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Production of Astaxanthin Using Modified E. coli Cells

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Abstract

There are several promising markets for naturally synthesized Astaxanthin, a carotenoid found in krill, shrimp, salmon, and other marine life that imparts to these creatures a pink coloring of the flesh and has demonstrated human health-promoting anti-inflammatory and antioxidant activity. Compared to Astaxanthin produced through chemical synthesis, Astaxanthin synthesized through natural fermentative processes (in yeast and algae) is esterified, imparting greater antioxidant activity as well as bioavailability and making it the preferred ingredient for nutraceutical formulations. Additionally, as consumer preferences trend toward natural production processes free from the use of petrochemical solvents which may pose hazards to health hazards, fermentative production coupled with an extraction process featuring more environment- and health-friendly solvents is desired. Recently, a strain of E.coli has been genetically optimized to produce Astaxanthin, offering a cheaper synthesis route compared with algal cultivation. Herein, the authors propose a process for the production of natural Astaxanthin through fermentation in genetically modified E.coli and recovery of the compound from biomass via supercritical CO_2 extraction. The fermentation seed train is composed of three pre-seed, two seed, and three production fed-batch fermenters. Biomass from the production stage is collected in a harvest/surge tank for continuous downstream processing. The biomass is concentrated in broth, the cells are lysed, and the slurry of lysed cells are dried. The lysis product is loaded with fructose and diatomaceous earth in order to produce biomass pellets that are appropriate for the extraction phase. Supercritical CO_2 with ethanol co-solvent is used to extract Astaxanthin from these pellets. Astaxanthin is heat-sensitive and has low accumulation in cells. Despite the expensive equipment necessary to preserve the structure and activity of the product and the low yearly production rate, the high selling price of Astaxanthin makes this process economically profitable, with an investor's rate of return of 125%, net present value of $468 million, and return on investment of 171%.

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 21, 2020

Dear Dr. Warren Seider and Professor Bruce Vrana,

The following report contains the design for a process that uses modified *E. coli* bacteria cells to produce the carotenoid astaxanthin. The process produces 55.44 MT of astaxanthin per year. This alternative natural production method has a greater growth rate than traditional algal growth methods. The design for this process includes a seed reactor scale up, batch fermentation, and a continuous downstream with separation, extraction, and purification of astaxanthin.

An economic analysis of this process shows its potential profitability. With lower downstream costs than typical energy intensive natural production methods and a product that may still be sold at a comparable price point, *E. coli* based astaxanthin production has shown to be highly lucrative. The plant has a return on investment of 171 percent, an investor's rate of return of 125 percent, and a net present value of $468MM. Given these tremendous results, we highly recommend this plant moves forward with the specifications in this report.

Thank you for your help with this project. Your assistance this past semester has been greatly appreciated.

Sincerely,

Christina Eng   Connor Gallagher   Maura Kimmel

Production of Astaxanthin Using Modified *E. coli* Cells

Christina Eng
Connor Gallagher
Maura Kimmel

Project Author: Dr. Richard Bockrath
Project Advisor: Dr. Warren Seider

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

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1. Abstract

There are several promising markets for naturally synthesized Astaxanthin, a carotenoid found in krill, shrimp, salmon, and other marine life that imparts to these creatures a pink coloring of the flesh and has demonstrated human health-promoting anti-inflammatory and antioxidant activity. Compared to Astaxanthin produced through chemical synthesis, Astaxanthin synthesized through natural fermentative processes (in yeast and algae) is esterified, imparting greater antioxidant activity as well as bioavailability and making it the preferred ingredient for nutraceutical formulations. Additionally, as consumer preferences trend toward natural production processes free from the use of petrochemical solvents which may pose hazards to health hazards, fermentative production coupled with an extraction process featuring more environment- and health-friendly solvents is desired. Recently, a strain of E.coli has been genetically optimized to produce Astaxanthin, offering a cheaper synthesis route compared with algal cultivation. Herein, the authors propose a process for the production of natural Astaxanthin through fermentation in genetically modified E.coli and recovery of the compound from biomass via supercritical CO_2 extraction. The fermentation seed train is composed of three pre-seed, two seed, and three production fed-batch fermenters. Biomass from the production stage is collected in a harvest/surge tank for continuous downstream processing. The biomass is concentrated in broth, the cells are lysed, and the slurry of lysed cells are dried. The lysis product is loaded with fructose and diatomaceous earth in order to produce biomass pellets that are appropriate for the extraction phase. Supercritical CO_2 with ethanol co-solvent is used to extract Astaxanthin from these pellets. Astaxanthin is heat-sensitive and has low accumulation in cells. Despite the expensive equipment necessary to preserve the structure and activity of the product and the low yearly production rate, the high selling price of Astaxanthin makes this process economically

profitable, with an investor's rate of return of 125%, net present value of $468 million, and return on investment of 171%.

2. Introduction

2.1 Project Origin

Astaxanthin is a keto-carotenoid pigment with strong antioxidant activity. It is in strong demand in the growing nutraceutical market. Research has shown astaxanthin to be beneficial to some cardiovascular, inflammatory, and neurodegenerative diseases. It has also shown anti-cancer properties (1). Cosmetically, recent studies have found it to carry a moisturizing effect that combats aging. Astaxanthin is also valued for its red pigmentation, provided by the conjugated double bonds along the center chain of the compound, shown in Figure 2.1. This vibrant pigment is often used to give farmed salmon a more natural pink appearance.

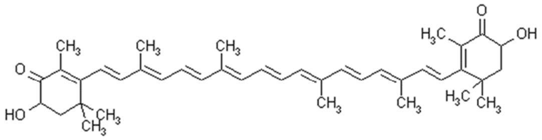


Figure 2.1 Chemical structure of astaxanthin (2).

Wild salmon flesh is typically a deep pink, acquired by their diet of crustaceans. Farmed salmon, however, would be naturally white on their diet of feed. In many cases consumers will not purchase white salmon. Research by the DSM has shown consumers will spend up to $1 per pound for darker fish. This leads fish farmers, whose product accounts for 70% of the U.S. fish market, to use feed supplements to attain a pinker salmon through the use of astaxanthin (3). The global astaxanthin market is estimated at around $250 million, with about $200 million coming from fish coloration use (3). In total, it accounts for one-fifth of fish feed costs.

Astaxanthin is typically produced commercially through *Haematococcus* algae. This production requires large culture ponds and expensive milling techniques to successfully crack the cell walls and retrieve astaxanthin. New research performed by Zhang has shown a route to optimize the production of astaxanthin via *Escherichia coli* (4). He produced a production rate of astaxanthin 16 times higher than current algae growth methods. Zhang's method also produced 100% pure enantiomer 3 S, 3'S-astaxanthin, which makes it preferable to synthetically produced astaxanthin that is known to have potentially harmful isomers.

2.2 Project Goals and Scope

The goal of this project was to design a process to produce 55 ton/yr of astaxanthin, utilizing the *E. coli* based fermentation data provided by Zhang. Recent marketing studies have shown natural astaxanthin sells for $4,000/kg, so entering at a price point of $3,500/kg it should be feasible to take 20% of the current market. Overall, the process must have an IRR of 25% or greater to be deemed profitable.

The process was to have a batch upstream fermentation component coupled with a continuous downstream. The most important downstream considerations were the separation of solids and liquids, cell disruption, drying, pelletization, and extraction. The solid astaxanthin produced must have solvent levels of less than 1 PPM. The project scope ends with the extraction of astaxanthin and does not include a product design. Additional considerations concerned an environmentally friendly plant and minimized energy consumption.

2.3 Objective Time Chart

Figure 2.3 shows the timeline of the significant design steps for this design project. In January, tasks included preliminary design following the first meeting with project author Dr.

Richard Bockrath and advisor Warren Seider. Then began the design of the fermentation process, to be completed in February. The remainder of February was spent designing the continuous downstream process and beginning the market analysis. In March, the equipment design was refined and a profitability analysis completed. In the final month of April, the report and presentation were created, edited, and finalized.

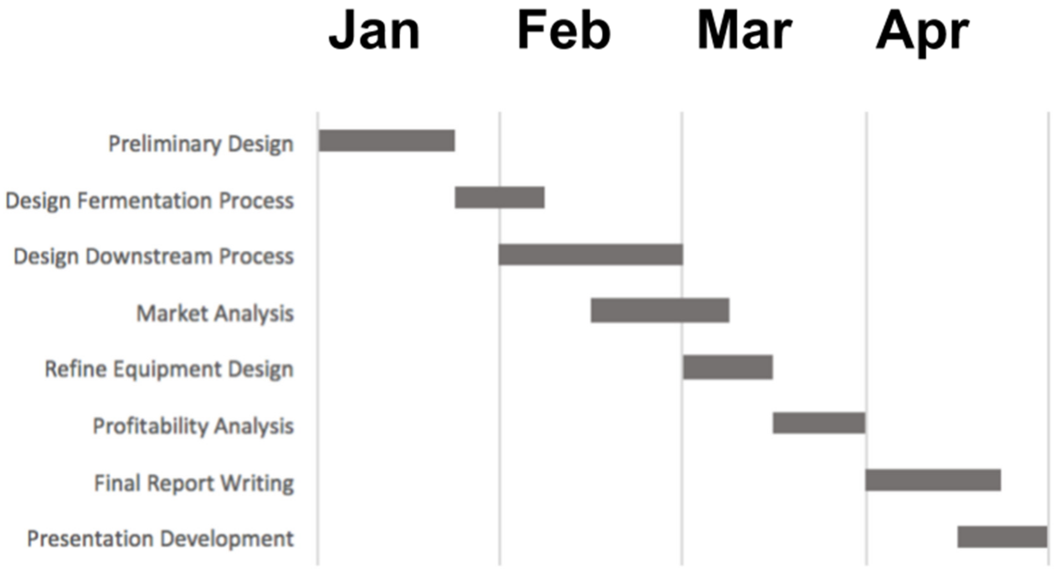


Figure 2.3 Process time chart

2.4 Project Charter

Project Name: Production of Astaxanthin Using Modified E. coli Cells

Project Leaders: Christina Eng, Connor Gallagher, Maura Kimmel

Specific Goals: The goal of this project is to develop a process to produce astaxanthin using genetically modified *E. coli* cells. This process should be quite profitable considering the high purchase price of astaxanthin.

Project Scope:

In Scope:

- Design seed train to grow *E. coli* from flasks to a production fermenter
- Design process for two production fermenters
- Downstream product purification processes including centrifugation, drying, and extraction
- Financial analysis including evaluation of cost, revenue, and profitability

Out of Scope:

- Genetic modification of *E. coli*
- Safety approval from governments where product will be manufactured
- Final product distribution

Deliverables

Detailed Process Design

- Process that produces 55 tons of astaxanthin yearly
- Batch upstream with a continuous downstream

Profitability Assessment

- Project IRR of 25% or greater

Timeline

- 1 year design, 1 year construction, 15 years of production

3. Innovation Map

N/A

4. Market and Competitive Assessment

Astaxanthin

The astaxanthin market is split between two major applications: aquaculture and nutraceuticals. In 2017 total revenue in the carotenoid market was $1.5 billion, with 55% coming from astaxanthin (5). The market is projected to grow between 4 and 5% per year. At the anticipated time of our initial production the market for astaxanthin is therefore conservatively estimated to be $965 million. 75% of this market is taken up by synthetic production, and the other 25% by natural production.

Synthetic astaxanthin is significantly weaker than natural astaxanthin in singlet oxygen quenching and free radical elimination. It is produced from petrochemicals and has not been demonstrated to be safe for human use. As such, it is used primarily in the salmon fish feed market (6). Recent studies have shown synthetic astaxanthin to sell for $2,000/kg, half the price of natural products.

Typical natural astaxanthin production is produced using Haematococcus pluvialis fermentation. This method has high production costs due to low growth rates and energy intensive extraction steps (1). Our natural product is produced via genetically modified *E. coli* fermentation. *E. coli* have weaker cell walls, significantly lessening the cost of cell lysis and extraction in our process. Coupled with a higher growth rate it should yield cheaper overall production costs. With lower costs, we plan to enter the market at a price point of $3,500, 12.5% cheaper than other natural products (5). At this cheaper price, we believe we can capture 20% of the current market (55 ton/yr)

5. Customer Requirements

N/A

6. CTQ Variable-Product Requirements

N/A

7. Product Concepts

N/A

8. Superior Product Concepts

N/A

9. Competitive Patent Analysis

N/A

10. Preliminary Process Synthesis

10.1 Seed Train Design

The sizing, numbering, and scheduling of fermenters in the seed train was the critical first step in designing the plant. Without a grasp of biomass concentrations and stages, one cannot progress to determine material costs and flow rates, heat exchanger duties, agitator speeds, or the like. And absent a sense of the rate at which astaxanthin-laden cells are available for further processing, the downstream design is unable to take shape.

At the recommendation of Dr. Bockrath, the project author, the upstream design was to consist of three stages, labeled pre-seed, seed, and production stages. The production stage is divided between a growth phase and production, or stationary, phase. The former is characterized exclusively by biomass growth, while the latter is the phase during which astaxanthin is synthesized. The production *phase* was locked in at a 26.5 hour batch time. The final biomass concentrations at each stage were additionally determined; for the pre-seed stage, 1 g/L; for the seed stage, 6 g/L; for the growth phase of the production stage, 24 g/L; and for the production phase of the production stage, 30 g/L, even in production conditions. The pre-seed fermenters were to be fed by 4 L of inoculum grown in the laboratory.

The industry convention of each seed train stage having volume ten times that of its predecessor was used to calculate the batch times of the seed and production stages. This calculation is not dependent on the actual size of the fermenters, but only on the scale-up factor. The result was a 19 hour batch time for the seed stage, and a 16.5 hour batch time for the production phase of the production stage. This brought the total batch time of the production fermenter to 43 hours; figuring in a ten-hour turnaround time, which would be adopted for each stage, this brought the batch time—and preferably the *cycle* time—to a duration of 53 hours. The batch time is how long a single batch at *any* stage takes, while the cycle time describes the duration of the bottleneck stage, the one that runs without interruption and is responsible for the output of product. Therefore, the batch time is stage-dependent, whereas the cycle time is a process-wide property. It is desired that the batch time of the production stage serves as the cycle time, since the production stage is the largest and therefore most expensive; ensuring that it runs "continuously" (without downtime, but in batch mode) justifies the expense.

Optimistically assuming that the cycle time is 53 hours, corresponding to the batch time for the production fermenter, the size of the production fermenter could be calculated based on the annual astaxanthin target rate, adjusted for downstream losses—approximately 59.8 tons/year. The only missing piece of information is the *number* of production fermenters. As the number of production fermenters increase, the cycle time for the upstream process decreases, as completed production batches are available more frequently. Increasing the number of production fermenters also decreases the necessary volume of a production fermenter. However, one obviously cannot arbitrarily increase the number of production fermenters to generate lower cycle times, since at some critical point the cost of purchasing and installing more fermenters outweighs the benefit of lower cycle times. In consultation with Dr. Bockrath, and after experimenting with different numbers of fermenters and their associated bare module costs, it was settled that three production fermenters—each of total size 225,000 L—provided an optimal arrangement of reasonable volume (and cost) and low enough cycle time. Moreover, the fact that a production fermenter of this size only constitutes 0.25 percent of the yearly demand of valuable astaxanthin means that no single production batch is unduly important in generating adequate product sales.

Knowing the volume of the production fermenter means the volumes of the seed and pre-seed fermenters can also be calculated. The seed fermenter is one tenth the size of the production fermenter, so it is 22,500 L. The pre-seed fermenter is one tenth the size of the seed fermenter, so it is 2,250 L. What remains is to calculate the number of seed and pre-seed fermenters necessary to accommodate the astaxanthin demand.

Following the recommendations in Biegler, Grossman, and Westerberg (1997), a mixed-integer nonlinear programming (MINLP) model for optimizing single-product batch campaigns was employed to determine the numbering of sub-production fermenter (40). This model and its application are described in detail in the paragraphs that follow. Biegler, Grossman, and Westerberg (1997) will hereinafter be referred to as BGM.

Let N_j be the number of fermenters in stage j. Accordingly, j ranges from 1 to 3, where $j = 1$ represents the pre-seed stage, $j = 2$ the seed stage, and $j = 3$ the production stage. Let V_j be the volume of the fermenters at stage j. Let C be the total purchase cost of all seed train fermenters. The objective function to be minimized is

$$C = \sum_{j=1}^{3} N_j V_j \tag{1}$$

since the purchase cost of fermenter vessels is calculated as a function of volume. BGM would recommend modifying this equation to

$$C = \sum_{j=1}^{3} N_j \alpha_j V_j^{\beta_j}$$

where α_j and β_j are a cost coefficient and exponent, respectively. One could, for instance, use the power law to estimate $\beta_j = \beta = 0.6$. Since the costing procedures used in this study come from a different text, with a different form for pressure vessels, the cost coefficient and exponent were assumed to be 1. Considering volumes for all of the fermenters are already known and the number of production fermenters—the largest expense by far—is fixed at this point, it was assumed that a linear relationship between volume and price was sufficient for the optimization procedure.

Let T_L be the cycle time for the process. This has been discussed at length above, but a mathematical definition is provided:

$$T_L = \min\left(\frac{\tau_j}{N_j}\right)$$

where τ_j is the batch time for stage *j*. Let *Q* be the annual demand of astaxanthin, and let *B* be the size of a batch of astaxanthin at the end of the seed train, *i.e.,* the astaxanthin present at the end of a production batch. The quotient of *Q* / *B* is the number of production batches required to meet demand. With this in mind, the constraint on (1) is

$$\frac{Q}{B} T_L \leq H \tag{2}$$

where *H* is the horizon time, a semi-arbitrary time over which the process is to be analyzed. Since *Q* was selected to be the *annual* demand of astaxanthin, *H* should accordingly be one year. In English, this constraint requires that, given the batch time for each stage, the number of fermenters at that stage is sufficient to ensure that the demand of astaxanthin is met in the time allocated to meet the demand. For illustration, if some N_j is too low, then T_L is too high, and the number of batches to meet demand, *Q* / *B*, take too long to finish. The goal of the optimization is to select the lowest-“cost” arrangement of fermenters such that the horizon constraint is met.

Performing MINLP is no simple task. For instance, neither (1) nor (2) is convexified, an important property to successfully utilize MINLP algorithms. (BGM details how to do this.) Perhaps more alarmingly, Python, the programming language ubiquitously used for optimization problems, lacks a standard, widely-used package to perform MINLP calculations. Luckily, the problem as framed in this Section has several properties that enable easier computation than would otherwise be necessary for a more general MINLP problem.

First of all, by virtue of industry sizing convention, all fermenters already have a volume associated with them. Therefore, there is no need to calculate V_{j}. This process would generally be computationally intensive, since V_{j} are continuous variables, requiring significantly more iterations to achieve optimal values. Second, N_3 is predetermined; $N_3 = 3$. Again, this reduces the number of computations necessary to find an optimum. Finally, it can be very safely assumed that N_{j} will not exceed, say, 5 fermenters/stage. If it is possible to find $N_{\mathrm{j}} \leq 5$ that satisfy the horizon constraint, then this arrangement must automatically minimize costs beyond what any arrangement $N_{\mathrm{j}} > 5$ could accomplish, since the volumes of the fermenters are fixed and a linear relationship between volume and cost has been assumed. These unique conditions make it eminently feasible to write a Python script that brute-force iterates over all 25 arrangements of pre-seed and seed fermenters where $N_j \leq 5$. This is a small enough sample that one can simply "eyeball" the correct answer. Such a Python script is provided in the Appendix. Note that, had all 25 arrangements failed to meet the horizon constraint (which did not happen), one would need only change (not increase, because $N_j \leq 5$ had already been proven not to work) the range over which the iteration occurs to find the optimum arrangement.

This method yielded 3 pre-seed fermenters and 2 seed fermenters. Implicit in the method and the design is the availability of common lines of transfer between every pair of fermenters between two stages; thus, any batch that is completed may be moved to any available fermenter at the next highest stage. The consequence of this is that, for example, pre-seed fermenter #1 may transfer its contents at the conclusion of the batch to seed fermenter #1 today but seed fermenter #2 tomorrow. There are no set "series" that the batches follow.

Before this method was attempted, batch scheduling in Excel was conducted. Three production fermenters were fixed in place, and sub-production fermenters were added as needed to complete the schedule. This procedure resulted in an identical arrangement of fermenters as MINLP. Process design is full of uncertainties, but this sizing, numbering, and scheduling of fermenters is not one of them in this proposed plant design.

10.2 Plant Locations

None of the raw materials, utilities, or equipment required to run the plant are geographically constrained. Nevertheless, it is not possible to correctly evaluate the finances of the proposed plant without selecting a location, at the very least to calculate taxes owed. Todaro and Vogel's *Fermentation and Biochemical Engineering Handbook* comments that "[t]he carbohydrate source, because of its large volume, is the only fermentation raw material which has any influence on plant location." (41) Consultants agreed that this is a valid way to select a plant location.

The carbohydrate source here is glucose, "usually made from corn starch." The search for corn commenced with enthusiasm. The world's leading corn producer is by far the United States (41), whose tax system and depreciation schedule are conveniently the ones with which the authors are most familiar. It was determined to purchase Clearsweet® 99% Refined Liquid Dextrose Corn Syrup, and tie the plant's location to Cargill Sweeteners Regional Office in Lancaster, PA, from which the fermentable corn syrup is ostensibly shipped. Therefore, it was decided to construct the plant in or around Lancaster, PA. Cargill Sweeteners has another

Regional Office in Naperville, IL, and the decision to construct the plant in Lancaster, PA, is likely equivalent to erecting the plant anywhere in the Midwest of the United States, especially in locations of high corn density or corn processing density. Information about Clearsweet is included in the Appendix, while further exploration of glucose sourcing is discussed in Section 21.

10.3 Cooling Options

From the bioprocess engineer's perspective, *E. coli*, and really most all microbes, have the irritating tendency, when left to themselves, to release considerable amounts of heat during the exponential growth phase. Consequently, fermenters must be appropriately cooled during the growth phase to keep conditions optimal for growth—namely, at a temperature of 37ºC. Three options were identified, in increasing order of complexity: cooling jacket, cooling coils, and external heat exchangers. An attempt to design a cooling jacket that would extract sufficient heat was conducted for each fermenter stage. Only the pre-seed fermenter has enough surface area to remove the heat generated during its batch. Continuously operated external heat exchangers were used to cool the seed and production fermenters; their specifications are extensively detailed in Sections 14, 15, and 16. Cooling coils were abandoned partly because of the authors' greater familiarity designing heat exchangers and the availability of ASPEN to quickly synthesize viable geometries. In addition, the necessity of agitators in the fermenters raised concerns that the agitators could be obstructed by the presence of cooling coils. It may be prudent to re-evaluate this design choice upon construction of the plant. Nevertheless, since heat exchangers are generally the more expensive investment, this selection is an appropriately conservative choice at this stage of design and evaluation.

10.4 Extraction Methods

Solid-liquid extraction involves the isolation of soluble components from a solid or semi-solid matrix (such as lysed cellular mass) using appropriate solvents. As part of our preliminary process synthesis, several different methods of extracting Astaxanthin from biomass were considered, including the use of organic solvents, edible oils, and supercritical carbon dioxide, and gas-expanded solvents. Here, we assume cell lysis achieved by passing a concentrated cell slurry through a homogenizer serves as an effective pre-treatment in breaking down the biomass matrix, such that the issue of extraction is solubility-, rather than diffusivity-limited.

Studies have indicated that Astaxanthin is thermally degradable, and that its antioxidant activity can be significantly diminished upon oxidation (7). Therefore, when evaluating extraction methods, we considered the heat and oxygen sensitivity of our product. Selection was also dictated by our compound being slightly polar (requiring solvents more polar than those typically used for non-polar carotenoids). A crucial consideration was whether the extraction method would leave any toxic or undesirable artefacts in the final extract product. Finally, the environmental impact of each method was also considered.

Several works (8) have described the use of dichloromethane, chloroform, hexane, isopropanol, and methanol-based solvents to extract Astaxanthin from *H. Pluvialis*. Hexane is often used in industrial extraction processes (9). However, these chemicals are not generally recognized as safe, and could raise health concerns if residual solvent remained in the final product. Additionally, high energy and costs are associated with separating the astaxanthin from the solvent via distillation and evaporation. Solvents generally recognized as safe (GRAS) include acetone and ethanol. Other solvents investigated, in the edible oils category, were soybean, grapeseed, corn, olive, and vegetable oil (10). The oil extracts were separated from the culture medium containing cell debris by simple gravity settling. These are environmentally friendly solvents, and use of gravitational settling would drastically reduce costs of specialized extraction equipment, however, the extraction was time-consuming, requiring, in one study, 48 hours of extraction to obtain upwards of 87.5% Astaxanthin recovery (11).

Supercritical CO_2 extraction offers several advantages: due to the low critical temperature of CO_2 the extraction can be operated at moderate temperatures, preve

nting the degradation of Astaxanthin. Furthermore, CO_2 is relatively cheap, inert, generally recognized as safe, and non-toxic. Supercritical fluid extraction generally offers shorter extraction times and requires lower solvent flow rates, reducing equipment and operational costs. Subcritical fluid extraction and gas-expanded ethanol extraction were also methods that appeared both cost-effective, non hazardous to health, and environmentally friendly. The use of gas-expanded ethanol, as described by Reyes, et. al (12) appeared to have an extraction recovery superior to that of S-CO_2. Pressurized fluid extraction, as described by Boonoun, et. al and Huang, et. al (13, 14) may even be performed without the use of energy-costly drying processes prior to extraction. However, there was insufficient research and implementation of such recent extraction technologies to base our technoeconomic analysis.

Although research on pressurized and gas-expanded (GXE) solvents seemed promising, there was a much smaller pool of literature describing the extraction of Astaxanthin using these methods. On the other hand, supercritical CO_2 extraction is already an established method in the decaffeination of coffee, and recovery of cannabis, fruit, and even carotenoid extracts. Consequently, there already exist manufacturers of positive displacement plunger pumps appropriate for use in supercritical CO_2 extraction processes. Entire literature reviews are available, detailing the determination of optimal supercritical CO_2 extraction conditions, including temperature, pressure, co-solvent concentration, and solvent / feed loading ratio. Because of its beneficial safety profile, abundance of literature on its use to extract Astaxanthin, established use in industry, and availability of specialty equipment, we decided to use supercritical CO_2 extraction instead of the other methods previously described.

11. Assembly of Database

11.1 Cost of Raw Materials

The raw materials needed for this process are summarized, with their prices per metric ton (MT), in Table 11.1 below.

Table 11.1. Raw Material Prices for Plant Operation.

Material Name	Cost ($USD/MT)
Glucose/dextrose	$400
Anhydrous ammonia	$250
Phosphoric acid	$800
Media salts and yeast extract (each individually)	$1,654
Carbon Dioxide	$30
Diatomaceous Earth	$24,300
Fructose	$10,000
Bleach (Sodium Hypochlorite)	$571
Ethylene Glycol	$123.3
Ethanol	$20,881
Astaxanthin Product	$3,500,000

Ethanol, diatomaceous earth, and fructose costs were all found using the resource SciFinder. Typically meant for small scale prices, these costs may in actuality be lower than as presented in Table 11.1. The cost of bleach was retrieved from a large scale cleaning product provider and it is sold by the barrel (15). Ethylene glycol cost was retrieved from forecasting data by ICIS, a petrochemical market intelligence provider (16). The cost of carbon dioxide was forecasted by Synapse Energy Economics for the next 30 years. In this process, the carbon dioxide will be recycled. Therefore the high estimate of the price of carbon dioxide in 2021 (the initial year of this process) was used to calculate CO2 costs (17).

The issues with SciFinder primarily featuring laboratory prices were insurmountable for the fermentation raw materials. Using SciFinder prices for glucose, anhydrous ammonia, and phosphoric acid, the plant was projected to rapidly slide into billions of dollars of debt, and consultants confirmed that these prices were unfounded in reality. Consequently, prices for glucose, anhydrous ammonia and phosphoric acid were estimated using Alibaba's website, the most accessible source for costing purchases of industrial size. Consultants expressed some degree of skepticism over these prices, saying that even these may be too high. Consider these figures, then, to be conservative estimates.

The astaxanthin product will be sold at a price of $3,500/kg, or $3.5 million/MT. This is a conservative price of natural astaxanthin, which typically sells for $4,000/kg, or $4 million/MT. Profitability analysis was conducted with a selling price of astaxanthin that will decrease with time, as competitors are expected to join the market.

11.2 Effective Chemical Equations

Provided with the problem statement, the following equations (Table 11.2) were used to quantify the material balances into and out of fermenters during growth and production phases. The growth equation is on a mass basis, whereas the production reaction is written on a typical mole basis.

Table 11.2. Effective Equations for Growth and Production Phases.

Growth	100 g $C_6H_{12}O_6$ + 8.5 g NH_3 + 4.7 g H_3PO_4 + 32 g O_2 → 50 g biomass + 54.9 g CO_2 + 39.1 g H_2O
Production	1.0 $C_6H_{12}O_6$ + 2.17 O_2 → 0.075 Astaxanthin + 3 CO_2 + 4.05 H_2O

11.3 Cell Growth Kinetics

Because the cells in use in the plant are genetically modified, it is necessary to make use of the kinetics data provided by Zhang, et al. in their paper on the multidimensional heuristic process for high-yield astaxanthin synthesis. The data utilized is copied from this paper and reproduced in Figure 11.1 below. This figure shows optical density (OD) and glucose concentrations as a function of time observed in the types of *E. coli* cells that will be used in this plant.

OD was taken as a proxy for biomass growth, as is common practice in bioprocess engineering. There are avid debates about the precise mathematical relationship between cell concentration and OD, even though it is typically calibrated experimentally for the spectrophotometer in use. No absolute values were used to calculate rate data in this study; only *ratios* of OD points on Figure 11.1 were used. This method is not without its own flaws, however. By taking ratios, the OD of blank medium is neglected, or assumed to be zero. This is approximately true in actuality, but it is important to note the uncertainty propagated through the following calculations as a consequence.

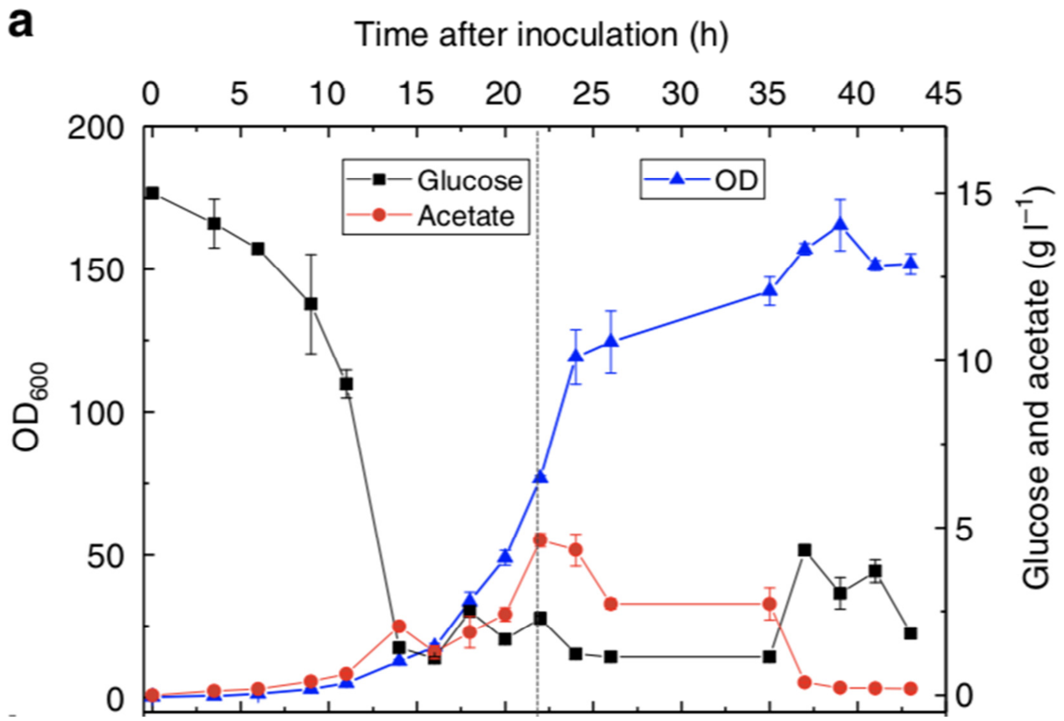


Figure 11.1. Time profiles of optical density and glucose as functions of time.

From Figure 11.1, it can be deduced that the cell growth rate is 0.224 hr^{-1}. This value was used to calculate batch times for the pre-seed and seed fermenters, as well as the growth phase of the production fermenter. The production/stationary phase for the production fermenter was taken as 26.5 hours without regard to the data in Figure 11.1. It was an assumption given in the project statement.

11.4 Peak Rates

The inclusion of glucose consumption data in Figure 11.1 provides a convenient source from which to approximate the peak rates of raw material consumption and off gas production during fermentation. Determination of peak rates is imperative for the design of the feed air compressor, fermenter vents, and heat exchangers. It was decided from the data in Figure 11.1

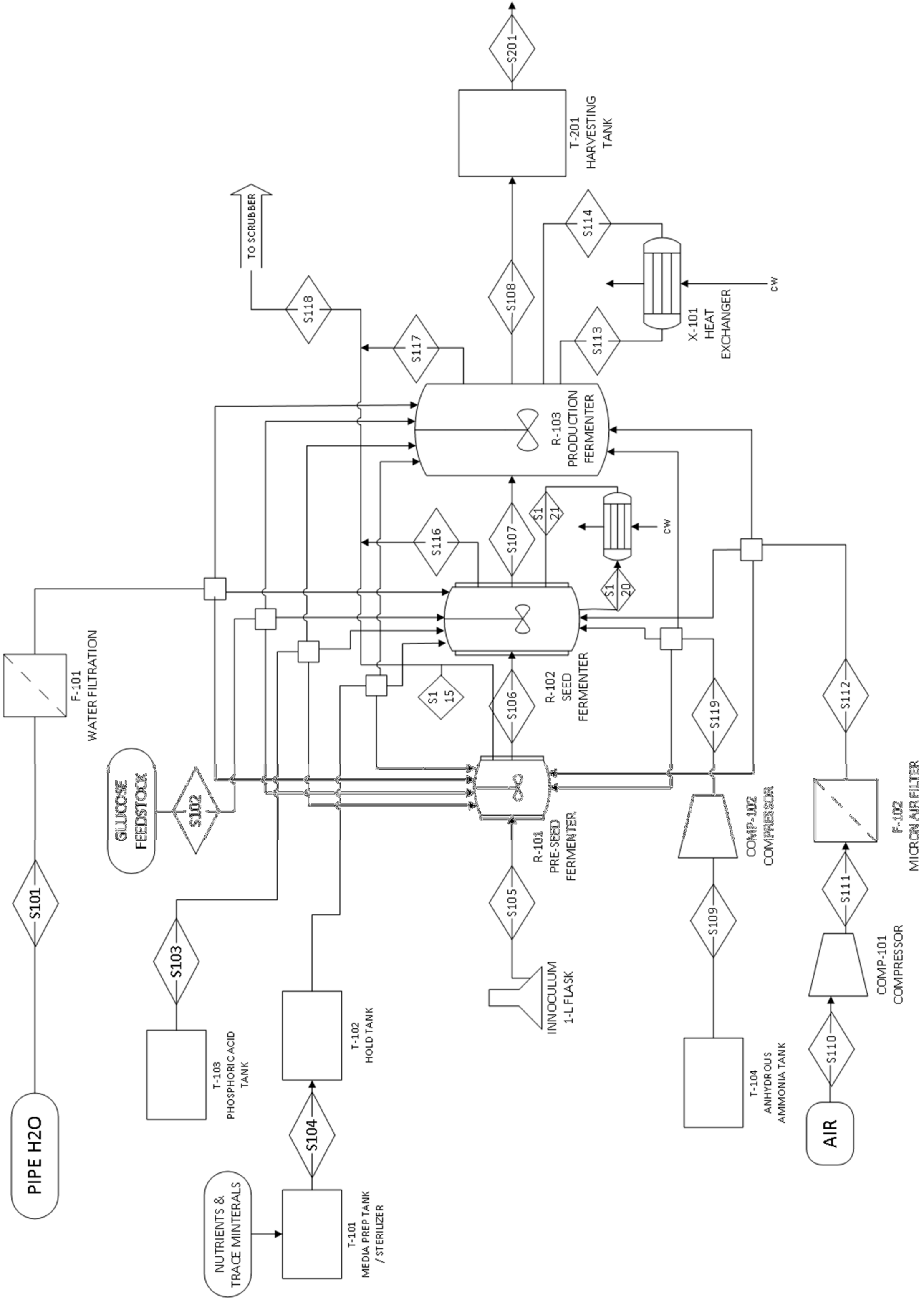
that the peak rate of biomass growth is 0.45 hr^{-1}, and peak consumption of glucose is 2.3 g/(L hr), both approximately twice the average rate for each of these respective measures. Consequently, peak rates for each of the feed components and product gases were taken to be twice their time-averaged values. Such a trend emulates a rule of thumb proffered by Prof. Vrana.

11.5 Cell Growth Thermodynamics

Without heats of formation for biomass and astaxanthin, performing Hess's Law calculations is not possible for the cell growth and production phases characteristic of this process. Instead, the well-known work of Cooney, et al. (1969) was consulted (43). The so-called Cooney constant is an empirically derived factor that converts the oxygen fed to a bioreactor to the heat generated by cells consuming that oxygen. Its actual value is 0.124 kcal/mmol O_2. Intended for the exponential growth phase, the Cooney constant was also used in this study's calculations for the production phase, to compensate for difficulties regarding the heat of formation of astaxanthin. A heat of formation for astaxanthin, -265 kJ/mol, *was* estimated using ProPred. Its use was rejected, however, because it implies that the production of astaxanthin is endothermic, unlikely for a reaction that resembles combustion. (The industrial consultants adamantly doubted the veracity of the figure, as well.) Therefore, the Cooney constant was used for the production phase. If anything, it results in an overestimate of the heat generated during the production phase, acceptable for this type of profitability study.

12. Process Flow Diagrams and Material Balances

Fig. 12.1 Section 1: Upstream (Seed Train) Design



Material Balance For Section 1 (Upstream, Fermentation)

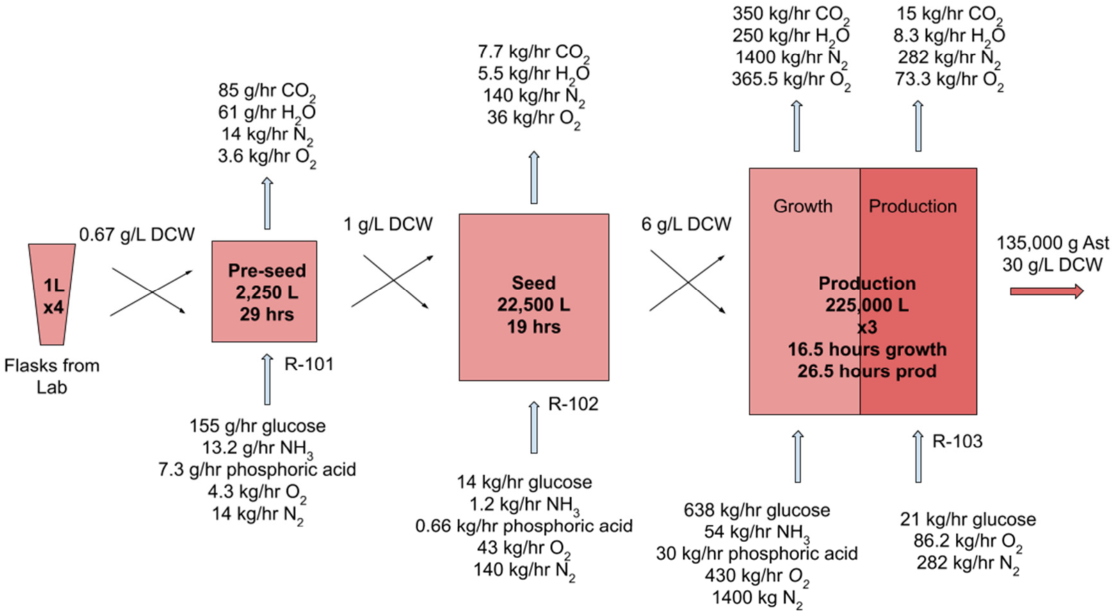


Fig. 12.2 Section 2: Downstream (Pre-Extraction)

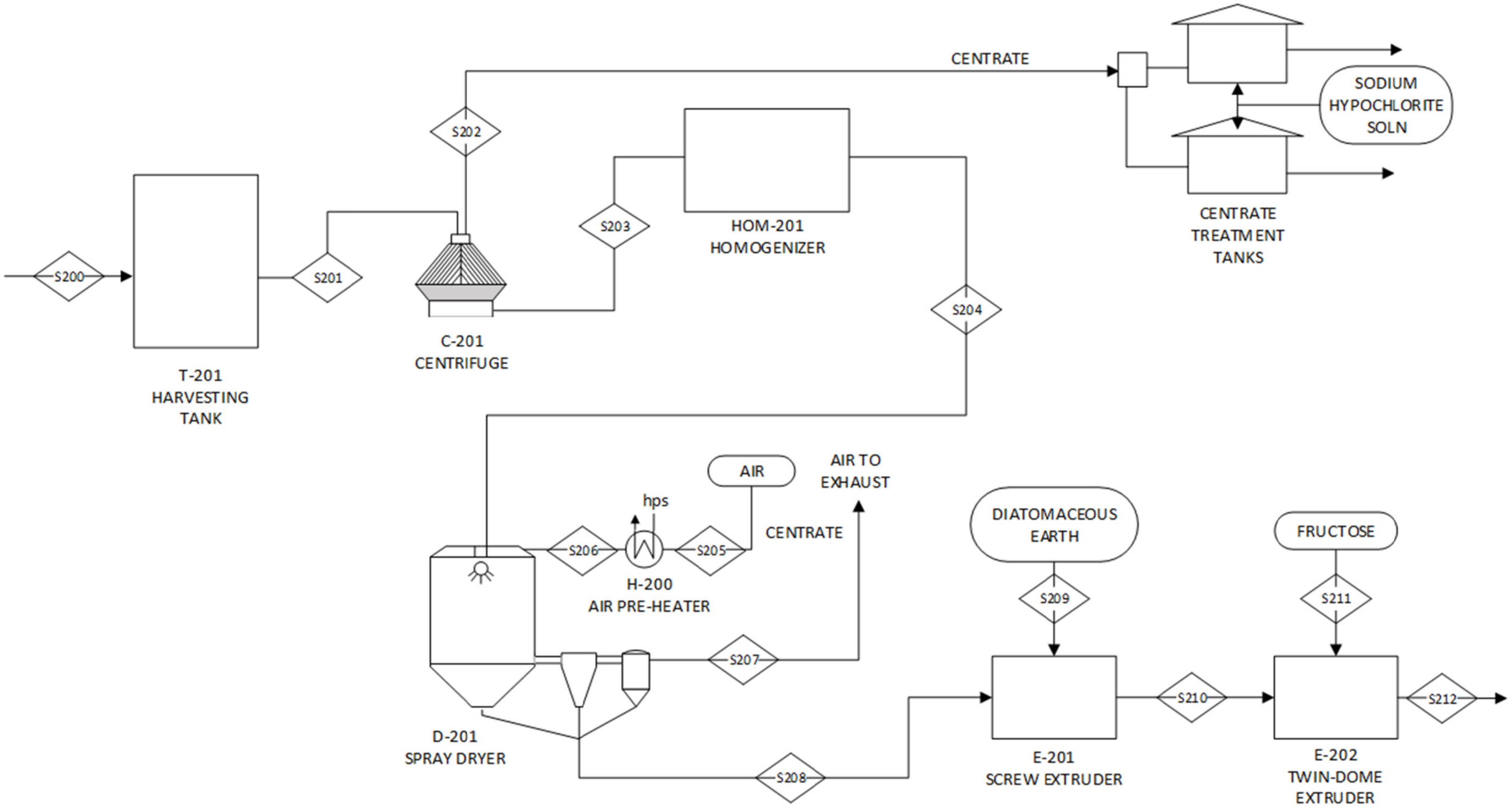


Fig. 12.3 Supercritical Extraction 1/2

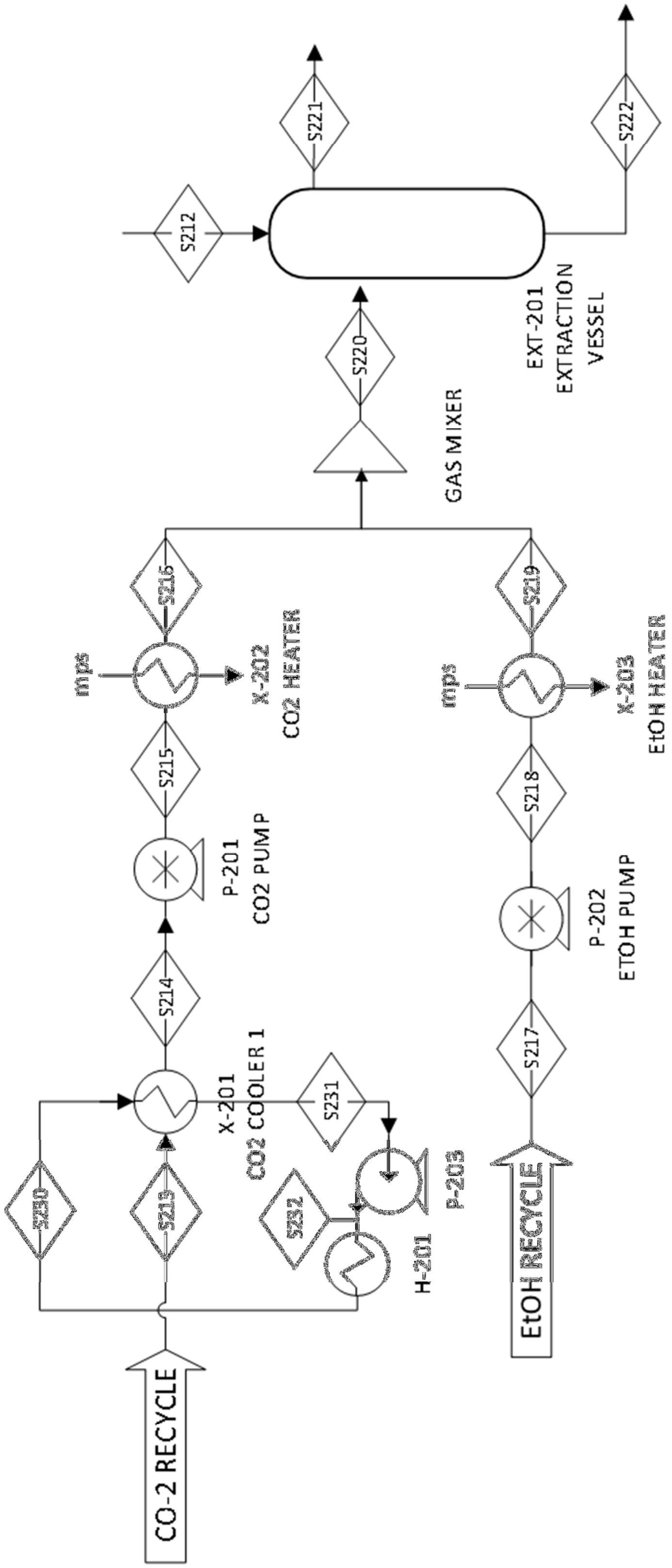


Fig. 12.4 Supercritical Extraction 2/2

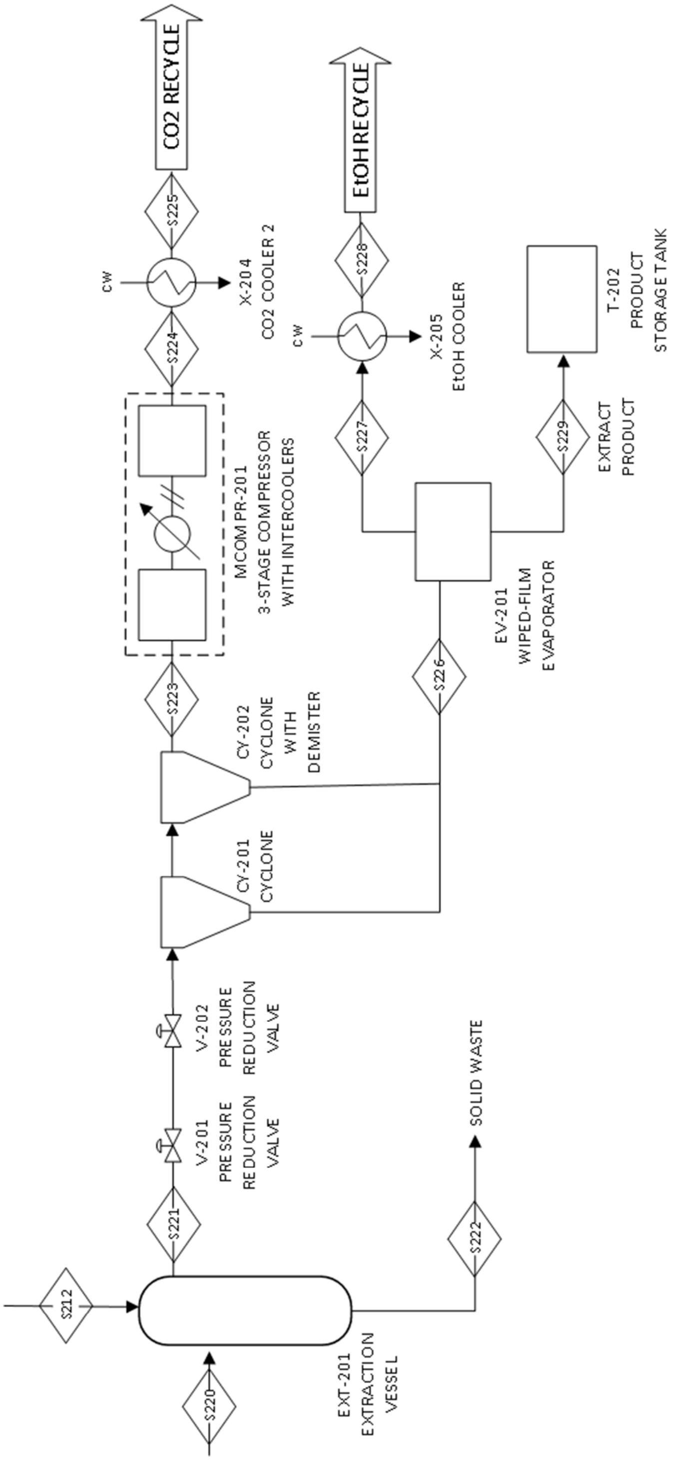


Table 12.1 Material Balance for Section 2 (Downstream)

Stream ID	S217	S218	S219	S220	S221	S222	S223	S224	S225	S226	S227	S228	S229	S230	S231	S232
Temperature	35.0	41.7	60.0	60.0	60.0	22.0	22.0	48.9	22.0	22.0	78.4	35.0	78.4	10.3	10.4	10.4
Pressure	1.0	300.0	300.0	300.0	300.0	1.0	1.0	65.0	65.0	1.0	1.0	1.0	1.0	1.0	1.0	1.0
Vapor Fraction	0.0	0.0	0.0	0	0	0.0	1.0	1.0	0.0	0.0	1.0	0.0	1.0	0.0	0.0	0.0
Mass Flow (kg/hr)	358.1	358.1	358.1	7162.4	7444.1	344.6	358.1	6804.3	6804.3	365.2	357.8	357.8	7.4	4000.0	4000.0	4000.0
Component Material Balance																
Water	0.0	0.0	0.0	0.0	0.0	16.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	1600.0	1600.0	1600.0
Cell Mass	0.0	0.0	0.0	0.0	274.6	274.6	0.0	0.0	0.0	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	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
Diatomaceous Earth	0.0	0.0	0.0	0.0	0.0	36.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
Fructose	0.0	0.0	0.0	0.0	0.0	18.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
Carbon Dioxide	0.0	0.0	0.0	6804.3	6804.3	0.0	6804.3	6804.3	6804.3	0.0	0.0	0.0	0.0	0.0	0.0	0.0
Ethanol	358.1	358.1	358.1	358.1	358.1	0.0	0.0	0.0	0.0	358.1	357.8	357.8	0.4	0.0	0.0	0.0
Ethylene Glycol	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	2400.0	2400.0	2400.0
Astaxanthin	0.0	0.0	0.0	0.0	7.0	0.0	0.0	0.0	0.0	7.0	0.0	0.0	7.0	0.0	0.0	0.0

Stream ID	S201	S202	S203	S204	S205	S206	S207	S208	S209	S210	S211	S212	S213	S214	S215	S216
Temperature (°C)	37.0	37.0	37.0	37.0	22.0	220.0	80.0	80.0	22.0	22.0	22.0	22.0	22.0	-2.3	20.8	60.0
Pressure (bar)	1.0	1.0	1.0	1.0	1.0	1.0	1.0	1.0	1.0	1.0	1.0	1.0	65.0	65.0	300.0	300.0
Vapor Fraction	0.0	0.0	0.0	0.0	1.0	1.0	1.0	1.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	
Mass Flow (kg/hr)	10495	8963.0	1524.0	1524.0	83448	83448	84658	299.1	36.0	335.1	18.0	353.1	6804.3	6804.3	6804.3	6804.3
Component Material Balance																
Water	10189.	8963.0	1226.0	1226.0	0.0	0.0	1210.0	16.0	0.0	16.0	0.0	16.0	0.0	0.0	0.0	0.0
Cell Mass	298.0	0.0	298.0	298.0	0.0	0.0	0.0	283.1	0.0	283.1	0.0	283.1	0.0	0.0	0.0	0.0
Air	0.0	0.0	0.0	0.0	83448	83448	83448	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
Diatomaceous Earth	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	36.0	36.0	0.0	36.0	0.0	0.0	0.0	0.0
Fructose	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	18.0	18.0	0.0	0.0	0.0	0.0
Carbon Dioxide	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	6804.3	6804.3	6804.3	6804.3
Ethanol	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
Ethylene Glycol	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
Astaxanthin	7.6	0.0	7.6	7.6	0.0	0.0	0.0	7.3	0.0	7.3	0.0	7.3	0.0	0.0	0.0	0.0

13. Process Descriptions

13.1 Feed Material Storage and Preparation

An array of raw materials must be introduced at each stage of fermentation in order to create optimal growth conditions for the *E. coli* cells. Moreover, because the production fermenters especially will demand high quantities and flow rates of these materials, it is imperative to both store and prepare these materials in similarly large quantities.

The carbon source in the fermentation process is glucose. The dextrose corn syrup that will be purchased is a liquid only at temperatures at or exceeding 50ºC, so it will be stored at that temperature. Below 50ºC, it is a solid, but this phase shift is reversible. Storage as a liquid was chosen to facilitate the high flow rates necessary at peak glucose consumption during the production stage. It is assumed that, at this temperature, the glucose is sterile.

The nitrogen source is ammonia, which will be obtained in anhydrous form. Though it is a vapor when introduced to the fermenter, it is stored just below its boiling point at –33ºC, both for safety and to reduce storage space. The ammonia must accordingly be vaporized before being introduced to the fermenter. It is assumed that this process will adequately sterilize the ammonia.

Phosphoric acid is additionally needed in relatively small quantities to keep fermenter conditions slightly acidic, which *E. coli* prefer. As per safety protocols, phosphoric acid must be stored in stainless steel 316—the form of stainless steel specifically used to resist highly corrosive materials, like phosphoric acid. Among the other raw materials needed to carry out the upstream fermentation, phosphoric acid is the least abundant.

As an aerobic process, both during growth and production phases, significant aeration of the fermenters is required. Air will be sourced from the plant's natural external environment via a rotary screw air compressor, sized according to the maximum aeration required during the production batch time. The compression demand results in an outlet air stream at high temperature, which in combination with low pressure drop HEPA filters, should prevent the introduction of adventitious air-borne organisms or viruses.

Though not present in the effective reaction equations either for growth or production, Zym media will also be prepared and introduced to each of the fermenters. Zym media provides the *E. coli* with trace metals, sulfur, phosphorus, and yeast extract, all of which are essential to amino acid and protein formation. Crucially, by virtue of its composition of ɑ-lactose, Zym is auto-inducing, meaning at high enough biomass concentrations, it will initiate astaxanthin production via the intracellular expression vector. This is why it is possible to conduct both growth and production phases in a single production fermenter. Rather than purchasing Zym media, it will be made in concentrated quantities, heated and stored at 80ºC, to preserve sterility, until it is needed in a fermenter. Media is transferred to the open fermenter, where it is diluted with water to the prescribed working volume.

13.2 Seed Train Growth

E. coli cells recombinantly optimized for astaxanthin production are grown in the laboratory to 1 L volumes at a concentration of 0.67 g cells/L. Four 1 L shaker flasks are then transferred to one of three pre-seed fermenters, each with 2,250 L total volume. These fermenters will already have been supplied with sterilized media diluted with water to a working volume of approximately 1,800 L. It is common for inexperienced students and veteran engineers alike to balk at the magnitude of this dilution—from 4 to 1,800 L. This *is* practical and *is* done in industry. The *caveat* is that, early on in the batch, it can be very difficult to ascertain by optical density measurements whether growth is occurring at all. It is expected that repeated batches at this stage will simultaneously instill operators with confidence that this approach is feasible and elicit improved monitoring procedures in the early hours of growth. Overall, this stage has a batch time of 29 hours, and a final biomass concentration of 1 g/L.

A completed pre-seed batch is fed to whichever of the two seed fermenters is available. The seed fermenters are 22,500 L in total volume, and have working volumes of 18,000 L. The batch time at this stage is 19 hours, a full 10 hours shorter than the pre-seed stage. The fact that the dilution from pre-seed to seed stage is merely a factor of 10 is responsible for the brevity of this stage, in comparison to its predecessor. The final biomass concentration is 6 g/L at the conclusion of the seed stage.

The resulting biomass and broth reaches its final upstream destination in one of three production fermenters. A production fermenter has total volume 225,000 L, with working volume 180,000 L. Its name, however, belies its initial purpose. For the first 16.5 hours of a production batch, biomass continues to grow, without producing appreciable astaxanthin, to a final concentration of 24 g/L.

During each of the seed train growth stages, glucose, ammonia, phosphoric acid, and air are fed at rates specified in Sections 12 and 15. The fermenter contents are maintained at 37ºC, the well-documented optimal temperature for *E. coli* growth. This is accomplished in the seed and production stages by an external shell-and-tube heat exchanger that cools fermenter broth to 32ºC and reintroduces it to the bioreactor. Since only a small fraction of the fermenter contents are cooled per minute, heat released during growth should adequately warm the cooled fluid and maintain a steady temperature in the reactor of 37ºC. In the pre-seed stage, a cooling jacket suffices, due to that fermenter’s smaller stature. It was initially attempted to use cooling jackets for the larger fermenters, but there is simply insufficient surface area to induce enough heat transfer out of those fermenters.

Each fermenter has a turnaround time of 10 hours, during which time the fermenter contents are drained and transferred to the next stage, the fermenter is sterilized, and sterilized media is diluted with water and, in the case of the seed and production fermenters, combined with the previous stage's contents to achieve the appropriate working volume of broth.

13.3 Production Stage Fermentation

Zhang's methods to maximize astaxanthin production cannot undermine the reality that *E. coli* only make astaxanthin in appreciable quantities during a production, or stationary, phase, when growth is at best *linear*, rather than exponential. Initiating the production phase requires several changes from growth conditions. The temperature is lowered to 30ºC. Ammonia and phosphoric acid cease to be fed. The aeration rate is cut by 80 percent. This combination of conditions actively inhibit growth, permitting metabolic energy to be channeled into synthesizing astaxanthin.

The production stage is still carried out in the production fermenter, so these conditions must be altered by a different set of utilities also associated with this fermenter, namely two heat exchangers—one for one-time use to cool the fermenter contents to 30ºC, and another for maintaining this temperature for the duration of the batch. This portion of the batch lasts for 26.5 hours. (This makes the production stage last, in total, for 43 hours.) The final titer of astaxanthin at the conclusion of this batch is 0.75 g/L, yielding a total of 135 kg of astaxanthin per batch, or about 0.25 percent of yearly demand. This final value has been an important design consideration, since it demonstrates that no single batch can financially cripple the plant if it fails. Biomass production does not cease altogether during the production phase; accordingly, it is assumed that *E. coli* reaches an approximate concentration of 30 g/L during the production phase.

13.4 Fermentation Product Storage

The biomass and broth exiting the production fermenter must be held in a surge tank prior to entering the continuous downstream process. It must have a capacity large enough to hold 3 production batches of biomass and the accompanying broth. It is not expected that the tank will ever hold 3 batches, however this size takes into consideration possible downtime for the downstream processes. At full capacity, the storage tank should only be 80% full of liquid. Therefore a 306,000 L surge tank is required to handle the fermentation product. A cone-roof tank is desirable for its ability to vent and prevent pressure changes due to fluctuating temperature. The preferred tank material is stainless steel to prevent degradation over time (18).

13.5 Solid-Liquid Separation

The solid-liquid separation step is the first step in the continuous downstream process. At this point, astaxanthin rich *E. coli* is suspended in fermentation broth. To procure the astaxanthin the biomass must be separated from the broth. Centrifugation is used to remove a substantial portion of the broth. Our firm recommends a disc stack centrifuge for this step. Disc stack centrifuges separate solid-liquid mixtures in a continuous process using high centrifugal forces. As the internal bowl spins, denser solids are forced outward while the less dense liquids form inner layers. Central plates, or the disc stack, provide settling area for that liquid and speeds up the separation process.

The concentration of solids on the outer edge of the bowl can then be collected continuously and is referred to as the cell paste. Separated liquid also leaves continuously via a port at the top of the centrifuge and is referred to as the centrate (19). Disc stack centrifuges are shown to give a cell paste that is 20 wt% dry cell weight. To prevent buildup of fermentation product in the surge tank, the disc stack centrifuge must separate 10,200 L/hr of fermentation product. The centrate leaving this process must be treated and disposed of. The cell paste continues through the downstream process.

13.5.2 Cell Disruption

Cell disruption is a method of breaking cell walls. *E. coli* has much weaker cell walls than algal cells, which are typically used in astaxanthin production. Because of this, cell disruption does not require elaborate operations. A homogenizer is used to shear the cell walls and release astaxanthin. Homogenizers are comprised of two elements. A compression block and a homogenizing valve. The compression block pumps the product to a high pressure and the impact of that product against the valve shears the cell (20). The homogenizer will need to handle a throughput of 1,226 L/hr.

13.6 Spray Drying

A spray dryer is used to reduce the moisture content of the biomass slurry leaving the homogenizer (stream 204) from 80% to 5% water by weight. It does so by atomizing the feed to 20μm via rotary atomizer, and contacting the droplets with hot air such that 1206 kg H2O / h is evaporated. The powder product is then collected from the bottom of the drying chamber, and additional fine powders are recovered from the outlet air stream using a cyclone, followed by a wet bag filter. The combined stream of recovered powder, S208 is then fed to the pelletizer. To account for loss of atomized feed that accumulates on dryer chamber walls, we assumed a 95% powder product recovery.

The spray dryer utilizes an open-cycle of air that flows co-current (Fig. 13.6) with the atomized feed. Ambient air is heated by a preheater to 220°, contacts the feed droplets, and leaves the drying chamber saturated with water vapor at 80 °C. These temperatures, as well as co-current flow which ensures our dried product contacts the air stream at its lowest temperature, were chosen in order to prevent degradation of Astaxanthin.

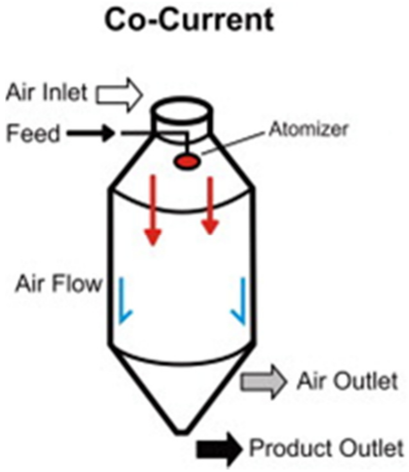


Fig. 13.6 Co-current flow of feed and air streams within the spray drying chamber (21)

Compared to the rotary drum dryer, the spray dryer requires less floor space, is more suitable for processing slurry feeds, and, most importantly, offers shorter drying/residence times, reducing the heat-induced degradation of our product. With a large evaporative load of 1206 kg H2O / h, and conical-base drying chamber volume of 581 m^3 (see section 15.15 for sizing and cost derivation), the unit will need to be custom ordered from GEA-Niro (22), using the largely customizable options available.

13.7 Pelletization

The dried biomass from the spray drier is a fluffy material, not fit for extraction. It must be condensed and compacted into solid pellets before astaxanthin can be recovered. There are two steps in the pelletization process, a grinding step and a binding step. During the grinding step a grinding agent is added to the biomass, and then that mixture is run through a screw extruder. The preferred grinding agent is diatomaceous earth. It is appealing because it is generally recognized as safe for human consumption, and carries a relatively low cost. The ideal amount of diatomaceous earth results in a pellet that is 10 wt% grinding agent.

After the grinding step, a binding agent is added and the mixture is pushed through a twin dome extruder. When forced through the die on the end of the extruder, the pellets will break off due to their own weight and may then undergo supercritical extraction. The preferred binding agent for this step is fructose. It is preferable due to its solubility in water and cost. The ideal amount of fructose results in a pellet that is 5 wt% binding agent (23).

13.8 Supercritical Fluid Extraction

To extract Astaxanthin from the biomass pellets, we will be taking advantage of the tunable solubilizing properties of supercritical carbon dioxide (S-CO_2). Carbon dioxide behaves as a supercritical fluid above its critical temperature (31.0 °C) and pressure (7.3773 MPa). Notably, at 60 °C, as the pressure of S-CO_2 is increased from 80 to 300 bar, the solubility (mole fraction of Astaxanthin in the supercritical fluid) increases from 0.80×10^{-5} to 4.89×10^{-5} (24). An advantage of using S-CO_2 extraction is that the extract can easily be separated from the supercritical fluid by depressurizing the system, causing the solubility of Astaxanthin in S-CO_2 to plummet. Compared to the use of traditional extraction methods utilizing organic or petrochemical process solvents, this method eliminates the need for complex separation steps and leaves behind no hazardous residues (25). Furthermore, the depressurized, then condensed stream of CO_2 can be recycled, making the extraction process more environmentally friendly.

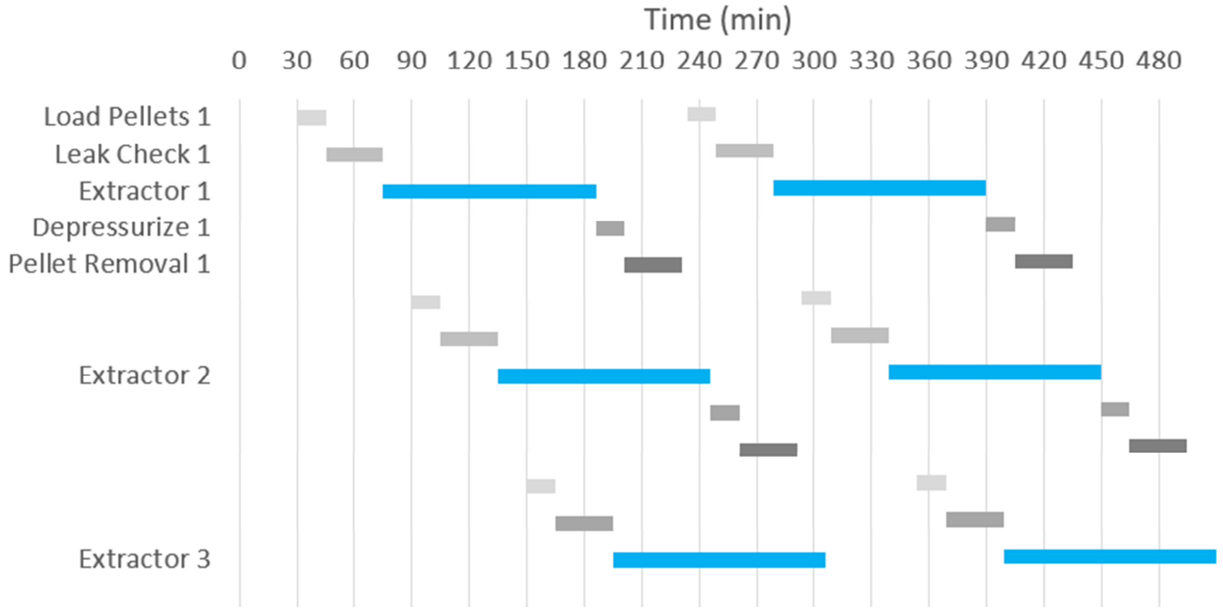
The extraction will be carried out at 60 °C and 30 MPa, with S-CO_2 and 5 wt % ethanol (EtOH) co-solvent. These conditions are based on those described by Valderrama, et. al, who report a 97% recovery in the extraction of Astaxanthin from disrupted *H.Pluvialis* algal biomatrix using S-CO_2 with 9.4 weight percent ethanol cosolvent and 20 kg solvent / kg feed loading ratio (26). The only modification is the co-solvent concentration, which is based on a report by Molino et. al indicating that an increase of EtOH concentrations above 5% has little effect on extraction efficiency (27).

Although use of a co-solvent necessitates additional separation steps to isolate the extract, overwhelming evidence from literature indicates that the increase in solubility of slightly polar molecules in S-CO_2 afforded by use of ethanol co-solvent is critical to obtaining high recoveries of Astaxanthin (28). Exclusion of the co-solvent would demand the use of a disproportionately high mass flow rate of CO_2, undermining one of the benefits of using supercritical extraction (Bockrath).

In the process flow diagram (Fig. 12.3) CO_2 and EtOH are shown to re-enter the process from their respective recycle streams S213 and S17, however, liquid CO_2 and EtOH may be initially fed from commercial cylinders at process start-up. In order for the CO_2 to remain liquid throughout the process of pumping to supercritical pressures, the pump inlet stream S213 must be cooled to -2 °C, through heat exchange with ethylene glycol refrigerant (60% Ethylene glycol, 40% water, by mass) in stream S230. The resulting stream 214, liquid CO_2 at 6.5 MPa and -2 °C, is then compressed to 30 MPa using a high pressure positive displacement pump. S215 is then heated to 60 °C through heat exchange with 150 psig steam, such that stream 216 is at optimal supercritical extraction conditions.The refrigerant stream S230 used to cool the CO_2 experiences a pressure drop of 0.0513 bar within X-201. Coolant pump P-203 is used to increase the pressure of the coolant to 1.013 bar and recirculate it back to the refrigerator H-203, which cools the diluted ethylene glycol back to -40 °C. Liquid EtOH co-solvent in S217 is compressed from 0.1 to 30 MPa, then heated to 60 °C and mixed with S216.

Batch extractions will be performed using three extraction vessels, with at most two vessels being used for extraction at a given time. Assuming that our overall downstream Astaxanthin recovery is 92% (5% loss in spray drying and 3% loss in extraction), the required processing rate of the pellet stream S212 is 358 kg / hr. An extraction time of 111 min was derived from extraction conditions described in Machmudah, et. al (29). Assuming one must reserve an additional 30 minutes to load pellets, 15 minutes for leak checks, and after extraction; 15 minutes to depressurize and 30 minutes to remove the pellets from the vessel, the total residence time for each extraction unit is 201 minutes (Bockrath). The scheduling of extraction vessel operation pictured in Fig. 13.8 achieves the desired product stream processing rate while enabling solvent to semi-continuously be fed into extraction vessels. The average and peak flow of solvent S220 are 6275 kg/hr and 7776 kg/hr, respectively.

Fig. 13.8.1 Schedule for Operation of 3 Batch Extraction Vessels



The supercritical fluid exiting the extraction vessel (S221) must be brought to supercritical temperature and pressure in order to separate the CO_2 from the Astaxanthin and ethanol. Typically, a pressure reduction valve is used to expand the stream and a cyclone is used to remove the particulates from gas through vortex separation. However, this process design is complicated by the likely event that fine particles of product are generated upon rapid expansion of the supercritical fluid and, rather than fully agglomerating upon impact with cyclone walls and draining with the liquid stream, are carried off with the gaseous CO_2. The specific design of this part of the process is beyond the scope of the current project, but the final design is likely to consist of two pressure reduction valves, two cyclones and a final demister (V202, V202, CY201, CY202) for highly efficient recovery of Astaxanthin from the gaseous stream.

The gaseous CO_2 stream (S223) must be condensed to a liquid state appropriate for pumping before being recycled. To perform this, CO_2 at atmospheric conditions is compressed to 6.5 MPa using a stainless steel, three-stage compressor with intercoolers MCOMPR-201 (operated with cooling water) that discharges the outlet gas at 120 °C. The gaseous CO_2 (S224) is then cooled to 22 °C and condensed using chilled water at 40 °F (~ 4 °C). The resulting liquid CO_2 (S225) is ready to be recycled back into the process. Ethanol is separated from the Astaxanthin extract using a wiped-film evaporator (EV-201) which was chosen for handling heat-sensitive products. The extract stream S229 leaving the evaporator contains an ethanol concentration of less than 1 ppm (mass), and is in accordance with FDA guidelines for good manufacturing practice for producing pharmaceuticals.

13.9 Product Storage

The astaxanthin product recovered from supercritical fluid extraction will be in a solid powder form. Astaxanthin is commonly stored at freezing temperatures to prevent degradation. At -20 □, solid astaxanthin is stable for up to two years. For product storage, cooled storage tanks will be used to house up to a weeks’ worth of product.

14. Energy Balance and Utility Requirements

14.1 Storage Tanks

The glucose, ammonia, phosphoric acid, and media storage tanks all require heating or cooling requirements, as shown below. For each of these calculations, it was assumed that each tank, modeled as a cylinder, has an aspect ratio of 3, and a heat transfer coefficient of 0.3 W/m^2K, due to insulation. Because of their size and temperature distance from room temperature, the glucose and media storage tanks have sizable utility requirements.

Material	Volume of tank (m^3)	Temperature (ºC)	Annual Duty (kWhr)
Glucose	110	55	13,133
Ammonia	17	–33	5,714
Phosphoric acid	4.5	37	757
Media	50	80	13,289

Corresponding utilities costs are tabulated in Section 19.

14.2 Fermenters

The heat generated in a single fermenter at each stage is summarized in Table 14.2 below. Heat generation was calculated using the Cooney constant, described in Section 11.

Fermenter Stage	Oxygen feed rate (kg/hr)	Heat generated (Btu/hr)	Heat duty of associated HX (Btu/hr)
Pre-seed	4.3	66,078	N/A
Seed	43	660,780	653,207
Production (growth)	430	6,607,797	6,631,220
Production (production)	86.2	1,324,633	1,473,650

14.3 Downstream Process Energy Usage

Equipment	Net Work / Heat Duty (kW)	Annual Duty (kW-h)
Spray Dryer	1628	12893760
Pre-heater	4624	36622080
CO2 Pump	59.43	470686
EtOH Pump	2.68	21226
CO2 Cooler #1	140.86	1115611
CO2 Cooler #2	43.61	345391
Etoh Cooler	14.37	113840
CO2 Heater	413.6	3275712
EtOH Heater	6.045	47876
3-Stage Compressor	1558.0	12339360
Coolant Pump	0.0081	64
Wiped Film Evaporator	102.50	811800
Centrifuge	55	435600
Homogenizer	11	87120
Pelletizer	7.5	59400
Product Cooling Tank	0.1	792

14.4 Downstream Electricity Use

Equipment	Electricity Use kW	Annual Electricity Use (kW-h)
Spray Dryer	1628	12894000
CO2 Pump	59.43	470700
EtOH Pump	2.68	21230
3-Stage Compressor	735.3	5824000
Wiped Film Evaporator	102.5	811900
Centrifuge	55	435600
Homogenizer	11	87120
Pelletizer	7.5	59400
Product Cooling Tank	0.1	792

14.5 Downstream, Other Utilities

Equipment	Utility	Utility / h	Utility / yr
Pre-heater	450 psig steam (kg)	6200	49104000
CO2 Heater	150 psig steam (kg)	600	4752000
EtOH Heater	50 psig steam (kg)	15	118800
CO2 Cooler #2	Chilled Water, 45 F (kg)	300	2376000
EtOH Cooler	Cooling Water, 90 F (kg)	670	5306400
Compressor Intercoolers	Cooling Water, 90 F (kg)	42450	336210000

15. Equipment List and Unit Descriptions

15.1 Air Compressor

The air compressor sources air from the plant's external environment to provide oxygen during cell growth and astaxanthin production. It is a rotary screw air compressor. Sized in order to accommodate more than 1.5 times the maximum aeration rate required during a production batch, the compressor can handle 5,000 m^3/hr of air input. The compressor pressurizes the air inlet stream to 210 kPa, or approximately 2 atm, which is the hydrostatic pressure at the bottom of the production fermenter. Assuming an approximate inlet temperature of about 10ºC, the outlet temperature due to compression is around 105ºC. *Perry's Handbook* describes this heating as an advantage in sterilization, so long as it is accompanied by filtration. The compressor weighs approximately 10,000 pounds, and has a bare-module cost of approximately $907,000. The compressor requires 241 kW of electricity to operate. Expectedly, this compressor is one of the more expensive pieces of the plant design.

15.2 HEPA Air Filter

To further sterilize the incoming air stream, a Puracel HEPA High-Temperature Stainless Steel Frame Box Filter will be purchased from manufacturer Air Filters Incorporated. Under United States regulations, the filter is rated as 99.97% efficient for particles greater than 0.3 microns in size. The filters can withstand temperatures up to 400ºF, and since it is being subjected to air at only 221ºF, this model should be more than sufficient. The dimensions of the filter are 24 in. x 24 in. x 12 in. The pressure drop across the air filter is considered to be negligible.

15.3 Air Inlet Heat Exchanger

A shell-and-tube heat exchanger is required to cool inlet air to 37ºC, the reactor temperature during growth. Cooling water at 25ºC will be flown at 100 gal/min through the tubes of the heat exchanger. There are four tube passes and one shell pass. The exchanger material is stainless steel 304. It was emphasized during the design that the pressure drop should be minimized, since the inlet air stream has already been pressurized by a compressor. This was accomplished, as the outlet air pressure of 1.953 atm, is only marginally reduced from 2 atm out of the compressor. The outer diameter of the tube is 3 in, whereas for the shell it is 33 in. There are 48 tubes and 8 baffles. This heat exchanger has a purchase cost of $55,724.

15.4 Glucose Storage Tank

A week's worth of Clearsweet 99% Refined Liquid Dextrose Corn Syrup, the glucose source, will be stored at 130ºF (55ºC) in a carbon steel, cone roof storage tank of 110 m^3 (~29,000 gal) in volume. Approximately 114,000 kg/week are discharged to the various fermenters, as about 10 full seed train runs are completed in this time frame. A storage tank of this size and shape has a purchase cost of $51,630.

15.5 Ammonia Storage Tank

Ammonia is by convention stored as a liquid at –33ºC, just below its boiling point, and atmospheric pressure. Just as with the storage tank for glucose, a week's worth of anhydrous ammonia is stored in a carbon steel, cone roof storage tank. With a density of 682 kg/m^3 at this temperature and pressure, the storage tank has a much smaller volume than its glucose counterpart, only needing 17,000 L (3,566 gal). The tank has a purchase cost of $19,811.

15.6 Ammonia Compressor

A compressor is needed to pressurize the ammonia feed prior to entering the fermentation vessels. At peak production, 330 kg/hr of ammonia must be compressed from 1atm to 2 atm, the same pressure as the bottom of the fermentation tanks. A positive displacement compressor with a 70% mechanical efficiency is adequate for this process. This compressor is estimated to cost $1,439,855. The yearly utility cost associated with the ammonia compressor is $10,210/yr.

15.7 Phosphoric Acid Storage Tank

85% phosphoric acid is stored at 37ºC (for ease of fermenter entry) and atmospheric pressure. A week's worth is stored at a time. An examination of the Safety Data Sheets for phosphoric acid dictates that storage should be confined to stainless steel 316 to cope with its potency. With a specific gravity of 1.680, a 4,500 L stainless steel 316 cone roof storage tank should be suitable. Accordingly, the purchase cost for such a tank is $21,050, assuming a materials factor of 2.1.

15.8 Media Preparation/Storage Tank

A storage tank to house prepared mineral media holds, again, a week's worth of the material. A stainless steel 304, cone roof storage tank of 50,000 L in volume is required to accomplish this. Each salt in the media recipe is purchased in solid form and dissolved in 40,000 L of water. (Thus, the tank is 80% empty.) The necessary volume was calculated according to the volume needed to completely dissolve the lowest solubility component of the media, ammonium sulfate. All other salts should have little difficulty dissolving completely; their concentrations in the storage tank are greatly smaller than saturation. The concentrations of salt required for the process were calculated on a production fermenter basis. In actuality, the media allotted for one run through the seed train will be allocated such that 5 percent is sent to the pre-seed fermenter, 45 percent to the seed fermenter, and 50 percent to the production fermenter. The media will be stored at 80ºC to ensure sterility. In total, the purchase cost for this tank is $58,574, assuming a materials factor of 1.7.

15.9 Pre-seed Fermenter (R-101)

The pre-seed fermenter, made from stainless steel 304, has a total volume of 2,250 L. Industrial fermenters typically have aspect ratios of 3:1; accordingly, the diameter of this fermenter is 0.98 m (3.23 feet), and the height is 2.95 m (9.69 feet). It is 80 percent full, making the working volume 1,800 L. The fluid level is therefore 2.36 m (7.74 feet). The batch time is 29 hours, and the final biomass concentration is 1 g/L. The aeration rate is, on average, 0.15 vvm. To aid with aeration and resolve any clumping issues, an agitator is necessary. A stainless steel Rushton impeller will be purchased. Its diameter will be half that of the fermenter; the impeller diameter is therefore 0.49 m. For each of the fermenters in this design, Seider et al. was used to estimate power requirements for the agitators. Since the *E. coli* growth is exothermic, it was assumed that a “reaction with heat transfer” is the closest approximation for a fermenter, and a power requirement of 3 hp / 1,000 gallons was assumed. Accordingly, the pre-seed agitator has a power requirement of 1.43 hp, or 1066 W. Finally, one must combat the aforementioned heat generation during microbial growth to maintain a constant temperature of 37ºC. A cooling jacket of 3.27 feet in diameter, fed with 200 gal/min of cooling water at room temperature, will satisfactorily cool the fermenter. This will be the only instance where a cooling jacket—the easy way out—can be successfully employed in the upstream process.

There will be three pre-seed fermenters in total. When a batch is completed, it will be transferred to either available seed fermenter. Ten hours of downtime is anticipated to transfer media and sterilize the fermenter.

15.10 Seed fermenter (R-102)

By industry convention, the seed fermenter, again made from stainless steel 304, is 10 times the size of its predecessor vessel. It has a total volume of 22,500 L, with a working volume of 18,000 L. Its diameter is 2.12 m, and its height 6.36 m; like the pre-seed fermenter, it has an aspect ratio of 3:1. The height of the fluid is 5.09 m. The batch time is 19 hours, achieving a final biomass concentration of 6 g/L.

Aeration during the growth phase continues as 0.15 vvm. Since this vessel is 10 times larger than the pre-seed, it will therefore require 10 times the air flow rate, demanding greater agitation and necessitating tenfold cooling, since heat generation is estimated using the Cooney constant. A larger Rushton impeller is purchased, with diameter 1.06 m and utilities of 14.3 hp, or 10.66 kW. Constructing a simple cooling jacket was attempted for this fermenter, but at peak aeration, the heat generation exceeds the capabilities of a cooling jacket. Accordingly, an external heat exchanger was designed. This heat exchanger will withdraw 450 L/min from the fermenter, cool it to 32ºC, and return it to the fermenter. The heat generated by the cell growth, combined with the small fraction of total broth that is withdrawn per minute, should ensure that the temperature in the reactor still remains constant at 37ºC.

There is a need for only two seed fermenters. When a batch is completed, the media will be transferred to one of three production fermenters, whichever is available. (There is guaranteed to be one available.) Ten hours of downtime is also anticipated for the seed fermenter before another batch is initiated.

15.11 Seed Heat Exchanger

The seed heat exchanger continuously withdraws at a rate of 450 L/min the contents of the seed fermenter, cools them to 30.7ºC, and returns them to the fermenter. On the advice of consultants, it is made of stainless steel 304, the same material as the seed fermenter. The seed broth flows through the tube side of the heat exchanger, which has outer diameter 1 inch and length 106.3 in. There are 88 tubes in triangular pitch, and a single tube pass. Cooling water flows through the shell side at 500 gpm. The shell side has inner diameter 12.44 inches and outer diameter 12.75 inches. There are 4 baffles. The heat duty is 653,207 Btu/hr, with an average heat transfer coefficient of 236.7 Btu/hr-ft^2-ºF. The purchase cost is $17,014. *Note that, for this and all other fermentation heat exchangers, the exchangers were designed using properties of water for the fermentation media.*

15.12 Production fermenter (R-103)

The production fermenter, the Broadway stage of the upstream design, is a 225,000 L behemoth with 180,000 L working volume. It is also made of stainless steel 304. Assuming an aspect ratio of 3:1, the diameter of the production fermenter is 4.57 m (15 feet), the height is 13.71 m (45 ft), and, at 80% full, the fluid height is about 11 m.

The production fermenter begins in its growth phase, which lasts 16.5 hours. The final biomass concentration at the end of this phase is 24 g/L. Aeration continues at 0.15 vvm, as it has in the previous stages of the seed train. The impeller has a diameter of 7.5 feet, and a power requirement of 143 hp, or 106.6 kW. Its speed is 125 RPM. Like the seed fermenter, cooling cannot be accomplished by a cooling jacket, so an external heat exchanger is set up to withdraw fluid at a rate of 4500 L/min, cool to 32ºC, and return to the fermenter. This should keep a constant optimal growth temperature of 37ºC. In summary, the growth phase of the production fermenter has nearly identical attributes as its predecessors, merely multiplied by the appropriate factor.

At the conclusion of the 16.5 hour growth phase, the production fermenter enters its production phase, during which the *E. coli* begin synthesizing astaxanthin in appreciable quantities. Feeds of phosphoric acid and ammonia, which were previously available during the growth phase, are halted. Aeration is reduced to only 0.03 vvm, and glucose is fed in significantly reduced quantities. The aim is to provide merely life-sustaining quantities of raw materials, to encourage anabolism of astaxanthin, at the expense of reproduction. Reproduction is, however, unavoidable, and the final biomass concentration is estimated at 30 g/L. The final titer of astaxanthin within the cells is desired to be 0.75 g/L. The production phase lasts 26.5 hours.

During the production phase, the temperature is reduced to 30ºC, below the optimal growth temperature of 37ºC. A heat exchanger, used a single time, is constructed to perform this cooling, and is described below. An additional heat exchanger continuously maintains the temperature at 30ºC, against the heat generated during production. It does so by withdrawing at the same rate—4500 L/min—and cooling to 27ºC, at which point the fluid is returned to the fermenter.

When the production batch has concluded, the contents are transferred to the downstream surge tank. The production fermenter, even with its much larger size, is still estimated to have a downtime of 10 hours for media transfer and sterilization. There are three production fermenters, and the plant has been designed so that a production batch is produced every 17.67 hours, amounting to about 10 completed seed train runs per week.

15.13 Production (Growth Phase) Heat Exchanger

The production heat exchanger for the growth phase continuously withdraws at a rate of 4500 L/min the contents of the production fermenter, cools them to 30.65ºC, and returns them to the fermenter. On the advice of consultants, it is made of stainless steel 304, the same material as the production fermenter. The contents of the fermenter flow through the tube side of the exchanger. The outer diameter of the tube side is 1 inch, and the tube length is 236 inches. There are 1236 tubes but 1 tube pass. Cooling water at 25ºC flows through the shell side. The shell side has inner diameter 48.23 inches and outer diameter 48.86 inches. There are 6 baffles. The heat duty is 6.63×10^6 Btu/hr, comparable to the heat generated in the production phase during this stage and phase, with an average heat transfer coefficient of 267 Btu/hr-ft^2-ºF. The purchase cost is $157,306.

15.14 Switch-to-Production Heat Exchanger

The growth batch lasts 16.5 hours, and the production batch lasts 26.5 hours; over the course of the single hour during which the transition occurs (the 0.5 hours associated with each stage), the production media will be withdrawn at a rate of 6,000 L/min and cooled to 30ºC via an external heat exchanger. (The flow rate of the production media allows each fluent element to be passed through the heat exchanger twice.) Cooling water at 20ºC will be flown at 1000 gpm.

This heat exchanger has only this single mandate, and is in use for a single hour per batch. Since the staggering of batch schedules ensures that no two production batches are switched from growth to production in the same hour, only one of these heat exchangers is needed for the entire plant. The production media will be sent through the tube side of the exchanger. The outer diameter of the tube side is 1 inch. The tube length is 236 inches, the number of tubes is 706, and the tubes are in triangular pitch. There is a single tube pass and a single shell pass. The shell outer diameter is approximately 38 inches. There are 8 baffles. The material is stainless steel 304. The average heat transfer coefficient is 194 Btu/hr-ft^2-ºF, and its duty is 9.8 x 10^6 Btu/hr. The purchase cost of the heat exchanger is $96,542.

15.15 Production (Production Phase) Heat Exchanger

This heat exchanger serves the same purpose as the production (growth phase) heat exchanger—withdraw production media, cool it, and recirculate it back into the reactor. Since the heat generation during the production phase is significantly reduced from that of the growth phase, the temperature difference between the reactor temperature, 30ºC, and the temperature at which the fluid re-enters the fermenter, 28.4ºC, need not be as large as in its neighbor, the growth phase heat exchanger. Fluid is withdrawn at a rate of 4,000 L/min, and cooling water at 15ºC is flown at 1000 gpm. The production media is sent through the tube side, which has 1 inch outer diameter, triangular pitch spacing, a length of 59 inches, and 129 tubes in total. There is one tube pass and one shell pass. The outer diameter of the shell is 18.11 inches. There are 2 baffles. The heat exchanger material is stainless steel 304, the same as the fermenter. Its duty is 1.47 x 10^6 Btu/hr, and the average heat transfer coefficient is 388 Btu/hr-sqft-F. The purchase cost is $23,493.

15.16 Spray Dryer

The spray dryer reduces the moisture content of the biomass slurry from 80% to 5% by atomizing the feed and contacting it with hot air. Inlet and outlet drying gas temperatures of 220 and 80 °C were chosen to avoid degradation of our product. The dryer will feature an open cycle of air, in which ambient air is preheated to the inlet temperature of 220 °C through heat exchange with steam at 450 psig, and discharged to the atmosphere after filtration. A co-current air-product flow will be utilized to ensure that, at the bottom of the chamber, the dry particles containing our thermolabile product are exposed to the air stream at its lowest temperature.

Calculations for the mass flow rate of air required are found in the Appendix, following methods described in Perry's Handbook for Chemical Engineers (30). The mass flow rate of air required was calculated to be 83,200 kg air / h (translating to 40,000 cfm of air). A quick scoping estimate of the spray dryer size can be made on the basis of this air flow rate assuming a 25 second residence time: we envision a cylindrical chamber with an equal height and diameter of 7.34 m, with a 60° conical bottom. A dryer with these design specifications, including associated particle collection and air filtration devices, can be custom ordered from GEA Niro (31). The heat duty of the preheater is 4,780 kW. Based on the evaporative load, required air flow rate, and cost evaluation method presented in Seider, et. al (2017), using stainless steel 304 as the primary equipment material wherever relevant, the estimated total cost of the dryer, cyclone and wet bag filter is $1,986,413.

The air preheater was designed as a shell-and-tube heat exchanger in Aspen, assuming material of construction to be stainless steel 304. In reality a finned-tube steam heater would be ideal.

15.17 Disc Stack Centrifuge

The disc stack centrifuge concentrates the dry cell weight in the broth to 20 wt%. The inlet to the centrifuge flows at a rate of 10,189 L/hr. There are two outlets, a centrate stream and a cell paste stream. The centrate consists of cell media and flows out at a rate of 8,962 L/hr. The centrate must be treated before disposal. The cell paste coming from the centrifuge flows at a rate of 1,226 L/hr and contains 0.25 kg/L of biomass. This cell paste continues through to the homogenization step. Crown Machinery manufactures a high capacity centrifuge that could handle these flow rates (32). The appropriate centrifuge would have a running load of 55 kW, operate at 4500 RPM and is estimated to cost around $163,000 (18).

15.18 Centrate Disposal

The 8,926 L/hr of centrate being removed from the fermentation product by the centrifuge must be treated before disposal. The centrate must be disposed of according to proper cell media disposal methods. The disposal system consists of two 23,000 L tanks, each of which will handle two hours of flow while being treated with 180 L of bleach. The capacity of each accounts for 20% empty space in the tanks. Cone-roofed tanks are preferred for their ability to adapt to pressure and temperature changes. Stainless steel 304 is the preferred material to avoid corrosion. The purchase cost for these two tanks is estimated at $146,838 (18).

15.19 Bleach Storage Tank

The bleach required for centrate disposal can be stored on a weekly basis. With 15,057 L of bleach required weekly, a cone-roofed storage vessel with a 19,000 L capacity is preferable. Stainless steel housing material is necessary, as sodium hypochlorite, the active ingredient in bleach will degrade a carbon-steel vessel (33). Using costing methods outlined in Seider et al. amounts to approximately $67,000 for this vessel (18).

15.20 Homogenizer

The homogenizer shears the *E. coli* cells to release the astaxanthin they contain. The feed to the homogenizer is the cell paste leaving the centrifuge. The machine has one input and one output, both flowing at 1,226 L/hr. GEA Niro's One11TS homogenizer is rated at max flow rates between 1,300 and 3,300 L/hr. That model requires 11 kW electrical utility to run and has a purchase cost of approximately $87,652 (20).

15.21 Diatomaceous Earth Storage Tank

The grinding agent for the pelletization step is diatomaceous earth. This material will be stored in a cone-roofed stainless steel 304 tank. It will be purchased on a biweekly basis, therefore requiring the storage tank hold up to 5,253 L of diatomaceous earth. A vessel 80% full at max is preferred, so for diatomaceous earth storage a 6,600 L tank is necessary.

15.22 Fructose Storage Tank

The binding agent for the pelletization step is fructose. The material will be purchased on a biweekly basis, with up to 3,575 L needing to be stored. A vessel 80% full at max is preferred, as is a cone-roofed stainless steel 304 tank type. The vessel must be 4,500 L total.

15.23 Twin Dome Granulator

The spray drying output is fluffy and must be condensed through pelletization. A twin dome granulator combines the grinding and binding step of pelletization into one process.

Biomass that is 5 wt% water exits the spray dryer at 306 kg/hr. The desired pellet consists of 5 wt% binding agent (fructose), 10 wt% grinding agent (diatomaceous earth), and less than 5 wt% water, with the rest being biomass. To satisfy this composition 36 kg of diatomaceous earth and 18 kg of fructose are added to the twin dome granulator by hour. The process consists of a screw extruder leading to a dome die. There is enough water initially present to absorb the fructose. The final pellet consists of 4.25 wt% water, and therefore a drying step can be omitted. LCI produces a twin dome extruder with an energy requirement of 7.5 kW (34). Using costing methods from Seider et al. (2017) and approximating the equipment as a screw extruder and pellet mill, the total bare module granulator cost can be estimated at approximately $452,500.

15.24 Positive Displacement Pump

P-201 is a high-pressure positive displacement (plunger) pump that compresses liquid CO_2 from 6.5 MPa to 30 MPa for use in supercritical fluid extraction. Such a pump was modeled with the aid of Aspen. The material of construction should be Stainless Steel 316 for its ability to withstand corrosion and cavitation erosion. Based on the costing guidelines presented in Seider et. al for a 59.43 brake horsepower reciprocating pump, assuming an efficiency of 0.72, the purchase cost of P-201 is $69,567 (18).

15.25 Ethanol Pump

P-202 is a centrifugal pump that compresses liquid EtOH from 6.5 MPa to 30 MPa for use in supercritical fluid extraction. The material of construction is stainless steel for its ability to withstand corrosion and cavitation erosion. Based on the costing guidelines presented in Seider et. al for a reciprocating pump with a break horsepower requirement of 2.68 kW, assuming an efficiency of 0.72, the purchase cost of the pump is $9575.

15.26 MCOMPR-201

MCOMPR-201 is a 3-stage compressor with intercoolers. It functions to compress the CO_2 gas recovered from the supercritical fluid extraction unit from 0.1 MPa to 6.5 MPa, such that this stream may later be cooled to the liquid phase, and brought back to supercritical temperature and pressure for another extraction. It was modeled as a 3-stage isentropic compressor in Aspen, using the ASME method, with an isentropic efficiency of 0.72. The material of construction is stainless steel 304. Assuming cooling water enters the intercoolers at 40°F and exits each stage at 120°F, the total amount of cooling water required is 42451 kg / hr, such that the final CO_2 outlet stream exits at 48.8 °C. The power consumption of the compressor is 735.3 kW, and the total heat duty of the intercoolers is -822.7 kW. The purchase cost of the entire compressor / intercooling unit, generated through Aspen's costing method, is \$1,692,900.

15.27 X-202, CO_2 Heater

X-202 is a stainless steel 304 heat exchanger which supports the supercritical fluid extraction process. It functions to heat the CO_2 (entering at 30 MPa) to 60°C, the temperature at which SFE takes place. The heat exchanger was modeled in Aspen, and has a heat duty of 1367 kW. 600 kg / hr of 150 psig steam will be used to heat the CO_2 stream, utilizing an actual heat exchange area of 20.3 sqft. The purchase cost, as generated by Aspen, is \$12,900. The bare module cost of the heater is \$40,893.

15.28 X-203, EtOH Heater

X-203 is a stainless steel 304 heat exchanger which supports the supercritical fluid extraction process. It functions to heat the EtOH (entering at 30 MPa) to 60°C, the temperature at which

SFE takes place. The heat exchanger was modeled in Aspen, and has a heat duty of 6.04 kW. 15 kg/hr of 50 psig steam will be used to heat the EtOH, utilizing an actual heat exchange area of 19 sqft. The installed cost of the heat exchanger, as tabulated by Aspen, is $11,000. The bare module cost is $34,870.

15.29 X-201, CO_2 Cooler #1

X-201 is a stainless steel (304) heat exchanger which supports the supercritical fluid extraction process. Its function is to cool the positive displacement pump inlet CO2 stream from 22 to -2 °C such that the stream does not rapidly expand and drop in density (and flow rate) as it is heated within the pump. The heat exchanger was modeled in Aspen, and has a heat duty of 140.86 kW. 4,000 kg/hr of 60% ethylene glycol, 40% water solution will be used to cool the CO_2 stream, utilizing an actual heat exchange area of 58.68 sqft. The purchase cost of the heat exchanger, as tabulated by Aspen, is $11100. The bare module cost is $35187.

15.30 X-205 EtOH Cooler

X-205 is a stainless steel (304) heat exchanger which supports the supercritical fluid extraction process. It functions to cool the liquid EtOH stream to 35°C so that it may be pumped to supercritical fluid extraction conditions of 30 MPa. The heat exchanger was modeled in Aspen, and has a heat duty of 14.4 kW. 670 kg / hr of 150 psig steam will be used to cool the EtOH stream, utilizing an actual heat exchange area of 34.7 sqft. The purchase cost, as tabulated by Aspen, is $11,800.00. The bare module cost is $37406.

15.31 X-204 CO_2 Cooler #2

X-204 is a stainless steel (304) heat exchanger which supports the supercritical fluid extraction process. It functions to cool CO_2 at 6.5 MPa to 22°C, where it exists as a liquid that can later be pumped. It has a heat duty of 43.71 kW, a purchase cost of $26,200, and a bare module cost of $83054.

15.32 Depressurizing Equipment (V-201, V-202, CY-201, CY-202)

The depressurization and gas/liquid separation equipment is likely to consist of two pressure reduction valves and two cyclones, the last cyclone equipped with a demister on its exit line for more efficient recovery of the Ethanol / Astaxanthin stream. Its cost will be estimated as 10 times the cost of a single cyclone for a total cost of $16800. The cost of a single cyclone was calculated based on methods presented in Seider, et. al. Vendors that sell cyclone-demister units include Bollfilter Protection Systems.

15.33 Extraction Vessel EXT-201

The extraction vessel was costed as a vertical pressure vessel made of Stainless Steel 304. To account for the vessel being used in a high-pressure system, the thickness of the walls was calculated to be 4.74 in. based on a design pressure of 4786 psig, which is 1.1x the operating pressure. To meet the desired stream processing rate, each extraction vessel must have a capacity of 360 kg of pellets. Assuming loose-bulk pellets with a density of 28.9 lb / ft^3, the volumetric capacity of 27.4 ft^3 suggests the use of a vessel 2 ft in diameter and 7 ft in height (accounting for 25% additional space above and below the feed bed).

15.34 Wiped-Film Evaporator EVAP-201

This was modeled in Aspen as a heater and flash vessel, and its cost was based on the area of heat transfer extracted from the flash vessel model. The heat duty is 102.5 kW. The area of heat transfer is 12.980 ft^2 and the bare module cost, based on methods described in Seider, et. al (2017) for a falling film evaporator, is $91,010.

15.35 Refrigerator H-201

The refrigerator is a cooling unit that supports the supercritical fluid extraction process. It functions to cool the 60% ethylene glycol coolant to -40 °C such that it can be recirculated to cool the pump inlet liquid CO_2 stream. The unit has a heat duty of -480,660 Btu/hr, equating to a refrigeration duty of 40.06 tons/hr. The bare module cost of this unit, assumed to be constructed of stainless steel 304 is $57,694.

15.36 Coolant Pump P-203

The coolant stream experiences a reduction in pressure of 0.0513 bar as it is passed through X-201. Coolant pump P-203 returns the pressure of this stream to atmospheric conditions and recirculates the coolant to the refrigerator. With a very small power consumption of 0.01 kW, the pump was costed as a centrifugal pump made of stainless steel 304, and has a bare module cost of $11,266.

15.37 Product Storage Tank

The astaxanthin product is in a solid powder form following the extraction process. It must be stored at -20℃ to prevent degradation. A week's worth of product may be stored in a

1,500 L cooled cone-roofed storage tank made of stainless steel 304. The power consumption to cool this tank is 0.01 kW and the tank itself has a bare module cost of $45,982.

16. Specification Sheets

SPECIFICATION SHEET Pre-Seed Fermenter				
Identification	Item ID: R-101		No. Req'd:	3
Function: Grow biomass to final concentration of 1 g/L.				
Operation: Batch, 29 hours, 10 hours turnaround				
Materials	In		Out	
Oxygen	4.3 kg/hr		3.6 kg/hr	
Nitrogen	14 kg/hr		14 kg/hr	
Glucose	155 g/hr			
Ammonia	13.2 g/hr			
Phosphoric acid	7.3 g/hr			
Water			61 g/hr	
Carbon dioxide			85 g/hr	
Design Data	Temperature: 37ºC			
	Pressure: 1 atm			
	Diameter: 0.98 m (3.23 feet)			
	Height: 2.95 m (9.69 feet); fluid height: 2.36 m (7.74 feet)			
	Volume: 2,250 L (1,800 L working volume)			
	Material: Stainless steel 304			
	Agitator: Stainless steel Rushton impeller, 0.49 m diameter			
	Agitator speed: 50 RPM			
	Cooling jacket diameter: 0.997 m (3.27 feet)			
Utilities	200 gpm cooling water (25ºC)			
	1066 W (1.43 hp) electricity for agitator			

Comments & Drawings: N/A

SPECIFICATION SHEET
Seed Fermenter

Identification	Item ID: R-102		No. Req'd:	2
Function: Grow biomass to final concentration of 6 g/L.				
Operation: Batch, 19 hours, 10 hours turnaround				

Materials	In	Out
Oxygen	43 kg/hr	36 kg/hr
Nitrogen	140 kg/hr	140 kg/hr
Glucose	14 kg/hr	
Ammonia	1.2 kg/hr	
Phosphoric acid	0.66 kg/hr	
Water		5.5 kg/hr
Carbon dioxide		7.7 kg/hr

Design Data	Temperature: 37ºC
	Pressure: 1 atm
	Diameter: 2.12 m (6.96 feet)
	Height: 6.36 m (20.87 feet); fluid height: 5.09 m (16.7 feet)
	Volume: 22,500 L (18,000 L working volume)
	Material: Stainless steel 304
	Agitator: Stainless steel Rushton impeller, 1.06 m diameter
	Agitator speed: 75 RPM
	Aeration rate: 0.15 VVM
Utilities	10.66 kW (14.3 hp) electricity for agitator

Comments & Drawings:

SPECIFICATION SHEET
Production Fermenter

Identification	Item ID: R-103		No. Req’d:	3
Function: Grow biomass to concentration of 24 g/L, at which time production of astaxanthin is initiated. Biomass has final concentration of 30 g/L.				
Operation: Batch, 16.5 hours growth, 26.5 hours production, 10 hours turnaround				
Materials	In growth	In production	Out growth	Out production
Oxygen	430 kg/hr	86.2 kg/hr	365.5 kg/hr	73.3 kg/hr
Nitrogen	1400 kg/hr	282 kg/hr	1400 kg/hr	282 kg/hr
Glucose	638 kg/hr	21 kg/hr		
Ammonia	54 kg/hr			
Phosphoric acid	30 kg/hr			
Water			250 kg/hr	8.3 kg/hr
Carbon dioxide			350 kg/hr	15 kg/hr
Astaxanthin				135 kg
Design Data	Temperature: 37ºC for growth, 30ºC for production			
	Pressure: 1 atm			
	Diameter: 4.57 m (15 feet)			
	Height: 13.71 m (45 feet); fluid height: 11 m (36 feet)			
	Volume: 225,000 L (180,000 L working volume)			
	Material: Stainless steel 304			
	Agitator: Stainless steel Rushton impeller, 2.286 m (7.5 feet)			
	Agitator speed: 125 RPM			
	Aeration rate: 0.15 VVM during growth, 0.03 VVM during production			
Utilities	106.6 kW (143 hp) electricity for agitator			
Comments & Drawings:				

SPECIFICATION SHEET
Seed Heat Exchanger

Identification	Item ID:		No. Req'd:	2
Function: To cool the contents of the seed fermenter as cells generate heat during growth.				
Operation: Continuous				
Materials	Inlet Temp	Inlet Pressure	Outlet Temp	Outlet Pressure
Cooling water	25ºC	1 atm	26.5ºC	1 atm
Seed media (tube)	37ºC	1 atm	30.7ºC	1 atm
Design Data	Flow rate of seed media: 450 L/min			
	Material: Stainless steel 304			
	Tube OD: 1 inch			
	Tube length: 106.3 inches, triangular pitch			
	No. Tubes: 88			
	Shell OD: 12.75 inches			
	Baffles: 4			
	Heat duty: 653,207 Btu/hr			
	Average heat transfer coefficient: 236.7 Btu/hr-ft^2-ºF			
Utilities	Cooling water (25ºC) at 500 gal/min			

Comments & Drawings: Purchase cost $17,014. Seed media modeled as water.

SPECIFICATION SHEET Production (Growth) Heat Exchanger				
Identification	Item ID:		No. Req'd:	3
Function: To cool the contents of the production fermenter during the growth phase.				
Operation: Continuous				
Materials	Inlet Temp	Inlet Pressure	Outlet Temp	Outlet Pressure
Cooling water	25ºC	1 atm	32.5ºC	1 atm
Seed media (tube)	37ºC	1 atm	30.65ºC	1 atm
Design Data	Flow rate of seed media: 4500 L/min			
	Material: Stainless steel 304			
	Tube OD: 1 inch			
	Tube length: 236 inches, triangular pitch			
	No. Tubes: 1236			
	Shell OD: 48.86 inches			
	Baffles: 6			
	Heat duty: 6.63 x 10^6 Btu/hr			
	Average heat transfer coefficient: 267 Btu/hr-ft^2-ºF			
Utilities	Cooling water (25ºC) at 1000 gal/min			

Comments & Drawings: Purchase cost $157,306. Production media modeled as water.

SPECIFICATION SHEET Production (Production) Heat Exchanger				
Identification	Item ID:		No. Req'd:	3
Function: To cool the contents of the production fermenter during the production phase.				
Operation: Continuous				
Materials	Inlet Temp	Inlet Pressure	Outlet Temp	Outlet Pressure
Cooling water	15ºC	1 atm	16.7ºC	1 atm
Seed media (tube)	30ºC	1 atm	28.4ºC	1 atm
Design Data	Flow rate of seed media: 4000 L/min			
	Material: Stainless steel 304			
	Tube OD: 1 inch			
	Tube length: 59 inches, triangular pitch			
	No. Tubes: 129			
	Shell OD: 18.11 inches			
	Baffles: 2			
	Heat duty: 1.47 x 10^6 Btu/hr			
	Average heat transfer coefficient: 388 Btu/hr-ft^2-ºF			
Utilities	Cooling water (25ºC) at 1000 gal/min			

Comments & Drawings: Purchase cost $23,493. Production media modeled as water.

SPECIFICATION SHEET **Switch-to-Production Heat Exchanger**				
Identification	Item ID:		No. Req'd:	1
Function: To transition the production fermenters from growth to production phase by lowering the temperature from 37ºC to 30ºC.				
Operation: Continuous, but one hour at a time.				
Materials	Inlet Temp	Inlet Pressure	Outlet Temp	Outlet Pressure
Cooling water	20ºC	1 atm	32ºC	1 atm
Seed media (tube)	37ºC	1 atm	30ºC	1 atm
Design Data	Flow rate of seed media: 6,000 L/min			
	Material: Stainless steel 304			
	Tube OD: 1 inch			
	Tube length: 236 inches, triangular pitch			
	No. Tubes: 706			
	Shell OD: 38 inches			
	Baffles: 8			
	Heat duty: 9.8 x 10^6 Btu/hr			
	Average heat transfer coefficient: 194 Btu/hr-ft^2-ºF			
Utilities	Cooling water (25ºC) at 1000 gal/min			

Comments & Drawings: Purchase cost $96,542. Production media modeled as water.

SPECIFICATION SHEET Air Compressor				
Identification	Item ID:		No. Req'd:	1
Function: To recover air from the plant's external environment and compress it to a pressure suitable for entry into the bottom of fermenters. Partially sterilizes incoming air by heating up air to 105ºC, which is in turn passed through high-temperature HEPA filters.				
Operation: Continuous				
Materials	Inlet Temp	Inlet Pressure	Outlet Temp	Outlet Pressure
Air	10ºC	1 atm	105ºC	2 atm
Design Data	Flow rate of air, at peak: 5000 m^3/hr			
	Peak conditions: 1.5 times maximum aeration of production batch			
	Type: Rotary screw air compressor			
	Material: Stainless steel 304			
	Weight: 10,000 lbs			
Utilities	241 kW of electricity			

Comments & Drawings: Bare module cost $907,000.

SPECIFICATION SHEET
Air Inlet Heat Exchanger

Identification	Item ID:		No. Req’d:	1
Function: To cool the filtered, compressed air that will be bubbled into the fermenters.				
Operation: Continuous				
Materials	Inlet Temp	Inlet Pressure	Outlet Temp	Outlet Pressure
Cooling water	25ºC	1 atm	16.7ºC	1 atm
Air (shell)	105ºC	2 atm	37ºC	1.953 atm
Design Data	Flow rate of air, at peak: 5000 m^3/hr			
	Material: Stainless steel 304			
	Tube OD: 3 inch			
	Tube length: 59 inches, triangular pitch			
	No. Tubes: 48			
	Shell OD: 33 inches			
	Baffles: 8			
Utilities	Cooling water (25ºC) at 100 gal/min			

Comments & Drawings: Purchase cost $55,724.

SPECIFICATION SHEET
Ammonia Compressor

Identification	Item ID:	COMP-102	No. Req'd:	1

Function : Increase the pressure of ammonia from 1 atm to 2 atm, prior to entering the fermenters.

Operation: continuous

Materials	Inlet	Outlet
Temperature °C	-33	29.1
Pressure (bar)	1.0133	2.0265
Max Ammonia Mass Flow (kg/hr)	330	30
Volumetric Flow Rate (L/hr)	381,837	240,281

Design Data	Mechanical efficiency: 70%
	Compressor Type: Positive displacement
Utilities	16.65 kW electricity required

Comments & Drawings: N/A

SPECIFICATION SHEET
Fermenter Product Surge Tank

Identification	Item ID:	T-201	No. Req'd:	1
Function : Hold batch fermenter product prior to continuous downstream processes				
Operation: continuous				

Materials	Inlet (S200)	Outlet (S201)
Temperature °C	30.65	25.0
Pressure (bar)	1.013	1.013
Component Mass Flow (kg/hr)	10,494	10,494
Water	10,189	10,189
Cell Mass	305.66	305.66

Design Data	Diameter: 3.2 m
	Height: 9.6 m
	Tank Type: Cone-roofed
	Material: Stainless Steel 304
	Volume: 305,660 L
	Working Volume: 244,528 L
Utilities	N/A

Comments & Drawings: N/A

SPECIFICATION SHEET
Centrifuge

Identification	Item ID:	C-201	No. Req’d:	1
Function : Remove excess fermentation broth from biomass.				
Operation: continuous				
Materials	Inlet (S201)		Outlet (S202)	Outlet (S203)
Component Mass Flow (kg/hr)	10,494		8,962	1,532
Water	10,189		8,962	1,226
Cell Mass	305.66		0	305.66
Design Data	Temperature: 25°C			
	Pressure: 1.013 bar			
	Maximum Throughput: 35,000 L/hr			
	Actual Throughput: 10,189 L/hr			
	Material: Stainless Steel 304			
	Bowl Speed: 4500 RPM			
Utilities	55 kW			

Comments & Drawings: N/A

SPECIFICATION SHEET **Homogenizer**				
Identification	Item ID:	HOM-201	No. Req'd:	1
Function : Shear *E. coli* cells, releasing astaxanthin product.				
Operation: continuous				
Materials	Inlet (S203)		Outlet (S204)	
Component Mass Flow (kg/hr)	1,532		1,532	
Water	1,226		1,226	
Cell mass	305.66		305.66	
Design Data	Temperature: 25 C			
	Pressure: 200 bar			
	Maximum Throughput:1,650 L/hr			
	Actual Throughput: 1,226 L/hr			
Utilities	11 kW electricity required			

Comments & Drawings: N/A

SPECIFICATION SHEET Twin Dome Extruder				
Identification	Item ID:	E-202	No. Req'd:	1
Function: Form fluffy biomass into pellets.				
Operation: Continuous				
Materials	Inlet (S210)	Inlet (S211)	Outlet (S212)	
Component Mass Flow (kg/hr)	320.9	17.98	359.6	
Biomass	290.38	0	290.38	
Water	15.28	0	15.28	
Fructose	0	17.98	17.98	
Diatomaceous Earth	35.96	0	35.96	
Design Data	Temperature: 25 ºC			
	Material: Stainless steel 304			
Utilities	7.5 kW electricity required			

Comments & Drawings: N/A

SPECIFICATION SHEET
Air Heater, Finned Steam Radiator

Identification	Item ID:	H-200	No. Req'd:	1
Function : Pre-heat incoming air at spray dryer inlet to 220°C.				
Operation: continuous				
Materials	Inlet	Outlet		
Temperature °C	22	220		
Pressure (bar)	1.013	1.013		
Component Mass Flow (kg/hr)				
Air	84658	84658		
Design Data	Type: Shell-and-Tube Heat Exchanger			
	Heat Duty: 4639 kW			
	Heat Exchange Area: 6469 sqft			
	Hot Fluid Location: Shell side			
	Tube OD / Pitch: 0.8 in / 1 in, Triangular Pitch			
	Shell OD: 48.97 in			
	14 Baffles, 12.6 in baffle spacing			
	1885 tubes, 1 tube pass			
Utilities	6200 kg/hr 450 psig steam			

Comments & Drawings: In actuality, a finned steam heater, rather than shell-and-tube heat exchanger may be more ideal for this application. Direct heating is not advised as nutraceutical-grade product should not come in contact with combustion byproducts in drying gas.

SPECIFICATION SHEET **Spray Dryer**				
Identification	Item ID:	D-201	No. Req'd:	1
Function : Reduce the moisture content of biomass slurry (S203) from 80% to 5%.				
Operation: continuous				
Materials	Inlet (S203)	Inlet (S206)	Outlet (S207)	Outlet (S208)
Temperature °C	37.0	220	80.0	80.0
Pressure (bar)	1.013	1.013	1.013	1.013
Component Mass Flow (kg/hr)				
Water	1226.0	0	1210.0	16.0
Cell Mass	298.0	0	0	283.1
Air	0	83448	83448.3	0
Design Data	Evaporative Load: 1210 kg/h			
	Diameter: 7.34 m			
	Height: 7.34 m			
	Conical (60°C) bottom			
	Material: Stainless Steel 304			
	Air Preheater Duty: 4639 kW			
	Dryer Energy Requirement: 1628.1 kW			
	Atomizer Type: Rotary, producing 20um diameter droplets			
	Air flow: open-cycle, co-current with product stream			
Utilities				
Comments & Drawings: N/A				

SPECIFICATION SHEET
Heat Exchanger, CO_2 Cooler

Identification	Item ID:	X-201	No. Req'd:	1
Function : Cool the inlet CO_2 stream of the positive displacement pump to -2 C such that during pumping (and simultaneous heating), the fluid remains in the liquid phase.				
Operation: continuous				
Streams	Hot In (S213)	Cold In (S230)	Hot Out (S214)	Cold Out (S231)
Temperature °C	22	-40	-2	10.34
Pressure (bar)	65	1.013	65	0.987
Materials (kg/hr)				
CO_2	6804.3	0	6804.3	0
Ethylene Glycol	0	4000	0	4000
Design Data	Type: Heat Exchanger			
	Heat duty: 139.4 kW			
	Hot Stream Location: Tube			
	Heat Exchange Area: 87.69 sqft			
	46 Tubes / 1 Pass, OD: 0.75 in., Triangular 0.9375 in. Pitch			
	Shell OD: 8.625 in,			
	14 Baffles, 17.5 in spacing			
	Material: Stainless Steel 304			
Utilities	4000 kg/h of coolant comprised of 60% Ethylene Glycol, 40% H_2O			

Comments & Drawings: N/A

SPECIFICATION SHEET
Heat Exchanger, CO_2 Heater

Identification	Item ID:	X-202	No. Req'd:	1
Function : Heat CO_2 above critical temperature for use as solvent in supercritical fluid extraction.				
Operation: continuous				
Streams	Hot In	Cold In (215)	Hot Out	Cold Out (S216)
Temperature °C	185.9	20.8	110.3	60
Pressure (bar)	11.36	65	11.34	64.52
Vapor Fraction	1	0	0	Supercritical
Materials (kg/hr)				
CO_2	0	6804.3	0	6804.3
Water	600	0	600	0
Design Data	Type: Heat Exchanger			
	Heat duty: 413.6			
	Hot Stream Location: Shell			
	Heat Exchange Area: 20.33 sqft			
	22 Tubes/ 2 passes, Tube OD: 0.75 in., Triangular 0.9375 in. Pitch			
	Shell OD: 6.625 in,			
	8 Baffles, 6.5 in spacing			
	Material: Stainless Steel 304			
Utilities	600 kg/h of 150 psig steam			

Comments & Drawings: N/A

SPECIFICATION SHEET **Heat Exchanger, EtOH Heater**				
Identification	Item ID:	X-203	No. Req’d:	1
Function : Heat EtOH co-solvent to 60 C for use in supercritical fluid extraction.				
Operation: continuous				
Streams	Hot In	Cold In (S218)	Hot Out	Cold Out (S219)
Temperature °C	182.2	41.7	99	60
Pressure (bar)	3.45	30	3.45	30
Vapor Fraction	1	0	.43	0
Materials (kg/hr)				
EtoH	0	358.2	0	358.2
Water	15	0	15	0
Design Data	Type: Heat Exchanger			
	Heat duty: 6.045 kW			
	Hot Stream Location: Shell			
	Heat Exchange Area: 19.0 sqft			
	26 Tubes/ 2 passes, Tube OD: 0.75 in., Triangular 0.9375 in. Pitch			
	Shell OD: 6.625 in,			
	12 Baffles, 12.8 in spacing			
	Material: Stainless Steel 304			
Utilities	15 kg/h of 50 psig steam			

Comments & Drawings: N/A

SPECIFICATION SHEET **Heat Exchanger, CO_2 Cooler #2**				
Identification	Item ID:	X-204	No. Req’d:	1
Function : Condense hot gaseous CO_2 in compressor outlet stream to liquid phase by cooling to 22C at 65 bar.				
Operation: continuous				
Streams	Hot In (S224)	Cold In	Hot Out (S225)	Cold Out
Temperature °C	48.88	7.22	22	7.22
Pressure (bar)	65	1.013	65	1.013
Vapor Fraction	1	0	0	0.21
Materials (kg/hr)				
CO_2	6804.3	0	6804.3	0
Water	0	300	0	300
Design Data	Type: Heat Exchanger			
	Heat duty: 43.61 kW			
	Hot Stream Location: Tube			
	Heat Exchange Area: 332.96 sqft			
	187 Tubes/ 1 passes, Tube OD: 0.75 in., Triangular 0.9375 in. Pitch			
	Shell OD: 16.14 in,			
	2 Baffles, 23.22 in spacing			
	Material: Stainless Steel 304			
Utilities	300 kg/hr of chilled water (entering at 45 F)			

Comments & Drawings: N/A

SPECIFICATION SHEET
Heat Exchanger, EtOH Cooler

Identification	Item ID:	X-205	No. Req'd:	1
Function : Cool recovered EtOH to moderate temperatures and liquid phase such that it can be pumped and recycled for use as a co-solvent in supercritical fluid extraction.				
Operation: continuous				
Streams	Hot In (S227)	Cold In	Hot Out (S228)	Cold Out
Temperature °C	77.4	25	34.57	43.46
Pressure (bar)	1.013	1.013	1.013	1.013
Vapor Fraction	0	0	0	0
Materials (kg/hr)				
EtOH	358.12	0	358.12	0
Water	0	670.0	0	670.00
Design Data	Type: Heat Exchanger			
	Heat duty: 14.37 kW			
	Hot Stream Location: Shell			
	Heat Exchange Area: 34.65 sqft			
	31 Tubes/ 1 pass, Tube OD: 0.75 in., Triangular 0.9375 in. Pitch			
	Shell OD: 6.625 in,			
	18 Baffles, 3.35 in spacing			
	Material: Stainless Steel 304			
Utilities	670 kg/hr of cooling water (enters at 25 °C)			

Comments & Drawings: N/A

SPECIFICATION SHEET **Positive Displacement Pump**				
Identification	Item ID:	P-201	No. Req'd:	1
Function : Raise the pressure of liquid CO_2 above critical pressure to use in supercritical fluid extraction.				
Operation: continuous				
Streams	In (S214)	Out (S215)		
Temperature °C	-2.3	20.8		
Pressure (bar)	65.0	300.0		
Vapor Fraction	0	0		
Materials (kg/hr)				
CO_2	6804.3	6804.3		
Design Data	Type: Positive Displacement Pump			
	Energy Requirement: 59.43 kW			
	Displacement: 5.053 cuft/min			
	Pressure head: 9358 ft			
	Material: stainless steel			
	Efficiency: 0.72			
Utilities	59.43 kW electricity			

Comments & Drawings: N/A

SPECIFICATION SHEET
Positive Displacement Pump, EtOH Pump

Identification	Item ID:	P-202	No. Req'd:	1
Function : Raise the pressure of EtOH co-solvent to a pressure suitable for supercritical fluid extraction with supercritical CO_2.				
Operation: continuous				
Streams	In (S217)	Out (S218)		
Temperature °C	35.0	41.7		
Pressure (bar)	1.013	300.0		
Vapor Fraction	0	0		
Materials (kg/hr)				
EtOH	358.1	358.1		
Design Data	Type: Positive Displacement Pump			
	Energy Requirement: 2.68 kW			
	Pump Efficiency: 0.72			
	Pressure head: 12117 ft			
	Material: stainless steel			
Utilities	2.68 kW electricity			

Comments & Drawings: N/A

SPECIFICATION SHEET **Centrifugal Pump, Coolant Pump**				
Identification	Item ID:	P-203	No. Req'd:	1
Function : Recirculate the coolant from the X-201 heat exchanger outlet to H-202 refrigerator.				
Operation: continuous				
Streams	In (S217)	Out (S218)		
Temperature °C	10.34	10.35		
Pressure (bar)	0.98	1.013		
Vapor Fraction	0	0		
Materials (kg/hr)				
Water	1600.0	1600.0		
Ethylene glycol	2400.0	2400.0		
Design Data	Type: Centrifugal Pump			
	Energy Requirement: 0.01 kW			
	Pump Efficiency: 0.85			
	Pressure head: 2.1 ft			
	Material: stainless steel			
Utilities	0.01 kW electricity			

Comments & Drawings: N/A

SPECIFICATION SHEET
Extraction Vessel

Identification	Item ID:	EXT-201	No. Req’d:	3
Function : Extraction vessel in which supercritical fluid extraction (at 30 MPa, 60 C) takes place.				
Operation: batch extraction				
Streams	In (S212)	In (S220)	Out (S221)	Out (S222)
Temperature °C	22.0	60.0	60	50
Pressure (bar)	0.98	1.013	300	300
Vapor Fraction	0	0	N/A	0
Materials (kg/hr)				
CO_2	0	6804.3	6804.3	0
EtOH	0	358.1	358.1	0
Water	16.0	0	0	16.0
Cell Mass	283.1	0	0	283.1
Diatomaceous Earth	36.0	0	0	36.0
Fructose	18.0	0	0	18.0
Astaxanthin	7.3	0	7.0	0
Design Data	Type: Vertical Pressure Vessel			
	Diameter: 2 ft			
	Height: 7 ft			
	Design Pressure: 4786 psig			
	Material: Stainless steel			
	Wall thickness: 4.74 in			
Utilities	N/A			
Comments & Drawings: Extraction is assumed to have 97% recovery of Astaxanthin product.				

SPECIFICATION SHEET
3-Stage Compressor

Identification	Item ID:	MCOMPR-201	No. Req'd:	1
Function : Increase the pressure of the recovered CO_2 stream (S223) to 65 bar. At this pressure, the CO_2 can later be cooled to the liquid state by X-204 and recycled in the supercritical fluid extraction process.				
Operation: continuous				
Streams	In (S223)	Out (S224)		
Temperature °C	22	48.888		
Pressure (bar)	1.01325	65		
Vapor Fraction	1	1		
Materials (kg/hr)				
CO_2	6804.3	6804.3		
Design Data	Type: 3-Stage Compressor with Intercooling			
	Compressor, Energy Required: 735.30 kW			
	Compression Ratio for each stage: 4.02			
	Intercoolers utilize cooling water (enter at 90F, exit at 120F)			
	Intercooler, heat duty: 822.7 kW			
	Material: Stainless steel			
Utilities	42451 kg/h of cooling water (entering at 90 F, leaving each stage at 120 F) for intercoolers. 735.30 kW electricity for compressor.			

Comments & Drawings:

SPECIFICATION SHEET **Wiped-Film Evaporator**				
Identification	Item ID:	EVAP-201	No. Req'd:	1
Function : Product recovery process after supercritical fluid extraction. Separates ethanol co-solvent from Astaxanthin product.				
Operation: continuous				
Streams	In (S226)	Out (S227)	Out (229)	
Temperature °C	22.0	78.4	78.37	
Pressure (bar)	1.0132	1.0132	1.0132	
Vapor Fraction	0.0	1	1	
Materials (kg/hr)				
Ethanol	358.1	357.8	0.3615	
Astaxanthin	7.0	0	7.0	
Design Data	Operates at boiling point of Ethanol (78.4 C)			
	Heat Duty: 102.5 kW			
	Flash Duty: 0.102 kW			
	Heat transfer area required: 12.98 ft^2			
	Material: Stainless steel			
Utilities	102.6 kW electricity			

Comments & Drawings: This evaporator was modeled in ASPEN as a heater and flash vessel. Its cost was derived from the heat transfer area required.

SPECIFICATION SHEET
Wet Gas Scrubber

Identification	Item ID:	N/A	No. Req’d:	1
Function : Remove pollutants from fermenter off-gas streams before they are released into the atmosphere.				
Operation: continuous				
Vendor: Pollution Systems				
Materials (kg/hr)	Input	Output		
Vent Gas (peak)	5247.4	5247.4		
Water	632.1	632.1		
Design Data	Type: Packed scrubber			
	Contact Liquid: Water			
	Removal Efficiency: 95%			
	Peak gas flow rate: 17,500 cfm			
	Inlet Loading: 32 lb/hr			
	Stack diameter / height: 28 in / 36 ft			
	Material: Stainless steel			
	30 Hp Scrubber Process Fan			
	150 gpm Recycle Pump			
Operating Conditions	Temp: 22 C□, Pressure: 1.013 bar			
Utilities	632.1 kg/h of process water			

Comments & Drawings: Peak vent-gas production: 3680.6 cfm.

17. Equipment Cost Summary

Table 17.1 lists the purchase costs of each piece of equipment designed or purchased. It also includes the bare module factor utilized for each piece of equipment, when applicable, and the associated bare module cost for a single piece of that equipment. The bare module factors were taken from Table 16.11 in Seider, et al. when available. Notably, a bare module factor of 4.0 was used for storage tanks (which is absent from Table 16.11 in Seider, et al.), in accordance with the equipment costing spreadsheet provided by the course. A default bare module of 3.21 was used as per the profitability spreadsheet guidelines when no bare module factor was available for that piece of equipment; for example, it is in use for the fermenter agitators.

Some of the rarer pieces of equipment required more creative pricing techniques. The homogenizer was approximated as a jet mill, with purchase cost \$87,652 and bare module factor 2.3. The pelletizer was approximated as a combination of a pellet mill, with purchase cost \$32,684 and crusher bare module factor 1.39, and screw extruder, with purchase cost \$268,924 and bare module factor 2.3.

Table 17.1. List of Equipment Purchased.

Equipment	Label	Purchase Cost (USD)	Number Purchased	Bare Module Factor	Bare Module Cost (USD)
Upstream Raw Materials					
Glucose storage tank		51,630	1	4.0	206,520
Ammonia storage tank		19,811	1	4.0	79,244
Ammonia compressor		669,700	1	2.15	1,439,855
Phosphoric acid storage tank		21,050	1	4.0	84,200
Media storage/prep tank		58,574	1	4.0	234,296
Air compressor		907,000	1	2.15	1,950,050
HEPA Filters		4,000	3	2.32	9,280
Fermentation					
Pre-seed fermenter (with jacket)		32,347	3	4.16	134,564
Seed fermenter		96,861	2	4.16	402,942
Production fermenter		307,328	3	4.16	1,278,484
Pre-seed agitator		21,700	3	3.21	69,657
Seed agitator		64,500	2	3.21	207,045
Production agitator		323,000	3	3.21	1,036,830

Seed HX		17,014	2	3.17	53,935
Production (growth) HX		157,306	3	3.17	498,660
Production (production) HX		23,493	3	3.17	74,473
Switch-to-Production HX		96,542	1	3.17	306,039
Gas scrubber vessel		77,799	1	4.16	323,644
Gas scrubber packing		12,925	1	4.16	53,768
Downstream (Pre-Extraction)					
Surge tank		279,110	1	4.0	1,116,440
Disk stack centrifuge		80,295	1	2.03	162,998
Centrate tanks		73,419	2	4.0	293,676
Bleach tank		67,120	1	4.0	268,480
Spray dryer		717,098	1	2.03	1,455,709
Wet bag filter		159,500	1	2.32	370,039
Pre-heater heat exchanger		213,520	1	3.17	676,858
Homogenizer		87,652	1	2.3	201,600
Pelletizer		-	1	-	448,978
Diatomaceous earth storage tank		39,018	1	4.0	156,072
Fructose storage tank		32,058	1	4.0	128,232

Extraction					
Multistage compressor with intercoolers		1,692,900	1	2.15	3,639,735
CO_2 Pump		69,567	1	3.3	229,572
EtOH Pump		9,575	1	3.3	31,598
CO_2 Heater		12,900	1	3.17	40,893
EtOH Heater		11,000	1	3.17	34,870
CO_2 Cooler		11,100	1	3.17	35,187
CO_2 Cooler 2		26,200	1	3.17	83,054
EtOH Cooler		11,800	1	3.17	37,406
Cyclones, valves, and demisters		-	-	-	168,367
Extraction vessel		238,065	1	4.16	990,351
Wiped-film evaporator		37,147	1	2.45	91,672
Transfer pump		3,414	1	3.3	11,266
Refrigerator		18,200	1	3.17	57,694
Product tank		18,246	1	4.0	72,984

The most significant investment costs predictably come from the production and seed fermenters and their associated agitators and the three compressors—the ammonia and air compressors, as well as the multistage compressors for extraction—with the surge tank, spray dryer, and extraction vessel also making sizable contributions. It is our opinion that these costs are largely fixed; what each of these pieces of equipment share in common is their massive size, and they are so large mainly out of necessity to appropriately complete their respective tasks. In other words, regardless of any changes that are made to the plant's process design, the owner is likely to be shackled with these costs. Where more progress could be made is perhaps the three distinct heat exchangers associated with the production fermenters, which total to more than $2 million. Further analysis of intraplant heat integration may be a valuable step to take to reduce these capital investments prior to plant construction.

18. Fixed Capital Investment Summary

The Profitability Analysis 4.0 spreadsheet, graciously provided by the course, was used to comprehensively compute the financials for this plant. Its outputs will be discussed in the following three sections.

The individual equipment costs detailed in Section 17 were input to the profitability spreadsheet and categorized as fabricated equipment, process machinery, spares, storage, or other. These categories are defined in Seider, et al. "Fabricated equipment" entails custom design; examples include heat exchangers, vessels, or columns. Fabricated equipment is primarily contrasted with process machinery, which is "selected from a vender-supplied list of standard sizes and often includes a driver." Examples of process machinery are pumps, compressors, and centrifuges. Even if no vendor was identified for these types of equipment, all pumps, compressors, and the like are identified as process machinery. While every effort was made to adequately categorize each piece of equipment, the reader should not consider these classifications as sacrosanct.

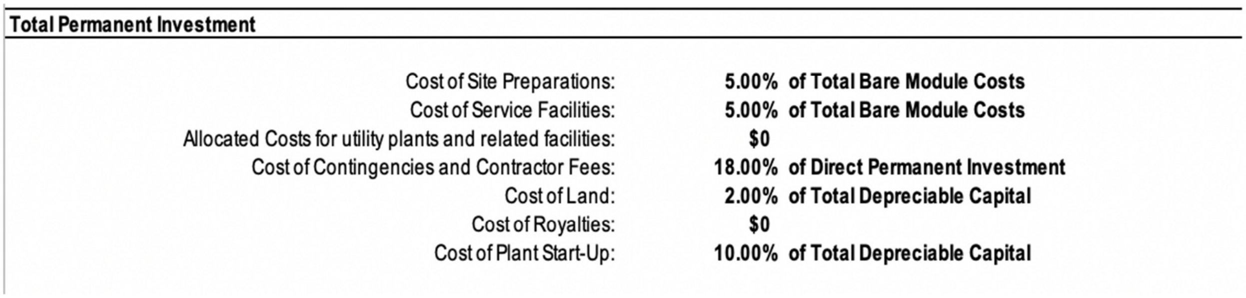
Table 18.1. The total bare module costs for the plant.

Total Bare Module Costs:				
Fabricated Equipment	$	10,741,632		
Process Machinery	$	12,675,562		
Spares	$	-		
Storage	$	2,991,514		
Other Equipment	$	-		
Catalysts	$	-		
Computers, Software, Etc.	$	-		
Total Bare Module Costs:			**$**	**26,408,707**

The total bare module costs, accounting for both purchase and installation of fixed equipment, nears $26.5MM. Process machinery, likely due to the compressors, make up a slightly larger fraction of the total bare module costs than fabricated equipment. Not to be ignored, storage costs near $3MM. No spares were accounted for in the profitability analysis, though multiple HEPA filters were purchased, and can be used as spares.

Having accounted for all bare module costs, it is now possible to estimate the total permanent investment for the plant. In lieu of estimating each component line-by-line, Guthrie's factored-cost methods are employed, which, for example, compute the cost of site preparation as some proportion of the total bare module cost. This method is facilitated by the profitability spreadsheet, which allows one to specify these factors, as shown in Table 18.2 for this plant.

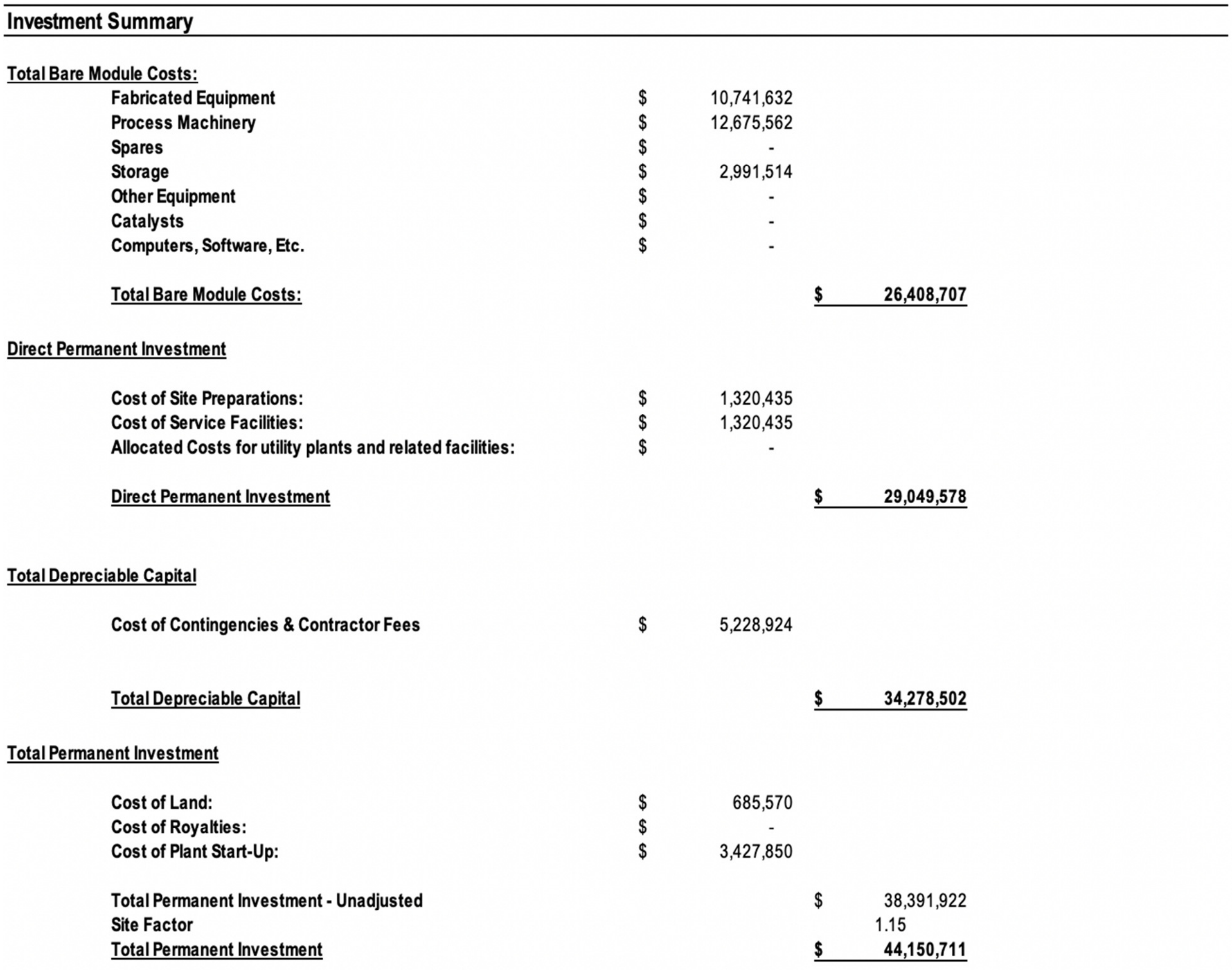
Table 18.2. Factored-Cost Method for Total Permanent Investment.



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:	**$0**	
Cost of Plant Start-Up:	**10.00%**	**of Total Depreciable Capital**

No values were changed from the default settings in the profitability spreadsheet. The establishment of utility plants has been abandoned, because it is estimated that the use of municipal utilities is cheaper. Moreover, no royalty obligations have been identified. With these factors in mind, the total permanent investment comes out to $38.4MM, with a more detailed breakdown provided in Table 18.3 below. A site factor of 1.15 was used, corresponding to the U.S. Midwest in Seider, et al. This is an admittedly generous classification of Lancaster, PA, but given that Lancaster is a borderline case and alternative locations are more firmly ensconced in the traditional "Midwest," it seems a reasonable selection. The site factor for the U.S. Northeast is marginally smaller, at 1.10, so this estimate is suitably conservative for an edge case like rural Pennsylvania.

Table 18.3. Total Permanent Investment for Plant.



Investment Summary				
Total Bare Module Costs:				
Fabricated Equipment	$	10,741,632		
Process Machinery	$	12,675,562		
Spares	$	-		
Storage	$	2,991,514		
Other Equipment	$	-		
Catalysts	$	-		
Computers, Software, Etc.	$	-		
Total Bare Module Costs:			**$**	**26,408,707**
Direct Permanent Investment				
Cost of Site Preparations:	$	1,320,435		
Cost of Service Facilities:	$	1,320,435		
Allocated Costs for utility plants and related facilities:	$	-		
Direct Permanent Investment			**$**	**29,049,578**
Total Depreciable Capital				
Cost of Contingencies & Contractor Fees	$	5,228,924		
Total Depreciable Capital			**$**	**34,278,502**
Total Permanent Investment				
Cost of Land:	$	685,570		
Cost of Royalties:	$	-		
Cost of Plant Start-Up:	$	3,427,850		
Total Permanent Investment - Unadjusted			$	38,391,922
Site Factor				1.15
Total Permanent Investment			**$**	**44,150,711**

19. Operating Cost- Cost of Manufacture

19.1 Raw Materials

The annual cost for each of the raw materials required to run the plant are included in Table 19.1 below. The prices assumed for each are documented in Table 11.1.

Table 19.1. Annual Costs of Each Raw Material in Use.

Material	Annual Cost (USD)	Cost (USD)/kg Astaxanthin
Glucose	\$2,034,817	\$37.00
Ammonia	\$102,395	\$1.86
Phosphoric acid	\$181,991	\$3.31
Media salts	\$1,043,204	\$18.97
Yeast extract (for media)	\$116,073	\$2.11
Bleach	\$450,082	\$8.18
Fructose	\$1,424,016	\$25.89
Diatomaceous earth	\$3,989	\$0.07

Per year, the plant spends approximately \$5.36 million on raw materials. Per kilogram of astaxanthin made, the plant spends about \$98, well short of the \$3,500/kg selling price. The largest cost by some margin is glucose, justifying selection of plant location on the basis of proximity to a glucose source, in Lancaster, PA.

In addition to the materials listed in Table 19.1, carbon dioxide, ethanol, and ethylene glycol are needed to operate the plant. However, they are one-time costs, since the materials are recycled. Over time, some of each of these components will be lost, but their exclusion from the profitability analysis is warranted since their combined cost is so low. (The one-time purchase is less than $10,000.)

19.2 Utilities

The utilities costs are summarized below in Table 19.2 below.

Table 19.2. Utilities costs, broken down by type of utility and location in the plant.

Utility/Usage	Cost (USD) / unit	Annual Cost (USD)
Electricity / Air compressor	$0.07/kWhr	$133,630
Electricity / Ammonia compressor	$0.07/kWhr	$10,210
Electricity / Upstream storage tanks	$0.07/kWhr	$2,302
Process water / Fermenters	$0.27 / m^3	$24,180
Cooling water / Pre-seed cooling jacket	$0.10/1,000 gal	$9,504
Cooling water / Seed HX	$0.10/1,000 gal	$23,760
Cooling water / Production (growth) HX	$0.10/1,000 gal	$43,029
Cooling water / Production (production) HX	$0.10/1,000 gal	$69,922
Cooling water / Switch-to-Production HX	$0.10/1,000 gal	$2,690
Electricity / Pre-seed agitator	$0.07/kWhr	$591
Electricity / Seed agitator	$0.07/kWhr	$5,910
Electricity / Production agitator	$0.07/kWhr	$59,100
Process water / Scrubber	$0.27 / m^3	$1,355
Electricity / Scrubber	$0.07/kWhr	$12,419
Electricity / Disk stack	$0.07/kWhr	$30,492

centrifuge		
Steam (450 psig) / Spray dryer	$8/1,000 lb	$866,046
Electricity / Spray dryer	$0.07/kWhr	$902,619
Electricity / Homogenizer	$0.07/kWhr	$6,099
Electricity / Pelletizer	$0.07/kWhr	$4,158
Steam (50 psig) / Extraction	$6/1,000 lb	$1,572
Steam (150 psig) / Extraction	$7/1,000 lb	$73,335
Chilled water / Extraction	$1.50/ton-day	$570,240
Cooling water / Extraction	$0.10/1,000 gal	$4,235,933
Electricity / Extraction	$0.07/kWhr	$442,085
Electricity / Astaxanthin storage	$0.07/kWhr	$222

Table 19.3. Aggregated Annual Utilities Costs by Type of Utility.

Utility	Annual Cost (USD)
Electricity	$1,609,837
Cooling water	$4,384,838
Chilled water	$570,240
Process water	$25,535
Steam (various pressures)	$940,953

Cooling water, liberally used in the upstream heat exchangers and downstream extraction, makes up the bulk of utility needs. Per year, the plant spends about $7.5 million on utilities, and utilities cost about $137 per kilogram of astaxanthin manufactured.

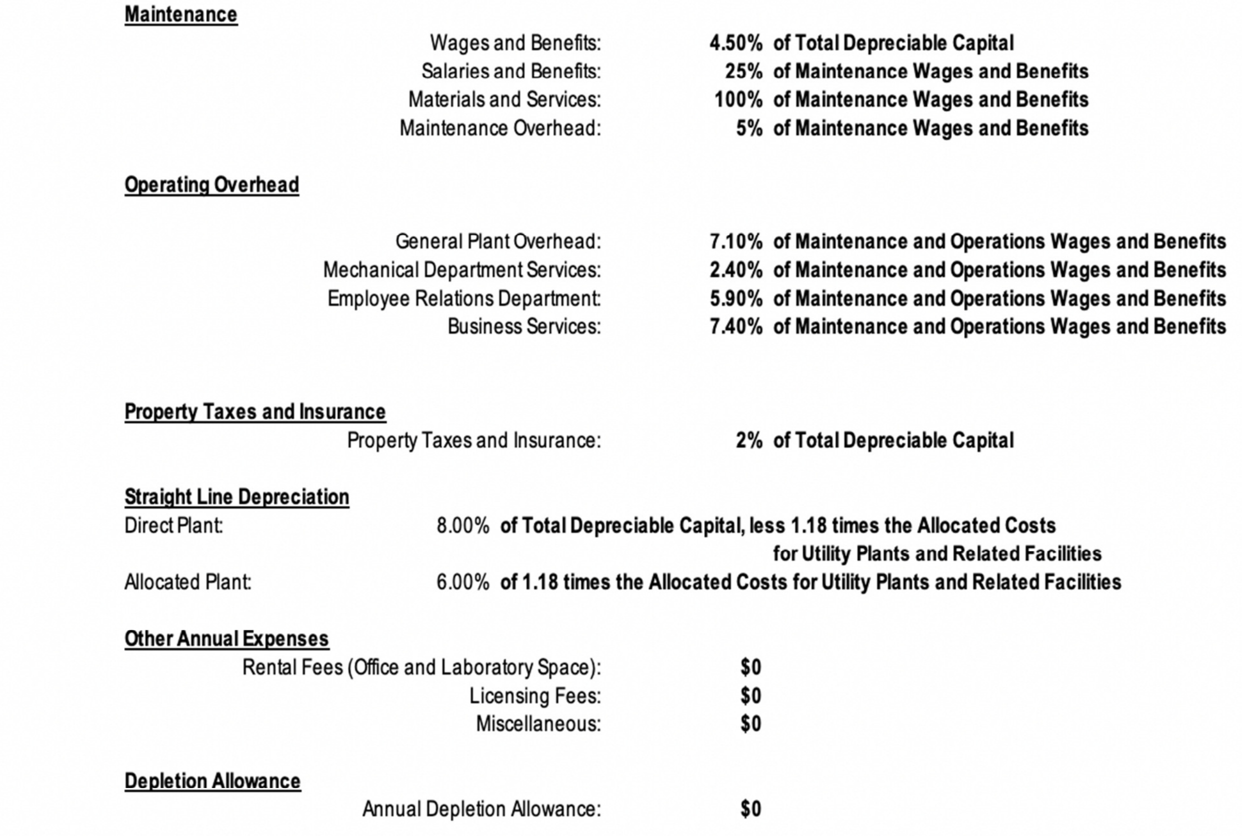
19.3 Labor

It is estimated that the plant will need 5 operators/shift, 3 engineers, and 5 laboratory technicians. There will be 5 shifts. Laboratory technicians are particularly important since the *E. coli* must be optimized for astaxanthin synthesis, per the methods of Zhang, et al. An operator is assumed to make $60,000/year, and engineers and laboratory technicians $200,000/year, including all supplies and overheads.

19.4 Remaining Fixed Costs

The remaining fixed costs are calculated using factored-cost methods, which have parameters described below in Table 19.4.

Table 19.4. Factors for Fixed Costs Outside of Operations Labor.

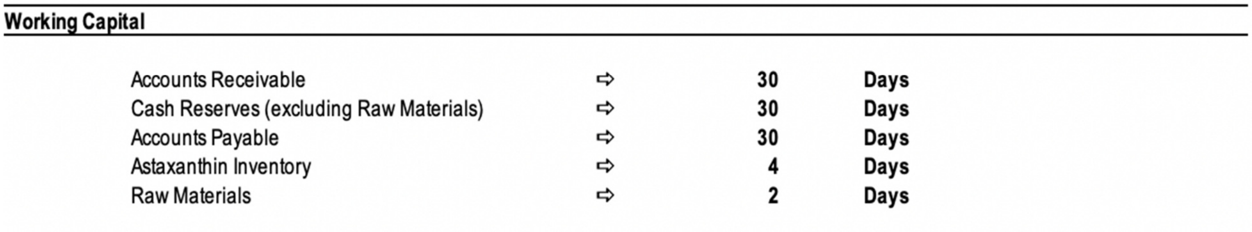


Maintenance		
Wages and Benefits:	**4.50%**	**of Total Depreciable Capital**
Salaries and Benefits:	**25%**	**of Maintenance Wages and Benefits**
Materials and Services:	**100%**	**of Maintenance Wages and Benefits**
Maintenance Overhead:	**5%**	**of Maintenance Wages and Benefits**
Operating Overhead		
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**
Property Taxes and Insurance		
Property Taxes and Insurance:	**2%**	**of Total Depreciable Capital**
Straight Line Depreciation		
Direct Plant:	8.00%	**of Total Depreciable Capital, less 1.18 times the Allocated Costs for Utility Plants and Related Facilities**
Allocated Plant:	6.00%	**of 1.18 times the Allocated Costs for Utility Plants and Related Facilities**
Other Annual Expenses		
Rental Fees (Office and Laboratory Space):	**$0**	
Licensing Fees:	**$0**	
Miscellaneous:	**$0**	
Depletion Allowance		
Annual Depletion Allowance:	**$0**	

19.5 Working Capital

The working capital required for the plant is similarly computed, with pre-ordained stocks of raw materials and cash reserves acquired to start-up the plant, detailed in Tables 19.5 and 19.6.

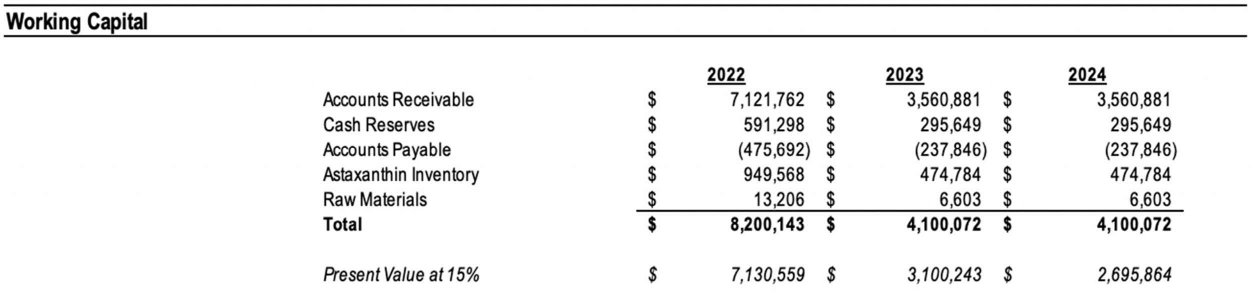
Table 19.5. Factored-Cost Method for Deriving Working Capital.



Working Capital

Accounts Receivable	⇨	**30**	**Days**
Cash Reserves (excluding Raw Materials)	⇨	**30**	**Days**
Accounts Payable	⇨	**30**	**Days**
Astaxanthin Inventory	⇨	**4**	**Days**
Raw Materials	⇨	**2**	**Days**

Table 19.6. Actual Accounting of Working Capital in Accordance with Table 19.5 Parameters.



Working Capital

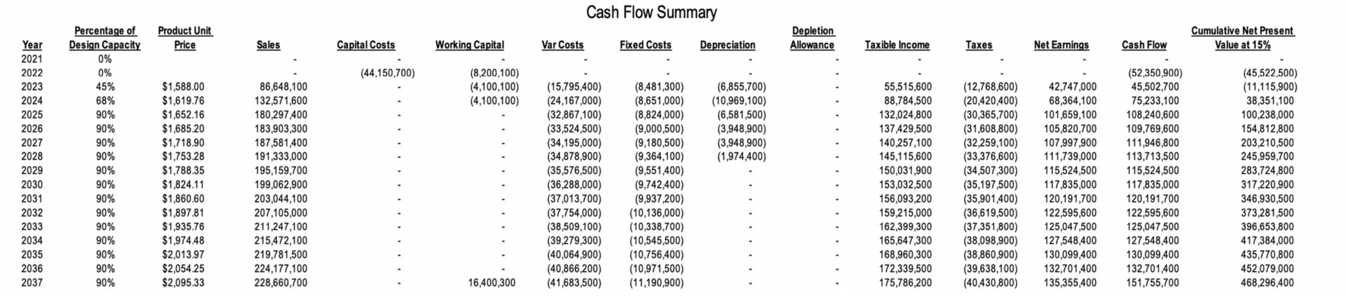
		2022		**2023**		**2024**
Accounts Receivable	$	7,121,762	$	3,560,881	$	3,560,881
Cash Reserves	$	591,298	$	295,649	$	295,649
Accounts Payable	$	(475,692)	$	(237,846)	$	(237,846)
Astaxanthin Inventory	$	949,568	$	474,784	$	474,784
Raw Materials	$	13,206	$	6,603	$	6,603
Total	**$**	**8,200,143**	**$**	**4,100,072**	**$**	**4,100,072**
Present Value at 15%	*$*	*7,130,559*	*$*	*3,100,243*	*$*	*2,695,864*

The total permanent investment, calculated in Table 18.3, and the working capital sum to the total capital investment. The total capital investment for this plant is just north of $57MM.

20. Profitability Analysis

It is assumed that design of the plant will commence in 2021, construction will take place in 2022, and production and sales begin in 2023. In 2023, the plant will operate at 45 percent capacity; in 2024, at 67.5 percent capacity; and for the remainder of the life of the plant (15 years of production), at 90 percent capacity. Assuming a discount rate of 15 percent for capital, a selling price of $3,500/kg in 2023, and a general inflation rate of 2 percent (commensurate with the Federal Reserve's dual mandate), the cash flow summary is provided in Table 20.1.

Table 20.1. Cash Flow Summary.

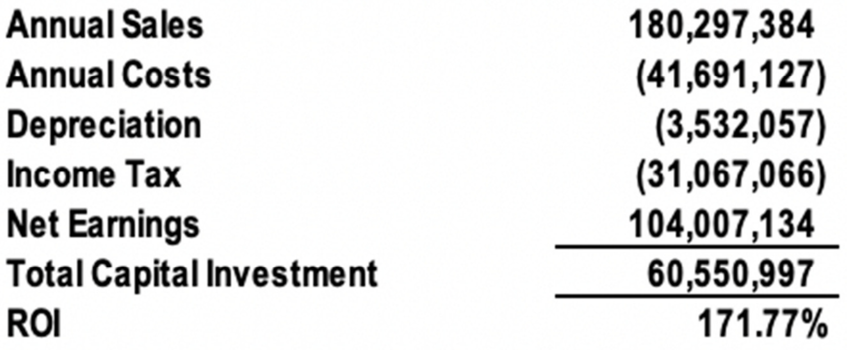


Cash Flow Summary

Year	Percentage of Design Capacity	Product Unit Price	Sales	Capital Costs	Working Capital	Var Costs	Fixed Costs	Depreciation	Depletion Allowance	Taxible Income	Taxes	Net Earnings	Cash Flow	Cumulative Net Present Value at 15%
2021	0%		-	-	-	-	-	-	-	-	-	-	-	-
2022	0%		-	(44,150,700)	(8,200,100)	-	-	-	-	-	-	-	(52,350,900)	(45,522,500)
2023	45%	$1,588.00	86,648,100	-	(4,100,100)	(15,795,400)	(8,481,300)	(6,855,700)	-	55,515,600	(12,768,600)	42,747,000	45,502,700	(11,115,900)
2024	68%	$1,619.76	132,571,600	-	(4,100,100)	(24,167,000)	(8,651,000)	(10,969,100)	-	88,784,500	(20,420,400)	68,364,100	75,233,100	38,351,100
2025	90%	$1,652.16	180,297,400	-	-	(32,867,100)	(8,824,000)	(6,581,500)	-	132,024,800	(30,365,700)	101,659,100	108,240,600	100,238,000
2026	90%	$1,685.20	183,903,300	-	-	(33,524,500)	(9,000,500)	(3,948,900)	-	137,429,500	(31,608,800)	105,820,700	109,769,600	154,812,800
2027	90%	$1,718.90	187,581,400	-	-	(34,195,000)	(9,180,500)	(3,948,900)	-	140,257,100	(32,259,100)	107,997,900	111,946,800	203,210,500
2028	90%	$1,753.28	191,333,000	-	-	(34,878,900)	(9,364,100)	(1,974,400)	-	145,115,600	(33,376,600)	111,739,000	113,713,500	245,959,700
2029	90%	$1,788.35	195,159,700	-	-	(35,576,500)	(9,551,400)	-	-	150,031,900	(34,507,300)	115,524,500	115,524,500	283,724,800
2030	90%	$1,824.11	199,062,900	-	-	(36,288,000)	(9,742,400)	-	-	153,032,500	(35,197,500)	117,835,000	117,835,000	317,220,900
2031	90%	$1,860.60	203,044,100	-	-	(37,013,700)	(9,937,200)	-	-	156,093,200	(35,901,400)	120,191,700	120,191,700	346,930,500
2032	90%	$1,897.81	207,105,000	-	-	(37,754,000)	(10,136,000)	-	-	159,215,000	(36,619,500)	122,595,600	122,595,600	373,281,500
2033	90%	$1,935.76	211,247,100	-	-	(38,509,100)	(10,338,700)	-	-	162,399,300	(37,351,800)	125,047,500	125,047,500	396,653,800
2034	90%	$1,974.48	215,472,100	-	-	(39,279,300)	(10,545,500)	-	-	165,647,300	(38,098,900)	127,548,400	127,548,400	417,384,000
2035	90%	$2,013.97	219,781,500	-	-	(40,064,900)	(10,756,400)	-	-	168,960,300	(38,860,900)	130,099,400	130,099,400	435,770,800
2036	90%	$2,054.25	224,177,100	-	-	(40,866,200)	(10,971,500)	-	-	172,339,500	(39,638,100)	132,701,400	132,701,400	452,079,000
2037	90%	$2,095.33	228,660,700	-	16,400,300	(41,683,500)	(11,190,900)	-	-	175,786,200	(40,430,800)	135,355,400	151,755,700	468,296,400

It is clear that the plant is highly profitable. Capital investment costs are recovered within the first year of production. In fact, in the year 2023, the plant has a net present value (NPV) of $38,351,100. Over the 15-year lifetime of the plant, it has a NPV in excess of $468 million. The return on investment (ROI), calculated in the third year of operation, is almost 172 percent, as computed in Table 20.2.

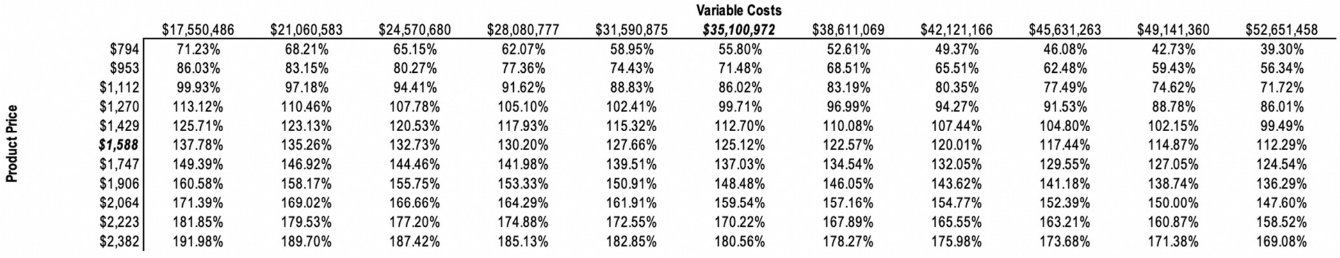
Table 20.2. Return on Investment in Third Year of Production.



Annual Sales	**180,297,384**
Annual Costs	**(41,691,127)**
Depreciation	**(3,532,057)**
Income Tax	**(31,067,066)**
Net Earnings	**104,007,134**
Total Capital Investment	**60,550,997**
ROI	**171.77%**

Finally, the investor's rate of return (IRR) is 125.12 percent. Deviations in IRR as a function of price or variable cost fluctuations are shown in Table 20.3 below. Take note that the price in this table is in pounds of astaxanthin ($1,588/lb astaxanthin). Table 20.3 illustrates the robustness of the economics for the plant; even if the variable costs increased by $17 million annually and the price of astaxanthin dropped to $1750/kg ($794/lb), the IRR would still be almost 40 percent.

Table 20.3. Sensitivity Analysis on IRR, with price in $/lb astaxanthin.



						Variable Costs					
Product Price		$17,550,486	$21,060,583	$24,570,680	$28,080,777	$31,590,875	***$35,100,972***	$38,611,069	$42,121,166	$45,631,263	$49,141,360	$52,651,458
	$794	71.23%	68.21%	65.15%	62.07%	58.95%	55.80%	52.61%	49.37%	46.08%	42.73%	39.30%
	$953	86.03%	83.15%	80.27%	77.36%	74.43%	71.48%	68.51%	65.51%	62.48%	59.43%	56.34%
	$1,112	99.93%	97.18%	94.41%	91.62%	88.83%	86.02%	83.19%	80.35%	77.49%	74.62%	71.72%
	$1,270	113.12%	110.46%	107.78%	105.10%	102.41%	99.71%	96.99%	94.27%	91.53%	88.78%	86.01%
	$1,429	125.71%	123.13%	120.53%	117.93%	115.32%	112.70%	110.08%	107.44%	104.80%	102.15%	99.49%
	$1,588	137.78%	135.26%	132.73%	130.20%	127.66%	125.12%	122.57%	120.01%	117.44%	114.87%	112.29%
	$1,747	149.39%	146.92%	144.46%	141.98%	139.51%	137.03%	134.54%	132.05%	129.55%	127.05%	124.54%
	$1,906	160.58%	158.17%	155.75%	153.33%	150.91%	148.48%	146.05%	143.62%	141.18%	138.74%	136.29%
	$2,064	171.39%	169.02%	166.66%	164.29%	161.91%	159.54%	157.16%	154.77%	152.39%	150.00%	147.60%
	$2,223	181.85%	179.53%	177.20%	174.88%	172.55%	170.22%	167.89%	165.55%	163.21%	160.87%	158.52%
	$2,382	191.98%	189.70%	187.42%	185.13%	182.85%	180.56%	178.27%	175.98%	173.68%	171.38%	169.08%

The high IRR is largely a product of the high selling price of astaxanthin. Because it is likely that such a lucrative process will garner significant competition over the life of the plant, it may be imprudent to simply rely on the sensitivity analysis in Table 20.3, which assumes a constant price over the life of the plant.

Consequently, another sensitivity analysis was conducted that accounts for decreasing prices at different rates. Table 20.4 details the profitability measures for annual price decreases of 1, 5, 10, and 20 percent. To be clear, for the 1 percent analysis, this means that the standard price of $3,500/kg was taken for 2023, but 99 percent of this figure was taken for 2024. 99 percent of the 2024 price was taken for 2025, and so on. The results show that the plant is highly resilient to price decreases. At 20 percent annual price decreases, the plant is still profitable, with an 83 percent ROI and 86 percent IRR. (Cash flow analysis shows that in this case the plant would start losing money around 2028-2029, when NPV is approximately $86MM.)

Table 20.4 Profitability Measures for Annual Price Decreases of 1, 5, 10, and 20 Percent.

Annual price decrease	**1 percent**	**5 percent**	**10 percent**	**20 percent**
NPV	$372MM	$272MM	$178MM	$58MM
ROI	158%	141%	121%	83%
IRR	120.32%	113.75%	105.18%	86.20%

21. Additional Considerations

Limitation of Consumer Base

The supercritical fluid extraction of Astaxanthin from a cell mass pellet requires the use of ethanol as a co-solvent. This poses a problem for potential Muslim consumers, however, as the presence of ethanol makes this a non-Halal (permitted) product in Islam.

Registered use of Genetically Modified Organisms

The EPA has established regulations specifically for microorganisms that require submission of a Microbial Commercial Activity Notice (MCAN) before they are used for commercial purposes (35). The use of the genetically modified strain of *E.coli* used to produce Astaxanthin will need to be registered and classified. This report will contain the microorganism's characteristics and genetic construction; byproducts of its manufacture, use, and disposal; health and environmental effects data; and other information, which may require additional laboratory research. Our analysis has assumed that the strain of *E.coli*, although genetically modified, does not significantly impact the environment or pose threats to human health.

Centrate Disposal

The centrate coming from the centrifuge must be treated before it can be disposed of. Proper procedure for the disposal of cell media involves treating the media for at least two hours with a hypochlorite solution in the amount of 10,000 ppm (36). To achieve this the centrate will be alternatively flowed into two tanks over the course of two hours, treated with hypochlorite bleach solution, and then disposed of. Each tank must handle 2 hours worth of centrate flow.

They will be 23,000 L in volume. Each will be cone-roofed in order to adapt to potential pressure changes caused by fluctuating temperature. Stainless steel tanks are preferred to avoid tank degradation (18).

Biomass Disposal

Cell inactivation is achieved in two forms within the downstream processing. The cells are lysed within the homogenizer, and then subjected to high temperatures, above that which causes irreversible denaturation of cellular DNA, within the spray dryer (37). There are two options for disposal of the dead cell mass collected from the extraction vessel. The first option is that it can be sold to farms to be used as animal feed. The presence of fructose and food-grade diatomaceous earth within the pellets will not pose any harm to the animals, and the latter has even added to livestock feed. However, additional research will be required to prove that the GMO is not an environmental or health hazard before the cell mass can be sold. Therefore, the second option, to send the dead cell mass to a landfill, at cost of $0.17/dry kg, is currently considered. The solid waste will be sent to a landfill for $58 / hr or $380,160 / year.

Fermenter Off-Gas Disposal

The gas exiting the exhaust vents of the fermenters is mainly composed of carbon dioxide, nitrogen, and oxygen. However, a packed bed chemical/gas scrubber, such as Model CS-17 from Pollution Systems (38), can be purchased system and used to filter out trace elements produced or exhausted during the fermentation, such as ammonia, chlorine or sulfur compounds, which may be hazardous if released directly to the environment. The total amount of energy required to run the 45 kW scrubber, including its fan, is 1,270,000,000 kJ annually.

Corn Grain Hammer Mill

It was additionally suggested during consultant meetings that an alternative to purchasing dextrose would be to invest in a corn grain hammer mill and operate this to produce a glucose source within the plant. Instead of purchasing glucose, we would purchase corn and process it to obtain the requisite glucose. A 2008 paper on *Industrial Crops and Products* (generously provided by Dr. Cohen) was consulted to estimate the capital and operating costs of such an addition to our process. The capital investment calculated in the paper is approximately $79 million; this additional investment, by itself, leaves the plant with a NPV greater than $300 million. What financially threatens the plant is the operating costs—an estimated $89 million in the 2008 paper. The corn grain hammer mill produces starch well in excess ($144 MT/hr) of the demands of our plant, so this value is likely a significant overestimate of what would be required to accompany the astaxanthin manufacture. Assuming a value of $89 million in operating costs, nonetheless, the plant is not profitable over the 15 year production life. That being said, it is just *barely* unprofitable; the reduction in operating costs to better match the needs of our plant are very likely to still yield a profitable design. Our final recommendation regarding the corn grain hammer mill is to (1) modify the standard design to require less corn and accommodate smaller outputs of glucose/starch, and (2) consider its use only if it is acceptable for the astaxanthin plant to have a lifetime in excess of 15 years.

22. Conclusions and Recommendations

The fermentative production of Astaxanthin via cultivation of genetically modified *E.coli* and supercritical fluid extraction with CO_2 and ethanol co-solvent was found to yield an ROI of 220%, making this a very economically profitable process. Further economic analysis is needed to gauge the additional costs of lab-scale optimization of Astaxanthin production in *E.coli*, GMO characterization, and technology licencing. We recommend that GMO characterization is completed to ensure that the *E.coli* are not an environmental or health hazard, such that the sale of dead cell mass (a waste product of the process) can be conducted to make additional profits. Furthermore, additional experimentation and optimization must be conducted to design the depressurization process equipment that separate CO_2 from Astaxanthin and ethanol following supercritical fluid extraction. As mentioned previously, the rapid expansion of supercritical fluid poses a complex problem for the agglomeration and recovery of Astaxanthin in the liquid ethanol phase, however the design of necessary equipment is out of the scope of this current evaluation.

An extraction method we had not moved forward with, on account of lack of available literature, is pressurized fluid extraction (PFE), otherwise known as subcritical fluid extraction. PFE uses temperatures and pressures in the range of 50–200 °C and 35–200 bar, below the supercritical state of the solvents, such that they remain in the liquid state. Extraction with dimethyl ether, DME, ($T_c = 127$ °C and $P_c = 53.7$ bar) is advantageous as DME is partially miscible in water, allowing for simultaneous extraction and removal of water from wet feeds (no need for drying of biomass prior to extraction). This method is environmentally friendly and cost effective, as the extraction takes place at room temperature and mild pressures, and final product recovery (removal of DME from the extract) can be achieved by evaporating the DME by depressurizing the collection vessel or by heating. For instance, Boonnoun, et. al, performed the extraction of Astaxanthin from H.Pluvialis at 20 C and 0.51 MPa, the saturated vapor pressure of DME. Although the study was conducted with undisrupted cells, leading to an extraction yield of 30%, yields of other carotenoids have been achieved by use of DME in cyanobacteria, suggesting that this method would be suitable for extraction from disrupted E.coli biomass. Another study, conducted by Huang, et. al, (39) achieved extraction efficiencies of 87.2% using dimethylaminocyclohexane (DMCHA) and removal of DMCHA by simple addition of H_2O and CO_2. A drawback of such studies was that they involved extractions on much longer time scales (up to 24 hours), and extraction was hindered by the need to permeabilize *H.Pluvialis* algal cell walls. However, if future research and experimentation indicates that extraction using subcritical fluids can be accomplished with shorter extraction times, this would be a very promising method to pursue.

23. Acknowledgements

We would like to thank Dr. Richard Bockrath, our project author, for providing us with precise guidance and enduring a great many emails and questions. Thank you to Dr. Warren Seider, our project advisor and process design professor, for sticking with us every step of the way, organizing meetings and materials, and teaching us everything we needed to know to complete the project professionally and accurately. We would also like to thank Professor Bruce Vrana for his continued responsiveness to questions and tireless efforts to keep our group, and the other ten groups, on schedule. We also owe a great debt to all of the CBE 459 industrial consultants who gave us sound advice and extra materials. In a semester interrupted by a once-in-a-lifetime public health event, this project progressed without significant interruption, in no small part to these hard-working and resolutely helpful individuals, and we hope they take pride in the results of this project as much as the authors do. Couldn’t have done it without you.

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Appendix A: Miscellaneous Calculations

Batch time calculations.

The growth rate of the genetically-optimized *E. coli* was taken as 0.224 hr^{-1}. The natural log term takes into account differences in input and output concentration as well as the dilution ratio, which is 10 except for the pre-seed fermenter, which instead is a dilution from 4 L flasks to a working volume of approximately 1800 L.

Pre-seed:

$$\Delta t = \frac{\ln(\frac{1}{0.67} * \frac{1780}{4})}{0.224\, hr^{-1}} = 29\, hrs$$

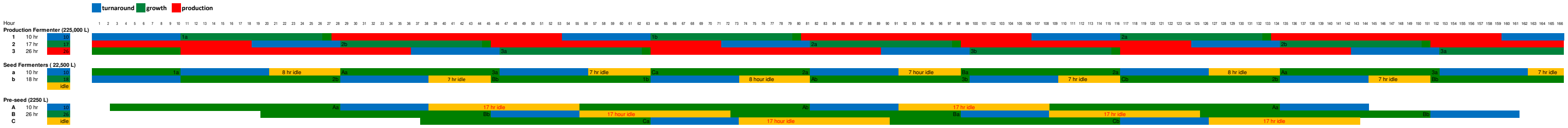
Seed:

$$\Delta t = \frac{\ln(\frac{6}{1} * 10)}{0.224\, hr^{-1}} = 19\, hrs$$

Production (growth):

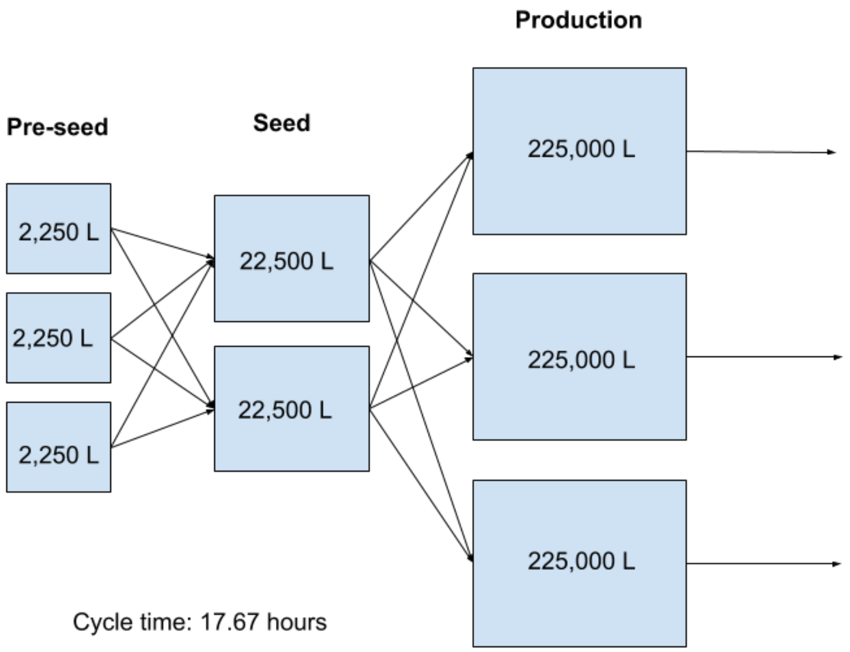
$$\Delta t = \frac{\ln\left(\frac{24}{6} * 10\right)}{0.224\, hr^{-1}} = 16.5\, hours$$

The batch time for the production phase of the production fermenter was a project given at 26.5 hours.


turnaround
growth
production
Hour
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 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 99 100 101 102 103 104 105 106 107 108 109 110 111 112 113 114 115 116 117 118 119 120 121 122 123 124 125 126 127 128 129 130 131 132 133 134 135 136 137 138 139 140 141 142 143 144 145 146 147 148 149 150 151 152 153 154 155 156 157 158 159 160 161 162 163 164 165 166
Production Fermenter (225,000 L)
1 10 hr 10
2 17 hr 17
3 26 hr 26
1a
1b
2a
2b
2a
2b
3a
3b
3a
Seed Fermenters (22,500 L)
a 10 hr 10
b 18 hr 18
idle
1a
8 hr idle
Aa
3a
7 hr idle
Ca
2a
7 hour idle
Ba
2a
8 hr idle
Aa
3a
7 hr idle
2b
7 hr idle
Bb
1b
8 hour idle
Ab
3b
7 hr idle
Cb
2b
7 hr idle
Bb
Pre-seed (2250 L)
A 10 hr 10
B 26 hr 26
C idle
Aa
17 hr idle
Ab
17 hr idle
Aa
Bb
17 hour idle
Ba
17 hr idle
Bb
Ca
17 hour idle
Cb
17 hr idle

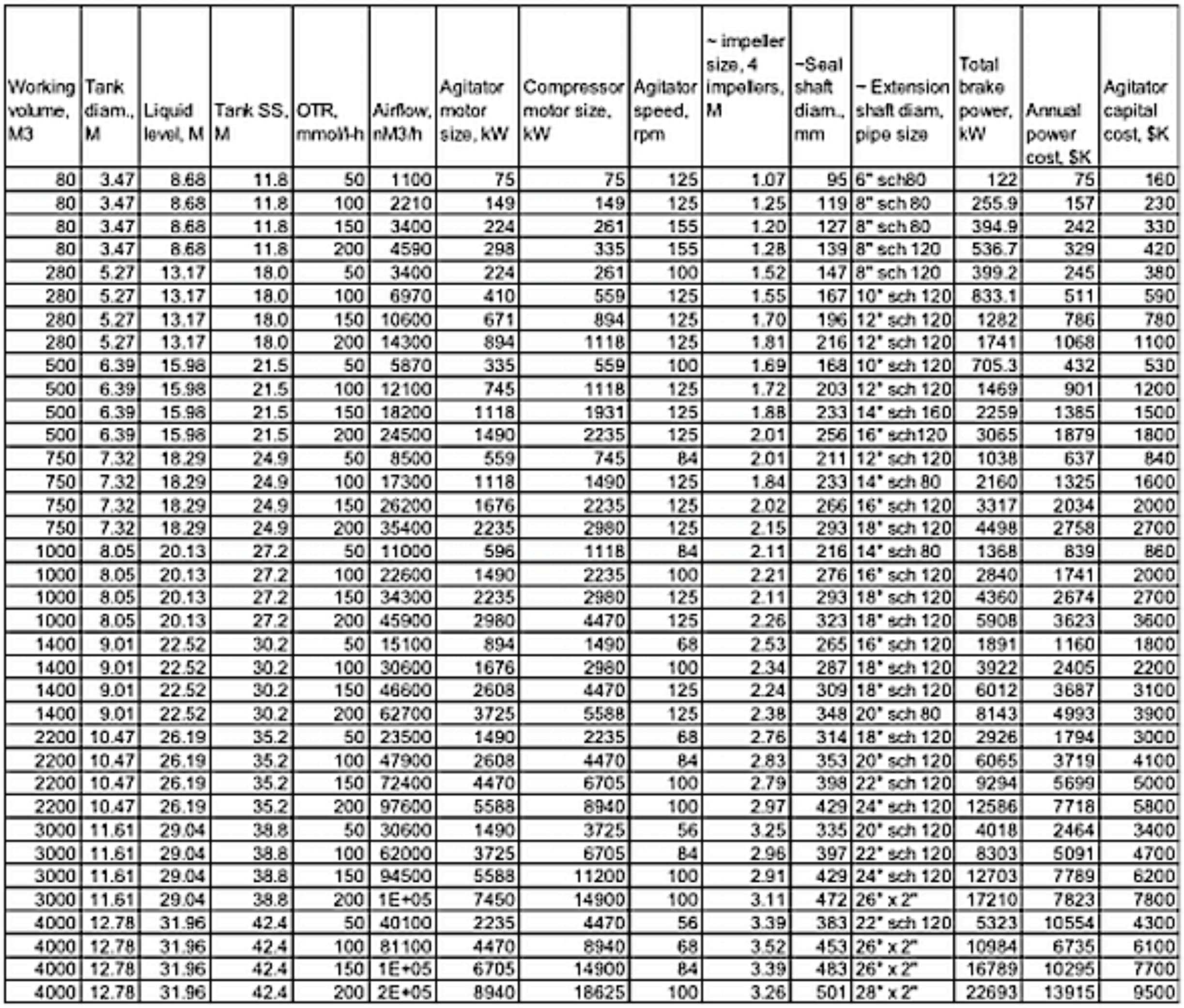
```

###############################################################
# CBE 459
# Spring 2020
# Group 4: Astaxanthin Manufacture
# This script performs an iterative MINLP procedure that the
# user can use to visually see the best arrangements of
# seed and pre-seed fermenters.
###############################################################

n3 = 3 # number of production fermenters
v3 = 225000 * 0.8 # working volume of production fermenter
v2 = v3 / 10 # volume of seed fermenter
v1 = v2 / 10 # volume of pre-seed fermenter

t1 = 39 # batch time, pre-seed
t2 = 29 # batch time, seed
t3 = 53 # batch time, production

H = 330 * 24 # horizon time, 7920 hours
Q = 60500 # astaxanthin demand, factoring in downstream
B = 0.75 * v3 / 1000 # astaxanthin made per production batch

for n1 in range(1, 6):
    for n2 in range(1, 6):
        print "########################################"
        print "## " + str(n1) + " pre-seed AND " + str(n2) + " seed ##"

        C = n1 * v1 + n2 * v2 + n3 * v3
        tau1 = float(t1) / n1
        tau2 = float(t2) / n2
        tau3 = float(t3) / n3
        tl = max(tau1, tau2, tau3)
        h1 = Q/B * tl

        print "Cost: " + str(C)
        print "Cycle time: " + str(tl)
        print h1
        print H
        if (h1 > H):
            print "Horizon constraint violated"

        print ""
```

Optimal seed train design. Common lines of transfer are shown between each of the stages, indicating that when a batch is completed, it goes to any available open fermenter at the next highest stage.



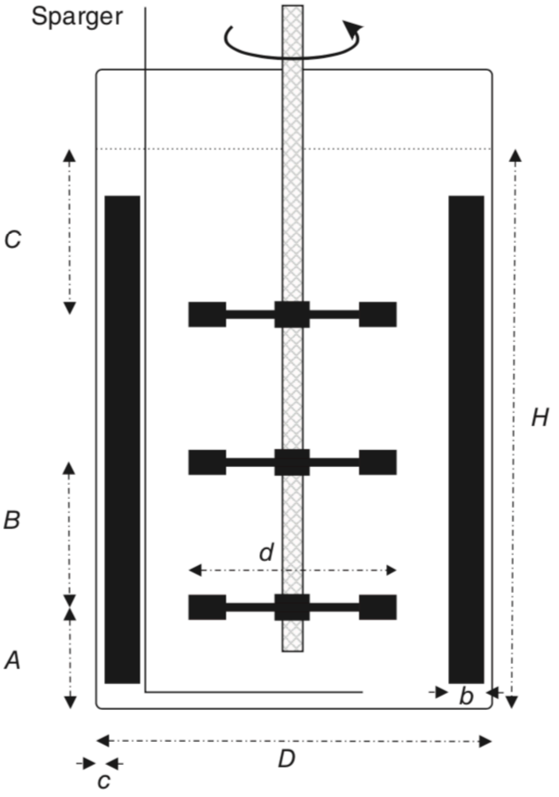
From *BioFuelsDigest.com*, article "Large Scale Microbial Production of Advanced Biofuels: How big can we go?" by Gregory Benz, 2014.
http://www.biofuelsdigest.com/bdigest/2014/12/07/large-scale-microbial-production-of-advanced-biofuels-how-big-can-we-go/

Table below gives characteristic measures of very large bioreactors, which were used to estimate aeration and agitation parameters for the production fermenter. Notably, the OUR of *E. coli* in this study was only about 24 mmol/L-h, which is why aeration was estimated to be 0.15 VVM during growth, instead of the ~0.22 VVM estimated by a number of bioreactors in this table.



Working volume, M3	Tank diam., M	Liquid level, M	Tank SS, M	OTR, mmol/l-h	Airflow, nM3/h	Agitator motor size, kW	Compressor motor size, kW	Agitator speed, rpm	~ impeller size, 4 impellers, M	~Seal shaft diam., mm	~ Extension shaft diam, pipe size	Total brake power, kW	Annual power cost, $K	Agitator capital cost, $K
80	3.47	8.68	11.8	50	1100	75	75	125	1.07	95	6" sch80	122	75	160
80	3.47	8.68	11.8	100	2210	149	149	125	1.25	119	8" sch 80	255.9	157	230
80	3.47	8.68	11.8	150	3400	224	261	155	1.20	127	8" sch 80	394.9	242	330
80	3.47	8.68	11.8	200	4590	298	335	155	1.28	139	8" sch 120	536.7	329	420
280	5.27	13.17	18.0	50	3400	224	261	100	1.52	147	8" sch 120	399.2	245	380
280	5.27	13.17	18.0	100	6970	410	559	125	1.55	167	10" sch 120	833.1	511	590
280	5.27	13.17	18.0	150	10600	671	894	125	1.70	196	12" sch 120	1282	786	780
280	5.27	13.17	18.0	200	14300	894	1118	125	1.81	216	12" sch 120	1741	1068	1100
500	6.39	15.98	21.5	50	5870	335	559	100	1.69	168	10" sch 120	705.3	432	530
500	6.39	15.98	21.5	100	12100	745	1118	125	1.72	203	12" sch 120	1469	901	1200
500	6.39	15.98	21.5	150	18200	1118	1931	125	1.88	233	14" sch 160	2259	1385	1500
500	6.39	15.98	21.5	200	24500	1490	2235	125	2.01	256	16" sch120	3065	1879	1800
750	7.32	18.29	24.9	50	8500	559	745	84	2.01	211	12" sch 120	1038	637	840
750	7.32	18.29	24.9	100	17300	1118	1490	125	1.84	233	14" sch 80	2160	1325	1600
750	7.32	18.29	24.9	150	26200	1676	2235	125	2.02	266	16" sch 120	3317	2034	2000
750	7.32	18.29	24.9	200	35400	2235	2980	125	2.15	293	18" sch 120	4498	2758	2700
1000	8.05	20.13	27.2	50	11000	596	1118	84	2.11	216	14" sch 80	1368	839	860
1000	8.05	20.13	27.2	100	22600	1490	2235	100	2.21	276	16" sch 120	2840	1741	2000
1000	8.05	20.13	27.2	150	34300	2235	2980	125	2.11	293	18" sch 120	4360	2674	2700
1000	8.05	20.13	27.2	200	45900	2980	4470	125	2.26	323	18" sch 120	5908	3623	3600
1400	9.01	22.52	30.2	50	15100	894	1490	68	2.53	265	16" sch 120	1891	1160	1800
1400	9.01	22.52	30.2	100	30600	1676	2980	100	2.34	287	18" sch 120	3922	2405	2200
1400	9.01	22.52	30.2	150	46600	2608	4470	125	2.24	309	18" sch 120	6012	3687	3100
1400	9.01	22.52	30.2	200	62700	3725	5588	125	2.38	348	20" sch 80	8143	4993	3900
2200	10.47	26.19	35.2	50	23500	1490	2235	68	2.76	314	18" sch 120	2926	1794	3000
2200	10.47	26.19	35.2	100	47900	2608	4470	84	2.83	353	20" sch 120	6065	3719	4100
2200	10.47	26.19	35.2	150	72400	4470	6705	100	2.79	398	22" sch 120	9294	5699	5000
2200	10.47	26.19	35.2	200	97600	5588	8940	100	2.97	429	24" sch 120	12586	7718	5800
3000	11.61	29.04	38.8	50	30600	1490	3725	56	3.25	335	20" sch 120	4018	2464	3400
3000	11.61	29.04	38.8	100	62000	3725	6705	84	2.96	397	22" sch 120	8303	5091	4700
3000	11.61	29.04	38.8	150	94500	5588	11200	100	2.91	429	24" sch 120	12703	7789	6200
3000	11.61	29.04	38.8	200	1E+05	7450	14900	100	3.11	472	26" x 2"	17210	7823	7800
4000	12.78	31.96	42.4	50	40100	2235	4470	56	3.39	383	22" sch 120	5323	10554	4300
4000	12.78	31.96	42.4	100	81100	4470	8940	68	3.52	453	26" x 2"	10984	6735	6100
4000	12.78	31.96	42.4	150	1E+05	6705	14900	84	3.39	483	26" x 2"	16789	10295	7700
4000	12.78	31.96	42.4	200	2E+05	8940	18625	100	3.26	501	28" x 2"	22693	13915	9500

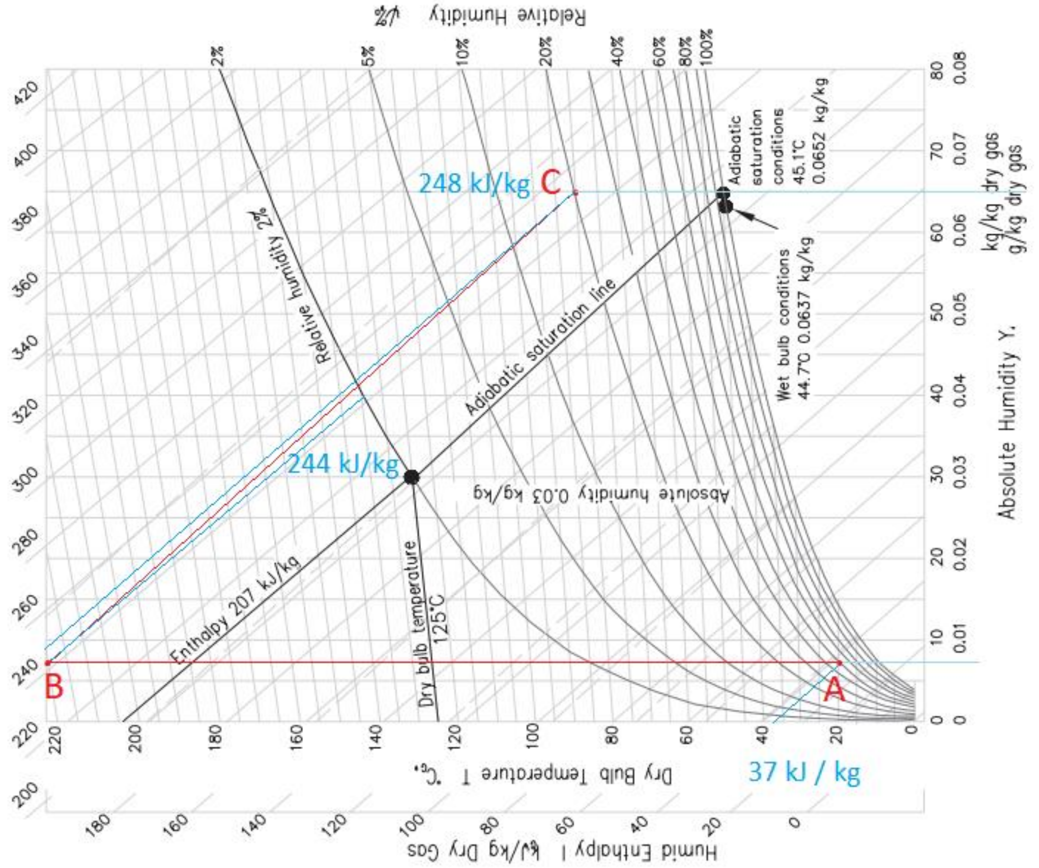
Taken from *Industrial Biotechnology: Products and Processes* (2016, Wittmann and Liao, Wiley Online Library) to estimate sizes of fermenters and agitators. Note that the book makes an error in the figure, calling the aspect ratio H/D = 1/3, when it is in fact H/D = 3 in the text.



A = Height from base to mid impeller
B = Distance between impellers
b = Baffle width
C = Distance between impeller and liquid level
c = Distance between baffles and vessel wall
D = Vessel diameter
d = Impeller diameter

A typical microbial fermenter vessel configuration:
3–6 baffles
$H/D = 1/3$
$d/D = 0.33–0.5$
$A/d = 0.5$
$B/d = 1$
$C/d = 0.5–1$
$b = D/10–D/12$
$c = D/72–D/50$

Figure 1.12 Standard design parameters for a microbial bioreactor vessel with typical values included. Parameters may vary based on the specific application.



Ambient air at point A, pre-heated air at point B, outlet air at point C.

To calculate required mass flow rate of air to evaporate 1210 kg/h water:

$$\frac{1210\frac{kg}{h}water}{(0.065-0.007)\,\frac{kg\,water}{kg\,air}} = 20862\ kg\ air.$$

Assuming 25% thermal efficiency, Required air = 4 * 20862 = 83448 kg air / hour.

Enthalpy balance:

$$83448\ kg\ air/hr * \frac{(224-227)kg}{kJ} = 1.73\ E\ 7\ kJ/hr$$

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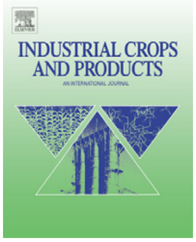

Engineering process and cost model for a conventional corn wet milling facility☆

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ABSTRACT

Conventional wet milling of corn is a process designed for the recovery and purification of starch and several coproducts (germ, gluten, fiber and steep liquor). The total starch produced by the wet milling industry in the USA in 2004 equaled 21.5 billion kg, including modified starches and starches used for sweeteners and ethanol production.

Process engineering and cost models for a corn wet milling process (for steeping and milling facilities) have been developed for a “generic” processing plant with a capacity of 2.54 million kg of corn per day (100,000 bu/day). The process includes grain cleaning, steeping, germ separation and recovery, fiber separation and recovery, gluten separation and recovery and starch separation. Information for the development of the models was obtained from a variety of technical sources including commercial wet milling companies, industry experts and equipment suppliers. The models were developed using process and cost simulation software (SuperPro Designer®) and include processing information such as composition and flow rates of the various process streams, descriptions of the various unit operations and detailed breakdowns of the operating and capital cost of the facility.

Based on the information from the model, we can estimate the cost of production per kilogram of starch using the input prices for corn and other wet milling coproducts. We have also used the model to conduct a variety of sensitivity studies utilizing modifications such as feedstock costs, corn compositional variations, and the sale of wet corn gluten feed. The model is also being used as a base-case for the development of models to test alternative processing technologies and to help in the scale-up and commercialization of new wet milling technologies.

This model is available upon request from the authors for educational, non-commercial and research uses.

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1. Introduction

Conventional wet milling of corn is a process designed for the recovery and purification of starch and several coproducts. The US corn wet milling industry can trace its beginnings back to 1844 when Thomas Kingsford, working at Wm. Colgate & Company in Jersey City, NJ, convinced his employer to try a new alkali process for extracting starch from corn. This plant became the world's first dedicated corn starch plant. Kingsford built his own corn wet milling facility a few years later in Oswego, NY (CRA, 2000). Many changes in processing and equipment have occurred over the last 160 years. The total starch produced by the wet milling industry in 2004 equaled 21.5 billion kg, including modified starches and starches used for sweeteners and ethanol production (CRA, 2005).

Prior to the 1880s, the corn refining industry simply discarded fiber, germ and protein from corn. Refiners began realizing the value of non-starch corn products to turn them into animal feed and extract corn oil from germ. These extractions not only decrease the production price of starch but also decrease starch loses and increased its quality.

Currently the end products of the wet milling process are starch slurry, germ, corn gluten feed and corn gluten meal. Starch is the primary product of the process (Blanchard, 1992). It is used with minimal further processing as a food additive or as an adhesive. Economically more important is its conversion to corn sweeteners and ethanol. Typical starch slurry leaving the mill house has 60% moisture content.

The germ is used for corn oil production and the resulting meal used for animal feed or added back to the corn gluten feed. Corn oil, the most valuable component of the corn kernel, is recovered from the germ by expelling or more often by solvent extraction. More than a million tonnes of corn oil are produced annually in the United States (Gunstone, 2006). Typical germ contains 48% oil, 13% protein, 12% starch, 2% ash and 3% moisture.

Corn gluten feed is the fiber rich component removed in the wet milling process. It is a high fiber, low protein feed used as energy, protein and fiber source for beef cattle. Corn gluten feed is produced by combining concentrated steepwater with the fiber during the separation process. This coproduct typically contains 60% fiber and 20% protein (White and Johnson, 2003).

Corn gluten meal is the high protein, low fiber fraction extracted during the wet milling process. It is used as an energy, protein, vitamin and mineral source for poultry and swine. The final corn gluten meal has typically 60% protein and 10% moisture (Blanchard, 1992; CRA, 1989).

Computer simulation models have been used successfully to understand processes and the physical and economical implications of experimental modifications. We developed engineering and economic models for the corn wet milling process (steeping and milling facilities) as research tools to help in the evaluation and optimization of the process and to aid in future process development. The models were developed using the software SuperPro Designer®, Version 7.0 (Intelligen Inc., Scotch Plains, NJ), based on previous models (Johnston et al., 2004) developed originally in Aspen Plus® (Aspen Technologies Inc., Cambridge, MA) and Microsoft Excel (Microsoft Corporation, Redmond, WA). Information on the corn wet milling process was obtained from various technical sources including commercial wet milling companies, industry experts and equipment suppliers.

2. Process model description

The conventional wet milling process includes many steps for the recovery and purification of starch and all coproducts (germ, gluten meal, and corn gluten feed). Our model is based on a "generic" processing plant with a capacity of 2.54 million kg of corn per day. The process and model is divided in six main sections, which include grain handling, steeping, germ separation and recovery, fiber separation and recovery, gluten separation and recovery and starch washing and recovery (Fig. 1). The unit operations in the model are identified by a number ID based on each one of the 6 sections (100's for grain handling, 200's for steeping, etc.) and the type of operation (one or two letters to identify equipment). All wet milling plants in US or around the world are quite similar in steeping and milling facilities. Depending upon the final end product (modified starch, glucose, High Fructose Corn Syrup (HFCS), ethanol or other fermentation products), downstream differences (after milling) exist in unit operations among wet milling plants. For greater usability and consistency in wet milling unit operations, this model was designed for only steeping and milling facilities (up to starch recovery). According to individual user requirements, additional downstream processes could be added if there is a need to model more specific products. Product yields generated from the model are shown in Table 1. The product yields are in line with information in Blanchard (1992) and from personal communication with Dorr Oliver (2002) and members of CRA. Table 2 shows the main unit operations and settings in the process model.

2.1. *Grain handling*

The corn is received in the facility and held in storage silos prior to cleaning. Small and large foreign matter in the corn is then removed to prevent clogging the screens, increasing viscosity during the process and affecting the quality of the finished products. This is represented in our model as a waste of 2.4% debris of total capacity. The silo in our model is sized to hold enough corn for 3 days of operation. Included in this area are also weighing and handling equipment. The cleaned corn is weighed and sent to steeping.

2.2. *Steeping*

The clean corn is soaked in a dilute SO_2 solution (steep acid), under controlled conditions of time and temperature. Steeping is the chemical processing step where the protein matrix is broken down to release the starch granules so they can be separated during subsequently milling. The objective of steeping is to facilitate the separation process by softening the kernel, increasing the moisture content of the grains and removing soluble matter. The overall efficiency of the wet milling process is dependent on the proper steeping of the corn. In practice, the steeping is done in a semi-continuous counter-

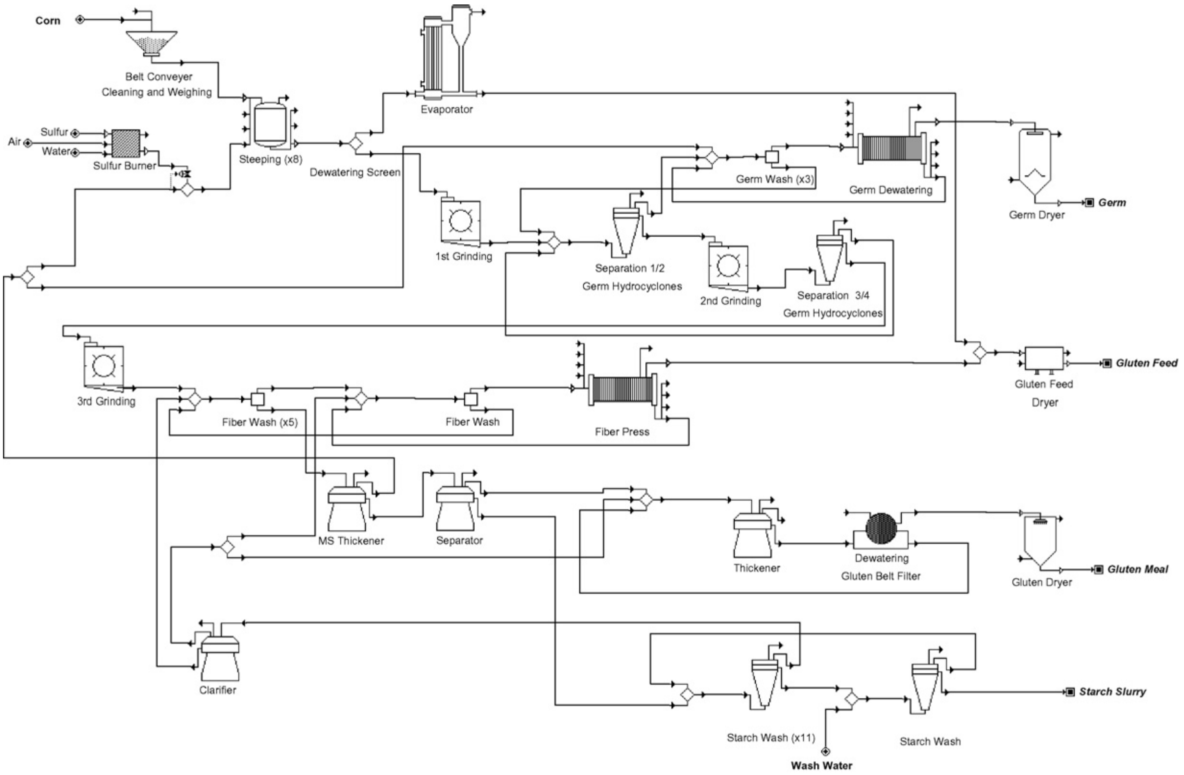

Fig. 1 – Simplified flow diagram of the corn wet milling process.

current system. During the steeping process the corn entering steeping is in contact with the most diluted, oldest SO_2 solution (light steepwater) and the oldest steeped corn is soaking in the most concentrated, freshest steepwater (steep acid). The corn does not move but the steepwater is transferred through the different tanks to go from the oldest steeped corn to the freshest. The water used for steeping is not fresh water but comes from downstream in the process; the SO_2 concentration is adjusted prior to steeping. The sulfur dioxide in our model is produced from burning elemental sulfur. During the steeping process, most of the soluble solids (about 69%) are removed and carried in the steepwater. This light steepwater (also called steep liquor) is concentrated to 50% solids, mixed with the corn fiber and dried to produce an animal feed as corn gluten feed. In our model, the corn is soaked in a group of eight stainless-steel tanks and held in the steep acid for a total of 36 h at 51 °C. The SO_2 concentration is 2000 ppm for the steep acid and 600 ppm for the light steepwater. The moisture content in the corn increases from 15 to 45% during steeping.

Table 1 – Corn wet milling product yields derived from the process model

Product	Yield (mass d.b. %)[a]
Dry germ	7.7
Gluten feed (steep water solids plus fiber)	19.4
Gluten meal	6.2
Starch	66.7

[a] Calculated on a dry weight basis after waste materials are removed.

2.3. *Germ separation and washing*

The germ is separated from the rest of the kernel after a coarse grinding. The swollen kernel is ground (first degermination) and the oil-rich corn germ is separated from the starchy slurry using four sets of hydrocyclones. The separation is based on the lower density of the germ, due to its high oil content, compared to the density of the slurry. The germ is retrieved from the overflow of the first set of hydrocyclones while the underflow continues the separation in the second set. After the first and second separations, the remaining slurry is ground again (second degermination) and any remaining germ is recovered by the last two sets of hydrocyclones. The overflows of all hydrocyclones, with the exception of the first set, are recycled to grind tanks to optimize the purity of the germ recovered. In order to achieve the desired separation, adjustments to the underflow-to-feed ratio (U/F) for the hydrocyclones are set. In our model, the U/F ratio has been set as 80, 70, 80 and 60% for the first, second, third and fourth hydrocyclones separations, respectively. The control of the specific gravity during germ separation is critical to proper recovery. Although it is possible to get a very clean separation of the germ from other components, the specific gravity is typically adjusted to allow some pericarp fiber (coarse fiber) to be co-recovered with the germ. This is done to aid in the oil extraction process because clean germ "slips" during expelling and decreases the oil extraction efficiency. The pure germ (overflow, separation 1) is washed in a series of screens using process water, dewatered to about

Table 2 – Overview of selected wet milling process equipment

Description	Detail
Belt conveyer	55.556 kg/s m loading rate/belt width
Cleaning	2.4% removed as debris
Steeping tanks	8 tanks 36 h residence time 90% volume 51 °C
Sulfur burner	2000 ppm of SO_2 in steeping tanks
Evaporator	Mechanical vapor recompression 50% solids steep liquor
Dewatering screen	DSM screen 50% overflow moisture content
Bauer mill—first grind	0.0087 kJ/s/(kg/h) specific power
Hydrocyclones 1/2	Primary germ separation
Bauer mill—second grind	0.0016 kJ/s/(kg/h) specific power
Hydrocyclones 3/4	Secondary germ separation
Screw press	Germ dewatering 50% final moisture content
Fluidized bed dryer	Germ dryer 0.07 kg natural gas/kg evaporated 3% moisture content
Disc mill—third grind	0.0116 kJ/s/(kg/h) specific power
Dewatering screen	Fiber wash DSM screens 76% overflow moisture content
Screw press	Fiber press 60% final moisture content
Rotatory dryer	Gluten feed dryer 0.07 kg natural gas/kg evaporated 10% final moisture content
Centrifuge	Clarifier 3744 l/min throughput 27% (w/w) solids in underflow
Centrifuge	Mill starch (MS) thickener 7378 l/min throughput 25% (w/w) Solids in underflow
Centrifuge	Primary separator 3012 l/min throughput 34% (w/w) solids in underflow
Centrifuge	Gluten thickener 2884 l/min throughput 16% (w/w) Solids in underflow
Rotatory drum vacuum filter	Gluten belt filter 60% final moisture content
Ring dryer	0.07 kg natural gas/kg evaporated 10% final moisture content
Hydrocyclone	Last stage of starch washing 4552 l/min throughput 1.3 kg fresh water/kg of dry corn

50% solids in a screw press and dried to a final moisture content of 3% in a fluidized bed dryer. In the model, the screens are represented by two-way component splitters and the screw press by a plate and frame filter. The dry germ is produced in our model at a rate of 7031 kg/h with a final lipid content of 43.7% on a dry weight basis and a protein content of 10.45%. The underflow of the last set of hydrocyclones (separation 4) continues the coproduct separation process.

2.4. *Fiber separation and recovery*

The degermed corn slurry from the germ separation is passed over the grit screen to separate water and loose starch and gluten (mill starch) from the fiber and bound starch and gluten. The mill starch is sent further in the process, for the separation of gluten and starch. The remaining solids (fiber and bound starch and gluten) are finely ground (third grind mill) to complete the dispersion of the starch; this milling is intended to free the starch with minimum fiber breakup. The ground slurry is then washed and separated in a series of tanks and fiber wash screens (six in our model), in a countercurrent fashion. The wash water (process water from the gluten thickener) is introduced in the last stage and it flows in a countercurrent fashion, finally coming out in the first screen with the free starch and gluten. The clean fiber is recovered in the last stage, dewatered first by a screen to a moisture content of 75% and then by a screw press to a final moisture of 60%. This fiber is usually combined with the concentrated steep liquor, dried in a rotary drum drier to 10% moisture and sold as corn gluten feed. The final corn gluten feed (19,000 kg/h in our model) has a protein content of approximately 20% on a dry weight basis.

2.5. *Gluten separation and recovery*

The gluten is separated from the starch by density differences in a disk stack centrifuge. Prior to the separation, the mill starch is degritted to remove any foreign particles such as sand, rust or pipe scale that might interfere with the centrifuges later in the process. The mill starch is then concentrated, to facilitate the separation, in a centrifuge called the mill-starch thickener. The thickened mill starch stream is passed to the primary centrifuge where the less-dense gluten (1.1 g/cm^3) is separated from the starch (1.6 g/cm^3). The purpose of the primary centrifuge is to obtain high-quality gluten in the overflow. The underflow, which contains the starch, some gluten and other impurities, is sent to the starch washing process. The gluten is then dewatered in three succeeding steps using a centrifuge (gluten thickener), a rotary vacuum belt filter and a ring dryer to a final moisture concentration of 10%. The gluten is sold, usually for animal feed, as corn gluten meal. The final corn gluten meal (6072 kg/h in our model) has a protein content of approximately 60% on a dry weight basis and contains xanthophylls that give it a yellow color.

2.6. *Starch washing and recovery*

The crude starch is washed in a series of small hydrocyclones, grouped in stages, in a countercurrent fashion. The wash water enters the system in the last washing stage, where the starch exits the process. During the washing, the underflow continues to the next stage while the overflow recycles back to the previous stage. The water with the impurities leaves the system at the first stage and it is recycled back to the middlings clarifier to concentrate the stream for further gluten and fiber separations. The overflow from the middlings clarifier contains the lowest concentration of solids in the system except for the fresh water. Our model shows the system with 12 hydrocyclone washing stages, each one with its own pump, using 2.3 kg of fresh water per kg of dry starch. The final starch slurry (144,000 kg/h) contains 60% moisture content with less than 1% of impurities.

3. Cost model description

A cost model of the dry grind ethanol process was developed to estimate the capital and production costs for the processing of corn in a wet milling facility. The data in the model was obtained from operators of wet milling facilities, equipment suppliers, pricing and cost data reported by trade organizations and government agencies and relevant publications. The assembling and analysis of this data was done using the cost estimating program in Superpro Designer®, using generally accepted methods for conducting conceptual economic evaluations for industrial processes (AACE International, 1990). The costs are not specific to any one plant since each facility has its own unique characteristics, but it is representative of currently operating wet milling processes in the United States with capital and operating costs typical of 2007.

The economic results in the model are linked to the physical flows and unit operations defined in the process simulation model. The results provide an understanding of the costs associated with the wet milling industry and help in the evaluation of the impact of wet milling costs due to changes in the composition and costs of the feedstock, products and processing operations of the process.

3.1. *Equipment and capital costs*

Corn wet milling facilities utilize milling equipment to break the structure of the corn kernel. Hydrocyclones, centrifuges and screens are used to separate components. Evaporators, presses, filters and dryers remove water. Tanks, conveyers and pumps are used to move and store the various process streams. Specialized equipment such as the sulfur burner is used to produce SO_2 needed for steeping. The proper application of much of this equipment to the wet milling industry is very specific and resides with the technology suppliers to this industry. The equipment sizing, materials of construction, and pricing for the development of this model came from these suppliers (Personal Communications, Andritz Sprout Inc., Barr-Rosin Inc., Dorr Oliver, 2002). Additional sources of equipment information for items such as tanks and pumps came from the Richardson's Process Plant Construction Estimating Standards, the Superpro Designer equipment cost estimating parameters and our internal cost database.

Equipment costs in the model can be changed by altering the number of pieces of equipment used, by substituting different prices or by changing the inputs, outputs and other characteristics of specific equipment items. If the user changes the process characteristics of an equipment item, the cost of that piece of equipment will be adjusted thru the use of a technique referred to as cost to capacity scaling factors. An understanding of this technique can be found in various texts on cost engineering (Jelen, 1970; Remer and Chai, 1990; Dysert, 2003).

Table 3 – Capital costs by system

System	Capital costs (US$ × 1000)
Corn handling and storage	
Corn storage	3,200
Corn handling	3,400
Steeping	
Steeping system	10,300
SO_2 generation	3,200
Steepwater evaporation	4,100
Germ separation	
Milling and separation	4,700
Washing	500
Drying	5,500
Fiber separation	
Separation and washing	10,100
Drying	8,700
Gluten separation	
Separation and washing	14,400
Drying	7,200
Starch washing	
Starch washing	4,000
Total capital cost	79,300

The capital cost of the facility has been developed from the costs of the individual equipment items. An installation factor of three times the equipment cost was used to develop the capital cost. This represents the cost of all the labor, materials and engineering for the wet milling processing unit and does not include costs for items such as laboratories, office buildings or railroad tracks to the facility. Table 3 presents the capital costs of the process by section and broken down by system. Working capital and cost of money during construction are not included in the capital equipment costs. Information found in Table 3 can help identify systems or unit operations, with high capital costs, for improvement and further study.

3.2. *Operating costs*

Shelled corn is the principal feedstock and it accounts for about three quarters of the facilities operating costs. Corn prices vary over time and by location and considerable care must be taken in selecting the appropriate value for use. Pricing information in the model is based on market prices published in 2007 by the United States Department of Agriculture (Baker and Allen, 2007). Water and sulfur are two other material inputs in the process. Process water is included at a rate of US$ 0.35 per 1000 l. A small amount of sulfur is consumed in the wet milling process to produce the SO_2 described in Section 2.2. The amount of sulfur consumed and its cost are included in the model. The total cost of sulfur is less than US$ 20,000 year^{-1}.

Natural gas, steam and electricity are the utilities required for the wet milling process. The utility that is required for each piece of equipment is calculated by the model. The steam is assumed to be generated using natural gas and the costs for both natural gas and steam are based on a natural gas price of US$ 0.3516 kg^{-1}. Electrical costs are estimated at a cost of US$ 0.014 MJ^{-1} (US$ 0.05 kWh^{-1}). The unit cost of utilities can be easily changed by the user as needed.

The cost of the plant operators to run the facility has been included at 5 people per shift at an all inclusive rate of US$ 50.00 h^{-1}. Additional operating costs included in the model include plant maintenance (6% of capital costs), insurance (1% of capital costs), local taxes (2% of capital costs) and miscellaneous facility expenses at 5% of capital costs.

3.3. *Product values*

Due to the variety of possible products derived from starch, the starch slurry is chosen as the primary product in the facility modeled. The starch slurry is suitable for further processing to different products, such as dried starch, modified starch, dextrins, sweeteners or ethanol. Corn germ and two protein rich animal feeds, corn gluten meal with a 60% protein content and corn gluten feed with a 20% protein content are also produced. They are considered as coproducts by the industry and in the model. Some facilities sell a limited amount of the concentrated corn steepwater, but in this model the concentrated steepwater is blended with fiber to produce corn gluten feed. Corn gluten feed and corn gluten meal are considered commodities and their market prices are published in various sources (Baker and Allen, 2007).

Market prices for corn germ, which is used for the extraction of corn oil, are not readily available, but can be calculated from the germ's protein and oil content and current market values of crude corn oil and corn based protein feeds (Johnston et al., 2005). In the model a market value of corn germ of US$ 0.296 kg^{-1} has been used based on a crude corn oil price of US$ 0.695 kg^{-1} (USDA, 2007) and a corn gluten feed price of US$ 0.08 kg^{-1} (Baker and Allen, 2007).

3.4. *Annual and unit production costs*

Annual production costs for the production of starch in a water slurry are calculated by adding together all the annual operating costs to produce the starch slurry and its coproducts and then reducing this number by the income received from the value of the coproducts of the starch production (Table 4). The annual production costs include a depreciation allowance of 10% of the capital cost to which is based on a 10 year effective operating life for the facility with no salvage value at the end of its life, and the operating costs described in Section 3.2.

Unit production costs are calculated by prorating the total annual starch production costs (total production costs less coproduct credits US$ 89,536,000 year^{-1}) over the total annual production (463,150,000 kg/year). Since the starch is produced as a slurry that is approximately 60% water, the unit production cost is based on the dry weight of the starch in the slurry and not the entire slurry. The unit production cost for the starch in our model is equal to US$ 0.194 kg^{-1}.

Table 4 shows the total raw material cost (US$ 111,414,000 year^{-1}) being 81% of the total operating costs. The corn cost represents more than 99% of the total raw material costs. The coproduct credits (US$ 47,808,000 year^{-1}) decrease the annual starch production costs by almost 35%.

Table 4 – Annual operating and production costs

	Annual cost (US$ × 1000)/year
Operating costs	
Raw materials	
Corn	111,018
Sulfur	19
Water	377
Total raw materials	111,414
Depreciation	7,933
Facility related costs	3,467
Utilities	
Natural gas	6,840
Steam	1,695
Electricity	4,015
Total utilities	12,550
Operations labor	1,980
Total operating costs	137,344
Coproduct credits	
Corn gluten meal	19,255
Corn gluten feed	12,071
Corn germ	16,482
Total coproduct credits	47,808
Annual starch production cost (operating costs minus coproduct credits)	89,536

3.5. *Sensitivities*

Technical models such as this one are useful in providing an understanding of the physical and economic constraints of a given process. They are also powerful tools for predicting the process outputs due to changes in process inputs. In this model, the impacts of pricing and minor compositional changes on the economics of the process are easy to modify. Unit operation changes, changes in feedstock flows and significant compositional changes in the process inputs are also possible but require a good understanding of the model's operation.

In our base model we used a corn price of US$ 0.132 kg^{-1} (US$ 3.36 bushel^{-1}) which was representative of corn prices in the spring of 2007. However, corn prices will differ by time period and by geographical location. The impact of the cost of corn on the cost of production of starch is shown in Fig. 2. The cost of starch production increases proportionately with the increase in the price of corn.

To further evaluate the engineering and cost models, we tested them using the composition of a corn with higher oil content (HOC) and analyze the outcome of the model comparing it to real yield data obtained experimentally in the lab. For this exercise, the composition of the corn had to be modified as well as the flow rates of other input streams (wash water, sulfur), the conditions of certain unit operations (evaporator, germ dryer and split after steeping). The HOC has a higher germ content (15% vs. 7.6% d.w.b.) and therefore lower fiber, gluten, soluble solids and starch content (61% vs. 66.5% d.w.b. for starch). The changes occurring throughout the process from the HOC increase the operating costs (mainly additional germ drying costs) by less than 0.3% (US$

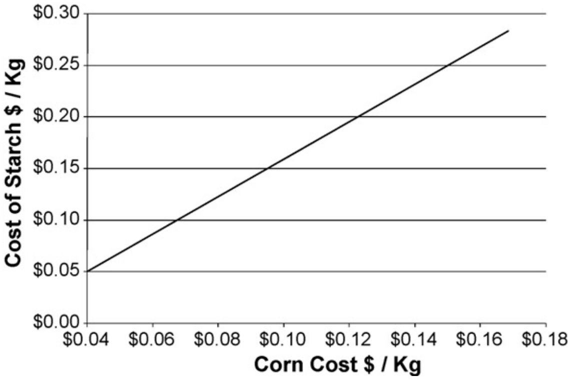

Fig. 2 – Impact of corn price on starch production cost.

267,000), but decreased the plant capital cost by 3.4%. The analysis of the impact on profitability shows the large increase in the sale of the germ more than offsets the increased operating costs and the decreased income from the starch, corn gluten feed and corn gluten meal. The net production income of the process (revenues less production costs) increases by US$ 3,427,000 or slightly over 3%.

4. Conclusions

A Technical Cost Model was developed for a corn wet milling processing plant with a capacity to process 2.54 million kg of corn per day. This model can be used in general as a tool to understand better the wet milling process and the cost issues associated with it. We use the model to conduct sensitivity studies using modifications like feedstock costs, variations in corn composition and sale of wet corn gluten feed. These and similar results contribute to the improvement of the process and the reduction of costs. Additionally, the process simulation model is currently being used to test alternative wet milling processing technologies and to predict the impact of those modifications.

Several areas have strong potential for future wet milling research. The removal of sulfur and the benefits/issues that this could have on processing and the coproduct composition could be very important for health and environmental effects. Another potential research area is the incorporation of membrane filtration technologies into the coproduct recovery processing and evaluation of the energy reduction potential they may offer. In each research area, the presented model should prove useful as a baseline comparison and a starting point for modifications.

4.1. *Model availability*

This model is available upon request from the authors for educational uses and non-commercial research to study the wet milling process and to show the impact of changes in the costs of starch and coproducts. It is not intended to replace a customized process design package. The model requires the use of SuperPro Designer®, Version 7.0 or later. A free copy of this program can be used to view the model and may be downloaded form the Intelligen website (www.intelligen.com).

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Appendix B: Vendor Specification Sheets

technical

Clearsweet® 99% Refined Liquid Dextrose Corn Syrup

Product Information

Cargill's Clearsweet® 99% Refined Liquid Dextrose Corn Syrup is a low ash, demineralized corn syrup with a high dextrose content. The high fermentability of this syrup makes it ideal for applications requiring a carbohydrate source such as fermentation for the food, pharmaceutical, and brewing industries.

REPRESENTATIVE CHEMICAL AND PHYSICAL DATA

Chemical and Physical Properties

Fermentables (%)	99.5
Refractive Index (20°C)	1.4629 – 1.4653
Refractive Index (45°C)	1.4581 – 1.4604
Total Solids (%)	70.5 – 71.5
Moisture (%)	28.5 – 29.5
Sulfated Ash (%)	0.05 max
pH (1:1)	3.5 – 5.0
pH (1:10)	4.0 – 6.0
Sulfur Dioxide (ppm)	2 max
Conductivity (30% DS)	65 micromhos
Calories/100g	284

Typical Carbohydrate Profile

(% Dry Basis)

Dextrose	99.0
Fructose	0.1
Maltose	0.6
Maltotriose	0.2
Higher Saccharides	0.1

Microbiological Limits

Mesophilic Bacteria	1,000 cfu/10g max
Yeast	100 cfu/10g max
Mold	100 cfu/10g max

Sensory Characteristics

Appearance	Clear Liquid
Taste	Sweet, Bland
Odor	Characteristic

Density and Viscosity Factors

Temp (°F)	Specific Gravity (Temp°F/60°F)	Pounds/ Gallon (Temp°F)	Pounds/ Gallon (DSB)	Viscosity (cP)
130	1.3239	11.04	7.84	41
140	1.3205	11.01	7.82	34
150	1.3170	10.98	7.80	28

Regulatory/Labeling Data

United States	
GRAS Affirmation	CFR 21 184.1865
Labeling	Corn Syrup; Dextrose
Canada	
FDR	B.18.016
Labeling	Glucose Syrup; Glucose

Shelf Life and Storage*

The recommended storage temperature range for Clearsweet® 99% Refined Liquid Dextrose Corn Syrup is 130 - 140°F. Syrups stored for extended periods (over 6 months) should be evaluated periodically for fitness of use.

*At below normal storage temperatures, e.g. room temperature, this product may be in a crystallized or "solid" state. It can be returned to its liquid form by applying heat. Please consult Cargill's *Storage and Handling Guidelines* for more details on proper handling.

contact

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Lancaster, PA 17603
Tel: 800-734-8215
Fax: 717-431-3120

400 E. Diehl Road, Suite 330
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Fax: 630-505-7846

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Updated 14-Aug-12

technical

Clearsweet® 99% Refined Liquid Dextrose Corn Syrup

Nutritional Information

Nutritional Profile

The following typical information may be of use in answering questions regarding ingredients in corn sweeteners. Unless specified otherwise, all values are reported on a commercial or "as is" basis.

Nutrient	Amount	Nutrient	Amount
Calories	284 cal/100 g	Cadmium	< 0.05 mg/100 g
Moisture	29.0 g/100 g	Caffeine	< 0.50 mg/100 g
Protein	0.01 g/100 g	Calcium	< 1.0 mg/100 g
Ash	0.05 g/100 g	Chloride	< 1.0 mg/100 g
Total Carbohydrate	71.0 g/100 g	Chromium	< 0.05 mg/100 g
Simple Sugar* (DSB**)	99.1 g/100 g	Copper	< 0.10 mg/100 g
Total Fat	< 0.10 g/100 g	Fluoride	< 0.10 mg/100 g
Dietary Fiber	< 0.10 g/100 g	Iron	< 0.05 mg/100 g
Cholesterol	< 0.10 mg/100 g	Lead	< 0.01 mg/100 g
Trans Fatty Acid	< 0.10 g/100 g	Magnesium	< 0.01 mg/100 g
Biotin	< 0.01 mg/100 g	Manganese	< 0.05 mg/100 g
Niacin	< 0.05 mg/100 g	Mercury	< 0.01 mg/100 g
Pantothenic Acid	< 0.30 mg/100 g	Molybdenum	< 0.10 mg/100 g
Riboflavin	< 0.01 mg/100 g	Phosphorus	< 0.01 mg/100 g
Thiamin	< 0.05 mg/100 g	Potassium	< 0.05 mg/100 g
Vitamin A	< 15 IU/100 g	Selenium	< 0.10 mg/100 g
Vitamin B_6	< 0.05 mg/100 g	Sodium	< 1.0 mg/100 g
Vitamin B_{12}	< 0.30 mg/100 g	Sulfur Dioxide	< 0.50 mg/100 g
Vitamin C	< 0.05 mg/100 g	Zinc	< 1.0 mg/100 g
Arsenic	< 0.10 mg/100 g		

* $DP_1 + DP_2$
** Dry Solids Basis

contact

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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.

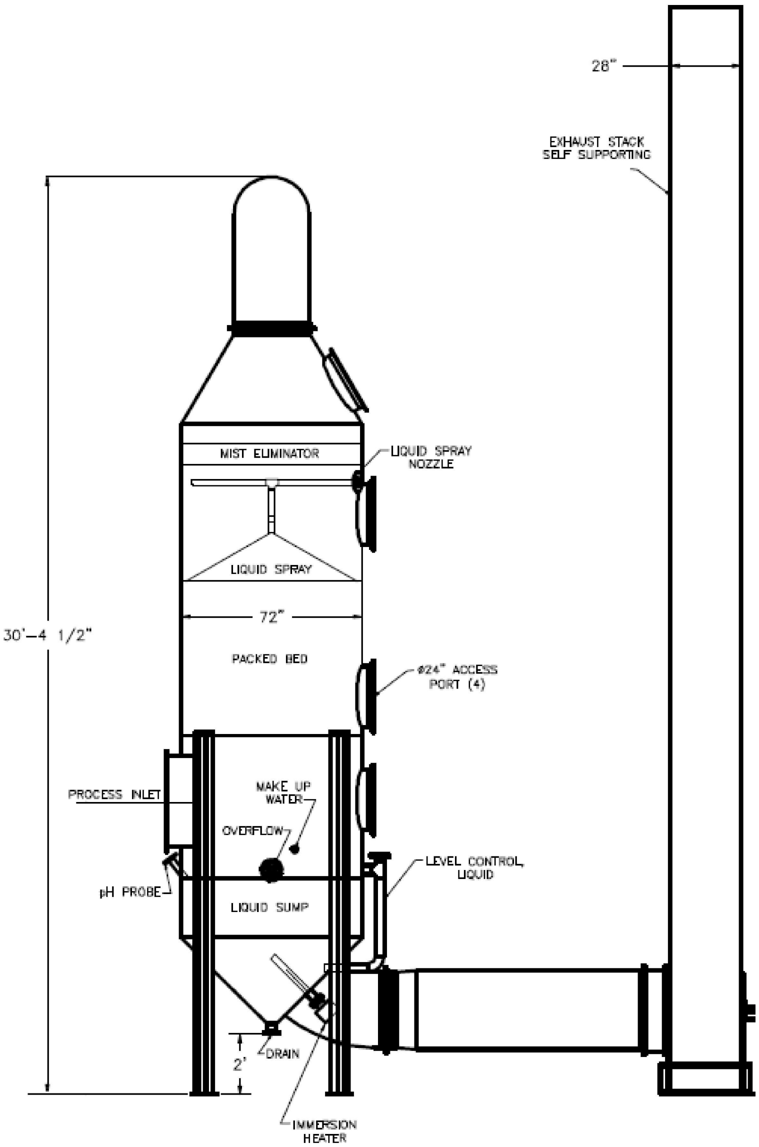
The buildup of salts in the scrubbing solution is limited using fresh makeup water and blowdown. The cleaned exhaust stream then passes through a mist eliminator where water droplets are removed. Finally, the cleaned air stream is discharged to the atmosphere.

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


28"
EXHAUST STACK
SELF SUPPORTING
MIST ELIMINATOR
LIQUID SPRAY
NOZZLE
LIQUID SPRAY
72"
30'-4 1/2"
PACKED BED
ø24" ACCESS
PORT (4)
PROCESS INLET
MAKE UP
WATER
OVERFLOW
LEVEL CONTROL,
LIQUID
pH PROBE
LIQUID SUMP
2'
DRAIN
IMMERSION
HEATER

CROWN
MACHINERY

2018 CATALOGUE

Crown Machinery Enterprise Introduction

Crown Machinery Inc. is a modern innovative high-tech centrifuge R&D and manufacture enterprise with four main branches in global work located in USA , South Korea, Philippine and China. Adhering to the concept of quality is the enterprise life, innovation is the driving force for the development, Conform to the trend of the development of modern industry, Fusion concept of global economic integration, Creative thinking, Integrate liquid separation processing industry leading enterprise in the upstream and downstream resources; Gather technical force; Together with the power of the global enterprise for business purposes; Dedicated to supply the clients complete separation solution.

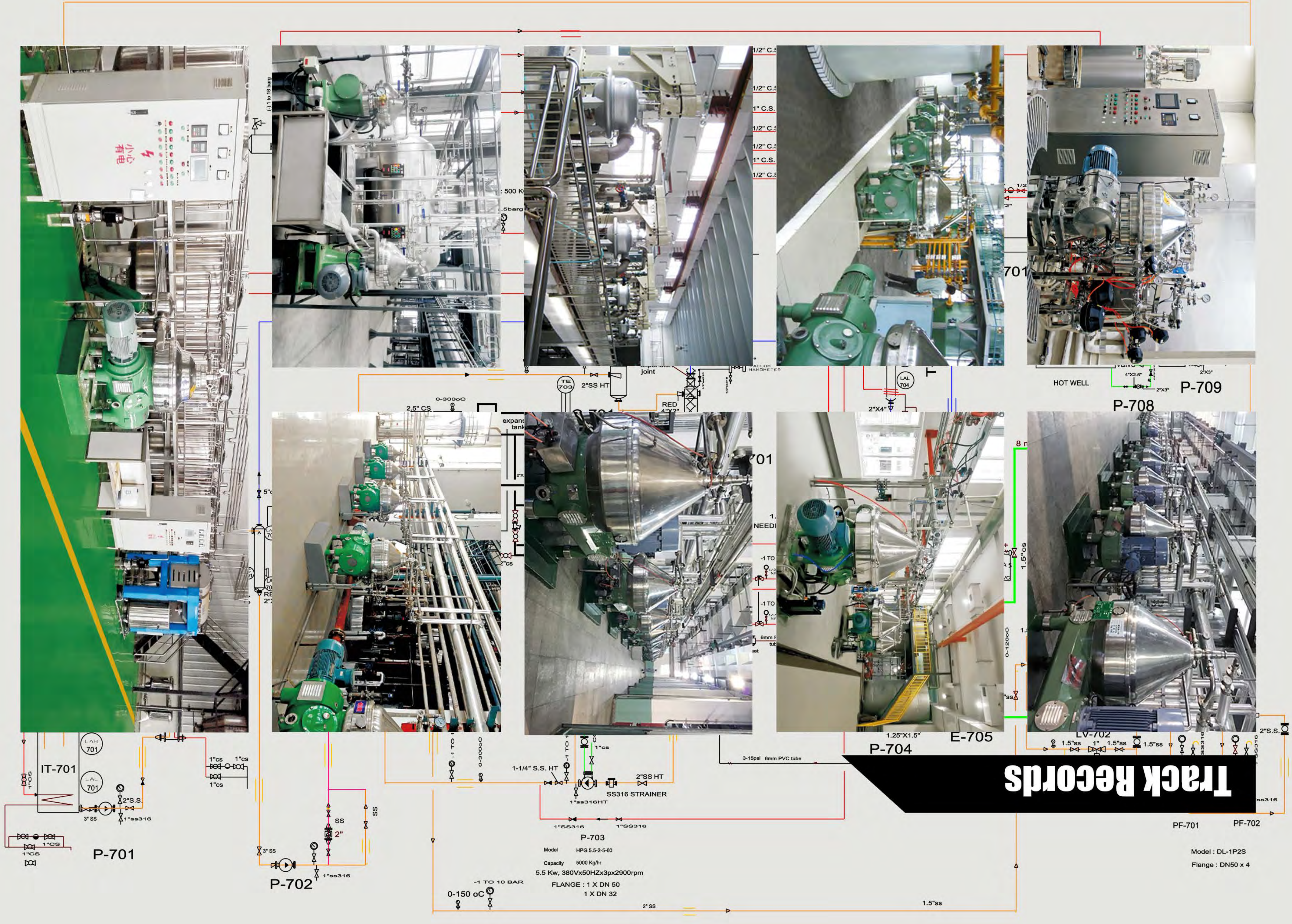
Our company assemble a number of skillful, talented professionals, introduction advanced of international centrifuge technology with 30 years experiences of the development and design, through adopting international advanced management method, we have developed very professional separator and centrifuge for edible oil , pharmaceutical , chemical , waste project and various liquid industry. Until now we have accumulated more than 500 clients in global world market and get wide good feedback for our products and service, as our enterprise name "Crown Machinery" described we would like to supply the products like the crown quality and service.

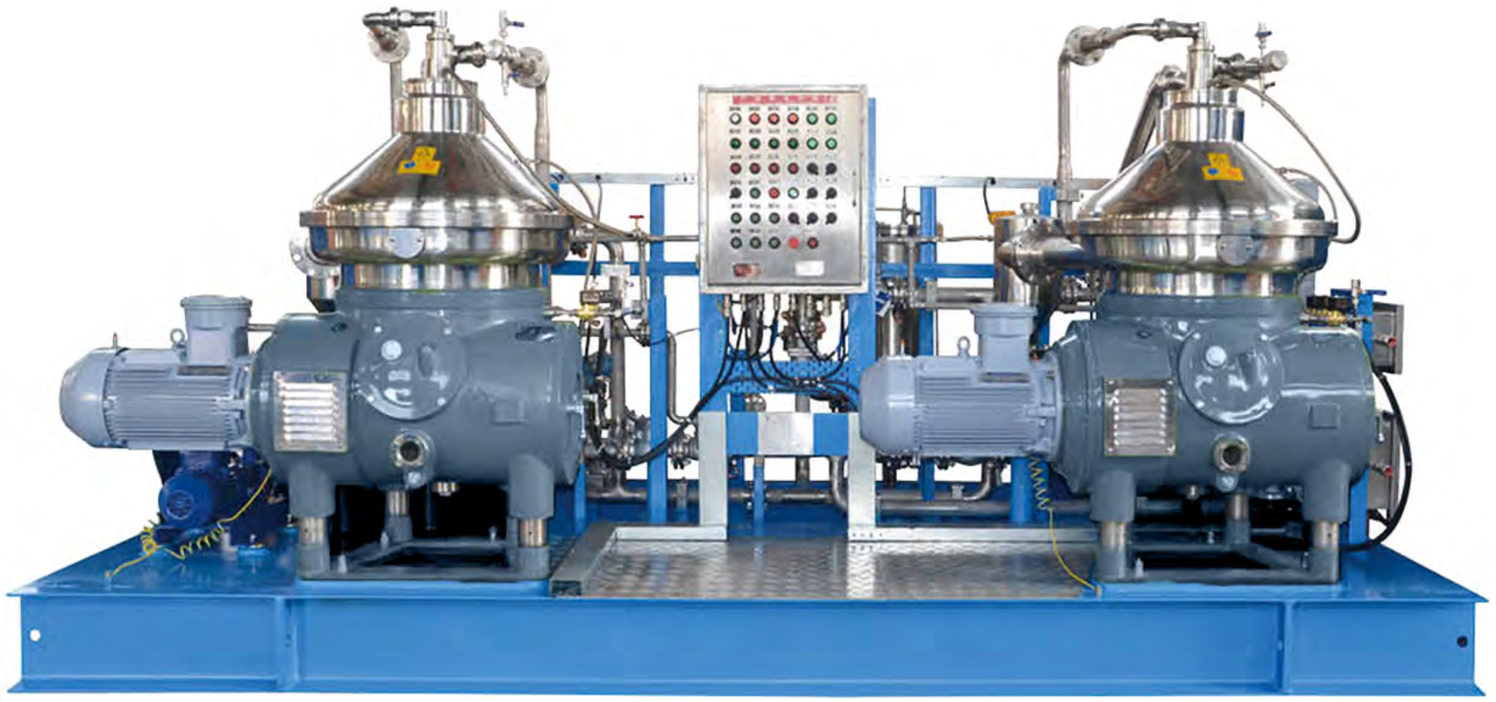
Nowadays, our USA branch mainly forwards the waste-water market; Manila branch mainly prompts the coconut products machinery in Asia-Pacific market; Our Korean branch also named the Hanil Science Medical Co.,Ltd. is focusing on the Bio-tech and Bio-pharmaceutical market; And Liaoyang Crown Machinery Co.,Ltd. in China works as the head-quarter of four branches to serve the machinery selection, sale, technology support and after-service job.

Up to now, we have successfully introduced many clients' final products such as coconut oil into Chinese market to achieve a mutually beneficial win-win situation. We do hope serving the client not only the products but also the wonderful experience to cooperate with us.

Welcome to contact and visit us.

Track Records





Disc Stack Centrifuge

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China

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Recoletos St., Intramuros Manila
Philippines

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Korea

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USA **+1 262 656 7680**
Korea **+82 42 825 4260**

📠 +86 419 2122006

Disc centrifuge is a type of separator that has a series of conical discs which provides a parallel configuration of centrifugation spaces. The disc centrifuge is used to remove solids (usually impurities) from liquids or to separate two liquid phases from each other by means of an enormously high centrifugal force. The denser solids or liquids which are subjected to these forces move outwards towards the rotating bowl wall while the less dense fluids moves towards the centre. The special plates nown as disc stacks) increase the surface settling area which speeds up the separation process. Different stack designs, arrangements and shapes are used for different processes depending on the type of feed present. The concentrated denser solid or liquid is then removed continuously manually or intermittently, depending on the design of the conical plate centrifuge.

TYPICAL APPLICATIONS

BEVERAGE / BREWING INDUSTRY

- Fruit and vegetable juices
- Pectin
- Citrus fruits and tropical fruits
- Essential oils
- Beer
- Wine
- Coffee and tea
- Potable alcohol

CHEMISTRY / BIOTECHNOLOGY

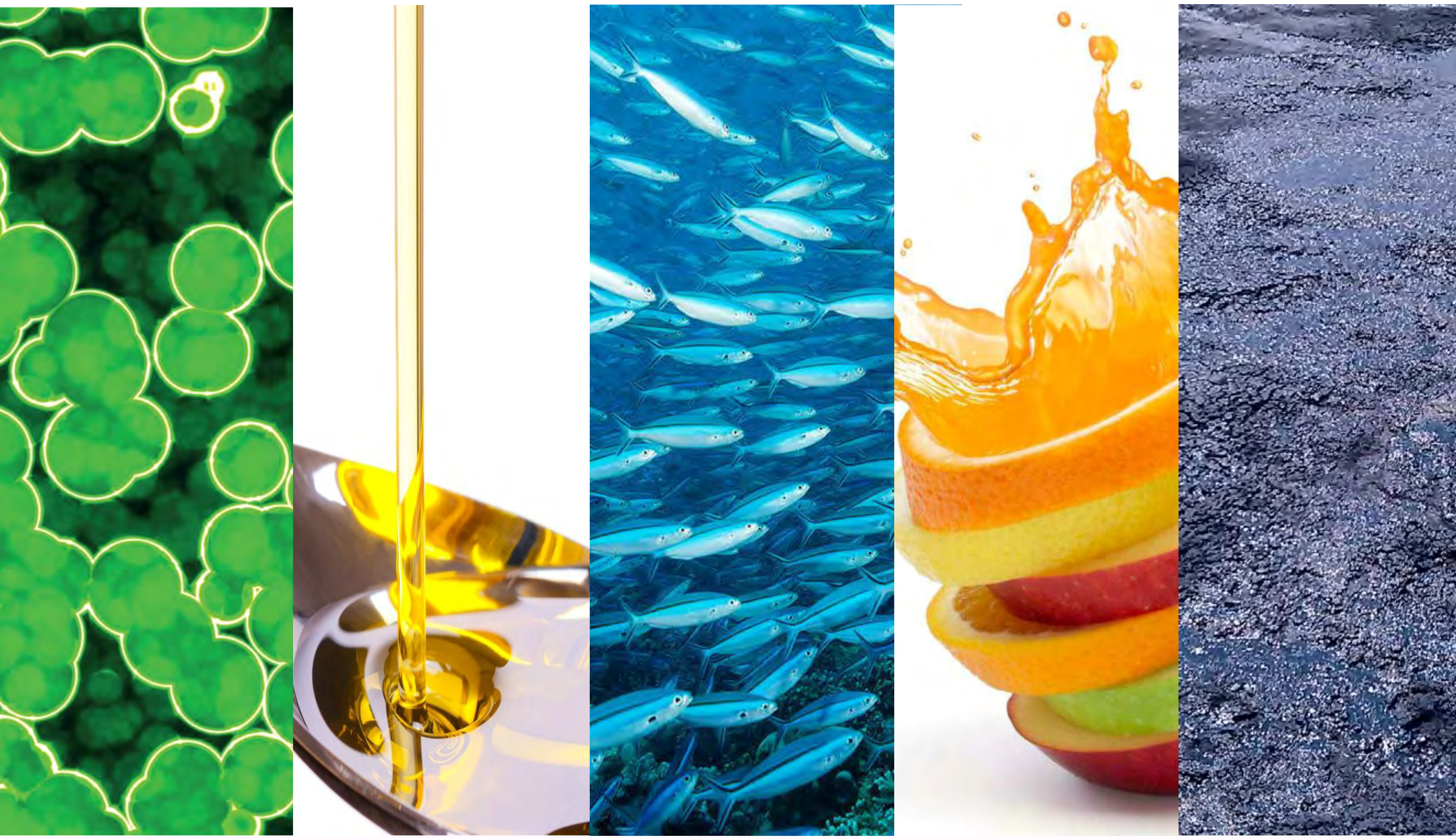
- Process waste water
- Production of vitamins
- Pigments
- Solvents
- Algae (food, cosmetics)
- Fermentation broths (industrial biotechnology)

FATS / OILS / BIOFUELS

- Biodiesel
- Algae (biofuels)
- Fish oil
- Animal fats
- Vegetable oil
- Coconut oil
- Olive oil
- Avocado oil
- Palm oil
- Soybean oil

INDUSTRIAL / MINERAL OILS

- Processing slop oil and oil residues
- Reservoir water



Complete Liquid Solution Provider

The various parameters have a significant influence on separation efficiency. Knowing these parameters means increasing the efficiency and yield of your separation process.

We support our customers along the entire process chain based on our 30 years mature separation experience and technology.

Advantages of Disc Stack Centrifuge:

- Easy maintenance and fair prices for service and maintenance thanks to compact machine design
- Individual and flexible adaptation to customer processes for optimal yields
- Full automatic discharge or manual discharge system can be chose.
- Customized solutions which can easily be integrated into existing processes and systems
- Optimum process layout: selection or combination of different separation equipment: decanters, disc stack centrifuges, belt presses and systems

DGS/DGC-700

DGS-400

DGS/DGC-300

DGS/DGC-500

Centrifuge Machine Standard Design

The machine has a main frame that consist a horizontal drive shaft with clutch and brake, worm gear, lubricating oil bath and vertical bowl spindle in the lower position.

The bowl is mounted on top of the spindle, fixed by the upper parts, the gasket, the collecting parts, and frame hood. The material feed into the bowl, by the effects of centrifugal force the liquid phase pumped out of machine through outlet pipe, meanwhile the solid phase adhere on the bowl wall, then were discharged automatically by operation water. The electric motor is of the variable frequency drive type or of controlled-torque type. All parts in contact with material are made of stainless steel.

Basic Equipment

Concentrator or purifier parts, inlet and outlet devices, revolution counter, set of erosion-protective parts, illuminated sight glass for liquid phase outlet, vibration sensor, vibration-isolating base plate, flange motor, set of tools and set of standard spare parts.

Optional Extras

Electric cabinet, frequency converter, discharge control panel, standard set of fittings, set of CIP valves and fittings and serviceability package for on-line viewing of separator status

Material Data

Bowl body, hood and locking ring S.S304 Solids cover and frame hood S.S304 Frame bottom parts green casting iron Inlet and outlet parts S.S304 Gasket and O-ring Nitrile rubber

Disc Centrifuge Main Parameter

Model	Bowl Speed (rpm)	Through-put Capacity (L/H)	Running Load (kw)	Dimensions (mm)		
				Width	Front-to-Back	Height
300	7302	300-500	4	950	950	1250
400	7070	1000-2000	7.5	1555	1130	1640
480	6600	3000	15	1780	1500	1900
500	6600	5000	18.5	1780	1500	1900
550	6000	10000	22	1800	1850	1900

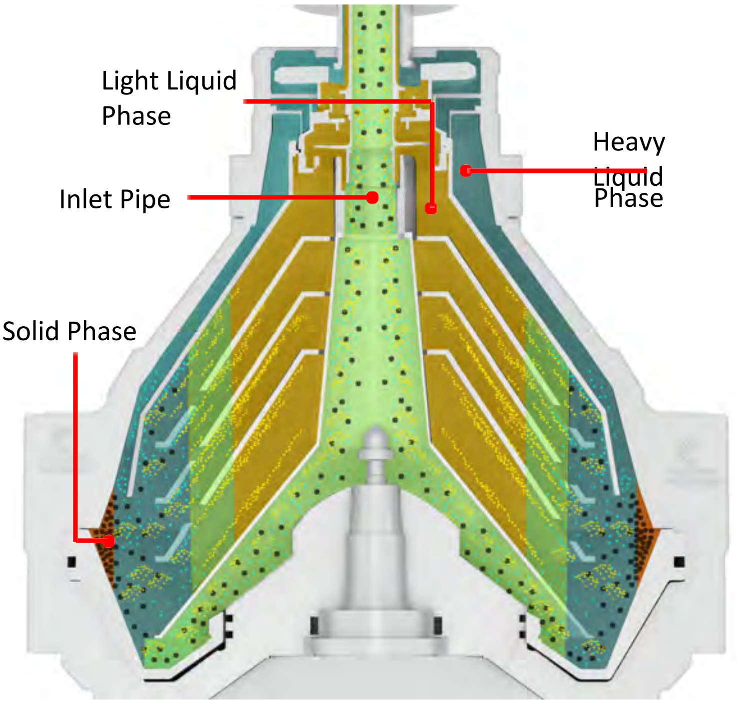
*Actual production capacity base on the raw materials.

DGS series Disc Centrifuge Operating principles

Whole separation process of a disc centrifuge accomplished through a rotating bowl, which is mounted on the top of the vertical axis driven by the motor at high-speed rotation. The bowl consist a paring of discs that are nestled together, and a small space between the disc.

Emulsion is added by a inlet pipe located in the center of the bowl. When the emulsion flows through the gap between the discs, the liquid phase of emulsion layered under the centrifugal force and form on the surface of the disc, then light liquid phase flow upward through disc, and heavy liquid phase upward flows close to the wall of bowl, and the separated liquids discharge from the outlet pipe. The solids particles automatically discharge for each batches.

* An emulsion is a mixture of two or more liquids that are immiscible indeed, possible with a certain amount of solid particles

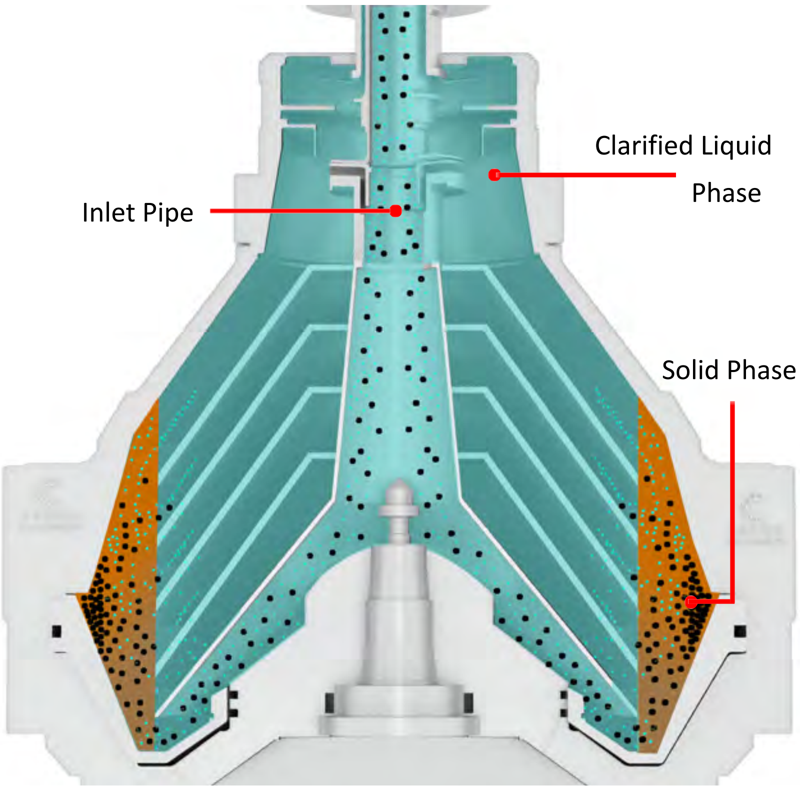


DGC series Disc Centrifuge Operating principles

Whole separation process of a disc centrifuge accomplished through a rotating bowl, which is mounted on the top of the vertical axis driven by the motor at high-speed rotation. The bowl consist a paring of discs that are nestled together, and a small space between the disc.

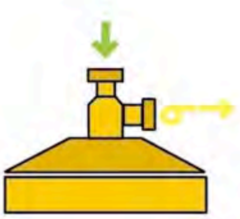
Suspension is added by a inlet pipe located in the center of the bowl. When the suspension flows through the gap between the discs, the solid particles settle under the centrifugal force on the disc to form a sediment, then it slides out of the disc surface and accumulates in the largest diameter of the bowl, and the separated liquid discharges from the outlet of bowl. The solids phase will automatically discharged for batches.

* A suspension is a heterogeneous mixture containing solid particles that are sufficiently large for sedimentation.



Liquid Feeding/Discharging Configuration

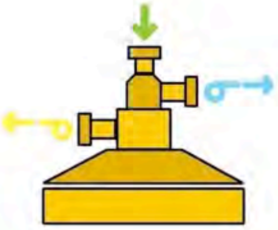
DGC series
One liquid Phase



🟢 Feeding Material

🟡 Liquid Phase

Flow with Pressure



DGS series
Two liquid Phase

🟢 Feeding Material

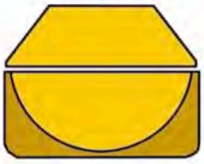
🟡 Light Phase

Flow with Pressure

🔵 Heavy Phase

Flow with Pressure

Discharging Method



Manual Discharging

Shut down and open the bowl, manually remove the inside sediment by labor.

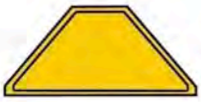
Automatic Discharging

Through Intermittent open lower parts of the bowl, sludge discharged automatically.

Continuous Discharging

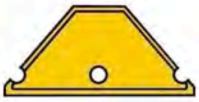
Sludge discharging achieve by the nozzles around the pericline.

Disc Separation



Clarification

Separate the solid particles form the liquid



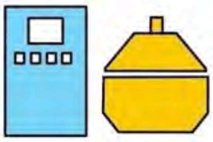
Separation

Separate a heavy liquid phase from major light liquid phase, meanwhile the suspended solid particles be separated as well. Maximum level of purified the light liquid phase.

Concentration

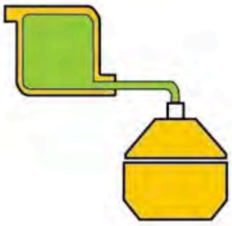
Separate a light liquid phase from major heavy liquid phase, meanwhile the suspended solid particles be separated as well. Maximum level of purified the heavy liquid phase.

Optional Components and Systems



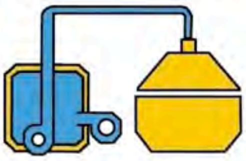
Electric Cabinet

Monitoring and adjustment of power , parameters setting and safety devices.



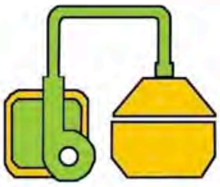
Gravity Feeding System

Ensure the material contnous and stable feeding to centrifuge.



CIP Cleaning System

Control the system clean the separation components automatically.



Feeding Pump

Ensure the flow of material to the centrifuge is stable and adjusted automatically.

CROWN
MACHINERY

2017 CATALOGUE

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Iran
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GEA One Series Homogenizers

Simple the best

engineering for
a better world

gea.com

Applications, benefits and features

Now the quality of the very best homogenizers is also available for simple processes and small-medium productions. The 3-piston homogenizers of One Series are manufactured to ensure easy maintenance and simple installation into small systems for dairy products, beverages and soft chemicals.

The simplest is the most reliable solution

The homogenizers from the One range combine convenience and absolute quality to deliver unmatched benefits.

Constant design innovation, optimization of production processes, material selection, excellent cost-quality ratio and unmatched performance make this the best solution available on the market.

- SIMPLE: One offers a simple design construction, complete with all the options needed to be easily integrated into the system, as ready-to-use and low maintenance units.
- FLEXIBLE: available in five versions, One homogenizers can meet any production need (from 300 l/h to 10.000 l/h up to 250 bar).
- RELIABLE: constant engineering improvement, excellent quality standard of our production and the competence of our personnel are the winning drivers to guarantee long lasting machine.
  We have had only one complaint in the last 10 years on the head block due to a sizing problem.

Applications

One is an ideal homogenizer for dairy products and beverages, such as:

- Pasteurized flavoured milk
- Milk cream
- Cheese
- Yogurt drink
- Dressing
- Ice cream
- Fruit juice
- Cloudy drink
- Butter oil
- Tomato products
- Liquid soap & detergent
- Wax emulsion

Main advantages

EASY TO USE

REDUCED MAINTENANCE COST

SAFE SANITARY DESIGN

LOW ENERGY CONSUMPTION

COMPLETE INSTALLATION PACKAGE

Technical features

Constructed to improve the process performance and reduce operating costs, One machines feature one-piece compression head made in forged duplex stainless steel and machined with CNC equipment to strict quality parameters. The limited number of parts and the simplified construction allow a simple use and maintenance.

The design and surface finish of the parts ensure safe sanitary execution for optimized CIP of machine parts that come into contact with the processed product. One homogenizers, available for one or two stages, come in a wide range to meet every production need. The objective is a product with constant, optimized quality that will open new market opportunities.

Absolute quality is our standard

The energy efficient solutions of One range homogenizers are the fit for purpose solutions for small and medium size process lines with reduced consumptions and optimized running costs. The 3-piston homogenizers, built according to EN ISO 9001:2008 Quality System and suitable for CIP and SIP, can reach a working pressure up to 250 bar. No compromise on quality: One is the modern answer to market demands, designed to give you everything you need at the right price.

Designed and manufactured to ensure flexibility, easy installation and maintenance, these machines can be easily integrated into small systems in dairy, beverages and soft chemicals productions.

Quality of GEA One TS and GEA One TF

A complete homogenizers family at your disposal. One Series can be divided in two size families: GEA One Homogenizers TS and GEA One Homogenizers TF. Both can be customized with two homogenizing stages and electric power board. As standard they are available with the following characteristics:

DUPLEX ALLOY MONOBLOCK

TUNGSTEN CARBIDE HOMOGENIZING VALVE

HIGH EFFICIENCY MOTORS

GEA One Homogenizers TS

Homogenizers from One TS are ideal machines for small production volumes up to 4.500 l/h, with top sanitary features and splash lubrication. Designed and constructed to ensure flexibility and ease of installation and maintenance, these machines can be easily integrated into small systems.

GEA One Homogenizers TF

Homogenizers from One TF series are fit for purpose solution for medium size process lines up to 10.000 l/h and variable speed drive. They feature a forced lubrication with gear pump and low oil pressure switch.

PERFORMANCES

Max Flow Rate l/h Pressure bar	One Homogenizer 7TS	One Homogenizer 11TS	One Homogenizer 15TS	One Homogenizer 37TF	One Homogenizer 75TF
100	1.050	3.300	4.500	8.000	10.000
130	1.050	2.500	3.400	8.000	10.000
150	1.050	2.200	3.000	7.500	10.000
180	1.050	1.800	2.500	6.500	10.000
200	850	1.650	2.200	6.000	10.000
210	850	1.550	2.100	5.700	10.000
240	800	1.300	1.800	5.000	10.000
250	650	1.300	1.800	4.000 - 4.800	9.800
Power kW	**7,5**	**11**	**15**	**37**	**75**

Technical specifications are preliminary and subject to modification without notice

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.

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gea.com/contact

Twin Dome Extruder

Model TDG-110G

TECHNICAL BULLETIN

LCI's TDG-110G Twin Dome Granulator is a production scale twin screw extruder, which converts wetted powders into pellets of a controlled size and shape ideally suited for spheronization.

Features

- Low pressure extrusion with negligible heat generation
- Screw transport ensures plug flow, first in first out
- Two 110 mm diameter screws
- Easy-to-clean clamshell design extrusion chamber for easy access to process parts
- Hydraulic clamping system for ease of disassembly
- Capable of producing 200-300 kg/hr
- Dome dies capable of producing 0.6-2.0 mm diameter extrudates
- 316 SS GMP design with high polish finish
- Caster mounted
- 10 hp inverter duty motor in 460 VAC/3/60
- Integrated controls on articulating arm / integrated electrical panel

Operation

The wet mix, which is typically prepared in a high shear granulator, is metered into the feed hopper by a feeder. Twin screws transport the wet mix to the extrusion zone where it is then wiped through a dome die (screen) to produce well formed, cylindrical extrudates of a controlled diameter. The extrudates break off by their own weight and are collected for the next processing step.

The patented hemispherical shaped dies result in evenly distributed extrusion forces.

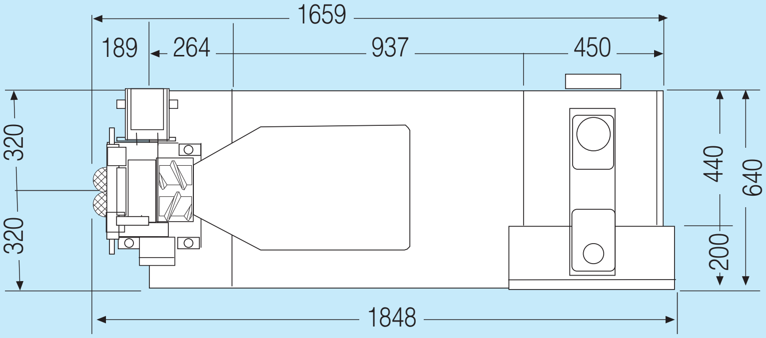
Integrated controls are mounted on an articulating arm.

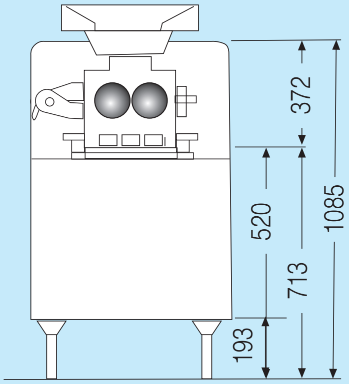
Left: The extrusion chamber with clamshell design opens quickly for easy cleaning and screen changing.

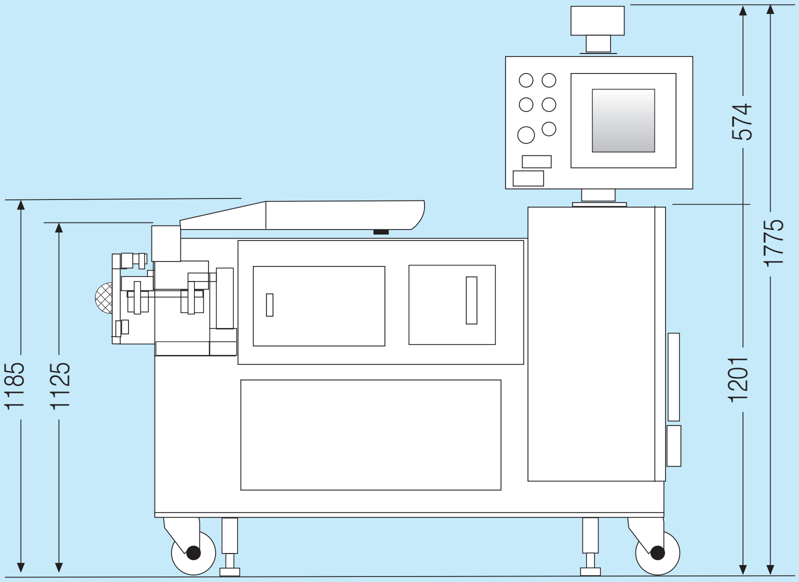
LCI Corporation is the exclusive distributor for Fuji Paudal Company, Ltd. in the Americas.

Twin Dome Granulator TDG-110G and Control Panel Dimensions (shown in mm)

Weight: 900kg





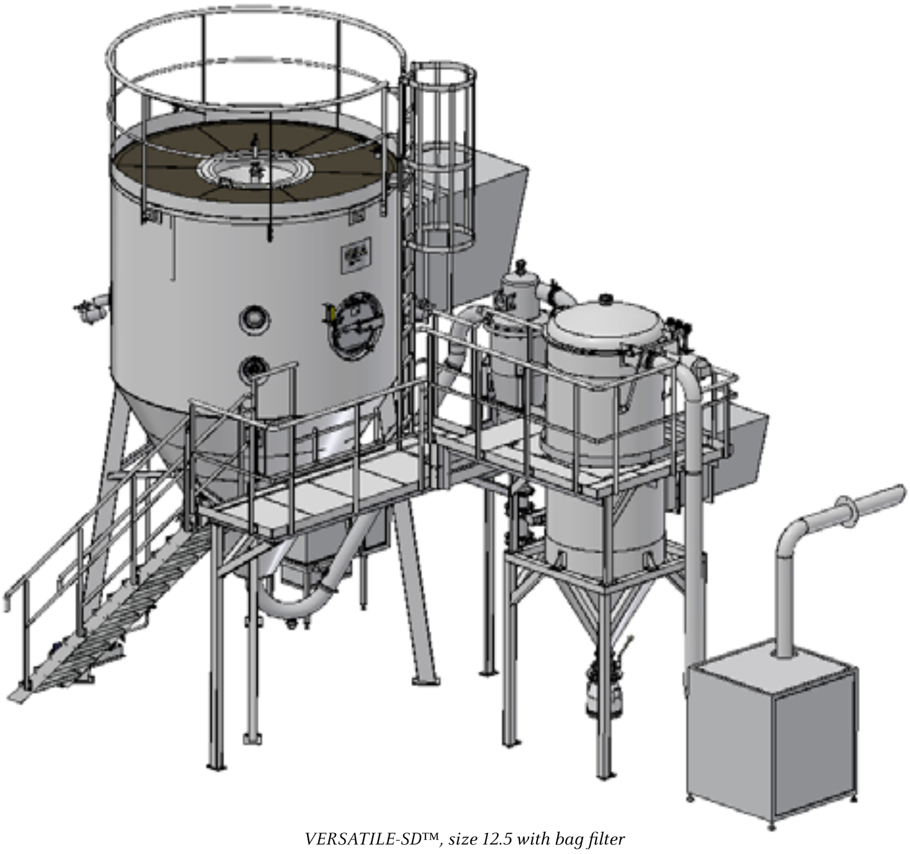


PRODUCTION MINOR™ and VERSATILE-SD™

GEA's PRODUCTION MINOR™ and VERSATILE-SD™ belong to a range of versatile production-scale spray drying units. They can be used in the production of a wide range of products – from advanced ceramics and catalysts to flavors, herbal extracts and food ingredients.

The PRODUCTION MINOR™ is a flexible spray dryer that can be used for both R&D – where a larger capacity is required – and for small-volume productions. The capacity of the PRODUCTION MINOR™ makes it suitable for a wide range of products. It is a standard spray dryer in a sanitary design with several modules and options available.

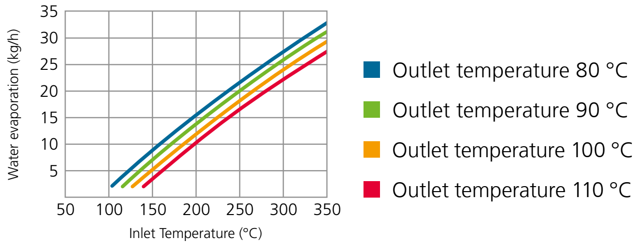
The VERSATILE-SD™ spray dryers are modular spray dryers designed to utilize the same modules and configurations for different capacity levels and product requirements. With several modules and options available, they can be designed to meet almost every customer requirement.



VERSATILE-SD™, size 12.5 with bag filter

PRODUCTION MINOR™

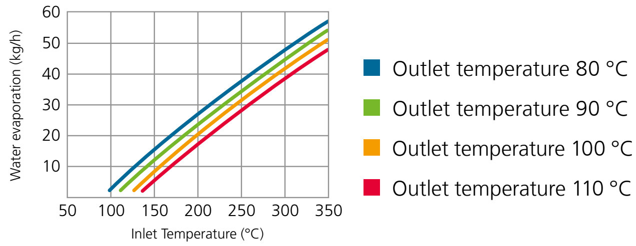
co-current two-fluid-nozzle atomization



Key figures	PRODUCTION MINOR™
Nominal main process gas flow (kg/h)	360
Water evaporation capacity (kg/h)	5 – 30
Typical mean particle size (μm)	10 – 90
Space requirements, L × W × H (m)	4.4 × 2 × 2.7

VERSATILE-SD™, size 6.3

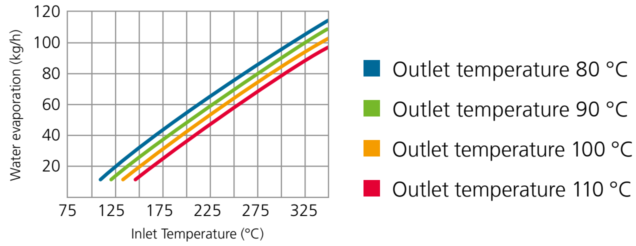
co-current pressure atomization



Key figures	VERSATILE-SD™ size 6.3
Nominal main process gas flow (kg/h)	630
Water evaporation capacity (kg/h)	10 – 55
Typical mean particle size (μm)	10 – 130
Space requirements, L × W × H (m)	5.5 × 4 × 6.3

VERSATILE-SD™, size 12.5

co-current pressure atomization



Key figures	VERSATILE-SD™ size 12.5
Nominal main process gas flow (kg/h)	1,250
Water evaporation capacity (kg/h)	20 – 110
Typical mean particle size (μm)	20 – 140
Space requirements, L × W × H (m)	6.5 × 4 × 6.8

Options

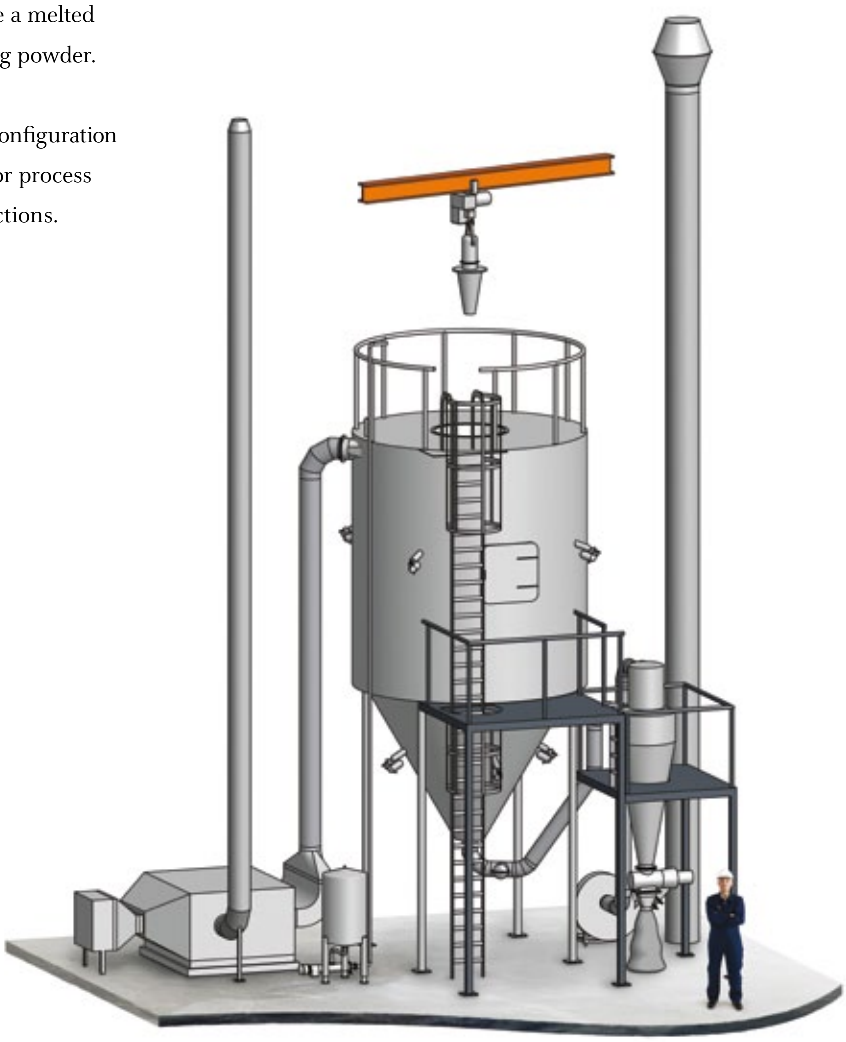
- Rotary atomizer
- Two-fluid nozzle
- Pressure nozzle
- Feed pump
- Pneumatic hammer
- Explosion membrane
- Explosion suppression system
- Bag filter
- Wet scrubber
- HEPA filter
- Double flap valve
- Fire extinguishing equipment
- Powder cooling systems
- Air broom
- JET SWEEP™
- External fluid bed
- Extra data logging
- CIP systems

VERSATILE-SD™

From pilots to industrial units

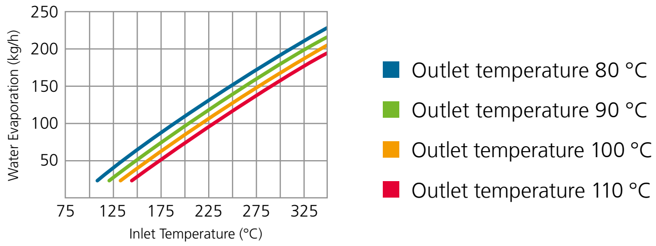
The VERSATILE-SD™ sizes 25 and 50 are standard, semi-industrial plants based on standard modules. The applications are many, thanks to the flexibility of the atomization system, even including spray congealing applications, where a melted feed stock is atomized and turned into a free-flowing powder.

To develop the right process and select the ideal size, configuration and equipment, the GEA test centers are available for process development and carrying out tests and trial productions.



VERSATILE-SD™, size 25

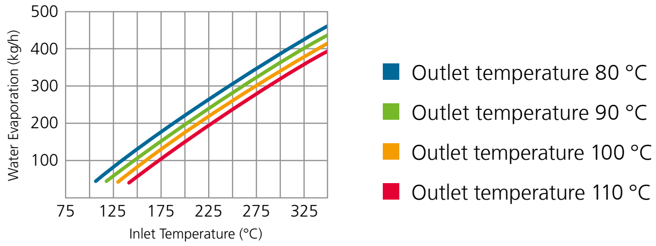
co-current atomization



Key figures	VERSATILE-SD™ size 25
Nominal main process gas flow (kg/h)	2,500
Water evaporation capacity (kg/h)	40 – 220
Typical mean particle size (µm)	40 – 150
Space requirements, L × W × H (m)	10 × 5 × 9

VERSATILE-SD™, size 50

co-current atomization



Key figures	VERSATILE-SD™ size 50
Nominal main process gas flow (kg/h)	5,000
Water evaporation capacity (kg/h)	80 – 440
Typical mean particle size (µm)	80 – 160
Space requirements, L × W × H (m)	11 × 6 × 11.7

Options

- Rotary atomizer
- Two-fluid nozzle
- Pressure nozzle
- COMBI-NOZZLE™
- Feed pump
- Pneumatic hammers
- Electrical heater, steam heater or indirect gas heater
- Explosion venting, flame arrester or suppression system
- Double-flap valve or rotary valve for powder discharge
- Bag filter or wet scrubber
- HEPA filter
- Fire extinguishing equipment
- Powder cooling systems
- Air broom
- JET SWEEP™
- External fluid bed
- Data logging
- CIP equipment

Appendix C: Material Safety Data Sheets

Safety Data Sheet

according to 29CFR1910/1200 and GHS Rev. 3

Ammonia

SECTION 1 : Identification of the substance/mixture and of the supplier

Product name : **Ammonia**

Manufacturer/Supplier Trade name:

Manufacturer/Supplier Article number: **S25164**

Recommended uses of the product and uses restrictions on use:

Manufacturer Details:

AquaPhoenix Scientific
9 Barnhart Drive, Hanover, PA 17331

Supplier Details:

Fisher Science Education
15 Jet View Drive, Rochester, NY 14624

Emergency telephone number:

Fisher Science Education Emergency Telephone No.: 800-535-5053

SECTION 2 : Hazards identification

Classification of the substance or mixture:

Corrosive
Skin corrosion, category 1B

Environmentally Damaging
Acute hazards to the aquatic environment, category 1

Irritant
Specific target organ toxicity following single exposure, category 3

STOT SE 3
AcAq Tox 1
Skin Corr. 1B

Signal word :Danger

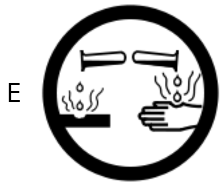
Hazard statements:
Causes severe skin burns and eye damage
May cause respiratory irritation
Very toxic to aquatic life
Precautionary statements:
If medical advice is needed, have product container or label at hand
Keep out of reach of children
Read label before use
Do not breathe dust/fume/gas/mist/vapours/spray
Avoid release to the environment
Wear protective gloves/protective clothing/eye protection/face protection
Use personal protective equipment as required

Do not eat, drink or smoke when using this product
Wash skin thoroughly after handling
IF INHALED: Remove victim to fresh air and keep at rest in a position comfortable for breathing
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 doctor/physician
IF ON SKIN (or hair): Remove/Take off immediately all contaminated clothing. Rinse skin with water/shower
IF SWALLOWED: Rinse mouth. Do NOT induce vomiting
Collect spillage
Specific treatment (see supplemental first aid instructions on this label)
Wash contaminated clothing before reuse
Store locked up
Store in a dry place
Store in a well ventilated place. Keep container tightly closed
Dispose of contents/container to ...

Combustible Dust Hazard: :
May form combustible dust concentrations in air (during processing).

Other Non-GHS Classification:

WHMIS



NFPA/HMIS

NFPA SCALE (0-4)

Health	3
Flammability	0
Physical Hazard	0
Personal Protection	X

HMIS RATINGS (0-4)

SECTION 3 : Composition/information on ingredients

Ingredients:		
CAS 1336-21-6	Ammonium Hydroxide, ACS	12.32 %
CAS 7732-18-5	Deionized Water	87 %
		Percentages are by weight

SECTION 4 : First aid measures

Description of first aid measures

After inhalation: Move exposed individual to fresh air. Loosen clothing as necessary and position individual in a comfortable position.Seek medical advice if discomfort or irritation persists.If breathing difficult, give oxygen.

After skin contact: Wash affected area with soap and water. Rinse/flush exposed skin gently using water for 15-20 minutes. Seek medical advice if discomfort or irritation persists.

After eye contact: Protect unexposed eye. Rinse/flush exposed eye(s) gently using water for 15-20 minutes. Remove contact lens(es) if able to do so during rinsing. Seek medical attention if irritation persists or if concerned.

After swallowing: Rinse mouth thoroughly. Do not induce vomiting. Have exposed individual drink sips of water. Seek medical attention if irritation, discomfort or vomiting persists.

Most important symptoms and effects, both acute and delayed:

Irritation, Nausea,Headache, Shortness of breath.;

Indication of any immediate medical attention and special treatment needed:

If seeking medical attention, provide SDS document to physician.

SECTION 5 : Firefighting measures

Extinguishing media

Suitable extinguishing agents: If in laboratory setting, follow laboratory fire suppression procedures. Use appropriate fire suppression agents for adjacent combustible materials or sources of ignition

For safety reasons unsuitable extinguishing agents:

Special hazards arising from the substance or mixture:

Combustion products may include carbon oxides or other toxic vapors.Thermal decomposition can lead to release of irritating gases and vapors.Avoid generating dust; fine dust dispersed in air in sufficient concentrations, and in the presence of an ignition source is a potential dust explosion hazard.

Advice for firefighters:

Protective equipment: Use NIOSH-approved respiratory protection/breathing apparatus.

Additional information (precautions): Move product containers away from fire or keep cool with water spray as a protective measure, where feasible.Use spark-proof tools and explosion-proof equipment.

SECTION 6 : Accidental release measures

Personal precautions, protective equipment and emergency procedures:

Wear protective equipment. Transfer to a disposal or recovery container.Use spark-proof tools and explosion-proof equipment.Use respiratory protective device against the effects of fumes/dust/aerosol. Keep unprotected persons away. Ensure adequate ventilation.Keep away from ignition sources. Protect from heat.Stop the spill, if possible. Contain spilled material by diking or using inert absorbent.

Environmental precautions:

Prevent from reaching drains, sewer or waterway. Collect contaminated soil for characterization per Section 13

Methods and material for containment and cleaning up:

If in a laboratory setting, follow Chemical Hygiene Plan procedures.Place into properly labeled containers for recovery or disposal. If necessary, use trained response staff/contractor.Dust deposits should not be allowed to accumulate on surfaces, as these may form an explosive mixture if they are released into the atmosphere in sufficient concentration. Avoid dispersal of dust in the air (i.e., clearing dust surfaces with compressed air). Collect solids in powder form using vacuum with (HEPA filter)

Reference to other sections:

SECTION 7 : Handling and storage

Precautions for safe handling:

Wash hands after handling. Follow good hygiene procedures when handling chemical materials. Do not eat, drink, smoke, or use personal products when handling chemical substances. If in a laboratory setting, follow Chemical Hygiene Plan.Use only in well ventilated areas.Avoid contact with eyes, skin, and clothing.

Conditions for safe storage, including any incompatibilities:

Provide ventilation for containers. Avoid storage near extreme heat, ignition sources or open flame. Store away from foodstuffs. Store away from oxidizing agents.Store in cool, dry conditions in well sealed containers. Store with like hazards

SECTION 8 : Exposure controls/personal protection

Control Parameters:	1336-21-6, Ammonium Hydroxide, ACGIH TLV: 17 mg/m3 1336-21-6 , Ammonium Hydroxide , OSHA PEL: 35 mg/m3 1336-21-6, Ammonium Hydroxide, OSHA TWA 25 ppm (18 mg/m3) ST 35 ppm (27 mg/m3) 1336-21-6, Ammonium Hydroxide, ACGIH TWA 25 ppm (18 mg/m3) ST 35 ppm (27 mg/m3)
Appropriate Engineering controls:	Emergency eye wash fountains and safety showers should be available in the immediate vicinity of use/handling.Provide exhaust ventilation or other engineering controls to keep the airborne concentrations of vapor or dusts (total/respirable) below the applicable workplace exposure limits (Occupational Exposure Limits-OELs) indicated above.Use under a fume hood. It is recommended that all dust control equipment such as local exhaust ventilation and material transport systems involved in handling of this product contain explosion relief vents or an explosion suppression system or an oxygen deficient environment.Ensure that dust-handling systems (such as exhaust ducts, dust collectors, vessels, and processing equipment) are designed in a manner to prevent the escape of dust into the work area (i.e., there is no leakage from the equipment).
Respiratory protection:	Use suitable respiratory protective device when high concentrations are present. Use suitable respiratory protective device when aerosol or mist is formed. For spills, respiratory protection may be advisable.
Protection of skin:	The glove material has to be impermeable and resistant to the product/ the substance/ the preparation being used/handled.Selection of the glove material on consideration of the penetration times, rates of diffusion and the degradation.
Eye protection:	Safety glasses with side shields or goggles.
General hygienic measures:	The usual precautionary measures are to be adhered to when handling chemicals. Keep away from food, beverages and feed sources. Immediately remove all soiled and contaminated clothing. Wash hands before breaks and at the end of work. Do not inhale gases/fumes/dust/mist/vapor/aerosols. Avoid contact with the eyes and skin.

SECTION 9 : Physical and chemical properties

Appearance (physical state,color):	Clear, colorless liquid.	**Explosion limit lower**: **Explosion limit upper**:	Not Determined Not Determined

Odor:	Ammonia-like	**Vapor pressure**:	115 at 20 C
Odor threshold:	Not Determined	**Vapor density**:	3.38
pH-value:	9	**Relative density**:	0.9
Melting/Freezing point:	- 72 C	**Solubilities**:	Infinite solubility in water.
Boiling point/Boiling range:	36 C	**Partition coefficient (n-octanol/water)**:	Not Determined
Flash point (closed cup):	Not Determined	**Auto/Self-ignition temperature**:	Not Determined
Evaporation rate:	Not Determined	**Decomposition temperature**:	Not Determined
Flammability (solid,gaseous):	Not Determined	**Viscosity**:	a. Kinematic:Not Determined b. Dynamic: Not Determined
Density: 0.9 g/cm3 at 20 °C			

SECTION 10 : Stability and reactivity

Reactivity:
Chemical stability: No decomposition if used and stored according to specifications.
Possible hazardous reactions:
Conditions to avoid: Store away from oxidizing agents, strong acids or bases.
Incompatible materials: Strong oxidizers, acids, gold, mercury, halogens, silv er, calcium hypochlorite bleaches.
Hazardous decomposition products: Ammonia and nitrogen oxides.

SECTION 11 : Toxicological information

Acute Toxicity:		
Oral:	LD50: 350 mg/kg (rat)	Ammonium Hydroxide (1336-21-6)
Chronic Toxicity: No additional information.		
Corrosion Irritation: No additional information.		
Sensitization:		No additional information.
Single Target Organ (STOT):		No additional information.
Numerical Measures:		No additional information.
Carcinogenicity:		No additional information.
Mutagenicity:		No additional information.
Reproductive Toxicity:		No additional information.

SECTION 12 : Ecological information

Ecotoxicity

Fish (acute 1336-21-6): 96 Hr LC50 Pimephales promelas: 8.2 mg/L

Crustacea (acute 1336-21-6): 48 Hr EC50 water flea: 0.66 mg/L; 48 Hr EC50 Daphnia pulex: 0.66 mg/L

Persistence and degradability: Readily degradable in the environment.
Bioaccumulative potential:
Mobility in soil:
Other adverse effects:

SECTION 13 : Disposal considerations

Waste disposal recommendations:

Product/containers must not be disposed together with household garbage. Do not allow product to reach sewage system or open water.It is the responsibility of the waste generator to properly characterize all waste materials according to applicable regulatory entities (US 40CFR262.11). Consult federal state/ provincial and local regulations regarding the proper disposal of waste material that may incorporate some amount of this product.

SECTION 14 : Transport information

UN-Number

2672

UN proper shipping name

Ammonia Solution

Transport hazard class(es)

Class:
8 Corrosive substances

Packing group:III
Environmental hazard:
Transport in bulk:
Special precautions for user:

SECTION 15 : Regulatory information

United States (USA)

SARA Section 311/312 (Specific toxic chemical listings):

Acute, Chronic

SARA Section 313 (Specific toxic chemical listings):

1336-21-6 Ammonium Hydroxide

RCRA (hazardous waste code):

None of the ingredients is listed

TSCA (Toxic Substances Control Act):

All ingredients are listed.

CERCLA (Comprehensive Environmental Response, Compensation, and Liability Act):

1336-21-6 Ammonium Hydroxide, ACS 1000

Proposition 65 (California):

Chemicals known to cause cancer:

None of the ingredients is listed

Chemicals known to cause reproductive toxicity for females:

None of the ingredients is listed

Chemicals known to cause reproductive toxicity for males:

None of the ingredients is listed

Chemicals known to cause developmental toxicity:

None of the ingredients is listed

Canada

Canadian Domestic Substances List (DSL):

All ingredients are listed.

Canadian NPRI Ingredient Disclosure list (limit 0.1%):

None of the ingredients is listed

Canadian NPRI Ingredient Disclosure list (limit 1%):

1336-21-6 Ammonium hydroxide

SECTION 16 : Other information

This product has been classified in accordance with hazard criteria of the Controlled Products Regulations and the SDS contains all the information required by the Controlled Products Regulations.Note:. The responsibility to provide a safe workplace remains with the user.The user should consider the health hazards and safety information contained herein as a guide and should take those precautions required in an individual operation to instruct employees and develop work practice procedures for a safe work environment.The information contained herein is, to the best of our knowledge and belief, accurate.However, since the conditions of handling and use are beyond our control, we make no guarantee of results, and assume no liability for damages incurred by the use of this material.It is the responsibility of the user to comply with all applicable laws and regulations applicable to this material.

GHS Full Text Phrases:

Abbreviations and acronyms:
IMDG: International Maritime Code for Dangerous Goods
PNEC: Predicted No-Effect Concentration (REACH)
CFR: Code of Federal Regulations (USA)
SARA: Superfund Amendments and Reauthorization Act (USA)
RCRA: Resource Conservation and Recovery Act (USA)
TSCA: Toxic Substances Control Act (USA)
NPRI: National Pollutant Release Inventory (Canada)
DOT: US Department of Transportation
IATA: International Air Transport Association
GHS: Globally Harmonized System of Classification and Labelling of Chemicals
ACGIH: American Conference of Governmental Industrial Hygienists
CAS: Chemical Abstracts Service (division of the American Chemical Society)
NFPA: National Fire Protection Association (USA)
HMIS: Hazardous Materials Identification System (USA)
WHMIS: Workplace Hazardous Materials Information System (Canada)
DNEL: Derived No-Effect Level (REACH)

Effective date : 12.31.2014
Last updated : 03.19.2015

SAFETY DATA SHEET

Issuing Date January 5, 2015 **Revision Date** June 12, 2015 **Revision Number** 1

1. IDENTIFICATION OF THE SUBSTANCE/PREPARATION AND OF THE COMPANY/UNDERTAKING

Product identifier

Product Name	**Clorox® Regular-Bleach$_1$**

Other means of identification

EPA Registration Number	5813-100

Recommended use of the chemical and restrictions on use

Recommended use	Household disinfecting, sanitizing, and laundry bleach
Uses advised against	No information available

Details of the supplier of the safety data sheet

Supplier Address
The Clorox Company
1221 Broadway
Oakland, CA 94612

Phone: 1-510-271-7000

Emergency telephone number

Emergency Phone Numbers	For Medical Emergencies, call: 1-800-446-1014 For Transportation Emergencies, call Chemtrec: 1-800-424-9300

2. HAZARDS IDENTIFICATION

Classification

This chemical is considered hazardous by the 2012 OSHA Hazard Communication Standard (29 CFR 1910.1200).

Skin corrosion/irritation	Category 1
Serious eye damage/eye irritation	Category 1

GHS Label elements, including precautionary statements

Emergency Overview

Signal word **Danger**

Hazard Statements
Causes severe skin burns and eye damage
Causes serious eye damage

Appearance Clear, pale yellow **Physical State** Thin liquid **Odor** Bleach

Precautionary Statements - Prevention

Wash face, hands and any exposed skin thoroughly after handling.
Wear protective gloves, protective clothing, face protection, and eye protection such as safety glasses.

Precautionary Statements - Response

Immediately call a poison center or doctor.
If swallowed: 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.
If inhaled: Remove person to fresh air and keep comfortable for breathing.
Specific treatment (see supplemental first aid instructions on this label).
If in eyes: Rinse cautiously with water for several minutes. Remove contact lenses, if present and easy to do. Continue rinsing.

Precautionary Statements - Storage

Store locked up.

Precautionary Statements - Disposal

Dispose of contents in accordance with all applicable federal, state, and local regulations.

Hazards not otherwise classified (HNOC)

Although not expected, heart conditions or chronic respiratory problems such as asthma, chronic bronchitis, or obstructive lung disease may be aggravated by exposure to high concentrations of vapor or mist.

Product contains a strong oxidizer. Always flush drains before and after use.

Unknown Toxicity
Not applicable.

Other information
Very toxic to aquatic life with long lasting effects.

Interactions with Other Chemicals
Reacts with other household chemicals such as toilet bowl cleaners, rust removers, acids, or products containing ammonia to produce hazardous irritating gases, such as chlorine and other chlorinated compounds.

3. COMPOSITION/INFORMATION ON INGREDIENTS

Chemical Name	CAS-No	Weight %	Trade Secret
Sodium hypochlorite	7681-52-9	5 - 10	*

* The exact percentage (concentration) of composition has been withheld as a trade secret.

4. FIRST AID MEASURES

First aid measures

General Advice	Call a poison control center or doctor immediately for treatment advice. Show this safety data sheet to the doctor in attendance.
Eye Contact	Hold eye open and rinse slowly and gently with water for 15 - 20 minutes. Remove contact lenses, if present, after the first 5 minutes, then continue rinsing eye. Call a poison control center or doctor for treatment advice.
Skin Contact	Take off contaminated clothing. Rinse skin immediately with plenty of water for 15-20 minutes. Call a poison control center or doctor for treatment advice.
Inhalation	Move to fresh air. If breathing is affected, call a doctor.
Ingestion	Have person sip a glassful of water if able to swallow. Do not induce vomiting unless told to do so by a poison control center or doctor. Do not give anything by mouth to an unconscious person. Call a poison control center or doctor immediately for treatment advice.
Protection of First-aiders	Avoid contact with skin, eyes, and clothing. Use personal protective equipment as required. Wear personal protective clothing (see section 8).

Most important symptoms and effects, both acute and delayed

Most Important Symptoms and Effects	Burning of eyes and skin.

Indication of any immediate medical attention and special treatment needed

Notes to Physician	Treat symptomatically. Probable mucosal damage may contraindicate the use of gastric lavage.

5. FIRE-FIGHTING MEASURES

Suitable Extinguishing Media
Use extinguishing measures that are appropriate to local circumstances and the surrounding environment.

Unsuitable Extinguishing Media
CAUTION: Use of water spray when fighting fire may be inefficient.

Specific Hazards Arising from the Chemical
This product causes burns to eyes, skin, and mucous membranes. Thermal decomposition can release sodium chlorate and irritating gases and vapors.

Explosion Data

Sensitivity to Mechanical Impact	None.
Sensitivity to Static Discharge	None.

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.

6. ACCIDENTAL RELEASE MEASURES

Personal precautions, protective equipment and emergency procedures

Personal Precautions	Avoid contact with eyes, skin, and clothing. Ensure adequate ventilation. Use personal protective equipment as required. For spills of multiple products, responders should evaluate the MSDSs of the products for incompatibility with sodium hypochlorite. Breathing protection should be worn in enclosed and/or poorly-ventilated areas until hazard assessment is complete.
Other Information	Refer to protective measures listed in Sections 7 and 8.

Environmental precautions

Environmental Precautions	This product is toxic to fish, aquatic invertebrates, oysters, and shrimp. Do not allow product to enter storm drains, lakes, or streams. See Section 12 for ecological Information.

Methods and material for containment and cleaning up

Methods for Containment	Prevent further leakage or spillage if safe to do so.
Methods for Cleaning Up	Absorb and containerize. Wash residual down to sanitary sewer. Contact the sanitary treatment facility in advance to assure ability to process washed-down material.

7. HANDLING AND STORAGE

Precautions for safe handling

Handling Handle in accordance with good industrial hygiene and safety practice. Avoid contact with skin, eyes, and clothing. Do not eat, drink, or smoke when using this product.

Conditions for safe storage, including any incompatibilities

Storage Store away from children. Reclose cap tightly after each use. Store this product upright in a cool, dry area, away from direct sunlight and heat to avoid deterioration. Do not contaminate food or feed by storage of this product.

Incompatible Products Toilet bowl cleaners, rust removers, acids, and products containing ammonia.

8. EXPOSURE CONTROLS/PERSONAL PROTECTION

Control parameters

Exposure Guidelines

Chemical Name	ACGIH TLV	OSHA PEL	NIOSH IDLH
Sodium hypochlorite 7681-52-9	None	None	None

ACGIH TLV: American Conference of Governmental Industrial Hygienists - Threshold Limit Value. OSHA PEL: Occupational Safety and Health Administration - Permissible Exposure Limits. NIOSH IDLH: Immediately Dangerous to Life or Health.

Appropriate engineering controls

Engineering Measures

Showers
Eyewash stations
Ventilation systems

Individual protection measures, such as personal protective equipment

Eye/Face Protection If splashes are likely to occur: Wear safety glasses with side shields (or goggles) or face shield.

Skin and Body Protection Wear rubber or neoprene gloves and protective clothing such as long-sleeved shirt.

Respiratory Protection If irritation is experienced, NIOSH/MSHA approved respiratory protection should be worn. Positive-pressure supplied air respirators may be required for high airborne contaminant concentrations. Respiratory protection must be provided in accordance with current local regulations.

Hygiene Measures Handle in accordance with good industrial hygiene and safety practice. Wash hands after direct contact. Do not wear product-contaminated clothing for prolonged periods. Remove and wash contaminated clothing before re-use. Do not eat, drink, or smoke when using this product.

9. PHYSICAL AND CHEMICAL PROPERTIES

Physical and Chemical Properties

Physical State	Thin liquid		
Appearance	Clear	**Odor**	Bleach
Color	Pale yellow	**Odor Threshold**	No information available

Property	**Values**	**Remarks/ Method**
pH	~12	None known
Melting/freezing point	No data available	None known
Boiling point / boiling range	No data available	None known
Flash Point	Not flammable	None known
Evaporation rate	No data available	None known
Flammability (solid, gas)	No data available	None known
Flammability Limits in Air		
Upper flammability limit	No data available	None known
Lower flammability limit	No data available	None known
Vapor pressure	No data available	None known
Vapor density	No data available	None known
Specific Gravity	~1.1	None known
Water Solubility	Soluble	None known
Solubility in other solvents	No data available	None known
Partition coefficient: n-octanol/water	No data available	None known
Autoignition temperature	No data available	None known
Decomposition temperature	No data available	None known
Kinematic viscosity	No data available	None known
Dynamic viscosity	No data available	None known
Explosive Properties	Not explosive	
Oxidizing Properties	No data available	

Other Information	
Softening Point	No data available
VOC Content (%)	No data available
Particle Size	No data available
Particle Size Distribution	No data available

10. STABILITY AND REACTIVITY

Reactivity

Reacts with other household chemicals such as toilet bowl cleaners, rust removers, acids, or products containing ammonia to produce hazardous irritating gases, such as chlorine and other chlorinated compounds.

Chemical stability

Stable under recommended storage conditions.

Possibility of Hazardous Reactions

None under normal processing.

Conditions to avoid

None known based on information supplied.

Incompatible materials

Toilet bowl cleaners, rust removers, acids, and products containing ammonia.

Hazardous Decomposition Products

None known based on information supplied.

11. TOXICOLOGICAL INFORMATION

Information on likely routes of exposure

Product Information .

Inhalation Exposure to vapor or mist may irritate respiratory tract and cause coughing. Inhalation of high concentrations may cause pulmonary edema.

Eye Contact Corrosive. May cause severe damage to eyes.

Skin Contact May cause severe irritation to skin. Prolonged contact may cause burns to skin.

Ingestion Ingestion may cause burns to gastrointestinal tract and respiratory tract, nausea, vomiting, and diarrhea.

Component Information

Chemical Name	LD50 Oral	LD50 Dermal	LC50 Inhalation
Sodium hypochlorite 7681-52-9	8200 mg/kg (Rat)	>10000 mg/kg (Rabbit)	-

Information on toxicological effects

Symptoms May cause redness and tearing of the eyes. May cause burns to eyes. May cause redness or burns to skin. Inhalation may cause coughing.

Delayed and immediate effects as well as chronic effects from short and long-term exposure

Sensitization No information available.

Mutagenic Effects No information available.

Carcinogenicity The table below indicates whether each agency has listed any ingredient as a carcinogen.

Chemical Name	ACGIH	IARC	NTP	OSHA
Sodium hypochlorite 7681-52-9	-	Group 3	-	-

IARC (International Agency for Research on Cancer)
Group 3 - Not Classifiable as to Carcinogenicity in Humans

Reproductive Toxicity No information available.

STOT - single exposure No information available.

STOT - repeated exposure No information available.
Chronic Toxicity Carcinogenic potential is unknown.
Target Organ Effects Respiratory system, eyes, skin, gastrointestinal tract (GI).

Aspiration Hazard No information available.

Numerical measures of toxicity - Product Information

The following values are calculated based on chapter 3.1 of the GHS document

ATEmix (oral)
54 g/kg
ATEmix (inhalation-dust/mist)
58 mg/L

12. ECOLOGICAL INFORMATION

Ecotoxicity
Very toxic to aquatic life with long lasting effects.

This product is toxic to fish, aquatic invertebrates, oysters, and shrimp. Do not allow product to enter storm drains, lakes, or streams.

Persistence and Degradability
No information available.

Bioaccumulation
No information available.

Other adverse effects
No information available.

13. DISPOSAL CONSIDERATIONS

Disposal methods
Dispose of in accordance with all applicable federal, state, and local regulations. Do not contaminate food or feed by disposal of this product.

Contaminated Packaging
Do not reuse empty containers. Dispose of in accordance with all applicable federal, state, and local regulations.

14. TRANSPORT INFORMATION

DOT	Not restricted.
TDG	Not restricted for road or rail.
ICAO	Not restricted, as per Special Provision A197, Environmentally Hazardous Substance exception.
IATA	Not restricted, as per Special Provision A197, Environmentally Hazardous Substance exception.
IMDG/IMO	Not restricted, as per IMDG Code 2.10.2.7, Marine Pollutant exception.

15. REGULATORY INFORMATION

Chemical Inventories

TSCA	All components of this product are either on the TSCA 8(b) Inventory or otherwise exempt from listing.
DSL/NDSL	All components are on the DSL or NDSL.

TSCA - United States Toxic Substances Control Act Section 8(b) Inventory
DSL/NDSL - Canadian Domestic Substances List/Non-Domestic Substances List

U.S. Federal Regulations

SARA 313
Section 313 of Title III of the Superfund Amendments and Reauthorization Act of 1986 (SARA). This product does not contain any chemicals which are subject to the reporting requirements of the Act and Title 40 of the Code of Federal Regulations, Part 372

SARA 311/312 Hazard Categories

Acute Health Hazard	Yes
Chronic Health Hazard	No
Fire Hazard	No
Sudden Release of Pressure Hazard	No
Reactive Hazard	No

Clean Water Act
This product contains the following substances which are regulated pollutants pursuant to the Clean Water Act (40 CFR 122.21 and 40 CFR 122.42)

Chemical Name	CWA - Reportable Quantities	CWA - Toxic Pollutants	CWA - Priority Pollutants	CWA - Hazardous Substances
Sodium hypochlorite 7681-52-9	100 lb			X

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)

Chemical Name	Hazardous Substances RQs	Extremely Hazardous Substances RQs	RQ
Sodium hypochlorite 7681-52-9	100 lb	-	RQ 100 lb final RQ RQ 45.4 kg final RQ

EPA Statement
This chemical is a pesticide product registered by the Environmental Protection Agency and is subject to certain labeling requirements under federal pesticide law. These requirements differ from the classification criteria and hazard information required for safety data sheets and for workplace labels of non-pesticide chemicals. Following is the hazard information as required on the pesticide label:

> **DANGER: CORROSIVE.** Causes irreversible eye damage and skin burns. Harmful if swallowed. Do not get in eyes, on skin, or on clothing. Wear protective eyewear and rubber gloves when handling this product. Wash thoroughly with soap and water after handling and before eating, drinking, chewing gum, using tobacco, or using the restroom. Avoid breathing vapors and use only in a well-ventilated area.

US State Regulations

California Proposition 65
This product does not contain any Proposition 65 chemicals.

U.S. State Right-to-Know Regulations

Chemical Name	New Jersey	Massachusetts	Pennsylvania	Rhode Island	Illinois
Sodium hypochlorite 7681-52-9	X	X	X	X	
Sodium chlorate 7775-09-9	X	X	X		

International Regulations

Canada
WHMIS Hazard Class
E - Corrosive material

16. OTHER INFORMATION

NFPA	**Health Hazard** 3	**Flammability** 0	**Instability** 0	**Physical and Chemical Hazards** -
HMIS	**Health Hazard** 3	**Flammability** 0	**Physical Hazard** 0	**Personal Protection** B

Prepared By	Product Stewardship 23 British American Blvd. Latham, NY 12110 1-800-572-6501
Revision Date	June 12, 2015
Revision Note	Revision Section 14.
Reference	1096036/164964.159

General Disclaimer

The information provided in this Safety Data Sheet is correct to the best of our knowledge, information and belief at the date of its publication. The information given is designed only as a guidance for safe handling, use, processing, storage, transportation, disposal, and release and is not to be considered a warranty or quality specification. The information relates only to the specific material designated and may not be valid for such material used in combination with any other materials or in any process, unless specified in the text.

End of Safety Data Sheet

SAFETY DATA SHEET

Carbon Dioxide

Airgas
an Air Liquide company

Section 1. Identification

GHS product identifier : Carbon Dioxide

Chemical name : Carbon dioxide, gas

Other means of identification : Carbonic, Carbon Dioxide, Carbonic Anhydride, R744, Carbon Dioxide USP

Product type : Gas.

Product use : Synthetic/Analytical chemistry and Medical use.

Synonym : Carbonic, Carbon Dioxide, Carbonic Anhydride, R744, Carbon Dioxide USP
SDS # : 001013

Supplier's details : Airgas USA, LLC and its affiliates
259 North Radnor-Chester Road
Suite 100
Radnor, PA 19087-5283
1-610-687-5253

24-hour telephone : 1-866-734-3438

Section 2. Hazards identification

OSHA/HCS status : This material is considered hazardous by the OSHA Hazard Communication Standard (29 CFR 1910.1200).

Classification of the substance or mixture : GASES UNDER PRESSURE - Liquefied gas
Simple asphyxiant.

GHS label elements

Hazard pictograms :

Signal word : Warning

Hazard statements : Contains gas under pressure; may explode if heated.
May displace oxygen and cause rapid suffocation.
May increase respiration and heart rate.

Precautionary statements

General : Read and follow all Safety Data Sheets (SDS'S) before use. Read label before use. Keep out of reach of children. If medical advice is needed, have product container or label at hand. Close valve after each use and when empty. Use equipment rated for cylinder pressure. Do not open valve until connected to equipment prepared for use. Use a back flow preventative device in the piping. Use only equipment of compatible materials of construction. Always keep container in upright position.

Prevention : Use and store only outdoors or in a well ventilated place.

Response : Not applicable.

Storage : Protect from sunlight. Store in a well-ventilated place.

Disposal : Not applicable.

Hazards not otherwise classified : In addition to any other important health or physical hazards, this product may displace oxygen and cause rapid suffocation.
May cause frostbite.

Section 3. Composition/information on ingredients

Substance/mixture	:	Substance
Chemical name	:	Carbon dioxide, gas
Other means of identification	:	Carbonic, Carbon Dioxide, Carbonic Anhydride, R744, Carbon Dioxide USP
Product code	:	001013

CAS number/other identifiers

CAS number : 124-38-9

Ingredient name	%	CAS number
Carbon Dioxide	100	124-38-9

Any concentration shown as a range is to protect confidentiality or is due to batch variation.

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	:	Immediately flush eyes with plenty of water, occasionally lifting the upper and lower eyelids. Check for and remove any contact lenses. Continue to rinse for at least 10 minutes. Get medical attention if irritation occurs.
Inhalation	:	Remove victim to fresh air and keep at rest in a position comfortable for breathing. If not breathing, if breathing is irregular or if respiratory arrest occurs, provide artificial respiration or oxygen by trained personnel. It may be dangerous to the person providing aid to give mouth-to-mouth resuscitation. Get medical attention if adverse health effects persist or are severe. If unconscious, place in recovery position and get medical attention immediately. Maintain an open airway. Loosen tight clothing such as a collar, tie, belt or waistband.
Skin contact	:	Flush contaminated skin with plenty of water. Remove contaminated clothing and shoes. Get medical attention if symptoms occur. Wash clothing before reuse. Clean shoes thoroughly before reuse.
Ingestion	:	As this product is a gas, refer to the inhalation section.

Most important symptoms/effects, acute and delayed

Potential acute health effects

Eye contact	:	No known significant effects or critical hazards.
Inhalation	:	No known significant effects or critical hazards.
Skin contact	:	No known significant effects or critical hazards.
Frostbite	:	Try to warm up the frozen tissues and seek medical attention.
Ingestion	:	As this product is a gas, refer to the inhalation section.

Over-exposure signs/symptoms

Eye contact	:	No specific data.
Inhalation	:	No specific data.
Skin contact	:	No specific data.
Ingestion	:	No specific data.

Indication of immediate medical attention and special treatment needed, if necessary

Notes to physician	:	Treat symptomatically. Contact poison treatment specialist immediately if large quantities have been ingested or inhaled.
Specific treatments	:	No specific treatment.

Section 4. First aid measures

Protection of first-aiders : No action shall be taken involving any personal risk or without suitable training. It may be dangerous to the person providing aid to give mouth-to-mouth resuscitation.

See toxicological information (Section 11)

Section 5. Fire-fighting measures

Extinguishing media

Suitable extinguishing media : Use an extinguishing agent suitable for the surrounding fire.

Unsuitable extinguishing media : None known.

Specific hazards arising from the chemical : Contains gas under pressure. In a fire or if heated, a pressure increase will occur and the container may burst or explode.

Hazardous thermal decomposition products : Decomposition products may include the following materials:
carbon dioxide
carbon monoxide

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. No action shall be taken involving any personal risk or without suitable training. Contact supplier immediately for specialist advice. Move containers from fire area if this can be done without risk. Use water spray to keep fire-exposed containers cool.

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.

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. Avoid breathing gas. Provide adequate ventilation. Wear appropriate respirator when ventilation is inadequate. Put on appropriate personal protective equipment.

For emergency responders : 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".

Environmental precautions : Ensure emergency procedures to deal with accidental gas releases are in place to avoid contamination of the environment. Inform the relevant authorities if the product has caused environmental pollution (sewers, waterways, soil or air).

Methods and materials for containment and cleaning up

Small spill : Immediately contact emergency personnel. Stop leak if without risk.

Large spill : Immediately contact emergency personnel. Stop leak if without risk. Note: see Section 1 for emergency contact information and Section 13 for waste disposal.

Section 7. Handling and storage

Precautions for safe handling

Protective measures : Put on appropriate personal protective equipment (see Section 8). Contains gas under pressure. Avoid breathing gas. Do not puncture or incinerate container. Use equipment rated for cylinder pressure. Close valve after each use and when empty. Protect cylinders from physical damage; do not drag, roll, slide, or drop. Use a suitable hand truck for cylinder movement.
Avoid contact with eyes, skin and clothing. Empty containers retain product residue and can be hazardous.

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. Workers should wash hands and face before eating, drinking and smoking. 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). Cylinders should be stored upright, with valve protection cap in place, and firmly secured to prevent falling or being knocked over. Cylinder temperatures should not exceed 52 °C (125 °F). Keep container tightly closed and sealed until ready for use. See Section 10 for incompatible materials before handling or use.

Section 8. Exposure controls/personal protection

Control parameters

Occupational exposure limits

Ingredient name	Exposure limits
Carbon Dioxide	**ACGIH TLV (United States, 3/2017). Oxygen Depletion [Asphyxiant].** STEL: 54000 mg/m³ 15 minutes. STEL: 30000 ppm 15 minutes. TWA: 9000 mg/m³ 8 hours. TWA: 5000 ppm 8 hours. **NIOSH REL (United States, 10/2016).** STEL: 54000 mg/m³ 15 minutes. STEL: 30000 ppm 15 minutes. TWA: 9000 mg/m³ 10 hours. TWA: 5000 ppm 10 hours. **OSHA PEL (United States, 6/2016).** TWA: 9000 mg/m³ 8 hours. TWA: 5000 ppm 8 hours. **OSHA PEL 1989 (United States, 3/1989).** STEL: 54000 mg/m³ 15 minutes. STEL: 30000 ppm 15 minutes. TWA: 18000 mg/m³ 8 hours. TWA: 10000 ppm 8 hours.

Appropriate engineering controls : Good general ventilation should be sufficient to control worker exposure to airborne contaminants.

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: safety glasses with side-shields.

Skin protection

Section 8. Exposure controls/personal 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. In the case of mixtures, consisting of several substances, the protection time of the gloves cannot be accurately estimated.
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.
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.
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.

Section 9. Physical and chemical properties

Appearance	
Physical state	: Gas. [Compressed gas.]
Color	: Colorless.
Odor	: Odorless.
Odor threshold	: Not available.
pH	: Not available.
Melting point	: Sublimation temperature: -79°C (-110.2 to °F)
Boiling point	: Not available.
Critical temperature	: 30.85°C (87.5°F)
Flash point	: [Product does not sustain combustion.]
Evaporation rate	: Not available.
Flammability (solid, gas)	: Not available.
Lower and upper explosive (flammable) limits	: Not available.
Vapor pressure	: 830 (psig)
Vapor density	: 1.53 (Air = 1) Liquid Density@BP: Solid density = 97.5 lb/ft3 (1562 kg/m3)
Specific Volume (ft ^{3}/lb)	: 8.7719
Gas Density (lb/ft ^{3})	: 0.114
Relative density	: Not applicable.
Solubility	: Not available.
Solubility in water	: Not available.
Partition coefficient: n-octanol/water	: 0.83
Auto-ignition temperature	: Not available.
Decomposition temperature	: Not available.
Viscosity	: Not applicable.
Flow time (ISO 2431)	: Not available.
Molecular weight	: 44.01 g/mole

Section 10. Stability and reactivity

Reactivity	: No specific test data related to reactivity available for this product or its ingredients.
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	: No specific data.
Incompatible materials	: No specific data.
Hazardous decomposition products	: Under normal conditions of storage and use, hazardous decomposition products should not be produced.
Hazardous polymerization	: Under normal conditions of storage and use, hazardous polymerization will not occur.

Section 11. Toxicological information

Information on toxicological effects

Acute toxicity

Not available.

Irritation/Corrosion

Not available.

Sensitization

Not available.

Mutagenicity

Not available.

Carcinogenicity

Not available.

Reproductive toxicity

Not available.

Teratogenicity

Not available.

Specific target organ toxicity (single exposure)

Not available.

Specific target organ toxicity (repeated exposure)

Not available.

Aspiration hazard

Not available.

Information on the likely routes of exposure : Not available.

Potential acute health effects

Eye contact	: No known significant effects or critical hazards.
Inhalation	: No known significant effects or critical hazards.
Skin contact	: No known significant effects or critical hazards.

Section 11. Toxicological information

Ingestion : As this product is a gas, refer to the inhalation section.

Symptoms related to the physical, chemical and toxicological characteristics

Eye contact : No specific data.

Inhalation : No specific data.

Skin contact : No specific data.

Ingestion : No specific data.

Delayed and immediate effects and also chronic effects from short and long term exposure

Short term exposure

Potential immediate effects : Not available.

Potential delayed effects : Not available.

Long term exposure

Potential immediate effects : Not available.

Potential delayed effects : Not available.

Potential chronic health effects

Not available.

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.

Numerical measures of toxicity

Acute toxicity estimates

Not available.

Section 12. Ecological information

Toxicity

Not available.

Persistence and degradability

Not available.

Bioaccumulative potential

Product/ingredient name	$LogP_{ow}$	BCF	Potential
Carbon Dioxide	0.83	-	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. Waste should not be disposed of untreated to the sewer unless fully compliant with the requirements of all authorities with jurisdiction. Empty Airgas-owned pressure vessels should be returned to Airgas. Waste packaging should be recycled. 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

	DOT	**TDG**	**Mexico**	**IMDG**	**IATA**
UN number	UN1013	UN1013	UN1013	UN1013	UN1013
UN proper shipping name	CARBON DIOXIDE	CARBON DIOXIDE	CARBON DIOXIDE	CARBON DIOXIDE	CARBON DIOXIDE
Transport hazard class(es)	2.2 	2.2 	2.2 	2.2 	2.2 
Packing group	-	-	-	-	-
Environmental hazards	No.	No.	No.	No.	No.

“Refer to CFR 49 (or authority having jurisdiction) to determine the information required for shipment of the product.”

Additional information

DOT Classification : **Limited quantity** Yes.
Quantity limitation Passenger aircraft/rail: 75 kg. Cargo aircraft: 150 kg.

TDG Classification : Product classified as per the following sections of the Transportation of Dangerous Goods Regulations: 2.13-2.17 (Class 2).
Explosive Limit and Limited Quantity Index 0.125
Passenger Carrying Road or Rail Index 75

IATA : **Quantity limitation** Passenger and Cargo Aircraft: 75 kg. Cargo Aircraft Only: 150 kg.

Special precautions for user : **Transport within user’s premises:** always transport in closed containers that are upright and secure. Ensure that persons transporting the product know what to do in the event of an accident or spillage.

Transport in bulk according to Annex II of MARPOL and the IBC Code : Not available.

Section 15. Regulatory information

U.S. Federal regulations : **TSCA 8(a) CDR Exempt/Partial exemption**: This material is listed or exempted.

Clean Air Act Section 112 (b) Hazardous Air Pollutants (HAPs) : Not listed

Section 15. Regulatory information

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
DEA List II Chemicals (Essential Chemicals)	Not listed

SARA 302/304

Composition/information on ingredients

No products were found.

SARA 304 RQ : Not applicable.

SARA 311/312

Classification : Refer to Section 2: Hazards Identification of this SDS for classification of substance.

State regulations

Massachusetts	This material is listed.
New York	This material is not listed.
New Jersey	This material is listed.
Pennsylvania	This material is listed.

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	This material is listed or exempted.
Canada	This material is listed or exempted.
China	This material is listed or exempted.
Europe	This material is listed or exempted.
Japan	**Japan inventory (ENCS)**: This material is listed or exempted. **Japan inventory (ISHL)**: This material is listed or exempted.
Malaysia	Not determined.
New Zealand	This material is listed or exempted.
Philippines	This material is listed or exempted.
Republic of Korea	This material is listed or exempted.
Taiwan	This material is listed or exempted.
Thailand	Not determined.
Turkey	This material is listed or exempted.
United States	This material is listed or exempted.
Viet Nam	Not determined.

Section 16. Other information

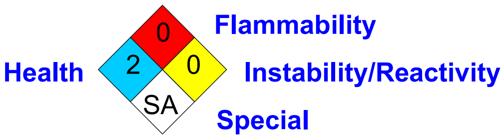
Hazardous Material Information System (U.S.A.)

Health	/	1
Flammability		0
Physical hazards		3

Caution: HMIS® ratings are based on a 0-4 rating scale, with 0 representing minimal hazards or risks, and 4 representing significant hazards or risks. Although HMIS® ratings and the associated label are not required on SDSs or products leaving a facility under 29 CFR 1910.1200, the preparer may choose to provide them. HMIS® ratings are to be used with a fully implemented HMIS® program. HMIS® is a registered trademark and service mark of the American Coatings Association, Inc.

The customer is responsible for determining the PPE code for this material. For more information on HMIS® Personal Protective Equipment (PPE) codes, consult the HMIS® Implementation Manual.

National Fire Protection Association (U.S.A.)



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Copyright ©2001, National Fire Protection Association, Quincy, MA 02269. This warning system is intended to be interpreted and applied only by properly trained individuals to identify fire, health and reactivity hazards of chemicals. The user is referred to certain limited number of chemicals with recommended classifications in NFPA 49 and NFPA 325, which would be used as a guideline only. Whether the chemicals are classified by NFPA or not, anyone using the 704 systems to classify chemicals does so at their own risk.

Procedure used to derive the classification

Classification	Justification
GASES UNDER PRESSURE - Liquefied gas	Expert judgment

History

Date of printing : 2/12/2018
Date of issue/Date of revision : 2/12/2018
Date of previous issue : 4/25/2017
Version : 0.03

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

References : Not available.

Notice to reader

Section 16. Other information

To the best of our knowledge, the information contained herein is accurate. However, neither the above-named supplier, nor any of its subsidiaries, assumes any liability whatsoever for the accuracy or completeness of the information contained herein.
Final determination of suitability of any material is the sole responsibility of the user. All materials may present unknown hazards and should be used with caution. Although certain hazards are described herein, we cannot guarantee that these are the only hazards that exist.

Diatomaceous Earth

SECTION 1 : Identification of the substance/mixture and of the supplier

Product name : **Diatomaceous Earth**

Manufacturer/Supplier Trade name:

Manufacturer/Supplier Article number: **S25298**

Recommended uses of the product and uses restrictions on use:

Manufacturer Details:

AquaPhoenix Scientific
9 Barnhart Drive, Hanover, PA 17331

Supplier Details:

Fisher Science Education
15 Jet View Drive, Rochester, NY 14624

Emergency telephone number:

Fisher Science Education Emergency Telephone No.: 800-535-5053

SECTION 2 : Hazards identification

Classification of the substance or mixture:

Health hazard
Specific target organ toxicity following repeated exposure, category 2

STOT RE 2

Signal word :Warning

Hazard statements:
May cause damage to organs through prolonged or repeated exposure
Precautionary statements:
If medical advice is needed, have product container or label at hand
Keep out of reach of children
Read label before use
Do not breathe dust/fume/gas/mist/vapours/spray
Get Medical advice/attention if you feel unwell
Dispose of contents and container to an approved waste disposal plant

Other Non-GHS Classification:

WHMIS

NFPA/HMIS

NFPA SCALE (0-4)

Health	1
Flammability	0
Physical Hazard	0
Personal Protection	X

HMIS RATINGS (0-4)

SECTION 3 : Composition/information on ingredients

Ingredients:		
CAS 68855-54-9	Kieselguhr, soda ash flux-calcined	>98 %
		Percentages are by weight

SECTION 4 : First aid measures

Description of first aid measures

After inhalation: Loosen clothing as necessary and position individual in a comfortable position.Move exposed to fresh air. Give artificial respiration if necessary. If breathing is difficult give oxygen.Get medical assistance if cough or other symptoms appear.

After skin contact: Rinse/flush exposed skin gently using soap and water for 15-20 minutes.Seek medical advice if discomfort or irritation persists.

After eye contact: Protect unexposed eye. Rinse/flush exposed eye(s) gently using water for 15-20 minutes. Remove contact lens(es) if able to do so during rinsing. Seek medical attention if irritation persists or if concerned.

After swallowing: Rinse mouth thoroughly. Do not induce vomiting. Have exposed individual drink sips of water. Seek medical attention if irritation, discomfort or vomiting persists.Never give anything by mouth to an unconscious person.

Most important symptoms and effects, both acute and delayed:

Irritation, Nausea,Headache, Shortness of breath.;

Indication of any immediate medical attention and special treatment needed:

If seeking medical attention, provide SDS document to physician.Physician should treat symptomatically.

SECTION 5 : Firefighting measures

Extinguishing media

Suitable extinguishing agents: Does not burn. Use extinguishing media appropriate for surrounding fire.

For safety reasons unsuitable extinguishing agents:

Special hazards arising from the substance or mixture:

Combustion products may include carbon oxides or other toxic vapors.Thermal decomposition can lead to release of irritating gases and vapors.

Advice for firefighters:

Protective equipment: Use NIOSH-approved respiratory protection/breathing apparatus.

Additional information (precautions): Avoid inhaling gases, fumes, dust, mist, vapor, and aerosols.Avoid contact with skin, eyes, and clothing.

SECTION 6 : Accidental release measures

Personal precautions, protective equipment and emergency procedures:

Wear protective equipment. Ensure that air-handling systems are operational.Ensure adequate ventilation.

Environmental precautions:

Prevent from reaching drains, sewer or waterway. Collect contaminated soil for characterization per Section 13. Should not be released into environment.

Methods and material for containment and cleaning up:

Keep in suitable closed containers for disposal.Wear protective eyeware, gloves, and clothing. Refer to Section 8.Always obey local regulations.Dust deposits should not be allowed to accumulate on surfaces, as these may form an explosive mixture if they are released into the atmosphere in sufficient concentration. Avoid dispersal of dust in the air (i.e., clearing dust surfaces with compressed air). Collect solids in powder form using vacuum with (HEPA filter). Evacuate personnel to safe areas.

Reference to other sections:

SECTION 7 : Handling and storage

Precautions for safe handling:

Minimize dust generation and accumulation. Follow good hygiene procedures when handling chemical materials. Refer to Section 8.Do not eat, drink, smoke, or use personal products when handling chemical substances. Avoid contact with eyes, skin, and clothing.

Conditions for safe storage, including any incompatibilities:

Store away from incompatible materials.Protect from freezing and physical damage.Keep away from food and beverages.Provide ventilation for containers. Avoid storage near extreme heat, ignition sources or open flame. Store in cool, dry conditions in well sealed containers. Store with like hazards

SECTION 8 : Exposure controls/personal protection

Control Parameters:	409-21-2, Carborundum, OSHA 15 mg/m3 TWA (total dust); 5 mg/m3 TWA (respirable fraction)
Appropriate Engineering controls:	Emergency eye wash fountains and safety showers should be available in the immediate vicinity of use/handling.Provide exhaust ventilation or other engineering controls to keep the airborne concentrations of vapor or dusts (total/respirable) below the applicable workplace exposure limits (Occupational Exposure Limits-OELs) indicated above.It is recommended that all dust control equipment such as local exhaust ventilation and material transport systems involved in handling of this product contain explosion relief vents or an explosion suppression system or an oxygen deficient environment.Ensure that dust-handling systems (such as exhaust ducts, dust collectors, vessels, and processing equipment) are designed in a manner to prevent the escape of dust into the work area (i.e., there is no leakage from the equipment).Use under a fume hood

Respiratory protection:	Not required under normal conditions of use. Where risk assessment shows air-purifying respirators are appropriate use a full-face particle respirator type N100 (US) or type P3 (EN 143) respirator cartridges as a backup to engineering controls.When necessary use NIOSH approved breathing equipment.
Protection of skin:	Select glove material impermeable and resistant to the substance.Select glove material based on rates of diffusion and degradation.Dispose of contaminated gloves after use in accordance with applicable laws and good laboratory practices.Use proper glove removal technique without touching outer surface. Avoid skin contact with used gloves.Wear protective clothing.
Eye protection:	Wear equipment for eye protection tested and approved under appropriate government standards such as NIOSH (US) or EN 166(EU).Safety glasses or goggles are appropriate eye protection.
General hygienic measures:	Perform routine housekeeping.Wash hands before breaks and at the end of work.Avoid contact with skin, eyes, and clothing.Before wearing wash contaminated clothing.

SECTION 9 : Physical and chemical properties

Appearance (physical state,color):	White Solid	**Explosion limit lower**: **Explosion limit upper**:	Not determined Not determined
Odor:	Not determined	**Vapor pressure**:	Not determined
Odor threshold:	Not determined	**Vapor density**:	Not determined
pH-value:	Not determined	**Relative density**:	3.21 g/cm3 at 20 °C
Melting/Freezing point:	Not determined	**Solubilities**:	insoluble
Boiling point/Boiling range:	decomposes	**Partition coefficient (n-octanol/water)**:	Not determined
Flash point (closed cup):	Not determined	**Auto/Self-ignition temperature**:	Not determined
Evaporation rate:	Not determined	**Decomposition temperature**:	Not determined
Flammability (solid,gaseous):	Not determined	**Viscosity**:	a. Kinematic:Not determined b. Dynamic: Not determined
Density: Not determined			

SECTION 10 : Stability and reactivity

Reactivity: Nonreactive under normal conditions.
Chemical stability: Stable under normal conditions.
Possible hazardous reactions: None under normal processing
Conditions to avoid: Incompatible Materials.
Incompatible materials: Strong acids.Strong bases.Oxidizing agents.
Hazardous decomposition products:

SECTION 11 : Toxicological information

Acute Toxicity:		
Oral:		LD50 - rat - > 2,000 mg/kg
Chronic Toxicity: No additional information.		
Corrosion Irritation: No additional information.		
Sensitization:		No additional information.
Single Target Organ (STOT):		No additional information.
Numerical Measures:		No additional information.
Carcinogenicity:		IARC : Category 2B: Possibly carcinogenic to humans.
Mutagenicity:		No additional information.
Reproductive Toxicity:		No additional information.

SECTION 12 : Ecological information

Ecotoxicity Persistence and degradability:
Bioaccumulative potential:
Mobility in soil:
Other adverse effects:

SECTION 13 : Disposal considerations

Waste disposal recommendations:

Contact a licensed professional waste disposal service to dispose of this material.Dispose of empty containers as unused product.Product or containers must not be disposed with household garbage.It is the responsibility of the waste generator to properly characterize all waste materials according to applicable regulatory entities (US 40CFR262.11).Chemical waste generators must determine whether a discarded chemical is classified as a hazardous waste. Chemical waste generators must also consult local, regional, and national hazardous waste regulations. Ensure complete and accurate classification.

SECTION 14 : Transport information

UN-Number

Not Regulated.

UN proper shipping name

Not Regulated.

Transport hazard class(es)
Packing group:Not Regulated
Environmental hazard:
Transport in bulk:
Special precautions for user:

SECTION 15 : Regulatory information

United States (USA)

SARA Section 311/312 (Specific toxic chemical listings):

None of the ingredients is listed

SARA Section 313 (Specific toxic chemical listings):

None of the ingredients is listed

RCRA (hazardous waste code):

None of the ingredients is listed

TSCA (Toxic Substances Control Act):

All ingredients are listed.

CERCLA (Comprehensive Environmental Response, Compensation, and Liability Act):

None of the ingredients is listed

Proposition 65 (California):

Chemicals known to cause cancer:

None of the ingredients is listed

Chemicals known to cause reproductive toxicity for females:

None of the ingredients is listed

Chemicals known to cause reproductive toxicity for males:

None of the ingredients is listed

Chemicals known to cause developmental toxicity:

None of the ingredients is listed

Canada

Canadian Domestic Substances List (DSL):

All ingredients are listed.

Canadian NPRI Ingredient Disclosure list (limit 0.1%):

68855-54-9 Diatomaceous earth

Canadian NPRI Ingredient Disclosure list (limit 1%):

None of the ingredients is listed

SECTION 16 : Other information

This product has been classified in accordance with hazard criteria of the Controlled Products Regulations and the SDS contains all the information required by the Controlled Products Regulations.Note:. The responsibility to provide a safe workplace remains with the user.The user should consider the health hazards and safety information contained herein as a guide and should take those precautions required in an individual operation to instruct employees and develop work practice procedures for a safe work environment.The information contained herein is, to the best of our knowledge and belief, accurate.However, since the conditions of handling and use are beyond our control, we make no guarantee of results, and assume no liability for damages incurred by the use of this material.It is the responsibility of the user to comply with all applicable laws and regulations applicable to this material.

GHS Full Text Phrases:

Abbreviations and acronyms:
IMDG: International Maritime Code for Dangerous Goods
IATA: International Air Transport Association
GHS: Globally Harmonized System of Classification and Labelling of Chemicals

ACGIH: American Conference of Governmental Industrial Hygienists
CAS: Chemical Abstracts Service (division of the American Chemical Society)
NFPA: National Fire Protection Association (USA)
HMIS: Hazardous Materials Identification System (USA)
WHMIS: Workplace Hazardous Materials Information System (Canada)
DNEL: Derived No-Effect Level (REACH)
PNEC: Predicted No-Effect Concentration (REACH)
CFR: Code of Federal Regulations (USA)
SARA: Superfund Amendments and Reauthorization Act (USA)
RCRA: Resource Conservation and Recovery Act (USA)
TSCA: Toxic Substances Control Act (USA)
NPRI: National Pollutant Release Inventory (Canada)
DOT: US Department of Transportation

Effective date : 10.24.2014
Last updated : 03.19.2015

SAFETY DATA SHEET

Creation Date 09-Jul-2009 **Revision Date** 18-Jan-2018 **Revision Number** 4

1. Identification

Product Name	**Ethanol, Anhydrous (Histological)**
Cat No. :	**A405-20; A405F-1GAL; A405P-4**
Synonyms	Grain alcohol, denatured; Ethyl alcohol, denatured; Ethyl hydroxide, denatured.
Recommended Use	Laboratory chemicals.
Uses advised against	Not for food, drug, pesticide or biocidal product use

Details of the supplier of the safety data sheet

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: 001-703-527-3887

2. Hazard(s) identification

Classification

This chemical is considered hazardous by the 2012 OSHA Hazard Communication Standard (29 CFR 1910.1200)

Flammable liquids	Category 2
Serious Eye Damage/Eye Irritation	Category 2
Specific target organ toxicity (single exposure)	Category 1
Target Organs - Central nervous system (CNS), Optic nerve, Respiratory system.	
Specific target organ toxicity - (repeated exposure)	Category 1
Target Organs - Kidney, Liver, spleen, Blood.	

Label Elements

Signal Word

Danger

Hazard Statements

Highly flammable liquid and vapor
Causes serious eye irritation
Causes damage to organs
Causes damage to organs through prolonged or repeated exposure

Precautionary Statements
Prevention
Obtain special instructions before use
Do not handle until all safety precautions have been read and understood
Use personal protective equipment as required
Wash face, hands and any exposed skin thoroughly after handling
Wear eye/face protection
Do not breathe dust/fume/gas/mist/vapors/spray
Do not eat, drink or smoke when using this product
Keep away from heat/sparks/open flames/hot surfaces. - No smoking
Keep container tightly closed
Ground/bond container and receiving equipment
Use explosion-proof electrical/ventilating/lighting/equipment
Use only non-sparking tools
Take precautionary measures against static discharge
Response
IF exposed: Call a POISON CENTER or doctor/physician
Skin
IF ON SKIN (or hair): Take off immediately all contaminated clothing. Rinse skin with water/shower
Eyes
IF IN EYES: Rinse cautiously with water for several minutes. Remove contact lenses, if present and easy to do. Continue rinsing
If eye irritation persists: Get medical advice/attention
Fire
In case of fire: Use CO2, dry chemical, or foam for extinction
Storage
Store locked up
Store in a well-ventilated place. Keep cool
Disposal
Dispose of contents/container to an approved waste disposal plant
Hazards not otherwise classified (HNOC)
Repeated exposure may cause skin dryness or cracking
Other hazards
Poison, may be fatal or cause blindness if swallowed. Vapor harmful. Cannot be made non-poisonous.
WARNING. Cancer and Reproductive Harm - https://www.p65warnings.ca.gov/.

3. Composition/Information on Ingredients

Component	CAS-No	Weight %
Ethyl alcohol	64-17-5	91-92
Methyl alcohol	67-56-1	<5
Methylisobutyl ketone	108-10-1	1.0 - 2.0
Ethyl acetate	141-78-6	1-2
Hexane	110-54-3	<1
Toluene	108-88-3	<0.1
Ligroine	8032-32-4	<0.1

4. First-aid measures

General Advice If symptoms persist, call a physician.

Eye Contact	Rinse immediately with plenty of water, also under the eyelids, for at least 15 minutes. Get medical attention.
Skin Contact	Wash off immediately with plenty of water for at least 15 minutes. If skin irritation persists, call a physician.
Inhalation	Move to fresh air. If not breathing, give artificial respiration. Get medical attention if symptoms occur.
Ingestion	Clean mouth with water and drink afterwards plenty of water.
Most important symptoms and effects	None reasonably foreseeable. Breathing difficulties. Inhalation of high vapor concentrations may cause symptoms like headache, dizziness, tiredness, nausea and vomiting
Notes to Physician	Treat symptomatically

5. Fire-fighting measures

Suitable Extinguishing Media	Use water spray, alcohol-resistant foam, dry chemical or carbon dioxide.
Unsuitable Extinguishing Media	Water may be ineffective, Do not use a solid water stream as it may scatter and spread fire
Flash Point	13.9 °C / 57 °F
Method -	No information available
Autoignition Temperature	362.8 °C / 685 °F
Explosion Limits	
Upper	18.0 vol %
Lower	3.3 vol %
Sensitivity to Mechanical Impact	No information available
Sensitivity to Static Discharge	No information available

Specific Hazards Arising from the Chemical
Flammable. Vapors may form explosive mixtures with air. Vapors may travel to source of ignition and flash back. Containers may explode when heated. Vapors may form explosive mixtures with air.

Hazardous Combustion Products
Carbon monoxide (CO) Carbon dioxide (CO_2)
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	Flammability	Instability	Physical hazards
3	3	0	N/A

6. Accidental release measures

Personal Precautions	Use personal protective equipment. Ensure adequate ventilation.
Environmental Precautions	Should not be released into the environment. See Section 12 for additional ecological information.
Methods for Containment and Clean Up	Soak up with inert absorbent material. Keep in suitable, closed containers for disposal.

7. Handling and storage

Handling	Wear personal protective equipment. Ensure adequate ventilation. Do not get in eyes, on skin, or on clothing. Avoid ingestion and inhalation.

Storage Keep containers tightly closed in a dry, cool and well-ventilated place. Keep away from heat and sources of ignition.

8. Exposure controls / personal protection

Exposure Guidelines

Component	ACGIH TLV	OSHA PEL	NIOSH IDLH	Mexico OEL (TWA)
Ethyl alcohol	STEL: 1000 ppm	(Vacated) TWA: 1000 ppm (Vacated) TWA: 1900 mg/m^3 TWA: 1000 ppm TWA: 1900 mg/m^3	IDLH: 3300 ppm TWA: 1000 ppm TWA: 1900 mg/m^3	TWA: 1000 ppm TWA: 1900 mg/m^3
Methyl alcohol	TWA: 200 ppm STEL: 250 ppm Skin	(Vacated) TWA: 200 ppm (Vacated) TWA: 260 mg/m^3 (Vacated) STEL: 250 ppm (Vacated) STEL: 325 mg/m^3 Skin TWA: 200 ppm TWA: 260 mg/m^3	IDLH: 6000 ppm TWA: 200 ppm TWA: 260 mg/m^3 STEL: 250 ppm STEL: 325 mg/m^3	TWA: 200 ppm TWA: 260 mg/m^3 STEL: 250 ppm STEL: 310 mg/m^3
Methylisobutyl ketone	TWA: 20 ppm STEL: 75 ppm	(Vacated) TWA: 50 ppm (Vacated) TWA: 205 mg/m^3 (Vacated) STEL: 75 ppm (Vacated) STEL: 300 mg/m^3 TWA: 100 ppm TWA: 410 mg/m^3	IDLH: 500 ppm TWA: 50 ppm TWA: 205 mg/m^3 STEL: 75 ppm STEL: 300 mg/m^3	TWA: 50 ppm TWA: 205 mg/m^3 STEL: 75 ppm STEL: 307 mg/m^3
Ethyl acetate	TWA: 400 ppm	(Vacated) TWA: 400 ppm (Vacated) TWA: 1400 mg/m^3 TWA: 400 ppm TWA: 1400 mg/m^3	IDLH: 2000 ppm TWA: 400 ppm TWA: 1400 mg/m^3	TWA: 400 ppm TWA: 1400 mg/m^3
Hexane	TWA: 50 ppm Skin	(Vacated) TWA: 50 ppm (Vacated) TWA: 180 mg/m^3 TWA: 500 ppm TWA: 1800 mg/m^3	IDLH: 1100 ppm TWA: 50 ppm TWA: 180 mg/m^3	TWA: 50 ppm TWA: 176 mg/m^3
Toluene	TWA: 20 ppm	(Vacated) TWA: 100 ppm (Vacated) TWA: 375 mg/m^3 Ceiling: 300 ppm (Vacated) STEL: 150 ppm (Vacated) STEL: 560 mg/m^3 TWA: 200 ppm	IDLH: 500 ppm TWA: 100 ppm TWA: 375 mg/m^3 STEL: 150 ppm STEL: 560 mg/m^3	TWA: 50 ppm TWA: 188 mg/m^3
Ligroine		(Vacated) TWA: 300 ppm (Vacated) TWA: 1350 mg/m^3 (Vacated) STEL: 400 ppm (Vacated) STEL: 1800 mg/m^3	TWA: 350 mg/m^3 Ceiling: 1800 mg/m^3	TWA: 300 ppm TWA: 1350 mg/m^3 STEL: 400 ppm STEL: 1800 mg/m^3

Legend

ACGIH - American Conference of Governmental Industrial Hygienists
OSHA - Occupational Safety and Health Administration
NIOSH IDLH: The National Institute for Occupational Safety and Health Immediately Dangerous to Life or Health

Engineering Measures Ensure adequate ventilation, especially in confined areas. Use explosion-proof electrical/ventilating/lighting/equipment. Ensure that eyewash stations and safety showers are close to the workstation location.

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 Long sleeved clothing.

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.
Hygiene Measures	Handle in accordance with good industrial hygiene and safety practice.

9. Physical and chemical properties

Physical State	Liquid
Appearance	Clear
Odor	Alcohol-like
Odor Threshold	No information available
pH	No information available
Melting Point/Range	< -90 °C / -130 °F
Boiling Point/Range	No information available 77.1 °C / 170.8 °F
Flash Point	13.9 °C / 57 °F
Evaporation Rate	3.6 (Butyl acetate = 1.0)
Flammability (solid,gas)	Not applicable
Flammability or explosive limits	
Upper	18.0 vol %
Lower	3.3 vol %
Vapor Pressure	48 mmHg
Vapor Density	1.5
Specific Gravity	0.785 - 0.792
Solubility	Soluble in water
Partition coefficient; n-octanol/water	No data available
Autoignition Temperature	362.8 °C / 685 °F
Decomposition Temperature	No information available
Viscosity	No information available

10. Stability and reactivity

Reactive Hazard	None known, based on information available
Stability	Stable under normal conditions.
Conditions to Avoid	Incompatible products. Excess heat. Keep away from open flames, hot surfaces and sources of ignition.
Incompatible Materials	Strong oxidizing agents, Acids, Acid anhydrides, Acid chlorides, Peroxides, Alkali metals
Hazardous Decomposition Products	Carbon monoxide (CO), Carbon dioxide (CO_2)
Hazardous Polymerization	Hazardous polymerization does not occur.
Hazardous Reactions	None under normal processing.

11. Toxicological information

Acute Toxicity

Product Information

Oral LD50	Based on ATE data, the classification criteria are not met. ATE > 2000 mg/kg.
Dermal LD50	Based on ATE data, the classification criteria are not met. ATE > 2000 mg/kg.
Vapor LC50	Based on ATE data, the classification criteria are not met. ATE > 20 mg/l.

Component Information

Component	LD50 Oral	LD50 Dermal	LC50 Inhalation
Ethyl alcohol	3450 mg/kg (Mouse)	Not listed	20000 ppm/10H (Rat)
Methyl alcohol	**Calc. ATE 60 mg/kg** LD50 > 1187 – 2769 mg/kg (Rat)	**Calc. ATE 60 mg/kg** LD50 = 17100 mg/kg (Rabbit)	**Calc. ATE 0.6 mg/L (vapours) or 0.5 mg/L (mists)** LC50 = 128.2 mg/L (Rat) 4 h

Methylisobutyl ketone	LD50 = 2080 mg/kg (Rat)	LD50 = 3000 mg/kg (Rabbit)	LC50 = 8.2 mg/L (Rat) 4 h
Ethyl acetate	10,200 mg/kg (Rat)	> 20 mL/kg (Rabbit) > 18000 mg/kg (Rabbit)	58 mg/l (rat; 8 h)
Hexane	LD50 = 25 g/kg (Rat)	LD50 = 3000 mg/kg (Rabbit)	LC50 = 48000 ppm (Rat) 4 h
Toluene	> 5000 mg/kg (Rat)	LD50 = 12000 mg/kg (Rabbit)	26700 ppm (Rat) 1 h
Ligroine	Not listed	Not listed	LC50 = 3400 ppm (Rat) 4 h

Toxicologically Synergistic Products No information available

Delayed and immediate effects as well as chronic effects from short and long-term exposure

Irritation Severe eye irritant

Sensitization No information available

Carcinogenicity The table below indicates whether each agency has listed any ingredient as a carcinogen.

Component	CAS-No	IARC	NTP	ACGIH	OSHA	Mexico
Ethyl alcohol	64-17-5	Group 1	Known	A3	X	Not listed
Methyl alcohol	67-56-1	Not listed	Not listed	Not listed	Not listed	Not listed
Methylisobutyl ketone	108-10-1	Group 2B	Not listed	A3	X	Not listed
Ethyl acetate	141-78-6	Not listed	Not listed	Not listed	Not listed	Not listed
Hexane	110-54-3	Not listed	Not listed	Not listed	Not listed	Not listed
Toluene	108-88-3	Not listed	Not listed	Not listed	Not listed	Not listed
Ligroine	8032-32-4	Not listed	Not listed	Not listed	Not listed	A3

IARC: (International Agency for Research on Cancer)	*IARC: (International Agency for Research on Cancer)* *Group 1 - Carcinogenic to Humans* *Group 2A - Probably Carcinogenic to Humans* *Group 2B - Possibly Carcinogenic to Humans*
NTP: (National Toxicity Program)	*NTP: (National Toxicity Program)* *Known - Known Carcinogen* *Reasonably Anticipated - Reasonably Anticipated to be a Human Carcinogen*
ACGIH: (American Conference of Governmental Industrial Hygienists)	*A1 - Known Human Carcinogen* *A2 - Suspected Human Carcinogen* *A3 - Animal Carcinogen* *ACGIH: (American Conference of Governmental Industrial Hygienists)*
Mexico - Occupational Exposure Limits - Carcinogens	*Mexico - Occupational Exposure Limits - Carcinogens* *A1 - Confirmed Human Carcinogen* *A2 - Suspected Human Carcinogen* *A3 - Confirmed Animal Carcinogen* *A4 - Not Classifiable as a Human Carcinogen* *A5 - Not Suspected as a Human Carcinogen*

Mutagenic Effects Mutagenic effects have occurred in experimental animals.

Reproductive Effects No information available.

Developmental Effects No information available.

Teratogenicity No information available.

STOT - single exposure Central nervous system (CNS) Optic nerve Respiratory system
STOT - repeated exposure Kidney Liver spleen Blood

Aspiration hazard No information available

Symptoms / effects,both acute and delayed Inhalation of high vapor concentrations may cause symptoms like headache, dizziness, tiredness, nausea and vomiting

Endocrine Disruptor Information No information available

Other Adverse Effects The toxicological properties have not been fully investigated.

12. Ecological information

Ecotoxicity

Contains a substance which is:. Toxic to aquatic organisms. The product contains following substances which are hazardous for the environment.

Component	Freshwater Algae	Freshwater Fish	Microtox	Water Flea
Ethyl alcohol	EC50 (72h) = 275 mg/l (Chlorella vulgaris)	Fathead minnow (Pimephales promelas) LC50 = 14200 mg/l/96h	Photobacterium phosphoreum:EC50 = 34634 mg/L/30 min Photobacterium phosphoreum:EC50 = 35470 mg/L/5 min	EC50 = 9268 mg/L/48h EC50 = 10800 mg/L/24h
Methyl alcohol	Not listed	Pimephales promelas: LC50 > 10000 mg/L 96h	EC50 = 39000 mg/L 25 min EC50 = 40000 mg/L 15 min EC50 = 43000 mg/L 5 min	EC50 > 10000 mg/L 24h
Methylisobutyl ketone	EC50: 400 mg/L/96h	LC50: 496 - 514 mg/L, 96h flow-through (Pimephales promelas)	EC50 = 79.6 mg/L 5 min	EC50: 4280.0 mg/L/24h EC50: 170 mg/L/48h EC50: 4280.0 mg/L/24h
Ethyl acetate	EC50 = 3300 mg/L/48h	Fathead minnow: LC50: 230 mg/l/ 96h Gold orfe: LC50: 270 mg/L/48h	EC50 = 1180 mg/L 5 min EC50 = 1500 mg/L 15 min EC50 = 5870 mg/L 15 min EC50 = 7400 mg/L 2 h	EC50 = 717 mg/L/48h
Hexane	Not listed	LC50: 2.1 - 2.98 mg/L, 96h flow-through (Pimephales promelas)	Not listed	EC50: 3.87 mg/L/48h
Toluene	EC50: = 12.5 mg/L, 72h static (Pseudokirchneriella subcapitata) EC50: > 433 mg/L, 96h (Pseudokirchneriella subcapitata)	50-70 mg/L LC50 96 h 5-7 mg/L LC50 96 h 15-19 mg/L LC50 96 h 28 mg/L LC50 96 h 12 mg/L LC50 96 h	EC50 = 19.7 mg/L 30 min	EC50: = 11.5 mg/L, 48h (Daphnia magna) EC50: 5.46 - 9.83 mg/L, 48h Static (Daphnia magna)
Ligroine	EC50: = 4700 mg/L, 72h (Pseudokirchneriella subcapitata)	Not listed	Not listed	Not listed

Persistence and Degradability Persistence is unlikely based on information available.

Bioaccumulation/ Accumulation No information available.

Mobility Will likely be mobile in the environment due to its volatility.

Component	log Pow
Ethyl alcohol	-0.32
Methyl alcohol	-0.74
Methylisobutyl ketone	1.19
Ethyl acetate	0.6
Hexane	4.11
Toluene	2.7

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.

Component	RCRA - U Series Wastes	RCRA - P Series Wastes
Methyl alcohol - 67-56-1	U154	-
Methylisobutyl ketone - 108-10-1	U161	-

Ethyl acetate - 141-78-6	U112	-
Toluene - 108-88-3	U220	-

14. Transport information

DOT

UN-No	UN1170
Proper Shipping Name	ETHANOL
Hazard Class	3
Packing Group	II

TDG

UN-No	UN1170
Proper Shipping Name	ETHANOL
Hazard Class	3
Packing Group	II

IATA

UN-No	UN1170
Proper Shipping Name	ETHANOL
Hazard Class	3
Packing Group	II

IMDG/IMO

UN-No	UN1170
Proper Shipping Name	ETHANOL
Hazard Class	3
Packing Group	II

15. Regulatory information

All of the components in the product are on the following Inventory lists: Australia Complete Regulatory Information contained in following SDS's X = listed China Canada The product is classified and labeled according to EC directives or corresponding national laws The product is classified and labeled in accordance with Directive 1999/45/EC Europe TSCA Korea Philippines U.S.A. (TSCA) Canada (DSL/NDSL) Europe (EINECS/ELINCS/NLP) Australia (AICS) Korea (ECL) China (IECSC) Japan (ENCS) Philippines (PICCS)

International Inventories

Component	TSCA	DSL	NDSL	EINECS	ELINCS	NLP	PICCS	ENCS	AICS	IECSC	KECL
Ethyl alcohol	X	X	-	200-578-6	-		X	X	X	X	X
Methyl alcohol	X	X	-	200-659-6	-		X	X	X	X	X
Methylisobutyl ketone	X	X	-	203-550-1	-		X	X	X	X	X
Ethyl acetate	X	X	-	205-500-4	-		X	X	X	X	X
Hexane	X	X	-	203-777-6	438-390-3		X	X	X	X	X
Toluene	X	X	-	203-625-9	-		X	X	X	X	X
Ligroine	X	X	-	232-453-7	-		X	-	X	X	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

Component	CAS-No	Weight %	SARA 313 - Threshold Values %
Methyl alcohol	67-56-1	<5	1.0
Methylisobutyl ketone	108-10-1	1.0 - 2.0	1.0
Hexane	110-54-3	<1	1.0
Toluene	108-88-3	<0.1	1.0

SARA 311/312 Hazard Categories See section 2 for more information

CWA (Clean Water Act)

Component	CWA - Hazardous Substances	CWA - Reportable Quantities	CWA - Toxic Pollutants	CWA - Priority Pollutants
Toluene	X	1000 lb	X	X

Clean Air Act

Component	HAPS Data	Class 1 Ozone Depletors	Class 2 Ozone Depletors
Methyl alcohol	X		-
Methylisobutyl ketone	X		-
Hexane	X		-
Toluene	X		-

OSHA Occupational Safety and Health Administration
Not applicable

CERCLA

Component	Hazardous Substances RQs	CERCLA EHS RQs
Methyl alcohol	5000 lb	-
Methylisobutyl ketone	5000 lb	-
Ethyl acetate	5000 lb	-
Hexane	5000 lb	-
Toluene	1000 lb 1 lb	-

California Proposition 65 Ethyl alcohol is only a considered a Proposition 65 developmental hazard when it is ingested as an alcoholic beverage This product contains the following proposition 65 chemicals

Component	CAS-No	California Prop. 65	Prop 65 NSRL	Category
Ethyl alcohol	64-17-5	Development (alcoholic beverages only)	-	Developmental Carcinogen
Methyl alcohol	67-56-1	Developmental	-	Developmental
Methylisobutyl ketone	108-10-1	Carcinogen Developmental	-	Developmental Carcinogen
Toluene	108-88-3	Developmental	-	Developmental

U.S. State Right-to-Know Regulations

Component	Massachusetts	New Jersey	Pennsylvania	Illinois	Rhode Island
Ethyl alcohol	X	X	X	X	X
Methyl alcohol	X	X	X	X	X
Methylisobutyl ketone	X	X	X	X	X
Ethyl acetate	X	X	X	-	X
Hexane	X	X	X	X	X
Toluene	X	X	X	X	X
Ligroine	-	X	X	-	-

U.S. Department of Transportation

Reportable Quantity (RQ):	N
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	Serious risk, Grade 3

16. Other information

Prepared By	Regulatory Affairs Thermo Fisher Scientific Email: EMSDS.RA@thermofisher.com
Creation Date	09-Jul-2009
Revision Date	18-Jan-2018
Print Date	18-Jan-2018
Revision Summary	This document has been updated to comply with the US OSHA HazCom 2012 Standard replacing the current legislation under 29 CFR 1910.1200 to align with the Globally Harmonized System of Classification and Labeling of Chemicals (GHS).

Disclaimer
The information provided in this Safety Data Sheet is correct to the best of our knowledge, information and belief at the date of its publication. The information given is designed only as a guidance for safe handling, use, processing, storage, transportation, disposal and release and is not to be considered a warranty or quality specification. The information relates only to the specific material designated and may not be valid for such material used in combination with any other materials or in any process, unless specified in the text

End of SDS

SAFETY DATA SHEET

Creation Date 02-Feb-2010 **Revision Date** 17-Jan-2018 **Revision Number** 4

1. Identification

Product Name	**Ethylene glycol**
Cat No. :	**E177-4; E177-20**
CAS-No	107-21-1
Synonyms	Monoethylene glycol; 1,2-Ethanediol
Recommended Use	Laboratory chemicals.
Uses advised against	Not for food, drug, pesticide or biocidal product use

Details of the supplier of the safety data sheet

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: 001-703-527-3887

2. Hazard(s) identification

Classification

This chemical is considered hazardous by the 2012 OSHA Hazard Communication Standard (29 CFR 1910.1200)

Acute oral toxicity	Category 4
Specific target organ toxicity (single exposure)	Category 3
Target Organs - Central nervous system (CNS).	
Specific target organ toxicity - (repeated exposure)	Category 2
Target Organs - Kidney, Liver.	

Label Elements

Signal Word
Warning

Hazard Statements
Harmful if swallowed
May cause drowsiness or dizziness
May cause damage to organs through prolonged or repeated exposure

Precautionary Statements
Prevention
Wash face, hands and any exposed skin thoroughly after handling
Do not eat, drink or smoke when using this product
Do not breathe dust/fume/gas/mist/vapors/spray
Use only outdoors or in a well-ventilated area
Response
Get medical attention/advice if you feel unwell
Inhalation
IF INHALED: Remove victim to fresh air and keep at rest in a position comfortable for breathing
Call a POISON CENTER or doctor/physician if you feel unwell
Ingestion
IF SWALLOWED: Call a POISON CENTER or doctor/physician if you feel unwell
Rinse mouth
Storage
Store in a well-ventilated place. Keep container tightly closed
Store locked up
Disposal
Dispose of contents/container to an approved waste disposal plant
Hazards not otherwise classified (HNOC)

WARNING. Reproductive Harm - https://www.p65warnings.ca.gov/.

3. Composition/Information on Ingredients

Component	CAS-No	Weight %
Ethylene glycol	107-21-1	>95

4. First-aid measures

Eye Contact	Rinse immediately with plenty of water, also under the eyelids, for at least 15 minutes. Get medical attention.
Skin Contact	Wash off immediately with plenty of water for at least 15 minutes. Get medical attention immediately if symptoms occur.
Inhalation	Move to fresh air. Do not use mouth-to-mouth method if victim ingested or inhaled the substance; give artificial respiration with the aid of a pocket mask equipped with a one-way valve or other proper respiratory medical device. Get medical attention immediately if symptoms occur. If not breathing, give artificial respiration.
Ingestion	Do not induce vomiting. Call a physician or Poison Control Center immediately.
Most important symptoms and effects	Breathing difficulties.
Notes to Physician	Treat symptomatically

5. Fire-fighting measures

Suitable Extinguishing Media	Use water spray, alcohol-resistant foam, dry chemical or carbon dioxide.

Unsuitable Extinguishing Media	No information available
Flash Point	111 °C / 231.8 °F
Method -	DIN 51758
Autoignition Temperature	413 °C / 775.4 °F
Explosion Limits	
Upper	15.30 vol %
Lower	3.20 vol %
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. Keep product and empty container away from heat and sources of ignition.

Hazardous Combustion Products

Carbon monoxide (CO) Carbon dioxide (CO_2)

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	Flammability	Instability	Physical hazards
2	1	1	N/A

6. Accidental release measures

Personal Precautions	Ensure adequate ventilation. Use personal protective equipment.
Environmental Precautions	Should not be released into the environment. See Section 12 for additional ecological information.
Methods for Containment and Clean Up	Soak up with inert absorbent material. Keep in suitable, closed containers for disposal.

7. Handling and storage

Handling	Wear personal protective equipment. Ensure adequate ventilation. Do not breathe vapors or spray mist. Avoid contact with skin, eyes and clothing.
Storage	Keep containers tightly closed in a dry, cool and well-ventilated place. Keep away from heat and sources of ignition.

8. Exposure controls / personal protection

Exposure Guidelines

Component	ACGIH TLV	OSHA PEL	NIOSH IDLH	Mexico OEL (TWA)
Ethylene glycol	TWA: 25 ppm STEL: 50 ppm STEL: 10 mg/m^3	(Vacated) Ceiling: 50 ppm (Vacated) Ceiling: 125 mg/m^3		Ceiling: 100 mg/m^3

Legend

ACGIH - American Conference of Governmental Industrial Hygienists
OSHA - Occupational Safety and Health Administration

Engineering Measures	Ensure adequate ventilation, especially in confined areas. Ensure that eyewash stations and safety showers are close to the workstation location.

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.
Hygiene Measures	Handle in accordance with good industrial hygiene and safety practice.

9. Physical and chemical properties

Physical State	Viscous liquid Liquid
Appearance	Colorless
Odor	Odorless
Odor Threshold	No information available
pH	5.5-7.5 50% aq. sol
Melting Point/Range	-13 °C / 8.6 °F
Boiling Point/Range	196 - 198 °C / 384.8 - 388.4 °F @ 760 mmHg
Flash Point	111 °C / 231.8 °F
Method -	DIN 51758
Evaporation Rate	No information available
Flammability (solid,gas)	Not applicable
Flammability or explosive limits	
Upper	15.30 vol %
Lower	3.20 vol %
Vapor Pressure	0.12 mmHg @ 20 °C
Vapor Density	2.14 (Air = 1.0)
Specific Gravity	1.113
Solubility	miscible
Partition coefficient; n-octanol/water	No data available
Autoignition Temperature	413 °C / 775.4 °F
Decomposition Temperature	> 500°C
Viscosity	21 cP (20°C)
Molecular Formula	C2 H6 O2
Molecular Weight	62.06

10. Stability and reactivity

Reactive Hazard	None known, based on information available
Stability	Hygroscopic.
Conditions to Avoid	Incompatible products. Excess heat. Exposure to moist air or water.
Incompatible Materials	Strong oxidizing agents, Strong acids, Strong bases, Aldehydes
Hazardous Decomposition Products	Carbon monoxide (CO), Carbon dioxide (CO_2)
Hazardous Polymerization	Hazardous polymerization does not occur.
Hazardous Reactions	None under normal processing.

11. Toxicological information

Acute Toxicity

Product Information

Component Information

Component	LD50 Oral	LD50 Dermal	LC50 Inhalation
Ethylene glycol	7712 mg/kg (Rat)	9530 µL/kg (Rabbit) 10600 mg/kg (Rat)	Not listed

Toxicologically Synergistic Products No information available

Delayed and immediate effects as well as chronic effects from short and long-term exposure

Irritation May cause eye, skin, and respiratory tract irritation

Sensitization No information available

Carcinogenicity The table below indicates whether each agency has listed any ingredient as a carcinogen.

Component	CAS-No	IARC	NTP	ACGIH	OSHA	Mexico
Ethylene glycol	107-21-1	Not listed	Not listed	Not listed	Not listed	Not listed

Mutagenic Effects No information available

Reproductive Effects No information available.

Developmental Effects No information available.

Teratogenicity No information available.

STOT - single exposure Central nervous system (CNS)

STOT - repeated exposure Kidney Liver

Aspiration hazard No information available

Symptoms / effects,both acute and delayed No information available

Endocrine Disruptor Information No information available

Other Adverse Effects The toxicological properties have not been fully investigated.

12. Ecological information

Ecotoxicity

Do not empty into drains. .

Component	Freshwater Algae	Freshwater Fish	Microtox	Water Flea
Ethylene glycol	EC50: 6500 - 13000 mg/L, 96h (Pseudokirchneriella subcapitata)	LC50: = 16000 mg/L, 96h static (Poecilia reticulata) LC50: 40000 - 60000 mg/L, 96h static (Pimephales promelas) LC50: = 40761 mg/L, 96h static (Oncorhynchus mykiss) LC50: = 41000 mg/L, 96h (Oncorhynchus mykiss) LC50: 14 - 18 mL/L, 96h static (Oncorhynchus mykiss) LC50: = 27540 mg/L, 96h static (Lepomis macrochirus)	Not listed	EC50: = 46300 mg/L, 48h (Daphnia magna)

Persistence and Degradability Persistence is unlikely

Bioaccumulation/ Accumulation No information available.

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
Ethylene glycol	5000 lb	-

California Proposition 65 This product does not contain any Proposition 65 chemicals

Component	CAS-No	California Prop. 65	Prop 65 NSRL	Category
Ethylene glycol	107-21-1	Developmental	-	Developmental

U.S. State Right-to-Know Regulations

Component	Massachusetts	New Jersey	Pennsylvania	Illinois	Rhode Island
Ethylene glycol	X	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 Slight risk, Grade 1

16. Other information

Prepared By
Regulatory Affairs
Thermo Fisher Scientific
Email: EMSDS.RA@thermofisher.com

Creation Date	02-Feb-2010
Revision Date	17-Jan-2018
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End of SDS

D-Fructose

SECTION 1 : Identification of the substance/mixture and of the supplier

Product name :	**D-Fructose**
Manufacturer/Supplier Trade name:	
Manufacturer/Supplier Article number:	**S25332**

Recommended uses of the product and uses restrictions on use:

Manufacturer Details:

AquaPhoenix Scientific
9 Barnhart Drive, Hanover, PA 17331

Supplier Details:

Fisher Science Education
15 Jet View Drive, Rochester, NY 14624

Emergency telephone number:

Fisher Science Education Emergency Telephone No.: 800-535-5053

SECTION 2 : Hazards identification

Classification of the substance or mixture:

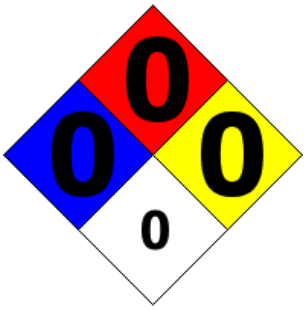
Not classified for physical or health hazards according to GHS.

Hazard statements:
Precautionary statements:
If medical advice is needed, have product container or label at hand
Keep out of reach of children
Read label before use
Do not eat, drink or smoke when using this product

Other Non-GHS Classification:

WHMIS

NFPA/HMIS



NFPA SCALE (0-4)

Health	**0**
Flammability	**0**
Physical Hazard	**0**
Personal Protection	**X**

HMIS RATINGS (0-4)

SECTION 3 : Composition/information on ingredients

Ingredients:

CAS 57-48-7	D-Fructose	100 %
		Percentages are by weight

SECTION 4 : First aid measures

Description of first aid measures

After inhalation: Loosen clothing as necessary and position individual in a comfortable position.Move exposed to fresh air. Give artificial respiration if necessary. If breathing is difficult give oxygen.Get medical assistance if cough or other symptoms appear.

After skin contact: Rinse/flush exposed skin gently using soap and water for 15-20 minutes.Seek medical advice if discomfort or irritation persists.

After eye contact: Protect unexposed eye. Rinse/flush exposed eye(s) gently using water for 15-20 minutes. Remove contact lens(es) if able to do so during rinsing. Seek medical attention if irritation persists or if concerned.

After swallowing: Rinse mouth thoroughly. Do not induce vomiting. Have exposed individual drink sips of water. Seek medical attention if irritation, discomfort or vomiting persists.Never give anything by mouth to an unconscious person.

Most important symptoms and effects, both acute and delayed:

Irritation, Nausea,Headache, Shortness of breath.;

Indication of any immediate medical attention and special treatment needed:

If seeking medical attention, provide SDS document to physician.Physician should treat symptomatically.

SECTION 5 : Firefighting measures

Extinguishing media

Suitable extinguishing agents: Substance is non-flammable. Use appropriate fire suppression agents for adjacent combustible materials or sources of ignition. Use water, dry chemical, chemical foam, carbon dioxide, or alcohol-resistant foam

For safety reasons unsuitable extinguishing agents:

Special hazards arising from the substance or mixture:

Combustion products may include carbon oxides or other toxic vapors.Thermal decomposition can lead to release of irritating gases and vapors.

Advice for firefighters:

Protective equipment: Use NIOSH-approved respiratory protection/breathing apparatus.

Additional information (precautions): Avoid inhaling gases, fumes, dust, mist, vapor, and aerosols.Avoid contact with skin, eyes, and clothing.

SECTION 6 : Accidental release measures

Personal precautions, protective equipment and emergency procedures:

Wear protective equipment. Ensure that air-handling systems are operational.Ensure adequate ventilation.

Environmental precautions:

Prevent from reaching drains, sewer or waterway. Collect contaminated soil for characterization per Section 13. Should not be released into environment.

Methods and material for containment and cleaning up:

Keep in suitable closed containers for disposal.Wear protective eyeware, gloves, and clothing. Refer to Section 8.Always obey local regulations.Dust deposits should not be allowed to accumulate on surfaces, as these may form an explosive mixture if they are released into the atmosphere in sufficient concentration. Avoid dispersal of dust in the air (i.e., clearing dust surfaces with compressed air). Collect solids in powder form using vacuum with (HEPA filter). Evacuate personnel to safe areas.

Reference to other sections:

SECTION 7 : Handling and storage

Precautions for safe handling:

Minimize dust generation and accumulation. Follow good hygiene procedures when handling chemical materials. Refer to Section 8.Do not eat, drink, smoke, or use personal products when handling chemical substances. Avoid contact with eyes, skin, and clothing.

Conditions for safe storage, including any incompatibilities:

Store away from incompatible materials.Protect from freezing and physical damage.Keep away from food and beverages.Provide ventilation for containers. Avoid storage near extreme heat, ignition sources or open flame. Store in cool, dry conditions in well sealed containers. Store with like hazards

SECTION 8 : Exposure controls/personal protection

Control Parameters:	No applicable occupational exposure limits
Appropriate Engineering controls:	Emergency eye wash fountains and safety showers should be available in the immediate vicinity of use/handling.Provide exhaust ventilation or other engineering controls to keep the airborne concentrations of vapor or dusts (total/respirable) below the applicable workplace exposure limits (Occupational Exposure Limits-OELs) indicated above.It is recommended that all dust control equipment such as local exhaust ventilation and material transport systems involved in handling of this product contain explosion relief vents or an explosion suppression system or an oxygen deficient environment.Ensure that dust-handling systems (such as exhaust ducts, dust collectors, vessels, and processing equipment) are designed in a manner to prevent the escape of dust into the work area (i.e., there is no leakage from the equipment).Use under a fume hood
Respiratory protection:	Not required under normal conditions of use. Where risk assessment shows air-purifying respirators are appropriate use a full-face particle respirator type N100 (US) or type P3 (EN 143) respirator cartridges as a backup to engineering controls.When necessary use NIOSH approved breathing equipment.
Protection of skin:	Select glove material impermeable and resistant to the substance.Select glove material based on rates of diffusion and degradation.Dispose of contaminated gloves after use in accordance with applicable laws and good laboratory practices.Use proper glove removal technique without touching outer surface. Avoid skin contact with used gloves.Wear protective clothing.
Eye protection:	Wear equipment for eye protection tested and approved under appropriate government standards such as NIOSH (US) or EN 166(EU).Safety glasses or goggles are appropriate eye protection.
General hygienic measures:	Perform routine housekeeping.Wash hands before breaks and at the end of work.Avoid contact with skin, eyes, and clothing.Before wearing wash contaminated clothing.

SECTION 9 : Physical and chemical properties

Appearance (physical state,color):	white crystalline powder	**Explosion limit lower**: **Explosion limit upper**:	Not determined Not determined
Odor:	Not Determined	**Vapor pressure**:	Not determined
Odor threshold:	Not determined	**Vapor density**:	Not determined
pH-value:	5.0 - 7 at 18 g/l at 25 °C (77 °F)	**Relative density**:	Not determined
Melting/Freezing point:	103 - 105 deg C	**Solubilities**:	freely soluble
Boiling point/Boiling range:	Not determined	**Partition coefficient (n-octanol/water)**:	Not determined
Flash point (closed cup):	Not determined	**Auto/Self-ignition temperature**:	Not determined
Evaporation rate:	Not determined	**Decomposition temperature**:	Not determined
Flammability (solid,gaseous):	Not determined	**Viscosity**:	a. Kinematic:Not determined b. Dynamic: Not determined
Density: Not determined			

SECTION 10 : Stability and reactivity

Reactivity: Nonreactive under normal conditions.
Chemical stability: Stable under normal conditions.
Possible hazardous reactions: None under normal processing
Conditions to avoid: Incompatible Materials.
Incompatible materials: Strong acids.Strong bases.Oxidizing agents.
Hazardous decomposition products:

SECTION 11 : Toxicological information

Acute Toxicity:		
Oral:	IUCLID	LD50 Rat 6450 mg/kg
Chronic Toxicity: No additional information.		
Corrosion Irritation: No additional information.		
Sensitization:		No additional information.
Single Target Organ (STOT):		No additional information.
Numerical Measures:		No additional information.
Carcinogenicity:		No additional information.
Mutagenicity:		No additional information.

Reproductive Toxicity:	No additional information.

SECTION 12 : Ecological information

Ecotoxicity Persistence and degradability:
Bioaccumulative potential:
Mobility in soil:
Other adverse effects:

SECTION 13 : Disposal considerations

Waste disposal recommendations:

Chemical waste generators must determine whether a discarded chemical is classified as a hazardous waste. Chemical waste generators must also consult local, regional, and national hazardous waste regulations. Ensure complete and accurate classification.

SECTION 14 : Transport information

UN-Number

Not Regulated.

UN proper shipping name

Not Regulated.

Transport hazard class(es)
Packing group:Not Regulated
Environmental hazard:
Transport in bulk:
Special precautions for user:

SECTION 15 : Regulatory information

United States (USA)

SARA Section 311/312 (Specific toxic chemical listings):

None of the ingredients is listed

SARA Section 313 (Specific toxic chemical listings):

None of the ingredients is listed

RCRA (hazardous waste code):

None of the ingredients is listed

TSCA (Toxic Substances Control Act):

All ingredients are listed.

CERCLA (Comprehensive Environmental Response, Compensation, and Liability Act):

None of the ingredients is listed

Proposition 65 (California):

Chemicals known to cause cancer:

None of the ingredients is listed

Chemicals known to cause reproductive toxicity for females:

None of the ingredients is listed

Chemicals known to cause reproductive toxicity for males:

None of the ingredients is listed

Chemicals known to cause developmental toxicity:

None of the ingredients is listed

Canada

Canadian Domestic Substances List (DSL):

All ingredients are listed.

Canadian NPRI Ingredient Disclosure list (limit 0.1%):

None of the ingredients is listed

Canadian NPRI Ingredient Disclosure list (limit 1%):

None of the ingredients is listed

SECTION 16 : Other information

This product has been classified in accordance with hazard criteria of the Controlled Products Regulations and the SDS contains all the information required by the Controlled Products Regulations.Note:. The responsibility to provide a safe workplace remains with the user.The user should consider the health hazards and safety information contained herein as a guide and should take those precautions required in an individual operation to instruct employees and develop work practice procedures for a safe work environment.The information contained herein is, to the best of our knowledge and belief, accurate.However, since the conditions of handling and use are beyond our control, we make no guarantee of results, and assume no liability for damages incurred by the use of this material.It is the responsibility of the user to comply with all applicable laws and regulations applicable to this material.

GHS Full Text Phrases:

Abbreviations and acronyms:
IMDG: International Maritime Code for Dangerous Goods
IATA: International Air Transport Association
GHS: Globally Harmonized System of Classification and Labelling of Chemicals
ACGIH: American Conference of Governmental Industrial Hygienists
CAS: Chemical Abstracts Service (division of the American Chemical Society)
NFPA: National Fire Protection Association (USA)
HMIS: Hazardous Materials Identification System (USA)
WHMIS: Workplace Hazardous Materials Information System (Canada)
DNEL: Derived No-Effect Level (REACH)
PNEC: Predicted No-Effect Concentration (REACH)
CFR: Code of Federal Regulations (USA)
SARA: Superfund Amendments and Reauthorization Act (USA)
RCRA: Resource Conservation and Recovery Act (USA)
TSCA: Toxic Substances Control Act (USA)
NPRI: National Pollutant Release Inventory (Canada)
DOT: US Department of Transportation

Effective date : 10.24.2014
Last updated : 03.19.2015

SECTION 1 : Identification of the substance/mixture and of the supplier

Product name :	**Phosphoric Acid,ACS**
Manufacturer/Supplier Trade name:	
Manufacturer/Supplier Article number:	**S25470B**

Recommended uses of the product and uses restrictions on use:

Manufacturer Details:

AquaPhoenix Scientific
9 Barnhart Drive, Hanover, PA 17331

Supplier Details:

Fisher Science Education
15 Jet View Drive, Rochester, NY 14624

Emergency telephone number:

Fisher Science Education Emergency Telephone No.: 800-535-5053

SECTION 2 : Hazards identification

Classification of the substance or mixture:

Corrosive
Skin corrosion, category 1B
Corrosive to metals, category 1

Corrosive to Metals 1
Skin Corrosion 1B

Signal word :Danger

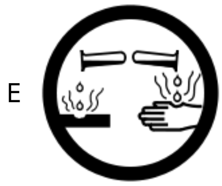
Hazard statements:
May be corrosive to metals
Causes severe skin burns and eye damage
Precautionary statements:
If medical advice is needed, have product container or label at hand
Keep out of reach of children
Read label before use
Do not breathe dust/fume/gas/mist/vapours/spray
Wash … thoroughly after handling
Wear protective gloves/protective clothing/eye protection/face protection
Keep only in original container
Do not eat, drink or smoke when using this product
Immediately call a POISON CENTER or doctor/physician
Specific treatment (see … on this label)
Wash contaminated clothing before reuse
IF SWALLOWED: Rinse mouth. Do NOT induce vomiting
IF ON SKIN (or hair): Remove/Take off immediately all contaminated clothing. Rinse skin with water/shower
IF INHALED: Remove victim to fresh air and keep at rest in a position comfortable for breathing
IF IN EYES: Rinse cautiously with water for several minutes. Remove contact lenses if present and easy to do. Continue rinsing

Store locked up
Store in a corrosive resistant/... container with a resistant inner liner
Dispose of contents/container to ...

Conc 10% to <25%:
Eye Irritation 2, Skin Irritation 2
Conc <10%:
Not classified for physical or health hazards under GHS.

Other Non-GHS Classification:

WHMIS



NFPA/HMIS

NFPA SCALE (0-4)

HMIS RATINGS (0-4)

SECTION 3 : Composition/information on ingredients

Ingredients:		
CAS 7664-38-2	Phosphoric Acid	>85 %
CAS 7732-18-5	Deionized Water	<15 %
		Percentages are by weight

SECTION 4 : First aid measures

Description of first aid measures

After inhalation: Seek medical attention immediately. Move exposed individual to fresh air. Loosen clothing as necessary and position individual in a comfortable position.

After skin contact: Remove contaminated clothing and wash before reuse or discard. Rinse skin with for 30 minutes with deluge of water or under a shower. Seek immediate medical attention.Wash affected area with soap and water.

After eye contact: Rinse immediately with plenty of water, also under the eyelids, for at least 30 minutes. Remove contact lens(es) if able to do so during rinsing. Seek medical attention immediately.Protect unexposed eye.

After swallowing: Seek medical attention immediately. Rinse mouth thoroughly. Do not induce vomiting. Have exposed individual drink sips of water.

Most important symptoms and effects, both acute and delayed:

Irritation, Nausea,Headache, Shortness of breath.May cause severe burns and ulcerations. May cause severe burn and irreversible eye injury. May cause gastrointestinal tract burns, corrosion and permanent tissue damage of the digestive tract and esophagus;

Indication of any immediate medical attention and special treatment needed:

If seeking medical attention, provide SDS document to physician.Wipe off contact areas with a dry cloth if possible, before flushing with water. Dispose of cloth by soaking in water. Neutralize the soaking solution with sodium hydroxide solution.

SECTION 5 : Firefighting measures

Extinguishing media

Suitable extinguishing agents: If in laboratory setting, follow laboratory fire suppression procedures. Use appropriate fire suppression agents for adjacent combustible materials or sources of ignition

For safety reasons unsuitable extinguishing agents:

Special hazards arising from the substance or mixture:

Hydrogen gas is released in contact with most metals. Combustion products may include carbon oxides or other toxic vapors.Combustion products may include phosphine, oxides of phosphorus, and hydrogen gas.

Advice for firefighters:

Protective equipment: Wear protective equipment Use respiratory protective device against the effects of fumes/dust/aerosol/vapor . Use NIOSH-approved respiratory protection/breathing apparatus.

Additional information (precautions): Move product containers away from fire or keep cool with water spray as a protective measure, where feasible.

SECTION 6 : Accidental release measures

Personal precautions, protective equipment and emergency procedures:

Wear protective equipment. Avoid contact with eyes, skin, and clothing. Use respiratory protective device against the effects of fumes/dust/aerosol. Keep unprotected persons away. Ensure adequate ventilation.Keep away from ignition sources. Protect from heat.Stop the spill, if possible. Contain spilled material by diking or using inert absorbent. Transfer to a disposal or recovery container.

Environmental precautions:

Prevent from reaching drains, sewer or waterway. Collect contaminated soil for characterization per Section 13

Methods and material for containment and cleaning up:

Absorb spillage to prevent material damage due to corrosiveness to metal. If in a laboratory setting, follow Chemical Hygiene Plan procedures.Place into properly labeled containers for recovery or disposal. If necessary, use trained response staff/contractor.Collect liquids using inert absorbent material.

Reference to other sections:

SECTION 7 : Handling and storage

Precautions for safe handling:

Wash hands after handling. Do not mix with bases. Use in a chemical fume hood.Follow good hygiene procedures when handling chemical materials. Do not eat, drink, smoke, or use personal products when handling chemical substances. If in a laboratory setting, follow Chemical Hygiene Plan.Use only in well ventilated areas.Prevent contact with eyes, skin, and clothing

Conditions for safe storage, including any incompatibilities:

Store away from oxidizing agents.Store in cool, dry conditions in well sealed containers. Keep container tightly sealed.Do not store under direct sun light. Do not pile up the containers. Do not store at temperatures close to freezing point.Container materials should be made of stainless steel 316-L, high-density polyethylene, or glass.Provide ventilation for containers. Avoid storage near extreme heat, ignition sources or open flame. Store away from foodstuffs.

SECTION 8 : Exposure controls/personal protection

Control Parameters:	7664-38-2, Phosphoric Acid, ACGIH TLV: 1 mg/m^3 as TWA 7664-38-2, Phosphoric Acid, ACGIH TLV 3 mg/m^3 as STEL 7664-38-2, Phosphoric Acid, OSHA PEL†: TWA 1 mg/m3 (See 29 CFR 1910.1000 Appendix G) 7664-38-2, Phosphoric Acid, NIOSH REL: TWA 1 mg/m3 7664-38-2, Phosphoric Acid, NIOSH REL ST: 3 mg/m3 7664-38-2, Phosphoric Acid, NIOSH IDLH: 1000 mg/m3
Appropriate Engineering controls:	Emergency eye wash fountains and safety showers should be available in the immediate vicinity of use/handling.Provide exhaust ventilation or other engineering controls to keep the airborne concentrations of vapor or dusts (total/respirable) below the applicable workplace exposure limits (Occupational Exposure Limits-OELs) indicated above.
Respiratory protection:	Use suitable respiratory protective device when high concentrations are present. Use suitable respiratory protective device when aerosol or mist is formed. For spills, respiratory protection may be advisable.
Protection of skin:	The glove material has to be impermeable and resistant to the product/ the substance/ the preparation being used/handled.Selection of the glove material on consideration of the penetration times, rates of diffusion and the degradation.
Eye protection:	Safety glasses with side shields or goggles.
General hygienic measures:	The usual precautionary measures are to be adhered to when handling chemicals. Keep away from food, beverages and feed sources. Immediately remove all soiled and contaminated clothing. Wash hands before breaks and at the end of work. Do not inhale gases/fumes/dust/mist/vapor/aerosols. Avoid contact with the eyes and skin.

SECTION 9 : Physical and chemical properties

Appearance (physical state,color):	Clear, colorless liquid	**Explosion limit lower**: **Explosion limit upper**:	Not determined Not determined
Odor:	Odorless	**Vapor pressure**:	Not determined
Odor threshold:	Not determined	**Vapor density**:	3.4
pH-value:	Not determined	**Relative density**:	1.680
Melting/Freezing point:	21°C	**Solubilities**:	Soluble in water
Boiling point/Boiling range:	158°C	**Partition coefficient (n-octanol/water)**:	Not determined
Flash point (closed cup):	Not determined	**Auto/Self-ignition temperature**:	Not determined

Evaporation rate:	Not determined	**Decomposition temperature**:	300°C
Flammability (solid,gaseous):	Not applicable	**Viscosity**:	a. Kinematic:Not determined b. Dynamic: Not determined
Density: Not determined **Additional property::**Hygroscopic. **Specific Gravity:**1.680 **Molecular Weight: :**98.00 g/mol			

SECTION 10 : Stability and reactivity

Reactivity:
Chemical stability: This hygroscopic substance pulls moisture from air. No decomposition if used and stored according to specifications.
Possible hazardous reactions:
Conditions to avoid: Metals. Exposure to moist air or water. Incompatible materials. Excess heat. Store away from oxidizing agents, strong acids or bases.
Incompatible materials: Metals. Bases . Alcohols. Amines. Halogenated agents. Organic peroxides. Amides. Azo. Diazo. Hydrazines. Chlorates. Carbamates. Esters. Fluorides. Phenols. Cresols . Organophosphates. Phosphothioates. Epoxides. Combustible and flammable materials. Explosives. Alkalines. Nitromethane. Sodium tetrahydroborate. Mercaptans. Aldehydes. Ketones. Glycols. Cyanides. Sulfides. Caustics. Strong acids.Carbides. Strong bases.Fulminates. Reducing agents. Nitrates. Acetic acid. Oxidizing agents
Hazardous decomposition products: Phosophine. Oxides of phosphorus. Hydrogen gas is released in contact with most metals.

SECTION 11 : Toxicological information

Acute Toxicity:		
Oral:	2600 mg/kg bw	LD50 for a 10% solution of 75.4% thermal phosphoric acid (rat)
Oral:	1530 mg/m3	LD50 oral-rat: (7764-38-2)
Inhalation:	>850mg/m3	LC50 inhalation-rat (1h) (7664-38-2)
Chronic Toxicity: No additional information.		
Corrosion Irritation:		
Dermal:	Section 2	Classified as a skin corrosion
Ocular:	Section 2 (eye damage is presumed with Skin 1 classification)	Eye Damage
Dermal:		May cause severe burns and ulcerations.
Ocular:		May cause severe burn and irreversible eye damage
Sensitization:		No additional information.
Single Target Organ (STOT):		No additional information.
Numerical Measures:		No additional information.

Carcinogenicity:	IARC: Not listed NTP: Not listed
Mutagenicity:	No additional information.
Reproductive Toxicity:	No additional information.

SECTION 12 : Ecological information

Ecotoxicity

Do not release to water: May release phosphates which will result in algae growth, increased turbidity, and depleted oxygen in the marine environment; at extremely high concentrations and/or quantities, this may be hazardous to fish or other marine organisms.

LpH50 (median lethal pH) (96h) phosphoric acid (bluegill sunfish): 3-3.25

Adult brook trout survived 5 months exposure to pH levels of 5.0 and above. Total egg production was not affected, but viability was significantly less at pH 5.0. Hatchability was significantly less at levels below pH 6.5. Growth and survival of alevins was reduced at the lower pH levels. : The data indicate that continuous exposure to pH levels below 6.5 result in significant reductions in egg hatchability and growth.

Algae: NOEC (EC50 >100 mg/l, the upper limit of toxic range) D. subspicatus : 100 mg/l

Persistence and degradability: Readily degradable in the environment.
Bioaccumulative potential: The phosphorus element is an essential nutrient for flora and fauna
Mobility in soil:
Other adverse effects:

SECTION 13 : Disposal considerations

Waste disposal recommendations:

Product/containers must not be disposed together with household garbage. Do not allow product to reach sewage system or open water.It is the responsibility of the waste generator to properly characterize all waste materials according to applicable regulatory entities (US 40CFR262.11). Consult federal state/ provincial and local regulations regarding the proper disposal of waste material that may incorporate some amount of this product.

SECTION 14 : Transport information

UN-Number

1805

UN proper shipping name

Phosphoric Acid Solution

Transport hazard class(es)

Class:
8 Corrosive substances

Packing group:III
Environmental hazard:Not listed as a Marine Polllutant
Transport in bulk:
Special precautions for user:

SECTION 15 : Regulatory information

United States (USA)

SARA Section 311/312 (Specific toxic chemical listings):

Acute

SARA Section 313 (Specific toxic chemical listings):

None of the ingredients is listed

RCRA (hazardous waste code):

None of the ingredients is listed

TSCA (Toxic Substances Control Act):

All ingredients are listed.

CERCLA (Comprehensive Environmental Response, Compensation, and Liability Act):

7664-38-2 Phosphoric acid 5000

Proposition 65 (California):

Chemicals known to cause cancer:

None of the ingredients is listed

Chemicals known to cause reproductive toxicity for females:

None of the ingredients is listed

Chemicals known to cause reproductive toxicity for males:

None of the ingredients is listed

Chemicals known to cause developmental toxicity:

None of the ingredients is listed

Canada

Canadian Domestic Substances List (DSL):

All ingredients are listed.

Canadian NPRI Ingredient Disclosure list (limit 0.1%):

None of the ingredients is listed

Canadian NPRI Ingredient Disclosure list (limit 1%):

7664-38-2 Phosphoric acid

SECTION 16 : Other information

This product has been classified in accordance with hazard criteria of the Controlled Products Regulations and the SDS contains all the information required by the Controlled Products Regulations.Note:. The responsibility to provide a safe workplace remains with the user.The user should consider the health hazards and safety information contained herein as a guide and should take those precautions required in an individual operation to instruct employees and develop work practice procedures for a safe work environment.The information contained herein is, to the best of our knowledge and belief, accurate.However, since the conditions of handling and use are beyond our control, we make no guarantee of results, and assume no liability for damages incurred by the use of this material.It is the responsibility of the user to comply with all applicable laws and regulations applicable to this material.

GHS Full Text Phrases:

Abbreviations and acronyms:
IMDG: International Maritime Code for Dangerous Goods
PNEC: Predicted No-Effect Concentration (REACH)

CFR: Code of Federal Regulations (USA)
SARA: Superfund Amendments and Reauthorization Act (USA)
RCRA: Resource Conservation and Recovery Act (USA)
TSCA: Toxic Substances Control Act (USA)
NPRI: National Pollutant Release Inventory (Canada)
DOT: US Department of Transportation
IATA: International Air Transport Association
GHS: Globally Harmonized System of Classification and Labelling of Chemicals
ACGIH: American Conference of Governmental Industrial Hygienists
CAS: Chemical Abstracts Service (division of the American Chemical Society)
NFPA: National Fire Protection Association (USA)
HMIS: Hazardous Materials Identification System (USA)
WHMIS: Workplace Hazardous Materials Information System (Canada)
DNEL: Derived No-Effect Level (REACH)

Effective date : 12.07.2014
Last updated : 03.23.2015

New Studier media for auto-induction and other applications

Reference: F.W. Studier (2005) Protein production by auto-induction in high-density shaking cultures. *Prot. Exp. Pur. 41*, 207-234.

ZYM-5052 medium for auto-induction

It is recommended to use this medium for auto-induction. The amount of kanamycin (when appropriate) used should be 100 μg/ml.

958 ml	ZY	tryptone yeast extract	1% 0.5%
20 ml	50 x M	Na_2HPO_4 KH_2PO_4 NH_4Cl Na_2SO_4	25 mM 25 mM 50 mM 5 mM
20 ml	50 x 5052	glycerol glucose α-lactose	0.5% 0.05% 0.2%
2 ml	1 M $MgSO_4$	$MgSO_4$	2 mM
0.2 ml	1000 x trace elements	trace elements	0.2 x

ZYP-5052 medium for auto-induction

This medium should be used when stronger buffering is needed. The amount of kanamycin (when appropriate) used should be at least 200μg/ml. Better even would be 400 μg/ml.

928 ml	ZY	tryptone yeast extract	1% 0.5%
50 ml	20 x P (formerly NPS)	Na_2HPO_4 KH_2PO_4 $(NH_4)_2SO_4$	50 mM 50 mM 25 mM
20 ml	50 x 5052	glycerol glucose α-lactose	0.5% 0.05% 0.2%
2 ml	1 M $MgSO_4$	$MgSO_4$	2 mM
0.2 ml	1000 x trace elements	trace elements	0.2 x

ZYM-505 medium for plasmid preparation

This medium is used to grow high-density cultures for plasmid preparations.

968 ml	ZY	tryptone yeast extract	1% 0.5%
20 ml	50 x M	Na_2HPO_4 KH_2PO_4 NH_4Cl Na_2SO_4	25 mM 25 mM 50 mM 5 mM
10 ml	100 x 505	glycerol glucose	0.5% 0.05%
2 ml	1 M $MgSO_4$	$MgSO_4$	2 mM
0.2 ml	1000 x trace elements	trace elements	0.2 x

MDG non-inducing medium

This medium is used for growing working cultures (such as overnight pre-cultures) and freezer cultures (glycerol stocks).

955 ml	sterile water		
20 ml	50 x M	Na_2HPO_4 KH_2PO_4 NH_4Cl Na_2SO_4	25 mM 25 mM 50 mM 5 mM
12.5 ml	40% glucose	glucose	0.5%
10 ml	25% aspartate	aspartate	0.25%
2 ml	1 M $MgSO_4$	$MgSO_4$	2 mM
0.2 ml	1000 x trace elements	trace elements	0.2 x

Stock solutions

ZY	1% tryptone	10 g/L
	0.5% yeast extract	5 g/L

Dissolve tryptone and yeast extract in the necessary amount of water to make up 1 L of total medium (*e.g.* 958 ml for ZYM-5052).

50 x M	1.25 M Na_2HPO_4-$7H_2O$	335 g/L
	1.25 M KH_2PO_4	170 g/L
	2.5 M NH_4Cl	134 g/L
	0.25 M Na_2SO_4	35.5 g/L

Add in sequence to 700 ml water and stir until all salts have been dissolved. Occasionally crystals appear but they can be re-dissolved in the microwave. The pH of a 20-fold dilution in water should be ~6.75.

20 x P	1.0 M Na_2HPO_4-$7H_2O$	268 g/L
	1.0 M KH_2PO_4	136 g/L
	0.5 M $(NH_4)_2SO_4$	66 g/L

Add in sequence to 770 ml water and stir until all salts have been dissolved. The pH of a 20-fold dilution in water should be ~6.75.

100 x 505	50% glycerol	500 g/L
	5% glucose	50 g/L

Add in sequence to 570 ml water and stir until all components have been dissolved.

50 x 5052	25% glycerol	250 g/L
	2.5% glucose	25 g/L
	10% α-lactose	100 g/L

Add in sequence to 730 ml water and stir until all components have been dissolved. Lactose is slow to dissolve. It may take two hours or more at room temperature. The process can be sped up by heating in microwave.

500 x $MgSO_4$	1 M $MgSO_4$-$7H_2O$	24.65 g/100 ml

For 100 ml stock solution dissolve 24.65 g $MgSO_4$ in 87 ml water.

Stock solutions (cont.)

80 x G	40% glucose	40 g/100 ml

To make 100 ml stock solution add 40 g glucose to 74 ml water and stir until all glucose has been dissolved. It may take 45 min or more at room temperature. The process can be sped up by heating in microwave.

100 x D	25% aspartate	25 g/100 ml

For 100 ml stock solution dissolve 25 g aspartic acid in 84 ml water and neutralize with 8 g NaOH (pH ~7).

1000x trace elements	50 mM $FeCl_3$ 20 mM $CaCl_2$ 10 mM $MnCl_2$ 10 mM $ZnSO_4$ 2 mM $CoCl_2$ 2 mM $CuCl_2$ 2 mM $NiCl_2$ 2 mM Na_2MoO_4 2 mM Na_2SeO_3 2 mM H_3BO_3

Preparation of 100 ml 1000x trace elements stock solution (in ~60 mM HCl).

Prepare stock solutions of the salts (except $FeCl_3$) as mentioned in the table below.

Dissolve 0.1 M $FeCl_3$ in 50 ml of 100x diluted concentrated HCl.

Add to 36 ml sterile water:

50 ml	0.1 M $FeCl_3$-$6H_2O$	2.70 g/100 ml
2 ml	1.0 M $CaCl_2$-$2H_2O$	15.8 g/100 ml
1 ml	1.0 M $MnCl_2$-$4H_2O$	19.8 g/100 ml
1 ml	1.0 M $ZnSO_4$-$7H_2O$	28.8 g/100 ml
1 ml	0.2 M $CoCl_2$-$6H_2O$	4.76 g/100 ml
2 ml	0.1 M $CuCl_2$-$2H_2O$	1.70 g/100 ml
1 ml	0.2 M $NiCl_2$-$6H_2O$	4.76 g/100 ml
2 ml	0.1 M Na_2MoO_4-$2H_2O$	2.42 g/100 ml
2 ml	0.1 M Na_2SeO_3	1.73 g/100 ml
2 ml	0.1 M H_3BO_3	0.62 g/100 ml

Chemicals

Compound	mol. weight	company	order number
tryptone		Sigma	T-9410
yeast extract		Sigma	Y-1000
glycerol	92.10	Roth	3783.1
glucose	180.16	Roth	X997.2
α-lactose-H_2O	360.32	Roth	6868.2
Na_2HPO_4-$7H_2O$	268.07	Sigma	S-9390
KH_2PO_4	136.09	Sigma	P-5379
NH_4Cl	53.49	Merck	1145.1000
Na_2SO_4	142.0	Sigma	S-6264
$(NH_4)_2SO_4$	132.1	OLS	2701316
$MgSO_4$-$7H_2O$	246.48	Roth	P072.2
aspartic acid	133.1	Sigma	A-8949
$FeCl_3$-$6H_2O$	270.30	Sigma	F-2877
$CaCl_2$-$2H_2O$	158.2	Sigma	C-3881
$MnCl_2$-$4H_2O$	197.91	Sigma	M-3634
$ZnSO_4$-$7H_2O$	287.56	Sigma	Z-4750
$CoCl_2$-$6H_2O$	237.95	Sigma	C-8661
$CuCl_2$-$2H_2O$	170.48	Sigma	C-6641
$NiCl_2$-$6H_2O$	237.72	Sigma	N-5756
Na_2MoO_4-$2H_2O$	241.9	Sigma	M-1003
Na_2SeO_3	172.9	Sigma	S-5261
H_3BO_3	61.83	Merck	165.1000

Appendix D: Patent

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(54) Title: METHOD FOR OBTAINING A LIPID-CONTAINING COMPOSITION FROM MICROBIAL BIOMASS

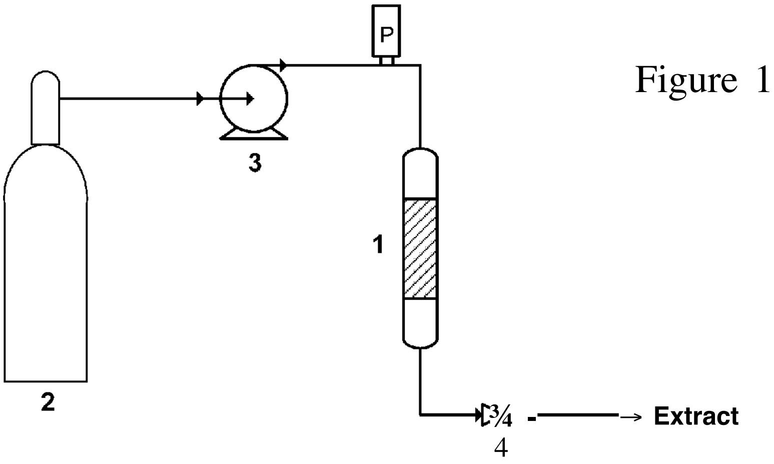

Figure 1

(57) Abstract: Methods are provided for pelletizing a microbial biomass, extracting a refined lipid composition from the pelletized biomass under supercritical conditions and distilling the refined lipid composition, at least once under short path distillation conditions, to obtain a lipid-containing fraction. Also disclosed are methods of making lipid-containing oil concentrates therefrom, by transesterifying and enriching the lipid-containing fraction.

WO 2012/1©9545 A2

TITLE

METHOD FOR OBTAINING A LIPID-CONTAINING COMPOSITION FROM MICROBIAL BIOMASS

This application claims the benefit of U.S. Provisional Application No. 61/441,836, filed February 11, 2011, U.S. Provisional Application No. 61/441,842, filed February 11, 2011, U.S. Provisional Application No. 61/441,849, filed February 11, 2011, U.S. Provisional Application No. 61/441,854, filed February 11, 2011, and U.S. Provisional Application No. 61/487,019, filed May 17, 2011, which are hereby incorporated by reference in their entirety

FIELD OF THE INVENTION

The present invention relates to methods for obtaining a lipid-containing fraction from microbial biomass. In particular, methods are provided for pelletizing a microbial biomass, extracting an extracted oil from the pelletized biomass and distilling the extracted oil, at least once under short path distillation conditions, to obtain a lipid-containing fraction. This lipid-containing fraction may be further enriched.

BACKGROUND OF THE INVENTION

Microorganisms such as filamentous fungi, yeast and algae produce a variety of lipids, including fatty acyls, glycerolipids, phospholipids, sphingolipids, saccharolipids, polyketides, sterol lipids and prenol lipids. It is advantageous to extract some of these lipids from the microbial cells in which they are produced, and thus a variety of processes have been implemented.

One class of lipids commonly extracted from microbes is glycerolipids, including the fatty acid esters of glycerol ("triacylglycerols" or "TAGs"). TAGs are the primary storage unit for fatty acids, and thus may contain long chain polyunsaturated fatty acids ("PUFAs"), as well as shorter saturated and unsaturated fatty acids and longer chain saturated fatty acids. There has been growing interest in including PUFAs, such as eicosapentaenoic acid ["EPA"; omega-3] and docosahexaenoic acid ["DHA"; omega-3], in pharmaceutical and dietary products. Means to efficiently and cost-effectively extract, refine and purify lipid compositions comprising PUFAs is therefore particularly desirable.

Many typical lipid isolation procedures involve disruption of the microbial cells (e.g., via mechanical, enzymatic or chemical means), followed by oil extraction using organic or green solvents. The disruption process releases the intracellular lipids from the microbial cells, which makes it readily accessible by the solvent during extraction. After extraction, the solvent is typically removed (e.g., by evaporation, for example by application of vacuum, change of temperature or pressure, etc.).

The resulting extracted oil is enriched in lipophilic components that accumulate in the lipid bodies. In general, the major components of the lipid bodies consist of TAGs, ergosterol esters, other sterol esters, free ergosterol and phospholipids. PUFAs present in lipid bodies are mainly as components of TAGs, diacylglycerols, monoacylglycerols, phospholipids and free fatty acids. The extracted oil may then be subsequently refined, to produce a highly purified TAG fraction enriched in PUFAs. Final specifications concerning the purified TAG fraction may be application-dependent, for example, depending on whether the oil is to be used as an additive or supplement (e.g., in food compositions, infant formulas, animal feeds, etc.), in cosmetic or pharmaceutical compositions, etc. Acceptable contaminant standards are either self-imposed (wherein a particular contaminant results in an undesirable property, e.g., haziness/cloudiness, odor) or determined by external nutrition councils (e.g., A Voluntary Monograph Of The Council for Responsible Nutrition [Washington, D.C.], March 2006, specifies the maximum acid, peroxide, anisidine, TOTOX, polychlorinated dibenzo-para-dioxin and polychlorinated dibenzofuran values for omega-3 EPA, omega-3 DHA and mixtures thereof).

U.S. Patent No. 6,727,373 discloses a microbial PUFA-containing oil with a high triglyceride content and a high oxidative stability. In addition, a method is described for the recovery of such oil from a microbial biomass derived from a pasteurized fermentation broth, wherein the microbial biomass is subjected to extrusion to form granular particles, dried, and the oil is then extracted from the dried granules using an appropriate solvent.

U.S. Patent No. 6,258,964 discloses a method of extracting liposoluble components contained in microbial cells, wherein the method requires drying microbial cells containing liposoluble components, simultaneously disrupting and molding the dried microbial cells into pellets by use of an extruder, and extracting the contained liposoluble components by use of an organic solvent.

U.S. Pat. Appl. Pub. No. 2009/0227678 discloses a process for obtaining lipid from a composition comprising cells and water, the process comprising contacting the composition with a desiccant, and recovering the lipid from the cells.

U.S. Patent No. 4,675,1 32 discloses a process for the concentration of PUFA moieties in a fish oil containing relatively low proportions of saturated and monounsaturated fatty acid moieties of the same chain length as the PUFA moieties to be concentrated, which comprises transesterifying fish oil glycerides with a lower alkanol to form a mixture of lower alkyl fatty acid esters, and extracting said esters with carbon dioxide (CO_2) under supercritical conditions.

A process flow diagram developed for a continuous countercurrent supercritical CO_2 fractionation process that produces high concentration EPA is disclosed by V.J. Krukonis et al. *(Adv. Seafood Biochem.,* Pap. Am. Chem. Soc. Annu. Meet. (1992), Meeting Date 1987, 169-1 79). The feedstock for the process is urea-crystallized ethyl esters of menhaden oil, and the basis for the design is a product concentration of 90% EPA (ethyl ester) at a yield of 90%.

Methods in which the distribution of TAGs, diacylglycerols, monoacylglycerols, and free fatty acids can be adjusted in a PUFA-containing lipid composition are sought. Methods for obtaining PUFA-containing lipid compositions which have improved oxidative stability are desired. Methods for obtaining PUFA-containing lipid compositions enriched in TAGs are also desired, as are economical methods for obtaining such compositions.

U.S. Patent 6,1 66,230 (GIST-Brocades) describes a process for treating a microbial oil comprising PUFAs (e.g., from *Mortierella alpina)* with a polar solvent to extract at least one sterol (e.g., desmosterol) that is soluble in the solvent and then separating at least some of the solvent containing the sterol from the oil, wherein the oil has a sterol content of less than 1.5%.

U.S. Patent 7,695,626 (Martek) describes a process for recovering neutral lipids comprising PUFAs from a microbial biomass (e.g., *Schizochytrium),* said process comprising the steps of contacting the biomass with a nonpolar solvent to recover lipid in an extraction process, refining and/or bleaching and/or deodorizing the lipid composition, adding a polar solvent to the lipid composition, cooling the mixture to selectively precipitate at least one other compound (e.g., trisaturated glycerides, phosphorus-containing materials, wax esters, saturated fatty acid containing sterol esters, sterols, squalene, hydrocarbons) and then removing this undesirable compound from the lipid composition.

Previous methods have not utilized techniques of short path distillation as an effective means to avoid exposing PUFAs, specifically highly unsaturated fatty acids, to high temperatures and remove ergosterol (ergosta-5,7,22-ίηβη-3β-οΙ; CAS Registry Number 57-87-4) contaminants from microbial oils.

Int'l. Appl. Pub. No. WO 201 1/080503 A2 discloses a chromatographic separation process for recovering a PUFA product, from a feed mixture, comprising introducing the feed mixture to a simulated or actual moving bed chromatography apparatus having a plurality of linked chromatography columns containing, as eluent, an aqueous alcohol, wherein the apparatus has a plurality of zones comprising at least a first zone and second zone, each zone having an extract stream and a raffinate stream from which liquid can be collected from said plurality of linked chromatography columns, and wherein (a) a raffinate stream containing the PUFA product together with more polar components is collected from a column in the first zone and introduced to a nonadjacent column in the second zone, and/or (b) an extract stream containing the PUFA product together with less polar components is collected from a column in the second zone and introduced to a nonadjacent column in the first zone, said PUFA product being separated from different components of the feed mixture in each zone. Various fish oil derived feedstocks were purified to produce 85 to greater than 98% EPA ethyl esters. Although Int'l. Appl. Pub. No. WO 2001/080503 A2 demonstrated processes to recover EPA and DHA in high purity from fish oils, the disclosure also states that suitable feed mixtures for fractionating may be obtained from "synthetic sources including oils obtained from genetically modified plants, animals and microorganisms including yeasts". Further, "genetically modified yeast is particularly suitable when the desired PUFA product is EPA".

SUMMARY OF THE INVENTION

In a first embodiment, the invention concerns a method comprising:

(a) pelletizing a microbial biomass having a moisture level and comprising oil-containing microbes;

(b) extracting the pelletized microbial biomass of step (a) to produce an extracted oil; and,

(c) distilling the extracted oil of step (b) at least once under short path distillation conditions, wherein said distillation produces a distillate fraction and a lipid-containing fraction.

In a second embodiment of the method, the oil-containing microbes are selected from the group consisting of yeast, algae, fungi, bacteria, euglenoids, stramenopiles and oomycetes. Preferably, the yeast is *Yarrowia.*

In a third embodiment of the method, the oil-containing microbes comprise at least one polyunsaturated fatty acid in the oil, wherein the polyunsaturated fatty acids are preferably selected from the group consisting of: linoleic acid, gamma-linolenic acid, eicosadienoic acid, dihomo-gamma-linolenic acid, arachidonic acid, docosatetraenoic acid, omega-6 docosapentaenoic acid, alpha-linolenic acid, stearidonic acid, eicosatrienoic acid, eicosatetraenoic acid, eicosapentaenoic acid, omega-3 docosapentaenoic acid and docosahexaenoic acid.

In a fourth embodiment of the method, the moisture level of the microbial biomass is in the range of about 1 to 10 weight percent.

In a fifth embodiment of the method, said step (a) pelletizing the microbial biomass comprises:

(1) mixing the microbial biomass and at least one grinding agent capable of absorbing oil to provide a disrupted biomass mix comprising disrupted microbial biomass;

(2) blending the disrupted biomass mix with at least one binding agent to provide a fixable mix capable of forming a solid pellet; and,

(3) forming said fixable mix into solid pellets to provide a pelletized microbial biomass.

Preferably, steps (1) mixing the microbial biomass and (2) blending at least one binding agent are performed in an extruder, are performed simultaneously, or are performed simultaneously in an extruder; and, step (3) forming said solid pellet from said fixable mix comprises a step selected from the group consisting of:

(i) extruding said fixable mix through a die to form strands;

(ii) drying and breaking said strands; and,

(iii) combinations of step (i) extruding said fixable mix through a die to form strands and step (ii) drying and breaking said strands.

In a sixth embodiment of the present method, the disrupted microbial biomass is produced in a twin screw extruder comprising: (a) a total specific energy input of about 0.04 to 0.4 KW/(kg/hr); (b) a compaction zone using bushing elements with progressively shorter pitch length; and, (c) a compression zone using flow restriction; wherein the compaction zone is prior to the compression zone within the extruder.

In a seventh embodiment of the method, the at least one grinding agent preferably has a property selected from the group consisting of:

(a) said at least one grinding agent is a particle having a Moh hardness of 2.0 to 6.0 and an oil absorption coefficient of 0.8 or higher as determined according to ASTM Method D1483-60;

(b) said at least one grinding agent is selected from the group consisting of silica and silicate; and,

(c) said at least one grinding agent is present at about 1 to 20 weight percent, based on the summation of the weights of microbial biomass, grinding agent and binding agent in the solid pellet.

The at least one binding agent preferably has a property selected from the group consisting of:

(a) said at least one binding agent is selected from water and carbohydrates selected from the group consisting of sucrose, lactose, fructose, glucose, and soluble starch; and,

(b) said at least one binding agent is present at about 0.5 to 10 weight percent, based on the summation of the weights of microbial biomass, grinding agent and binding agent in the solid pellet.

In an eighth embodiment of the method, the pellets have a property selected from the group consisting of:

(a) said pellets have an average diameter of about 0.5 to about 1.5 mm and an average length of about 2.0 to about 8.0 mm; and,

(b) said pellets comprise about 70 to about 98.5 weight percent of microbial biomass comprising oil-containing microbes, about 1 to about 20 weight percent of at least one grinding agent capable of absorbing oil and about 0.5 to 10 weight percent of at least one binding agent, based on the summation of the weights of microbial biomass, grinding agent and binding agent in the solid pellet.

In a ninth embodiment of the method, the extracting is performed with an organic solvent to produce an extracted oil and said extracted oil is is degummed and optionally bleached prior to said step (c) distilling the extracted oil.

In a tenth embodiment of the method, the extracting comprises:

(1) processing the pelletized microbial biomass with a solvent comprising liquid or supercritical fluid carbon dioxide, wherein said pelletized microbial biomass comprising oil-containing microbes further comprises at least one polyunsaturated fatty acid in the oil, to obtain:

(i) an extract comprising a lipid fraction substantially free of phospholipids; and,

(ii) a residual biomass comprising phospholipids; and,

(2) fractionating the extract obtained in step (1), part (i) at least once to obtain an extracted oil having a refined lipid composition comprising at least one polyunsaturated fatty acid, wherein the refined lipid composition is enriched in triacylglycerols relative to the oil composition of pelletized microbial biomass that is not processed with a solvent.

In an eleventh embodiment of the method, the extracted oil of step (b) comprises a sterol fraction, the distillate fraction of step (c) comprises the sterol and the lipid-containing fraction of step (c) comprises a reduced amount of the sterol when compared to the amount of the sterol in the extracted oil that has not been subjected to short path distillation. The sterol fraction may comprise one or more sterols selected from the group consisting of: stigmasterol, ergosterol, brassicasterol, campesterol, β-sitosterol and desmosterol.

In a twelfth embodiment of the method, the extracted oil having a refined lipid composition comprising at least one polyunsaturated fatty acid and enriched in triacylglycerols relative to the oil composition of pelletized microbial biomass that is not processed with a solvent further comprises a sterol fraction of at least 300 mg/100 g. Upon distillation at least once under short path distillation conditions, a distillate fraction is produced comprising the sterol and a lipid-containing fraction is produced comprising triacylglycerols and a reduced amount of sterol when compared to the amount of sterol in the extracted oil having a refined lipid composition that has not been subjected to short path distillation.

In a thirteenth embodiment, the method further comprises:

(d) transesterifying the lipid-containing fraction of step (c); and,

(e) enriching the transesterified lipid-containing fraction of step (d) to obtain an oil concentrate.

In a fourteenth embodiment, the oil-containing microbes accumulate in excess of 25% of their dry cell weight as microbial oil; and, the microbial oil comprises 30 to 70 weight percent of eicosapentaenoic acid, measured as a weight percent of total fatty acids, and is substantially free of docosahexaenoic acid; and, the enriching of step (e) is by a combination of at least two processes, said first process comprising fractional distillation and said second process selected from the group consisting of: urea adduct formation, liquid chromatography, supercritical fluid chromatography, simulated moving bed chromatography, actual moving bed chromatography and combinations thereof; and, the oil concentrate is an eicosapentaenoic acid concentrate comprising at least 70 weight percent of eicosapentaenoic acid, measured as a weight percent of oil, and substantially free of docosahexaenoic acid.

BIOLOGICAL DEPOSITS

The following biological materials have been deposited with the American Type Culture Collection (ATCC), 10801 University Boulevard, Manassas, VA 201 10-2209, and bear the following designations, accession numbers and dates of deposit.

Biological Material	Accession No.	Date of Deposit
Yarrowia lipolytica Y4128	ATCC PTA-8614	August 23, 2007
Yarrowia lipolytica Y8412	ATCC PTA-10026	May 14, 2009
Yarrowia lipolytica Y8259	ATCC PTA-10027	May 14, 2009

The biological materials listed above were deposited under the terms of the Budapest Treaty on the International Recognition of the Deposit of Microorganisms for the Purposes of Patent Procedure. The listed deposit will