4-19-2022

Cellular Agriculture

Christina Kim
University of Pennsylvania

Amanda Kishun
University of Pennsylvania

Fahmida Lubna
University of Pennsylvania

Cellular Agriculture

Abstract

Cellular agriculture is a field of biotechnology focused on the production of animal products using cells grown *in vitro* . Traditional meat production consumes vast amounts of water, arable land, and feed crops, as well as driving deforestation, emitting large amounts of greenhouse gases, and creating large potential reservoirs for zoonotic diseases. As the global demand for meat increases, continuing to scale up the industry for slaughtered meat could have disastrous consequences for the environment. Growing cells in bioreactors creates the potential to drastically decrease land requirements, feed requirements, and other environmental impacts. For example, hindgut fermentation of feed, the main source of methane emissions from cattle farming, can be eliminated entirely by supplying the cells with pure glucose.

This report proposes a process to produce 35 million pounds per year of a cultured ground beef product. The process starts with a starter colony of bovine muscle satellite cells, which are proliferated, differentiated to bovine muscle fiber, and then dewetted, mixed with plant-based fat, and extruded to the final product. Bubble column bioreactors are used for the seed train, final proliferation, and differentiation steps in order to adequately oxygenate large process volumes without threatening cell viability. The process shows profitability at a price of $100 per pound of product. The plant has a return on investment of 217%, an investor's rate of return of 223%, and a cumulative net present value of about $2 billion over the plant's lifespan.

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 19, 2022

Dear Dr. Bomyi Lim, Prof. Bruce Vrana, and Dr. Jeffrey Cohen,

This report proposes a process to produce 35 million pounds per year of a cultured meat product for human consumption. The process design includes proliferation and differentiation of stem cells on microcarrier substrate in bubble column bioreactors, then downstream processing steps including microcarrier dissolution, cell dewetting, mixing with plant-based fat, and extrusion to a final ground beef product.

The economic and profitability analyses of this product show profitability at a price of $100/lb of ground beef. The plant has a return on investment of 217%, an investor's rate of return of 223%, and a cumulative net present value of about $2 billion over the lifespan of the plant. The economic analysis can be further expanded with considerations for final packaging and shipping to consumers, which were outside of the scope of this project. With continued research into optimizing cell lines and growth media for cultured meat, we anticipate that the economic viability of the process will continue to improve.

Thank you all for your support and guidance over the past semester. We greatly appreciate all of your experience and insight, and your assistance has been invaluable.

Sincerely,

Christina Kim Amanda Kishun Fahmida Lubna

Cellular Agriculture

Christina Kim

Amanda Kishun

Fahmida Lubna

Project Author: Dr. Jeffrey D. Cohen

Project Advisor: Dr. Bomyi Lim

University of Pennsylvania School of Engineering and Applied Science

Department of Chemical & Biomolecular Engineering

April 19, 2022

Table of Contents

Abstract

Cellular agriculture is a field of biotechnology focused on the production of animal products using cells grown *in vitro*. Traditional meat production consumes vast amounts of water, arable land, and feed crops, as well as driving deforestation, emitting large amounts of greenhouse gases, and creating large potential reservoirs for zoonotic diseases. As the global demand for meat increases, continuing to scale up the industry for slaughtered meat could have disastrous consequences for the environment. Growing cells in bioreactors creates the potential to drastically decrease land requirements, feed requirements, and other environmental impacts. For example, hindgut fermentation of feed, the main source of methane emissions from cattle farming, can be eliminated entirely by supplying the cells with pure glucose.

This report proposes a process to produce 35 million pounds per year of a cultured ground beef product. The process starts with a starter colony of bovine muscle satellite cells, which are proliferated, differentiated to bovine muscle fiber, and then dewetted, mixed with plant-based fat, and extruded to the final product. Bubble column bioreactors are used for the seed train, final proliferation, and differentiation steps in order to adequately oxygenate large process volumes without threatening cell viability. The process shows profitability at a price of $100 per pound of product. The plant has a return on investment of 217%, an investor's rate of return of 223%, and a cumulative net present value of about $2 billion over the plant's lifespan.

List of Tables and Figures

Section 1: Introduction and Objective

a. Project Motivation

Meat is the staple protein source in most humans' diets and, given the rise in the human population, current methods of meat production may no longer be able to scale with future demand. The environmental impacts of increasing meat production are also potentially devastating: livestock is responsible for 14.5% of global greenhouse gas emissions, with 44% of those emissions being methane (Gerber et al., 2013). Globally, 77% of agricultural land is being used for meat and dairy livestock, while only supplying 18% of calories produced (Ritchie, 2019). Beef production alone is responsible for 41% of deforestation each year (Ritchie, 2021).

The growing field of cellular agriculture, a field of biotechnology focused on the production of animal cells *in vitro*, offers potential for a sustainable alternative. Growing isolated tissue rather than an entire animal substantially reduces the resource requirements and air, soil, and water pollution for meat production. Furthermore, industrial cultured meat production would also decrease the chances of foodborne illnesses, and decrease antibiotic resistance in humans as *in vitro* meat does not require antibiotics.

As opposed to plant-based meat alternatives, cultured meat contains real animal tissue like muscle and fat. Therefore, with the proper structure, cultured meat products can look, feel, and taste identical to their traditional counterparts - but without the need to raise and slaughter a living, thinking animal. Slaughter-free, eco-friendly meat products can appeal to some vegetarians as well as meat-eaters, converging different customer bases together.

The basic production method for cultured meat involves three steps: proliferation, differentiation, and processing. First, a starter colony of stem cells is grown on microcarriers

through a series of reactors of increasing size, allowing the cells to divide and proliferate. Next, the growth medium (the nutrient broth in which the cells grow) is adjusted to induce the stem cells to stop dividing and instead differentiate into the desired tissues. Finally, the differentiated cells are dewetted and processed into the final product. Here, it was decided to culture beef muscle fiber, mix it with plant-based fat, and extrude it to produce cultured ground beef.

b. Project Goals

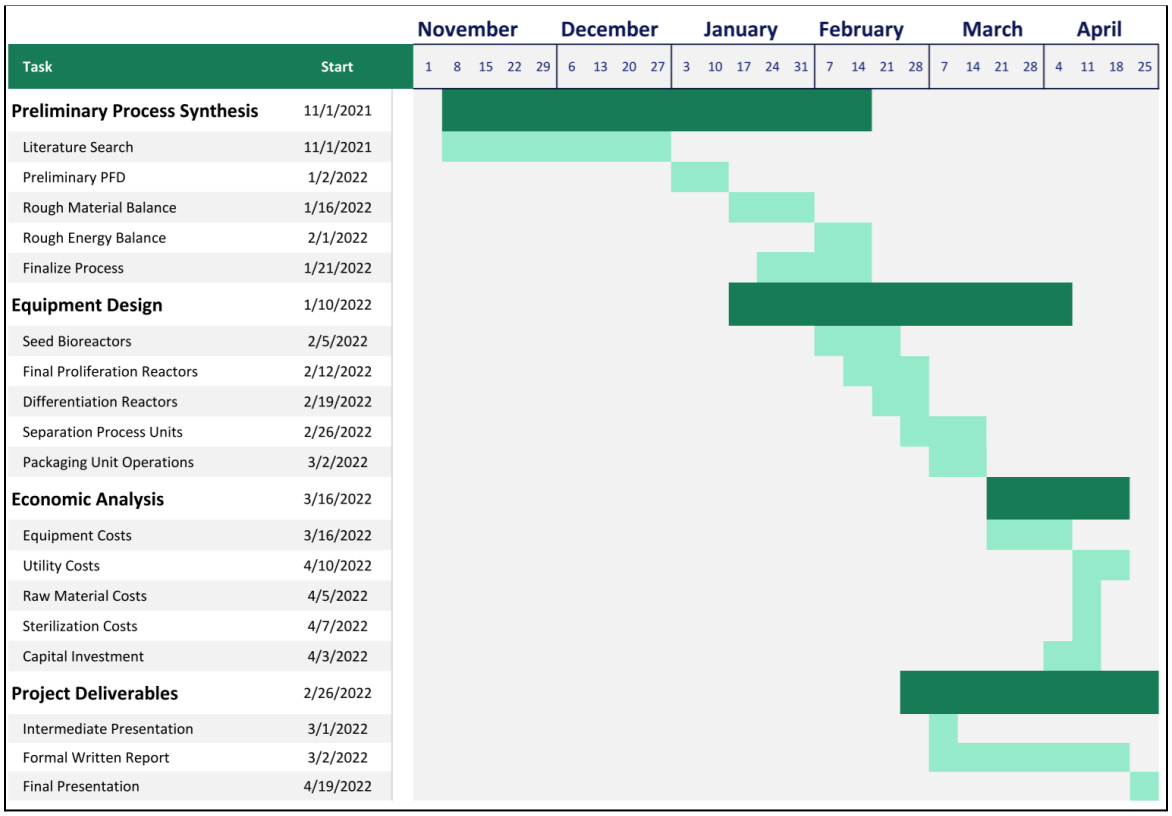
The goal of this project is to develop a plant design for manufacturing a cultured meat product for human consumption. Due to the lack of existing regulations on cultured meat products in the United States, the authors have decided to focus on adhering to the standards set forth by the United States Department of Agriculture and Food and Drug Administration regarding slaughtered meat.

To provide a viable alternative to current slaughter meat production, the manufacturing goal from the project statement is to produce 35 million pounds of cultured meat product per year using an aerobic upstream process and a primarily plant-based growth medium.

c. Time Chart

The timeline for meeting set deadlines in completing the project is outlined below. In late December and early January, the team met with project author Dr. Jeffrey Cohen and project advisor Dr. Bomyi Lim to discuss the current literature and make early design decisions such as product type (e.g. pork vs beef, sausage vs burger) and current technologies regarding lab-grown meat. The month of February was used to design and produce a process flow diagram including the upstream and downstream process units. Early March was dedicated to report writing and delivering a presentation to the department indicating the team's progress since the start of the

semester. The remainder of March was used to document the utilities and raw materials needed to meet production goals. April's deliverables focused on economic analysis and finalizing the report to meet the project deadline.



Task	Start
Preliminary Process Synthesis	11/1/2021
Literature Search	11/1/2021
Preliminary PFD	1/2/2022
Rough Material Balance	1/16/2022
Rough Energy Balance	2/1/2022
Finalize Process	1/21/2022
Equipment Design	1/10/2022
Seed Bioreactors	2/5/2022
Final Proliferation Reactors	2/12/2022
Differentiation Reactors	2/19/2022
Separation Process Units	2/26/2022
Packaging Unit Operations	3/2/2022
Economic Analysis	3/16/2022
Equipment Costs	3/16/2022
Utility Costs	4/10/2022
Raw Material Costs	4/5/2022
Sterilization Costs	4/7/2022
Capital Investment	4/3/2022
Project Deliverables	2/26/2022
Intermediate Presentation	3/1/2022
Formal Written Report	3/2/2022
Final Presentation	4/19/2022

Figure 1.1: Objective time-chart. This image outlines the deliverables and how the team achieved the project deadlines.

d. Project Deliverables

The project deliverables include an intermediate presentation, final written report, and final presentation. The scope of this project involves the creation of a plant design for the industrial manufacture of 35 million pounds of a cultured beef product with a primarily plant-based growth medium. Bovine muscle satellite cells (BMSc) were the cell line chosen for this project after a literature search on lab-grown meat products. The group also considered chicken and porcine cell lines but dismissed them due to the lack of research on their growth

kinetics. Current research offers the most information on the proliferation and differentiation of bovine cell lines, specifically BMSc.

With that in mind, a full process flow diagram is included to outline the many steps required for the production process from upstream manufacturing to downstream manufacturing and various utilities along the way. The report further details the equipment specifications and costs associated with each of the operations to match the needs of the BMSc line. To provide an in-depth understanding of the economics of the process, the location is specified to be in the Midwest: Illinois. The plant location was influenced by the accessibility of required materials in the area; namely, Illinois is one of the United States' top producers of corn and soy (Grant, 2022), which are required in large quantities to feed the cells. The raw material costs, total capital investment, and profitability analysis are also taken into account to recommend a product price in order to deliver a return on investment.

Apart from the scientific and financial considerations explored in this report, there are also environmental, ethical, and regulatory standards to be considered. Beef generally has one of the highest environmental impacts compared to other meats and the report offers a comparison of the costs associated with *in vivo* and *in vitro* production. The purpose of the report is to provide a conclusive analysis of the process to take laboratory-scale production into industrial manufacture of an *in vitro* cultured beef product.

Section 2: Market Analysis

The Food and Agriculture Organization (FAO) of the United Nations projected that annual global demand for meat would reach 455 million metric tons or 1 trillion pounds by 2050, representing a 76% increase since 2005 (Silva, 2018). As of 2020, current global meat production is 328 million metric tons, or 724 billion pounds per year (Shahbandeh, 2022). With the projected future demand for meat far exceeding the current supply, an alternative production method is critical.

Not only does the rise of cellular agriculture provide a solution to meat supply and demand, but it also represents a new market. The cultured meat market was valued at $1.64 million in 2021, estimated to reach $2.79 billion by 2030 (Allied Analytics, 2021), thereby indicating the robust growth that the industry is expected to undergo. In the past ten years alone, the costs associated with producing cultured meat products have substantially decreased. For example, the first cultured meat burger manufactured in 2013 cost $332,000 in research funds to produce (Kelland, 2013), whereas a cultured chicken nugget cost a mere $50 to fabricate in 2019 (Shanker, 2019). Given the rapid growth of cellular agriculture exhibited in the past few years, production costs would likely decrease as the manufacturing processes become more robust and scaled up.

Section 3: Competitive Analysis

As of the writing of this report, there are no cultured meat products approved for sale in the United States. However, in 2019 the US Department of Agriculture's Food Safety & Inspection Service (USDA FSIS) and the Food and Drug Administration (FDA) announced an agreement on how to jointly regulate cultured meat (US FDA, 2020). Under this joint framework, the FDA will oversee cell proliferation and differentiation before transferring oversight to the FSIS; the FSIS will oversee downstream processing from cell harvest to final product packaging and labeling (USDA Press, 2021). This agreement paves the way for cultured meat companies to seek approval to operate in the United States in the near future.

In other regions, there are several companies that are beginning to bring their products to consumers (see **Table 3.1** for a summary). The most notable, Eat Just's GOOD Meat, has received approval in Singapore for cultured chicken nuggets and was the first to sell a cultured meat product to consumers in December 2020 (Gilchrist, 2021). Currently, there are no direct competitors to this project's specific product, cultured beef, on the market, but two of the most prominent companies in that space are Israel-based Aleph Farms and Netherlands-based Mosa Meat. Aleph Farms, founded in 2017, announced the world's first cultured steak in 2018 (Ashkenazi, 2021) and in February 2022 unveiled a 65,000 m^2 pilot facility they plan to use to continue scaling up their steak production process (Aleph Farms, 2022). Mosa Meat, founded in 2016 by the researchers who demonstrated the first cultured burger in 2013, was approved in March 2022 to allow the public to begin sampling their cultured burger in controlled settings in the Netherlands (De Lorenzo, 2022).

Table 3.1. Summary of notable competitors in the cultured meat space.

Company Name	Location	Year Founded	Main Product	Latest Progress
Eat Just	United States	2011	Chicken	Approved for sale in Singapore (Dec. 2020)
Aleph Farms	Israel	2017	Beef Steak	65,000 m^2 pilot facility (Feb. 2022)
Mosa Meat	Netherlands	2016	Beef Burgers	Approved for tastings in the Netherlands (Mar. 2022)

Section 4: Customer Requirements

The final product is for human consumption and therefore must comply with the regulatory requirements for meat for human consumption set forth by the United States Department of Agriculture (USDA) and the Food and Drug Administration (FDA). The final product is ground beef with 80% protein content provided by bovine muscle cells and 20% fat content from hydrogenated vegetable oil. The decision to focus on guidelines set forth by the United States government is associated with the lack of existing regulatory standards for a cultured beef product.

The USDA's Food Safety and Inspection Service (FSIS) is responsible for inspecting all meat products that are to be shipped and sold across state borders (USDA FSIS, 2016b) under the Federal Meat Inspection Act. While many states have their own governing bodies for meat sold within their own borders, this project focuses on a nationwide product that would fall under the federal government's jurisdiction. Given that the product is ground beef to be sold to a distributor responsible for packaging and form, this product does not need to be graded according to federal regulations.

Nevertheless, the product must still be inspected to ensure that it is sanitary, safe for human consumption, and correctly packaged. The process outlined in this report falls under the first subchapter of the Federal Meat Inspection Act: Inspection Requirements; Adulteration & Misbranding, (USDA FSIS, 2016a). Due to the non-slaughter nature of a cultured beef product, the amendments and sections that apply to this project regard sanitation and labeling of the product alone. Sections 607-609 of the Code calls for the proper labeling, marking, and containment of the product by which this process will abide in that an inspector will supervise

during the day and night to guarantee adherence to sanitary guidelines (Federal Meat Inspection Act, 1906).

Though the product is primarily cultured beef, the addition of hydrogenated vegetable oil as a fat component requires that the product follow FDA guidelines under the Compliance Policy Guideline Sec 565.100, FDA Jurisdiction Over Meat and Poultry Products. To prevent multiple government agencies from re-inspecting the same product, the FDA can use their jurisdiction under the Federal Food, Drug, and Cosmetic Act to inspect rather than the USDA's FSIS. However, the nature of the product indicates that the USDA will have the final say in determining whether the product is safe for human consumption, (US Office of Regulatory Affairs, 2018).

Section 5: Preliminary Process synthesis

The novelty of cellular agriculture presents a challenge in that many of the existing processes focus on lab-scale production which needs to be scaled up to meet the stated manufacturing goals. Any existing large-scale processes are kept private as industry secrets, thereby increasing the difficulty of the project. The team utilized current lab-scale methods in the early upstream processes and scaled up using standard industry practices for adherent cells, which require attaching to a surface to grow optimally. This surface was provided by microcarriers, small beads used in cell culture in order to give cells something to adhere to while also increasing surface area for mass and energy exchange with the cell culture medium. Fortunately, many of the downstream processing units follow industry standards for the processing and storage of the finished product. The challenges presented throughout the project are highlighted below.

The first challenge in which the group was presented was to determine the type of cultured meat product to produce. After reviewing the available research, it was noted that cultured beef is the meat product on which most lab-scale *in vitro* meat projects were focused on. Due to the availability of this data and research, the team chose to industrialize current lab-scale technologies for cultured ground beef. Another reason for this choice is that it allows for a thorough comparison between *in vitro* and *in vivo* methods to produce one of the most environmentally costly meat products: beef.

The bovine muscle satellite cell (BMSc) line was decided upon as they can only be differentiated into a limited number of tissue types, depending on the medium used for differentiation (Williams et al., 2012), reducing the potential for non-desired tissue types in the

final product. This process focuses on the differentiation of these satellite cells into muscle cells to create a protein-rich cell product. In the final steps of downstream processing, hydrogenated vegetable oil is used as a fat source to develop a protein to fat ratio of 80:20.

a. Scale-up of Lab-scale Processes to Industrial-scale

When scaling laboratory technology to industrial size process equipment, factors such as enthalpy of growth, sterility, and separation processes become more important. While cell growth times remain stable between the two processes, the amount of heat produced on the industrial scale becomes much more problematic. Therefore, the addition of cooling jackets to production bioreactors was a necessary step in preventing cell death due to overheating. The enthalpy change associated with growth kinetics must be considered differently between the two cases as an ice bath is not a suitable means for keeping internal process temperature consistent at 36.5℃ when reactors are hundreds of thousands of liters large. As a result of such reactor sizes, maintaining sterility proves difficult.

If reactor temperatures rise or fall beyond the optimal growth temperatures, cell death can complicate sterility between batches. The goal of the proliferation and differentiation phases of the process is to keep the cells alive until the downstream dewatering step. The purpose of this goal is to guarantee the longest possible shelf life of the product once ready for sale. Not only is sterility important in increasing the longevity of the cultured beef, but also to satisfy the aforementioned USDA guidelines on sanitary production. This challenge differs in production scale from lab-scale as the majority of laboratory equipment is single-use and/or can be disassembled for cleaning and sterilization, and therefore does not require the clean-in-place necessary at industrial scale to prevent cross-contamination between batches.

Another aspect that influences sterility and cell death is the oxygen uptake rate of the cells and determining the mass transfer coefficient (k_La) for optimal oxygen transfer rate. At insufficient dissolved oxygen concentrations, hypoxia may result in significant cell death compromising the entire batch. At excessive oxygen concentrations, the volumetric mass transfer ratio becomes significantly smaller thereby decreasing the efficiency of the sparge oxygen delivery. As opposed to laboratory scale, where shaking can provide sufficient aeration, the industrialization of these processes includes the addition of gas sparges in all bioreactors. These considerations are taken into account and further explained in Section 7: Process Synthesis.

The determination of a fed-batch approach to meet manufacturing goals differs from current research in that many experiments regarding cultured meat are conducted in a batch modality. As a result of the cell requiring microcarriers to grow, it was concluded that a continuous approach would not be optimal or even feasible. The inclusion of microcarriers and differing requirements at the proliferation and differentiation stages would complicate a continuous model. Therefore, a fed-batch approach would allow varying growth media compositions to optimize growth and limit any sterility concerns between batches. Increased cleaning and clear delineations between batches would minimize the sterility concerns and maximize outputs in line with current regulations.

b. Plant-Based Growth Media

This project also involved developing a primarily plant-based growth medium. However, traditional growth media require a significant amount of fetal bovine serum (FBS), a byproduct of cattle slaughter. FBS contains nutrients, hormones, and other growth factors critical for cell growth, mimicking the complex native environment surrounding mammalian cells (van der Valk et al., 2010). BMSc proliferation medium typically contains up to 20% FBS (Khasawneh et al.,

2019), while differentiation medium uses 2% FBS or similar animal products such as horse serum (Xu et al., 2018). Reducing the use of FBS provides us with an opportunity to differentiate the product from prior work.

The base for the plant-based medium was based on cell glucose and glutamine requirements as cell energy and growth requirements are based on these metrics. To obtain these requirements, a cell culture medium containing both corn grain hydrolysate and soybean hydrolysate was used to match the glucose and amino acid profiles needed for optimal growth.

Not only are these components necessary for cellular respiration, but also for the generation of more cells. As a result, the hydrocarbon source for glucose chosen was corn grain hydrolysate due to its high reducing sugar content, (Huang et al., 2017). The amino acid source for glutamine, which proves to be the cells' limiting amino acid (Quang & Zakardas, 1989), is soybean hydrolysate due to its high protein content. Combining the two plant hydrolysates satisfies upstream plant-based growth media requirements.

The use of FBS complicates the goal of an entirely slaughter-free meat alternative. However, this process sought to minimize the use of FBS wherever possible. Based on recent research, the FBS requirement of this proliferation medium can be reduced to 10% by weight, and it was determined that FBS could be eliminated entirely from the differentiation medium (Will et al., 2015). While additives differ between proliferation and differentiation media, the plant-based growth media primarily featured the corn grain and soybean hydrolysates as the main nutrient source for the cells.

Initially, the team planned to introduce a hammer mill and enzymatic hydrolysis unit for both the corn grain and soybean to minimize the costs associated with the raw materials for this

plant. The location of the plant in Illinois, where corn grain and soybean production are among the highest in the nation, indicated that allocating for hydrolysates would lower the total capital investment required. However, ultimately allocating on-site processing of raw corn and soy proved beyond the scope of the project.

c. Separation of Media and Microcarriers from Cell Slurry

Due to the adherent nature of the BMSc line, the use of microcarriers are critical in meeting the optimal growth density in the bioreactors to meet manufacturing goals. Current research shows that Cytodex I microcarriers are the best option for BMSc growth (Luining, 2015). Cytodex I microcarriers are composed of dextran, which provides a nontoxic supportive structure for the cells to proliferate and differentiate on during the upstream process. However, its inclusion in the final product would compromise the taste and texture of the cultured beef product. Therefore, its exclusion in the downstream processing steps is necessary. This section discusses the various methods explored in research to remove the cells from the beads and how this process intends on separating them.

Trypsin and EDTA are two industry-known chemicals that can remove cells from the surface of a microcarrier. The costs associated with trypsin, an enzyme known to dislodge adherent cells from the surface of the microcarriers, were far too high to consider and would not solve the problem of ridding the cell solution from the microcarrier waste. Also, trypsin may increase cell loss on the surface of a microcarrier as it is quite ineffective when compared to EDTA (Rourou et al., 2013). While adding EDTA to a cell slurry decreased cell loss, the concentration of EDTA increases cell death as EDTA is a known cytotoxic chemical (Luining, 2015).

Microcarriers also complicate the downstream separation steps. Not only would the downstream processing units need to include a solid-liquid separation device, but also a solid-solid separation unit. Solid-solid separation devices are much more difficult to design, especially if the desired solid is significantly smaller than the solid it is to be separated from. In this case, a simple deadend filtration device would not be sufficient due to the small size difference between the cells and the microcarriers. Therefore, additional separation units would be necessary to achieve the desired product. The affinity of the cells to the microcarriers further complicates the issue.

However, there is an alternative to releasing the cells from the microcarriers: dissolving the microcarrier beads entirely. Dextranase is known to be capable of dissolving dextran microcarriers while preserving cell viability (Lindskog et al., 1987), and was thus selected as the dissolving agent. Although dissolving the microcarriers meant that they could not be recycled for future batches, it minimized cell losses as there would be no surface for the cells to re-adhere to and removed the problem of having to design a solid-solid separation device. By introducing dextranase to break down the microcarrier before the solid-liquid separation device, the assumption can be made that none of the dextran microcarriers would remain to be removed in the vacuum rotary drum to separate the waste metabolites and unspent media from the cell slurry. As a result, a solid-solid separation device would no longer be required. Note that it may be possible to improve the economics of this project if an effective solid-solid separation device were to be implemented as it would allow for the recycling of beads and reduce raw materials.

Section 6: Process Flow Diagram and Material Balance

a. Process Flow Diagram

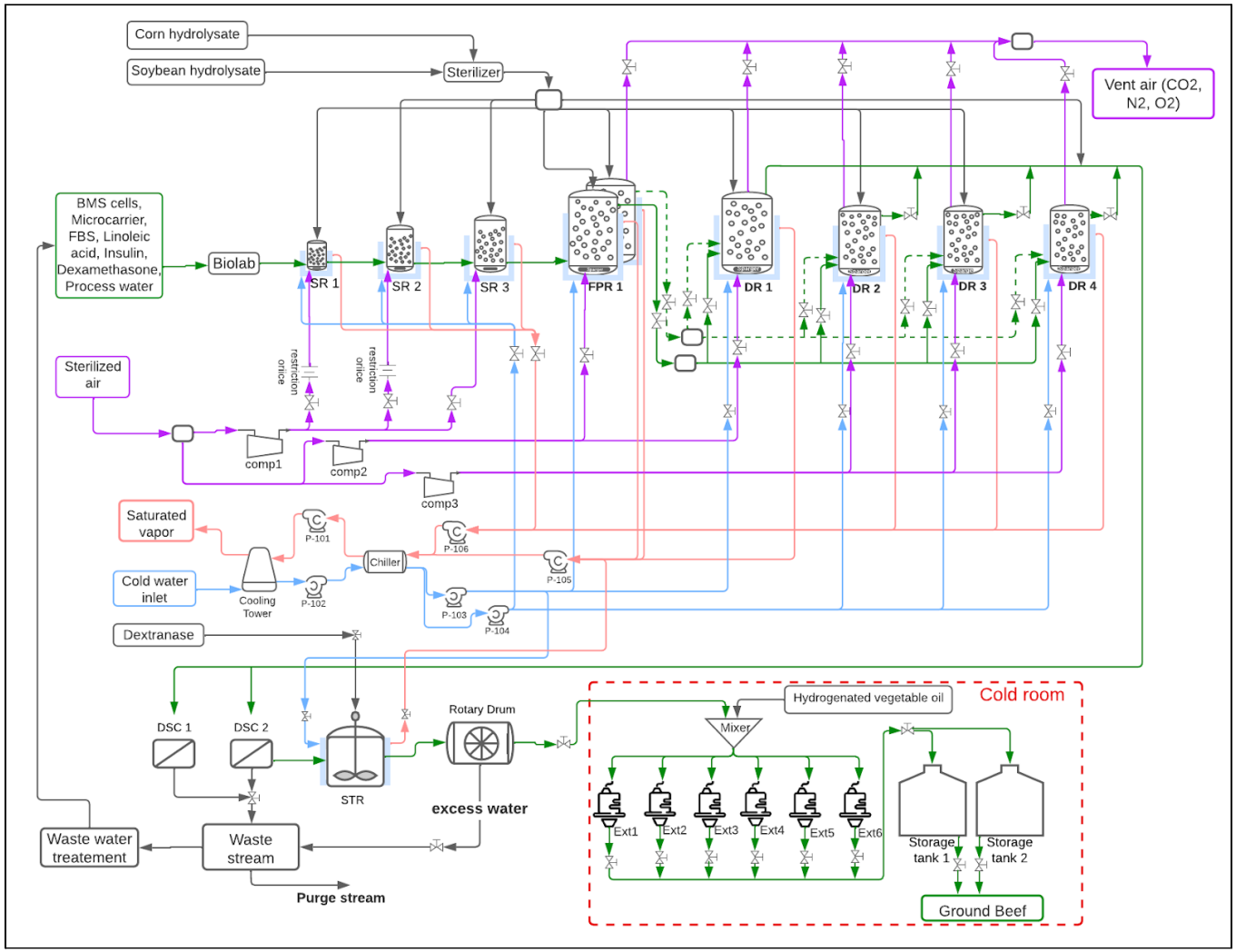


Figure 6.1. Process flow diagram. The upstream and downstream processes for cultured beef. Stream numbers are included in Section 9.

b. Material Balances

The material balances for a fed-batch system as proposed by this project are outlined in the following tables. The fed-batch system proposed by this process utilizes two important features of a batch and continuous process. The charge requirements are added to the bioreactor at the start of each batch. The continuous feeds are added throughout the process time that each batch spends in each bioreactor. As a result, the following tables are designated according to bioreactor charge requirements, continuous feeds throughout the process, and the overall annual requirement of each raw material to produce 35 million pounds of ground beef product per year.

Table 6.1 indicates the amount of each substance needed to attain an optimal growth density of 60 million cells per mL and maintain this density throughout the differentiation stage of the process. The bioreactors are named according to the process flow diagram shown previously, but there is a distinction between final proliferation reactor (FPR) 1 at full harvest and half harvest. This difference denotes when the contents of the entire reactor are sent to differentiation reactor (DR) 1 and when only half of its contents are sent to DR 2, DR 3, or DR 4 as they operate at half the capacity of DR 1. While the exact same proliferation reactor is used, half of it is harvested three times, each time allowing the contents to double again between harvests, until the fourth and final harvest takes the full contents of the FPR and sends it to DR 1. The purpose of the multiple harvests is to maximize the amount of product that can be produced from one batch, as proliferation is extremely time-intensive, taking 31 days from the beginning of the seed train to the first half harvest.

Note that insulin, linoleic acid, and dexamethasone are not included in the proliferation stages of the process. The addition of insulin, linoleic acid, and dexamethasone, along with the

cessation of adding FBS, induces the stem cells to begin differentiating (Will et al., 2015). This serum-free differentiation medium was taken from a review in which various serum-free media are explored and compared to the performance of cells in FBS-containing media (Will et al., 2015). In addition, Cytodex I microcarriers are not included in the differentiation stages, as cell division halts during differentiation, and thus additional microcarriers no longer need to be added.

Table 6.1: Batch bioreactor charge requirements for cell proliferation/differentiation

	Soybean Hydrolysate [lbs/batch]	Corn Grain Hydrolysate [lbs/batch]	FBS [lbs/batch]	Insulin [lbs/batch]	Linoleic Acid [lbs/batch]	Dexamethasone [lbs/batch]	Cytodex 1 [lbs/batch]
SR 1	0.002	0.001	0.0004	-	-	-	1.6
SR 2	0.05	0.03	0.009	-	-	-	40
SR 3	0.37	0.23	0.07	-	-	-	700
FPR 1 (Full Harvest)	44	28	8.0	-	-	-	88,000
FPR 1 (Half Harvest)	44	14	6.5	-	-	-	44,000
DR 1	44	28	-	0.22	0.47	0.19	-
DR 2	22	14	-	0.11	0.24	0.09	-
DR 3	22	14	-	0.11	0.24	0.09	-
DR 4	22	14	-	0.11	0.24	0.09	-

Batch requirements differ from the continuous feed supplied to the reactors in that it provides the necessary reactor initial concentrations to induce an optimal growth environment. Therefore, **Table 6.2** summarizes the necessary additions that need to be made to ensure that this growth environment is kept stable. The important factors include glucose, glutamine, and water such that optimal growth can occur. Only process water, soybean hydrolysate, and corn grain hydrolysate are added continuously. These are the three main components that the cells need to undergo cellular respiration and grow under specified conditions. As a result of the generous

charge amounts of growth regulators such as FBS and Cytodex I microcarriers, and differentiation inducers such as insulin, linoleic acid, and dexamethasone, the cells grow and differentiate in their bioreactors in the presence of all the necessary components. While it would be possible to charge the vessels with all necessary media from the start, it would not allow for control of the optimal growth density. By not controlling the chosen cell density, the cells may overpopulate the bioreactors leading to possible cell exhaustion and death.

Table 6.2: Continuous feed bioreactor requirements for cell proliferation/differentiation

	Process Water [lbs/hr]	**Soybean Hydrolysate [lbs/hr]**	**Corn Grain Hydrolysate [lbs/hr]**
SR 1	0.003	0.004	0.003
SR 2	0.07	0.10	0.07
SR 3	0.54	1.98	1.3
FPR 1 (Full Harvest)	39	150	99
FPR 1 (Half Harvest)	20	76	50
DR 1	140	52	4
DR 2	69	26	2
DR 3	69	26	2
DR 4	69	26	2

A fed-batch system in which components are added to each bioreactor for varying amounts of time results in a complex equation for determining the yearly requirements for each material in the production of a lab-grown meat product. **Table 6.3** briefly summarizes these calculations in which each requirement is determined. The charge amount is first added to the product of the material flow rate and time spent in the reactor per batch. Then, this sum is multiplied by the number of batches produced per year. The major components of the material balance are the process and cooling water, soybean hydrolysate, corn grain hydrolysate,

microcarriers, air, and cooling water as their requirements are well into the millions of pounds per year. Also, the starter cells, FBS, linoleic acid, insulin, and dexamethasone are necessary for adequate growth and differentiation as previously noted.

Table 6.3: Yearly requirements for cell proliferation/differentiation

Component	Annual Requirement	Units
Process Water	6.4	million lbs
Starter Cells	8.3	lbs
Soybean Hydrolysate	110	million lbs
Corn Grain Hydrolysate	75	million lbs
Microcarriers	8.6	million lbs
Air	940	million lbs
FBS	1.1	thousand lbs
Linoleic Acid	46	lbs
Insulin	21	lbs
Dexamethasone	18	lbs

While **Table 6.3** outlines the total yearly requirement of the upstream processes alone, **Table 6.4** focuses on the total annual requirements for all raw materials and cooling water utilities that enter and exit the overall process. The necessary additions here include the amount of dextranase, hydrogenated vegetable oil, and cooling water to produce 35 million pounds of cultured beef product.

The amount of process water added to the bioreactors is less than the amount lost as a result of the supernatant purge of 10% to prevent ammonia produced during growth and differentiation from accumulating in the system. This idea continues for any of the added growth supplements such as soybean and corn grain hydrolysates, FBS, linoleic acids, insulin, and dexamethasone. The evaporated process water is included in the annual waste leaving in the air

stream as it results from the dry air traveling through the bioreactors and becoming saturated. The cooling process also evaporates water in the cooling tower and chiller which are also included in the exiting air for the process.

Due to the use of the hydrolysates in energy production and cell mass production, there is no clear way to distinguish how the cells are using them thereby resulting in a whole-process mass balance that does not close. Using these hydrolysates, the process is able to grow 28 million pounds of cells from 8.3 pounds of starter BMSc and supplement it with 7 million pounds of hydrogenated vegetable oil to achieve the desired fat to protein ratio. It should also be noted that the microcarriers and dextranase enter and leave the process in the same amount as they are tools used to achieve growth and therefore, not part of the final product. As a result, 35 million pounds of an *in vitro* beef product are formed per year.

Table 6.4: Yearly requirements for overall process

Component	Annual Intake	Annual Waste	Units
Process Water	6.4	9.1	million lbs
Starter Cells	8.3	-	lbs
Soybean Hydrolysate	110	11	million lbs
Corn Grain Hydrolysate	75	7.5	million lbs
Microcarriers	8.6	8.6	million lbs
Air	94	140	million lbs
Nitrogen	72	72	million lbs
Oxygen	22	9.6	million lbs
Carbon Dioxide	-	17	million lbs
Water Vapor	-	35	million lbs
FBS	1.1	0.11	thousand lbs
Linoleic Acid	46	4.6	lbs
Insulin	21	4.6	lbs
Dexamethasone	18	1.8	lbs
Dextranase	8.6	8.6	million lbs
Hydrogenated Vegetable Oil	7	-	million lbs
Cooling Water	29	-	million lbs
Cultured Beef Product	-	35	million lbs

c. Timeline of Key Steps in the Process and Batch Scheduling

The biggest obstacle in batch scheduling was in ensuring efficient usage of the bioreactors. In order to meet the production goal of 35 million pounds per year of the final product, 28 million pounds per year of bovine muscle cells must be produced. With two final proliferation reactors, each with a working volume of 225 m^3 and harvested 4 times (see Section 7 for more detail), each batch produces approximately 739,000 pounds of cells; the process is able to produce about 39 batches per year, for 28.6 million pounds of cells produced (thus leaving 2% excess in case of quality control issues).

In order to meet the required number of batches, the seed and final proliferation bioreactors run concurrently. The three seed train bioreactors are run such that the first step takes the longest (7.14 days), followed by the second (7.08 days), and then the third (6.35 days). This ensures that seed train steps do not finish earlier than those that come after them; while excess downtime is undesirable, it is vital to keep cells moving between reactors, where they have adequate oxygen and nutrients, rather than creating a need for storage that may compromise cell viability.

The slowest step by far is the initial growth in the final proliferation reactors, at 10.5 days. While the length of time is undesirable in terms of batch efficiency, the delay does allow us to have both of the final proliferation reactors share the same set of seed and differentiation reactors. Offsetting the start time of each final proliferation reactor by approximately 8 days allows for this reduction in total bioreactors needed, as well as staggers the timing for when cells arrive at downstream processing. See **Figure 6.1** for a Gantt chart demonstrating the full schedule.

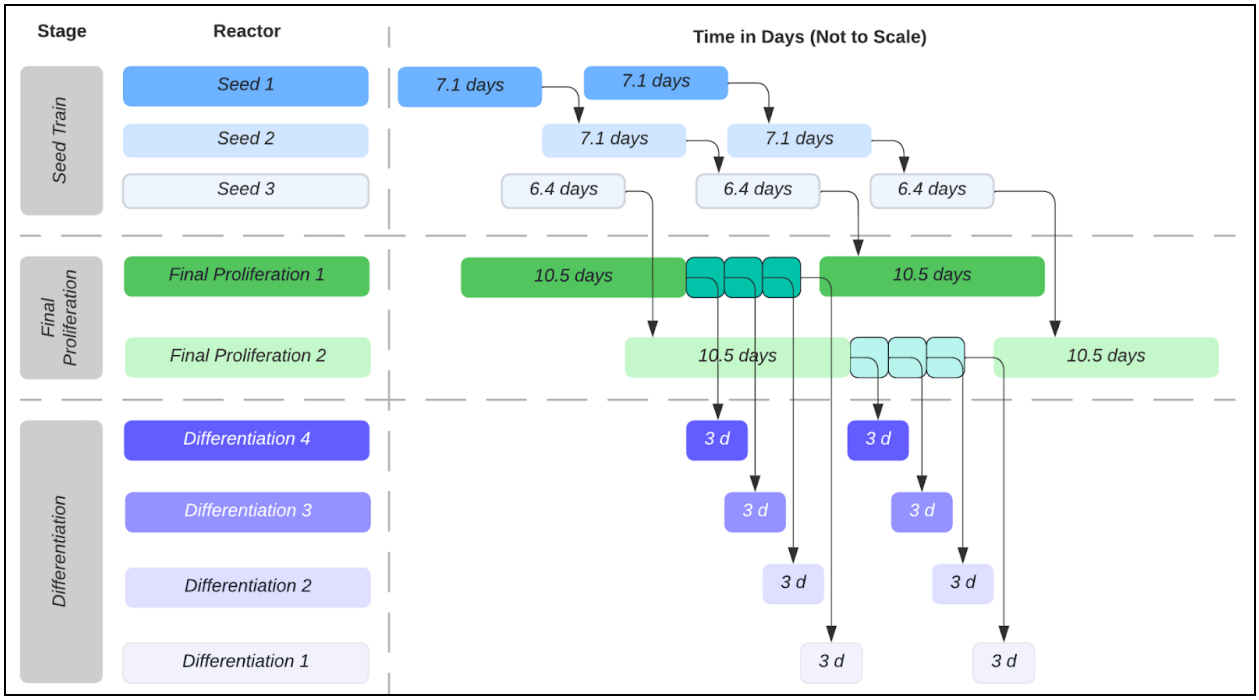


Figure 6.2. Bioreactor Gantt chart. For the final proliferation reactors, the unlabeled boxes following each 10.5-day proliferation indicate the subsequent 3 doublings as the final proliferation reactors are harvested and the cells sent to differentiation. The gaps between time blocks represent cleaning (see "Clean-in-Place" in Section 7) and other routine maintenance.

Section 7: Process Synthesis

a. Process Summary

The cultured meat process can be broadly divided into two categories: upstream and downstream processing. The upstream component of the manufacturing plant focuses on the cultivation of the cells to be used as the protein component whereas the downstream component involves the dewatering, mixing, extrusion, and storage of the finished product.

However, each of the process units involved in the entire manufacturing process must also be maintained under certain conditions and cleaned thoroughly. Due to the heat generation associated with cell growth, an intricate cooling system is needed for the process to maintain ideal growth conditions. As previously mentioned, the industrialization of a laboratory process brings about concerns of sterility, necessitating the inclusion of a clean-in-place (CIP) procedure for all process units which come into contact with the cell product.

The upstream process, oxygen transfer, cooling jacket network, downstream process, and clean-in-place process are outlined in the following subsections.

b. Upstream Process

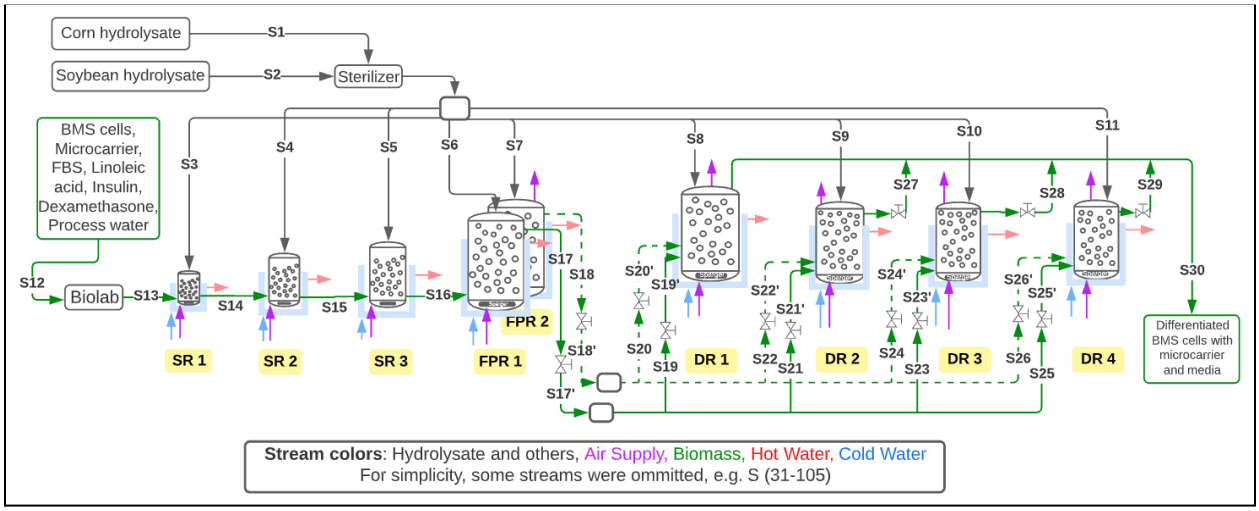


Figure 7.1. **PFD: upstream process units.** Upstream process units and the associated streams for proliferation and differentiation. The network of sterile air supplies is also included.

Upstream processing refers to the proliferation and differentiation of the cells, producing a mixture of growth medium and cells attached to microcarriers that is sent to downstream processing. For this process, bovine muscle satellite cells (BMSc) were used due to a greater amount of information available about them in the literature. This cell line is adherent, meaning it must be grown attached to a solid substrate; Cytodex 1 was selected, small dextran beads that have been shown to best encourage BMSc growth (Luining, 2015). Following 31 days of proliferation (see **Figure 6.1**), the BMSc must then be differentiated to muscle fiber final product formation. This step is accomplished by serum starvation (the depletion of nutrients from fetal bovine serum that occurs as the cells divide) and the addition of small concentrations of dexamethasone, linoleic acid, and insulin (Will et al., 2015). After 72 hours (Will et al., 2015), the BMS cells will have finished differentiating to muscle fiber and are ready to be sent to downstream processing.

For the bioreactors, bubble columns are chosen rather than the more traditional stirred tanks currently used in most industrial cell culture. In its simplest form, a bubble column reactor

consists of a tall, thin cylinder with a gas sparger on the bottom and a vent on top. Gas bubbles traveling up from the sparger keep the medium circulating and provide oxygen to the entire tank. This reactor type was chosen in order to adequately oxygenate the largest reactors; mammalian cells have a relatively large oxygen requirement as opposed to the bacteria and yeast primarily grown in fermenters of this size scale. If a stirred tank had been chosen, the amount of stirring required to uniformly oxygenate the cell growth medium would have placed the cells at risk of injury or death from shear stress. Bubble column reactors also have the advantage of being relatively simple to design and build. With the number of bioreactors required for the plant, this simplicity helps us save on construction costs and reduces possible points of failure in the design (eg, a mechanical agitator).

Due to the large size of the final proliferation reactors, 300 m^3, a three-step seed train is required in order to expand the starting cells to a concentration high enough to inoculate the largest reactors. The first step, inoculating the first seed reactor, begins with an allocated, on-site cell culture lab. It is assumed that there is steady access to high volumes of concentrated, frozen cell starter such that each batch begins with 150 mL of starter at a concentration of 65 million cells/mL, as described in a recent article in the Journal of Chemical Technology & Biotechnology (Wong et al., 2021). The full seed train consists of a series of 3 seed reactors of 0.01, 0.33, and 2.49 m^3 volumes, leading up to the cells being sent to one of two final proliferation reactors of 300 m^3 volume.

Each final proliferation reactor is harvested four times - thrice taking half the total culture volume before allowing the cells to double again, and for the fourth and final harvest draining the reactor entirely, in a method adapted from Guan et al. (2021). Each harvest is sent to a differentiation reactor, so that four total are required: three of 150 m^3, and one of 300 m^3 volume.

Due to the slow doubling time of the cell line, 36.6 hours (Simsa et al, 2019), the two final proliferation reactors can be scheduled offset from one another such that the same set of seed and differentiation reactors can be used for both (see **Figure 6.1** for a bioreactor scheduling chart).

c. Oxygen Transfer

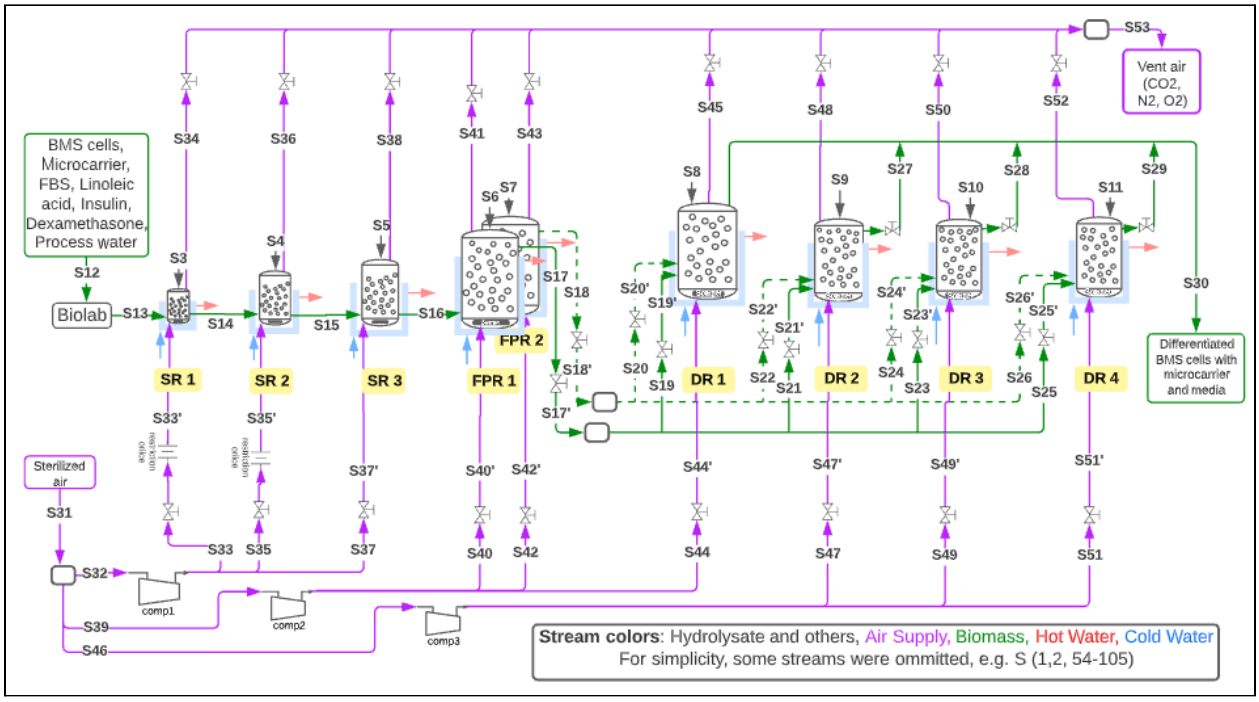


Figure 7.2. PFD: sterile air supply. Sterile Air Supply for Aerobic Bioreaction.

The upstream process includes aerobic bioreaction which uses oxygen to convert sugar molecules to hydrocarbons for cell growth. To maintain proper growth conditions, a minimum amount of oxygen should be present depending on the cell population in the reactor (i.e. more oxygen is required at higher cell density). The oxygen requirement also depends on the oxygen transfer rate (OTR) and the oxygen uptake rate (OUR). OTR refers to the absorption of oxygen from gas bubbles to liquid while OUR is defined as the consumption of oxygen from liquid to the cell. The OTR that is supplied by the sparger gas must equal the OUR at a steady-state to maintain a dissolved concentration of oxygen in the liquid. A key concept to consider is the

liquid boundary layer formed around the air bubbles which limits the OTR into the bioreactor media. If oxygen is not properly transferred to the media from the air, it can not be consumed by the cells as needed so it is crucial to obtain a mass transfer coefficient (k_La) for a given air supply. It is also important to determine the pressure difference between dissolved oxygen in air and media which acts as a driving force for OTR. This process uses a 10% dissolved oxygen setpoint and a solubility of 0.03 grams of oxygen per kg of water (Engineering Toolbox, 2008) to create an OTR driving force. As shown in **Figure 7.2,** the process is set to supply sterile air with 21% saturated oxygen to all the bioreactors. However, the flow rate of air supplied to the reactors vary depending on the oxygen demand which is determined by a combination of equations discussed in **Appendix C**.

To summarize the method, the specific OUR of mammalian cells, 0.20 pmol O_2/cell/hr (Goudar et al., 2011), and the peak viable cell density, 60 million cells/mL (Mizukami et al., 2013), in each bioreactor is used to obtain the OUR (mmol O_2/L/hr). Equating OUR to OTR and using Fick's first law of diffusion, the k_La requirement is determined. Then, a correlation between k_La and gas superficial velocity in a bubble column reactor is used to obtain the sparge gas rate that would meet the oxygen demand (Zedníková et al., 2018).

The gas bubbles need to overcome the pressure difference to reach the top of the reactor with the help of compressors. Three compressors are used to pressurize the air for three different supply networks. The first compressor (Comp 1) supplies air to the seed train reactors (SR1, SR2, and SR3). Note that streams S33' and S35' include restriction orifices to reduce the pressure since sparge gas rates for SR1 and SR2 are significantly lower than SR3 (see **Table 9.3**). The second compressor (Comp 2) supplies air to both the final proliferation reactors (FPR 1, 2)

and the full-sized differentiation reactor (DR1). Lastly, the third compressor (Comp 3) supplies sterile air to the three half-sized differentiation reactors (DR 2, 3, 4).

During the aerobic bioreaction, carbon dioxide is created as a byproduct in a 1:1 mole ratio with oxygen. Therefore, generated carbon dioxide, unreacted inert nitrogen, and the remaining oxygen leave the reactor through the vent as depicted in **Figure 7.2.**

d. Cooling Jacket Network

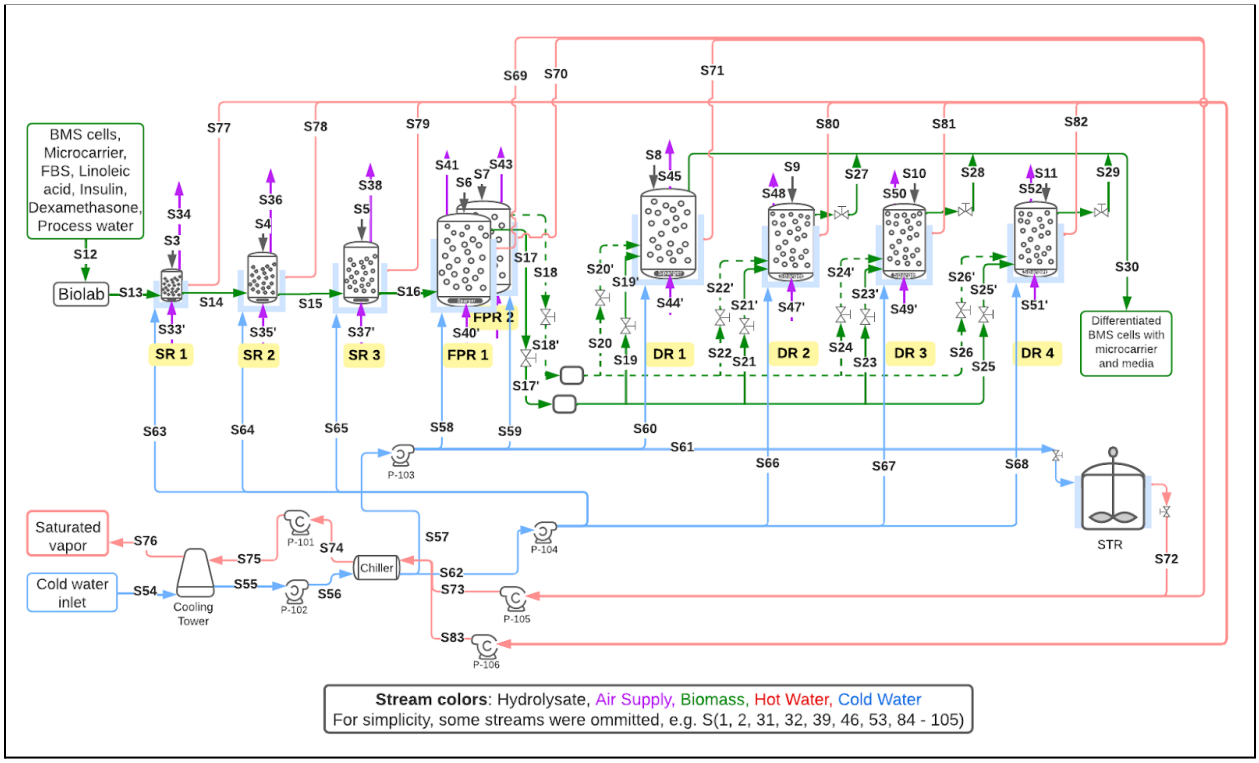


Figure 7.3. Cooling Tower and Chiller Network. The diagram includes streams supplying chilled water at 22℃ (blue) to all the bioreactors and warm water at 26.5℃ (red) back to the chiller.

Throughout the entire proliferation and differentiation process, metabolic activities in the bubble column reactors generate heat that, left unchecked, will hinder cell growth and viability (Guan & Kemp, 1999). It is crucial to remove the additional heat generated from cell growth and maintain the content inside the reactors at a constant temperature of 36.5℃ to keep the cells

viable (“Cell Culture Environment,” n.d.). To do so, a cooling tower is installed onsite to supply cold water to all the bioreactors through annular cooling jackets. Heat transfer is typically more efficient in mechanically agitated reactors due to a thinner boundary layer on the reactor surface as a result of higher process fluid velocity (Mahir et al., 2021). However, the optimal cell growth condition for this process demands the use of bubble column reactors which have lower heat transfer.

The addition of cooling jackets around each reactor will help the heat transfer process by introducing a temperature difference. The annular cooling vessels will be made up of 304 stainless steel, which has a thermal conductivity of 16.2 W/m*K (Azo Materials, 2005). The relatively high thermal conductivity and proper jacket thickness will provide sufficient surface area for proper heat transfer. A thorough energy balance on the cooling jacket is further discussed in Section 9.

Figure 7.3 shows the overall cooling jacket network for the plant. Depicted in blue lines, chilled water at 22℃ is supplied to each bioreactor. The cooling jackets remain in contact with the walls of each reactor, allowing heat transfer to occur from process volume to the jacket. The heat generated from cell growth is transferred to the cooling water, raising its temperature to 26.5℃. The red lines depict the warm water that carries the generated heat from the bioreactors back to the chiller.

For most of the year, a cooling tower alone is adequate to provide cooling water for the plant. However, the temperature of Illinois in the summer necessitates a chiller onsite. Over the last decade, the highest temperature experienced in Illinois ranged from 34℃ to 41℃, which is significantly higher than the cooling water temperature that is required for the process. A chiller can take the water from the cooling tower and bring it down to the desired temperature before

reaching the bioreactor cooling jackets. It should be noted that the cooling jacket network may be modified to include heat exchangers that can heat the water in winter if necessary.

e. Downstream Process

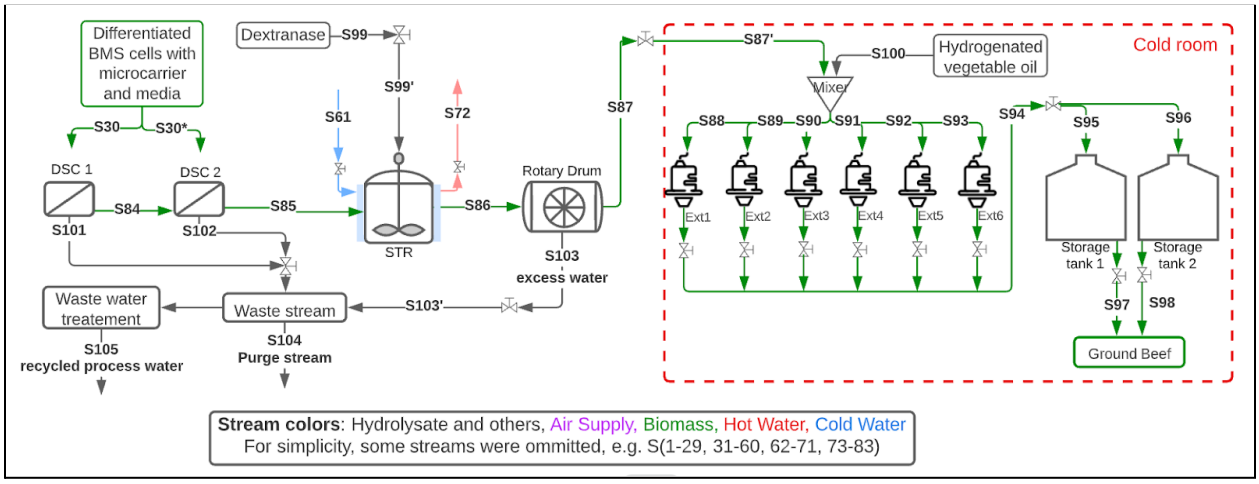


Figure 7.4. PFD: downstream process units. Downstream process units and their associated waste and feed streams which includes the product of the upstream process units and follows the process through to the production of the final product: ground beef.

Downstream processing refers to the separation, dewatering, and packaging steps of cultured meat production. Following the differentiation bioreactors, the first step is a disk stack centrifuge to dewater and remove the bulk of the liquid medium resulting in a concentrated cell slurry containing microcarriers. A disk stack centrifuge was chosen as the ideal separation unit in that it allowed for a sufficient amount of growth media to be removed. It also clarified the media such that it could be sterilized and reused for subsequent batches so long that sufficient nutrients were added.

Next, the cell suspension enters a stirred-tank reactor (STR) to allow for the addition of dextranase. This step is required to dissolve the microcarriers and release the adherent cells for further cell separation from the suspension. The STR plays an important role in the dissolution of the microcarriers as it allows for the dextranase to homogenize within the solution and minimize

any gradients that may occur. This homogenization is critical to reducing the amount of dextranase needed to disintegrate all microcarriers present in the cell mixture.

Dextranase is an expensive enzyme whose function is to dissolve the dextran beads without harming the cells. The decision to place the dewatering step before the dissolution of the microcarriers stems from this cost. To be effective in dissolving the dextran beads, the amount of dextran added needs to be in a 1:1 ratio with the weight of microcarriers. Dewatering the slurry before its addition was necessary to ensure that the water content of the exit stream leaving the differentiation reactors was limited. Therefore, less dextranase would need to be added to the stirred-tank reactor that followed. The stirred tank helps to homogenize the mixture of cells, microcarriers, and dextranase to fully dissolve the dextran beads and dislodge the cells thereby solving two problems at once.

The final separation step is a rotary drum vacuum dryer with minimized heat to prevent cell death and produce a cake-like consistency of cells. The inclusion of this step is to remove any excess media and process water to ensure a texture of the product that is analogous to slaughter meat. The specification of the rotary drum to be a vacuum is to physically separate any remaining liquid from the cell product without the potential for shearing the cells. This specification also prevents cell death as a result of excessive temperatures common to other types of drum dryers. As a result of this step, the cells are now a dry, dense protein.

Maintaining sterility and cleanliness according to FDA and USDA guidelines, all successive steps in the downstream process are completed in a cold room to prevent spoilage and prepare the product for sale and transport. While the meat can be stored at 0°F for three to four months, the cold room is kept at a temperature of 10°F. This temperature would be sufficient to

finish processing and store the product for 3 days onsite. This decision would allow distributers to determine how to package and market the product according to their own standards.

To prepare the final product, the cells are mixed with hydrogenated vegetable oil to achieve an 80% protein to 20% fat content by weight. To achieve the desired ratio, hydrogenated vegetable oil is fed to the mixer in a 1:4 ratio to cell product following its exit from the rotary drum vacuum dryer. As a result, a total of 7 million pounds of hydrogenated vegetable oil is added to the 28 million pounds of cells produced to achieve a total mass product for sale of 35 million pounds. The mixture is given a ground beef consistency in a twin shell tumbler to ensure that the product is homogenous.

Following the mixer, the aggregate feeds into three extruders operating in parallel to one another. The inclusion of six screw extruders to the process diagram allows for downstream processing while the others are being cleaned between batches.

Finally, the product is stored in two identical storage vessels in which each will contain a three-day supply of product due to the meat product's relatively short shelf life. A three-day storage supply of product is optimal as each batch of cells are produced three days within one another as a result of the batch scheduling system described in Section 6.

f. Clean-In-Place (CIP)

In order to maintain sterility, each process unit that touches the product undergoes a clean-in-place (CIP) cycle following each batch. For all units, the CIP cycle begins with a greywater wash, followed by a 2% NaOH caustic wash, and finally a virgin hot water wash, as advised by the industrial consultants. 100% of the virgin hot water is reused as greywater, while 90% of the caustic wash is reused for the next batch. The remaining 10% of the used caustic wash is neutralized using sulfuric acid before disposal.

The final step is sterilization. For the seed, proliferation, differentiation, and stirred tank reactors, sterilization is accomplished using 15 psig saturated steam, bringing the interior surface to 121°C for 15 minutes. For the remaining downstream process units - the rotary drum dryer, centrifuges, extruders, and storage tanks - sterilization is accomplished using a 0.2% peracetic acid (PAA) solution. For each piece of equipment, the flow rate of cleaning liquid and cycle time for each cleaner was determined using the specifications for commercially available CIP equipment (Alfa Laval, n.d.). A full table for the amounts of steam, hot water, and chemicals required is provided in **Appendix D**.

To mix the cleaning chemicals, NaOH is purchased as dry powder and mixed with pure water at a ratio of 2 grams NaOH to 100 grams of water. PAA is purchased as a 15% solution and diluted with pure water to form a 0.2% solution.

Section 8a: Equipment List and Unit Descriptions

a. Seed Reactors (SR)

The seed train system serves to proliferate a small number of starter cells to a number sufficient to inoculate the 300 m^3 final proliferation reactors. Each seed reactor (SR) is constructed with 304 stainless steel with an attached cooling jacket to counter the enthalpy associated with growing the cells. SR 1 serves to proliferate 9.75 billion cells to 250 billion cells in a 0.013 m^3 bubble column reactor within 7.14 days. SR 2 grows this amount of cells to 6.25 trillion cells in a larger bubble column with a volume of 0.33 m^3 in 7.08 days. Finally, SR 3 achieves a final cell count of 112 trillion cells within 6.4 days in a 2.5 m^3 bubble column reactor. After achieving the desired cell concentration of 60 million cells per mL, the cells move on to the final proliferation reactors.

b. Final Proliferation Reactors (FPR)

The two final proliferation reactors act on an offset parallel schedule, fed by the same set of 3 seed reactors (see Figure 6.1 for a Gantt chart). These reactors are also identical in size and function in that they are both 300 m^3 volume bubble column 304 stainless steel reactors and are equipped with a cooling jacket for the reasons aforementioned. 304 stainless steel was chosen on the advice of the industrial consultants in order to balance bioreactor manufacturing costs with the ability to maintain a sterile cell growth environment. The proliferation reactors are intended to grow the cells to a peak viable cell density (VCD) of 60 million cells per mL and achieve a final cell count of 112 trillion cells. Upon reaching peak VCD, half of the contents of the reactor are sent to a half-size differentiation reactor (DR) before the remaining contents are allowed to

double. This is repeated until 3 half-size DRs have been filled and the FPR is filled a final time before being sent to a full-size DR to complete the upstream process.

c. Differentiation Reactors (DR)

There are two sizes of bubble column reactors used for optimizing the differentiation process. All four of the differentiation reactors are built using 304 stainless steel and a cooling jacket to absorb any heat released by the cells during respiration. DR 1 is the largest bioreactor at 300 m^3 for the final harvest of cells produced in the full proliferation reactors. DR 2, DR 3, and DR 4 are equivalent in size at 150 m^3 and are designed to differentiate only half of the contents of the final proliferation reactors.

d. Compressors (Comp)

Though there are only three compressors responsible for supplying air to all nine reactors at once, the compressors each supply air to 3 reactors depending on the reactor sizes. The three seed reactors receive their gas sparge from the air compressed by Comp 1. As a result, the work required is a mere 1.9 horsepower in comparison to Comp 2 and Comp 3. Comp 2 supplies air to all of the full-sized reactors (FPR 1, FPR 2, and DR 1) resulting in a network requirement of 660 horsepower to deliver the air at the required pressure needed to overcome the pressure within each column. Comp 3 supplies air to the remaining half-sized differentiation reactors requiring a total input of 330 horsepower.

e. Cooling Tower and Chiller

The addition of the cooling tower in the process flow sheet allows us to reuse the cooling water being supplied to the jackets insulating each of the reactors. The inlet temperature of the water entering the cooling jackets is 22.0°C and returns to the tower at 26.5°C to be cooled to

22.0℃ once again. Through the process of evaporative cooling in the tower, the utility water attains the desired temperature and only a fraction of additional cooling is purchased.

Due to the low ambient air temperatures in Illinois year-round, it was determined that a chiller would only need to be in use during the summer months of June, July, and August to achieve the desired temperature in the cooling water exiting the tower.

f. Pumps (P)

Pumps are required to circulate water in the bioreactor cooling jacket network. Six cast-iron centrifugal pumps with a vertical split case and a flow rate range of 50-3500 gallons per minute are used. Pumps P-101, P-105, and P-106 supply warm water at 26.5℃ and a pressure drop of 2 psig to the cooling tower, chiller, and chiller respectively. Pump P-102 supplies cold water to the chiller at 22℃ with a pressure drop of 19 psig. P-103 and P-104 supply cold water at 22℃ to full-sized reactors at a pressure drop of 17 psig and to the half-sized reactors at a pressure drop of 13 psig, respectively.

g. Disc Stack Centrifuges (DSC)

Two identical disc stack centrifuges operate in parallel to one another. When one is in operation, the other is set to be sanitized between batches such that the cell product is produced as continuously as possible. These centrifuges are designed in 304 stainless steel to allow for adequate cleaning in accordance with the clean-in-place procedure. Its 250 m^3 size allows for the separation of the solid and liquid phases of the 225 m^3 entering from the upstream process.

h. Stirred Tank Reactor (STR)

The 304 stainless steel stirred tank reactor operates to evenly distribute the added dextranase to the process and ensure that no dextran microcarriers remain in the final product. Due to the decreased volume of the cell precipitate coming out of the DSC, the STR is only a fraction of the initial volume of cell slurry entering the downstream process at 75 m^3. This reactor also has a cooling jacket to ensure that the cells are provided adequate cooling and prevent cell death and resulting contamination. Clean-in-place and steam sterilization procedures are maintained between batches.

i. Vacuum Rotary Drum Dryer (Rotary Drum)

The vacuum rotary drum dryer serves to dewater the cell slurry and form a cake-like consistency with the cells using a pressure difference. The dryer operates at 36.5℃ and 0 psia to achieve the vacuum effect. The cells will adhere to the drum and excess water will be removed during its rotation to be later mixed with the hydrogenated vegetable oil and extruded. The dryer has an effective area of 4.65 m^2 and is constructed using 304 stainless steel such that cleaning procedures can be performed.

j. Mixer

The mixer has a volume of 5.66 m^3 and is constructed of 304 stainless steel to be cleaned and sterilized between batch scheduling. It propels a final product into the extruders with a formulation of 80% cell concentrate and 20% hydrogenated vegetable oil. The cake-like consistency that exits the rotary drum is now completed with the oil to give a texture akin to traditional ground beef.

k. Screw Extruders (Ext)

The six identical screw extruders are included for completeness, but only three are in operation at a time. This design is such that the three extruders out of operation can be cleaned and sterilized while the others are extruding product to ensure continuous production. They are designed to extrude 4,000 lb/hr and operate in the cold room at 10°F and atmospheric pressure.

l. Storage Tanks

Two storage tanks, equal in size, material, and construction, are used to contain the product in its final form for 3 days in the cold room. As a result, they are operating at 10°F and atmospheric pressure. The identical vessels are 416 m^3 and composed of 304 stainless steel and follow the clean-in-place procedure outlined in Section 7.

Section 8b: Equipment Specification Sheets

a. Seed Reactors

Seed Bioreactor 1		
	Item	Bubble Column Reactor
	PFD Identifier	SR 1
	No. Required	1
Function:	Proliferate starter cells to desired density	
Operation:	Fed-Batch	
Operating Conditions:		
Temperature (°C)		36.5
Pressure (psig)		0
Design Data:		
Height (m)		0.68
Diameter (m)		0.16
Volume (m^3)		0.013
Thickness (mm)		80
Pressure Rating (psig)		30
Gas Sparge Rate (m^3/hr)		0.87
Material of Construction		304 Stainless Steel
Utilities:	Cooling Jacket	

Seed Bioreactor 2		
	Item	Bubble Column Reactor
	PFD Identifier	SR 2
	No. Required	1
Function:	Proliferate cells to desired density	
Operation:	Fed-Batch	
Operating Conditions:		
Temperature (°C)		36.5
Pressure (psig)		0
Design Data:		
Height (m)		1.9
Diameter (m)		0.47
Volume (m^3)		0.33
Thickness (mm)		80
Pressure Rating (psig)		40
Gas Sparge Rate (m^3/hr)		7.4
Material of Construction		304 Stainless Steel
Utilities:	Cooling Jacket	

Seed Bioreactor 3	**Item**	Bubble Column Reactor
	PFD Identifier	SR 3
	No. Required	1
Function:	Proliferate cells to desired density	
Operation:	Fed-Batch	
Operating Conditions:		
Temperature (°C)		36.5
Pressure (psig)		0
Design Data:		
Height (m)		0.93
Diameter (m)		3.7
Volume (m^3)		2.5
Thickness (mm)		80
Pressure Rating (psig)		50
Gas Sparge Rate (m^3/hr)		28
Material of Construction		304 Stainless Steel
Utilities:	Cooling Jacket	

b. Final Proliferation Reactors

Final Proliferation Reactor	**Item**	Bubble Column Reactor
	PFD Identifier	FPR 1/FPR 2
	No. Required	2
Function:	Proliferate cells to desired density	
Operation:	Fed-Batch	
Operating Conditions:		
Temperature (°C)		36.5
Pressure (psig)		0
Design Data:		
Height (m)		18
Diameter (m)		4.6
Volume (m^3)		300
Thickness (mm)		80
Pressure Rating (psig)		140
Gas Sparge Rate (m^3/hr)		686
Material of Construction		304 Stainless Steel
Utilities:	Cooling Jacket	

c. Differentiation Reactors

Differentiation Reactor	**Item**	Bubble Column Reactor
	PFD Identifier	DR 1
	No. Required	1
Function:	Proliferate cells to desired density	
Operation:	Fed-Batch	
Operating Conditions:		
Temperature (°C)		36.5
Pressure (psig)		0
Design Data:		
Height (m)		18
Diameter (m)		4.6
Volume (m^3)		300
Thickness (mm)		80
Pressure Rating (psig)		140
Gas Sparge Rate (m^3/hr)		686
Material of Construction		304 Stainless Steel
Utilities:	Cooling Jacket	

Differentiation Reactor	**Item**	Bubble Column Reactor
	PFD Identifier	DR 2/DR 3/DR 4
	No. Required	3
Function:	Proliferate cells to desired density	
Operation:	Fed-Batch	
Operating Conditions:		
Temperature (°C)		36.5
Pressure (psig)		0
Design Data:		
Height (m)		14
Diameter (m)		3.6
Volume (m^3)		150
Thickness (mm)		80
Pressure Rating (psig)		80
Gas Sparge Rate (m^3/hr)		432
Material of Construction		304 Stainless Steel
Utilities:	Cooling Jacket	

d. Compressors

Compressor	**Item**		Compressor
	PFD Identifier		Comp 1
	No. Required		1
Function:	Compress air to desired pressure		
Operation:	Continuous		
Streams:		S32	S32’
Inlet/Outlet		Inlet	Outlet
Pressure (psig)		0	27.3
Mass Flow (lb/hr)		98	98
Molar Flow (lbmol/hr)		3.4	3.4
Mole Fraction			
Nitrogen		0.79	0.79
Oxygen		0.21	0.21
Volumetric Flow (m^3/hr)		102	36.4
Design Data:			
Net Work (kWh)			1.4
Notes:	Provides gas sparge to SR 1, SR 2, SR 3		

Compressor	**Item**		Compressor
	PFD Identifier		Comp 2
	No. Required		1
Function:	Compress air to desired pressure		
Operation:	Continuous		
Streams:		S39	S39’
Inlet/Outlet		Inlet	Outlet
Pressure (psig)		0	373
Mass Flow (lb/hr)		5,545	5,545
Molar Flow (lbmol/hr)		191.5	191.5
Mole Fraction			
Nitrogen		0.79	0.79
Oxygen		0.21	0.21
Volumetric Flow (m^3/hr)		52,271	2,060
Design Data:			
Net Work (kWh)			491
Notes:	Provides gas sparge to PFR 1, PFR 2, DR 1		

Compressor			
	Item		Compressor
	PFD Identifier		Comp 3
	No. Required		1
Function:	Compress air to desired pressure		
Operation:	Continuous		
Streams:		S46	S46’
Inlet/Outlet		Inlet	Outlet
Pressure (psig)		0	296
Mass Flow (lb/hr)		3,494	3,494
Molar Flow (lbmol/hr)		121	121
Mole Fraction			
Nitrogen		0.79	0.79
Oxygen		0.21	0.21
Volumetric Flow (m^3/hr)		26,116	1,297
Design Data:			
Net Work (kWh)			246
Notes:	Provides gas sparge to DR 2, DR 3, DR 4		

e. Cooling Tower and Chiller

Cooling Tower					
	Item				Cooling Tower
	PFD Identifier				Cooling Tower
	No. Required				1
Function:	Deliver cold water for cooling jacket				
Operation:	Continuous				
Streams:		S75	S54	S76	S55
Inlet/Outlet		Inlet	Inlet	Outlet	Outlet
Temperature (°C)		26.5	25	26.5	22
Pressure (psig)		14.7	14.7	14.7	34.1
Mass flow (lb/sec)		408	411	411	408
Design Data:					
Height (m)					18
Diameter (m)					4.6
Volume (m^3)					300
Thickness (mm)					80
Pressure Rating (psig)					432
Evaporation Rate (lb/sec)					3
Material of Construction					Carbon Steel
Utilities:	Cooling Jacket				
Notes:	Cooling tower receives hot water at 26.5°C from the bioreactors through the chiller at a rate of 408 lb/s, but water evaporated at 3 lb/s, releasing the heat transferred from the overall cell growth				

Chiller					
	Item				Chiller
	PFD Identifier				Chiller
	No. Required				1
Function:	Stabilize cooling water inlet temperature to 22°C in extreme weather				
Operation:	Continuous				
Streams:		S56	S73/S83	S57/S62	S74
Inlet/Outlet		Inlet	Inlet	Outlet	Outlet
Temperature (°C)		22	26.5	22	22
Pressure (psig)		14.7	14.7	17.1	17.1
Mass flow (lb/sec)		408	408	408	408
Design Data:					
Cooling Capacity (tons)					3,490
Chiller Size (tons)					4,190
Net Work (kWh)					12,200
Material of Construction					Carbon Steel
Utilities:	Cooling Jacket				
Notes:	Additional water is added to account for the evaporation rate during the summer months				

f. Pumps

Pump			
	Item		Pump
	PFD Identifier		P-101
	No. Required		1
Function:	Deliver warm water from the chiller to the cooling tower		
Operation:	Continuous		
Operating Conditions:			
Temperature (°C)			26.5
Pressure drop (psig)			2.4
Pump head (m)			1.7
Efficiency			0.9
Streams:		S74	S75
Inlet/Outlet		Inlet	Outlet
Pressure (psig)		17.1	14.7
Capacity (lb/sec)		408	408
Design Data:			
Net Work (kWh)			0.03

Pump

	Item		Pump
	PFD Identifier		P-103
	No. Required		1
Function:	Deliver cold water to full-sized bioreactors		
Operation:	Continuous		
Operating Conditions:			
Temperature (°C)			22
Pressure drop (psig)			17.1
Pump head (m)			25.8
Efficiency			0.9
Streams:		S57	S58-61
Inlet/Outlet		Inlet	Outlet
Pressure (psig)		17.1	34.1
Capacity (lb/sec)		274	274
Design Data:			
Net Work (kWh)			0.35
Notes:	Supplies cold water at 22°C to FPR 1, FPR 2, DR 1, and STR		

Pump

	Item		Pump
	PFD Identifier		P-102
	No. Required		1
Function:	Deliver cold water from cooling tower to chiller		
Operation:	Continuous		
Operating Conditions:			
Temperature (°C)			22
Pressure drop (psig)			19.5
Pump head (m)			13.7
Efficiency			0.9
Streams:		S55	S56
Inlet/Outlet		Inlet	Outlet
Pressure (psig)		34.1	14.7
Capacity (lb/sec)		408	408
Design Data:			
Net Work (kWh)			0.28

Pump

	Item	Pump
	PFD Identifier	P-104
	No. Required	1
Function:	Deliver cold water to seed train and half-sized bioreactors	
Operation:	Continuous	
Operating Conditions:		
Temperature (°C)		22
Pressure drop (psig)		13.1
Pump head (m)		20.1
Efficiency		0.9
Streams:	S62	S63-68
Inlet/Outlet	Inlet	Outlet
Pressure (psig)	17.1	30.1
Mass flow (lb/sec)	133	133
Design Data:		
Net Work (kWh)		0.13
Notes:	Supplies cold water at 22°C from the chiller to seed train, DR 2, DR 3, and DR 4	

Pump

	Item	Pump
	PFD Identifier	P-105
	No. Required	1
Function:	Deliver hot water from full-sized bioreactors	
Operation:	Continuous	
Operating Conditions:		
Temperature (°C)		26.5
Pressure drop (psig)		2.4
Pump head (m)		15.4
Efficiency		0.9
Streams:	S69-72	S73
Inlet/Outlet	Inlet	Outlet
Pressure (psig)	17.1	14.7
Capacity (lb/sec)	272	272
Design Data:		
Net Work (kWh)		0.21
Notes:	Supplies hot water at 26.5°C from FPR 1, FPR 2, DR 1, and STR to the chiller	

Pump

	Item		Pump
	PFD Identifier		P-106
	No. Required		1
Function:	Deliver hot water from half-sized bioreactor and seed train		
Operation:	Continuous		
Operating Conditions:			
Temperature (°C)			26.5
Pressure drop (psig)			2.4
Pump head (m)			12.6
Efficiency			0.9
Streams:		S77-82	S83
Inlet/Outlet		Inlet	Outlet
Pressure (psig)		17.1	14.7
Capacity (lb/sec)		132	132
Design Data:			
Net Work (kWh)			0.08
Notes:	Supplies hot water at 26.5°C from DR 2, DR 3, DR 4 and seed trains to the chiller		

g. Disc Stack Centrifuges

Disc Stack Centrifuge	**Item**			Disc Stack Centrifuge
	PFD Identifier			DSC 1/DSC 2
	No. Required			2
Function:	Remove the majority of growth media from cell slurry			
Operation:	Continuous			
Streams:		S30/S30'	S101/S102	S84/S85
Inlet/Outlet		Inlet	Outlet	Outlet
Temperature (°C)		36.5	36.5	36.5
Pressure (psig)		0	0	0
Mass Flow (lb/hr)		498,515	371,250	127,265
Volumetric Flow (m^3/hr)		225	169	56
Design Data:				
Volume (m^3)				250
Net Work (kWh)				67
Material of Construction				304 Stainless Steel
Notes:	Supernatant is recycled and treated while 10% is purged due to [NH_3]			

h. Stirred Tank Reactor

Stirred Tank Reactor	**Item**			Stirred Tank
	PFD Identifier			Reactor
	No. Required			STR
				1
Function:	Distribute Dextranase within cell slurry to dissolve Cytodex I microcarriers			
Operation:	Batch			
Streams:		S99’	S84/S85	S86
Inlet/Outlet		Inlet	Inlet	Outlet
Temperature (°C)		36.5	36.5	36.5
Pressure (psig)		0	0	0
Design Data:				
Height (m)				2.9
Diameter (m)				11.5
Volume (m^3)				75
Thickness (mm)				80
Pressure Rating (psig)				80
Net Work (kWh)				1.08
Material of Construction				304 Stainless Steel
Utilities:	Cooling Jacket			

i. Vacuum Rotary Drum

Vacuum Rotary Drum Dryer	**Item**	Vacuum Rotary Drum Dryer		
	PFD Identifier	Rotary Drum		
	No. Required	1		
Function:	Dewater cell slurry to form a cake-like consistency using a pressure difference			
Operation:	Batch			
Streams:		S86	S87	S103
Inlet/Outlet		Inlet	Outlet	Outlet
Temperature (°C)		36.5	36.5	36.5
Pressure (psig)		0	0	0
Design Data:				
Effective Area (m^2)				4.65
Net Work (kWh)				5.22
Pressure (psig)				-14.7
Material of Construction				304 Stainless Steel
Notes:	Purpose of using a pressure difference instead of heat is to prevent cell death			

j. Mixer

Mixer				
	Item			Twin Shell Tumbler
	PFD Identifier			Mixer
	No. Required			1
Function:	Propels final product into storage vessels with a ground meat consistency Continuous			
Operation:				
Streams:		S87'	S100	S88-S103
Inlet/Outlet		Inlet	Inlet	Outlet
Temperature (°F)		10	10	10
Pressure (psig)		0	0	0
Mass flow (lb/hr)		8,545	2,137	10,682
Mass Fraction				
Dehydrated Cell Mixture		1	0	0.80
Hydrogenated Vegetable Oil		0	1	0.20
Volumetric Flow (m^3/hr)		3.76	1.06	
Design Data:				
Volume (m^3)				5.66
Net Work (kWh)				18
Material of Construction				304 Stainless Steel
Notes:	Steam-sterilized and cleaned-in-place between batches			

k. Screw Extruders

Screw Extruder			
	Item		Screw Extruder
	PFD Identifier		Ext1/Ext2/Ext3/Ext4/Ext5/Ext6
	No. Required		6
Function:	Propels final product into storage vessels with a ground meat consistency		
Operation:	Continuous		
Streams:		S88-93	S88'-S93'
Inlet/Outlet		Inlet	Outlet
Temperature (°F)		10	10
Pressure (psig)		0	0
Design Data:			
Flow Rate (lb/hr)			4,000
Net Work (kWh)			110
Material of Construction			304 Stainless Steel
Notes:	Three operate while the other set is cleaned such that product can continuously be extruded		

l. Storage Tanks

Storage Tank		
	Item	Tank
	PFD Identifier	Storage Tank 1/2
	No. Required	2
Function:	Contain product for transport	
Operating Conditions:		
Temperature (°F)		10
Pressure (psig)		0
Design Data:		
Height (m)		8.3
Diameter (m)		8
Volume (m^3)		416
Pressure Rating (psig)		0-30
Material of Construction		304 Stainless Steel
Notes:	Stores finished product for 3 days in cold room while awaiting shipment	

Section 9: Energy Balance and Utility Requirement

a. Cooling Water and Chilling

To maintain the bioreactor process temperature at 36.5℃, chilled water at 22℃ is circulated through the cooling jackets around each bioreactor at varying flow rates depending on the reactor size. The temperature difference between the jacket and the reactor content drives the rate of heat transfer, while the flow rate controls the amount of heat that is transferred.

The specific heat of bioreaction for CHO cells was used to model the enthalpy of cell growth for bovine muscle satellite cells (BMSc). According to research on CHO cell growth, a comparison between consumption of glucose and glutamine with respect to oxygen uptake rate provides a heat flux range of 20 to 25 picoWatts per cell (Guan & Kemp, 1999). A smaller heat flux resulted in better metabolic activities which led to the selection of 20 picoWatts/cell as the specific heat of bioreaction of BMSc. Given the peak viable cell density (VCD) and the volume of each bioreactor, the total number of cells was obtained. The product of the total cell population and the specific heat of reaction provided the amount of heat generated from each bioreactor at maximum VCD. The cooling jacket was then specified to remove the heat generated to maintain a constant process temperature.

The overall heat transfer coefficient was approximated to be about 227 watts/m^2/K based on the typical range of 150-500 watts/m^2/K for liquid-free convection with steam jackets around a stirred tank. While bubble column reactors have free convection of liquid, their main source of agitation comes from air bubbles passing through the column instead of a stirrer. So, the chosen heat transfer coefficient was smaller than 500 watts/m^2/K. In addition, an aspect ratio of 3:1 between the process height and diameter was used to size each bioreactor and determine the surface area that would be in contact with the cooling jacket. A minimum difference of 10°C was

maintained between the process temperature (T_P) and the cooling jacket outlet temperature (T_{co}) to ensure adequate heat transfer. Using this constraint, the coolant flow rate was interactively changed until the coolant outlet temperature converged to the desired value. The calculated values for cooling jacket size and energy requirements are shown in **Table 9.1**.

Table 9.1. Bioreactor cooling jacket fluid requirements

	Seed Reaction 1	Seed Reaction 2	Seed Reaction 3	Half Size BCR (3x)	Full Size BCR (4x)
Viable Cell Density, VCD [millions cells/mL]	25	25	60	60	60
Specific heat of bioreaction [picoWatts/cell]	20	20	20	20	20
Total cells	2.50E+11	6.30E+12	1.10E+14	6.80E+15	1.40E+16
Heat generated, $q_{process}$ [Watts]	5	125	2,244	135,000	270,000
Reactor diameter, d [m]	0.16	0.47	0.93	3.63	4.57
Bioreactor process height, h_r [m]	0.49	1.42	2.75	10.9	13.7
Bioreactor aspect ratio	3	3	3	3	3
Bioreactor process Volume, V_P [m^3]	0.01	0.25	1.87	112.5	225
Cooling jacket area, A_{CJ} [m^2]	0.27	2.27	8.72	134	213
Heat transfer coefficient, $U_{Overall}$ [Watts/m^2/K]	227	227	227	227	227
Coolant inlet temperature , T_{ci} [°C]	22	22	22	22	22
Coolant outlet temperature , T_{co} [°C]	26.5	26.5	26.5	26.5	26.5
Process temperature, T_p [°C]	36.5	36.5	36.5	36.5	36.5
Log mean temperature difference, ΔT_{lm} [°C]	-12.1	-12.1	-12.1	-12.1	-12.1
Coolant flow rate, Q_{cj} [L/min]	2.3	19.9	76.4	1176	1868

In addition to the coolant flow rate, an approximate power requirement was also obtained using an estimate for an industrial cooling tower, operating in crossflow with propeller fans (SPX Cooling, 2016). The cooling tower from SPX Cooling Technologies requires 20,000 kWh propeller fan energy to cool 400 tons of water from 35°C to 30°C if constantly operated at full capacity for 1200 hours. The cooling tower for this process can store 1.3 million pounds or 650

US tons of water and need to cool it from 26.5℃ to 22℃. Although the amount of water varies for both tower designs, the change in temperature is approximately the same which allows for a rough estimate of the energy requirement for this cooling tower. The energy balance in **Table 9.5** uses the energy consumption of 20,000 kW for the cooling tower.

For the chiller energy requirement, an outline provided by the Cary Company was used to estimate the ideal chiller size and cooling capacity (Cary Company, n.d.). The chiller is assumed to operate primarily in summer for about 122 days when the atmospheric temperature of Illinois is higher than 25℃. Over the last decade, the maximum temperature in Illinois ranged from 34 to 41℃ (Current Results, 2021) so an average of 38℃ was used for chiller energy calculation. Based on the outline, the chiller would need 12,200 kWh to chill water from 38℃ to 22℃ at a rate of 408 pounds per second as included in **Table 9.5.**

For the scope of this project, it was encouraged to use an estimated energy requirement for the cooling tower and the chiller rather than designing all the units. However, note that the amount of cooling water, size of the unit, material used, etc. would significantly influence the power requirement to run.. Therefore, a rigorous calculation of energy consumption on the cooling tower and chiller based on fans, motors, operating capacity, and operation time should be considered in the future.

b. Pump Requirements

Table 9.2 includes the energy consumption required for each pump to supply cooling water to the respective bioreactors. The cast-iron centrifugal pumps are assumed to operate at a 90% efficiency with varying capacity or mass flowrate and pressure drop. The pump head was calculated using equation 14.2 from Seider et al. and the power input was determined using a shaft power calculation formula (Alluri, 2018).

The cooling tower height was assumed to be similar to the full-sized bubble column reactors, resulting in a 34.1 psig hydraulic pressure of water leaving the tower. P-102 brings that water pressure down to 14.7 psig before it enters the chiller. P104 supplies water at a lower pressure of 30.1 psig to the half-sized bioreactors due to their smaller process height. An approximated distance of 300 ft was used for the pipe network connecting the chiller and the bubble column reactors to the cooling tower. The pressure drop across the 300 ft horizontal pipe was estimated for a circular glavanized steel pipe with 6 inch diameter and 0.004 inch surface roughness. The estimated pressure drop of 2.4 psig gave an inlet pressure of 17.1 psig for the remaining pumps except P-102. The hot water from the bioreactors is collected at the top of the process height from full and half-sized bubble column reactors and sent to P-105 and P-106, respectively, at 17.1 psig. The pressure is then reduced to 14.7 psig before entering the chiller. The power input was determined to be the product of flowrate, pump head, water density, gravitational constant divided by the percent efficiency.

Table 9.2. Cooling water pump energy requirements

	Temp (°C)	Static head, dh [m]	Inlet pressure [psig]	Outlet pressure [psig]	Pressure head, H [m]	Mass flowrate [lb/s]	Pump Power Input [Watts]
P101 (chiller to cooler)	26.5	0.0	17.1	14.7	1.7	408	33
P102 (cooler to chiller)	22.0	0.0	34.1	14.7	13.7	408	276
P103 (chiller to all full - cold)	22.0	13.7	17.1	34.1	25.8	274	349
P104 (chiller to all half - cold)	22.0	10.9	17.1	30.1	20.1	133	132
P105 (all full to chiller - hot)	26.5	13.7	17.1	14.7	15.4	272	206
P106 (all half to chiller - hot)	26.5	10.9	17.1	14.7	12.5	132	82

c. Compressor Requirements

The compressors supply sterile air filtered from the plant's external environment to each bioreactor for aerobic cell growth. The energy requirement for each compressor was based on the

volumetric flow rate of air and the outlet pressure requirement of air supply to reach the top of the bioreactor height. The second compressor (Comp 2 on **Figure 6.1**) requires about 491 kWh to supply air to the full-sided bubble column reactors while comp 3 needs 246 kWh. Note that the energy requirements for air supply to seed reactor 1 and 2 were significantly low due to small sizes and were not included in the overall energy balance in **Table 9.5.**

Table 9.3. Air compressor energy requirements

	Compressor 1			Compressor 2	Compressor 3
	Seed Reactor 1	**Seed Reactor 2**	**Seed Reactor 3**	**FPR 1, FPR 2, DR 1**	**DR 2, DR 3, DR 4**
Sparge gas rate [L/min]	14	123	469	11434	7203
Total mass (cell & liquid) [Kg]	10.1	250	15,895	1,912,500	956,250
Total reactor volume [m^3]	0.01	0.33	2.49	300	150
Total density in bioreactor [Kg/m^3]	750	750	6,390	6,360	6,380
Gravitational constant [m/s^2]	9.81	9.81	9.81	9.81	9.81
Bioreactor process height, h_p [m]	0.49	1.42	2.78	13.7	10.9
P2 - P1 [Pa]	3,590	10,400	174,000	858,000	681,000
Outlet pressure, P2 [Pa]	105,000	112,000	275,000	958,000	782,000
Work required [kWh]	0	0.02	1.42	491	246

d. Cold Room Refrigeration

In order to prevent product spoilage, all process steps downstream of the vacuum rotary drum are carried out in a cold room. A cold room temperature of 10°F (-12°C) was selected to keep the product cold without rendering it too hard to work with. Assuming a 15 x 10 x 10 meter (50x30x30 ft) cold room insulated using polystyrene, an online calculator from the industrial refrigeration company Alfa LU-VE was used to calculate the power requirements to maintain the desired cold room temperature 24 hours per day (Alfa LU-VE, n.d.). Approximately 48 kW are required for cooling, giving a specific cooling capacity of 32 W/m^3 (see **Table 9.4** for a full

summary of cooling requirements). A full summary of the cold room specifications is listed in **Appendix E**.

Table 9.4. Cold room energy requirements

Cooling Requirement	kW
Transmission Losses	12.6
Ventilation Losses	5.9
Other Heat Sources	3.1
Cooling Down	26.5
Total Required Cooling Capacity	**48.1**

e. Overall Energy Balance Summary

Table 9.5 summarizes the overall energy consumption for the process along with the list of units and their quantity. The downstream process units consist of a disc stack centrifuge, stirred tank reactor, vacuum rotary drum dryer, mixer, and screw extruders each with their own energy requirements. The disc stack centrifuge energy requirement was based off of Alfa Laval's CH 900 industrial disc stack separation device at 67 kWh ("Alfa Laval CH 900," n.d.). The STR's energy usage of 1.08 kWh was modeled from an online source utilizing the vessel size and speed of agitation. Similarly, the rotary drum and mixer power requirements were formulated upon the effective area and volume to be 5.22 kWh and 18 kWh, respectively. Lastly, the screw extruders' energy consumption proved to be 110 kWh each following a commercially available product model (IDAH, n.d.).

Table 9.5. Overall energy balance

Energy Balance			
	Energy (kWh)		Number of Units
Cooling Tower	-	20,000	1
Chiller	-	12,200	1
	-	-	-
Pump (P101)	0.03	-	1
Pump (P102)	0.28	-	1
Pump (P103)	0.35	-	1
Pump (P104)	0.13	-	1
Pump (P105)	0.21	-	1
Pump (P106)	0.08	-	1
Total energy required for pumps		**1.08**	-
Compressor 1	1.42	-	1
Compressor 2	491	-	1
Compressor 3	246	-	1
Total energy required for compressors		**738**	-
Disc Stack Centrifuges	-	67	2
STR	-	1.08	1
Vacuum Rotary Drum	-	5.22	1
Mixer	-	0.75	1
Screw Extruder	-	110	3
Cold Room	-	2,850	1
Total energy required for the process		**36,259**	-
Total energy added to the process		**36,259**	-

f. Wastewater Treatment

One of the biggest concerns in this process is the amount of water required - the cell medium is generally 5% cells by volume, which with the amount of cells produced per year creates a yearly water demand of millions of pounds. Therefore, it is highly desirable to be able to treat and recycle process water such that 90% can be reused after each batch, and the remaining 10% purged. Towards that end, it was decided to allocate a wastewater treatment plant

onsite so that after each batch, the supernatant from the disk stack centrifuges can be sent to treatment and recycled.

After separating the used growth medium from the harvested cells, the biggest concern for wastewater treatment is ammonia removal. Ammonia is a byproduct of cell growth that is cytotoxic at high concentration; purging part of the recycle stream prevents its accumulation in the system. Ammonia is also highly water soluble, requiring involved separation processes such as reverse osmosis or treatment in bioreactors with nitrogen-removing microbes. Therefore, the allocated wastewater treatment plant is specified to employ primary (physical screening), secondary (biological treatment), and tertiary (specialized treatments like reverse osmosis) treatments (Seider et al., 2017).

Section 10: Economic Analysis

a. General Information on the Process Economics

An economic analysis of the cultured ground beef manufacturing process was conducted using the Profitability Analysis 4.0 spreadsheet provided in section 17.8 of Seider et al., created by Brian K. Downey (2008). The spreadsheet provides estimates and rigorous profitability measures based on process specifications. In the following sections, the total capital investment, production cost, cash flow, profitability, and specifications for this process will be discussed.

The cellular agriculture process located in Illinois will yield 35 million lbs of ground beef from a manufacturing facility that would operate for 300 days a year as shown in **Table 10.1**. The product will be priced at $100 per pound of ground beef to generate profit and continue the manufacturing process. Although slaughtered ground beef price is significantly lower at $5 per pound (US Bureau of Labor Statistics, 2022), it is reasonably comparative to the price of current cultured meat products. According to a 2021 techno-economic analysis by the Good Food Institute (GFI), the current production price of cultured meat ranges from $70 to $10,000 per pound (Vergeer et al., 2021; Fassler, 2021), which is projected to reach $2.50 per pound by 2030 with large-scale production. Therefore, a set price of $100 per pound is a good starting price for the cultured ground beef. However, the ultimate goal for this process would be to lower the production cost by optimization and to provide a selling price that is competitive with slaughtered ground beef.

Table 10.1: General economic information: overall process

General Information	
Process Title:	**Cellular Agriculture**
Product:	**Ground Beef**
Plant Site Location:	**Illinois**
Site Factor:	**1.15**
Operating Hours per Year:	**7200**
Operating Days Per Year:	**300**
Operating Factor:	**0.8219**
Product Information	
This Process will Yield	
	4,861 lb of Ground Beef per hour
	116,667 lb of Ground Beef per day
	35,000,000 lb of Ground Beef per year
Price	**$100.00 /lb**

Table 10.2 documents an estimated chronological cycle of this process. Upon completion of plant design by 2023 and construction by 2024, production would begin in 2025 and continue for the next 14 years. A large portion of the total permanent investment (60%) would be distributed for construction and the remaining amount would be distributed throughout the first three years of production. (Further discussion of the total permanent investment is included in Section 11.) The manufacturing plant is set to start at 80% of the design capacity by the first year of production and take 2 years to reach 100% production capacity. A 5-year Modified Accelerated Cost Recovery System (MACRS) is selected for this process along with a 4% inflation rate for the product price.

Table 10.2. Manufacturing process chronology

Chronology					
Year	Action	Distribution of Permanent Investment	Production Capacity	Depreciation 5 year MACRS	Product Price
2023	Design		0.0%		
2024	Construction	60%	0.0%		
2025	Production	20%	80.0%	20.00%	$100.00
2026	Production	15%	90.0%	32.00%	$103.71
2027	Production	5%	100.0%	19.20%	$107.56
2028	Production		100.0%	11.52%	$111.55
2029	Production		100.0%	11.52%	$115.69
2030	Production		100.0%	5.76%	$119.98
2031	Production		100.0%		$124.43
2032	Production		100.0%		$129.05
2033	Production		100.0%		$133.83
2034	Production		100.0%		$138.80
2035	Production		100.0%		$143.95
2036	Production		100.0%		$149.29
2037	Production		100.0%		$154.83
2038	Production		100.0%		$160.57
2039	Production		100.0%		$166.53

b. Equipment Cost Summary

Table 10.3 lists the total cost of each piece of equipment that needs to be fabricated or purchased. It also includes the quantity that is associated with the price and the estimated bare module factor (F_{BM}) for each piece of equipment. The F_{BM} was obtained from table 16.11 in Seider, et al. for certain equipment. However, for the storage tank, F_{BM} was not available in the table so an estimate of 1.1 for a spherical, fixed roof storage tank was used based on published values (Higgins et al., 2017). In addition, commercially available bubble column reactor sizes ranged mainly from 0.01 m^3 to 20 m^3 which was not sufficient to reach the production goal. The manufacturing plant requires 0.01 m^3 to 300 m^3 bioreactors which would occasionally undergo high-pressure steam sterilization. Therefore, the bubble column reactors were considered as onsite fabricated pressure vessels for cost estimation.

Table 10.3: Equipment cost estimates

Equipment Name	Type	Quantity	Purchase Cost	Bare Module Factor	Bare Module Cost
Bubble Column Reactors	Fabricated Equipment	7	$6,704,000	4.2	$27,887,000
Seed Reactors	Fabricated Equipment	3	$82,000	4.2	$343,000
Rotary Drum Vacuum Dryer	Fabricated Equipment	1	$207,000	2.3	$481,000
Centrifugal Pumps	Process Machinery	6	$104,000	3.3	$343,200
Compressors	Process Machinery	3	$1,014,000	2.1	$2,129,000
Mixer	Process Machinery	1	$78,000	2.0	$156,000
Storage Tank	Storage	2	$234,000	1.1	$258,000
HEPA Filter	Other Equipment	3	$3,585	2.32	$8,317
Submicron Filter	Other Equipment	3	$720	2.32	$1,670
Extruder	Other Equipment	6	$1,194,000	1.4	$1,383,000
Disc Stack Centrifuge	Other Equipment	2	$1,975,000	2.0	$4,010,000
Cold Room	Other Equipment	1	$33,300	3.21	$107,000
Wastewater Treatment	Other Equipment	1	$1,115,000	3.21	$3,579,150
Total					**$40,686,338**

The four main types of equipment that drove up the investment cost are the bubble column reactors, compressors, and disc stack centrifuges. The purchase cost for each full-size (300 m^3) bubble column reactor was approximately $1,200,000 and a half-size column was about $600,000. With an F_{BM} of 4.2, the total bare module cost of 4 full-size and 3 half-size columns contribute $28 million to the investment. The cost may be reduced by decreasing the column size or the number of columns required for this process. However, this would only be possible through modification of the cell line and/or other cell growth conditions, which is discussed in Section 14.

The bare module cost of the cold room was estimated by surveying the manufacturing cost of industrial cold room storage. A typical above-ground cold room storage unit costs $33,300 to build, including HVAC system, insulation, power supply and backup (Allied Buildings, n.d.). This estimated fabrication cost along with the bare module factor of 3.21 from the profitability analysis spreadsheet provided a total bare module cost of $107,000 to build a cold room onsite. In order to maintain the product at 10°F, refrigeration costs come out to $2 per ton ($0.001/lb) of material stored as listed in **Table 12.4** below (Seider et al., 2017).

The cost associated with the wastewater treatment was estimated using a purchase cost equation for a tertiary wastewater treatment plant (Seider et al., 2017). (See Section 9 for a more complete discussion on wastewater treatment requirements.) In order to process 25 gallons per minute as the process requires, the bare module cost of the waste treatment plant was calculated at approximately $3.6 million.

Section 11: Total Permanent Investigation or Total Fixed Capital

The individual equipment cost listed in **Table 10.2** is used as input in the profitability spreadsheet to obtain the total bare module cost of about $40,700,000. Each piece of equipment was categorized based on the description provided in Seider, et al. The bubble column reactors, the seed reactors, and the rotary drum were characterized as "fabricated equipment" due to their customized size requirement for large-scale production. The fabricated machinery requires process machinery such as pumps, compressors, and mixers which could be supplied from vendors given the standard size. The storage tanks were categorized as storage and the remaining pieces of equipment were lumped together as "other equipment." Although a strong effort was made to determine the proper equipment size and quotes from vendors, the equipment categorization is still subject to change based on new information. **Table 11.1** lists the total cost of each equipment type along with the total bare module cost.

Table 11.1. Total bare module cost summary

Total Bare Module Costs:			
Fabricated Equipment	$	28,711,000	
Process Machinery	$	2,628,923	
Spares	$	-	
Storage	$	258,000	
Other Equipment	$	9,089,138	
Catalysts	$	-	
Computers, Software, Etc.	$	-	
Total Bare Module Costs:			$ 40,687,061

Once the total bare module cost was calculated, it could be used to obtain the total permanent investment for the process using the factors listed in **Table 11.2**. The percentage of each factor specified in the table below was obtained from the profitability analysis spreadsheet and Seider et al.

Table 11.2. Assumptions used for total permanent investment calculation

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

After allocating 5% of the total bare module cost to site preparation and 5% to service facilities, the direct permanent investment (DPI) for this process was calculated to be about $44,800,000 as shown in **Table 11.3**. 18% of the DPI was allocated to cover contingencies and contractor fees and the total depreciable capital (TDC) was found to be $52,800,000. The TDC is crucial as it accounts for a portion of maintenance costs due to equipment usage, tax, and insurance. A 2% land cost and a 10% plant start-up cost of TDC are then added to give an unadjusted total permanent investment (TPI) of $59,100,000. Since the plant is located in Illinois, an investment site factor of 1.15 (Seider et al., 2017) was used to adjust the TPI and get a final TPI of $68,000,000 for the manufacturing process.

Table 11.3. Investment summary

Total Bare Module Costs:			
Fabricated Equipment	$	28,711,000	
Process Machinery	$	2,628,923	
Spares	$	-	
Storage	$	258,000	
Other Equipment	$	9,089,138	
Catalysts	$	-	
Computers, Software, Etc.	$	-	
Total Bare Module Costs:			**$ 40,687,061**
Direct Permanent Investment			
Cost of Site Preparations:	$	2,034,353	
Cost of Service Facilities:	$	2,034,353	
Allocated Costs for utility plants and related facilities:	$	-	
Direct Permanent Investment			**$ 44,755,767**
Total Depreciable Capital			
Cost of Contingencies & Contractor Fees	$	8,056,038	
Total Depreciable Capital			**$ 52,811,805**
Total Permanent Investment			
Cost of Land:	$	1,056,236	
Cost of Royalties:	$	-	
Cost of Plant Start-Up:	$	5,281,180	
Total Permanent Investment - Unadjusted			$ 59,149,221
Site Factor			1.15
Total Permanent Investment			**$ 68,021,604**

Section 12: Cost of Manufacturing

a. Raw Material Cost

The cost of each raw material required to run the manufacturing process is included in **Table 12.1** below. It also includes the required ratio of raw material to ground beef on a pound per pound basis. The product of the ratio and the cost of each raw material was calculated and

totaled over all the material to obtain a total weighted raw material cost of $68 per pound of ground beef.

Table 12.1. Raw material costs

Raw Materials						
	Raw Material:	**Unit:**	**Required Ratio:**	**Cost of Raw Material:**		
1	Bovine muscle satellite cell	lb	0.80 lb per lb of Ground Beef	$	0.09	per lb
2	Corn grain hydrolysate	lb	2.1 lb per lb of Ground Beef	$	9.7	per lb
3	Soybean hydrolysate	lb	3.1 lb per lb of Ground Beef	$	2.3	per lb
4	Microcarriers	lb	0.25 lb per lb of Ground Beef	$	129	per lb
5	FBS	lb	0.000030 lb per lb of Ground Beef	$	435	per lb
6	Linoleic acid	lb	0.0000013 lb per lb of Ground Beef	$	0.001	per lb
7	Insulin	lb	0.00000061 lb per lb of Ground Beef	$	947,000	per lb
8	Dexamethasone	lb	0.00000051 lb per lb of Ground Beef	$	213,000	per lb
9	Dextranase	lb	0.25 lb per lb of Ground Beef	$	23	per lb
10	Hydrogenated vegetable oil	lb	0.20 lb per lb of Ground Beef	$	8.2	per lb
	Total Weighted Average:				**$67.8**	**per lb of Ground Beef**

b. Byproduct Cost

Carbon dioxide is generated from aerobic cell growth in each bioreactor and is considered the only byproduct of this process, as shown in **Table 12.2**. Based on the current design, generated CO_2 would be vented out of the system into the atmosphere. In the future, this CO_2 could potentially be captured in order to monetize the byproduct and reduce carbon footprint.

Table 12.2. Byproduct costs

Byproducts						
	Byproduct:	**Unit:**	**Ratio to Product**	**Byproduct Selling Price**		
1	Carbon dioxide	lb	0.30 lb per lb of Ground Beef	$	-	per lb
	Total Weighted Average:			**$**	**-**	**per lb of Ground Beef**

c. Utility Cost

The cost along with the required ratio of each utility to ground beef is summarized in **Table 12.3**. The high-pressure steam, hot water, NaOH, H_2SO_4, and PAA are required for the

clean-in-place (CIP) process to sterilize each unit that comes into direct contact with the product. The cost of water in Illinois (Gregory et al., 2017) was used to estimate the cost per pound of water supply. An overall energy requirement from **Table 9.4** was used to calculate the required ratio of electricity per pound of ground beef and the estimated cost of electricity in Illinois was used (Electricity Local, n.d.). The utility cost per pound for the wastewater treatment and the refrigeration at 10°F were obtained from Table 17.1 in Seider et al. Note that hot water and refrigeration utility costs are shown as zero but the exact values are $0.0002 and $0.001 per lb respectively.

Table 12.3. Utility costs

Utilities					
	Utility:	Unit:	Required Ratio	Utility Cost	
1	High Pressure Steam	lb	0.01 lb per lb of Ground Beef	$ 0.01	per lb
2	Hot water	lb	0.09 lb per lb of Ground Beef	$ 0.00	per lb
3	Process Water	lb	0.18 lb per lb of Ground Beef	$ 0.09	per lb
4	Cooling Water	lb	0.84 lb per lb of Ground Beef	$ 0.09	per lb
5	Electricity	kWh	6 kWh per lb of Ground Beef	$ 0.06	per kWh
6	Refrigeration	lb per day	1 lb per day per lb of Ground Beef	$ 0.00	per lb per day
7	Waste water treatment	lb	2.4 lb per lb of Ground Beef	$ 0.2	per lb
8	NaOH (dry powder)	lb	0.0001 lb per lb of Ground Beef	$ 2.3	per lb
9	96% H2SO4	lb	0.0000002 lb per lb of Ground Beef	$ 16.8	per lb
10	15% PAA	lb	0.0004 lb per lb of Ground Beef	$ 2.3	per lb
	Total Weighted Average:				$1 per lb of Ground Beef

d. Fixed Cost Summary

Besides the raw material and utility costs, there is a fixed cost to run the manufacturing facility. The fixed cost depends on several factors such as cost of labor, maintenance, operation overhead, property tax, depletion, etc. **Table 12.4** includes all the factors and the assumptions – obtained from the profitability spreadsheet – used to calculate the total fixed cost.

Table 12.4. Factors in total fixed cost calculation

Fixed Costs		
Operations		
Operators per Shift:	1 (assuming 5 shifts)	
Direct Wages and Benefits:	$40 /operator hour	
Direct Salaries and Benefits:	15% of Direct Wages and Benefits	
Operating Supplies and Services:	6% of Direct Wages and Benefits	
Technical Assistance to Manufacturing:	$60,000.00 per year, for each Operator per Shift	
Control Laboratory:	$65,000.00 per year, for each Operator per Shift	
Maintenance		
Wages and Benefits:	4.50% of Total Depreciable Capital	
Salaries and Benefits:	25.00% of Maintenance Wages and Benefits	
Materials and Services:	100.00% of Maintenance Wages and Benefits	
Maintenance Overhead:	5.00% 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:	2.00% of Total Depreciable Capital	
Straight Line Depreciation		
Direct Plant:	8.00% of Total Depreciable Capital, less	# times the Allocated Costs for Utility Plants and Related Facilities
Allocated PI	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	

Table 12.5 includes the summary of the cost associated with each factor and yields a total fixed cost of $8,440,000 for this process.

Table 12.5. Fixed cost summary

Operations			
	Direct Wages and Benefits	$	416,000
	Direct Salaries and Benefits	$	62,400
	Operating Supplies and Services	$	24,960
	Technical Assistance to Manufacturing	$	300,000
	Control Laboratory	$	325,000
	Total Operations	**$**	**1,128,360**
Maintenance			
	Wages and Benefits	$	2,376,531
	Salaries and Benefits	$	594,133
	Materials and Services	$	2,376,531
	Maintenance Overhead	$	118,827
	Total Maintenance	**$**	**5,466,022**
Operating Overhead			
	General Plant Overhead:	$	244,884
	Mechanical Department Services:	$	82,778
	Employee Relations Department:	$	203,495
	Business Services:	$	255,231
	Total Operating Overhead	**$**	**786,387**
Property Taxes and Insurance			
	Property Taxes and Insurance:	$	1,056,236
Other Annual Expenses			
	Rental Fees (Office and Laboratory Space):	$	-
	Licensing Fees:	$	-
	Miscellaneous:	$	-
	Total Other Annual Expenses	**$**	**-**
Total Fixed Costs		**$**	**8,437,004**

e. Variable Cost Summary

While the fixed cost for the manufacturing process remains constant over the production years, the cost of raw materials, utilities, and general expenses vary with the production rate of the plant. These expenses are referred to as the variable cost which is calculated using factors and assumptions listed in **Table 12.6** and the values summarized in **Table 12.7**. The total variable cost for this process was calculated to be $3 billion, almost $2 billion of which comes from the annual raw material cost.

Table 12.6. Factors in variable cost calculation

General Expenses	
Selling / Transfer Expenses:	3.00% of Sales
Direct Research:	4.80% of Sales
Allocated Research:	0.50% of Sales
Administrative Expense:	2.00% of Sales
Management Incentive Compensation:	1.25% of Sales

Table 12.7. Variable cost summary

Variable Cost Summary			
Variable Costs at 100% Capacity:			
General Expenses			
Selling / Transfer Expenses:		$	105,000,000
Direct Research:		$	168,000,000
Allocated Research:		$	17,500,000
Administrative Expense:		$	70,000,000
Management Incentive Compensation:		$	43,750,000
Total General Expenses		$	404,250,000
Raw Materials	$67.774571 per lb of Ground Beef		$2,372,110,001
Byproducts	$0.000000 per lb of Ground Beef		$0
Utilities	$0.801049 per lb of Ground Beef		$28,036,708
Total Variable Costs		**$**	**2,804,396,709**

f. Total Capital Investment

The total capital investment is estimated using the working capital calculation described in section 17.3 by Seider et al. The working capital included funds, in addition to the fixed capital and the startup funds, needed for the plant to run properly until payment is received from the customer. Subtracting the accounts payable from the sum of cash reserves, accounts receivable, and inventory yields the working capital. **Table 12.8** includes the assumed period for each factor that is needed to calculate the working capital and **Table 12.9** summarizes the total capital investment of $190 million for this process.

Table 12.8. Assumptions in working capital calculation

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

Table 12.9. Total capital investment summary

Working Capital

	2024	2025	2026
Accounts Receivable	$ 230,136,986	$ 28,767,123	$ 28,767,123
Cash Reserves	$ 2,398,272	$ 299,784	$ 299,784
Accounts Payable	$(157,817,866)	$ (19,727,233)	$ (19,727,233)
Ground Beef Inventory	$ 30,684,932	$ 3,835,616	$ 3,835,616
Raw Materials	$ 10,398,290	$ 1,299,786	$ 1,299,786
Total	**$ 115,800,614**	**$ 14,475,077**	**$ 14,475,077**
Present Value at 15%	*$ 100,696,186*	*$ 10,945,238*	*$ 9,517,598*

Total Capital Investment **$ 189,180,626**

Section 13: Profitability Analysis

a. Cash Flow

The cash flow for this process with MACRS depreciation for a 5-year class life, calculated over an estimated life of 17 years including design (2023) and construction (2024) is summarized in **Table 13.1.** It is also graphically represented in **Figure 13.1**. The cash flow is an important financial factor in understanding the profitability of the proposed manufacturing process. It is generally referred to as the net passage of money going into and out of the company, with all of the costs as negative and after-tax profit and depreciation as positive. Negative values in the table are enclosed with parentheses. For this process, there is no net earning during the design and construction period. From 2025 to 2039, there are production and sales that would lead to a net positive cash flow for the company and make the process profitable.

Table 13.1. 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%
2023	0%		-	-	-	-	-	-	-	-	-	-	-	-
2024	0%		-	(40,813,000)	(115,800,600)	-	-	-	-	-	-	-	(156,613,600)	(136,185,700)
2025	80%	$100.00	2,800,000,000	(13,604,300)	(14,475,100)	(2,243,517,400)	(8,437,000)	(10,562,400)	-	537,483,300	(198,868,800)	338,614,500	321,097,400	106,610,100
2026	90%	$103.71	3,266,865,000	(10,203,200)	(14,475,100)	(2,617,595,800)	(8,750,000)	(16,899,800)	-	623,619,400	(230,739,200)	392,880,200	385,101,700	359,820,700
2027	100%	$107.56	3,764,517,400	-	-	(3,016,342,900)	(9,074,600)	(10,139,900)	-	728,960,000	(269,715,200)	459,244,800	469,384,700	628,192,900
2028	100%	$111.55	3,904,181,000	-	-	(3,128,249,300)	(9,411,300)	(6,083,900)	-	760,436,500	(281,361,500)	479,075,000	485,158,900	869,402,600
2029	100%	$115.69	4,049,026,100	-	-	(3,244,307,300)	(9,760,500)	(6,083,900)	-	788,874,400	(291,883,500)	496,990,900	503,074,800	1,086,895,700
2030	100%	$119.98	4,199,245,000	-	-	(3,364,671,100)	(10,122,600)	(3,042,000)	-	821,409,400	(303,921,500)	517,487,900	520,529,900	1,282,582,200
2031	100%	$124.43	4,355,037,000	-	-	(3,489,500,400)	(10,498,100)	-	-	855,038,500	(316,364,200)	538,674,200	538,674,200	1,458,675,700
2032	100%	$129.05	4,516,608,900	-	-	(3,618,960,900)	(10,887,600)	-	-	886,760,400	(328,101,300)	558,659,000	558,659,000	1,617,481,500
2033	100%	$133.83	4,684,175,100	-	-	(3,753,224,300)	(11,291,500)	-	-	919,659,200	(340,273,900)	579,385,300	579,385,300	1,760,696,700
2034	100%	$138.80	4,857,958,000	-	-	(3,892,469,000)	(11,710,500)	-	-	953,778,600	(352,898,100)	600,880,500	600,880,500	1,889,851,900
2035	100%	$143.95	5,038,188,200	-	-	(4,036,879,600)	(12,144,900)	-	-	989,163,700	(365,990,600)	623,173,200	623,173,200	2,006,327,400
2036	100%	$149.29	5,225,105,000	-	-	(4,186,647,800)	(12,595,500)	-	-	1,025,861,700	(379,568,800)	646,292,900	646,292,900	2,111,368,000
2037	100%	$154.83	5,418,956,400	-	-	(4,341,972,400)	(13,062,800)	-	-	1,063,921,200	(393,650,800)	670,270,300	670,270,300	2,206,096,500
2038	100%	$160.57	5,619,999,700	-	-	(4,503,059,600)	(13,547,400)	-	-	1,103,392,700	(408,255,300)	695,137,400	695,137,400	2,291,525,000
2039	100%	$166.53	5,828,501,700	-	144,750,800	(4,670,123,100)	(14,050,000)	-	-	1,144,328,500	(423,401,600)	720,927,000	865,677,700	2,384,035,500

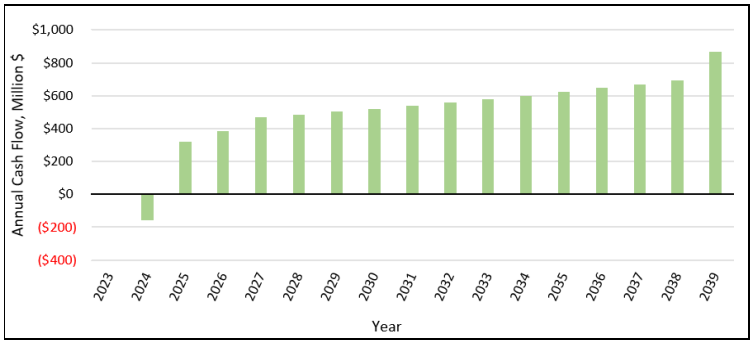


Figure 13.1. Annual cash flows.

b. Return on Investment and Sensitivity Analysis

To obtain the net present value (NPV) of this project, each cash flow in **Table 13.1** was discounted to its present value using a 15% interest rate. The capital investment costs could be recovered and the plant would have an NPV of $107,000,000 within the first year of production. Over the 17-year lifetime of the plant, it would generate a cumulative NPV of $2 billion. In addition, the return on investment (ROI) in the third year of production would be almost 217%, meaning the net earning would be nearly twice the total capital investment as shown in **Table 13.2**.

Table 13.2. Return on investment in the third year of production

The Internal Rate of Return (IRR) for this project is	223.27%
The Net Present Value (NPV) of this project in 2023 is	$2,384,035,500

ROI Analysis (Third Production Year)

Annual Sales	3,764,517,435
Annual Costs	(3,025,417,588)
Depreciation	(5,441,728)
Income Tax	(271,453,504)
Net Earnings	462,204,615
Total Capital Investment	212,772,372
ROI	217.23%

The internal rate of return (IRR) was calculated to be 223%; it is the interest rate that would give an NPV of zero. Usually, the profitability of alternative processes is compared using these values such that the largest IRR and the smallest NPV are desired. **Table 13.3** includes a sensitivity analysis that calculates IRR based on deviations in the initial product price and the variable cost. Even at the current variable cost of $2.8 billion, the product price could be decreased to $90/lb of ground beef and still maintain a positive IRR of 130%. This analysis is important in evaluating the profit margin for a competitive product price. As discussed in section 10, the lowest price for cultured meat product based on GFI's techno-economic analysis is $70 per pound. To compete in the same market, the ground beef price of $100 per pound must decrease. For example, with a reduced price of $50 per pound, there must be a 40% decrease in the variable cost (new variable cost of $1.7 billion) to maintain a minimum positive IRR of 72%. Since raw material contributes to the largest share of variable cost, optimization of raw material usage may bring the cost down.

Table 13.3. Sensitivity analysis on IRR with varying price and variable cost

		Variable Costs										
		$1,402,198,355	$1,682,638,025	$1,963,077,696	$2,243,517,367	$2,523,957,038	***$2,804,396,709***	$3,084,836,380	$3,365,276,051	$3,645,715,722	$3,926,155,393	$4,206,595,064
Product Price	$50.00	405.94%	72.69%	Negative IRR	Negative IRR	Negative IRR	Negative IRR	Negative IRR	Negative IRR	Negative IRR	Negative IRR	Negative IRR
	$60.00	535.47%	317.56%	101.69%	Negative IRR	Negative IRR	Negative IRR	Negative IRR	Negative IRR	Negative IRR	Negative IRR	Negative IRR
	$70.00	597.17%	436.16%	275.49%	115.82%	Negative IRR	Negative IRR	Negative IRR	Negative IRR	Negative IRR	Negative IRR	Negative IRR
	$80.00	633.23%	505.63%	378.13%	250.87%	124.17%	-14.08%	Negative IRR	Negative IRR	Negative IRR	Negative IRR	Negative IRR
	$90.00	656.89%	551.22%	445.60%	340.07%	234.70%	129.68%	24.35%	Negative IRR	Negative IRR	Negative IRR	Negative IRR
	$100.00	673.60%	583.44%	493.30%	403.21%	313.18%	223.27%	133.58%	44.13%	Negative IRR	Negative IRR	Negative IRR
	$110.00	686.03%	607.41%	528.80%	450.22%	371.66%	293.16%	214.75%	136.50%	58.46%	Negative IRR	Negative IRR
	$120.00	695.64%	625.94%	556.25%	486.57%	416.91%	347.28%	277.69%	208.17%	138.76%	69.51%	-7.22%
	$130.00	703.29%	640.69%	578.10%	515.52%	452.95%	390.39%	327.86%	265.36%	202.92%	140.56%	78.31%
	$140.00	709.53%	652.72%	595.92%	539.12%	482.33%	425.55%	368.78%	312.04%	255.32%	198.64%	142.02%
	$150.00	714.71%	662.71%	610.71%	558.72%	506.74%	454.76%	402.79%	350.83%	298.89%	246.97%	195.08%

Section 14: Other Important Considerations

a. Environmental Comparisons

Water pollution in traditional farming methods for cultivating beef results from nitrogen and phosphorous runoff from farmlands into freshwater sources and the use of freshwater in hydrating cattle. Potential water pollution in the case of cellular agriculture occurs after the cells and media are centrifuged when process water is purged to remove any of the created ammonia. In both cases, the effects of meat production negatively affects the surrounding water supply. This section serves to compare the effects of both production methods to determine which results in less water pollution in the Midwest.

Blue water usage refers to water that is used for animals drinking on a farm as well as crop irrigation. This usage is estimated to be 112 L per kg of carcass weight in the Midwestern region of the United States. The use of blue water for these purposes results in excreted waste in animals which pollutes water systems via runoff. It is estimated that 24.3 grams of nitrogen pollution is produced per kilogram of carcass weight of cattle and 0.38 milligrams of phosphorus is polluted per kilogram of carcass weight. For 35 million pounds of cattle meat to be produced, 1.8 billion liters of blue water is required which results in 390 million pounds of nitrogen and 6,000 pounds of phosphorus entering the waterways as a pollutant (Rotz et al., 2019).

Due to the recycle of process water, only 10% of the annual requirement is purged and emitted as waste. Therefore, of the 91 million pounds of sterile process water required throughout all the bioreactors, only 9.1 million pounds of water is purged yearly as waste due to its ammonia content. This equates to nearly 4.1 million liters of water, and even if the same percentage of ammonia was produced from this usage, only 905,000 pounds of nitrogen is released as waste. When considering the amount of water evaporated in the bioreactors, 6.4

million pounds, then the total blue water requirement is a mere 2.9 million liters. Despite initial concerns of the water consumption of the process, it is clear that cellular agriculture results in decreased water pollution.

Another form of pollution in which cattle farming plays a serious role is air pollution. Traditional cattle farming results in the formation of greenhouse gases (GHG) such as methane and carbon dioxide (CO_2). Methane production results from the hindgut fermentation that occurs within cattle when eating plants, but this is not produced within the cell culture process, where cells are fed pure glucose, and therefore not included in the analysis. Carbon dioxide, however, is produced in both processes as a result of cellular respiration.

20.6 kilograms of carbon dioxide is produced for each kilogram of carcass weight produced in traditional farming (Rotz et al., 2019). Therefore, to traditional prepare 35 million pounds of carcass weight, then 330 million pounds of CO_2 is emitted. To compare, only 17 million pounds of CO_2 is emitted as a result of the industrialized production of a cultured beef product. To conclude, the environmental harms of producing a cultured beef product as opposed to raising cattle for slaughter are significantly reduced. These reductions are most noticeable in the water and air pollution metrics previously mentioned. It is also important to note that there is no soil pollution as a result of this process whereas there is clear soil pollution from farming agriculture due to waste produced by animals on farmlands.

b. Monetary Considerations

The fossil energy consumption of traditional beef cattle production in the midwest US is about 49MJ per kg of carcass weight (CW) (Rotz et al., 2018). The fossil fuel includes fuel, natural gas and electricity required for cattle production. Although it is not directly comparable to the electricity usage for the cultured ground beef plant as carcass weight includes the weight

of bones, viscera, and other non-meat tissue, it conceptualizes the vast energy requirement for cattle production. It takes approximately 31.5 kWh to produce one pound of traditional beef (Save on Energy, 2019); in contrast, the ground beef product from this process requires about 7 kWh per pound, accounting for electricity and refrigeration energy requirements.

c. Ethical Considerations

The largest ethical concern for the process is the reliance on animal slaughter for two things: fetal bovine serum, and initial harvesting of bovine muscle satellite cells. The proposed process, while minimizing FBS wherever possible, does not manage to eliminate it entirely. However, research is continuing in that direction; for example, a recent preprint proposed a serum-free proliferation medium, "Beefy9," which yielded a doubling time of 39 hours, only slightly more than the 36.6 hours in the current process (Stout et al., 2021). The process also assumes access to a biolab with cryogenically frozen cell starters ready to go at any time. Currently, such a cell bank would require periodic slaughter of cattle in order to harvest more BMSc, as natural cell lines cannot divide indefinitely. Future research into immortalizing cell lines for cultured meat production is needed before slaughter can be eliminated from the creation of starter cell colonies.

d. Plant Location

The largest motivating factor in selecting the location of the plant was ready access to raw materials. In order to feed the cells, the process consumes hundreds of millions of pounds of corn grain and soybean hydrolysates per year. Illinois is the United States' top producer of soy and number 2 producer of corn (Grant, 2022), making it an attractive location for the plant. Being in the northern part of the Midwest, Illinois also has the advantage of relatively mild springs and summers, reducing the amount of energy needed to cool/chill the water for the cooling jacket network.

Section 15: Conclusion & Recommendations

Traditional beef production methods are among some of the worst environmentally costly meat production methods currently available. The current FDA- and USDA-approved alternatives are plant-based and do not appeal to all meat-eating consumers. The field of cellular agriculture fills this market gap with a promise to culture meat products *in vitro* and minimize animal slaughter. Though this project hoped to eliminate the need altogether, slaughter is still required for the use of FBS to attain a viable growth density during the proliferation stages of the upstream process, and for the initial harvesting of BMSc. The utilization of reduced FBS in proliferation only (and none in differentiation) minimizes the amount of animal harm produced by this process and ultimately differentiates this process from other production methods. Nevertheless, advancements in the cell doubling time, raw material cost reduction, and further development of a suspension BMSc line can enhance the project's feasibility by reducing costs and time needed to produce 35 million pounds of meat.

The slow doubling time of 36.6 hours for BMSc line influences the number of batches produced per year and the equipment sizes. For this process, the doubling time yields 39 batches a year to produce 35 million pounds of ground beef using 300m^3 proliferation bubble column reactors and a combination of 300m^3 and 150 m^3 differentiation bubble column reactors. Due to the large size requirement, the bioreactors have to be fabricated on site, costing more that prefabricated industrial bubble column reactors. For example, the estimated bare module cost of a 300 m^3 bubble column was about $5 million whereas a 50 m^3 prefabricated reactor costs $40,000 ("Bubble Column Reactor," n.d.). With a lower doubling time and the same production goal, the equipment sizes can be reduced, potentially eliminating the necessity for custom,

on-site fabrication. If equipment sizes are kept the same, a lower doubling time can provide more batches per year resulting in more production per facility per year.

A lower doubling time can be achieved by directed evolution using a chemostat. Directed evolution is a common method in cell engineering that mimics the process of natural selection towards a desired trait (Wides & Milo, 2018). A chemostat can be used to modify the dilution rate by changing the continuous flow rate of media added to the reactor. If the dilution rate exceeds the maximum cell growth rate, cells will be washed out of the chemostat through the volume that is continuously removed. For this project, it is hypothesized that a chemostat could be used with high dilution rate to induce directed evolution on the BMSc line, such that cells with a lower growth rate would be washed out and lead to a adapted cell line with a higher growth rate and thus lower doubling time. Further research and experiments would be required to evaluate the feasibility of this method. If the hypothesized experiment leads to a lower doubling time for the BMSc cell lines, it would help reduce the overall cost of this process through smaller equipment sizes.

Another consideration for cost reduction is in raw material cost. For the process, the final raw material cost comes out to $68 per pound of ground beef, driven up largely by the high costs of the corn grain and soybean hydrolysates. Although it may not be possible to reduce the amount of hydrolysates without completely changing the cell line, it may be beneficial to allocate the enzymatic hydrolysis and hammer milling equipment onsite. The allocation of these process units on the site would decrease the costs associated with purchasing hydrolysates. As a result, the costs associated with the raw materials are reduced when purchasing raw corn grain and raw soybean. However, adding more process units would increase the capital and operation

costs of the plant, so it would be worthwhile to complete a detailed economic analysis of the processes to determine if the costs associated far outweigh the purchase cost of hydrolysates.

Although hydrolysates significantly increases the final product cost, microcarriers (Cytodex 1) are the biggest culprit for the high cost as it is responsible for 47.5% of the raw material cost per pound of product. There are two potential avenues to explore regarding the reduction of these costs: use an alternative separation method to reuse and recycle microcarriers to reduce the amount necessary per year or engineer a suspension cell line that does not require microcarrier usage at all.

The former allows for the process to stay virtually unchanged in all aspects except to the addition of the STR to dissolve the dextran Cytodex I microcarriers using dextranase. It is recommended that future research considers different solid-solid separation methods to extract the cells from the beads without causing cell death. This research would have the potential to improve the process financial analysis in that there may not be such a large annual usage of Cytodex I beads. As a result, the total amount of beads and thereby, total cost associated with them, may be reduced with this addition. However, another alternative rids the process of microcarriers altogether.

The latter method would allow for a microcarrier free environment where cells would grow freely in suspension. This would not only eliminate dextranase and the cost asscoiated with replacing dissolved Cytodex 1, but also simplify the process water recycling process. However, it is challenging to evolve an adherent cell line to a suspension cell line due to the different growth mechanisms. HEK293 is an example of human cell line that was genetically engineered for suspension culture, but it involved an in-depth genome engineering process

altering gene expressions that regulated cell adhesion (Malm et al., 2020). Genetically modifying the BMSc in a similar manner would require prolonged research and development, but the resulting improvements to the process may be worth the investment.

Overall, if cultured beef is to become truly competitive as an alternative to traditional slaughter meat, many improvements are required to the existing process. The current available cell line, directly animal-derived bovine muscle satellite cells, is nowhere near as optimized as more traditional cell culture lines such as Chinese hamster ovary or HEK293. Advancements are needed in immortalizing the cell line to eliminate the need for slaughter for BMSc harvest, reducing the doubling time, and potentially creating a bovine stem cell that can be easily cultured in suspension. Further, completely eliminating slaughter from the cultured meat process requires advancements in serum-free medium. Raw material costs also need to decrease in order to bring down prices, especially with regards to the corn and soy hydrolysates that make up the bulk of the cell medium. Cheaper alternatives, either in nutrient sourcing or in allocating corn and soy processing, need to be sought in order to decrease these costs.

However, even with all of the challenges facing a potential cultured meat process currently, the industry is still very new and quickly advancing. The current process, though requiring a fairly expensive price point for the final product, provides a promising starting point for cultured meat scaleup.

Section 16: Acknowledgments

First, our group would like to thank Dr. Jeffrey D. Cohen, our project author, for helping us tirelessly throughout the design process. His extensive knowledge of the pharmaceutical industry, cell growth mechanics and scale-up considerations proved extremely valuable in defining our project. His consistent support in answering our questions guided us throughout the semester. We also thank Dr. Bomyi Lim, our faculty advisor, for lending us your support, advice, and feedback. Your kind words of encouragement boasted our spirit and kept us motivated. Thank you to Prof. Bruce Vrana for organizing the course and keeping us on track. We would also like to thank all of the industrial consultants who have donated their time and expertise to assisting us on this project. This process has been challenging, rigorous, and rewarding, and completing it would not have been possible without all of your help.

Section 17: Bibliography

Alfa Laval. (n.d.). *Full tank cleaning product line.* Retrieved April 17, 2022, from https://www.alfalaval.us/microsites/tank-cleaning/tank-cleaning-devices/full-product-line

Alfa Laval CH 900: Modularized disc stack separation system for industrial use. (n.d.). Alfa Laval. https://www.alfalaval.com/globalassets/documents/products/separation/centrifugal-separators/separators/ch-series/product_leaflet_ch_900_system_en.pdf

Alfa LU-VE. (n.d.). *Cold room calculator.* LU-VE Group. Retrieved April 17, 2022, from https://alfa.luvegroup.com/en/coldroomcalc.html

Aleph Farms. (2022, February 17). *Aleph Farms expands as we gear up for global commercialization*. https://www.aleph-farms.com/blog/new-facility

Allied Analytics. (2021). *Cultured meat market by type and end user: Global opportunity analysis and industry forecast 2022-2030.* Research and Markets. https://www.researchandmarkets.com/reports/5341573/cultured-meat-market-by-type-and-end-user-global

Allied Buildings. (n.d.). *How much does it cost to build a cold storage warehouse?* Retrieved April 17, 2022 from https://www.alliedbuildings.com/how-much-does-it-cost-to-build-a-cold-storage-warehouse/

Alluri, S. (2018). *Pump power calculation formula: Specific speed of a centrifugal pump.* Sugar Process Technologies. https://www.sugarprocesstech.com/pump-power-calculation/

Ashkenazi, S. (2021, February 9). Israel's Aleph Farms prints first-ever ribeye steak. *Globes.* https://en.globes.co.il/en/article-israels-aleph-farms-prints-first-ever-ribeye-steak-1001360113

Azo Materials. (2005, May 18). *Grade 304 stainless steel: Properties, fabrication and applications.* https://www.azom.com/article.aspx?ArticleID=2867

Bubble column reactor. (n.d.). Alibaba. Retrieved April 17, 2022 from https://www.alibaba.com/showroom/bubble-column-reactor.html

The Cary Company. (n.d.). *How to properly size an industrial chiller*. Retrieved April 17, 2022 from https://www.thecarycompany.com/insights/articles/how-to-properly-size-an-industrial-chiller

Cell culture environment. (n.d.). ThermoFisher Scientific. Retrieved April 17, 2022, from https://www.thermofisher.com/us/en/home/references/gibco-cell-culture-basics/cell-culture-environment.html

Current Results. (2021). *Chicago - Highest temperature for each year.* https://www.currentresults.com/Yearly-Weather/USA/IL/Chicago/extreme-annual-chicago-high-temperature.php

De Lorenzo, D. (2022, March 17). Dutch Parliament approves cultured meat tasting in the Netherlands. *Forbes.* https://www.forbes.com/sites/danieladelorenzo/2022/03/17/dutch-parliament-approves-cultured-meat-tasting-within-the-netherlands/?sh=2828369a60bf

Engineering Toolbox. (2008). *Solubility of gases in water vs. temperature.* https://www.engineeringtoolbox.com/gases-solubility-water-d_1148.html

Electricity Local. (n.d.). *Illinois electricity rates & consumption*. Retrieved April 17, 2022 from https://www.electricitylocal.com/states/illinois/#ref

Fassler, J. (2021, September 9). Lab-grown meat is supposed to be inevitable. The science tells a different story. *The Counter.* https://thecounter.org/lab-grown-cultivated-meat-cost-at-scale/

The Federal Meat Inspection Act, Pub. L. No. 59–382, 34 Stat. 669. (1906). https://uscode.house.gov/view.xhtml?path=/prelim@title21/chapter12&edition=prelim

Gerber, P.J., Steinfeld, H., Henderson, B., Mottet, A., Opio, C., Dijkman, J., Falcucci, A. & Tempio, G. (2013). *Tackling climate change through livestock – A global assessment of emissions and mitigation opportunities.* Food and Agriculture Organization of the United Nations (FAO), Rome. https://www.fao.org/3/i3437e/i3437e.pdf

Gilchrist, K. (2021, March 1). This multibillion-dollar company is selling lab-grown chicken in a world-first. *CNBC.* https://www.cnbc.com/2021/03/01/eat-just-good-meat-sells-lab-grown-cultured-chicken-in-world-first.html

Goudar, C. T., Piret, J. M., & Konstantinov, K. B. (2011). Estimating cell specific oxygen uptake and carbon dioxide production rates for mammalian cells in perfusion culture. *Biotechnology progress, 27*(5), 1347-1357. https://doi.org/10.1002/btpr.646

Grant, D. (2022, January 28). Illinois top soybean producer, second for corn. *The Telegraph.* https://www.thetelegraph.com/news/article/Illinois-top-soybean-producer-second-for-corn-16814546.php

Gregory, T., O'Connell, P.M., & Reyes, C. (2017, December 27). Precious resource, private profits: Rates and tempers rise as towns look to companies to manage water systems. *Chicago Tribune.* https://graphics.chicagotribune.com/news/lake-michigan-drinking-water-rates/privatization.html

Guan, X., Zhou, J., Du, G., & Chen, J. (2021). Bioprocessing technology of muscle stem cells: implications for cultured meat. *Trends in Biotechnology.* https://doi.org/10.1016/j.tibtech.2021.11.004

Guan, Y. H., & Kemp, R. B. (1999). On-line heat flux measurements improve the culture medium for the growth and productivity of genetically engineered CHO cells. *Cytotechnology, 30*(1-3), 107–120. https://doi.org/10.1023/A:1008038515285

Higgins, M., Neal, R., Gade, N., & Eastwood, R. (2017, March 9). *Manufacturing facility for nylon-6,6.* AIChE. https://shareok.org/bitstream/handle/11244/52274/oksd_HigginsNeal_HT_2017.pdf?sequence=1&isAllowed=y

Huang, W., Wang, E., Chang, J., Wang, P., Yin, Q., Liu, C., Zhu, Q., and Lu, F. (2017). Effect of physicochemical pretreatments and enzymatic hydrolysis on corn straw degradation and reducing sugar yield. *BioRes, 12*(4), 7002-7015.

IDAH. (n.d.). *High quality extruder - Single screw extruder (for aqua feed and pet food).* https://idah.com/products/single-screw-extruder

Kelland, H. (2013, August 6). First taste of test-tube burger declared 'close to meat'. *Reuters.* https://www.reuters.com/article/us-science-meat-in-vitro/first-taste-of-test-tube-burger-declared-close-to-meat-idUSBRE9740PL20130806

Khasawneh, R. R., Al Sharie, A. H., Abu-El Rub, E., Serhan, A. O., & Obeidat, H. N. (2019). Addressing the impact of different fetal bovine serum percentages on mesenchymal stem cells biological performance. *Molecular biology reports, 46*(4), 4437–4441. https://doi.org/10.1007/s11033-019-04898-1

Lindskog, U., Lundgren, B., Billig, D., & Lindner, E. (1987). Alternatives for harvesting cells grown on microcarriers: effects on subsequent attachment and growth. *Developments in biological standardization, 66*, 307–313. PMID: 3582760.

Luining, D. (2015). *Optimization of bovine satellite cell proliferation on microcarriers*. [Master's thesis, Leiden University]. https://www.researchgate.net/publication/299603637_OPTIMIZATION_OF_BOVINE_SATELLITE_CELLS_PROLIFERATION_ON_MICROCARRIERS

Mahir, M., El Maakoul, A., Khay, I., Saadeddine, S., & Bakhouya, M. (2021). An investigation of heat transfer performance in an agitated vessel. *Processes, 9*(3), 468. https://doi.org/10.3390/pr9030468

Malm, M., Saghaleyni, R., Lundqvist, M., Giudici, M., Chotteau, V., Field, R., ... & Rockberg, J. (2020). Evolution from adherent to suspension: systems biology of HEK293 cell line development. *Scientific reports, 10*(1), 1-15. https://doi.org/10.1038/s41598-020-76137-8

Mizukami, A., Orellana, M. D., Caruso, S. R., de Lima Prata, K., Covas, D. T., & Swiech, K. (2013). Efficient expansion of mesenchymal stromal cells in a disposable fixed bed culture system. *Biotechnology progress, 29*(2), 568–572. https://doi.org/10.1002/btpr.1707

Quang, N., & Zarkadas, C. G. (1989). Comparison of the amino acid composition and connective tissue protein contents of selected bovine skeletal muscles. *Journal of Agricultural and Food Chemistry, 37*(5), 1279-1286. https://doi.org/10.1021/jf00089a017

Ritchie, H. (2021, February 23). *Cutting down forests: what are the drivers of deforestation?* Our World in Data. https://ourworldindata.org/what-are-drivers-deforestation

Ritchie, H. (2019, November 11). *Half of the world's habitable land is used for agriculture.* Our World in Data. https://ourworldindata.org/global-land-for-agriculture

Rotz, C. A., Asem-Hiablie, S., Place, S., & Thoma, G. (2019). Environmental footprints of beef cattle production in the United States. *Agricultural systems, 169*(1-13). https://doi.org/10.1016/j.agsy.2018.11.005

Rourou, S., Riahi, N., Majoul, S., Trabelsi, K., & Kallel, H. (2013). Development of an in situ detachment protocol of Vero cells grown on Cytodex1 microcarriers under animal component-free conditions in stirred bioreactor. *Applied biochemistry and biotechnology, 170*(7), 1724-1737. https://doi.org/10.1007/s12010-013-0307-y

Save on Energy. (2019, December 6). *American food production requires more energy than you'd think.* https://www.saveonenergy.com/learning-center/post/american-food-production-requires-energy/

Seider, W.D., Lewin, D.R., Seader, J.D., Widagdo, S., Gani, R., & Ng, K.M. (2017). *Product and process design principles.* (4th ed.). John Wiley & Sons.

Shahbandeh, M. (2022, April 14). *Global production of meat 2016-2020*. Statista. https://www.statista.com/statistics/237644/global-meat-production-since-1990/

Shanker, D. (2019, October 22). These $50 chicken nuggets were grown in a lab. *Bloomberg Businessweek.*

https://www.bloomberg.com/news/articles/2019-10-22/clean-meat-just-chicken-nuggets-grown-in-a-lab-coming-soon

Silva, G. (2018, December 3). *Feeding the world in 2050 and beyond – Part 1: Productivity challenges.* Michigan State University Extension: Agriculture. https://www.canr.msu.edu/news/feeding-the-world-in-2050-and-beyond-part-1

Simsa, R., Yuen, J., Stout, A., Rubio, N., Fogelstrand, P., & Kaplan, D. L. (2019). Extracellular heme proteins influence bovine myosatellite cell proliferation and the color of cell-based meat. *Foods, 8*(10), 521. https://doi.org/10.3390/foods8100521

Stout, A. J., Mirliani, A. B., White, E. C., Yuen, J. S., & Kaplan, D. L. (2021). Simple and effective serum-free medium for sustained expansion of bovine satellite cells for cell cultured meat. *bioRxiv.* https://doi.org/10.1101/2021.05.28.446057

SPX Cooling Technologies. (2016). *Cooling tower energy and its management.* https://spxcooling.com/wp-content/uploads/H-001B.pdf

US Bureau of Labor Statistics. (2022). *Average retail food and energy prices, U.S. and Midwest region*. https://www.bls.gov/regions/mid-atlantic/data/averageretailfoodandenergyprices_usandmidwest_table.htm

US FDA. (2020, October 6). *Food made with cultured animal cells.* https://www.fda.gov/food/food-ingredients-packaging/food-made-cultured-animal-cells

USDA FSIS. (2016, January 21). *Federal Meat Inspection Act.* https://www.fsis.usda.gov/policy/food-safety-acts/federal-meat-inspection-act

USDA FSIS. (2016, February 29). *Ground beef and food safety.* https://www.fsis.usda.gov/food-safety/safe-food-handling-and-preparation/meat/ground-beef-and-food-safety

USDA Press. (2021, September 2). *USDA seeks comments on the labeling of meat and poultry products derived from animal cells.* https://www.usda.gov/media/press-releases/2021/09/02/usda-seeks-comments-labeling-meat-and-poultry-products-derived

US Office of Regulatory Affairs. (2018, August 24). *CPG Sec 565.100 FDA jurisdiction over meat and poultry products.* https://www.fda.gov/regulatory-information/search-fda-guidance-documents/cpg-sec-565100-fda-jurisdiction-over-meat-and-poultry-products

van der Valk, J., Brunner, D., De Smet, K., Svenningsen, Å. F., Honegger, P., Knudsen, L. E., ... & Gstraunthaler, G. (2010). Optimization of chemically defined cell culture

media–replacing fetal bovine serum in mammalian in vitro methods. *Toxicology in vitro, 24*(4), 1053-1063. https://doi.org/10.1016/j.tiv.2010.03.016

Vergeer, R., Sinke, P., & Odegard, I. (2021). *TEA of cultivated meat. Future projections for different scenarios*. CE Delft. https://cedelft.eu/publications/tea-of-cultivated-meat/

Wides, A., & Milo, R. (2018). Understanding the dynamics and optimizing the performance of chemostat selection experiments. *arXiv preprint arXiv:1806.00272*. https://doi.org/10.48550/arXiv.1806.00272

Will, K., Schering, L., Albrecht, E., Kalbe, C., & Maak, S. (2015). Differentiation of bovine satellite cell-derived myoblasts under different culture conditions. *In Vitro Cellular & Developmental Biology-Animal, 51*(9), 885-889. https://doi.org/10.1007/s11626-015-9916-9

Williams, L. A., Davis-Dusenbery, B. N., & Eggan, K. C. (2012). SnapShot: Directed differentiation of pluripotent stem cells. *Cell, 149*(5), 1174–1174.e1. https://doi.org/10.1016/j.cell.2012.05.015

Wong, H. E., Chen, C., Le, H., & Goudar, C. T. (2021). From chemostats to high-density perfusion: the progression of continuous mammalian cell cultivation. *Journal of Chemical Technology & Biotechnology.* https://doi.org/10.1002/jctb.6841

Xu, J., Liu, D., Yin, H., Tong, H., Li, S., & Yan, Y. (2018). Fatty acids promote bovine skeletal muscle satellite cell differentiation by regulating ELOVL3 expression. *Cell and tissue research, 373*(2), 499-508. https://doi.org/10.1007/s00441-018-2812-3

Zedníková, M., Orvalho, S., Fialová, M., & Ruzicka, M. C. (2018). Measurement of volumetric mass transfer coefficient in bubble columns. *ChemEngineering, 2*(2), 19. https://doi.org/10.3390/chemengineering2020019

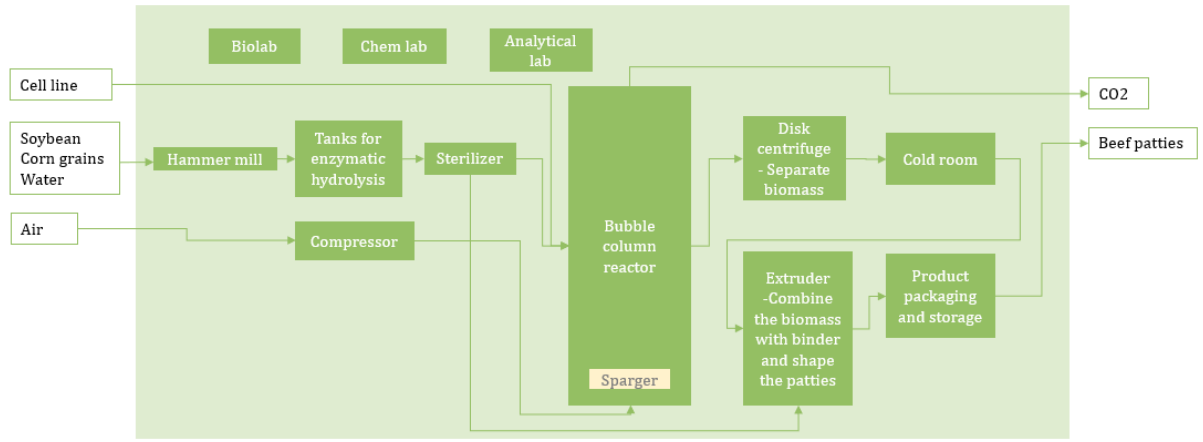
Section 18: Appendices

Appendix A: Acronyms & Abbreviations

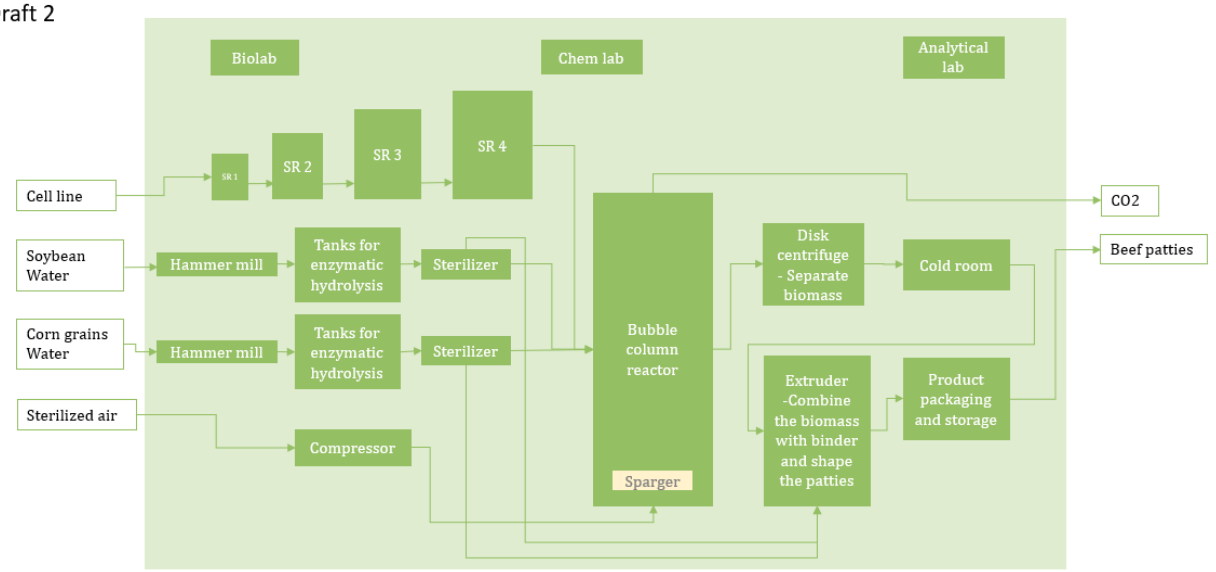
Abbreviation	Full Term
BCR	bubble column reactor
BMSc	bovine muscle satellite cell
CIP	clean-in-place
Comp	compressor
DR	differentiation reactor
DSC	disc stack centrifuge
EDTA	ethylenediaminetetraacetic acid
Ext	extruder
FBS	fetal bovine serum
FDA	Food and Drug Administration
FPR	final proliferation reactor
FSIS	Food Safety & Inspection Service
GFI	Good Food Institute
OTR	oxygen transfer rate
OUR	oxygen uptake rate
P	pump
PAA	peracetic acid
SR	seed reactor
STR	stirred tank reactor
USDA	United States Department of Agriculture
VCD	viable cell density

Appendix B: Preliminary Versions of the PFD

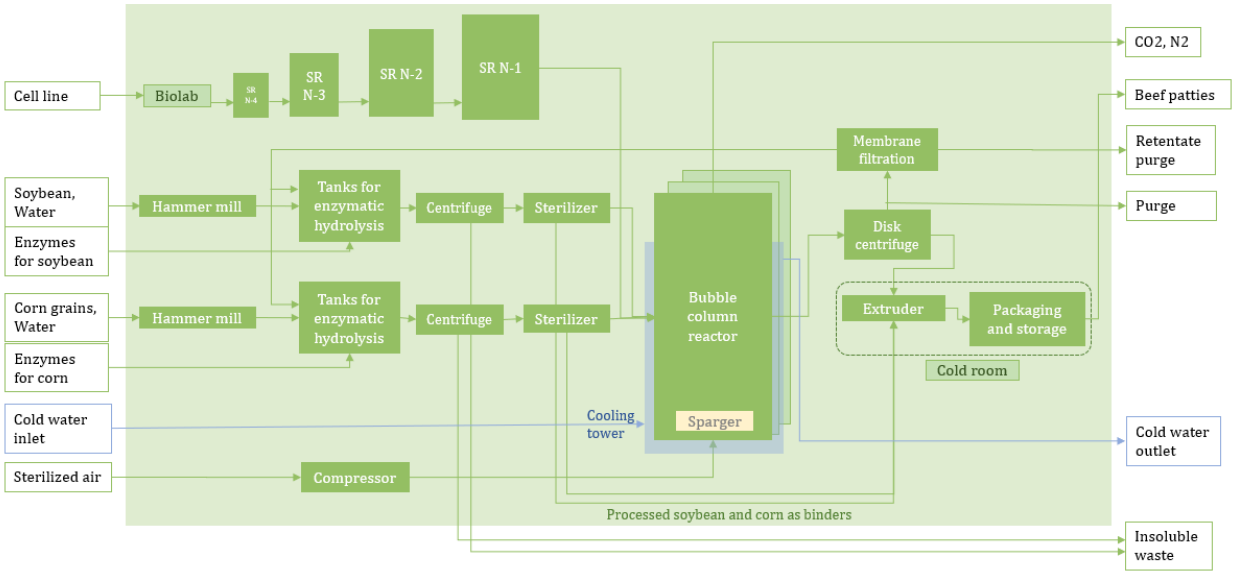
Draft 1



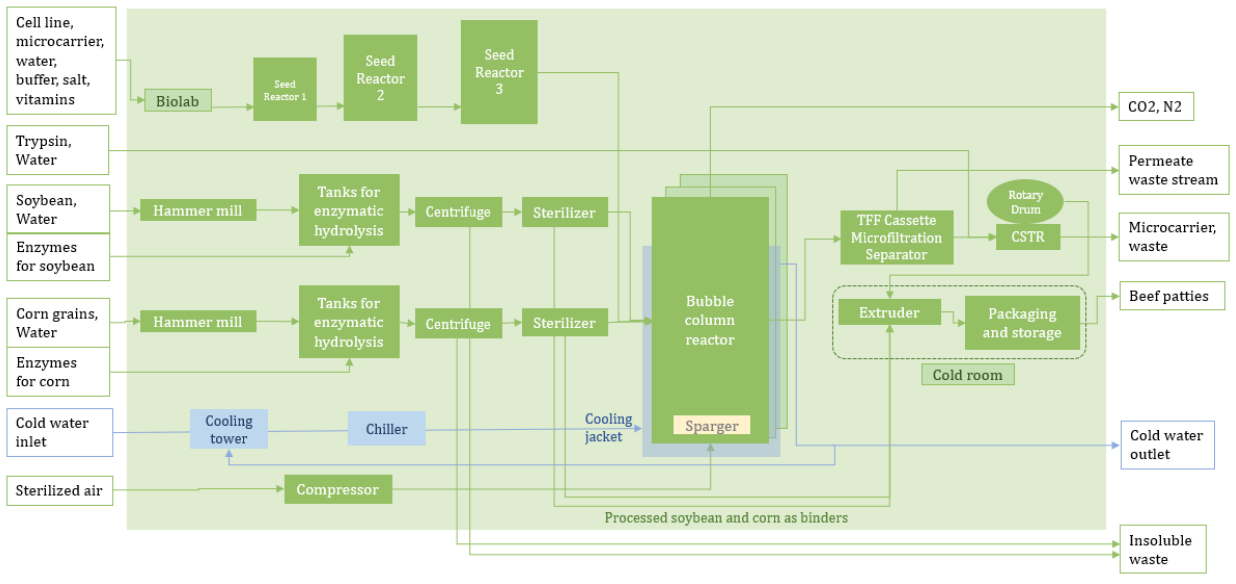
Draft 2

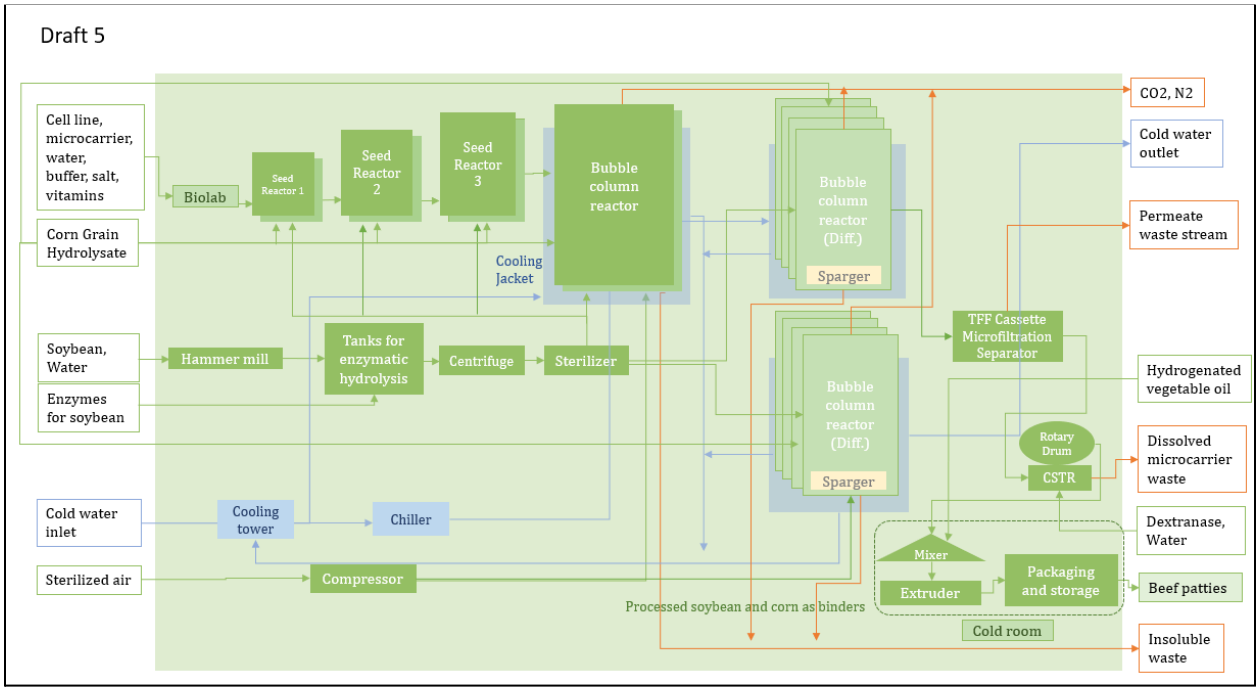


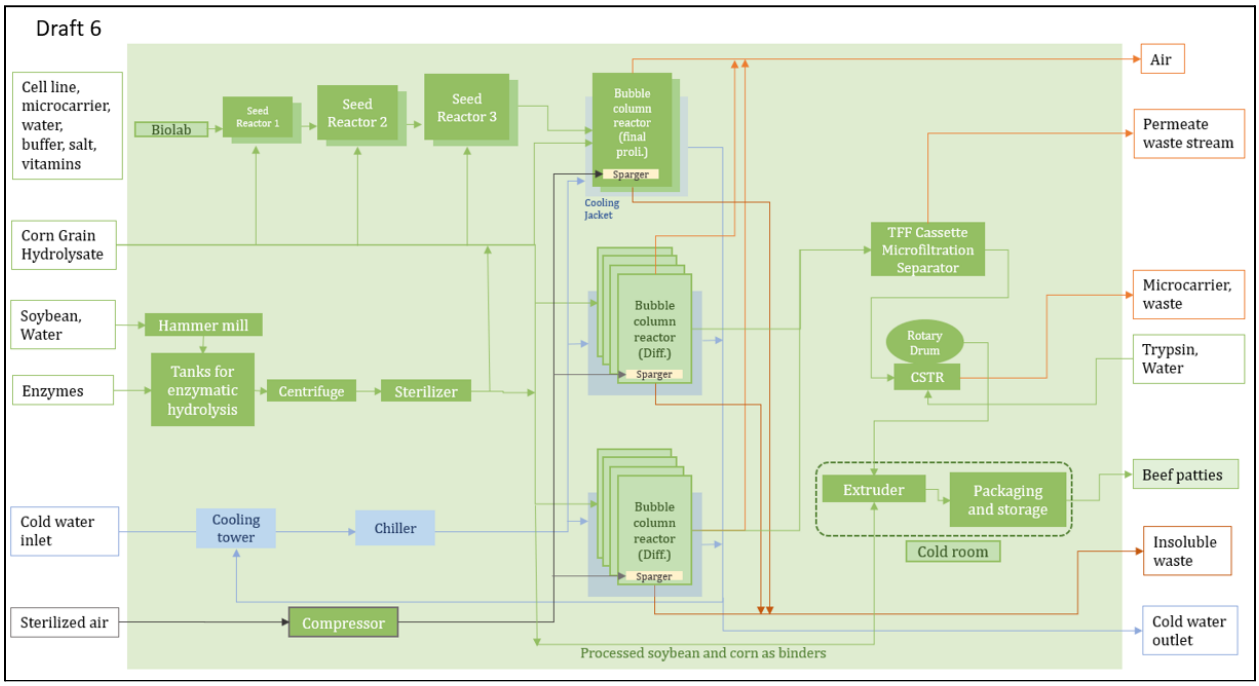
Draft 3

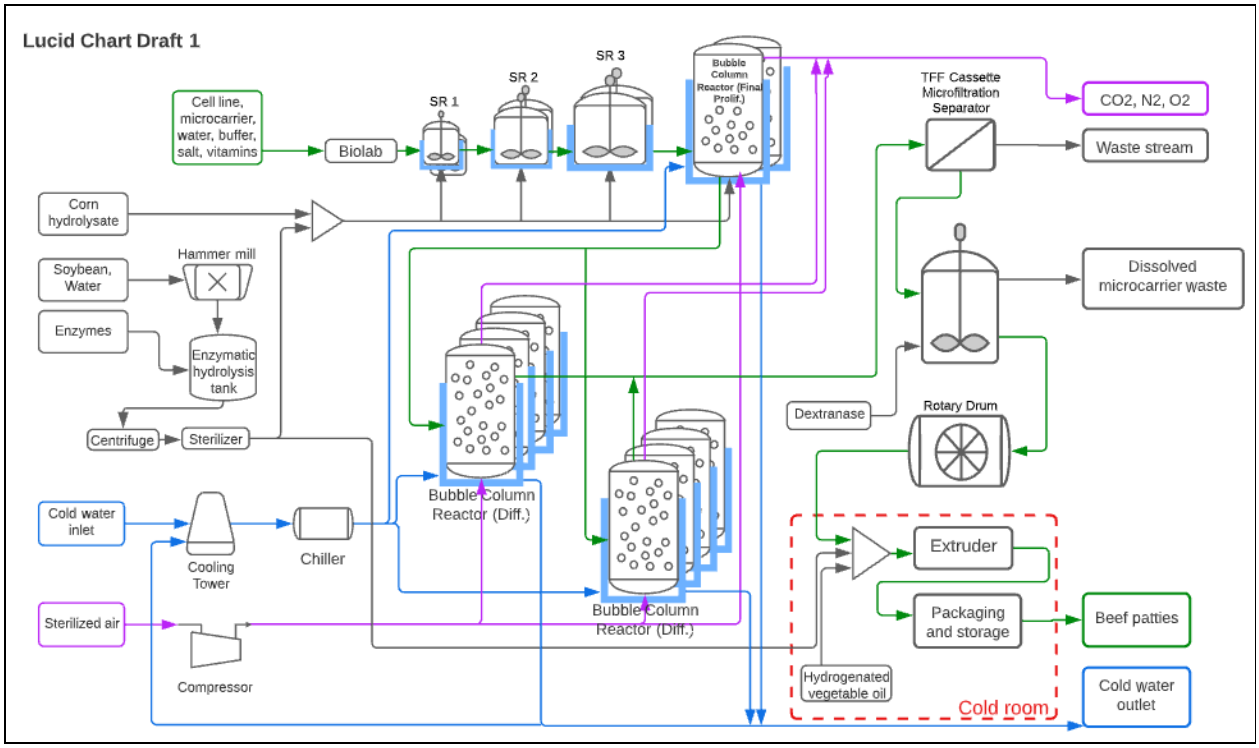


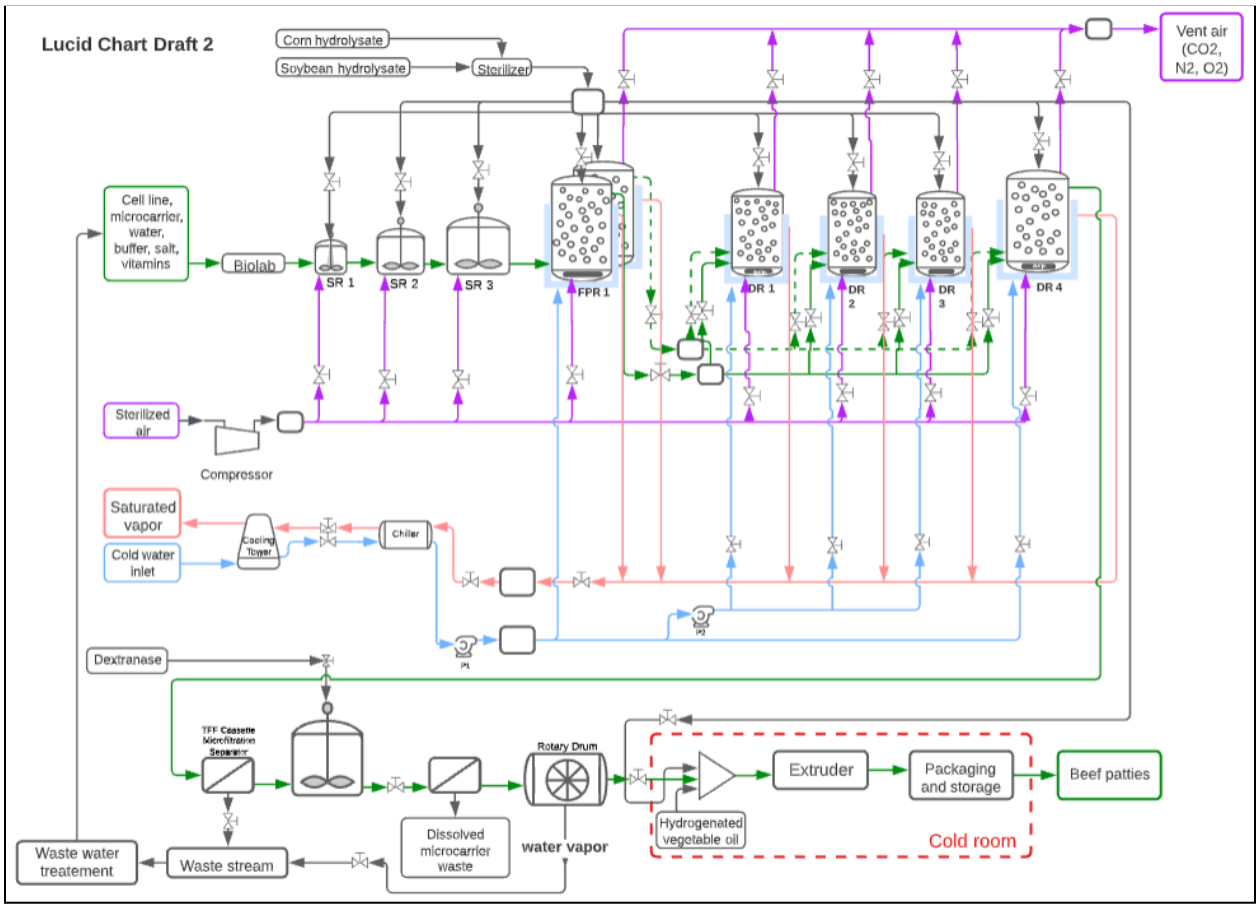
Draft 4




Draft 5
Cell line, microcarrier, water, buffer, salt, vitamins
Biolab
Seed Reactor 1
Seed Reactor 2
Seed Reactor 3
Bubble column reactor
Bubble column reactor (Diff.)
Sparger
Cooling Jacket
Corn Grain Hydrolysate
Soybean, Water
Hammer mill
Tanks for enzymatic hydrolysis
Centrifuge
Sterilizer
Enzymes for soybean
Bubble column reactor (Diff.)
Sparger
TFF Cassette Microfiltration Separator
Rotary Drum
CSTR
Cold water inlet
Cooling tower
Chiller
Sterilized air
Compressor
Mixer
Extruder
Packaging and storage
Cold room
Processed soybean and corn as binders
CO2, N2
Cold water outlet
Permeate waste stream
Hydrogenated vegetable oil
Dissolved microcarrier waste
Dextranase, Water
Beef patties
Insoluble waste


Draft 6
Cell line, microcarrier, water, buffer, salt, vitamins
Biolab
Seed Reactor 1
Seed Reactor 2
Seed Reactor 3
Bubble column reactor (final proli.)
Sparger
Cooling Jacket
Corn Grain Hydrolysate
Soybean, Water
Hammer mill
Tanks for enzymatic hydrolysis
Centrifuge
Sterilizer
Enzymes
Bubble column reactor (Diff.)
Sparger
TFF Cassette Microfiltration Separator
Rotary Drum
CSTR
Cold water inlet
Cooling tower
Chiller
Sterilized air
Compressor
Bubble column reactor (Diff.)
Sparger
Extruder
Packaging and storage
Cold room
Processed soybean and corn as binders
Air
Permeate waste stream
Microcarrier, waste
Trypsin, Water
Beef patties
Insoluble waste
Cold water outlet


Lucid Chart Draft 1
Cell line, microcarrier, water, buffer, salt, vitamins
Biolab
SR 1
SR 2
SR 3
Bubble Column Reactor (Final Prolif.)
TFF Cassette Microfiltration Separator
CO2, N2, O2
Waste stream
Corn hydrolysate
Soybean, Water
Hammer mill
Enzymes
Enzymatic hydrolysis tank
Dissolved microcarrier waste
Centrifuge
Sterilizer
Dextranase
Rotary Drum
Bubble Column Reactor (Diff.)
Cold water inlet
Cooling Tower
Chiller
Extruder
Packaging and storage
Beef patties
Sterilized air
Compressor
Hydrogenated vegetable oil
Cold water outlet
Cold room


Lucid Chart Draft 2
Corn hydrolysate
Soybean hydrolysate
Sterilizer
Vent air (CO2, N2, O2)
Cell line, microcarrier, water, buffer, salt, vitamins
Biolab
SR 1
SR 2
SR 3
FPR 1
DR 1
DR 2
DR 3
DR 4
Sterilized air
Compressor
Saturated vapor
Cold water inlet
Cooling Tower
Chiller
Dextranase
TFF Cassette Microfiltration Separator
Rotary Drum
Extruder
Packaging and storage
Beef patties
Hydrogenated vegetable oil
Cold room
Waste water treatement
Waste stream
Dissolved microcarrier waste
water vapor

Appendix C: Calculations

a. Material Balance

i. Process Water and Evaporation of Process Water

Process Water	Seed 1	Seed 2	Seed 3	Proliferation	Differentiation 1	Differentiation 2		
cross sectional area (m2)	2.06E-02	1.76E-01	6.73E-01	1.64E+01	1.03E+01	1.64E+01		
fraction volume water	9.50E-01	9.50E-01	9.50E-01	9.50E-01	9.50E-01	9.50E-01		
Process height (m)	4.86E-01	1.42E+00	2.78E+00	1.37E+01	1.09E+01	1.37E+01		
volume water (m^3)	9.50E-03	2.38E-01	1.78E+00	2.14E+02	1.07E+02	2.14E+02		
mass water needed (kg)	9.50E+00	2.38E+02	1.78E+03	2.14E+05	1.07E+05	2.14E+05		
mass water (lbs)	2.09E+01	5.24E+02	3.92E+03	4.71E+05	2.36E+05	4.71E+05		
# of reactors	1.00E+00	1.00E+00	1.00E+00	2.00E+00	3.00E+00	1.00E+00		
total water (lbs)	2.09E+01	5.24E+02	3.92E+03	9.43E+05	7.07E+05	4.71E+05	**2.36E+06**	**lbs of process water required in tanks per batch**
	8.11E+02	2.03E+04	1.52E+05	3.65E+07	2.74E+07	1.83E+07	9.14E+07	**lbs of process water required in tanks per year**
Evap. Process Water	Seed 1	Seed 2	Seed 3	Proliferation	Differentiation 3	Differentiation 1		
cross sectional area (m2)	2.06E-02	1.76E-01	6.73E-01	1.64E+01	1.03E+01	1.64E+01		
Vapor Pressure (atm)	6.01E-02	6.01E-02	6.01E-02	6.01E-02	6.01E-02	6.01E-02		
Headspace Pressure (atm)	1.00E+00	1.00E+00	1.00E+00	1.00E+00	1.00E+00	1.00E+00		
Headspace height (m)	1.62E-01	4.73E-01	9.26E-01	4.57E+00	3.63E+00	4.57E+00		
volume water (m^3)	2.00E-04	5.01E-03	3.75E-02	4.51E+00	2.25E+00	4.51E+00		
mass water needed (kg)	2.00E-01	5.01E+00	3.75E+01	4.51E+03	2.25E+03	4.51E+03		
mass water (lbs)	4.42E-01	1.10E+01	8.26E+01	9.94E+03	4.97E+03	9.94E+03		
# of reactors	1.00E+00	1.00E+00	1.00E+00	1.00E+00	1.00E+00	1.00E+00		
total water (lbs)	4.42E-01	1.10E+01	8.26E+01	9.94E+03	4.97E+03	9.94E+03		Total water supply rate after evap
water supply rate (lbs/hr)	**2.58E-03**	**6.50E-02**	**5.42E-01**	**3.93E+01**	**6.90E+01**	**1.38E+02**		**8.88E+02** lbs/min
corn supply rate (lbs/min)	**6.19E-08**	**1.56E-06**	**1.30E-05**	**9.43E-04**	**1.66E-03**	**3.31E-03**		6.39E+06 lbs/yr
	3.42E+01	**8.55E+02**	**6.40E+03**	**7.70E+05**	**3.85E+05**	**7.70E+05**		

ii. Bioreactor Charge Requirements

Hydrolysate Charge	Seed 1	Seed 2	Seed 3	Proliferation	Proliferation 2	Proliferation 3	Proliferation 4	Differentiation 1/2	Differentiation 1/2	Differentiation 1/2	Differentiation 1
cells to be made	2.40E+11	6.00E+12	1.06E+14	1.33E+16	6.72E+15	6.72E+15	6.72E+15	6.72E+15	6.72E+15	6.72E+15	1.34E+16
Amount of Soy (lbs)	1.96E-03	4.91E-02	3.67E-01	4.42E+01	4.42E+01	4.42E+01	4.42E+01	2.21E+01	2.21E+01	2.21E+01	4.42E+01
Amount of Corn (lbs)	1.25E-03	3.13E-02	2.34E-01	2.82E+01	1.41E+01	1.41E+01	1.41E+01	1.41E+01	1.41E+01	1.41E+01	2.82E+01
Amount of FBS (lbs)	3.57E-04	8.93E-03	6.68E-02	8.04E+00	6.47E+00	6.47E+00	6.47E+00				
Amount of Insulin (lbs)								1.10E-01	1.10E-01	1.10E-01	2.20E-01
Amount of Lineloic Acid (lbs)								2.35E-01	2.35E-01	2.35E-01	4.71E-01
Amount of Dexamethasone (lbs)								9.24E-02	9.24E-02	9.24E-02	1.85E-01
Amount of Cytodex 1 Beads	1.59E+00	3.96E+01	6.99E+02	8.81E+04	4.44E+04	4.44E+04	4.44E+04				

iii. Bioreactor Feed Requirements

Raw Materials	Seed 1	Seed 2	Seed 3	Proliferation	Proliferation 2	Proliferation 3	Proliferation 4	Differentiation 1/2	Differentiation 1/2	Differentiation 1/2	Differentiation 1
cells to be made	2.40E+11	6.00E+12	1.06E+14	1.33E+16	6.72E+15	6.72E+15	6.72E+15	6.72E+15	6.72E+15	6.72E+15	1.34E+16
Yx/glu (E9 cells/E-3 mol)	4.13E-01	4.13E-01	4.13E-01	4.13E-01	4.13E-01	4.13E-01	4.13E-01				
mglu (E-15 mol/(cell*hr)								6.92E+01	6.92E+01	6.92E+01	6.92E+01
Yx/gln (E9 cells/E-3 mol)	5.73E-01	5.73E-01	5.73E-01	5.73E-01	5.73E-01	5.73E-01	5.73E-01				
a1 for mgln (E-15 mol/(cell*hr)								3.20E+00	3.20E+00	3.20E+00	3.20E+00
a2 for mgln (E-3 mol/L)								2.10E+00	2.10E+00	2.10E+00	2.10E+00
glucose required (mmol)	5.82E+02	1.45E+04	2.56E+05	3.23E+07	1.63E+07	1.63E+07	1.63E+07				
charge glucose (lbs)	2.64E-03	6.61E-02	4.94E-01	5.95E+01	5.95E+01	5.95E+01	5.95E+01	2.97E+01	2.97E+01	2.97E+01	5.95E+01
maintenance glucose required (lbs/hr)	**1.33E-03**	**3.35E-02**	**6.64E-01**	**5.04E+01**	**2.53E+01**	**2.53E+01**	**2.53E+01**	**1.02E+00**	**1.02E+00**	**1.02E+00**	**2.05E+00**
maintenance corn grain hydrolysate requi	**2.62E-03**	**6.60E-02**	**1.31E+00**	**9.93E+01**	**4.98E+01**	**4.98E+01**	**4.98E+01**	**2.02E+00**	**2.02E+00**	**2.02E+00**	**4.03E+00**
overall glucose added after charge (lbs)	**2.28E-01**	**5.70E+00**	**1.01E+02**	**1.27E+04**	**6.40E+03**	**6.40E+03**	**6.40E+03**	**7.37E+01**	**7.37E+01**	**7.37E+01**	**1.47E+02**
glutamine required (mmol)	4.19E+02	1.05E+04	1.85E+05	2.33E+07	1.17E+07	1.17E+07	1.17E+07				
charge glutamine (lbs)	3.87E-04	9.67E-03	7.24E-02	8.71E+00	8.71E+00	8.71E+00	8.71E+00	4.35E+00	4.35E+00	4.35E+00	8.71E+00
maintenance glutamine required (lbs/hr)	**7.86E-04**	**1.98E-02**	**3.89E-01**	**2.96E+01**	**1.49E+01**	**1.49E+01**	**1.49E+01**	**5.13E+00**	**5.13E+00**	**5.13E+00**	**1.03E+01**
maintenance soybean hydrolysate (lbs/hr)	**3.99E-03**	**1.00E-01**	**1.98E+00**	**1.50E+02**	**7.56E+01**	**7.56E+01**	**7.56E+01**	**2.60E+01**	**2.60E+01**	**2.60E+01**	**5.21E+01**
overall glutamine added after charge (lbs)	**1.35E-01**	**3.36E+00**	**5.93E+01**	**7.48E+03**	**3.77E+03**	**3.77E+03**	**3.77E+03**	**3.69E+02**	**3.69E+02**	**3.69E+02**	**7.38E+02**

b. k_La requirement for Oxygen Transfer Rate and Sparge Gas Rate

To determine the mass transfer coefficient (k_La) for each bioreactor, the initial step is to establish a relationship between oxygen transfer rate (OTR), k_La and the concentration of dissolved oxygen in the liquid and gas phase. Modification of Fick's first law of diffusion gives the desired relation.

Fick's First Law of Diffusion:

$$J = - D_{i/media} * \Delta C_i/\Delta X \quad (1)$$

Where, J = Diffusion flux

$D_{i/media}$ = Diffusion coefficient of species i in media

$\Delta C_i/\Delta X$ = concentration gradient of species i

For flux of oxygen through the air bubble, equation 1 can be modified by replacing $D_{o2/media}/\Delta X$ with $k_La_{O2/media}$ such that ΔX is considered the boundary layer thickness around the air bubble. With this modification, the updated equation yields

$$OTR = k_La_{O2} * (C_{O2/gas} - C_{o2/liq}) = k_La_{O2} * (P_{O2/gas} - P_{o2/liq}) * H_{O2/media} \quad (2)$$

where, $P_{O2/gas}$ and $P_{O2/liq}$ = pressure of dissolved oxygen in the gas and liquid

$H_{O2/media}$ = solubility of oxygen in media

On the other hand, oxygen uptake rate (OUR) is given by the specific oxygen uptake rate (sOUR) of a cell and the peak viable cell density (VCD) at which the cells can grow without complication.

$$OUR = sOUR * VCD \quad (3)$$

By equating OUR and OTR and isolating K_La, the desired equation for K_La requirement is obtained as shown by equation 4.

$$k_La_{O2/media} = \frac{sOUR * VCD}{(P_{O2/gas} - P_{o2/liq})*H_{O2/media}} \quad (4)$$

Once the K_La requirement is obtained for all the bioreactors, a correlation developed by Zedníková et al is used to obtain the gas holdup (ε_G) in a bubble column based on required K_La. The gas holdup then gives the superficial velocity of the sparge gas (u_G) in the reactor. Using the cross sectional area of the bioreactor and the calculated u_G one can obtain the volumentrc sparge gas rate to meet the OTR requirements.

Correlation	Operating Conditions
$\varepsilon_G = 0.765u_G^{0.603}$	$D = 0.19$m, $u_G = 20$–200 mm/s
$k_La = 1.144u_G^{0.943}$	1 m clear liquid height perforated plate
$k_La = 1.723\varepsilon_G^{1.561}$	pure heterogeneous flow regime T = 25 °C, atmospheric pressure

Figure C.1. Correlations used to the sparge gas rate calculation (Zedníková et al. 2018)

Table C.1. Example Calculation for K_La Requirment and Sparge Gas Rate

Seed reactor 3 - KLa estimation		
Inputs	Units	Values w/ 21 mol% O2
VCD	x 10^6 cells/mL	60
Dissolved O2 set point	% sauration O2	10
p_o2/liq	mmHg O2	15.9
% O2 in sparge gas	mol % O2	21
sOUR, qo2	pmol O2/cell/hr	0.2
OUR = OTR	mmol O2/L/hr	12
H_o2/media	g O2/L/760 mmHg O2	0.033
H_o2/media	mmol O2/L/mmHg O2	0.001
O2 KLa required	1/hr	61.6
For Seed - Gas sparge rate estimate for bubble column		
Inputs	Units	Values (21% O2)
O2 KLa required	1/hr	61.6
O2 KLa required	1/sec	0.017
Vessel I. D.	m	0.162
Vessel cross sectional area	m^2	0.021
eps_g, Gas holdup	vol gas/vol liq	0.052
Ug, Sparge gas superficial velocity	m/s	0.012
Ug, Sparge gas superficial velocity	m/min	0.697
Sparge gas rate	standard L/min	14

The carbon dioxide production for the process was calculated using mass balance and a 1:1 mole ratio between O_2 and CO_2 from the aerobic respiration equation. Table C.2 summarizes the calculation for yearly CO_2 production rate.

Table C.2. Summary of Yearly CO_2 Production Rate

Total O2 added to process	16,613,511	lb/yr
Total O2 consumed or CO2 produced	7,670	L/min
Total O2 consumed or CO2 produced	**9,443,238**	**lb/yr**
Total NO2 in outlet	**50,174**	**lb/yr**
Total O2 left in outlet	**7,170,273**	**lb/yr**

c. Compressor Energy Requirement Calculation

Table C.3. Example Calculation for Work Required to Supply Air

Seed reactor 1 - Electric work required		
Variable	**Values**	**Unit**
Density of Air at STP	1.2	kg/m^3
Specific volume	0.8	m^3/kg
Sparge gas rate	14.5	L/min
Sparge gas rate	0.0	m^3/s
VCD	25000000.0	cells/mL
Density of cell	25.0	$cells/m^3$
Reactor vol	0.014	m^3
Working vol of bioreactor	0.010	m^3
Total cells	0.3	cells
Mass of cells	3.16E-11	kg
Density of water	1000.0	kg/m^3
Mass of water	10.1	kg

Total mass in bioreactor	10.1	kg
Total density in bioreactor	750.0	kg/m^3
Gravitational constant	9.8	m/s^2
Bioreactor process height	0.5	m
Inlet Pressure, P1	101325	Pa
P2 - P1	3588	$kg/m*s^2$
Outlet pressure, P2	104913	Pa
Work required	0.9	J/s

d. CIP/SIP Calculation

Upstream

	Diameter (m)	Height (m)	Surface Area (m2)	Volume (m3)	Number	Total Mass (kg)	J to 121 C	kg steam to 121 C	heat loss (J/s)	kg steam for 15 min	Total Steam per Batch (kg)	
Seed 1	1.62E-01	6.48E-01	3.71E-01	3.71E-04	1	2.96E+00	1.25E+05	4.74E-02	6.34E+03	2.16E+00	2.21E+00	1.88E+02
Seed 2	4.73E-01	1.89E+00	3.17E+00	3.17E-03	1	2.53E+01	1.07E+06	4.05E-01	5.42E+04	1.85E+01	1.89E+01	1.61E+03
Seed 3	9.26E-01	3.70E+00	1.21E+01	1.21E-02	1	9.69E+01	4.10E+06	1.55E+00	2.07E+05	7.06E+01	7.21E+01	6.14E+03
FP/Full-size Diff	4.57E+00	1.83E+01	2.95E+02	2.95E-01	3	2.36E+03	9.98E+07	3.77E+01	5.05E+06	1.72E+03	1.76E+03	4.99E+04
Half-size Diff	3.63E+00	1.45E+01	1.86E+02	1.86E-01	3	1.49E+03	6.29E+07	2.38E+01	3.18E+06	1.08E+03	1.11E+03	3.14E+04

Saturated steam	https://www.engineer			Batches per year	38.72237402		boiler feed water: $2.00/1000 gal		50 psig steam:$13.20/1000 kg		2.96E+03	
pressure (psig)	15											
temp (F)	179		SIP		**Steam per Year**	114,542.15	kg/yr	**1,511.96**	**$/yr**			
temp (K)	355				**Hot Water**	100,074.10	gal/yr	**200.15**	**$/yr**	834,618.02	lb/yr	
enthalpy (Btu/lb)	1137		CIP	need x10 at startup	**Water for NaOH**	10,007.41	gal/yr	**20.01**	**$/yr**	83,461.80	lb/yr	
enthalpy (J/kg)	2.64E+06				**NaOH (dry powder)**	**3,964.47**	**$/yr**			1,693.56	lb/yr	
					96% H2SO4	**58.15**	**$/yr**			3.46	lb/yr	

GammaJet Models												
Vessel Size	**Flow Rate**	**Cycle Time**										
250-1250 m3	8-31 m3/hr	8-12 min	FP/full diff/half diff		1 m3 = 260 gal							
500-30,000 L	2-8 m3/hr	5-10 min	seed 3									
up to 0.76 m	0.5-2 m3/hr	2-5 min	seed 1/2									

	flow rate (m3/hr)	flow (m3/min)	cycle time (min)	m3/cleaner/batch	**gal/cleaner/batch**	m3/cleaner/yr	**gal/cleaner/yr**	**CIP time (min/batch)**			
Seed 1 (.16 m)	.8 (interpolating)	0.013	5	0.065	16.9	2.516954311	654.4081209	15			
Seed 2 (.47 m)	1.4 (interp)	0.023	5	0.115	29.9	4.453073012	1157.798983	15		96% H2SO4: $37/kg	
Seed 3 (2500 L)	2.4 (interp)	0.04	10	0.4	104	15.48894961	4027.126898	30		NaOH: $5.15/kg	
FP/Full-size Diff (3	8	0.13	12	4.68	1216.8	181.2207104	47117.3847	36			
Half-size Diff (150	8	0.13	12	4.68	1216.8	181.2207104	47117.3847	36			

	hot H2O gal/yr	**2% NaOH gal/yr**	**NaOH kg/yr**	NaOH mol/yr	H2SO4 mol/yr	96% H2SO4 kg/yr	**96% H2SO4 $/yr**
Seed 1	654.4081209	65.44081209	5.033908622	0.2013563449	0.1006781724	0.01027756344	0.3802698472
Seed 2	1157.798983	115.7798983	8.906146024	0.3562458409	0.1781229205	0.01818338147	0.6727851142
Seed 3	4027.126898	402.7126898	30.97789921	1.239115969	0.6195579843	0.06324654423	2.340122136
FP/Full-size Diff	47117.3847	4711.73847	362.4414208	14.49765683	7.248828416	0.7399845675	27.379429
Half-size Diff	47117.3847	4711.73847	362.4414208	14.49765683	7.248828416	0.7399845675	27.379429

Downstream

	kg Steam per Batch	steam lb/batch	steam lb/yr								
STR	5.86E+02	1.29E+03	4.99E+04								
Saturated steam	https://www.engineer			Batches per year	38.72237402		boiler feed water: $2.00/1000 gal		50 psig steam:$13.20/1000 kg		
pressure (psig)	15										
temp (F)	179		SIP		**Steam per Year**	49,910.22	lb/yr	299.46	$/yr		
temp (K)	355				**Hot Water**	130,116.47	gal/yr	260.23	$/yr	1,085,171.36	lb/yr
enthalpy (Btu/lb)	1137			need x10 at startup	**Water for NaOH**	13,011.65	gal/yr	26.02	$/yr	108,517.14	lb/yr
enthalpy (J/kg)	2.64E+06		CIP		**NaOH (dry powder)**	2,201.97	lb/yr	24,948.33	$/yr		
					96% H2SO4	4.50	lb/yr	365.95	$/yr		
					water for PAA	1,071,064.13	lb/yr	256.85	$/yr		
					15% PAA	14,468.95	lb/yr	32,962.84	$/yr		
GammaJet Models											
Vessel Size	**Flow Rate**	**Cycle Time**									
250-1250 m3	8-31 m3/hr	8-12 min	FP/full diff/half diff		1 m3 = 260 gal						
500-30,000 L	2-8 m3/hr	5-10 min	seed 3		1 gal water = 8.34 lb						
up to 0.76 m	0.5-2 m3/hr	2-5 min	seed 1/2								
19k-95k L	3.5-19 m3/hr	8-20 min									
	flow rate (m3/hr)	flow (m3/min)	cycle time (min)	m3/cleaner/batch	**gal/cleaner/batch**	m3/cleaner/yr	**gal/cleaner/yr**	**CIP time (min/batch)**			
6 extruders (100 m3)	4	0.067	12	4.824	1254.24	186.7967323	48567.15039	36			
2 DSC (225 m3)	8	0.13	12	3.12	811.2	120.8138069	31411.5898	36		96% H2SO4: $37/kg	
2 tanks (400 m3/110k	10	0.17	12	4.08	1060.8	157.987286	41076.69436	36		NaOH: $5.15/kg	
STR (75m3)	3	0.05	12	0.6	156	23.23342441	6040.690347	36		15% PAA: $19/gal	
RDV (2 ft)	.6 (interp)	0.01	5	0.05	13	1.936118701	503.3908622	15			
mixer (30 m3)	1.5	0.025	10	0.25	65	9.680593504	2516.954311	30			
	hot H2O gal/yr	**2% NaOH gal/yr**	**NaOH kg/yr**	NaOH mol/yr	H2SO4 mol/yr	96% H2SO4 kg/yr	**96% H2SO4 $/yr**	0.2% PAA gal/yr	15% PAA gal/yr	15% PAA $/yr	
6 extruders (100 m3)	48567.15039	4856.715039	373.5934645	14.94373858	7.47186929	0.7627533234	28.22187296	48567.15039	647.5620051	12303.6781	
2 DSC (225 m3)	31411.5898	3141.15898	241.6276139	9.665104554	4.832552277	0.493323045	18.25295266	31411.5898	418.8211974	7957.60275	
2 tanks (400 m3/110k	41076.69436	4107.669436	315.974572	12.63898288	6.319491439	0.6451147511	23.86924579	41076.69436	547.6892581	10406.0959	
STR (75m3)	6040.690347	604.0690347	46.46684882	1.858673953	0.9293369764	0.09486981634	3.510183205	6040.690347	80.54253795	1530.308221	
RDV (2 ft)	503.3908622	50.33908622	3.872237402	0.1548894961	0.07744474803	0.007905818028	0.292515267	503.3908622	6.711878163	127.5256851	
mixer (30 m3)	2516.954311	251.6954311	19.36118701	0.7744474803	0.3872237402	0.03952909014	1.462576335	2516.954311	33.55939081	637.6284255	

Appendix D: Full Clean-in-Place Specifications

	Virgin Hot Water (gal/yr)	2% NaOH (gal/yr)	15 psig Steam (lb/yr)	0.2% Peracetic Acid (gal/yr)
SR 1	650	65	190	-
SR 2	1,200	120	16,000	-
SR 3	4,000	400	6,100	-
FPR 1&2, DR 1 (each)	16,000	1,600	50,000	-
DR 2-4 (each)	16,000	1,600	31,000	-
STR	6,000	600	13,000	-
DSC 1&2 (each)	16,000	1,600	-	16,000
Rotary Drum	500	50	-	500
Mixer	6,200	620	-	6,200
Ext 1-6 (each)	8,100	810	-	8,100
Storage Tanks (each)	21,000	2,100	-	21,000

Appendix E: Full Cold Room Refrigeration Specifications

Room temperature	-12.00	°C
Temp. difference inside / outside room	37.00	°C
Ventilation loss factor	Normal	
Running time installation	24	Hrs/day
Length x Width x Height	15.00 x 10.00 x 10.00	m
Room volume	1500.00	m^3
Insulation material	Polystyrene	
Thickness	150.00	mm
Floor insulation	0.00	mm
K-value insulation panel	0.23	W/m^2K
Cooler fans	250.00 / 24	Watt / Hrs/day
Illumination	15.00 / 24	Watt / Hrs/day
Persons	2 / 24	number / Hrs/day
Other heat sources	0.00 / 0	Watt / Hrs/day
Products	Meat minced meat	
Storage quantity	160000.00	kg
Stock shift	1500.00	kg
Entering temperature	36.50	°C
Cool down/congel. time	6	hr
Heat loads		
Transmission losses	12640	Watt
Ventilation losses	5906	Watt
Other heat sources	3100	Watt
Cooling down/congel.	26516	Watt
Respiration	0	Watt
Subtotal	48162	Watt
With a running time per day of 24 hrs the total required cooling capacity is 48.2 kW		
The specific capacity is	32.1	W/m^3

Appendix F: Safety Data Sheets (SDS)

SAFETY DATA SHEET

Dexamethasone Solid Formulation

Version	Revision Date:	SDS Number:	Date of last issue: 04/09/2021
2.6	08/27/2021	2533225-00009	Date of first issue: 02/23/2018

SECTION 1. IDENTIFICATION

Product name : Dexamethasone Solid Formulation
Other means of identification : No data available

Manufacturer or supplier's details

Company name of supplier : Merck & Co., Inc
Address : 2000 Galloping Hill Road
Kenilworth - New Jersey - U.S.A. 07033
Telephone : 908-740-4000
Emergency telephone : 1-908-423-6000
E-mail address : EHSDATASTEWARD@merck.com

Recommended use of the chemical and restrictions on use

Recommended use : Veterinary product

Restrictions on use : Not applicable

SECTION 2. HAZARDS IDENTIFICATION

GHS classification in accordance with the Hazardous Products Regulations

Reproductive toxicity : Category 1B

GHS label elements

Hazard pictograms :

Signal Word : Danger

Hazard Statements : H360D May damage the unborn child.

Precautionary Statements :

Prevention:

P201 Obtain special instructions before use.
P202 Do not handle until all safety precautions have been read and understood.
P280 Wear protective gloves, protective clothing, eye protection and face protection.

Response:

P308 + P313 IF exposed or concerned: Get medical attention.

Storage:

P405 Store locked up.

Disposal:

P501 Dispose of contents and container to an approved waste disposal plant.

Other hazards

Dust contact with the eyes can lead to mechanical irritation.
Contact with dust can cause mechanical irritation or drying of the skin.
May form explosive dust-air mixture during processing, handling or other means.

SECTION 3. COMPOSITION/INFORMATION ON INGREDIENTS

Substance / Mixture : Mixture

Components

Chemical name	Common Name/Synonym	CAS-No.	Concentration (% w/w)
Starch	Sago starch	9005-25-8	>= 30 - < 60 *
Dexamethasone	No data available	50-02-2	>= 0.1 - < 1 *

* Actual concentration or concentration range is withheld as a trade secret

SECTION 4. FIRST AID MEASURES

General advice : In the case of accident or if you feel unwell, seek medical advice immediately.
When symptoms persist or in all cases of doubt seek medical advice.

If inhaled : If inhaled, remove to fresh air.
Get medical attention.

In case of skin contact : In case of contact, immediately flush skin with soap and plenty of water.
Remove contaminated clothing and shoes.
Get medical attention.
Wash clothing before reuse.
Thoroughly clean shoes before reuse.

In case of eye contact : If in eyes, rinse well with water.
Get medical attention if irritation develops and persists.

If swallowed : If swallowed, DO NOT induce vomiting.
Get medical attention.
Rinse mouth thoroughly with water.

Most important symptoms and effects, both acute and delayed : May damage the unborn child.
Contact with dust can cause mechanical irritation or drying of the skin.
Dust contact with the eyes can lead to mechanical irritation.

Protection of first-aiders : First Aid responders should pay attention to self-protection, and use the recommended personal protective equipment when the potential for exposure exists (see section 8).

Notes to physician : Treat symptomatically and supportively.

SECTION 5. FIRE-FIGHTING MEASURES

Suitable extinguishing media : Water spray
Alcohol-resistant foam
Carbon dioxide (CO2)
Dry chemical

Unsuitable extinguishing media : None known.

Specific hazards during fire fighting	:	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. Exposure to combustion products may be a hazard to health.
Hazardous combustion products	:	Carbon oxides
Specific extinguishing methods	:	Use extinguishing measures that are appropriate to local circumstances and the surrounding environment. Use water spray to cool unopened containers. Remove undamaged containers from fire area if it is safe to do so. Evacuate area.
Special protective equipment for fire-fighters	:	In the event of fire, wear self-contained breathing apparatus. Use personal protective equipment.

SECTION 6. ACCIDENTAL RELEASE MEASURES

Personal precautions, protective equipment and emergency procedures	:	Use personal protective equipment. Follow safe handling advice (see section 7) and personal protective equipment recommendations (see section 8).
Environmental precautions	:	Avoid release to the environment. Prevent further leakage or spillage if safe to do so. Retain and dispose of contaminated wash water. Local authorities should be advised if significant spillages cannot be contained.
Methods and materials for containment and cleaning up	:	Sweep up or vacuum up spillage and collect in suitable container for disposal. Avoid dispersal of dust in the air (i.e., clearing dust surfaces with compressed air). 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. Local or national regulations may apply to releases and disposal of this material, as well as those materials and items employed in the cleanup of releases. You will need to determine which regulations are applicable. Sections 13 and 15 of this SDS provide information regarding certain local or national requirements.

SECTION 7. HANDLING AND STORAGE

Technical measures	:	Static electricity may accumulate and ignite suspended dust causing an explosion. Provide adequate precautions, such as electrical grounding and bonding, or inert atmospheres.
Local/Total ventilation	:	If sufficient ventilation is unavailable, use with local exhaust ventilation.
Advice on safe handling	:	Do not get on skin or clothing. Do not breathe dust. Do not swallow.

Avoid contact with eyes.
Handle in accordance with good industrial hygiene and safety practice, based on the results of the workplace exposure assessment
Keep container tightly closed.
Minimize dust generation and accumulation.
Keep container closed when not in use.
Keep away from heat and sources of ignition.
Take precautionary measures against static discharges.
Take care to prevent spills, waste and minimize release to the environment.

Conditions for safe storage : Keep in properly labeled containers.
Keep tightly closed.
Store in accordance with the particular national regulations.

Materials to avoid : Do not store with the following product types:
Strong oxidizing agents
Organic peroxides
Explosives
Gases

SECTION 8. EXPOSURE CONTROLS/PERSONAL PROTECTION

Ingredients with workplace control parameters

Components	CAS-No.	Value type (Form of exposure)	Control parameters / Permissible concentration	Basis
Starch	9005-25-8	TWA	10 mg/m³	CA AB OEL
		TWA (Total dust)	10 mg/m³	CA BC OEL
		TWA (respirable dust fraction)	3 mg/m³	CA BC OEL
		TWAEV (total dust)	10 mg/m³	CA QC OEL
		TWA	10 mg/m³	ACGIH
Dexamethasone	50-02-2	TWA	10 µg/m3 (OEB 3)	Internal
	Further information: Skin			
		Wipe limit	100 µg/100 cm²	Internal

Engineering measures : All engineering controls should be implemented by facility design and operated in accordance with GMP principles to protect products, workers, and the environment.
Containment technologies suitable for controlling compounds are required to control at source and to prevent migration of the compound to uncontrolled areas (e.g., open-face containment devices).
Minimize open handling.

Personal protective equipment

Respiratory protection : If adequate local exhaust ventilation is not available or exposure assessment demonstrates exposures outside the recommended guidelines, use respiratory protection.

Filter type : Particulates type

Hand protection

Material	:	Chemical-resistant gloves
Remarks	:	Consider double gloving.
Eye protection	:	Wear safety glasses with side shields or goggles. If the work environment or activity involves dusty conditions, mists or aerosols, wear the appropriate goggles. Wear a faceshield or other full face protection if there is a potential for direct contact to the face with dusts, mists, or aerosols.
Skin and body protection	:	Work uniform or laboratory coat. Additional body garments should be used based upon the task being performed (e.g., sleevelets, apron, gauntlets, disposable suits) to avoid exposed skin surfaces. Use appropriate degowning techniques to remove potentially contaminated clothing.
Hygiene measures	:	If exposure to chemical is likely during typical use, provide eye flushing systems and safety showers close to the working place. When using do not eat, drink or smoke. Wash contaminated clothing before re-use. The effective operation of a facility should include review of engineering controls, proper personal protective equipment, appropriate degowning and decontamination procedures, industrial hygiene monitoring, medical surveillance and the use of administrative controls.

SECTION 9. PHYSICAL AND CHEMICAL PROPERTIES

Appearance	:	powder
Color	:	white
Odor	:	No data available
Odor Threshold	:	No data available
pH	:	No data available
Melting point/freezing point	:	No data available
Initial boiling point and boiling range	:	No data available
Flash point	:	Not applicable
Evaporation rate	:	Not applicable
Flammability (solid, gas)	:	May form explosive dust-air mixture during processing, handling or other means.
Flammability (liquids)	:	No data available
Upper explosion limit / Upper	:	No data available

flammability limit		
Lower explosion limit / Lower flammability limit	:	No data available
Vapor pressure	:	Not applicable
Relative vapor density	:	Not applicable
Relative density	:	No data available
Density	:	No data available
Solubility(ies)		
Water solubility	:	No data available
Partition coefficient: n-octanol/water	:	Not applicable
Autoignition temperature	:	No data available
Decomposition temperature	:	No data available
Viscosity		
Viscosity, kinematic	:	Not applicable
Explosive properties	:	Not explosive
Oxidizing properties	:	The substance or mixture is not classified as oxidizing.
Molecular weight	:	No data available
Particle size	:	No data available

SECTION 10. STABILITY AND REACTIVITY

Reactivity	:	Not classified as a reactivity hazard.
Chemical stability	:	Stable under normal conditions.
Possibility of hazardous reactions	:	May form explosive dust-air mixture during processing, handling or other means. Can react with strong oxidizing agents.
Conditions to avoid	:	Heat, flames and sparks. Avoid dust formation.
Incompatible materials	:	Oxidizing agents
Hazardous decomposition products	:	No hazardous decomposition products are known.

SECTION 11. TOXICOLOGICAL INFORMATION

Information on likely routes of exposure

Inhalation
Skin contact

Ingestion
Eye contact

Acute toxicity
Not classified based on available information.

Components:

Starch:

Acute oral toxicity	:	LD50 (Rat): > 5,000 mg/kg
Acute dermal toxicity	:	LD50 (Rabbit): > 2,000 mg/kg

Dexamethasone:

Acute oral toxicity	:	LD50 (Rat): > 2,000 mg/kg
		LD50 (Mouse): > 6,500 mg/kg
Acute toxicity (other routes of administration)	:	LD50 (Rat): 14 mg/kg Application Route: Subcutaneous

Skin corrosion/irritation
Not classified based on available information.

Components:

Dexamethasone:

Species	:	Rabbit
Result	:	Mild skin irritation

Serious eye damage/eye irritation
Not classified based on available information.

Components:

Starch:

Species	:	Rabbit
Result	:	No eye irritation

Dexamethasone:

Species	:	Rabbit
Result	:	Mild eye irritation

Respiratory or skin sensitization

Skin sensitization
Not classified based on available information.

Respiratory sensitization
Not classified based on available information.

Components:

Starch:

Test Type	:	Maximization Test
Routes of exposure	:	Skin contact
Species	:	Guinea pig
Result	:	negative

Germ cell mutagenicity

Not classified based on available information.

Components:

Starch:

Genotoxicity in vitro	:	Test Type: Bacterial reverse mutation assay (AMES) Result: negative

Dexamethasone:

Genotoxicity in vitro	:	Test Type: Bacterial reverse mutation assay (AMES) Result: negative Test Type: in vitro test Test system: mouse lymphoma cells Result: negative
Genotoxicity in vivo	:	Test Type: Micronucleus test Species: Mouse Application Route: Oral Result: negative

Carcinogenicity

Not classified based on available information.

Reproductive toxicity

May damage the unborn child.

Components:

Dexamethasone:

Effects on fetal development	:	Test Type: Development Species: Mouse Application Route: Subcutaneous Developmental Toxicity: LOAEL: 6 mg/kg body weight Result: Specific developmental abnormalities., Cleft palate Species: Rabbit Application Route: Intramuscular Developmental Toxicity: NOAEL: 0.025 mg/kg body weight Result: Specific developmental abnormalities. Species: Rabbit Application Route: Intramuscular Developmental Toxicity: LOAEL: >= 0.062 mg/kg body weight Result: Specific developmental abnormalities.

Species: Rat
Application Route: Subcutaneous
Developmental Toxicity: LOAEL: >= 0.02 mg/kg body weight
Result: Skeletal and visceral variations ., Retardations.

Reproductive toxicity - Assessment : May damage the unborn child.

STOT-single exposure

Not classified based on available information.

STOT-repeated exposure

Not classified based on available information.

Components:

Dexamethasone:

Routes of exposure	:	Oral
Target Organs	:	Adrenal gland, Immune system, thymus gland
Assessment	:	May cause damage to organs through prolonged or repeated exposure.

Repeated dose toxicity

Components:

Starch:

Species	:	Rat
NOAEL	:	>= 2,000 mg/kg
Application Route	:	Skin contact
Exposure time	:	28 Days
Method	:	OECD Test Guideline 410

Dexamethasone:

Species	:	Rat
NOAEL	:	0.0015 mg/kg
Application Route	:	Oral
Exposure time	:	7 d
Target Organs	:	Liver
Remarks	:	Significant toxicity observed in testing

Species	:	Rat
LOAEL	:	0.003 mg/kg
Application Route	:	Oral
Exposure time	:	90 d
Target Organs	:	Blood, Adrenal gland, thymus gland
Remarks	:	Significant toxicity observed in testing

Species	:	Rat
LOAEL	:	0.125 mg/kg
Application Route	:	Oral
Exposure time	:	6 Weeks
Target Organs	:	Adrenal gland
Remarks	:	Significant toxicity observed in testing

Species: Rat
Application Route: Subcutaneous
Developmental Toxicity: LOAEL: >= 0.02 mg/kg body weight
Result: Skeletal and visceral variations ., Retardations.

Reproductive toxicity - Assessment : May damage the unborn child.

STOT-single exposure

Not classified based on available information.

STOT-repeated exposure

Not classified based on available information.

Components:

Dexamethasone:

Routes of exposure	:	Oral
Target Organs	:	Adrenal gland, Immune system, thymus gland
Assessment	:	May cause damage to organs through prolonged or repeated exposure.

Repeated dose toxicity

Components:

Starch:

Species	:	Rat
NOAEL	:	>= 2,000 mg/kg
Application Route	:	Skin contact
Exposure time	:	28 Days
Method	:	OECD Test Guideline 410

Dexamethasone:

Species	:	Rat
NOAEL	:	0.0015 mg/kg
Application Route	:	Oral
Exposure time	:	7 d
Target Organs	:	Liver
Remarks	:	Significant toxicity observed in testing

Species	:	Rat
LOAEL	:	0.003 mg/kg
Application Route	:	Oral
Exposure time	:	90 d
Target Organs	:	Blood, Adrenal gland, thymus gland
Remarks	:	Significant toxicity observed in testing

Species	:	Rat
LOAEL	:	0.125 mg/kg
Application Route	:	Oral
Exposure time	:	6 Weeks
Target Organs	:	Adrenal gland
Remarks	:	Significant toxicity observed in testing

Species	:	Rat
LOAEL	:	0.4 mg/kg
Application Route	:	Oral
Exposure time	:	3 Months
Target Organs	:	Immune system
Remarks	:	Significant toxicity observed in testing

Species	:	Dog
LOAEL	:	8 mg/kg
Application Route	:	Oral
Exposure time	:	3 Months
Target Organs	:	Immune system
Remarks	:	Significant toxicity observed in testing

Aspiration toxicity

Not classified based on available information.

Experience with human exposure

Components:

Dexamethasone:

Ingestion	:	Target Organs: Immune system Target Organs: Adrenal gland Target Organs: Bone Symptoms: muscle weakness

SECTION 12. ECOLOGICAL INFORMATION

Ecotoxicity

Components:

Dexamethasone:

Toxicity to daphnia and other aquatic invertebrates	:	EC50 (Daphnia magna (Water flea)): > 56 mg/l Exposure time: 48 h Method: OECD Test Guideline 202
Toxicity to algae/aquatic plants	:	EC50 (Pseudokirchneriella subcapitata (green algae)): > 9.2 mg/l Exposure time: 72 h Method: OECD Test Guideline 201 NOEC (Pseudokirchneriella subcapitata (green algae)): 9.2 mg/l Exposure time: 72 h Method: OECD Test Guideline 201
Toxicity to fish (Chronic toxicity)	:	NOEC (Pimephales promelas (fathead minnow)): 0.033 mg/l Exposure time: 32 d Method: OECD Test Guideline 210
Toxicity to microorganisms	:	EC50: > 1,000 mg/l Exposure time: 3 h

Test Type: Respiration inhibition
Method: OECD Test Guideline 209

NOEC: 1,000 mg/l
Exposure time: 3 h
Test Type: Respiration inhibition
Method: OECD Test Guideline 209

Persistence and degradability

Components:

Dexamethasone:

Biodegradability : Result: Not readily biodegradable.
Biodegradation: 50 %
Exposure time: 3.54 d
Method: OECD Test Guideline 314

Bioaccumulative potential

Components:

Dexamethasone:

Partition coefficient: n-octanol/water : log Pow: 1.83

Mobility in soil

No data available

Other adverse effects

No data available

SECTION 13. DISPOSAL CONSIDERATIONS

Disposal methods

Waste from residues : Dispose of in accordance with local regulations.
Contaminated packaging : Empty containers should be taken to an approved waste handling site for recycling or disposal.
If not otherwise specified: Dispose of as unused product.

SECTION 14. TRANSPORT INFORMATION

International Regulations

UNRTDG
Not regulated as a dangerous good

IATA-DGR
Not regulated as a dangerous good

IMDG-Code
Not regulated as a dangerous good

Transport in bulk according to Annex II of MARPOL 73/78 and the IBC Code
Not applicable for product as supplied.

Domestic regulation

TDG
Not regulated as a dangerous good

Special precautions for user
Not applicable

SECTION 15. REGULATORY INFORMATION

The ingredients of this product are reported in the following inventories:

AICS	:	not determined
DSL	:	not determined
IECSC	:	not determined

SECTION 16. OTHER INFORMATION

Full text of other abbreviations

ACGIH	:	USA. ACGIH Threshold Limit Values (TLV)
CA AB OEL	:	Canada. Alberta, Occupational Health and Safety Code (table 2: OEL)
CA BC OEL	:	Canada. British Columbia OEL
CA QC OEL	:	Québec. Regulation respecting occupational health and safety, Schedule 1, Part 1: Permissible exposure values for airborne contaminants
ACGIH / TWA	:	8-hour, time-weighted average
CA AB OEL / TWA	:	8-hour Occupational exposure limit
CA BC OEL / TWA	:	8-hour time weighted average
CA QC OEL / TWAEV	:	Time-weighted average exposure value

AIIC - Australian Inventory of Industrial Chemicals; ANTT - National Agency for Transport by Land of Brazil; ASTM - American Society for the Testing of Materials; bw - Body weight; CMR - Carcinogen, Mutagen or Reproductive Toxicant; DIN - Standard of the German Institute for Standardisation; DSL - Domestic Substances List (Canada); ECx - Concentration associated with x% response; ELx - Loading rate associated with x% response; EmS - Emergency Schedule; ENCS - Existing and New Chemical Substances (Japan); ErCx - Concentration associated with x% growth rate response; ERG - Emergency Response Guide; GHS - Globally Harmonized System; GLP - Good Laboratory Practice; IARC - International Agency for Research on Cancer; IATA - International Air Transport Association; IBC - International Code for the Construction and Equipment of Ships carrying Dangerous Chemicals in Bulk; IC50 - Half maximal inhibitory concentration; ICAO - International Civil Aviation Organization; IECSC - Inventory of Existing Chemical Substances in China; IMDG - International Maritime Dangerous Goods; IMO - International Maritime Organization; ISHL - Industrial Safety and Health Law (Japan); ISO - International Organisation for Standardization; KECI - Korea Existing Chemicals Inventory; LC50 - Lethal Concentration to 50 % of a test population; LD50 - Lethal Dose to 50% of a test population (Median Lethal Dose); MARPOL - International Convention for the Prevention of Pollution from Ships; n.o.s. - Not Otherwise Specified; Nch - Chilean Norm; NO(A)EC - No Observed (Adverse) Effect Concentration; NO(A)EL - No Observed (Adverse) Effect Level; NOELR - No Observable Effect Loading Rate; NOM - Official Mexican Norm; NTP - National Toxicology Program; NZIoC - New Zealand Inventory of Chemicals; OECD - Organization for Economic Co-operation and Develop-

ment; OPPTS - Office of Chemical Safety and Pollution Prevention; PBT - Persistent, Bioaccumulative and Toxic substance; PICCS - Philippines Inventory of Chemicals and Chemical Substances; (Q)SAR - (Quantitative) Structure Activity Relationship; REACH - Regulation (EC) No 1907/2006 of the European Parliament and of the Council concerning the Registration, Evaluation, Authorisation and Restriction of Chemicals; SADT - Self-Accelerating Decomposition Temperature; SDS - Safety Data Sheet; TCSI - Taiwan Chemical Substance Inventory; TDG - Transportation of Dangerous Goods; TECI - Thailand Existing Chemicals Inventory; TSCA - Toxic Substances Control Act (United States); UN - United Nations; UNRTDG - United Nations Recommendations on the Transport of Dangerous Goods; vPvB - Very Persistent and Very Bioaccumulative; WHMIS - Workplace Hazardous Materials Information System

Sources of key data used to compile the Material Safety Data Sheet	:	Internal technical data, data from raw material SDSs, OECD eChem Portal search results and European Chemicals Agency, http://echa.europa.eu/
Revision Date	:	08/27/2021
Date format	:	mm/dd/yyyy

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 is designed only as a guidance for safe handling, use, processing, storage, transportation, disposal and release and shall not be considered a warranty or quality specification of any type. The information provided relates only to the specific material identified at the top of this SDS and may not be valid when the SDS material is used in combination with any other materials or in any process, unless specified in the text. Material users should review the information and recommendations in the specific context of their intended manner of handling, use, processing and storage, including an assessment of the appropriateness of the SDS material in the user's end product, if applicable.

CA / Z8

SAFETY DATA SHEET

Version 6.2
Revision Date 04/15/2022
Print Date 04/16/2022

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

1.1 Product identifiers

Product name : Dextranase, from *Chaetomium erraticum*

Product Number : D0443
Brand : Sigma
CAS-No. : 9025-70-1

1.2 Relevant identified uses of the substance or mixture and uses advised against

Identified uses : Laboratory chemicals, Synthesis of substances

1.3 Details of the supplier of the safety data sheet

Company : Sigma-Aldrich Inc.
3050 SPRUCE ST
ST. LOUIS MO 63103
UNITED STATES

Telephone : +1 314 771-5765
Fax : +1 800 325-5052

1.4 Emergency telephone

Emergency Phone # : 800-424-9300 CHEMTREC (USA) +1-703-527-3887 CHEMTREC (International) 24 Hours/day; 7 Days/week

SECTION 2: Hazards identification

2.1 Classification of the substance or mixture

Not a hazardous substance or mixture.

2.2 GHS Label elements, including precautionary statements

Not a hazardous substance or mixture.

2.3 Hazards not otherwise classified (HNOC) or not covered by GHS - none

SECTION 3: Composition/information on ingredients

3.1 Substances

Synonyms : 1,6-α-D-Glucan 6-glucanohydrolase

CAS-No. : 9025-70-1
EC-No. : 232-803-9

SECTION 4: First aid measures

4.1 Description of first-aid measures

If inhaled
If breathed in, move person into fresh air. If not breathing, give artificial respiration.

In case of skin contact
Wash off with soap and plenty of water.

In case of eye contact
Flush eyes with water as a precaution.

If swallowed
Never give anything by mouth to an unconscious person. Rinse mouth with water.

4.2 Most important symptoms and effects, both acute and delayed
The most important known symptoms and effects are described in the labelling (see section 2.2) and/or in section 11

4.3 Indication of any immediate medical attention and special treatment needed
No data available

SECTION 5: Firefighting measures

5.1 Extinguishing media

Suitable extinguishing media
Use water spray, alcohol-resistant foam, dry chemical or carbon dioxide.

5.2 Special hazards arising from the substance or mixture
Nature of decomposition products not known.

5.3 Advice for firefighters
Wear self-contained breathing apparatus for firefighting if necessary.

5.4 Further information
No data available

SECTION 6: Accidental release measures

6.1 Personal precautions, protective equipment and emergency procedures
Avoid dust formation. Avoid breathing vapors, mist or gas.
For personal protection see section 8.

6.2 Environmental precautions
Do not let product enter drains.

6.3 Methods and materials for containment and cleaning up
Sweep up and shovel. Keep in suitable, closed containers for disposal.

6.4 Reference to other sections
For disposal see section 13.

SECTION 7: Handling and storage

7.1 Precautions for safe handling

Advice on protection against fire and explosion
Provide appropriate exhaust ventilation at places where dust is formed.Normal measures for preventive fire protection.

Hygiene measures
General industrial hygiene practice.
For precautions see section 2.2.

7.2 Conditions for safe storage, including any incompatibilities

Storage conditions
Keep container tightly closed in a dry and well-ventilated place.

Storage stability
Recommended storage temperature
2 - 8 °C

Storage class
Storage class (TRGS 510): 13: Non Combustible Solids

7.3 Specific end use(s)
Apart from the uses mentioned in section 1.2 no other specific uses are stipulated

SECTION 8: Exposure controls/personal protection

8.1 Control parameters

Ingredients with workplace control parameters
Contains no substances with occupational exposure limit values.

8.2 Exposure controls

Appropriate engineering controls
General industrial hygiene practice.

Personal protective equipment

Eye/face protection
Use equipment for eye protection tested and approved under appropriate government standards such as NIOSH (US) or EN 166(EU).

Skin protection
Handle with gloves. Gloves must be inspected prior to use. Use proper glove removal technique (without touching glove's outer surface) to avoid skin contact with this product. Dispose of contaminated gloves after use in accordance with applicable laws and good laboratory practices. Wash and dry hands.

Full contact
Material: Nitrile rubber
Minimum layer thickness: 0.11 mm
Break through time: 480 min
Material tested:Dermatril® (KCL 740 / Aldrich Z677272, Size M)

Splash contact
Material: Nitrile rubber

Minimum layer thickness: 0.11 mm
Break through time: 480 min
Material tested:Dermatril® (KCL 740 / Aldrich Z677272, Size M)

data source: KCL GmbH, D-36124 Eichenzell, phone +49 (0)6659 87300, e-mail sales@kcl.de, test method: EN374
If used in solution, or mixed with other substances, and under conditions which differ from EN 374, contact the supplier of the EC approved gloves. This recommendation is advisory only and must be evaluated by an industrial hygienist and safety officer familiar with the specific situation of anticipated use by our customers. It should not be construed as offering an approval for any specific use scenario.

Body Protection
Choose body protection in relation to its type, to the concentration and amount of dangerous substances, and to the specific work-place., The type of protective equipment must be selected according to the concentration and amount of the dangerous substance at the specific workplace.

Respiratory protection
Respiratory protection is not required. Where protection from nuisance levels of dusts are desired, use type N95 (US) or type P1 (EN 143) dust masks. Use respirators and components tested and approved under appropriate government standards such as NIOSH (US) or CEN (EU).

Control of environmental exposure
Do not let product enter drains.

SECTION 9: Physical and chemical properties

9.1 Information on basic physical and chemical properties

a)	Appearance	Form: liquid
b)	Odor	No data available
c)	Odor Threshold	No data available
d)	pH	No data available
e)	Melting point/freezing point	No data available
f)	Initial boiling point and boiling range	No data available
g)	Flash point	()No data available
h)	Evaporation rate	No data available
i)	Flammability (solid, gas)	No data available
j)	Upper/lower flammability or explosive limits	No data available
k)	Vapor pressure	No data available
l)	Vapor density	No data available
m)	Density	No data available

	Relative density	No data available
n)	Water solubility	No data available
o)	Partition coefficient: n-octanol/water	No data available
p)	Autoignition temperature	No data available
q)	Decomposition temperature	No data available
r)	Viscosity	No data available
s)	Explosive properties	No data available
t)	Oxidizing properties	No data available

9.2 Other safety information
No data available

SECTION 10: Stability and reactivity

10.1 Reactivity
No data available

10.2 Chemical stability
Stable under recommended storage conditions.

10.3 Possibility of hazardous reactions
No data available

10.4 Conditions to avoid
No data available

10.5 Incompatible materials
Strong oxidizing agents

10.6 Hazardous decomposition products
In the event of fire: see section 5

SECTION 11: Toxicological information

11.1 Information on toxicological effects

Acute toxicity
Oral: No data available
Inhalation: No data available
Dermal: No data available
No data available

Skin corrosion/irritation
No data available

Serious eye damage/eye irritation
No data available

Respiratory or skin sensitization
Prolonged or repeated exposure may cause allergic reactions in certain sensitive individuals.

Germ cell mutagenicity
No data available

Carcinogenicity

IARC: No ingredient of this product present at levels greater than or equal to 0.1% is identified as probable, possible or confirmed human carcinogen by IARC.

ACGIH: No ingredient of this product present at levels greater than or equal to 0.1% is identified as a carcinogen or potential carcinogen by ACGIH.

NTP: No ingredient of this product present at levels greater than or equal to 0.1% is identified as a known or anticipated carcinogen by NTP.

OSHA: No component of this product present at levels greater than or equal to 0.1% is on OSHA's list of regulated carcinogens.

Reproductive toxicity
No data available
No data available

Specific target organ toxicity - single exposure
No data available

Specific target organ toxicity - repeated exposure
No data available

Aspiration hazard
No data available

11.2 Additional Information

To the best of our knowledge, the chemical, physical, and toxicological properties have not been thoroughly investigated.

SECTION 12: Ecological information

12.1 Toxicity
No data available

12.2 Persistence and degradability
No data available

12.3 Bioaccumulative potential
No data available

12.4 Mobility in soil
No data available

12.5 Results of PBT and vPvB assessment
PBT/vPvB assessment not available as chemical safety assessment not required/not conducted

12.6 Endocrine disrupting properties
No data available

12.7 Other adverse effects
No data available

SECTION 13: Disposal considerations

13.1 Waste treatment methods

Product
Offer surplus and non-recyclable solutions to a licensed disposal company.

Contaminated packaging
Dispose of as unused product.

SECTION 14: Transport information

DOT (US)
Not dangerous goods

IMDG
Not dangerous goods

IATA
Not dangerous goods

Further information
Not classified as dangerous in the meaning of transport regulations.

SECTION 15: Regulatory information

SARA 302 Components
No chemicals in this material are subject to the reporting requirements of SARA Title III, Section 302.

SARA 313 Components
This material does not contain any chemical components with known CAS numbers that exceed the threshold (De Minimis) reporting levels established by SARA Title III, Section 313.

SARA 311/312 Hazards
No SARA Hazards

Massachusetts Right To Know Components
No components are subject to the Massachusetts Right to Know Act.

Pennsylvania Right To Know Components

Dextranase	CAS-No. 9025-70-1	Revision Date

New Jersey Right To Know Components

Dextranase	CAS-No. 9025-70-1	Revision Date

California Prop. 65 Components

This product does not contain any chemicals known to the State of California to cause cancer, birth, or any other reproductive defects.

SECTION 16: Other information

Further information

The above information is believed to be correct but does not purport to be all inclusive and shall be used only as a guide. The information in this document is based on the present state of our knowledge and is applicable to the product with regard to appropriate safety precautions. It does not represent any guarantee of the properties of the product. Sigma-Aldrich Corporation and its Affiliates shall not be held liable for any damage resulting from handling or from contact with the above product. See www.sigma-aldrich.com and/or the reverse side of invoice or packing slip for additional terms and conditions of sale.

The branding on the header and/or footer of this document may temporarily not visually match the product purchased as we transition our branding. However, all of the information in the document regarding the product remains unchanged and matches the product ordered. For further information please contact mlsbranding@sial.com.

Version: 6.2 Revision Date: 04/15/2022 Print Date: 04/16/2022

SAFETY DATA SHEET

1. Identification of the substance/mixture and of the company/undertaking

Identification of the substance/preparation

Product code 10439001
Product name ES Cell FBS
Fetal Bovine Serum, Qualified

Company/Undertaking Identification

Life Technologies
5791 VAN ALLEN WAY
PO BOX 6482
CARLSBAD, CA 92008
+1 760 603 7200

INVITROGEN CORPORATION NEW ZEALAND LIMITED
18 - 24 BOTHA ROAD
PENROSE
AUCKLAND 1006 PAR NEW ZEALAND
+011 64 9 579 3024
0800 636 327

INVITROGEN AUSTRALIA PTY LIMITED
30-32 COMPARK CIRCUIT
MULGRAVE VIC 3170
Silver Tower-Beijing Office
Room 1711 Beijing Silver Tower
#2 DongSanHuan North Rd, Beijing 100027
P.R.China
Tel: +86-010-8446 1800 Fax: +86-010-6410 6617
Guangzhou Office
Room1010-1013 South Tower
Guangzhou World Trade Center Complex
371-376 HuanShi Dong Rd
Guangzhou 510095, China
Tel: +86-020-8760 9229 Hotline: 800 830 2001 Fax: +86-020-8775 0687

24 hour Emergency Response (Transport): 866-536-0631
301-431-8585
Outside of the U.S. ++1-301-431-8585

For in vitro diagnostic use. CAUTION: Not for human or animal therapeutic use.

2. Hazards identification

Australian MSDS Statement Classified as non-hazardous according to the criteria of Worksafe Australia.

GHS - Classification

Signal word
not hazardous

Health hazard
not hazardous

Physical hazards
not hazardous

European Union
Not hazardous

EU Specific Hazard Statements

R-phrase(s)
None

S-phrase(s)
None

Principle Routes of Exposure/ Potential Health effects

Eyes	May cause eye irritation with susceptible persons.
Skin	May cause skin irritation in susceptible persons.
Inhalation	May be harmful by inhalation.
Ingestion	May be harmful if swallowed.

Specific effects

Carcinogenic effects	none
Mutagenic effects	none
Reproductive toxicity	none
Sensitisation	none
Target Organ Effects	None under normal use conditions

3. COMPOSITION/INFORMATION ON INGREDIENTS

The product contains no substances which at their given concentration, are considered to be hazardous to health. We recommend handling all chemicals with caution.

4. FIRST AID MEASURES

Skin contact	Rinse with plenty of water. If symptoms arise, call a physician.
Eye contact	Rinse immediately with plenty of water, also under the eyelids, for at least 15 minutes. If symptoms persist, call a physician.
Ingestion	Never give anything by mouth to an unconscious person. If symptoms persist, call a physician. Do not induce vomiting without medical advice.
Inhalation	Move to fresh air. If symptoms persist, call a physician. If not breathing, give artificial respiration.
Notes to physician	Treat symptomatically.

5. FIRE-FIGHTING MEASURES

Suitable extinguishing media	Water spray. Carbon dioxide (CO2). Foam. Dry chemical.
Special protective equipment for firefighters	Wear self-contained breathing apparatus and protective suit.

6. ACCIDENTAL RELEASE MEASURES

Personal precautions	Use personal protective equipment.
Methods for cleaning up	Soak up with inert absorbent material.

Environmental precautions

Prevent further leakage or spillage if safe to do so.

See Section 12 for additional information.

7. HANDLING AND STORAGE

Handling	Avoid contact with skin, eyes and clothing. Wear personal protective equipment.
Storage	Keep in a dry, cool and well-ventilated place.

8. Exposure controls/personal protection

Exposure limits

We are not aware of any national exposure limit.

Engineering measures Ensure adequate ventilation, especially in confined areas.

Personal protective equipment

Respiratory protection	In case of insufficient ventilation wear suitable respiratory equipment.
Hand protection	Impervious gloves.
Eye protection	Safety glasses with side-shields.
Skin and body protection	Lightweight protective clothing.
Hygiene measures	Handle in accordance with good industrial hygiene and safety practice.

Environmental exposure controls	Prevent product from entering drains.

9. PHYSICAL AND CHEMICAL PROPERTIES

General Information

Form	liquid	
Appearance	No information available	
Odour	No information available	
Boiling Point/Range	**°C** No data available	**°F** No data available
Melting point/range	**°C** No data available	**°F** No data available
Flash point	**°C** No data available	**°F** No data available
Autoignition temperature	**°C** No data available	**°F** No data available
oxidising properties	No information available	
Water solubility	soluble	

10. STABILITY AND REACTIVITY

Stability	Stable under normal conditions.
Materials to avoid	No dangerous reaction known under conditions of normal use.
Hazardous decomposition products	None under normal use.
polymerisation	Hazardous polymerisation does not occur.

11. Toxicological information

Acute toxicity

Not hazardous

Principle Routes of Exposure/ Potential Health effects

Eyes	May cause eye irritation with susceptible persons.
Skin	May cause skin irritation in susceptible persons.
Inhalation	May be harmful by inhalation.
Ingestion	May be harmful if swallowed.
Carcinogenic effects	none.
Mutagenic effects	none.
Reproductive toxicity	none.
Sensitisation	none.
Target Organ Effects	None under normal use conditions

12. ECOLOGICAL INFORMATION

Ecotoxicity effects	No information available.
Mobility	No information available.

Biodegradation	Inherently biodegradable.
Bioaccumulation	Does not bioaccumulate.

13. DISPOSAL CONSIDERATIONS

Dispose of in accordance with local regulations.

14. TRANSPORT INFORMATION

IATA

Proper shipping name	Not classified as dangerous in the meaning of transport regulations.
Hazard class	None
Subsidiary Class	None
Packing group	None
UN-No	None

15. Regulatory information

International Inventories

International Inventories Complies

Industrial Safety and Health Law
This product complies with ISHL
Toxic Chemicals Control Law

Japan

Industrial Safety and Health Law
This product complies with ISHL

Korea
Complies

16. OTHER INFORMATION

Reason for Revision (M)SDS sections updated

For in vitro diagnostic use. CAUTION: Not for human or animal therapeutic use.

The above information was acquired by diligent search and/or investigation and the recommendations are based on prudent application of professional judgment. The information shall not be taken as being all inclusive and is to be used only as a guide. All materials and mixtures may present unkown hazards and should be used with caution. Since the Company cannot control the actual methods, volumes, or conditions of use, the Company shall not be held liable for any damages or losses resulting from the handling or from contact with the product as described herein. THE INFORMATION IN THIS MSDS DOES NOT CONSTITUTE A WARRENTY, EXPRESSED OR IMPLIED, INCLUDING ANY IMPLIED WARRANTY OF MERCHANTABILITY OR FITNESS FOR ANY PARTICULAR PUPOSE.

End of Safety Data Sheet

Safety Data Sheet (SDS)
OSHA HazCom Standard 29 CFR 1910.1200(g) and GHS Rev 03.

Issue date 04/28/2015 Reviewed on 05/01/2018

Trade name: Shortening, Fats or Oils

* 1 Identification

· **Product identifier**

· **Trade name: Shortening, Fats or Oils**
· **CAS Number:**
68334-28-1
· **Relevant identified uses of the substance or mixture and uses advised against**
No further relevant information available.

· **Details of the supplier of the safety data sheet**
· **Manufacturer/Supplier:**
Ag Processing Inc
12700 West Dodge
Rd. Omaha, NE 68154
Phone: 402-496-7809
· **Emergency telephone number:** 402-496-6688

* 2 Hazard(s) identification

· **Classification of the substance or mixture**
The substance is not classified according to the Globally Harmonized System (GHS).

· **Label elements**
· **GHS label elements** Non-Regulated Material
· **Hazard pictograms** Non-Regulated Material
· **Signal word** Non-Regulated Material
· **Hazard statements** Non-Regulated Material
· **Unknown acute toxicity:**
100 percent of the mixture consists of ingredient(s) of unknown toxicity.

· **Classification system:**
· **NFPA ratings (scale 0 - 4)**

Health = 0
Fire = 1
Reactivity = 0

· **HMIS-ratings (scale 0 - 4)**

Health = 0
Fire = 1
Reactivity = 0

· **Hazard(s) not otherwise classified (HNOC):** None known

* 3 Composition/information on ingredients

· **Chemical characterization: Substances**
· **CAS No. Description**
68334-28-1 Partially Hydrogenated Vegetable Oil

4 First-aid measures

· **Description of first aid measures**
· **General information:** No special measures required.
· **After inhalation:** Supply fresh air; consult doctor in case of complaints.

(Contd. on page 2)

Safety Data Sheet (SDS)

OSHA HazCom Standard 29 CFR 1910.1200(g) and GHS Rev 03.

Issue date 04/28/2015 Reviewed on 05/01/2018

Trade name: Shortening, Fats or Oils

· ***After skin contact:***
Wash with soap and water.
Normal first aid procedures for treating burns should be employed if skin comes in contact with hot oil.
Dermatitis could result from prolonged residence on skin of allergy sensitive individuals.
· ***After eye contact:*** Rinse opened eye for several minutes under running water.
· ***After swallowing:*** If swallowed and symptoms occur, consult a doctor.
· ***Information for doctor:***
· ***Most important symptoms and effects, both acute and delayed:*** No further relevant information available.
· ***Indication of any immediate medical attention and special treatment needed***
No further relevant information available.

5 Fire-fighting measures

· ***Extinguishing media***
· ***Suitable extinguishing agents:***
CO2, extinguishing powder or water spray. Fight larger fires with water spray or alcohol resistant foam.
· ***For safety reasons unsuitable extinguishing agents:*** Water with full jet
· ***Special hazards arising from the substance or mixture***
Rags soaked in any solvent present a fire hazard and should always be stored in UL listed or Factory Mutual approved, covered containers. Improperly stored rags can create conditions that lead to oxidation. Oxidation under certain conditions can lead to spontaneous combustion.
· ***Advice for firefighters***
· ***Protective equipment:***
As in any fire, wear self-contained breathing apparatus pressure-demand (NIOSH approved or equivalent), and full protective gear to prevent contact with skin and eyes.

6 Accidental release measures

· ***Personal precautions, protective equipment and emergency procedures*** Product is slippery when spilled.
· ***Environmental precautions:*** Do not allow to enter sewers/ surface or ground water.
· ***Methods and material for containment and cleaning up:***
Absorb with liquid-binding material (ie. sand, diatomite, acid binders, universal binders, sawdust).
Dispose of the collected material according to regulations.
· ***Reference to other sections***
See Section 7 for information on safe handling.
See Section 8 for information on personal protection equipment.
See Section 13 for disposal information.

7 Handling and storage

· ***Handling:***
· ***Precautions for safe handling*** No special measures required.
· ***Information about protection against explosions and fires:*** No special measures required.
· ***Conditions for safe storage, including any incompatibilities***
· ***Storage:***
· ***Requirements to be met by storerooms and receptacles:*** No special requirements.
· ***Information about storage in one common storage facility:*** Not required.
· ***Further information about storage conditions:*** None.
· ***Specific end use(s)*** No further relevant information available.

(Contd. on page 3)

Safety Data Sheet (SDS)
OSHA HazCom Standard 29 CFR 1910.1200(g) and GHS Rev 03.

Issue date 04/28/2015 Reviewed on 05/01/2018

Trade name: Shortening, Fats or Oils

* 8 Exposure controls/personal protection

· **Additional information about design of technical systems:** No further data; see section 7.

· **Control parameters**

· **Components with occupational exposure limits:**
68334-28-1 Partially Hydrogenated Vegetable Oil (100%)
Under normal uses and conditions, edible oils and fats pose no health hazard. If aspirated as an oil mist the respiratory system may be affected. Oil mist is considered a nuisance particulate by the American Conference of Governmental Industrial Hygienists (ACGIH) who recommend a TLV of 10 ppm

· **Additional information:** The lists that were valid during the creation of this SDS were used as basis.

· **Exposure controls**
· **Personal protective equipment:**
· **General protective and hygienic measures:**
Keep away from foodstuffs, beverages and feed.
Immediately remove all soiled and contaminated clothing and wash before reuse.
Breathing equipment: Not required.
· **Protection of hands:** Not required
· **Material of gloves**
The selection of the suitable gloves does not only depend on the material, but also on further marks of quality and varies from manufacturer to manufacturer.
· **Penetration time of glove material:** Not applicable

(Contd. on page 4)

Safety Data Sheet (SDS)
OSHA HazCom Standard 29 CFR 1910.1200(g) and GHS Rev 03.

Issue date 04/28/2015 Reviewed on 05/01/2018

Trade name: Shortening, Fats or Oils

* 9 Physical and chemical properties

· **Information on basic physical and chemical properties**
· **General Information**
· **Appearance:**
Form: Solid or Liquid
Color: White to Yellow
· **Odor:** Light vegetable oil
· **Odor threshold:** Not determined.

· **pH-value:** Not determined.

· **Change in condition**
Melting point/Melting range: 3.2-130 °F (typical)
Boiling point/Boiling range: >300°C @ 0.05 mm Hg
>250°C @ 0.001 mm Hg

· **Flash point:** Not applicable.

· **Flammability (solid, gaseous):** Not applicable.

· **Ignition temperature:**

Decomposition temperature: Not determined.

· **Auto igniting:** Not determined.

· **Danger of explosion:** Product does not present an explosion hazard.

· **Explosion limits:**
Lower: Not determined.
Upper: Not determined.

· **Vapor pressure:** Not determined.

· **Density @ 20 °C (68 °F):** 0.698-0.921 g/cm³ (5.825-7.686 lbs/gal) (liquid)
· **Relative density** Not determined.
· **Vapor density** Not determined.
· **Evaporation rate** Not determined.

· **Solubility in / Miscibility with**
Water: Insoluble.

· **Partition coefficient (n-octanol/water):** Not determined.

· **Viscosity:**
Dynamic: Not determined.
Kinematic: Not determined.
Organic solvents: 0.0 %

Solids content: 100.0 %
· **Other information** No further relevant information available.

(Contd. on page 5)

Safety Data Sheet (SDS)

OSHA HazCom Standard 29 CFR 1910.1200(g) and GHS Rev 03.

Issue date 04/28/2015 Reviewed on 05/01/2018

Trade name: Shortening, Fats or Oils

10 Stability and reactivity

· **Reactivity** No further relevant information available.
· **Chemical stability** Stable under normal conditions.
· **Thermal decomposition / conditions to be avoided:** No decomposition if used according to specifications.
· **Possibility of hazardous reactions** No dangerous reactions known.
· **Conditions to avoid** No further relevant information available.
· **Incompatible materials:** No further relevant information available.
· **Hazardous decomposition products:** No dangerous decomposition products known.

* 11 Toxicological information

· **Information on toxicological effects**
· **Acute toxicity:**
· **Primary irritant effect:**
· **on the skin:** No irritating effect.
· **on the eye:** No irritating effect.
· **Additional toxicological information:**
When used and handled according to specifications, the product does not have any harmful effects according to our experience and the information provided to us.
The substance is not subject to classification.

· **Carcinogenic categories**
· **IARC (International Agency for Research on Cancer)** Substance is not listed.

· **NTP (National Toxicology Program)**
Substance is not listed.
· **OSHA-Ca (Occupational Safety & Health Administration)**
Substance is not listed.

12 Ecological information

· **Toxicity**
· **Aquatic toxicity:** No further relevant information available.
· **Persistence and degradability** No further relevant information available.
· **Behavior in environmental systems:**
· **Bioaccumulative potential** No further relevant information available.
· **Mobility in soil** No further relevant information available.
· **Additional ecological information:**
· **General notes:** Not known to be hazardous to water.
· **Results of PBT and vPvB assessment**
· **PBT:** Not applicable.
· **vPvB:** Not applicable.
· **Other adverse effects** No further relevant information available.

13 Disposal considerations

· **Waste treatment methods**
· **Recommendation:**
Small quantities can be disposed of with household waste.
Recycle or dispose with household trash.

(Contd. on page 6)

Safety Data Sheet (SDS)
OSHA HazCom Standard 29 CFR 1910.1200(g) and GHS Rev 03.

Issue date 04/28/2015 Reviewed on 05/01/2018

Trade name: Shortening, Fats or Oils

· **Uncleaned packagings:**
· **Recommendation:** Disposal must be made according to official regulations.

* 14 Transport information

· **UN-Number**	
· **DOT, ADN, IMDG, IATA**	Non-Regulated Material
· **ADR**	Non-Regulated Material
	Not Regulated
· **UN proper shipping name**	
· **DOT, ADR, ADN, IMDG, IATA**	Non-Regulated Material
· **Transport hazard class(es)**	
· **DOT, ADR, ADN, IMDG, IATA**	
· **Class**	Non-Regulated Material
· **Packing group**	
· **DOT, ADR, IMDG, IATA**	Non-Regulated Material
· **Environmental hazards:**	Not applicable.
· **Special precautions for user**	Not applicable.
· **Transport in bulk according to Annex II of MARPOL73/78 and the IBC Code**	Not applicable.
· **UN "Model Regulation":**	-

* 15 Regulatory information

· **Safety, health and environmental regulations/legislation specific for the substance or mixture**
· **Sara**

· **Section 355 (extremely hazardous substances):**
Substance is not listed.

· **Section 313 (Specific toxic chemical listings):**
Substance is not listed.

· **TSCA (Toxic Substances Control Act):**
Substance is listed.

· **California Proposition 65**

· **Chemicals known to cause cancer:**
Substance is not listed.

· **Chemicals known to cause reproductive toxicity for females:**
Substance is not listed.

· **Chemicals known to cause reproductive toxicity for males:**
Substance is not listed.

· **Chemicals known to cause developmental toxicity:**
Substance is not listed.

· **Carcinogenic categories**

· **EPA (Environmental Protection Agency)**
Substance is not listed.

· **TLV (Threshold Limit Value established by ACGIH)**
Substance is not listed.

(Contd. on page 7)

Safety Data Sheet (SDS)

OSHA HazCom Standard 29 CFR 1910.1200(g) and GHS Rev 03.

Issue date 04/28/2015 Reviewed on 05/01/2018

Trade name: Shortening, Fats or Oils

· **NIOSH-Ca (National Institute for Occupational Safety and Health)**

Substance is not listed.

· **GHS label elements**
Non-Regulated Material
· **Hazard pictograms**
Non-Regulated Material

· **Signal word**
Non-Regulated Material

· **Hazard statements**
Non-Regulated Material

· **National regulations:**

Substance is not listed.

· **State Right to Know**

Substance is not listed.

· **Chemical safety assessment:** A Chemical Safety Assessment has not been carried out.

16 Other information

The information and recommendations in this safety data sheet are, to the best of our knowledge, accurate as of the date of issue. Nothing herein shall be deemed to create warranty, expressed or implied and shall not establish a legally valid contractual relationship. It is the responsibility of the user to determine applicability of this information and the suitability of the material or product for any particular purpose.

· **Date of preparation / last revision** 04/28/2015 /
· **Abbreviations and acronyms:**
ADR: The European Agreement concerning the International Carriage of Dangerous Goods by Road
ADN: The European Agreement concerning the International Carriage of Dangerous Goods by Inland Waterways
IMDG: International Maritime Code for Dangerous Goods
DOT: US Department of Transportation
IATA: International Air Transport Association
ACGIH: American Conference of Governmental Industrial Hygienists
CAS: Chemical Abstracts Service (division of the American Chemical Society)
NFPA: National Fire Protection Association (USA)
HMIS: Hazardous Materials Identification System (USA)
Eye Irrit. 2B: Serious eye damage/eye irritation, Hazard Category 2B
STOT SE 3: Specific target organ toxicity - Single exposure, Hazard Category 3
· ***Data compared to the previous version altered.***
SDS created by MSDS Authoring Services www.msdsauthoring.com (877) 204-9106

Novo Nordisk Pharmatech A/S

SAFETY DATA SHEET

Product name:	Insulin Human AF	Page:	1/8
Revision Date:	2015-08-11	Print date:	2015-08-21
		SDS-ID:	GB-EN/4.0

SECTION 1: IDENTIFICATION OF THE SUBSTANCE/MIXTURE AND OF THE COMPANY/UNDERTAKING

1.1. Product identifier

Product name:	Insulin Human AF
CAS-No.:	11061-68-0
EC No.:	234-279-7
Container size:	HDPE plastic bottles. 1 g, 10 g, 50 g, 100 g, 1 kg.

1.2. Relevant identified uses of the substance or mixture and uses advised against

Application:	For Cell Culture Medium Use. For Further Manufacturing Use Only. Not for Therapeutic Use.

1.3. Details of the supplier of the safety data sheet

Supplier:	Novo Nordisk Pharmatech A/S København svej 216 DK-4600 Køge Tel:+45 56 67 10 00 www.novonordiskpharmatech.com
Responsible for safety data sheet authoring:	SDS_info@dhigroup.com

1.4. Emergency telephone number

Emergency telephone:	+ 45 56 67 10 00 (Only during office hours)

SECTION 2: HAZARDS IDENTIFICATION

2.1. Classification of the substance or mixture

The product is not classified.

2.2. Label elements

The substance/mixture does not meet the criteria for classification and labelling.

2.3. Other hazards

PBT/vPvB:	Not relevant.
Other:	Dust may form explosive mixture with air. May cause irritation to skin, eyes and respiratory system.

SECTION 3: COMPOSITION/INFORMATION ON INGREDIENTS

3.1. Substances

SAFETY DATA SHEET

GHS/CLP:

%:	CAS-No.:	EC No.:	REACH Reg. No:	Chemical name:	Hazard classification:	Notes:
100	11061-68-0	234-279-7	-	Insulin (human)	-	

SECTION 4: FIRST AID MEASURES

4.1. Description of first aid measures

Inhalation:	Move into fresh air and keep at rest.
Skin contact:	Rinse with water. If skin irritation occurs: Get medical advice/attention.
Eye contact:	Rinse the eye with water immediately. Contact physician if irritation persists.
Ingestion:	Rinse mouth and drink plenty of water. Keep person under observation. If uncomfortable: Seek hospital and bring along these instructions.

4.2. Most important symptoms and effects, both acute and delayed

Symptoms/effects:	See section 11 for more detailed information on health effects and symptoms.

4.3. Indication of any immediate medical attention and special treatment needed

Medical attention/treatments:	Not known.

SECTION 5: FIREFIGHTING MEASURES

5.1. Extinguishing media

Extinguishing media:	Use fire-extinguishing media appropriate for surrounding materials.

5.2. Special hazards arising from the substance or mixture

Specific hazards:	Dust may form an explosive mixture in the atmosphere.

5.3. Advice for firefighters

Protective equipment for fire-fighters:	Selection of respiratory protection for fire fighting: follow the general fire precautions indicated in the workplace.

SECTION 6: ACCIDENTAL RELEASE MEASURES

6.1. Personal precautions, protective equipment and emergency procedures

Personal precautions: Follow precautions for safe handling described in this safety data sheet.

6.2. Environmental precautions

Environmental precautions: Avoid release to the environment.

6.3. Methods and material for containment and cleaning up

Methods for cleaning up: Collect spillage with shovel, broom or the like and reuse, if possible. Dispose of large amounts of spillage/waste according to agreement with local authorities. Flush contaminated area with plenty of water. Use spark-proof tools and explosion-proof equipment.

6.4. Reference to other sections

References: For personal protection, see section 8.
For waste disposal, see section 13.

SECTION 7: HANDLING AND STORAGE

7.1. Precautions for safe handling

Safe handling advice: Observe good chemical hygiene practices. Avoid inhalation of dust and contact with skin and eyes.

Technical measures: Do not smoke or use open fire or other sources of ignition. Use spark-proof tools and explosion-proof equipment.

Technical precautions: Provide adequate ventilation. Provide easy access to water supply and eye wash facilities.

7.2. Conditions for safe storage, including any incompatibilities

Technical measures for safe storage: No special precautions.

Storage conditions: Insulin Human must be kept in a deep freezer at -20°C ± 5°C in tightly closed original packing.

7.3. Specific end use(s)

Specific use(s): Not relevant.

SAFETY DATA SHEET

SECTION 8: EXPOSURE CONTROLS/PERSONAL PROTECTION

8.1. Control parameters

Occupational exposure limits:

CAS-No.:	Chemical name:	As:	Exposure limits:	Type:	Notes:	References:
-	Dusts, respirable dust	-	4 mg/m3	TWA	-	EH40
-	Dusts, total inhalable dust	-	10 mg/m3	TWA	-	EH40

8.2. Exposure controls

Engineering measures:	Provide adequate ventilation. Observe occupational exposure limits and minimise the risk of inhalation of dust.
Personal protection:	Personal protection equipment should be chosen according to the CEN standards and in discussion with the supplier of the personal protective equipment.
Respiratory equipment:	In case of inadequate ventilation or risk of inhalation of dust, use suitable respiratory equipment with particle filter (type P2).
Hand protection:	Risk of contact: Wear protective gloves. PVC gloves are recommended. The most suitable glove must be chosen in consultation with the gloves supplier, who can inform about the breakthrough time of the glove material.
Eye protection:	During dust-raising work: Wear goggles/face shield.
Hygiene measures:	Wash hands after contact.
Environmental Exposure Controls:	Not available.

SECTION 9: PHYSICAL AND CHEMICAL PROPERTIES

9.1. Information on basic physical and chemical properties

Form:	Powder.
Colour:	White.
pH:	Not relevant.
Melting point / freezing point:	Not available.
Relative density:	Not available.
Solubility:	Not available.
Decomposition temperature (°C):	Not available.

9.2. Other information

Other data:	Not relevant.

SAFETY DATA SHEET

SECTION 10: STABILITY AND REACTIVITY

10.1. Reactivity

Reactivity: None known.

10.2. Chemical stability

Stability: Stable under the prescribed storage conditions.

10.3. Possibility of hazardous reactions

Hazardous Reactions: None known.

10.4. Conditions to avoid

Conditions/materials to avoid: None specific.

10.5. Incompatible materials

Incompatible materials: Not known.

10.6. Hazardous decomposition products

Hazardous decomposition products: None in particular.

SECTION 11: TOXICOLOGICAL INFORMATION

11.1. Information on toxicological effects

Inhalation: Dust may irritate throat and respiratory system and cause coughing.
Inhalation of dust of insulin crystals may result in a mild temporary decrease of the blood sugar level (hypoglycemia).

Skin contact: Powder may irritate skin.

Eye contact: Direct contact is irritating.

Ingestion: No harmful effects expected in amounts likely to be ingested by accident.

Specific effects: None known.

SECTION 12: ECOLOGICAL INFORMATION

12.1. Toxicity

Ecotoxicity: The product is not expected to be hazardous to the environment.

12.2. Persistence and degradability

Degradability: The degradability of the product has not been stated.

12.3. Bioaccumulative potential

Bioaccumulative potential: No data available on bioaccumulation.

12.4. Mobility in soil

Mobility: No data available.

12.5. Results of PBT and vPvB assessment

PBT/vPvB: Not relevant.

12.6. Other adverse effects

Other adverse effects: None known.

SECTION 13: DISPOSAL CONSIDERATIONS

13.1. Waste treatment methods

Dispose of waste and residues in accordance with local authority requirements.

Waste from residues: EWC-code: 16 03 06

SECTION 14: TRANSPORT INFORMATION

The product is not covered by international regulation on the transport of dangerous goods (IMDG, IATA, ADR/RID).

14.1. UN number

UN-No: -

14.2. UN proper shipping name

Proper Shipping Name: -

14.3. Transport hazard class(es)

Class: -

14.4. Packing group

PG: -

14.5. Environmental hazards

Marine pollutant: -

Environmentally Hazardous substance: -

14.6. Special precautions for user

Special precautions: None known.

14.7. Transport in bulk according to Annex II of MARPOL and the IBC Code

Transport in bulk: Not relevant.

SAFETY DATA SHEET

SECTION 15: REGULATORY INFORMATION

15.1. Safety, health and environmental regulations/legislation specific for the substance or mixture

National regulation:

Regulation (EC) No 1907/2006 of the European Parliament and of the Council of 18 December 2006 concerning the Registration, Evaluation, Authorisation and Restriction of Chemicals (REACH), establishing a European Chemicals Agency, amending Directive 1999/45/EC and repealing Council Regulation (EEC) No 793/93 and Commission Regulation (EC) No 1488/94 as well as Council Directive 76/769/EEC and Commission Directives 91/155/EEC, 93/67/EEC, 93/105/EC and 2000/21/EC, with amendments.
Regulation (EC) No 1272/2008 of the European Parliament and of the Council of 16 December 2008 on classification, labelling and packaging of substances and mixtures, amending and repealing Directives 67/548/EEC and 1999/45/EC, and amending Regulation (EC) No 1907/2006 with amendments.
The Chemicals (Hazard Information and Packaging for Supply) Regulations 2009 (S.I 2009 No. 716).
The Control of Substances Hazardous to Health Regulations 2002 (S.I 2002 No. 2677) with amendments.
EH40/2005, Workplace exposure limits 2005, with amendments.
The List of Wastes (England) (Amendment) Regulations 2005. (SI 2005 No. 895).

15.2. Chemical Safety Assessment

CSA status: Not relevant.

SECTION 16: OTHER INFORMATION

The user must be instructed in the proper work procedure and be familiar with the contents of these instructions.

The following sections contain revisions or new statements:
1, 3.

The information on this data sheet represents our current data and is reliable provided that the product is used under the prescribed conditions and in accordance with the application specified on the packaging and/or in the technical guidance literature. Any other use of the product which involves using the product in combination with any other product or any other process is the responsibility of the user.

Made by DHI - Environment and Toxicology, Agern Allé 5, DK-2970 Hørsholm, Denmark.
www.dhigroup.com.

ThermoFisher SCIENTIFIC

SAFETY DATA SHEET

Creation Date 12-Sep-2014 **Revision Date** 24-Dec-2021 **Revision Number** 5

1. Identification

Product Name **Linoleic acid**

Cat No. : **AC215040000; AC215040050; AC215040250; AC215041000**

CAS No 60-33-3
Synonyms (Z,Z)-9,12-Octadecadienoic acid; Linolic acid

Recommended Use Laboratory chemicals.
Uses advised against Food, drug, pesticide or biocidal product use.

Details of the supplier of the safety data sheet

Company
Fisher Scientific Company
One Reagent Lane
Fair Lawn, NJ 07410
Tel: (201) 796-7100

Acros Organics
One Reagent Lane
Fair Lawn, NJ 07410

Emergency Telephone Number For information **US** call: 001-800-ACROS-01 / **Europe** call: +32 14 57 52 11
Emergency Number **US**:001-201-796-7100 / **Europe:** +32 14 57 52 99
CHEMTREC Tel. No.**US**:001-800-424-9300 / **Europe**:001-703-527-3887

2. Hazard(s) identification

Classification
Classification under 2012 OSHA Hazard Communication Standard (29 CFR 1910.1200)

This chemical is not considered hazardous by the 2012 OSHA Hazard Communication Standard (29 CFR 1910.1200)

Label Elements

Hazard Statements

Precautionary Statements
Hazards not otherwise classified (HNOC)
None identified

3. Composition/Information on Ingredients

Component	CAS No	Weight %
Linoleic acid	60-33-3	>95

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 Remove 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.

Notes to Physician Treat symptomatically

5. Fire-fighting measures

Suitable Extinguishing Media Water spray, carbon dioxide (CO2), dry chemical, alcohol-resistant foam.

Unsuitable Extinguishing Media No information available

Flash Point > 112 °C / > 233.6 °F

Method - No information available

Autoignition Temperature No information available

Explosion Limits

Upper No data available

Lower No data available

Sensitivity to Mechanical Impact No information available

Sensitivity to Static Discharge No information available

Specific Hazards Arising from the Chemical
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
0	1	1	N/A

6. Accidental release measures

Personal Precautions Ensure adequate ventilation. Use personal protective equipment as required.

Environmental Precautions Should not be released into the environment.

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/face protection. Ensure adequate ventilation. Avoid ingestion and inhalation. Do not get in eyes, on skin, or on clothing.

Storage. Keep container tightly closed in a dry and well-ventilated place. Protect from direct sunlight. Keep under nitrogen. To maintain product quality: Keep refrigerated. Incompatible Materials. Bases. Strong oxidizing agents. Reducing Agent.

8. Exposure controls / personal protection

Exposure Guidelines This product does not contain any hazardous materials with occupational exposure limitsestablished by the region specific regulatory bodies.

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 No protective equipment is needed under normal use conditions.

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

9. Physical and chemical properties

Physical State	Liquid
Appearance	Clear
Odor	No information available
Odor Threshold	No information available
pH	No information available
Melting Point/Range	-5 °C / 23 °F
Boiling Point/Range	229 - 230 °C / 444.2 - 446 °F @ 16 mmHg
Flash Point	> 112 °C / > 233.6 °F
Evaporation Rate	No information available
Flammability (solid,gas)	Not applicable
Flammability or explosive limits	
Upper	No data available
Lower	No data available
Vapor Pressure	No information available
Vapor Density	No information available
Specific Gravity	0.900
Solubility	Insoluble in water
Partition coefficient; n-octanol/water	No data available
Autoignition Temperature	No information available
Decomposition Temperature	No information available
Viscosity	No information available
Molecular Formula	C18 H32 O2
Molecular Weight	280.45

10. Stability and reactivity

Reactive Hazard None known, based on information available

Stability Air sensitive.

Conditions to Avoid Excess heat. Exposure to air. Exposure to light. Incompatible products.

Incompatible Materials Bases, Strong oxidizing agents, Reducing Agent

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 No acute toxicity information is available for this product

Component Information

Toxicologically Synergistic Products No information available

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

Irritation No information available

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
Linoleic acid	60-33-3	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 None known

STOT - repeated exposure None known

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. .

Persistence and Degradability Insoluble in water May persist based on information available.

Bioaccumulation/ Accumulation No information available.

Mobility Is not likely mobile in the environment due its low water solubility.

13. Disposal considerations

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

14. Transport information

DOT Not regulated
TDG Not regulated
IATA Not regulated
IMDG/IMO Not regulated

15. Regulatory information

United States of America Inventory

Component	CAS No	TSCA	TSCA Inventory notification - Active-Inactive	TSCA - EPA Regulatory Flags
Linoleic acid	60-33-3	X	ACTIVE	-

Legend:
TSCA US EPA (TSCA) - Toxic Substances Control Act, (40 CFR Part 710)
X - Listed
'-' - Not Listed

TSCA 12(b) - Notices of Export Not applicable

International Inventories
Canada (DSL/NDSL), Europe (EINECS/ELINCS/NLP), Philippines (PICCS), Japan (ENCS), Japan (ISHL), Australia (AICS), China (IECSC), Korea (KECL).

Component	CAS No	DSL	NDSL	EINECS	PICCS	ENCS	ISHL	AICS	IECSC	KECL
Linoleic acid	60-33-3	X	-	200-470-9	X	X	X	X	X	KE-26285

KECL - NIER number or KE number (http://ncis.nier.go.kr/en/main.do)

U.S. Federal Regulations

SARA 313 Not applicable

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

CWA (Clean Water Act) Not applicable

Clean Air Act Not applicable

OSHA - Occupational Safety and Health Administration Not applicable

CERCLA Not applicable

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

U.S. State Right-to-Know Regulations Not applicable

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 No information available

Authorisation/Restrictions according to EU REACH

Safety, health and environmental regulations/legislation specific for the substance or mixture

Component	CAS No	OECD HPV	Persistent Organic Pollutant	Ozone Depletion Potential	Restriction of Hazardous Substances (RoHS)
Linoleic acid	60-33-3	Listed	Not applicable	Not applicable	Not applicable

Component	CAS No	Seveso III Directive (2012/18/EC) - Qualifying Quantities for Major Accident Notification	Seveso III Directive (2012/18/EC) - Qualifying Quantities for Safety Report Requirements	Rotterdam Convention (PIC)	Basel Convention (Hazardous Waste)
Linoleic acid	60-33-3	Not applicable	Not applicable	Not applicable	Annex I - Y34

16. Other information

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

Creation Date 12-Sep-2014
Revision Date 24-Dec-2021
Print Date 24-Dec-2021
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

NATIONAL PEROXIDE LIMITED, KALYAN

MATERIAL SAFETY DATA SHEET for PERACETIC ACID (15%w/w)

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

1.1. Identification of the substance/preparation

Product name	:	PERACETIC ACID - 15 %w/w
Chemical Name	:	Peracetic Acid
Synonyms	:	PAA, Peroxyethanoic acid, Peracetic acid
Molecular formula	:	CH_3-COOOH
Molecular Weight	:	76.05 g/mol

1.2. Use of the Substance/Preparation

Recommended use	:	Pesticide Cleaning Agent Oxidising Agent

1.3. Company/Undertaking Identification

Address	:	National Peroxide Limited, NRC Road, Village Vadavali, P.O. Mohone, Kalyan – 421102, Thane Dist., Maharashtra State, India.
Telephone	:	91 251 2278024, 2278076, 2278000
Email address	:	mktg@naperol.com

1.4. Emergency telephone number

Telephone	:	**+91 9594640688 (Emergency 24 Hour)**

2. HAZARDS IDENTIFICATION

Appearance	:	Liquid
Colour	:	Colorless
Odour	:	Pungent

Main effects

- Oxidising.
- Contact with combustible material may cause fire.
- Causes sever burns.
- Harmful by inhalation, in contact with skin and if swallowed.

Inhalation

- Inhalation of vapours is irritating to the respiratory system, may cause throat pain and cough.
- Breathing difficulties
- Repeated or prolonged exposure: Risk of sore throat, nose bleeds, chronic bronchitis.

Eye contact

- Severe eye irritation
- Redness
- Lachrymation
- Swelling of tissue
- May cause irreversible eye damage.
- Small amounts splashed into eyes can cause irreversible tissue damage and blindness.

Skin contact

- Severe skin irritation
- Redness
- Swelling of tissue
- Causes burns.

Ingestion

- Paleness and cyanosis of the face.
- If ingested, severe burns of the mouth and throat, as well as a danger of perforation of the oesophagus and the stomach.
- Risk of shock.
- Excessive fluid in the mouth and nose, with risk of suffocation.
- Risk of throat (o)edema and suffocation.
- Bloating of stomach, belching.
- Nausea
- Bloody vomiting
- Cough
- Breathing difficulties
- Risk of chemical pneumonitis and pulmonary (o)edema.

3. COMPOSITION/INFORMATION ON INGREDIENTS

Peracetic acid
CAS-No. : 79-21-0
Concentration : **appr. 15.0 %**

Hydrogen peroxide
CAS-No. : 7722-84-1
Concentration : **appr. 23.0 %**

Acetic acid
CAS-No. : 64-19-7
Concentration : **appr. 17.0 %**

4. FIRST AID MEASURES

4.1. Inhalation

- In case of accident by inhalation: remove casualty to fresh air and keep at rest.
- Victim to lie down in the recovery position, cover and keep him warm.
- Oxygen or artificial respiration if needed.
- Call a physician immediately.

4.2. Eye contact

- Rinse immediately with plenty of water, also under the eyelids, for at least 15 minutes.
- In the case of difficulty of opening the lids, administer an analgesic eye wash (oxybuprocaine).
- Consult with an ophthalmologist immediately in all cases.
- Take the Victim immediately to hospital.

4.3. Skin contact

- Take off contaminated clothing and shoes immediately.
- Wash off immediately with plenty of water.
- Keep warm and in a quiet place.
- Wash contaminated clothing before reuse.
- Consult a physician.

4.4. Ingestion

The following actions are recommended:
- Call a physician immediately.
- Take victim immediately to hospital.

If victim is conscious:
- If swallowed, rinse mouth with water (only if the person is conscious).
- Do NOT induce vomiting.

If victim is unconscious but breathing:
- Artificial respiration and/or oxygen may be necessary.

5. FIRE-FIGHTING MEASURES

5.1. Suitable extinguishing media

- Water
- Water spray

5.2. Extinguishing media which must not be used for safety reasons

- None.

5.3. Special exposure hazards in a fire

- Oxidising
- Oxygen released in thermal decomposition may support combustion
- Contact with combustible material may cause fire.
- Contact with flammables may cause fire or explosions.
- Risk of explosion if heated under confinement.

5.4. Hazardous decomposition products

- Oxygen
- The release of other hazardous decomposition products is possible.

5.5 Special protective equipment for fire-fighters

- Evacuate personnel to safe areas.
- In the event of fire, wear self-contained breathing apparatus.
- When intervention in close proximity wear acid resistant over suit.
- Clean contaminated surface thoroughly.

5.6. Other information

- Keep product and empty container away from heat and sources of ignition.
- Keep containers and surroundings cool with water spray.
- Approach from upwind.

6. ACCIDENTAL RELEASE MEASURES

6.1. Personal precautions

- Keep people away from and upwind of spill/leak.
- Refer to protective measures listed in sections 7 and 8.
- Isolate the area.
- Keep away from Incompatible products.
- Prevent further leakage or spillage if safe to do so.
- In case of contact with combustible material, keep material wet with plenty of water.

6.2. Environmental precautions

- The product should not be allowed to enter drains, water courses or the soil.
- If the product contaminates rivers and lakes or drains inform respective authorities.

6.3. Methods for cleaning up

- Dam up.
- Soak up with inert absorbent material.
- Dilute with plenty of water.
- Do not add chemical products.
- Treat recovered material as described in the section "Disposal considerations".
- Never return spills in original containers for re-use.

7. HANDLING AND STORAGE

7.1. Handling

- Use only in well-ventilated areas.
- Keep away from heat.
- Keep away from Incompatible products.
- May not get in touch with:

- Organic materials
- Use only equipment and materials which are compatible with the product.
- Before all operations, passivate the piping circuits and vessels according to the procedure recommended by the producer.
- Never return unused material to storage receptacle.
- Use only in an area with adequate water supply
- Containers and equipment used to handle the product should be used exclusively for that product.

7.2. Storage

- Keep in a cool, well-ventilated place.
- Keep away from heat.
- Keep away from Incompatible products.
- Keep away from combustible material.
- Store in a receptacle equipped with a vent.
- Store in original container.
- Keep container closed.
- Keep in a bunded area.
- Regularly check the condition and temperature of the containers.
- Information about special precautions needed for bulk handling is available on request.

7.3. Specific use(s)

- For further information, please contact: Supplier

7.4. Packaging material

- Aluminium 99,5 %
- Stainless steel 304L / 316L
- Approved grades of HDPE.

7.5. Other information

- Refer to protective measures listed in sections 7 and 8.
- Do not confine the product in a circuit, between closed valves, or in a container without a vent.

8. EXPOSURE CONTROLS / PERSONAL PROTECTION

8.1. Exposure Limit Values

Peracetic acid

- WEL (TWA = 1 ppm)

Hydrogen peroxide

- WEL (TWA = 1 ppm, TWA = 1.4 mg/m^3)
- WEL (STEL = 2 ppm, STEL = 2.8 mg/m^3)
- TLV (NOHSC) (TWA = 1 ppm, TWA = 1.4 mg/m^3)

Acetic acid

- WEL (TWA = 10 ppm, TWA = 25 mg/m_3)
- WEL (STEL = 15 ppm, TWA = 38 mg/m_3)
- TLV (NOHSC) (TWA = 10 ppm; TWA = 25 mg/m_3)

8.2. Exposure Controls

- Ensure adequate ventilation.
- Apply technical measures to comply with the occupational exposure limits.
- Refer to protective measures listed in sections 7 and 8.

8.2.1. Occupational exposure controls

8.2.1.1. Respiratory protection

- In case of emissions, face mask with type NIOSH approved respiratory protection.
- Self-contained breathing apparatus in medium confinement/insufficient oxygen/in case of large uncontrolled emissions/in all circumstances when the mask and cartridge do not give adequate protection.
- Use only respiratory protection that conforms to international/ national standards.

8.2.1.2. Hand protection

- Protective gloves - impervious chemical resistant:
- PVC
- Rubber gloves
- Take note of the information given by the producer concerning permeability and break through times, and of special workplace conditions (mechanical strain, duration of contact).

8.2.1.3. Eye protection

- Chemical resistant goggles must be worn.
- If splashes are likely to occur, wear:
- Tightly fitting safety goggles
- Face-shield

8.2.1.4. Skin and body protection

- Protective suit
- If splashes are likely to occur, wear:
- Wear : Apron, Boots - Suitable material : Butyl rubber products

8.2.1.5. Hygiene measures

- Use only in an area equipped with a safety shower.
- Eye wash bottle with pure water
- When using do not eat, drink or smoke.
- Handle in accordance with good industrial hygiene and safety practice.

8.2.2. Environmental exposure controls

- Dispose of rinse water in accordance with local and national regulations.

9. PHYSICAL AND CHEMICAL PROPERTIES

9.1. General Information (appearance, odour)

Appearance	:	liquid
Colour	:	colorless
Odour	:	pungent

9.2. Important Health Safety and Environmental Information

pH	:	<1
Boiling point/range	:	*Remarks*: not applicable, Thermal decomposition.
Flash point	:	*Remarks*: not applicable, flammable vapours may occur above Self-Accelerating decomposition temperature (SADT).
Flammability	:	*Lower explosion limit*: *Remarks*: not applicable.
Explosive properties	:	*Remarks*: With certain materials (see section 10).
	:	*Remarks*: In case of heating:
Oxidizing properties	:	*Remarks*: yes
Relative density / Density	:	1.1
Solubility	:	Water *Remarks*: completely miscible
	:	Polar organic solvents *Remarks*: soluble
	:	Aromatic solvents *Remarks*: slightly soluble
Partition coefficient (in octanol/water)	:	*log Pow*: -1.25

9.3. Other data

Melting Point	:	ca. -30 °C (-22°F)

Decomposition Temperature : >= 55 °C (131°F)
Remarks: Self-Accelerating decomposition temperature (SADT)

10. STABILITY AND REACTIVITY

10.1. Stability

- Potential for exothermic hazard
- Stable under recommended storage conditions.

10.2. Conditions to avoid

- Contamination
- To avoid thermal decomposition, do not overheat.
- Keep at temperature not exceeding: 55 °C (131 °F)

10.3. Materials to avoid

- Acids, bases, metals, Salts of metals, reducing agents, organic materials, flammable materials

10.4. Hazardous decomposition products

- Oxygen
- The release of other hazardous decomposition products is possible.

11. TOXICOLOGICAL INFORMATION

Toxicological data

Acute oral toxicity

- LD50, rat, 330 mg/kg (7% solution)

Acute inhalation toxicity

- LC50, 1 h, rat, 590 mg/m3 (Peracetic acid)

Acute dermal irritation / corrosion

- LD50, rabbit, 1,410 mg/kg (10% solution)

Skin irritation

- Rabbit, Corrosive

Eye irritation

- Rabbit, Risk of serious damage to eyes. (4% solution)
- ***Irritation (other route)***
- Inhalation, mouse, Irritating to respiratory system., RD 50 = 22-24 mg/m3 (Peracetic Acid)

Sensitization

- Guinea pig, Did not cause sensitization on laboratory animals.

Chronic toxicity

- Oral, Prolonged exposure, rat, no systemic effect
- Dermal, Repeated exposure, guinea pig, irritant effects

Carcinogenicity

- Animal testing did not show any carcinogenic effects.

Genetic toxicity in vitro

- In vitro tests have shown mutagenic effects.

Genetic toxicity in vivo

- Animal testing did not show any mutagenic effects.

Possible hazards (summary)

- Corrosive effects

12. ECOLOGICAL INFORMATION

12.1. Ecotoxicity effects

Acute toxicity

- Fishes, Salmo gairdneri, LC_{50}, 96 h, 13 mg/l
  Remarks: fresh water
- Fishes, Pimephales platessa, NOEC, 56 mg/l
- Crustaceans, Daphnia magna, EC50, 48 h, 3.3 mg/l
  Remarks: fresh water
- Crustaceans, Daphnia magna, NOEC, 1 mg/l
- Crustaceans, Crangon crangon, EC50, 96 h, 126.8 mg/l (12 % solution)
  Remarks: salt water
- Crustaceans, Crangon crangon, NOEC, 56 mg/l

Chronic toxicity

- Fishes, various species, LC50
  Remarks: no data available
- NOEC
  Remarks: no data available
- Algae, various species, EC50, 72 - 96 h, 0.7 - 16 mg/l

12.2. Mobility

- Air, Volatility
  Remarks: not significant
- Water
  Remarks: Solubility, Mobility.
- Soil/sediments, adsorption
  Remarks: non-significant

12.3. Persistence and degradability

Abiotic degradation

- Air
  Result: The product can be degraded by abiotic (e.g. chemical or photolytic) processes
- Water, t 1/2 (Hydrolysis) ca. 120 h
  Result: Chemical degradation
- Soil 99 %, < 0.5 h (1 % solution)
  Result: Chemical degradation

Biodegradation

- aerobic, Tested according to: Closed Bottle test, 28 d
  Remarks: non-biodegradable.
- aerobic, Tested according to: ready biodegradability/MITI, from 2 mg/l, > 70 %, 28 d
  Remarks: Readily biodegradable.
- Anaerobic.
  Remarks: no data available
- Effects on waste water treatment plants, 90 mg/l
  Remarks: inhibitory action
- Effects on waste water treatment plants
  Remarks: BOD increase of treated effluent by acetic acid formation

12.4. Bioaccumulative potential

- Log Pow -1.25
  Result: Does not bioaccumulate.

12.5. Other adverse effects

- no data available

12.6. Possible hazards (summary)

- Toxic to aquatic organisms.
- Nevertheless, hazard for the environment is limited due to product properties:
- Inherently biodegradable.

- Does not bioaccumulate.

13. DISPOSAL CONSIDERATIONS

13.1. Waste from residues / unused products

- In accordance with local and national regulations.
- Limited quantity
- Dilute with plenty of water.
- Flush into sewer with plenty of water.
- Large quantities:
- Contact manufacturer.

13.2. Packaging treatment

- Empty containers.
- Clean container with water.
- Dispose of rinse water in accordance with local and national regulations.
- Do not rinse the dedicated containers.
- The empty and clean containers are to be reused in conformity with regulations.

14. TRANSPORT INFORMATION

UN-No **3109**

IATA-DGR

Class	5.2
Sub-risks	CORROSIVE
Packing group	II
ICAO-Labels	5.2 + 8
Proper shipping name:	ORGANIC PEROXIDE TYPE F LIQUID (PEROXYACETIC ACID, TYPE F STABILISED)

IMDG

Class	5.2
Sub-risks	Corrosive
Packing group	II
IMO-Labels	5.2 + 8
Proper shipping name:	ORGANIC PEROXIDE TYPE F LIQUID (PEROXYACETIC ACID, TYPE F STABILISED)

15. REGULATORY INFORMATION

15.1. Label

- Hazardous components which must be listed on the label: Hydrogen peroxide
- Classified as hazardous according to criteria of NOHSC.

Symbol(s)	C	Corrosive
	O	Oxidising
R-phrase(s)	R8	Contact with combustible material may cause fire.
	R20/21/22	Harmful by inhalation, in contact with skin and if swallowed.
	R35	Causes sever burns.
S-phrase(s)	S 1/2	Keep locked up and out of the reach of children.
	S 3/7	Keep container tightly closed in a cool place.
	S14	Keep away from combustible material: Acids, Reducing agents, Salts of metals.
	S36/37/39	Wear suitable protective clothing, gloves and eye/face protection.
	S45	In case of accident or if you feel unwell, seek

Medical advice immediately (show the label wherever possible).

15.2. Other information

- The percentage concentration of the solution has to be indicated next to the product name.

15.3. Inventory Information

- One or more components not listed on inventory.
-

16. OTHER INFORMATION

16.1. Ratings

NEPA (National Fire Protection Association)
Health = 3 Flammability = 1 Instability = 2 Special =OX
HMIS (Hazardous Material Information System)
Health = 3 Fire = 1 Reactivity = 2 PPE: Supplied by User; dependent on local conditions

16.2. Text of phrases mentioned

- WEL	WORKPLACE EXPOSURE LIMIT.
- TWA	TIME WEIGHTED AVERAGE.
- STEL	SHORT TERM EXPOSURE LIMIT.
- NOHSC	NATIONAL OCCUPATIONAL HEALTH AND SAFETY COMMISSION

16.3. Revisions

- Rev. No. 10 / 01/04/2012 – IMS First Issue
- Rev. No. 11 / 01/07/2021 – Telephone nos.

The information given corresponds to the current state of our knowledge and experience of the product, and is not exhaustive. This applies to product which conforms to the specification, unless otherwise stated. In this case of combinations and mixtures one must make sure that no new dangers can arise. In any case, the user is not exempt from observing all legal, administrative and regulatory procedures relating to the product, personal hygiene, and protection of human welfare and the environment.

SAFETY DATA SHEET

Version 6.5
Revision Date 10/28/2021
Print Date 04/16/2022

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

1.1 Product identifiers

Product name : Sodium hydroxide

Product Number : S8045
Brand : SIGALD
Index-No. : 011-002-00-6
CAS-No. : 1310-73-2

1.2 Relevant identified uses of the substance or mixture and uses advised against

Identified uses : Laboratory chemicals, Synthesis of substances

1.3 Details of the supplier of the safety data sheet

Company : Sigma-Aldrich Inc.
3050 SPRUCE ST
ST. LOUIS MO 63103
UNITED STATES

Telephone : +1 314 771-5765
Fax : +1 800 325-5052

1.4 Emergency telephone

Emergency Phone # : 800-424-9300 CHEMTREC (USA) +1-703-527-3887 CHEMTREC (International) 24 Hours/day; 7 Days/week

SECTION 2: Hazards identification

2.1 Classification of the substance or mixture

GHS Classification in accordance with 29 CFR 1910 (OSHA HCS)

Corrosive to Metals (Category 1), H290
Skin corrosion (Category 1A), H314
Serious eye damage (Category 1), H318
Short-term (acute) aquatic hazard (Category 3), H402

For the full text of the H-Statements mentioned in this Section, see Section 16.

2.2 GHS Label elements, including precautionary statements

Pictogram

Signal word Danger

Hazard statement(s)	
H290	May be corrosive to metals.
H314	Causes severe skin burns and eye damage.
H402	Harmful to aquatic life.
Precautionary statement(s)	
P234	Keep only in original container.
P260	Do not breathe dusts or mists.
P264	Wash skin thoroughly after handling.
P273	Avoid release to the environment.
P280	Wear protective gloves/ protective clothing/ eye protection/ face protection.
P301 + P330 + P331	IF SWALLOWED: Rinse mouth. Do NOT induce vomiting.
P303 + P361 + P353	IF ON SKIN (or hair): Take off immediately all contaminated clothing. Rinse skin with water/ shower.
P304 + P340 + P310	IF INHALED: Remove person to fresh air and keep comfortable for breathing. Immediately call a POISON CENTER/ doctor.
P305 + P351 + P338 + P310	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/ doctor.
P363	Wash contaminated clothing before reuse.
P390	Absorb spillage to prevent material damage.
P405	Store locked up.
P406	Store in corrosive resistant container with a resistant inner liner.
P501	Dispose of contents/ container to an approved waste disposal plant.

2.3 Hazards not otherwise classified (HNOC) or not covered by GHS - none

SECTION 3: Composition/information on ingredients

3.1 Substances

Synonyms	:	Caustic soda
Formula	:	NaOH
Molecular weight	:	40.00 g/mol
CAS-No.	:	1310-73-2
EC-No.	:	215-185-5
Index-No.	:	011-002-00-6

Component	Classification	Concentration
sodium hydroxide		
	Met. Corr. 1; Skin Corr. 1A; Eye Dam. 1; Aquatic Acute 3; H290, H314, H318, H402 Concentration limits: >= 0.4 %: Met. Corr. 1, H290; >= 5 %: Skin Corr. 1A, H314; 2 - < 5 %: Skin Corr. 1B, H314; 0.5 - < 2 %: Skin Irrit. 2, H315; 0.5 - < 2 %: Eye Irrit. 2,	<= 100 %

	H319;	

For the full text of the H-Statements mentioned in this Section, see Section 16.

SECTION 4: First aid measures

4.1 Description of first-aid measures

General advice
First aiders need to protect themselves. Show this material safety data sheet to the doctor in attendance.

If inhaled
After inhalation: fresh air. Call in physician.

In case of skin contact
In case of skin contact: Take off immediately all contaminated clothing. Rinse skin with water/ shower. Call a physician immediately.

In case of eye contact
After eye contact: rinse out with plenty of water. Immediately call in ophthalmologist. Remove contact lenses.

If swallowed
After swallowing: make victim drink water (two glasses at most), avoid vomiting (risk of perforation). Call a physician immediately. Do not attempt to neutralise.

4.2 Most important symptoms and effects, both acute and delayed
The most important known symptoms and effects are described in the labelling (see section 2.2) and/or in section 11

4.3 Indication of any immediate medical attention and special treatment needed
No data available

SECTION 5: Firefighting measures

5.1 Extinguishing media

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

Unsuitable extinguishing media
For this substance/mixture no limitations of extinguishing agents are given.

5.2 Special hazards arising from the substance or mixture
Sodium oxides
Not combustible.
Ambient fire may liberate hazardous vapours.

5.3 Advice for firefighters
Stay in danger area only with self-contained breathing apparatus. Prevent skin contact by keeping a safe distance or by wearing suitable protective clothing.

5.4 Further information
Suppress (knock down) gases/vapors/mists with a water spray jet. Prevent fire extinguishing water from contaminating surface water or the ground water system.

SECTION 6: Accidental release measures

6.1 Personal precautions, protective equipment and emergency procedures
Advice for non-emergency personnel: Avoid inhalation of dusts. Avoid substance contact. Ensure adequate ventilation. Evacuate the danger area, observe emergency procedures, consult an expert.
For personal protection see section 8.

6.2 Environmental precautions
Do not let product enter drains.

6.3 Methods and materials for containment and cleaning up
Cover drains. Collect, bind, and pump off spills. Observe possible material restrictions (see sections 7 and 10). Take up dry. Dispose of properly. Clean up affected area. Avoid generation of dusts.

6.4 Reference to other sections
For disposal see section 13.

SECTION 7: Handling and storage

7.1 Precautions for safe handling
For precautions see section 2.2.

7.2 Conditions for safe storage, including any incompatibilities

Storage conditions
No metal containers.
Tightly closed. Dry.

Storage class
Storage class (TRGS 510): 8B: Non-combustible, corrosive hazardous materials

7.3 Specific end use(s)
Apart from the uses mentioned in section 1.2 no other specific uses are stipulated

SECTION 8: Exposure controls/personal protection

8.1 Control parameters

Ingredients with workplace control parameters

Component	CAS-No.	Value	Control parameters	Basis
sodium hydroxide	1310-73-2	C	2 mg/m3	USA. ACGIH Threshold Limit Values (TLV)
		C	2 mg/m3	USA. NIOSH Recommended Exposure Limits
		TWA	2 mg/m3	USA. Occupational Exposure Limits (OSHA) - Table Z-1 Limits for Air Contaminants
		C	2 mg/m3	USA. OSHA - TABLE Z-1 Limits for Air Contaminants - 1910.1000
		C	2 mg/m3	California permissible exposure limits for chemical contaminants (Title 8, Article 107)

Derived No Effect Level (DNEL)

Application Area	Routes of exposure	Health effect	Value
Workers	Inhalation	Long-term local effects	1 mg/m3
Consumers	Inhalation	Long-term local effects	1 mg/m3

8.2 Exposure controls

Appropriate engineering controls
Immediately change contaminated clothing. Apply preventive skin protection. Wash hands and face after working with substance.

Personal protective equipment

Eye/face protection
Use equipment for eye protection tested and approved under appropriate government standards such as NIOSH (US) or EN 166(EU). Tightly fitting safety goggles

Skin protection
This recommendation applies only to the product stated in the safety data sheet, supplied by us and for the designated use. When dissolving in or mixing with other substances and under conditions deviating from those stated in EN374 please contact the supplier of CE-approved gloves (e.g. KCL GmbH, D-36124 Eichenzell, Internet: www.kcl.de).
Full contact
Material: Nitrile rubber
Minimum layer thickness: 0.11 mm
Break through time: 480 min
Material tested:KCL 741 Dermatril® L

This recommendation applies only to the product stated in the safety data sheet, supplied by us and for the designated use. When dissolving in or mixing with other substances and under conditions deviating from those stated in EN374 please contact the supplier of CE-approved gloves (e.g. KCL GmbH, D-36124 Eichenzell, Internet: www.kcl.de).
Splash contact
Material: Nitrile rubber
Minimum layer thickness: 0.11 mm
Break through time: 480 min

Material tested:KCL 741 Dermatril® L

Body Protection
protective clothing

Respiratory protection
required when dusts are generated.
Our recommendations on filtering respiratory protection are based on the following standards: DIN EN 143, DIN 14387 and other accompanying standards relating to the used respiratory protection system.

Control of environmental exposure
Do not let product enter drains.

SECTION 9: Physical and chemical properties

9.1 Information on basic physical and chemical properties

a)	Appearance	Form: pellets Color: white
b)	Odor	odorless
c)	Odor Threshold	Not applicable
d)	pH	ca.> 14 at 100 g/l at 20 °C (68 °F)
e)	Melting point/freezing point	Melting point/range: 318 °C (604 °F)
f)	Initial boiling point and boiling range	1,390 °C 2,534 °F at 1,013 hPa
g)	Flash point	()Not applicable
h)	Evaporation rate	No data available
i)	Flammability (solid, gas)	The product is not flammable.
j)	Upper/lower flammability or explosive limits	No data available
k)	Vapor pressure	< 24 hPa at 20 °C (68 °F)
l)	Vapor density	1.38 - (Air = 1.0)
m)	Density	2.13 g/cm3 at 20 °C (68 °F)
	Relative density	No data available
n)	Water solubility	1,090 g/l at 20 °C (68 °F)
o)	Partition coefficient: n-octanol/water	Not applicable for inorganic substances
p)	Autoignition temperature	No data available
q)	Decomposition temperature	No data available
r)	Viscosity	No data available
s)	Explosive properties	No data available

t) Oxidizing properties none

9.2 Other safety information

Relative vapor density 1.38 - (Air = 1.0)

SECTION 10: Stability and reactivity

10.1 Reactivity
No data available

10.2 Chemical stability
The product is chemically stable under standard ambient conditions (room temperature) .

10.3 Possibility of hazardous reactions
Violent reactions possible with:
Acetone
Chlorine
Ethylene oxide
Fluorine
Hydrogen halides
Hydrazine hydrate
hydroxylamine
Acid anhydrides
Acrolein
Acid chlorides
Acids
sulfuric acid
Chloroform
Water
hydrogen peroxide
anhydrides
phosphides
halogen-halogen compounds
trichloroethene
can decompose violently in contact with:
Organic Substances
hydrogen sulphide
Risk of ignition or formation of inflammable gases or vapours with:
powdered aluminium
Ammonium salts
persulfates
Sodium borohydride
phosphorus
Oxides of phosphorus
Halogenated hydrocarbon
Light metals
Metals
Risk of explosion/exothermic reaction with:
Bromine
Calcium
in powder form
furfuryl alcohol
Nitromethane

Peroxides
organic nitro compounds
Nitriles
Acrylic monomers
Chloroform
with
Acetone
Nitrobenzene
with
Methanol
Nitrobenzene
with
salts
magnesium
Zinc
and
Tin
(in the presence of atmospheric oxygen and/or moisture)

10.4 Conditions to avoid
no information available

10.5 Incompatible materials
Aluminum, brass, Metals, metal alloys, Zinc, Tin

10.6 Hazardous decomposition products
In the event of fire: see section 5

SECTION 11: Toxicological information

11.1 Information on toxicological effects

Acute toxicity
Oral: No data available
Symptoms: If ingested, severe burns of the mouth and throat, as well as a danger of perforation of the esophagus and the stomach.
Inhalation: No data available
Inhalation: Corrosive to respiratory system.
Symptoms: burns of mucous membranes, Cough, Shortness of breath, Possible damages:, damage of respiratory tract
Dermal: No data available
No data available

Skin corrosion/irritation
Skin - Rabbit
Result: Causes burns.
Remarks: (Regulation (EC) No 1272/2008, Annex VI)

Serious eye damage/eye irritation
Eyes - Rabbit
Result: Causes serious eye damage.
(OECD Test Guideline 405)
Remarks: (Regulation (EC) No 1272/2008, Annex VI)
Causes serious eye damage.

Respiratory or skin sensitization
Patch test: - In vitro study
Result: negative
Remarks: (ECHA)

Germ cell mutagenicity
No data available

Carcinogenicity

IARC: No ingredient of this product present at levels greater than or equal to 0.1% is identified as probable, possible or confirmed human carcinogen by IARC.

NTP: No ingredient of this product present at levels greater than or equal to 0.1% is identified as a known or anticipated carcinogen by NTP.

OSHA: No component of this product present at levels greater than or equal to 0.1% is on OSHA's list of regulated carcinogens.

Reproductive toxicity
No data available

Specific target organ toxicity - single exposure
No data available

Specific target organ toxicity - repeated exposure
No data available

Aspiration hazard
No data available

11.2 Additional Information

RTECS: WB4900000
burning sensation, Cough, wheezing, laryngitis, Shortness of breath, spasm, inflammation and edema of the larynx, spasm, inflammation and edema of the bronchi, pneumonitis, pulmonary edema, Material is extremely destructive to tissue of the mucous membranes and upper respiratory tract, eyes, and skin., To the best of our knowledge, the chemical, physical, and toxicological properties have not been thoroughly investigated.

Other dangerous properties can not be excluded.

Handle in accordance with good industrial hygiene and safety practice.

SECTION 12: Ecological information

12.1 Toxicity

Toxicity to fish	LC50 - Gambusia affinis (Mosquito fish) - 125 mg/l - 96 h Remarks: (ECOTOX Database)
Toxicity to daphnia and other aquatic invertebrates	EC50 - Ceriodaphnia (water flea) - 40.4 mg/l - 48 h Remarks: (ECHA)
Toxicity to bacteria	EC50 - Photobacterium phosphoreum - 22 mg/l - 15 min Remarks: (External MSDS)

12.2 Persistence and degradability
The methods for determining the biological degradability are not applicable to inorganic substances.

12.3 Bioaccumulative potential
No data available

12.4 Mobility in soil
No data available

12.5 Results of PBT and vPvB assessment
PBT/vPvB assessment not available as chemical safety assessment not required/not conducted

12.6 Endocrine disrupting properties
No data available

12.7 Other adverse effects
Harmful effect due to pH shift.
Forms corrosive mixtures with water even if diluted.
Neutralisation possible in waste water treatment plants.
Discharge into the environment must be avoided.

SECTION 13: Disposal considerations

13.1 Waste treatment methods

Product
Waste material must be disposed of in accordance with the national and local regulations. Leave chemicals in original containers. No mixing with other waste. Handle uncleaned containers like the product itself. See www.retrologistik.com for processes regarding the return of chemicals and containers, or contact us there if you have further questions.

SECTION 14: Transport information

DOT (US)
UN number: 1823 Class: 8 Packing group: II
Proper shipping name: Sodium hydroxide, solid
Reportable Quantity (RQ): 1000 lbs
Poison Inhalation Hazard: No

IMDG
UN number: 1823 Class: 8 Packing group: II EMS-No: F-A, S-B
Proper shipping name: SODIUM HYDROXIDE, SOLID

IATA
UN number: 1823 Class: 8 Packing group: II
Proper shipping name: Sodium hydroxide, solid

SECTION 15: Regulatory information

SARA 302 Components

This material does not contain any components with a section 302 EHS TPQ.

SARA 313 Components
This material does not contain any chemical components with known CAS numbers that exceed the threshold (De Minimis) reporting levels established by SARA Title III, Section 313.

SARA 311/312 Hazards
No SARA Hazards

Massachusetts Right To Know Components
No components are subject to the Massachusetts Right to Know Act.

SECTION 16: Other information

Further information
The above information is believed to be correct but does not purport to be all inclusive and shall be used only as a guide. The information in this document is based on the present state of our knowledge and is applicable to the product with regard to appropriate safety precautions. It does not represent any guarantee of the properties of the product. Sigma-Aldrich Corporation and its Affiliates shall not be held liable for any damage resulting from handling or from contact with the above product. See www.sigma-aldrich.com and/or the reverse side of invoice or packing slip for additional terms and conditions of sale.

The branding on the header and/or footer of this document may temporarily not visually match the product purchased as we transition our branding. However, all of the information in the document regarding the product remains unchanged and matches the product ordered. For further information please contact mlsbranding@sial.com.

Version: 6.5 Revision Date: 10/28/2021 Print Date: 04/16/2022

SAFETY DATA SHEET

Version 6.10
Revision Date 08/02/2021
Print Date 04/16/2022

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

1.1 Product identifiers

Product name : Sulfuric acid

Product Number : 339741
Brand : Aldrich
Index-No. : 016-020-00-8
CAS-No. : 7664-93-9

1.2 Relevant identified uses of the substance or mixture and uses advised against

Identified uses : Laboratory chemicals, Synthesis of substances

1.3 Details of the supplier of the safety data sheet

Company : Sigma-Aldrich Inc.
3050 SPRUCE ST
ST. LOUIS MO 63103
UNITED STATES

Telephone : +1 314 771-5765
Fax : +1 800 325-5052

1.4 Emergency telephone

Emergency Phone # : 800-424-9300 CHEMTREC (USA) +1-703-527-3887 CHEMTREC (International) 24 Hours/day; 7 Days/week

SECTION 2: Hazards identification

2.1 Classification of the substance or mixture

GHS Classification in accordance with 29 CFR 1910 (OSHA HCS)

Corrosive to Metals (Category 1), H290
Skin corrosion (Category 1A), H314
Serious eye damage (Category 1), H318

For the full text of the H-Statements mentioned in this Section, see Section 16.

2.2 GHS Label elements, including precautionary statements

Pictogram

Signal word Danger

Hazard statement(s)	
H290	May be corrosive to metals.
H314	Causes severe skin burns and eye damage.
Precautionary statement(s)	
P234	Keep only in original container.
P264	Wash skin thoroughly after handling.
P280	Wear protective gloves/ protective clothing/ eye protection/ face protection.
P301 + P330 + P331	IF SWALLOWED: Rinse mouth. Do NOT induce vomiting.
P303 + P361 + P353	IF ON SKIN (or hair): Take off immediately all contaminated clothing. Rinse skin with water/ shower.
P304 + P340 + P310	IF INHALED: Remove person to fresh air and keep comfortable for breathing. Immediately call a POISON CENTER/ doctor.
P305 + P351 + P338 + P310	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/ doctor.
P363	Wash contaminated clothing before reuse.
P390	Absorb spillage to prevent material damage.
P405	Store locked up.
P406	Store in corrosive resistant container with a resistant inner liner.
P501	Dispose of contents/ container to an approved waste disposal plant.

2.3 Hazards not otherwise classified (HNOC) or not covered by GHS - none

SECTION 3: Composition/information on ingredients

3.1 Substances

Formula	:	H_2O_4S
Molecular weight	:	98.08 g/mol
CAS-No.	:	7664-93-9
EC-No.	:	231-639-5
Index-No.	:	016-020-00-8

Component	Classification	Concentration
sulphuric acid		
	Met. Corr. 1; Skin Corr. 1A; Eye Dam. 1; H290, H314, H318 Concentration limits: >= 0.3 %: Met. Corr. 1, H290; >= 15 %: Skin Corr. 1A, H314; 5 - < 15 %: Skin Irrit. 2, H315; 5 - < 15 %: Eye Irrit. 2, H319;	<= 100 %

For the full text of the H-Statements mentioned in this Section, see Section 16.

SECTION 4: First aid measures

4.1 Description of first-aid measures

General advice
First aiders need to protect themselves. Show this material safety data sheet to the doctor in attendance.

If inhaled
After inhalation: fresh air. Call in physician.

In case of skin contact
In case of skin contact: Take off immediately all contaminated clothing. Rinse skin with water/ shower. Call a physician immediately.

In case of eye contact
After eye contact: rinse out with plenty of water. Immediately call in ophthalmologist. Remove contact lenses.

If swallowed
After swallowing: make victim drink water (two glasses at most), avoid vomiting (risk of perforation). Call a physician immediately. Do not attempt to neutralise.

4.2 Most important symptoms and effects, both acute and delayed
The most important known symptoms and effects are described in the labelling (see section 2.2) and/or in section 11

4.3 Indication of any immediate medical attention and special treatment needed
No data available

SECTION 5: Firefighting measures

5.1 Extinguishing media

Suitable extinguishing media
Use water spray, alcohol-resistant foam, dry chemical or carbon dioxide.

5.2 Special hazards arising from the substance or mixture
Sulfur oxides
Not combustible.
Ambient fire may liberate hazardous vapours.

5.3 Advice for firefighters
Stay in danger area only with self-contained breathing apparatus. Prevent skin contact by keeping a safe distance or by wearing suitable protective clothing.

5.4 Further information
Suppress (knock down) gases/vapors/mists with a water spray jet. Prevent fire extinguishing water from contaminating surface water or the ground water system.

SECTION 6: Accidental release measures

6.1 Personal precautions, protective equipment and emergency procedures
Advice for non-emergency personnel: Do not breathe vapors, aerosols. Avoid substance contact. Ensure adequate ventilation. Evacuate the danger area, observe emergency procedures, consult an expert.
For personal protection see section 8.

6.2 Environmental precautions
Do not let product enter drains.

6.3 Methods and materials for containment and cleaning up
Cover drains. Collect, bind, and pump off spills. Observe possible material restrictions (see sections 7 and 10). Take up with liquid-absorbent and neutralising material (e.g. Chemizorb® H⁺, Merck Art. No. 101595). Dispose of properly. Clean up affected area.

6.4 Reference to other sections
For disposal see section 13.

SECTION 7: Handling and storage

7.1 Precautions for safe handling
For precautions see section 2.2.

7.2 Conditions for safe storage, including any incompatibilities

Storage conditions
No metal containers.
Tightly closed.
Storage class (TRGS 510): 8B: Non-combustible, corrosive hazardous materials

7.3 Specific end use(s)
Apart from the uses mentioned in section 1.2 no other specific uses are stipulated

SECTION 8: Exposure controls/personal protection

8.1 Control parameters

Ingredients with workplace control parameters

Component	CAS-No.	Value	Control parameters	Basis
sulphuric acid	7664-93-9	TWA	0.2 mg/m3	USA. ACGIH Threshold Limit Values (TLV)
		TWA	1 mg/m3	USA. OSHA - TABLE Z-1 Limits for Air Contaminants - 1910.1000
		TWA	1 mg/m3	USA. Occupational Exposure Limits (OSHA) - Table Z-1 Limits for Air Contaminants

8.2 Exposure controls

Appropriate engineering controls
Change contaminated clothing and immerse in water. Preventive skin protection Wash hands and face after working with substance.

Personal protective equipment

Eye/face protection
Use equipment for eye protection tested and approved under appropriate government standards such as NIOSH (US) or EN 166(EU). Tightly fitting safety goggles

Skin protection
This recommendation applies only to the product stated in the safety data sheet, supplied by us and for the designated use. When dissolving in or mixing with other substances and under conditions deviating from those stated in EN374 please contact the supplier of CE-approved gloves (e.g. KCL GmbH, D-36124 Eichenzell, Internet: www.kcl.de).
Full contact
Material: Viton®
Minimum layer thickness: 0.7 mm
Break through time: 480 min
Material tested:Vitoject® (KCL 890 / Aldrich Z677698, Size M)

This recommendation applies only to the product stated in the safety data sheet, supplied by us and for the designated use. When dissolving in or mixing with other substances and under conditions deviating from those stated in EN374 please contact the supplier of CE-approved gloves (e.g. KCL GmbH, D-36124 Eichenzell, Internet: www.kcl.de).
Splash contact
Material: butyl-rubber
Minimum layer thickness: 0.7 mm
Break through time: 120 min
Material tested:Butoject® (KCL 898)

Body Protection
Acid-resistant protective clothing

Respiratory protection
required when vapours/aerosols are generated. Our recommendations on filtering respiratory protection are based on the following standards: DIN EN 143, DIN 14387 and other accompanying standards relating to the used respiratory protection system.

Control of environmental exposure
Do not let product enter drains.

SECTION 9: Physical and chemical properties

9.1 Information on basic physical and chemical properties

a)	Appearance	Form: clear, liquid Color: colorless
b)	Odor	odorless
c)	Odor Threshold	Not applicable
d)	pH	1.2 at 5 g/l
e)	Melting point/freezing point	Melting point: 10.31 °C (50.56 °F)
f)	Initial boiling point and boiling range	290 °C 554 °F - lit.
g)	Flash point	()No data available
h)	Evaporation rate	No data available
i)	Flammability (solid, gas)	No data available
j)	Upper/lower	No data available

	flammability or explosive limits	
k)	Vapor pressure	1.33 hPa at 145.8 °C (294.4 °F)
l)	Vapor density	3.39 - (Air = 1.0)
m)	Density	1.84 g/cm3 at 25 °C (77 °F) - lit.
	Relative density	No data available
n)	Water solubility	soluble
o)	Partition coefficient: n-octanol/water	Not applicable for inorganic substances
p)	Autoignition temperature	No data available
q)	Decomposition temperature	No data available
r)	Viscosity	No data available
s)	Explosive properties	No data available
t)	Oxidizing properties	No data available

9.2 Other safety information

Surface tension	55.1 mN/m at 20 °C (68 °F)
Relative vapor density	3.39 - (Air = 1.0)

SECTION 10: Stability and reactivity

10.1 Reactivity
No data available

10.2 Chemical stability
The product is chemically stable under standard ambient conditions (room temperature) .

10.3 Possibility of hazardous reactions
A risk of explosion and/or of toxic gas formation exists with the following substances:
Water
Alkali metals
alkali compounds
Ammonia
Aldehydes
acetonitrile
Alkaline earth metals
alkalines
Acids
alkaline earth compounds
Metals
metal alloys
Oxides of phosphorus
phosphorus
hydrides
halogen-halogen compounds
oxyhalogenic compounds

permanganates
nitrates
carbides
combustible substances
organic solvent
acetylidene
Nitriles
organic nitro compounds
anilines
Peroxides
picrates
nitrides
lithium silicide
iron(III) compounds
bromates
chlorates
Amines
perchlorates
hydrogen peroxide

10.4 Conditions to avoid
no information available

10.5 Incompatible materials
animal/vegetable tissuesContact with metals liberates hydrogen gas.

10.6 Hazardous decomposition products
In the event of fire: see section 5

SECTION 11: Toxicological information

11.1 Information on toxicological effects

Acute toxicity
LD50 Oral - Rat - male and female - 2,140 mg/kg
Remarks: (ECHA)
Inhalation: Corrosive to respiratory system.
Dermal: No data available

Skin corrosion/irritation
Skin - Rabbit
Result: Extremely corrosive and destructive to tissue.
Remarks: (IUCLID)

Serious eye damage/eye irritation
Causes serious eye damage.

Respiratory or skin sensitization
No data available

Germ cell mutagenicity
Test Type: Ames test
Test system: Salmonella typhimurium
Result: negative
Remarks: (HSDB)

Carcinogenicity

No data available

IARC: No ingredient of this product present at levels greater than or equal to 0.1% is identified as probable, possible or confirmed human carcinogen by IARC.

NTP: No ingredient of this product present at levels greater than or equal to 0.1% is identified as a known or anticipated carcinogen by NTP.

OSHA: No component of this product present at levels greater than or equal to 0.1% is on OSHA's list of regulated carcinogens.

Reproductive toxicity
No data available

Specific target organ toxicity - single exposure
No data available

Specific target organ toxicity - repeated exposure
No data available

Aspiration hazard
No data available

11.2 Additional Information

RTECS: WS5600000
Material is extremely destructive to tissue of the mucous membranes and upper respiratory tract, eyes, and skin., spasm, inflammation and edema of the larynx, spasm, inflammation and edema of the bronchi, pneumonitis, pulmonary edema, burning sensation, Cough, wheezing, laryngitis, Shortness of breath, Headache, Nausea, Vomiting, Pulmonary edema. Effects may be delayed.
To the best of our knowledge, the chemical, physical, and toxicological properties have not been thoroughly investigated.

After inhalation of aerosols: damage to the affected mucous membranes. After skin contact: severe burns with formation of scabs. After eye contact: burns, corneal lesions. After swallowing: severe pain (risk of perforation!), nausea, vomiting and diarrhoea. After a latency period of several weeks possibly pyloric stenosis.

Other dangerous properties can not be excluded.

Handle in accordance with good industrial hygiene and safety practice.

Stomach - Irregularities - Based on Human Evidence

Stomach - Irregularities - Based on Human Evidence

SECTION 12: Ecological information

12.1 Toxicity

Toxicity to daphnia and other aquatic invertebrates	static test EC50 - Daphnia magna (Water flea) - > 100 mg/l - 48 h (OECD Test Guideline 202)
Toxicity to algae	static test ErC50 - Desmodesmus subspicatus (green algae) - > 100 mg/l - 72 h (OECD Test Guideline 201)

12.2 Persistence and degradability
The methods for determining the biological degradability are not applicable to inorganic substances.

12.3 Bioaccumulative potential
No data available

12.4 Mobility in soil
No data available

12.5 Results of PBT and vPvB assessment
PBT/vPvB assessment not available as chemical safety assessment not required/not conducted

12.6 Other adverse effects
Biological effects:
Harmful effect due to pH shift.
Caustic even in diluted form.
Does not cause biological oxygen deficit.
Endangers drinking-water supplies if allowed to enter soil and/or waters in large quantities.
Neutralisation possible in waste water treatment plants.
Discharge into the environment must be avoided.

SECTION 13: Disposal considerations

13.1 Waste treatment methods

Product
Waste material must be disposed of in accordance with the national and local regulations. Leave chemicals in original containers. No mixing with other waste. Handle uncleaned containers like the product itself. See www.retrologistik.com for processes regarding the return of chemicals and containers, or contact us there if you have further questions.

SECTION 14: Transport information

DOT (US)
UN number: 1830 Class: 8 Packing group: II
Proper shipping name: Sulfuric acid
Reportable Quantity (RQ): 1000 lbs
Poison Inhalation Hazard: No

IMDG
UN number: 1830 Class: 8 Packing group: II EMS-No: F-A, S-B
Proper shipping name: SULPHURIC ACID

IATA
UN number: 1830 Class: 8 Packing group: II
Proper shipping name: Sulphuric acid

SECTION 15: Regulatory information

SARA 302 Components

	CAS-No.	Revision Date
sulphuric acid	7664-93-9	2007-07-01

SARA 313 Components
The following components are subject to reporting levels established by SARA Title III, Section 313:

	CAS-No.	Revision Date
sulphuric acid	7664-93-9	2007-07-01

SARA 311/312 Hazards
Acute Health Hazard, Chronic Health Hazard

Massachusetts Right To Know Components
No components are subject to the Massachusetts Right to Know Act.

SECTION 16: Other information

Further information
The above information is believed to be correct but does not purport to be all inclusive and shall be used only as a guide. The information in this document is based on the present state of our knowledge and is applicable to the product with regard to appropriate safety precautions. It does not represent any guarantee of the properties of the product. Sigma-Aldrich Corporation and its Affiliates shall not be held liable for any damage resulting from handling or from contact with the above product. See www.sigma-aldrich.com and/or the reverse side of invoice or packing slip for additional terms and conditions of sale.

The branding on the header and/or footer of this document may temporarily not visually match the product purchased as we transition our branding. However, all of the information in the document regarding the product remains unchanged and matches the product ordered. For further information please contact mlsbranding@sial.com.

Version: 6.10 Revision Date: 08/02/2021 Print Date: 04/16/2022