally, the costs for air and water are not included as these are incorporated into the utilities costs. Finally, the cost of methylomonas bacteria is not included as this is incorporated in great detail in the research royalties sensitivity study.

Table 19.1. Costs of all chemical reactants and amount required annually.

Raw Material	Ratio (MT per MT product)	Cost ($USD/MT)	Yearly Requirement (MT)	Annual Cost ($USD)
Methanol	2.290	$432	20,212	$8,731,780
Anhydrous ammonia	0.098	$473	862	$407,479
Disodium phosphate	0.102	$800	899	$719,563
Potassium phosphate	0.035	$1,000	309	$309,444
Magnesium sulfate	0.025	$100	220	$21,964
Calcium chloride	0.014	$200	123	$24,604
Iron(II) sulfate	0.010	$100	88	$8,837

19.2 Utilities

The yearly requirements and costs for utilities including steam, process water, cooling water, and electricity were presented in detail in Section 14. The standards prices for these utilities according to the Product and Process Design Principles textbook were utilized. The

largest utilities costs include the cost of steam for the sterilization process and the cost of electricity for the dryer. The costs for utilities are presented again in Table 19.2.

Table 19.2. Annual utility requirements and costs.

Utility	Ratio (MT/MT Product)	Cost ($USD/MT)	Yearly Requirement (MT)	Yearly Cost ($USD)
High Pressure Steam	98.3	$17.6	867,536.9	$15,268,650
Cooling Water	277.71	$0.027	2,451,227.3	$66,183
Process Water	16.66	$0.27	147,053.0	$39,704
Electricity	4,479.93 kW-hr	$0.07/kW-hr	39,534,178.6 kW-hr	$2,767,392

19.3 Variable Costs and Working Capital

The variable costs for this plant include selling, research, and administrative expenses as well as working capital for accounts receivable, accounts payable, cash reserves, and raw material and product inventories. The general expenses were all set to follow general recommendations with the exception of the research expenses. Due to the research needed to genetically modify bacteria, the direct research expenses were set to a baseline value 8%, though a more detailed sensitivity analysis will be presented in Section 20.3. Additionally, due to the highly volatile nature of the weather in Alberta, Canada and the six week continuous process time of our bioreactors, the raw material inventories were increased to 7 days and the product inventory was increased to 30 days to ensure adequate supply in the case of any unforeseen delay in manufacturing. A summary of the variable cost and working capital can be seen in Figure 19.3.

Variable Costs			
General Expenses:			
Selling / Transfer Expenses:		3.00%	of Sales
Direct Research:		8.00%	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	⇨	30	Days
Cash Reserves (excluding Raw Materials)	⇨	30	Days
Accounts Payable	⇨	30	Days
SCP/Astaxanthin Inventory	⇨	30	Days
Raw Materials	⇨	7	Days

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

19.4 Total Variable Cost Summary

The variable costs for the process, including research expenses, working capital, raw material costs, and utilities costs can be evaluated together to calculate the total annual variable costs for this plant. The output of total variable cost from the Profitability Spreadsheet can be seen in Figure 19.4. It can be seen that the total variable cost is $49,861,698 per year at 100% operating capacity.

Variable Cost Summary

Variable Costs at 100% Capacity:			
General Expenses			
Selling / Transfer Expenses:			$ 4,369,068
Direct Research:			$ 11,650,848
Allocated Research:			$ 728,178
Administrative Expense:			$ 2,912,712
Management Incentive Compensation:			$ 1,820,445
Total General Expenses			$ 21,481,251
Raw Materials	$1,158.53	per MT of SCP/Astaxanthin	$10,225,684
Byproducts	$0.00	per MT of SCP/Astaxanthin	$0
Utilities	$2,056.87	per MT of SCP/Astaxanthin	$18,154,762
Total Variable Costs			**$ 49,861,698**

Working Capital

	2023	2024	2025
Accounts Receivable	$ 10,234,392	$ 568,577	$ 568,577
Cash Reserves	$ 2,232,792	$ 124,044	$ 124,044
Accounts Payable	$ (1,994,407)	$ (110,800)	$ (110,800)
SCP/Astaxanthin Inventory	$ 10,234,392	$ 568,577	$ 568,577
Raw Materials	$ 167,673	$ 9,315	$ 9,315
Total	**$ 20,874,843**	**$ 1,159,714**	**$ 1,159,714**
Present Value at 15%	*$ 13,725,548*	*$ 663,070*	*$ 576,583*

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

19.5 Operations, Maintenance, and Overhead

After determining the total bare module cost of all equipment, plant operations, maintenance, and overhead were calculated into the fixed cost of the plant. It was determined that 5 operators would be needed per shift for each of the 4 shifts. These operators would work to run the biological and chemical laboratories, ensure safe upkeep of the control systems used to regulate methanol and ammonia flow into the process, manually monitor the batch seed train

process running every three weeks, and to manage the super sacks of final product for transport. The remaining values of operations, maintenance, and overhead, including wages, salaries, and benefits, maintenance and plant overhead, and business services were left at the default values as there were no specific indications that our plant would need to deviate from these general recommendations. A summary of operations, maintenance, and overhead inputs can be seen in Figure 19.5.

Fixed Costs		
Operations		
	Operators per Shift:	**5 (assuming 4 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:	**$60,000.00 per year, for each Operator per Shift**
	Control Laboratory:	**$65,000.00 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. Operations, maintenance, and overhead inputs from Profitability Analysis Spreadsheet.

19.6 Other Fixed Costs

Other fixed costs for consideration in this process include property taxes, straight line depreciation, and licensing fees. Due to the extremely remote plant location of Alberta, Canada and thus the lower property tax rate, the property taxes and insurance were estimated to be only 1.5% of the depreciable capital, which is a very conservative estimate even for this plant location

(31). Additionally, straight line depreciation was estimated to be only 4% according to the Capital Cost Allowance in Canada for Class 1 buildings (32). A licensing fee of $200,000 was added for licensing the patent and research about the genetically modified organisms and obtaining approval by the Canadian Food Inspection Agency. A summary of inputs for these other fixed costs can be seen in Figure 19.6.

Property Taxes and Insurance

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

Straight Line Depreciation

Direct Plant:	4.00%	**of Total Depreciable Capital, less 1.18 times the Allocated Costs for Utility Plants and Related Facilities**
Allocated Plant:	4.00%	**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:	**$200,000**
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 cost from the Profitability Spreadsheet can be seen in Figure 19.7, which includes operations, maintenance, overhead, and property taxes, and depreciation. It can be seen that the total fixed cost is $13,817,917 per year.

Fixed Cost Summary

Operations		
Direct Wages and Benefits	$	1,664,000
Direct Salaries and Benefits	$	249,600
Operating Supplies and Services	$	99,840
Technical Assistance to Manufacturing	$	1,200,000
Control Laboratory	$	1,300,000
Total Operations	**$**	**4,513,440**
Maintenance		
Wages and Benefits	$	2,970,249
Salaries and Benefits	$	742,562
Materials and Services	$	2,970,249
Maintenance Overhead	$	148,512
Total Maintenance	**$**	**6,831,573**
Operating Overhead		
General Plant Overhead:	$	399,475
Mechanical Department Services:	$	135,034
Employee Relations Department:	$	331,958
Business Services:	$	416,354
Total Operating Overhead	**$**	**1,282,822**
Property Taxes and Insurance		
Property Taxes and Insurance:	$	990,083
Other Annual Expenses		
Rental Fees (Office and Laboratory Space):	$	-
Licensing Fees:	$	200,000
Miscellaneous:	$	-
Total Other Annual Expenses	**$**	**200,000**
Total Fixed Costs	**$**	**13,817,917**

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

20. Profitability Analysis

20.1 Baseline Cash Flow Summary and Profitability

The profitability of the entire process over the 10 year lifespan was evaluated taking into account the fixed costs, variable costs, investments, and working capital as well as the annual product sales. For the baseline process, an intermediate product selling price of $16,500/MT was assumed, with royalties for genetic modification set at $5,000,000 upfront and 4% of annual sales. This process was found to be extremely profitable, with an Internal Rate of Return (IRR) of 41.86% and a Net Present Value (NPV) of $129,424,200. Additionally, the Return on Investment (ROI) after the 3rd production year was found to be 56.01%. This extremely positive financial outlook is reassurance that in the case of contaminated batches, increases in raw material costs, or unexpected equipment failure, the process for the production of SCP/astaxanthin would still be highly profitable. The overall cash flow summary for this process over the 10 year lifespan can be seen in Figure 20.1.1.

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%
2020	0%		-	-	-	-	-	-	-	-	-	-	-	-
2021	0%		-	-	-	-	-	-	-	-	-	-	-	-
2022	0%		-	(78,926,200)	-	-	-	-	-	-	-	-	(78,926,200)	(59,679,500)
2023	0%		-	-	(20,874,800)	-	-	-	-	-	-	-	(20,874,800)	(73,405,100)
2024	86%	$16,500.00	124,518,400	-	(1,159,700)	(42,631,800)	(13,817,900)	(2,640,200)	-	65,428,500	(17,338,600)	48,090,000	49,570,500	(45,063,000)
2025	90%	$16,902.60	134,643,200	-	(1,159,700)	(46,098,200)	(14,155,100)	(2,640,200)	-	71,749,700	(19,013,700)	52,736,000	54,216,500	(18,107,800)
2026	95%	$17,315.02	145,187,900	-	-	(49,708,400)	(14,500,500)	(2,640,200)	-	78,338,800	(20,759,800)	57,579,000	60,219,200	7,926,600
2027	95%	$17,737.51	148,730,400	-	-	(50,921,300)	(14,854,300)	(2,640,200)	-	80,314,700	(21,283,400)	59,031,300	61,671,500	31,111,200
2028	95%	$18,170.31	152,359,500	-	-	(52,163,800)	(15,216,700)	(2,640,200)	-	82,338,800	(21,819,800)	60,519,000	63,159,200	51,758,100
2029	95%	$18,613.66	156,077,000	-	-	(53,436,600)	(15,588,000)	(2,640,200)	-	84,412,200	(22,369,200)	62,043,000	64,683,200	70,145,100
2030	95%	$19,067.83	159,885,300	-	-	(54,740,400)	(15,968,300)	(2,640,200)	-	86,536,300	(22,932,100)	63,604,200	66,244,400	86,519,700
2031	95%	$19,533.09	163,786,500	-	-	(56,076,100)	(16,358,000)	(2,640,200)	-	88,712,200	(23,508,700)	65,203,500	67,843,700	101,102,200
2032	95%	$20,009.70	167,782,900	-	-	(57,444,300)	(16,757,100)	(2,640,200)	-	90,941,200	(24,099,400)	66,841,800	69,482,000	114,088,900
2033	95%	$20,497.93	171,876,800	-	23,194,300	(58,846,000)	(17,166,000)	(2,640,200)	-	93,224,600	(24,704,500)	68,520,100	94,354,600	129,424,200

Figure 20.1.1. Cash Flow Summary from the Profitability Analysis Spreadsheet for the plant with a 10 year lifespan.

As mentioned in the introductory sections, astaxanthin production was originally considered as an additional product for this process because of its high market prices. In order to compare the new process, with astaxanthin, and the original process developed by Norferm,

without astaxanthin, a profitability analysis was performed. Without astaxanthin production, even though SCP can be produced roughly three times faster due to a faster cell doubling time, the IRR is negative. The net present value of the project in 2020 is $-127,000,000 and the ROI is -27.87%. A cash flow analysis is shown below in Figure 20.1.2. Clearly, the process is much more profitable after the addition of astaxanthin.

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%
2020	0%		-	-	-	-	-	-	-	-	-	-	-	-
2021	0%		-	-	-	-	-	-	-	-	-	-	-	-
2022	0%		-	(75,926,200)	-	-	-	-	-	-	-	-	(75,926,200)	(57,411,100)
2023	0%		-	-	(4,874,800)	-	-	-	-	-	-	-	(4,874,800)	(60,616,400)
2024	86%	$1,500.00	33,858,000	-	(270,800)	(45,266,100)	(13,817,900)	(2,640,200)	-	(27,866,200)	7,384,500	(20,481,700)	(18,112,300)	(70,972,100)
2025	90%	$1,536.60	36,611,000	-	(270,800)	(48,946,700)	(14,155,100)	(2,640,200)	-	(29,131,000)	7,719,700	(21,411,300)	(19,041,900)	(80,439,300)
2026	95%	$1,574.09	39,478,300	-	-	(52,780,000)	(14,500,500)	(2,640,200)	-	(30,442,400)	8,067,200	(22,375,200)	(19,735,000)	(88,971,300)
2027	95%	$1,612.50	40,441,500	-	-	(54,067,800)	(14,854,300)	(2,640,200)	-	(31,120,800)	8,247,000	(22,873,800)	(20,233,600)	(96,577,800)
2028	95%	$1,651.85	41,428,300	-	-	(55,387,100)	(15,216,700)	(2,640,200)	-	(31,815,700)	8,431,200	(23,384,600)	(20,744,300)	(103,359,200)
2029	95%	$1,692.15	42,439,100	-	-	(56,738,500)	(15,588,000)	(2,640,200)	-	(32,527,600)	8,619,800	(23,907,800)	(21,267,600)	(109,404,800)
2030	95%	$1,733.44	43,474,700	-	-	(58,122,900)	(15,968,300)	(2,640,200)	-	(33,256,900)	8,813,100	(24,443,800)	(21,803,600)	(114,794,300)
2031	95%	$1,775.74	44,535,400	-	-	(59,541,100)	(16,358,000)	(2,640,200)	-	(34,003,900)	9,011,000	(24,992,900)	(22,352,600)	(119,598,800)
2032	95%	$1,819.06	45,622,100	-	-	(60,993,900)	(16,757,100)	(2,640,200)	-	(34,769,200)	9,213,800	(25,555,300)	(22,915,100)	(123,881,800)
2033	95%	$1,863.45	46,735,300	-	5,416,500	(62,482,200)	(17,166,000)	(2,640,200)	-	(35,553,100)	9,421,600	(26,131,500)	(18,074,800)	(126,819,500)

Figure 20.1.2. Cash flow summary from the Profitability Analysis Spreadsheet for Norferm process with only SCP production.

20.2 Product Selling Price Sensitivity Analysis

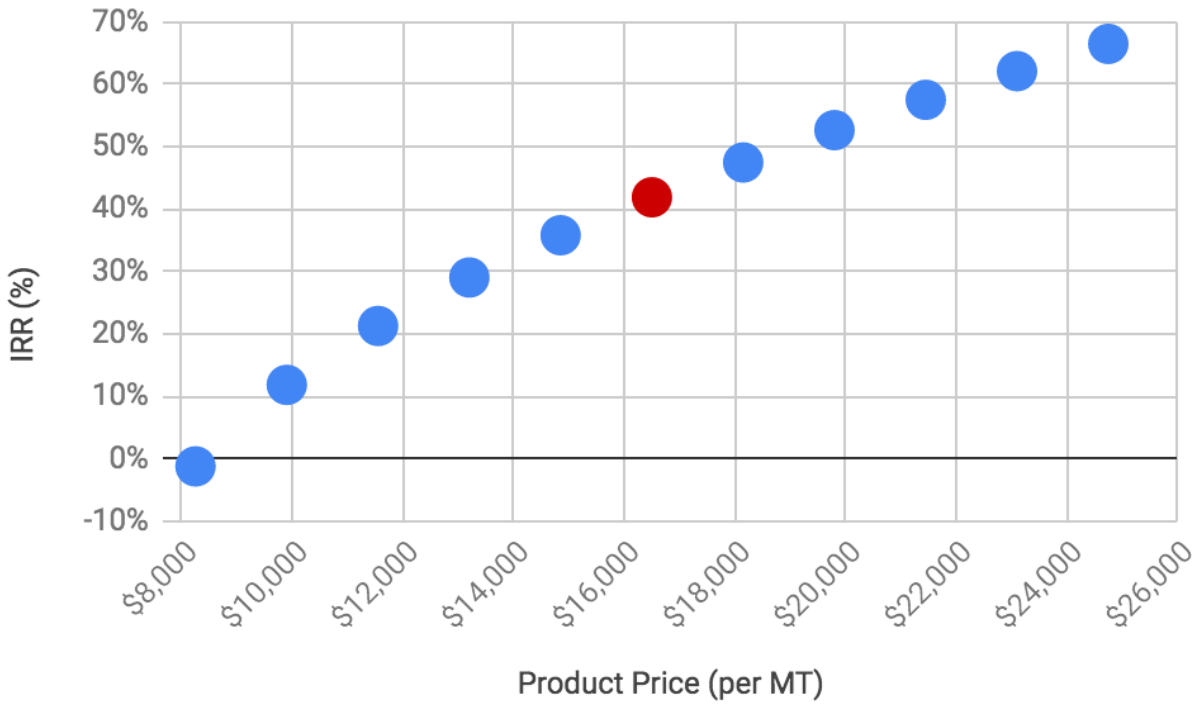


Figure 20.2.1. Relationship between product price per metric ton and the IRR of the process. The red dot represents the product price used in baseline analysis.

Figure 20.2.1 shows the sensitivity of the Internal Rate of Return with respect to the product price per MT. The selected price is shown in red. The IRR is very sensitive to changes in product pricing. An increase in price could drive the IRR up significantly. The selected price is in the middle of the price range shown. This price was selected originally based off of a weighted calculation of the market price of SCP and the market price of astaxanthin, given that our product is 0.3% by weight of astaxanthin. Since the market price of astaxanthin has quite a wide range depending on if it is produced naturally or synthetically, the price of $16,500 was calculated based off of a very conservative natural astaxanthin price. It is possible that if the demand is high enough, the price could be reevaluated after a few years of production. Given the market opportunities in the fish feed market for astaxanthin, it is possible that the product price could be further evaluated in the future.

20.3 Genetic Modification Royalties Sensitivity Analysis

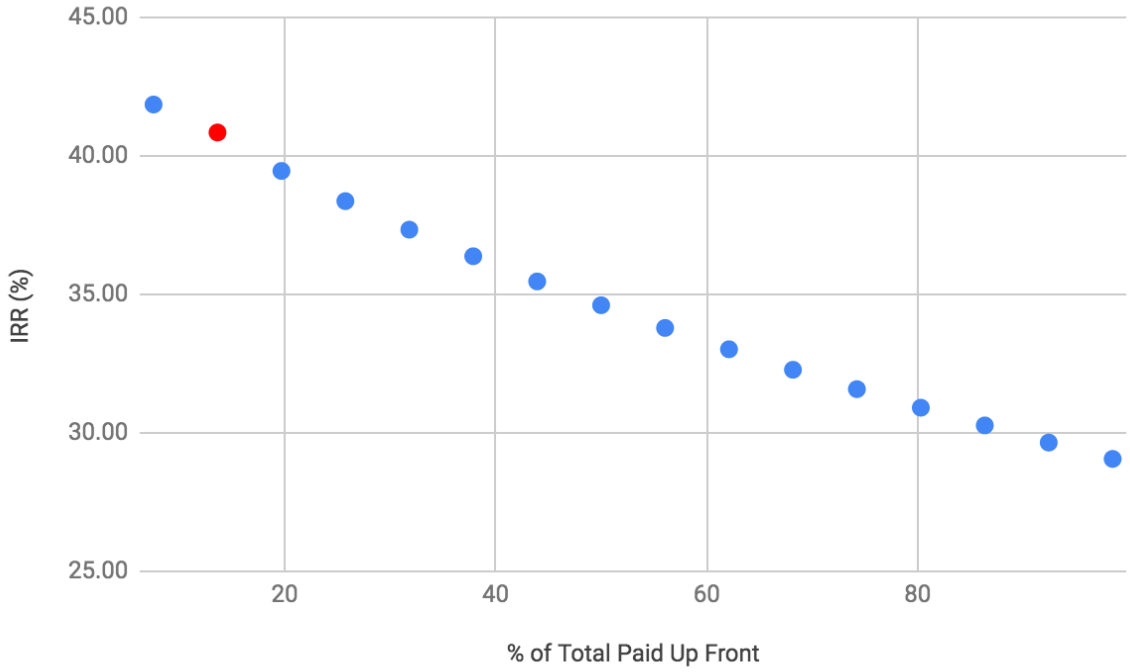


Figure 20.3.1. Relationship between the percent of total genetic modification royalties paid up front and IRR for the process. The red dot represents the percent of genetic modification royalties paid upfront in the baseline analysis.

The sensitivity of the IRR with respect to the percent of the total amount paid for rights to the genetically modified bacteria used in the process is shown in Figure 20.3.1. This sensitivity was considered because the laboratory that is hired for scientific research to allow the process to create astaxanthin as a co-product is highly valuable to this process, and often decisions must be made about whether to pay royalties in a lump sum upfront or as a portion of the profits over many years. The total amount paid over the course of 10 years of production was kept constant throughout the trials of the sensitivity analysis. The analysis shows that paying less royalties up front and a higher percentage of the sales to the rights provider is economically beneficial. This information is important for price negotiations with the organization leasing the rights to the

genetically modified bacteria. The red dot shows the data used in the base case profitability analysis. This represents a $5,000,000 one time royalties payment and a Direct Research cost of 8% of total sales. It is important to keep in mind that the lowest Direct Research rate possible is 4% of total sales since this is considered the general recommendation for a typical plant design. The data represented in this figure ranges from an upfront payment of $1,000,000 and an annual rate of 8.26% to an upfront payment of $65,000,000 and an annual rate of 4.07%. The royalty payment plan chosen is very economically beneficial, giving an IRR of 41.9%.

20.4 Methanol Carbon Efficiency Sensitivity Analysis

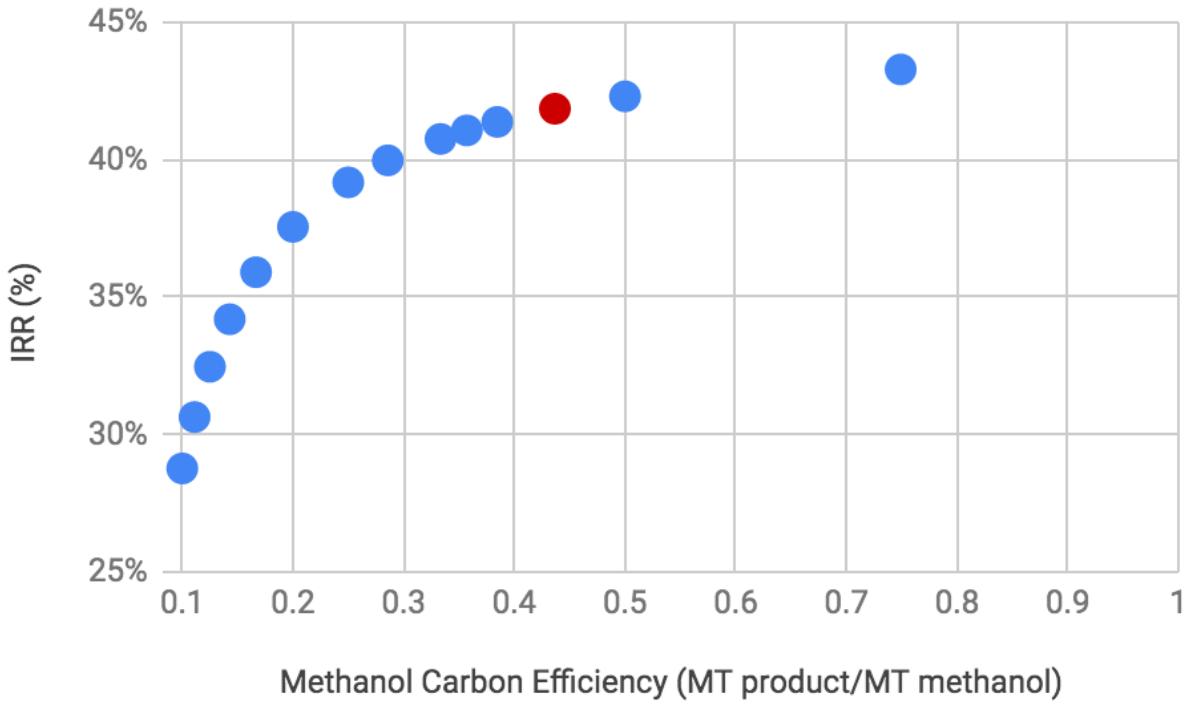


Figure 20.4.1. Relationship between the carbon efficiency of the methanol and the IRR of the process. The red dot represents the methanol carbon efficiency used in baseline analysis.

Figure 20.4.1 shows the sensitivity of the IRR relative to the carbon efficiency of the methanol. The red point represents the 0.44 MT product to MT methanol ratio used when designing the process, a ratio originally obtained from a patent describing SCP production from

methylomonas bacteria. The maximum efficiency of the methanol is 0.75 MT product to MT methanol based solely off of mass balances; however, this is unreasonable to obtain due to inherent inefficiencies in the process. Increasing the methanol carbon efficiency would not make a huge difference in the IRR of the process; however, if the methanol efficiency was much lower, it may have a more influential negative effect. It is probably not beneficial to spend time and financial resources on improving the carbon efficiency of the methanol. Other routes should be used to increase the profits of the plant.

21. Additional Considerations

21.1 Plant Location

During the preliminary research stages of this report, it became apparent that the location of the plant housing this process could have large ramifications on its feasibility and economic potential. Therefore, an appropriate amount of research was devoted to selecting the ideal site for the operation. This location was found the be the Canadian city of Medicine Hat.

Located in the Alberta province of Canada, Medicine Hat fulfills many of the criteria considered when selecting the location of the plant. Canada is the fourth largest producer of farmed salmon in the world, ensuring a demand for nutritious aquaculture feed and pigmentation for salmon farming which is easily accessible (33). The climate in Medicine Hat is ideal for a highly exothermic process such as this. With the highest average monthly temperature being 20°C and the coldest months having an average temperature well below freezing, this cool climate could alleviate a large portion of the cooling requirements from the cooling towers to providing the cooling water necessary for this operation (34).

Because of the genetic modification needed to produce SCP with astaxanthin, one of the most important criteria for selecting a location was the regulatory policies regarding genetically modified organisms that would apply there. Thankfully, Canada is one of the most accepting countries in the world toward genetically modified food product or animal feed (35). There is still a regulatory process required to gain approval for a genetically modified product. This is discussed later in Section 21.5. The final consideration used to select Medicine Hat is the proximity to sources of raw materials. Methanex has a large methanol plant located in Medicine Hat, Alberta so proximity to this location would provide the plant with a source for its largest

needed raw material (18). The second largest needed raw material is ammonia, and there is a large ammonia and fertilizer producer about 200 km away in Carseland, Alberta (19). The proximity of both of these sources of raw materials could greatly reduce the cost and difficulty in acquiring them.

21.2 Environmental Impact

Potential ways that the environment could be negatively impacted by the plant designed are through carbon dioxide emissions, energy usage, and biological contaminants.

Carbon Dioxide and Energy Usage

Under the *Canadian Environmental Protection Act of 1999* from the Environment and Climate Change Canada (ECCC), reporting is required for greenhouse gas emissions of certain amounts. In 2017, the minimum amount of emissions per year necessary for a report decreased from 50,000 to 10,000 MT. However, the process designed for this project emits around 5,700 MT of carbon dioxide per year, much less than the required reporting amount (36). It should be noted that this value does not include the carbon dioxide from the energy production needed to run the plant.

Even though the plant does not produce enough carbon dioxide to report to the ECCC, it is incredibly important for companies in Canada to appear eco-friendly to gain positive public perception (37). For this reason, the plant will be open to adapting new technology to help reduce greenhouse gas emissions.

Biological Contaminants

In order to eliminate the biological hazards that could leave through the gas outlet stream in each seed reactor and the continuous phase, a scrubber will be installed. The efficacy of this

scrubber will be tested frequently in order to ensure the 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.

21.3 Canadian Food Inspection Agency Approval

The Canadian Food Inspection Agency (CFIA) is a department within the Government of Canada focused on mitigating risks to food safety. The CFIA typically collaborates with industry, consumers, and other government and municipal organizations to protect Canadians from food related health risks. In the *Food and Drugs Act*, the CFIA includes in the definition of novel foods "a food that is derived from a plant, animal or microorganisms that has been genetically modified" (38). Because of this definition, any GMO food must go through a close approval process by the Minister. Resultantly, the CFIA is responsible for the regulation of any plants or livestock feed with GMOs. The CFIA is also responsible for setting standards on Canadian food labels so they will not be misleading to consumers, though providing GMO labelling is entirely voluntary throughout Canada (39).

In the *Feeds Regulations Act*, the CFIA lists all ingredients and flavoring that have been evaluated and approved for use in livestock feed in Canada (40). Approved on this list are various dried bacterial fermentation products which must be labelled with guarantees of minimum crude protein content, anhydrous ammonia, ammonium phosphate dibasic, calcium chloride, ferrous sulfate heptahydrate, magnesium phosphate, and disodium phosphate (40). This covers the majority of the reactants and products in the process designed. The CFIA has also published detailed requirements for carotenoid based coloring agents, such as astaxanthin, in salmonid fish and poultry feed. It is stated that astaxanthin should not exceed a concentration of

80 mg/MT of salmonid fish feed and provides detailed explanations of the regulations, labelling, and approval process for these products (41).

21.4 Toxicology and Animal Testing

Although thorough toxicology testing and animal testing are not within the scope of this project, it is important to understand the types of regulations that exist for fish feed in Canada. Given this project, it is assumed that the genetically modified methanotrophic bacteria has already been thoroughly tested for toxicology and animal safety and approved by the CFIA. Under the *Feeds Act* administered by the Canadian Food Inspection Agency (CFIA), "all feeds must be safe to livestock; to humans (by the potential transfer of residues into human food and via worker/bystander exposure) and to the environment" (40).

The CFIA requires that feeds must be shown to be effective for their intended purpose. Therefore, the efficacy of the astaxanthin to make the salmon and other fish more pink and natural-looking must be proven before the product can be sold. Additionally, the ideal concentration of astaxanthin in the fish meal must be stated on the packaging. Because the final product has too much astaxanthin in it to feed directly to the fish, it is important that the fish meal company knows the correct amount of additional fish meal to dilute the product with.

The CFIA also requires a pre-market assessment. This assessment consists of new ingredient approval and authorization and also product registration. Because this process is new to Canada, the composition of the product, manufacturing process, and use of the final product must go through assessment and approval. This involves a risk assessment completed by the CFIA.

21.5 Genetically Modified Organisms

The methanotrophic bacteria to be used in this process must be genetically engineered to produce astaxanthin. Because of this, the product would very likely be considered a genetically modified organism (GMO) to be used in food products. It has been stated previously that Canadian policies and opinions are very friendly to GMO products, but there is still a thorough approval process that must be completed in order to sell these SCPs as animal feed. The regulation of GMOs in Canada is overseen by Health Canada, a government agency under the Canadian Food Inspection Agency (42).

Health Canada outlines a 7 to 10 year process for the testing and approval of a new GM food or feed stock. The first step is a preliminary consultation with the Novel Foods Section of the Food Directorate to outline the process and clarify the documentation that will be needed to complete subsequent steps. The second step is to submit a proposal for a new GM food to Health Canada, including sufficient background research and documentation regarding the GMO in question. Next, the agency will assign scientific investigators to perform a battery of tests to determine the safety of the new product in several areas, including the potential for the production of new toxins in the product, the potential for the introduction of new allergenic effects in the product, and the potential for any unforeseen negative side-effects of the genetic modification. The evaluators will also identify the major and minor constituents of the GM food, such as protein content and vitamin content, and compare the nutritional and compositional aspects of the GM food to its unmodified state.

After testing, Health Canada will then request any additional documentation regarding the GMO that it deems requires further information. When all documents have been submitted

and testing is complete, the investigators will synthesize a final report summarizing their findings and recommendations to submit to Health Canada for review. This report is used to create a Health Canada Food Rulings Proposal, which is then reviewed by all senior members of the Food Directorate before a decision is made. If all the findings are deemed acceptable, the agency will send a "Letter of No Objection" to the proprietor of the new GMO stating that the product may be sold within Canada. Finally, a statement of this decision will be posted on the Health Canada website (43).

Another consideration in the selling of GMOs in foods is the labelling of products that contain GMOs. In Canada, foods that contain GMOs are only required to be labelled as such if there is a possible health or safety risk associated with the genetic modification of that product. Otherwise, the labelling of GM foods in Canada is a completely voluntary process (42).

21.6 Municipal Waste Treatment

It is critical to the success of this plant that proper regulations are followed for waste treatment and disposal. Due to the alternating six week campaign periods of the continuous bioreactors, every three weeks the waste from the 150,000 L bioreactors will need to be disposed of, as well as the wastewater from the CIP system following the campaign period ending. Resultantly, proximity and direct access to a municipal waste center is of high importance. At the end of each continuous reactor cycle, the reactor contents will be sent through the centrifuge to isolate the remaining product, and the supernatant will be sterilized for environmental safety precautions, drained from the system, and sent to the municipal waste center. In the case of a contaminated batch, feed flow of ammonia and methanol will be ceased, and the chemical and

biological potential of the contents will be exhausted. The batch will then be allowed to run through the drainage system, packaged, and sent to the municipal waste center.

To gain the support of the municipal waste center, the plant must earn permission to send them our waste and carefully follow all protocols. Alberta has 66 waste incinerators for hazardous, non-hazardous, and inert waste streams and well as a large Energy-from-Waste facility aimed to generate heat from non-hazardous waste to power a nearby plant, located about 300 km from our plant location in Wainwright, Alberta (44). By following the guidelines laid out in the *Environmental Protection and Enhancement Act* and the *Waste Control Regulation* by the Alberta Environment and Parks government division, approval and government support for waste disposal will be ensured (45).

21.7 Genetic Engineering Techniques

It has been shown that much of the success of this project hinges on the ability of the bacterial cells to produce astaxanthin. While the genetic engineering required to produce these cells will be handled by a third party, and the exact mechanism of that engineering is outside the scope of this report, it is important to confirm the feasibility of such a construct before advancing further in this project.

In the current market, commercially available astaxanthin is acquired through chemical synthesis or harvested from algae which naturally produce it. Both of these methods are limited, as low yields in algae extraction and purity concerns in chemical synthesis make large scale production of astaxanthin from either process difficult. Because of this, the goal of engineering bacterium capable of mass producing astaxanthin has been the focus of several recent studies.

The carotenoid pathway is a well-studied mechanism through which microorganisms produce carotenoids of various lengths, such as astaxanthin. In a 2016 study, it was shown that the carotenoid pathway in *Corynebacterium glutamicum* bacterium, which naturally produces the 50 carbon decaprenoxanthin carotenoid, could be reprogrammed to produce astaxanthin. This was achieved by silencing specific genes active in the carotenoid pathway in order to produce β-carotene, a key building block of astaxanthin. β-carotene is then processed by two enzymes, β-carotene ketolase and hydroxylase, to produce astaxanthin (46). In 2017, a group of researchers took this further by trying to balance the expression of these two enzymes in order to maximize the production of astaxanthin in *E. Coli*. Here, they were able to achieve a dry weight astaxanthin to cell ratio of 0.74%, and an astaxanthin to carotenoid purity of 96.6% (47).

The genetic engineering utilized in these studies is well known and relatively simple to perform. With this information, it seems safe to assume that the ability to engineer methanotrophic bacteria to produce sufficient quantities of astaxanthin which is central to this project is a very realistic proposal.

22. Conclusions and Recommendations

Based on the design criteria and economic analysis of this process, it was found that the process of creating SCP and astaxanthin using methanol as the fuel source is highly profitable. The genetic modification of methylomonas bacteria in order to allow for astaxanthin as a co-product was found to be the distinguishing feature to ensure the economic success of this process in contrast with the Norferm process which failed economically. Additionally, the use of methanol rather than methane is a key feature of this process that allowed for increased process safety, ease of raw material delivery, and lower process cooling requirements, without leading to a significant change in the process economics. The process was found to be most profitable with the genetic modification royalties paid as a portion of sales over many years instead of as an upfront lump sum. Within reasonable limits, in the case of equipment failure and replacement, batch contamination, an increase in raw material costs, or a decrease in product value, the process would still remain well over the general guideline of a 15% IRR.

Should this plant remain in operation for many years to come, it would be highly beneficial to investigate getting the product approved in other countries such as Norway and Chile. These countries make up a large majority of the farmed salmon market but have much stricter guidelines on GMOs, which is the main reason they were not considered in scope for the original product market. Additionally, it may be beneficial down the line to consider adding a separation process to create a pure astaxanthin product since the human market for astaxanthin as a nutraceutical is growing rapidly. This would require significant capital costs for separation equipment, an even stricter product approval process for direct human consumption, and more detailed design work to ensure adequate product purity in separation, but may ultimately prove

financially beneficial depending on the direction of the market in the near future. A third area for future exploration involves purifying and selling off the carbon dioxide byproduct for use in other industries, such as beverage carbonation or dry ice production. This would have to be analyzed financially to determine if the profits from selling the carbon dioxide would outweigh the cost input of purifying and preparing the carbon dioxide for sale. One expected difficulty with selling this byproduct is that the current gas vent stream is only 13% carbon dioxide and 80% nitrogen from the large air flow input to the process, so an extensive separation system would be required to purify the carbon dioxide product.

23. Acknowledgements

We would like to acknowledge Dr. Jeffrey Cohen, Dr. Bomyi Lim, and Professor Bruce Vrana for their input and expertise throughout the course of this project. We would also like to thank all the other industrial consultants who met with us over the course of the semester. In particular, we would like to thank Dr. Cohen for proposing this project and offering his support constantly throughout the semester.

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Appendix A: Calculations

A.1 Continuous Cooling Requirements

The enthalpy of fermentation given was -23.9 kJ/g dry cell mass. However, this enthalpy of fermentation was based on methane as a carbon source. In order to convert this value to account for the decision to use methanol as the carbon source, the ratio of the heat of combustion of methane and methanol was used.

$$\Delta H_R(methane) = -55.51\ \frac{kJ}{g}$$
$$\Delta H_R(methanol) = -22.68\ \frac{kJ}{g}$$
$$Enthalpy\ of\ fermentation\ (methanol) = -23.9 \frac{kJ}{g\ dry\ mass} \times \frac{-22.68 \frac{kJ}{g}}{-55.51 \frac{kJ}{g}} = -9.76\ \frac{kJ}{g\ dry\ mass}$$

A.2 Heat Exchanger Design

Equations governing the design of the continuous heat exchanger to cool the fermenter contents.

1. To find the Log Mean Temperature Difference.

$$\Delta T_1 = \Delta T_{hot,in} - \Delta T_{cold,out} = 33 - 22 = 11°C$$
$$\Delta T_2 = \Delta T_{hot,out} - \Delta T_{cold,in} = 22 - 12 = 10°C$$

$$Log\ Mean\ Temperature\ Difference = \Delta T_{lm} = \frac{\Delta T_1 - \Delta T_2}{ln(\frac{\Delta T_1}{\Delta T_2})} = \frac{11-10}{ln(\frac{11}{10})} = 10.49°C$$

2. Find the enthalpy generation rate.

$$Q = biomass\ production\ rate \times specific\ heat\ of\ fermentation$$
$$Q = 1{,}094 \frac{kg\ dry\ cells}{hr} \times 9.76 \frac{kJ}{g\ dry\ biomass} \times \frac{1{,}000\ g}{1\ kg} = 10{,}682{,}848\ \frac{kJ}{hr}$$

3. Find heat exchange area.

$$A_{total} = \frac{Q}{U \Delta T_{lm}} = \frac{10{,}682{,}848 \frac{kJ}{hr} \times \frac{1{,}000\ J}{1\ kJ} \times \frac{1\ hr}{3{,}600\ sec}}{567 \frac{W}{m^2 °C} \times 10.49°C} = 498.42\ m^2$$

4. Find the area per cooling tube.

$$A/tube = \pi \times cooling\ tube\ diameter \times cooling\ tube\ length = \pi \times 3.81\ cm \times \frac{1m}{100\ cm} \times 9.14\ m = 1.09 \frac{m^2}{tube}$$

5. Find number of tubes required

$$\#\ tubes = \frac{A_{total}}{A/tube} = \frac{498.42 m^2}{1.09 \frac{m^2}{tube}} = 455.39\ tubes$$

6. Find the coolant flow rate.

$$Coolant\ Flow\ Rate = \frac{Q \times \varrho}{c_p \times \Delta T_{coolant}} = \frac{10{,}682{,}848 \frac{kJ}{hr} \times \frac{1{,}000\ J}{1\ kJ} \times \frac{1\ L}{1\ kg} \times \frac{1\ hr}{60\ min}}{4{,}184 \frac{J}{kgK} \times 10°C} = 4{,}255.4\ \frac{L}{min}$$

7. Find the recycle flow rate.

$$Recycle\ Flow\ Rate = \frac{Q \times \varrho}{c_p \times \Delta T_{media}} = \frac{10{,}682{,}848 \frac{kJ}{hr} \times \frac{1{,}000\ J}{1\ kJ} \times \frac{1\ L}{1\ kg} \times \frac{1\ hr}{60\ min}}{4{,}184 \frac{J}{kgK} \times 11°C} = 3{,}868.6\ \frac{L}{min}$$

A.3 Mass Balance Calculations

In order to perform batch and continuous mass balances, a few initial calculations were performed based on essential assumptions.

Molecular Makeup of Cell

In order to determine raw material demands, the elemental makeup of a cell was assumed (12):

Element	Fraction by mass
C	0.5
H	0.2
O	0.14
N	0.08
P	0.03
S	0.01
K	0.01
Mg	0.005
Ca	0.005
Fe	0.002

Carbon Efficiency of Methanol

From patent investigation, processes of this nature normally have methanol efficiency of 0.44 g of dry cell mass per g of methanol fed (9). This value was used to determine the amount of methanol fed to the reactor. The methanol that was not used directly in the cell mass instead became carbon dioxide.

Excess Oxygen

To ensure enough oxygen supply, 50% excess air is supplied to the reactor.

Gasvent Water Evaporation

Some water will leave in the gasvent stream in both the batch and continuous reactors. It is assumed that because the gasvent stream contains both nitrogen and oxygen, the solubility of water in the gasvent stream behaves similarly to water in air. From standard steam tables, 0.032 kg of water per kg of air leave in the saturated gasvent stream at the reactor temperature and pressure.

Centrifuge Assumption

After discussing with consultants, it was assumed that the centrifuge product contains 75% by weight water and 25% by weight cell mass product.

Water Mass Balance

The overall mass balance for water is:
$$into\ reactor = out\ through\ centrifuge + gasvent - generated$$

This mass balance assumes that ammonia was the only hydrogen source, not water (for simplicity of calculation).

Mineral Consumption

The mass balance calculations assume that all minerals are consumed in cell growth. Additionally, the minerals were not considered elemental balances for hydrogen or oxygen because they exist in negligible amounts.

A.4 Seed Train/Batch Start Up Batch Times

An example calculation for Seed Train Reactor 1 is shown.

1. Find specific growth rate from given doubling time of 1.5 hrs.

$$\mu = \frac{ln(\frac{X}{Xo})}{t_{doubling}} = \frac{ln(2)}{1.5\ hr} = 0.462\frac{1}{hr}$$

$X = final\ cell\ mass$

$X_o = initial\ cell\ mass$

$\mu = specific\ growth\ rate$

2. Find maximum cell mass in bioreactor given peak cell density of 35 g/L.

$$X = \varrho \times V = 35\frac{g}{L} \times 0.008\ L = 0.28\ g$$

$\varrho = peak\ cell\ density$

$V = reactor\ usable\ volume$

3. Find time for batch growth.

For cell growth in the log phase (assuming lag phase is negligible):

$$t_{batch} = \frac{ln(\frac{X}{Xo})}{\mu} = \frac{ln(\frac{0.28\ g}{0.014\ g})}{0.462\frac{1}{hr}} = 6.48\ hr$$

A.5 Seed Train/Batch Start Up Variable Methanol Flow Rates

The following procedure was used to develop a relationship between time and the methanol flow rate for each seed train reactor for the batch start up period. As mentioned in the report, this is important because the methanol concentration in the reactor cannot exceed 1% by weight.

Where X is the mass of cells and M is the mass of methanol.

t (hr)	ln(X)=ln(Xo +μt)	X weight (g)	M weight (g)	M flow rate in (g/hr)	Total M in	ln(M)
0	ln(0.014)+0.462 x 0	0.014	X/0.44	M/0.2	sum(M values)	ln(sum(M values))
0.2	ln(0.014)+0.462 x 0.2	$e^{ln(X)}$	X/0.44	M/0.2	sum(M values)	ln(sum(M values))
0.4	ln(0.014)+0.462 x 0.4	$e^{ln(X)}$	X/0.44	M/0.2	sum(M values)	ln(sum(M values))
etc.	ln(0.014)+0.462 x t	$e^{ln(X)}$	X/0.44	M/0.2	sum(M values)	ln(sum(M values))

The total methanol in found via this method must match that determined in the mass balance within 2% to ensure accuracy.

The natural logarithmic relationship is found by finding the slope and y intercept of the ln(M) vs t data. The relationship gives an equation of the form:

$$M\ flow\ rate = initial\ M\ flow\ rate\ e^{slope\ t}$$

An example with values is provided below for the first seed reactor.

t (hr)	ln(X)	X weight (g)	M weight (g)	M flow rate in (g/hr)	Total M in (g)	ln(M)
0	-4.2687	0.0140	0.0318	0.0154		-4.1734
0.2	-4.1763	0.0154	0.0349	0.0169	0.0031	-4.0810
0.4	-4.0839	0.0168	0.0383	0.0185	0.0034	-3.9886
0.6	-3.9915	0.0185	0.0420	0.0203	0.0037	-3.8962
0.8	-3.8991	0.0203	0.0460	0.0223	0.0041	-3.8038
1	-3.8067	0.0222	0.0505	0.0244	0.0045	-3.7114
1.2	-3.7143	0.0244	0.0554	0.0268	0.0049	-3.6190
1.4	-3.6219	0.0267	0.0608	0.0294	0.0054	-3.5266
1.6	-3.5295	0.0293	0.0666	0.0323	0.0059	-3.4342
1.8	-3.4371	0.0322	0.0731	0.0354	0.0065	-3.3418
2	-3.3447	0.0353	0.0802	0.0388	0.0071	-3.2494
2.2	-3.2523	0.0387	0.0879	0.0426	0.0078	-3.1570
2.4	-3.1599	0.0424	0.0964	0.0467	0.0085	-3.0646
2.6	-3.0675	0.0465	0.1058	0.0512	0.0093	-2.9722
2.8	-2.9751	0.0510	0.1160	0.0561	0.0102	-2.8798
3	-2.8827	0.0560	0.1272	0.0616	0.0112	-2.7874
3.2	-2.7903	0.0614	0.1396	0.0675	0.0123	-2.6950
3.4	-2.6979	0.0673	0.1531	0.0741	0.0135	-2.6026
3.6	-2.6055	0.0739	0.1679	0.0813	0.0148	-2.5102
3.8	-2.5131	0.0810	0.1841	0.0891	0.0163	-2.4178
4	-2.4207	0.0889	0.2020	0.0977	0.0178	-2.3254
4.2	-2.3283	0.0975	0.2215	0.1072	0.0195	-2.2330
4.4	-2.2359	0.1069	0.2429	0.1176	0.0214	-2.1406
4.6	-2.1435	0.1172	0.2665	0.1290	0.0235	-2.0482
4.8	-2.0511	0.1286	0.2923	0.1415	0.0258	-1.9558

5	-1.9587	0.1410	0.3205	0.1552	0.0283	-1.8634
5.2	-1.8663	0.1547	0.3516	0.1702	0.0310	-1.7710
5.4	-1.7739	0.1697	0.3856	0.1866	0.0340	-1.6786
5.6	-1.6815	0.1861	0.4229	0.2047	0.0373	-1.5862
5.8	-1.5891	0.2041	0.4639	0.2245	0.0409	-1.4938
6	-1.4967	0.2239	0.5088	0.2463	0.0449	-1.4014
6.2	-1.4043	0.2455	0.5580	0.2701	0.0493	-1.3090
6.4	-1.3119	0.2693	0.6121	0.2894	0.0540	-1.2399
6.5	-1.2657	0.2820	0.6410	0.2894	0.0289	-1.2399
				TOTAL MEOH IN	**0.6092**	**SLOPE** 0.4597

$$M\ flow\ rate = 0.01540\ [\tfrac{g}{hr}]e^{\,0.4597[\frac{1}{hr}]\,t[hr]}$$

A.6 Seed Train/Batch Start Up Variable Cooling Requirements

An example for the Seed Train Reactor 6 heat exchanger for cooling is shown.

1. To find the Log Mean Temperature Difference.

$$\Delta T_{\,1} = \Delta T_{\,hot,in} - \Delta T_{\,cold,out} = 33 - 22 = 11°\text{C}$$
$$\Delta T_{\,2} = \Delta T_{\,hot,out} - \Delta T_{\,cold,in} = 26 - 12 = 14°\text{C}$$

$$Log\ Mean\ Temperature\ Difference = \Delta T_{\,lm} = \frac{\Delta T_{\,1} - \Delta T_{\,2}}{ln(\frac{\Delta T_{\,1}}{\Delta T_{\,2}})} = \frac{11-14}{ln(\frac{11}{14})} = 12.4°\text{C}$$

2. Find the maximum enthalpy generation rate.

$$Q = biomass\ production\ rate\ maximum\ \times specific\ heat\ of\ fermentation$$
$$Q = 125.45\frac{kg\ dry\ cells}{hr} \times 9.76\frac{kJ}{g\ dry\ biomass} \times \frac{1000\ g}{1\ kg} = 1,224,975\frac{kJ}{hr}$$

3. Find heat exchange area.

$$A_{\,total} = \frac{Q}{U\Delta T_{\,lm}} = \frac{1,224,975\frac{kJ}{hr} \times \frac{1,000\ J}{1\ kJ} \times \frac{1\ hr}{3600\ sec}}{567\frac{W}{m^{\,2}°\text{C}} \times 9.76°\text{C}} = 48.24\ m^{\,2}$$

4. Find the area per cooling tube.

$$A/tube = \pi \times cooling\ tube\ diameter \times cooling\ tube\ length = \pi \times 3.81\ cm \times \frac{1m}{100\ cm} \times 9.14\ m = 1.09 \frac{m^2}{tube}$$

5. Find number of tubes required.

$$\#\ tubes = \frac{A_{total}}{A/tube} = \frac{48.24 m^2}{1.09 \frac{m^2}{tube}} = 45\ tubes$$

6. Find the maximum coolant flow rate after 6.35 hrs.

$$Coolant\ Flow\ Rate = \frac{Q \times \varrho}{c_p \times \Delta T_{coolant}} = \frac{1{,}224{,}975 \frac{kJ}{hr} \times \frac{1{,}000\ J}{1\ kJ} \times \frac{1\ L}{1\ kg} \times \frac{1\ hr}{60\ min}}{4{,}184 \frac{J}{kgK} \times 10°C} = 487.96\ \frac{L}{min}$$

7. Find the maximum recycle flow rate after 6.35 hrs.

$$Recycle\ Flow\ Rate = \frac{Q \times \varrho}{c_p \times \Delta T_{media}} = \frac{1{,}224{,}975 \frac{kJ}{hr} \times \frac{1{,}000\ J}{1\ kJ} \times \frac{1\ L}{1\ kg} \times \frac{1\ hr}{60\ min}}{4{,}184 \frac{J}{kgK} \times 7°C} = 697.09\ \frac{L}{min}$$

8. Find the minimum enthalpy generation rate.

$$Q = biomass\ production\ rate\ maximum \times specific\ heat\ of\ fermentation$$
$$Q = 5.42 \frac{kg\ dry\ cells}{hr} \times 9.76 \frac{kJ}{g\ dry\ biomass} \times \frac{1{,}000\ g}{1\ kg} = 52{,}934 \frac{kJ}{hr}$$

9. Find the new Log Mean Temperature Difference.

$$\Delta T_{lm} = \frac{Q}{U \times A_{total}} = \frac{52{,}934 \frac{kJ}{hr} \times \frac{1{,}000\ J}{1\ kJ} \times \frac{1\ hr}{3600\ sec}}{567 \frac{W}{m^2 °C} \times 48{,}24\ m^2} = 0.54°C$$

10. Use guess and check to find T_{co}.

$$T_{co} = 32.99°C$$

11. Find the minimum coolant flow rate.

$$Coolant\ Flow\ Rate = \frac{Q \times \varrho}{c_p \times \Delta T_{coolant}} = \frac{52{,}934 \frac{kJ}{hr} \times \frac{1{,}000\ J}{1\ kJ} \times \frac{1\ L}{1\ kg} \times \frac{1\ hr}{60\ min}}{4{,}184 \frac{J}{kgK} \times 21°C} = 10.04\ \frac{L}{min}$$

12. Find the minimum recycle flow rate.

$$Recycle\ Flow\ Rate = \frac{Q \times \varrho}{c_p \times \Delta T_{media}} = \frac{52{,}934 \frac{kJ}{hr} \times \frac{1{,}000\ J}{1\ kJ} \times \frac{1\ L}{1\ kg} \times \frac{1\ hr}{60\ min}}{4{,}184 \frac{J}{kgK} \times 7°C} = 30.12\ \frac{L}{min}$$

13. Develop a relationship between time and flow rate for coolant and recycle.

Relationship is proportional to growth rate- natural log relationship.

T (hr)	Coolant FR (L/min)	ln(coolant FR)	Recycle FR (L/min)	ln(recycle FR)
0	10.04	2.31	30.12	3.46
6.35	487.96	6.19	697.09	6.55
	slope	0.612	slope	0.495

$Coolant\ FR = 10.04[\frac{L}{min}]\ e^{\,0.612\ [\frac{1}{min}]t[min]}$

In MT/hr,

$Coolant\ FR = 0.602[\frac{MT}{hr}]\ e^{\,36.69\ [\frac{1}{hr}]t[hr]}$

$Recycle\ FR = 30.12[\frac{L}{min}]\ e^{\,0.495\ [\frac{1}{min}]t[min]}$

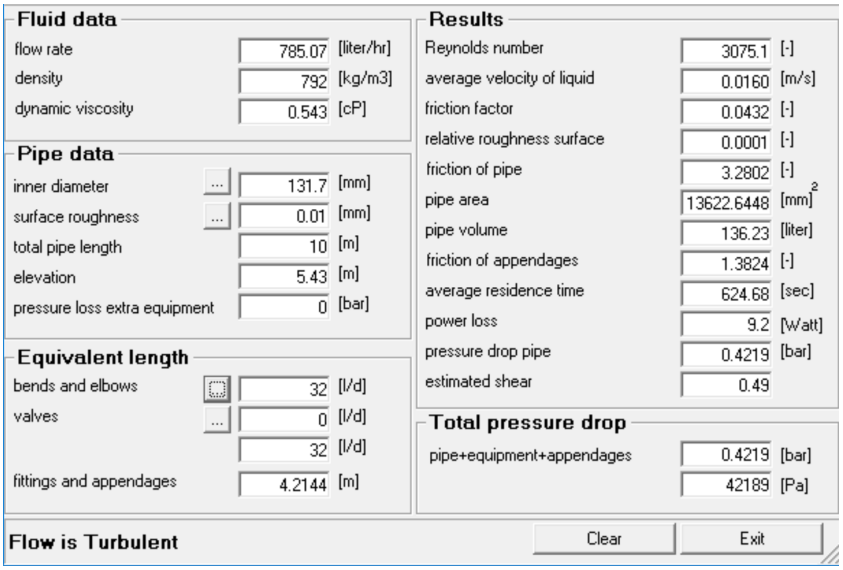
In MT/hr,

$Recycle\ FR = 1.81[\frac{MT}{hr}]\ e^{\,29.68\ [\frac{1}{hr}]t[hr]}$

A.7 Pump and Compressor Requirements

The pumps and piping were designed using dP Pump Designer software. The software took inputs such as the volumetric flow rate, the density of the material, and the viscosity of the material. The software used these inputs to calculate the pressure drop, Reynold's number, and power loss of the corresponding pump.

Below is an example pump calculation using the dP software. The pump detailed here corresponds to streams S-030 and S-031 carrying methanol from the splitters to the 150,000 L fermenters in the continuous process. As can be seen, the inner pipe diameter is assumed to be 131.7 mm, and the pipe length is assumed to be 10 m with two 90 degree bends.



Calculation of Head

In order to use the Equipment Costing Spreadsheet provided by the university professors, the head in units of feet was needed.

$$Head\,(ft) = Pressure\ Drop\,(bar) \times \frac{100{,}000\,Pa}{1\,bar} \times \frac{3.28\,ft}{1\,m} \times \frac{1}{density\,(\frac{kg}{m^3})} \times 9.81\,\frac{m}{s^2}$$

$$Head\,(ft) = 0.3108(bar) \times \frac{100{,}000\,Pa}{1\,bar} \times \frac{3.28\,ft}{1\,m} \times \frac{1}{792\,(\frac{kg}{m^3})} \times 9.81\,\frac{m}{s^2} = 13.12\,ft$$

Compressor Requirements

$Pressure\ at\ Bottom\ of\ Tank = Pressure\ of\ Gas\ above\ tank + Pressure\ of\ Liquid\ in\ tank$
$Pressure\ of\ Gas\ above\ tank = 1.22\ bar$
$Pressure\ of\ Liquid\ in\ tank = \varrho \times g \times h$
Where ρ = the density of the liquid (kg/m^3)
g = the acceleration due to gravity (m/s^2)
h = the height of the liquid (m)
$\varrho \times g \times h = (1000\,\frac{kg}{m^3}) \times (9.81\,\frac{m}{s^2}) \times 7.5\,m\ =\ 73575\,Pa\ =\ 0.74\,bar$
$Pressure\ at\ Bottom\ of\ Tank = 1.22\,bar + 0.74\,bar = 1.96\,bar$

To ensure the compression is sufficient, 20% excess compression was required:
$1.96\,bar + 1.96 \times 0.20 = 2.35\,bar$

A.8 Raoult's Law Calculations for Gas Vent

Raoult's Law:

$yP = xP^{*}$

Concentration in reactor cannot exceed 1% by mass.

$$y_{methanol} = \frac{x_{methanol} \times P^{*}}{P} = \frac{0.01 \frac{g\ methanol}{g\ water} \times 103 \frac{1\ mole\ methanol}{32\ g\ methanol} \times \frac{18\ g\ water}{1\ mole\ water} \times 189\ mmHg}{103\ mmHg} = 0.010 -- negligible$$

A.9 Sterilization Loop Design

The heat exchangers in the sterilization loop were designed using the method described in A.2. The method to determine the dimensions of the holding tube is below.

$$L = \frac{R_T V}{A}$$

Where $R_T = residence\ time$

$V = volumetric\ flow\ rate$

$A = area\ of\ pipe$

$$L = \frac{2.5\ min \times 0.273 \frac{m^3}{min}}{0.0177\ m^2} = 38.56\ m$$

A.10 Utilities Calculations

When calculating the utilities requirements for this process, the yearly operation time for each unit which required utilities was taken into account. The example calculation shown below was used to find the cooling water requirements for the external heat exchanger for the 10,000 L seed train batch reactor.

Yearly Cooling water Requirement for Seed6 Heatex

$$200 \frac{L}{min} \times 60 \frac{min}{hr} \times 7 \frac{hrs}{operation} \times 16 \frac{operations}{year} \times 0.001 \frac{MT}{L} = 1344.0 \frac{MT}{year}\ cooling\ water$$

The yearly cooling water demand for all units was then summed to calculate the total yearly cooling water requirements.

Yearly cooling water requirements

150,000 L Continuous Heatex + Seed6 Heatex + 150,000 L Batch Heatex + Cooler

$$1979271.6\ \frac{MT}{year} + 1344.0\ \frac{MT}{year} + 26648.2\ \frac{MT}{year} + 443963.5\ \frac{MT}{year} = 2451227.3\ \frac{MT}{year}\ \textit{cooling water}$$

Appendix B: Equipment Costing Sources

Equipment	Flowsheet Label	Cost Source
Cell Preparation		
Cell Bank	N/A	K2 Scientific Site Listing
1 mL Test Tubes	N/A	Fisher Scientific Site Listing
Storage		
Methanol Storage Tank	T-02	Equipment Costing Spreadsheet
Mineral Growth Media Storage Tank	T-03	Equipment Costing Spreadsheet
Ammonia Storage Tank	T-04	Equipment Costing Spreadsheet
Seed Train Process		
Pump	S-008	Equipment Costing Spreadsheet
Pump	S-009	Equipment Costing Spreadsheet
Pump	S-011	Equipment Costing Spreadsheet
Ammonia Vaporizer	P-01	Algas Email Quote
Compressor	S-012	Equipment Costing Spreadsheet
Compressor	S-013	Equipment Costing Spreadsheet
Air Compressor	P-02	Equipment Costing Spreadsheet
Compressor	S-014	Equipment Costing Spreadsheet
Coarse Air Filter	F-01	Camfil Email Quote
Compressor	S-015	Equipment Costing Spreadsheet
Submicron Air Filter	F-02	Camfil Email Quote
Compressor	S-016	Equipment Costing Spreadsheet
10 mL Reactor*	R-01	Sartorius Bioreactors Email Quote (6/10 Rule)
100 mL Reactor*	R-02	Sartorius Bioreactors Email Quote (6/10 Rule)
2 L Reactor*	R-03	Sartorius Bioreactors Email Quote (6/10 Rule)
25 L Reactor*	R-04	Sartorius Bioreactors Email Quote (6/10 Rule)
500 L Reactor*	R-05	Sartorius Bioreactors Email Quote (6/10 Rule)
10,000 L Reactor*	R-06	Sartorius Bioreactors Email Quote (6/10 Rule)
Pump	S-073	Equipment Costing Spreadsheet
Cooler	H-09	Equipment Costing Spreadsheet
Pump	S-074	Equipment Costing Spreadsheet
Batch Process/Continuous Process		
Gas Compressor	P-03, P-04	Equipment Costing Spreadsheet

Compressor	S-035, S-037	Equipment Costing Spreadsheet
Pump	S-030, S-031	Equipment Costing Spreadsheet
150,000 L Reactor	R-07, R-08	Paul Mueller Company Email Quote (6/10 Rule)
Pump	S-038/P-05, S-041/P-06	Equipment Costing Spreadsheet
Batch Heat Exchanger	H-01, H-02 during batch	Equipment Costing Spreadsheet
Heat Exchanger	H-01, H-02	Equipment Costing Spreadsheet
Pump	S-044, S-051	Equipment Costing Spreadsheet
Centrifuge	C-01, C-02	Table 16.32 in Product and Process Design Text
Pump	S-045, S-052	Equipment Costing Spreadsheet
Pump	S-060, S-061	Equipment Costing Spreadsheet
Pump	S-058, S-059	Equipment Costing Spreadsheet
Pump	S-070	Equipment Costing Spreadsheet
Pump	S-071, S-072	Equipment Costing Spreadsheet
Pump	S-046, S-053	Equipment Costing Spreadsheet
Economizer	H-03, H-06	Equipment Costing Spreadsheet
Sterilizer	H-04, H-07	Equipment Costing Spreadsheet
Cooler	H-05, H-08	Equipment Costing Spreadsheet
Scrubber	F-03	Equipment Costing Spreadsheet (using packed column)
Pump	S-069	Equipment Costing Spreadsheet
Spares		
Pumps	N/A	Equipment Costing Spreadsheet
Compressors	N/A	Equipment Costing Spreadsheet
Heat Exchanger	N/A	Equipment Costing Spreadsheet
Scrubber	N/A	Equipment Costing Spreadsheet (using packed column)
Product Purification		
Dryer	D-01	Table 16.32 in Product and Process Design Text
Product Bin	B-01	Wamgroup Email Quote
Product Bags	N/A	Bag Corp Store Site Listing
Cleaning		
CIP System		Sani-Matic Email Quote

*Seed train bioreactors were priced individually using the 6/10 rule because a total quote for all 6 bioreactors in sum was given by the company.

Appendix C: Detailed Pump Specifications

Stream No.	Material	Source	Destination	Pressure Drop (bar)	Power Loss (W)	Head (ft)
S-008	MeOH	Storage Tank	Splitter	0.311	13.6	13.12
S-030	MeOH	Splitter	Fermenter	0.422	9.2	17.81
S-031	MeOH	Splitter	Fermenter	0.422	9.2	17.81
S-009	Mineral/Water	Storage Tank	Splitter	0.533	21.8	17.81
S-058	Mineral/Water	Splitter	Media Mixer	0.392	8	13.12
S-059	Mineral/Water	Splitter	Media Mixer	0.392	8	13.12
S-038	Reactor Culture	Fermenter	Heatex1	0.223	1,624.1	7.45
S-041	Reactor Culture	Fermenter	Heatex2	0.223	1,624.1	7.45
S-044	Reactor Culture	Fermenter	Centrifuge	0.196	101.2	6.57
S-051	Reactor Culture	Fermenter	Centrifuge	0.196	101.2	6.57
S-045	Spent Media	Centrifuge	Media Mixer	0.196	89.3	6.57
S-052	Spent Media	Centrifuge	Media Mixer	0.196	89.3	6.57
S-046	Mineral/Water	Media Mixer	Economizer	0.196	99.7	6.57
S-053	Mineral/Water	Media Mixer	Economizer	0.196	99.7	6.57
S-069	Water	Tap Water	Scrubber	0.196	6.9	6.56
S-070	Scrubber Water	Scrubber	Water Splitter	0.196	6.9	6.56
S-71	Scrubber Water	Water Splitter	Media Mixer	0.196	3.5	6.56
S-72	Scrubber Water	Water Splitter	Media Mixer	0.196	3.5	6.56
S-060	Wet Cell Mass	Centrifuge	Spray Dryer	0.196	11.9	6.56
S-061	Wet Cell Mass	Centrifuge	Spray Dryer	0.196	11.9	6.56

Stream No.	Material	Source	Destination	Flow rate (L/hr)	Reynold's No.	Average Velocity (m/s)
S-008	MeOH	Storage Tank	Splitter	1,570.16	6,150.2	0.032
S-030	MeOH	Splitter	Fermenter	785.08	3,075.1	0.016
S-031	MeOH	Splitter	Fermenter	785.08	3,075.1	0.016
S-009	Mineral/Water	Storage Tank	Splitter	1,474.20	3,958.9	0.030
S-058	Mineral/Water	Splitter	Media Mixer	737.10	1,979.5	0.015
S-059	Mineral/Water	Splitter	Media Mixer	737.10	1,979.5	0.015
S-038	Reactor Culture	Fermenter	Heatex1	262,352.50	449,337.6	2.180
S-041	Reactor Culture	Fermenter	Heatex2	262,352.50	449,337.6	2.180
S-044	Reactor Culture	Fermenter	Centrifuge	18,547.30	31,766.4	0.154
S-051	Reactor Culture	Fermenter	Centrifuge	18,547.30	31,766.4	0.154
S-045	Spent Media	Centrifuge	Media Mixer	16,363.10	28,025.5	0.136
S-052	Spent Media	Centrifuge	Media Mixer	16,363.10	28,025.5	0.136
S-046	Mineral/Water	Media Mixer	Economizer	18,271.60	31,294.2	0.152
S-053	Mineral/Water	Media Mixer	Economizer	18,271.60	31,294.2	0.152
S-069	Water	Tap Water	Scrubber	1,272.40	3,417.0	0.026
S-070	Scrubber Water	Scrubber	Water Splitter	1,272.40	3,417.0	0.026
S-71	Scrubber Water	Water Splitter	Media Mixer	636.20	1,708.5	0.013
S-72	Scrubber Water	Water Splitter	Media Mixer	636.20	1,708.5	0.013
S-060	Wet Cell Mass	Centrifuge	Spray Dryer	2,184.20	5,865.6	0.045
S-061	Wet Cell Mass	Centrifuge	Spray Dryer	2,184.20	5,865.6	0.045

Appendix D: Vendor Specification Sheets

Algas-SDI™

ISO 9001 Certified

...Innovative liquid vaporizing and gas mixing solutions

Azeovaire-AA

Anhydrous Ammonia Steam Heated Vaporizer; Models A480SAA through A6600SAA

- *Safely and efficiently vaporizes anhydrous ammonia for SCR, SNCR and FGC systems as well as other industrial and commercial applications.*
- *100% turndown capability.*
- *Stainless steel, float activated high liquid NH_3 level shutdown switch.*
- *Two safety devices to ensure dry superheated NH_3.*
- *Air actuated stainless steel inlet shutoff valve.*
- *Proven reliability in SCR systems around the world.*
- *DCS Interface.*

Features

- *NH_3 pressure vessel is fabricated in accordance with ASME Pressure Vessel Code, Section VIII, Division 1.*
- *NH_3 pressure vessel rating: 250 PSIG at 650°F (17.6 Kg/CM^2 at 343°C).*
- *ASME and U.L. stamped external relief valve: 250 PSIG (17.6 Kg/CM^2).*
- *Explosion Proof configuration meets Class I, Division 1, Group D as defined by NFPA pamphlet 70.*
- *Fully insulated.*
- *Pressure balanced NH_3 supply/delivery operation.*
- *Steam trap with strainer.*
- *Steam temperature gauge.*
- *All exposed metal surfaces are mechanically cleaned, primed and painted.*
- *Complete with all operating and safety controls. Ready to connect to plant facilities.*
- *Factory tested and packaged.*

Options

- *Aqueous ammonia configuration available.*
- *350 psig pressure rating.*
- *Post-weld heat treat.*
- *Vapor temperature transmitter.*

Revised 04.16.01

ECLIPSE™ Innovative Thermal Solutions

Specifications

	MODEL	A480SAA	A960SAA	A1200SAA	A1680SAA	A2475SAA	A3300SAA	A4950SAA	A6600SAA
Vaporization Capacity*	lbs/hr kg/hr	480 218	960 436	1,200 545	1,680 764	2,475 1,125	3,300 1,500	4,950 2,250	6,600 3,000
Heat Exchanger Surface Area	ft^2 m^2	16 1.5	31 2.9	39 3.6	58 5.4	83 7.7	110 10.2	167 15.5	267 20.5
Shipping Weight	lbs kg	700 318	1,100 506	1,150 529	1,750 805	2,000 920	2,400 1,090	3,000 1,364	3,400 1,545
Heat Exchanger Design Pressure	psig kg/cm^2	250 17.6	250 17.6	250 17.6	250 17.6	250 17.6	250 17.6	250 17.6	250 17.6
Heat Exchanger Test Pressure	psig kg/cm^2	375 26.3	375 26.3	375 26.3	375 26.3	375 26.3	375 26.3	375 26.3	375 26.3
Steam Requirements	psig kg/cm^2	← 15-150 psig 1-10 kg/cm^2 →							
Electrical	Voltage, 1ph Frequency	← 110/50 Hz 110/60 Hz				220/50 Hz 208-240/60 Hz →			

- *Vaporization capacity ratings at 100 F (37.8 C) saturated liquid ammonia inlet, 15 F superheated ammonia outlet with 15 psig saturated steam. Higher pressures or lower temperatures may decrease capacity. For larger models, please contact ASDI for more information.*

STEAM CONSUMPTION REQUIRED FOR NH_3 VAPORIZATION

AZEOVAIRE® Steam Heated NH_3 vaporizers require a quantity of steam proportional to usage. A simple rule when estimating steam consumption is 1lb. of steam to vaporize 1.8 lbs. of NH_3.

For example, an **AZEOVAIRE®** Model A1200SAA with a maximum ammonia vaporization capacity of 1200 lbs/Hr, but with a plant usage of only 1100 lbs/Hr will consume approximately 611 lbs. of steam per hour.

STEAM CONSUMPTION		
Model	Vaporization Capacity (Lbs/Hr)	Steam Required @ 100% Load (Lbs/Hr)
A480SAA	480	266
A960SAA	960	532
A1200SAA	1200	664
A1680SAA	1680	930
A2475SAA	2475	1370
A3300SAA	3300	1827
A4950SAA	4950	2741
A6600SAA	6600	3654

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Your ASDI distributor:

ECLIPSE™ Innovative Thermal Solutions
Algas • SDI

Form: AZSAA0401

SPXFLOW

MagMixer® MBE Series

HIGH POWER MIXER FOR LIFE SCIENCE

Lightnin®

About Lightnin

A World Leader in Industrial Mixing since 1923, Lightnin has over 90 years of unrivaled experience in industrial mixing technology, process knowledge, and technological innovation. Lightnin enjoys a global reputation for durable, long-lasting mixers, agitators, aerators, and flocculators for fluid process systems. We offer a full spectrum of impeller designs for diverse applications. In addition, we offer a worldwide service network, mixer repair, gearbox repair, and replacement parts programs. Look to Lightnin for knowledge, technology, and service excellence.

About SPX FLOW

Based in Charlotte, North Carolina, SPX FLOW is a leading global supplier of highly engineered flow components, process equipment and turn-key systems, along with the related aftermarket parts and services, into the food and beverage, power and energy and industrial end markets. SPX FLOW has more than $2 billion in annual revenues and approximately 8,000 employees with operations in over 35 countries and sales in over 150 countries around the world. To learn more about SPX FLOW, please visit our website at www.spxflow.com

A Standard for Cleanability, Durability and Performance - MagMixer® MBE

MAGMIXER MBE SERIES

The big advantage of Lightnin's magnetic agitator is the complete separation of the interior of the tank from the outside. In contrast to conventional agitators, there is no shaft penetrating the tank and therefore no mechanical seal. This eliminates the risks of leaks and microbial contamination and the need for special maintenance that are associated with conventional agitators. We have developed our magnetic agitators with special emphasis on optimizing their cleanability, which is essential for sterile processes. The MBE Series conforms to EC 1935 and AMSE BPE confirming the design of these agitators are qualified for such applications.

Bottom-mounted magnetic agitators are state of the art for low-viscosity liquids in pharmaceutical and biotechnology production. The compact design, low maintenance and high reliability guarantee trouble-free production. Using a bottom-mounted agitator also frees up space on the tank lid for sensors, valves and sight glasses.

Magnetic Impellers

MBE: OPEN DESIGN & OPTIMAL FLOW THROUGH ROTOR

Open design with excellent cleaning: hub and magnetic rotor are connected only by the impeller blades.

The ceramic bearings are oversized (in diameter and height), product-lubricated and consist of outer (silicon carbide) and inner (zirconium oxide) bearing. This results in exceptional stability, good emergency running properties and particle generation below detectable levels.

Ease of maintenance - ceramic bearing parts can be replaced by users on site; no spare rotor needed.

A very large gap between the rotor and the containment shell maximizes flow through the gap and minimizes shear stress.

CFD-engineered mixing: fluid is drawn from above and pumped radially. Perfect for mixing solid powders into liquids; rapid breakdown of temperature and concentration gradients, ensures good heating and cooling.

MBE: MAGNETIC IMPELLERS

New and stronger magnetic materials enable us to reach a higher transferable torque for the same geometry of the drive and rotor. Lightnin offers a wide range of drive sizes with operating torques from 30 Ncm to 300 Nm (2.65lbf.in to 2655lbf.in) to suit a wide range of applications.

Lightnin MBE magnetic impellers ensure that the optimal formulation of the product is achieved throughout the whole volume. When the product is being transferred out of the vessel, the homogeneity of the mixed product is maintained reliably down to the last drop.

The open design enables easy cleaning (CIP) and sterilization (SIP). Lightnin experts will help you select the best impeller variant for your process, the best drive unit and the most suitable installation option (welded or Plug In containment shell).

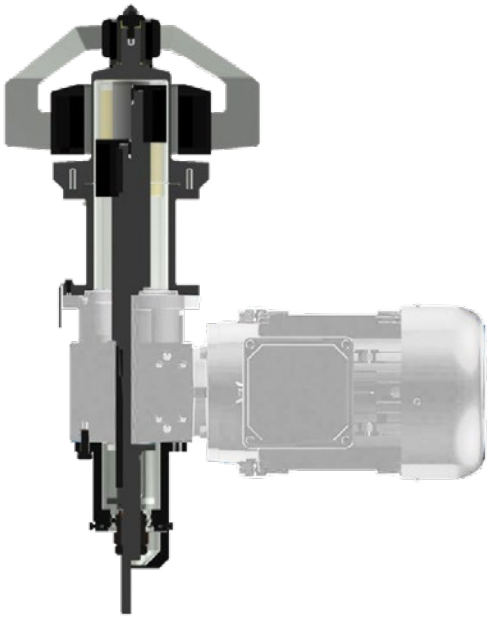
Typical Applications

SPX FLOW provides advanced Lightnin mixing solutions for additional applications including Vaccine Production, Manufacturing of active ingredients, Solutions for Injection and Infusion and many more.

Design Features & Functions of Bottom-Mounted Magnetic Agitators

BEARINGS

- Zirconium oxide inner bearing: less risk of breakage, resistant to damage by sudden settling of the rotor
- Silicon carbide outer bearing: with channels in the face side for better lubrication of the bottom contact surface and enhanced cleaning (CIP)
- Run-dry capability with the rotor type MBE: Magnetic lifting of the impeller reduces the load on the bearing surface so that the agitator can be kept running while the vessel is emptied (mixing down to the last drop)



LOWERING DEVICE

Agitators size from MBE200 and upwards are supplied with a special lowering mechanism that withdraws the drive magnet out of the containment shell.

BENEFITS OF THE RETRACTABLE DRIVE MAGNET

Controlled removal and safe insertion of the impeller due to withdrawl of the drive magnet. The drive unit remains in position while the magnetic drive rotor is lowered out of the containment shell. Avoids damage to the ceramic bearings. Improved safety: The device protects against the crushing hazard involved in placing the agitator head on the bearing, and thus meets the demand of the EC Machinery Directive for designed protection against injury.

REMOVABLE CONTAINMENT SHELL (PLUG-IN)

As an alternative to a version with a flange for welding into the vessel, the agitator can be supplied with a removable containment shell. This can facilitate maintenance. This practical Plug In solution is becoming more popular and makes it easier to switch from shaft-driven agitators with mechanical seals to magnetic agitators.

ELECTROPOLISHING OF STAINLESS STEEL SURFACES

As well as mechanical polishing to two levels, we also electropolish as standard so that we can meet the growing demand for the highest possible surface quality.

Reliable and easy to maintain

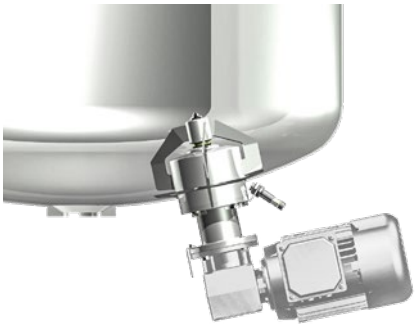
EXTRA LARGE PLUG IN FLANGE

The Plug In flange is also available with a large-diameter flange which allows the whole agitator head to be extracted from the vessel through the bottom opening.

IMPELLER SPEED MONITORING

Sometimes a problem inside the tank or an operating error can lead to forces on the agitator head that exceed the maximum tranmissible torque, so that the magnetic coupling decouples. In this situation the agitator head stops turning although the drive is still running. To monitor this issue, we offer an optional contact-free rpm sensor for the agitator head.

For safety reasons, the speed sensor is included as standard in agitators for use in ATEX Zone 0 (directive 2014/34/EU)



ATTACHMENT OF THE DRIVE SHELL BY TRICLAMP

Attaching the drive with a TriClamp fitting enables it to be removed quickly without tools, eg: when using the agitator with an autoclavable tank. As another alternative, bayonet fittings are also possible.

EXTRACTOR TOOL

An optional tool for the removable containment shell (Plug In). The tool enables the containment shell to be removed from the tank easily and gently.

Technical Specifications

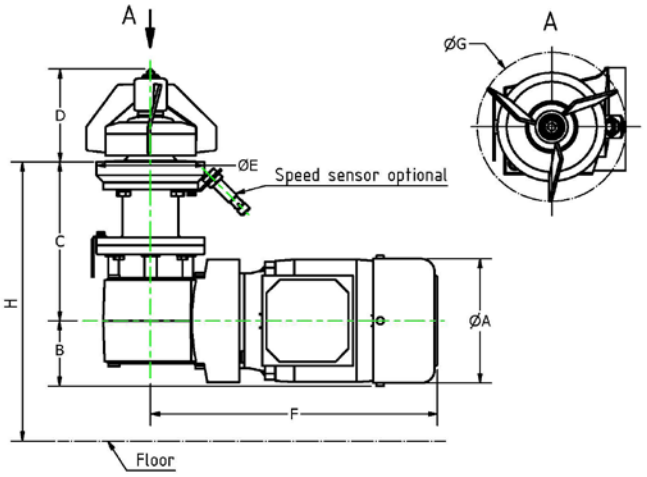
WETTED MATERIALS

Male Bearing	Standard	ZrO2
	Optional	SSiC
		TC-6N
		TC-NB
Female Bearing	Standard	SSiC
	Optional	TC-6N
		TC-NB
Material	Standard	316L (1.4435)
	Optional	904L (1.4539)
		AL-6XN (1.4529)
		Hastelloy-C22
O-Rings	Standard	EPDM
	Optional	FEP
		Silicone
		Viton
Standards	Compliant	FDA, USP Class VI
		AD 2000-W2, ASME 2.2
		BPE 2016
		Non animal origin polishing compounds used
Certificates	Standard	2.2 & 3.1 Mat. Certificates
	Optional	3.2 Mat. Certificates
Surface Finish	Standard	20Ra (<0.5µm)
	Optional	Min. 12Ra (0.3µm)
Other	Standard	Max. Viscosity: 500cp
	Standard	Max. Temp.: 280F (138°C)
	Standard	Dry Running for 15 minutes at reduced speed
	Standard	pH range: 1-14
	Optional	Impeller removal tool

MOUNTING OPTIONS

Welded in Tank Plate - Tank plate welded to vessel by customer, Mixer bolts to plate, impeller sits on tank plate bearing post

Plug in Tank Plate - Modified tank plate welded to vessel by customer, Mixer with coupling and bearing inserts into tank plate, Impeller sits on bearing



TANK PLATE / FLANGE

Type	Standard	Welded Flange
	Optional	Plug In Flange
		Special Sized Welded Flange
		Special Sized Plug In Flange
Accessories	Optional	Removal Tool for Plug In Flange
Material	Standard	316L (1.4435)
	Optional	904L (1.4539)
		AL-6XN (1.4529)
		Hastelloy-C22
Pressure Rating	Standard	Full Vacuum to 5 BarG
Other		Delivery of Tank plate ahead of unit

DRIVE UNITS

Paint	Standard	FDA compliant White Epoxy RAL9003
	Optional	FDA compliant other colors
Protection Class I	Standard	IP55
	Optional	IP65
		IP66
		IP67
Protection Class II	Standard	IE2 (up to 0.55kW/0.75Hp)
	Optional	IE3 (0.75kW/1.0Hp and above)
Other Drive Options		ATEX Category 2 Stainless Steel
		Tri-Clamp connection

MAGMIXER MBE SERIES

SPX FLOW Size	Impeller Diameter (mm/in)	Motor Power (kW)	Motor Power (Hp)	Min. Speed (rpm)	Max Speed (rpm)	Max Mixing Volume @ 1mPas (L/G)
MBE 12D	80/3.15	0.09	0.121	170	1000	140/37
MBE 25D	105/4.13	0.18	0.241	170	950	350/92
MBE 25	130/5.12	0.18	0.241	110	550	500/132
MBE 50	165/6.49	0.37	0.496	110	520	1,200/317
MBE 75	190/7.48	0.55	0.738	85	460	2,500/660
MBE 100	250/9.84	0.75	1.006	85	340	14,500/3,830
MBE 200	300/11.81	1.50	2.012	85	300	28,000/7,397
MBE 300	350/13.78	2.20	2.95	85	285	31,000/8,189
MBE 550	400/15.74	4.00	5.364	85	270	25,000/6,604
MBE 750	450/17.72	5.50	7.376	85	270	40,000/10,567
MBE 1000	500/19.69	7.50	10.06	85	236	60,000/15,850

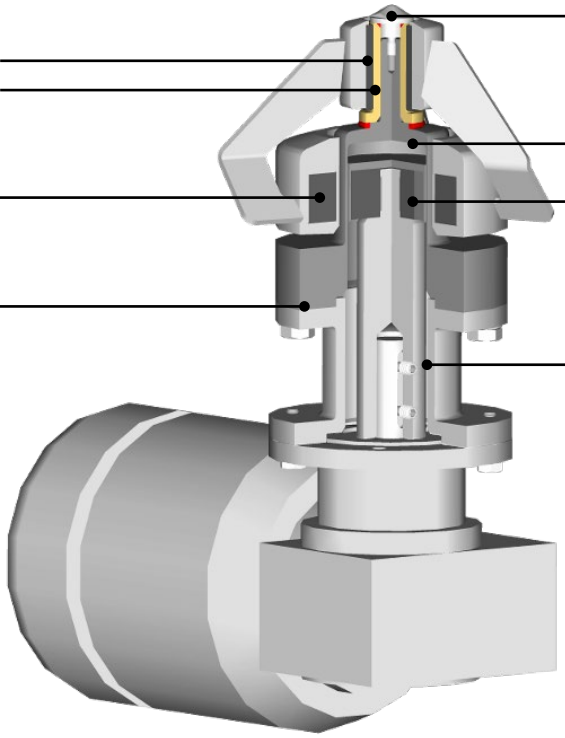
DRIVE UNITS

BEARINGS
Female bearing SiC (Silicon Carbide)
Male bearing ZrO_2 (Zirconium Oxide)
Options available

AGITATOR IMPELLER
with integrated NeFeB magnets

WELD PLATE
Welds to vessel wall
(Removable Plug In option available)

GEARED MOTOR
White FDA Compliant Epoxy Paint
IP55, IE3 Motor - Voltage to suit
ATEX option available
IP65, IP66, IP67 available
Stainless Steel drive option available



RETAINING BOLT
with sealing O-ring

O-RING

DRIVE MAGNETS

DRIVE PEDESTAL
316SS Standard

SPXFLOW

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LIG-B-971 Version: 04/2018

sartorius stedim biotech

ambr® 15 cell culture
The Industry Standard Single-Use Advanced Micro Bioreactor System

turning science into solutions

Bioreactor System for Cell Line Development and Process Optimization

ambr® 15 is an automated microscale bioreactor system that replicates classical laboratory scale bioreactors. The benchtop system comprises disposable micro bioreactor vessels, an automated workstation and user friendly software. ambr 15 offers automated parallel processing and control of 24 or 48 bioreactor experiments using just one operator. Designed to be installed in a standard laminar airflow biological safety cabinet for aseptic operation. ambr 15 provides efficient, consistent and scalable bioreactor experiments compared to classical laboratory bioreactors.*

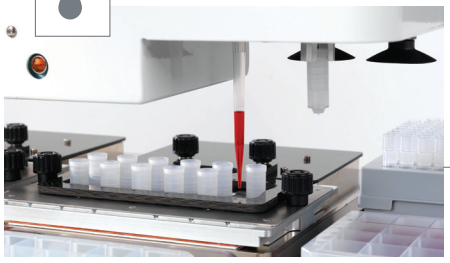
ambr® 15 transforms the way scientists undertake cell line development in the 21st century

- **Improves early clone selection decisions** by providing a predictive bioreactor model with pH and DO control
- **Is the established industry standard** with 50 systems installed in the top 20 biopharma companies
- **Increases lab productivity** by managing multiple (24 or 48) bioreactor development experiments in parallel.
- **Reduces the cost per experiment** by saving substantial amounts on facility space, capital, labour, media and consumables.
- **Helps create better cell lines faster** by automating vessel set-up, feeding, base addition and sampling.
- **Simplifies adoption of Quality by Design (QbD) principles** enabling large multifactorial experimental designs (DoE) in real time.

* See separate ambr 15 Performance Data Sheet

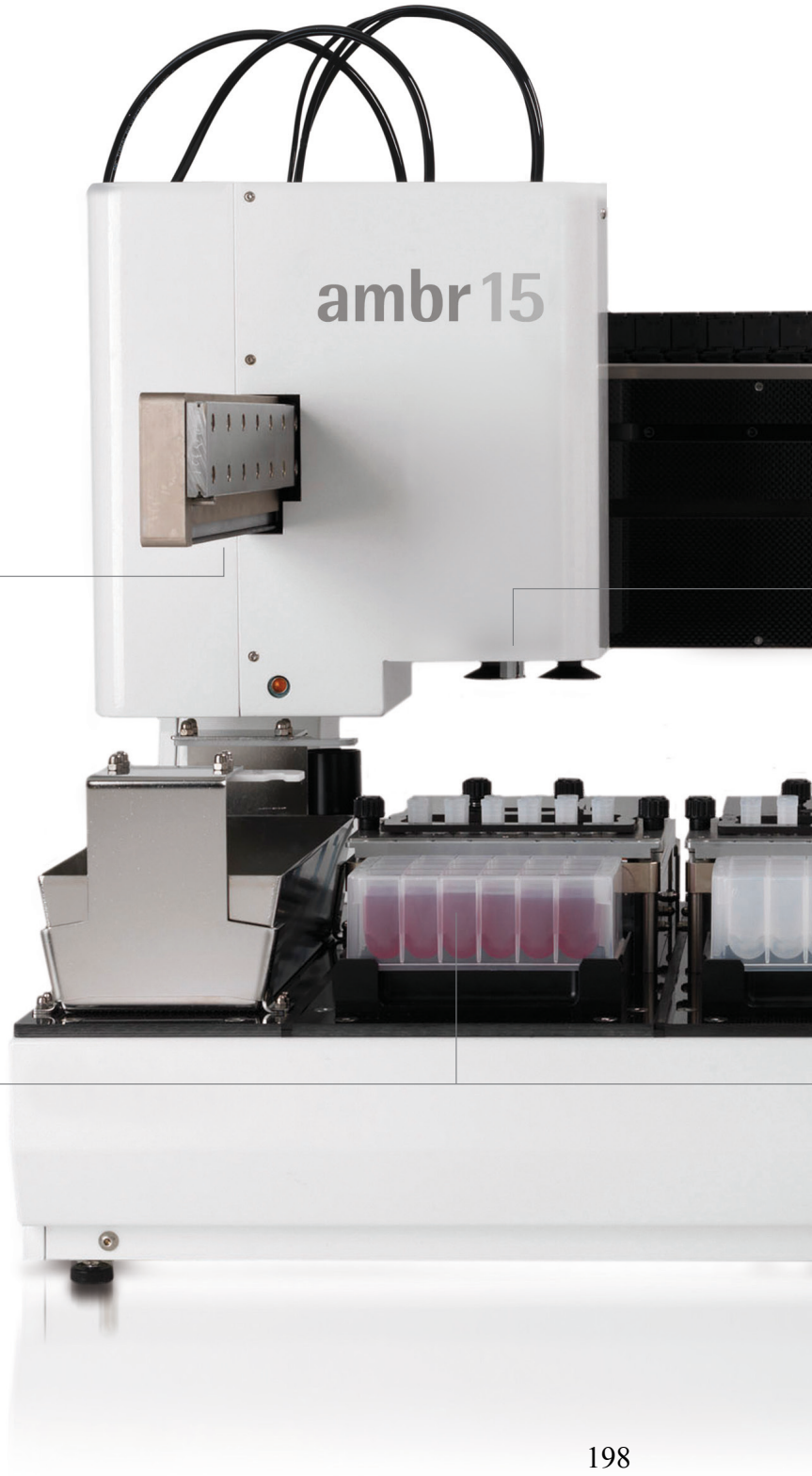

Flexsafe STR
sartorius stedim

ambr® 15 Combines Disposable Micro Bioreactor Vessels, an Automated Workstation and Easy-to-Use Software



Liquid handling unit
For media, feed and reagent addition plus sampling from the bioreactor vessels. Unit includes dual pipette heads, automated vessel decapper and plate delidder.

Culture stations
Each culture station can run up to 12 micro bioreactor vessels and controls temperature and impeller speed. Gas mixture is controlled and delivered independently to each vessel.



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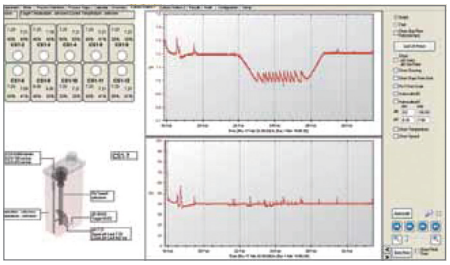

Software
Software enables easy experiment construction. It controls and monitors all experiments and records data and events with a full audit trail.

Sterile working environment
System fits within most standard biological safety cabinets.

Labware lid management
Lids are automatically removed prior to liquid transfer and replaced afterwards.

Disposable tips
Sterile disposable tips are used to eliminate cross contamination. Two sizes of high grade tips are available to transfer liquid volumes accurately and precisely.

Image shows ambr 15 cell culture, 24 vessel system

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Functions

ambr® 15 automatically controls, feeds and samples 24 or 48 bioreactor vessels in parallel

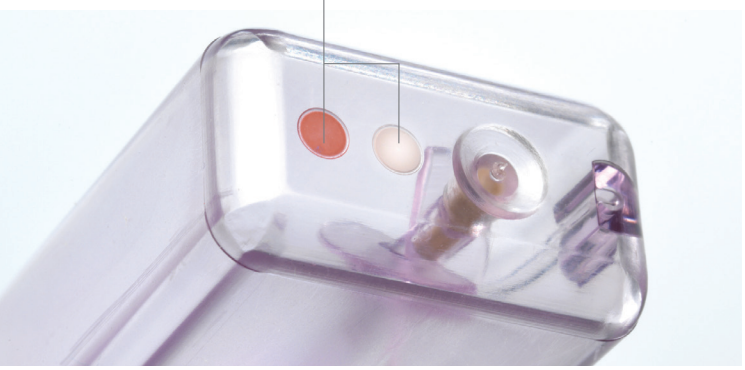
ambr® 15 cell culture
Fully automated parallel processing, control and evaluation of all bioreactor experiments with:

- Online monitoring and closed-loop control of pH and DO
- Independent control of:
  - O_2
  - CO_2
  - N_2

  for each vessel
- Full control of impeller speed and bioreactor temperature
- Optional integration with Beckman Coulter Vi-CELL XR or Cedex HiRes cell viability analysers

ambr® 15 cell culture micro bioreactor vessel
Mimics the characteristics of a classical lab scale bioreactor to enable optimal cell growth and productivity:

- pH and DO sensors for continuous monitoring and control
- Integral impeller for rapid, efficient mixing
- Sparge tube delivers gas to the impeller mixing zone
- 10 to 15 mL culture volume with sample port to allow addition of reagents & feeds or removal of samples



Micro bioreactor vessel
The core disposable micro bioreactor technology includes sensors, stirring impeller, gas sparging and sample port.

1 Gas sparge tube
2 Sample port
3 pH and DO sensor spots
4 Impeller

Applications

ambr 15 cell culture can be used as a microscale model to unblock bottlenecks in a range of development applications such as:

- Cell line selection
- Media development
- Media feed strategies
- Process optimization
- QbD and DoE studies

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Specifications subject to change without notice.
Publication No. : SBI1522-e141101 Order No. : 85037-548-41 Ver. 04 | 2016

sartorius stedim biotech

ambr® 250 modular
Single-Use Benchtop Bioreactor with Simplified Operation for Increased Productivity

turning science into solutions

New Advanced Benchtop Bioreactor System for Parallel Microbial and Cell Culture

ambr® 250 modular is an innovative new high performance benchtop bioreactor system for parallel microbial or cell culture in 100 - 250 mL single-use vessels. The system utilizes the same advanced stirred tank bioreactor technology pioneered in the original ambr® 250 high throughput system. The system comprizes a series of elegantly designed benchtop modules enabling 1 to 8 bioreactors to be operated in parallel and a control module with intuitive system software accessed via a user-interface screen.

Productivity

Each single-use bioreactor vessel is fully integrated with sensors, liquid reservoirs and syringe pumps which make it possible for experiments to be set up and turned around rapidly. A single user can operate up to 8 bioreactors at a time.

Scalability

Because the bioreactor vessels are geometrically similar to larger bioreactors, all processes on the system can be scaled up to those of large bioreactors making for optimum scalability.

Ease-of Use

Due to the unique integrated single-use design of the vessel, with probes, pumps and reservoirs the user can focus on the process and not the set-up. There is no need to connect multiple tubes or filters or to autoclave the vessels and accessories.

Expandable

The modularity of the system means that it can be extended to meet the needs of an expanding company.

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New ambr® 250 modular

ambr® 250 modular System Combines 1 to 8 Bioreactor Stations and a Control Module with System Software

The modular design is expandable up to 8 bioreactors.

Bioreactor module
Holds 2 bioreactor stations. Up to 4 modules can be connected to the controller.

Fast-loading peristaltic pump
Accessible for each bioreactor, provides an alternative route for feeding and harvesting. This is in addition to the 5 integrated syringe pumps in each bioreactor.

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Chilled liquid reservoir
Chills liquids to temperatures between 6-8°C ensuring temperature sensitive media can be maintained.

3 step 'Easy Connect' installation for all gas, liquid and sensor connections
- Step 1 - slot in
- Step 2 - secure with clamp
- Step 3 - secure pH connector

Enabling quick set-up and rapid turnaround.

Touch screen user-interface
Enabling easy control and supervision of multiple bioreactors.

Bioreactor controller
Manages all processes including pH, temperature and DO for up to 8 bioreactors. Fully integrated foam control.

Optional off-gas analyzer
Monitors and controls processes using data from individual bioreactors' exhaust gases.

ambr® 250 modular Bioreactor Vessels

Each bioreactor is fully integrated with 5 liquid reservoirs and proprietary single-use syringe pumps. The integration simplifies experimental set-up, eliminates any need for vessel sterilization, and significantly reduces any error due to manual handling.

Visible liquid addition lines
Allow view of liquid during auto-priming.

Vessel scalability
Vessels are geometrically similar to standard bench and pilot scale bioreactors, enabling straightforward scale-up.

Single-use mammalian or microbial bioreactors
- 100 - 250 mL working volume.
- Dual pitch blade or Rushton impellers.
- Spot based DO sensor.
- Disposable pH electrode.
- Integrated gas inlet filters.
- Sparge and headspace gassing options.
- Integrated exhaust gas condenser.

Integrated liquid reservoirs
- 2 Integrated 125 mL reservoirs per bioreactor
- 3 Integrated 50 mL. Reservoirs per bioreactor.

Single-use syringe pumps
Each reservoir is integrated to its own high precision syringe pump allowing for highly consistent and accurate liquid delivery.

208

Fully integrated liquid lines and reservoirs
Allowing for rapid experimental set-up and turnaround.

Gas tube
Gases can either be delivered into the headspace or sparged into the media. These delivery systems are independent and can function in parallel.

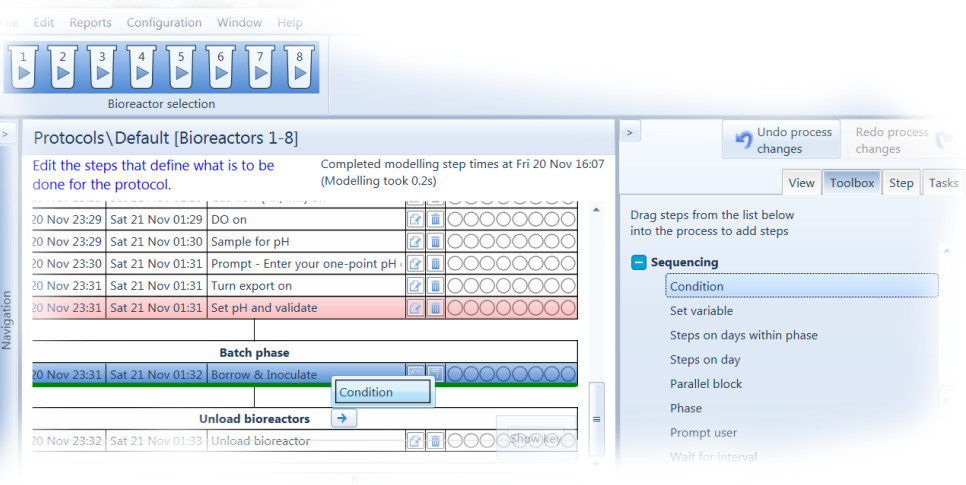
Septum cap
Allows for rapid liquid additions with a syringe.

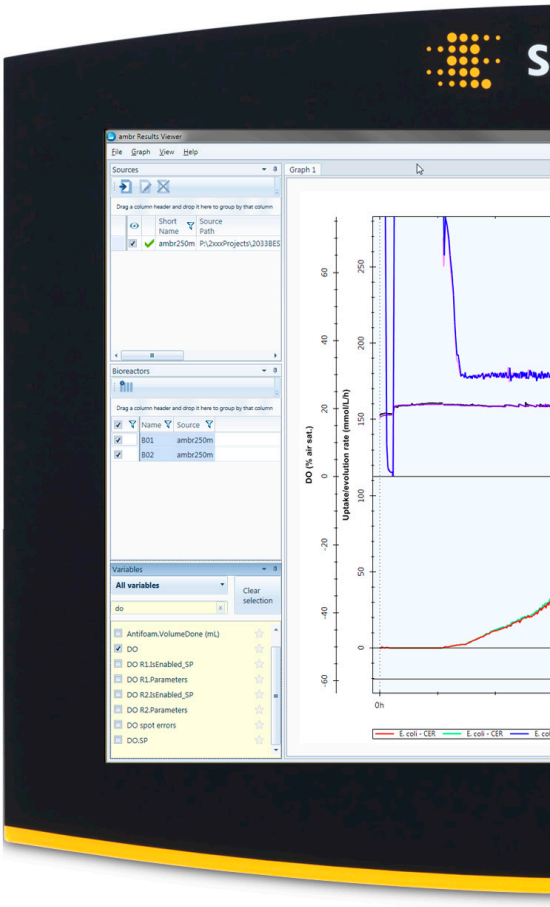
Single-use pH and DO sensors
Both pre-calibrated, the DO spot measures 0 - 200% and the standard pH probe has a measurement range of 2 - 8.5.

Double impeller - Rushton or pitch-blade
For microbial or mammalian vessels respectively.

Software

ambr® 250 modular is supplied with the same advanced software as the established ambr 250 high throughput system. Users can design new and complex experiments and analyze results easily and quickly.





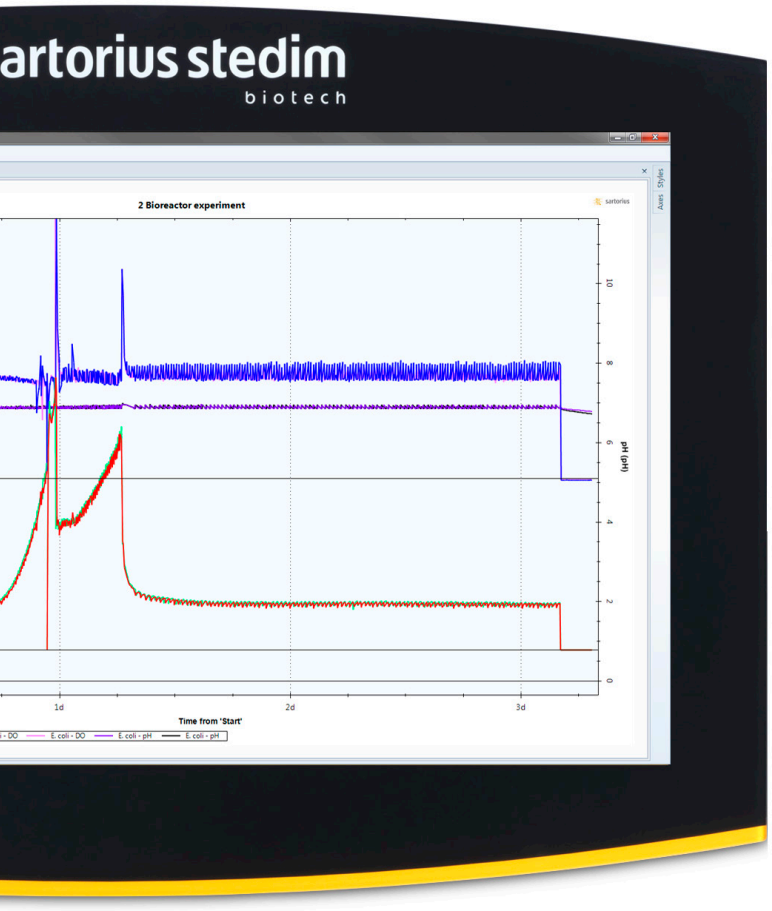
ambr 250 modular software encompasses 4 applications all of which enable export and import of data to | from different sources.

Process Definition
Simple 'drag and drop' process steps for quick and easy definition of experiments.

Runtime
Monitoring and adjustment of all process parameters in real time, for each individual bioreactor.

Experiment Viewer
Historian application which enables users to fully analyze experiments looking at audit trails, all variables and completed recipes.

Results Viewer
Comparison of plots from experiments run at different times - allows parameters from multiple experiments to be plotted together on the same graph.



Functions

ambr® 250 modular automatically controls and adds liquids to 1 to 8 bioreactor | fermenter vessels in parallel

Bioreactor controller

- Three gasses per bioreactor with mass flow sensor:
- Mammalian
  - O_2
  - CO_2
  - N_2 | air
- Microbial
  - O_2
  - Air
  - N_2
- Five positive displacement liquid pumps per bioreactor for high precision at low flow rates.
- Individual bioreactor temperature control with heating and cooling.
- Individual impeller speed control per bioreactor.
- Optional off-gas analysis by BioPAT ambr Xgas for CO_2 and O_2, also uses OUR and CER measurements.

Applications

ambr® 250 modular is configurable for microbial or mammalian cell culture and is used in R&D across biopharm and industrial biotech for the following applications:

- Process optimization.
- Process characterization.
- Process robustness experimentation in support of QbD studies.
- Process scale-down model.

Scalability

Single-Use from Cell Line and Process Development to Production Scale

- Geometrical similarity of vessel design
- Consistent mixing and gassing strategies
- Reliable single-use platform

ambr® 250 modular

BIOSTAT® B

Univessel® SU 2L

BIOSTAT® STR 50

Similar geometry and sensors -

Process development

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ambr® 250 modular

BIOSTAT® B

UniVessel® Glass
0.5 – 10 L working volume

Biostatl® Cplus
5 - 30 L working volume

Also scalable to glass and stainless steel bioreactors

BIOSTAT® STR 200

BIOSTAT® STR 500

BIOSTAT® STR 1000

scaling up from 0.25 L to 1000 L

Production

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Specifications subject to change without notice.
Publication No. : SBI1545-e160301 Order No. : 85037-551-30 Ver. 05 | 2016

sartorius stedim biotech

BIOSTAT STR® Bioreactors and Flexsafe STR® Bags
A Perfect Match for True Scalability in Single Use

turning science into solutions

Single-Use Production Platform of the Future

The BIOSTAT STR® fully scalable, single-use bioreactor family offers working volumes from 12.5 L to 2000 L and is based on conventional stirred-tank design. It is now available in its second generation with improved hardware design, Flexsafe STR® bags for excellent cell growth and robustness and single-use, non-invasive biomass monitoring.

Cell Growth

The complete control of our Flexsafe® bag production process – from the resin to the final sterilized bag – guarantees you consistent lot-to-lot cell growth performance, even of the most sensitive cell lines.

Scalability

Classic stirred-tank design from the ambr® 250 to the BIOSTAT STR® 2000. Simplify your scale-up and scale-down, reduce risks in your process transfers and easily switch between reusable and single-use bioreactors.

Connectivity

Easily connect your BIOSTAT STR® to our BioPAT® MFCS or third party supervisory software like DeltaV™. Straightforward integration into existing automation infrastructure provides you with data consistency across scales and throughout the entire development process.

Continuous Processing

Get the best solution for high-cell density, intensified cultures with combining efficient oxygen transfer, mixing and CO_2 stripping with integrated advanced analytics for high level and automated monitoring

- BioPAT® ViaMass for viable biomass
- BioPAT® Trace for glucose | lactate

Microcarrier Cultures

Benefit from our flexible stirrer and sparger options. Successfully grow your shear sensitive cells on microcarriers and ensure excellent cell growth and viability.


STR
sartorius stedim
BIOSTAT STR

Accelerate Your Development – Rely on Proven Scalability

Sartorius Stedim Biotech offers classic stirred-tank design in single-use bioreactors, from ambr® 250 to BIOSTAT STR® 2000.

- Geometrical similarity of vessel design
- Consistent mixing and gassing strategies
- Reliable single-use sensor platform

Simplify your scale-up and scale-down studies
Easily switch between reusable and single-use bioreactors
Mitigate risks during process transfers

ambr® 250
100 – 250 mL working volume

UniVessel® SU 2 L
0.6 – 2 L working volume

BIOSTAT STR® 50
12.5 – 50 L working volume

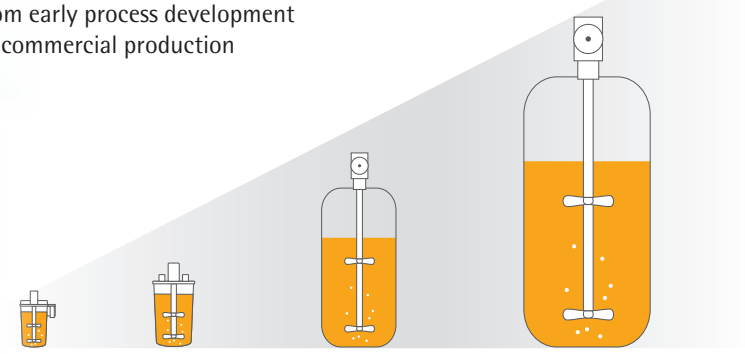
BIOSTAT STR® 200
50 – 200 L working volume

Cell line Development

Process Development

- Mammalian, insect and stem cell culture
- High cell density cultures exceeding 150 million cells/mL
- Adherent cell culture on microcarriers
- Low to medium cell density microbial culture

Seamless scalability in single use
from early process development
to commercial production



BIOSTAT STR® 500
125 – 500 L working volume

BIOSTAT STR® 1000
250 – 1,000 L working volume

BIOSTAT STR® 2000
500 – 2,000 L working volume

Production

- Antibodies and recombinant proteins
- Vaccines
- Cell and gene therapy

For further details please have a look at:
www.sartorius.com/biostat-str

Advanced Functionality

The BIOSTAT STR® bioreactor system is comprised of a stainless steel bag holder, with new, improved design, a single-use Flexsafe STR® bag with advanced single use sensor solutions and a superior control system that can easily be connected to third party SCADA systems such as Delta V™.

Easy Installation Even at 2,000 L Scale

Install our bioreactor bags in next to no time thanks to our large door, the fixed motor position and an easily accessible filter holder position. Prevent tube and cable spaghetti with our cable and tube management system.

Larger Sensor Window

Increase your sensor and connection options. Use standard re-usable sensors via special insertion devices. Connect external cell retention devices.

Advanced Single-Use Sensors

Run your processes in an intensified, automated and predictive manner with our advanced analytics solutions. Use BioPAT® ViaMass to monitor cell growth and exert a tight control onto feeding and harvesting in intensified processing. With BioPAT® Trace you are able to improve your product titer and quality with controlling and maintaining a low-glucose concentration.

Advanced Aeration Concept

Attain high oxygen transfer rates and minimize foaming with our innovative sparging element that combines a 0.8 mm ring sparger and a 150 µm micro-sparger with defined hole sizes. Remove excessive CO_2 using a stripping gas flow. Benefit from optimal gas bubble size and homogeneous distribution.

Proven Stirrer Design

Achieve excellent fluid flow dynamics – comparable to classic stirred-tank bioreactors - with two impellers on a magnetically coupled center-line shaft. Choose between Rushton and 3-blade segment impeller designs.

Local Controller with Full Connectivity

Reduce operator training effort and mitigate human error with our GAMP compliant DCU local control platform and intuitive design of the human machine interface. Now enabling also straightforward integration into 3rd party SCADA systems including Delta V™.

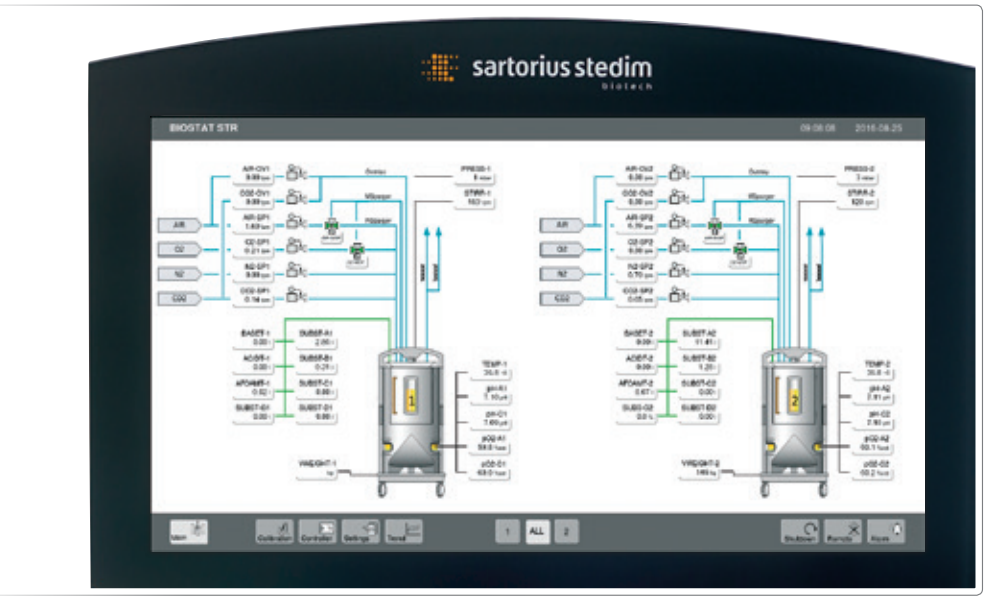
Increased Double Wall

Cool down your culture harvest at the end of your process faster than ever due to the increased heat exhange area of our bag holder.

Intuitive and Industry-Proven Local Control

Benefit from our industry-proven, robust and intuitive-use local control platform for our BIOSTAT® bioreactors, SARTOFLOW® crossflow filtration units and FlexAct® configurable systems. Available in its fourth generation, with new, improved features.

- Minimizes training and enables you to start your process right away
- Increases operator flexibility
- Mitigates human error



Centralized User | Password Management Utilizing Windows® Domain Login

Central management of user rights for your bioprocess equipment has never been easier – the BIOSTAT STR® can connect directly to your user management data implemented on your Microsoft® server and your user can work with their well-known user names and passwords.

Import and Export Process Relevant Settings

Easily store your individual process settings and user authentication data and transfer them between BIOSTAT STR® systems.

Integration and Connectivity at Its Best

Our BIOSTAT STR® provides the right interfaces and tools to connect to supervisory control and data acquisition systems (SCADA) or distributed control systems (DCS) – regardless if you chose to work with our BioPAT® MFCS as a ready-to-use solution or connect to your specific company SCADA or DCS solution.

BioPAT® MFCS – Turnkey SCADA Solution

Specialized for bioprocesses, BioPAT® MFCS is designed as a "plug-and-play" tool for advanced SCADA functionalities. It is ideally suited for capturing, storing and visualizing process data of all BIOSTAT® and ambr® bioreactors and other process equipment.

One source bundle with full Sartorius responsibility and lifecycle concept

Specifically tailored for biopharmaceutical industry

Cost-effective & flexible automation platform

Setpoints | Actuator Access

DCU Modbus fieldbus | DCU OPC interface

Process Values | Alarms

Siemens Simatic PCS 7

Emerson Process Management DeltaV™

Rockwell Automation

For straightforward integrations of your BIOSTAT STR® into a DeltaV™ network, we provide a Modbus mapping of your system in digital format. Once imported into your DeltaV™ configuration, it enables you to access process values, set controller parameters or even access actuators in a direct manner – e.g. pumps or valves.

Conventional Stirred-Tank Design

Ease of Use

Large doors provide easy access for safe bag installation. Our ease-of-use concept enables fast and straightforward installation even of large-scale bags up to 2,000 L. The torospherical Flexsafe STR® bags fit perfectly into the bagholder – preventing creases and crinkles.

Lifting device for easy installation of 2,000 L bags

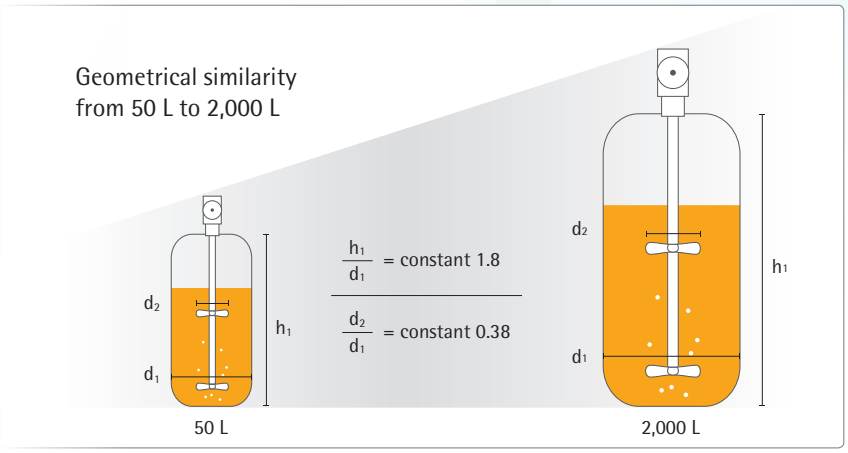
Easy Implementation Flexible Combinations

Gamma-sterilized Flexsafe STR® bags are ready to use. Sterile connection and disconnection devices, like our BioWelder® and BioSealer® can be used for safe connections and liquid transfer. Needle free sampling ports allow easy and convenient sampling without any risk of contamination.

BioWelder®

Easy and safe installation of a 2,000 L Flexsafe STR® bag

Watch Video:
www.sartorius.com/biostat-str



Configurable Flexsafe STR® Bag Design

The bags feature a 1:4 turndown ratio. Flexsafe STR® bags are individually configurable, offering multiple options for tubing, connectors, spargers and agitation devices to cover all process demands. Pre-configured standard bags are readily available from stock – the choice is all yours.

Tubing Options	Choose between silicone and C-Flex® 374 tubing in various diameters. Internal dip tubes for inoculation or carrier-free harvesting.
Sparger Type	Choose between just using the 0.8 mm ring sparger or the 150 µm micro-sparger part of our innovative sparging element or a combination of both.
Impeller Type	Choose between two 3-blade segment impellers, two 6-blade disk impellers (Rushton) or a combination of both.
Connections	Choose among Luer, MPC, Opta® sterile connectors and tube welding.
Sensors	Choose between single-use and conventional pH and DO probes. Monitor viable biomass with our single-use BioPAT® ViaMass capacitance probe and improve your product titer, quality and ensure glycolisation homogeneity with our BioPAT® Trace.

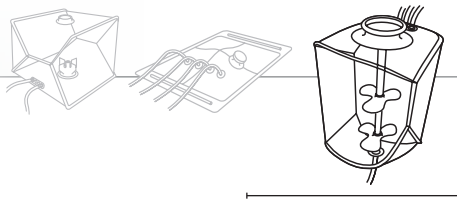
BioPAT® Trace

BioPAT® ViaMass

Opta® Connectors

Extensive bioreactor characterization data on kLa, mixing time and power input available.
www.sartorius.com/bpi-supplement

New Flexsafe® Bag Family



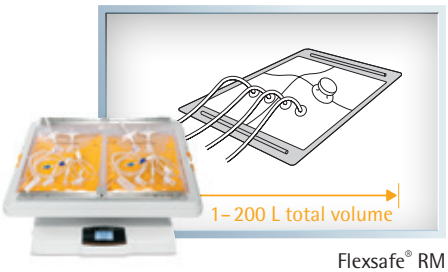
50 L Flexsafe STR® 2,000 L

Safe and Convenient Single-Use Processing

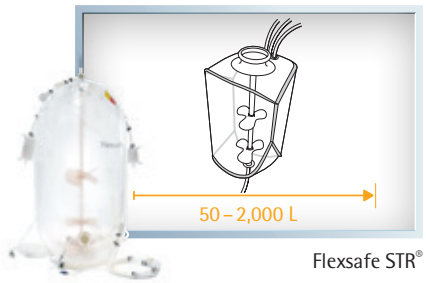
Flexsafe® – One Film Across All Single-Use Applications

Flexsafe® meets your requirements for outstanding robustness and ease of use throughout all steps of single-use processing – from rocking motion or stirred tank bioreactor cell culture, through to large-scale mixing to shipping of drug products. In addition, Flexsafe® reduces time and expense for process validation, extractable and leachable studies, toxicology assessment and stability studies.

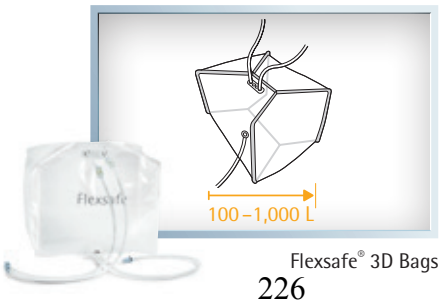
Seed | Production | 3D Storage & Shipping



Flexsafe® RM



Flexsafe STR®



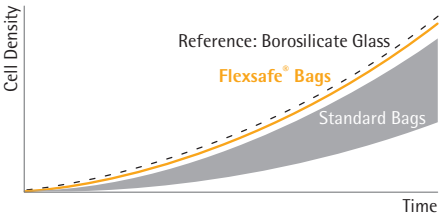
Flexsafe® 3D Bags

One for All

Use our Flexsafe STR® bags for clinical or commercial production of your valuable antibody, recombinant protein or vaccine. Team up with our Flexsafe® RM bags in your seed train. Benefit from the same PE film material across all of your cell culture steps.

Cell Growth

Flexsafe® ensures excellent and reproducible growth behavior of the most sensitive production cell lines. The complete control of our raw materials, the extrusion process and the bag assembly guarantees consistent lot-to-lot cell growth performance.

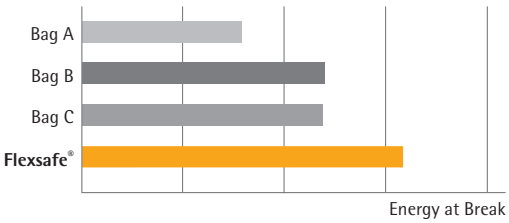


We optimized the resin and minimized the additive package, in collaboration with our resin and film suppliers.

Independent labs have confirmed that Flexsafe® bags are free of cytotoxic leachables. No bDtBPP is identified in WFI extracts of Flexsafe® bags.

Robustness

The thickness, strength and flexibility of the new polyethylene film enhances the mechanical robustness of Flexsafe® – making it ideal for all bioprocessing applications. The strength of Flexsafe® significantly reduces the risk of accidental damage to the bag due to inappropriate handling. Its flexibility enables convenient installation and self-deployment of the bag in its container.



Assurance of Supply

Global SUPPLY GUARANTEE

Flexsafe® provides you with an unprecedented assurance of supply and enables robust business continuity plans. Our strategic partnership with resin and film suppliers ensures full traceability of raw materials and control over the entire manufacturing process from the resins to the final assembled bags.

New Flexsafe® Bag Family

Watch Video:
www.sartorius.com/sartorius/en/EUR/flexsafe

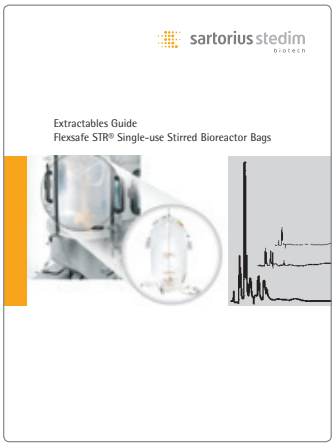
Unparalleled Safety in Single-Use Technology

Unlike stainless steel bioreactors, single-use bioreactors are made, shipped, stored and installed for every run. Therefore, we have developed a holistic safety concept that governs the entire process from manufacturing to using single-use bioreactor bags to produce drugs for human use, from end to end. Especially, testing the integrity of bioreactor bags at the point of use, helps to mitigate potential contamination and biosafety risks. This is even more important for processes requiring higher biosafety levels, e.g. in vaccine production, where protection of operators and the environment are of great concern.

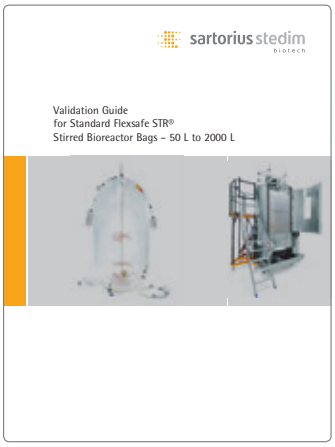
Bio

Development	Production	Transportation
– User requirement specification (URS) – Rigorous mechanical, chemical and biological testing of components – Qualification of bioreactor bags according to applicable guidelines – Generation of extractable and leachable data – Extensive application-based robustness testing of entire bioreactor bags	– ISO 9001-certified production – State-of-the-art quality system according to applicable cGMP requirements – Incoming goods inspections – Qualified components with proven cell culture performance – ISO 7 cleanroom bag assembly – Qualified manufacturing procedures – Validated gamma irradiation procedures – Stringent quality control and product release	– Innovative packaging concept for safe transportation and storage – Cleanroom compliant packaging – Easy and safe unpacking without cutting objects

Comprehensive Supporting Documentation Available



Extractables Guide



Validation Guide

Learn more about our single-use bioreactor bag qualification approach.
www.sartorius.com/bpi-supplement

safety

Installation	Operation	Deinstallation
– System designed for convenient installation to reduce operator manipulation	– Pressure measurement and control keeps bag pressure within permissible range	– Aseptic disconnection
– Detailed instruction and video installation guide	– Single-use exhaust cooler and back-up exhaust filter to prevent filter blockage	– Convenient dismantling
– Aseptic connector technology	– Overheating protection to maintain film material properties	
– Bag Tester for post-installation, point-of-use leak test	– Completely closed bag with magnetic coupling and single-use sensors	
– Tubing and cable management for easy, safe and clean set up	– Spill containment tray with option to connect to kill tank	

Sartocheck® 4 plus Bag Tester

Post-installation, pre-use testing takes safety to highest level

– Patented techology for non-destructive point-of-use bag test

– Tests the bag and the manual connections simultaneously

– Fully validated, automatic and fast test method based on pressure drop

– First bag tester for single-use bioreactors and specifically designed for BIOSTAT STR®

Reduce your risk of batch losses due to operator failures and incorrect handling.

Post-installation bag testing

Watch Video:
http://microsite.sartorius.com/sartocheck/video.html

Maximized Process Security

To keep your biopharmaceutical process robust and reliable, we provide a comprehensive range of services to ensure the highest reliability and uptime of your BIOSTAT STR®, regulatory compliance and best quality of results. From installation and qualification to regular preventative maintenance: Our service team will be happy to assist you on site and will be with you quickly thanks to our worldwide service network.

Installation and Commissioning

Safe and proper operation of your equipment – right from the start

Qualification (SAT)

Compliance with GMP requirements, easy integration into your quality management system

Operator Training

Quality through greater expertise: Sartorius trains the personnel operating our equipment

Installation Phase

Utilization Phase

Repairs and Spare Parts

In the event of service requests, we are quickly at your side with the necessary spare parts – worldwide

Maintenance and Contracts

Optimal equipment operation and protection against potential downtimes

Calibration

Accurate results in the long term and compliance with regulatory requirements

Service Contracts for the Entire System Lifecycle

With our Bioprocess Service Program, Sartorius offers service contracts to protect your equipment through its entire lifetime. Based on your specific risk assessment and requirements, you can choose between three Service Level Agreements: Essential, Advanced and Comprehensive. Protect your BIOSTAT STR® by choosing the appropriate service contract. For maximum productivity and minimum downtimes.

Essential	Advanced	Comprehensive
You benefit from:	You benefit from:	You benefit from:
– A plannable annual maintenance	– A plannable annual maintenance	– A plannable annual maintenance
– A fast support at the technical helpdesk within one business day and priority on-site-response	– Technical helpdesk reaction time within 8 hours and 72 hours on-site response	– Technical helpdesk reaction time within 4 hours and 48 hours on-site response
– In case of repair: A discount on all time and material based cost elements	– In case of repair: labor and travel costs are covered, a discount of 10% on spare parts	– In case of repair: all costs are covered

Your Benefits

- Process stability and minimized downtime
- Maximized system uptime, higher profitability
- Optimized total cost of ownership

For further details and the dedicated datasheets, please have a look at our website: www.sartorius.com/service

Technical Specifications

BIOSTAT STR® 50

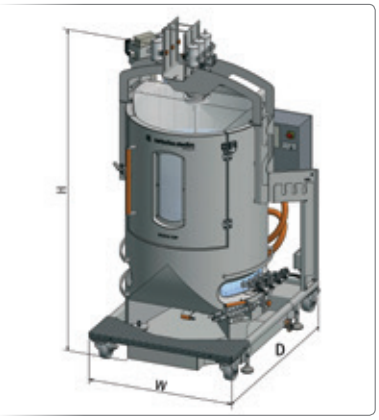
BIOSTAT STR® 200

BIOSTAT STR® 500

BIOSTAT STR® 1000

BIOSTAT STR® 2000

Bag Holder with TCU



	BIOSTAT STR® 50	BIOSTAT STR® 200	BIOSTAT STR® 500	BIOSTAT STR® 1000	BIOSTAT STR® 2000
Material	AISI 304L stainless steel				
Dimensions W × D × H	588 × 880 × 1952 mm 23.2 × 34.6 × 77 in.	788 × 980 × 1978 mm 31 × 38.6 × 78 in.	1033 × 1380 × 2312 mm 40.7 × 54.3 × 91 in.	1225 × 1380 × 2692 mm 48.2 × 54.3 × 106 in.	3400 × 2750 × 3900 mm 134 × 108.3 × 153.5 in. (incl.platform)
Footprint	0.52 m^2 5.6 ft^2	0.77 m^2 8.3 ft^2	1.43 m^2 15.4 ft^2	1.69 m^2 18.2 ft^2	9.35 m^2 100.6 ft^2 (incl.platform)
Weight	185 kg 408 lb	285 kg 629 lb	575 kg 1,268 lb	765 kg 1,687 lb	2,045 kg 4,509 lb (incl.platform)
Packaging dimensions	1450 × 1150 × 1900 mm 57.1 × 45.3 × 74.8 in.	1450 × 115 × 1900 mm 57.1 × 45.3 × 74.8 in.	1700 × 1200 × 2200 mm 67 × 47.2 × 86.6 in.	1700 × 1500 × 2540 mm 67 × 59 × 100 in.	
Installed on skid	•	•	•	•	•
Double wall	•	•	•	•	•
Electro-polished	•	•	•	•	•
Single front door	1	1	1	1	1
Holder for gas filters	•	•	•	•	•
Viewing window	1	1	1	1	1
Lateral window for sensors and ports	2	2	2	2	2
Top drive motor	•	•	•	•	•

• available – not available ( ) optional, needs to be ordered separately

Control Tower

BIOSTAT STR® 50 &
BIOSTAT STR® 200 Twin

	BIOSTAT STR® 50	BIOSTAT STR® 200	BIOSTAT STR® 500	BIOSTAT STR® 1000	BIOSTAT STR® 2000
Material	AISI 304 stainless steel				
Dimensions W × D × H	800 × 910 × 1708 mm 31.5 × 35.8 × 67.2 in.				
Footprint	0.73 m^2 7.86 ft^2				
Weight	182 kg 402 lb				
Packaging dimension	1450 × 1150 × 1900 mm 57.1 × 45.3 × 74.8 in.				
Single version	•	•	•	•	•
Twin version*	•	•	–	–	–
Installed on skid	•	•	•	•	•
Color touch screen	19"	19"	19"	19"	19"
Safety measurement and shut-off	•	•	•	•	•
Different user level log in	(•)	(•)	(•)	(•)	(•)
Logbook function	(•)	(•)	(•)	(•)	(•)
Uninterrupted power supply (UPS) 24 V	(•)	(•)	(•)	(•)	(•)

* Twin configuration to accommodate extra-low space requirements. Ideal for seed train and small-scale production.

Facility and Utility Requirements

	BIOSTAT STR® 50	BIOSTAT STR® 200	BIOSTAT STR® 500	BIOSTAT STR® 1000	BIOSTAT STR® 2000
Power Supply Power | Frequency | Consumption					
208 VAC | 60 Hz | 23 A	•	•	•	•	•
400 VAC | 50 Hz | 18 A	•	•	•	•	•
Gas Supply	Gas specification according to ISO 8573-1: dry, free of oil and dust				
Compressed air (bar)	2	2	2	4	4
Gas pressure O_2, N_2, CO_2 (bar)	2	2	4	4	4
Water Supply					
Cooling water – supply pressure (bar)	1.5	1.5	1.5	1.5	2
Drain for water	25 lpm	25 lpm	25 lpm	25 lpm	40 lpm
Temperature	min. 8°C min. 46°F	min. 8°C min. 46°F	min. 8°C min. 46°F	min. 8°C min. 46°F	min. 8°C min. 46°F
Degree of hardness	max. 12 dH	max. 12 dH	max. 12 dH	max. 12 dH	max. 12 dH
Environmental Requirements					
Ambient temperature	5 – 40°C 41 – 104°F				
Relative humidity range	< 85%				

• available – not available ( ) optional, needs to be ordered separately

Process Control

	BIOSTAT STR® 50	BIOSTAT STR® 200	BIOSTAT STR® 500	BIOSTAT STR® 1000	BIOSTAT STR® 2000
Agitation Module					
Max. stirrer speed*	240 rpm	150 rpm	110 rpm	90 rpm*	70 rpm
Temperature Control Unit					
Type	Heating | cooling	Heating | cooling	Heating | cooling	Heating | cooling	Heating | cooling
Heating kW | cooling HP					
Temperature control, double wall	8°C (46°F) above cooling water up to 40°C (104°F)	8°C (46°F) above cooling water up to 40°C (104°F)	8°C (46°F) above cooling water up to 40°C (104°F)	8°C (46°F) above cooling water up to 40°C (104°F)	8°C (46°F) above cooling water up to 40°C (104°F)
Over-temperature protection	•	•	•	•	•
Connection to pressure rated cooling water	•	•	•	•	•
Aeration Module	4-gas mix (O_2, N_2, CO_2, air) with 3 outlets				
Rotameters (Flow Meters)					
– Outlets	3	3	3	3	3
– Accuracy of rotameters	± 5% full-scale	± 5% full-scale	± 5% full-scale	± 5% full-scale	± 5% full-scale
For sparger line	ring, micro or combi-sparger (O_2, N_2, CO_2, air)				
– Flow rates (lpm)**	0.7 – 5.5	1.0 – 23	5.0 – 55	12 – 104	30 – 210
For overlay line	(CO_2, air)				
– Flow rates (lpm)**	0.7 – 5.5	1.0 – 23	5.0 – 55	12 – 104	30 – 210
Mass Flow Controllers (MFC)					
– Number of MFCs	up to 6	up to 6	up to 6	up to 6	up to 6
– Accuracy of MFC	± 1% full-scale	± 1% full-scale	± 1% full-scale	± 1% full-scale	± 1% full-scale
For sparger line	Ring sparger, micro- or combi-sparger (O_2, N_2, CO_2, air)				
– Flow rates (lpm)**	(0.025 – 5)	(0.1 – 20)	(0.25 – 50)	(0.5 – 100)	1.0 – 200
For overlay line	(CO_2, air)				
– Flow rates (lpm)**	(0.025 – 5)	(0.1 – 20)	(0.25 – 50)	(0.5 – 100)	1.0 – 200
Softkey to switch air | O_2 between ring and micro-sparger	•	•	•	•	•
Advanced DO controller	•	•	•	•	•
Pump Module					
Max. 6 built-in pumps					
WM314 fixed speed	(1 to 4)	(1 to 4)	(1 to 4)	(1 to 4)	(1 to 4)
WM314 speed-controlled	(0 to 4)	(0 to 4)	(0 to 4)	(0 to 4)	(0 to 4)
External Pumps					
Speed-controlled	(up to 4)	(up to 4)	(up to 4)	(up to 4)	(up to 4)

* Valid for 2 × 3-blade impellers. In case of 1 × 3-blade and 1 × 6-blade impellers, the maximum stirrer speed may be reduced depending on the filling level. At maximum filling level, the stirrer speed is limited to 70 rpm at 1,000 L scale.

** Alternative lower flow ranges are available upon request.

• available – not available ( ) optional, needs to be ordered separately

Process Control Continued

	BIOSTAT STR® 50	BIOSTAT STR® 200	BIOSTAT STR® 500	BIOSTAT STR® 1000	BIOSTAT STR® 2000
Sensors & Measurement					
Temperature probe Pt 100	•	•	•	•	•
– Measurement range	0 – 150 °C 32 – 302 °F	0 – 150 °C 32 – 302 °F	0 – 150 °C 32 – 302 °F	0 – 150 °C 32 – 302 °F	0 – 150 °C 32 – 302 °F
pH, single-use	•	•	•	•	•
– Measurement range	6.0 – 8.0	6.0 – 8.0	6.0 – 8.0	6.0 – 8.0	6.0 – 8.0
– Recalibration function	•	•	•	•	•
pH, electro-chemical, reusable	•	•	•	•	•
– Measurement range	4-10	4-10	4-10	4-10	4-10
DO, single-use	•	•	•	•	•
– Measurement range	0 – 110%	0 – 110%	0 – 110%	0 – 110%	0 – 110%
– Recalibration function	•	•	•	•	•
DO optical or polarographic, reusable	•	•	•	•	•
– Measurement range	0 – 100%	0 – 100%	0 – 100%	0 – 100%	0 – 100%
Load cells	(•)	(•)	•	•	•
Balance substrate	(up to 4)	(up to 4)	(up to 4)	(up to 4)	(up to 4)
BioPAT® ViaMass	•	•	*	*	*
BioPAT® Trace (Glucose | Lactate sensor)	•	•	•	•	•
– Accuracy	0.1 g/L	0.1 g/L	0.1 g/L	0.1 g/L	0.1 g/L
Accessories					
Ladder | Platform	–	–	(•)	(•)	(•)
Filter line IN	•	•	•	•	•
Filter line OUT	•	•	•	•	•
SU exhaust cooler	(•)	(•)	(•)	(•)	–
Holder for conventional probes	(•)	(•)	(•)	(•)	(•)

Communication | Interface of DCU Control Tower

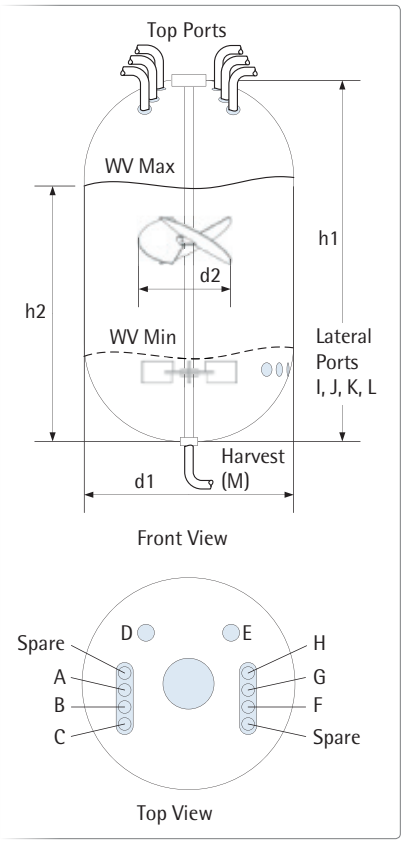
	BIOSTAT STR® 50	BIOSTAT STR® 200	BIOSTAT STR® 500	BIOSTAT STR® 1000	BIOSTAT STR® 2000
RS232	up to 4	up to 4	4	4	5
Industrial Ethernet	•	•	•	•	•
Analog IN	4	4	4	4	4
Analog OUT	4	4	4	4	4
DeltaV™ connectivity	(•)	(•)	(•)	(•)	(•)

• available – not available ( ) optional, needs to be ordered separately * coming soon on Flexsafe STR® 500 | 1000 | 2000 L

Flexsafe STR® Bags

Geometrical Data of Flexsafe STR® Bags

Flexsafe STR®	50 L	200 L	500 L	1,000 L	2,000 L
Total volume [L]	68	280	700	1,300	2,800
Max. working volume [L]	50	200	500	1,000	2,000
Min. working volume [L]	12.5	50	125	250	500
Turndown ratio	1:4	1:4	1:4	1:4	1:4
Bag diameter, d_1 [mm]	370	585	815	997	1,295
Bag height, h_1 [mm]	666	1,055	1,467	1,800	2,330
Ratio, h_1/d_1	1.8	1.8	1.8	1.8	1.8
Liquid height, h_2 [mm]	480	783	1,005	1,360	1,670
Ratio, h_2/d_1	1.3	1.34	1.23	1.36	1.29
Impeller diameter, d_2 [mm]	143	225	310	379	492
Ratio, d_2/d_1	0.38	0.38	0.38	0.38	0.38
Distance between impellers [mm]	186	300	403	493	640
Volume bottom impeller, fully immersed V_1 [L]	12.5	50	125	250	395
Volume top impeller, touches surface V_2 [L]	28	100	245	450	930
Volume top impeller, fully immersed V_3 [L]	32	130	330	610	1,240
Bag packaging dimensions W × D × H (mm | in.)	395 × 395 × 1170 | 15.5 × 15.5 × 46	395 × 595 × 1440 | 15.5 × 23.4 × 56.7	764 × 1153 × 1320 | 30.1 × 45.4 × 52	764 × 1153 × 1440 | 30.1 × 45.4 × 56.7	995 × 1195 × 1715 | 39.2 × 47 × 67.5



Example of Flexsafe STR® Bag

Example of Flexsafe STR® 200 L Basic Configuration

Pos.	Designation	Tubing Size | Material	Tubing Termination	Remarks
A	Overlay aeration	½" × ¾", Si(Pt)	Opta connector	Without spare port
B	Substrate line 1	⅜" × ⅝"	MPC quick coupling	
C	Substrate line 2	⅜" × ⅝"	MPC quick coupling	
D	Sparger aeration	½" × ¾", Si(Pt)	Opta connector	Without spare port
E	Substrate 3-4	¼" × 7/16", C-Flex®*; ⅜" × ⅝", C-Flex®*	MPC quick coupling	2 lines via Y, dip tube
F	Base addition	⅛" × ¼", C-Flex®*	Clave connector	
G	Antifoam addition	¼" × ⅜", C-Flex®*	Clave connector	
H	Gas out (exhaust)	½" ID, Si(Pt)	Opta connector	2 lines via Y
I	Temperature sensor	Si(Pt)	N/A	Reusable sensor (Pt100)
J | K	DO | pH Sensor	N/A	N/A	Optical sensor
L	Small-volume sampling	⅛" × ⅛", Si(Pt)	Clave connector	
M	Bottom-drain harvest	½" × ¾", C-Flex®*	MPC quick coupling	

* C-Flex® is a registered trademark of Saint-Gobain Performance Plastics Corporation.

Ordering Information

Description	Order No.
BIOSTAT STR® Bioreactor System	
System will be configured according to process requirements based on broad choice of configuration options. Please contact your sales representative.	
Flexsafe STR® Single-use Bioreactor Bag	
Standard Design	
Flexsafe STR® 50 L	FRS132668
Flexsafe STR® 200 L	FRS132670
Flexsafe STR® 500 L	FRS124057
Flexsafe STR® 1,000 L	FRS124058
Flexsafe STR® 2,000 L	FRS132367
Customer-specific designs: Please contact your sales representative.	
Bag Tester Fleece*	
For 50 L STR®	DZ050L-S2SIT
For 200 L STR®	DZ200L-S2SIT
For 500 L STR®	DZ500L-S2SIT
For 1,000 L STR®	DZ001K-S2SIT
For 2,000 L STR®	DZ002K-S2SIT
Accessories	
Filter line IN 50 | 200 L	DS200L-SBFLI
Filter line OUT 50 | 200 L	DS200L-SBFLO
Filter line IN 500 | 1,000 L	DS001K-SBFLI
Filter line OUT 500 | 1,000 L	DS001K-SBFLO
Filter line IN 2,000 L	DS002K-SBFLI
Filter line OUT 2,000 L	DS002K-SBFLO
Lab Cart (STR® 2,000)	1ZG---0032
Lifting device & adapter (STR® 2,000)	2ZG---0009
Autoclave tray for re-usable probes	1ZG---0034
Single-Use Exhaust Cooler	
SU exhaust cooler STR® 50 | 200	DS200L---EC
SU exhaust cooler STR® 500 | 1,000	DS001K---EC

* Fleece for old bag holder still available – DZ050L-SBSIT, DZ200L-SBSIT, DZ500L-SBSIT, DZ001K-SBSIT

Fleeces for Gen 2 hardware are now available.

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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.

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.

Our Philosophy is Simple:

We are committed to meeting and exceeding our customers' expectations of value by providing high quality equipment, excellent service, and complete process solutions.

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.

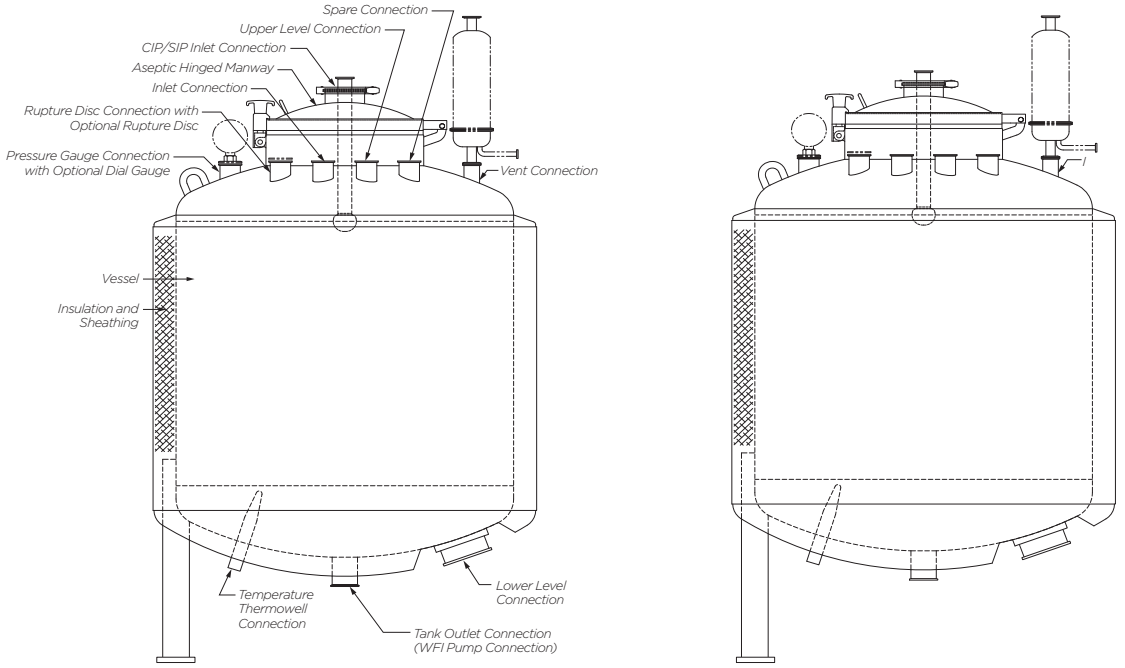
The equipment can be fully tested in our shop at elevation closely meeting the actual utility parameters at the installation site. We can even ship these systems using our own fleet of trucks.

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.

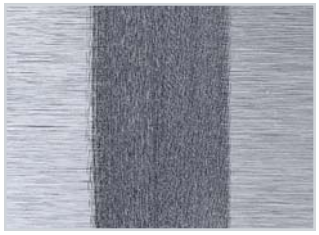
No. 7 Finish. Highly reflective surface obtained with the equivalent of 320 grit abrasive. Minimal grit lines. Used where product contact surfaces are critical. Typically used with a No. 7 weld finish.

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.

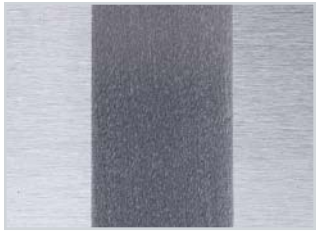
Weld Finishes

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.

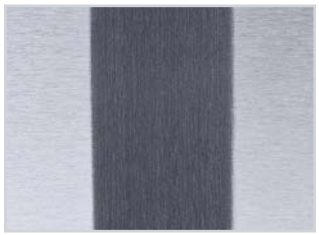


Course Grind (No. 2) Industrial

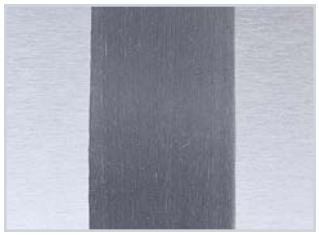
Medium Grind (No. 3)



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.

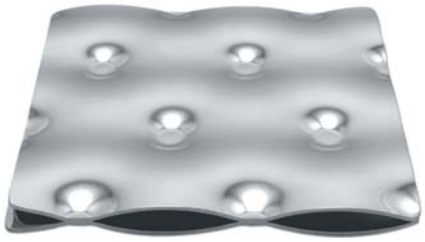
Ultra-Fine Grind (No. 7) 320 Grit. A highly reflective, sanitary surface with minimal grit lines. Normally used to provide excellent product release and cleanability. Use where sanitary product contact surfaces are most critical. Target Ra is 15.

**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

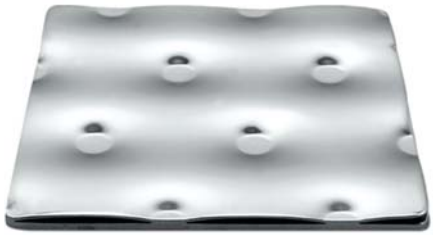
Most commonly utilized in immersion applications, double-embossed Mueller Temp-Plate heat transfer surface helps maximize heating and cooling by using both sides of the heat transfer plate.

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

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www.paulmueller.com • Email: biopharm@paulmueller.com

OPAKFIL 2V

V-Bank Filter

ADVANTAGES

- Fully incinerable
- 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	M6-F7: 200 Pa, F8-F9 300 Pa
Maximum airflow	1,25 x nominal flow
Temperature max	70°C
RH. max	100%
Mounting/Frames	Front and side access housings and frames are available.

Model Name	EN779	ISO16890	Dimensions WxHxD (mm)	Air Flow/pressure drop (m^3/h/Pa)	Media area (m^2)	Weight (kg)	Energy consumption	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%	592x592x268	3400/100	8	3	1359	C	56	56	66	66	87
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	3400/230	8	3		E	83	83	87	87	95
2V9	F9	ePM1 80%	592x490x268	2800/230	7	2,5		E					
2V9	F9	ePM1 80%	592x287x268	1700/230	4	2		E					

Energy Consumption, kWh/year: Calculated according to Eurovent Guideline 4/21-2018
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 m^3/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
Maximum airflow	Nominal flow rate (if not, efficiency drops)
Temperature max	70°C
RH. max	100%
Mounting/Frames	FKB, 4N, CamSafe2

Model Name	EN1822	Dimensions WxHxD (mm)	Air Flow/pressure drop (m^3/h/Pa)	Media area (m^2)	Weight (kg)	Media Type
VGXXL13-762X610X292-M	H13	762x610x292	6000/380	46	14	
VGXL14-610x610x292-M	H14	610x610x292	4000/310	38	11	
VGXXL13-610x610x292-M	H13	610x610x292	5000/380	38	11	
VGXL13-610X610X292-M	H13	610x610x292	4000/250	38	11	
VGXL12-610x610x292-M	E12	610x610x292	4000/250	38	11	
VGXXL11-610x610x292-M	E11	610x610x292	5000/250	33	11	
VGXXL10-610x610x292-M	E10	610x610x292	5000/230	33	11	
VGXL13-595x595x292-M	H13	595x595x292	3200/250	37	11	
VGXL13-305x610x292-M	H13	305x610x292	1500/250	15	5	
VGXL12-305x610x292-M	E12	305x610x292	1500/245	15	5	
VGXXL11-305x610x292-M	E11	305x610x292	2000/250	13	5	
VGXXL10-305x610x292-M	E10	305x610x292	2000/230	13	5	eXtreme Carbon Impregnated
VGXL14-305x610x292-M	H14	305x610x292	1500/310	15	5	
VGXL13-289x595x292-M	H13	289x595x292	1300/250	15	5	

Type M = Gasket on one side

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.

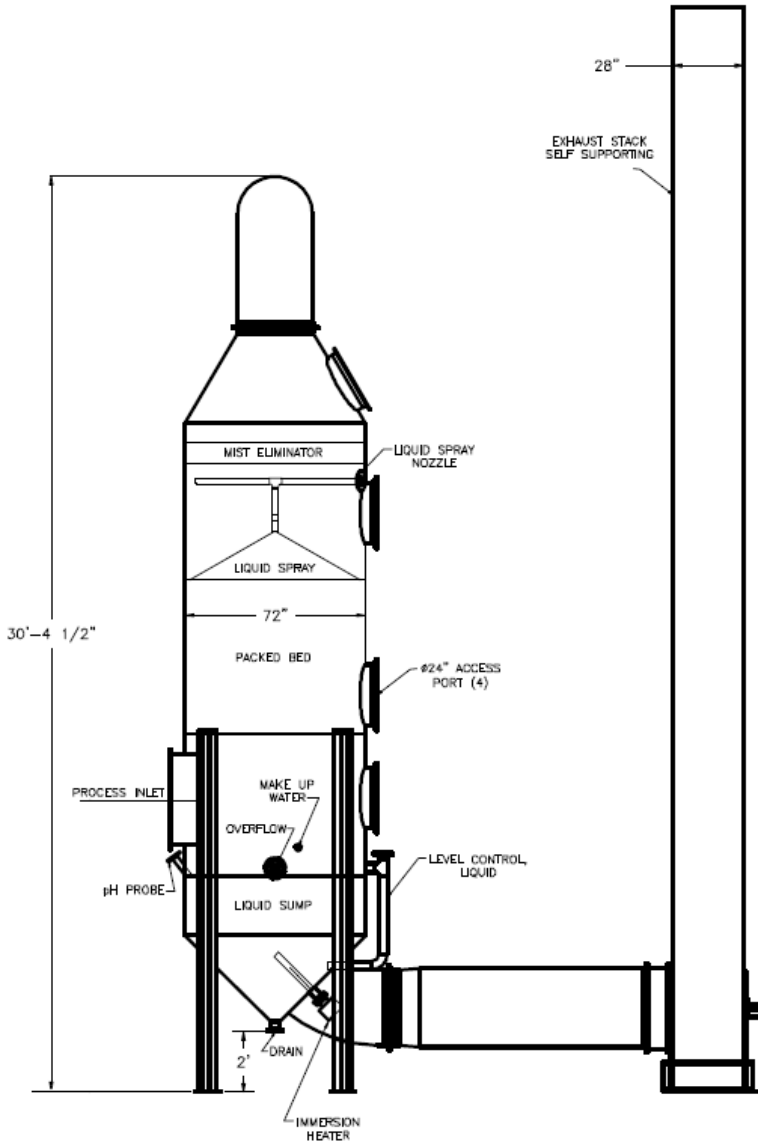
Base System Components

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



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

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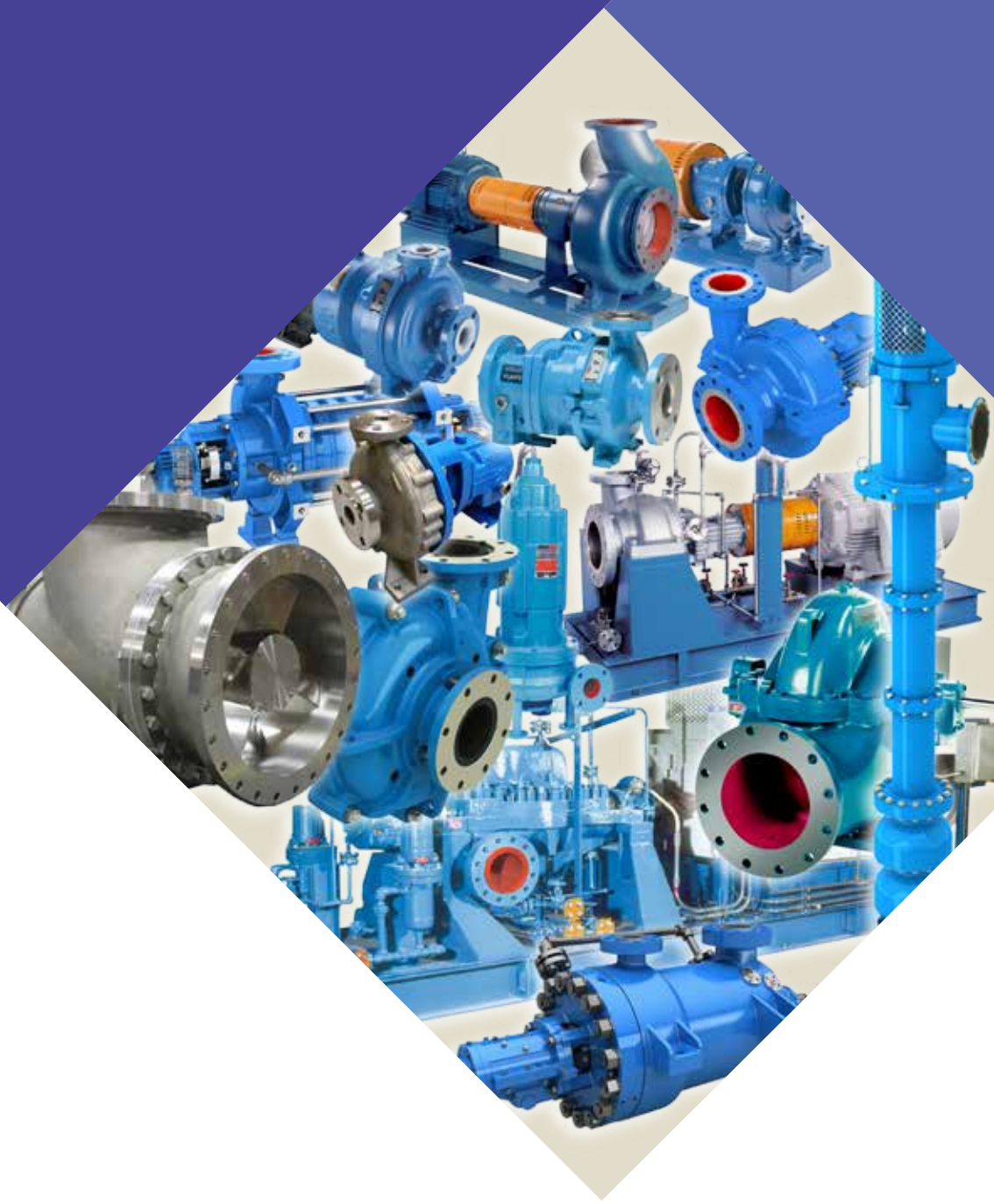
Email: Sales@PollutionSystems.com
Web: www.PollutionSystems.com

GOULDS PUMPS

Pump Selection Guide



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ITT

ENGINEERED FOR LIFE

Pump Selection Guide

Goulds Pumps... Serving the World's Industries

Goulds Pumps presents this 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 3 where the full line of Goulds Pumps is listed by pump type. For more details about your selection, refer to the page indicated. Contact your nearest Goulds Pumps 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 specializes in high alloys for our chemical pumps ranging from 316SS to Zirconium and other special alloys as requested. 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 for users with a preference for a metric pump. Other superior pumps include the 3500XD enhanced performance medium consistency stock pump and a complete line of double suction and LoPulse fan pumps.

Mining and Minerals

Goulds Pumps' 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, potash, soda ash, salt, gold and aggregate industries throughout the world.

Goulds Pumps 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.

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.

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.

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.

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.

Pump Selection Chart

ITT Goulds Pumps 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.

Pump Category	Goulds Model	Pump Type	Chemical	Pulp & Paper	Mining & Minerals	Power Generation	Oil Refining & Gas Processing	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														19
Paper Stock/ Process	3175	Paper Stock/Process														4
	3180/3185	Paper Stock/Process														4
	3181/3186	High Temperature														4
	3500XD	Heavy-Duty Paper Stock														4
Chemical Process	3171	Vertical Sump and Process														5
	CV3171	Non-Clog Vertical Sump Process														5
	NM3171	FRP Vert. Sump/Process														5
	3196	ANSI Chemical Process														6
	LF3196	Low Flow ANSI Process														6
	HT3196	ANSI High-Temperature Process														6
	CV3196	Non-Clog Process														6
	3796	Self-Priming Process														7
	3996	ANSI In-Line Process														7
	3296 EZMAG	ANSI Metallic Sealless Process														7
	NM3196	ANSI FRP Process														8
	3298	ANSI Tefzel® Lined Sealless														8
	SP3298	ANSI Tefzel® Lined Sealless														8
	3198	ANSI PFA Tefzel® Lined Process														8
	V3298	Tefzel® Lined Sealless														8
	3299	ANSI PFA Teflon® Lined Sealless														7
	IC	ISO Chemical Process														9
	ICB	Close-Coupled ISO Process														9
	ICP	High-Temperature ISO Magnetic Drive														10
	ICM	ISO Metallic Magnetic Drive														9
	ICMB	Close-Coupled ISO Sealless														9
	ICMP	High-Temperature ISO Magnetic Drive														10
API 610 ISO 13709	API 3171	Industrial Duty Vertical Sump														11
	3700/3710	1-Stage, Overhung (OH2)														11
	3910	Vertical In-Line (OH3)														11
	3610	Axially Split, 1-Stage (BB1)														10
	3620	Radially Split, 1-Stage (BB2)														10
	3640	Radially Split, 2-Stage (BB2)														10
	3600	Axially Split, Multistage (BB3)														11
	7200CB	Barrel Multistage (BB5)														11
Sump/ Abrasives/ Solids Handling	Trash Hog	Solids Handling, Self-Priming														12
	VHS VJC	Vertical Cantilever														12
																12
	HSU HSUL JCU	Submersible														12
																12
																12
	VRS	Abrasive Slurry R.L. Cantilever														14
Abrasives Slurry/Solids Handling	JC	Medium-Duty Abrasive Slurry														13
	SRL	Rubber-Lined Abrasive Slurry														14
	SRL-C	Rubber-Lined Abrasive Slurry														14
	SRL-S	Rubber-Lined Abrasive Slurry														14
	SRL-XT	Rubber-Lined Abrasive Slurry														14
	5500	Severe Duty Abrasive Slurry														13
	HS	Non-Clog Solids Handling														13
	VRS	Abrasive Slurry R.L. Cantilever														15
	XHD	Severe Duty Slurry														13
Multistage/ Axial Flow/ Double Suction	AF	Axial Flow														16
	3311	High-Pressure Multistage														16
	3393	High-Pressure Multistage														14
	3935	Diffuser-Type Multistage														15
	3400 Series	Single Stage, Double Suction														15/16
	3355	Multistage														15
	3316	Two-Stage														14
Vertical Mixed and Axial Flow	WCAX-GP	Wet Pit Pumps														17
	YDD-GP															17
	WCA-GP															17
	WCB-GP															17
	WCC-GP															17
	WCE-GP															17
	WCL-GP															17
	WMCC-GP															17
	WMCE-GP															17
	WCAG-GP															17
	VIC	Vertical Turbine/Can Type														17
	VIT	Vertical Industrial Turbine														17
	VIS	Vertical Submersible														18
	VMP	Vertical Marine														18

* TEFZEL® and TEFLON® are registered trademarks for fluoropolymer resins, films and fibers made by DuPont.

Ideally Suited for Service Indicated

Process Pumps

Model 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* 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

Materials: All Iron / 316SS Trim, 316SS, 317SS, CD4MCu

Model 3500XD
Medium Consistency Systems

Thick stock pulp is pumped with the model 3500XD enhanced performance medium consistency pumping system. System includes engineered standpipe, control valve, dilution system and level transmitter. A patented air separation device removes air from the pulp to improve mixing effectiveness. Bleaching chemicals and oxygen are mixed in-line with the Model 3501 mixer with Double Shear™ rotor, optimized injection port, and unique low pressure drop casing design.

- Consistencies from 8% to 16%
- Capacities to 900 m³/h | 4,000 GPM
- Pressures to 22 bar | 325 PSIG

Applications:

- O^2 Delignification Reactor
- D Stage Feed Pumping
- EOP Stage Pumping
- High Density Tower

Materials: From 316SS to Titanium

Model 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* condition monitoring as standard.

3181 with ANSI flanges

- Capacities to 3000 m 3 /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 m 3 /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

Model 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* 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

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: All Iron / 316SS Trim, 316SS, 317SS, CD4MCu

Vertical Sump & Process

Model 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

Model 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

Model 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/hr |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.

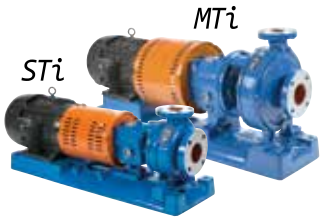
See page 3 table for list of eight color-coded market designations.

ANSI Process Pumps

Model 3196
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

Model HT 3196
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

Model LF 3196
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

Model CV 3196
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

ANSI/Sealless Process Pumps

Model 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

Model 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

Sealless Process Pumps

Model 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/hr | 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

Model 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

See page 3 table for list of eight color-coded market designations.

Sealless Process Pumps

Model 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/hr | 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: Tefzel® (ETFE)

Model 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: Tefzel® (ETFE)

Model 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: Tefzel® (ETFE) Construction

Sealed Lined & Non-Metallic

Model 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* 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: PFA Teflon®

Model 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

ISO Process Pumps

Sealed

Model IC
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

Model 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

Sealless

Model 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

Model 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

See page 3 table for list of eight color-coded market designations.

ISO/API Process Pumps

Sealed

Model 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

Sealless

Model 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

API 610 Process Pumps

Model 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/hr | 50,000 GPM
- Heads to 215 m | 700 ft
- Temperatures to 150° C | 300° F - optional 205°C | 400°F
- Pressures to 21 bar | 300 PSIG - optional 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

Models 3620 and 3640 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

API 610 Process Pumps

Model 3600 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/hr | 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

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

Model 3700 & 3710 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

Model 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.

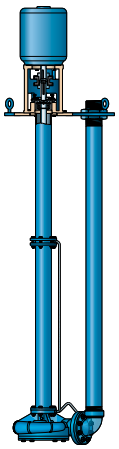
- Capacities to 1,360 m³/h | 6,000 GPM
- Heads to 230 m | 750 ft
- Temperatures to 340° C | 650°F
- Pressures to 42 bar | 600 PSIG

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

Model 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

See page 3 table for list of eight color-coded market designations.

Sump / Abrasives / Solids Handling

Model 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, CD4MCu, 316SS

Trash Hog®
Solids Handling Self-Priming
Goulds Trash Hog is designed for superior solids handling capability, optimum pump performance, and extreme ease of maintenance for a wide range of industrial, pulp & paper, mining, and wastewater services. Whether handling sludge, debris or plant wastes, there's no other pump that compares to the Trash Hog.

- Capacities to 1,363 m³/h | 6,000 GPM
- Heads to 43 m | 140 ft
- Temperatures to 107° C | 225° F
- Pressures to 6 bar | 85 PSIG
- Suction Lifts to 7.6 m | 25 ft
- Spherical solids to 76 mm | 3 inches

Applications:

- Pulp & Paper Industry – Black Liquor Sump, Paper Machine Floor Sump, Knotters Discharge Pump, White Water Service
- General Industry – Wash Down Sump, Food Wastes, Fish Farming, Rendering Wastes, Machine Coolant Sump
- Mining & Metal Fabrication – Mine Dewatering, Mill Scale Runoff, Cutting Oil Transfer, Construction Site Dewatering

Materials: Cast Iron, Stainless Steel, CD4MCu, High Chrome Iron Fitted

Models 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

Abrasives / Solids Handling

Model 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

Model 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, CD4MCu, Endura Chrome

Model 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, CD4MCu

Model 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, CD4MCu, Endura Chrome

See page 3 table for list of eight color-coded market designations.

Abrasives / Solids Handling
Multistage / Axial Flow / Double Suciton

Models 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

Model 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

Multistage / Axial Flow / Double Suction

Model 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.

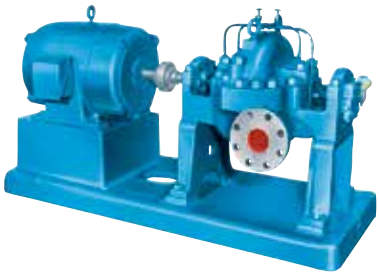
- Capacities to 750 m³/h | 3,300 GPM
- Heads to 1,000 m | 3,300 ft
- Temperatures to 177° C | 350° F
- Pressures to 114 bar | 1,650 PSIG

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

Model 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, Bronze, 316SS

Multistage / Axial Flow / Double Suction

Model 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

Model 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: Cast Iron, Stainless Steel, Stainless Fitted

Goulds Model 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, All Bronze,
Cast Iron / Stainless Steel, All Stainless Steel (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, All Bronze,
Cast Iron / Stainless Steel, All Stainless Steel (1724 kPa)

See page 3 table for list of eight color-coded market designations.

Multistage / Axial Flow / Double Suction

Goulds Model 3420
Large Capacity

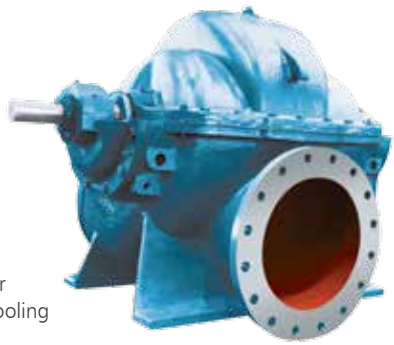
- Capacities to 14,762 m³/h | 65,000 GPM
- Heads to 122 m | 400 ft
- Temperatures to 135°C | 275°F
- Working Pressures to 1379 kPa | 200 PSIG

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, All Bronze,
Cast Iron / Stainless Steel, All Stainless Steel (1724 kPa)

Goulds Model 3498
Extra Large Capacity



- Capacities to 51,098 m³/h | 225,000 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, All Bronze,
Cast Iron / Stainless Steel, All Stainless Steel (1724 kPa)

Model 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.

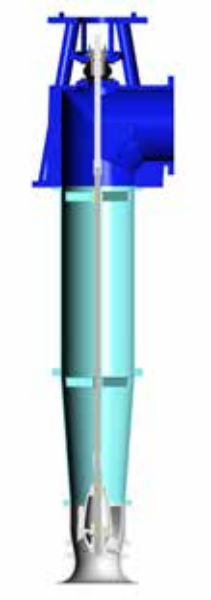
- Capacities to 68,000 m³/h | 300,000 GPM
- Heads to 9.2 m | 30 ft
- Temperatures to 176° C | 350° F
- Available in cast iron, austenitic stainless steels, duplex alloys, nickel, nickel-chrome alloys, nickel-chrome-moly alloys, titanium and other alloys as required for the service
- Available in 6 - 66 inch sizes (larger sizes on application)

Materials: Cast Iron, 304SS, 316SS, CD4MCu, Nickel, Monel, Alloy 20, UHB-904L, Titanium, Hastelloy, Sanicro 28

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

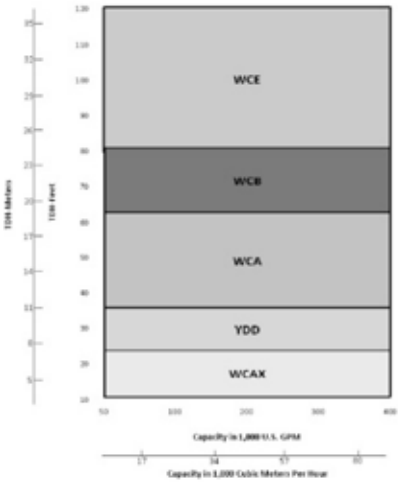
Vertical Mixed & Axial Flow

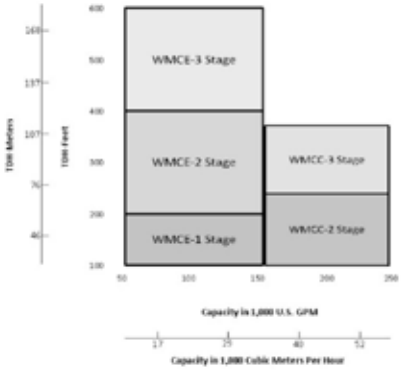


Models WCAX-GP, YDD-GP, WCA-GP, WCB-GP, WCC-GP, WCE-GP, WCL-GP, WMCC-GP, WMCE-GP, WCAG-GP

Vertical Mixed & Axial Flow

Custom designed for maximum reliability and high efficiency.





Materials: Bronze Fitted, All Bronze, SS Fitted, Ni Resist, All SS

Model VIC
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 m^3/Hr | 70,000 GPM
- Heads to 1,067 m | 3,500 ft
- Pressures to 176 kg/cm^2 | 2,500 psi
- Bowl sizes from 152.4 mm to 1,400 mm | 6" to 55"
- Temperatures to 260°C | 500° 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

Model VIT
Vertical Pumps

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 m^3/Hr |70,000 GPM
- Heads to 1,067 m | 3,500 ft
- Pressures to 176 kg/cm^2 | 2,500 psi
- Bowl sizes from 152.4 mm to 1,400 mm| 6" to 55"
- Temperatures to 260°C | 500° 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

See page 3 table for list of eight color-coded market designations.

Multistage / Axial Flow / Double Suction

Model 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 m^3/hr | 70,000 GPM
- Heads to 1,067m | 3,500 ft
- Pressures to 176 kg/cm^2 | 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

Model 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 m^3/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

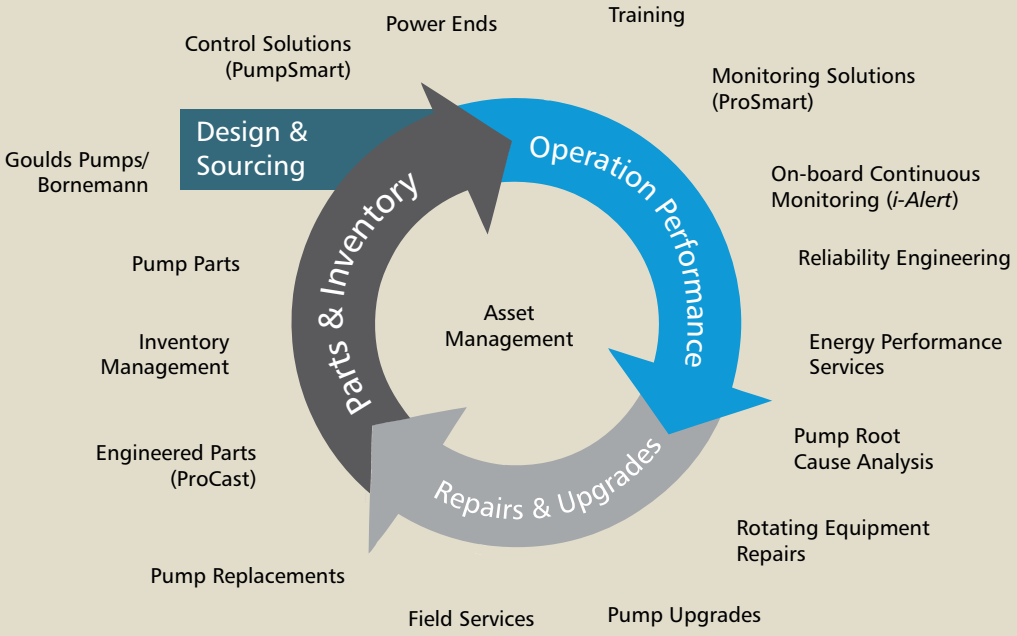
See page 3 table for list of eight color-coded market designations.

PRO services

Reliability has no quitting time.

Building on over 160 years of Goulds Pumps experience, **PRO Services** provides an array of services focused on reducing equipment total cost of ownership (TCO) and increasing plant output, including predictive monitoring, maintenance contracts, field service, engineered upgrades, inventory management, and overhauls for pumps and other rotating equipment. www.ittproservices.com

Your total Solution For Equipment Life Cycle Optimization







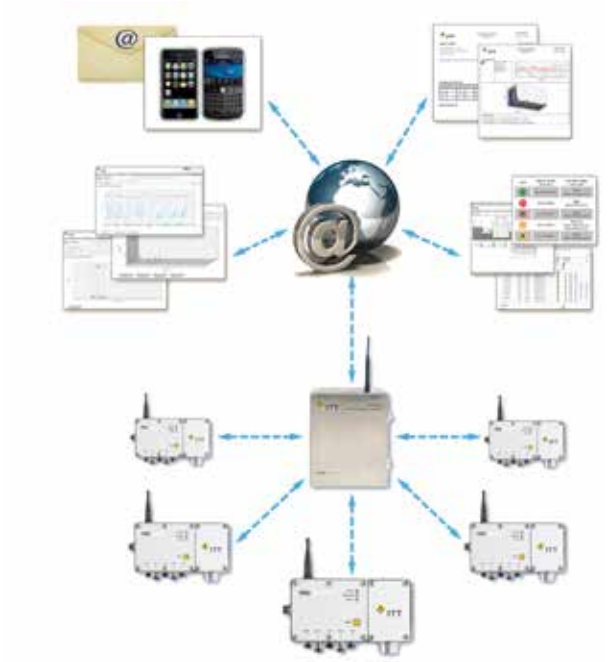
ProSmart®
Predictive Condition Monitoring

Our ProSmart systems provide continuos, predictive monitoring for all of your rotating equipment at an exceptionally low price. With ProSmart, the focus of your Predictive Maintenance Program can change from data collection to analysis and improvement activities. In addition, by continuosly monitoring your equipment, ProSmart can proactively warn you of on setting machinery problems.

Features

- **Minimizing Process Downtime**
  Early warning and advanced diagnostics enable maintenance activities to be planned instead of reactive
- **Advanced Diagnostic Tools**
  Tying together both machinery health and process conditions, ProSmart speeds your root cause diagnosis.
- **Automatic Notification of Machinery Issues**
  Resources focus only on machines in need, maximizing productivity.
- **Continuous Monitoring of Machine Health**
  Automated data collection and analysis every 5 seconds; saving you time from routine data collection.

How it Works



Web application
Elminates software installation and management costs.

Hosted Interface
The ProNet user interface provides the ability to view, analyze, and store data in a secure environment anywhere in the world.

With online reports that range from supervisory overviews to detailed analysis, ProSmart provides benefits to each level of your organization.

Wireless Architecture
Reduces installation costs and complexity.

Communication Module
As the gateway to the Internet, ProSmart CM provides a secure connection to the ProNet application via LAN, DSL, cellular, or 802.11 wireless routers.

Data Monitor
Integrated processing capabilities allow 155 channels of information to be collected every 5 seconds, 24/7/365.

Machine Level
ProSmart can be used to provide continuous machinery monitoring of all your rotating equipment. Standard process signals can be integrated for greater diagnostic capabilities.

i-ALERT®2

Bluetooth® SMART Equipment Health Monitor

The i-*ALERT*®*2* Equipment Health Monitor is a Bluetooth Smart-enabled condition monitor that allows customers to identify potential problems before they become costly failures. It tracks vibration, temperature and run-time hours and wirelessly syncs that data with a smartphone or tablet through the i-*ALERT2* mobile app.

- Wirelessly sync real-time and historical data directly to smart mobile device with the i-ALERT2 Mobile App.
- Scan multiple machines at the same time from a safe distance up to 30 meters/100 feet.
- Rugged, Safe & Reliable; IP67 water & dust resistant
- Rated for any industrial environment; Intrinsically Safe, Class 1 Div. 1 ATEX Zone 0

Monitor
Track vibration, temperature, & run-time hours 24/7/365.

Alarm
Checks every five minutes & alarms if equipment is outside normal operating parameters.

Trend
Stores data once per hour & on alarm for 30 days. Stores the weekly average, minimum & maximum up to 5 years.

Analyze
Diagnose machine faults with vibration tools Fast Fourier Transform & Time Waveform analysis.

PumpSmart®
Control Solutions

The award-winning PumpSmart is an intelligent flow system that works with any pump, utilizing our smart VFD controller and our proprietary control software to provide advanced process control, enhanced reliability through failure prevention, reduced life cycle costs and significantly lower energy costs - up to 65%. PumpSmart Performance Services can assess your complete pumping systems, units or plant to identify all the ways you can improve your pumping life-cycle costs; from process control strategies to system design to maintenance practices.

Features (LV)

- 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 sychronized 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 (MV)

- Pump Protection & Predictive Monitoring
  Takes intelligent control of your pumping system to ensure it operates within the parameters neede for maximized output PLUS it can also prevent 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 of pumping systems.

  Provides better operation and less down-time due to process pump visibility.

  Patented logic can improve overall system visibility and predictive monitoring.

PumpSmart®
Engineered Solutions

Features

- 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.lenntech.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 has 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' 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 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 _____

ITT
ENGINEERED FOR LIFE

Member of Hydraulic Institute

B.PSG.en-US.2015-09

STNX decanter centrifuge

High-performance decanter centrifuge for starch production

Applications

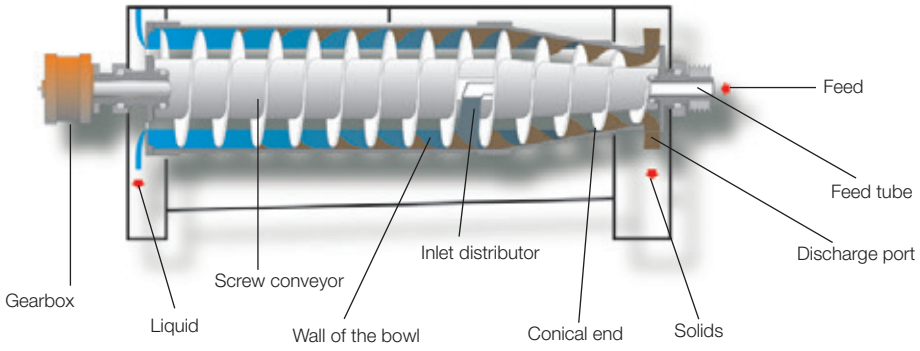
Alfa Laval STNX decanter centrifuges are used for a wide range of different starch processing operations. These include dewatering of heat-coagulated potato protein and maize gluten, 2-phase and 3-phase wheat starch and protein splitting, and fruit juice separation and fibre dewatering in the potato and tapioca starch industries.
The STNX range provides the starch industry with the most cost-effective, high-performance solution, with the lowest power consumption and life cycle costs available.

Design

Alfa Laval designed the STNX range of decanter centrifuges with a focus on performance, easy access, reliability and low noise levels.
The rotating assembly is supported on a compact welded box beam frame with main bearings at both ends. The in-line motor is flanged or foot-mounted on the decanter with adjustable brackets for belt tension adjustment. The bowl is driven at the conical end by an electric motor using a V-belt transmission.
The bowl, conveyor, casing, inlet tube, outlets and other parts that come in contact with the process media are made of AISI 316 and duplex stainless steel.

Operating principles

Separation takes place in a horizontal cylindrical bowl equipped with a screw conveyor. The product is fed into the bowl through a stationary inlet tube and is then smoothly accelerated by an inlet rotor. Centrifugal force immediately makes the solids build up on the wall of the bowl.
The conveyor rotates in the same direction as the bowl, but at a different speed, thus moving the solids towards the conical end of the bowl. The solids leave the bowl through the solids discharge openings into the casing.
Separation takes place throughout the entire length of the cylindrical part of the bowl, and the clarified liquid leaves the bowl by flowing over adjustable plate dams into the casing.



Direct Drive

Direct Drive is a unique system developed by Alfa Laval for automatic control of the differential speed between the bowl and the conveyor. This makes it easy to maintain the best possible balance between liquid clarity and solids dryness, irrespective of variations in the feed.

Direct Drive comprises a new type of gearbox and variable frequency drive, which do not expose the bowl drive to parasitic braking power loss. The electrical installation is straightforward, power consumption is kept to a minimum, and accurate control is achieved within a wide range of differentials.

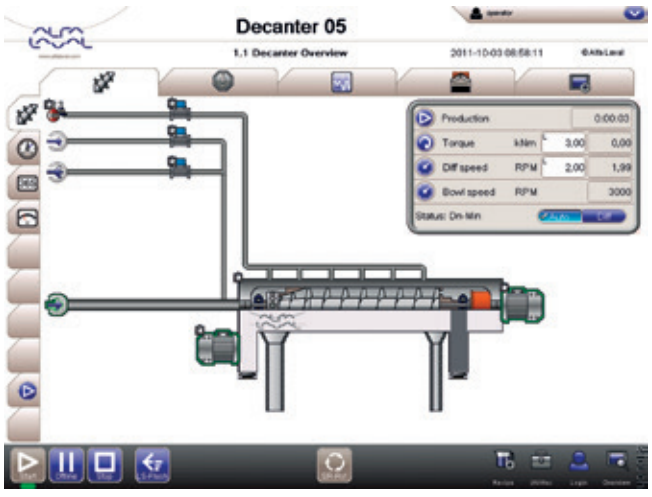
2Touch controls add value

Every STNX decanter centrifuge is equipped with a pre-installed, factory-tested 2Touch control package. The system is designed for SCADA/DCS system integration.

The combination of 2Touch control system and STNX separation technology ensures that you get the most out of any STNX installation, while keeping installation, commissioning, operation and maintenance costs to a minimum.

The system monitors temperature and vibration and also features adaptive controls, which allow intelligent and smooth adjustment of the torque and speed of the conveyor.

Additional enhancement packages are available for the 2Touch control package. These include:



- Automatic clean out in the event of a blackout or complete power failure
- Maintenance and training aids, including manuals in PDF format and videos about routine maintenance procedures
- Process module for continual polymer regulation and dosing optimization
- Remote monitoring, response and reporting

Process optimization

The STNX decanter centrifuge can be adjusted to suit specific requirements by varying the

- bowl speed to obtain the required G-force for optimized separation
- conveying speed to optimize the balance between liquid clarity and solids dryness
- pond depth in the bowl to optimize the balance between liquid clarity and solids dryness
- feed flow – the STNX design is capable of handling a wide range of flow rates.

Power consumption becomes power reduction

The bowl can be equipped with specially developed power plates or tubes that harness and exploit hydraulic energy to reduce power consumption still further.

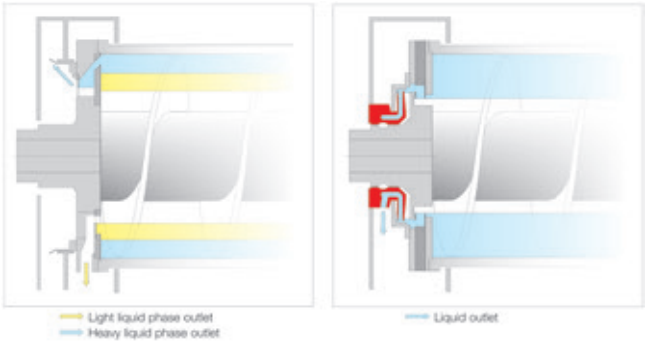
Some of the discharge velocity from the centrate is captured and re-directed by these patented power plates in order to contribute to the bowl rotation. This results in a reduction in the velocity of the discharged liquid, which in turn reduces overall power requirements by 15-20%.

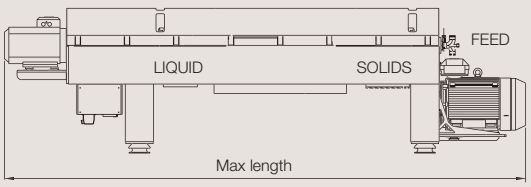
Reduced power consumption supports sustainable production and helps you live up to new environmental regulations – such as such as reductions in allowable CO_2 emissions.

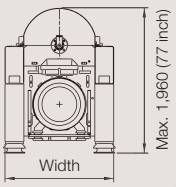
Options

Alfa Laval STNX decanter centrifuges are available with a range of unique design options. These include a paring disc to minimize foam, plough tiles to improve process performance and reduce operational conveyor torques, pulp wash facilities, process rinse features and full cleaning-in-place (CIP), as well as FDA-approved elastomers and seals.

All STNX decanter centrifuges are available in 3-phase versions suitable for the splitting process. They are ATEX-compliant and are also available for zones 1, 2 and 22.

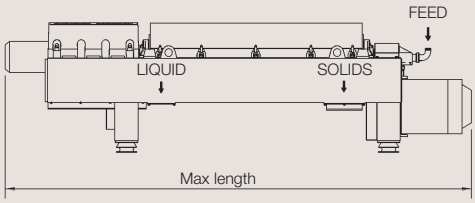


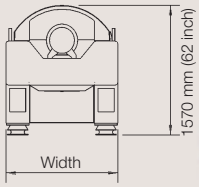




STNX 3651	4300 mm (168 inch)
STNX 4451	5020 mm (198 inch)
STNX 6541	6452 mm (255 inch)
STNX 7241	6900 mm (272 inch)

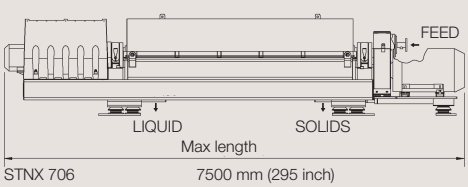
990 mm (39 inch)
1060 mm (42 inch)
1450 mm (57 inch)
1510 mm (60 inch)

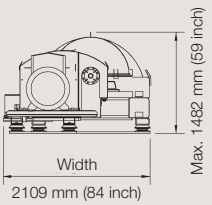




STNX 912	3013 mm (119 inch)
STNX 438	4735 mm (187 inch)
STNX 944	6000 mm (236 inch)

785 mm (31 inch)
1190 mm (47 inch)
1300 mm (51 inch)





STNX 706	7500 mm (295 inch)

2109 mm (84 inch)

Technical specifications

Design	Bowl Diameter (mm)	Max. Bowl speed (rpm)	Max. G-force	Weight (kg)	Installed power (KW)	Sound Pressure Level[1] (dB(A) re. 20µPa)
STNX 912	280 (11 inch)	4400	3030	1500 (3300 lbs)	18.5-26 (25-35 hp)	81
STNX 3651	360 (14 inch)	4200	3550	2300 (5071 lbs)	22-48 (35-70 hp)	82
STNX 4451	440 (17 inch)	3800	3551	3200 (7040 lbs)	15-66 (20-80 hp)	81
STNX 438	480 (19 inch)	3650	3574	5000 (11000 lbs)	44.5-90 (70-120 hp)	85
STNX 944	575 (23 inch)	2900	2703	7000 (15400 lbs)	75-160 (100- 200 hp)	86
STNX 6541	650 (25 inch)	3100	3491	6500 (14300 lbs)	83-205 (109-273 hp)	83
STNX 7241	720 (28 inch)	2900	3384	8600 (18959 lbs)	100-296 (134-395 hp)	84
STNX 706	740 (29 inch)	2800	3243	13000 (28860 lbs)	132-250 (150-300 hp)	89

[1]Declared A-weighted emission sound pressure level in free field over a reflecting plane at 1 m. distance from the decanter operating at maximum bowl speed, tested with water and closed outlet.

How to contact Alfa Laval
Up-to-date Alfa Laval contact details for all countries are always available on our website at www.alfalaval.com

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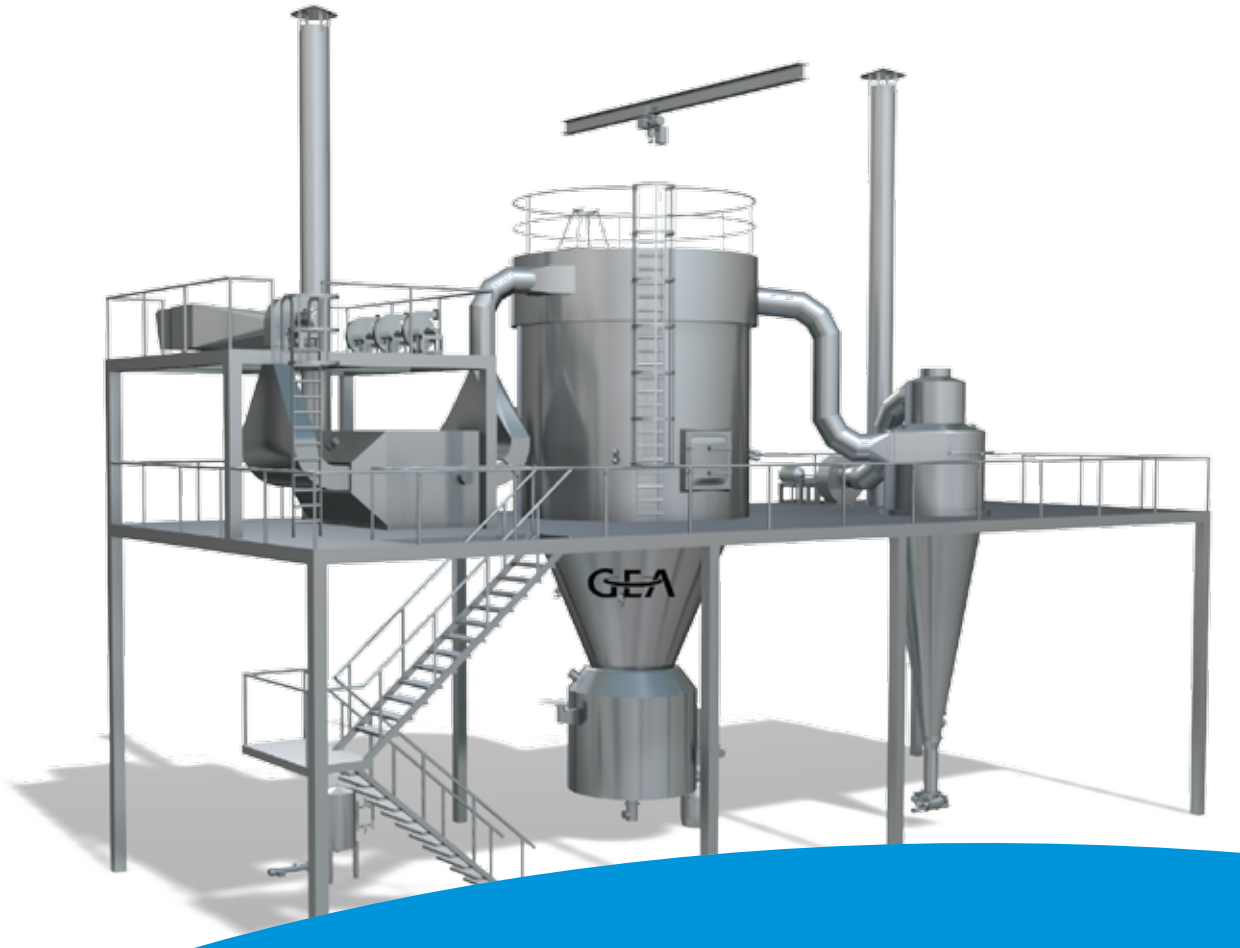


VERSATILE-SD®
Versatile Spray Dryer, FSD®

Designed for food

engineering for
a better world

Expedite your time to market

GEA offers a range of spray dryers designed specifically for R&D, product development and small-volume production. As a pioneer in all aspects of spray drying, with more than 10,000 contracted and installed plants worldwide, we can help you to choose the most suitable equipment, assess each project on its individual needs, and tailor both the process and the spray dryer to match your specific requirements.

However, we also understand that not every plant needs to be custom built. That is why we have designed standard spray drying units that are flexible, compact and easy to install.

To optimize your process development projects and shorten your time to market, the VERSATILE-SD® (VSD) and the Versatile Spray Dryer, FSD® (VFSD) have been designed to fast-track product development without compromising powder quality. These units are perfect for both large and small companies that wish to launch a new food concept or produce small batches of high-value products, saving time and cost on the design, purchase and installation of the spray drying unit.

Flexible, safe and easy to clean

Not only are the VSD and VFSD cost-effective, compact and easy to install, they are also extremely versatile. Five sizes with five standard configurations and different exhaust systems provide the flexibility needed to produce different foods, flavors and other products at a variety of production rates.

Being modular, these standard units can be quickly reconfigured or expanded, offering fast turnaround times between product changeovers. As with all GEA equipment, the VSD and VFSD are equipped with the ultimate in hygiene, safety and monitoring features, including optional clean-in-place (CIP) functionality.

Consistent and reliable

With quality control being a key concern, the VSD and VFSD have been developed to be reliable and consistent in delivering products of the desired quality. The control system and HMI (interface) allow specific product and parameter settings to provide both repeatability and easy operation.

More than just a spray dryer

We help our customers to configure the right product recovery system and develop the right process for their product, assessing factors such as organoleptic properties and powder functionality. We can also advise on plant configuration, auxiliary equipment and environmental aspects.

Spray dryers can also be used for spray congealing applications.

GEA test centers are available for process development, tests and trial runs.

Versatile Spray Dryer, VERSATILE-SD®

The VSD spray dryer processes single-particle powder products. The flexible atomization system has a wide range of functions that atomize and convert a liquid feed into a fine powder according to your specifications.

Key benefits

- Ready-to-use standard units for easy product selection, delivery and installation
- Available with five different exhaust systems
- Available in five different sizes with water evaporation capacities from 6 to 320 kg/h
- Easy to operate, clean and switch between batches
- Handles food products at low air inlet temperatures, producing a fine powder
- Explosion protection system
- Optional features include CIP, electrical air heat booster, etc.

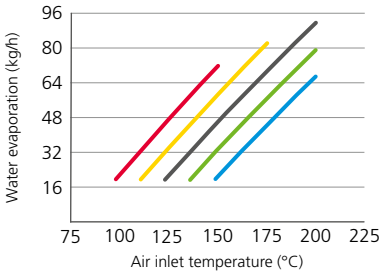
All VSD configurations include the following: an indirect gas heater, a rotary atomizer, a swan-neck chamber and an exhaust system with the following options: cyclone, bag filter, cyclone plus cyclone, cyclone plus bag filter or cyclone plus scrubber.

The VSD is available in five different sizes ranging from a VERSATILE-SD® size 6.3 to a VERSATILE-SD® size 80. We can also offer smaller-scale units such as our trusted MOBILE MINOR® and PRODUCTION MINOR®.

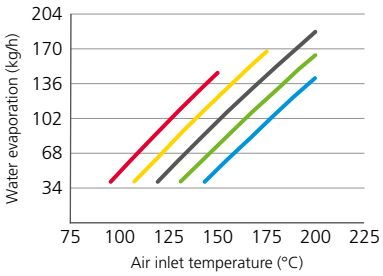
VERSATILE-SD®

Size	VSD-25	VSD-50	VSD-80
Water evaporation capacity (kg/h)	20-90	40-185	60-320
Typical mean particle size (µm)	20-100	20-100	20-100
Space requirements L x W x H (m)	13 x 6 x 11	14.5 x 6.7 x 12.5	19 x 7.5 x 14.5

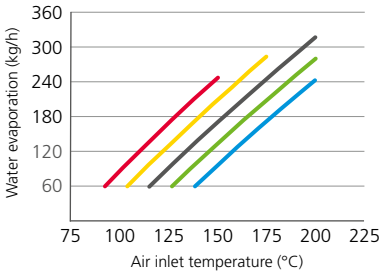
VSD-25 co-current atomization



VSD-50 co-current atomization



VSD-80 co-current atomization



*VSDs can be configured for temperatures above 200 °C

Air outlet temperature

- 70 °C
- 80 °C
- 90 °C
- 100 °C
- 110 °C

Versatile Spray Dryer, FSD®

The VFSD spray drying plant is a multistage dryer with an integrated fluid bed under the drying chamber. By combining spray drying and fluid bed technology it provides the same performance as a dryer with external fluid beds – but with a much smaller footprint.

The VFSD dries product into larger agglomerated particles. Drying and agglomeration take place in the same chamber producing coarse, dustless, free-flowing powders. This technology is particularly appropriate for sticky, hygroscopic and/or heat-sensitive food applications.

Agglomeration improves the powder's ability to flow and disperse without forming lumps. The fluid bed process can also be used to improve a powder's bulk density or particle size to make a more homogeneous product with a lower segregation tendency.

Key benefits

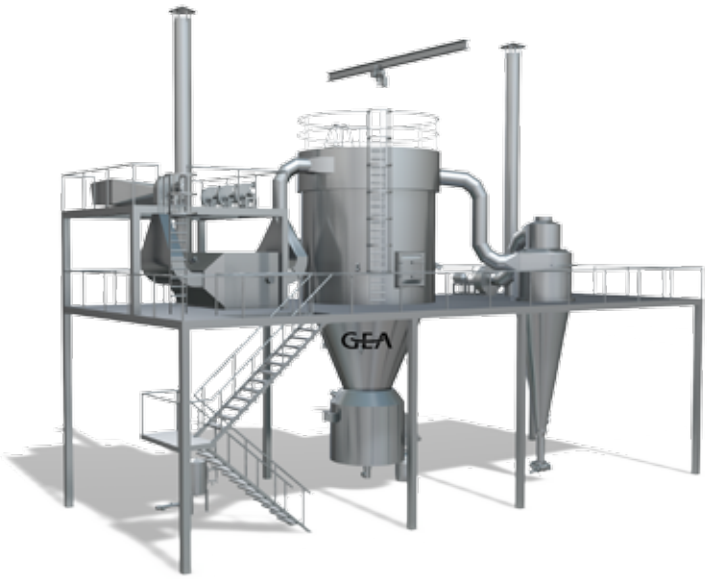
- Ready-to-use standard units for easy product selection, delivery and installation
- Available with five different exhaust systems
- Available in six different sizes with water evaporation capacities from 4 to 265 kg/h
- Easy to operate, clean and switch between batches
- Handles foodstuffs to produce large agglomerated powder particles
- Explosion protection system
- Optional features include CIP, electrical air heat booster, etc.

Versatile Spray Dryer, FSD®

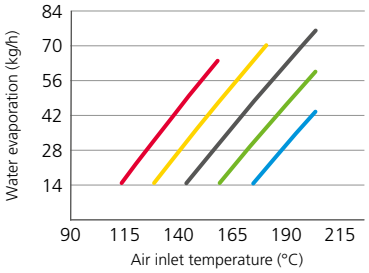
Size	VFSD-25	VFSD-50	VFSD-80
Water evaporation capacity (kg/h)	15-77	30-165	40-265
Typical mean particle size (µm)	100-250	100-250	100-250
Space requirements L x W x H (m)	13.5 x 6 x 11	16 x 6.7 x 12	19 x 7.5 x 13.1

All VFSD configurations include the following: an indirect gas heater, pressure nozzle atomization, a chamber with an integrated 'wraparound' static fluid bed for gentle drying and cooling prior to powder discharge and bagging, and an exhaust system with the following options: cyclone, bag filter, cyclone plus bag filter, cyclone plus cyclone or cyclone plus scrubber.

The VFSD is available in six different sizes ranging from a versatile FSD® size 4.0 to a FSD® size 80. We also offer the smaller-scale FSD MINOR® for R&D purposes.



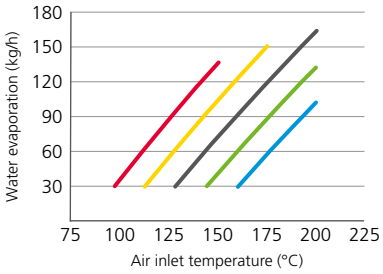
VFSD-25 co-current atomization



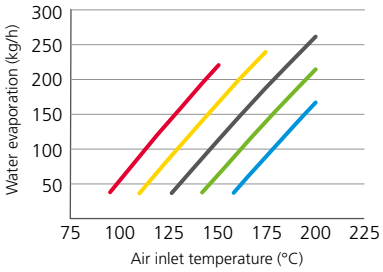
Air outlet temperature

- 70 °C
- 80 °C
- 90 °C
- 100 °C
- 110 °C

VFSD-50 co-current atomization



VFSD-80 co-current atomization



*VFSDs can be configured for temperatures above 200 °C

engineering for
a better world

We live our values.

Excellence • Passion • Integrity • Responsibility • GEA-versity

GEA is a global technology company with multi-billion euro sales operations in more than 50 countries. Founded in 1881 the company is one of the largest providers of innovative equipment and process technology. GEA is listed in the STOXX® Europe 600 Index. In addition, the company is included in selected MSCI Global Sustainability Indexes.

GEA Denmark
GEA Process Engineering A/S
Gladsaxevej 305
DK-2860 Soeborg
Tel +45 39 54 54 54
Fax +45 39 54 58 00
gea.com/contact
gea.com/food

SOLIDS DISCHARGING EQUIPMENT

BA
Bin Activators

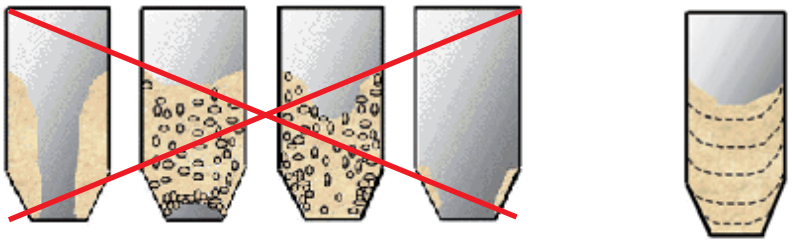
WAMGROUP

EXTRAC®

BA
Bin Activators
SOLIDS DISCHARGING EQUIPMENT

The BA Bin Activator is a discharging device which, thanks to controlled vibration, ensures continuous down-flow of the material from silos and hoppers.

Using this device ensures product extraction with optimum “Mass Flow”.



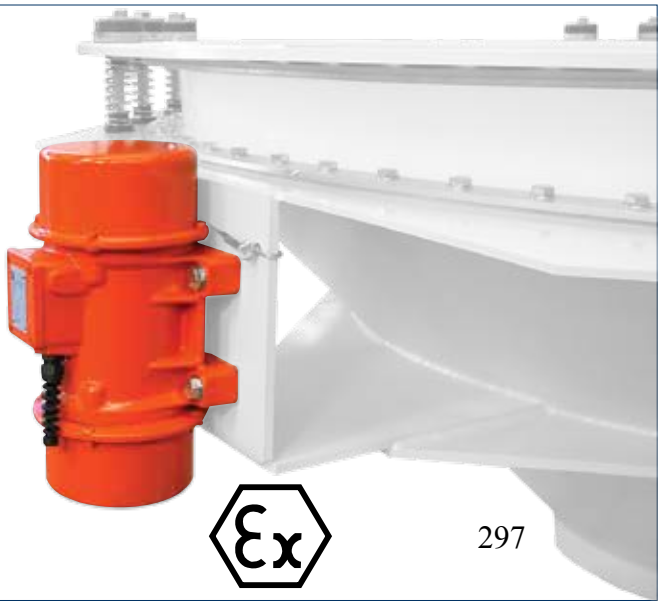
Ratholing *Bridging* *Segregation* *Incomplete emptying* *Mass Flow*

- Ensures uniform descent of the product (MASS FLOW) inside the silo
- Avoids bridging in the discharge cone
- Prevents “ratholing” and segregation of the material to be discharged
- Stops dangerous flushing
- Prevents economic loss due to plant downtimes

- Turned cone constructed using industrial techniques
- Seamless discharging cone
- High flowability
- Maximum resistance to stress
- Available with food-grade painting and in 304L SS

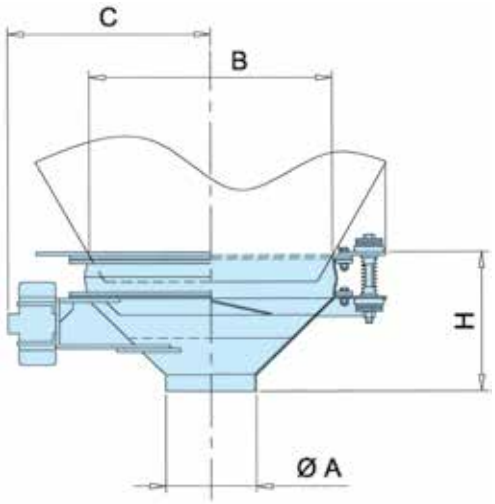
- Suspensions pivoting at 360°
- Maximum efficiency
- Minimum vibration energy consumption
- Minimum vibrations transmitted to silo

- Electric vibrator with adjustable masses, ATEX certified for zone 21



ATEX version zone 22

Negative pressure version for silos

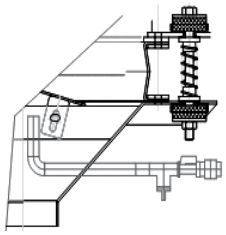


TYPE	Size	Ø A STD	B	C	H	Motors	kg
BA040	400	114	380	427	330	1	59
BA060	600	168	580	519	408	1	80
BA075	750	219	730	609	456	1	99
BA090	900	219	880	684	531	1	134
BA100	1,000	273	980	734	555	1	146
BA125	1,250	273	1,230	937	730	1	290
BA150	1,500	323	1,480	1,120	774	1	475
BA180	1,800	323	1,780	1,194	924	2	726
BA210	2,100	406	2,080	1,420	1,033	2	881
BA235	2,350	406	2,330	1,547	1,166	2	1,255
BA250	2,500	406	2,480	1,705	1,307	2	1,530
BA300	3,000	406	2,980	1,955	1,568	2	2,456

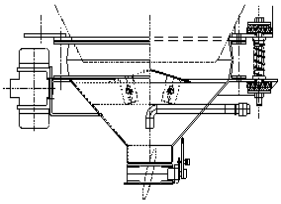
Dimensions in mm

OPTIONS & ACCESSORIES

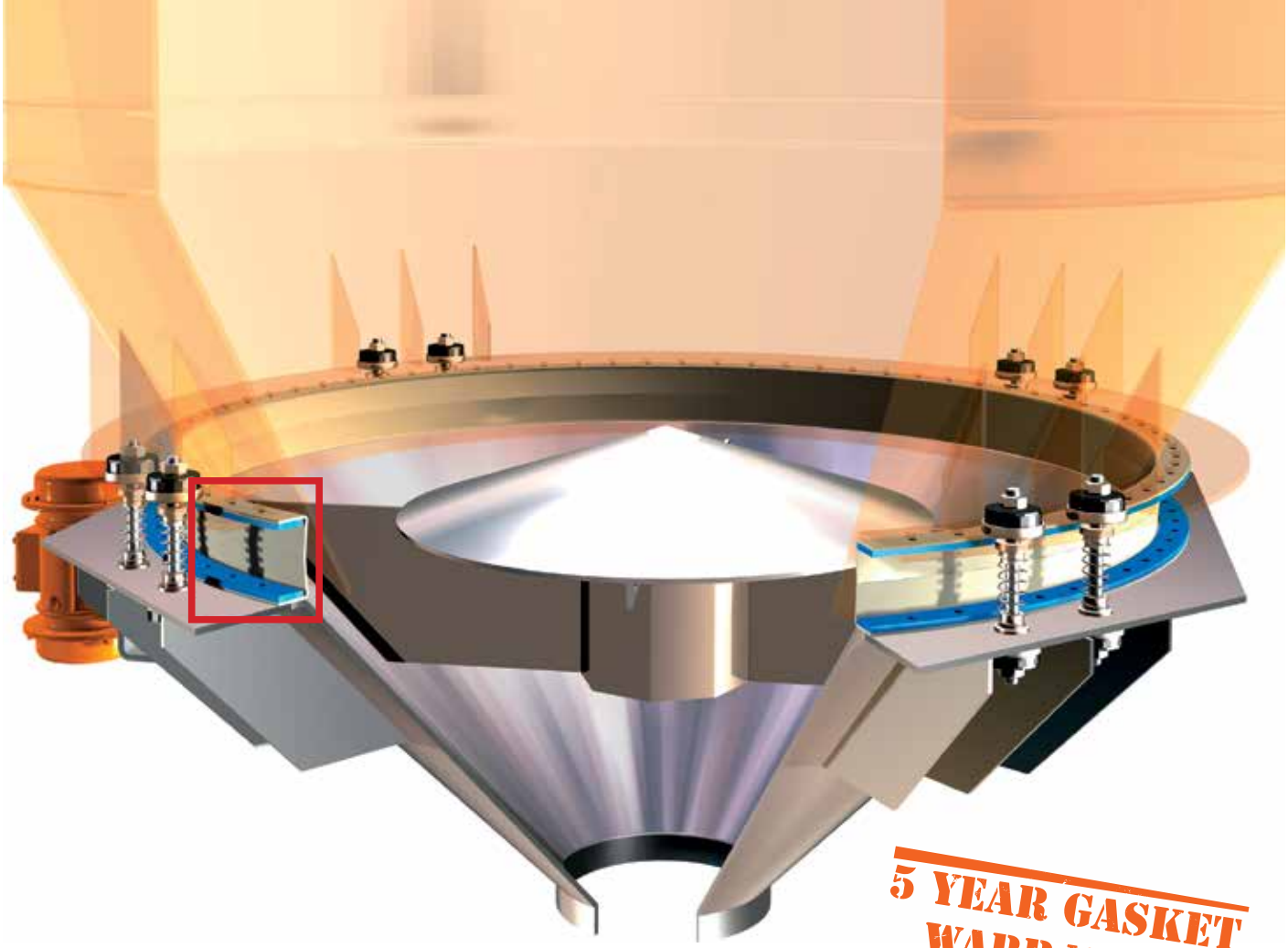
Large range of baffle plates



Decompressor



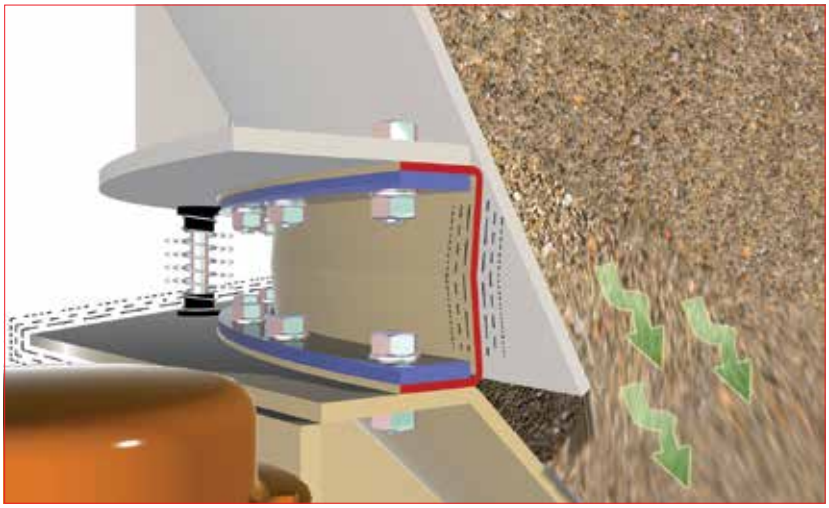
Pneumatic accelerator



5 YEAR GASKET WARRANTY*

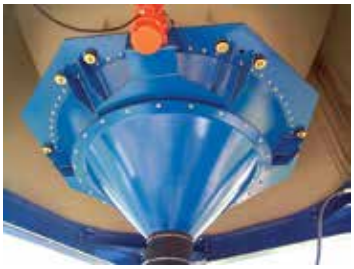
GASKET WITH MAXIMUM RELIABILITY:

- Seamless one-piece without joints for maximum resistance to stress
- Double flange for perfect fixing and optimum sealing
- Construction in wear-resistant SINT® food-grade engineering polymer
- Design studied to prevent stagnation, ideal for food and perishable products



** The validity of the warranty is conditioned by strict respect of the conditions of use described in the Installation, Use & Maintenance Manual.*

APPLICATIONS




- Food
- Flour milling
- Animal feed milling
- Plastics
- Chemicals
- Pharmaceuticals
- Glass
- Fertilizers
- Waste water treatment
- Foundries
- Premixed adhesives for ceramics industry

WAMGROUP®

WAM® FLITECH® MAP® EXTRAC® SPECO® TOREX® OLI® RONCUZZI® TECNO CM

EXTRAC®

Bio-Pharm

SANI-MATIC®

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

UltraFlow 45

UltraFlow 110

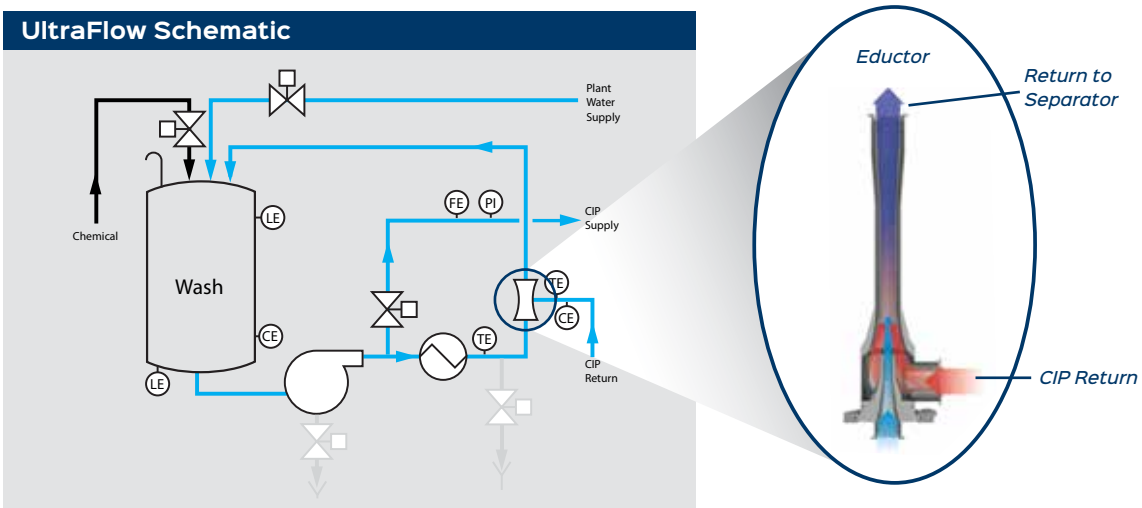
The Sani-Matic UltraFlow is a self-contained, compact and portable Clean-In-Place (CIP) System programmed to accommodate a variety of recirculated CIP applications. Designed for critical cleaning, the UltraFlow meets cGMP and ASME BPE standards.

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

- Operation and maintenance manuals
- Recommended spare parts (RSP) list
- Mechanical Bill of Materials (BOM)
- Instrumentation calibration procedures
- Material Test Reports (MTRs)
- Weld maps and weld logs (including qualification and inspection records)
- Inspection test results, reports and certificates
- Component vendor documentation
- As-built General Assembly (GA) drawings
- As-built Process and Instrumentation Diagrams (P&ID)
- As-built electrical drawings

Optional

- Functional Specifications (FS)
- Configuration Specification (CS)
- Factory Acceptance Test (FAT)
- Site Acceptance Test (SAT)
- Installation and Operation Qualification (IQ/OQ)
- Traceability matrix
- Instrument data sheets
- Cleaning and passivation certificate
- Borescope Inspection Video
- Hydrostatic test certificate
- 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) @ 460 VAC, 3-Phase, 60 Hz	15 kW, 50 amps (standard) or 30 kW, 68 amps (optional) @ 460 VAC, 3-Phase, 60 Hz
• Electrical Power (with optional steam heat)	11 amps @ 460 VAC, 3-Phase, 60 Hz	27 amps @ 460 VAC, 3-Phase, 60 Hz
- 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

Cleaning Confidence.

Repeatable results you can count on every time you clean your process parts and equipment.
That's Cleaning Confidence from Sani-Matic.

SANI-MATIC®

sanimatic.com

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Appendix E: Material Safety Data Sheets

SECTION 1: Identification of the substance/mixture and of the company/undertaking

1.1 Product identifier
Material Name
Methanol
Synonyms
Methyl alcohol, wood alcohol, methyl hydroxide
Chemical Family
Alcohols
Registration status
01-2119433307-44-0031 EC #: 200-659-6. CAS #: 67-56-1.
1.2 Relevant identified uses of the substance or mixture and uses advised against
Identified uses
Industrial use: Manufacture of substance. Distribution of substance. Formulation & (re)packing of substances and mixtures. Use as a fuel. Use in cleaning agents. Use as laboratory reagent. Water treatment chemicals, wastewater. Professional use: Use as a fuel. Use in cleaning agents. Use as laboratory reagent. Use in oil and gas field drilling and production operations. Consumer use: Consumer use of cleaning agents and de-icers. Consumer use of fuels.
Uses advised against
None identified
1.3 Details of the supplier of the safety data sheet

Methanex Europe SA/NV I
Waterloo Office Park - Building P
Drève Richelle 161 - box 31
B-1410 Waterloo
Belgium
Phone: +(32) 2 352 06 70
E-mail: reach@methanex.com
Fax: +(32) 2 352 06 99

1.4 Emergency telephone number
+44 (0) 1235 239 670 (24h/7d)

SECTION 2: Hazards identification

2.1 Classification of the substance or mixture
Classification according to Regulation (EC) No 1272/2008 [CLP]
Flammable Liquids - Category 2
Acute Toxicity - Oral - Category 3
Acute Toxicity - Dermal - Category 3
Acute Toxicity - Inhalation - Vapor - Category 3
Specific Target Organ Toxicity - Single Exposure - Category 1 (optic nerve , central nervous system)
2.2 Label elements
Labeling according to Regulation (EC) No. 1272/2008 [CLP]
Hazard symbols

Signal word
Danger
Hazard statements
H225 Highly flammable liquid and vapor.
H301 Toxic if swallowed.
H311 Toxic in contact with skin.
H331 Toxic if inhaled.
H370 Causes damage to organs.
Precautionary statements
Prevention
P233 Keep container tightly closed.
P210 Keep away from heat, hot surfaces, sparks, open flames and other ignition sources. No smoking.
P240 Ground/Bond container and receiving equipment.
P241 Use explosion-proof electrical/ventilating/lighting equipment.
P243 Take action to prevent static discharges.
P242 Use non-sparking tools.
P271 Use only outdoors or in a well-ventilated area.
P280 Wear protective gloves/protective clothing/eye protection/face protection.
P260 Do not breathe dust/fume/gas/mist/vapors/spray.
P264 Wash thoroughly after handling.
P270 Do not eat, drink or smoke when using this product.
Response
P370+P378 In case of fire: Use appropriate media to extinguish.
P308+P311 If exposed or concerned: Call a POISON CENTER or doctor/physician.
P304+P340 IF INHALED: Remove person to fresh air and keep comfortable for breathing.
P303+P361+P353 IF ON SKIN (or hair): Take off immediately all contaminated clothing. Rinse skin with water/shower.
P361+P364 Take off immediately all contaminated clothing and wash it before reuse.
P301+P310 IF SWALLOWED: Immediately call a POISON CENTER/doctor.
P330 Rinse mouth.
P311 Call a POISON CENTER or doctor.
P321 Specific treatment (see label).
Storage
P403+P233 Store in a well-ventilated place. Keep container tightly closed.
P235 Keep cool.
P405 Store locked up.
Disposal
P501 Dispose of contents/container in accordance with local/regional/national/international regulations.
2.3 Other hazards
If swallowed there is a risk of blindness.

SECTION 3: Composition / information on ingredients

3.1 SUBSTANCES

CAS EC No Registration No	Component Name Synonyms	1272/2008 (CLP)	Percent
67-56-1 200-659-6	Methanol	Annex VI, Table 3: Flam. Liq. 2 - H225	100

--		Acute Tox. (Oral) 3 - H301 Acute Tox. (Vapour) 3 - H331 Acute Tox. (Gas) 3 - H331 Acute Tox. (Dermal) 3 - H311 Acute Tox. (Dust/Mist) 3 - H331 STOT SE 1 - H370 STOT SE 2 - H371	

Component Related Regulatory Information
Specific concentration limit (SCL): STOT SE 1; H370: C≥10%. STOT SE 2; H371: 3% ≤ C < 10%.

SECTION 4: First aid measures

4.1 Description of first aid measures
Inhalation
IF INHALED: Remove person to fresh air and keep comfortable for breathing. Administer oxygen if breathing is difficult. Immediately call a POISON CENTER or doctor.
Skin
IF ON SKIN (or hair): Remove/take off immediately all contaminated clothing. Wash with plenty of water. Immediately call a POISON CENTER or doctor. Wash contaminated clothing before reuse.
Eyes
IF IN EYES: Immediately flush eyes with water for at least 15 minutes, while holding eyelids open. Remove contact lenses, if present and easy to do. Continue rinsing. If irritation develops and persists, get medical attention.
Ingestion
IF SWALLOWED: Rinse mouth. Do NOT induce vomiting. Never give anything by mouth to an unconscious person. Seek medical attention immediately.
4.2 Most Important Symptoms/Effects
Acute
Poison. May be fatal if swallowed. If swallowed there is a risk of blindness. Toxic if swallowed, in contact with skin or if inhaled. Ingestion causes nausea, weakness and central nervous system effects, headache, vomiting, dizziness, symptoms of drunkenness. Coma and death due to respiratory failure may follow severe exposures: Medical treatment necessary. A latent period of several hours may occur between exposure and the onset of symptoms.
Delayed
Causes damage to organs through prolonged or repeated exposure.
4.3 Indication of Immediate Medical Attention and Special Treatment
Treat symptomatically and supportively. The severity of symptoms depends upon the length and concentration of the exposure. If ingested, get immediate medical attention. Antidote: Fomepizole enhances elimination of metabolic formic acid. Antidote should be administered by qualified medical personnel.
Note to Physicians
Treat symptomatically. The severity of outcome following methanol ingestion may be more related to the time between ingestion and treatment, rather than the amount ingested. Therefore, there is a need for rapid treatment of any ingestion exposure. Call a POISON CENTER.

SECTION 5: Firefighting measures

5.1 Extinguishing media
Suitable extinguishing media
Carbon dioxide, regular dry powder, water spray, alcohol resistant foam, sand. Use water spray to cool fire fire-exposed containers. Water will not cool methanol below its flash point. Collect spillage.
Unsuitable Extinguishing Media
Do not use high-pressure water streams.
5.2 Special hazards arising from the substance or mixture

Highly flammable liquid and vapor. Mixtures >20% methanol with water: flammable. May form explosive mixture with air. Vapors are heavier than air and may travel along the ground to some distant source of ignition and flash back. Containers may rupture or explode if exposed to heat. Dangerous gases may accumulate in confined spaces. Toxic.

Combustion

Releases toxic gases, vapors. Carbon monoxide, carbon dioxide, formaldehyde.

5.3 Advice for firefighters

Methanol: Burns with invisible flame. Flame may not be visible in daylight. Cool containers with water spray until well after the fire is out.

Fire Fighting Measures

Do not allow run-off from fire-fighting to enter drains or water courses. Keep unnecessary people away, isolate hazard area and deny entry.

Protective Equipment and Precautions for Firefighters

Wear full protective firefighting gear including self-contained breathing apparatus (SCBA) for protection against possible exposure.

SECTION 6: Accidental release measures

6.1 Personal precautions, protective equipment and emergency procedures

Wear appropriate personal protective equipment. Move container from fire area if it can be done without risk. Do not breathe gas/fume/vapor/spray. Avoid contact with eyes and skin.

6.2 Environmental precautions

Avoid release to the environment. Biodegradable at low concentrations. Soluble in water. When released, this product is expected to evaporate. Contact authorities in the event of pollution of soil and aquatic environment or discharge into drains. Dispose in accordance with all applicable federal, state/regional and local laws and regulations.

6.3 Methods and Materials for Containment and Cleaning Up

Wear suitable protective clothing and eye/face protection. Stop leak if this can be done without risk. Do not touch or walk through spilled material. Evacuate the area promptly and keep upwind of the spilled material. Ensure adequate ventilation. Avoid inhalation of mists or vapors. Avoid contact with eyes, skin and clothing. Remove all sources of ignition. Avoid friction, static electricity and sparks. Small spills: Absorb with sand or other non-combustible material. Use non-sparking tools and equipment. Collect spilled material in appropriate container for disposal. Clean contaminated surface thoroughly. Large spills: Contain the released material by diking the containment area with absorbent. A vapor suppressing foam may be used to reduce vapors. Collect spilled material in appropriate container for reuse or disposal.

6.4 Reference to other sections

Safe handling: see section 7. Personal protection equipment (PPE): see section 8. Disposal: see section 13.

SECTION 7: Handling and storage

7.1 Precautions for safe handling

Use in a well ventilated area. Wear personal protective clothing and equipment, see Section 8. Eliminate all sources of ignition. No smoking. Do not enter confined spaces unless adequately ventilated. Clean up contamination/spills as soon as they occur. Decontaminate personnel, spill area and all tools and equipment. Use explosion-proof equipment. Use good industrial hygiene practices in handling this material. Wash hands and other exposed areas with mild soap and water before eating, drinking or smoking and leaving work. Empty containers may contain residual amounts of this product; therefore, empty containers should be handled with care. Do not breathe vapor.

7.2 Conditions for safe storage, including any incompatibilities

Store in a well-ventilated place. Keep container tightly closed.

Keep cool.

Store locked up.

Keep/Store only in original container. Keep out of direct sunlight, and away from heat, water, and incompatible materials. Ground/Bond container and receiving equipment. Provide appropriate fire extinguishers and spill cleanup equipment in or near storage area. Store at room temperature. Store in a dry area. Store in fireproof room. Keep unauthorized personnel away.

Incompatible Materials

Lead, Aluminum, zinc, oxidizing agents, strong acids, strong bases, polyethylene, PVC (Polyvinyl chloride), nitrile

7.3 Specific end use(s)

Industrial use: Manufacture of substance. Distribution of substance. Formulation & (re)packing of substances and mixtures. Use as a fuel. Use in cleaning agents. Use as laboratory reagent. Water treatment chemicals, wastewater. Professional use: Use as a fuel. Use in cleaning agents. Use as laboratory reagent. Use in oil and gas field drilling and production operations. Consumer use: Consumer use of cleaning agents and de-icers. Consumer use of fuels.

SECTION 8: Exposure controls/personal protection

8.1 Control parameters

Component Exposure Limits

Methanol	**67-56-1**
EU (IOELV):	200 ppm TWA ; 260 mg/m3 TWA
	Possibility of significant uptake through the skin
ACGIH:	200 ppm TWA
	250 ppm STEL
Austria:	200 ppm TWA [TMW]; 260 mg/m3 TWA [TMW]
	800 ppm STEL [KZW] 4 X 15 min ; 1040 mg/m3 STEL [KZW] 4 X 15 min
	skin notation
Belgium:	200 ppm TWA ; 266 mg/m3 TWA
	250 ppm STEL ; 333 mg/m3 STEL
	Skin
Bulgaria	200 ppm TWA ; 260 mg/m3 TWA
	Skin notation
Croatia	200 ppm TWA [GVI]; 260 mg/m3 TWA [GVI]
	Skin Notation
Cyprus	200 ppm TWA ; 260 mg/m3 TWA
	Skin-potential for cutaneous absorption
Czech Republic	250 mg/m3 TWA

	1000 mg/m3 Ceiling
	Potential for cutaneous absorption
Denmark.	200 ppm TWA ; 260 mg/m3 TWA
	Potential for cutaneous absorption
Estonia	200 ppm TWA ; 260 mg/m3 TWA
	250 ppm STEL ; 350 mg/m3 STEL
	Skin notation
Finland:	200 ppm TWA ; 270 mg/m3 TWA
	250 ppm STEL ; 330 mg/m3 STEL
	Potential for cutaneous absorption
France:	200 ppm TWA [VME] (restrictive limit); 260 mg/m3 TWA [VME] (restrictive limit)
	1000 ppm STEL [VLCT]; 1300 mg/m3 STEL [VLCT]
	Risk of cutaneous absorption
Germany (TRGS):	200 ppm TWA AGW (The risk of damage to the embryo or fetus can be excluded when AGW and BGW values are observed) exposure factor 4 ; 270 mg/m3 TWA AGW (The risk of damage to the embryo or fetus can be excluded when AGW and BGW values are observed) exposure factor 4
	skin notation
Germany (DFG):	200 ppm TWA MAK ; 270 mg/m3 TWA MAK
	800 ppm Peak ; 1080 mg/m3 Peak
	skin notation
Greece:	200 ppm TWA ; 260 mg/m3 TWA
	250 ppm STEL ; 325 mg/m3 STEL
	skin - potential for cutaneous absorption
Hungary	260 mg/m3 TWA [AK]
	potential for cutaneous absorption
Ireland:	200 ppm TWA ; 260 mg/m3 TWA
	600 ppm STEL (calculated); 780 mg/m3 STEL (calculated)

	Potential for cutaneous absorption
Italy:	200 ppm TWA Media Ponderata nel Tempo ; 260 mg/m3 TWA Media Ponderata nel Tempo
	skin - potential for cutaneous absorption
	200 ppm TWA ; 262 mg/m3 TWA
	Skin - potential for cutaneous absorption
Latvia	200 ppm TWA ; 260 mg/m3 TWA
	skin - potential for cutaneous exposure
Lithuania	200 ppm TWA [IPRD]; 260 mg/m3 TWA [IPRD]
	Skin notation
Luxembourg	200 ppm TWA; 260 mg/m3 TWA
Malta	200 ppm TWA ; 260 mg/m3 TWA
	possibility of significant uptake through the skin
Netherlands:	133 mg/m3 TWA ; 100 ppm TWA
	skin notation
Poland	100 mg/m3 TWA [NDS]
Portugal:	200 ppm TWA [VLE-MP] (indicative limit value); 260 mg/m3 TWA [VLE-MP] (indicative limit value)
	250 ppm STEL [VLE-CD]
	skin - potential for cutaneous exposure (indicative limit value)
Romania	200 ppm TWA ; 260 mg/m3 TWA
Slovak Republic	200 ppm TWA ; 260 mg/m3 TWA
	Potential for cutaneous absorption
Slovenia	200 ppm TWA ; 260 mg/m3 TWA
Spain:	200 ppm TWA [VLA-ED] (indicative limit value); 266 mg/m3 TWA [VLA-ED] (indicative limit value)
	skin - potential for cutaneous exposure
Sweden:	200 ppm LLV ; 250 mg/m3 LLV

	250 ppm Indicative STLV ; 350 mg/m3 Indicative STLV
	Skin notation
United Kingdom:	200 ppm TWA ; 266 mg/m3 TWA
	250 ppm STEL ; 333 mg/m3 STEL
	Potential for cutaneous absorption

Component Biological Exposure Limits

Methanol	**67-56-1**
ACGIH:	15 mg/l Medium: urine Time: end of shift Parameter: Methanol (background, nonspecific)
Czech Republic	15 mg/l Medium: urine Time: end of shift Parameter: Methanol (background, nonspecific)

Derived No Effect Levels (DNELs)

DNEL long-term dermal (systemic): 40 mg/kg bw/day. DNEL long-term inhalative (systemic): 260 mg/m3. DNEL short-term dermal (systemic): 40 mg/kg bw/day. DNEL short-term inhalative (systemic): 260 mg/m3.

Predicted No Effect Concentrations (PNECs)

PNEC aquatic, freshwater: 154 mg/L. PNEC aquatic, marine water, PNEC aquatic, intermittent release: 1540 mg/L. PNEC sediment, freshwater, PNEC sewage treatment plant (STP): 100 mg/L.

8.2 Exposure Controls

Engineering controls

Provide adequate local exhaust ventilation to maintain worker exposure below exposure limits. Use explosion-proof electrical/ventilating/lighting equipment. Handle substance within a closed system. Ground/Bond container and receiving equipment. Maintain eye wash fountain and quick-drench shower in work area.

Eye/face protection

Use eye protection according to EN 166, designed to protect against liquid splashes.

Skin Protection

Wear appropriate chemical resistant clothing (EN ISO 6529).

Respiratory Protection

Any supplied-air respirator with a full facepiece that is operated in a pressure-demand or other positive-pressure mode (EN 137). Respirator selection must be based on known or anticipated exposure levels, the hazards of the product and the safe working limits of the selected respirator.

Glove Recommendations

Wear suitable gloves tested to EN374, butyl rubber.

SECTION 9: Physical and chemical properties

9.1 Information on basic physical and chemical properties

Appearance	clear	**Physical State**	liquid
Odor	alcohol odor	**Color**	colorless
Odor Threshold	4.2 - 5960 ppm	**pH**	Not applicable
Melting Point	-97.8 °C	**Boiling Point**	64.7 °C

Boiling Point Range	Not available	**Freezing point**	-97.6 °C
Evaporation Rate	4.1 (butyl acetate = 1)	**Flammability (solid, gas)**	Not applicable
Autoignition Temperature	464 °C	**Flash Point**	11 °C
Lower Explosive Limit	5.5 %	**Decomposition temperature**	Not available
Upper Explosive Limit	36.5 %	**Vapor Pressure**	12.8 kPa (@ 20 °C)
Vapor Density (air=1)	1.1 (@ 20 °C)	**Specific Gravity (water=1)**	792 kg/m
Water Solubility	Not available	**Partition coefficient: n-octanol/water**	-0.77 (log value)
Viscosity	0.8 cP (20 °C, dynamic)	**Kinematic viscosity**	Not available
Solubility (Other)	Not available	**Density**	0.791 - 0.793 at 20 °C
VOC	100 %	**Molecular Weight**	32.04 (g/mol)
Critical Temperature	239.4 °C	**Oxidising properties**	Not oxidising
Explosive properties	Vapors may form explosive mixtures with air		

Solvent Miscibility
Miscible
Miscible with water.

SECTION 10: Stability and reactivity

10.1 Reactivity
Containers may rupture or explode if exposed to heat.
10.2 Chemical stability
Stable under normal conditions of use. In use may form flammable/explosive vapor-air mixture. Product is hygroscopic.
10.3 Possibility of hazardous reactions
Will not polymerize.
10.4 Conditions to avoid
Avoid heat, flames, sparks and other sources of ignition. Containers may rupture or explode if exposed to heat.
10.5 Incompatible materials
Lead, Aluminum, zinc, oxidizing agents, strong acids, strong bases, polyethylene, PVC (Polyvinyl chloride), nitrile
10.6 Hazardous decomposition products
Heat, carbon monoxide, carbon dioxide, flammable gases, formaldehyde

SECTION 11: Toxicological information

11.1 Information on toxicological effects

Acute and Chronic Toxicity

Poison. May be fatal if swallowed. If swallowed there is a risk of blindness. Toxic if swallowed, in contact with skin or if inhaled.

Component Analysis - LD50/LC50

The components of this material have been reviewed in various sources and the following selected endpoints are published:

Methanol (67-56-1)

Oral LD50 Rat 5600 mg/kg
Dermal LD50 Rabbit 15800 mg/kg
Inhalation LC50 Rat 64000 ppm 4 h

Product Toxicity Data

Acute Toxicity Estimate

Dermal	300 mg/kg
Inhalation - Vapor	3 mg/L
Oral	100 mg/kg

Irritation/Corrosivity Data

May cause irritation to eyes, skin and respiratory tract.

Respiratory Sensitization

No data available.

Dermal Sensitization

No data available.

Germ Cell Mutagenicity

No data available.

Component Carcinogenicity

None of this product's components are listed by IARC or DFG.

Toxicity for reproduction

No data available.

Specific Target Organ Toxicity - Single Exposure

optic nerve, central nervous system

Specific Target Organ Toxicity - Repeated Exposure

No target organs identified.

Aspiration hazard

No data available.

SECTION 12: Ecological information

12.1 Toxicity

Avoid release to the environment.

Component Analysis - Aquatic Toxicity

Methanol	**67-56-1**
Fish:	LC50 96 h Pimephales promelas 28200 mg/L [flow-through]; LC50 96 h Pimephales promelas >100 mg/L [static]; LC50 96 h Oncorhynchus mykiss 19500 - 20700 mg/L [flow-through]; LC50 96 h Oncorhynchus mykiss 18 - 20 mL/L [static]; LC50 96 h Lepomis macrochirus 13500 - 17600 mg/L [flow-through]
Algae:	EC50 72 hr Selenastrum capricornutum 22000 mg/l

Invertebrate:	EC50 48 hr Daphnia >10000 mg/l

12.2 Persistence and degradability
Rapidly degradable.
12.3 Bioaccumulative potential
No indication of bioaccumulation potential.
12.4 Mobility in soil
mobile
Bioconcentration factor (BCF)
Bioconcentration factor (BCF): < 10
12.5 Results of PBT and vPvB assessment
No components of this material are listed.
Not fulfilling PBT and vPvB criteria.
12.6 Other adverse effects
No additional information.

SECTION 13: Disposal considerations

13.1 Waste treatment methods
Waste disposal according to directive 2008/98/EC, covering waste and dangerous waste. Incineration is the preferred disposal method.
Waste codes/waste designations according to LoW: EWC-code: 07 01 04*.
Empty product containers may contain product residue. Recycle if possible.
Prevent entry into sewers, drains, ditches, underground or confined spaces and waterways.
Dispose in accordance with all applicable federal, state/regional and local laws and regulations.

SECTION 14: Transport information

		ADR	RID	ICAO	IATA	ADN	IMDG
14.1	UN Number	UN1230	UN1230	UN1230	UN1230	UN1230	UN1230
14.2	UN Proper Shipping Name	METHANOL	METHANOL	METHANOL	METHANOL	METHANOL	METHANOL
14.3	Transport Hazard Class(es)	3 Risks: 6.1	3 Risks: 6.1	3 Risks: 6.1	3 Risks: 6.1	3 Risks: 6.1	3 Risks: 6.1
14.4	Packing Group	II	II	II	II	II	II
14.5	Environmental Hazards	--	--	--	--	--	--
14.6	Special Precautions For User	--	--	--	--	--	--

14.7	Transport in Bulk According to Annex II of MARPOL and the IBC Code	--	--	--	--	--	--
14.8	Further information	ADR Tunnel Code Restrictions: D/E	--	--	--	--	--

International Bulk Chemical Code
This material contains one or more of the following chemicals required by the IBC Code to be identified as dangerous chemicals in bulk.

Methanol	**67-56-1**
IBC Code:	Category Y

SECTION 15: Regulatory information

15.1 Safety, health and environmental regulations/legislation specific for the substance or mixture
REACH Candidate List of Substances of Very High Concern (SVHC) for Authorization (Article 59(1)) - Reg. (EU) No. 1907/2006
No components of this material are listed.
EU - REACH (1907/2006) - Annex XVII Restrictions of Certain Dangerous Substances, Mixtures and Articles
No components of this material are listed.
EU - Substances Depleting the Ozone layer (1005/2009)
No components of this material are listed.
EU - Persistent Organic Pollutants (850/2004)
No components of this material are listed.
EU - Export and Import Restrictions (689/2008) - Chemicals and Articles Subject to Export Ban
No components of this material are listed.
EU - Seveso III Directive (2012/18/EU) - Qualifying Quantities of Dangerous Substances
No components of this material are listed.
EU - Plant Protection Products (1107/2009/EC)
No components of this material are listed.
EU - Biocides (528/2012/EU)
No components of this material are listed.
EU – Water Framework Directive (2000/60/EC)
No components of this material are listed.
EU - Limitation of Emissions of Volatile Organic Compounds Due to the Use of Organic Solvents in Certain Activities and Installations (1999/13/EC)
No components of this material are listed.
EU - Detergent Regulation (648/2004/EC)
No components of this material are listed.
Germany Regulations
Germany Water Classification - Product
hazard class 2 - hazard to waters

Germany Water Classification - Component
Methanol (67-56-1)
ID Number 145, hazard class 2 - hazard to waters
Denmark Regulations

Methanol	**67-56-1**
	Solvents
	Properties of concern with regard to the List of hazardous substances

Component Analysis - Inventory
Methanol (67-56-1)

US	CA	EU	AU	PH	JP - ENCS	JP - ISHL	KR KECI - Annex 1	KR KECI - Annex 2	KR - REACH CCA	CN	NZ	MX	TW	VN (Draft)
Yes	DSL	EIN	Yes	Yes	Yes	No	Yes	No	No	Yes	Yes	Yes	Yes	No

15.2 Chemical Safety Assessment
For this substance a chemical safety assessment has been carried out.

SECTION 16: Other information

16.1 Indication of changes
05-07-2018 - Update to Section(s) 3, 7, 12, 16. Template update. Additional translation.
Preparation Date
New SDS: 14 September 2016
Revision date
05 July 2018
16.2 Key / Legend
ACGIH - American Conference of Governmental Industrial Hygienists; ADR - European Road Transport; AU - Australia; BOD - Biochemical Oxygen Demand; C - Celsius; CA - Canada; CA/MA/MN/NJ/PA - California/Massachusetts/Minnesota/New Jersey/Pennsylvania*; CAS - Chemical Abstracts Service; CERCLA - Comprehensive Environmental Response, Compensation, and Liability Act; CFR - Code of Federal Regulations (US); CLP - Classification, Labelling, and Packaging; CN - China; CPR - Controlled Products Regulations; DFG - Deutsche Forschungsgemeinschaft; DOT - Department of Transportation; DSD - Dangerous Substance Directive; DSL - Domestic Substances List; EC – European Commission; EEC - European Economic Community; EIN - European Inventory of (Existing Commercial Chemical Substances); EINECS - European Inventory of Existing Commercial Chemical Substances; ENCS - Japan Existing and New Chemical Substance Inventory; EPA - Environmental Protection Agency; EU - European Union; F - Fahrenheit; F - Background (for Venezuela Biological Exposure Indices); IARC - International Agency for Research on Cancer; IATA - International Air Transport Association; ICAO - International Civil Aviation Organization; IDL - Ingredient Disclosure List; IDLH - Immediately Dangerous to Life and Health; IMDG - International Maritime Dangerous Goods; ISHL - Japan Industrial Safety and Health Law; IUCLID - International Uniform Chemical Information Database; JP - Japan; Kow - Octanol/water partition coefficient; KR KECI Annex 1 - Korea Existing Chemicals Inventory (KECI) / Korea Existing Chemicals List (KECL); KR KECI Annex 2 - Korea Existing Chemicals Inventory (KECI) / Korea Existing Chemicals List (KECL) , KR - Korea; LD50/LC50 - Lethal Dose/ Lethal Concentration; LEL - Lower Explosive Limit; LLV - Level Limit Value; LOLI - List Of LIsts™ - ChemADVISOR's Regulatory Database; MAK - Maximum Concentration Value in the Workplace; MEL - Maximum Exposure Limits; MX – Mexico; Ne- Non-

specific; NFPA - National Fire Protection Agency; NIOSH - National Institute for Occupational Safety and Health; NJTSR - New Jersey Trade Secret Registry; Nq - Non-quantitative; NSL – Non-Domestic Substance List (Canada); NTP - National Toxicology Program; NZ - New Zealand; OSHA - Occupational Safety and Health Administration; PEL- Permissible Exposure Limit; PH - Philippines; RCRA - Resource Conservation and Recovery Act; REACH- Registration, Evaluation, Authorisation, and restriction of Chemicals; RID - European Rail Transport; SARA - Superfund Amendments and Reauthorization Act; Sc - Semi-quantitative; STEL - Short-term Exposure Limit; TCCA – Korea Toxic Chemicals Control Act; TDG - Transportation of Dangerous Goods; TLV - Threshold Limit Value; TSCA - Toxic Substances Control Act; TW – Taiwan; TWA - Time Weighted Average; UEL - Upper Explosive Limit; UN/NA - United Nations /North American; US - United States; VLE - Exposure Limit Value (Mexico); VN (Draft) - Vietnam (Draft); WHMIS - Workplace Hazardous Materials Information System (Canada)

16.3 Key literature references and sources for data

Available upon request.

16.4 Methods Used for Classification of Mixture According to Regulation (EC) No 1272/2008

Available upon request.

16.5 Relevant H- and EUH-phrases (Number and full text) and Notes

H225 Highly flammable liquid and vapor
H301 Toxic if swallowed
H311 Toxic in contact with skin
H331 Toxic if inhaled

16.6 Training advice

Read the Safety Data Sheet before handling product.

16.7 Further Information

Disclaimer:

The information above is believed to be accurate and represents the best information currently available to us. Users should make their own investigations to determine the suitability of the information for their particular purposes. This document is intended as a guide to the appropriate precautionary handling of the material by a properly trained person using this product. Methanex Corporation and its subsidiaries make no representations or warranties, either express or implied, including without limitation any warranties of merchantability, fitness for a particular purpose with respect to the information set forth herein or the product to which the information refers. Accordingly, Methanex Corp. will not be responsible for damages resulting from use of or reliance upon this information.

SAFETY DATA SHEET

Ammonia, Anhydrous

Section 1. Identification

Product identifier : Ammonia, Anhydrous

Chemical name : Ammonia

SDS # : **302**

Other means of identification

Synonyms : **This safety data sheet applies to the following:**

AMM – Anhydrous Ammonia Agricultural Grade 82-0-0
AMM – Anhydrous Ammonia Commercial Grade
AMMR – Anhydrous Ammonia Refrigeration Grade
AMMMET – Anhydrous Ammonia Metallurigal Grade

Product code(s) : **AMM; AMMR; AMMMET**

Product type : Liquefied compressed gas.

Relevant identified uses of the substance or mixture and uses advised against

Identified uses
Fertilizer. Manufacture of specialty fertilizers. Manufacture of chemicals.

Uses advised against	**Reason**
Reserved for industrial and professional use only. Product is not intended for consumer use.	Risk assessment.

Supplier's details : PCS Sales (USA), Inc. (A Subsidiary of Nutrien Ltd.)
1101 Skokie Blvd.
Suite 400
Northbrook, IL 60062
T 1-800-524-0132

PCS Sales (Canada), Inc. (A Subsidiary of Nutrien Ltd.)
Suite 500
122 1st Avenue South
Saskatoon, Saskatchewan Canada S7K 7G3
T 1-800-542-0132

sds@nutrien.com - www.nutrien.com

Emergency telephone number (with hours of operation) : Nutrien North American
24 HOUR EMERGENCY TELEPHONE NUMBERS:

English:
Transportation Emergencies: 1-800-792-8311 Medical Emergencies: 1-303-389-1653

French or Spanish:
Tranportation or Medical Emergencies: 1-303-389-1654

Section 2. Hazard identification

Classification of the substance or mixture : FLAMMABLE GASES - Category 2
GASES UNDER PRESSURE - Liquefied gas
CORROSIVE TO METALS - Category 1
ACUTE TOXICITY (inhalation) - Category 3
SKIN CORROSION - Category 1B
SERIOUS EYE DAMAGE - Category 1

OSHA/HCS status : This material is considered hazardous by the OSHA Hazard Communication Standard (29 CFR 1910.1200).

GHS label elements

Hazard pictograms :

Signal word : Danger

Hazard statements : Flammable gas.
Contains gas under pressure; may explode if heated.
May be corrosive to metals.
Toxic if inhaled.
Causes severe skin burns and eye damage.

Precautionary statements

General : Not applicable.

Prevention : Wear protective gloves. Wear eye or face protection. Wear protective clothing. Keep away from heat, hot surfaces, sparks, open flames and other ignition sources. No smoking. Keep only in original packaging. Use only outdoors or in a well-ventilated area. Avoid breathing gas. Wash hands thoroughly after handling.

Response : IF INHALED: Remove person to fresh air and keep comfortable for breathing. Immediately call a POISON CENTER or physician.
IF SWALLOWED: Immediately call a POISON CENTER or physician. Rinse mouth. Do NOT induce vomiting.
IF ON SKIN (or hair): Take off immediately all contaminated clothing. Rinse skin with water. Wash contaminated clothing before reuse. Immediately call a POISON CENTER or physician.
IF IN EYES: Rinse cautiously with water for several minutes. Remove contact lenses, if present and easy to do. Continue rinsing. Immediately call a POISON CENTER or physician.
Leaking gas fire: Do not extinguish, unless leak can be stopped safely. In case of leakage, eliminate all ignition sources.
Absorb spillage to prevent material damage.

Storage : Store locked up. Protect from sunlight. Store in a well-ventilated place. Store in a corrosion resistant container with a resistant inner liner.

Disposal : Dispose of contents and container in accordance with all local, regional, national and international regulations.

Supplemental label elements : None known.

Other hazards which do not result in classification : Very toxic to aquatic life.

Section 3. Composition/information on ingredients

Substance/mixture : Substance

Ingredient name	% (v/v)	CAS number
Ammonia	99.5 - 99.98	7664-41-7
Water	0 - 0.5	7732-18-5

Any concentration shown as a range is to protect confidentiality or is due to batch variation.

Section 3. Composition/information on ingredients

There are no additional ingredients present which, within the current knowledge of the supplier and in the concentrations applicable, are classified as hazardous to health or the environment and hence require reporting in this section.

Occupational exposure limits, if available, are listed in Section 8.

Section 4. First-aid measures

Description of necessary first aid measures

Eye contact : CORROSIVE. Begin eye irrigation immediately. All eye exposures to anhydrous ammonia require medical evaluation following decontamination. Immediately rinse eyes with large quantities of water or saline for a minimum 20 minutes, longer irrigation time is preferred if possible, due to the chemical reaction that occurs - see Notes to Physician below. If possible, remove contact lenses being careful not to cause additional eye damage. If the initial water supply is insufficient, keep the affected area wet with a moist cloth and transfer the person to the nearest place where rinsing can be continued for the recommended length of time. Call an ambulance for transport to hospital. Continue eye irrigation during transport. For additional advice call the medical emergency number on this safety data sheet or your poison center or doctor.

Inhalation : CORROSIVE. If gases or vapors exceed the IDLH or are present in unknown concentrations, rescuers must wear self-contained breathing apparatus and a suit resistant to gases (Level B). In the U.S., OSHA Hazwoper requirements under 29CFR1910.120 overrule the lesser protection requirements given in the anhydrous ammonia standard, 1910.111.
REMOVE PERSON TO FRESH AIR. Watch closely for signs of wheezing and breathing difficulties. Maintain an open airway. If not breathing, begin CPR. Oxygen may be administered by trained personnel. Affected persons who have stopped breathing or are having difficulty breathing or are unconscious need immediate medical attention. Symptoms may be delayed after exposure to anhydrous ammonia. The exposed person may need to be kept under medical surveillance for 24 - 48 hours. Call an ambulance for transport to hospital. For additional advice call the medical emergency number on this SDS or your poison center or doctor.

Skin contact : CORROSIVE. Causes severe burns. Contact with rapidly expanding gas from evaporating liquid or compressed gas may cause cold burns or frostbite. Immediately begin rinsing the affected areas with water. Remove contaminated clothing and shoes. Affected areas should be rinsed for a minimum 20 minutes, longer irrigation time is preferred if possible, due to the chemical reaction that occurs - see Notes to Physician below. Luke-warm water is recommended for prolonged irrigation to prevent hypothermia. Conscious persons without breathing difficulties may benefit from continued irrigation in a fixed shower or bathing facility prior to hospital transport. Call an ambulance for transport to hospital. Continue skin irrigation during transport. For additional advice call the medical emergency number on this safety data sheet or your poison center or doctor.

Ingestion : CORROSIVE. May cause severe burns to the mouth, throat, and stomach. If the affected person requires cardiopulmonary resuscitation, avoid mouth to mouth contact. Do not induce vomiting. If vomiting occurs, attempt to keep head lower than the chest so that vomit does not enter the lungs. For signs of breathing difficulties, refer to the INHALATION section. Call an ambulance for transportation to hospital. For additional advice, call the medical emergency number on this safety data sheet or your poison center or doctor.

Most important symptoms/effects, acute and delayed

Potential acute health effects

Eye contact : Corrosive to eyes on contact. Causes serious eye damage. Eye contact can result in temporary or permanent corneal damage and/or blindness. The full extent of damage to the eyes may not be known for 1 week after injury.

Inhalation : Toxic if inhaled. Corrosive to the respiratory system. May cause severe breathing difficulties.

Section 4. First-aid measures

Skin contact : Corrosive to the skin. Causes severe burns. Contact with rapidly expanding gas may cause cold burns or frostbite.

Ingestion : Will cause cold burns and will evaporate causing massive inhalation overexposure. Corrosive to the digestive tract. May cause burns to the mouth, throat and stomach.

Over-exposure signs/symptoms

Eye contact : Adverse symptoms may include the following:
pain
watering
redness
loss of vision

Inhalation : Adverse symptoms may include the following:
Exposure to airborne concentrations above statutory or recommended exposure limits may cause irritation of the nose, throat and lungs.
coughing
respiratory tract irritation
wheezing and breathing difficulties

Skin contact : Adverse symptoms may include the following:
pain or irritation
redness
blistering may occur
Signs of frostbite: redness, blistering may occur

Ingestion : Adverse symptoms may include the following:
bloating
difficulty swallowing
throat and stomach pain
nausea or vomiting
respiratory tract irritation
wheezing and breathing difficulties

Indication of immediate medical attention and special treatment needed, if necessary

Notes to physician : Anhydrous ammonia reacts with moisture to produce ammonium hydroxide. Ammonium hydroxide rapidly penetrates skin's stratum corneum layer, eyes, and mucous membranes causing liquefaction necrosis. In addition, anhydrous ammonia is a cryogenic liquid or compressed gas. Venting or evaporation can cause frostbite. The extent of injury depends on duration of exposure and concentration of gas or liquid. Do not attempt to use chemicals to neutralize the exposure. Gas inhalation may cause delayed pulmonary symptoms (acute lung injury). The exposed person may need to be kept under medical surveillance for 24-48 hours. 24 Hr Medical Emergency telephone number for professional support: English: 1-303-389-1653; French or Spanish: 1-303-389-1654.

Specific treatments : Corrosive hydroxyl ions generated by the production of ammonium hydroxide rapidly penetrate the skin, eyes, and mucous membranes. Outcomes can be improved by minimizing time to decontamination and extending decontamination times to reduce tissue damage. Expert opinion indicates extended decontamination is required to remove corrosive chemicals. Skin and eye decontamination should be performed for a minimum of 20 minutes, longer irrigation time is preferred if possible. Extended decontamination times may be required depending on the exposure. To avoid hypothermia, irrigation water should be maintained at a comfortable temperature. If the patient is not in extremis, it may be necessary to delay transport to emergency care facilities to ensure adequate decontamination time. However, early patient transport may be necessary depending on patient's condition or the availability of water. If possible, continue skin and/or eye irrigation during emergency medical transport. Double-bag contaminated clothing and personal belongings of the patient.

Protection of first-aiders : No action shall be taken involving any personal risk or without suitable training. Depending on the situation, the rescuer should wear an appropriate mask, gloves, protective clothing and a respirator or self-contained breathing apparatus. Mouth-to-mouth resuscitation of oral exposure patients is not recommended. First-aiders with contaminated clothing should be properly decontaminated.

Section 4. First-aid measures

See toxicological information (Section 11)

Section 5. Fire-fighting measures

Extinguishing media

Suitable extinguishing media : In case of fire, use water spray.

Unsuitable extinguishing media : Do not use water jet. Do not direct water into spilled anhydrous ammonia. Ammonia is a cryogenic liquid which cools on evaporation limiting vapor release. Water used for fire fighting at supplied temperatures will raise the temperature of ammonia resulting in greater evaporation.

Specific hazards arising from the chemical : Contains gas under pressure. Flammable gas. In a fire or if heated, a pressure increase will occur and the container may burst, with the risk of a subsequent explosion.

Hazardous thermal decomposition products : Emits toxic fumes when heated to decomposition. Decomposition products may include the following materials:
nitrogen oxides

Special protective actions for fire-fighters : Promptly isolate the scene by removing all persons from the vicinity of the incident if there is a fire. Contact supplier immediately for specialist advice. No action shall be taken involving any personal risk or without suitable training. Refer to protective measures listed in sections 7 and 8.

Eliminate all ignition sources if safe to do so. Approach release from upwind. Stop leak if safe to do so. Cool containing vessels with flooding quantities of water until well after fire is out. Move containers from fire area if this can be done without risk. This product is likely to volatilize rapidly into the air because of its high vapor pressure. Do not direct water into spilled anhydrous ammonia. Ammonia is a cryogenic liquid which cools on evaporation limiting vapor release. Water used for fire fighting at supplied temperatures will raise the temperature of ammonia resulting in greater evaporation. Use water spray to keep fire-exposed containers cool. Use water spray curtain to divert vapor drift. Contain and collect the water used to fight the fire for later treatment and disposal.

Special protective equipment for fire-fighters : Fire-fighters should wear appropriate protective equipment and self-contained breathing apparatus (SCBA) with a full face-piece operated in positive pressure mode. Refer to protective measures listed in sections 7 and 8. If gases or vapors are present, rescuers must wear self-contained breathing apparatus and a suit resistant to gases (Level A) under U.S. OSHA requirements. The requirements of 29CFR 1910.120 have been deemed to overrule the lesser protection requirements given in 1910.111. Fully-encapsulating, vapor-protective clothing should be worn for spills and leaks without fire.

Remark : Product will burn with difficulty if kept between the Lower Explosive Limit of 16% and Upper Explosive Limit of 25%. This product is generally regarded as non-flammable due to the difficulty of ignition. However, the presence of oil or other combustible materials will increase the fire hazard, and may ignite with explosive force under favorable conditions.

If mixed with chlorine or hypochlorites, it may form nitrogen trichloride which may explode spontaneously in air.

Section 6. Accidental release measures

Personal precautions, protective equipment and emergency procedures

For non-emergency personnel : No action shall be taken involving any personal risk or without suitable training. Evacuate surrounding areas. Keep unnecessary and unprotected personnel from entering. Shut off all ignition sources. No flares, smoking or flames in hazard area. Do not breathe gas. Provide adequate ventilation. Wear appropriate respirator when ventilation is inadequate. Put on appropriate personal protective equipment.

Section 6. Accidental release measures

For emergency responders : Fully-encapsulating, vapor-protective clothing should be worn for spills and leaks without fire. Self-contained breathing apparatus (SCBA) should be used to avoid inhalation of the product. If specialized clothing is required to deal with the spillage, take note of any information in Section 8 on suitable and unsuitable materials. See also the information in "For non-emergency personnel".

Refer to Emergency Response Guidebook, Guide 125 for further information regarding spill control and Isolation/Protective Action Distances Guidelines.

Do not direct water into spilled anhydrous ammonia. Ammonia is a cryogenic liquid which cools on evaporation limiting vapor release. Water used for fire fighting at supplied temperatures will raise the temperature of ammonia resulting in greater evaporation.
Community Emergency Response Instructions for Sheltering-in-Place:

* Stay indoors (unless evacuation has been called by local authorities)
* Close all windows and doors, seal with duct tape or wet towels
* Shut off furnace, exhaust fans, fireplaces, and air conditioners
* Wait for and follow advice from local police or authorities
* If the smell is very strong, breath through a wet cloth and turn on any nearby showers to absorb airborne vapors

Note: see Section 1 for emergency contact information and Section 13 for waste disposal.

Environmental precautions : Ensure emergency procedures to deal with accidental gas releases are in place to avoid contamination of the environment. Use water spray curtain to divert vapor drift. Do not direct water into spilled anhydrous ammonia. Ammonia is a cryogenic liquid which cools on evaporation limiting vapor release. Water used for fire fighting at supplied temperatures will raise the temperature of ammonia resulting in greater evaporation. Collect contaminated fire-fighting water separately. It must not enter the sewage system.

Avoid dispersal of spilled material and runoff and contact with soil, waterways, drains and sewers. Inform the relevant authorities if the product has caused adverse impacts (sewers, waterways, soil or air).

Methods and materials for containment and cleaning up

Small spill : Immediately contact emergency personnel. Note: see Section 1 for emergency contact information and Section 13 for waste disposal.
Use personal protective equipment as required. Stop leak if without risk. Use spark-proof tools and explosion-proof equipment.

Large spill : Immediately contact emergency personnel. Note: see Section 1 for emergency contact information and Section 13 for waste disposal. Evacuate area. Refer to Emergency Response Guidebook, Guide 125 for further information regarding spill control and Isolation/Protective Action Distances Guidelines.

Section 7. Handling and storage

Precautions for safe handling

Protective measures : Put on appropriate personal protective equipment (see Section 8). Contains gas under pressure. Do not get in eyes or on skin or clothing. Do not breathe gas. Use only with adequate ventilation. Wear appropriate respirator when ventilation is inadequate. Do not enter storage areas and confined spaces unless adequately ventilated. Store and use away from heat, sparks, open flame or any other ignition source. Use explosion-proof electrical (ventilating, lighting and material handling) equipment. Use only non-sparking tools. Empty containers retain product residue and can be hazardous. Do not puncture or incinerate container. Workers must be trained in the safe handling and use of this product.

Section 7. Handling and storage

Advice on general occupational hygiene : Eating, drinking and smoking should be prohibited in areas where this material is handled, stored and processed. Remove contaminated clothing and protective equipment before entering eating areas. See also Section 8 for additional information on hygiene measures.

Conditions for safe storage, including any incompatibilities : Store in accordance with local regulations. Store in a segregated and approved area. Store away from direct sunlight in a dry, cool and well-ventilated area, away from incompatible materials (see Section 10). Store locked up. Eliminate all ignition sources. Keep container tightly closed and sealed until ready for use. Refer to NFPA 400 Hazardous Materials Code for further information on the safe storage and handling of hazardous materials.
Ensure compliance with OSHA 29CFR1910.111 Storage and handling of anhydrous ammonia and 1910.119 Process safety management of highly hazardous chemicals requirements, if applicable.
All anhydrous ammonia retail sites in Canada must be compliant with the standards outlined in Fertilizer Canada's Fertilizer Safety & Security Council Ammonia Code of Practice. The Code applies to agricultural ammonia including road and rail transportation, storage and handling of products and outlines best practices applicable to the distribution, storage and handling of anhydrous ammonia to ensure safety and security.

Section 8. Exposure controls/personal protection

Control parameters

Occupational exposure limits

Ingredient name	Exposure limits
Canadian Regulations: Ammonia	**CA Alberta Provincial (Canada, 4/2009).** 8 hrs OEL: 17 mg/m³ 8 hours. 8 hrs OEL: 25 ppm 8 hours. 15 min OEL: 35 ppm 15 minutes. 15 min OEL: 24 mg/m³ 15 minutes. **CA British Columbia Provincial (Canada, 4/2014).** TWA: 25 ppm 8 hours. STEL: 35 ppm 15 minutes. **CA Ontario Provincial (Canada, 1/2013).** TWA: 25 ppm 8 hours. TWA: 17 mg/m³ 8 hours. STEL: 35 ppm 15 minutes. STEL: 24 mg/m³ 15 minutes. **CA Quebec Provincial (Canada, 1/2014).** TWAEV: 25 ppm 8 hours. TWAEV: 17 mg/m³ 8 hours. STEV: 35 ppm 15 minutes. STEV: 24 mg/m³ 15 minutes. **CA Saskatchewan Provincial (Canada).** TWA: 25 ppm 8 hours. STEL: 35 ppm 15 minutes. **CA Manitoba Provincial (Canada).** TWA: 25 ppm STEL: 35 ppm
U.S. Federal Regulations: Ammonia	**ACGIH TLV (United States, 4/2014).** TWA: 25 ppm 8 hours. TWA: 17 mg/m³ 8 hours. STEL: 35 ppm 15 minutes. STEL: 24 mg/m³ 15 minutes. **OSHA PEL 1989 (United States, 3/1989).** STEL: 35 ppm 15 minutes.

Section 8. Exposure controls/personal protection

	STEL: 27 mg/m³ 15 minutes. **NIOSH REL (United States, 10/2013).** TWA: 25 ppm 10 hours. TWA: 18 mg/m³ 10 hours. STEL: 35 ppm 15 minutes. STEL: 27 mg/m³ 15 minutes. **OSHA PEL (United States, 2/2013).** TWA: 50 ppm 8 hours. TWA: 35 mg/m³ 8 hours.
Water	None assigned.

Appropriate engineering controls : Use only with adequate ventilation. Use process enclosures, local exhaust ventilation or other engineering controls to keep worker exposure to airborne contaminants below any recommended or statutory limits. The engineering controls also need to keep gas, vapor or dust concentrations below any lower explosive limits. Use explosion-proof ventilation equipment.

Environmental exposure controls : Emissions from ventilation or work process equipment should be checked to ensure they comply with the requirements of environmental protection legislation. In some cases, fume scrubbers, filters or engineering modifications to the process equipment will be necessary to reduce emissions to acceptable levels.

Individual protection measures

Hygiene measures : Wash hands, forearms and face thoroughly after handling chemical products, before eating, smoking and using the lavatory and at the end of the working period. Appropriate techniques should be used to remove potentially contaminated clothing. Wash contaminated clothing before reusing. Ensure that eyewash stations and safety showers are close to the workstation location.

Eye/face protection : Safety eyewear complying with an approved standard should be used when a risk assessment indicates this is necessary to avoid exposure to liquid splashes, mists, gases or dusts. If contact is possible, the following protection should be worn, unless the assessment indicates a higher degree of protection: chemical splash goggles.
If inhalation hazards exist, a full-face respirator may be required instead.

Skin protection

Hand protection : Chemical-resistant, impervious gloves complying with an approved standard should be worn at all times when handling chemical products if a risk assessment indicates this is necessary. Considering the parameters specified by the glove manufacturer, check during use that the gloves are still retaining their protective properties. It should be noted that the time to breakthrough for any glove material may be different for different glove manufacturers. Recommended:
butyl rubber
nitrile rubber
neoprene rubber
Viton®
Viton®/butyl rubber

Contact your personal protective equipment manufacturer to verify the compatibility of the equipment for the intended purpose.

Body protection : Personal protective equipment for the body should be selected based on the task being performed, the risks involved, the materials of construction and its design, and should be approved by a specialist before handling this product. Contact your personal protective equipment manufacturer to verify the compatibility of the equipment for the intended purpose.

Under emergency conditions, or where contact with high concentration gas is probable, a chemically resistant, gas tight, encapsulating suit with positive pressure self contained breathing apparatus is required. For accidental splash protection against the liquid, chemically resistant impervious coveralls or a chemical resistant suit should be worn.

Section 8. Exposure controls/personal protection

Other skin protection : Appropriate footwear and any additional skin protection measures should be selected based on the task being performed and the risks involved and should be approved by a specialist before handling this product. Recommended: Impervious rubber safety boots. Contact your personal protective equipment manufacturer to verify the compatibility of the equipment for the intended purpose.

Respiratory protection : Based on the hazard and potential for exposure, select a respirator that meets the appropriate standard or certification. Respirators must be used according to a respiratory protection program to ensure proper fitting, training, and other important aspects of use. Respirator selection must be based on known or anticipated exposure levels, the hazards of the product and the safe working limits of the selected respirator. Use a NIOSH approved chemical cartridge or canister respirator with a full facepiece for ammonia concentrations up to 300 PPM. Use a positive pressure SCBA for concentrations above 300 PPM, for emergency response, or for entry into unknown concentrations. For U.S. work sites where respiratory protection is required, ensure that a respiratory protection program meeting 29 CFR 1910.134 requirements is in place.

Thermal hazards : Contact with rapidly expanding gas may cause cold burns or frostbite. Wear cold insulating gloves underneath impervious chemical resistant gloves.

Section 9. Physical and chemical properties

Appearance

Physical state : Gas. [Compressed gas.]

Color : Colorless.

Odor : Pungent. Ammoniacal. [Strong]

Odor threshold : Variable. ~ 17 ppm

pH : 11.6 [Conc. (% w/w): 1.7%]

Melting point : -77.7°C (-107.9°F)

Boiling point : -33°C (-27.4°F)

Flash point : Not available.

Evaporation rate : Not available.

Flammability (solid, gas) : Slightly flammable in the presence of the following materials or conditions: open flames, sparks and static discharge and heat. Gas may accumulate in low or confined areas or travel a considerable distance to a source of ignition and flash back, causing fire or explosion.
Product will burn with difficulty if kept between the Lower Explosive Limit of 16% and Upper Explosive Limit of 25%. This product is generally regarded as non-flammable due to the difficulty of ignition. However, the presence of oil or other combustible materials will increase the fire hazard, and may ignite with explosive force under favorable conditions.

Lower and upper explosive (flammable) limits : Lower: 16%
Upper: 25%

Vapor pressure : 843 kPa (6323 mm Hg) [20°C]
2032.5 kPa (15244.8 mm Hg) [50°C]

Vapor density : Vapor Density: Variable, depending on temperature. 0.77 kg/m³ @ STP

Relative density : Not available.

Solubility : Easily soluble in the following materials: cold water.
Soluble in the following materials: hot water.
Partially soluble in the following materials: Methanol.

Solubility in water : 540 g/l

Partition coefficient: n-octanol/water : 0.23

Auto-ignition temperature : 651°C (1203.8°F)

Decomposition temperature : Not available.

Viscosity : Not available.

Section 10. Stability and reactivity

Reactivity : Reactive with acids
Incompatible with halogens, hydrogen peroxide, chlorinated hydrocarbons, fluorine, nitric acid, oxidizing agents and sulfuric acid.
Incompatible with copper alloys, copper, and zinc.

Chemical stability : The product is stable.

Possibility of hazardous reactions : Under normal conditions of storage and use, hazardous reactions will not occur.

Conditions to avoid : Avoid all possible sources of ignition (spark or flame). Do not pressurize, cut, weld, braze, solder, drill, grind or expose containers to heat or sources of ignition.

Incompatible materials : Extremely reactive or incompatible with acids. Highly reactive with oxidizing agents and reducing agents. Forms explosive compounds with many heavy metals such as mercury or silver. May react explosively with chlorine, hypochlorites such as bleach or chlorinating chemicals and other halogens such as bromine, iodine, fluorine or their compounds.

Highly corrosive to copper and its alloys. Slightly corrosive to aluminum and zinc. Very slightly corrosive to mild steel. Non-corrosive to glass or stainless steel (304 or 316). Do not use copper, brass, bronze, or galvanized steel in contact with ammonia. Do not use brazed joints in ammonia service. Contact your sales representative or a metallurgical specialist to ensure compatability with your equipment.

Hazardous decomposition products : Under normal conditions of storage and use, hazardous decomposition products should not be produced.

Section 11. Toxicological information

Information on toxicological effects

Acute toxicity

Product/ingredient name	Result	Species	Dose	Exposure
Ammonia, anhydrous	LC50 Inhalation Gas.	Rat	9500 ppm	1 hours
	LC50 Inhalation Gas.	Rat	2000 ppm	4 hours
	LC50 Inhalation Vapor	Rat - Male, Female	11590 mg/m³	1 hours
	LC50 Inhalation Vapor	Rat	7040 mg/m³	30 minutes
	LC50 Inhalation Vapor	Rat	18600 mg/m³	5 minutes
	LD50 Oral	Rat - Male	350 mg/kg	-
Water	LD50 Oral	Rat	>90 g/kg	-

Conclusion/Summary : Corrosive to the respiratory tract. Corrosive to the digestive tract.

Irritation/Corrosion

Not available.

Conclusion/Summary

Skin : Causes severe skin burns and eye damage.

Eyes : Causes severe skin burns and eye damage.

Respiratory : Corrosive to the respiratory tract.

Sensitization

Not available.

Conclusion/Summary

Skin : No known significant effects or critical hazards.

Respiratory : Non-sensitizer to lungs.

Section 11. Toxicological information

Mutagenicity

Product/ingredient name	Test	Experiment	Result
Ammonia	OECD 471 Bacterial Reverse Mutation Test OECD 474 Mammalian Erythrocyte Micronucleus Test	Experiment: In vivo Subject: Bacteria Experiment: In vivo Subject: Mammalian-Animal	Negative Negative

Conclusion/Summary : No mutagenic effect.

Carcinogenicity

Not available.

Conclusion/Summary : No known significant effects or critical hazards.

Reproductive toxicity

Product/ingredient name	Maternal toxicity	Fertility	Development toxin	Species	Dose	Exposure
Ammonia	Negative	-	Negative	Rabbit - Female	Oral: 100 mg/kg	-

Conclusion/Summary : No known significant effects or critical hazards.

Teratogenicity

Not available.

Conclusion/Summary : No known significant effects or critical hazards.

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 : Inhalation

Potential acute health effects

Eye contact : Corrosive to eyes on contact. Causes serious eye damage. Eye contact can result in temporary or permanent corneal damage and/or blindness. The full extent of damage to the eyes may not be known for 1 week after injury.

Inhalation : Toxic if inhaled. Corrosive to the respiratory system. May cause severe breathing difficulties.

Skin contact : Corrosive to the skin. Causes severe burns. Contact with rapidly expanding gas may cause cold burns or frostbite.

Ingestion : Will cause cold burns and will evaporate causing massive inhalation overexposure. Corrosive to the digestive tract. May cause burns to the mouth, throat and stomach.

Symptoms related to the physical, chemical and toxicological characteristics

Eye contact : Adverse symptoms may include the following:
pain
watering
redness
loss of vision

Section 11. Toxicological information

Inhalation : Adverse symptoms may include the following:
Exposure to airborne concentrations above statutory or recommended exposure limits may cause irritation of the nose, throat and lungs.
coughing
respiratory tract irritation
wheezing and breathing difficulties

Skin contact : Adverse symptoms may include the following:
pain or irritation
redness
blistering may occur
Signs of frostbite: redness, blistering may occur

Ingestion : Adverse symptoms may include the following:
bloating
difficulty swallowing
throat and stomach pain
nausea or vomiting
respiratory tract irritation
wheezing and breathing difficulties

Delayed and immediate effects and also chronic effects from short and long term exposure

Short term exposure

Potential immediate effects : See above.

Potential delayed effects : See above.

Long term exposure

Potential immediate effects : See above.

Potential delayed effects : See below.

Potential chronic health effects

Conclusion/Summary : Adverse effects are typically the result of acute overexposure. These effects may be long term or permanent in nature. There is no known effect from chronic exposure to this product.

General : No known significant effects or critical hazards.

Carcinogenicity : No known significant effects or critical hazards.

Mutagenicity : No known significant effects or critical hazards.

Teratogenicity : No known significant effects or critical hazards.

Developmental effects : No known significant effects or critical hazards.

Fertility effects : No known significant effects or critical hazards.

Other information : The odor recognition threshold for ammonia ranges from 0.7 PPM for persons with an acute sense of smell to over 50 PPM for acclimatized individuals. Generally, concentrations of up to 25 PPM are tolerated although unpleasant and pungent. Above this concentration, irritation of the eyes, nose and throat may begin. The extent of irritation increases with increasing ammonia concentration, and decreases with acclimatization.

NIOSH has established 300 PPM as the concentration immediately dangerous to life and health (IDLH), which is defined as the concentration above which self-rescue may be difficult or impossible due to physiological effects. At concentrations over 1000 PPM increasing chest tightness, brochospasm and severe eye and skin irritation may result. Delayed effects such as chemical pneumonitis and pulmonary edema may develop several hours after exposure. Exposure to high concentrations (>5,000 ppm) may cause death. Effects may be more pronounced at lower concentrations in children, the elderly, and persons with impaired lung function.

Section 12. Ecological information

Toxicity

Product/ingredient name	Result	Species	Exposure
Ammonia	Acute EC50 29.2 mg/l Marine water	Algae - Ulva fasciata - Zoea	96 hours
	Acute LC50 2080 µg/l Fresh water	Crustaceans - Gammarus pulex	48 hours
	Acute LC50 0.53 ppm Fresh water	Daphnia - Daphnia magna	48 hours
	Acute LC50 300 µg/l Fresh water	Fish - Hypophthalmichthys nobilis	96 hours
	Chronic NOEC 1 mg/l Fresh water	Algae - Skeletonema costatum	3 days
	Chronic NOEC 0.204 mg/l Marine water	Fish - Dicentrarchus labrax	62 days
	Acute LC50 0.89 mg/l	Fish	96 hours
	Acute LC50 450 µg/l Fresh water	Fish - Oncorhynchus tshawytscha - Underyearling	96 hours
	Chronic LOEL 0.022 mg/l	Fish	73 days
	Chronic NOEC 0.79 mg/l Fresh water	Daphnia	96 hours

Conclusion/Summary : Very toxic to aquatic life.

Persistence and degradability

Conclusion/Summary : Not persistent. Readily biodegradable

Product/ingredient name	Aquatic half-life	Photolysis	Biodegradability
Ammonia	-	-	Readily

Bioaccumulative potential

Product/ingredient name	$LogP_{ow}$	BCF	Potential
Ammonia	0.23	-	low
Water	-1.38	-	low

Mobility in soil

Soil/water partition coefficient (K_{OC}) : Not available.

Other adverse effects : No known significant effects or critical hazards.

Section 13. Disposal considerations

Disposal methods : The generation of waste should be avoided or minimized wherever possible. Disposal of this product, solutions and any by-products should at all times comply with the requirements of environmental protection and waste disposal legislation and any regional local authority requirements. Dispose of surplus and non-recyclable products via a licensed waste disposal contractor. Empty pressure vessels should be returned to the supplier. Incineration or landfill should only be considered when recycling is not feasible. This material and its container must be disposed of in a safe way. Empty containers or liners may retain some product residues. Do not puncture or incinerate container.

Section 14. Transport information

	TDG Classification	DOT Classification	Mexico Classification	IMDG	IATA
UN number	1005	UN1005	UN1005	UN1005	UN1005
UN proper shipping name	Ammonia, anhydrous	Ammonia, anhydrous	Amoniaco, anhidro	Ammonia, anhydrous	Ammonia, anhydrous

Section 14. Transport information

Transport hazard class(es)	2.3 (8) 	2.2 Domestic or 2.3 International See below. 	2.3 	2.3 (8) 	2.3 (8) 
Packing group	-	-	-	-	-
Environmental hazards	No.	No.	No.	Yes.	No.
Additional information	**ERAP Index** 3000 **Passenger Carrying Ship Index** Forbidden **Passenger Carrying Road or Rail Index** Forbidden **TDG Classification:** Product classified as per the following sections of the Transportation of Dangerous Goods Regulations: 2.3 and Schedule I	Inhalation hazard zone D **Reportable quantity** 100 lbs / 45.4 kg Packages of less than the reportable quantity are not subject to Hazmat transportation requirements. **Packaging instruction** **Passenger aircraft** Quantity limitation: Forbidden. Packaging instructions: 304, 314, 315 **Cargo aircraft** Quantity limitation: Forbidden. **Special provisions** 13, T50	**Special provisions** 23	The marine pollutant mark is not required when transported in sizes of ≤5 L or ≤5 kg.	The environmentally hazardous substance mark may appear if required by other transportation regulations.

Special precautions for user : Ammonia shipments between the U.S. and Canada, including empty or residue railcars or trucks is regulated under agreement. Changes in Transport Canada's Transportation of Dangerous Goods Regulations has changed the classification of Anhydrous Ammonia from Class 2.2 to Class 2.3. **Shipment of anhydrous ammonia within Canada using the DOT green 2.2 Placard are prohibited. Shipments originating in Canada going to the United States are to be placarded with the White UN 1005 Anhydrous Ammonia Placard.** DOT rules allow shipments to proceed between the U.S. and Canada with this placard. **Domestic shipments within the U.S. must continue to use the green DOT 2.2**

Section 14. Transport information

Non-flammable compressed gas placard.

Transport in bulk according to Annex II of MARPOL and the IBC Code : Not available.

Section 15. Regulatory information

Canadian lists

Canadian NPRI : The following components are listed: Ammonia (total)

CEPA Toxic substances : The following components are listed: Ammonia dissolved in water

Canada inventory : All components are listed or exempted.

International regulations

Chemical Weapon Convention List Schedules I, II & III Chemicals

Not listed.

Montreal Protocol (Annexes A, B, C, E)

Not listed.

Stockholm Convention on Persistent Organic Pollutants

Not listed.

Rotterdam Convention on Prior Informed Consent (PIC)

Not listed.

UNECE Aarhus Protocol on POPs and Heavy Metals

Not listed.

Inventory list

Australia : All components are listed or exempted.

China : All components are listed or exempted.

Europe : All components are listed or exempted.

Japan : All components are listed or exempted.

Malaysia : All components are listed or exempted.

New Zealand : All components are listed or exempted.

Philippines : All components are listed or exempted.

Republic of Korea : All components are listed or exempted.

Taiwan : All components are listed or exempted.

Turkey : Not determined.

U.S. Federal Regulations: : **TSCA 8(a) CDR Exempt/Partial exemption**: Not determined
TSCA 8(b) Active inventory: All components are listed or exempted.
Clean Water Act (CWA) 311: ammonia, anhydrous
Clean Air Act (CAA) 112 regulated toxic substances: ammonia, anhydrous

Clean Air Act Section 112 (b) Hazardous Air Pollutants (HAPs) : Not listed

Clean Air Act Section 602 Class I Substances : Not listed

Clean Air Act Section 602 Class II Substances : Not listed

DEA List I Chemicals (Precursor Chemicals) : Not listed

Section 15. Regulatory information

DEA List II Chemicals (Essential Chemicals) : Not listed

SARA 302/304 Composition/information on ingredients

Name	%	EHS	SARA 302 TPQ		SARA 304 RQ	
			(lbs)	(gallons)	(lbs)	(gallons)
Ammonia	99.5 - 99.98	Yes.	500	-	100	-

SARA 304 RQ : 100 lbs / 45.4 kg

SARA 311/312

Classification : Fire hazard
Sudden release of pressure
Immediate (acute) health hazard

Composition/information on ingredients

Name	%	Fire hazard	Sudden release of pressure	Reactive	Immediate (acute) health hazard	Delayed (chronic) health hazard.
Ammonia	99.5 - 99.98	Yes.	Yes.	No.	Yes.	No.

SARA 313

	Product name	CAS number	%
Form R - Reporting requirements	Ammonia, anhydrous	7664-41-7	100
Supplier notification	Ammonia, anhydrous	7664-41-7	100

SARA 313 notifications must not be detached from the SDS and any copying and redistribution of the SDS shall include copying and redistribution of the notice attached to copies of the SDS subsequently redistributed.

State regulations

Massachusetts : The following components are listed: Ammonia

New York : The following components are listed: Ammonia

New Jersey : The following components are listed: Ammonia

Pennsylvania : The following components are listed: Ammonia

California Prop. 65 : This product, as manufactured, does NOT contain any substance in concentrations known to the state of California to cause cancer, birth defects or other reproductive harm. Nutrien cannot guarantee the downstream compliance of any product once out of Nutrien custody.

Section 16. Other information

History

Date of issue/Date of revision : 1/22/2019

Date of previous issue : 8/31/2017

Version : 3.1

Indicates information that has changed from previously issued version.
General format change.

Section 16. Other information

Key to abbreviations : ATE = Acute Toxicity Estimate
BCF = Bioconcentration Factor
GHS = Globally Harmonized System of Classification and Labelling of Chemicals
IATA = International Air Transport Association
IBC = Intermediate Bulk Container
IMDG = International Maritime Dangerous Goods
LogPow = logarithm of the octanol/water partition coefficient
MARPOL = International Convention for the Prevention of Pollution From Ships, 1973 as modified by the Protocol of 1978. ("Marpol" = marine pollution)
UN = United Nations
HPR = Hazardous Products Regulations

Procedure used to derive the classification

Classification	Justification
FLAMMABLE GASES - Category 2	Weight of evidence
GASES UNDER PRESSURE - Liquefied gas	Weight of evidence
CORROSIVE TO METALS - Category 1	Weight of evidence
ACUTE TOXICITY (inhalation) - Category 3	Weight of evidence
SKIN CORROSION - Category 1B	Weight of evidence
SERIOUS EYE DAMAGE - Category 1	Weight of evidence

References : Transportation of Dangerous Goods Act and Clear Language Regulations, current edition at time of SDS preparation, Transport Canada;
Hazardous Products Act and Regulations, current revision at time of SDS preparation, Health Canada;
Domestic Substances List, current revision at time of SDS preparation, Environment Canada;
29 CFR Part 1910, current revision at time of SDS preparation, U.S. Occupational Safety and Health Administration;
40 CFR Parts 1-799, current revision at time of SDS preparation, U.S. Environmental Protection Agency;
49 CFR Parts 1-199, current revision at time of SDS preparation, U.S. Department of Transport;
Mexican Official Standard NOM-018-STPS-2015, Harmonised System for the Identification and Communication of Hazards and Risks by Hazardous Chemicals in the Workplace;
NORMA Oficial Mexicana NOM-010-STPS-2014, Agentes químicos contaminantes del ambiente laboral-Reconocimiento, evaluación y control.
Mexican Official Standard NOM-002-SCT / 2011, List of the most commonly transported hazardous substances and materials;
Threshold Limit Values for Chemical Substances, current edition at time of SDS preparation, American Conference of Governmental Industrial Hygienists;
NFPA 400, National Fire Codes, National Fire Protection Association, current edition at time of SDS preparation;
NFPA 704, National Fire Codes, National Fire Protection Association, current edition at time of SDS preparation;
Corrosion Data Survey, Sixth Edition, 1985, National Association of Corrosion Engineers;
ERG 2016, Emergency Response Guidebook, U.S. Department of Transport, Transport Canada, and the Secretariat of Transportation and Communications of Mexico
Hazardous Substances Data Bank, current revision at time of SDS preparation, National Library of Medicine, Bethesda, Maryland
Integrated Risk Information System, current revision at time of SDS preparation, U. S. Environmental Protection Agency, Washington, D.C.
Pocket Guide to Chemical Hazards, current revision at time of SDS preparation, National Institute for Occupational Safety and Health, Cincinnati, Ohio ;
Agency for Toxic Substances and Disease Registry Databank, current revision at time of SDS preparation, U.S. Department of Health and Human Services, Atlanta, Georgia
National Toxicology Program, Report on Carcinogens, Division of the National Institute of Environmental Health Sciences, Research Triangle Park, North Carolina.
Registry of Toxic Effects of Chemical Substances. National Institute for

Section 16. Other information

Occupational Safety and Health, Cincinnati, Ohio
California Code of Regulations, Title 27, Div 4, Chapter 1, Proposition 65 Aug 30, 2018 rev and current updates
The Fertilizer Institute, Product Toxicology Testing Program Results, TFI, Washington , D.C., 2003

Notice to reader

DISCLAIMER AND LIMITATION OF LIABILITY

The information and recommendations contained in this Safety Data Sheet ("SDS") relate only to the specific material referred to herein (the "Material") and do not relate to the use of such Material in combination with any other material or process. The information and recommendations contained herein are believed to be current and correct as of the date of this SDS. HOWEVER, THE INFORMATION AND RECOMMENDATIONS ARE PRESENTED WITHOUT WARRANTY, REPRESENTATION OR LICENSE OF ANY KIND, EXPRESS OR IMPLIED, WITH RESPECT TO THEIR ACCURACY, CORRECTNESS OR COMPLETENESS, AND THE SELLER, SUPPLIER AND MANUFACTURER OF THE MATERIAL AND THEIR RESPECTIVE AFFILIATES (COLLECTIVELY, THE "SUPPLIER") DISCLAIM ALL LIABILITY FOR RELIANCE ON SUCH INFORMATION AND RECOMMENDATIONS. This SDS is not a guarantee of safety. A buyer or user of the Material (a "Recipient") is responsible for ensuring that it has all current information necessary to safely use the Material for its specific purpose.

FURTHERMORE, THE RECIPIENT ASSUMES ALL RISK IN CONNECTION WITH THE USE OF THE MATERIAL. THE RECIPIENT ASSUMES ALL RESPONSIBILITY FOR ENSURING THE MATERIAL IS USED IN A SAFE MANNER IN COMPLIANCE WITH APPLICABLE ENVIRONMENTAL, HEALTH, SAFETY AND SECURITY LAWS, POLICIES AND GUIDELINES. THE SUPPLIER DOES NOT WARRANT THE MERCHANTABILITY OF THE MATERIAL OR THE FITNESS OF THE MATERIAL FOR ANY PARTICULAR USE AND ASSUMES NO RESPONSIBILITY FOR INJURY OR DAMAGE CAUSED DIRECTLY OR INDIRECTLY BY OR RELATED TO THE USE OF THE MATERIAL.

SAFETY DATA SHEET

SECTION 1 - SUBSTANCE IDENTITY AND COMPANY INFORMATION

Product Name: Various Microbial Cultures at Biosafety Level 1 or 2 or 3
ATCC Catalog #: Various

COMPANY INFORMATION:
AMERICAN TYPE CULTURE COLLECTION
PO BOX 1549
MANASSAS, VA 20108

FOR INFORMATION CALL: 800-638-6597 or 703-365-2700
AFTER-HOURS CONTACT: 703-365-2710
CHEMTREC EMERGENCY: 800-424-9300 or 703-527-3887

SECTION 2 - COMPOSITION/INFORMATION ON INGREDIENTS

Either freeze dried, frozen or growing cells shipped in liquid cell culture medium (a mixture of components that may include, but is not limited to: inorganic salts, vitamins, amino acids, carbohydrates and other nutrients dissolved in water). Frozen Cultures may also contain a 5%-10% solution of Dimethyl sulfoxide as a cryoprotectant.

SECTION 3 - HAZARD IDENTIFICATION

HMIS Rating: N/A
NFPA Rating: N/A

This substance is not hazardous as defined by OSHA 29CFR 1910.1200 however this product should be handled according to good lab practices, with proper personal protective equipment, proper engineering controls and within the parameters of the purchaser's safety program.

Health Hazards

ATCC recommends that all ATCC microbial cultures be handled by qualified microbiologists using appropriate safety procedures and precautions. Detailed discussions of laboratory safety procedures are provided in **Laboratory Safety: Principles and Practice** (Fleming et al) and in the U.S. Government Publication, **Biosafety in Microbiological and Biomedical Laboratories.** This publication is available in its entirety in the Center for Disease Control Office of Health and Safety's web site at http://www.cdc.gov/biosafety/publications/bmbl5/index.htm .

Information on the classification of human etiologic agents on the basis of hazard can be found as Appendix B in the NIH **Guidelines for Research Involving Recombinant DNA Molecules** at http://grants.nih.gov/grants/policy/recombinentdnaguidelines.htm.

SECTION 4 - FIRST AID MEASURES

Report to your Safety Office and Seek Medical Attention as Soon as Possible

Ingestion: If person is unconscious seek emergency medical attention; never give anything by mouth to an unconscious person.
If the person is conscious wash mouth out with water and call a physician then administer three cups of water. Do not induce vomiting unless directed to do so by a physician.

Inhalation: If person is unconscious seek emergency medical attention, if person is conscious remove to fresh air and call a physician.

Dermal exposure: Immediately wash skin with water followed by washing with soap and water. Remove all contaminated clothing.

Eye exposures: Flush eyes with water for at least 15 minutes with eyelids separated and call a physician.

SECTION 5 - FIRE FIGHTING MEASURES

Flammability: Data not available
Suitable Extinguishing Media: Water spray, carbon dioxide, dry chemical powder, Halon (where regulations permit), or appropriate foam.
Firefighting Protective Equipment: Wear self-contained breathing apparatus and protective clothing to prevent inhalation, ingestion, skin and eye contact.
Specific Hazard(s): Responders should take into consideration the biohazard risk associated with responding to a fire in the area where the material may be stored or handled.

SECTION 6 - ACCIDENTAL RELEASE MEASURES

Procedure(s) of Personal Precaution(s): At a minimum use PPE listed in Section 8.
Wear laboratory coat, gloves and eye protection. Avoid all contact.
Methods for Cleaning Up
Patient/Victim: Wash with soap and water. Work clothes should be laundered separately. Launder contaminated clothing before re-use. Do not take clothing home.
Equipment/Environment: Allow aerosols to settle; wearing protective clothing, gently cover spill with paper towel and apply 1% sodium hypochlorite, starting at perimeter and working towards the center; allow sufficient contact time before cleanup (30 min).

Note: The use of additional PPE may be necessary for cleaning solutions.

SECTION 7 - HANDLING AND STORAGE

Handle and store according to instructions on product information sheet and label.
Special Requirements:

SAFETY DATA SHEET

Follow established laboratory procedures when handling material.

SECTION 8 - EXPOSURE CONTROLS/PERSONAL PROTECTION

Use Personal Protective Equipment: Including Eye Protection, Chemical Resistant Gloves, and appropriate clothing to prevent skin exposure. In addition, a Respiratory protection program that complies with OSHA 29 CFR 1910.134 and ANSI Z88.2 requirements or European Standard EN 149 must be followed whenever workplace conditions warrant respirator use.

Engineering Controls: The use and storage of this material requires user to maintain and make available appropriate eyewash and safety shower facilities. Use appropriate ventilation method to keep airborne concentrations a low as possible.

Exposure Limits: No exposure limits for this material have been established by ACGIH, NIOSH, or OSHA.

SECTION 9 - PHYSICAL AND CHEMICAL PROPERTIES

Data Not Available

SECTION 10 - STABILITY AND REACTIVITY

Hazardous polymerization will not occur.

SECTION 11 - TOXICOLOGICAL INFORMATION

Route of Exposure

Eye Contact: Data not available.
Skin Contact: Data not available.
Skin Absorption: Data not available.
Inhalation: Data not available.
Ingestion: Data not available.
Parenteral Exposure: Data not available.

Sensitization
Skin: Data not available
Respiratory: Data not available

Target Organ(s) or System(s): Data not available
Signs and Symptoms of Exposure
Skin and Mucous Membranes: Data not available
Respiratory: Data not available
Gastrointestinal: Data not available

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Toxicity Data:	Data not available
Effects of Long Term or Repeated Exposure:	Data not available
Chronic Exposure–Teratogen:	Data not available
Chronic Exposure–Mutagen:	Data not available
Chronic Exposure–Reproductive Hazard:	Data not available

SECTION 12 - ECOLOGICAL INFORMATION

No ecological information available.

SECTION 13 - DISPOSAL CONSIDERATIONS

Decontaminate all wastes before disposal (steam sterilization, chemical disinfection, and/or incineration).

Dispose of in accordance with applicable regulations.

SECTION 14 - TRANSPORT INFORMATION

Contact ATCC for transport information.

SECTION 15 - REGULATORY INFORMATION

Contact ATCC for regulatory information.

SECTION 16 - OTHER INFORMATION

DATE REVISED: JUNE 1, 2016

THE INFORMATION PRESENTED IN THIS DOCUMENT IS BELIEVED TO BE CORRECT BASED UPON DATA AVAILABLE TO ATCC. USERS SHOULD MAKE AN INDEPENDENT DECISION REGARDING THE ACCURACY OF THIS INFORMATION BASED ON THEIR NEEDS AND DATA AVAILABLE TO THEM. ALL SUBSTANCES AND MIXTURES MAY PRESENT UNKNOWN HAZARDS AND ALL NECESSARY SAFETY PRECAUTIONS SHOULD BE TAKEN. ATCC ASSUMES NO LIABILITY RESULTING FROM USING OR COMING IN CONTACT WITH THIS SUBSTANCE.

Date of Issue:
17 April 2015

SAFETY DATA SHEET

1. SUBSTANCE AND SOURCE IDENTIFICATION

Product Identifier

SRM Number: 2186-II
SRM Name: Disodium Hydrogen Phosphate
Other Means of Identification: Not applicable.

Recommended Use of This Material and Restrictions of Use

This Standard Reference Material (SRM) is intended for use in calibration of pH meters to be used for the measurement of pD in deuterium oxide. A unit of SRM 2186-II consists of one bottle containing 30 g of disodium hydrogen phosphate powder.

Company Information

National Institute of Standards and Technology
Standard Reference Materials Program
100 Bureau Drive, Stop 2300
Gaithersburg, Maryland 20899-2300

Telephone: 301-975-2200
FAX: 301-948-3730
E-mail: SRMMSDS@nist.gov
Website: http://www.nist.gov/srm

Emergency Telephone ChemTrec:
1-800-424-9300 (North America)
+1-703-527-3887 (International)

2. HAZARDS IDENTIFICATION

Classification

Physical Hazard: Not classified.
Health Hazard: Not classified.

Label Elements

Symbol: No Symbol
Signal Word: No Signal Word

Hazard Statement(s): Not applicable.

Precautionary Statement(s): Not applicable.

Hazards Not Otherwise Classified: Not applicable.

Ingredients(s) with Unknown Acute Toxicity: Not applicable.

3. COMPOSITION AND INFORMATION ON HAZARDOUS INGREDIENTS

Substance: Disodium hydrogen phosphate

Other Designations: Sodium phosphate, dibasic; disodium phosphate; disodium acid orthophosphate; soda phosphate; disodium phosphoric acid; Na_2HPO_4

Components are listed in compliance with OSHA's 29 CFR 1910.1200; for the actual values see the Certificate of Analysis.

Hazardous Component(s)	CAS Number	EC Number (EINECS)	Nominal Mass Concentration (%)
Disodium hydrogen phosphate	7558-79-4	231-448-7	100

4. First Aid Measures

Description of First Aid Measures:

Inhalation: If adverse effects occur, remove to uncontaminated area. If not breathing, give artificial respiration or oxygen by qualified personnel. Seek immediate medical attention.

Skin Contact: Wash skin with soap and water for at least 15 minutes.

Eye Contact: Flush eyes with water for at least 15 minutes. If necessary, seek medical attention.

Ingestion: If a large amount is swallowed, seek medical attention.

Most Important Symptoms/Effects, Acute and Delayed: May cause irritation of the eyes, respiratory system, and skin.

Indication of any immediate medical attention and special treatment needed, if necessary: If any of the above symptoms are present, seek medical attention if needed.

5. Fire Fighting Measures

Fire and Explosion Hazards: Negligible fire hazard. See Section 9, "Physical and Chemical Properties" for flammability properties.

Extinguishing Media:
Suitable: Use extinguishing media appropriate for the surrounding area.
Unsuitable: None listed.

Specific Hazards Arising from the Chemical: None listed.

Special Protective Equipment and Precautions for Fire-Fighters: Avoid inhalation of material or combustion byproducts. Wear full protective clothing and NIOSH approved self-contained breathing apparatus (SCBA).

NFPA Ratings (0 = Minimal; 1 = Slight; 2 = Moderate; 3 = Serious; 4 = Severe)

Health = 1 Fire = 0 Reactivity = 0

6. Accidental Release Measures

Personal Precautions, Protective Equipment and Emergency Procedures: Keep unnecessary personnel away. Use suitable protective equipment; see Section 8, "Exposure Controls and Personal Protection".

Methods and Materials for Containment and Clean up: Notify safety personnel of spills. Collect spilled material in appropriate container for disposal. Avoid generating dust. Clean up residue with a high-efficiency particulate filter vacuum.

7. Handling and Storage

Safe Handling Precautions: Avoid generating dust. See Section 8, "Exposure Controls and Personal Protection".

Storage: Store and handle in accordance with all current regulations and standards. Keep separated from incompatible substances (see Section 10, "Stability and Reactivity").

8. Exposure Controls and Personal Protection

Exposure Limits: This material is a particulate matter and adequate inhalation/respiratory protection should be used to minimize exposure. No occupational exposure limits have been established for disodium hydrogen phosphate. The exposure limits for Particulates Not Otherwise Regulated are applicable.

OSHA (PEL): 15 mg/m^3 (TWA, total particulates)
5 mg/m^3 (TWA, respirable particulates)

Engineering Controls: Provide local exhaust or process enclosure ventilation system. Ensure compliance with applicable exposure limits.

Personal Protection: In accordance with OSHA 29 CFR 1910.132, subpart I, wear appropriate Personal Protective Equipment (PPE) to minimize exposure to this material.

Respiratory Protection: If workplace conditions warrant a respirator, a respiratory protection program that meets OSHA 29CFR 1910.134 must be followed. Refer to NIOSH 42 CFR 84 for applicable certified respirators.

Eye/Face Protection: Wear splash resistant safety goggles with a face shield. An eyewash station should be readily available near areas of use.

Skin and Body Protection: Personal protective equipment for the body should be selected based on the task being performed and the risks involved and should be approved by a specialist before handling this product. Chemical-resistant gloves should be worn at all times when handling chemicals.

9. PHYSICAL AND CHEMICAL PROPERTIES

Descriptive Properties

Appearance (physical state, color, etc.):	colorless, white powder
Molecular Formula:	Na_2HPO_4
Molar Mass (g/mol):	141.96
Odor:	odorless
Odor threshold:	not available
pH:	9.1 (1 % solution)
Evaporation rate:	not applicable
Melting point/freezing point:	not available
Relative Density as specific gravity (water = 1):	2.066 at 16 °C (dihydrate)
Vapor Pressure (mmHg):	not applicable
Vapor Density (air = 1):	not applicable
Viscosity (cP):	not applicable
Solubility(ies):	soluble in water (12.5 %); very slight soluble in alcohol
Partition coefficient (n-octanol/water):	not available
Particle Size:	not available

Thermal Stability Properties

Autoignition Temperature:	not available
Thermal Decomposition:	not available
Initial boiling point and boiling range:	not available
Explosive Limits, LEL (Volume %):	not available
Explosive Limits, UEL (Volume %):	not available
Flash Point (°C):	not available
Flammability (solid, gas):	not available

10. STABILITY AND REACTIVITY

Reactivity: Stable at normal temperatures and pressure.

Stability: X Stable _____ Unstable

Possible Hazardous Reactions: No data available.

Conditions to Avoid: Generating dust.

Incompatible Materials: Metals and acids.

Fire/Explosion Information: See Section 5, "Fire Fighting Measures".

Hazardous Decomposition: Thermal decomposition will produce sodium monoxide and oxides of phosphorus.

Hazardous Polymerization: _____ Will Occur X Will Not Occur

11. TOXICOLOGICAL INFORMATION

Route of Exposure: X Inhalation X Skin _____ Ingestion

Symptoms Related to the Physical, Chemical and Toxicological Characteristics: May cause irritation of the eyes, respiratory system, and skin.

Potential Health Effects (Acute, Chronic and Delayed):

Inhalation: Acute: irritation; chronic: no information available.

Skin Contact: Acute and chronic: irritation, chronic exposure may also cause dermatitis.

Eye Contact: Acute: mild irritation; chronic: no information available.

Ingestion: Acute: irritation, changes in blood pressure, nausea, vomiting, diarrhea, stomach pain, irregular heartbeat, bluish skin color, blood disorders, and coma; chronic: no information available.

Numerical Measures of Toxicity:

Acute Toxicity: Not classified.
Rat, Oral LD50: 17 g/kg

Skin Corrosion/Irritation: Not classified.
Rabbit, Skin: 500 mg (24 h) - mild

Serious Eye Damage/ Eye Irritation: Not classified.
Rabbit, Eyes: 500 mg (24 h) - mild

Respiratory Sensitization: Not classified; no data available.

Skin Sensitization: Not classified; no data available.

Germ Cell Mutagenicity: Not classified; no data available.

Carcinogenicity: Not classified.

Listed as a Carcinogen/Potential Carcinogen _____ Yes X No
Sodium hydrogen phosphate is not listed by NTP, IARC or OSHA as a carcinogen/potential carcinogen.

Reproductive Toxicity: Not classified; no data available.

Specific Target Organ Toxicity, Single Exposure: Not classified; no data available.

Specific Target Organ Toxicity, Repeated Exposure: Not classified; no data available.

Aspiration Hazard: Not classified; no data available.

12. ECOLOGICAL INFORMATION

Ecotoxicity Data:

Invertebrate: Water flea (*Daphnia magna*) LC50 (freshwater, static, 21 °C to 25 °C): 1154 mg/L (25 h)

Persistence and Degradability: No data available.

Bioaccumulative Potential: No data available.

Mobility in Soil: No data available.

Other Adverse effects: No data available.

13. DISPOSAL CONSIDERATIONS

Waste Disposal: Dispose of waste in accordance with all applicable federal, state, and local regulations.

14. TRANSPORTATION INFORMATION

U.S. DOT and IATA: Not regulated by DOT or IATA.

15. REGULATORY INFORMATION

U.S. Regulations:

CERCLA Sections 102a/103 (40 CFR 302.4): 5000 lbs (2270 kg) final RQ

SARA Title III Section 302 (40 CFR 355.30): Not regulated.

SARA Title III Section 304 (40 CFR 355.40): Not regulated.

SARA Title III Section 313 (40 CFR 372.65): Not regulated.

OSHA Process Safety (29 CFR 1910.119): Not regulated.

SARA Title III Sections 311/312 Hazardous Categories (40 CFR 370.21):

ACUTE HEALTH:	No.
CHRONIC HEALTH:	No.
FIRE:	No.
REACTIVE:	No.
PRESSURE:	No.

State Regulations: California Proposition 65: Not listed.

U.S. TSCA Inventory: Listed.

TSCA 12(b), Export Notification: Not listed.

Canadian Regulations: WHMIS Information: Not provided for this material.

16. OTHER INFORMATION

Issue Date: 17 April 2015

Sources: ChemAdvisor, Inc., SDS *Sodium Phosphate, Dibasic*, 20 March 2015.

CDC; NIOSH; *NIOSH Pocket Guide to Chemical Hazards*; Department of Health and Human Services (DHHS), Centers for Disease Control and Prevention (CDC), National Institute for Safety and Health; *Particulates not otherwise regulated*, 04 April 2011; available at http://www.cdc.gov/niosh/npg/npgd0480.html (accessed Apr 2015).

Hazardous Substances Data Bank (HSDB), National Library of Medicine's TOXNET system, *Disodium Hydrogen Phosphate CAS No. 7558-79-4*; available at http://toxnet.nlm.nih.gov (accessed Apr 2015).

Key of Acronyms:

ACGIH	American Conference of Governmental Industrial Hygienists	NRC	Nuclear Regulatory Commission
ALI	Annual Limit on Intake	NTP	National Toxicology Program
CAS	Chemical Abstracts Service	OSHA	Occupational Safety and Health Administration
CERCLA	Comprehensive Environmental Response, Compensation, and Liability Act	PEL	Permissible Exposure Limit
CFR	Code of Federal Regulations	RCRA	Resource Conservation and Recovery Act
DOT	Department of Transportation	REL	Recommended Exposure Limit
EC50	Effective Concentration, 50 %	RM	Reference Material
EINECS	European Inventory of Existing Commercial Chemical Substances	RQ	Reportable Quantity
EPCRA	Emergency Planning and Community Right-to-Know Act	RTECS	Registry of Toxic Effects of Chemical Substances
IARC	International Agency for Research on Cancer	SARA	Superfund Amendments and Reauthorization Act
IATA	International Air Transportation Agency	SCBA	Self-Contained Breathing Apparatus
IDLH	Immediately Dangerous to Life and Health	SRM	Standard Reference Material
LC50	Lethal Concentration, 50 %	STEL	Short Term Exposure Limit
LD50	Lethal Dose, 50 %	TLV	Threshold Limit Value
LEL	Lower Explosive Limit	TPQ	Threshold Planning Quantity
MSDS	Material Safety Data Sheet	TSCA	Toxic Substances Control Act
NFPA	National Fire Protection Association	TWA	Time Weighted Average
NIOSH	National Institute for Occupational Safety and Health	UEL	Upper Explosive Limit
NIST	National Institute of Standards and Technology	WHMIS	Workplace Hazardous Materials Information System

Disclaimer: Physical and chemical data contained in this SDS are provided only for use in assessing the hazardous nature of the material. The SDS was prepared carefully, using current references; however, NIST does not certify the data in the SDS. The certified values for this material are given in the NIST Certificate of Analysis.

Users of this SRM should ensure that the SDS in their possession is current. This can be accomplished by contacting the SRM Program: telephone (301) 975-2200; fax (301) 948-3730; e-mail srmmsds@nist.gov; or via the Internet at http://www.nist.gov/srm.

Date of Issue:
26 January 2017

SAFETY DATA SHEET

1. SUBSTANCE AND SOURCE IDENTIFICATION

Product Identifier

SRM Number: 200b
SRM Name: Potassium Dihydrogen Phosphate (Fertilizer Standard)
Other Means of Identification: Not applicable.

Recommended Use of This Material and Restrictions of Use

This Standard Reference Material (SRM) is a highly purified and homogeneous lot of crystalline potassium dihydrogen phosphate (KH_2PO_4). It is intended primarily for use as a working standard in the calibration and standardization of procedures employed in the fertilizer industry for the determination of potassium and phosphorus. A unit of SRM 200b consists of one bottle containing 90 g of crystalline potassium dihydrogen phosphate.

Company Information

National Institute of Standards and Technology
Standard Reference Materials Program
100 Bureau Drive, Stop 2300
Gaithersburg, Maryland 20899-2300

Telephone: 301-975-2200
FAX: 301-948-3730
E-mail: SRMMSDS@nist.gov
Website: http://www.nist.gov/srm

Emergency Telephone ChemTrec:
1-800-424-9300 (North America)
+1-703-527-3887 (International)

2. HAZARDS IDENTIFICATION

Classification

Physical Hazard: Not classified.
Health Hazard: Not classified.

Label Elements
Symbol
No Symbol/Pictogram

Signal Word
No signal word.

Hazard Statement(s):
No hazard statements.

Precautionary Statement(s):
No precautionary statements

Hazards Not Otherwise Classified: Not applicable.

Ingredients(s) with Unknown Acute Toxicity: Not applicable.

3. COMPOSITION AND INFORMATION ON HAZARDOUS INGREDIENTS

Substance: Potassium dihydrogen phosphate

Other Designations: Potassium phosphate monobasic; potassium acid phosphate; potassium diphosphate; potassium orthophosphate.

Components are listed in compliance with OSHA's 29 CFR 1910.1200; for the actual values see the NIST Certificate of Analysis.

Hazardous Component(s)	CAS Number	EC Number (EINECS)	Nominal Mass Concentration (%)
Potassium dihydrogen phosphate	7778-77-0	231-913-4	100

4. FIRST AID MEASURES

Description of First Aid Measures:

Inhalation: If adverse effects occur, remove to uncontaminated area. If not breathing, give artificial respiration or oxygen by qualified personnel. Seek immediate medical attention.

Skin Contact: Wash skin with soap and water for at least 15 minutes. Thoroughly clean and dry contaminated clothing before reuse.

Eye Contact: Flush eyes with water for at least 15 minutes. If necessary, seek medical attention.

Ingestion: If a large amount is swallowed, get medical attention.

Most Important Symptoms/Effects, Acute and Delayed: May cause irritation.

Indication of any immediate medical attention and special treatment needed, if necessary: If any of the above symptoms are present, seek medical attention if needed.

5. FIRE FIGHTING MEASURES

Fire and Explosion Hazards: Negligible fire hazard. See Section 9, "Physical and Chemical Properties" for flammability properties.

Extinguishing Media:
Suitable: Use extinguishing agents appropriate for surrounding fire.
Unsuitable: None listed.

Specific Hazards Arising from the Chemical: None listed.

Special Protective Equipment and Precautions for Fire-Fighters: Avoid inhalation of material or combustion byproducts. Wear full protective clothing and NIOSH approved self-contained breathing apparatus (SCBA).

NFPA Ratings (0 = Minimal; 1 = Slight; 2 = Moderate; 3 = Serious; 4 = Severe)

Health = 1 Fire = 0 Reactivity = 0

6. ACCIDENTAL RELEASE MEASURES

Personal Precautions, Protective Equipment and Emergency Procedures: Use suitable protective equipment; see Section 8, "Exposure Controls and Personal Protection".

Methods and Materials for Containment and Clean up: Collect spilled material in appropriate container for disposal. Avoid generating dust.

7. HANDLING AND STORAGE

Safe Handling Precautions: Minimize dust generation. See Section 8, "Exposure Controls and Personal Protection".

Storage: Store the unused portion of this material in the original tightly-capped bottle in a dry environment at normal laboratory temperatures. Store and handling in accordance with all current regulations and standards. Keep separated from incompatible substances (See Section 10, "Stability and Reactivity").

8. EXPOSURE CONTROLS AND PERSONAL PROTECTION

Exposure Limits: No occupational exposure limits have been established for potassium dihydrogen phosphate. This material is a particulate matter and adequate inhalation/respiratory protection should be used to minimize exposure. OSHA Particulates Not Otherwise Regulated (PNOR) exposure limits apply.

OSHA (PEL): 15 mg/m^3 (TWA, total dust)
5 mg/m^3 (TWA, respirable fraction)

NIOSH (REL): 15 mg/m^3 (TWA, total dust)
5 mg/m^3 (respirable fraction)

Engineering Controls: Provide local exhaust or process enclosure ventilation system. Ensure compliance with applicable exposure limits.

Personal Protection: In accordance with OSHA 29 CFR 1910.132, subpart I, wear appropriate Personal Protective Equipment (PPE) to minimize exposure to this material.

Respiratory Protection: If workplace conditions warrant a respirator, a respiratory protection program that meets OSHA 29CFR 1910.134 must be followed. Refer to NIOSH 42 CFR 84 for applicable certified respirators.

Eye/Face Protection: Wear splash resistant safety goggles with a face shield. An eye wash station should be readily available near areas of use.

Skin and Body Protection: Personal protective equipment for the body should be selected based on the task being performed and the risks involved and should be approved by a specialist before handling this product. Chemical-resistant gloves should be worn at all times when handling chemicals.

9. PHYSICAL AND CHEMICAL PROPERTIES

	Potassium Dihydrogen Phosphate
Descriptive Properties:	
Appearance (physical state, color, etc.):	colorless to white, crystalline powder
Molecular Formula:	KH_2PO_4
Molar Mass (g/mol):	136.09
Odor:	odorless
Odor threshold:	not available
pH (solution):	4 to 4.5 (5 %)
Evaporation rate:	not applicable
Melting point/freezing point (ºC):	253 (487.4 ºF)
Relative Density (g/mL):	2.338 (water = 1)
Vapor Pressure (mmHg):	not available
Vapor Density (air = 1):	not available
Viscosity (cP):	not applicable
Solubility(ies):	water soluble (33 % at 25 °C); insoluble in alcohol
Partition coefficient (n-octanol/water):	not available
Particle Size:	not available
Thermal Stability Properties:	
Autoignition Temperature (ºC):	not applicable
Thermal Decomposition (ºC):	not available
Initial boiling point and boiling range (ºC):	not applicable
Explosive Limits, LEL (Volume %):	not applicable
Explosive Limits, UEL (Volume %):	not applicable
Flash Point (ºC):	not applicable
Flammability (solid, gas):	not available

10. STABILITY AND REACTIVITY

Reactivity: Stable at normal temperatures and pressure.

Stability: X Stable _____ Unstable

Possible Hazardous Reactions: None listed.

Conditions to Avoid: Avoid generating dust.

Incompatible Materials: Bases, and metals.

Fire/Explosion Information: See Section 5, "Fire Fighting Measures".

Hazardous Decomposition: Miscellaneous decomposition products.

Hazardous Polymerization: _____ Will Occur X Will Not Occur

11. TOXICOLOGICAL INFORMATION

Route of Exposure: X Inhalation _____ Skin X Ingestion

Symptoms Related to the Physical, Chemical and Toxicological Characteristics: Exposure may cause irritation.

Potential Health Effects (Acute, Chronic and Delayed):

Inhalation: Acute: mild irritation of mucous membranes, with sore throat and cough; chronic: no data available.

Skin Contact: Acute: prolonged contact may cause irritation; chronic: dermatitis.

Eye Contact: Acute: mild irritation; chronic: no data available.

Ingestion: Acute: large doses may cause nausea, diarrhea, cramps; chronic: same symptoms as acute exposure; bone and joint pain are also possible.

Numerical Measures of Toxicity:

Acute Toxicity: Not classified.
Rat, Oral LD50: 3200 mg/kg
Rabbit, Skin LD50: >4640 mg/m^3

Skin Corrosion/Irritation: Not classified. No data available.

Serious Eye damage/Eye irritation: Not classified. No data available.

Respiratory Sensitization: Not classified; no data available.

Skin Sensitization: Not classified; no data available.

Germ Cell Mutagenicity: Not classified; no data available.

Carcinogenicity: Not classified.

Listed as a Carcinogen/Potential Carcinogen _____ Yes X No
Potassium dihydrogen phosphate is not listed by IARC, NTP or OSHA as a carcinogen.

Reproductive Toxicity: Not classified.
Rat, Oral TDLo: 6846 mg/kg (pregnant 1 d to 22 d)

Specific Target Organ Toxicity, Single Exposure: Not classified; no data available.

Specific Target Organ Toxicity, Repeated Exposure: Not classified; no data available.

Aspiration Hazard: Not classified; no data available.

12. ECOLOGICAL INFORMATION

Ecotoxicity Data:

Invertebrate: Polychaete worm (*Capitella capitata*) LC50: 2400 µg/L (28 d), static

Mollusk: Zebra mussel, adult, length 1.5-2.0 cm (*Dreissena polymorpha*) LC50: 137 000 µg/L (24 h) fresh water at 10 °C, pH 7, static

Persistence and Degradability: No data available.

Bioaccumulative Potential: No data available.

Mobility in Soil: No data available.

Other Adverse effects: No data available.

13. DISPOSAL CONSIDERATIONS

Waste Disposal: Dispose of waste in accordance with all applicable federal, state, and local regulations.

14. TRANSPORTATION INFORMATION

U.S. DOT and IATA: Not regulated by DOT or IATA.

15. REGULATORY INFORMATION

U.S. Regulations:

CERCLA Sections 102a/103 (40 CFR 302.4): Not regulated.

SARA Title III Section 302 (40 CFR 355.30): Not regulated.

SARA Title III Section 304 (40 CFR 355.40): Not regulated.

SARA Title III Section 313 (40 CFR 372.65): Not regulated.

OSHA Process Safety (29 CFR 1910.119): Not regulated.

SARA Title III Sections 311/312 Hazardous Categories (40 CFR 370.21):

ACUTE HEALTH:	No.
CHRONIC HEALTH:	No.
FIRE:	No.
REACTIVE:	No.
PRESSURE:	No.

State Regulations:

California Proposition 65: Not listed.

U.S. TSCA Inventory: Listed.

TSCA 12(b), Export Notification: Not listed.

Canadian Regulations:

WHMIS Information: Not provided for this material.

16. OTHER INFORMATION

Issue Date: 26 January 2017

Sources: ChemADVISOR, Inc., MSDS *Potassium Phosphate Monobasic*, 09 December 2015.

Hazardous Substances Data Bank, National Library of Medicine, *Monopotassium Dihydrogen Phosphate* CAS# 7778-77-0, available at https://toxnet.nlm.nih.gov/cgi-bin/sis/htmlgen?HSDB (accessed Jan 2017).

29 CFR Occupational Health and Safety Office (OSHA) 1910.1000, *Limits for Air Contaminants*, Table Z-1; available at http://www.osha.gov/pls/oshaweb/owadisp.show_document?p_table=STANDARDS&p_id=9992 (accessed Jan 2017).

Center for Disease Control (CDC) NIOSH Pocket Guide to Chemical Hazards, *Particulates not otherwise regulated*; available at http://www.cdc.gov/niosh/npg/npgd0480.html (accessed Jan 2017).

Key of Acronyms:

ACGIH	American Conference of Governmental Industrial Hygienists	NIOSH	National Institute for Occupational Safety and Health
ALI	Annual Limit on Intake	NIST	National Institute of Standards and Technology
CAS	Chemical Abstracts Service	NRC	Nuclear Regulatory Commission
CEN	European Committee for Standardization	NTP	National Toxicology Program
CERCLA	Comprehensive Environmental Response, Compensation, and Liability Act	OSHA	Occupational Safety and Health Administration
CFR	Code of Federal Regulations	PEL	Permissible Exposure Limit
CPSU	Coal Mine Dust Personal Sample Unit	RCRA	Resource Conservation and Recovery Act
DOT	Department of Transportation	REL	Recommended Exposure Limit
EC50	Effective Concentration, 50 %	RM	Reference Material
EINECS	European Inventory of Existing Commercial Chemical Substances	RQ	Reportable Quantity
EPCRA	Emergency Planning and Community Right-to-Know Act	RTECS	Registry of Toxic Effects of Chemical Substances
IARC	International Agency for Research on Cancer	SARA	Superfund Amendments and Reauthorization Act
IATA	International Air Transport Association	SCBA	Self-Contained Breathing Apparatus
IDLH	Immediately Dangerous to Life and Health	SRM	Standard Reference Material
ISO	International Organization for Standardization	STEL	Short Term Exposure Limit
LC50	Lethal Concentration, 50 %	TDLo	Toxic Dose Low
LD50	Lethal Dose, 50 %	TLV	Threshold Limit Value
LEL	Lower Explosive Limit	TPQ	Threshold Planning Quantity
MSDS	Material Safety Data Sheet	TSCA	Toxic Substances Control Act
NFPA	National Fire Protection Association	TWA	Time Weighted Average
MSHA	Mine Safety and Health Administration	UEL	Upper Explosive Limit
		WHMIS	Workplace Hazardous Materials Information System

Disclaimer: Physical and chemical data contained in this SDS are provided only for use in assessing the hazardous nature of the material. The SDS was prepared carefully, using current references; however, NIST does not certify the data in the SDS. The certified values for this material are given in the NIST Certificate of Analysis.

Users of this SRM should ensure that the SDS in their possession is current. This can be accomplished by contacting the SRM Program: telephone (301) 975-2200; fax (301) 948-3730; e-mail srmmsds@nist.gov; or via the Internet at http://www.nist.gov/srm.

ISO9001:2000 Certified

Material Safety Data Sheet
Magnesium Sulfate, Heptahydrate, ACS

Section 1 - Chemical Product and Company Identification

MSDS Name:
Magnesium Sulfate, Heptahydrate, ACS
Catalog Numbers:
LC16490
Synonyms:
Epsom salts
Company Identification:
LabChem Inc
200 William Pitt Way
Pittsburgh, PA 15238
Company Phone Number:
(412) 826-5230
Emergency Phone Number:
(800) 424-9300
CHEMTREC Phone Number:
(800) 424-9300

Section 2 - Composition, Information on Ingredients

CAS#	Chemical Name:	Percent
10034-99-8	Magnesium sulfate heptahydrate	100

Section 3 - Hazards Identification

Emergency Overview

Appearance: *Colorless solid*
Caution! May cause eye, skin, and respiratory tract irritation. May cause central nervous system depression. The toxicological properties of this material have not been fully investigated.
Target Organs: Central nervous system, gastrointestinal system

Potential Health Effects

Eye:
May cause mild eye irritation.
Skin:
May cause skin irritation.
Ingestion:
May cause gastrointestinal irritation with nausea, vomiting and diarrhea. The toxicological properties of this substance have not been fully investigated.
Inhalation:
May cause respiratory tract irritation. The toxicological properties of this substance have not been fully investigated.
Chronic:
Exposure to high concentrations may cause central nervous system depression.

Section 4 - First Aid Measures

Eyes:
Flush eyes with plenty of water for at least 15 minutes, occasionally lifting the upper and lower eyelids. Get medical aid.

Skin:
Get medical aid. Flush skin with plenty of water for at least 15 minutes while removing contaminated clothing and shoes. Wash clothing before reuse.

Ingestion:
Never give anything by mouth to an unconscious person. Get medical aid. Do NOT induce vomiting. If conscious and alert, rinse mouth and drink 2-4 cupfuls of milk or water.

Inhalation:
Remove from exposure and move to fresh air immediately. If not breathing, give artificial respiration. If breathing is difficult, give oxygen. Get medical aid.

Notes to Physician:
Treat symptomatically and supportively.

Antidote:
The use of calcium gluconate to precipitate the oxalate should be determined by only qualified medical personnel.

Section 5 - Fire Fighting Measures

General Information:
As in any fire, wear a self-contained breathing apparatus in pressure-demand, MSHA/NIOSH (approved or equivalent), and full protective gear. Substance is noncombustible.

Extinguishing Media:
Use extinguishing media most appropriate for the surrounding fire.

Autoignition Temperature:
Not applicable.

Flash Point:
Not applicable.

NFPA Rating:
CAS# 10034-99-8: H- 1, F- 0, I- 0

Explosion Limits:
Lower: n/a Upper: n/a

Section 6 - Accidental Release Measures

General Information:
Use proper personal protective equipment as indicated in Section 8.

Spills/Leaks:
Vacuum or sweep up material and place into a suitable disposal container. Clean up spills immediately, observing precautions in the Protective Equipment section. Avoid generating dusty conditions. Provide ventilation.

Section 7 - Handling and Storage

Handling:

Wash thoroughly after handling. Remove contaminated clothing and wash before reuse. Use with adequate ventilation. Minimize dust generation and accumulation. Avoid contact with eyes, skin, and clothing. Keep container tightly closed. Avoid ingestion and inhalation.

Storage:

Store in a cool, dry place.

Section 8 - Exposure Controls, Personal Protection

Engineering Controls:

Facilities storing or utilizing this material should be equipped with an eyewash facility and a safety shower. Use adequate ventilation to keep airborne concentrations low.

Exposure Limits:

Chemical Name:	ACGIH	NIOSH	OSHA
Magnesium sulfate heptahydrate	none listed	none listed	none listed

OSHA Vacated PELs:

Magnesium sulfate heptahydrate: No OSHA Vacated PELs are listed for this chemical.

Personal Protective Equipment

Eyes:

Wear appropriate protective eyeglasses or chemical safety goggles as described by OSHA's eye and face protection regulations in 29 CFR 1910.133 or European Standard EN166.

Skin:

Wear appropriate protective gloves to prevent skin exposure.

Clothing:

Wear appropriate protective clothing to prevent skin exposure.

Respirators:

A respiratory protection program that meets OSHA's 29 CFR 1910.134 and ANSI Z88.2 requirements or European Standard EN 149 must be followed whenever workplace conditions warrant respirator use.

Section 9 - Physical and Chemical Properties

Physical State:	Solid
Color:	Transparent to white
Odor:	Odorless
pH:	5.0 – 8.2 (5% solution)
Vapor Pressure:	Not available
Vapor Density:	Not available
Evaporation Rate:	Not available
Viscosity:	Not available
Boiling Point:	Not available
Freezing/Melting Point:	Not available
Decomposition Temperature:	1124°C

Material Safety Data Sheet
Magnesium Sulfate, Heptahydrate, ACS

Solubility in water: Soluble
Specific Gravity/Density: 1.678
Molecular Formula: $MgSO_4 \cdot 7H_2O$
Molecular Weight: 246.48

Section 10 - Stability and Reactivity

Chemical Stability:
Stable under normal temperatures and pressures.
Conditions to Avoid:
Dust generation, excess heat.
Incompatibilities with Other Materials:
No significant incompatibilities identified with common materials and contaminants.
Hazardous Decomposition Products:
Oxides of sulfur.
Hazardous Polymerization:
Will not occur.

Section 11 - Toxicological Information

RTECS:
CAS# 10034-99-8: OM4508000
LD50/LC50:
CAS# 10034-99-8: Not available.
Carcinogenicity:
CAS# 10034-99-8: Not listed by ACGIH, IARC, NTP, or California Prop 65.
California: None
Epidemiology:
No information found
Teratogenicity:
No information found
Reproductive:
No information found
Mutagenicity:
No information found
Neurotoxicity:
No information found

Section 12 - Ecological Information

No information found.

Section 13 - Disposal Considerations

Dispose of in accordance with Federal, State, and local regulations.

Section 14 - Transport Information

US DOT

Shipping Name: Not regulated
Hazard Class:
UN Number:
Packing Group:

Section 15 - Regulatory Information

US Federal

TSCA:

CAS# 10034-99-8 is not on the TSCA Inventory because it is a hydrate. It is considered to be listed if the CAS number for the anhydrous form is on the inventory (40CFR720.3(u)(2)).

SARA Reportable Quantities (RQ):

None of the chemicals in this material have an RQ.

CERCLA/SARA Section 313:

No chemicals are reportable under Section 313.

OSHA - Highly Hazardous:

None of the chemicals in this product are considered highly hazardous by OSHA.

US State

State Right to Know:

CAS# 10034-99-8 is not present on state lists from California, Pennsylvania, Minnesota, Massachusetts, Florida, or New Jersey.

California Regulations:

None of the chemicals in this product are listed.

European/International Regulations

Canadian DSL/NDSL:

CAS# 10034-99-8 is listed on Canada's DSL List.

Canada Ingredient Disclosure List:

CAS# 10034-99-8 is not listed on the Canadian Ingredient Disclosure List

Section 16 - Other Information

MSDS Creation Date: June 28, 2007
Revision Date: None

Information in this MSDS is from available published sources and is believed to be accurate. No warranty, express or implied, is made and LabChem Inc. assumes no liability resulting from the use of this MSDS. The user must determine suitability of this information for his application.

ISO9001:2000 Certified

Material Safety Data Sheet
Calcium chloride, ACS

Section 1 - Chemical Product and Company Identification

MSDS Name:
Calcium chloride, ACS
Catalog Numbers:
LC12725
Synonyms:
Calcium dichloride dihydrate.
Company Identification:
LabChem, Inc.
200 William Pitt Way
Pittsburgh, PA 15238
Company Phone Number:
(412) 826-5230
Emergency Phone Number:
(800) 424-9300
CHEMTREC Phone Number:
(800) 424-9300

Section 2 - Composition, Information on Ingredients

CAS#	Chemical Name:	Percent
10035-04-08	Calcium chloride dihydrate	100%

Section 3 - Hazards Identification

Emergency Overview

Appearance: *white solid*

Warning! May be harmful if swallowed. May cause severe respiratory and digestive tract irritation with possible burns. May cause severe eye and skin irritation with possible burns. May cause cardiac disturbances. Hygroscopic (absorbs moisture from air).

Target Organs: Eyes.

Potential Health Effects

Eye:
May cause severe eye irritation and possible eye burns.
Skin:
Causes skin irritation and possible burns, especially if the skin is wet or moist.
Ingestion:
May cause severe gastrointestinal irritation with nausea, vomiting and possible burns. May cause cardiac disturbances. May be harmful if swallowed. In very severe cases, seizures, rapid respiration, slow heartbeat, or death may result.
Inhalation:
May cause severe irritation of the upper respiratory tract with pain, burns, and inflammation.

Chronic:
Effects may be delayed.

Section 4 - First Aid Measures

Eyes:
Immediately flush eyes with plenty of water for at least 15 minutes, occasionally lifting the upper and lower lids until no evidence of chemical remains. Get medical aid at once.

Skin:
Immediately flush skin with plenty of soap and water for at least 15 minutes while removing contaminated clothing and shoes. Get medical aid.

Ingestion:
Do not induce vomiting. If victim is conscious, give 2-4 glasses of water or milk. Get medical aid at once.

Inhalation:
Give artificial respiration if necessary. Move victim to fresh air. Keep victim warm and at rest. Get medical aid at once. Do not use mouth-to-mouth resuscitation.

Notes to Physician:
Treat symptomatically and supportively.

Section 5 - Fire Fighting Measures

General Information:
As in any fire, wear a self-contained breathing apparatus in pressure-demand, MSHA/NIOSH (approved or equivalent), and full protective gear.

Extinguishing Media:
For small fires, use dry chemical, carbon dioxide, water spray or alcohol-resistant foam.

Autoignition Temperature:
No information found.

Flash Point:
No information found.

NFPA Rating:
Health-2; flammability-0; reactivity-0

Explosion Limits:
Lower: n/a Upper: n/a

Section 6 - Accidental Release Measures

General Information:
Use proper personal protective equipment as indicated in Section 8.

Spills/Leaks:
Vacuum or sweep up material and place into a suitable disposal container. Clean up spills immediately. Avoid creating airborne particles. Provide ventilation.

Section 7 - Handling and Storage

Handling:

Wash thoroughly after handling. Use with adequate ventilation. Do not get on skin or in eyes. Do not ingest or inhale. Always use cool water when dissolving calcium chloride. Heat evolved is significant. Avoid breathing dust, vapor, mist, or gas.

Storage:

Store capped at room temperature. Protect from heat and incompatibles.

Section 8 - Exposure Controls, Personal Protection

Engineering Controls:

Facilities storing or utilizing this material should be equipped with an eyewash facility and a safety shower. Use adequate general or local exhaust ventilation to keep airborne concentrations below the permissible exposure limits.

Exposure Limits:

Chemical Name:	ACGIH	NIOSH	OSHA
Calcium chloride	none listed	none listed	none listed

OSHA Vacated PELs:

Calcium chloride: No OSHA Vacated PELs are listed.

Personal Protective Equipment

Eyes:

Do not wear contact lenses when working with chemicals. An eye wash fountain should be available in the immediate work area. Wear appropriate protective eyeglasses or chemical safety goggles as described in 29 CFR 1910.133.

Skin:

Wear appropriate protective gloves to prevent skin exposure.

Clothing:

Wear appropriate protective clothing to prevent skin exposure.

Respirators:

Follow the OSHA respirator regulations found in 29 CFR 1910.134. Use a NIOSH/MSHA approved respirator if exposure limits are exceeded or if irritation or other symptoms are experienced.

Section 9 - Physical and Chemical Properties

Physical State: Solid
Color: White
Odor: Odorless
pH: 4.5-8.5 (5% solution at 25°C)
Vapor Pressure: Not available
Vapor Density: Not available

Evaporation Rate:	Not available
Viscosity:	Not available
Boiling Point:	Not available
Freezing/Melting Point:	175°C
Decomposition Temperature:	Not available
Solubility in water:	Soluble
Specific Gravity/Density:	Not available
Molecular Formula:	CaCl2.2H2O
Molecular Weight:	147.01

Section 10 - Stability and Reactivity

Chemical Stability:
Stable under normal storage and handling conditions.
Conditions to Avoid:
Dust generation, excess heat, exposure to water or moist air
Incompatibilities with Other Materials:
Bromine trifluoride, Furan-2-peroxycarboxylic acid. Solutions attack some metals.
Hazardous Decomposition Products:
Calcium oxide, hydrogen chloride.
Hazardous Polymerization:
Has not been reported.

Section 11 - Toxicological Information

RTECS:
CAS# 10035-04-8: EV9810000
LD50/LC50:
CAS# 10035-04-8:
Oral, mouse: LD50 = 1940 mg/Kg;
Oral, rabbit: LD50 = 1384 mg/Kg;
Oral, rat: LD50 = 1 g/Kg.
Carcinogenicity:
CAS# 10035-04-8: Not listed by ACGIH, IARC, NTP, or CA Proposition 65.
Epidemiology:
No information available.
Teratogenicity:
No information available.
Reproductive:
No information available.
Mutagenicity:
Mutagenic effects have occurred in experimental animals.
Neurotoxicity:
No information available.

Section 12 - Ecological Information

No information available.

Section 13 - Disposal Considerations

Dispose of in accordance with Federal, State, and local regulations.

Section 14 - Transport Information

US DOT

Shipping Name: Not regulated.
Hazard Class:
UN Number:
Packing Group:

Section 15 - Regulatory Information

US Federal

TSCA:

CAS# 10035-04-8 is not listed on the TSCA inventory because it is a hydrate. It is considered to be listed if the CAS number for the anhydrous form is on the inventory (40 CFR 720.3(u)(2)).

CAS# 10043-52-4 (anhydrous) is listed on the TSCA inventory. Does not have a Significant New Use Rule.

SARA Reportable Quantities (RQ):

CAS# 10035-04-8 does not have a RQ.

CERCLA/SARA Section 313:

Not reportable under Section 313.

OSHA - Highly Hazardous:

Not considered highly hazardous by OSHA.

US State

State Right to Know:

CAS# 10035-04-8 is not listed on the following state right to know lists: California, Florida, New Jersey, Pennsylvania, Minnesota, and Massachusetts.

California Regulations:

Not listed.

European/International Regulations

Canadian DSL/NDSL:

CAS# 10035-04-8 is listed on Canada's DSL List.

Canada Ingredient Disclosure List:

CAS# 10035-04-8 is not listed on the Ingredient Disclosure List.

Material Safety Data Sheet
Calcium chloride, ACS

Section 16 - Other Information

MSDS Creation Date: July 24, 2006
Revision Date: None

Information in this MSDS is from available published sources and is believed to be accurate. No warranty, express or implied, is made and LabChem Inc. assumes no liability resulting from the use of this MSDS. The user must determine suitability of this information for his application.

Material Safety Data Sheet

Creation Date 21-Dec-2009 **Revision Date** 21-Dec-2009 **Revision Number** 1

1. PRODUCT AND COMPANY IDENTIFICATION

Product Name **Ferrous sulfate heptahydrate**

Cat No. **I146-3; I146-10; I146-500; I146-500LC; I149-3**

Synonyms Iron (II) sulfate heptahydrate (Crystalline/Certified ACS/USP/FCC)

Recommended Use Laboratory chemicals

Company
Fisher Scientific
One Reagent Lane
Fair Lawn, NJ 07410
Tel: (201) 796-7100

Emergency Telephone Number
CHEMTREC®, Inside the USA: 800-424-9300
CHEMTREC®, Outside the USA: 703-527-3887

2. HAZARDS IDENTIFICATION

WARNING!

Emergency Overview

Harmful if swallowed. May cause central nervous system effects. Irritating to eyes and skin. May cause irritation of respiratory tract.

Appearance Blue green **Physical State** Solid **Odor** odorless

Target Organs Eyes, Skin, Central nervous system (CNS), Liver, Kidney, Blood

Potential Health Effects

Acute Effects
Principle Routes of Exposure

Eyes Irritating to eyes.
Skin Irritating to skin. May be harmful in contact with skin.
Inhalation May cause irritation of respiratory tract. May be harmful if inhaled. Inhalation may cause central nervous system effects.
Ingestion Harmful if swallowed. May cause central nervous system effects. Ingestion may cause gastrointestinal irritation, nausea, vomiting and diarrhea.

Chronic Effects Tumorigenic effects have been reported in experimental animals.. Experiments have shown reproductive toxicity effects on laboratory animals. May cause adverse liver effects. May cause adverse kidney effects.

See Section 11 for additional Toxicological information.

Aggravated Medical Conditions No information available.

3. COMPOSITION/INFORMATION ON INGREDIENTS

Haz/Non-haz

Component	CAS-No	Weight %
Iron (II) Sulfate heptahydrate	7782-63-0	> 99
Ferrous sulfate	7720-78-7	-

4. FIRST AID MEASURES

Eye Contact Rinse immediately with plenty of water, also under the eyelids, for at least 15 minutes. Obtain medical attention.

Skin Contact Wash off immediately with plenty of water for at least 15 minutes. Obtain medical attention.

Inhalation Move to fresh air. If breathing is difficult, give oxygen. Do not use mouth-to-mouth resuscitation if victim ingested or inhaled the substance; induce artificial respiration with a respiratory medical device. Get medical attention immediately if symptoms occur.

Ingestion Do not induce vomiting. Call a physician or Poison Control Center immediately.

Notes to Physician Treat symptomatically.

5. FIRE-FIGHTING MEASURES

Flash Point Not applicable
Method No information available.

Autoignition Temperature No information available.

Explosion Limits
Upper No data available
Lower No data available

Suitable Extinguishing Media Use water spray, alcohol-resistant foam, dry chemical or carbon dioxide.

Unsuitable Extinguishing Media No information available.

Hazardous Combustion Products No information available.

Sensitivity to mechanical impact No information available.
Sensitivity to static discharge No information available.

Specific Hazards Arising from the Chemical
Thermal decomposition can lead to release of irritating gases and vapors.

Protective Equipment and Precautions for Firefighters
As in any fire, wear self-contained breathing apparatus pressure-demand, MSHA/NIOSH (approved or equivalent) and full protective gear

NFPA **Health** 2 **Flammability** 1 **Instability** 1 **Physical hazards** N/A

6. ACCIDENTAL RELEASE MEASURES

Personal Precautions Use personal protective equipment. Ensure adequate ventilation. Avoid dust formation. Avoid contact with skin, eyes and clothing.

Environmental Precautions Should not be released into the environment.

Methods for Containment and Clean Up Sweep up or vacuum up spillage and collect in suitable container for disposal. Avoid dust formation.

7. HANDLING AND STORAGE

Handling Wear personal protective equipment. Ensure adequate ventilation. Avoid contact with skin, eyes and clothing. Avoid dust formation. Do not breathe dust.

Storage Keep containers tightly closed in a dry, cool and well-ventilated place.

8. EXPOSURE CONTROLS / PERSONAL PROTECTION

Engineering Measures Ensure adequate ventilation, especially in confined areas. Ensure that eyewash stations and safety showers are close to the workstation location.

Exposure Guidelines This product does not contain any hazardous materials with occupational exposure limits established by the region specific regulatory bodies.

NIOSH IDLH: *Immediately Dangerous to Life or Health*

Personal Protective Equipment

Eye/face Protection Wear appropriate protective eyeglasses or chemical safety goggles as described by OSHA's eye and face protection regulations in 29 CFR 1910.133 or European Standard EN166

Skin and body protection Wear appropriate protective gloves and clothing to prevent skin exposure

Respiratory Protection Follow the OSHA respirator regulations found in 29 CFR 1910.134 or European Standard EN 149. Use a NIOSH/MSHA or European Standard EN 149 approved respirator if exposure limits are exceeded or if irritation or other symptoms are experienced

9. PHYSICAL AND CHEMICAL PROPERTIES

Physical State	Solid
Appearance	Blue green
Odor	odorless
Odor Threshold	No information available.
pH	3 - 5 (5 % Solution)
Vapor Pressure	No information available.
Vapor Density	No information available.
Viscosity	No information available.
Boiling Point/Range	300°C / 572°F
Melting Point/Range	64°C / 147.2°F
Decomposition temperature °C	> 300°C
Flash Point	Not applicable
Evaporation Rate	negligible
Specific Gravity	1.898
Solubility	Partly soluble in water
log Pow	No data available

9. PHYSICAL AND CHEMICAL PROPERTIES

Molecular Weight	278.01
Molecular Formula	FeSO4.7H2O

10. STABILITY AND REACTIVITY

Stability	Air sensitive. Moisture sensitive.
Conditions to Avoid	Avoid dust formation. Incompatible products. Excess heat. Exposure to air. Exposure to moist air or water.
Incompatible Materials	Strong oxidizing agents, Strong bases
Hazardous Decomposition Products	Sulfur oxides, Thermal decomposition can lead to release of irritating gases and vapors
Hazardous Polymerization	Hazardous polymerization does not occur
Hazardous Reactions .	None under normal processing.

11. TOXICOLOGICAL INFORMATION

Acute Toxicity

Component Information

Component	LD50 Oral	LD50 Dermal	LC50 Inhalation
Iron (II) Sulfate heptahydrate	1520 mg/kg (Mouse)	Not listed	Not listed
Ferrous sulfate	237 mg/kg (Rat)	Not listed	Not listed

Irritation	Irritating to eyes and skin
Toxicologically Synergistic Products	No information available.

Chronic Toxicity

Carcinogenicity	There are no known carcinogenic chemicals in this product
Sensitization	No information available.
Mutagenic Effects	Mutagenic effects have occurred in experimental animals.
Reproductive Effects	Experiments have shown reproductive toxicity effects on laboratory animals.
Developmental Effects	No information available.
Teratogenicity	Teratogenic effects have occurred in experimental animals..

Other Adverse Effects Tumorigenic effects have been reported in experimental animals.. See actual entry in RTECS for complete information.

Endocrine Disruptor Information No information available

12. ECOLOGICAL INFORMATION

Ecotoxicity

Do not empty into drains.

Component	Freshwater Algae	Freshwater Fish	Microtox	Water Flea
Ferrous sulfate	Not listed	Not listed	Not listed	EC50 48 h 152 mg/L

Persistence and Degradability No information available

Bioaccumulation/ Accumulation No information available

Mobility No information available

13. DISPOSAL CONSIDERATIONS

Waste Disposal Methods 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 to ensure complete and accurate classification

14. TRANSPORT INFORMATION

DOT Not regulated

TDG Not regulated

IATA Not regulated

IMDG/IMO Not regulated

15. REGULATORY INFORMATION

International Inventories

Component	TSCA	DSL	NDSL	EINECS	ELINCS	NLP	PICCS	ENCS	AICS	CHINA	KECL
Iron (II) Sulfate heptahydrate	-	-	-	-	-		X	-	X	X	-

15. REGULATORY INFORMATION

Ferrous sulfate	X	X	-	231-753-5	-		X	X	X	X	KE-21121 X

Legend:
X - Listed
E - Indicates a substance that is the subject of a Section 5(e) Consent order under TSCA.
F - Indicates a substance that is the subject of a Section 5(f) Rule under TSCA.
N - Indicates a polymeric substance containing no free-radical initiator in its inventory name but is considered to cover the designated polymer made with any free-radical initiator regardless of the amount used.
P - Indicates a commenced PMN substance
R - Indicates a substance that is the subject of a Section 6 risk management rule under TSCA.
S - Indicates a substance that is identified in a proposed or final Significant New Use Rule
T - Indicates a substance that is the subject of a Section 4 test rule under TSCA.
XU - Indicates a substance exempt from reporting under the Inventory Update Rule, i.e. Partial Updating of the TSCA Inventory Data Base Production and Site Reports (40 CFR 710(B).
Y1 - Indicates an exempt polymer that has a number-average molecular weight of 1,000 or greater.
Y2 - Indicates an exempt polymer that is a polyester and is made only from reactants included in a specified list of low concern reactants that comprises one of the eligibility criteria for the exemption rule.

U.S. Federal Regulations

TSCA 12(b) Not applicable

SARA 313
Not applicable

SARA 311/312 Hazardous Categorization

Acute Health Hazard	No
Chronic Health Hazard	No
Fire Hazard	No
Sudden Release of Pressure Hazard	No
Reactive Hazard	No

Clean Water Act

Component	CWA - Hazardous Substances	CWA - Reportable Quantities	CWA - Toxic Pollutants	CWA - Priority Pollutants
Iron (II) Sulfate heptahydrate	X	-	-	-
Ferrous sulfate	X	1000 lb	-	-

Clean Air Act
Not applicable

OSHA
Not applicable

CERCLA
This material, as supplied, contains one or more substances regulated as a hazardous substance under the Comprehensive Environmental Response Compensation and Liability Act (CERCLA) (40 CFR 302)

Component	Hazardous Substances RQs	CERCLA EHS RQs
Iron (II) Sulfate heptahydrate	1000 lb	-
Ferrous sulfate	1000 lb	-

California Proposition 65
This product does not contain any Proposition 65 chemicals.

State Right-to-Know

Component	Massachusetts	New Jersey	Pennsylvania	Illinois	Rhode Island
Iron (II) Sulfate heptahydrate	X	-	X	-	-
Ferrous sulfate	X	X	X	-	-

U.S. Department of Transportation
Reportable Quantity (RQ): Y
DOT Marine Pollutant N
DOT Severe Marine Pollutant N

U.S. Department of Homeland Security
This product does not contain any DHS chemicals.

Other International Regulations

Mexico - Grade No information available

Canada

This product has been classified in accordance with the hazard criteria of the Controlled Products Regulations (CPR) and the MSDS contains all the information required by the CPR.

WHMIS Hazard Class
D1B Toxic materials
D2B Toxic materials

16. OTHER INFORMATION

Prepared By Regulatory Affairs
Thermo Fisher Scientific
Tel: (412) 490-8929

Reviewed
2013.01.18
14:51:01 -05'00'

Creation Date 21-Dec-2009

Print Date 21-Dec-2009

Revision Summary "***", and red text indicates revision

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End of MSDS

Appendix F: Patents

US009932598B2

(12) **United States Patent**
Palsson et al.

(10) **Patent No.: US 9,932,598 B2**
(45) **Date of Patent: Apr. 3, 2018**

(54) **METABOLIC ENGINEERING OF MICROBIAL ORGANISMS**

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(*) Notice: Subject to any disclaimer, the term of this patent is extended or adjusted under 35 U.S.C. 154(b) by 351 days.

(21) Appl. No.: **13/957,340**

(22) Filed: **Aug. 1, 2013**

(65) **Prior Publication Data**

US 2014/0038296 A1 Feb. 6, 2014

Related U.S. Application Data

(60) Provisional application No. 61/742,218, filed on Aug. 2, 2012.

(51) **Int. Cl.**

C12N 15/70	(2006.01)
C12P 7/56	(2006.01)
C12N 9/02	(2006.01)
C12N 9/10	(2006.01)

(52) **U.S. Cl.**

CPC ***C12N 15/70*** (2013.01); ***C12N 9/0008*** (2013.01); ***C12N 9/1029*** (2013.01); ***C12P 7/56*** (2013.01); ***C12Y 102/04001*** (2013.01); ***C12Y 203/01054*** (2013.01)

(58) **Field of Classification Search**

CPC C12N 15/70; C12N 9/0008; C12N 9/1029; C12Y 102/04001; C12Y 203/01054; C12P 7/56

See application file for complete search history.

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(57) **ABSTRACT**

Microbial strains with desirable carbohydrate productions characteristics and methods of making and using the same are provided herein.

18 Claims, 5 Drawing Sheets

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Figure 1

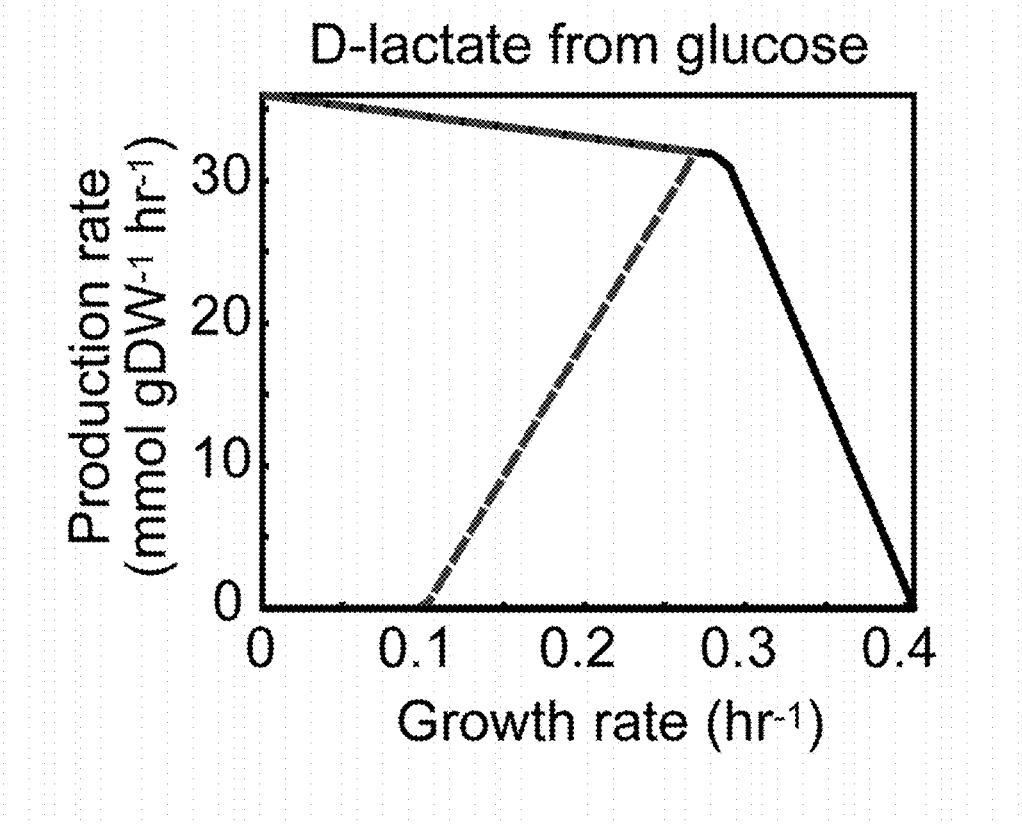

D-lactate from glucose
Production rate (mmol gDW-1 hr-1)
30
20
10
0
0
0.1
0.2
0.3
0.4
Growth rate (hr-1)

Figure 2

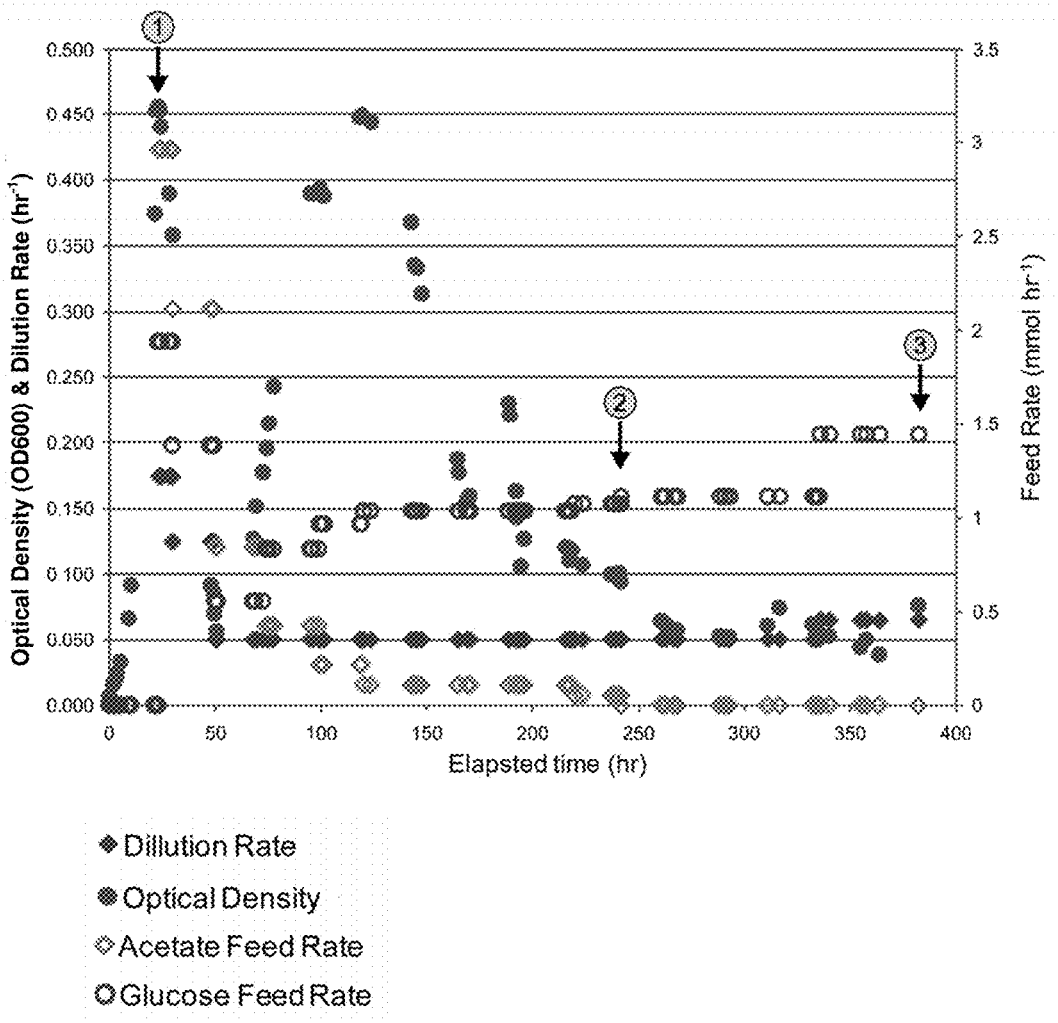

Optical Density (OD600) & Dilution Rate (hr-1)
Feed Rate (mmol hr-1)
Elapsted time (hr)
0.500
0.450
0.400
0.350
0.300
0.250
0.200
0.150
0.100
0.050
0.000
3.5
3
2.5
2
1.5
1
0.5
0
0
50
100
150
200
250
300
350
400
1
2
3
Dillution Rate
Optical Density
Acetate Feed Rate
Glucose Feed Rate

Figure 3

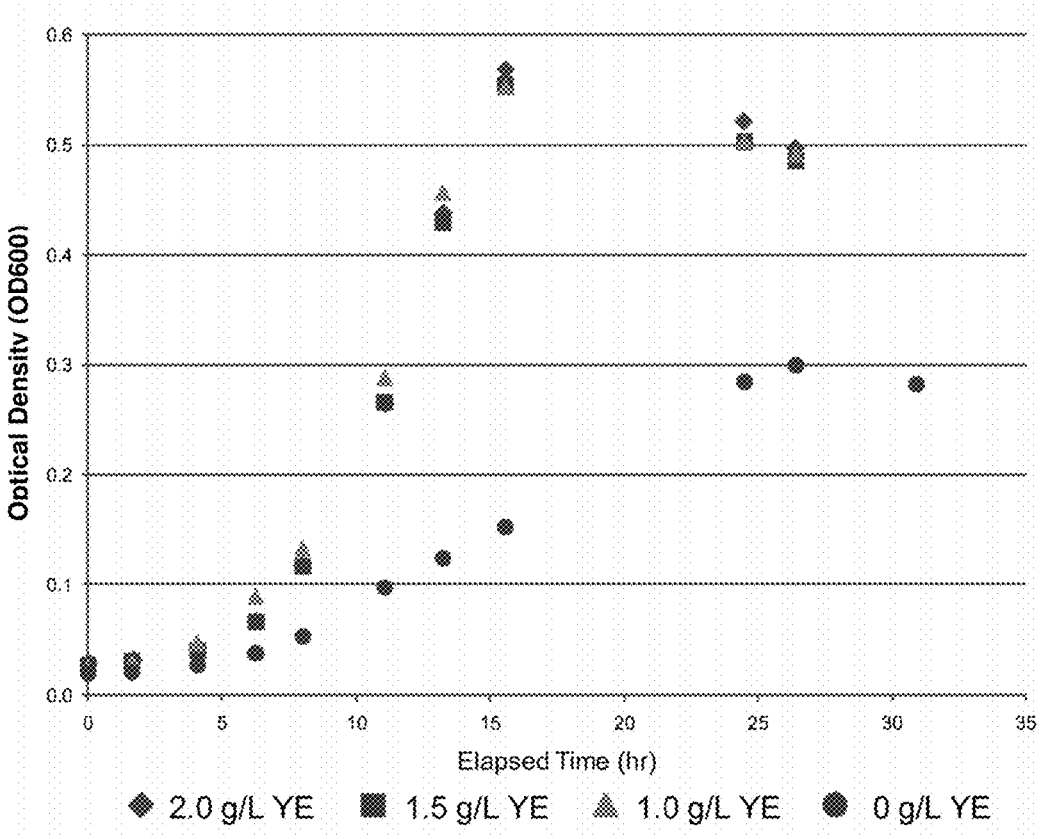

Optical Density (OD600)
0.0
0.1
0.2
0.3
0.4
0.5
0.6
0
5
10
15
20
25
30
35
Elapsed Time (hr)
2.0 g/L YE
1.5 g/L YE
1.0 g/L YE
0 g/L YE

Figure 4

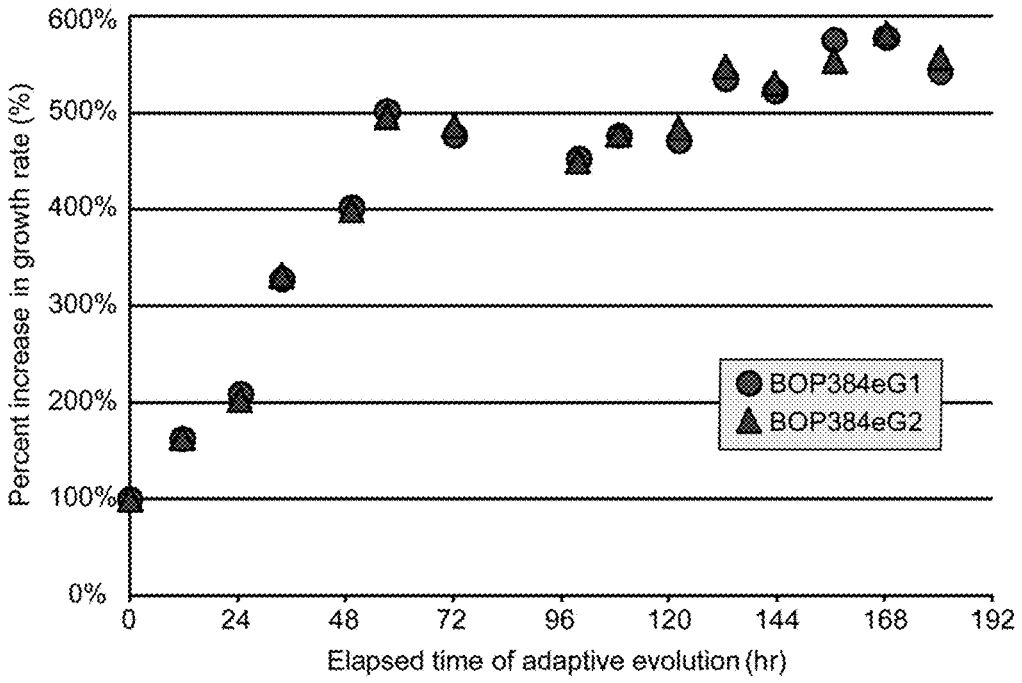
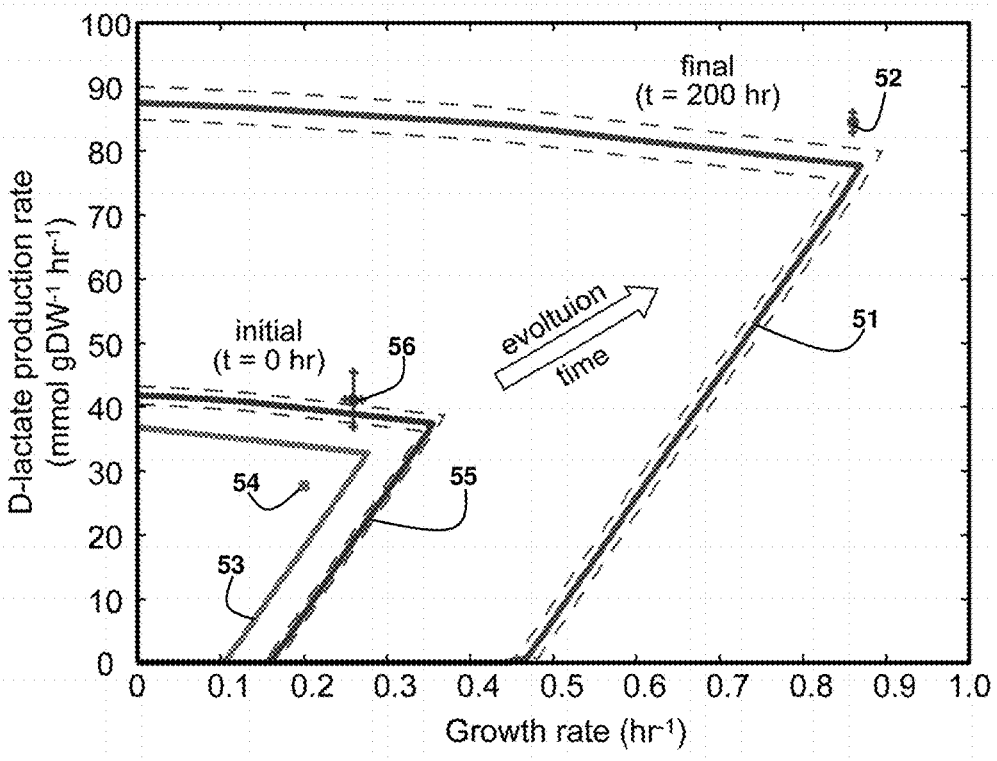

600%
500%
400%
300%
200%
100%
0%
Percent increase in growth rate (%)
0
24
48
72
96
120
144
168
192
Elapsed time of adaptive evolution (hr)
BOP384eG1
BOP384eG2

Figure 5


D-lactate production rate
(mmol gDW-1 hr-1)
Growth rate (hr-1)
final
(t = 200 hr)
initial
(t = 0 hr)
evolution
time
51
52
53
54
55
56
0
10
20
30
40
50
60
70
80
90
100
0
0.1
0.2
0.3
0.4
0.5
0.6
0.7
0.8
0.9
1.0

METABOLIC ENGINEERING OF MICROBIAL ORGANISMS

RELATED APPLICATIONS

The present application claims the benefit of U.S. Provisional Application No. 61/742,218, filed on Aug. 2, 2012, which is hereby incorporated by reference in its entirety. The present application is related to U.S. Provisional Application No. 61/401,017, filed Aug. 4, 2010, and U.S. Provisional Application No. 61/273,426, filed Aug. 3, 2009, each of which is hereby incorporated by reference in its entirety.

SEQUENCE LISTING AND TABLES IN ELECTRONIC FORMAT

The present application is being filed along with a Sequence Listing in electronic format. The Sequence Listing is provided as a file entitled UCSD033001ASEQUENCE.txt, last saved Aug. 1, 2013, created on Jul. 31, 2013, which is 53,033,897 bytes in size. The information in the electronic format of the Sequence Listing is incorporated herein by reference in its entirety.

BACKGROUND

Through the process of metabolic engineering, microbial organisms can be engineered for the production of various desirable compounds. Such engineered microbial strains and their products can be useful. For example, some microbial strains can produce chemicals from renewable feedstocks rather than from nonrenewable petroleum. Metabolic engineering has been practiced for many years, and the traditional approaches have included strategies such as random mutagenesis with selection for an over-producer or over-expression of genes either directly responsible for secondary metabolite production or genes indirectly involved in increasing metabolite production. Provided herein are engineered microbial strains with useful carbohydrate production characteristics. In some embodiments, the engineered microbial strains are designed using a genome-scale metabolic model so as to systematically select a strain with a desired phenotype.

FIELD

The field relates generally to genetically engineered microbial organisms. In some embodiments, microbial organisms genetically engineered for high-yield production of carbohydrates are provided.

SUMMARY

In one embodiment, a genetically engineered microbial organism comprising a genetic modification which substantially reduces pyruvate format lyase (PFL) activity and a genetic modification which substantially reduces pyruvate dehydrogenase (PDH) activity is provided. In some aspects of this embodiment, the production of carbohydrates comprises producing D-lactate from a glucose precursor. In some aspects of this embodiment, the genetic modification which substantially reduces PFL activity reduces PFL activity by at least about 70%. In some aspects of this embodiment, the genetic modification which substantially reduces PDH activity reduces PDH activity by at least about 70%. In some aspects of this embodiment, the genetic modification which substantially reduces PFL activity reduces PFL activity by at least about 90%, and wherein the genetic modification which substantially reduces PDH activity reduces PDH activity by at least about 90%. In some aspects of this embodiment, the genetic modification which substantially reduces PFL activity eliminates PFL activity, and wherein the genetic modification which substantially reduces PDH activity eliminates PDH activity. In some aspects of this embodiment, the microbial organism has undergone adaptive evolution. In some aspects of this embodiment, the genetically engineered microbial organism further comprises a genetic modification that increases that increases the mutation rate of the microbial organism at least about 2-fold per generation. In some aspects of this embodiment, the genetic modification that increases the mutation rate comprises a loss-of-function mutation in the mutS gene. In some aspects of this embodiment, the genetic modification which substantially reduces PFL activity is selected from the group consisting of: a hypomorphic mutation in each of pflABfocA and pflDC, a phenotypic null mutation in each of pflABfocA and pflDC, a deletion of each of pflABfocA and pflDC, a hypomorphic mutation in pflABfocA and a deletion of pflDC, a deletion of pflABfocA and a hypomorphic mutation in pflDC, a hypomorphic mutation in pflABfocA and a phenotypic null mutation in pflDC, a null mutation in pflABfocA and a hypomorphic mutation in pflDC, a deletion of in pflABfocA and a null mutation in pflDC, and a phenotypic null mutation in pflABfocA and a deletion of pflDC. In some aspects of this embodiment, the genetic modification which substantially reduces PDH activity is selected from the group consisting of a hypomorphic mutation in aceEF, a null mutation in aceEF, and a deletion of aceEF. In some aspects of this embodiment, the genetic modification which substantially reduces PFL activity comprises a deletion of each of pflABfocA and pflDC, and wherein the genetic modification eliminating PDH activity comprises a deletion of aceEF. In some aspects of this embodiment, the organism comprises a genotype of: pflABfocA pflDC aceEF xylFGH rbsACB alsBAC mutS. In some aspects of this embodiment, the organism is of one of the BOP384eG1 strain or BOP384eG2 strain. In some aspects of this embodiment, the genetically engineered microbial further comprises deletion of each of a native xylGFH, rbsACB, and alsBAC operon. In some aspects of this embodiment, the genetically engineered microbial organism further comprises at least one mutation selected from the group consisting of: 1022011 C→T, 111897 G→A, 1135244 A→G, 1137595 A→G, 1163111 A→G, 1247873 G→A, 1260197 C→T, 1274794 T→C, 1386732 A→G, 1435247 Δ1 bp, 1440978 G→A, 1580390 A→, 1604028 T→C, 1610530+C, 1679479 T→C, 1682336 A→G, 1729483 G→A, 1760136 G→A, 1866695 G→A, 1929016 G→A, 1950262 G→A, 1976527 Δ776 bp, 2071288 G→A, 2085304+G, 2098010 T→C, 2257220 A→G, 2358479+C, 2405257 G→C, 2534334 Δ1::IS186 (−)+6 bp::Δ1, 2620968 Δ1 bp, 2662540 T→C, 2732557+C, 2740321 T→C, 2763809+C, 2767188 G→A, 2782127 A→G, 2809146+A, 2824039 T→C, 2826646 G→A, 2844070 G→A, 2926442+T, 2927497 G→A, 2932138 TA, 2965591 T→C, 2975919 T→C, 3114125 C→T, 3176882 G→A, 3218853 T→C, 3246033 G→A, 3268091 C→T, 3268123 CA, 3268165 C→T, 3279141 C→T, 3302829 Δ1 bp, 3315496 A→G, 3335733 C→T, 34111+T, 3429378 T→C, 345189 A→G, 3506796 T→C, 3526004 T→C, 3548297 T→C, 379237 Δ1 bp, 379237 Δ2 bp, 386281 C→T, 3955730 G→A, 3957957 C→T, 3978813 C→A, 3982057 C→T, 3983344 C→T, 3990407 Δ8 bp, 407943 A→G, 4105271 A→G, 4153541 A→G, 42111 C→A, 4234068 T→G, 4294403+CG,

4306572 3434 bp→82 bp, 4444833 A→G, 4503718 G→A, 454725 C→T, 4604230+G, 4604346 Δ1 bp, 507894 T→C, 533362 A→G, 547694 A→G, 547831+G, 558493 G→T, 560913+G, 619171 A→G, 653396 T→C, 696062 G→A, 700027 C→T, 700286+C, 700599+C, 700679+G, 751964 C→T, 760544 C→T, 844446 C→T, 852434+A, 922473+G, 961467 C→T, and 963462 A→G, with reference to SEQ ID NO: 1. In some aspects of this embodiment, the microbial organism comprises the mutations annotated in any one of SEQ ID NOs: 2-9. In some aspects of this embodiment, the genetically engineered microbial organism has a steady-state glucose uptake rate of at least about 30 mmol per gDW per hour under standard conditions in 1 g per liter yeast extract medium, for example at least about 30 mm per gDW per hour, 35, 40, 45, 50, 55, 60, 65, 70, 75, 80, 85, 90, 95, or 100 mm per gDW per hour. In some aspects of this embodiment, the microbial organism is a recombinant *E. coli*.

Another embodiment includes a method of producing a genetically engineered microbial organism that has a steady-state glucose uptake rate of at least about 30 mmol per gDW per hour in a medium comprising 1 g per liter yeast extract, and the method includes performing at least one cycle of adaptive evolution on a culture of a microbial organism as described herein. In some aspects of this embodiment, the adaptive evolution comprises at least one of steady-state evolution or serial passage exponential evolution. In some aspects of this embodiment, the adaptive evolution comprises at least 14 days of adaptive evolution. In some aspects of this embodiment, the adaptive evolution comprises at least 16 days of adaptive evolution. In some aspects of this embodiment, the steady-state glucose uptake is at least about 40 mmol per gDW per hour under standard conditions in 1 g per liter yeast extract medium.

Another embodiment includes a genetically engineered microbial organism comprising a genetic modification which reduces pyruvate format lyase (PFL) activity and a genetic modification which reduces pyruvate deghydrogenase (PDH) activity, in which the genetically engineered microbial organism has a steady-state glucose uptake rate of at least about 30 mmol per gDW per hour under standard conditions in 1 g per liter yeast extract medium.

BRIEF DESCRIPTION OF THE DRAWINGS

FIG. **1** is a graph illustrating the predicted production envelopes for strains constructed according to some embodiments herein. This production envelope was determined to be a superior design out of a pool of designs that was computationally generated. The glucose uptake rate is 18 mmol gDW^{-1} hr^{-1} (a typical wild-type anaerobic uptake rate) and minimal medium conditions were used

FIG. **2** is a graph illustrating continuous culture adaptive evolution of strain BOP374 to remove acetate auxotrophy according to some embodiments herein. Three different time points are denoted, (1) the time at which continuous culture was initiated, (2) the point at which acetate feed to the culture was ended, and (3) the time at which a strain was collected. The dilution rate (hr^{-1}), optical density (OD600), and feed rates for glucose and acetate (mmol hr^{-1}) are given. As the acetate feed was decreased, the cellular density decreased. At point 3, the evolution resulted in isolation of a strain that could grow solely on glucose.

FIG. **3** is a graph illustrating growth characteristics of microbial cells according to some embodiments herein. BOP384, the lactate production strain was examined for its growth on glucose minimal medium with and without supplementation of yeast extract (YE) at different levels. All levels of supplementation had a similar growth profile and final optical density, both greater than those for no supplementation.

FIG. **4** is a graph illustrating the percent increase of growth rate by adaptively evolved strains according to some embodiments herein. Growth rate over an initial unevolved lactate production strain (e.g. strain BOP384) is shown over time for strains evolved on glucose. The strain was evolved in duplicate in parallel evolutions. The initial growth rates were determined each on glucose and the same starting strain was used for both evolutions. Both duplicates (BOP384eG1 and BOP384eG2) possessed a similar growth rate path to the end phenotype and similar final endpoint growth rates.

FIG. **5** is a graph illustrating the predicted production envelopes and experimental production measurements for unevolved and end point lactate production strains (BOP384, BOP384eG1, and BOP384eG2). The production envelopes are given based on experimentally measured substrate uptake rates (mmol gDW^{-1} hr^{-1}), solid lines (averages) and dashed lines (considering standard deviation). Also plotted are the experimentally measured values for the lactate production rates (mmol gDW^{-1} hr^{-1}) and growth rates (hr^{-1}). Experimental values are given in Table IV. The line 51 and point 52 are for the endpoint strain (with error bars); line 53 is the unevolved strain without supplementation (single measurement 54); and line 55 and point 56 are for the unevolved strain with yeast extract supplementation. For the endpoint, the optimal growth rate value for the envelopes lies near the experimentally determined endpoint growth rate and lactate production rate. The evolution resulted in a significant increase in production and growth rates.

DETAILED DESCRIPTION

According to some embodiments herein, microbial organisms are provided. The microbial organism can be configured to produce a carbohydrate from a precursor. In some embodiments, the microbial organism includes mutations eliminating format lyase (PFL) activity and pyruvate dehydrogenase (PDH) activity. In some embodiments, the microbial organism is *E. coli*. In some embodiments, the microbial organism further undergoes adaptive evolution, and organisms with desired carbohydrate production characteristics are selected. To expedite adaptive evolution, the microbial organism can comprise at least one genetic modification that increases the mutation rate. In some embodiments, the mutation is a mutS mutation. In some embodiments, the microbial organism has at least a pflABfocA pflDC aceEF genotype. Optionally, the microbial organism can include additional mutations. In some embodiments the microbial organisms has a pflABfocA pflDC aceEF xylFGH rbsACB alsBAC mutS genotype.

Metabolic engineering is a growing field for which new methods are being developed to generate microbial products rapidly and efficiently. According to some embodiments, two approaches that can aid this process are analysis of growth-coupled production using constraint-based modeling, and adaptive evolution for strain optimization. In some embodiments, the constraint-based modeling may comprise the modeling described in U.S. Provisional Application No. 61/742,218, filed on Aug. 2, 2012, U.S. Provisional Application No. 61/401,017, filed Aug. 4, 2010, now expired, or U.S. Provisional Application No. 61/273,426, filed Aug. 3, 2009, the disclosure of each of which are hereby incorporated by reference in their entireties. In some embodiments

the two approaches are combined, for example by selecting a strain identified from a model-driven analysis of the production of native microbial metabolites (for example, *Escherichia coli* metabolites, see Feist et al. 2010), evolving it using adaptive evolution, and characterizing its production capabilities. As such, in some embodiments, an engineered microbial strain is provided. In some embodiments adaptive evolutions results in a substantial increase in growth rate and/or substrate consumption rate for this strain. In some embodiments, the production phenotype of this strain is similar to the computationally predicted phenotype. In some embodiments, the production phenotype of this strain is similar to the computationally predicted phenotype, for example exhibiting substantial increases in growth rate and/or substrate consumption rate, and/or product production rate.

Carbohydrates

Lactic acid (and its corresponding lactate ions, for example D-Lactate) is an industrially relevant chemical with many practical uses. In some embodiments, lactic acid or a derivative thereof is useful in the food and beverage industry as an acidulant or as a preservative, or in polylactic acid (PLA), a biodegradable plastic.

A variety of carbohydrates can be produced by microbial organisms from a variety of precursors (also referred to herein as "substrates") under a variety of conditions. In some embodiments, at least one of D-Lactate, Glycerol, L-Alanine, L-Serine, Pyruvate, Fumarate, L-Malate, Succinate, 2-Oxoglutarate, or L-Glutamate is produced by a microbial organism. In some embodiments, the carbohydrate is produced under aerobic conditions. In some embodiments, the carbohydrate is produced under anaerobic conductions. In some embodiments, the carbohydrate is produced from a precursor, for example glucose or xylose. In some embodiments, D-lactate is produced from a glucose precursor.

Genetic Modifications

A variety of genetic modifications, for example mutations, can be provided according to some embodiments herein. A genetic locus in a microbial organism can produce one or more gene products. In some embodiments, the gene product is a nucleic acid, for example a ribosomal RNA. In some embodiments, the gene product is a polypeptide.

A genetic modification affecting one or more genetic loci can cause a loss-of-function in the gene product, a gain-of-function in the gene product, and/or can cause the gene product to adopt a new function. In some embodiments, a loss-of-function mutation is provided that reduces, but does not eliminate activity of the gene product (this class of mutation can also be referred to herein a "hypomorphic" mutation or "partial loss-of-function" mutation). In some embodiments, a loss-of-function mutation substantially reduces gene product activity. As used herein, "substantially reduces" gene product activity and variations of this root term refer to at least a 50% reduction in gene product activity compared to wild-type, for example, a 50%, 55%, 60%, 65%, 70%, 75%, 80%, 81%, 82%, 83%, 84%, 85%, 86%, 87%, 88%, 89%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99%, 99.5%, 99.9%, 99.99%, or 99.999% reduction. In some embodiments, a loss-of-function mutation eliminates gene product activity (this class of mutation can also be referred to herein as a "null" mutation). In some embodiments, a null mutation eliminates at least the gene-product-encoding sequence of a genetic locus. In some embodiments a null mutation eliminates the entire genetic locus. While a deletion of a genetic locus is a type of null mutation, null mutations can also encompass other sorts of genetic modifications. In some embodiments, a null mutation does not eliminate gene-product-encoding sequence, but prevents expression of the gene product (for example, by eliminating a promoter, a translation start sequence, or introducing an early stop codon). In some embodiments, a null mutation does not eliminate gene-product-encoding sequence or expression of a gene product, but eliminates or substantially eliminates activity of the gene product (for example, by mutating one or more catalytic residues from a protein). This latter class of null mutation may also be referred to herein as a "phenotypic null," or variations of this root term. In some embodiments, a loss-of-function mutation is a dominant negative mutation.

In addition to mutation of a genetic locus, other genetic modifications can also reduce or eliminate gene activity. By way of non-limiting example, antisense oligonucleotides can reduce or eliminate activity of the target gene. In some embodiments, a genetic modification for reducing gene expression comprises at least one antisense oligonucleotide. In some embodiments, an antisense oligonucleotide comprises an RNA complementary to at least a portion of a transcript of a target gene. In some embodiments, a microbial cell is genetically modified to express an antisense RNA directed to at least one transcript of the target gene. Additional exemplary genetic modifications that can be used to reduce gene activity in accordance with some embodiments herein include ribozymes, transcriptional repressors, inducible promoters, proteases directed to polypeptide encoded by a target gene, and the like.

In some embodiments, a mutation or genetic modification eliminates the activity of two or more gene products. In some embodiments, a mutation deletes an operon. In some embodiments, a mutation eliminates activity of one gene, and as a result, also eliminates activity of a second gene (for example, if the products of gene A and gene B function as a dimer, the elimination of either of gene A activity or gene B activity can also eliminate activity of the other gene).

A variety of techniques for making mutations are known to the skilled artisan. In some embodiments, a desired mutation is introduced via homologous recombination. A variety of vectors can be used for homologous mutation, for example phage or viral vectors, plasmid vectors, artificial chromosomes, and the like. In some embodiments, the vector is a pKD46, pKD13, or pCP20 plasmid, or variant thereof. In some embodiments, homologous sequences on a vector flank a genetic locus that can be used to identify homologous recombinants, for example an antibiotic resistance marker (for example, but not limited to kanamycin, chloramphenicol, or ampicillin resistance) or metabolic enzyme that permits an auxotroph to survive in a particular minimal medium. In some embodiments, mutations are introduced into at least a genome are random, and mutant microbial organisms having the desired mutations are selected.

In some embodiments, a host genome or portion thereof is synthesized, and introduced into a microbial organism. In some embodiments an entire host genome having the desired genetic features is synthesized and inserted into a microbial cell (see, e.g. Gibson et al., Science 2: 329, pp. 52-56, hereby incorporated by reference in its entirety).

Metabolic Engineering

In some embodiments, a systems biology based approach to metabolic engineering comprises growth-coupled design, in which the production of a metabolite by a microbial strain increases as growth rate increases (Burgard et al. 2003). Some traditional strain designs have relied on genetic manipulations that alter the metabolism in a way that typically redirects metabolic flux from producing biomass to

producing a specific desired product. Without being limited by any particular theory, such strains can be highly unstable, and if left unsupported, mutations that increase growth rate at the cost of production can occur, and the strain can lose productivity over time. Furthermore, it is unusual for the increased secretion of a metabolic by-product alone to simultaneously increase growth rate. However, according to some embodiments, genome-scale metabolic models and constraint-based analysis methods can predict genetic manipulations that couple production objectives to a selection pressure (i.e., growth rate). Laboratory adaptive evolution can then be used as a tool to optimize in vivo strain designs.

OptKnock (Burgard et al. 2003) and OptGene (Patil et al. 2005) are two in silico algorithms, either or both of which can be used for designing growth-coupled strains according to some embodiments herein. Several OptKnock designs for the production of lactic acid have been constructed and adaptively evolved in vivo, and it was found that the experimental results closely agreed with the computational predictions (Fong et al. 2005). An in silico screen of the growth-coupled design potential of *E. coli* was conducted utilizing the genome-scale metabolic reconstruction and model iAF1260 and the aforementioned design algorithms (Feist et al. 2010). As such, in some embodiments, an iAF1260 model of *E. coli* is provided. According to some embodiments herein, a growth-coupled design for producing a carbohydrate is identified. In some embodiments, the carbohydrate is D-lactate. This design may be based on computationally predicted properties such as high predicted product yield, ability to produce and secrete only one compound (homofermentation), and use of well characterized metabolic pathways to produce the targeted product. According to some embodiments herein, computationally driven growth-coupled strain design processes are followed by construction of a strain in vivo, optimization of this strain through the adaptive evolution process, and characterization of its production phenotype.

Processes developed in accordance with some embodiments herein can result in generation of a computationally-predicted production strain design. In some embodiments, a metabolic reconstruction of a microbial organism is provided. By way of non-limiting example, organisms for which metabolic reconstructions are available are listed in Feist et al. 2008a (hereby incorporated by reference in its entirety), for example *Bacillus subtilis* (described in Oh et al. 2007, hereby incorporated by reference in its entirety). In some embodiments, a strain design is based on a metabolic reconstruction of *E. coli*. In some emboidments, a strain design is based on a metabolic reconstruction of *B. subtilis*.

In some embodiments, a compound, precursor, and/or production strain design is selected from the designs identified from the screen of native compounds used in Feist et al. 2010. The methods described herein can be readily applied to generate further strains of *E. coli* or other microbial organisms. In some embodiments, a previously-described strain design is used as a starting point for a strain design according to embodiments herein. For example, the analyzeGCdesign algorithm can apply a penalty for knockouts and can be applied to reduce the total number of genetic loci mutated in a strain design. As such, in some embodiments, the analyzeGCdesign algorithm is applied to an existing strain design. In some embodiments, the analyzeGCdesign algorithm has a knockout penalty of 90%. In some embodiments, the analyzeGCdesign algorithm is used to "streamline" a particular strain design so as to minimize the number of mutations for arriving at a desired characteristic.

Adaptive Evolution

In some embodiments, the microbial strain undergoes adaptive evolution. As used herein, adaptive evolution refers to obtaining one or more organisms with one or more desired characteristics through selection for the desired characteristics. It is contemplated that unless explicitly stated otherwise herein, "selection for a desired characteristic" and variations of this root phrase encompasses both positive selection for a desired characteristic and selection against an undesired characteristic. In some embodiments, the selected characteristic comprises at least one of growth rate, consumption of substrate, production rate of carbohydrate, or production efficiency of carbohydrate. In some embodiments, direct selection for the desired characteristic is performed. In some embodiments, at least one desired characteristic is coupled to at least one other characteristic, and selection is performed for the other characteristic. In some embodiments, the other characteristic is also a desired characteristic. In some embodiments, the other characteristic is a neutral characteristic (for example a marker, or a reporter molecule).

It is contemplated herein the coupling of microbial growth and target molecule production can facilitate adaptive evolution. In some embodiments, production of target molecule (for example carbohydrate) by a strain is growth-coupled to biomass production. Without being limited by any particular theory, the strain can be required to produce target molecule in order to produce biomass, so that achievement of faster growth can require secretion of the target molecule. As such, selection for faster growth can also select for increased secretion of target molecule.

Serial passage selection (also referred to as "serial passage exponential" or "SPE" selection) can result in optimization of the strain for target production as well as growth rate. In some embodiments, adaptive evolution comprises serial passage selection. In some embodiments, growth is coupled to target product secretion, and selection is performed for growth. The selection can be performed by serial passage selection. Protocols for serial passage are described in Fong, et al. 2005. Biotechnol Bioeng 91(5):643-8; Ibarra et al., Nature 420:186-9; Fong et al. 2003. Journal of Bacteriology 185(21):6400-8; Herring et al., 2006. Nat Genet. 38(12):1406-1412; and Fong and Palsson, 2004. Nat Genet. 36(10):1056-58, each of which is hereby incorporated by reference in its entirety. Serial passage selection can comprise passaging cells so that the cells remain in exponential growth phase, and never (or almost never) reach stationary phase. By way of non-limiting example, cells can be transferred when they reach a certain optical density that is characteristic of the cells being in exponential growth phase. In some embodiments, serial passage selection can comprise sufficiently large transfer volumes so as to reduce the chance of fixation of hitchhiker mutations. In some embodiments, serial passage selection is performed in batch cultures. In some embodiments, serial passage selection is performed to select for microbial organisms with increased carbohydrate production. In some embodiments, serial passage selection is performed to select for microbial organisms with increased D-lactate production. In some embodiments, at least 2 rounds of serial passage selection are performed, for example 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, 20, 30, 40, 50, 60, 70, 80, 90, 100, 150, 200, 250, 300, 350, 400, 450, 500, 600, 700, 800, 900, or 1000 rounds of serial passage selection, including ranges between any two of the listed values.

In some embodiments, adaptive evolution includes steady state evolution. Steady state evolution can comprise gradually increasing the strength of a selective pressure in order to select for at least one desired characteristic. In some embodiments steady state evolution is provided to alleviate auxotropy for one or more metabolites. In some embodiments, concentrations of metabolite are gradually decreased in continuous culture. In some embodiments, the continuous culture is performed for at least 1 day, for example 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, 20, 21, 22, 23, 24, 25, 26, 27, 28, 29, 30, 31, 32, 33, 34, 35, 36, 37, 38, 39, 40, 41, 42, 43, 44, 45, 46, 47, 48, 49, 50, 60, 70, 80, 90 or 100 days, including ranges between any two of the listed values.

Without being limited by any particular theory, in some circumstances, adaptive evolution can be accelerated by increasing the mutation rate. As such, in some embodiments a microbial stain is engineered to have an increased mutation rate. In some embodiments, the microbial strain comprises a mutation that yields an increased mutation rate. By way of non-limiting example, the microbial strain can comprise the mutS mutation, which has been associated with increased mutation rate. In some embodiments, the microbial strain comprises a mutation (or combination of mutations) that increase the mutation rate by at least 1.2×, for example about 1.2×, 1.5×, 2×, 3×, 4×, 5×, 6×, 7×, 8×, 9×, 10×, 20×, 30×, 40×, 50×, 60×, 70×, 80×, 90×, 100×, 1000×, or 10000× compared to a reference strain lacking the mutation (or mutations) that increase the mutation rate. In some embodiments, after a strain with one or more desired characteristics is obtained through adaptive evolution, one or more mutations that increase the mutation rate are removed. In some embodiments, the mutations are replaced with wild-type sequence or functionally wild-type sequence. In some embodiments, the mutations are reverted.

Microbial Organisms

Microbial organisms and strains thereof are provided according to some embodiments herein. As used herein, "microbial organism," "microorganism," and the like refers to single cell prokaryotic, eukaryotic, and archaea. Exemplary microbial organisms include, but are not limited to, *Escherichia coli, Bacillus* species, *Pseudomonas* species, *Salmonella* species, *Rhodococcus* species, *Lactobacillus* species, *Enterococcus* species, *Alcaligenes* species, *Klebsiella* species, *Paenibacillus* species, *Arthrobacter* species, *Corynebacterium* species, *Brevibacterium* species, *Saccharomyces* species, *Pichia* species, *Candida* species, *Hansenula* species, *Cyanobacteria* species, *Bacillus subtilis, Bacillus licheniformis, Alcaligenes eutrophus, Paenibacillus macerans, Rhodococcus erythropolis, Pseudomonas putida, Lactobacillus plantarum, Enterococcus faecium, Enterococcus gallinarium*, and *Enterococcus faecalis*. In some embodiments, the microbial organism is a *Bacillus* species, for example *B. subtilis*. In some embodiments, the microbial organism is *E. coli*.

In some embodiments, a genetically engineered microbial organism comprising a genetic modification which substantially reduces pyruvate format lyase (PFL) activity and a genetic modification which substantially reduces pyruvate dehydrogenase (PDH) activity is provided. Without being limited by any particular theory, in some embodiments, removal of only two metabolic reactions can provide higher carbohydrate uptake than removal of three or more metabolic reactions. In some embodiments, the strain comprises a pflABfocA pflDC aceEF genotype or a genotype conferring a phenotype corresponding to that resulting from a pflABfocA pflDC aceEF genotype. According to some embodiments herein, a pflABfocA pflDC aceEF strain comprises one or more additional genetic modifications. In some embodiments, the strain comprises one or more antibiotic resistance markers such as kanR (also notated herein as "kan+"). In some embodiments, the strain comprises mutations in additional genetic loci, for example the operons that permit growth on xylose (for example, by knockouts of one or more of the xylFGH, rbsABC, or alsBAC operons).

In some embodiments, a microbial strain as shown in Table II is provided. In some embodiments, the strain is one of BOP330, BOP336, BOP338, BOP370, BOP372, BOP374, BOP 374e, BOP384, BOP384e1, BOP384eG2. In some embodiments, the strain is BOP374e. In some embodiments, the strain is BOP384eG1. In some embodiments, the strain is BOP384eG2. Any of strains BOP374e, BOP384eG1, and BOP384eG2 or any of the other strains described herein can be deposited in an acceptable depository under the Budapest Treaty on the International Recognition of the Deposit of Microorganisms for the Purposes of Patent Procedure.

In some embodiments, a genetically engineered microbial organism comprising a genetic modification which substantially reduces pyruvate format lyase (PFL) activity and a genetic modification which substantially reduces pyruvate dehydrogenase (PDH) activity is provided, and further comprises at least one of the mutations listed in Table VIa. In some embodiments, the strain further comprises at least one mutation selected from the group consisting of (each numerical position listed is with reference to SEQ ID NO: 1): C→T, 111897 G→A, 1135244 A→G, 1137595 A→G, 1163111 A→G, 1247873 G→A, 1260197 C→T, 1274794 T→C, 1386732 A→G, 1435247 Δ1 bp, 1440978 G→A, 1580390 A→G, 1604028 T→C, 1610530+C, 1679479 T→C, 1682336 A→G, 1729483 G→A, 1760136 G→A, 1866695 G→A, 1929016 G→A, 1950262 G→A, 1976527 Δ776 bp, 2071288 G→A, 2085304+G, 2098010 T→C, 2257220 A→G, 2358479+C, 2405257 G→C, 2534334 Δ1:: IS186 (−)+6 bp::Δ1, 2620968 Δ1 bp, 2662540 T→C, 2732557+C, 2740321 T→C, 2763809+C, 2767188 G→A, 2782127 A→G, 2809146+A, 2824039 T→C, 2826646 G→A, 2844070 G→A, 2926442+T, 2927497 G→A, 2932138 T→A, 2965591 T→C, 2975919 T→C, 3114125 C→T, 3176882 G→A, 3218853 T→C, 3246033 G→A, 3268091 C→T, 3268123 C→A, 3268165 C→T, 3279141 C→T, 3302829 Δ1 bp, 3315496 A→G, 3335733 C→T, 34111+T, 3429378 T→C, 345189 A→G, 3506796 T→C, 3526004 T→C, 3548297 T→C, 379237 Δ1 bp, 379237 Δ2 bp, 386281 C→T, 3955730 G→A, 3957957 C→T, 3978813 C→A, 3982057 C→T, 3983344 C→T, 3990407 Δ8 bp, 407943 A→G, 4105271 A→G, 4153541 A→G, 42111 C→A, 4234068 T→G, 4294403+C→G, 4306572 3434 bp-→82 bp, 4444833 A→G, 4503718 G→A, 454725 C→T, 4604230+G, 4604346 Δ1 bp, 507894 T→C, 533362 A→G, 547694 A→G, 547831+G, 558493 G→T, 560913+G, 619171 A→G, 653396 T→C, 696062 G→A, 700027 C→T, 700286+C, 700599+C, 700679+G, 751964 C→T, 760544 C→T, 844446 C→T, 852434+A, 922473+G, 961467 C→T, and 963462 A→G. It is noted that SEQ ID NO: 1 represents a genomic sequence of wild-type *E. coli* K-12 MG1655, but that it is contemplated that any or all of the indicated mutations (or a corresponding mutation in a respective host genome) can readily be applied to a different microbial strain or species as described herein. In some embodiments, the microbial strain comprises at least 2 of these mutations, for example 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, 20, 21, 22, 23, 24, 25, 26, 27, 28, 29, 30, 31, 32, 33, 34, 35, 36, 37, 38, 39, 40, 41, 42, 43, 44, 45, 46, 47, 48, 49,

50, 51, 52, 5, 54, 55, 56, 57, 58, 59, 60, 91, 62, 63, 64, 65, 66, 67, 68, 69, 70, 71, 72, 73, 74, 75, 76, 77, 78, 79, 80, 81, 82, 83, 84, 85, 86, 87, 88, 89, 90, 91, 92, 93, 94, 95, 96, 97, 98, 99, 100, 101, 102, or 103 of these mutations, including ranges between any two of the listed values. In some embodiments, the microbial strain comprises all of the indicated mutations in one of BOP384' Day 0 (see SEQ ID NO: 2), BOP384G1D' Day 3 (see SEQ ID NO: 3), BOP384G1H' Day 5 (see SEQ ID NO: 4), BOP384G1N' Day 8 (see SEQ ID NO: 5), BOP384G1S' Day 10 (see SEQ ID NO: 6), BOP384G1X' Day 13 (see SEQ ID NO: 7), BOP384eG1' (isolate 1) Day 14 (see SEQ ID NO: 8), or BOP384eG1' (isolate 2) Day 14 (see SEQ ID NO: 9) as shown in Tables VIb and VIc. In some embodiments, the microbial strain comprises at least one fewer mutation than the set of mutations identified in BOP384' Day 0 (see SEQ ID NO: 2), BOP384G1D' Day 3 (see SEQ ID NO: 3), BOP384G1H' Day 5 (see SEQ ID NO: 4), BOP384G1N' Day 8 (see SEQ ID NO: 5), BOP384G1S' Day 10 (see SEQ ID NO: 6), BOP384G1X' Day 13 (see SEQ ID NO: 7), BOP384eG1' (isolate 1) Day 14 (see SEQ ID NO: 8), or BOP384eG1' (isolate 2) Day 14 (see SEQ ID NO: 9) as shown in Tables VIb and VIc, for example at least one, two, three, four, five, six, seven, eight, nine, or ten fewer mutations. In some embodiments, the microbial strain comprises the set of mutations identified in BOP384' Day 0 (see SEQ ID NO: 2), BOP384G1D' Day 3 (see SEQ ID NO: 3), BOP384G1H' Day 5 (see SEQ ID NO: 4), BOP384G1N' Day 8 (see SEQ ID NO: 5), BOP384G1S' Day 10 (see SEQ ID NO: 6), BOP384G1X' Day 13 (see SEQ ID NO: 7), BOP384eG1' (isolate 1) Day 14 (see SEQ ID NO: 8), or BOP384eG1' (isolate 2) Day 14 (see SEQ ID NO: 9) as shown in Tables VIb and VIc, plus at least one additional mutation identified in Table VIa, for example one, two, three, four, five, six, seven, eight, nine, or ten additional mutations identified in Table VIa.

In some embodiments, a microbial strains as shown in Table II undergoes adaptive evolution. In some embodiments, a pflABfocA pflDC aceEF strain or a strain having a phenotype corresponding to that of a pflABfocA pflDC aceEF strain undergoes adaptive evolution. In some embodiments, a BOP338 strain undergoes adaptive evolution. In some embodiments, a BOP374e strain undergoes adaptive evolution.

It can be useful for a microbial strain to have desirable uptake and/or production characteristics under growing conditions that are adaptable to an industrial scale. In some embodiments, microbial strains as described herein have desirable production characteristics (e.g. product yield and volumetric productivity) at growth densities in a range consistent with industrial production. Exemplary descriptions of microbial strain production at industrial scales can be found in Chang et al. 1999; Dien et al. 2001; Zhou et al. 2003; and Zhu et al. 2007)

According to some embodiments herein, microbial strains with carbohydrate uptake and/or production capabilities near, at, or above the theoretical maximum are provided. In some embodiments, the theoretical production maximum of a microbial strain is as provided in Feist et al., 2010. In some embodiments, lactate production strains as provided herein provide superior performance in terms of production rate, growth rate, and/or byproduct formation as compared to other lactate-producing strains. In some embodiments, lactate production strains as provided herein provide superior performance in terms of production rate, growth rate, and/or byproduct formation as compared to the theoretical maximum. Exemplary characteristics of lactate-producing strains can be found in Fong et al., 2005, Chang et al. 1999; Dien et al. 2001; Zhou et al. 2003; Zhu et al. 2007.

In some embodiments, a strain is provided having an uptake rate of at least about 30 mmol per grams dry weight per hour (mmol gDW^{-1} hr^{-1}) under standard conditions in 1 g per liter yeast extract medium, for example about 30 mmol gDW^{-1} hr^{-1}, 31, 32, 33, 34, 35, 36, 37, 38, 39, 40, 41, 42, 43, 44, 45, 46, 47, 48, 49, 50, 51, 52, 53, 54, 55, 56, 57, 58, 59, 60, 61, 62, 63, 64, 65, 66, 67, 68, 69, 70, 71, 72, 73, 74, 75, 76, 77, 78, 79, 80, 81, 82, 83, 84, 85, 86, 87, 88, 89, 90, 91, 92, 93, 94, 95, 96, 97, 98, 99, or 100 mmol gDW^{-1} hr^{-1}, including ranges between any two of the listed values. In some embodiments, the strain may provide more than 100 mmol gDW^{-1} hr^{-1}. In some embodiments, the uptake rate is at least about 20.7 mmol gDW^{-1} hr^{-1}. In some embodiments, the uptake rate is at least about 43.1 mmol gDW^{-1} hr^{-1}. In some embodiments, the substrate for uptake is glucose. In some embodiments, D-lactate is produced from the glucose.

In some embodiments, a strain is provided having a production rate of at least about 30 mmol per grams dry weight per hour (mmol gDW^{-1} hr^{-1}) under standard conditions in 1 g per liter yeast extract medium, for example about 30 mmol gDW^{-1} hr^{-1}, 31, 32, 33, 34, 35, 36, 37, 38, 39, 40, 41, 42, 43, 44, 45, 46, 47, 48, 49, 50, 51, 52, 53, 54, 55, 56, 57, 58, 59, 60, 61, 62, 63, 64, 65, 66, 67, 68, 69, 70, 71, 72, 73, 74, 75, 76, 77, 78, 79, 80, 81, 82, 83, 84, 85, 86, 87, 88, 89, 90, 91, 92, 93, 94, 95, 96, 97, 98, 99, 100, 110, 120, 130, 140, 150, 160, 170, 180, 190 or 200 mmol gDW^{-1} hr^{-1}, including ranges between any two of the listed values. In some embodiments, the strain may provide more than 200 mmol gDW^{-1} hr^{-1}. In some embodiments, the substrate is glucose and the product is D-lactate. In some embodiments, the glucose uptake rate is 18 mmol gDW^{-1} hr^{-1} and minimal medium conditions are used.

In some embodiments, a strain is provided having a growth rate of at least about 0.2 hr^{-1} is provided, for example a growth rate of about 0.2 hr^{-1}, 0.25, 0.26, 0.3, 0.35, 0.4, 0.45, 0.5, 0.55, 0.6, 0.65, 0.7, 0.75, 0.8, 0.85, 0.86, 0.9, 0.95, 1, 1.05, 1.1, 1.15, 1.2, 1.25, 1.3, 1.35, 1.4, 1.45, 1.5, 1.55, 1.6, 1.65, 1.7, 1.75, 1.8, 1.85, 1.9, 1.95, or 2 hr^{-1}, including ranges between any two of the listed values. In some embodiments, a strain having a growth rate of more than 2 hr^{-1} is provided.

Additional Alternative Embodiments

Traditional metabolic engineering can require a high level of organism familiarity and biological intuition. However, with systems biology methods and genome-scale metabolic models, it is possible to reliably and systematically predict the phenotype of microbial organisms (Park et al. 2008). Successful cases of systems biology driven strain designs for *E. coli* include production of the metabolites lycopene (Alper et al. 2005a; Alper et al. 2005b), lactic acid (Fong et al. 2005), succinic acid (Lee et al. 2005; Lee et al. 2008; Wang et al. 2006), and amino acids (Lee et al. 2007; Park et al. 2007). These cases demonstrated in *E. coli* as well as those for additional organisms have been reviewed (Feist and Pals son 2008; Park et al. 2008) and represent examples of metabolic engineering driven by systems biology.

Although lactate production strains have been constructed previously (Chang et al. 1999; Dien et al. 2001; Zhou et al. 2003; Zhu et al. 2007), strains according to some embodiments herein can differ in one or more of the following aspects: (i) strains according to some embodiments herein can be generated without any recombinant DNA, thus

streamlining the construction and fermentation process (e.g., no induction of a transgene is required), (ii) strains according to some embodiments herein do not require complex fermentations, such as two-stage aerobic and anaerobic fermentations; (iii.) strains according to some embodiments herein have the potential for continuous processing.

Model-driven design also has an advantage in that it offers a means to quickly predict the effect of additional knockouts and supplementation of medium, which was mostly speculative and performed by trial and error in other studies. One specific prediction from modeling was that acetyl-CoA could be generated in the pflABfocA, pflDC, aceEF knockout strain through pathways detailed in the model that were previously speculated not to be utilized (Zhu et al. 2007). The weaning of the strain off of acetate supplementation provides evidence that this is indeed possible; however the necessity of yeast extract for continued growth indicates that further testing is needed. Modeling predicted that the lactate produced here is optically pure D-lactate, as the predicted active enzyme is that of the D-lactate specific ldhA gene. Optically pure lactate is preferred for polymer generation (Hofvendahl and Hahn-Hagerdal 2000) and can be directly assayed in these strains. The next step for the evaluation of this lactate producing strain is evolution under continuous processing conditions and higher substrate conditions. Strains that are growth coupled are, in theory, suitable for continuous culture as they will not be outperformed by mutants that exhibit faster growth under such conditions. This continuous processing potential may significantly impact the field of metabolic engineering.

EXAMPLES

Example 1: Computational Model and in Silico Design

The metabolic reconstruction of *E. coli* iAF1260 (Feist et al. 2007) with the minor changes described (Feist et al. 2010) was utilized. This model has been functionally tested and verified against experimental data to accurately predict growth rates, metabolite excretion rates, and growth phenotypes on a number of substrate and genetic conditions (Feist et al. 2007). For all simulations, the reactions CAT, SPODM, and SPODMpp (oxidative stress reactions) and the FHL reaction were constrained to zero for reasons previously established (Feist et al. 2007). Flux balance analysis (Price et al. 2004) was used for computing optimal phenotypes using iAF1260 and the biomass objective function, BOFCORE, with the reported maintenance energies presented with the reconstruction (Feist et al. 2007). All computations were performed using the MATLAB (The MathWorks Inc., Natick, Mass.) and the COBRA Toolbox (Becker et al. 2007) software packages with TOMLAB (Tomlab Optimization Inc., San Diego, Calif.) solvers.

Model conditions were set to computational minimal medium as previously defined (Feist et al. 2007). Minimal medium with yeast extract supplementation (for experimental comparison) was simulated by allowing amino acid and nucleotide base uptake rates for simulations in amounts proportional to that required for supporting a given experimentally determined growth rate computationally. In modeling terms, a given uptake rate for an amino acid or nucleotide base was equal to the stoichiometric coefficient of that component in the biomass objective function multiplied by the experimental growth rate. The growth and non-growth associated maintenance modeling parameters identified in model development for growth on glucose were used for all design calculations (Feist et al. 2007).

In order to improve computationally identified growth coupled knockout strains and reduce the number of knockouts necessary, a COBRA Toolbox function (Becker et al. 2007) called analyzeGCdesign (see Supplementary Files) was created. This function uses a simple algorithm and objective function to find a better growth coupled solution, given an OptKnock or OptGene solution (or any set of knockouts) as an input (Feist et al. 2010).

Example 2: Selection of Strain Designs

From the pool of computational designs (Feist et al. 2010), the production of D-lactate from glucose was identified as a production target as lactate was predicted to be a homofermentation product with a high yield. The original designs generated from the computational analysis gave two designs for three- and five-reaction knockouts that could result in the high yield phenotype (Table I). A sensitivity analysis using the analyzeGCdesign algorithm with a penalty for knockouts of 90% was performed on each of the designs to determine if the high yield could be sustained or improved with fewer metabolic interventions (i.e., knockouts). This analysis returned a two reaction removal design (reactions PFL, pyruvate formate lyase, and PDH, pyruvate dehydrogenase) as the optimal design for the given knockout penalty and maximization of yield starting from the three knockout design, and a single reaction removal (ALCD2x, alcohol dehydrogenase (ethanol)) starting from the five knockout design. The PFL and PDH design for the production of lactic acid from glucose was found to be a novel design based on a literature search, although similar designs exist (Zhou et al. 2003; Zhu et al. 2007), and it was selected for construction.

TABLE I

Results of computational analysis of strain designs for production of D-lactate from glucose.

OptKnock Design	Production Rate (mmol gDW^{-1} hr^{-1})	Yield (wt %)	By-products	By-product Rate (mmol gDW^{-1} hr^{-1})	By-product Yield (wt %)	Growth Rate (hr^{-1})	analyzeGC design Max Yield - 90% KO Penalty
PDH, PFL, PGI	36.158	90.4%	succinate	0.087	0.3%	0.261	PFL, PDH
			CO_2	0.51	0.6%		

TABLE I-continued

Results of computational analysis of strain designs for production of D-lactate from glucose.

OptKnock Design	Production Rate (mmol gDW^{-1} hr^{-1})	Yield (wt %)	By-products	By-product Rate (mmol gDW^{-1} hr^{-1})	By-product Yield (wt %)	Growth Rate (hr^{-1})	analyzeGC design Max Yield - 90% KO Penalty
ALCD2x, ATPS4rpp, G6PDH2r, GHMT2r, PGI	38.429	96.1%	acetate succinate CO_2	0.138 0.033 0.196	0.2% 0.1% 0.2%	0.100	ALCD2x

Example 3: Strain Construction

The starting strain was wild-type *E. coli* K-12 MG1655 (ATCC 700926). This strain has been extensively characterized physiologically (Fong et al. 2005; Fong et al. 2003; Ibarra et al. 2002; Reed et al. 2006), and its genome has been resequenced (Herring et al. 2006. Nat Genet. 38(12):1406-1412, hereby incorporated by reference in its entirety). A genomic sequence of wild-type *E. coli* K-12 MG1655 is provided as SEQ ID NO: 1. The computational model iAF1260 is based on the K-12 MG1655 genome. Gene disruptions were performed using homologous recombination of PCR-amplified linear fragments (Datsenko and Wanner 2000). During the gene deletion process, strains were grown aerobically in LB liquid medium and on 1.0% agar plates and the antibiotics kanamycin, chloramphenicol, and ampicillin were used for section. The plasmids pKD46, pKD13, and pCP20 were used in this process. Strains were preserved at −80° C. and were given a systematic "BOP" tag and number (for example, BOP27) under a standard strain identifier protocol. Knockout genotypes were confirmed by PCR using pairs of locus-specific primers.

Three operons were removed to completely eliminate the activity of the two reactions in the design. Two operons for the PFL reaction, pflABfocA, the main isozyme and additionally the transporter that allows passage of the reaction by-product formate, and pflDC, the minor isozyme. Removal of one operon, aceEF, encoding the core of the pyruvate dehydrogenase catalyzing enzyme, eliminated the PDH reaction. These gene deletions were defined in the gene to protein to reaction associations in iAF1260 (Feist et al. 2007). The resultant strain was labeled BOP338 (Table II). The operons xylFGH, rbsACB, and alsBAC were also deleted in order to improve the strain's ability to grow on xylose, although it was only grown on glucose in this study. This strain was labeled BOP374 (Table II).

TABLE II

Strains according to some embodiments herein (SS—steady-state evolution, SPE—serial passage exponential)

Strain	Parent	Evolution	Genotype
BOP27	N/A		MG1655 ATCC#47076
BOP330	BOP27		pflABfocA kan +
BOP336	BOP331		pflABfocA pflDC kan +
BOP338	BOP337		pflABfocA pflDC aceEF kan +
BOP370	BOP339		pflABfocA pflDC aceEF xylFGH kan +
BOP372	BOP371		pflABfocA pflDC aceEF xylFGH rbsACB kan +
BOP374	BOP372		pflABfocA pflDC aceEF xylFGH rbsACB alsBAC kan +
BOP374e	BOP374	SS, 16 days	pflABfocA pflDC aceEF xylFGH rbsACB alsBAC kan +
BOP384	BOP374e		pflABfocA pflDC aceEF xylFGH rbsACB alsBAC mutS kan +
BOP384eG1	BOP384	SPE, 14 days	pflABfocA pflDC aceEF xylFGH rbsACB alsBAC mutS kan +
BOP384eG2	BOP384	SPE, 14 days	pflABfocA pflDC aceEF xylFGH rbsACB alsBAC mutS kan +

After construction of the production strain, it was analyzed for growth properties under the minimal medium conditions for which it was designed to be evolved and optimized in. Minimal media for cultures was selected as follows: M9 minimal was selected as it has been demonstrated that lactic acid can be made with the given defined minimal nutrients (Fong et al. 2005). Yeast extract (Sigma, Catalog #8013-01-2) and sodium acetate were used as supplementation where specified. This analysis was done in batch mode and the results are shown in Table III. The screen revealed that the lactate production strain constructed was auxotrophic for acetate. This indicates that the strain was not able to make the necessary acetyl-CoA required for generation of lipids. With the strain containing PFL and PDH knockouts, this result was not unexpected. These growth rates could potentially increase with adaptation to these conditions. Furthermore, supplementation with yeast extract, a widely used culture supplement (see below) also increased the growth rate in a few instances examined, but could not support growth as the only supplement along with glucose (data not shown).

TABLE III

Initial growth characteristics of production strains (NG—no growth)

Strain	Culture Conditions	Supplement	Aerobicity	Growth Rate (hr^{-1})
BOP338	4 g/L glucose M9	none	aerobic	NG
BOP338	4 g/L glucose M9	1 g/L acetate	aerobic	0.37
BOP374	4 g/L glucose M9	none	aerobic	NG
BOP374	4 g/L glucose M9	1 g/L acetate	aerobic	0.43
BOP374	4 g/L glucose M9	none	anaerobic	NG
BOP374	4 g/L glucose M9	1 g/L acetatean	aerobic	0.16

Examples 4-5

Continuous Culture Evolutions

Continuous culture was performed in a 1.0 L New Brunswick BioFlo fermentor. M9 medium was used in the fermentor as specified. The agitation rate was 500 RPM and was constant for the run. Temperature was maintained at 37° C. Culture pH was maintained with 5% NaOH and was maintained at 7.0. Dissolved oxygen was also monitored for the evolution and was maintained at zero. The volume of medium in the fermentor was maintained at 1.0 L unless noted otherwise. To maintain anoxic conditions in the fermentor, 5% CO2 balance N_2 gas was supplied to the fermentor at 1 VVM. For the weaning off of acetate, the glucose concentration was 4.0 g/L and the acetate was at 2.0 g/L. Feed rates are presented in the results section as a function of time. Samples were removed aseptically and optical density measurements were taken. Samples were analyzed by HPLC.

Products were identified and quantified by HPLC using an Aminex 87-H ion exchange column at 65° C. The mobile phase was 5 mM H2SO4 at an isocratic flow of 0.5 mL/minute. Sample injection volume was 10 μL. Products were identified by retention time using utilizing ultraviolet detection at 210 nm and refractive index detection at 30° C. internal temperature and 45° C. external temperature and quantified by relating peak area to those of standards.

Adaptive evolution was conducted in 100 mL flasks with M9 medium supplemented with 4.0 g/L glucose. Other supplements are stated in the Results section. Cultures were maintained at 37° C. in an anaerobic chamber with the atmospheric gas being a mixture of 7.5% H2/10% CO2 with balance N_2. Experiments were designed to keep cells growing in exponential growth phase. To do this, the inoculum volume was changed for each passage throughout the course of evolution, and passages were performed at an optical density of approximately 0.2. Cultures were frozen and stored at −80° C. at regular intervals throughout adaptive evolution, approximately every other day.

Example 4: Adaptive Evolution to Alleviate Acetate Auxotrophy

Adaptive evolution was also used to alleviate the acetate auxotrophy. BOP374 was evolved in a 1.0 L chemostat anaerobically with a stepwise decreasing acetate feed rate (FIG. **2**). To do this, the strain was initially inoculated into the fermentor in a batch mode with a glucose and acetate mixture, after the culture grew up to an appreciable density (point 1), the culture was run in continuous culture mode for 16 days, during which the acetate feed rate was sequentially lowered until the strain was growing solely on glucose minimal medium (point 2). At the end of the evolution, a colony was isolated from the fermentor and was designated and preserved with a new strain number (point 3). Continuous culture was chosen for the evolution as it allowed for automation and monitoring of the process along with a straightforward procedure to drop auxotrophy feed rates. The evolved strain harvested at the end of the evolution was confirmed to have the same genotype as the starting strain (in terms of gene knockouts) and was designated BOP374e.

Example 5: Adaptive Evolution to Optimize Production Phenotypes

In order to increase the rate of mutation and subsequently reduce the time necessary to evolve strains to an optimal phenotype, the mutS gene was removed from strain BOP374e to generate the mutator strain BOP384 (Table II). The mutS gene is involved in DNA mismatch repair (Acharya et al. 2003; Schlensog and Bock 1991), thus its removal can cause a substantial increase in the mutation rate of *E. coli* (Giraud et al. 2001; Shaver et al. 2002).

Initial characterization of the lactate production strain revealed that supplementation of the medium with yeast extract (YE) was necessary to sustain anaerobic growth during the adaptive evolution process. Strains growing with supplementation all possessed approximately the same growth profile and production characteristics when compared to the unsupplemented culture (FIG. **3**). For the supplemented cultures, the maximum growth rate was 0.26±0.01 hr-1. The culture without supplementation had a maximum growth rate of 0.20 hr-1 during exponential phase. The overall batch product yields, Yp/s, are given in Table IV. After BOP384 growing with no supplementation was passed to fresh medium (with the same initial composition, no supplementation) under anaerobic conditions, growth ceased in numerous attempts after approximately two to three culture doublings (results not shown), further justifying the choice of medium supplementation with yeast extract

BOP384 was evolved in duplicate on glucose using the established batch serial passage adaptive evolution process to keep the population in exponential growth phase (see Materials and Methods). The starting strain was unevolved BOP384 supplemented with 1.0 g/L YE (characteristics are shown in Table IV). Both of the duplicate evolutions resulted in endpoint strains with very similar growth and production profiles. The percent increase over the initial 0.26±0.01 hr-1 growth rate after the first 200 hours of the entire evolution (which was about 14 days total) was roughly 500%, as shown in FIG. **4**. There was a rapid increase in the growth rate during the adaptation with the cultures reaching their final growth rates in about 2.5 days. This adaptation period of 2.5 days is much less than that of *E. coli* in other adaptive evolution experiments in which strains reached their final growth rate in 10-30 days (Fong and Palsson 2004; Ibarra et al. 2002). The final growth rate anaerobically was much higher than observed in earlier studies. In total, the evolution process was carried out for slightly over 14 days until the growth rate stopped increasing. Single colonies were isolated from the final cultures of each evolution (after 14 days), their knockout genotypes were confirmed by PCR, and they were designated as BOP384eG1 and BOP384eG2.

Strains BOP384eG1 and BOP384eG2 were cultured from frozen stocks and examined for their production capabilities. It is noted that the growth rates of the indicated populations are illustrated reported in FIG. **4**, whereas clonal isolates are exampled and reported in Table IV. Furthermore, growth rates from FIG. **4** may include overestimates due to characteristic dilution error that can occur when calculating the expected small inoculation sizes. Nonetheless, the increase in growth phenotype is significant.

Example 6: Growth Rates, Culture Doublings, and Division Events of Engineered Microbial Strains

Cell concentration in cultures was determined by measuring the optical density at 600 nm (OD600) using a Biomate 3 spectrophotometer (Thermo Scientific, USA). A value of 1.55*1012 cells L-10D600^{-1} was used to calculate cell numbers with a dry cell weight of 2.9*10-13 gDW cell^{-1} (Neidhardt 1996). Total biomass can be calculated as 0.45 gDW L^{-1} OD600^{-1}. Growth rates of batch cultures during exponential growth were determined using at least three cell and metabolite concentration data points.

The mutations that accumulate during adaptive evolution occur randomly during cell division, so it was useful to calculate the total number of cell doublings that have occurred at any time. The formula used for this was D=(2^G−1)*I where D is the total number of cellular division events, I is the initial number of cells, and G is the number of cell divisions per initial cell (the number of generations). One doubling occurred for every new cell in the culture.

Characterization of the final lactate producing strains indicated evolution to a production phenotype in agreement with computational predictions. Table IV contains data from the characterization of the endpoint strains. The final production rate of lactate was 84.4±1.5 mmol gDW^{-1} hr^{-1} and additionally succinate was made a rate of 4.3±0.3 mmol gDW^{-1} hr^{-1}. This correlated to a 97.9±1.2% and 6.5±0.3% wt % product yield at steady-state during the exponential growth phase for lactate and succinate, respectively. Overall percent product yields for lactate and succinate were 98.4±3.4% and 3.4±2.8% wt %, respectively. The overall yield was monitored throughout the evolution and was consistent at these values after 2.5 days of evolution. The steady-state production rate of lactate increased over 2 fold for the endpoint strain compared to the unevolved strain. The glucose consumption rate also has a similar 2 fold increase, and the production rate of succinate increased 7 fold. The steady-state wt % yield for lactate was approximately the same and that of succinate increased approximately 3.5 fold. The overall wt % yields were approximately the same for both lactate and succinate. The summation of the wt % yields over 100% indicate that some of the supplemented yeast extract was contributing to the lactate and/or succinate production. Overall, 93.8% of the total product generated during fermentation at steady-state was lactate, close to the homofermentative criteria set for the strain in the computational selection of strain designs.

The data from the unevolved and evolved strains (including strains according to some embodiments herein) on glucose analyzed with the iAF1260 model is shown in FIG. **5**. The computational predictions and experimentally evolved endpoint measurements display good agreement. For the measurement of unevolved, unsupplemented growth on glucose, the initial phenotype is suboptimal with the production rate and growth rate less than the optimally predicted point. Coincidentally, the glucose uptake rate of 18.4 is almost identical to that observed for anaerobic growth of *E. coli* in an earlier modeling and experimental evaluation of growth (Varma and Palsson 1994) and gives confidence in the single measurement. Supplementation with yeast extract for unevolved glucose growth displays near optimal behavior as predicted with the model at the measured uptake rate. This demonstrates that yeast extract allows more incoming carbon (glucose and yeast extract content) to be used for lactate production and for biomass generation. The endpoint predictions and experimental measurements are in good agreement, with the experimentally measured growth rate and lactate production rate contributing to a point very near the optimal growth rate. The increased growth and production rates are due to the over 2 fold increase in the glucose uptake rate. This characterization of an adaptively evolved production strain demonstrates that a computationally designed strain can result in an experimentally verified production phenotype in good agreement with modeling predictions.

The endpoint strains (see Table IV and FIG. **5**) display superior performance in terms of production rate, growth rate, and by-product formation over previously generated lactate production strains (Fong et al. 2005). In comparison to lactate production studies on an industrial scale, the steady-state and overall yields generated in this study of 0.98 g g^{-1} are at the same level or above previously reported values of 0.9 g g^{-1} (Chang et al. 1999), 0.93 g g^{-1} (Dien et al. 2001), 1.0 g g^{-1} (Zhou et al. 2003), and 0.86 g g^{-1} (Zhu et al. 2007). Furthermore, even at the relatively low cellular densities used for this process, a volumetric productivity of 1.7 g L^{-1} hr^{-1} was achieved, comparing favorably with previous studies where cell densities were driven roughly an order of magnitude higher and productivity values of 0.7-3.5 g L^{-1} hr^{-1} were reported (Chang et al. 1999; Dien et al. 2001; Zhou et al. 2003; Zhu et al. 2007). The overall product yield is very close to theoretical maximum for a growing strain (Feist et al. 2010), and is even above the theoretical value when considering supplementation.

TABLE IV

Characterization of the lactate production strain BOP384 prior to and after evolution (% $Y_{p/s}$—percent production yield, Supp.—supplement, YE—yeast extract).

Evolution Status	Supp.	Growth Rate (hr^{-1})	Product/ Substrate	Production/ Consumption Rate (mmol gDW^{-1} hr^{-1})	% $Y_{p/s}$ Steady-State (wt %)	% $Y_{p/s}$ (wt %)
unevolved	1, 1.5, 2 g/L YE	0.26 ± 0.01	glucose	20.7 ± 0.7		
			lactate	41.1 ± 4.2	99.7 ± 0.1%	101.3 ± 6.4%
			succinate	0.6 ± 0.1	1.8 ± 0.4%	3.7 ± 0.5%
unevolved	none	0.2	glucose	18.4		
			lactate	27.7	75.30%	97.30%
			succinate	0	0%	3.90%
evolved	1 g/L YE	0.86 ± 0.00	glucose	43.1 ± 1.3		
			lactate	84.4 ± 1.5	97.9 ± 1.2%	*98.4 ± 3.4%
			succinate	4.3 ± 0.3	6.5 ± 0.3%	*3.4 ± 2.8%

*Overall yield monitored throughout evolution and was consistent after 2.5 days.

Example 7: Genomic Sequencing of Selected Microbial Strains

Clonal isolates of each of the strains indicated in Table V were taken along the course of the roughly 14 day evolution, sequenced, and compared to the wild type and starting strains. The following is a list of isolates which were sequenced:

TABLE V

Strains selected for sequencing Strain selected for sequencing (timepoint in evolution is indicated, if applicable)
'BOP27'-Wild Type-ATCC 700926
'BOP384' Day 0
'BOP384G1D' Day 3
'BOP384G1H' Day 5
'BOP384G1N' Day 8
'BOP384G1S' Day 10
'BOP384G1X' Day 13
'BOP384eG1' isolate 1 Day 14
'BOP384eG1' isolate 2 Day 14

Genomic DNA was isolated using Promega's Wizard DNA Purification Kit. The quality of DNA was assessed with UV absorbance ratios using a Nano drop. DNA was quantified using Qubit dsDNA High Sensitivity assay. Paired-end resequencing libraries were generated using Illumina's Nextera XT kit with 1 ng of input DNA total. The sequencing results were analyzed using the breseq analysis software.

Sequences of each strain that was sequenced were compared to a wild-type reference sequence, SEQ ID NO: 1. Mutations identified in the analysis, including the affected gene, if any, and the affected protein, if any, are shown in Table VIa. The mutations identified in each sequenced strain (recovered at various timepoints) and the location of the mutations are shown in Tables VIb and VIc in which "TRUE" indicates the presence of a mutation in a particular strain and a "blank" entry indicates that the wildtype sequence is present at that location in a particular strain. Thus, from Tables VIb and VIc, the complete genomic sequence of each strain may be determined. The mutations found in each of the following strains are annotated in SEQ ID NOs: 2-9, as indicated in Tables VIb and VIc.: BOP384' Day 0 (see SEQ ID NO: 2), BOP384G1D' Day 3 (see SEQ ID NO: 3), BOP384G1H' Day 5 (see SEQ ID NO: 4), BOP384G1N' Day 8 (see SEQ ID NO: 5), BOP384G1S' Day 10 (see SEQ ID NO: 6), BOP384G1X' Day 13 (see SEQ ID NO: 7), BOP384eG1' (isolate 1) Day 14 (see SEQ ID NO: 8), or BOP384eG1' (isolate 2) Day 14 (see SEQ ID NO: 9).

TABLE VIa

Mutations Identified from Genomic Sequencing

Mutation Position & Nucleotide change (with reference to SEQ ID NO: 1)	Gene	Protein change
1022011 C→T	yccS	A366T (GCT→ACT)
111897 G→A	yacF	Q235* (CAG→TAG)
1135244 A→G	flgH	D153G (GAC→GGC)
1137595 A→G	flgJ/flgK	intergenic (+60/-6)
1163111 A→G	thiK	H210R (CAC→CGC)
1247873 G→A	dhaM	A155A (GCC→GCT)
1260197 C→T	prs	R301H (CGT→CAT)
1274794 T→C	narL	S87G (AGC→GGC)
1386732 A→G	tpx	L35P (CTG→CCG)
1435247 Δ1 bp	micC	intergenic (-330/+37)
1440978 G→A	ldhA/ydbH	intergenic (-111/-97)
1580390 A→G	ydeN	S53S (AGT→AGC)
1604028 T→C	lsrB	G318G (GGT→GGC)
1610530 + C	yneH	coding (746/927 nt)
1679479 T→C	folM	D160D (GAT→GAC)
1682336 A→G	tus	E18E (GAA→GAG)
1729483 G→A	lhr	P791P (CCG→CCA)
1760136 G→A	sufC	P134L (CCG→CTG)
1866695 G→A	yeaG	M588I (ATG→ATA)
1929016 G→A	purT	M38M (GTG→ATG)
1950262 G→A	yecE/yecN	intergenic (+25/-28)

TABLE VIa-continued

Mutations Identified from Genomic Sequencing

Mutation Position & Nucleotide change (with reference to SEQ ID NO: 1)	Gene	Protein change
1976527 Δ776 bp	flhD/insB-5	
2071288 G→A	flu	D576N (GAT→AAT)
2085304 + G	yoeI/yeeY	intergenic (-166/+49)
2098010 T→C	gnd	Y428C (TAC→TGC)
2257220 A→G	pscK	G33G (GGT→GGC)
2358479 + C	yfaW	coding (958/1206 nt)
2405257 G→C	lrhA/yfbQ	intergenic (-594/-326)
2534334 Δ1 :: IS186 (-) + 6 bp :: Δ1	crr	coding (479-484/510 nt)
2620968 Δ1 bp	purN/ppk	intergenic (+74/-98)
2662540 T→C	yfhR	G43G (GGT→GGC)
2732557 + C	yfiH	coding (500/732 nt)
2740321 T→C	yfiR	V142A (GTC→GCC)
2763809 + C	yfjM/rnlA	intergenic (-11/-131)
2767188 G→A	yfjQ	D168N (GAT→AAT)
2782127 A→G	ypjB	pseudogene (907/1374 nt)
2809146 + A	mpTRUE	coding (355/531 nt)
2824039 T→C	srlA	G62G (GGT→GGC)
2826646 G→A	gutM	V2M (GTA→ATA)
2844070 G→A	hycE	P142S (CCG→TCG)
2926442 + T	sdaC	coding (192/1290 nt)
2927497 G→A	sdaC	G416D (GGT→GAT)
2932138 T→A	fucA/fucP	intergenic (-428/-119)
2965591 T→C	ptsP	N289S (AAC→AGC)
2975919 T→C	lysA	T335A (ACC→GCC)
3114125 C→T	yghJ	V1004M (GTC→ATC)
3176882 G→A	tolC	R249H (CGC→CAC)
3218853 T→C	ygjG	R446R (CGT→CGC)
3246033 G→A	yqjA	S80N (AGC→AAC)
3268091 C→T	yhaC/rnpB	intergenic (+467/+556)
3268123 C→A	yhaC/rnpB	intergenic (+499/+524)
3268165 C→T	yhaC/rnpB	intergenic (+541/+482)
3279141 C→T	agaW/agaA	pseudogene (49/555 nt)
3302829 Δ1 bp	mtr	coding (1011/1245 nt)
3315496 A→G	nusA	L18P (CTA→CCA)
3335733 C→T	mlaC	D61N (GAT→AAT)
34111 + T	carB/caiF	intergenic (+73/-189)

TABLE VIa-continued

Mutations Identified from Genomic Sequencing

Mutation Position & Nucleotide change (with reference to SEQ ID NO: 1)	Gene	Protein change
3429378 T→C	rimN	E20E (GAA→GAG)
345189 A→G	yahN	Y125H (TAT→CAT)
3506796 T→C	yhfW	H347R (CAC→CGC)
3526004 T→C	yrfF	V505A (GTG→GCG)
3548297 T→C	malP	K733K (AAA→AAG)
379237 Δ1 bp	frmR/yaiO	intergenic (-132/+56)
379237 Δ2 bp	frmR/yaiO	intergenic (-132/+55)
386281 C→T	tauC	G29G (GGC→GGT)
3955730 G→A	ilvY	R38R (CGC→CGT)
3957957 C→T	ppiC/yifO	intergenic (-121/+78)
3978813 C→A	rffM/yifK	intergenic (+94/-97)
3982057 C→T	aslB	L359L (CTC→CTT)
3983344 C→T	aslA	V229V (GTG→GTA)
3990407 Δ8 bp	cyaA	coding (1232-1239/2547 nt)
407943 A→G	ykiA	pseudogene (111/342 nt)
4105271 A→G	fieF	A260A (GCA→GCG)
4153541 A→G	argC	D173G (GAC→GGC)
42111 C→A	caiT/fixA	intergenic (-180/-292)
4234068 T→G	yjbE	V47G (GTC→GGC)
4294403 + CG	gltP/yTRUEO	intergenic (+586/+56)
4306572 3434 bp→82 bp	alsC	
4444833 A→G	ytfN	D900G (GAT→GGT)
4503718 G→A	yjhC	V137M (GTT→ATT)
454725 C→T	tig	I123I (ATC→ATT)
4604230 + G	leuP	noncoding (80/87 nt)
4604346 Δ1 bp	leuQ	noncoding (79/87 nt)
507894 T→C	ybaQ/copA	intergenic (+111/+205)
533362 A→G	gcl	T75A (ACT→GCT)
547694 A→G	ylbE_1	pseudogene (139/252 nt)
547831 + G	ylbE_1	pseudogene (2/252 nt)
558493 G→T	sfmC	L99L (CTG→CTT)
560913 + G	sfmD	coding (1994/2604 nt)
619171 A→G	fepC	G84G (GGT→GGC)
653396 T→C	dpiA	Y104Y (TAT→TAC)
696062 G→A	glnW/glnU	intergenic (-9/+26)
700027 C→T	nagC	C264Y (TGC→TAC)
700286 + C	nagC	coding (532/1221 nt)

TABLE VIa-continued

Mutations Identified from Genomic Sequencing

Mutation Position & Nucleotide change (with reference to SEQ ID NO: 1)	Gene	Protein change
700599 + C	nagC	coding (219/1221 nt)
700679 + G	nagC	coding (139/1221 nt)
751964 C→T	ybgD	A19T (GCA→ACA)
760544 C→T	sucA	C872C (TGC→TGT)
844446 C→T	ybiO	P86P (CCG→CCA)
852434 + A	mntR	coding (29/468 nt)
922473 + G	clpS/clpA	intergenic (+17/-14)
961467 C→T	rpsA	M84M (CTG→TTG)
963462 A→G	ihfB/ycaI	intergenic (+127/-81)

TABLE VIb

Mutations Identified from Genomic Sequencing

Mutation Position & Nucleotide change	BOP27' (Wild Type) ATCC 700926	BOP384' Day 0 (see SEQ ID NO: 2)	BOP384G1D' Day 3 (see SEQ ID NO: 3)	BOP384G1H' Day 5 (see SEQ ID NO: 4)	BOP384G1N' Day 8 (see SEQ ID NO: 5)
1022011 C→T			TRUE		
111897 G→A					
1135244 A→G					TRUE
1137595 A→G					
1163111 A→G					TRUE
1247873 G→A				TRUE	
1260197 C→T					
1274794 T→C					
1386732 A→G					
1435247 Δ1 bp					
1440978 G→A					
1580390 A→G				TRUE	
1604028 T→C					
1610530 + C					TRUE
1679479 T→C		TRUE			
1682336 A→G					
1729483 G→A					
1760136 G→A			TRUE		
1866695 G→A					
1929016 G→A			TRUE		
1950262 G→A					TRUE
1976527 Δ776 bp		TRUE	TRUE	TRUE	TRUE
2071288 G→A					
2085304 + G					
2098010 T→C					
2257220 A→G					
2358479 + C				TRUE	
2405257 G→C		TRUE	TRUE	TRUE	TRUE
2534334 Δ1 :: IS186 (—) + 6 bP :: Δ1		TRUE	TRUE	TRUE	TRUE
2620968 Δ1 bp					
2662540 T→C					TRUE
2732557 + C					
2740321 T→C		TRUE	TRUE	TRUE	TRUE
2763809 + C					
2767188 G→A					
2782127 A→G					
2809146 + A					
2824039 T→C					
2826646 G→A					TRUE
2844070 G→A					

TABLE VIb-continued

Mutations Identified from Genomic Sequencing					
Mutation Position & Nucleotide change	BOP27' (Wild Type) ATCC 700926	BOP384' Day 0 (see SEQ ID NO: 2)	BOP384G1D' Day 3 (see SEQ ID NO: 3)	BOP384G1H' Day 5 (see SEQ ID NO: 4)	BOP384G1N' Day 8 (see SEQ ID NO: 5)
2926442 + T				TRUE	
2927497 G→A					TRUE
2932138 T→A		TRUE	TRUE	TRUE	TRUE
2965591 T→C					
2975919 T→C					
3114125 C→T					
3176882 G→A					
3218853 T→C					
3246033 G→A				TRUE	
3268091 C→T					
3268123 C→A					
3268165 C→T					
3279141 C→T		TRUE	TRUE	TRUE	TRUE
3302829 Δ1 bp					
3315496 A→G		TRUE			
3335733 C→T				TRUE	
34111 + T					TRUE
3429378 T→C			TRUE		
345189 A→G			TRUE		
3506796 T→C					
3526004 T→C					
3548297 T→C				TRUE	
379237 Δ1 bp					TRUE
379237 Δ2 bp					
386281 C→T					
3955730 G→A		TRUE			
3957957 C→T	TRUE	TRUE	TRUE	TRUE	TRUE
3978813 C→A					
3982057 C→T					
3983344 C→T					
3990407 Δ8 bp		TRUE	TRUE	TRUE	TRUE
407943 A→G					
4105271 A→G					
4153541 A→G		TRUE	0/47		
42111 C→A					
4234068 T→G					
4294403 + CG	TRUE	TRUE	TRUE	TRUE	TRUE
4306572		TRUE	TRUE	TRUE	TRUE
3434 bp→82 bp					
4444833 A→G					
4503718 G→A					
454725 C→T			TRUE	TRUE	
4604230 + G				TRUE	
4604346 Δ1 bp					
507894 T→C		TRUE	TRUE	TRUE	TRUE
533362 A→G				TRUE	
547694 A→G	TRUE	TRUE	TRUE	TRUE	TRUE
547831 + G	TRUE	TRUE	TRUE	TRUE	TRUE
558493 G→T					
560913 + G					
619171 A→G					
653396 T→C					
696062 G→A					
700027 C→T					
700286 + C					TRUE
700599 + C				TRUE	
700679 + G			TRUE		
751964 C→T		TRUE			
760544 C→T					
844446 C→T					TRUE
852434 + A					
922473 + G				TRUE	
961467 C→T			TRUE		
963462 A→G					

TABLE VIc

Mutations Identified from Genomic Sequencing

Mutation Position & Nucleotide change	BOP384G1S' Day 10 (see SEQ ID NO: 6)	BOP384G1X' Day 13 (see SEQ ID NO: 7)	BOP384eG1' (isolate 1) Day 14 (see SEQ ID NO: 8)	BOP384eG1' (isolate 2) Day 14 (see SEQ ID NO: 9)
1022011 C→T				
111897 G→A			TRUE	
1135244 A→G				
1137595 A→G		TRUE	TRUE	TRUE
1163111 A→G				
1247873 G→A				
1260197 C→T	TRUE			
1274794 T→C		TRUE	TRUE	TRUE
1386732 A→G			TRUE	
1435247 Δ1 bp		TRUE		
1440978 G→A		TRUE	TRUE	TRUE
1580390 A→G				
1604028 T→C		TRUE		
1610530 + C				
1679479 T→C				
1682336 A→G		TRUE	TRUE	TRUE
1729483 G→A		TRUE	TRUE	TRUE
1760136 G→A				
1866695 G→A			TRUE	
1929016 G→A				
1950262 G→A	TRUE			
1976527 Δ776 bp	TRUE	TRUE	TRUE	TRUE
2071288 G→A		TRUE	TRUE	TRUE
2085304 + G		TRUE	TRUE	
2098010 T→C		TRUE	TRUE	TRUE
2257220 A→G			TRUE	
2358479 + C				
2405257 G→C	TRUE	TRUE	TRUE	TRUE
2534334 Δ1::IS186 (—) + 6 bp::Δ1	TRUE	TRUE	TRUE	TRUE
2620968 Δ1 bp	TRUE			
2662540 T→C	TRUE			
2732557 + C		TRUE	TRUE	
2740321 T→C	TRUE	TRUE	TRUE	TRUE
2763809 + C	TRUE			
2767188 G→A	TRUE			
2782127 A→G			TRUE	
2809146 + A		TRUE	TRUE	
2824039 T→C			TRUE	
2826646 G→A	TRUE			
2844070 G→A		TRUE	TRUE	TRUE
2926442 + T				
2927497 G→A	TRUE	TRUE	TRUE	TRUE
2932138 T→A	TRUE	TRUE	TRUE	TRUE
2965591 T→C		TRUE		
2975919 T→C		TRUE	TRUE	TRUE
3114125 C→T	TRUE			
3176882 G→A			TRUE	
3218853 T→C	TRUE			
3246033 G→A				
3268091 C→T	TRUE			
3268123 C→A	TRUE			
3268165 C→T	TRUE			
3279141 C→T	TRUE	TRUE	TRUE	TRUE
3302829 Δ1 bp	TRUE			
3315496 A→G				
3335733 C→T				
34111 + T	TRUE	TRUE	TRUE	
3429378 T→C				
345189 A→G				
3506796 T→C			TRUE	
3526004 T→C		TRUE	TRUE	TRUE
3548297 T→C				
379237 Δ1 bp		TRUE	TRUE	
379237 Δ2 bp	TRUE			
386281 C→T		TRUE		TRUE
3955730 G→A				
3957957 C→T	TRUE	TRUE	TRUE	TRUE
3978813 C→A			TRUE	
3982057 C→T		TRUE		

TABLE VIc-continued

Mutations Identified from Genomic Sequencing				
Mutation Position & Nucleotide change	BOP384G1S' Day 10 (see SEQ ID NO: 6)	BOP384G1X' Day 13 (see SEQ ID NO: 7)	BOP384eG1' (isolate 1) Day 14 (see SEQ ID NO: 8)	BOP384eG1' (isolate 2) Day 14 (see SEQ ID NO: 9)
3983344 C→T	TRUE			
3990407 Δ8 bp	TRUE	TRUE	TRUE	TRUE
407943 A→G		TRUE		
4105271 A→G			TRUE	
4153541 A→G				
42111 C→A			TRUE	
4234068 T→G	TRUE			
4294403 + CG	TRUE	TRUE	TRUE	TRUE
4306572 3434 bp→82 bp	TRUE	TRUE	TRUE	TRUE
4444833 A→G		TRUE	TRUE	TRUE
4503718 G→A	TRUE			
454725 C→T				
4604230 + G				
4604346 Δ1 bp		TRUE		
507894 T→C	TRUE	TRUE	TRUE	TRUE
533362 A→G				
547694 A→G	TRUE	TRUE	TRUE	TRUE
547831 + G	TRUE	TRUE	TRUE	TRUE
558493 G→T			TRUE	
560913 + G		TRUE	TRUE	TRUE
619171 A→G			TRUE	
653396 T→C		TRUE	TRUE	TRUE
696062 G→A		TRUE		
700027 C→T		TRUE	TRUE	TRUE
700286 + C	TRUE			
700599 + C				
700679 + G				
751964 C→T				
760544 C→T		TRUE	TRUE	TRUE
844446 C→T	TRUE			
852434 + A			TRUE	
922473 + G				
961467 C→T				
963462 A→G			TRUE	

General Comments

With respect to the use of substantially any plural and/or singular terms herein, those having skill in the art can translate from the plural to the singular and/or from the singular to the plural as is appropriate to the context and/or application. The various singular/plural permutations may be expressly set forth herein for sake of clarity.

It will be understood by those within the art that, in general, terms used herein, and especially in the appended claims (e.g., bodies of the appended claims) are generally intended as "open" terms (e.g., the term "including" should be interpreted as "including but not limited to," the term "having" should be interpreted as "having at least," the term "includes" should be interpreted as "includes but is not limited to," etc.). It will be further understood by those within the art that if a specific number of an introduced claim recitation is intended, such an intent will be explicitly recited in the claim, and in the absence of such recitation no such intent is present. For example, as an aid to understanding, the following appended claims may contain usage of the introductory phrases "at least one" and "one or more" to introduce claim recitations. However, the use of such phrases should not be construed to imply that the introduction of a claim recitation by the indefinite articles "a" or "an" limits any particular claim containing such introduced claim recitation to embodiments containing only one such recitation, even when the same claim includes the introductory phrases "one or more" or "at least one" and indefinite articles such as "a" or "an" (e.g., "a" and/or "an" should be interpreted to mean "at least one" or "one or more"); the same holds true for the use of definite articles used to introduce claim recitations. In addition, even if a specific number of an introduced claim recitation is explicitly recited, those skilled in the art will recognize that such recitation should be interpreted to mean at least the recited number (e.g., the bare recitation of "two recitations," without other modifiers, means at least two recitations, or two or more recitations). Furthermore, in those instances where a convention analogous to "at least one of A, B, and C, etc." is used, in general such a construction is intended in the sense one having skill in the art would understand the convention (e.g., "a system having at least one of A, B, and C" would include but not be limited to systems that have A alone, B alone, C alone, A and B together, A and C together, B and C together, and/or A, B, and C together, etc.). In those instances where a convention analogous to "at least one of A, B, or C, etc." is used, in general such a construction is intended in the sense one having skill in the art would understand the convention (e.g., "a system having at least one of A, B, or C" would include but not be limited to systems that have A alone, B alone, C alone, A and B together, A and C together, B and C together, and/or A, B, and C together, etc.). It will be further understood by those within the art that virtually any disjunctive word and/or phrase presenting two or more alternative terms, whether in the description, claims, or drawings, should be understood to

contemplate the possibilities of including one of the terms, either of the terms, or both terms. For example, the phrase "A or B" will be understood to include the possibilities of "A" or "B" or "A and B."

In addition, where features or aspects of the disclosure are described in terms of Markush groups, those skilled in the art will recognize that the disclosure is also thereby described in terms of any individual member or subgroup of members of the Markush group.

As will be understood by one skilled in the art, for any and all purposes, such as in terms of providing a written description, all ranges disclosed herein also encompass any and all possible sub-ranges and combinations of sub-ranges thereof. Any listed range can be easily recognized as sufficiently describing and enabling the same range being broken down into at least equal halves, thirds, quarters, fifths, tenths, etc. As a non-limiting example, each range discussed herein can be readily broken down into a lower third, middle third and upper third, etc. As will also be understood by one skilled in the art all language such as "up to," "at least," "greater than," "less than," and the like include the number recited and refer to ranges which can be subsequently broken down into sub-ranges as discussed above. Finally, as will be understood by one skilled in the art, a range includes each individual member. Thus, for example, a group having 1-3 articles refers to groups having 1, 2, or 3 articles. Similarly, a group having 1-5 articles refers to groups having 1, 2, 3, 4, or 5 articles, and so forth.

While various aspects and embodiments have been disclosed herein, other aspects and embodiments will be apparent to those skilled in the art. The various aspects and embodiments disclosed herein are for purposes of illustration and are not intended to be limiting, with the true scope and spirit being indicated by the following claims.

While the present invention has been described in some detail for purposes of clarity and understanding, one skilled in the art will appreciate that various changes in form and detail can be made without departing from the true scope of the invention.

The term "comprising" as used herein is synonymous with "including," "containing," or "characterized by," and is inclusive or open-ended and does not exclude additional, unrecited elements or method steps.

All numbers expressing quantities of ingredients, reaction conditions, and so forth used in the specification are to be understood as being modified in all instances by the term "about." Accordingly, unless indicated to the contrary, the numerical parameters set forth herein are approximations that may vary depending upon the desired properties sought to be obtained. At the very least, and not as an attempt to limit the application of the doctrine of equivalents to the scope of any claims in any application claiming priority to the present application, each numerical parameter should be construed in light of the number of significant digits and ordinary rounding approaches.

The above description discloses several methods and materials of the present invention. This invention is susceptible to modifications in the methods and materials, as well as alterations in the fabrication methods and equipment. Such modifications will become apparent to those skilled in the art from a consideration of this disclosure or practice of the invention disclosed herein. Consequently, it is not intended that this invention be limited to the specific embodiments disclosed herein, but that it cover all modifications and alternatives coming within the true scope and spirit of the invention.

The foregoing description and Examples detail certain embodiments. It will be appreciated, however, that no matter how detailed the foregoing may appear in text, the invention may be practiced in many ways and the invention should be construed in accordance with the appended claims and any equivalents thereof.

REFERENCES

All references discussed herein, including the references below, are incorporated herein by reference in their entirety and are hereby made a part of this specification. To the extent publications and patents or patent applications incorporated by reference contradict the disclosure contained in the specification, the specification is intended to supersede and/or take precedence over any such contradictory material.

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SEQUENCE LISTING

The patent contains a lengthy "Sequence Listing" section. A copy of the "Sequence Listing" is available in electronic form from the USPTO web site (http://seqdata.uspto.gov/?pageRequest=docDetail&DocID=US09932598B2). An electronic copy of the "Sequence Listing" will also be available from the USPTO upon request and payment of the fee set forth in 37 CFR 1.19(b)(3).

What is claimed is:

1. A genetically engineered recombinant *E. coli* comprising a genetic modification of pflABfocA and a genetic modification of pflDC which genetic modifications substantially reduce pyruvate formate lyase (PFL) activity, and a genetic modification which substantially reduces pyruvate dehydrogenase (PDH) activity, wherein the genetically engineered recombinant *E. coli* comprises a genotype of: pflABfocA pflDC aceEF xylFGH rbsACB alsBAC mutS.

2. The genetically engineered recombinant *E. coli* of claim **1**, wherein the genetically engineered recombinant *E. coli* is configured for producing carbohydrates, wherein producing carbohydrates comprises producing D-lactate from a glucose precursor.

3. The genetically engineered recombinant *E. coli* of claim 1, wherein the genetic modifications which substantially reduce PFL activity reduce PFL activity by at least about 70%.

4. The genetically engineered recombinant *E. coli* of claim 1, wherein the genetic modification which substantially reduces PDH activity reduces PDH activity by at least about 70%.

5. The genetically engineered recombinant *E. coli* of claim 1, wherein the genetic modification which substantially reduces PFL activity reduces PFL activity by at least about 90%, and wherein the genetic modification which substantially reduces PDH activity reduces PDH activity by at least about 90%.

6. The genetically engineered recombinant *E. coli* of claim 1, wherein the genetic modifications which substantially reduce PFL activity eliminate PFL activity, and wherein the genetic modification which substantially reduces PDH activity eliminates PDH activity.

7. The genetically engineered recombinant *E. coli* of claim 1, wherein the genetically engineered recombinant *E. coli* has undergone adaptive evolution.

8. The genetically engineered recombinant *E. coli* of claim 1, further comprising a genetic modification that increases the mutation rate of the genetically engineered recombinant *E. coli* at least about 2-fold per generation.

9. The genetically engineered recombinant *E. coli* of claim 8, wherein the genetic modification that increases the mutation rate comprises a loss-of-function mutation in the mutS gene.

10. The genetically engineered recombinant *E. coli* of claim 1, wherein the genetic modifications which substantially reduce PFL activity are selected from the group consisting of: a hypomorphic mutation in each of pflABfocA and pflDC, a phenotypic null mutation in each of pflABfocA and pflDC, a deletion of each of pflABfocA and pflDC, a hypomorphic mutation in pflABfocA and a deletion of pflDC, a deletion of pflABfocA and a hypomorphic mutation in pflDC, a hypomorphic mutation in pflABfocA and a phenotypic null mutation in pflDC, a null mutation in pflABfocA and a hypomorphic mutation in pflDC, a deletion of in pflABfocA and a null mutation in pflDC, and a phenotypic null mutation in pflABfocA and a deletion of pflDC.

11. The genetically engineered recombinant *E. coli* of claim 1, wherein the genetic modification which substantially reduces PDH activity is selected from the group consisting of a hypomorphic mutation in aceEF, a null mutation in aceEF, and a deletion of aceEF.

12. The genetically engineered recombinant *E. coli* of claim 1, wherein the genetic modifications which substantially reduce PFL activity comprise a deletion of each of pflABfocA and pflDC, and wherein the genetic modification eliminating PDH activity comprises a deletion of aceEF.

13. The genetically engineered recombinant *E. coli* of claim 1, wherein the genetically engineered recombinant *E. coli* is of one of the BOP384eG1 strain or BOP384eG2 strain.

14. The genetically engineered recombinant *E. coli* of claim 1, further comprising deletion of each of a native xylGFH, rbsACB, and alsBAC operon.

15. The genetically engineered recombinant *E. coli* of claim 1, further comprising at least one mutation selected from the group consisting of: 1022011 C→T, 111897 G→A, 1135244 A→G, 1137595 A→G, 1163111 A→G, 1247873 G→A, 1260197 C→T, 1274794 T→C, 1386732 A→G, 1435247 Δ1 bp, 1440978 G→A, 1580390 A→G, 1604028 T→C, 1610530+C, 1679479 T→C, 1682336 A→G, 1729483 G→A, 1760136 G→A, 1866695 G→A, 1929016 G→A, 1950262 G→A, 1976527 Δ776 bp, 2071288 G→A, 2085304+G, 2098010 T→C, 2257220 A→G, 2358479+C, 2405257 G→C, 2534334 Δ1::IS186 (−)+6 bp::Δ1, 2620968 Δ1 bp, 2662540 T→C, 2732557+C, 2740321 T→C, 2763809+C, 2767188 G→A, 2782127 A→G, 2809146+A, 2824039 T→C, 2826646 G→A, 2844070 G→A, 2926442+T, 2927497 G→A, 2932138 T→A, 2965591 T→C, 2975919 T→C, 3114125 C→T, 3176882 G→A, 3218853 T→C, 3246033 G→A, 3268091 C→T, 3268123 C→A, 3268165 C→T, 3279141 C→T, 3302829 Δ1 bp, 3315496 A→G, 3335733 C→T, 34111+T, 3429378 T→C, 345189 A→G, 3506796 T→C, 3526004 T→C, 3548297 T→C, 379237 Δ1 bp, 379237 Δ2 bp, 386281 C→T, 3955730 G→A, 3957957 C→T, 3978813 C→A, 3982057 C→T, 3983344 C→T, 3990407 Δ8 bp, 407943 A→G, 4105271 A→G, 4153541 A→G, 42111 C→A, 4234068 T→G, 4294403+CG, 4306572 3434 bp→82 bp, 4444833 A→G, 4503718 G→A, 454725 C→T, 4604230+G, 4604346 Δ1 bp, 507894 T→C, 533362 A→G, 547694 A→G, 547831+G, 558493 G→T, 560913+G, 619171 A→G, 653396 T→C, 696062 G→A, 700027 C→T, 700286+C, 700599+C, 700679+G, 751964 C→T, 760544 C→T, 844446 C→T, 852434+A, 922473+G, 961467 C→T, and 963462 A→G, with reference to SEQ ID NO: 1.

16. The genetically engineered recombinant *E. coli* of claim 2, wherein the genetically engineered recombinant *E. coli* has a steady-state glucose uptake rate of at least about 30 mmol per gDW per hour under standard conditions in 1 g per liter yeast extract medium.

17. A genetically engineered, recombinant *E. coli* comprising a genetic modification of pflABfocA and a genetic modification of pflDC which genetic modifications reduce pyruvate formate lyase (PFL) activity, and a genetic modification which reduces pyruvate deghydrogenase (PDH) activity, wherein said genetically engineered recombinant *E. coli* has a steady-state glucose uptake rate of at least about 30 mmol per gDW per hour under standard conditions in 1 g per liter yeast extract medium,

the genetically engineered recombinant *E. coli* further comprising at least one mutation selected from the group consisting of: 1022011 C→T, 111897 G→A, 1135244 A→*G, 1137595 A→G, 1163111 A→G, 1247873 G→A, 1260197 C→T, 1274794 T→C, 1386732 A→G, 1435247 Δ1 bp, 1440978 G→A, 1580390 A→G, 1604028 T→C, 1610530+C, 1682336 A→G, 1729483 G→A, 1760136 G→A, 1866695 G→A, 1929016 G→A, 1950262 G→A, 2071288 G→A, 2085304+G, 2098010 T→C, 2257220 A→G, 2358479+C, 2620968 Δ1 bp, 2662540 T→C, 2732557+C, 2763809+C, 2767188 G→A, 2782127 A→G, 2809146+A, 2824039 T→C, 2826646 G→A, 2844070 G→A, 2926442+T, 2927497 G→A, 2965591 T→C, 2975919 T→C, 3114125 C→T, 3176882 G→A, 3218853 T→C, 3246033 G→A, 3268091 C→T, 3268123 C→A, 3268165 C→T, 3302829 Δ1 bp, 3335733 C→T, 34111+T, 3429378 T→C, 345189 A→G, 3506796 T→C, 3526004 T→C, 3548297 T→C, 379237 Δ1 bp, 379237 Δ2 bp, 386281 C→T, 3978813 C→A, 3982057 C→T, 3983344 C→T, 407943 A→G, 4105271 A→G, 42111 C→A, 4234068 T→G, 4444833 A→G, 4503718 G→A, 454725 C→T, 4604230+G, 4604346 Δ1 bp, 533362 A→G, 558493 G→T, 560913+G, 619171 A→G, 653396 T→C, 696062 G→A, 700027 C→T, 700286+C, 700599+C, 700679+G,

760544 C→T, 844446 C→T, 852434+A, 922473+G, 961467 C→T and 963462 A→G, with reference to SEQ ID NO: 1.

18. A genetically engineered recombinant *E. coli* comprising a genetic modification of pflABfocA and a genetic modification of pflDC which genetic modifications substantially reduce pyruvate formate lyase (PFL) activity, and a genetic modification which substantially reduces pyruvate dehydrogenase (PDH) activity, the genetically engineered recombinant *E. coli* comprising deletion of each of a native xylGFH, rbsACB, and alsBAC operon.

* * * * *

United States Patent [19]

Präve et al.

[11] **4,166,004**

[45] **Aug. 28, 1979**

[54] **PROCESS FOR THE PREPARATION OF SINGLE CELL PROTEIN USING *METHYLMONAS CLARA* ATCC 31226**

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[21] Appl. No.: **817,870**

[22] Filed: **Jul. 21, 1977**

[30] **Foreign Application Priority Data**

Jul. 24, 1976 [DE] Fed. Rep. of Germany 2633451

[51] **Int. Cl.**[2] **C12B 1/00**

[52] **U.S. Cl.** **435/253**; 435/804; 435/822

[58] **Field of Search** 195/49, 96, 115

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Primary Examiner—Thomas G. Wiseman

Attorney, Agent, or Firm—Curtis, Morris & Safford

[57] **ABSTRACT**

A process for the preparation of a biomass is disclosed by cultivation of bacteria of the genus Methylomonas under aerobic conditions in a nutrient medium containing methanol as the sole carbon source, nitrogen sources and essential mineral salts, which comprises using a strain of the species *Methylomonas clara* ATCC 31226. The single cell protein thus obtained has a low content of nucleic acids, fats and carbohydrates and is especially suitable to prepare food and feed.

2 Claims, No Drawings

PROCESS FOR THE PREPARATION OF SINGLE CELL PROTEIN USING *METHYLMONAS CLARA* ATCC 31226

The present invention relates to a process for the preparation of single cell protein (biomass) having a high portion of protein and containing moreover fats, carbohydrates and vitamins. The biomass serves as basic material for the preparation of human food and animal feed.

Known processes for the preparation of biomass by means of strains of bacteria, which grow on methanol as the only source of carbon, frequently result in products containing pigments, biopolymers or undesired metabolites such as polyhydroxybutyric acid and thus, they are suitable as foodstuffs for humans and animals to a very limited degree only. The odor, the flavor or the toxic properties of such accompanying substances may even exclude the intended use of the biomass.

A novel strain of bacteria of the genus Methylomonas has now been found which is capable of utilizing methanol, methane or dimethylamine as sole source of carbon. This strain has been designated *Methylamonas clara* FH-B-5460 and is on deposit at the American Type Culture Collection under ATCC 31226.

The process for the preparation of a biomass (single cell protein) according to the invention by cultivating bacteria of the genus Methylomonas under aerobic conditions in a nutrient medium containing methanol as source of carbon, nitrogen sources and mineral salts, comprises using a strain of bacteria of the genus *Methylomonas clara* and maintaining the methanol concentration in a range between 5 and 150 ppm, calculated on the weight of the nutrient medium. Methanol is the sole source of carbon.

Preference is given to a methanol conentration in the range of from 5 to 100 ppm and, for continuous operation, a concentration in the range of from 5 to 30 ppm is particularly preferred.

The process is carried out in well aerated fermenters which hold a nutrient medium comprising in addition to methanol as the sole carbon source salts such as potassium nitrate, ammonium sulfate, ammonium phosphate or ammonia as nitrogen source. It moreover contains phosphates, for example potassium dihydrogen phosphate or disodium hydrogenphosphate and magnesium and potassium salts and finally trace elements which can be found in tap water, especially iron, copper and molybdenum salts.

The process is advantageously carried out at a temperature in the range of from 30° to 42° C., preferably of from 35° to 39° C.

The process according to the invention may be carried out in known fermentation vessels, for example in aerated agitator vessels or in modern fermenters such, for example, as loop reactors, in continuous or in discontinuous operation.

The liquid nutrient medium in the fermenter is aerated with 0.1 to 1.5 liters of air per liter of nutrient medium and minute (vvm).

The methanol concentration to be maintained can be controlled continuously by various measures, for example by measuring the nitrogen consumption, the portion of biomass in the suspension or preferably by measuring the carbon dioxide release. Using the latter method, a quick reaction to the lack of carbon, indicated by a decreasing CO_2 release, is possible, and thus the amount of methanol to be added can be calculated accurately. Alternatively the methanol concentration control can be carried out especially advantageously by measuring the gaseous methanol by means of a flame ionization detector.

The pH of the culture suspension consisting of the nutrient medium and the growing cell mass ranges between 4.0 and 9.0, preferably between 6.0 and 7.2. If the pH of the culture suspension falls below the required value, it may be readjusted by the addition of an adequate quantity of alkali metal, for example of sodium hydroxide or of potassium hydroxide solution. Similarily, a too high pH may be readjusted by the addition of acids, for example, of hydrochloric acid or sulfuric acid.

The biomass is separated in usual manner, for example by centrifugation while repeatedly washing with water, using optionally decantors and separators. Thus there is obtained a pasty biomass containing of from 75 to 90% by weight of water.

Drying can be carried out in various ways, for example by means of drum dryers, fluidized bed dryers or spray dryers. The product thus obtained contains only from 1 to 4% by weight of water and of from 80 to 90% by weight of crude protein. This crude protein has a content of amino acids ranging between 75 and 78% by weight, the portion of essential amino acids amounting to about 50% by weight of the total content of amino acids. The product has a low content of nucleic acid, fats and carbohydrates, the content of nucleic acid ranging between about 10 and 14% by weight, of fats between about 5 and 10% by weight and of carbohydrates between about 5 and 10% by weight.

The biomass (single cell protein) thus obtained contains no pigments, no toxic substances, no reserve substances such as biopolymers, no polyhydroxybutyric acid, unwanted odorants or secondary metabolites.

The dried biomass obtained according to the invention is therefore especially suitable as protein source for the preparation of human food and animal feed.

The novel species *Methylomonas clara* FH-B-5460 ATCC 31226 is characterized as follows:

I. Growth attitude:

(a) no growth on: glucose agar or glucose medium, bouillon agar or bouillon nutrient medium, gelatin, peptone agar or medium, litmus milk, potato nutrient medium, amino acid nutrient medium.

(b) growth on: methanol-containing synthetic nutrient medium.

II. Morphology:

(a) small rods of 0.5 to 1.5 μ, motile by means of polar flagella, no spores, no cysts.

(b) circular colonies, transparent, slightly vitreous.

(c) no pigments.

III. Physiology:

(a) growth at:

20° C.	±
25° C.	+
30° C.	++
35° C.	+++
37° C.	++++
39° C.	+++
41° C.	+

optimum growth temperature:	37° C.
optimum pH value:	6.8 to 7.0
gram negative.	
(b) growth factors	not required
formation of polyhydroxybutyric acid	–
formation of indoles	–
formation of acetone	–
reduction of NO_3	+

-continued

cytochromoxidase	+
catalase	+
isocitrate dehydrogenase	+
malate dehydrogenase	+
oxidase	+
formation of H_2S	−
liquefaction of gelatine	−
hydrolysis of starch	−
formatin of citric acid	−
coagulation of milk	−
Growth on	
ammonium salts	+
ureas	−
acetate	−
dimethylamine	+
trimethylamine	−
monomethylamine	−
formiate	−
formaldehyde	−
sugar, polysaccharides	−
alcohols (except methanol)	−
fixation of N_2	+
fixation of CO_2	

Several known species of Methylomonas and other methanol utilizing microorganisms are described in Bergey's Manual of Determinative Bacteriology, 8th edition, the Williams & Wilkens Company, Baltimore, 1974. Further, related strains are disclosed in various publications. The characteristic properties of the novel species *Methylomonas clara*, in comparison with that of known species, are given in the following Table 1. It can be seen from the table that the Pseudomonas species differ from the novel species in that they form polyhydroxybutyric acid and in that they do not grow on methane or dimethylamine. The known Methylomonas species differ from the novel species by their temperature optimum, the formation of pigments, the formation of polyhydroxybutyric acid and by their coccal form. A further important difference between the novel species and the known species is the hexulose phosphate pathway. This pathway is energetically favored in the case of the novel species in comparison with the serine pathway in the course of the methanol utilization.

In the following table the symbol G+ G(%) at the bottom of the first column indicates the guanine and the cytosine portion of the total content of the pyrimidine bases.

In column 2 there is characterized the novel species *Methylomonas clara* ATCC 31226.

Columns 3 to 7 and column 11 show related strains, which are described in Bergey's Manual of Determinative Bacteriolog, 8th edition, 1974, The Williams & Wilkens Company/Baltimore.

Column 8 to 11 show related species selected from various publications, namely *Pseudomonas methanolica*, disclosed in U.S. Pat. No. 3,755,082; Pseudomonas AM 1, disclosed in J. Bact. 114 (1), 390 (1973); *Pseudomonas methylotropa*, disclosed in German Offenlegungsschrift No. 2,261,164.

Table 1

	Methylomonas clara FH-B-5460 ATCC 31226	*Methylomonas methanica*	*Methylomonas methano-oxidans*	*Methylomonas methanoni-trificans*	*Methylococcus capsala-ticus*
single cell shape[micron]	0.5 × 1.5 rods	0.6 × 1.0 rods	1 × 3 rods	1 × 2 2 × 4 rods	1 × 1 cocci
motility	+		+	+	−
formation of pigments	−	+	−	+	−
pHBS	−	−	−	+	−
fixation of N_2	+	−		+	
fixation of CO_2	+				
NO_3 as nitrogen source	+	+			+
temperature opt. °C.	37°	30°	30°		37°
growth on amino acids	−				±
methanol as C source	+		+	+	+
methane as C source	+	+	+	+	+
ethanol as C source		−	−	+	
hexulose phosphate pathway	+	+	+	+	+
serine pathway	−				
formaldehyde					
formiate					
trimethylamide	+				
dimethylamide					
G + C (%)		50–54	50–54	50–54	62,5

	Methylosinus methylocystis	*Ps. methanolica*	*Pseudomonas* AM 1	*Ps. methylotropha*	*Methylomonas methanica*
motility				+	
formation of pigments					±
pHBS		+	+	−	
fixation of N_2					
fixation of CO_2					
NO_3 as nitrogen source					
temperture opt. °C.					
growth on amino acids					
methanol as C source		+	+	+	+
methane as C source		−	−	−	+
ethanol as C source			+	−	−
hexulose phosphate pathway					
serine pathway	+				
formaldehyde				−	
formiate				−	−
trimethylamide				−	−
dimethylamide					

Table 1-continued

G + C (%)	62,5	54	52,1

The following examples illustrate the invention:

EXAMPLE 1

The strain *Methylomonas clara* FH-B-5460 ATCC 31226 is cultivated on slants containing

18 g of	gar
2.0 ml of	H_3PO_4 of 85% strength
3.0 ml of	NH_4OH of 12% strength
0.01 g of	Na_2HPO_4
1.2 g of	H_2SO_4
0.8 g of	$MgSO_4 . 7 H_2D$
0.03 g of	$FeSO_4 . 7 H_2O$
10.0 ml of	methanol (added prior to filling the tubes)
1.0 ml of	solution of trace elements, containing $CuSO_3$, H_3BO_3, $MnSO_4$, $ZnSO_4$, Na_2MoO_4
1 l of	water

pH adjustion prior to sterilization to 6.7.

The slants are heated for 30 minutes in an autoclave, to a temperature of 120° C. Thereafter they are inoculated with *Methylomonas clara* and kept at a temperature of 37° C. for a period of 2 days. The cell mass of two of the slants is suspended by means of physiological sodium chloride solution and transferred by inoculation to the next stage.

This stage is a shaken culture (preculture), which is placed in a 2 liter Erlenmeyer flask holding 1 liter of nutrient solution (having the same composition as above, but containing no agar). 3.3 ml of methanol (filtered under sterile conditions) are added thereto. This culture is shaken for three days at a temperature of 37° C. in agigator vessels which have an amplitude of 4 cm, at a speed of 220 rpm. After 24 and 48 hours, respectively, 3.3 ml of methanol are added.

The following stage (main culture) is achieved in a fermenter fed with about 20 liters of nutrient solution (having the same composition as above, but containing no agar). After sterilization for 30 minutes, at a temperature of 120° C., under 1.2 to 1.4 bars, 2 liters of preculture are inoculated into the fermenter. Fermentation in the fermenter equipped with flat paddle mixers, an airing ring and three baffles is carried out under the following conditions:

temperature	37° C.	
aeration	10 liters/minute	0.5 vvm
pressure	0.2 bars	
number of revolutions per minute	500	
pH	6.6	

20 ml of ethanol are added and further amounts of 20 ml are added each time when the CO_2 release of the cells decreases. The methanol concentration amounts only to at most 0.1% by volume thereby.

After 22 hours the 20 liters of culture suspension of the main culture are transferred by inoculation to a prefermenter having a capacity of 200 liters. This fermenter is equipped and is treated in the same way as the main culture. Fermentation is carried out under the following conditions:

temperature	37° C.	
aeration	6 to 8 m^3/h	0.5 to 0.75 vvm
pressure	0.2 bars	
rpm	380	
pH	6.7	

The pH is maintained in a range of from 6.7 to 6.8 by means of sterile ammonium hydroxide of 10% strength. The methanol supply is controlled by measuring the methanol concentration in the waste air by means of a flame ionization detector, each time, when the methanol concentration falls below 50 ppm, further quantities of methanol are added.

A concentration of methanol in the waste air of 60 vpm corresponds to a concentration of methanol in the solution of 0.22%.

After 20 hours of fermentation a main fermenter is inoculated with 200 liters of culture suspension from the prefermenter; it contains 2000 liters of nutrient solution and works under the following conditions:

temperature	37° C.	
aeration	60 to 80 m^3/h	0.5 to 0.75 vvm
pressure	0.2 bars	
rpm	170	
pH	6.7	

The methanol feed is carried out in the manner described for the 200 liter prefermenter. The methanol concentration in the nutrient solution is in the range of from 50 to 80 ppm. The cell mass is worked up after a fermentation period of 22 hours. To do this, the pH of the culture suspension is brought to a value of 4.0 by the addition of dilute sulfuric acid and the cell mass is separated by centrifugation in separators at a speed of 400 rpm. Alternatively it may be separated by centrifugation at a pH of from 6.5 to 6.8. The separated cell mass (moisture content 80%) is washed with water and then dried to a dry content of 25% in the separator. Thereafter the cell mass is dried in a spray dryer at an entrance temperature of from 120° to 150° C. The powder obtained still has a moisture content of from 1.5 to 3.5% and contains

85% of crude protein (N×6.25)
74% of amino acids, comprising about 50% of essential amino acids
8 to 12% of nucleic acids
6 to 8% of crude fat
5 to 6% of crude ash
(the percentages are to be understood as percentages by weight).

EXAMPLE 2

The strain *Methylomonas clara* FH-B-5460 ATCC 31226 is cultivated as in Example 1.

The main fermenter is intended for use in continuous fermentation and has a capacity of 3000 liters. It is permanently aerated and works under the following conditions:

temperature	37° C.	
aeration	80 Nm^3/h	0.67 vvm
pressure	0.1 bar	

-continued

rpm	390
pH	6.8

The main fermenter contains 2000 liters of nutrient solution (of the same composition as in Example 1, but containing no agar) and is inoculated with 200 liters of culture suspension of the prefermenter. The methanol concentration is measured in the manner described hereinbefore and controlled by the addition of methanol/water at a ratio by volume of 40:60 to maintain an average free conentration of methanol in the range of from 10 to 50 ppm in the nutrient solution.

Continuous operation is started, when 7 to 10 kg of cell mass/1 have been produced.

The nutrient medium is added at a throughput rate of 0.25/h. This rate is increased to 0.33/h, which corresponds to about 650 l/h, after a period of about 12 hours. The mean free concentration of methanol is maintained between 10 and 20 ppm. The corresponding quantity of nutrient medium, on which bacteria have grown, is drawn off, kept for a period of 1 to 2 hours in an intermediate placed vessel without adding methanol and is then submitted to the cell separation and drying processes as described in Example 1.

The cell mass obtained has a moisture content of from 2 to 4% and contains

81% of curde protein (N×6.25)

70% of amino acids, among which about 50% of essential amino acids

8 to 10% of nucleic acids

5 to 10% of crude fat

5 to 10% of crude ash

What is claimed is:

1. A method for preparing a biomass which comprises cultivating a strain of the species *Methylmonas clara* ATCC 31226 under aerobic conditions in a nutrient medium containing a nitrogen source, essential mineral salts, and methanol at a concentration between 5 ppm and 150 ppm, by weight of the nutrient medium, as the sole carbon source.

2. A method as in claim 1 which is performed continuously and wherein the concentration of methanol is between 5 ppm and 30 ppm.

* * * * *

United States Patent [19]

Wagner et al.

[11] **4,048,013**

[45] **Sept. 13, 1977**

[54] **PROCESS FOR PRODUCING SINGLE-CELL PROTEIN FROM METHANOL USING METHYLOMONAS SP. DSM 580**

[75] Inventors: **Fritz Wagner**, Stockheim; **Hermann Sahm**, Wolfenbuttel, both of Germany

[73] Assignee: **Gesellschaft fur Molekularbiologische Forschung mbH**, Braunschweig, Germany

[21] Appl. No.: **694,792**

[22] Filed: **June 10, 1976**

[51] **Int. Cl.**[2] **C12B 1/00**

[52] **U.S. Cl.** **195/49**; 195/96

[58] **Field of Search** 195/49, 96; 426/656

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Assistant Examiner—Thomas G. Wiseman
Attorney, Agent, or Firm—Haseltine, Lake & Waters

[57] **ABSTRACT**

A process for preparing a single cell protein from methanol is described. This process utilizes Methylomonas sp. DSM 580 as the obligate methanol-assimilating bacterium for the preparation of the single cell protein.

8 Claims, No Drawings

PROCESS FOR PRODUCING SINGLE-CELL PROTEIN FROM METHANOL USING METHYLOMONAS SP. DSM 580

The increasing demand for protein for human food and animal feed makes it necessary to develop new processes for producing lowcost protein with high nutritive value. During the past few years, much research was directed towards production of protein by microorganisms capable of utilizing gaseous hydrocarbons such as methane or liquid hydrocarbons resulting from petroleum fractions as a source of carbon and energy. Gaseous hydrocarbons, however, have the disadvantage of low solubility in an aqueous medium and explosion hazards associated with working under aerobic cultural conditions. Liquid hydrocarbons have the disadvantage of low water solubility too, which results in an increased energy consumption to disperse this substrate in the medium into small drops. Furthermore, it is necessary to refine the biomass which is produced on fluid hydrocarbons by solvent treatment in the final stage.

In the last few years, great interest has been shown in the development of processes for cultivation of microorganisms on a large scale with methanol as a particularly suitable source of carbon and energy. This substrate has the advantage that it can be easily and cheaply produced in a chemically defined form out of synthesis gas, which is obtainable from a very wide range of natural resources such as natural gas, petroleum and coal.

In DT-OS (German Offenugungschrift) 2,040,358, a process for producing single-cell protein is described using alcohols, aldehydes, ketones, carbonic acids and their derivatives as carbon sources formed by oxidation of liquid alkanes.

In DT-AS (German Auslegesschrift) 2,152,039, production of bacterial biomass in a culture medium containing methanol is described employing the following bacteria strains: Protaminobacter ruber var. machidanus ATCC 21 611, ATCC 21 612, ATCC 21 613 or ATCC 21 614.

In DT-OS 2,311,066, a process for producing single-cell proteins with the aerobic microorganism Methylomonas methanolica NRRL-B- 5458 cultivated in a medium containing methanol as the only carbon source is described.

In DT-OS 2,059,277, a microbial process for producing protein is described utilizing the following bacteria strains under aerobic conditions and methanol as the sole source of carbon: Pseudomonas methanica, Pseudomonas sp. ATCC 21 438, Pseudomonas sp. ATCC 21439, Pseudomonas sp. PRL-W 4, Corynebacterium sp. ATCC 21232, Corynebacterium sp. ATCC 21 235 and Corynebacterium sp. ATCC 21236.

In DT-OS 2,407,740, a process for cultivation of microorganisms is described, which comprises a mixed culture consisting of a facultative methanol-assimilating bacterium and several non-methanol assimilating bacteria. The methanol-assimilating bacterium is nonmobile and grows on methanol as well as on other organic compounds, for example, glucose and glycerine.

In DT-OS 2418385, a process for producing a product rich in protein is described employing a nonpink pigmented bacterial strain, which is a derivative of the microorganism Pseudomonas extorquens (NCIB Nr. 9399).

Until now, no obligate methanol assimilating bacterium has been employed for the production of protein from methanol, and only facultative methylotrophic microorganisms have been utilized. This stage of technology has been overcome by the invention using an obligate methylotrophic bacterium as a source of single-cell protein.

From a soil sample collected at the Rheinufer Ludwigshafen, an obligate methanol-assimilating bacterium was isolated by enrichment culture. 1 g of the soil sample was suspended in 100 ml of a mineral salt medium of the following composition: KH_2PO_4, 3.75 g; Na_2HPO_4, 2.5 g; $(NH_4)_2SO_4$, 4.0 g; $MgSO_4.7H_2O$, 0.5 g; $Ca(NO_3)_2.4H_2O$, 0.025 g; $FeSO_4.7H_2O$, 0.005 g; $ZnSO_4.H_2O$, 0.005 g, in 1000 ml distilled water, pH 7, with 1% (v/v) methanol and incubated in 500 ml flasks on a rotary shaker at 100 rpm at 30° C. The inoculated medium was cultured for five days and then 0.1 ml of it was streaked on nutrient agar plates with the same medium except with 2% agar. After repeated selections of single colonies from agar plates and cultivation in liquid cultures a pure bacterium, designated Methylomonas sp., with the Nr. DSM 580, was obtained. It is an object of the present invention to provide a process for a methanol-based production of single-cell protein. The process for production of single-cell protein according to the invention comprises inoculating a sterile liquid medium with a culture of the obligate methanol-assimilating bacterium Methylomonas sp. DSM 580 containing assimilable sources of nitrogen, methanol as the sole source of carbon and energy, essential mineral salts and, if necessary, growth-promoting agents. The fermentation is carried out under aerobic conditions providing the system with air or oxygen enriched air, the cultivating temperature is from 20°–45° C. After culturing, the microbial cells are removed out of the three phase system and dried, presenting a biomass with a crude protein content from 60–70% (w/w), a nucleic acid content from 2–17% (w/w), an ash content of 3–6% (w/w) and a lipid content from 3–8% (w/w). The culture fluid removed in the separation step may be recycled back in the process.

The microorganism suitable for use in the present invention is the methanol-assimilating bacterium Methylomonas sp. DSM 580 with the following morphological and cultural characteristics:

1. Cell morphology: Non spore forming short rod measuring approximately 0.5 × 1.5 μm, highly motile with a single polar flagellum. In liquid culture the cells are slightly pink, (while after centrifugation the cell pellet is intensively pink).
2. Colony characteristics: Translucent, nonpigmented, circular, smooth, 1 – 2 mm diameter after 2–3 days incubation.
3. Staining reaction: gram - negative
4. Physiology: aerobic, catalase positive, methanol dehydrogenase positive, hexose - phosphate-synthetase positive, hydroxypyruvate - reductase negative.
5. Growth characteristics:

	min.	optimum	maximum
Temperature (° C)	20	33–36	45
pH	4.5	6.5–7.5	9.5
Methanol conc. % (v/v)		0.5–1.5	5.0

The described bacterium Methylomonas sp. is deposited at the German collection of microorganisms, Göttingen, with the number DSM 580.

Methylomonas sp. DSM 580 is an obligate methylotrophic bacterium i.e., only methanol supports its growth. Growth was not observed when C_1-compounds other than methanol or when some other substrates were tested (Table I). Table I: Growth of Methylomonas sp. DSM 580 on various carbon sources in mineral salt medium under aerobic conditions.

Substrate	Growth[1)]
Methane	–
Methanol	+
Methylamine	–
Formaldehyde	–
Sodium Formate	–
Ethanol	–
Propanol-(1)	–
Propanol-(2)	–
Sodium Acetate	–
Sodium Lactate	–
Sodium Pyruvate	–
Sodium Succinate	–
Sodium Citrate	–
Glucose	–
Fructose	–
Serine	–

[1)] + growth – no growth

Furthermore, it was determined that Methylomonas sp. DSM 580 is cultivated in a batch culture with an initial methanol concentration from 0.5 - 5% (v/v), preferably from 2–3% (v/v) and that Methylomonas sp. DSM 580 is cultivated in a batch culture where the methanol concentration is maintained from 0.01–2.0% (v/v), by an automatic control system and 25% (v/v) methanol is consumed.

It was also determined that Methylomonas sp. DSM 580 is cultivated in a continuous culture at a dilution rate from 0.1–0.5 vol/vol/h under chemostatic or turbidostatic conditions.

Furthermore, it was determined that, throughout the fermentation, a constant pH was adjusted in the range of from 4.5–9.0 by adding alkali or acids.

It was also determined that Methylomonas sp. DSM 580 is cultivated at a temperature from 20°–45° C, preferably from 33°–36° C.

Furthermore, it was determined that throughout the fermentation the fermenter is supplied with air or oxygen enriched air with an aeration rate from 0.5–1.5 vol/vol/min and that the gas mixture has an oxygen content of 20–60% v/v).

It was also determined that the aqueous culture medium contains ammonium and/or nitrate salts of inorganic acids and/or urea as assimilable sources of nitrogen and the essential cations such as sodium, potassium, magnesium, calcium, iron, zinc, manganese and the anions such as phosphate, sulfate, nitrate, chloride and growth promoting agents.

As used herein, nutrient substances are chemical compounds, which contain, besides the carbon and energy source, anions and cations which are taken up by the microorganisms and are necessary for their growth. Growth promoting agents are natural or synthetic compounds, which are required by the microorganisms but could not be synthetized by these organisms in sufficient amounts.

The invention is now illustrated in the following examples:

EXAMPLE 1

A 80 l capacity fermenter is charged with 50 l mineral salt medium (composition: $(NH_4)_2SO_4$, 200 g; KH_2PO_4, 150 g; Na_2HPO_4 125 g; $MgSO_4.7H_2O$, 25 g; $Ca(NO_3)_2.4H_2O$, 1.25 g; $FeSO_4.7H_2O$, 0.25 g; $ZnSO_4.H_2O$, 0.25 g; KCl, 0.25 g in 50 l distilled water) and sterilized at 121° C for 10 minutes, cooled to 15° C, aseptically mixed with 1000 ml methanol, inoculated with 500 ml of a culture of Methylomonas sp. DSM 580 and incubated at 35° C for 28 hours under stirring with a turbostirrer with a stirring rate of 300 rpm and a aeration rate of 0.7 vol/vol/min. Throughout the fermentation the pH is automatically maintained at 6.4 by the addition of a 6% ammonium hydroxide solution. After 22 hours of fermentation, the medium is cooled to 15° C and adjusted to pH 3 by sulfuric acid and the precipitated biomass is recovered by filtration, washed with water and then dried (324 g cell dry weight). The cell composition of the dried biomass is: 71% crude protein, 9% nucleic acids, 4% ash and 7% lipid.

EXAMPLE 2

A 340 l fermenter, fitted with an "intensor," produced by Biologische Verfahrenstechnik AG, Basle, is charged with 200 l mineral salt medium (compounds: 500 g $(NH_4)_2SO_4$, 500 g NH_4NO_3, 600 g KH_2PO_4, 500 g Na_2HPO_4, 140 g $MgSO_4.7H_2O$, 15 g $Ca(NO_3)_2.4H_2O$, 6 g $FeSO_4.7H_2O$, 2 g KCl in 200 l tap water), the pH is adjusted to 6.8, sterilized for 15 minutes at 121° C, cooled down to 33° C, 2000 ml methanol were added aseptically, inoculated with 4000 ml culture of Methylomonas sp. DSM 580 grown for 18 hours in the same medium, and incubated at 33° C with the aeration rate of 0.5% (v/v) in the culture medium, methanol concentration was kept constant at 0.5% (v/v) by measuring continuously the vaporconcentration of methanol, which is in relation with the concentration of methanol in the medium; furthermore the pH is maintained at 6.8 by automatic addition of 12% (v/v) ammoniumsolution. After an incubation of 25 hours the cell concentration is 14 g dry weight/l, now the fermenter is aerated with oxygen enriched air with an oxygen content of 40% (v/v), while the aeration rate is the same as above. After 60 hours, the fermenter is cooled down to 15° C, the cells were harvested by a high-speed continuous flow centrifuge at 10,000 g and dried. Under these process-conditions, the cell yield is 0.44 g dry weight/g methanol and these dried cells contain 76% crude protein, 6.5% nucleic acids, 5% ash and 4.5% lipid.

EXAMPLE 3

A 80 l fermenter with an intensor as described in Example 2 is charged with 50 l culture medium the same as in Example 1, inoculated with 1000 ml culture of Methylomonas sp. DSM 580, which was grown 15 hours at 35° C, cultivated at 35° C, an aeration rate of 0.5 vol/vol/min, the stirrer speed of 1200 rev/min and a constant pH at 7.0. The continuous culture is started after 18 hours with a dilution rate of 0.05, which is increased after 48 hours to 0.1, and then within 120 hours by steps to 0.35. Under these conditions, a steady state is possible, the cell yield is 0.46 g dry weight/g methanol and the production is 10.8 g/l/h. The cell composition is 72% crude protein, 4.5% nucleic acids, 3.5% ash and 5.5% lipid. Under steady state conditions, 50% of the culture filtrate is recycled back and the added culture medium is reduced to this amount.

The present invention generally possesses the advantage that for the first time an obligate methylotrophic baterium is used as process organism with a significant higher productivity than other known facultative methylotrophic microorganisms have. Furthermore, no undesirable mutation of this microorganism involved in the present process can occur which may affect the ability of growing on methanol. This bacterium is very stable, its metabolism is reduced to a minimum and genetic variations would cause death of the cells. Another advantage of the present invention is that the key enzymes of the dissimilation and assimilation pathways of methanol are constitutive in Methylomonas sp. DSM 580. In the case of methanol-limited conditions in continuous culture, the nucleic acid content in the cells is very low and there is practically no loss of methanol through evaporation.

We claim:

1. A process for the production of single-cell protein from methanol, which comprises placing, in an aerated fermenter with or without a mechanical stirrer, a growing submerged culture of the obligate methanol-utilizing bacterium Methylomonas sp. DSM 580, produced under aerobic conditions, cultivating it in the presence of methanol, used as the sole source of carbon and energy, and mineral salts, in the presence or absence of growth promoting agents, this system further provided with air or oxygen enriched air at a cultivating temperature from 20° to 45° C, thereafter removing the cell mass out of the three phase system and drying, resulting in a biomass with a crude protein content of at least 60–76% (w/w), a nucleic acid content of 2–17% (w/w), an ash content of 4–6% (w/w), a lipid content of 3–8 (w/w), the culture fluid, after removing the cell mass, is capable of being, at least partially, recycled.

2. A process according to claim **1** for the production of single cell protein from methanol which comprises placing, in an aerated fermenter with or without a mechanical stirrer, a growing submerged culture of the obligate methanol-utilizing bacterium Methylomonas sp. DSM 580, produced under aerobic conditions, cultivating it in the presence of methanol used as the sole source of carbon and energy, the aqueous culture medium containing compounds selected from ammonium salts, nitrate salts, urea and mixtures thereof as nitrogen source, salts selected from those containing cations, selected from sodium, potassium, magnesium, calcium, iron, and manganese, anions selected from phosphate, sulfate, nitrate and chloride in the presence or absence of growth promoting agents, this system being provided with air or oxygen enriched air, at a cultivating temperature from 20° to 45° C, thereafter removing the cell mass of the three phase system and drying, resulting in a biomass with a crude protein content of at least 60–76% (w/w), a nucleic acid content of 2–17% (w/w), an ash content of 3–6% (w/w), a lipid content of 3–8% (w/w) the culture fluid, after removing the cell mass is capable of being, at least partially, recycled.

3. A process according to claim **2** in which Methylomonas sp. DSM 580 is cultivated in a batch-culture with an initial methanol concentration from 0.5–5% (v/v), preferably from 2–3% (v/v).

4. A process according to claim **2** in which Methylomonas sp. DSM 580 was cultivated in a batch-culture, where the methanol concentration is maintained at from 0.01–2% (v/v) by an automatic control system and 25% methanol (v/v) is consumed.

5. A process according to claim **2** in which Methylomonas sp. DSM 580 is produced continuously in a chemostatic or turbidostatic culture at a dilution rate of from 0.1–0.5 v/v/h.

6. A process according to claim **2** in which the pH is adjusted automatically in the range from 4.5–9.0 by adding alkali or acids throughout the fermentation.

7. A process according to claim **2** in which the fermenter is supplied with air or oxygen enriched air throughout the fermentation with an aeration rate from 0.5–1.5 v/v/min and the gas mixture has an oxygen content of 20–60% (v/v).

8. A process according to claim **2** in which the fermentation vessel is supplied with air or oxygen enriched air, with an aeration rate from 0.1–0.2 v/v/min and the gas mixture has an oxygen content of 20–60% (v/v).

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