Design of an Insulin Glargine Manufacturing Plant to Increase Affordability and Accessibility of Diabetes Medication in the Sub-Saharan Region of Africa

A Technical Report submitted to the Department of Chemical Engineering

Presented to the Faculty of the School of Engineering and Applied Science

University of Virginia • Charlottesville, Virginia

In Partial Fulfillment of the Requirements for the Degree

Bachelor of Science, School of Engineering

Gabriella Recce

Spring, 2024

Technical Project Team Members

Jack Dunleavy

Vadaysha Jefferson

Joon Kim

On my honor as a University Student, I have neither given nor received unauthorized aid on this assignment as defined by the Honor Guidelines for Thesis-Related Assignments

Eric Anderson, Department of Chemical Engineering

Design of an Insulin Glargine Manufacturing Plant to Increase Affordability and Accessibility of Diabetes Medication in the Sub-Saharan Region of Africa

Table of Contents

1. Summary

This project aims to design an insulin glargine manufacturing facility that is able to supply affordable insulin to 6 million people in sub-Saharan Africa. To meet this goal of supporting 25% of the diabetic population of Africa, 3 tonnes of insulin must be produced annually. The upstream and downstream processes have been designed with an overall protein yield of 32.52%. One batch will produce ~ 10.28 kg of insulin, therefore 272 batches per year are needed to hit our target. We begin and finish a batch every 28.5 hours. At a cost per unit of insulin of $0.05, our annual revenue will be $3.83 billion dollars. Our process has been determined to be economically viable with an internal rate of return of 60% based on a discounted cash flow analysis using a discount rate of 20%.

2. Introduction

Before the discovery of insulin therapy, individuals with type 1 diabetes had limited options and often faced starvation diets to survive (Mazur, 2011). Advancements in insulin therapy have significantly improved life expectancies for people with type 1 diabetes. Despite these advancements, diabetes has become a global epidemic, affecting over 420 million individuals according to the World Health Organization. The accessibility and affordability of insulin remain major challenges across the globe, particularly in Sub-Saharan Africa, where the limited access can result in life expectancies as low as one year for children with type 1 diabetes (Peer, 2014). Sub-Saharan Africa, which faces additional challenges such as infectious diseases, lack of diabetes education, and government constraints on patient treatment and insulin distribution, is particularly impacted by global supply chain and production issues of the medicine. To address these challenges, this project aims to produce and distribute insulin glargine, a long-acting insulin, in Sub-Saharan Africa. Insulin glargine plays a crucial role in diabetes management, helping individuals maintain stable blood sugar levels. Unlike fast-acting insulin, it lasts approximately 24 hours, offering convenience and better blood sugar control throughout the day.

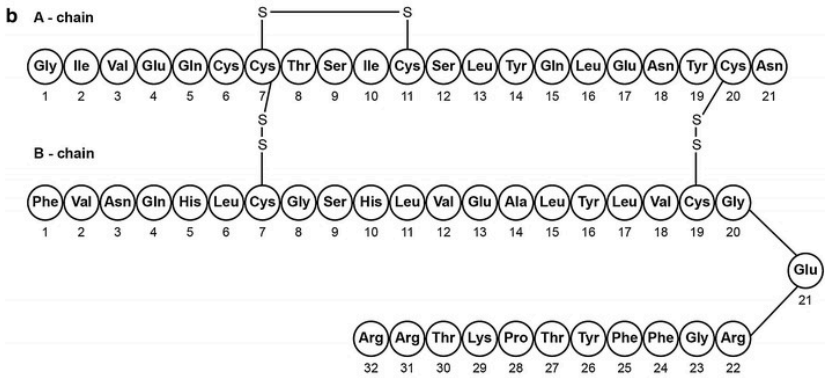
The project aims to manufacture insulin glargine using a well-established biotechnological process involving genetically engineered *Escherichia coli* (*E. coli*) bacteria. The process encompasses various unit operations, including fermentation, cell harvesting, filtration, chromatography, concentration, sterilization, and purification. *E. coli* is chosen as the host cell due to its advantages of high yield, cost-effectiveness, and ease of handling. To create the desired slow-release effect of insulin glargine, specific modifications to the amino acid chain must be made during production. As a long-acting form of insulin, insulin glargine helps manage

the body's general needs and lasts typically for 24 hours as opposed to fast-acting forms of insulin which help reduce blood glucose levels at meal times and lasts for a shorter duration of time (Beran et al., 2016).

The current standard process for insulin production relies on genetically engineered *E. coli* bacteria. For the upstream portion of this project, *E. coli* will be the desired product, for "using *E. coli* as the expression system for large-scale recombinant insulin production possesses the advantages of high growth rate, simple media requirement, ease of handling, high yield, and cost effectiveness" (Siew & Zhang, 2021). Similar to other recombinant proteins, *E. coli* will be modified to express the A and B chains of insulin. To create the slow-release and long-acting effect of insulin glargine, modifications to insulin's amino acid chain, including asparagine to glycine on the A chain at position 21 and adding two arginines to positions 31 and 32 on the B chain, need to be made during the production process.

Figure 2.1.a

Insulin Glargine Amino Acid Chain (Hilgenfeld et al., 2014)



This change causes the insulin to act for up to 24 hours after injection and allows for the insulin to remain soluble at a pH of 4.0, which is the pH of the solution that the insulin resides

(Cunningham & Freeman, 2022). As a result, the insulin analog will precipitate after the initial injection into the body's pH of ~7.4, slowing its absorption and extending its duration of action (Hilgenfeld et al., 2014). Insulin glargine acts by binding to insulin receptors (IR), which are tyrosine kinase receptors with two extracellular alpha domains and two intracellular catalytic beta domains (Cunningham & Freeman, 2022). After insulin binds, the beta catalytic domains undergo a conformational change to activate the tyrosine kinase domains and auto-phosphorylate the beta subunits. The beta subunits activate phosphatidylinositol-4,5-bisphosphate 3-kinase (PI3k) which triggers an intracellular signaling cascade and eventually ignites the activity of the glucose transporter type 4 (GLUT4) receptor, mitogen-activated protein kinase (MAPK) pathway, and the protein kinase C (PKC) pathway. All three pathways are involved with the uptake of glucose into fat and muscle cells, thus regulating the blood glucose levels in the body.

The drug will be delivered via an injectable form of insulin glargine. This allows for compact storage, large scale distribution, and safe administration. The insulin glargine will be added to a saline formulation and sold in 10 mL vials across the region. This formulation will also contain zinc, m-Cresol, glycerol, and polysorbate 20 along with the solution. Zinc is included to maintain stability of the insulin structure, specifically the way the protein is folded. m-Cresol is used as a preservative to prevent growth of microorganisms. Glycerin is added as a preservative and humectant. Polysorbate 20 is added for its high safety characterization as an excipient. The vials contain 1000 units of insulin glargine and will last about a month.

The target market for this project is in the sub-Saharan region of Africa, a continent where 24 million people are living with diabetes (World Health Organization, 2022). For Type 2 Diabetes Mellitus (T2DM), a patient usually requires 0.5 units/kg of insulin glargine a day. (Straight Healthcare, n. d.) If the average weight of a person living in Africa is 70 kg, then 35

units of insulin are needed per day. Since 100 units of insulin is equivalent to 3.64 mg, the plant must produce 2790 kg/year or ~3 tonnes/year. This amount would provide sufficient insulin glargine to 6 million people, which is 25% of diabetic patients in Africa. This target size was chosen based on the feasibility of creating a manufacturing plant, the availability of the market, and constraints on scale-up and production. Although 25% is an ambitious goal, it can be achieved for there are few insulin manufacturing sites and stakeholders in this region. The competition in this region should be lower and the demand is high. For example, Novo Nordisk, one of the leading manufacturers of insulin, just made a deal in September 2023 with the South African manufacturer, Aspen, to expand and reach the needs of 1.1 million people which only equates to 17% of our target market (Cullinan, 2023). Our goal is to service the needs of the other 83% of our target population.

3. Previous Work

Two main sources were used as references to model our design project. The first source is a research paper titled, “Recombinant Insulin Glargine Production Process Using *Escherichia Coli*”, written by Hwang et al. in 2016. This paper describes the process of synthesizing insulin glargine on a laboratory scale. We scaled up this study to an industrial scale and based our design process on the parameters listed in the paper. Our second source was a previous capstone project at UVA done by Iudica et al. in 2023. In this project, the students designed an insulin glargine manufacturing process using a different design and process flow. We used this source as an example for formatting, design, and overall presentation of the capstone project. Additionally, previous research was done during the Fall 2023 semester in CHE4474 to gather information about our product, design our flow process, and create a rough economic estimate of our facility and process. This information was compiled as a deliverable and was used as a starting point for our design project.

4. Discussion

I. Process Flow Diagram

The process to produce insulin glargine involves different unit operations, auxiliary equipment, and intricate steps. The process flow diagram, depicted in Figure 4.1.a, illustrates the equipment layout for this process. The system includes an upstream process, consisting of fermenters with equipment tags F100-F104, and a downstream process starting at P105 and ending with FR101. A complete list of equipment with their equipment tag and description can be found in Table 4.1.a.

Figure 4.1.a

Process Flow Diagram

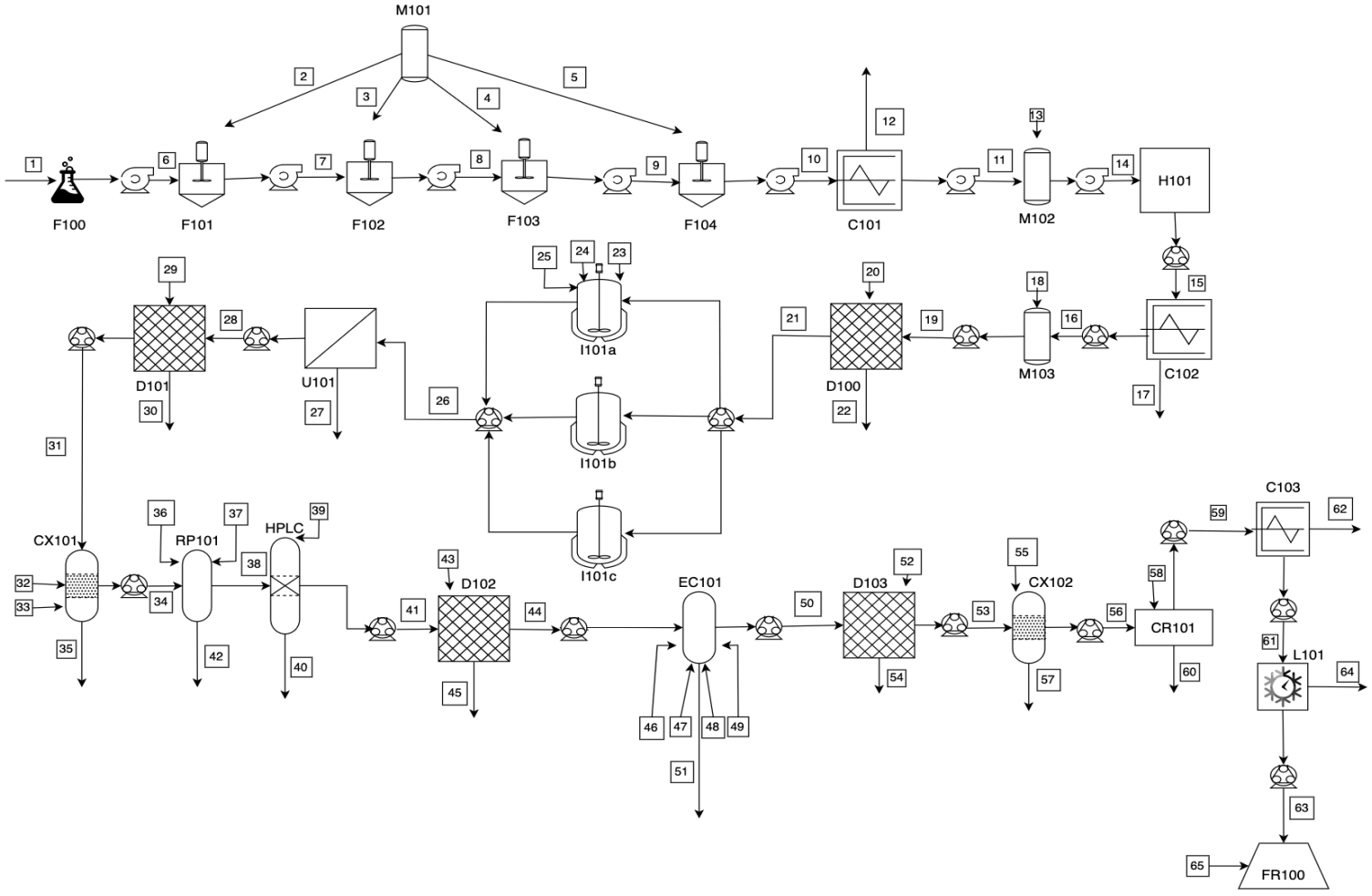


Table 4.1.a

Equipment Tags and Description

Equipment Tag	Equipment Description
F100	1 L Shake Flask
F101	10 L Fermenter
F102	100 L Fermenter
F103	1000 L Fermenter
F104	10000 L Fermenter
P101-P122	Pumps
M101 - M103	Mixing Tanks
HT100-HT118	Holding Tanks
C101	Centrifuge
H101	High-Pressure Homogenizer
C102	Centrifuge
D100	Diafiltration
I101	Incubator
U101	Ultrafiltration
D101	Diafiltration
CX101	Cation Exchange Chromatography
RP101	Reverse Phase Chromatography
HPLC	High-Performance Liquid Chromatography
D102	Diafiltration
EC101	Enzymatic Cleavage Vessel
D103	Diafiltration
CX102	Cation Exchange Chromatography
CR101	Crystallizer
C103	Centrifuge
L101	Lyophilizer
FR101	Formulation

II. Material Balance

The goal of our project is to manufacture ~3 tonnes of insulin glargine a year to supply life-sustaining medicine to 25% of the diabetic population in Africa, or roughly 6 million people. To create the material balance, we started with this number and worked backwards with the percent recoveries of each piece of equipment involved in the upstream and downstream processes. The insulin yield for each piece of equipment is highlighted in Table 4.2.a. The equipment design is based off of these recoveries and is further explained in the Equipment Design section of the report. The amount of supplemental materials and concentrations that are used with each piece of equipment were scaled up from the report written by Hwang et al. (2016).

After the insulin glargine is produced from fermentation in the upstream process, the downstream equipment is used to separate and purify the protein. The first unit operation is the centrifuge (C101) which removes media from the cells. The centrifuge has a 99% recovery, so some protein was lost in the waste stream. The stream leaving the centrifuge was assumed to be 50% w/w solids. The cells are then resuspended in a mixing tank (M102) that contains a buffer (sucrose, Tris, EDTA, sodium chloride, and water for injection (WFI)). The concentrations for every buffer used in this process were referenced from Hwang et al. (2016). The mixing tanks have an assumed recovery of 100%. To disrupt the cells, the slurry goes through the high-pressure homogenizer (H101) which has a 86% recovery rate. The cell debris and protein leave the equipment to be centrifuged once more to separate the desired protein and remove the buffer. The same assumptions and calculations were used for all centrifugation steps, so C102 operates similarly to C101.

A second mixing tank (M103) was used to resuspend the cell debris and protein in a buffer solution (Tris, EDTA, lysozyme, Triton X-100, urea, and WFI). The buffer was exchanged with another buffer containing urea, glycine, and WFI in the diafiltration unit (D100). With a

98% recovery, the diafiltration unit filtered out the remaining cellular debris and original buffer before the rest of the solution was sent to the incubator (I101).

The protein is refolded in the incubator with a buffer (urea, glycine, and WFI), media (β-mercaptoethanol), and a pH adjuster (HCl) added to the solution. The volume for the pH adjusters were found using Equation 4.2.a.

$$pH = -log(\frac{Mol\ H - Mol\ OH}{Combined\ Volume}) \quad (4.2.a)$$

Only 75% of the protein refolds, so the exit stream contains both folded and unfolded protein (Kim et al., 2015). The solution is sent to an ultrafiltration unit (U101) with a 98% recovery to filter out the misfolded protein. The next diafiltration (D101) step was calculated the same way as the previous diafiltration step.

The protein then goes through a series of purification steps, the first one being cation-exchange chromatography, taking place in CX101. Various buffers containing urea, acetic acid, sodium chloride, and WFI were used to equilibrate, load, and elute the chromatography columns. The concentrations of the solutions were found in Hwang et al. (2016), but the volumes were scaled depending on the size of each respective column. The CX101 column is 1000 L, and a total of 2 column volumes (CV) are used to elute the material in the column, while 8 other CVs are needed to load and equilibrate the column. The protein is captured in a single column volume. After the cation-exchange chromatography, the protein is further purified using reverse-phase chromatography in RP101. Both chromatography steps have a recovery of 90%. The material balance for the reverse-phase column was similar to the cation-exchange column, however the column size is only 200 L, so multiple units are needed to elute the total volume of solution. The volume of the equilibration and elution solutions containing acetonitrile, acetic acid, and WFI were calculated for the entire batch of protein rather than just for one column. Additionally, routine samples are taken from the batch to be tested in the high-performance liquid chromatography (HPLC) column to check for purity during these steps.

After chromatography, the protein goes through another diafiltration (D102) step to wash the product with WFI. This diafiltration step was calculated similarly to the previous diafiltration steps. The solution then enters the enzymatic cleavage vessel (EC101) with a buffer (borate, citraconic anhydride, and WFI), media (trypsin), and pH adjusters (NaOH and acetic acid) added to the solution. The enzymatic cleavage vessel has a recovery of 90% and uses the enzyme, trypsin, to cleave the ends of the protein. The solution then goes through a buffer exchange in the final diafiltration step (D103) to suspend the protein in an elution buffer containing urea, acetic acid, and WFI. The solution is eluted through a second cation-exchange chromatography (CX102) before entering the crystallizer.

The crystallizer (CR101) crystallizes the protein in the presence of zinc and has a 97% recovery. This step is also used for further purification because only pure insulin will form crystals. The crystals are then sent to be lyophilized in the lyophilizer (L101), which was assumed to remove all liquids and freeze the API before being sent to the formulation unit (FR101). The formulation unit combines the purified insulin glargine with an injectable solution. The final product is packaged in 10 mL vials containing insulin glargine, zinc, m-Cresol, glycerol, polysorbate 20, and WFI.

Table 4.2.a

Insulin Glargine Overall Yield

Equipment Tag	Equipment Description	Insulin Yield (kg/batch)	Insulin Yield (kg/year)	Step Yield	Cumulative Yield
F100	1 L Shake Flask	N/A	N/A	100%	100.00%
F101	10 L Fermenter	N/A	N/A	100%	100.00%
F102	100 L Fermenter	N/A	N/A	100%	100.00%
F103	1000 L Fermenter	N/A	N/A	100%	100.00%
F104	10000 L Fermenter	31.49	8596.77	100%	100.00%
P101-P123	Pumps	31.49	8596.77	100%	100.00%
M101 - M103	Mixing Tanks	31.49	8596.77	100%	100.00%
C101	Centrifuge	30.86	8424.78	98%	98.00%
H101	High-Pressure Homogenizer	24.69	6740.37	86%	78.41%
C102	Centrifuge	24.22	6612.06	98%	76.91%
D100	Diafiltration	23.74	6481.02	98%	75.39%
I101	Incubator	17.80	4859.40	75%	56.53%
U101	Ultrafiltration	17.45	4763.85	98%	55.41%
D101	Diafiltration	17.10	4668.30	98%	54.30%
CX101	Cation Exchange Chromatography	15.39	4201.47	90%	48.87%
RP101	Reverse Phase Chromatography	13.85	3781.05	90%	43.98%
D102	Diafiltration	13.57	3704.61	98%	43.09%
EC101	Enzymatic Cleavage Vessel	12.21	3333.33	90%	38.77%
D103	Diafiltration	11.97	3267.81	98%	38.01%
CX102	Cation Exchange Chromatography	10.77	2940.21	90%	34.20%
CR101	Crystallizer	10.45	2852.85	97%	33.19%
C103	Centrifuge	10.24	2795.52	98%	32.52%
L101	Lyophilizer	10.24	2795.52	100%	32.52%
FR101	Formulation	10.24	2795.52	100%	32.52%

III. Cell Growth Model

The upstream process begins with fermenting *E. coli* in a shake flask before the cell culture is moved into four fermentors via a seed train design. The purpose of the seed train design is to achieve a final concentration of 18 g/L of dry cell mass in each fermentor based on the literature by Hwang et al. (2016). In order to do this, the microbial growth kinetics were modeled by the Monod Equation which can be seen in Equation 4.3.a.

$$\mu = \frac{\mu_{max} S}{Ks + S} \quad (4.3.a)$$

The specific growth rate, μ, can be calculated using parameters μ_{max} and K_s. The maximum specific growth rate was found to be $\mu_{max} = 0.425\ h^{-1}$, (Iudica et al., 2023) and after reviewing literature by Poccia et al. (2014), the half saturation constant was found to be 0.01 g/L. After obtaining these parameters the change in cells and change in substrate concentration could be calculated using the following equations.

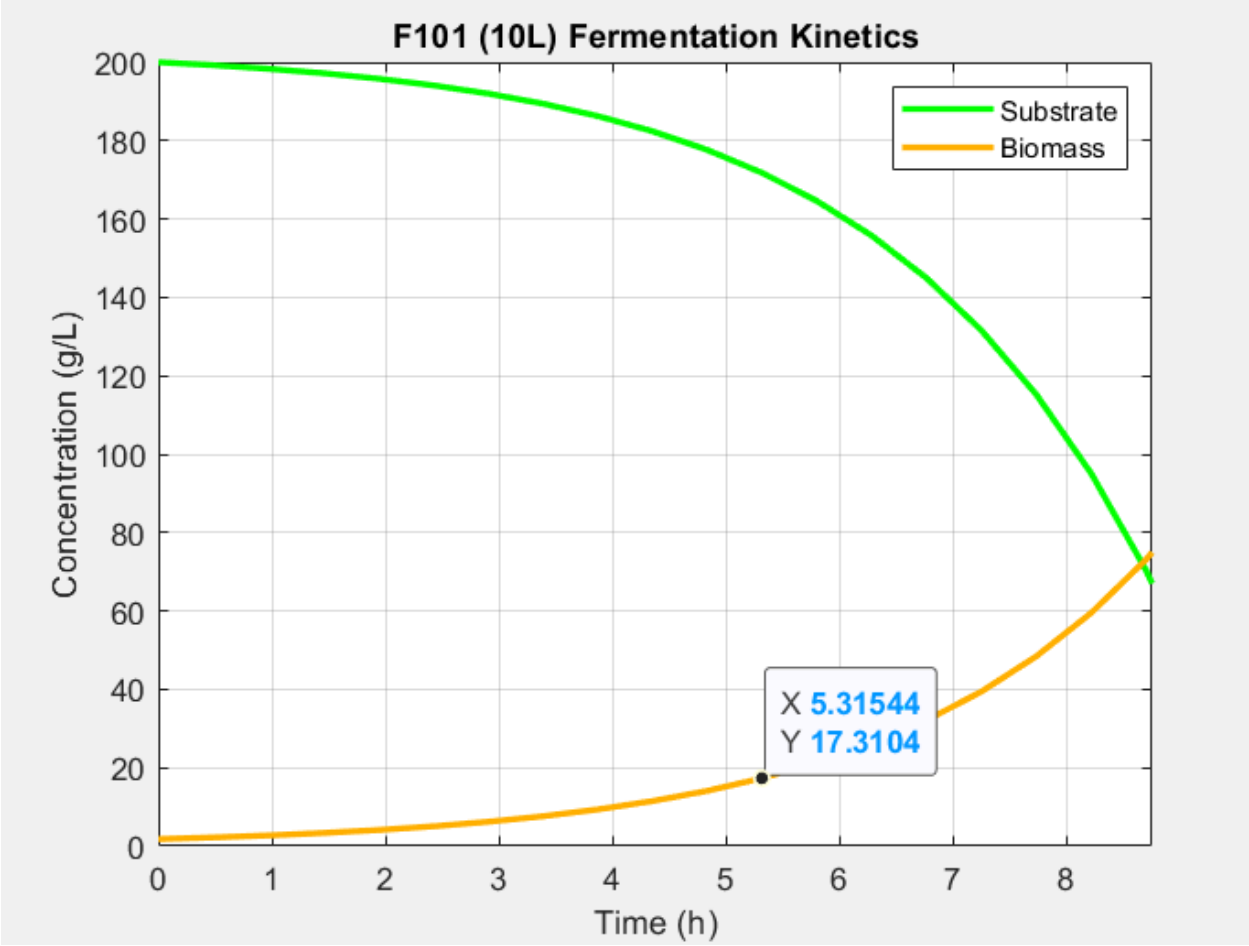
$$\frac{dX}{dt} = \mu X \quad (4.3.b)$$

$$\frac{dS}{dt} = -\frac{1}{Y_{X/S}} \frac{dX}{dt} \quad (4.3.c)$$

The first three fermentors will be run as batch fermentations, and the first fermentor after the shake flask will have a volume of 10 L. Starting conditions for the fermentation include an initial substrate concentration (S_o) of 200 g/L, an initial cell concentration (X_o) of 1.8 g/L, and a yield coefficient ($Y_{X/S}$) of 0.55 g/g within a five and half hour period. Figure 4.3.a conveys this information in the form of a cell growth curve.

Figure 4.3.a

Batch Fermentation Cell Growth Curve



The fourth fermentor in this process runs as a fed-batch fermentation and is 10,000 L. It has an initial concentration of 1.8 g/L and takes five and a half hours to run. However, it is continuously supplied with additional glucose and LB broth from mixing tank M101. The fermentor will start with 800 L from the previous fermentor F103, and 7,200 L of fermentation media will be supplied to it from M101.

IV. Seed Train and Bioreactor Design

i. Tank Geometry

The purpose of the seed train design is to increase the overall number of *E. Coli* cells in preparation for the final fermentation (Hernández Rodríguez et al., 2013). This works by increasing the size of each fermentor by a factor of 10; the seed train starts with a 1 L shake flask and is followed by a 10 L, 100 L, 1000 L, and 10,000 L fermentor. The final concentration for each fermentor is 18 g of cells per liter of fermentation broth and from this, about 3.633 g of insulin can be recovered. In order to produce 2790 kg of insulin a year, 1,536,000 L of fermentation broth are required; this translates to running 272 batches annually.

While designing the four fermentors, a working volume of 80% was used to achieve good mixing and agitation and prevent overflow (Prpich, 2023b). For example, the 10,000 L fermentor will only be filled with 8,000 L of fermentation broth. To calculate the dimensions of each fermentor standard geometry was used. This encompassed using a 1:1 ratio for the diameter to height of the reactor. This information is conveyed in Table 4.4.a.

Table 4.4.a.

Geometry of Fermentors

Equipment Tag	Tank Volume (L)	Working Volume (L)	Height of Tank (m)	Diameter (m)	Height of Liquid (m)
F101	10	8	0.234	0.234	0.187
F102	100	80	0.503	0.503	0.402
F103	1000	800	1.084	1.084	0.867
F104	10000	8000	2.335	2.335	1.868

ii. Agitation Specifications and Oxygen Requirements

When determining the oxygen requirements for a fermentor the microbial species being used will determine the specific oxygen demand (q_{O2}). This seed train design uses *E. Coli* as the microbial for cell growth which has a specific oxygen demand of 0.32 g O_2/g cells-h (Prpich, 2023c). This value can be related to the oxygen uptake rate (OUR), which determines how much oxygen the cells are using at a given moment, as stated in Equation 4.4.a.

$$OUR = q_{O2}X \quad (4.4.a)$$

For our process we chose to make OUR equal to the oxygen transfer rate in order to keep up with oxygen demands when the cells are in the maximum growth phase.

$$OTR = k_L a(C^*_{O2} - C_{O2}) \quad (4.4.b)$$

The oxygen transfer rate uses the difference in the solubility of oxygen C^*_{O2} and the concentration of oxygen in the system with the kLa, which takes mass transfer into account, to indicate how much oxygen is being transferred into the cells; our system has a OTR of 0.9 g O_2 /L-h.

In order to determine the amount of oxygen needed to supply the fermentors and ensure good mixing a kLa value was calculated; for this seed train design the kLa found was 200 h^{-1}. This value was determined by following a series of six steps which include: calculating a Reynolds number, finding a Power Number (N_p) based on the impeller type Rushton and solving for the power of an ungassed system (P), calculating standard geometry, calculating a correcting factor and determining the number of impellers, calculating the aeration number (Na), and lastly calculating the total power (P_{total}). The first step according to George Prpich was to calculate the

Reynolds Number by using Equation 4.4.c, where N is the impeller rotational speed (s^{-1}), D_i is the impeller diameter (m), ϱ is the liquid density (kg/m^3), and μ is the liquid viscosity (kg/m·s) (Prpich, 2023a).

$$Re = \frac{ND_i^2\rho}{\mu} \quad (4.4.c)$$

Water is the liquid used inside of the fermentors and will flow at turbulent speeds. Due to this a Reynolds number of 10,000 or more is needed to insure good mixing (Prpich, 2023a). The liquid density assumed is 1000 kg/m^3, while the viscosity is 1000 kg/m˙s. The next step is to calculate the Power Number (N_p) in Equation 4.4.b where P is the power in ungassed systems (W).

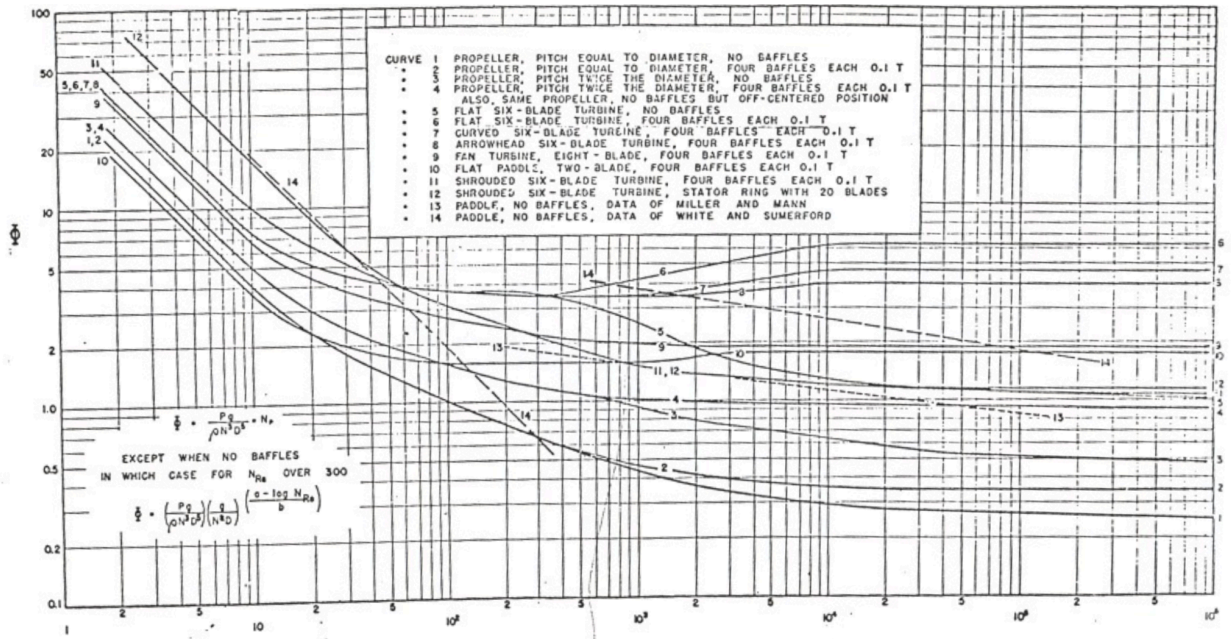
$$N_p = \frac{P}{\rho N^3 D_i^5} \quad (4.4.d)$$

The purpose of the power number (N_p) is to depict how a fluid's characterization affects power transfer within a reactor. In order to determine the N_p Figure 4.4.a can be utilized; it correlates the Reynolds number to the power number. All of the reactors in the seed train design have a power number of 6.2. Once the power number has been determined, the equation can be rearranged to solve for the power (P) in the ungassed system using Equation 4.4.e (Prpich, 2023a).

$$P = N_p\rho N^3 D_i^5 \quad (4.4.e)$$

Figure 4.4.a

Power Number vs. Reynolds Number



Note. Compares the power number to the Reynolds number based on impeller type. From *Chem. Eng. Prog.* Vol. 46, Nos 8 and 9 by Rushton, J.H. et al., 1950.

By using standard geometry, assumptions can be made such as the height of the fermentor and diameter of the tank being three times the diameter of the impeller. The height of the impeller can also be assumed to be equal to the diameter of the impeller, while the area of the tank is equivalent to the diameter of the tank squared times pi divided by four (Prpich, 2023a). Figure 4.4.b shows some standard geometry correlations.

Figure 4.4.b

Standard Geometry Correlations

Type of impeller	D_t/D_i	H_L/D_i	H_i/D_i	Baffle plates	
				N_b	W_b/D_t
Flat-blade turbine L_i/D_i=0.25,W_i/D_i=0.2	3	3	1	4	0.10
Paddle $W_i/D_i = 0.25$	3	3	1	4	0.10
Marine propeller Pitch = D_i	3	3	1	4	0.10

To determine the minimum and maximum number of impellers the reactor can use, Equation 4.4.d uses the height of the liquid (H_L) in the reactor and the diameter of the impeller (D_i) (Davis, 2010). In the seed train design fermentors F101-F103 use one impeller, while fermentor F104 uses two impellers.

$$\frac{H_L - Di}{Di} > n > \frac{H_L - 2*Di}{2*Di} \qquad (4.4.f)$$

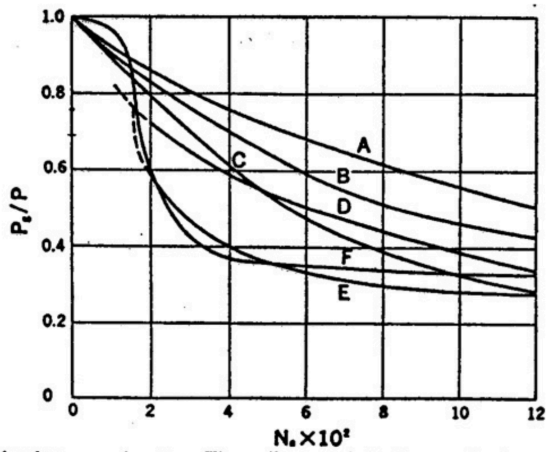
Since aerobic fermentation is favorable to increase *E. Coli* growth, we will supply oxygen to each of our fermenters. *E. Coli* requires 19.8 ± 1.7 mmol O_2/(h per g cells) (Andersen & von Meyenburg, 1980). The next step would be to use Equation 4.4.g to calculate the Aeration Number (Na), where Q_g is the aeration rate (m^3/s), N is the impeller rotational speed (s^{-1}), and Di is the diameter of the impeller (m).

$$N_a = \frac{Q_g}{ND_i^3} \qquad (4.4.g)$$

The ratio of gassed to ungassed power (Pg/P) is determined using Figure 4.4.c, and the curve used for our system was Curve F.

Figure 4.4.c

Pg/P vs Na Curve



The aeration number on the X-axis helps to indicate if air is being supplied at a good rate, and if you have reduced power consumption. Figure 4.4.d also indicates what fraction of the power is being lost due to cavities from bubble formation. To find the total power, the gassed power (Pg) is calculated in Equation 4.4.h. where n_i is the number of impellers, and f_c is a correction factor based on tank geometry (Prpich, 2023a). Since we are using standard geometries for our fermenters, the correction factor will be 1.

$$P_{tot} = P_g = (\frac{P_g}{P}) * n_i * f_c * P \qquad (4.4.h)$$

The power total is equivalent to the amount of power needed in a gassed system. It will determine what type of motor is needed for the fermentor. Using these parameters, a kLa of 200 h^{-1} was found using Equation 4.4.g. where V is the volume of the tank and D_t is the tank diameter.

$$k_L a = \frac{0.0333}{D_t^4} * (\frac{P_g}{V})^{0.541} * Q_g^{0.541/\sqrt{D_t}} \qquad (4.4.i)$$

The final oxygen requirements, including Reynolds number, k_La, oxygen supply, and gassed power, for our fermenters are shown in Table 4.4.b.

Table 4.4.b

Oxygen Requirements

Tank Volume (L)	**Reynolds Number**	**k_La (h^{-1})**	**Oxygen Supply (g)**	**Gassed Power (W)**
10	83304	200	316.8	37.9
100	133575	200	3168.0	72.5
1000	348070	200	31680.0	455.2
10000	1767059	200	316800.0	42532.6

V. Separations Design

i. Centrifugation (C101-C103)

Disc stack centrifugation operates on the principle of sedimentation, exploiting the varying densities of components within a liquid medium to effectively separate particles of different sizes and masses. This technique capitalizes on centrifugal force generated by rapid rotation, compelling denser particles towards the periphery while lighter particles remain closer to the center. This also allows for collection from an outlet on the outer wall of the bowl, as well as from the outlet on the top of the device. The distinctive design of disc stack centrifuges, characterized by a series of closely spaced discs arranged in a stack within a rotating chamber, enhances separation efficiency by maximizing surface area for particle interaction and minimizing particle settling distance.

Our first centrifuge (C101) comes immediately following the end of the fermentation process (F100-F104) - specifically the Alfa Laval FOPX 610 centrifuge, a common choice in industrial settings for its reliability and continual process capability. With an operation speed of about 4000 RPM, operation is estimated to separate with a yield of up to 99%, based on the 1998 Alfa Laval equipment manual, and calculations from the capstone project by Iudica et. al. This model has a processing capability of ~10,000 L/hr, which results in a processing time of about 0.8 hours from the fermentation output stream as stated in the Alfa Laval equipment manual (Alfa Laval, 1998). The majority of the solution processed is discarded as 7857 L of waste, and the outlet stream consists of 142 kg of *E. Coli* in 142 L growth media. This separation occurs due to the heavier cells being pushed to the edge of the bowl, while the majority of the liquid is removed from the top of the centrifuge as waste. The slurry is sent to a mixing tank for washing and resuspension in order to prepare for cell lysis.

The same disc stack centrifuge model will be used to process the output from the homogenizer (C102). For each batch, a total of 2852 L containing 117 kg *E. Coli* will be processed in approximately 17 minutes. The *E. Coli* at this point in the process has now been separated, and 24.69 kg of protein is in the homogenizer outlet stream as well, separate from the cell debris. With a 99% yield, 24.4 kg of protein per batch will be retained while 116.7 kg per batch of cell debris is spun out. The separation is facilitated through the solubilizing of desired protein in the buffer, while the rest of the cellular material is left undissolved. The volume leaving the centrifuge is approximately 2702 L and contains 24.22 kg of protein. The product stream for C102 is the lighter component collected in the middle of the centrifuge this time, while the cell debris is collected through the outer port to be discarded as waste.

Following the crystallization step, a final separation via centrifuge (C103) will be employed to prepare the product for lyophilization (L101). The incoming stream from the crystallizer is 10.45 kg of insulin in 6250 L of Buffer 5. Optimal performance as previously described with a feed stream of approximately 2000L/hr results in a processing time of about 3 hours from the crystallizer output stream. The protein has now crystallized and is not solubilized within Buffer 5. The centrifuge removes 5779 L of Buffer 5 through the top port to be discarded as waste, while the remaining 10.24 kg of insulin in 102.8 L of Buffer 5 is collected through the port on the outside wall for further processing. This is the last separation step in the process before the final product is sent to be lyophilized and formulated as an injectable.

ii. Mixing Tanks (M101-M103)

Mixing tanks are used to resuspend cells after centrifugation and to wash the desired protein with different buffers in the process. In our process there are three mixing tanks (M101, M102, M103) that are made of stainless steel. The ratio of tank diameter to tank height is held constant at 3 for each of the mixing tanks (Nienow, 1997).

Since *E. coli* is not considered as a shear-sensitive bacteria, Rushton impellers were the chosen agitators to ensure proper mixing (Mirro & Voll, 2009). The impeller diameter is set at ⅓ of the tank's diameter (Perry et al., 2008). The number of impellers required is based on the impeller diameter and the height of the liquid in the tank, described in Equation 4.4.d from a previous section.

The optimal spacing for impellers is between 1 to 2 *Di*. The spacing between the last impeller to the bottom of the tank is equal to *Di*, and the spacing between the top impeller to the surface of the liquid should be 1.5 *Di* or more (Davis, 2010). These specifications were taken into consideration when calculating the number of impellers needed for each tank. The tip speed for the impellers was set under 3.2 m/s to protect the cells from shear force damage (Junker, 2004). Additionally, the tip speed was set to maintain a turbulent Reynolds number to hold a constant power number. Baffles are used in mechanically stirred tanks to promote the stability of power drawn by the impeller and to avoid the fluid swirling, thus enhancing mixing (Kamla et al., 2017). A set of 4 evenly spaced baffles were put into each tank with widths of 1/12th of the tank diameter (Perry et al., 2008).

The mixing time for each tank is based on Equation 4.5.a from Perry et al. 2008, where *Dt* is the diameter of the tank, *H* is the height of the tank, ω is the impeller speed, and *Di* is the diameter of the impeller.

$$t_{mix} = 5 * \pi * Dt^2 * \frac{H}{4} * \frac{1}{0.92*\omega*Di^2*Dt} \qquad (4.5.a)$$

The first mixing tank (M101) is used for the fermentation broth that will be supplied to each fermentation tank (F101-F104). The fermentation media will enter each fermenter at 72% of the volume capacity to account for foaming and agitation. Glucose will be supplied to the shake flask at 200 g, and will increase by a factor of 10 for each successive fermentation. The specifications of the mixing tank will be found in Table 4.5a. A second mixing tank, M102, is used for cell resuspension after centrifugation in C101. The cells collected from centrifugation will be resuspended in the resuspension buffer, Buffer 1, listed in Table 5.5.b. After the mixing is complete, the 142.56 kg of cells and the buffer solution will go to the high-pressure homogenizer (H101). The third and final mixing tank (M103) is used for resuspension and washing of the inclusion bodies in a washing solution (Buffer 2) after centrifugation (C102). After washing is complete, the solution will go to diafiltration (D101). The dimensions of each tank are shown in Table 4.5.a.

Table 4.5.a

Mixing Tank Dimensions

Tag Number	**M101**	**M102**	**M103**
Capacity, L	10,000	5,000	6,000
Working Volume, L	8,000	3,000	4,000
Diameter, m	2.40	1.50	2.00
Height, m	7.20	4.50	6.00
Baffle Width, m	0.20	0.13	0.16
Impeller Diameter, m	0.80	0.50	0.67
Rotational Speed, rps	1.27	2.03	1.53
Mixing Time, s	90.00	57.00	75.00

iii. High-Pressure Homogenizer (H101)

High-pressure homogenization (HPH) is a well-established comminution technique that is widely used in the production of fine emulsions or in the disruption of microorganisms, targeting either their inactivation or the release of intracellular products (Kluge et al., 2012). During this process, liquid is passed through a narrow gap under high pressure where the different processing parameters lead to changes in globule/particle size (Yadav et al., 2019). Specifically for misfolded proteins in *E. coli*, HPH can be a critical unit operation influencing inclusion body (IB) quality and, subsequently, refolding yields (Ebner et al., 2023).

For our process we will be using a Hy-Drive HS-3-085-04 Production Homogenizer (UHPH, 2024). This equipment can process volumes up to 1000 L/hr and features both Cleaning-in-Place (CIP) and Steaming-in-Place (SIP) capability. The volumetric flow rate is dependent on the input pressure; therefore, this equipment will be set at 1400 bar to have an adequate flow rate of 330 L/hr. Researchers observed that when the pressure range is between 1000 and 1500 bar, the maximum protein release and drastic reduction of cell size was observed after the first pass through the HPH. In subsequent passes, micronization of cell debris was observed but without much variation in protein release (Ramanan et al., 2009). Therefore only one pass through the HPH is needed and will result in product recovery of 86% (Eggenreich et al., 2020). It will take 15 hours to process the 2,852 L of Buffer 1 and insulin glargine protein from the resuspension mixing tank (M101). The output stream will release 24.69 kg of the insulin precursor, and the sludge will be sent to centrifugation (C102). The power consumption of the high-pressure homogenizer is 23 kW (UHPH, 2024).

iv. Diafiltration (D100-D103)

Diafiltration is a continuous system where a solvent is added to offset concentration effects and achieve high protein recovery purity and yield. The process exchanges small-molecule components by continuously replacing the initial solvent that passes through the ultrafiltration membranes with a new solvent. The process stream along with the solvent are fed through the membrane using a pump, while the insulin glargine protein is retained because it is too large to pass through the ultrafiltration membrane. The solvent used for diafiltration, however, can flow through the membrane.

The overall process contains four diafiltration steps: the first step (D100) after washing in a mixing tank (M103), the second step (D101) after the ultrafiltration step (U101), the third step (D102) after reverse phase HPLC (RP101), and the last diafiltration step (D103) after the enzymatic cleavage vessel (EC101). The process will use the MaxCell Ultrafiltration Hollow Fiber Tangential Flow Cartridge, UF-3-C-85, with a nominal molecular weight cutoff (NMWC) of 3 kDa (Cytiva, 2023). The molecular weight of insulin glargine is 6,063 Da, so the protein will be too big to pass through the membrane. The hollow fiber membrane has a membrane area of 13 m^2 and can produce a permeate flow rate (Q_P) ranging from 14.011 L/min to 112.087 L/min depending on the pump speed (Cytiva, 2023).

For the first diafiltration step (D101), the process feed consists of the insulin glargine protein, Buffer 1, Buffer 2, and growth media. Buffer 3 is added as the solvent. The total volume of the process feed yields the initial volume (V_0) of about 3,421 L. To optimize the processing time, the diafiltration volume (V_D) is first found using Equation 4.5.b.

$$V_D = -\frac{V_0 ln((\frac{C}{C_0})_{protein})}{1-\sigma_{protein}} \quad (4.5.b)$$

The protein yield, $(\frac{C}{C_0})_{protein}$, is 0.98 and the rejection coefficient, $\sigma_{protein}$, is 0.995 for all diafiltration steps. The rejection coefficient is a measure of the fraction of material interest

that is rejected by the membrane. The rejection coefficient of the insulin glargine protein is about 1 because the molecular weight of the insulin glargine protein exceeds the NMWC of the membrane. A high rejection coefficient means that a small amount of protein will pass through the membrane. It is assumed that the rejection coefficient of the buffer, σ_{buffer}, is 0 because the molecular weight of the buffers are below the NMWC of the membrane used in the process.

Using Equation 4.5.b, the diafiltration volume for this process is 13,824 L. 7,475 L of Buffer 3 is retained and moved into the incubators. Based on the specifications for the MaxCell Ultrafiltration Cartridge, it was determined that a permeate flow rate of 112.087 L/min yielded the best process time. The permeate flux, u_p, given by Equation 4.5.c, was found to be 0.00862 m/s. For diafiltration, it is assumed that the permeate flux is approximately equal to the initial permeate flux ($u_{p,0}$).

$$u_p = \frac{Q_P}{A} = u_{p,0} \qquad (4.5.c)$$

With the initial permeate flux and the diafiltration volume known, the processing time for this diafiltration step is 2.05 hours, as shown by Equation 4.5.d.

$$t = \frac{V_D}{Au_{p,0}} \qquad (4.5.d)$$

For this diafiltration system, the inlet pressure is set at 3.4 bar and the temperature must be between 25-80 °C (Cytiva, 2023). Upon the completion of the process, the insulin glargine protein (23.74 kg/batch) in solution with some excess of Buffer 3 is sent to the incubators. The rest of the solvents are removed and sent off as waste.

The second diafiltration step (D101) comes after the first ultrafiltration step (U101), where 45,217 L are fed into the system. This initial volume consists of the process stream containing insulin glargine, Buffer 3, Buffer 4, and Adjuster A. WFI is added as the solvent. Using the same equations and process above (Equations 4.5.b - 4.5.d) to calculate the processing

time, the diafiltration volume was found to be 182,703 L. The volumes were calculated using the same parameter assumptions described in the description for the first diafiltration unit.

Using Equation 4.5.d, the processing time was calculated to be 27.1 hours. The system operates under the same conditions described in the description for the first diafiltration unit. Upon completion, the insulin glargine protein (17.1 kg/batch) and some WFI are moved to the cation exchanger (CX101). The remainder of the solvents are removed and sent off as waste.

The third diafiltration step (D102) comes after the reverse phase high performance liquid chromatography step (RP101), where 3,250 L are fed into the system. This initial volume consists of the process stream containing insulin glargine and Buffer 7. WFI is added as the solvent. Using the same equations and process above to calculate the processing time, the diafiltration volume was found to be 13,132 L. The volumes were calculated using the same parameter assumptions described in the description for the first diafiltration unit.

Using Equation 4.5.d, the processing time was calculated to be 1.95 hours. The system operates under the same conditions described in the description for the first diafiltration unit. Upon completion, the insulin glargine protein (13.57 kg/batch) and some WFI are moved to the enzymatic cleavage vessel (EC101). The remainder of the solvents are removed and sent off as waste.

The fourth diafiltration step (D103) comes after the enzymatic cleavage step (EC101), where 350 L are fed into the system. This initial volume consists of the process stream containing insulin glargine, Buffer 8, Adjuster C, Adjuster D, and media. Buffer 5 is added as the solvent. Using the same equations and process above to calculate the processing time, the diafiltration volume was found to be 1,414 L. The volumes were calculated using the same parameter assumptions described in the description for the first diafiltration unit.

Using Equation 4.5.d, the processing time was calculated to be 12.6 minutes. The system operates under the same conditions described in the description for the first diafiltration unit.

Upon completion, the insulin glargine protein (11.97 kg/batch) and some excess of Buffer 5 are moved to the cation exchanger (CX102). The remainder of the solvents are removed and sent off as waste

v. Incubation (I101)

After diafiltration, the substance is incubated in an incubator (I101) in 0.1 mM β-mercaptoethanol at 4°C for 48 hrs for refolding. After the refolding reaction has occurred, the pH of the solution is adjusted 4.5 with 5 N HCl. The protein yield after refolding is 75% (Kim et al., 2015).

We will incubate the solution in 20,000 L tanks that are designed similarly to the mixing tanks. The incubators have a diameter of 3 meters and a height of 9 meters. Four baffles are distributed evenly around the tank with a width of 0.25 m. 3 impellers with a width of 1 m will be set at an impeller speed of 1 rev/second, resulting in a mixing time of 113 seconds. Since the incubators need to process about 50,000 L of solution, 3 incubators are needed for this process. After incubation, the refolded protein is sent to an ultrafiltration unit to filter out misfolded proteins and other cellular debris.

vi. Ultrafiltration (U101)

Similar to diafiltration, ultrafiltration can be run continuously, but there is no solvent feed because the volume going into the membrane is not being replaced by a buffer, but instead is used to remove debris from the solution. Ultrafiltration separates macrosolutes that are retained from smaller molecules that pass through an ultrafiltration membrane.

The process includes only one ultrafiltration step, U101, which occurs directly after incubation. The MaxCell Ultrafiltration Hollow Fiber Tangential Flow Cartridge, UFP-10-C-85, will be used in the ultrafiltration process. Unlike in diafiltration, the NMWC of this membrane is 10 kDa (Cytiva, 2023). The NMWC for the ultrafiltration membrane design is different from that

of diafiltration because insulin must be able to pass through the membrane while blocking undesired components such as cell debris from passing through. The molecular weight of insulin glargine is 6,063 Da, so the insulin will be able to pass through the membrane. The hollow fiber membrane has a membrane area of 13 m^2 and can produce an average permeate flow rate ranging from 14.011 L/min to 112.087 L/min depending on the pump speed (Cytiva, 2023).

The process feed in U101 consists of the insulin glargine, Buffer 3, Buffer 4, Media A, and Adjuster A with a total combined volume of 49,149 L. The insulin and buffers flow through the membrane, so the concentration of insulin in the permeate will be equivalent to the concentration of insulin in the original solution, so the rejection coefficient (σ) is zero demonstrated by Equation 4.5.e.

$$\sigma_{protein} = 1 - \frac{C_P}{C} \quad (4.5.e)$$

For the ultrafiltration system, the inlet pressure must be 3.4 bar and the temperature must be between 25-80°C (Cytiva, 2023). The system will run until the chosen step yield, 92.4% of the solution, flows through the membrane. Since the concentration of the permeate will be equivalent to the concentration of the feed, the permeate flow rate will be 112.087 L/min. The unit will be able to process 92.4% of the original 49,149 L of solution in 6.75 hours using a permeate flow rate of 112.087 L/min. The permeate product will be 45,220 L/batch consisting of a mixture of insulin glargine, Buffer, 3, Buffer 4, and Adjuster A. The retentate waste stream will be 3,932 L/batch consisting of a small amount of insulin glargine, Buffer 3, Buffer 4, Media A, and Adjuster A. The recovered insulin (17.45 kg/batch) passes through to the second diafiltration system (D101). The realistic recovery of the insulin will be lower than the number calculated given that the amount of insulin in the waste stream should be proportionate to the volumetric ratio of the waste stream itself because of the lack of a large driving force.

vii. Cation Exchange Chromatography (CX101-CX102)

Insulin has a positive charge - cation exchange chromatography (CEX) functions on the principle that insulin has a higher affinity for the stationary phase than the contaminants in the feed stream. These impurities, such as other proteins, small peptide chains, cell debris, etc. will either flow through or attach to the resin. For those attached to the resin, a salt gradient in the inlet feed stream will slowly ramp up - those that are less tightly bound to the resin will wash off until finally the insulin can elute and is collected. Compared to other purification steps in the process, CEX separates DNA and proteins more closely characteristic of the insulin protein itself. The loading and elution of insulin from the column is also accompanied by washing, equilibration, and regeneration of the column.

According to Hwang et al. (2016), the column utilized for purifying insulin glargine should be filled with SP Sepharose Fast Flow resin as the stationary phase. According to specifications from the manufacturer, the resin has a dynamic binding capacity of 55 mg protein/mL buffer and a recommended flow velocity of 90 cm/h (Cytiva, 2023). The column specifications are ~1000L with 1.3 m diameter, and 0.5 m bed depth. The equilibration buffer will contain 7M urea and 0.25 M acetic acid. After washing the column with this buffer, the bound proteins will be eluted with elution buffers containing 7 M urea, 0.25 M acetic acid, and 1 M sodium chloride with a 0-1 M sodium chloride gradient.

From the recommended flow velocity - for the column described, that is a 1.27 m^3/hr flow rate or 1270 L/hr [cm/h = (cm^3/h)/(cm^2) or Flow Velocity = (Volumetric FR)/(Cross Sectional Area)]. Residence time is then. $\tau = (\pi \times (0.65^2) \times 0.5)/1.27 = 0.52$ hr or 31 minutes. Research indicates that, with our increased residence time, yields of this chromatography step can be estimated at ~90% (Cytiva, 2023). These yields were found in conditions that had: 2 CV (Column Volume = 1000 L) buffer for elution, 8 CV for equilibration, washing, regeneration steps, and 1 CV for collection of the purified protein.

The first column (CX101) is used to process the 17.1 kg of insulin per batch, from the diafiltration unit D101, 2 CV worth would be 2000 L, 1.58 hr time frame. A total of 17.1 kg protein in 2 CV buffer, gives an inlet concentration of ~0.0086 kg/L. This being collected in 1 CV, would indicate an outlet concentration of 0.017 kg/L, or 0.0154 kg/L from 90% yield, resulting in an outlet composition of 15.39 kg in 1000L. The total time per batch which has 10 CV for elution, equilibration, washing, and regeneration means that 10,000 L / 1,270 L/hr = 7.87 hr + residence time gives about 8.87 hours total to complete a full batch cycle. The other 1.54 kg of protein is eluted with the other 10,000 L of buffer and is discarded as waste.

After leaving the enzymatic cleavage vessel, a second cation exchange chromatography column (CX102) is employed to separate the transformed insulin from the amino acid chains that were clipped. The column will also ideally remove any protein incorrectly transformed and any other major impurities. This step aids in purification and also prepares the slurry for an optimal crystallization in the next step. The column itself will have the same dimensions as the previous column. The equilibration buffer will be the same - 7M urea and 0.25 M acetic acid. After washing the column with this buffer, the bound proteins will be eluted with elution buffers containing 7 M urea, 0.25 M acetic acid, and 1 M sodium chloride with a 0-1 M sodium chloride gradient.

CX102 receives 11.97 kg of protein in 10000 L of Buffer 5 from D103. Elution will be done with 2 column volumes of buffer, using a linear gradient of NaCl. The purified insulin product will be collected in a single column volume of buffer. Pre-elution and post-elution wash and regeneration cycles will consist of 8 column volumes of the buffer. The total time for the elution will be approximately 1.6 hours and the whole column cycle about 9 hours. The expected yield is about 90%, with a composition of 10.77 kg insulin in 1000 L of buffer. The waste stream is composed of 1.2 kg of protein in 10,000 L of buffer.

viii. Reverse Phase HPLC Column (RP101)

After washing the column with this buffer, the bound proteins will be eluted with elution buffers containing 7 M urea, 0.25 M acetic acid, and 1 M sodium chloride. The eluent containing at least 60% purity of insulin glargine is put through Prep-HPLC (RP101) following the Cation-Exchange protocol (CX101) to further purify the product by partitioning stationary molecules from the mobile phase. Reversed-phase chromatography separates molecules based on their hydrophobicity where hydrophilic molecules elute first. Quickly eluting product impurities include C-peptide, N-terminal signal sequences, dipeptides, aggregated insulin, misfolds, miscleaves, deamidated insulin, and any residual proinsulin (Siew & Zhang, 2021). Strongly hydrophobic molecules, such as insulin, will filter last (Schluter, 2000). Two solvents will be used to collect the protein linearly, solvent A containing 0.25 M acetic acid and 15% acetonitrile (ACN), and solvent B containing 0.25 M acetic acid and 45% ACN (Hwang et al., 2016)

The Prep-HPLC column will be 200 L with a length 0.25 m and a diameter of 0.97 m. It is equipped with Zorbax C8 (particle size 5μm) that has a binding capacity of 50 mg/mL (Bioclone, 2009). The column was equilibrated at a flow rate of 3 ml/min (0.18 L/hr) with 10 CV solvent A and the collected protein was loaded onto the column at a flow rate of 1 ml/min. This flow rate corresponds to a flow velocity of 1.25 m/hr. Residence time is then $\tau = (\pi \times (0.485^2) \times 0.25)/.18 = 1.03$ hr or ~ 62 minutes. After washing at a flow rate of 3 ml/min with 10 CV solvent A, bound proteins were eluted at a flow rate of 3 mL/min with 6 CV solvent A and B by application of a linear gradient (0-70% solution B). The cycle time for 1 column is around 6.18 hours plus one hour for cleaning-in-place, totaling 7.18 hours.

ix. High Performance Liquid Chromatography (HPLC)

Samples from the Prep-HPLC will be sent to the HPLC to further test for purity of the insulin glargine. Five 20 µl samples per batch will be sent through a Protein & Peptide C4 analytical column (250 mm x 4.6 mm, particle size 5µm). Buffer 6 contains 50 mM NaH2PO4·H20, 0.1 M NaClO4 with a 2.5 pH, and Solvent B will linearly increase from a concentration of 10-80% ACN. The eluent flows at a rate of 1 ml/min and the peak absorbance is monitored by a UV light detector to determine purity (Hwang et al., 2016).

x. Enzymatic Cleavage Vessel (EC101)

The function of this vessel (batch reactor) will be to conduct the reaction in which the enzyme, trypsin, cleaves arginine and lysine residue at the carboxy end of insulin (Siew & Zhang, 2021). Cleaving the residue from the terminal ends of the protein is paramount for the formation of mature insulin. This vessel will act as an incubator for the reaction, and it will be operated under the following conditions: a temperature of 25 °C, a pH of 8.5, and a run time of 5 hours. As the reactor mixes the enzyme and insulin protein, it will help accelerate the rate of reaction. At the two hour mark, 9 units of trypsin per mg of protein will be added to the reactor. About 13.57 kg of insulin and 0.034 kg of trypsin are inputted for the reaction to take place. WFI and Buffer 8, containing borate and citraconic anhydride, are also needed to complete the reaction. The conversion rate of the reaction is 90% and will lead to a recovery of 12.21 kg of insulin.

Table 4.5.b

Enzymatic Cleavage Vessel Dimensions

Reactor Volume (L)	150.000
Reactor Diameter (m)	0.576
Reactor Area (m^2)	0.260
Reactor Height (m)	0.576
Impeller Diameter (m)	0.192
Impeller Speed (RPM)	250.000
Number of Impellers	2.000

xi. Crystallizer Tank (CR101)

There are multiple modes of crystallization. The most common process of crystallization in industry is evaporative crystallization which operates off of the principle of evaporation through heat. However, since insulin is a protein, evaporative crystallization incurs the risk of denaturing the insulin. The proposed method for crystallization is through an anti-solvent. An antisolvent allows crystals to form within a solvent. In this process, an 18% solution of zinc-chloride will be used to form insulin crystals, isolating the insulin from impurities that do not co-crystallize. Insulin self-associates to form hexamers of insulin peptide in the presence of zinc ions. A hexameric form protects from physical and chemical degradation during storage, giving insulin a longer storage life.

The crystallizer chosen for this process is the Crystallization Package - 200L 316L SST Ai Dual-Jacketed Reactor. The crystallizer has a volume of 200 L, a dimension of 1.09m x 0.89m x 2.29m, a 35 L cooling jacket, and operates at a pH of 5.4-6.2 at a temperature of 5°C. Solutions are able to hold more of its solute at higher temperatures which is why lower temperatures are typically required for crystals to form. Equation 4.5.f is used to calculate the heat, power, and time.

$$Q = UA\Delta T \quad (4.5.f)$$

A 50% ethylene glycol and water solution is used as the coolant rather than just water due to its low freezing point of -36.8°C compared to water's freezing point at 0°C. A cooling jacket is the most economical and common method of cooling, but another source of cooling may be required for the crystallization process of insulin. If the temperature of the coolant in the jacket is too low, ice can form on the surfaces lowering the heat transfer properties of the unit. A proposed method for cooling is using cooling coils on the inlet stream to lower the feed stream while using a jacket to maintain low temperatures during the crystallization process.

The initial feed stream is composed of 10.77 kg of insulin glargine and 2082 L of Buffer 5. 18% of a zinc chloride solution in WFI is added to the crystallizer until the insulin in solution reaches a concentration of 0.1% (Hwang et al. 2016). In order to achieve this concentration, 110.45 L of the zinc chloride solution must be added to the crystallizer. 8.71 kg of insulin along with 2082 L of Buffer 5, 3.75 L of the zinc chloride solution, and 4,168 L of water passes through to the centrifuge, requiring a total of 12 hours.

xii. Lyophilizer (L101)

The final protein quantity is then lyophilized for formulation. The lyophilizer works by removing all the moisture from the product by freezing and then pulling a vacuum, which allows the frozen liquid to sublimate from the product. The insulin will be dissolved in acetic acid via a diafiltration unit, so the lyophilizer has to decrease the pressure enough to sublime organic solvents, leaving the resulting dry product in a stable conformation. Our proposed model is the Vikumer lyophilizer model FD-50L, which can condense up to 500 kgs of product, freeze to -85°C, and reach a final vacuum pressure of 1×10^{-5} bar.

xiii. Formulation (FR101)

After the lyophilizer, the insulin is then sent to formulation where it is put into a solution containing zinc, m-Cresol, glycerol, WFI, and polysorbate 20. The final quantities of each ingredient can be found in Table 5.5.a. An overage of 7% was accounted for in the fill amount. Zinc is included to maintain stability of the insulin structure, specifically the way the protein is folded. m-Cresol is used as a preservative to prevent growth of microorganisms. Glycerin is added as a preservative and humectant. Polysorbate 20 is added for its high safety characterization as an excipient (Kim et al., 2021). The solution is packaged into 10 mL vials using the Shanghai ALWELL Filling Line to fill 239,285 vials per batch. The formulation machine can fill 16,000 vials per hour, so there will be 4 machines in parallel to have a packaging time of 3.7 hours.

VI. Schedule for Batch Operations

The schedule for batch operations includes both the schedule for the upstream and downstream processes. The upstream process will take a total of 35 hours, including process time, CIP/SIP, drain and fill times, for one batch. The downstream process will take a total of 153 hours to complete one batch. Overall, the one batch process from start to finish will take a total of 188 hours, or 7.85 days. We need to run 272 upstream and downstream processes with a 28.5 hour buffer in between batch production in order to meet our desired production goal.

We will need multiple sets of equipment to batch overlap some of the time-extensive processes. The process requires 2 duplicates for each incubator (I101a, I101b, I101c) and 4 formulation units which will run simultaneously. There will be no additional equipment needed for the upstream process or the remainder of the downstream process as there is no overlap between batches. A more detailed batch schedule is detailed in the final recommendations section.

VII. CIP/SIP Requirements

Instead of using single-use equipment, we will be using cleaning in place (CIP) and steaming in place (SIP) procedures to clean and sterilize our equipment after each batch. CIP includes rinsing with WFI, 0.5% NaOH (caustic), and a final rinse with WFI to wash away residue and sanitize the equipment. SIP sterilizes the equipment by deploying hot steam inside the equipment to increase sterility assurance and product safety (Pai, 2022). Each piece of equipment in our process, excluding the pumps, will need to be cleaned and sanitized and are listed in Table 4.7.a. This table also features the clean, steam, and drain times required for each piece of equipment based on its design.

i. Cleaning in Place

The batch equipment, including tanks and fermenters, will be cleaned using Glacier Tanks Fixed CIP Spray Ball line which features 360° spray coverage and multiple sizes. The Tri Clamp B300 model will be used to clean tanks larger than 5000L and the Tri Clamp B200 model will be used to clean smaller tanks (Glacier Tanks LLC, n.d.). To properly clean our equipment, 10% of the tank volume is needed for the caustic cleaning solution, whereas 5% of the tank volume will be allocated to WFI rinses (*How much CIP detergent needed?*, 2022). The solutions are set at a velocity of 1.5 m/s and a temperature of 60°C. For non-continuous unit operations, the time needed for the pre-rinse, caustic wash, and final rinse were determined from Equation 4.7.a based on our specific spray nozzle (*CIP and Sanitation of a Process Plant*, 2011). Equations 4.7.b and 4.7.c were used to determine WFI rinse volume and time. The drain time was calculated using Equation 4.7.d and the assumed average of our pump's operating flow rates at 3600 L/hr (Peters et al., 2002). The 10,000 L bioreactor, F104, was used in the example equations below.

$$Flow\ rate\ (L/h)\ =\ Diameter\ (m)\ *\ 3.14\ *\ 1490 \quad (4.7.a)$$

$$0.05\ *\ 10,000\ L\ working\ vol\ =\ 500\ L\ WFI\ (rinse\ 1) \quad (4.7.b)$$

$$Water\ Rinse\ Time\ (min)\ =\ \frac{500L * 60min}{2.335m * 3.14 * 1490} = 2.75\ min\ WFI\ (rinse\ 1) \quad (4.7.c)$$

$$Drain\ Time\ (min)\ =\ \frac{500L\ WFI}{3600\ L/hr}\ *\ \frac{60\ min}{1\ hr} =\ 3.62\ min\ WFI\ drain \quad (4.7.d)$$

For the unit operations that run continuously, the WFI and caustic rinse time and volume were calculated based on the equipment's flow rates and exposure time. The caustic rinse exposure time is 20 minutes while the WFI rinse exposures are 10 minutes (Weincek, 2006). Equation 4.7.e calculates the rinse volume for WFI for the first diafiltration unit, D100. The drain time for all continuous unit operations was assumed to be 0.00 minutes.

$$WFI\ Rinse\ Volume\ (L)\ =\ 6725\ L/hr\ *\ \frac{1\ hr}{60\ min} *\ 10\ min\ =\ 1120.87\ L\ WFI\ rinse$$

(4.7.e)

ii. Steaming in Place

After the cleaning in place, the Steam in Place cycle begins to completely sterilize the equipment. The steam will be set at 121°C and 10 psig and the exposure time will be at 30 minutes (*Sigma Aldrich*, 2020). The volume of steam needed for the batch unit operations will be calculated using the exposure time and Equation 4.7.f. Equation 4.7.f models the steam volume equation for F104. The volume of steam needed for the continuous equipment will be calculated using the exposure time and the flowrate of the system. This is modeled for D100 in Equation 4.7.g.

$$Volume\ Steam\ (L)\ =\ 2.335\ m\ *\ 3.14\ *\ 1490\ *\ \frac{1\ hr}{60\ min}\ *\ 30\ min\ =\ 5462.27\ L$$

(4.7.f)

$$Volume\ Steam\ (L)\ =\ 6725\ L/hr\ *\ \frac{1\ hr}{60\ min}\ *\ 30\ min\ =\ 3362.5\ L$$ (4.7.g)

Table 4.7.a

Clean in Place and Steam in Place Volumes and Total Times

Equipment Tag	Total Time (min)	Total Caustic (L)	Total WFI (L)	Total Steam (L)
F101	70.08	1.00	1.00	547.39
F102	70.38	10.00	10.00	1176.66
F103	71.77	100.00	100.00	2535.80
F104	78.24	1000.00	1000.00	5462.26
C101	60.00	7000.00	3500.00	10462.26
M102	82.82	1000.00	1000.00	3508.95
H101	60.00	195.00	97.5.00	165.00

C102	60.00	7000.00	3500.00	9678.60
M103	74.81	500.00	500.00	4678.60
D100	60.00	2312.08	1156.04	3362.50
I101	82.82	6000.00	6000.00	21053.70
U101	60.00	2312.08	1156.04	3362.50
D101	60.00	2312.08	1156.04	3362.50
CX101	60.00	100.00	100.00	0.05
RP101	60.00	20.00	20.00	0.09
D102	60.00	2312.08	1156.04	3362.50
EC101	70.50	1000.00	1000.00	1347.43
D103	60.00	2312.08	1156.04	3362.50
CX102	60.00	100.00	100.00	0.05
CR101	70.35	1041.00	1041.00	2549.83
C103	60.00	7000.00	3500.00	10462.26
L101	60.00	5000.00	5000.00	17544.74
Total Times & Volumes	1451.79	48627.40	32249.70	107986.23

VIII. Media Sterilization Requirements

There will be a continuous sterilization model put in place in order to sterilize the growth media before it is used in the fermentation process. The liquid will be heated to 121°C by injecting steam into the medium, which is a common form of wet-heat sterilization. This will ensure that all microorganisms, including bacterial spores, are killed. A cooling system will then bring the temperature down to 37°C for fermentation. The continuous sterilization equipment is from the Actini Group, which can sterilize up to 40,000 L per hour, which exceeds the capacity of our needed media requirements.

IX. Heat Requirements

i. Heat Generation

Throughout this process there are several points at which the temperature drops or increases; to ensure these temperature changes are reached safely adding cooling and warming jackets was necessary. The pieces of equipment with changes in heat include the fermentors, incubators, the enzymatic cleavage vessel as well as the crystallizer; the gassed power for each piece of equipment was calculated to obtain the heat generation. Equation 4.9.a depicts how the gassed power was calculated for fermentor F104.

$$Gassed\ Power\ (W)\ =\ 0.5\ *\ 2\ Impellers\ *\ 1\ *\ 42532.6\ Watts\ =\ 42532.6\ W \quad (4.9.a)$$

For the fermentors, the heat generated was the sum of heat from cells and from the impeller. As stated by Shuler and Kargi, the total heat evolution in a batch fermentation can be calculated by using the oxygen uptake rate, Q_{O2} (Shuler & Kargi, 2002). The oxygen uptake rate for the fermentors is equal to $Q_{O2,max}$ which is $0.9\frac{g}{L*h}$. From there there Q_{GR} represents the heat from cells and can be used to calculate the heat generated. A sample calculation for fermentor F104 is provided in equation 4.9.d, and Table 4.9.a lists heat generation for all pieces of equipment.

$$Heat\ Generated\ (W)\ =\ Heat\ from\ Cells\ +\ Heat\ from\ Impeller \quad (4.9.b)$$

$$Heat\ from\ Cells = Q_{GR}\ (kcal/h)\ =\ 0.12Q_{O2} \quad (4.9.c)$$

$$Heat\ Generated\ (W)\ =\ 251040\ \text{W}\ +\ 42532.6\ W = 293572.6\ \text{W} \quad (4.9.d)$$

Table 4.9a

Heat Generated by Equipment

Equipment Tag	Heat Generated (kW)
F101	0.289
F102	2.548
F103	25.559
F104	293.572
I101a	6.000
I101b	6.000
I101c	6.000
EC101	0.332
CR101	0.200

ii. Heat Equipment Design

Several pieces in this process require jackets to cool or warm the insulin to operating conditions. The enzymatic cleavage vessel and fermentors F101 to F-104 require jackets to warm them to 25 °C and 37 °C respectively. In this case, water can be used as the agent to transfer heat into the fermentors and enzymatic cleavage vessel. Cooling jackets are needed for the incubator and crystallizer; these pieces' contents need to be cooled to 4 °C and 5 °C. To facilitate this process the cooling agent of 50% ethylene glycol and water solution is needed because the target temperature is too close to the freezing point of water, thus water would be inadequate because it could potentially form ice on the vessel's walls. The cooling jackets can be treated as shell and tube heat exchangers in regards to the tanks and from there the size of each jacket can be found. To calculate the area for each piece the following equation was used:

$$A = \frac{Q}{U_o * \Delta T_{lm}} \quad (4.9.e)$$

The amount of power, Q, needed for the fermentors can be obtained by adding the heat generated by cells to the heat produced from the rotation of the impeller.

$$Q = Heat\ Generated\ from\ Cells\ (W) + Heat\ of\ Gassed\ System\ (W)$$

Subsequently ΔT_{lm} can be calculated as follows:

$$\Delta T_{lm} = \frac{T_2 - T_1}{ln\left(\frac{T_H - T_1}{T_H - T_2}\right)} \quad (4.9.f)$$

Lastly the overall heat transfer coefficient is needed to find the area. It can be calculated with equation 4.9.g.

$$U_0 = \left(\frac{1}{h_0} + \frac{r_0}{k_{steel}} * ln\left(\frac{r_0}{r_i}\right) + \frac{1}{h_i} * \frac{r_0}{r_i}\right)^{-1} \quad (4.9.g)$$

For our cooling jackets to be effective, the required area must be smaller than the jacket area covering each of the tanks. The jacketed area can be seen by 4.9.h. In the equation, h_T represents the wetted height, and D_T represents the diameter of the tank.

$$A_{jacket} = \pi h_T D_T \qquad (4.9.h)$$

The mass and volumetric flows of ethylene glycol can be calculated by 4.9.i and Equation 4.9.j respectively. Q is the heat generated, T_2 and T_1 are the outlet and inlet temperatures, and C_P is the heat capacity of ethylene glycol. To get the volumetric flow rate, the mass flow rate is divided by the density of ethylene glycol.

$$m_c = \frac{Q}{(T_2 - T_1)C_P} \qquad (4.9.i)$$

$$V = \frac{m_c}{\rho} \qquad (4.9.j)$$

The inlet and outlet temperatures, required area, jacket area, and volumetric flow rates for each of the fermenters, mixing tanks, and incubators were calculated using equations 4.9.h through 4.9.j; furthermore, all the actual cooling jacket areas were found to be greater than the required area, making the cooling jacket designs sufficient for our process. It is important to note that for the incubators (I101a, I101b, I101c) and crystallizer (CR101) the inlet cooling temperatures are at freezing temperatures (-10 and 0°C). Ethylene glycol is fed as an anti freezing agent to prevent any ice crystals from forming on the surface of the tank which would affect its conductive properties.

Table 4.9.b

Primary Vessel Parameters

	Warming Agent/ Coolant inlet temperature (°C)	**Warming Agent/ Coolant outlet temperature [°C]**	**Area of Jacket [m^2]**	**Q [W]**	**T_{lm} [℃]**	**Uo**
F101	100	99.6	0.197	288.9	79.80	17.78
F102	100	99.6	1.458	2548.3	79.80	21.90
F103	100	99.6	15.680	25559.2	79.80	20.43
F104	100	99.6	180.097	293572.6	79.80	20.43
EC101	100	99.21	0.204	332.3	79.61	20.41
I101a	-10	7.746	21.368	9251.8	15.812	17.81
I101b	-10	7.746	21.368	9251.8	15.812	17.81
I101c	-10	7.746	21.368	9251.8	15.812	17.81
CR101	0	0.59	0.577	200.0	19.71	17.58

X. Ancillary Equipment

i. Pumps

There are multiple pumps required for the operation of the insulin manufacturing facility. The liquid media throughout the different operations containing cells and proteins need external forces to facilitate movement to the different unit operations. In order to determine the amount of power required by each pump, Equation 4.10.a was used:

$$Power = Differential\ Pressure * Volumetric\ Flow\ Rate \quad (4.10.a)$$

Power is in units of watts (W), differential pressure in units of pascals (Pa), and volumetric flow rate in units of cubic meter per second (m^3/s). A few considerations for the pump calculations include pressure differences, gravity head, and frictional losses due to pipe movement, heat exchangers, and control valves. The pumps operate using electrical motor drives, operating at a 70% efficiency and a 90% electrical driver efficiency.

There are different types of pumps available for use, but for the proposed plant, a combination of centrifugal and peristaltic pumps will be used. Centrifugal pumps are very versatile and adaptable in their applications. They can move large quantities of liquids but operate with a medium to high shear rate. Peristaltic pumps are more energy efficient and can efficiently move high viscosity fluids. They are used in high pressure applications and operate with a low shear rate. The upstream process will utilize 16 centrifugal pumps due to the large amounts of liquids involved in the process. The downstream process will utilize 52 peristaltic pumps due to the smaller amounts of liquids involved as well as the shear rate. Downstream is concerned with proteins so appropriate consideration should be given to prevent the denaturation of the protein products. Because of the nature of the centrifugal pumps, extra frictional loss will be involved in the power calculation for centrifugal pumps compared to the calculations of the peristaltic pumps. The specifications for the pumps can be found in Table 4.10.a.

Table 4.10.a

Pump Pressure, Flow Rate, and Power Specifications

Equipment Tag	Differential Pressure (Bar)	Volumetric Flow Rate (L/s)	Power (kW)
P100	1.01	1.00	1.61E-01
P101	3.04	1.00	4.83E-01
P102	1.01	1.00	1.61E-01
P103	1.01	1.00	1.61E-01
P104	1.01	1.00	1.61E-01
P105	1.01	2.78	4.47E-01
P106	1.45	1.00	2.31E-01
P107	1.40	1.00	2.22E+02
P108	1.40	2.78	6.17E+02
P109	1.60	1.00	2.54E-01
P110	3.91	1.87	1.16E+00
P111	3.91	1.00	6.21E-01
P112	3.91	1.87	1.16E+00
P113	1.01	1.87	3.00E-01
P114	2.90	1.00	4.60E-01
P115	5.07	1.00	8.04E-02
P116	3.40	1.87	1.01E+00
P117	3.40	1.00	5.40E-01
P118	3.40	1.87	1.01E+00
P119	2.90	1.00	4.60E-01
P120	5.07	1.00	8.04E-02
P121	5.07	2.78	2.23E-01
P122	5.07	1.00	8.04E-02

ii. Holding Tanks

Multiple holding tanks are needed throughout our process to hold media, buffers, waste, and adjusters. The holding tanks are designed similarly to the mixing tanks found in the Separations Design section. The capacity of each tank was determined based on the total volume of each substance needed throughout the process. The power needed for each tank was calculated from Equation 4.10.b:

$$Power = Np * \rho * D^5 * N^3 \quad (4.10.b)$$

Power is described as multiplying the power number (Np), the density of the liquid inside the holding tank (ρ) in kg/m^3, the impeller speed (N) in rev/s, and the impeller diameter (D) in m (Perry et al., 2008). Our holding tanks are made of stainless steel and each one features 3 impellers used for mixing except for HT105, HT110, HT112, and HT113 which have 2 impellers. In order to decrease foam production, the waste streams with cells and ACN do not require mixing. The specifications of each holding tank can be found in Table 4.10.b.

Table 4.10.b

Holding Tank Dimensions and Parameters

Equipment Tag	Solution	Capacity (L)	Diameter (m)	Height (m)	Baffle Width (m)	Impeller diameter (m)	Rotation Speed (rev/s)	Mixing Time (s)	Power (kW)
HT100	WFI	500,000	3.24	9.7	0.27	1.080	0.94	122.19	6.04
HT101	Buffer 1	3,000	1.50	4.5	0.13	0.500	2.04	56.57	2.37
HT102	Buffer 2	1,000	1.00	3.0	0.08	0.333	3.06	37.71	1.05
HT103	Buffer 3	20,000	2.00	6.0	0.17	0.667	1.53	75.43	62.48
HT104	Buffer 4	50,000	2.50	7.5	0.21	0.833	1.22	94.28	102.76
HT105	Adjuster A	50	0.25	0.8	0.02	0.083	12.22	9.43	0.05
HT106	Buffer 5	25,000	2.00	6.0	0.17	0.667	1.53	75.43	24.43
HT107	Adjuster B	500	0.50	1.5	0.04	0.167	6.11	18.86	0.36
HT108	Solvent A	5,000	1.50	4.5	0.13	0.500	2.04	56.57	1.56
HT109	Solvent B	3,000	1.50	4.5	0.13	0.500	2.04	56.57	1.18
HT110	Buffer 6	2	0.13	0.4	0.01	0.042	24.45	4.71	0.02
HT111	Buffer 8	500	0.50	1.5	0.04	0.167	6.11	18.86	0.20
HT112	Adjuster C	50	0.25	0.8	0.02	0.083	12.22	9.43	0.10
HT113	Adjuster D	50	0.25	0.8	0.02	0.083	12.22	9.43	0.04
HT114	Media	300	0.50	1.5	0.04	0.167	6.11	18.86	0.21
HT115	Caustic NaOH	50,000	2.50	7.5	0.21	0.833	1.22	94.28	34.20
HT116	Waste w/ cells	10,000	1.50	4.5	0.13	0.500	2.04	56.57	0.00
HT117	Waste w/ ACN	20,000	1.75	5.3	0.15	0.583	1.75	66.00	0.00
HT118	Other Waste	100,000	2.50	7.5	0.21	0.833	1.22	94.28	807.36

XI. Waste Treatment

Within our process are multiple different kinds of waste to be disposed of, each through different means. First, is cellular debris from the *E. Coli* used to express our protein. The cellular material will be separated from the fermentation broth after homogenization, and consolidated before being sent to a steam autoclave unit to neutralize the biological hazard. The autoclaved materials will be either incinerated or sent to a hazardous waste treatment facility that we contract out. The buffer salts and stabilizers will be separated from the organics through a precipitation or filtration method. They will be collected and sealed in drums to be sent to a proper waste facility for disposal. Some of these buffer ingredients can be sent to a landfill, but some will need to go to the hazardous waste treatment facility.

For buffer ingredients that cannot be precipitated out, they will be neutralized and released to public sewage where possible. In an ideal setting for disposal of organic solvents - they would first be put through a distillation column to try and recycle as much solvent as possible. Acetonitrile, for example, would try to be recovered as much as possible through this process. While this would be an ideal form of processing for this waste, the design of a waste facility was considered out of scope for this project but is mentioned for accuracy. Any solvent unable to be recaptured, will be neutralized using some form of pH adjustment before being sent to a hazardous waste facility. Some solvents, however, have the capability to be released into the public sewage system following pH treatments depending on local and national regulatory guidelines. Finally, misfolded proteins/amino acids will first be denatured in bleach or caustic before being sealed in a mixture of cement/lime and disposed of to either a landfill or our contracted waste treatment facility. The waste flows and associated costs can be found in Table 5.5.a and the economics section.

5. Final Recommendations

I. Upstream

The fermentor design for our process uses a seed train configuration in order to obtain the same final concentration of cells (18 g/L), while maximizing the number of cells we can produce. After receiving cells from shake flasks, the first fermentor in our process is F101 and has a volume of 10 L, fermentor F102 is 100L, F103 is 1,000 L, and F104 is 10,000 L; each fermentor operates at 37 ℃ it takes about 5.5 hours for each fermentor to reach a final concentration of 18 g cells per liter of fermentation media. Cells are fed with glucose, oxygen, ampicillin and fermentation media based on the size of each fermentor. The fermentation media was supplied from the first mixing tank (M101) which consists of LB Broth that acts as a source of nutrition for the cells. The tank will be 10,000 L with a working volume of 8,000 L. It will have a diameter of 2.4 m and a height of 7.2 m. Four baffles with a width of 0.2 m and three impellers with a diameter of 0.8 m will be rotating at a speed of 1.27 rps to mix the contents. The total mixing time is 90 seconds, and then the fermentation media will enter each fermenter at 72% of the volume capacity to account for foaming and agitation. Each fermentor has a working volume of 80%, and was designed to achieve a kLa of 200 in order to maintain 30% oxygenation at all times.

II. Downstream

Our first separation is a centrifuge (C101) that comes immediately following the end of the fermentation process (F100-F104) - specifically the Alfa Laval FOPX 610. This model has a processing capability of ~10,000L/hr, which results in a processing time of about 0.8 hours from the fermentation output stream. The majority of the solution processed is discarded as 7857L of waste, and the outlet stream consists of 142 kg of *E. coli* in 142L growth media.The slurry is sent to a mixing tank for washing and resuspension in order to prepare for cell lysis.

A second mixing tank, M102, is used for cell resuspension after centrifugation in C101. The cells collected from centrifugation will be resuspended in the resuspension buffer, Buffer 1. The tank has a capacity of 5,000 L with a working volume of 3,000 L. It will have a diameter of 1.5 m and a height of 4.5 m. Four baffles with a width of 0.125 m and three impellers with a diameter of 0.5 m will be rotating at a speed of 2.03 rps to mix the contents. After a mixing time of 57 seconds and the mixing is complete, the 142.56 kg of cells and the buffer solution will go to the high-pressure homogenizer (H101).

Following centrifugation, the High-Pressure Homogenizer (H101) will be used to disrupt the cells to release the desired protein, insulin glargine. The Hy-Drive HS-3-085-04 Production Homogenizer will be used to process a flow rate of 330 L/hr at 1400 bar. It will take 15 hours to process the 2,852 L of Buffer 1 and 142.56 kg of cells. This lysing technique will release the insulin precursor inside the cell, where it will be further separated from the cellular debris once it is sent to centrifugation (C102). The total power consumption of H101 is 23 kW.

The same disc stack centrifuge model will be used to process the output from the homogenizer (C102). For each batch, a total of 2852 L containing 117 kg *E. coli* will be processed in approximately 17 minutes. 24.4 kg of protein per batch will be retained while 116.7 kg per batch of cell debris is spun out. The volume leaving the centrifuge is approximately 2702 L and contains 24.22kg of solubilized protein. The cell debris is collected through the outer port to be discarded as waste.

The third and final mixing tank (M103) is used for resuspension and washing of the inclusion bodies in a washing solution (Buffer 2) after centrifugation (C102). The components of Buffer 2 are listed in Table 5.5.b. After washing is complete, the solution will go to diafiltration (D101). The tank has a capacity of 6,000 L with a working volume of 4,000 L. It will have a diameter of 2.0 m and a height of 6.0 m. Four baffles with a width of 0.16 m and three impellers

with a diameter of 0.67 m will be rotating at a speed of 1.53 rps to mix the contents. After a mixing time of 75 seconds and washing is complete, the solution will go to diafiltration (D101).

Diafiltration units are used to exchange buffers. In the diafiltration processes, the process feeds are exchanged for an added solvent. For the first diafiltration step (D100), the process feed consists of the insulin glargine protein, Buffer 1, Buffer 2, and growth media. Buffer 3 is added as the solvent. The total volume of the process feed yields the initial volume (V_0) of about 3,421 L. The diafiltration volume for this process is 13,824 L. 7,475 L of Buffer 3 is retained and moved into the incubators. A permeate flow rate of 112.087 L/min yielded the best process time, and the permeate flux was found to be 1.44×10^{-4} m/s. The processing time for this diafiltration step is 2.05 hours. Upon the completion of the process, the insulin glargine protein (23.74 kg/batch) in solution with some excess of Buffer 3 is sent to the incubators. The rest of the solvents are removed and sent off as waste.

Incubation is needed to refold the insulin glargine after its intensive journey through the high-pressure homogenizer and diafiltration units. The solution is incubated in an incubator (I101) with 0.1 mM β-mercaptoethanol at 4°C for 48 hrs for refolding. After the refolding reaction has occurred, the pH of the solution is adjusted to 4.5. The solution is incubated in 20,000 L tanks that are designed similarly to the mixing tanks. The incubators have a diameter of 3 meters and a height of 9 meters. Four baffles are distributed evenly around the tank with a width of 0.25 m. Three impellers with a width of 1 m will be set at an impeller speed of 1 rev/second, resulting in a mixing time of 113 seconds. Since the incubators need to process about 50,000 L of solution, and are a rate limiting step in our production schedule, 3 incubators are needed for this process. After incubation, the refolded protein is sent to an ultrafiltration unit to filter out misfolded proteins and other cellular debris.

Ultrafiltration is used to separate insulin from cell debris. The process feed in the ultrafiltration unit consists of insulin glargine, Buffer 3, Buffer 4, Media A, and Adjuster A with a total combined volume of 49,149 L. The permeate product is 45,220 L/batch consisting of a mixture of insulin glargine, Buffer, 3, Buffer 4, and Adjuster A. The retentate waste stream will be 3,932 L/batch consisting of a mixture of a small amount of insulin glargine, Buffer 3, Buffer 4, Media A, and Adjuster A. The recovered insulin (17.45 kg/batch) passes through to the second diafiltration system (D101).

The second diafiltration step (D101) comes after the first ultrafiltration step (U101), where 45,217 L are fed into the system. This initial volume consists of the process stream containing insulin glargine, Buffer 3, Buffer 4, and Adjuster A. WFI is added as the solvent. The diafiltration volume was found to be 182,703 L. The processing time was calculated to be 27.1 hours. Upon completion, the insulin glargine protein (17.1 kg/batch) and some WFI are moved to the cation exchanger (CX101). The remainder of the solvents are removed and sent off as waste.

The next step is cation exchange chromatography. The column is filled with SP Sepharose Fast Flow resin as the stationary phase. The column specifications are 1000L with 1.3m diameter, and 0.5m bed depth. The equilibration buffer will contain 7M urea and 0.25 M acetic acid. After washing the column with this buffer, the bound proteins will be eluted with elution buffers containing 7 M urea, 0.25 M acetic acid, and 1 M sodium chloride with a 0-1 M sodium chloride gradient. The first column (CX101) is used to process the 17.1 kg of insulin per batch from the diafiltration unit D101. The elution will occur in a 1.58 hr time frame. The outlet stream will consist of 15.39kg in 1000 L buffer to be sent to RP101. The other 1.54 kg of protein is eluted with the other 10000 L of buffer and is discarded as waste.

The Reverse-Phase Chromatography unit (RP101) further purifies the product by partitioning stationary molecules from the mobile phase. Reversed-phase chromatography separates molecules based on their hydrophobicity where hydrophilic molecules elute first. The

HPLC column will be 200 L with a length 0.25m and a diameter of 0.97 m. It is equipped with Zorbax C8 (particle size 5μm) that has a binding capacity of 50 mg/mL. The column is equilibrated at a flow rate of 3 ml/min (0.18 L/hr) with 10 CV Buffer A and the collected protein was loaded onto the column at a flow rate of 1 ml/min, corresponding to a residence time is 1.03 hr or ~ 62 minutes. The cycle time for 1 column is around 6.18 hours plus one hour for cleaning-in-place, totaling 7.18 hours. Samples from the reverse-phase chromatography column will be sent to the HPLC to further test for purity of the insulin glargine. Five 20 μl samples per batch will be sent through a Protein & Peptide C4 analytical column with dimensions of 250 mm x 4.6 mm, and a particle size of 5μm. A UV light detector will be used to monitor peak absorbance to determine purity.

The third diafiltration step (D102) comes after the reverse phase high performance liquid chromatography step, where 3,250 L are fed into the system. This initial volume consists of the process stream containing insulin glargine and Buffer 7. WFI is added as the solvent. The diafiltration volume was found to be 13,132 L. The processing time was calculated to be 1.95 hours. Upon completion, the insulin glargine protein (13.57 kg/batch) and some WFI are moved to the enzymatic cleavage vessel (EC101). The remainder of the solvents are removed and sent off as waste.

This vessel will act as an incubator for the reaction to cleave arginine and lysine residues from the protein. It will be operated under the following conditions: a temperature of 25 °C, a pH of 8.5, and a run time of 5 hours. At the two hour mark, 9 units of trypsin per mg of protein will be added to the reactor. Additionally WFI and Buffer 8 containing borate and citraconic anhydride are added.

The fourth diafiltration step (D103) comes after the enzymatic cleavage step (EC101), where 133 L are fed into the system. This initial volume consists of the process stream containing insulin glargine, Buffer 8, Adjuster C, Adjuster D, and media. Buffer 5 is added as the

solvent. The diafiltration volume was found to be 539 L. The processing time was calculated to be 4.80 minutes. Upon completion, the insulin glargine protein (11.97 kg/batch) and some excess of Buffer 5 are moved to the cation exchanger (CX102). The remainder of the solvents are removed and sent off as waste.

After leaving the transformation tank and undergoing a buffer exchange, a second cation exchange chromatography column (C102) is employed. CX102 receives 11.97 kg of protein in 10000 L of Buffer 5 from D103. The total time for the elution will be approximately 1.6 hours and the whole column cycle about 9 hours. The outlet stream sent to the crystallizer consists of 10.77 kg insulin in 1000 L of buffer. The waste stream is composed of 1.2 kg of protein in 10000 L of buffer.

Next the product stream is sent to the crystallizer (CR101) - the method for crystallization is through an anti-solvent. The unit must operate at a temperature of 5°C with ethylene glycol as the coolant. In this process, an 18% solution of zinc-chloride will be used to form insulin crystals, isolating the insulin from impurities that do not co-crystallize. The initial feed stream is composed of 6,718 L of insulin glargine and Buffer 5. 18% of a zinc chloride-WFI solution is added to the crystallizer until insulin reaches a concentration of 0.1%. In order to achieve this concentration, 110.45 L of the zinc chloride solution must be added to the crystallizer. 8.71 kg of insulin passes through to the centrifuge, requiring a total of 12 hours.

Following the crystallization step, a final separation via centrifuge (C103) will be employed to prepare the product for lyophilization (L101). The incoming stream from the crystallizer is 10.45 kg of insulin in 6250 L of Buffer 5. The centrifuge removes 5779 L of Buffer 5 through the top port to be discarded as waste, while the remaining 10.24 kg of insulin in 102.8 L of Buffer 5 is collected through the port on the outside wall in a 3 hour operating time.. This is the last separation step in the process before the final product is sent to be lyophilized and formulated as an injectable.

The final protein quantity is then lyophilized in the Vikumer lyophilizer model FD-50L and sent for formulation. The lyophilizer will freeze the product to -85°C, and reach a final vacuum pressure of 1 x 10^{-5} bar to ensure that the organic solvent has sublimed. A total of 10.28 kg of insulin will be lyophilized and sent for formulation. After the lyophilizer, the insulin is then sent to formulation where it is put into a solution containing zinc, m-Cresol, glycerol, and polysorbate 20. The solution is packaged into 10 mL vials using the Shanghai ALWELL Filling Line to fill 239,285 vials per batch. The formulation machine can fill 16,000 vials per hour, so there will be 4 machines in parallel to have a packaging time of 3.7 hours.

III. Heat Requirements

This process has nine cooling/warming jackets in place to account for temperature fluctuations. There are cooling jackets on the three incubators and crystallizer, while there are warming jackets on the four fermentors and the enzymatic cleavage vessel. It is recommended to use a 50% ethylene glycol and water solution for the cooling jackets due to the low temperatures of 4℃ and 5 ℃ needed. For the warming jackets it is recommended to use water as the heating agent to reach temperatures 25℃ and 37℃.

IV. Ancillary Equipment

The process requires 68 pumps to supply feed streams to unit operations and to assist flows. 16 centrifugal pumps will be used for the upstream equipment to process large volumes of media, while 52 peristaltic pumps will be used for the downstream process.

Multiple holding tanks are also needed throughout the process to supply various buffers, adjusters, media, and WFI to the unit operations. A total of 18 holding tanks were designed with volumes ranging from 5-500,000 L to hold various materials such as WFI, pH adjusters, cleaning materials, and waste products. The holding tanks also have impellers to ensure that the buffers are mixed to the desired compositions and the solutions are not held stagnant.

V. Stream Table

The stream table, Table 5.5.a, includes all of the inputs and outputs for each piece of equipment in our process. This accounts for input, adjusters, buffers, media, waste, and output streams. The name of our buffers are accounted for in the Location column and are used throughout the contents column to reduce repetition. For example, Buffer 1 containing sucrose, Tris, EDTA, NaCl, and WFI will just be named Buffer 1 for the remainder of the process. A complete list of buffers and their components can be found in Table 5.5.b. An additional note is that Buffer 8 is composed of Solvent A and Solvent B and is just found in the HPLC waste stream. Buffer 7 is not listed, for it is a waste stream combination of Buffer 5 and Buffer 6 and is not used within the process. Additionally, the buffers were noted as individual components in the stream table. For example, if a buffer became diluted in a waste stream, the waste stream would have components of that buffer and a greater amount of WFI listed, rather than a new diluted buffer component. Additionally, the gas outlet streams for the fermentations were captured in a fumigation system and are not listed as individual streams in this process. The design of the fumigation system was considered outside the scope of this project.

Table 5.5.a

Stream Table

Stream Number	Location	Contents	Flow Rate	
1	F100 In	Fermentation Media (LB)	0.80	L/batch
		Cells	0.06	g/batch
		Glucose	200.00	g/batch
		Oxygen	n/a	n/a
		Ampicillin	0.04	g/batch
2	F101 In M101 Out	LB Broth	7.20	L/batch
		Glucose	2.00	kg/batch
		Oxygen	49.50	g/batch
		Ampicillin	0.40	g/batch
3	F102 In M101 Out	LB Broth	72.00	L/batch
		Glucose	20.00	kg/batch
		Oxygen	495.00	g/batch
		Ampicillin	4.00	g/batch
4	F103 In M101 Out	LB Broth	720.00	L/batch
		Glucose	2000.00	kg/batch
		Oxygen	4950.00	g/batch
		Ampicillin	40.00	g/batch
5	F104 In M101 Out	LB Broth	7200.00	L/batch
		Glucose	2000.00	kg/batch
		Oxygen	49500.00	g/batch
		Ampicillin	400.00	g/batch
6	F101 In Shake Flask Out	Growth Media	0.80	L/batch
		Cells	1.44	g/batch
7	F102 In	Growth Media	8.00	L/batch

	F101 Out	Cells	144.00	g/batch
8	F103 In F102 Out	Growth Media	80.00	L/batch
		Cells	14400.00	g/batch
9	F104 In F103 Out	Growth Media	800.00	L/batch
		Cells	1440.00	kg/batch
10	Fermentation Out C101 Feed	Cell Mass	144.00	kg/batch
		Growth Media	8000.00	L/batch
11	M102 Input C101 Output	Cells	142.56	kg/batch
		Growth Media	142.56	L/batch
12	C101 Waste	Cells	1.44	kg/batch
		Growth Media	7857.44	L/batch
13	M102 Input (Buffer 1)	Sucrose	285.20	kg/batch
		Tris	34.55	kg/batch
		EDTA	41.67	kg/batch
		NaCl	33.33	kg/batch
		WFI	2852.00	L/batch
14	H101 Input M102 Output	Cells	142.56	kg/batch
		Buffer 1	2852.00	L/batch
		Growth Media	142.56	L/batch
15	C102 Input H101 Output	Protein	24.69	kg/batch
		Cell Debris	117.87	kg/batch
		Buffer 1	2852.00	L/batch
		Growth Media	142.56	L/batch
16	M103 Input C102 Output	Protein	24.22	kg/batch
		Cell Debris	1.18	kg/batch
		Buffer 1	2709.00	L/batch
		Growth Media	135.46	L/batch
17	C102 Waste	Protein	0.47	kg/batch

		Cell Debris	116.69	kg/batch
		Buffer 1	142.60	L/batch
		Growth Media	7.10	L/batch
18	M103 Input (Buffer 2)	Tris (20 mM)	1.76	kg/batch
		EDTA (1mM)	0.21	kg/batch
		Lysozyme (0.02%)	0.15	kg/batch
		Triton X-100 (1%)	7.27	kg/batch
		Urea (0.5 M)	21.82	kg/batch
		WFI	726.60	L/batch
19	D100 Input M103 Output	Protein	24.22	kg/batch
		Cell Debris	1.18	kg/batch
		Buffer 1	2566.40	L/batch
		Buffer 2	726.60	L/batch
		Growth Media	128.36	L/batch
20	D100 Input (Buffer 3)	Urea	1643.00	kg/batch
		Glycine	2.57	kg/batch
		WFI	13305.00	L/batch
21	I101 Input D100 Output	Protein	23.74	kg/batch
		Buffer 3	7475.30	L/batch
22	D100 Waste	Protein	0.48	kg/batch
		WFI	7475.30	L/batch
		Buffer 3	5829.70	L/batch
23	I101Input (Buffer 4)	Urea	269.00	kg/batch
		Glycine	5.60	kg/batch
		WFI	47480.00	L/batch
24	I101 Media Input	β-mercaptoethanol	0.37	kg/batch
25	I101 pH Input (Adjuster A)	HCl	9.75	kg/batch
		WFI	23.74	L/batch

26	U101 Input I101 Output	Protein	17.80	kg/batch
		Buffer 3	1645.60	L/batch
		Buffer 4	47480.00	L/batch
		Media A	0.37	kg/batch
		Adjuster A	23.74	L/batch
27	U101 Waste	Protein	0.35	kg/batch
		Buffer 3	131.60	L/batch
		Buffer 4	3798.40	L/batch
		Media A	0.37	kg/batch
		Adjuster A	1.89	L/batch
28	D101 Input U101 Output	Protein	17.45	kg/batch
		Buffer 3	1514.00	L/batch
		Buffer 4	43681.60	L/batch
		Adjuster A	21.85	L/batch
29	D101 WFI Input	WFI	182703.00	L/batch
30	D101 Waste	Protein	0.35	kg/batch
		Buffer 3	1514.00	L/batch
		Buffer 4	43681.60	L/batch
		Adjuster A	21.85	L/batch
		WFI	180993..00	L/batch
31	CX101 Input D101 Output	Protein	17.10	kg/batch
		WFI	1710.00	L/batch
32	CX101 Input (Buffer 5)	Urea	3363.00	kg/batch
		Acetic Acid	120.10	kg/batch
		WFI	11000.00	L/batch
33	CX101 Adjuster	Sodium Chloride	175.32	kg/batch
34	RP101 Input CX101 Output	Protein	15.39	kg/batch
		Buffer 5	1000.00	L/batch

35	CX101 Waste	Protein	1.54	kg/batch
		Buffer 5	10000.00	L/batch
36	RP101 Solvent Input (Solvent A)	Acetic Acid	30.03	kg/batch
		ACN	300.00	kg/batch
		WFI	2000.00	L/batch
37	RP Solvent Input (Solvent B)	Acetic Acid	30.03	kg/batch
		ACN	900.00	kg/batch
		WFI	1200.00	L/batch
38	HPLC Input RP101 Output	Protein	2.60E-05	kg/batch
		Buffer 5	2.00E-04	L/batch
39	HPLC Input (Buffer 6)	NaH2PO4*H2O	6.90E-08	kg/batch
		NaClO4	1.20E-07	L/batch
		ACN	1.00E-04	L/batch
		WFI	1.00E-04	L/batch
40	HPLC Waste	Protein	2.60E-05	kg/batch
		Buffer 5	2.00E-04	L/batch
		Buffer 6	2.00E-04	L/batch
41	RP101 Output D102 Input	Protein	13.85	kg/batch
		Buffer 7	3250.00	L/batch
42	RP101 Waste	Protein	0.28	kg/batch
		Buffer 7	50.00	L/batch
43	D102 WFI	WFI	13000.00	L/batch
44	D102 Output EC101 Input	Protein	13.57	kg/batch
		WFI	200.00	L/batch
45	D102 Waste	Protein	0.29	kg/batch
		WFI	12700.00	L/batch
		Buffer 7	50.00	L/batch
46	EC101 Input (Buffer 8)	Borate	0.24	kg/batch

		Citraconic Anhydride	59.85	kg/batch
		WFI	300.00	L/batch
47	EC101 Media Input	Trypsin	0.03	kg/batch
48	EC101 (Adjuster C)	NaOH	10.56	kg/batch
		WFI	20.00	L/batch
49	EC101 (Adjuster D)	Acetic Acid	3.29	kg/batch
		WFI	30.00	L/batch
50	EC101 Output D103 Input	Protein	13.57	kg/batch
		Buffer 8	300.00	L/batch
		Adjuster C	20.00	L/batch
		Adjuster D	30.00	L/batch
		Media	0.04	kg/batch
51	D103 Input (Buffer 5)	Urea	3363.00	kg/batch
		Acetic Acid	120.10	kg/batch
		WFI	11000.00	L/batch
532	D103 Output CX102 Input	Protein	11.97	kg/batch
		Buffer 5	9900.00	L/batch
53	D103 Waste	Protein	1.60	kg/batch
		Buffer 8	300.00	L/batch
		Adjuster C	20.00	L/batch
		Adjuster D	30.00	L/batch
		Media	0.04	kg/batch
		Buffer 5	1100.00	L/batch
54	CX102 Adjuster	Sodium Chloride	175.32	kg/batch
55	CX102 Output CR101 Input	Protein	10.77	kg/batch
		Buffer 5	2082.00	L/batch
56	CX102 Waste	Protein	1.20	kg/batch
		Buffer 5	6718.00	L/batch

57	CR101 Media	Zinc Chloride (18%)	20.80	kg/batch
		WFI	110.45	L/batch
58	CR101 Output C103 Input	Protein	10.45	kg/batch
		Buffer 5	6250.00	L/batch
		Media	3.75	L/batch
59	CR101 Waste	Protein	0.32	kg/batch
		Buffer 5	468.00	L/batch
		Media	106.70	L/batch
60	C103 Output L101 Input	Protein	10.24	kg/batch
		Buffer 5	102.80	L/batch
61	C103 Waste	Protein	0.21	kg/batch
		Buffer 5	5779.20	L/batch
62	L101 Output FR101 Input	Protein	10.28	kg/batch
63	L101 Waste	Buffer 5	5779.20	L/batch
64	FR101 Media Input	Zinc	0.72	kg/batch
		m-Cresol	6.46	L/batch
		Glycerol	38.05	L/batch
		Polysorbate 20	0.47	kg/batch

Table 5.5.b

Buffer Table

Buffer	Components	Quantity	
Buffer 1	Sucrose	285.20	kg/batch
	Tris	34.55	kg/batch
	EDTA	41.67	kg/batch
	NaCl	33.33	kg/batch
	WFI	2852.00	L/batch
Buffer 2	Tris (20 mM)	1.76	kg/batch
	EDTA (1mM)	0.21	kg/batch
	Lysozyme (0.02%)	0.15	kg/batch
	Triton X-100 (1%)	7.27	kg/batch
	Urea (0.5 M)	21.82	kg/batch
	WFI	726.60	L/batch
Buffer 3	Urea	1643.00	kg/batch
	Glycine	2.57	kg/batch
	WFI	13305.00	L/batch
Buffer 4	Urea	269.00	kg/batch
	Glycine	5.60	kg/batch
	WFI	47480.00	L/batch
Buffer 5	Urea	3363.00	kg/batch
	Acetic Acid	120.10	kg/batch
	WFI	11000.00	L/batch
Buffer 6	NaH2PO4*H2O	6.90E-08	kg/batch
	NaClO4	1.20E-07	L/batch
	ACN	1.00E-04	L/batch
	WFI	1.00E-04	L/batch
Buffer 8	Borate	0.24	kg/batch
	Citraconic Anhydride	59.85	kg/batch
	WFI	300.00	L/batch

VI. Schedule for Batch Operations

i. Upstream Schedule

There are 6 total unit operations in the upstream production process for insulin glargine: the shake flask (F100), mixing tank (M101), and the four fermentation units (F101, F102, F103, F104). Table 4.6.a below indicates the time each step takes to complete the process, cleaning and steaming in place, draining, and filling. One point of note is that the time required to transport the mixture from each unit along the seed train is not known because the distance between fermenters is beyond the scope of this project.

Table 4.6.a

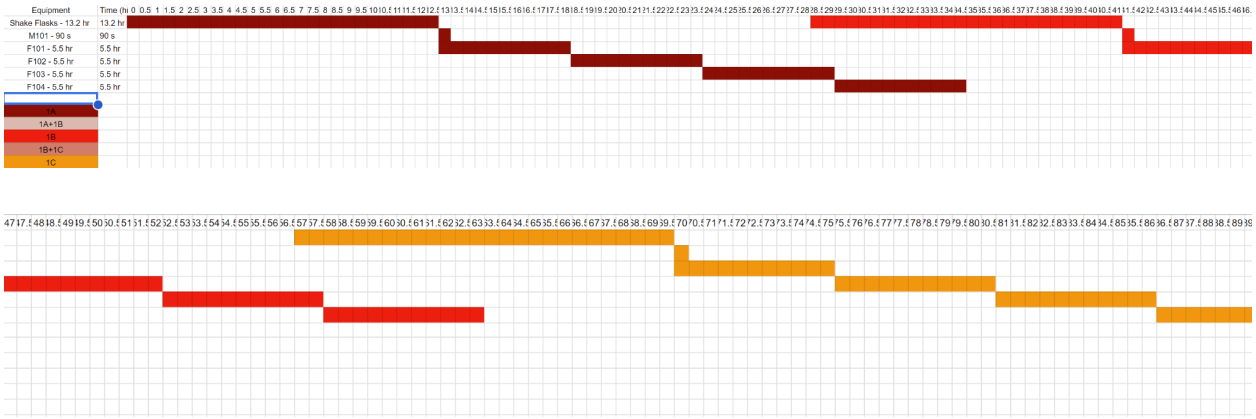
Upstream Process, CIP/SIP, Drain and Fill Times

Equipment Tag	Process Time (hr)	CIP/SIP Time (hr)	Drain Time (hr)	Fill Time (hr)	Total (hr)
F100	13.2	n/a	0.00	0.00	13.20
F101	5.5	1.16	0.00	0.00	6.66
F102	5.5	1.17	0.02	0.02	6.71
F103	5.5	1.19	0.16	0.16	7.01
F104	5.5	1.30	1.66	1.66	10.12
				Total (hr)	43.73 (1.82 days)

Starting at the shake flasks (F100) and going along the seed train to the 10,000 L fermenter (F104), the process takes 13 hours to complete. The drain and fill times reported in Table 4.6.a for F101 and F102 are 0 because the amount of time it takes to drain 1 L and 10 L of the mixture is insignificant at our assumed flow rate. We assume a flow rate exiting and entering the fermenter to be 3,600 L/hr based on industry standards for industrial pumps (Peters et al., 2002). The batch schedule for 3 batches in the upstream process is shown in Figure 4.6.a. The schedule below does not require duplicate units for the upstream process.

Figure 4.6.a

Upstream Batch Schedule



The first batch starts on an arbitrary date and runs for approximately 35 hours or about 1.45 days. The batches are buffered by 28.5 hours. This separation is the most economical and efficient batch schedule for the downstream production of insulin glargine while meeting production demands which will be discussed in the next section. Upon completion, the batch is sent to the first unit in the downstream process, C101. 28.5 hours after Batch 2, the cycle starts over and repeats with Batch 1.

ii. Downstream Schedule

For the downstream process, there are nineteen total types of unit operations used to purify and produce insulin glargine. For some of the unit operations, duplicates are required to meet production demands. Table 4.6.b below indicates the time each step takes to complete the process, cleaning and steaming in place, draining, and filling. Similar to the upstream process, the transportation time between processes was not determined.

Table 4.6.b

Downstream Process, CIP/SIP, Drain and Fill Times

Equipment Tag	Process Time (hr)	CIP/SIP Time (hr)	Drain Time (hr)	Fill Time (hr)	Total (hr)
C101	0.80	1.00	n/a	n/a	1.80
M102	n/a	1.38	n/a	n/a	1.38
H101	15.00	1.00	n/a	n/a	16.00
C102	0.25	1.00	n/a	n/a	1.25
M103	n/a	1.25	n/a	n/a	1.25
D100	2.00	1.00	3.73	3.73	10.46
I101	48.00	1.38	15.27	15.27	79.91
U101	6.08	1.00	13.65	13.65	35.10
D101	27.10	1.00	12.18	12.18	52.46
CX101	9.00	1.00	0.48	0.48	10.95
RP101	7.20	1.00	0.28	0.28	8.76
D102	1.95	1.00	3.61	3.61	10.17
EC101	5.00	1.18	0.14	0.14	6.45
D103	n/a	1.00	3.06	3.06	7.12
CX102	9.00	1.00	2.75	2.75	15.50
CR101	12.00	1.17	0.58	0.58	14.33
C103	3.00	1.00	n/a	n/a	4.00
L101	2.50	1.00	0.03	0.03	3.56
				Total (hr)	280.45 (11.69 days)

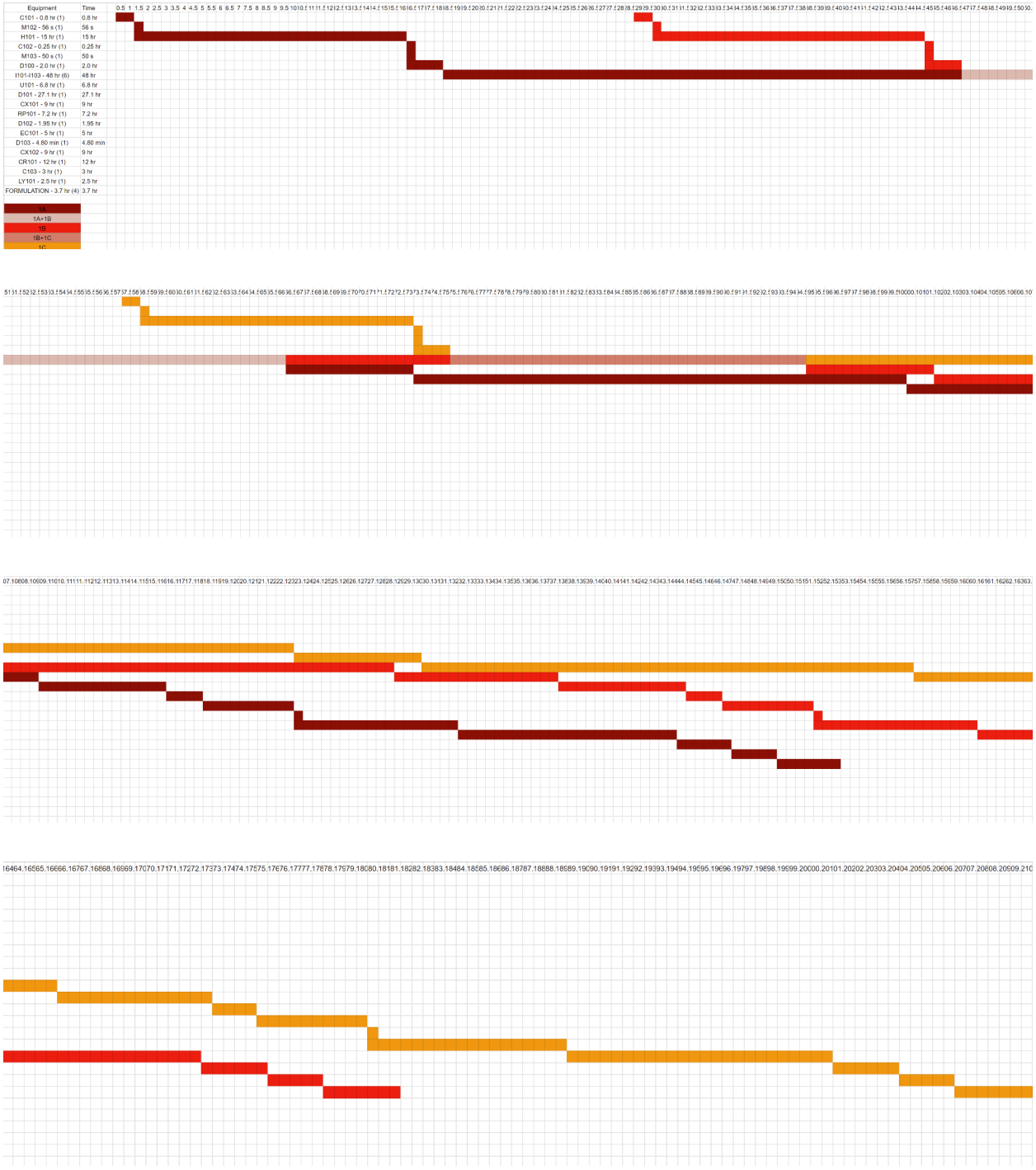
The downstream process begins after upstream finishes at F104. The process moves along different purification and separation processes to isolate the insulin glargine protein from buffers, media, pH adjusters, and cell debris. The downstream process alone will be completed in about 153.5 hours or about 6.40 days, and the insulin glargine product is freeze-dried in a

lyophilizer, L101. Table 4.6.b illustrates some operations data for processing, drain, and fill times, some of which is unknown indicated by “n/a”. Equipment D-103, M-101, and M-102 do not show a time for process, drainage, and filling because these tests require small sample times which makes the times insignificant. It is significant, however, to take into account the amount of time to clean and steam the units. For the centrifuges (C101, C102, C103) and the high-pressure homogenizer (H101), there are no drain and fill times since the two processes run continuously.

The batch schedule for the downstream process is shown in Figure 4.6.b. The figure depicts three batches with overlap in the incubators with 3 incubators and 4 lyophilizers running simultaneously.

Figure 4.6.b

Downstream Batch Schedule



Once F104 is completed, the batch is transported to the first centrifugation process (C101) where the downstream process begins. This process is more dense than the upstream

process because it has nineteen different steps, and each step has varying process times. Based on the schedule, 6 incubators and 4 formulation units are required because of overlap to meet production demands. As shown in Table 4.6.b, incubation takes the longest amount of time to complete the process, clean, drain, and fill. Disregarding the cleaning and steaming times, the downstream process takes a total of about 153 hours. Combined with the upstream process, the total process takes 188 hours or about 7.85 days if run as a single batch.

As stated in the upstream process, the batches are buffered by 28.5 hours. After configuring numerous different batch schedules, a 28.5 hour separation was determined to be the most efficient because this number minimizes the number of duplicate equipment units. Any time below this timeframe would have increased the capital cost, since duplicate units would be required due to overlap in run time and cleaning time. Additionally any time above 28.5 hours would have made the process output inefficient. A time above 31.5 hours would fail to meet the production demands of 272 batches. After the initial 188 hours, a batch will be completed every 28.5 hours following the previous batch. From this information, 271 batches will be completed in 7,723.5 hours after the initial 188 hours for a total of 7911.5 hours, or approximately 330 days. 31.5 hours leads to a total time of 8,724.5 hours, or approximately 364 days. The 28.5 hour buffer minimizes equipment cost while allowing for additional downtime that can be used for maintenance or holidays.

VII. CIP/SIP

The process will include cleaning-in-place and steaming-in-place protocol systems for sterilization and sanitation of the equipment. After the unit operation is complete, a clean-in-place process begins first with a WFI rinse. Caustic NaOH at 60°C will be sprayed for a total of 20 minutes of exposure. A final 10 minute WFI rinse will conclude the cleaning-in-place process. After the CIP process is complete, steaming-in-place will release a hot steam at 121°C and 10 psig to further sterilize the equipment. The batch equipment, including tanks and

fermenters, will be cleaned using Glacier Tanks Fixed Spray Ball which features 360° spray coverage and multiple sizes.

VIII. Waste Treatment

Three types of waste will be produced from this process and will be contained in holding tanks before being sent to their respective sanitation sites. The individual waste streams are listed in Table 5.5.a, and the three waste containers are listed in Table 4.10.b. The first type of waste will be streams that contain *E. coli* cells, and these streams will be sent through a continuous sanitation system at 135°C until it is eventually deposited into holding tank HT116. From here, the decontaminated waste will be sent to the public sewer system. The second waste stream, containing acetonitrile, will be collected in holding tank HT117 and sent to the local industrial toxic waste collectors. Finally, all other waste streams will be neutralized to a pH of 7 from the caustic NaOH employed during the CIP process, and will be held in holding tank 118 before being sent to the public sewer system. The additional NaOH from CIP will be neutralized with sulfuric acid before also being sent to the public sewer system. A further analysis of waste treatment cost and disposal can be found in the economics section.

IX. Process Economics

i. Capital Costs

The capital costs for our manufacturing site include construction, land, equipment, and piping. The total primary equipment cost was calculated to be $8,336,270, found in Table 5.9.a, and the ancillary equipment costs totaled $1,464,346, found in Table 5.9.b. Prices for equipment were based on manufacturer supplied prices, inquiry to industry professionals, and estimates based on known pricing of similar equipment. The quantity of equipment was based on the requirements to produce 272 batches in a calendar year, along with back ups for equipment with maintenance needs or replacement.

For construction, just the site for our plant will cost $5,633,356 based on land cost of 400,000Br/m^2 and an 800m^2 size plot for our site (Addis Abba, 2023). The land price per square meter was estimated using the higher cost land estimates due to the space and development that may be needed. The square meterage was based on manufacturing facilities with similar production requirements to ours, such as Eli Lilly's Technology Center South. The rest of the construction costs for the site can be found in Table 5.9.c. All of the construction costs within Table 5.9.c were based on a ratio given by a heuristics table in Peter, Timmerhaus, and Wests' *Plant Design and Economics for Chemical Engineers* (2003). Our plant is a mainly fluid processing plant, so the costs were scaled according to those values.

Table 5.9.a

Primary Equipment Cost

Equipment	Quantity*	Price/Unit (USD)	Total Cost
1 L Shake Flask	2	$35	$70
10 L Fermenter	2	$100	$200
100 L Fermenter	2	$500	$1,000
1,000 L Fermenter	2	$3,000	$6,000
10,000 L Fermenter	2	$20,000	$40,000
Centrifuge	4	$130,000	$520,000
High-pressure Homogenizer	2	$100,000	$200,000
Ultrafiltration Unit	2	$200,000	$400,000
Diafiltration Unit	6	$200,000	$1,200,000
Incubator	6	$75,000	$450,000
CEX Column	8	$400,000	$3,200,000
RP-HPLC Column	3	$50,000	$150,000
Prep HPLC Column	20	$20,000	$400,000
Enzymatic Cleavage Vessel	2	$22,000	$44,000
Crystallizer	2	$100,000	$200,000
Lyophilizer	8	$200,000	$1,600,000
Total Primary Equipment Cost			$8,336,270

*Includes spare equipment in case of maintenance/emergency

Table 5.9.b

Ancillary Equipment Cost

Equipment	Quantity	Price/Unit (USD)	Total Cost
Centrifugal Pumps	16	$2,050	$32,800
Peristaltic Pumps	52	$1,258	$65,416
500000 L WFI Tank	1	$600,000	$600,000
3000 L Holding Tank	3	$7,000	$21,000
1000 L Holding Tank	2	$3,000	$6,000
20000 L Holding Tank	3	$40,000	$120,000
50000 L Holding Tank	2	$50,000	$100,000
50 L Holding Tank	4	$200	$800
25000 L Holding Tank	2	$45,000	$90,000
500 L Holding Tank	3	$800	$2,400
5000 L Holding Tank	2	$9,000	$18,000
300 L Holding Tank	2	$650	$1,300
2 L Holding Tank	2	$75	$150
10000 L Holding Tank	2	$20,000	$40,000
100000 L Holding Tank	2	$125,000	$250,000
Total Ancillary Equipment Cost			$1,464,346

Table 5.9.c

Construction Costs

Capital Investment Cost	% Delivered Equipment Cost	Total Cost
Direct Costs		
Equipment Installation	47	$4,606,290
Instrumentation and Controls	36	$3,528,222
Piping (installed)	68	$6,664,419
Electrical Systems (installed)	11	$1,078,068
Buildings (including services)	18	$1,764,111
Yard Improvements	10	$980,062
Service Facilities (Installed)	70	$6,860,431
Indirect Costs		
Engineering and Supervision	33	$3,234,203
Construction Expenses	41	$4,018,253
Legal Expenses	4	$392,025
Contractor's Fee	22	$2,156,136
Contingency	44	$4,312,271
Total Capital Investment Costs		$39,594,489

ii. Plant Operating Costs

The operating costs for our manufacturing site include raw materials, utilities, employee wages, taxes, and import fees. The total raw materials cost for our process, including WFI, comes out to about $300M annually; this annual total also includes things such as chromatography column resins and DF/UF membranes that may need to be replaced. These costs were sourced from various manufacturing wholesale sites, discussion with industry professionals, and personal experiences working with given equipment. Because raw materials includes WFI, as it is used in the production process, the only cost under utility is the power consumption which includes heating and cooling. The total power cost, along with all other annual operating costs, can be found in Table 5.9.d. These line items and their costs were chosen in part in accordance with the guidance found in Chapter 6 of Towler and Sinnott's 2008 release of Principles, Practice and Economics of Plant and Process Design.

The major operating cost for our manufacturing plant is taxes, due to Ethiopia having a standard 30% corporate tax rate. Based on our market and product pricing, this will be 72% of our annual operating costs at $1.14B. The power costs, while potentially unstable, are relatively cheap however and are only estimated to be $1.8M annually. Shipping and distribution costs, which mainly concerns fees and taxes related to import of our raw materials, comes out to be $120,000,000. All of these costs can be found in Table 5.9.e.

Table 5.9.d

Raw Materials Costs

Equipment	Material	Amount/ batch	Unit	Amount/ year	Unit	Unit Price	Total Price
Fermentation	LB Broth (0.25g/L)	2000.00	kg	544000	kg	$125.00	$75,000,000
	Glucose	2222.00	kg	604384	kg	$3.50	$2,115,344
	Ampicillin	0.45	kg	121	kg	$45.00	$5,447
	WFI	8000.00	L	2176000	L	$0.75	$1,632,000
	Oxygen	246.30	m^3	669960	m^3	$0.11	$7,370
M102 Buffer (Buffer 1)	Sucrose	285.20	kg	77574	kg	$14.00	$1,086,042
	Tris	34.55	kg	9398	kg	$52.00	$488,675
	EDTA	41.67	kg	11334	kg	$78.00	$884,071
	NaCl	33.33	kg	9066	kg	$0.30	$2,720
	WFI	2852.00	L	775744	L	$0.75	$581,808
M103 Buffer (Buffer 2)	Tris (20 mM)	1.76	kg	479	kg	$52.00	$24,893
	EDTA (1mM)	0.21	kg	58	kg	$78.00	$4,498
	Lysozyme (0.02%)	0.15	kg	39	kg	$241.80	$9,537
	Triton X-100 (1%)	7.27	L	1977	L	$100.00	$197,744
	Urea (0.5 M)	21.82	kg	5935	kg	$43.00	$255,195
	WFI	726.60	L	197635	L	$0.75	$148,226
D100 Buffer (Buffer 3)	Urea	1643.00	kg	446896	kg	$43.00	$19,216,528
	Glycine	2.57	kg	699	kg	$111.00	$77,593
	WFI	13305.00	L	3618960	L	$0.75	$2,714,220
I101 Buffer (Buffer 4)	Urea	269.00	kg	73168	kg	$43.00	$3,146,224
	Glycine	5.60	kg	1523	kg	$111.00	$169,075
	B-mercaptoethanol	0.37	L	101	L	$184.00	$18,568
	WFI	47480.00	L	12914560	L	$0.75	$9,685,920
I101 pH Adjuster A	HCl	9.75	L	2652	L	$10.25	$27,183
	WFI	23.74	L	6457	L	$0.75	$4,843
D101 WFI	WFI	174726.00	L	47525472	L	$0.75	$35,644,104
CX101 Buffer	Urea	3363.00	kg	914736	kg	$43.00	$39,333,648

(Buffer 5)	Acetic Acid	120.10	L	32667	L	$32.14	$1,049,924
	WFI	11000.00	L	2992000	L	$0.75	$2,244,000
CX101 Adjuster	Sodium Chloride	175.32	kg	47687	kg	$0.30	$14,306
RP101 Solvent A	Acetic Acid	30.03	L	8168	L	$32.14	$262,525
	ACN	300.00	L	81600	L	$50.00	$4,080,000
	WFI	2000.00	L	544000	L	$0.75	$408,000
RP101 Solvent B	Acetic Acid	30.03	L	8168	L	$32.14	$262,525
	ACN	900.00	L	244800	L	$50.00	$12,240,000
	WFI	1200.00	L	326400	L	$0.75	$244,800
HLPC Buffer (Buffer 6)	NaH2PO4*H2O	0.69	kg	188	kg	$15.00	$2,815
	NaClO4	0.12	L	33	L	$50.00	$1,632
	ACN	0.50	L	136	L	$50.00	$6,800
	WFI	2.00	L	544	L	$0.75	$408
D102 WFI	WFI	13000.00	L	3536000	L	$0.75	$2,652,000
EC101 Buffer (Buffer 8)	Borate	0.24	kg	64	kg	$43.00	$2,749
	Citraconic Anhydride	59.85	kg	16279	kg	$700.00	$11,395,440
	WFI	300.00	L	81600	L	$0.75	$61,200
EC101 Media Input	Trypsin	0.03	kg	9	kg	$313.30	$2,897
EC101 Adjuster C	NaOH	10.56	kg	2872	kg	$0.60	$1,723
	WFI	20.00	L	5440	L	$0.75	$4,080
EC101 Adjuster D	Acetic Acid	3.29	L	895	L	$32.14	$28,761
	WFI	30.00	L	8160	L	$0.75	$6,120
D103 Buffer (Buffer 5)	Urea	3363.00	kg	914736	kg	$43.00	$39,333,648
	Acetic Acid	120.10	L	32667	L	$32.14	$1,049,924
	WFI	11000.00	L	2992000	L	$0.75	$2,244,000
CX102 Adjuster	Sodium Chloride	175.32	kg	47687	kg	$0.30	$14,306
CR101 Media	Zinc Chloride (18%)	20.80	kg	5658	kg	$240.00	$1,357,824
	WFI	110.45	L	30042	L	$0.75	$22,532
FR101	Zinc	0.72	kg	196	kg	$2.59	$507

	m-Cresol	6.46	L	1757	L	$0.17	$299
	Glycerol	38.05	L	10350	L	$4.30	$44,503
	Polysorbate 20	0.47	kg	128	kg	$6.46	$826
	Vials	238295.00	vials	64816240	vials	$0.20	$12,963,248
CIP	NaOH	24310.00	kg	6612320	kg	$0.60	$3,967,392
	WFI	32249.00	L	8771728	L	$0.75	$6,578,796
	Steam	107986.00	L	29372192	L	$0.00	$276,099

The total quantity of waste to be disposed of is approximately 78 million liters per year. Based on waste incineration prices, the total cost of waste management annually will be about $5.8M. These prices are for american based waste management contracts, so the yearly cost could be even more expensive in our location which may not have the infrastructure for our waste management needs (Energy Justice Network, 2020).

Table 5.9.e

Total Annual Operating Costs

Line Item	**Cost**
Raw Materials	$297,300,714
Labor	$1,500,000
Taxes	$1,149,750,000
Waste Treatment	$5,800,000
Power	$1,820,000
Shipping & Distribution	$120,000,000
TOTAL	$1,576,150,714

iii. Discounted Cash Flow Analysis

A discounted cash flow analysis was performed to estimate the potential expected value of future cash based on our initial investments. The major cash flows associated with the plant include initial capital costs (including equipment, land, and construction), taxes, operating costs (raw materials, employee salaries, and utilities) and revenue generated from our product. Our plant is located in Ethiopia, where the standard corporate tax (CIT) is 30% (Worldwide Tax Summary, 2023). Our construction is estimated to occur over a period of 2 years to complete. The capital costs will accrue during this period, and will be paid out during the lifespan of the plant.

We intend to sell our product at a price point of $0.05 per unit. A few of the major competitors within the insulin distribution space include Eli Lilly, Novo Nordisk, and Sanofi. While prices do fluctuate over time, these manufacturers have sold long-acting insulin products for $350, $400, and $450 per vial respectively (McQueen & Li, 2023) . Based on the assumption that an individual would need approximately 1,000 units monthly, or one vial, our product would cost only $50 a month. The goal of our production is to increase accessibility of insulin to individuals in lower income regions of Africa, and our price point reflects this. With this price, our yearly revenue will come to $3.83 billion.

Our economic analyses were performed using a discount rate of 20% found in Figure 5.9.a. This is due to insulin already being an established product with a well characterized production and distribution model (Stasior et al., 2018). The DCF values were calculated using Equation 5.9.a. The variable CF represents cash flow, the variable i represents the discount rate, and the variable N represents the number of time periods.

$$\sum \frac{(CF_N)}{(1+i)^N} \qquad (5.9.a)$$

Figure 5.9.a

Discounted Cash Flow over Plant Lifespan (Discount Rate = 20%)

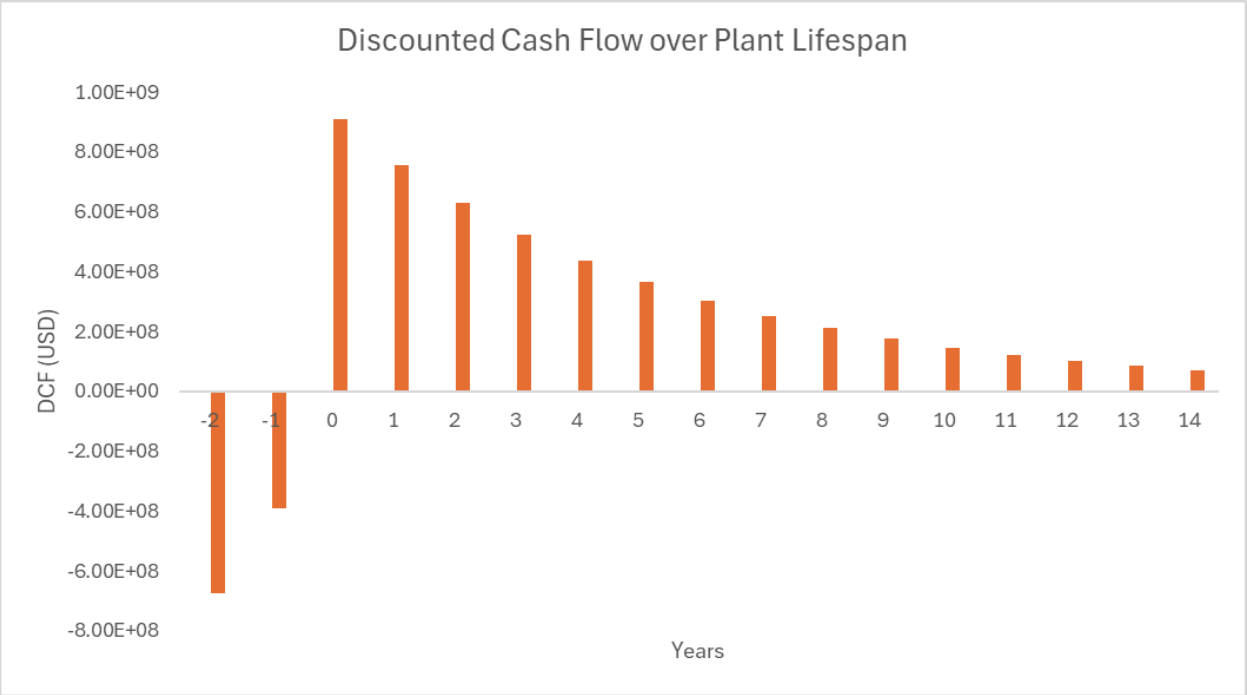
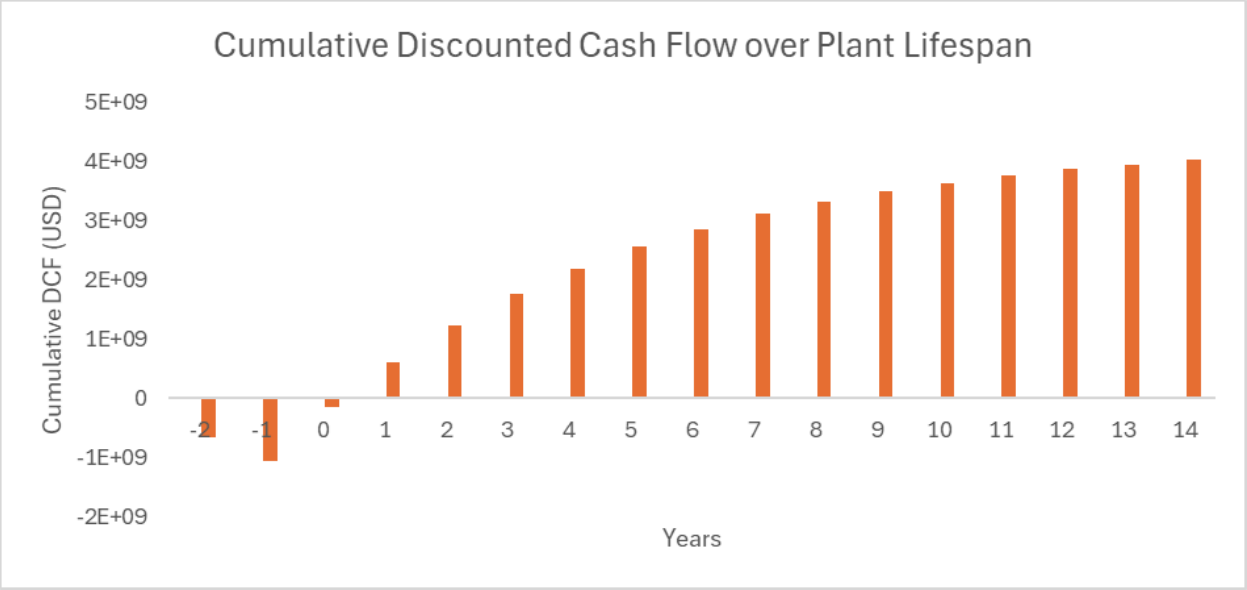


Figure 5.9.b

Cumulative Discounted Cash Flow over Plant Lifespan (Discount Rate = 20%)



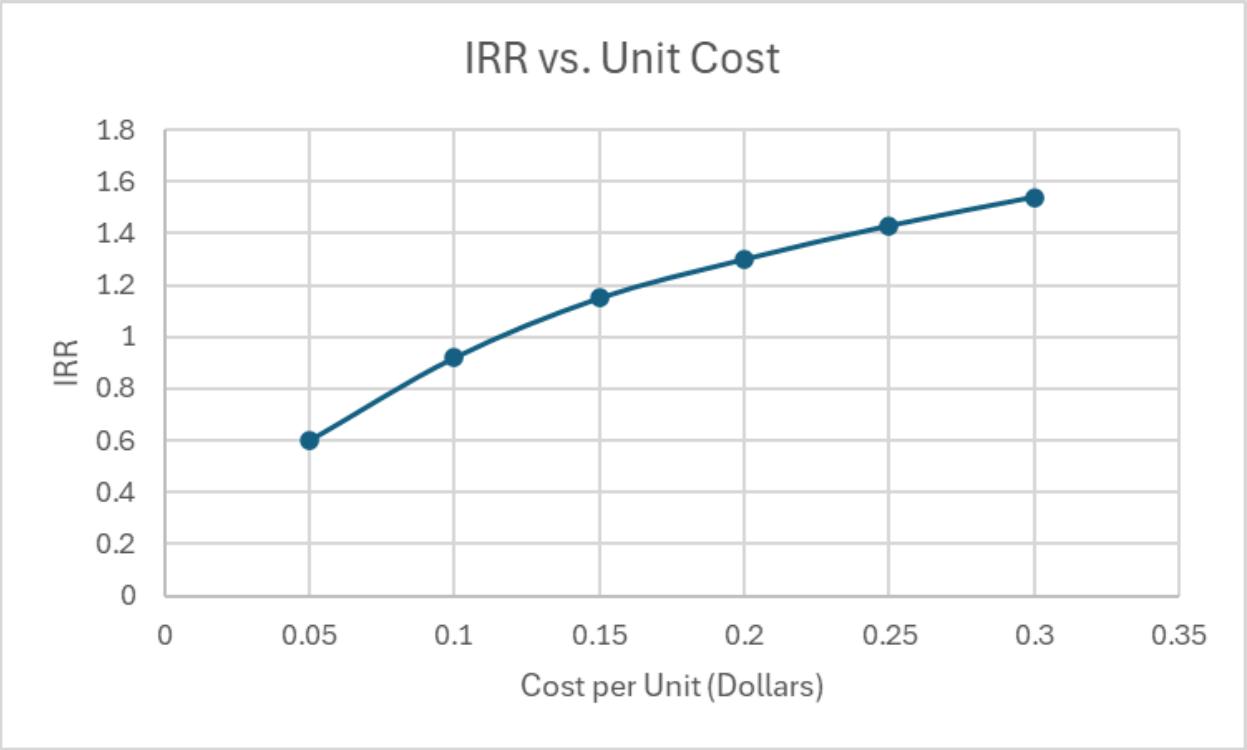
The internal rate of return (IRR) in a discounted cash flow DCF analysis is the discount rate that makes the net present value (NPV) of future cash flows equal to zero. In other words, it is the rate of return at which the present value of cash inflows equals the present value of cash outflows. Our IRR is only about 60% - this is on the low end relative to what a typical pharmaceutical manufacturing plant might hope to achieve. This is due to our extremely low unit cost in order to ensure the target market is able to purchase their medicine.

iv. Risk Analysis

The economics reported for this plan are based on estimates and general heuristics provided to estimate some of these costs. In reality, the cost of production and the potential profit from the product could vary wildly depending on a variety of factors. Figure 5.9.c shows how the IRR would change with the cost of our insulin units. At a price point competitive but relatively close to our competitors, around $0.2-0.3 per unit, our IRR increases by almost 100% relative to our IRR with a $0.05 per unit cost. While this may seem like it would deter investors from supporting this site, our goal is to provide medicine to people who have historically had difficulty in accessing it. Our current models still show the capability to profit, and our efforts investment-wise would be focused on targeting foundations and organizations that support these projects such as the Bill and Melinda Gates foundation.

Figure 5.9.c

Internal Rate of Return vs. Unit Cost of Insulin Glargine



Figures 5.9.d and Figure 5.9.e show the discounted cash flow and cumulative discounted cash flow if instead a 40% discount rate was used. This is on the higher end of the discount rates to estimate a pharmaceutical business model, specifically representing a project at the early stages of development (Stasior et al., 2018). The net present value of the site would still be approximately $800M. The lifespan of the plant in terms of profitability would decrease, as seen in the dropoff of net present value at a 40% discount rate, but could still absolutely be a profitable endeavor depending on the goals and objectives of our shareholders.

Figure 5.9.d

Discounted Cash Flow over Plant Lifespan (Discount Rate = 40%)

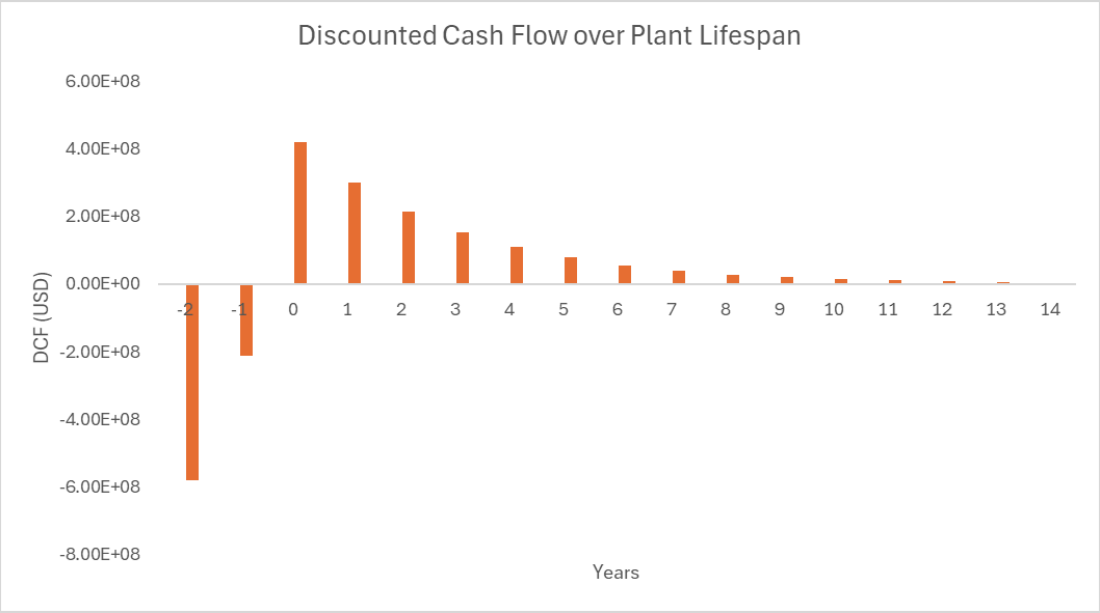
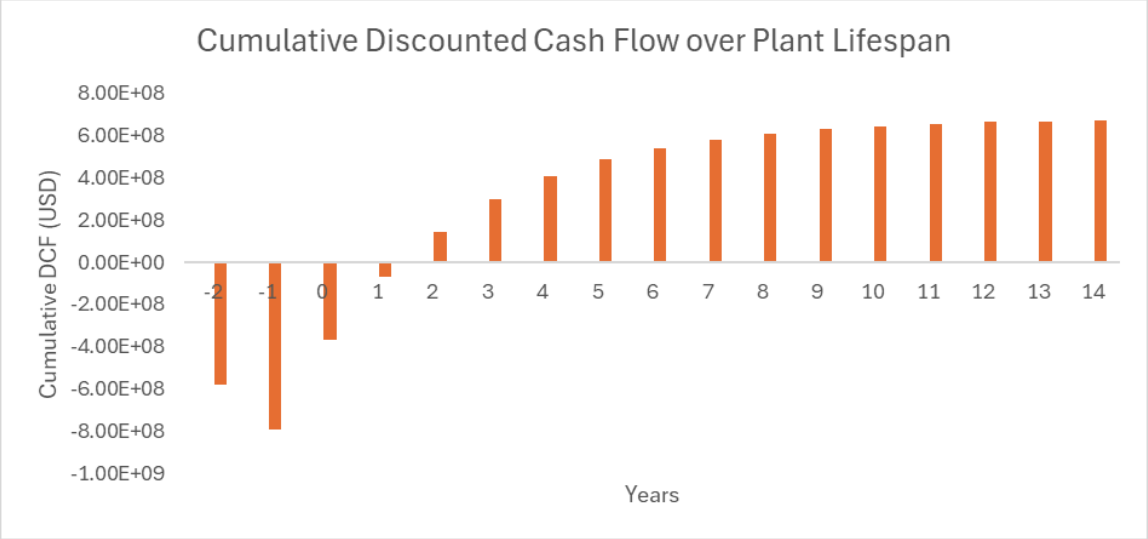


Figure 5.9.e

Cumulative Discounted Cash Flow over Plant Lifespan (Discount Rate = 40%)



Two significant economic risks for establishing an insulin manufacturing plant in Ethiopia are energy accessibility and political instability. Energy accessibility poses a challenge due to the country's intermittent power supply and limited access to reliable utilities, which could disrupt manufacturing operations and increase operational costs (U.S. Department of Commerce,

2024). Political instability, including civil unrest or changes in government policies, may lead to regulatory uncertainty, delays in approvals, and potential supply chain disruptions. Mitigating these risks will require investing in alternative energy sources, such as renewable energy systems, to ensure uninterrupted operations. Due to our proximity to the coast, the utilization of wind turbines or even solar energy could provide security in terms of access to consistent sources of energy that we would need to operate our plant. Additionally, engaging with local stakeholders, monitoring political developments closely, and maintaining contingency plans will be essential to navigate the complexities of the political landscape and ensure the plant's resilience in the face of potential instability.

X. Safety, Health, Environmental, Social and Ethical Considerations

i. Safety and Health Considerations

Our first priority is the safety of the people involved with the manufacturing process and who will receive our product. There will be many people employed at our facility, including operators, technicians, engineers, administration, human resources, maintenance, and support staff. The biggest safety concern is the chemicals and equipment that are involved in the insulin glargine manufacturing process. The hazardous chemicals in our process include acetic acid, sulfuric acid, urea, acetonitrile, sodium hydroxide, citraconic anhydride, and hydrochloric acid. The hazards associated with these chemicals include toxicity, flammability and corrosivity. It is important that these chemicals are handled and stored properly to avoid these potential hazards and mitigate any associated risks. Procedures listed by each chemical's Safety Data Sheet (SDS) should be followed and the proper personal protective equipment (PPE) should be used when handling these chemicals. Additionally, protective measures should be followed when handling the equipment in the production line. Risks associated with the manufacturing equipment include but are not limited to, high pressures, high temperatures, elevated surfaces, and rotating equipment. Safety measures such as relief valves, alarm systems, safeguards, cooling jackets, Lock Out/Tag Out procedures, and warning labels have been put into place to protect our employees and improve our process. Finally, proper education and training will be provided and instilled in each person involved with the manufacturing process to reduce risk and ensure confidence and safety.

Moreover, the patients who receive our insulin glargine process are also an important safety consideration. Protective measures such as sterilization, filtration, and chromatography are included in the production process to ensure the purity of our product. Our starting materials are sustainable sourced and we implemented WFI for a safe injectable. Additionally, cleaning and sanitizing procedures are consistently followed as every batch goes through our process. Finally,

our site will follow the proper regulations set by the Food and Drug Administration (FDA) and the South African Health Products Regulatory Authority (SAHPRA) to comply with good manufacturing practices. Regular safety inspections and purity testing will also be conducted to maintain our product's integrity.

ii. Environmental Concerns

The environmental concerns involved in our production process include the potential release of the materials used in the manufacturing process, as well as the overall generated waste. There is a potential that some waste streams might include active *E. coli* cells and could fail to be sterilized. Other waste streams contain hazardous chemical waste, such as urea, that include further protocols for disposal. If either of these materials were to enter the surrounding environment, they could potentially cause environmental issues and contamination. To mitigate these risks, our waste streams are sterilized and sent to the respective African government authorities for proper disposal and incineration. Additionally, extensive education and training for our employees, as well as regular equipment maintenance, is provided to mitigate the risks associated with material leakage or release. Both waste treatment and safety equipment expenses are considered in the economic analysis and operating cost.

iii. Social Ethical Considerations

The social and ethical concerns with this project include building an American company's manufacturing plant in a different country, the indigenous peoples' perspective of diabetes medication, documentation and statistics, as well as working with the local government and distributing fair wages and support to our employees.

We are building our manufacturing plant in Ethiopia, which is home to the second largest population in Africa, and has a growing economy and workforce. There are several other foreign companies that have manufacturing plants in this region, including H&M and Primark. Although there is a high demand for work, it is important to follow the customs and regulations that are

established in this country to ensure a successful working environment for both American and Ethiopian people. Additionally, the goal of this project is to supply affordable and accessible insulin glargine to the people of sub-Saharan Africa. The price set for our insulin is significantly lower than that of our competitors, and should be manageable for the people living in impoverished regions. Ethiopia's central location and interconnectedness also provides adequate accessibility via roadways and waterways.

Another consideration is the indigenous peoples' perspective of diabetes medications and management. Insulin glargine is one of the higher-priced medications, and has to compete with other needed medicines such as anti-retroviral drugs, tuberculosis treatment, and malaria prevention programs (Gill et al., 2009). Additionally, traditional healers are an essential part of the African healthcare system. Cooperation and introduction of the insulin glargine would have to be integrated into their healing practices in order for our drug to be accepted. Proper education about the efficacy of the drug, the African diabetes epidemic, as well as the economic opportunity of this manufacturing plant is also needed to gain trust in the area and in our product. Additionally, there are difficulties with safe and sanitary distribution of our product, including safe injection processes. Proper handling and injection training is vital to ensure the safety of our patients.

Documentation and statistics are also important to consider. Since there is a lack of documentation about patients in Africa, it could be difficult to keep track of our distribution and who our product is helping. Therefore, it is important to keep detailed records of our manufacturing process, the personnel involved, as well as patient care and distribution so that we can show the impact of our manufacturing site and protect the people who are using our product.

Finally, it is important to support our employees with fair wages, good working conditions, and comprehensive training about our materials, processes, and product. We will pay our employees the standard Ethiopian wages and benefits. Our plant will be designed to

accommodate all people regardless of disability or traveling restrictions, and all of our employees will undergo extensive safety, ethical, and diversity training to ensure that our people are safe and supported.

6. Conclusion and Final Recommendations

The purpose of this project is to design an insulin glargine manufacturing process in sub-Saharan Africa so it is accessible and affordable for the people living there. Our goal is to supply insulin to 6 million people, which is 25% of the diabetic population in Africa. This report includes a detailed upstream and downstream process that will produce $2.79 * 10^6$ tonnes of insulin glargine per year, thus meeting our target and supplying life-sustaining and affordable medication for the people of Africa.

To reach our goal, 272 batches would need to be completed every year with each batch producing 10.24 kg of insulin glargine. The upstream process is estimated to take 43.7 hours or 1.8 days, while the downstream process is estimated to take 346.5 hours or 14.4 days. With this process time, it would not be possible to have 272 batches made in a year, especially with planned maintenance, projected equipment deep-cleaning or replacement, and company holidays and shutdowns. Therefore, we decided to have additional incubators and lyophilizers, which were the rate limiting steps in our production schedule, in order to meet our goal in a timely manner thus rendering our project successful. After considering capital costs, operating costs, and selling each unit of our final product for $0.05, the plant will produce $3.8 billion in annual revenue, with an IRR of 60% over 15-20 years of operation. Therefore, our project is deemed economically feasible and favorable.

Since this project was designed and completed in less than a year, some technical and logical assumptions were made to streamline the process. If further research were to be done on this project, there are a few recommendations that could be made to increase the accuracy of the design and results. Our first recommendation would be to have extensive research done on the infrastructure present in Ethiopia. Our inspiration for choosing Ethiopia for our manufacturing site was to promote and boost the economy of a country that was outside of South Africa's already established infrastructure and economy. By introducing a new company into Ethiopia, we

would be able to create jobs, fuel the economy, support our employees and the people living there, and increase the capital value of the area. However, there are safety concerns such as political unrest that are presently in that country that could potentially affect production and distribution efforts. Since our product is used to sustain life, there is little room for disruption or failure to complete orders. More research could be conducted on the present economic and political situations in African countries to determine the best area for a manufacturing site.

Another recommendation would be to investigate the tradeoffs between using a CIP/SIP system versus single-use equipment. Initially, we decided to install a CIP/SIP system due to the volume of solution that would be processed throughout our system; however, single-use equipment provides many benefits such as sterility, less cleaning materials needed, and a shorter process time. Analysis of the waste products of CIP/SIP versus the single-use plastic would also need to be conducted to determine which system is better for the environment, as well as waste treatment or recycling costs.

Other assumptions were made surrounding the operating costs and equipment costs. Many of the raw materials were priced based on small amounts, since the bulk prices of these materials were not easily found online. Even with these higher priced items, our process is still profitable. However, we assume that there would be a discounted bulk price for some of the materials we used in the process. Additionally, some of the equipment was priced and found on Alibaba, a site known for selling almost anything for a much lower cost than its competitors, and the quality and dependability of that equipment could potentially be deemed as questionable. Therefore, we recommend further research be done on large-scale industrial equipment listed in the sections above to get quotes from credible suppliers and comparable and competitive prices.

Our last recommendation would be to further investigate the cost-benefit analysis of our formulation process. Our project focused on formulating our product into 10 mL vials that would be sold to a supplier or third-party vendor. Further research could be conducted to determine if

manufacturing single-use prefilled syringes would be economically favorable, rather than 10 mL vials without syringes. Additionally, for our patients and buyers, the cost of purchasing our product versus buying a prefilled syringe could be compared to determine which option is cheaper.

7. Acknowledgements

We would like to thank Professor Eric Anderson for his support, guidance, and assistance throughout this project. We would also like to thank the professors from the University of Virginia Chemical Engineering Department, notably Professor George Prpich and Professor Nicholas Vecchiarello.

8. References

Addis Abba. (2023, June 28). Ethiopia: Skyrocketing land prices soar to unprecedented heights, leaving homebuyers, investors astounded. allAfrica.com. https://allafrica.com/stories/202306270286.html

Admin. (2022, October 2). How much CIP detergent needed?. *TechNotes*. https://technotes.alconox.com/detergents/solujet/how-much-cip-detergent-needed/#:~:text=A%3A%20A%20good%20rule%20of,In%2DPlace%20(CIP).

Alfa Laval (1998) FOPX-610 Separator Manual. Alfa Laval Separations. http://www.fdm.com.pe/wp-content/uploads/2018/11/ALFA-LAVAL-MANUAL-FOPX-610.pdf

Anderson, K., & von Meyenburg, K. (1980). Are growth rates of Escherichia coli in batch cultures limited by respiration? *Journal of Bacteriology*, 144(1), 114-123.

Beran, D., Ewen, M., & Laing, R. (2016). Constraints and challenges in access to insulin: a global perspective. The lancet. *Diabetes & endocrinology*, 4(3), 275–285. https://doi.org/10.1016/S2213-8587(15)00521-5

C8 magnetic beads. Bioclone. (2009). http://www.bioclone.us/C8-Reversed-phased-magnetic-beads-particle-resin-matrix.html

CIP and Sanitation of Process Plant. SPX Flow. (2011). https://www.spxflow.com/assets/pdf/apv-cip-systems-22003-05-02-2013-gb.pdf

Cullinan, K. (2023, September 29). New insulin production deal is a boost for Africa's diabetes challenge. *Health Policy Watch*.

https://healthpolicy-watch.news/new-insulin-production-deal-is-a-boost-for-africas-diabetes-challenge/#:~:text=A%20new%20deal%20between%20Danish,production%20within%20just%20one%20year.

Cunningham AM, Freeman AM. Glargine Insulin. [Updated 2022 Dec 28]. In: StatPearls [Internet]. *Treasure Island (FL): StatPearls Publishing*; 2023 Jan-. Available from: https://www.ncbi.nlm.nih.gov/books/NBK557756/

Cytiva. (2023). MaxCell ultrafiltration hollow fiber tangential flow cartridges. https://www.cytivalifesciences.com/en/us/shop/bioprocessing-filtration/tangential-flow-filtration/hollow-fiber-cartridges/maxcell-ultrafiltration-hollow-fiber-tangential-flow-cartridges-p-00501

Davis, R. (2010). Design and scale-up of production scale stirred tank ... Design and Scale-Up of Production Scale Stirred Tank Fermentors. https://digitalcommons.usu.edu/cgi/viewcontent.cgi?article=1533&context=etd

Ebner, J., Sedlmayr, V., & Klausser, R. (2023). High Pressure Homogenization for Inclusion Body Isolation. In J. Kopp & O. Spadiut (Eds.), Inclusion Bodies (pp. 9-22). *Methods in Molecular Biology*, vol. 2617. Humana: New York, NY. https://doi.org/10.1007/978-1-0716-2930-7_9

Eggenreich, B., Wurm, D. J., Rajamanickam, V., Klausser, R., Slouka, C., & Spadiut, O. (2020). High pressure homogenization is a key unit operation in inclusion body processing. *Journal of Biotechnology*, 324(Supplement), 100022. https://doi.org/10.1016/j.btecx.2020.100022

Energy Justice Network. (2020). Trash Incineration is the Most Expensive Way to Manage Waste. https://www.energyjustice.net/incineration/expensive-waste

General purpose freeze dryers - drying bulk material. Vikumer Freeze Dry. (2023, December 13). https://vikumer.com/multi-purpose-tray-freeze-dryers/

Gill, G. V., Mbanya, J. C., Ramaiya, K. L., & et al. (2009). A sub-Saharan African perspective of diabetes. *Diabetologia*, 52, 8–16. https://doi.org/10.1007/s00125-008-1167-9

Glacier Tanks, LLC. CIP Spray Balls | Rotating & Fixed. (n.d.). https://www.glaciertanks.com/tank-systems/tank-accessories/cip-spray-balls.html

Hernández Rodríguez, T., Pörtner, R., & Frahm, B. (2013). Seed train optimization for suspension cell culture. *BMC Proceedings*, 7(Suppl 6), P9. https://doi.org/10.1186/1753-6561-7-S6-P9

Hilgenfeld, R., Seipke, G., Berchtold, H., & Owens, D. R. (2014). The evolution of insulin glargine and its continuing contribution to diabetes care. *Drugs*, 74(8), 911–927. https://doi.org/10.1007/s40265-014-0226-4

Hwang, H. G., Kim, K. J., Lee, S. H., Kim, C. K., Min, C. K., Yun, J. M., Lee, S. U., & Son, Y. J. (2016). Recombinant Glargine Insulin Production Process Using Escherichia coli. Journal of microbiology and biotechnology, 26(10), 1781–1789. https://doi.org/10.4014/jmb.1602.02053

Insulin dosing - straight healthcare. (n.d.). https://www.straighthealthcare.com/insulin-dosing.html

Iudica, B. (2023). Design of an Insulin Glargine Manufacturing Facility in Singapore to Target the Rise of Diabetes Cases in Asian-Pacific Countries; The Failure of the Affordable Insulin Now Act for the Uninsured. Charlottesville, VA: University of Virginia, School of Engineering and Applied Science, BS (Bachelor of Science), 2023. Retrieved from https://doi.org/10.18130/9hkh-7065

Junker, B. H. (2004). Scale-up methodologies for Escherichia coli and yeast fermentation processes. *Journal of Bioscience and Bioengineering*, 97(6), 347-364. https://doi.org/10.1016/S1389-1723(04)70218-2

Kamla, Y., Bouzit, M., Ameur, H., et al. (2017). Effect of the Inclination of Baffles on the Power Consumption and Fluid Flows in a Vessel Stirred by a Rushton Turbine. *Chinese Journal of Mechanical Engineering*, 30, 1008–1016. https://doi.org/10.1007/s10033-017-0158-5

Kim, J., Kwak, S., Park, M. S., Rhee, C. H., Yang, G. H., Lee, J., Son, W. C., & Kang, W. H. (2021). Safety verification for polysorbate 20, pharmaceutical excipient for intramuscular administration, in Sprague-Dawley rats and New Zealand White rabbits. *PloS one*, 16(8), e0256869. https://doi.org/10.1371/journal.pone.0256869

Kluge, J., Muhrer, G., & Mazzotti, M. (2012). High pressure homogenization of pharmaceutical solids. *The Journal of Supercritical Fluids*, 66, 380-388. https://doi.org/10.1016/j.supflu.2012.01.009

Mazur A. Why were "starvation diets" promoted for diabetes in the pre-insulin period? *Nutr J*. 2011 Mar 11;10:23. doi: 10.1186/1475-2891-10-23. PMID: 21396115; PMCID: PMC3062586.

McQueen, M., Li, D. (2023). How Much Does Insulin Cost?. GoodRx Health. https://www.goodrx.com/healthcare-access/research/how-much-does-insulin-cost-compare-brands?label_override=undefined

Mirro, R., & Voll, K. (2009, January 1). Which impeller is right for your cell line? - bioprocess ... Which Impeller is Right for Your Cell Line. https://bioprocessintl.com/analytical/cell-line-development/which-impeller-is-right-for-your-cell-line-183538/

Nienow, A. W. (1997). Mixing: Theory and Practice (Chapter 18, Section 6.6). Butterworth-Heinemann.

Pai, A. (2022, September 26). Understanding CIP & SIP in Pharma - TSA process equipments. TSA Process Equipments - Solving Process and High Purity Challenges for Pharma and Biotech. https://www.tsaprocessequipments.com/understanding-cip-sip-in-pharma/#:~:text=SIP%20is%20referred%20to%20as,disassemble%20and%20re%2Dassemble%20them.

Peer, N., Kengne, A.-P., Motala, A. A., & Mbanya, J. C. (2014). Diabetes in the Africa region: An update. *Diabetes Research and Clinical Practice*, 103(2), 197-205. https://doi.org/10.1016/j.diabres.2013.11.006

Perry, R. H., Green, D. W., & Maloney, J. O. (Eds.). (2008). *Perry's Chemical Engineers' Handbook (9th ed.)*. McGraw-Hill.

Peters, M. S., Timmerhaus, K. D., & West, R. E. (2002). *Plant design and economics for chemical engineers (5th ed.*). McGraw-Hill Professional.

Poccia, M., Beccaria, A., & Dondo, R. (2014). Modeling the microbial growth of two Escherichia coli strains in a multi-substrate environment. *Brazilian Journal of Chemical Engineering*, 31, 347-354. https://doi.org/10.1590/0104-6632.20140312s00002587

Prpich, G. (2023a). Mixing and kLa Prediction [Lecture Notes]. UVA Canvas. https://canvas.its.virginia.edu/courses/59242/pages/scale-up

Prpich, G. (2023b). Aerobic Bioreactor Design Algorithm [Lecture Notes]. UVA Canvas. https://canvas.its.virginia.edu/courses/59242/pages/scale-up

Prpich, G. (2023c). Oxygen Transfer [Lecture Notes]. UVA Canvas. https://canvas.its.virginia.edu/courses/59242/pages/scale-up

Ramanan, R. N., Tey, B. T., Ling, T., & Ariff, A. (2009). Classification of pressure range based on the characterization of Escherichia coli cell disruption in high pressure homogenizer. *American Journal of Biochemistry and Biotechnology*, 5, 21-29. https://doi.org/10.3844/ajbb.2009.21.29

Rushton, J. H. (1950). Power characteristics of mixing impellers part 1. *Chem. Eng. Prog.*, *46*, 395-404.

Schlüter, H. (2000). Chapter 3 - Reversed-Phase Chromatography. In M. Kastner (Ed.), *Journal of Chromatography Library* (Vol. 61, pp. 147-234). Elsevier. https://doi.org/10.1016/S0301-4770(08)60531-X

Shiloach, J., Kaufman, J., Guillard, A. S., & Fass, R. (1996). Effect of glucose supply strategy on acetate accumulation, growth, and recombinant protein production by Escherichia coli BL21 (λDE3) and Escherichia coli JM109. *Biotechnology and bioengineering*, 49(4), 421-428.

Shuler, M. L., & Kargi, F. (2002). Bioprocess engineering: Basic concepts (2nd ed). Prentice Hall.

Shuler, M., & Kargi, F. (2018). Bioprocess Engineering: Basic concepts. Prentice Hall PTR

Siew, Y. Y., & Zhang, W. (2021). Downstream processing of recombinant human insulin and its analogues production from E. coli inclusion bodies. *Bioresources and bioprocessing*, 8(1), 65. https://doi.org/10.1186/s40643-021-00419-w

Sigma Aldrich. (2020). Steam-in-place-sterilization-filtration-trains- ... https://www.sigmaaldrich.com/deepweb/assets/sigmaaldrich/marketing/global/documents/166/381/steam-in-place-sterilization-filtration-trains-tn5022en-ms.pdf

Sterilizers (industrial range). Actini Group. (2024, January 18). https://www.actini.com/products/sterilisateur-de-medias/

Towler, G., & Sinnot R.K. (2008). Principles, Practice and Economics of Plant and Process Design. Butterworth-Heinemann.

UHPH - production homogenizers - features and benefits. High Pressure Production Homogenizers - UHPH. (n.d.). https://www.homogenisingsystems.com/production_high_pressure_homogenizers.html

U.S. Department of Commerce. (2024). Ethiopia - Energy. International Trade Administration | Trade.gov. https://www.trade.gov/country-commercial-guides/ethiopia-energy

Walker, G. M. (2014). *Fermentation (industrial): Media for industrial fermentations*. In C. A. Batt & M. L. Tortorello (Eds.), Encyclopedia of Food Microbiology (Second Edition) (pp. 769-777). Academic Press. https://doi.org/10.1016/B978-0-12-384730-0.00107-5

Weincek, M. (2006). Biotech CIP Cycle Development: Case Study examples ... CIP Cycle Development. https://ispe.org/sites/default/files/attachments/public/Sept-Oct-2006.pdf

World Health Organization. (2022, November 14). African region tops world in undiagnosed diabetes: Who analysis. African region tops world in undiagnosed diabetes: WHO analysis. https://www.afro.who.int/news/african-region-tops-world-undiagnosed-diabetes-who-analysis

Worldwide Tax Summary Online. (2023). Ethiopia - Corporate Taxes. PricewaterhouseCoopers. https://taxsummaries.pwc.com/ethiopia/corporate/taxes-on-corporate-income#:~:text=Corporate%20income%20tax%20(CIT)%20is,income%20reduced%20by%20allowable%20expenses.

Yadav, K. et al. (2019, Dec 11). High Pressure Homogenizer in Pharmaceuticals: Understanding Its Critical Processing Parameters and Applications. *Journal of Pharmaceutical Innovation*. doi: 10.1007/s12247-019-09413-4 [Original source: https://studycrumb.com/alphabetizer]