Design of an Insulin Glargine Manufacturing Facility in Singapore to Target the Rise of Diabetes Cases in Asian-Pacific Countries

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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

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1. Summary

This project aims to produce insulin glargine efficiently and affordably for the Asia-Pacific region. The upstream and downstream processes have been designed with an overall protein yield of 48.96%. One batch from a 20,000L fermenter will produce 35.54 kg of insulin glargine. Thus, to reach our target of 4%, or 9 million people, of the diabetic population in the Asia-Pacific region, 764 batches per year must be produced. We begin and finish a batch every 18 hours. With one full upstream and downstream system, we can achieve 453 batches per year. Thus, we will have two full upstream and downstream systems to achieve 906 batches per year. We will have 142 batches in excess to account for errors and shutdowns. Each batch will bring in about $6.3 million in revenue for a total annual profit of $2.5 billion. Our process has been determined to be economically viable with an internal rate of return of 242%.

2. Introduction

The prevalence of diabetes in Asia is rising; 60% of diabetes cases are in Asia with the majority of these cases in India and China (Ramachandran et al., 2012). Thus, it is appropriate to produce insulin for the Asian market, specifically developing nations where more than 80% of the world cases of type 2 diabetes occur (Ramchandran et al., 2012). Further, insulin prices are high, and it can be difficult for patients in developing countries to manage the high out-of-pocket costs, as diabetics in lower economic groups spend 25-34% of their income on treatment (Ramachandran et al., 2012). High prices are attributed to a variety of factors including a vulnerable population willing to pay thousands of dollars for a lifesaving drug, only a select few companies producing insulin, and patent abuse through evergreening (Rajkumar, 2020). To target this market and reduce distribution difficulties to developing countries, an insulin manufacturing plant will be built in Singapore to serve the developing nations in the surrounding area.

Our insulin glargine product is slow-release; produced via recombinant DNA technology using a strain of *Escherichia coli* (DrugBank, 2022). Insulin is rendered long-acting by replacing asparagine with glycine in position 21 of the A-chain and by carboxy-terminal extension of the B-chain by 2 arginine residues (Bolli, 1999). The arginine amino acids shift the isoelectric point from 5.4 to 6.7, making the molecule less soluble in physiological blood; this allows the product to crystallize before dissolving, rendering it “slow-release”.

The target market for our drug is the type 2 diabetic population in Asia. 227 million people in the Asia-Pacific region live with type 2 diabetes (World Health Organization, 2020). We will attempt to provide insulin glargine to 4% of this population, or 9 million people. This target was chosen over the course of the project as the capacity of a reasonably sized facility was determined. To achieve more than 4% of the market, more than two full upstream and

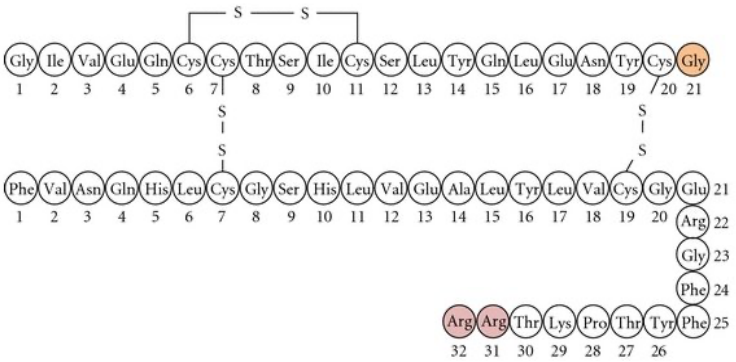
downstream systems of our determined scale would be necessary. Thus, we determined 4% of the target market to be the limit of the project, as we are a new manufacturer entering a competitive market.

To determine the amount of insulin glargine we will need to produce to support 9 million people, we assumed the average patient weighs 75 kg and uses 3 units of insulin glargine per kg body weight every day (Zinman et al., 2011). With this assumption, we will need to produce $7.46*10^{11}$ units/year which is equivalent to 27 tonne/year (3.64 mg for 100 units).

The process for producing insulin glargine is similar to other recombinant proteins. The protein is produced in a culture of *E. coli*. Various downstream steps such as centrifugation, high-pressure homogenization, ultrafiltration, diafiltration, and chromatography are used to separate and purify the protein. The process differs from other protein production processes, as it involves two incubation steps that change the precursor to active insulin glargine. The first incubation refolds the protein to ensure the three disulfide bonds of insulin glargine are produced. Figure 2.1.a shows the amino acid sequence of insulin glargine and the disulfide bonds produced during the refolding step.

Figure 2.1.a

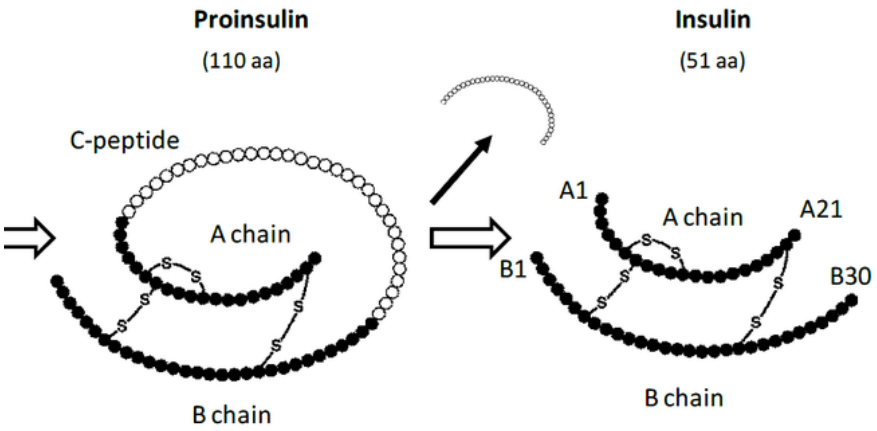
Insulin Glargine Amino Acid Sequence (Uehata et al., 2011)



The second incubation converts the insulin glargine precursor to active insulin glargine. This process is more complex than the refolding step, as the amino acid sequence needs to be cleaved in specific places to achieve the active form of insulin glargine. The first step of the process is citraconylation in which citraconic anhydride is used to acylate primary amino groups and free lysine residues to protect them from cleavage (Hwang et al., 2016). The amino acid sequence can then be cleaved with trypsin to produce the A chain and B chain of insulin glargine. The conversion of the amino acid sequence can be seen in Figure 2.1.b. Note the amino acid in the figure is insulin and not insulin glargine. The conversion process is the same for both proteins, so the figure was deemed acceptable in this instance. The only difference shown in the figure is insulin glargine has 32 amino acids in the B chain instead of 30. The last step in the conversion process is to deacylate the protein with glacial acetic acid to return the modified amino groups and lysine residues to their native state.

Figure 2.1.b

Conversion into Active Insulin Glargine (Khudhair, 2019)



3. Previous Work

Two major reference sources were used in this design project. The first source is a lab-scale study on the process of producing insulin glargine by Hwang et al. in 2016. We modified and scaled up the procedure used in this study to produce our insulin glargine product. A second source of previous work is the Wilson et al. capstone project conducted at UVA in 2015. In this project, students designed a process for producing insulin glargine using yeast. We have used this project as a reference for a scaled-up insulin glargine manufacturing process. Last semester, we conducted preliminary research on our desired product, an analysis of the desired scale of our process, and a rough economic appraisal of our production process. This research was used as a starting point for the design work in this report.

4. Discussion

I. Process Flow Diagram

The process of producing insulin glargine contains an array of different steps and equipment. In Figure 4.1.a, the process flow diagram provides an overview of all equipment and streams relevant to the process. The equipment and streams are labeled with the appropriate tags as described in Table 4.1.a. In the process flow diagram, the upstream process consists of the seed train and fermentation (F-101 to F-105), and the downstream process begins with P-105 and continues until the desired product is lyophilized in LY-101.

Figure 4.1.a

Process Flow Diagram

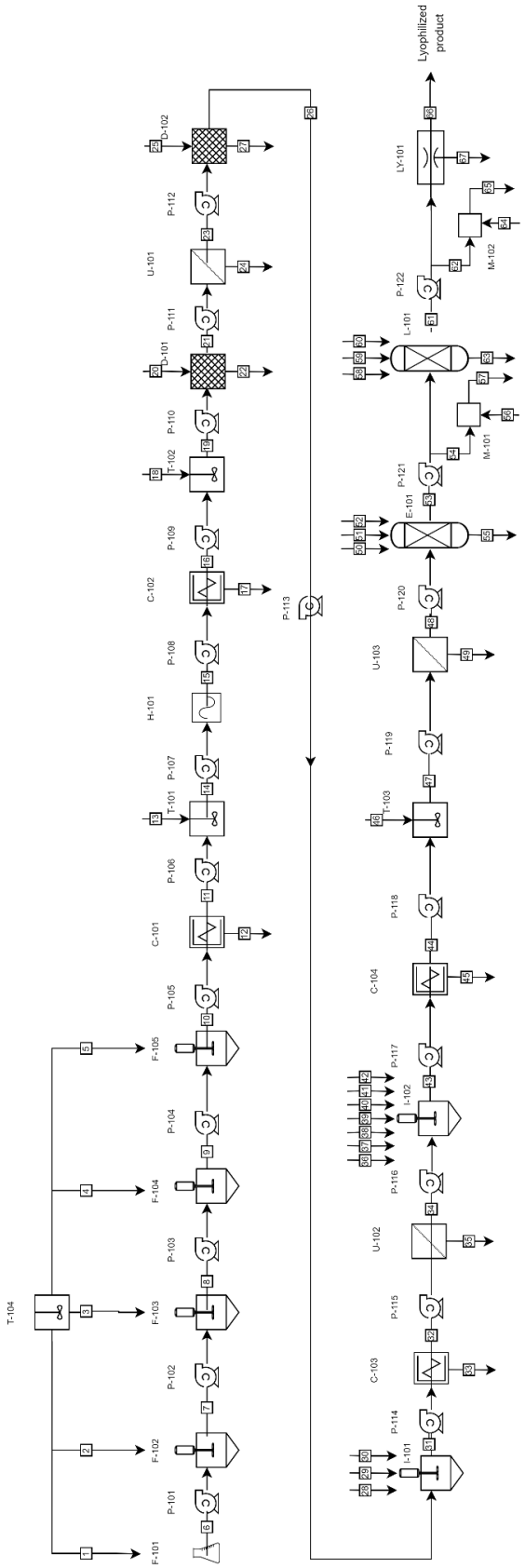


Table 4.1.a

Equipment Tags

Equipment Tag	Description
F-101	2L Shake Flask
F-102	20L Fermenter
F-103	200L Fermenter
F-104	2,000L Fermenter
F-105	20,000L Fermenter
P-101-122	Pumps
C-101	Centrifuge
T-101-104	Tanks
H-101	High-Pressure Homogenizer
C-102	Centrifuge
D-101	Diafiltration
U-101	Ultrafiltration
D-102	Diafiltration
I-101	Incubator
C-103	Centrifuge
U-102	Ultrafiltration
I-102	Incubator
C-104	Centrifuge
U-103	Ultrafiltration
E-101	Cation Exchange Chromatography
M-101-102	HPLC
L-101	Prep-HPLC
LY-101	Lyophilizer

II. Material Balance

To produce the material balance, we used our desired yearly production of 27 tonnes/year to achieve our goal of reaching 4% of the target market and worked backward to find the protein yield of each step in the process using the percent recovery of each step. The percent recoveries were determined based off of literature values and work of previous projects. The equipment design, explained in further sections, is based on these recoveries. The protein yield of each unit is described in Table 4.2.a. After we calculated the insulin glargine yield, the amount of each supplemental material was determined using concentrations found in Hwang et al. (2016).

After the insulin glargine is produced in the upstream process, the downstream units separate and purify the protein. The first unit of the downstream process is centrifugation (C-101) to remove the media from the cells. We made the assumption that after centrifugation the solid stream exiting the centrifuge would have 50% w/w solids. Our centrifuge has a recovery of 99%, so some protein was lost in the waste stream of the centrifuge.

A mixing tank (T-101) is used to resuspend the cells in a buffer (sucrose, Tris, EDTA, sodium chloride, and water for injection, WFI). The buffer concentrations were found in Hwang et al. (2016). The cells are then disrupted using a high-pressure homogenizer (H-101) which has a recovery of 96.1%, as not all of the cells are properly disrupted. A collection of cell debris and protein in the buffer leaves H-101 to be centrifuged to collect the protein and remove the buffer. The same assumptions were used for all centrifugation steps, so the material streams around C-102 were calculated the same way as C-101.

A second tank was used to resuspend the cell debris and protein in a buffer solution (Tris, EDTA, lysozyme, triton, urea, and WFI). Again, the concentration of the buffer solutions was found in Hwang et al. (2016). A buffer exchange takes place in a diafiltration unit (D-101) to

concentrate the cells and protein in WFI. We made the assumption that after 6 control volumes of buffer were added to the diafiltration, 99.5% of the original buffer was exchanged with the new buffer (Schwartz, 2003). The remaining buffer was deemed negligible. The solution then goes through an ultrafiltration (U-101) unit to remove the cell debris from the protein in the solution. We assumed all cell debris was removed from the solution. A second diafiltration (D-102) unit is used to exchange the buffer again to a buffer made up of urea, glycine, and WFI. The calculations were conducted the same way as for D-101.

The protein is then refolded in an incubator (I-101) with a buffer (urea, glycine, and WFI), media (β-mercaptoethanol), and a pH adjuster (HCl) added to the solution. The concentrations of the inputs were found in Hwang et al. (2016), but the volumes of the pH adjusters needed to be determined using the following Equation 4.2.a:

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

Only 82.1% of the protein refolds, so the output of the incubator includes both unfolded and refolded protein (Hwang et al., 2016). The next steps, centrifugation (C-103) and ultrafiltration (U-102) were calculated the same way as the previous centrifugation and ultrafiltration steps.

A second incubation (I-102) is used to convert the protein into active insulin glargine. Media (trypsin), buffer (sodium tetraborate, boric acid, and WFI), and multiple pH adjusters (NaOH, citraconic anhydride, HCl, and ZnCl) are added during this incubation. All of the concentrations were found in Hwang et al. (2016). The paper did not mention a specific volume for the buffer, so the volume of the buffer was assumed to be equivalent in volume to other buffer additions in the process (30 ml/g protein). The conversion of the protein into active insulin glargine was assumed to be 100%. A centrifuge (C-104) is used to collect the insulin glargine

after conversion. The calculations were conducted in the same way as the other centrifuges. A final tank (T-103) was used to resolve the insulin glargine into a buffer solution (urea, acetic acid, and WFI) before cation chromatography, and a final ultrafiltration (U-103) system is used to filter any remaining debris from the solution. The cell debris removed from ultrafiltration is assumed to be zero.

The protein solution goes through cation exchange chromatography next to remove any large contaminants such as DNA and other proteins. Various buffers containing urea, acetic acid, sodium chloride, and WFI were used to equilibrate and elute the chromatography column. The concentrations of the solutions were found in Hwang et al. (2016), but the volumes were scaled up according to the size of our column. The volumes of the CEX solutions needed per batch are based on the entire protein amount, but the column will be loaded twice per batch. Thus, only half of the solutions will be run through the column at a time. The eluted protein will be tested with an analytical HPLC unit for purity after both chromatography steps. The volumes and concentrations of solutions needed for HPLC were kept the same as in the Hwang et al. study because it is an analytical unit and does not require scale-up (2016).

After CEX, the protein will enter the Prep-HPLC column. The material balances for this column were calculated in the same way as the CEX column. Here, five columns will be used, and each column will be loaded five times because only 100 L columns are available for Prep-HPLC. The volumes of the equilibration and elution solutions containing acetonitrile, acetic acid, and WFI are for the entire batch of protein, not one cycle of Prep-HPLC. After final purification, the solution will be lyophilized in LY-101. It was assumed that the lyophilizer will remove all liquids and freeze all of the API as the final product.

Table 4.2.a

Insulin Glargine Overall Yield

Equipment Tag	Description	Insulin Yield (kg/year)	Step Yield	Cumulative Yield
F-101	2L Shake Flask	55443.69	100.00%	100.00%
F-102	20L Fermenter	55443.69	100.00%	100.00%
F-103	200L Fermenter	55443.69	100.00%	100.00%
F-104	2,000L Fermenter	55443.69	100.00%	100.00%
F-105	20,000L Fermenter	55443.69	100.00%	100.00%
P-101-122	Pumps	55443.69	100.00%	100.00%
C-101	Centrifuge	54889.25	99.00%	99.00%
T-101-104	Tanks	54889.25	100.00%	99.00%
H-101	High-Pressure Homogenizer	52748.57	96.10%	95.14%
C-102	Centrifuge	52221.09	99.00%	94.19%
D-101	Diafiltration	51176.66	98.00%	92.30%
U-101	Ultrafiltration	47287.24	92.40%	85.29%
D-102	Diafiltration	46341.49	98.00%	83.58%
I-101	Incubator	38046.37	82.10%	68.61%
C-103	Centrifuge	37665.90	99.00%	67.93%
U-102	Ultrafiltration	34803.29	92.40%	62.77%
I-102	Incubator	34803.29	100.00%	62.77%
C-104	Centrifuge	34455.26	99.00%	62.14%
U-103	Ultrafiltration	31836.66	92.40%	57.42%
E-101	Cation Exchange Chromatography	29926.46	94.00%	53.97%
L-101	Prep-HPLC	27143.30	90.70%	48.95%
LY-101	Lyophilizer	27143.30	100.00%	48.95%

III. Cell Growth Model

The upstream processing of this project consists of a seed train of five bioreactors to produce our desired protein. To design the fermentations, a cell growth model is needed to determine the necessary substrate concentration and time to produce our desired cell density for downstream processing. We aim to produce 18 g/L dry cell weight, as this is the cell density used in the insulin glargine production process we are referencing, Hwang et al. (2016).

Microbial growth kinetics were modeled using the Monod model (Equation 4.3.a) in which μ is the specific growth rate, μ_{max} is the maximum specific growth rate, S is the substrate concentration, and K_s is the Monod saturation constant.

$$\mu = \frac{\mu_{max} S}{K_s + S} \tag{4.3.a}$$

The maximum specific growth rate and the Monod saturation constant are constants for the specific cell strain; thus, in this model, the specific growth rate of the system is dependent on the substrate concentration. To determine the constants, μ_{max} and K_s, for our specific cells, we fit data from Shiloach et al. about the cell growth of *E. coli* JM109 cells and substrate requirements to the Monod model (1996). μ_{max} was found to be 0.425 h^{-1}, and K_s was found to be 0 g/L. Other studies in literature found similar results for μ_{max} and K_s (Senn et al., 1994).

After the constants were determined, a cell growth curve was created for each fermentation by solving Equations 4.3.b and 4.3.c simultaneously where $Y_{X/S}$ is the change in cell concentration divided by the change in substrate concentration and X is the cell concentration.

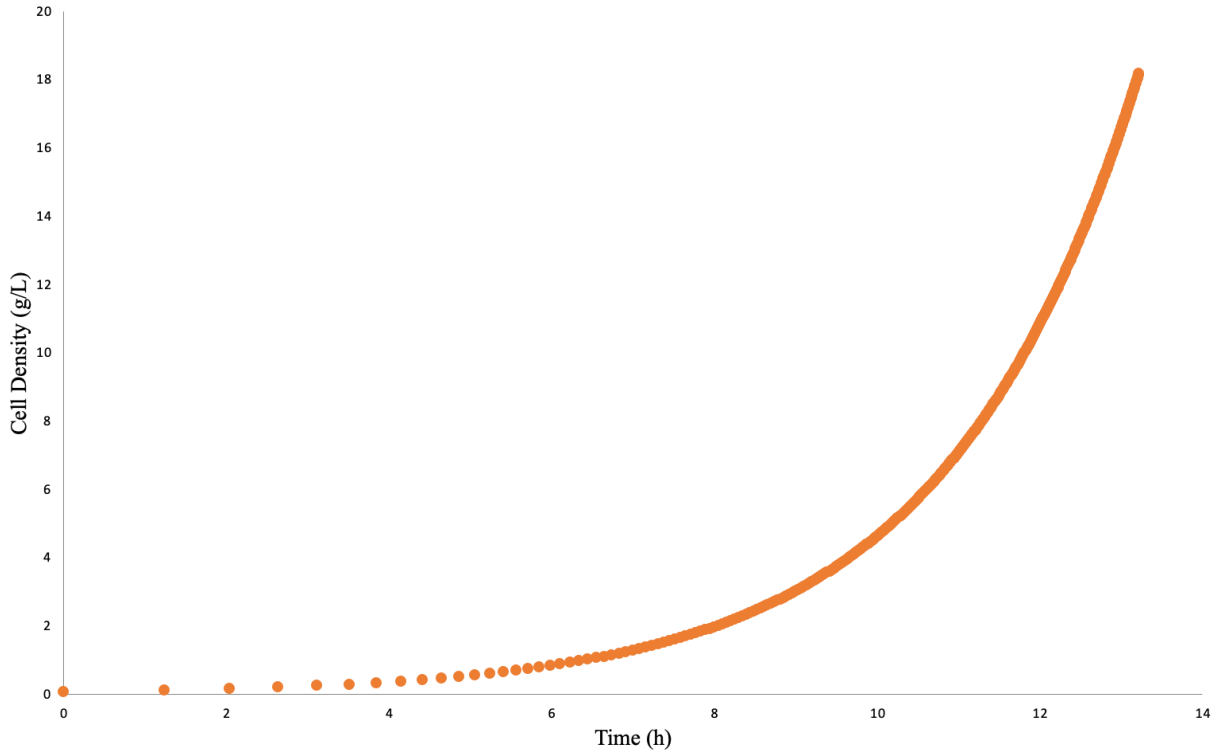
$$\frac{dS}{dt} = -\frac{1}{Y_{X/S}}\frac{dX}{dt} \tag{4.3.b}$$

$$\frac{dX}{dt} = \mu X \qquad (4.3.c)$$

The first four fermentations of the seed train will be run as batch fermentations. We will begin with a 2 L fermentation with a starting cell concentration of 0.066 g/L and a starting substrate concentration of 200 g/L. The following cell growth curve was obtained for the fermentation (Figure 4.3.a).

Figure 4.3.a

Batch Fermentation Cell Growth Curve

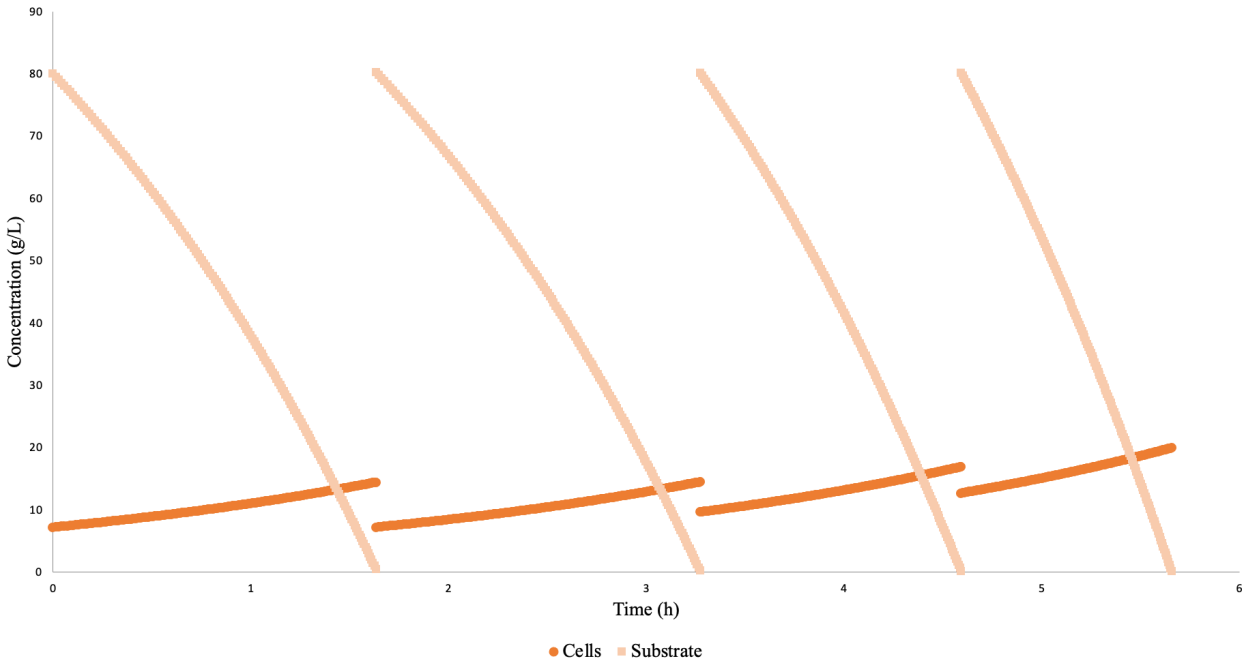


The last fermentation, 20,000 L, will be run as fed-batch. In a fed-batch fermentation, media and substrate are added into the reactor periodically to replace the depleted substrate concentrations. Fed-batch fermentation was chosen, as it can produce a higher cell density than batch fermentation (Mahmoodi & Nassireslami, 2022). We will begin with 5,000 L and feed four

times to a final volume of 20,000 L. Each feed will replenish the glucose concentration. The cell growth and substrate depletion correspond with this process as shown in Figure 4.3.b.

Figure 4.3.b

Fed-batch Model of Biomass and Substrate Concentration



IV. Seed Train and Bioreactor Design

i. Tank Geometry

As mentioned in the cell growth section, the fermentations will be run until the *E. coli* cells reach a concentration of 18 g/L. We assume that 52.4% of the dry cell weight in *E. coli* is protein (Stouthamer, 1973) and 38.52% of the protein is insulin glargine (Hwang, H. et al., 2016). We are also aiming to recover 49% of the insulin glargine we produce. Therefore, to produce 27 tonne of insulin glargine per year, about 15 million L of fermentation solution needs to be produced every year. The size of the final fermentation bioreactor was then chosen to produce a reasonable number of batches while conforming to the limits of *E. coli* fermentation (Xu et al., 1999). A 20,000 L reactor would need to produce 764 batches per year, so we decided to design two 20,000 L reactors to achieve a total of 764 batches per year.

The seed train was then designed according to a scale factor of 10 (Kern, S. et al., 2013). Thus, the seed train consists of four bioreactors (2 L, 20 L, 200 L, 2,000 L) which feed to a final production fermenter of 20,000 L. The volumes listed thus far in the report are the working volumes of the reactors. The working volume was chosen to be 80% of the total volume of the reactor to avoid spillover from agitation and foaming (Eyer, 2016). Once the volume of the reactors was determined, the height and diameter of the reactors were determined using an aspect ratio of 3. The aspect ratio in standard geometry is 1, but a higher aspect ratio is often used in stirred tank reactors for microbiological cultures (Jagani et al., 2010). The geometry of the tanks is shown in Table 4.4.a.

Table 4.4.a

Geometry of Fermenter Tanks

Tank Volume (L)	Working Volume (L)	Height (m)	Diameter (m)	Height of Liquid (m)
25000	20000	6.59	2.20	5.27
2500	2000	3.06	1.02	2.45
250	200	1.42	0.47	1.14
25	20	0.66	0.22	1.14
2.5	2	0.31	0.10	0.24

ii. Agitation Specifications

E. coli is not shear-sensitive (Mirro & Voll, 2009), and Rushton impellers are often used for aerobic fermentations (Jagani et al., 2010). Thus, Rushton impellers were chosen for our process. The impeller diameter was chosen to be half of the tank diameter, and the baffle width was chosen to be 0.1 of the tank diameter (Davis, 2010). Each tank will have four baffles, and Equation 4.4.a was used to determine the number of impellers needed for each tank (Davis, 2010).

$$\frac{H_L - D_i}{D_i} > n > \frac{H_L - 2*D_i}{2*D_i} \quad (4.4.a)$$

The spacing of the impellers will be 1 impeller diameter between impellers, 1 impeller diameter between the bottom of the tank and the bottom impeller, and 1.5 impeller diameters between the top of the liquid and the top impeller (Davis, 2010).

The tip speed will be set to 5 m/s. This speed should not harm the *E. coli* cells (G. Prpich, personal communication, February 15, 2023). The agitation rate, N, was determined using Equation 4.4.b (Prpich, 2021).

$$N = \frac{Tip\ Speed}{\Pi * D_i} \quad (4.4.b)$$

The mixing time was approximated using Equation 4.4.c (Green & Perry, 2008).

$$t_m = 5 * \Pi * D_t^2 * \frac{h}{4} * \frac{1}{0.92 * N * D_i^2 * D_t} \quad (4.4.c)$$

The agitation specifications are shown in Table 4.4.b.

Table 4.4.b

Agitation Specifications

Tank Volume (L)	Number of Impellers	Impeller Diameter (m)	Baffle Width (m)	Tip Speed (m/s)	Agitation Rate (RPM)	Mixing Time (s)
25000	3	1.10	0.22	5	87	35
2500	3	0.51	0.10	5	187	16
250	3	0.24	0.05	5	403	8
25	3	0.11	0.02	5	869	4
2.5	3	0.05	0.01	5	1873	2

iii. Oxygen Requirements

The final consideration for fermenter design is oxygen requirements for aerobic fermentation. *E. coli* cells require oxygen for cell growth; thus, we will need to supply oxygen to our bioreactors during fermentation. *E. coli* typically require 20 mmol O_2/(g cells h) (Varma et al., 1993). The oxygen uptake rate was then determined using Equation 4.4.d where X is the cell concentration in g/L and q_{O_2} is the oxygen required in mol/(g cells h) (Wilson et al., 2015).

$$OUR = X * q_{O_2} \quad (4.4.d)$$

We assume that oxygen transfer is the rate-limiting step in fermentation, so the oxygen transfer rate is equal to the oxygen uptake rate (Wilson et al., 2015). Equation 4.4.e can then be used to determine the necessary k_La of the system.

$$OUR = k_L a\,(C^* - C_L) \quad (4.4.e)$$

We will also assume that the dissolved oxygen concentration in the media, C_L, is negligible compared to the solubility of oxygen, C^* (Wilson et al., 2015). The solubility of oxygen can be calculated using Equation 4.4.f (Wilson et al., 2015).

$$C^* = \frac{P_{tot}}{H} * y_A \quad (4.4.f)$$

We will be using pure oxygen instead of air as our oxygen source to maximize the amount of oxygen in the fermentation while keeping a lower gas flow rate. Thus, y_A will be 1. H is Henry's constant which is 769.23 atmL/mol (Shapley, n.d.). The total pressure of the system is assumed to be 1 atm. Using the above equations, the k_La needed for our system was calculated to be 0.077 s^{-1}.

Once the necessary k_La is calculated, the oxygen flow rate necessary for the system can be calculated using the following steps. First, the Reynolds number needs to be calculated using Equation 4.4.g where ρ is the density of the media and μ is the viscosity of the media. The properties of the media were assumed to be equivalent to the properties of water. It should be noted that all of the Reynolds numbers suggested the tanks were turbulent (Table 4.4.c); thus, the tanks have sufficient mixing.

$$Re = \frac{N*D_i^2*\rho}{\mu} \tag{4.4.g}$$

Using Reynold's number, the power number is determined using the Rushton curve. Ungassed power can then be calculated using Equation 4.4.h.

$$P = N_p \rho N^3 D_i^5 \tag{4.4.h}$$

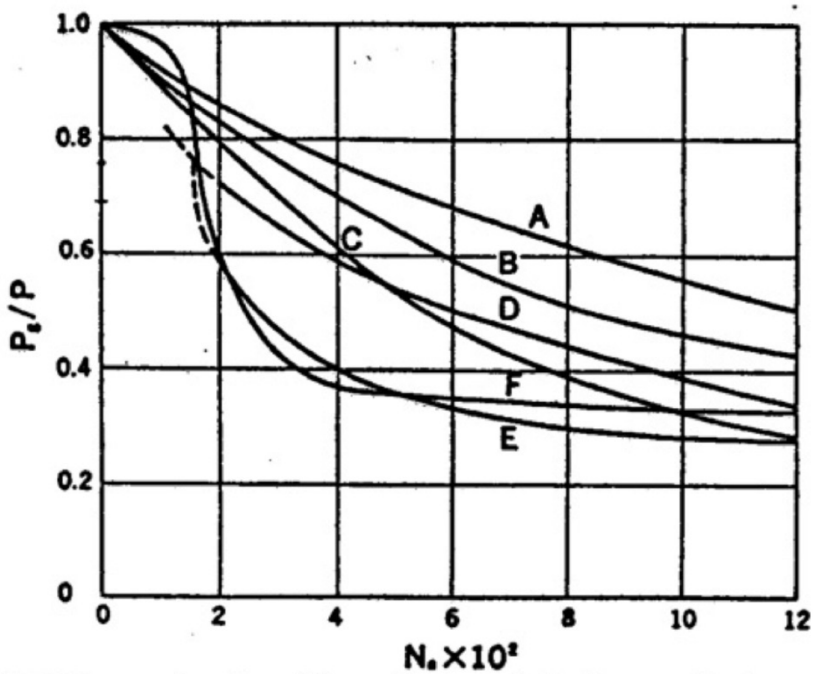
Aeration number is calculated using Equation 4.4.i where Q_g is the oxygen volumetric flow rate. An initial guess of the flow rate is used, and the equations are iterated with different flow rates until the final k_La of the system matches the necessary k_La, 0.077 s^{-1}.

$$N_a = \frac{Q_g}{ND_i^3} \tag{4.4.i}$$

The ratio of gassed to ungassed power, P_g/P, is then determined using the following curve (Figure 4.4.a) (Prpich, 2021). Curve F was used for our system.

Figure 4.4.a

P_g/P vs N_a Curve



The gassed power, P_g, is then calculated using Equation 4.4.j where n_i is the number of impellers and f_c is a correction factor based on tank geometry.

$$P_g = (P_g/P) * n_i * P * f_c \quad (4.4.j)$$

Finally, k_La is calculated using Equation 4.4.k (Prpich, 2021). vs, the superficial gas exit speed, is calculated by dividing the gas volumetric flow rate by the cross-sectional area of the tank (Wilson et al., 2015).

$$k_La = 0.026(P_g/V)^{0.4} vs^{0.5} \quad (4.4.k)$$

The necessary oxygen flow rate will provide a k_La equivalent to the necessary k_La, 0.077 s^{-1}. The oxygen requirements for our fermenters are shown in Table 4.4.c.

Table 4.4.c

Oxygen Requirements

Tank Volume (L)	Re	k_La (s^{-1})	Oxygen Supply (L/min)	Gassed Power (W)
25000	1700000	0.077	4,500	43000
2500	810000	0.077	300	21000
250	370000	0.077	30	4500
25	170000	0.077	4	1060
2.5	81000	0.077	0.4	229

V. Separations Design

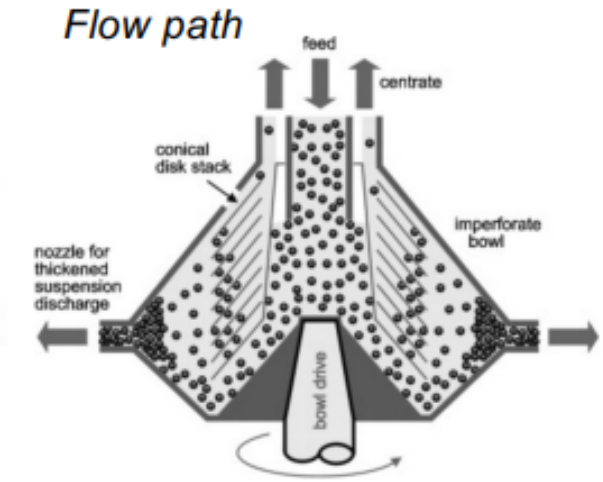
i. Centrifugation

Centrifugation is the process that uses centrifugal force to separate components in a mixture based on their size and density (Ohlendieck, 2010). The centrifugation device rotates around an axis, which generates the centrifugal force. Through rotation, a precipitate is formed from the denser solids in the liquid mixture, which tend to settle on the outer edge of the device. Meanwhile, the less dense liquid is forced toward the axis of rotation, which is called the supernatant.

We use a disk stack centrifuge to achieve continuous separation. Disk stack centrifuges are the most common industrial centrifuges because they can apply high centrifugal forces, which reduces the separation time (Amaro et al., 2017). This type of centrifuge is composed of a bowl surrounding a series of discs (Tarleton et al., 2007). As shown in Figure 4.5.a, the feed flows through the top of the centrifuge and down to the bottom of the bowl.

Figure 4.5.a

Continuous disk stack centrifuge flow path (Carta, 2022).



As the centrifuge rotates about an axis, the less dense liquid moves upward through the series of discs towards the center of rotation, while the denser solids are blocked by the series of discs and moved to the outer wall. The liquid supernatant is removed at the top of the apparatus near the feed input, while the precipitate is removed by an outlet on the outer wall of the bowl. The supernatant can be recycled back into the centrifuge again to achieve further separation depending on the desired recovery of the process.

There are four centrifugation steps throughout our process: after fermentation (C-101), after cell disruption (C-102), after the first incubation (C-103), and after the second incubation (C-104). Each centrifugation step uses the same model of disk stack centrifuge, the Alfa Laval MOPX 213, which can run continuously (Dolphin Centrifuge, 2020). This model has a volumetric flow rate capacity of 20,400 L/hr, a motor power of 16 kW, and a bowl speed of 4,140 rpm (Dolphin Centrifuge, 2020).

The first centrifugation step (C-101) comes after fermentation (F-105), where the fermentation cells used for insulin production are in solution with the LB growth media. The cells, being of greater density than the liquid media, are harvested through the outlet on the outer wall of the bowl, while the media is removed from the top of the apparatus as waste. There is 20,000 L of the mixture of media and cells going into the disk stack centrifuge from fermentation, so based on the specs of the Alfa Laval MOPX 213, the centrifuge can process this mixture in 0.978 hours (58.7 minutes). The step yield for this process is 99%, so 356.07 kg/batch of the harvested cells in solution with a small amount of the media are sent to a mixing tank (T-101) for resuspension. The 1% of the uncollected cells (3.62 kg/batch) in solution with the majority of the media is sent off as waste disposal.

The second centrifugation step (C-102) comes after cell disruption (H-101) using high-pressure homogenization to release the insulin precursor. The solution from this step contains a mixture of insulin glargine protein, cell debris, and Buffer 13. The volume of the mixture going into the centrifuge is 7,480 L, so the centrifuge can process this in 0.366 hours (21.9 minutes). With a 99% step yield, the precipitate, containing the denser insulin glargine protein (68.38 kg/batch) and cell debris is collected and sent to another mixing tank (T-102) for washing. The less-dense supernatant containing Buffer 13 and some cell debris is removed and treated as waste.

The third centrifugation step (C-103) comes after the first incubation step (I-101). The mixture coming out of I-101 is a mixture of insulin glargine protein, misfolded insulin glargine protein, Buffers 25 and 28, Media 29, and Adjustor 30. The volume of this mixture totals 130,000 L, and the centrifuge processes this mixture in 6.37 hours. For this centrifugation with a 99% step yield, the supernatant containing the refolded peptide fusion glargine protein in solution (49.32 kg/batch) with Buffers 25 and 28, Media 29, and Adjustor 30, is collected at the top of the bowl. The supernatant mixture is sent to an ultrafiltration step (U-102) to further separate the glargine protein. The misfolded protein precipitates out with a pH adjustment during the incubation, so the misfolded protein is discarded from the outer wall of the centrifuge and labeled as waste.

The fourth and final centrifugation step (C-104) comes after the second incubation step (I-102). The mixture coming out of I-102 is a mixture of insulin glargine protein, Buffers 25, 28, and 36, Medias 29 and 39, Adjustors 30, 38, 40, 41, and 42, and citraconic anhydride. The volume of this mixture is 126,000 L, and the centrifuge is able to process this volume in 6.19 hours. In this step, the insulin glargine protein is the densest component in the mixture, so it

precipitates and collects on the outer wall of the bowl. This protein (45.12 kg/batch) is collected and sent to a mixing tank (T-103). The supernatant, containing the rest of the mixture listed above is removed from the top of the bowl and discarded as waste.

ii. Mixing Tank

Mixing tanks (T-101, T102, T103) are used in the process to resuspend cells and wash the protein precipitate with different buffers. Tanks are stainless steel and have the volume capacity to meet the total buffer solution volume requirements in the process. The ratio of tank diameter to tank height is maintained at 3, as this is the basic stirred tank design (Green & Southard, 2019).

Turbine impeller agitators were designed to provide better mixing. The ratio of the impeller diameter to the diameter of the tank is set to 0.5, to avoid insufficient fluid movement or waste power. The number of impellers used in each tank is decided based on liquid height and impeller diameter (Equation 4.5.a), where H_L is the liquid height in the tank, and D_i is the diameter of the impeller (Davis, 2010).

$$\frac{H_L - D_i}{D_i} > n > \frac{H_L - 2*D_i}{2*D_i} \quad (4.5.a)$$

The required spacing between each impeller is between 1 and 2 D_i. The spacing from the lowest impeller to the bottom of the tank is 1 D_i, and the spacing from the liquid surface to the uppermost impeller is 1.5 D_i or more. These ratios were used to determine the distribution of impellers for each tank (Davis, 2010). The impeller tip speed is set under 3.2 m/s to avoid cell damage during the resuspension process (Junker, 2004). And for the mixing process, the impeller tip speed is also set at a value that gives an impeller Reynolds number that falls into the turbulent range, in order to obtain a constant power number, which can be used for future energy cost analysis (Green & Southard, 2019). Baffles were also designed and placed evenly around the tank to disrupt the bulk fluid flow for better mixing. The baffles are designed to be 0.1 D_t wide, 0.5 D_i from the bottom of the tank, and a sixth of the baffle width offset from the tank wall (Green & Southard, 2019).

The mixing time for each process is estimated based on Equation 4.5.b, where D_t is the diameter of the tank, D_i is the diameter of the impeller, ω is the impeller speed, and H is the height of the tank (Green & Perry, 2008).

$$t_{mix} = 5 * \pi * D_t^{\,2} * \frac{H}{4} * \frac{1}{0.92*\omega*D_i^{\,2}*D_t} \quad (4.5.b)$$

The first mixing tank, T-101, following C-101, is used for cell resuspension. The underflow is resuspended in the resuspension buffer (Buffer 13). After resuspension is complete, the cells (356.07 kg/batch) and buffer solution are transported to the high-pressure homogenizer. A second mixing tank, T-102, is used for resuspension of the inclusion bodies in washing solution (Buffer 18) after centrifugation (C-102). After washing is complete, the mixture (68.38 kg/batch of protein) goes to diafiltration (D-101). The third mixing tank, T-103 is used to redissolve the insulin glargine in Buffer 46 before chromatography. The mixed solution (45.12 kg/batch of protein) then goes to ultrafiltration (U-103). The dimensions of each tank are shown in Table 4.5.a.

Table 4.5.a

Tank Dimensions

Tag Number	T-101	T-102	T-103
Capacity, L	7300	10900	1500
Diameter, m	1.5	1.67	0.86
Height, m	4.5	5	2.58
Baffle width, m	0.15	0.17	0.086
Impeller diameter, m	0.75 (3 impellers)	0.83 (3 or 4 impellers)	0.43 (3 or 4 impellers)
Rotation Speed, rev/s	1	1	1
Mixing time, s	51	51	51

iii. High-Pressure Homogenizer

In the pharmaceutical industry, high-pressure homogenization is used for size reduction, mixing, and stabilization of dispersions (Yadav et al., 2019). The process produces high local stresses that reduce the particle size of the liquid mixture passing through the apparatus under high pressure (Yadev et al., 2019). High-pressure homogenization is one technique that falls under the cell disruption processes, which is the process of obtaining intracellular fluid by opening the cell wall of the particles in solution.

We chose to use high-pressure homogenization (H-101) as our cell disruption process because it is widely used in the pharmaceutical industry since it is easy to scale up and produce high-quality products (Lammari et al., 2021). For our process, we are using the Ariete NS3030 Homogenizer and High-Pressure Pump (GEA, 2023). This is a stainless-steel device that is suitable for Cleaning in Place (CIP) and Steaming in Place (SIP) (GEA, 2023). This model has many options in terms of pressure and maximum volumetric flow rate capacities ranging from 100 bar to 1,500 bar and 7,500 L/hr to 330 L/hr (GEA, 2023). The volumetric flow rate capacity is dependent on the input pressure, so for our process we will operate at 1,000 bar, which will give us a maximum volumetric flow rate of 650 L/hr. Research was conducted on high-pressure homogenization for *E.coli* cells and it was found that pressures between 1,000 bar and 3,000 bar were the best for bacterial inactivation, i.e., stabilization of dispersions (Diels et al., 2008). We chose to operate at 1,000 bar because it falls within this pressure range, and we also needed a flow rate that wasn't too slow, thus increasing the process time, so 650 L/hr met this condition. Under these conditions, we can process the 7,480 L of Buffer 13 and insulin glargine protein from the resuspension mixing tank (T-101) in 11.5 hours. Upon completion, this disruption technique releases the insulin precursor (69.07 kg/batch), and the mixture is sent to

centrifugation (C-102). The power consumption of the high-pressure homogenizer is 30 kW (GEA, 2023).

iv. Diafiltration

Diafiltration is a continuous system where a solvent is added to offset concentration effects and achieve protein recovery of high purity and high yield (Eugene et al., 2019). The process exchanges small-molecule components by continuously replacing the initial solvent that passes through the ultrafiltration membranes with a new solvent (Carta, 2022). The process stream and the solvent are fed into the membrane, and the insulin glargine protein can be retained, as it cannot pass through the ultrafiltration membrane because it is too large. Meanwhile, the solvent used for diafiltration can flow through the membrane. The mixture is fed through the membrane using a pump.

Our process contains two diafiltration steps: the first step (D-101) after washing in a mixing tank (T-102) and the second step (D-102) after the first ultrafiltration step (U-101). We will use the MaxCell Ultrafiltration Hollow Fiber Tangential Flow Cartridge, UF-3-C-85, membranes with a nominal molecular weight cutoff (NMWC) of 3 kDa (Cytiva, 2023). Since the molecular weight of insulin glargine is 6,063 Da, the protein will be too big for it to pass through the membrane. This type of hollow fiber membrane has a membrane area (A) 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 (D-101), the process feed consists of the insulin glargine protein and Buffer 18, and the solvent being added is WFI. These two have a combined initial volume (V_o) of 76,500 L. To optimize the processing time, the diafiltration volume (V_D) is first found and given by Equation 4.5.c.

$$V_D = - \frac{V_o * ln((\frac{C}{C_o})_{protein})}{1 - \sigma_{protein}} = V_P \quad (4.5.c)$$

The protein yield, $(\frac{C}{C_o})_{protein}$, 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 of interest being rejected by the membrane, so 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 hardly any of the protein is able to pass through the membrane. It is assumed that the rejection coefficient of the buffer, σ_{buffer}, is 0 because the molecular weight of Buffer 18 is below the NMWC of the membrane used in this process.

Using Equation 4.5.c, the diafiltration volume for this process is 309,000 L. For diafiltration, it is assumed that the process operates on a constant volume basis, meaning that the diafiltration volume is equal to the permeate volume (V_P). Thus, making the diafiltration flow rate (Q_D) and the permeate flow rate equal to each other. Based on the specs 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.d, was found to be 1.44 x 10^{-4} m/s. For diafiltration, it is assumed that the permeate flux is approximately equal to the initial permeate flux ($u_{p,0}$).

$$u_p = \frac{Q_P}{A} = u_{p,0} \tag{4.5.d}$$

With the initial permeate flux and the diafiltration volume known, the processing time for this diafiltration step is 46.0 hours, as shown by Equation 4.5.e.

$$t = \frac{V_D}{A * u_{p,0}} \tag{4.5.e}$$

For this diafiltration system, the inlet pressure must be at 3.4 bar and the temperature must be between 25 ºC - 80 ºC (Cytiva, 2023). Upon completion, the insulin glargine protein

(67.01 kg/batch) in solution with some of the WFI and cell debris is collected and sent to the first ultrafiltration system (U-101). The rest of the solvents, WFI, Buffer 18, and cell debris, are removed and sent off as waste.

The second diafiltration step (D-102) comes after the first ultrafiltration step (U-101), where 70,700 L is initially going into the system. This initial volume consists of the process stream containing WFI and the insulin glargine protein from ultrafiltration and the solvent containing Buffer 25. Using the same equations and process above (4.5.c - 4.5.e) to calculate the processing time, the diafiltration volume was found to be 286,000 L. This is again assuming that the rejection coefficient of the buffer is 0 since the molecular weight of Buffer 25 is lower than the NMWC. As the buffer can pass through the hollow fiber membrane, the insulin glargine protein again will not be able to pass through the filtration membrane since its molecular weight is greater than the NMWC. Thus, the rejection coefficient is 0.995 because although we will recover 98% of the protein, some will still manage to pass through the membrane. A rejection coefficient of 1 assumes that none of the proteins will pass through the membrane, making the recovery 100%, which is not realistic in diafiltration processes.

Since we are using the same membrane area, 13 m^2, and the same permeate flow rate, 112.087 L/min, the initial permeate flux for D-102 will stay the same, 1.44 x 10^{-4} m/s (Cytiva, 2023). By using Equation 4.5.e, the processing time was found to be 42.5 hours. This system also requires an inlet pressure of 3.4 bar and the temperature must be between 25 ^oC - 80 ^oC (Cytiva, 2023). Upon completion, the insulin glargine protein (60.68 kg/batch) and some of Buffer 25 are taken as the product and moved to incubation (I-101). The majority of Buffer 25, WFI, and some of the non-recovered proteins are sent off as waste.

v. Ultrafiltration

Similar to diafiltration, ultrafiltration can be run continuously, but there is no solvent feed; volume going into the membrane is not being replaced by a buffer, instead we run the system to remove any debris from the solution. Ultrafiltration separates macrosolutes that are retained from smaller molecules that pass through an ultrafiltration membrane (Carta, 2022).

For our ultrafiltration systems, we will only use one stage per process, so the membrane area will stay the same throughout all ultrafiltration processes. Our process contains three ultrafiltration steps: the first step (U-101) after the first diafiltration step (D-101), the second step (U-102) after the third centrifugation step (C-103), and the third step (U-103) after the mixing tank (T-103). We will use the MaxCell Ultrafiltration Hollow Fiber Tangential Flow Cartridge, UFP-10-C-85, for all ultrafiltration steps (Cytiva, 2023). Unlike in diafiltration, the NMWC of this membrane is 10 kDa (Cytiva, 2023). The NMWC for the ultrafiltration membrane design is not the same as diafiltration because the membrane should allow the insulin to flow through while blocking the undesired components such as cell debris. Since the molecular weight of insulin glargine is 6,063 Da, the insulin will be able to pass through the membrane. This type of 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).

For the first ultrafiltration step (U-101), the process feed consists of the insulin glargine protein, cell debris, and WFI. These have a combined initial volume of 10,900 L. Because the insulin and buffer will flow through the membrane, the concentration of insulin in the permeate will be equivalent to the concentration of insulin in the original solution, thus the rejection coefficient (σ) is zero demonstrated by Equation 4.5.f.

$$\sigma = 1 - \frac{C_p}{C} \quad (4.5.f)$$

For this first ultrafiltration system, the inlet pressure must be at 3.4 bar and the temperature must be between 25 ºC - 80 ºC (Cytiva, 2023). The system will run until 92.4%, the chosen step yield, of the solution flows through the membrane. Because the concentration of the permeate will be equivalent to the concentration of the feed, the permeate flow rate will be 77 L/min. We will be able to process 92.4% of the original 10,900 L of solution in 2.37 hours using a permeate flow rate of 77 L/min. The permeate product will be 10,000 L and collected as a mixture of insulin and WFI, while the retentate waste stream will be 830 L and consist of all the cell debris and some of the unrecovered insulin in solution with WFI. The recovered insulin (61.92 kg/batch) in solution is sent off to the second diafiltration system (D-102).

The second ultrafiltration step (U-102) comes after the third centrifugation step (C-103), where 130,000 L is initially going into the system. This initial volume consists of the insulin glargine protein, Buffers 25 and 28, Media 29, and Adjuster 30. The permeate volume was found to be 120,000 L, so the retentate volume will be 9,900 L. We will be able to process 92.4% of the original 130,000 L in 28.1 hours using a permeate flow rate of 77 L/min. Again, the system requires an inlet pressure of 3.4 bar and the temperature must be between 25 ºC - 80 ºC (Cytiva, 2023). The product, permeate stream contains the recovered insulin glargine protein (45.57 kg/batch) and some of the buffers, media, and adjuster inputs. This stream is sent to the second incubation step (I-102). The retentate waste stream contains a small amount of the unrecovered insulin, buffers, media, and adjusters from the input.

The third ultrafiltration step (U-103) comes after a mixing tank (T-103), where 1,400 L is initially going into the system. This initial volume consists of the insulin glargine protein in solution with Buffer 46. Again, the same membrane area and average permeate flow rate are

used. The permeate volume was found to be 1,300 L, so the retentate volume will be 100 L. The processing time for this ultrafiltration step was found to be 0.303 hours (18.2 minutes) using a permeate flow rate of 77 L/min. The pressure and temperature conditions mentioned previously are used again for this step (Cytiva, 2023). The product permeate stream containing the recovered insulin (41.69 kg/batch) in solution with some of Buffer 46 is sent through cation-ion exchange chromatography (E-101), while the retentate containing the rest of Buffer 46 and a small amount of the unrecovered insulin is sent off as waste.

vi. Incubation

The incubators were designed similarly to the mixing tanks for the refolding, conversion, and precipitation of the insulin glargine. We will incubate in 20,000 L tanks, which means we will need multiple incubators to meet the volume requirements of the incubation steps.

The first incubator (I-101) is used for the refolding process (described in the introduction). In this process, the insulin glargine precursor will be incubated for 48 hours to refold, under 4°C and pH 4.5. The incubator has a diameter of 2.03 m and a height of 6.12 m. Four baffles are distributed evenly around the tank, with a width of 0.2 m, and 3 impellers are used in the tank. The impeller speed is set to 1 rev/s and the time for complete mixing would be around 51 seconds. This step needs to process 130,000 L of buffer solution, so there need to be at least 7 incubators applied for this process. After incubation, the refolded protein (49.82 kg/batch), unfolded protein, buffers, media, and pH adjuster are sent to the third centrifuge (C-103).

The second incubator, I-102, is used for conversion and precipitation (described in the introduction). In the conversion step, the insulin glargine precursor is converted to active insulin glargine through peptide sequence cleavage. The temperature is kept around 25℃ for 12 hours, and the pH is adjusted from 8.5 (for the first 7 hours) to 2.5 (for the rest 5 hours). Then the temperature is set to 4℃, and the pH is adjusted to 6.1 for 16 hours of precipitation with zinc chloride, as the zinc ion can influence the mechanism of insulin precipitation. This incubator has the same equipment specifics and mixing time as the first incubator, I-101. For this step, we need to process around 130,000 L of solution, so 7 incubators are needed. After incubation, the converted protein (45.57 kg/batch), buffers, media, and pH adjusters are sent to the fourth centrifuge (C-104).

vii. Cation Exchange Chromatography (CEX)

Cation Exchange Chromatography (E-101) is used to purify the insulin glargine from other similarly sized impurities. Previous purification steps removed larger cell debris, but CEX is used to remove DNA and other proteins in the system. The desired protein, insulin glargine, is mixed with buffer solution and carries positive charges due to the lower pH of the buffer solution than the isoelectric point of insulin glargine. For a complete chromatography process cycle, five steps are needed: loading, washing, eluting, cleaning in place, and equilibration. The insulin glargine and buffer solution are first loaded into the CEX column, and insulin glargine will bind with solute-binding materials inside the column (stationary phase), and other unbound components will be washed as impurities. Then the elution buffer is fed to remove the insulin glargine from the stationary phase and collect it as pure. The next two steps, cleaning in place and equilibration, prepare the column to be ready for the next process cycle (Carta & Jungbauer, 2010; Carta, 2022).

Based on Hwang et al., a column used for insulin glargine purification should be packed with SP Sepharose Fast Flow resin (2016). This resin has a binding capacity of 70 mg/ml; thus, we will use 35 mg/ml as its dynamic binding capacity (Carta, personal conversion, 2023). Currently, we assume one CEX column volume will be 600 L, and residence time (L/u) is calculated to be 10 min. The column will need to be loaded twice to process 41.7 kg of protein in each batch. The time required for each step in one process cycle is calculated from required column volume times with residence time, and cleaning in place is assumed to take 60 mins (Carta, personal conversion, 2023).

The dimension of the column is calculated based on Equations 4.5.g - 4.5.j and residence time, where ΔP is the pressure drop across the column, B_0 is the hydraulic permeability, η is the

viscosity, ε is the extra particle void fraction, r_p is the radius of packing material particle, η_0 is the solvent viscosity, [η] is the intrinsic viscosity of insulin glargine, C_f is the feed concentration of insulin glargine, and L is the column length (Carta, 2022).

$$L * u = \frac{\Delta P * B_0}{\eta} \tag{4.5.g}$$

$$B_0 = \frac{1}{37.5} * \frac{\varepsilon^3}{(1-\varepsilon)^2} * r_p^{\,2} \tag{4.5.h}$$

$$\frac{\eta}{\eta_0} \sim 1 + [\eta] * C_f \tag{4.5.i}$$

$$d_c = \sqrt{\frac{Column\ volume}{L * \frac{\pi}{4}}} \tag{4.5.j}$$

The pressure drop is set to be 3 bar, as this is the maximum pressure drop for the packing materials (Cytiva, 2023). The length of CEX is calculated to be 1.24 m and the diameter is 0.5 m, with 2 loads to meet the volume requirement (Carta, Personal conversation, 2023). The required time for one column to complete 1 cycle is around 4.57 hours. Based on the 94% recovery, total cycle time, column volume, feed in volume, feed in insulin concentration, and Equation (4.5.k), the productivity of one CEX column is calculated to be 0.238 g/L*min (Carta, 2022).

$$Productivity = \frac{Recovery * V_f * C_f}{CV * T_{cycle}} \tag{4.5.k}$$

The purified protein solution (39.19 kg/batch of protein) will be transported to the Preparative High Performance Liquid Chromatography unit (L-101) for further purification. Samples will be transported to the High Performance Liquid Chromatography unit (M-101) to monitor the purity of the protein outlet from CEX.

viii. Preparative High Performance Liquid Chromatography (Prep-HPLC)

Prep-HPLC (L-101) following CEX (E-101), is used for further purification by removing all undesired proteins and peptides, such as misfolded insulin, and cleaved protein fragments. This is achieved by using the hydrophobic interaction between targeted proteins in the feed solution and the packed resin in the column (Carta, 2022). The elution is carried out with the concentration change of acetonitrile (ACN) in a linear manner, to produce the desired purity of insulin glargine (Hwang et al., 2016; Kroeff et al., 1989).

The calculation steps for Prep-HPLC are similar to CEX. We will use a 100 L column with a length of 0.52 m and a diameter of 0.5 m (Carta, Personal conversion, 2023). The Zorbax resin has a binding capacity of 85 mg/ml; thus, we will use 20% of that, 17 mg/ml, as its dynamic binding capacity (Carta, personal conversion, 2023). We will need 5 columns each processing 5 loads to process our 39.2 kg of protein. The cycle time for 1 column is around 7.19 hours. We will have a productivity of 0.82 g/L*min to reach 90.7% recovery of insulin glargine. The purified protein (35.54 kg/batch of protein) will then go to the lyophilizer (LY-101) to freeze-dry the final product. Samples of the purified protein will also be sent to an HPLC unit (M-102) to check the purity.

ix. High Performance Liquid Chromatography

High performance liquid chromatography (HPLC) is used to analyze the purity of the protein solutions leaving both the chromatography units (M-101 and M-102). We will test the purity of the solution five times using 20μL samples. We will use a Protein & Peptide C4 analytical column (250 mm × 4.6 mm column, particle size of 5 μm) (Hwang et al., 2016). Buffers of 50mM $NaH_2PO_4 \cdot H_2O$, 0.1M $NaClO_4$, and varying concentrations of ACN (10%-80%) will be used to elute the column at 1 ml/min (Hwang et al., 2016). The UV detector will monitor the absorbance of the protein to determine the purity of the samples.

x. Lyophilizer

After the final purification step of preparative high performance liquid chromatography, the product is ready to be lyophilized and sold to a secondary company to formulate. Lyophilizing works by first freezing the product and then reducing the pressure until the liquid sublimates leaving the frozen dry product (LYOVACTM Pharma Freeze Dryer, 2021). The proposed model, GEA LYOVAC FCM 800, fits 49,875 vials of 30 mm diameter (0.015 m radius), and each of the shelves has 125 mm clearance (GEA, 2021). To fit in the lyophilizer, we chose a height of 0.11 m for our vials. We used Equation 4.5.l, the volume of the vials times the number of vials, to calculate the volume of product we can lyophilize in one batch.

$$49875 \text{ vials} * \pi * 0.015m^2 * 0.11m = 3.71m^3 \quad (4.5.l)$$

Each batch will have 14,400L of product, so the proposed lyophilizer is large enough to freeze-dry the batch in four cycles. The insulin glargine will be in solution with acetonitrile and acetic acid, so the lyophilizer must lower the pressure enough to sublimate the solvents. The lyophilizer freezes to -75 C and then reduces the pressure to 0.01 mbar which is sufficient to sublimate the organic solvents (Denoulet, B., 2019). The lyophilizer pump will be connected to a fume hood to collect the sublimated organic solvents.

VI. Schedule for Batch Operations

i. Upstream Schedule

There are five total unit operations in the upstream production process for insulin glargine. Table 4.6.a below indicates the time each step takes to complete the process, cleaning and steaming in place, draining, and filling. It is important to note 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 and SIP, and Drain Times

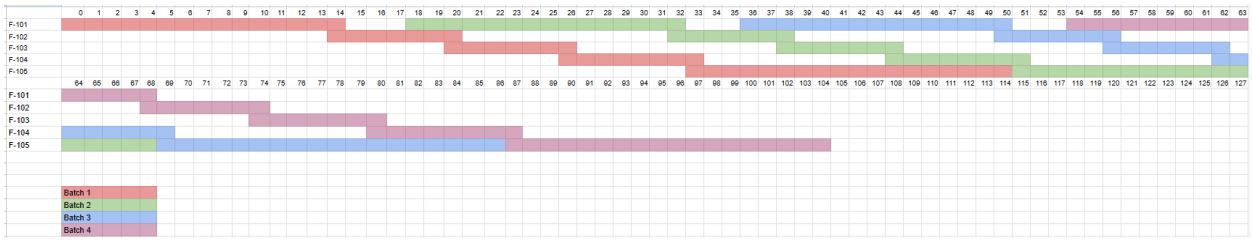
	Process Time (hr)	**CIP/SIP Time (hr)**	**Drain Time (hr)**	**Fill Time (hr)**	**Total (hr)**
F-101	13.2	0.548	0	0	13.7
F-102	5.42	0.608	0	0	6.03
F-103	5.42	0.746	0.04	0.04	6.25
F-104	5.42	1.14	0.4	0.4	7.36
F-105	5.42	2.72	4	4	16.1
				Total (hr)	49.5 (2.06 days)

Starting at the 2.5 L fermenter (F-101) and going along the seed train to the 25,000 L fermenter (F-105), the process takes 49.5 hours to complete, which is about 2.06 days. The drain and fill times reported in Table 4.6.a for F-101 and F-102 are 0 because the amount of time it takes to drain 2 L and 20 L of the mixture is insignificant at our assumed flow rate. We assume a flow rate exiting and entering the fermenter to be 5,000 L/hr because this is a commonly used flow rate for industrial pumps (Wilson et al., 2015).

The batch schedule for upstream is shown in Figure 4.6.a. Figure 4.6.a below only depicts one upstream system, but we will have two upstream systems running simultaneously.

Figure 4.6.a

Upstream Batch Schedule



We start our batches on an arbitrary date and they run for almost two days. The batches are separated by 18 hours. This 18-hour delay between batches was determined by configuring the most economical and efficient batch schedule for the downstream production of insulin glargine which will be discussed in the next section. Upon completion, the batch is sent to the first process in downstream, C-101. 18 hours into Batch 4, the cycle starts over and repeats with Batch 1.

ii. Downstream Schedule

For downstream, there are 20 total unit operations used to purify and produce insulin glargine. Table 4.6.b below indicates the time each step takes to complete the process, cleaning and steaming in place, draining, and filling. Resembling the upstream process, the transportation time between processes has not been determined.

Table 4.6.b

Downstream Process, CIP and SIP, and Drain Times

	Process Time (hr)	**CIP/SIP Time (hr)**	**Drain Time (hr)**	**Fill Time (hr)**	**Total (hr)**
C-101	0.978	1	n/a	n/a	1.98
T-101	0.0142	1.56	1.5	1.5	4.57
H-101	11.5	1	n/a	n/a	12.5
C-102	0.366	1	n/a	n/a	1.37
T-102	0.0142	1.26	2.19	2.19	5.65
D-101	46.0	1	14.9	14.9	76.8
U-101	2.37	1	2.12	2.12	7.61
D-102	42.5	1	3.04	3.04	49.6
I-101	48	9.4	24.3	24.3	106
C-103	6.37	1	n/a	n/a	7.37
U-102	28.1	1.78	24.3	24.3	78.5
I-102	28	1.77	10.6	10.6	51.0
C-104	6.19	1	n/a	n/a	7.19
T-103	0.0142	0.651	0.28	0.28	1.23
U-103	0.303	1	0.28	0.28	1.86
E-101	9.14	1	27.9	27.9	65.9
M-101	n/a	1	n/a	n/a	1
L-101	36.0	1	13.9	13.9	64.8
M-102	n/a	1	n/a	n/a	1
LY-101	1.5	1	2.88	2.88	8.26
				Total (hr)	554 (23.1 days)

The downstream process begins after upstream has finished at C-101. The process moves along different purification and separation processes to isolate the insulin glargine protein from buffers, media, pH adjusters, and cell debris. After nearly 554 hours, or 23.1 days, the downstream system should be complete, and an insulin glargine product is freeze-dried in a lyophilizer, LY-101. Table 4.6.b illustrates some operations having a “n/a” for the processing time, drain time, and fill time. The High Performance Liquid Chromatography (HPLC) units, M-101 and M-102, do not show a time for process, drainage, and filling because these two tests require very small sample sizes, thus making the times insignificant. For these two steps, it is only important to take into account the amount of time to clean and steam the units. For the centrifuges and the high-pressure homogenizer, C-101 - C-104 and H-101 respectively, there are no drain and fill times because these two processes are run continuously.

The batch schedule for downstream is shown in Figure 4.6.b. Once again, the figure only depicts one system, but there are two downstream systems running simultaneously.

Figure 4.6.b

Downstream Batch Schedule

Once F-105 is completed, the batch is transported to the first centrifugation process, C-101, where downstream begins. This process is more dense than upstream because it has 20 different steps, and each step has varying process times. Based on the schedule, we need to have multiple sets of equipment for one step because there is overlap. We need to have four unit operations for the first diafiltration step (D-101), the first incubation step (I-101), the second ultrafiltration step (U-102), the cation exchange chromatography step (E-101), and the prep-HPLC step (L-101). As shown in Table 4.6.b, these five steps take the longest amount of time to complete the process, clean, drain, and fill. As such, the four batches will overlap for diafiltration between hours 80 and 102. For incubation, they overlap between hour 214 and hour 266. The ultrafiltration step has overlap between hour 328 and 352. The cation exchange chromatography step has four batches overlapping between hours 468 and 480. For prep-HPLC, the batches overlap between hours 534 and 544. We will also need to have three unit operations for the second diafiltration (D-102) and the second incubation step (I-102). These steps also have a long process time, but not quite as long as the five mentioned before, thus we only require three units for these steps instead of four. For the second diafiltration step, there is overlap between hours 146 and 160 and hours 164 and 178. For the second incubator, there is an overlap between hours 388 and 402 and hours 406 and 420.

As mentioned in the upstream process, the batches are separated by 18 hours. After configuring numerous different batch schedules, an 18-hour separation was found to be the most efficient because we only need multiple units for seven operations. Any time below 18 hours would require more equipment for different operations which would have increased our capital costs, and any time above 18 hours would have made our process output too slow. This time also

allows room for us to add in the amount of time it takes to transfer materials from one process to another.

VII. CIP/SIP Requirements

Our process will be cleaned using clean-in-place and steam-in-place systems rather than using single-use equipment. Each piece of equipment listed in Table 4.7.a will need to be cleaned, which includes all of the equipment in our process besides the pumps. The batch equipment (fermenters, tanks) will be cleaned using Alfa Laval's LKRK F-version fixed static spray ball with 360° spray coverage (Alfa Laval, n.d.). The tanks less than 2000 L will use the smaller size, LKRK-64, and the tanks 2000L or greater will use the larger size, LKRK-94. The numbers in Table 4.7.a represent the total time for both WFI rinses, all drain times, caustic rinse time, and steam-in-place time. The time and quantity for the CIP and SIP processes were determined based on the type of equipment as outlined below.

i. Clean in Place

After the product flows through a given piece of equipment, a clean-in-place process ensues using water for injection (WFI) and 0.5% NaOH at 60° (caustic). The process of CIP begins with a WFI rinse, followed by a caustic rinse, and finishes with another WFI rinse.

The volume of WFI rinse for the bioreactors, tanks, and incubators is to be 5% of the working volume and the caustic rinse volume is to be 10% of the working volume to ensure adequate cleaning (*How much CIP detergent needed?,* 2022). The time required for WFI and caustic rinse were calculated using the equation (Equation 4.7.a) for a spray valve appropriate for our process (Hasting, 2008). For example, Equations 4.7.b and 4.7.c were used to calculate the WFI rinse volume and time for F-105, the 25,000 L bioreactor. Additionally, the drain times were calculated for these non-continuous unit operations. Equation 4.7.d shows the drain time for

WFI rinse calculated for F-105 with the assumption that our pumps are operating at a flow rate of 5,000 L/hour as stated in previous sections.

$$Flow\ Rate\ (L/hr)\ =\ diameter\ of\ tank\ (m)\ *\ 3.14\ *\ 1490 \quad (4.7.a)$$

$$20,000\ L\ working\ vol\ *\ 0.05\ =\ 1000\ L\ WFI\ rinse\ 1 \quad (4.7.b)$$

$$Water\ Rinse\ Time\ (min)\ =\ \frac{1000\ L*60\ min}{2.2\ m*3.14*1490}\ =\ 5.83\ min\ WFI\ rinse\ 1 \quad (4.7.c)$$

$$Drain\ Time\ (min)\ =\frac{1000\ L\ WFI}{5000\ L/hr} * \frac{60\ min}{1\ hr}\ =\ 12\ min\ WFI\ drain\ 1 \quad (4.7.d)$$

All other unit operations are run continuously, so the WFI rinse time and volume were based on the flow rates with which we are running the unit operations using exposure time. The WFI rinse exposure times are 5 minutes, and the caustic rinse exposure time is 20 minutes (Featherstone, 2015). For example, Equation 4.7.e was used to calculate the WFI rinse volume for C-101, the first centrifuge. Drain time was assumed to be 0.00 minutes for all continuous unit operations.

$$WFI\ Rinse\ Volume\ (L)\ =\ 20441.4\ L/hr\ *\frac{1\ hr}{60\ min} *\ 5\ min\ =\ 1703.45\ L\ WFI\ rinse\ 1 \quad (4.7.e)$$

ii. Steam in Place

After the Clean in Place cycle is complete, the Steam in Place cycle begins. The steam used will be clean steam at 121°C and 15 psig (*Clean and pure steam systems biopharmaceutical industry,* 2010). The steam exposure time will be maintained at 30 minutes for all unit operations to ensure complete sterilization (*Clean and pure steam systems biopharmaceutical industry,* 2010). The volume of steam used for the bioreactors, incubators, and tanks will be calculated using the spray valve equation (Equation 4.7.a) and the exposure time. For example, the steam volume calculation for F-105 can be seen in Equation 4.7.f. The volume of steam used for the continuous equipment will be calculated using the flowrate of the system as well as the exposure time, as seen in Equation 4.7.g for C-101. The time and volume requirements for all CIP and SIP steps are shown in Table 4.7.a.

$$Volume\ Steam\ (L) = 2.2\ m * 3.14 * 1490 * \frac{1\ hr}{60\ min} * 30\ min = 5146.46\ L \quad (4.7.f)$$

$$Volume\ Steam\ (L) = 20441.4\ L/hr * \frac{1\ hr}{60\ min} * 30\ min = 10220.7\ L \quad (4.7.g)$$

Table 4.7.a

Clean in Place and Steam in Place Time and Volume Requirements for all Equipment

Equipment Tag	Total Time (min)	Total Caustic (L)	Total WFI (L)	Total Steam (L)
F-101	32.86	0.20	0.20	233.93
F-102	36.46	2.00	2.00	514.65
F-103	44.77	20.00	20.00	1099.47
F-104	68.46	200.00	200.00	2386.09
F-105	163.07	2000.00	2000.00	5146.46
C-101	60.00	6813.80	3406.90	10220.70
T-101	93.43	730.00	730.00	2011.80
T-102	75.75	1090.00	1090.00	233.93
T-103	39.04	150.00	150.00	233.93
H-101	60.00	216.67	108.33	325.00
C-102	60.00	6813.80	3406.90	10220.70
D-101	60.00	2241.74	1120.87	3362.61
U-101	60.00	2241.74	1120.87	3362.61
D-102	60.00	2241.74	1120.87	3362.61
I-101	563.97	14600.00	14600.00	233.93
C-103	60.00	6813.80	3406.90	10220.70
U-102	107.08	2241.74	1120.87	3362.61
I-102	105.95	2000.00	2000.00	233.93
C-104	60.00	6813.80	3406.90	10220.70
U-103	60.00	2241.74	1120.87	3362.61
E-101	60.00	0.02	0.01	0.03
M-101	60.00	0.00	0.00	0.01
L-101	60.00	0.01	0.00	0.00
M-102	60.00	0.00	0.00	0.01
LY-101	60.00	10690.00	21380.00	10690.00
Total Times & Volumes	**32.86 min**	**70162.79 L**	**61512.50 L**	**81039.00 L**

VIII. Media Sterilization Requirements

A continuous sterilization model is used for media sterilization before use in the fermentation process. Media will be sterilized at a high temperature for a short-exposure time to avoid damage to the nutrients. Temperature is set at 121℃, as this is the temperature where most thermal sterilizations take place. The cooling section in the model will then cool down the sterilized media to 37℃ for fermentation (Shuler & Kargi, 2002). The continuous sterilization model will be purchased from Actini, and the highest capacity is 20,000 L/h, which satisfies our project media quantity requirement for the fermentation process (Actini, 2023).

IX. Heat Requirements

i. Heat Generation

It is vital to calculate heat generation of the equipment in our process to assess which unit operations require heat to be removed via a cooling jacket so as to not damage our product. We analyzed all equipment that would have significant heat generation and would not be self-cooled; this includes fermenters, tanks, and incubators as seen in Table 4.9.a. The incubator, tank, and fermenter heat generation were calculated as gassed power using Equation 4.3.j, and a sample calculation can be seen in Equation 4.9.a for the gassed power of F-105.

$$Gassed\ Power\ (W)\ =\ 0.4\ *\ 3\ Impellers\ *\ 30172.50\ Watts\ *\ 1.2 = 43448.40\ \text{W} \quad (4.9.a)$$

The total fermenter heat generations were calculated using Equation 4.9.b, and a sample calculation can be seen in Equation 4.9.c for F-105. Biomass-specific heat production, Q, was found in a study using E. coli and glucose at similar concentrations (Leiseifer, 1989). Table 4.9.a lists the heat generation for each piece of equipment.

$$Heat\ Generated\ (W)\ =\ Heat\ from\ Cell\ Growth\ +\ Heat\ from\ Impeller$$

$$=\ Q\ *\ 18 g/L\ *\ Bioreactor\ Volume\ (L)\ +\ Gassed\ Power \quad (4.9.b)$$

$$Heat\ Generated\ (W)\ =\ 0.652\ W/g\ *\ 18\ g/L\ *\ 20,000\ L\ +\ 43000\ W$$

$$= 277720\ \text{W F-105} \quad (4.9.c)$$

Table 4.9.a

Heat Generated by Equipment

Equipment Tag	Heat Generated (kW)
F-101	0.25
F-102	1.3
F-103	6.9
F-104	44
F-105	280
T-101	1.0
T-102	2.0
T-103	0.070
I-101	5.5
I-102	5.5

ii. Heat Equipment Design

Cooling jackets are used for controlling the temperature of the fermenters, mixing tanks, and incubators. The cooling liquid inside the jacket will be ethylene glycol, a common cooling agent in industrial usage (Anderson, Personal communication, 2023). It was important to use ethylene glycol as our cooling liquid as opposed to water because some processes operate at 4°C, which is very close to the freezing point of water. Ethylene glycol has a freezing point of -12.69°C (PubChem, 2023). The required area for each cooling jacket was determined by Equation 4.9.d (Carta, 2021).

$$A_{req} = \frac{Q}{U_o * \Delta T_{lm}} \quad (4.9.d)$$

In the equation above, Q is the heat generated, listed in Table 4.9.a. Here ΔT_{lm} is calculated by Equation 4.9.e, where T_1 is the inlet temperature of the coolant, T_2 is the outlet temperature of the coolant, and T_H is the temperature of the liquid inside the tank.

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

The overall convective heat transfer coefficient, U_0, is calculated by Equation 4.9.f, where h_0 is the convective heat transfer coefficient of ethylene glycol, h_i is the convective heat transfer coefficient of liquid inside the tank, which is assumed to be water. The inner radius of the tank, r_i, and the outer radius of the tank, r_o, which we assume all the tanks to have a wall thickness of 0.02 meters. The thermal conductivity of the tank wall, k_{steel}, is 16.3 $\frac{W}{m*K}$ for 316 stainless steel (FineTubes, 2015).

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

Here, the convective heat transfer coefficient of liquid inside the tank is calculated by Equation 4.9.g, where a is 0.54, b is 0.667, m' is 0.14, k_{water} is the thermal conductivity of water at bulk temperature, D_T is the diameter of the tank, μ is the viscosity of water at bulk temperature, and μ_s is the viscosity of water at tank wall temperature, which is the same as the inlet temperature of ethylene glycol. In addition, Re is calculated by Equation 4.9.h, and Pr is calculated by Equation 4.9.i, where D_i is the diameter of the impeller, N is the rotation speed of the impeller, ρ is the density of water (Carta, 2021; Green & Southard, 2019).

$$h_i = k_{water} * a * Re^b * Pr^{1/3} * \left(\frac{\mu}{\mu_s}\right)^{m'} * \frac{1}{D_T} \quad (4.9.g)$$

$$Re = \frac{D_i^2 * N * \rho}{\mu} \quad (4.9.h)$$

$$Pr = \frac{Cp * \mu}{k_{water}} \quad (4.9.i)$$

The equation to calculate the convective heat transfer coefficient of ethylene glycol is given by Equation 4.9.j. The Nusselt number, Nu, is given by Equation 4.9.k for ethylene glycol in a coiled tube around the incubator. The thermal conductivity, k_{glycol}, is ethylene glycol at bulk temperature, and the diameter, D_{tube}, is of the tube, which is assumed to be 0.5 meters. The Re and Pr are given by Equations 4.9.l and 4.9.m respectively. The Reynolds number of ethylene glycol is calculated using the velocity of ethylene glycol, v, which is assumed to be 1.25 m/s (Anderson, Personal communication, 2023).

$$h_o = \frac{Nu * k_{glycol}}{D_{tube}} \quad (4.9.j)$$

$$Nu = 0.023 * Re^{0.8} * Pr^{0.4} \quad (4.9.k)$$

$$Re = \frac{v * \rho * D_{tube}}{\mu} \quad (4.9.l)$$

$$Pr = \frac{C_p * \mu}{k_{glycol}} \quad (4.9.m)$$

For our cooling jackets to be effective, the required area must be smaller than the jacket area that covers each of the tanks. The jacket area can be seen by Equation 4.9.n. Here, h_T represents the height of the tank, and D_T again represents the diameter of the tank.

$$A_{jacket} = \pi * h_T * D_T \quad (4.9.n)$$

The mass and volumetric flows of ethylene glycol can also be calculated, given by Equation 4.9.o and Equation 4.9.p respectively. Again, 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.

$$m_c = \frac{Q}{(T_2 - T_1) * C_p} \quad (4.9.o)$$

$$V = \frac{m_c}{\rho} \quad (4.9.p)$$

The inlet and outlet temperatures, required area, jacket area, and volumetric flow rates for each of the fermenters, mixing tanks and incubators can be seen in Table 4.9.b. All the actual cooling jacket areas are greater than the required area, thus making the cooling jacket designs sufficient for our process. It is important to note that for mixing tanks T-102 and T-103 and for incubators I-101 and I-102 (last 16 hours), the inlet cooling temperature is 0°C. Extra precaution must be taken when monitoring these processes so that the mixture does not freeze and create a slush when mixing or incubating. If needed, the ethylene glycol can be fed as a defrosting agent periodically to prevent any solids from forming; similar to how refrigerators operate.

Table 4.9.b

Cooling Jacket Requirements

Cooling Jacket	Coolant inlet temperature, ℃	Coolant outlet temperature, ℃	Required jacket area, m^2	Actual jacket area, m^2	Volumetric Flow Rate, L/s
F-101	4	33	0.08	0.10	11.8
F-102	4	33	0.38	0.46	61.2
F-103	4	33	2.04	2.10	325
F-104	4	25	8.98	9.81	2890
F-105	2	15	44.6	45.5	30000
T-101	4	35	0.37	21.2	44.0
T-102	0	2	3.58	26.2	1420
T-103	0	2	0.13	6.97	49.8
I-101	0	2	9.84	39.2	3910
I-102 (1st 12 hr)	4	23	2.96	148	400
I-102 (last 16 hr)	0	2	9.76	148	3910

X. Ancillary Equipment

i. Pumps

As seen in our overall process flow diagram (Figure 4.1.a), our process requires the use of 22 pumps throughout our system. These pumps are used to facilitate product and feed throughout the system to ensure that the process can remain on schedule. For our process, we will be using Alfa Laval's SX2WLD rotary lobe pump (Alfa Laval, 2015). Rotary lobe pumps are positive displacement pumps that use lobes rotating around shafts to move liquids. The shafts that the lobes are attached to rotate in opposite directions, causing cavities to open and collapse within the pump, which moves the product from the inlet of the pump to the outlet of the pump. This pump style has been chosen because of the control of outlet flow rate, gentle pump action that minimizes product damage, ease of cleaning using CIP/SIP methods, and ease of maintenance (CSI, 2021). We have chosen Alfa Laval's SX2WLD because the pump is specifically designed for the transportation of fluid in biotechnology and pharmaceuticals, and the specific model fits our process qualifications of appropriate flow rate while minimizing pressure differential. Equation 4.10.a uses the data in the specification sheet for SX2WLD to show that the maximum flow rate of the pump is compatible with our process flow rate of 5000 L/h, and Equation 4.10.b shows the speed that the pump must operate at to reach our process' desired flow rate of 5000 L/h. The specification sheet lists the displacement as 0.181 liters/revolution, the differential pressure as 7 bar, and the maximum speed as 1000 revolutions/minute (Alfa Laval, 2015).

$$Maximum\ Flow\ Rate = 0.181\ L/rev * 1000\ rev/min * 60\ min/hr$$
$$= 10860\ L/hr \quad (4.10.a)$$

$$Pump\ Speed\ =\ \frac{5000\ L/h}{60\ min/hr\ *\ 0.181\ L/rev} = 460.41\ rev/min \qquad (4.10.b)$$

The differential pressure and flowrate through each of the pumps is the same, so the power consumption of each of the pumps is the same. Using equations and plots from Fristam (n.d.), the following power from each pump can be seen in Equation 4.10.d, calculated using Equation 4.10.c.

$$Power\ (kW)\ =\ \frac{(2*P+V)*n*C}{1000\frac{W}{kW}} \qquad (4.10.c)$$

where P is differential pressure in bar, V is viscosity factor from the viscosity factor graph, n is the speed of the pump in revolutions/minute, and C is the flow displacement in liters/revolution.

$$Power\ (kW)\ =\ \frac{(2*7\ bar+1.8)*460.41\ rev/min*0.181\ L/rev}{1000\frac{W}{kW}} =\ 1.32\ kW \qquad (4.10.d)$$

ii. Holding Tanks

Due to the large nature of our process, multiple holding and mixing tanks must be designed for the various feed inputs we have throughout our process. The design of the holding and mixing tanks is the same as the design of mixing tanks in the Separations Design section, and each holding and mixing tank's capacity is determined by the total required volume of caustic NaOH, ferment media, buffer solution, pH adjuster, and waste through the process. The power requirement in watts for the tank's impellers is calculated based on Equation 4.10.e.

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

Here, Np is the power number, which obtained from the graph of Power number vs Impeller Reynolds number, ρ is the density of the liquid inside the holding tank in kg/m^3, N is the impeller speed in rev/s and D is the impeller diameter in m (Green & Southard, 2019). The dimensions of each tank for different sterilization solutions, fermenter media, buffer solutions, pH adjusters, and waste are shown in Table 4.10.a. All tanks have 3 impellers for mixing, except the tanks for the media and buffers 56 and 64 which have 2 impellers, and the only waste stream that requires mixing is the neutralized waste.

Table 4.10.a

Tank Dimensions

Equipment Tag	Solution	Capacity L	Diameter m	Height m	Baffle width m	Impeller diameter m	Rotation Speed rev/s	Mixing time s	Power kW
HT-101	**Caustic NaOH**	72,000	3.13	9.38	0.31	1.56	1	51	46.36
HT-102	**Cell media**	30,000	1.17	7	0.23	1.17	1	51	10.78
HT-103	**Buffer 13**	7,200	1.45	4.35	0.15	0.73	1	51	1
HT-104	**Buffer 18**	11,000	1.67	5.01	0.17	0.84	1	51	2.02
HT-105	**Buffer 25**	61,000	2.96	8.87	0.3	1.48	1	51	35
HT-106	**Buffer 28&60**	120,000	3.70	11.12	0.37	1.85	1	51	108.63
HT-107	**Buffer 36 & 46**	1,400	0.84	2.52	0.08	0.42	1	51	0.07
HT-108	**Buffer 50 & 51**	140,000	3.90	11.71	0.39	1.95	1	51	140.45
HT-109	**Buffer 52**	7,300	1.46	4.37	0.15	0.73	1	51	1.02
HT-110	**Buffer 56 & 64**	1	0.08	0.23	0.008	0.038	1	51	$3.72*10^{-7}$
HT-111	**Buffer 59**	20,500	2.06	6.17	0.2	1.03	1	51	52.98
HT-112	**Adjustor 30 & 40**	1,900	0.93	2.79	0.09	0.47	1	51	0.11
HT-113	**Adjustor 42**	3,300	1.12	3.36	0.11	0.56	1	51	0.27
HT-114	**Adjustor 38 & 41**	50	0.28	0.83	0.03	0.14	1	51	$2.52*10^{-4}$
HT-115	**Waste with cells**	12,000	1.72	5.16	N/A	N/A	N/A	N/A	N/A
HT-116	**Waste with ACN**	70,000	3.10	9.29	N/A	N/A	N/A	N/A	N/A
HT-117	**Other waste**	472,000	5.85	17.56	0.59	2.93	1	51	1064

XI. Waste Treatment

Waste streams are treated differently based on their composition and pH values. For waste streams that contain *E.coli* cells, the liquid will be decontaminated through the continuous decontamination system from Actini, at 135℃, for at least 2 mins. The decontamination system has a maximum capacity of around 190,000 L/day (7,917 L/h) to meet the waste quantity. Then the decontaminated waste will then be sent to the public sewer system. Waste streams containing acetonitrile will be collected separately and sent to toxic industrial waste collectors approved by the Singapore government such as AlphaChem Technology (S) Pte Ltd for incineration (National Environment Agency, 2023). All of the other waste streams are collected and neutralized to a pH of about 7 using a caustic NaOH solution (0.5% wt/v). We will use the caustic from our cleaning in place. The streams can then be sent to the public sewer system (Anderson, Group meeting, 2023). The total volume needed of caustic NaOH solution for the neutralization process is around 3,000,000 L per year. The remaining NaOH from CIP will be neutralized with sulfuric acid before going into the public sewer system, as it is the least expensive acid to use (Digital Analysis Corporation, 2023).

5. Final Recommendations

I. Fermentation

The upstream processing of this project consists of a seed train of four stainless steel bioreactors and one final production fermenter. Media will first be sterilized using a continuous sterilization unit from Actini in which the media will be heated to 121℃ and then cooled back to 37℃ for the fermentations. The first fermentation will be 2L with a starting *E. coli* JM109 concentration of 0.066 g/L and a starting glucose concentration of 200 g/L. Each fermentation will proceed at 37℃ with about 0.2 vvm oxygen until a cell concentration of 18 g/L is achieved. Four baffles and three Rushton impellers with a tip speed of 5m/s will ensure adequate mixing in the tanks. A cooling jacket with ethylene glycol will remove heat from the reactors to maintain a temperature of 37℃. The 2L fermentation will take about 13.2 hours. The cell suspension from the first fermentation will be transferred to the next 25 L bioreactor. The initial cell concentration of the 25 L reactor will then be 1.8 L and will ferment with 200 g/L glucose until the cell concentration reaches 18 g/L. This fermentation will take 5.4 hours. The next two fermenters, 250 L and 2,500 L, will proceed according to the same steps. Both of these fermentations will also use 200 g/L glucose and take 5.4 hours.

The final fermentation will be operated as fed-batch. The contents of the 2,000 L reactor will be placed in the 25,000 L reactor along with 3,000 L of media and 80 g/L glucose. The reactor will be fed continuously with media at a rate of 52 L/min and the appropriate mass of glucose to achieve a constant 80 g/L glucose in the fermenter. The final concentration of cells in the 20,000 L reactor will be 18 g/L after 5.4 hours. This cell suspension will be sent to the downstream process for protein acquisition and purification. The entire fermentation process will take 34.8 hours.

II. Centrifugation

We use four different centrifugation steps in the downstream process (C-101, C-102, C-103, and C-104). We use disk stack centrifuges in all steps to achieve efficient and effective continuous separations. We use an Alfa Laval MOPX 213, which is a continuous disk stack centrifuge for all centrifugation steps. This model has a volumetric flow rate capacity of 20,400 L/hr, so with our pumps operating at 5,000 L/hr, this model can effectively run under these conditions.

The first centrifugation step (C-101) comes after fermentation (F-105), where the *E.coli* cells are harvested from the LB growth media. The process time for this centrifugation step that processes 20,000 L of media is 0.978 hours (58.7 minutes). The harvested cells in solution with some of the fermentation media will be sent to a mixing tank (T-101) for resuspension. Meanwhile, the media waste from fermentation will be sent off as waste.

The second centrifugation step (C-102) takes place after cell disruption (H-101) and separates the insulin glargine protein from the buffer. This centrifugation step will process 7,480 L of solution, equating to a process time of 0.366 hours (21.9 minutes). The precipitate, containing the denser insulin glargine protein will be sent to another mixing tank (T-102) for washing. The less-dense supernatant containing the buffer solution will be sent off as waste.

The third centrifugation step (C-103) follows the first incubation step (I-101) and separates the refolded peptide fusion glargine protein from the mixture. This step processes 130,000 L of buffers and pH adjusters, which will have a process time of 6.37 hours. The supernatant containing the refolded peptide fusion glargine protein will be sent to the second ultrafiltration step (U-102). The precipitate containing the buffers and pH adjusters will be sent off as waste.

The fourth centrifugation step (C-104) comes after the second incubation step (I-102), where the insulin glargine protein is collected, while the solution is discarded as waste. This centrifugation step processes 126,000 L of buffers, media, and pH adjusters, and will have a process time of 6.19 hours. The insulin glargine protein will be collected in the precipitate and sent to the third mixing tank (T-103). The supernatant waste containing the buffers, pH adjusters, and media will be sent off as waste.

III. Mixing Tanks

Mixing tanks (T-101, T-102, and T-103) are used in the process to resuspend cells and wash the protein precipitate with different buffers. Tanks are stainless steel and have the volume capacity to meet the total buffer solution volume requirements in the process. All tanks will use Rushton impellers and baffles to aid mixing.

The first mixing tank, T-101, following C-101, is used for cell resuspension in the buffer. T-101 will be 7,300 L with a height of 4.5 m and a diameter of 1.5 m. Four baffles with a width of 0.15 m and three impellers with a diameter of 0.75 m will be used in T-101, and the impeller rotation speed will be set at 1 rev/s. The total mixing time will be 51 seconds, and a cooling jacket will be used for maintaining the temperature of the liquid in the tank to 37℃. After resuspension is complete, the cells and buffer solution are transported to the high-pressure homogenizer (H-101).

The second mixing tank, T-102, is used for the resuspension of the inclusion bodies in the washing solution after centrifugation (C-102). T-102 will be 10,900 L with a height of 5 m and a diameter of 1.67 m. Four baffles with a width of 0.17 m and three or four impellers with a diameter of 0.83 m will be used in T-102, and the impeller rotation speed will be set at 1 rev/s. The total mixing time will be 51 seconds, and a cooling jacket will be used for maintaining the temperature of the liquid in the tank to be 4℃. After washing is complete, the mixture will be sent to diafiltration (D-101).

The third mixing tank, T-103, is used for resolving the insulin glargine in the buffer after precipitation before chromatography purification. T-103 will be 1,500 L with a height of 2.58 m and a diameter of 0.86 m. T-103 will have four baffles with a width of 0.086 m and three or four impellers with a diameter of 0.43 m, and the impeller rotation speed will be set at 1 rev/s. The

total mixing time will be 51 seconds, and a cooling jacket will be used for maintaining the temperature of the liquid in the tank to be 4℃. After washing is complete, the mixture will be sent to ultrafiltration (U-103).

IV. High-Pressure Homogenizer

The high-pressure homogenization (H-101) will be used to disrupt the cells and release the inclusion bodies one of which is insulin glargine. We will use the Ariete NS3030 Homogenizer and High Pressure Pump and will operate at 1,000 bar, which will give a maximum volumetric flow rate of 650 L/hr. Under these conditions, it will take 11.5 hours to disrupt cells from the mixing tank (T-101). This disruption technique releases the insulin precursor, and the mixture is sent to centrifugation (C-102). The power consumption of the high-pressure homogenizer is 30 kW.

V. Diafiltration

Diafiltration is used for offset concentration effects and to achieve protein recovery of high purity and high yield. We will use two diafiltration steps: the first step (D-101) after washing in a mixing tank (T-102) and the second step (D-102) after the first ultrafiltration step (U-101). We will use the MaxCell Ultrafiltration Hollow Fiber Tangential Flow Cartridge, UF-3-C-85, membranes with a nominal molecular weight cutoff (NMWC) of 3 kDa. This type of hollow fiber membrane has a membrane area of 13 m^2 and will have a rejection coefficient of around 0.995 and a rejection coefficient of the buffer around 0 for all diafiltration steps.

In the first diafiltration step (D-101), the protein yield is 0.98 and the rejection coefficient for the buffer is 0. This step is used to exchange the washing solution with WFI. The diafiltration volume for this process is 309,000 L and the permeate flow rate of 112.087 L/min (permeate flux is 1.44×10^{-4} m/s) yielded the best process time, 46.0 hours. For this diafiltration system, the inlet pressure will be at 3.4 bar and the temperature will be between 25 ^oC - 80 ^oC. Upon completion, the insulin glargine protein in solution with some of the WFI and cell debris will be sent to the first ultrafiltration system (U-101). The rest of the solvents, WFI, Buffer 18, and cell debris, will be removed and sent off as waste.

The second diafiltration step (D-102) comes after the first ultrafiltration step (U-101) and exchanges the WFI with a solubilization buffer. The diafiltration volume for this step is around 286,000 L. D-102 will have the same permeate flow rate and initial permeate flux as the D-101 and the processing time will be 42.5 hours. The inlet pressure and temperature will be kept the same as D-101. Upon completion, the insulin glargine protein and some of Buffer 25 will be sent to incubation (I-101). The majority of Buffer 25, WFI, and some of the non-recovered proteins will be sent off as waste.

VI. Ultrafiltration

Ultrafiltration steps are used to separate macrosolutes that are retained from smaller molecules that pass through an ultrafiltration membrane. We use one stage per process for our ultrafiltration systems, which does not change the membrane area. There are three ultrafiltration steps: the first step (U-101) after the first diafiltration step (D-101), the second step (U-102) after the third centrifugation step (C-103), and the third step (U-103) after the mixing tank (T-103). We will use the MaxCell Ultrafiltration Hollow Fiber Tangential Flow Cartridge, UFP-10-C-85, for all the ultrafiltration steps. The nominal molecular weight cutoff (NMWC) of this membrane is 10 kDa. The membrane area is 13 m^2.

The first ultrafiltration step (U-101) has a yield of 0.924, which stays constant through all the three steps. The permeate volume is 10,000 L and the retentate volume is 830 L. The membrane produces an average permeate flow rate of 77 L/min. This yielded the best process time, 2.37 hours. The inlet pressure will be at 3.4 bar and the temperature will be between 25^{o}C and 80^{o}C. The recovered protein solution is sent to the second diafiltration system (D-102). The cell debris and unrecovered protein in the solution with WFI will be sent off as waste.

The second ultrafiltration step (U-102) produces a permeate volume of 120,000 L and a retentate volume of 9,900 L. The process time for this step is 28.1 hours and requires the same inlet pressure and operating temperature as the previous step. The recovered insulin glargine protein solution will be sent to the second incubation step (I-102). The waste stream consisting of unrecovered protein and a buffer, media, and adjuster solution is sent off as waste.

The third ultrafiltration step (U-103) produces a permeate volume of 1,300 L and a retentate volume of 100 L. The process time will be 0.303 hours (18.2 minutes). The same pressure and temperature conditions previously will be used again for this step, and the

recovered protein solution will be sent to cation exchange chromatography (E-101). Unrecovered protein in solution with a buffer will be sent off as waste.

VII. Incubation

Once the insulin glargine precursor is washed and separated from debris, incubators will be used to refold, convert, and precipitate the insulin glargine into its active state. Multiple incubators will be used, and all are designed to have a capacity of 20,000 L.

The first incubator (I-101) will be used for the refolding process. I-101 will have a diameter of 2.03 m and a height of 6.12 m. Four baffles will be distributed evenly around the tank, with a width of 0.2 m, and 3 impellers with a diameter of 1.02 m. The impeller speed is set to 1 rev/s and the time for complete mixing will be around 51 seconds. At least 7 incubators will be applied for this process. In the first step of the refolding process, the inclusion body solution from D-102 is diluted with a refolding buffer and β-mercaptoethanol is added to the incubation. A cooling jacket will be used to maintain the temperature of the incubators at 4℃ for 48 hours of incubation. The pH of the solution will be changed to 4.5 and the solution will be sent to the centrifuge (C-103).

The second incubator, I-102, is used for conversion and precipitation. The incubator has the same equipment specifics and the same mixing time as the incubators I-101. At least 7 incubators are needed. The first step of the conversion process is to add a borate buffer to the refolded protein solution from U-102. The pH of the solution is changed to 8.5 and citraconic anhydride is added to the solution. A cooling jacket will keep the temperature around 25℃ for 2 hours. Then trypsin is added for the peptide cleavage and the temperature is kept at 25℃ for the next 5 hours. The pH of the solution will be adjusted to 2.5 and incubated for 5 hours at 25℃ for deacylation. The last part of the incubation is precipitation in which zinc chloride is added to the solution. The temperature will be set to 4℃, and the pH will be adjusted to 6.1 for 16 hours of precipitation. The mixture will then be sent to a centrifuge (C-104) for separation.

VIII. Cation Exchange Chromatography

Once the insulin glargine is converted to its active form. Cation Exchange Chromatography (E-101) will be used to purify the insulin glargine from other similarly sized impurities. The column in CEX used for insulin glargine purification should be packed with SP Sepharose Fast Flow resin (2016), and we assume one CEX column volume will be 600 L. The length of CEX is 1.24 m and the diameter is 0.5 m, with 2 loads to meet the volume requirement. The required time for one column to complete 1 cycle is around 4.57 hours, and the productivity of one CEX column is calculated to be 0.238 g/L*min to reach 94% recovery.

The purified protein solution will be transported to the Preparative High Performance Liquid Chromatography unit (L-101) for further purification. Samples will be transported to the High Performance Liquid Chromatography unit (M-101) to monitor the purity of the protein outlet from CEX.

IX. Preparative High Performance Liquid Chromatography

The Prep-HPLC (L-101) will be used for further purification by removing all undesired proteins and peptides. The column used will be 100 L and will be packed with Zorbax resin, and the length of the column is 0.52 m with a diameter of 0.5 m. We will need 5 columns each processing 5 loads. The cycle time for 1 column is around 7.19 hours, and we will have a productivity of 0.82 g/L*min to reach 90.7% recovery of insulin glargine. Purified protein will then go to the lyophilizer (LY-101) to freeze-dry the final product. Samples of the purified protein will also be sent to an HPLC unit (M-102) to check the purity.

X. High Performance Liquid Chromatography

High performance liquid chromatography (HPLC) will be used to analyze the purity of the protein solutions leaving the chromatography units (M-101 and M-102). A Protein & Peptide C4 analytical column (250 mm × 4.6 mm column, particle size of 5 μm) will be used for both processes. The purity of each solution will be tested five times using 20μL samples. The UV detector will monitor the protein's absorbance to determine the purity of the samples.

XI. Lyophilization

After the final purification step, the product will be lyophilized in four cycles using the unit GEA LYOVAC FCM 800. The lyophilizer will freeze the product to -75 C and then reduce the pressure to 0.01 mbar to sublimate the solvents. 49,875 vials of 30 mm diameter and 110 mm height will be lyophilized each cycle. After lyophilization, the API will be sold to a secondary company to formulate.

XII. Ancillary Equipment

The process requires 22 pumps to add feed streams to unit operations. The process will use Alfa Laval's SX2WLD rotary lobe pumps. These pumps are positive displacement pumps that use gentle pump action to minimize product damage.

The process requires multiple holding and mixing tanks for the various feed inputs throughout the process. A total of 17 types of tanks have been designed; ranging from 1-472,000 L and hold components including, but not limited to: NaOH, fermentation media, buffers, pH adjusters, and waste. All but two tanks have 3 impellers; many of these tanks are used for mixing our buffers and media to their desired compositions.

XIII. Stream Table

The stream table, Table 5.12.a, includes all of the inputs and outputs of each step in the production process. Buffers, adjusters, and media which proceed through multiple steps are listed according to the stream number where their makeup is described. For example, Buffer 13 corresponds to the buffer listed in Stream 13. The composition of Buffer 13 is the same as the buffer in Stream 13. We used this method to reduce redundancy in the streams.

Table 5.12.a

Stream Table

Stream Number	Location	Content	Flow Rate	
1	F-101 Input	LB Broth	2.00	L/batch
		Glucose	400.00	g/batch
		Cells	0.13	g/batch
		Oxygen	299.52	g/batch
		Ampicillin	0.10	g/batch
2	F-102 Input	LB Broth	20.00	L/batch
		Glucose	4000.00	g/batch
		Oxygen	1249.23	g/batch
		Ampicillin	1.00	g/batch
3	F-103 Input	LB Broth	200.00	L/batch
		Glucose	40000.00	g/batch
		Oxygen	12492.29	g/batch
		Ampicillin	10.00	g/batch
4	F-104 Input	LB Broth	2000.00	L/batch
		Glucose	400000.00	L/batch
		Oxygen	124922.88	g/batch
		Ampicillin	100.00	g/batch
5	F-105 Input	LB Broth	20000.00	L/batch

		Glucose	4000000.00	g/batch
		Oxygen	12492288.00	g/batch
		Ampicillin	1000.00	g/batch
6	F-101 Output F-102 Input	Growth Media	2.00	L/batch
		Cells	0.04	kg/batch
7	F-102 Output F-103 Input	Growth Media	20.00	L/batch
		Cells	0.36	kg/batch
8	F-103 Output F-104 Input	Growth Media	200.00	L/batch
		Cells	3.60	kg/batch
9	F-104 Output F-105 Input	Growth Media	2000.00	L/batch
		Cells	36.00	kg/batch
10	Fermentation Output C-101 Feed	Cell Mass	359.68	kg/batch
		Growth Media	20000.00	L/batch
11	C-101 Output T-101 Input	Cells	356.07	kg/batch
		Growth Media	356.07	L/batch
12	C-101 Waste	Cells	3.62	kg/batch
		Growth Media	19643.93	L/batch
13	T-101 Buffer Input	Sucrose	712.13	kg/batch
		Tris	86.27	kg/batch
		EDTA	104.06	kg/batch
		Sodium Chloride	83.23	kg/batch
		WFI	7121.31	L/batch
14	T-101 Output H-101 Input	Cells	356.07	kg/batch
		Buffer 13	7121.31	L/batch
		Growth Media	356.07	L/batch
15	H-101 Output C-102 Input	Protein	69.07	kg/batch
		Cell Debris	287.00	kg/batch
		Buffer 13	7121.31	L/batch
		Growth Media	356.07	L/batch
16	C-102 Output T-102 Input	Protein	68.38	kg/batch

		Cell Debris	284.13	kg/batch
		Buffer 13	16.79	L/batch
		Growth Media	335.72	L/batch
17	C-102 Waste	Protein	0.69	kg/batch
		Cell Debris	2.87	kg/batch
		Buffer 13	6785.59	L/batch
		Growth Media	339.28	L/batch
18	T-102 Buffer Input	Tris	25.62	kg/batch
		EDTA	3.09	kg/batch
		Lysozyme	2.86	kg/batch
		Triton	113.16	kg/batch
		Urea	317.58	kg/batch
		WFI	10575.30	L/batch
19	T-102 Output D-101 Input	Protein	68.38	kg/batch
		Cell Debris	284.13	kg/batch
		Buffer 18	10575.30	L/batch
		Buffer 13	335.72	L/batch
		Growth Media	16.79	L/batch
20	D-101 WFI Input	WFI	63451.80	L/batch
21	D-101 Output U-101 Input	Protein	67.01	kg/batch
		Cell Debris	278.44	kg/batch
		WFI	10575.30	L/batch
22	D-101 Waste	Protein	1.37	kg/batch
		Cell Debris	5.68	kg/batch
		Buffer 18	10575.30	L/batch
		WFI	52876.50	L/batch
		Growth Media	16.79	L/batch
		Buffer 13	335.72	L/batch
23	U-101 Output D-102 Input	Protein	61.92	kg/batch
		WFI	10097.30	L/batch

24	U-101 Waste	Protein	5.09	kg/batch
		Cell Debris	278.44	kg/batch
		WFI	830.51	L/batch
25	D-102 Buffer Input	Urea	5355.24	kg/batch
		Glycine	8.34	kg/batch
		WFI	60583.78	L/batch
26	D-102 Output I-101 Input	Protein	60.68	kg/batch
		Buffer 25	10097.30	L/batch
27	D-102 Waste	Protein	1.24	kg/batch
		WFI	10097.30	L/batch
		Buffer 25	50486.48	L/batch
28	I-101 Buffer Input	Urea	4306.95	kg/batch
		Glycine	89.72	kg/batch
		WFI	119517.98	L/batch
29	I-101 Media Input	β-mercaptoethanol	10.13	kg/batch
30	I-101 pH Adjuster Input	HCl	43.97	kg/batch
		WFI	240.92	L/batch
31	I-101 Output C-103 Input	Protein	49.82	kg/batch
		Misfolded Protein	10.86	kg/batch
		Buffer 25	10097.30	L/batch
		Buffer 28	119517.98	L/batch
		Media 29	10.13	kg/batch
		Adjuster 30	240.92	L/batch
32	C-103 Output U-102 Input	Protein	49.32	kg/batch
		Buffer 25	10097.13	L/batch
		Buffer 28	119507.31	L/batch
		Media 29	10.13	kg/batch
		Adjuster 30	240.90	L/batch
33	C-103 Waste	Misfolded Protein	10.86	kg/batch
		Buffer 25	0.17	L/batch
		Buffer 28	10.67	L/batch
		Media 29	0.00	kg/batch

		Adjustor 30	0.02	L/batch
34	U-102 Output I-102 Input	Protein	45.57	kg/batch
		Buffer 25	9329.74	L/batch
		Buffer 28	110424.75	L/batch
		Media 29	9.36	kg/batch
		Adjuster 30	222.59	L/batch
35	U-102 Waste	Protein	3.75	kg/batch
		Cell Debris	0.00	kg/batch
		Buffer 25	767.38	L/batch
		Buffer 28	9082.56	L/batch
		Media 29	0.77	kg/batch
		Adjuster 30	18.31	L/batch
36	I-102 Buffer Input	Boric Acid	1.69	kg/batch
		Sodium Tetraborate	10.43	kg/batch
		WFI	1367.19	L/batch
37	I-102 Input	Citraconic Anhydride	194.14	kg/batch
38	I-102 pH Adjustment Input	NaOH	5.92	kg/batch
		WFI	14.80	L/batch
39	I-102 Media Input	Trypsin	0.16	kg/batch
40	I-102 pH Adjustment Input	HCl	5.71	kg/batch
		WFI	1567.36	L/batch
41	I-102 pH Adjustment Input	ZnCl	19.89	kg/batch
		WFI	110.49	L/batch
42	I-102 pH Adjustment Input	NaOH	1294.39	kg/batch
		WFI	3235.98	L/batch
43	I-102 Output C-104 Input	Protein	45.57	kg/batch
		Buffer 25	9329.74	L/batch
		Buffer 28	110424.75	L/batch
		Media 29	9.36	kg/batch
		Adjuster 30	222.59	L/batch
		Buffer 36	1367.19	L/batch
		Adjuster 37	194.14	kg/batch

		Media 38	0.16	kg/batch
		Adjuster 39	14.80	L/batch
		Adjuster 40	1567.36	L/batch
		Adjuster 41	110.49	L/batch
		Adjuster 42	3235.98	L/batch
44	C-104 Output T-103 Input	Protein	45.12	kg/batch
		Buffers, Media, Adjusters, and Citraconic Anhydride 43	45.12	L/batch
45	C-104 Waste	Protein	0.46	kg/batch
		Buffers, Media, Adjusters, and Citraconic Anhydride 43	126431.45	L/batch
46	T-103 Buffer Input	Urea	569.05	kg/batch
		Acetic Acid	20.32	kg/batch
		WFI	1353.52	L/batch
47	T-103 Output U-103 Input	Protein	45.12	kg/batch
		Buffers, Media, Adjusters, and Citraconic Anhydride 43	45.12	L/batch
		Buffer 46	1353.52	L/batch
48	U-103 Output E-101 Input	Protein	41.69	kg/batch
		Buffer 46	41.69	L/batch
		Buffers, Media, Adjustors, and Citraconic Anhydride 43	1250.65	L/batch
49	U-103 Waste	Protein	3.43	kg/batch
		Buffer 46	102.87	L/batch
		Cell Debris	3.43	kg/batch
		Buffers, Media, Adjustors, and Citraconic Anhydride 43	0.00	L/batch
50	E-101 Buffer Input	Urea	5045.04	kg/batch
		Acetic Acid	180.16	kg/batch
		WFI	12000.00	L/batch

51	E-101 Buffer Input	Urea	50450.40	kg/batch
		Acetic Acid	1801.56	kg/batch
		WFI	120000.00	L/batch
52	E-101 Buffer Input	Urea	3027.02	kg/batch
		Acetic Acid	108.09	kg/batch
		Sodium Chloride	294.54	kg/batch
		WFI	7200.00	L/batch
53	E-101 Output L-101 Input	Protein	39.19	kg/batch
		Buffer 52	7200.00	L/batch
54	M-101 Input	Protein	2.60E-05	kg/batch
		Buffer 52	2.00E-04	L/batch
55	E-101 Waste	Protein	2.50	kg/batch
		Buffer 46	41.69	L/batch
		Buffer 50	1250.65	L/batch
		Buffer 51	12000.00	L/batch
		Buffers, Media, Adjustors, and Citraconic Anhydride 43	120000.00	L/batch
56	M-101 Buffer Input	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
57	M-101 Waste	Protein	2.60E-05	kg/batch
		Buffer 52	2.00E-04	L/batch
		Buffer 56	2.00E-04	L/batch
58	L-101 Solvent Input	Acetic Acid	360.31	kg/batch
		ACN	3600.00	L/batch
		WFI	20400.00	L/batch
59	L-101 Solvent Input	Acetic Acid	360.31	kg/batch
		ACN	3600.00	L/batch
		WFI	20400.00	L/batch
60	L-101 Solvent Input	Acetic Acid	216.19	kg/batch
		ACN	4320.00	L/batch

		WFI	10080.00	L/batch
61	L-101 Output LY-101 Input	Protein	35.54	kg/batch
		Buffer 60	14400.00	L/batch
62	M-102 Input	Protein	2.40E-05	kg/batch
		Buffer 60	2.00E-04	L/batch
63	L-101 Waste	Protein	3.65	kg/batch
		Buffer 52	7200.00	L/batch
		Buffer 58	24000.00	L/batch
		Buffer 59	24000.00	L/batch
64	M-102 Buffer Input	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
65	M-102 Waste	Protein	2.40E-05	kg/batch
		Buffer 60	2.00E-04	L/batch
		Buffer 64	2.00E-04	L/batch
66	LY-101 Output	Protein	35.54	kg/batch
67	LY-101 Waste	Buffer 60	14400.00	L/batch

XIV. Schedule for Batch Operations

The schedule for batch operations includes both the upstream and downstream processes. The upstream process will take 49.5 hours to complete one batch, which includes the process, CIP/SIP, drain, and fill times. The downstream process will take 554 hours to complete one batch, which also includes the process, CIP/SIP, drain, and fill times. All told, the manufacturing process of insulin glargine will take about 604 hours to complete, which is around 25.2 days. We will run two upstream and downstream systems with an 18-hour start time between batches.

We will be required to use multiple sets of equipment in the downstream process due to batch overlap in some of the longer processes. Four units will be required for the first diafiltration step (D-101), the second ultrafiltration step (U-102), the first incubation step (I-101), the cation exchange chromatography step (E-101), and the prep-HPLC step (L-101). Three units will be required for the second incubation step (I-102) and the second diafiltration step (D-102). There will be no additional equipment needed for the upstream process as there is no overlap between batches.

XV. CIP/SIP

The process will use clean-in-place and steam-in-place systems. After the product exits a unit operation, a clean-in-place process ensues beginning with a WFI rinse, followed by a 0.5% NaOH at 60°C rinse, and finishing with another WFI rinse to clean the equipment. After the clean-in-place cycle is complete, a steam-in-place cycle ensues using clean steam at 121°C and 15 psig to sterilize the equipment. The batch-style equipment (fermenters, tanks) will be cleaned using Alfa Laval's LKRH F-version fixed static spray ball with 360° coverage while the cleaning and sterilizing agents will just run through the continuous-style equipment.

XVI. Waste Treatment

Three types of waste will be produced. First, streams that contain *E.coli* cells will be decontaminated through a continuous decontamination system from Actini, at 135°C, for 2 mins. The decontaminated waste will then be sent to the public sewer system. Second, waste streams containing acetonitrile will be collected and sent to toxic industrial waste collectors. Finally, all other waste streams are collected and neutralized to a pH of 7 using a caustic NaOH solution from the CIP step. The neutralized streams will then be sent to the public sewer system. The remaining NaOH from CIP will be neutralized with sulfuric acid before going into the public sewer system.

XVII. Process Economics

i. Plant Capital Cost

Capital costs are accrued at the beginning of plant operation. They are one-time expenses to prepare the plant for operation such as land, purchased equipment, and piping. To begin our capital cost estimate, equipment cost was calculated.

The total main equipment cost is $67.5 million as shown in Table 17.1.a, and the total ancillary equipment cost is $31.5 million as shown in Table 17.1.b. In total, $99.0 million worth of capital was spent on purchased equipment. Prices from the purchased equipment were found using available prices on websites, estimating from previous projects, or using custom estimates based on previous personal projects.

Table 17.1.a

Main Equipment Cost

Category	Model	Quantity	Cost per Unit	Total Cost
Fermentation	Xcellerex XDR 10	2	$5,200	$10,400
	Xcellerex XDR	2	$6,670	$13,340
	Xcellerex XDR 200	2	$9,680	$19,360
	Xcellerex XDR 2000	2	$56,300	$112,600
	Custom Fermenter	2	$1,500,000	$3,000,000
Centrifugation	Alfa Laval MOPX 213	8	$750,000	$6,000,000
Mixing Tanks	Custom 1500 L Stainless Steel Tank	2	$45,000	$90,000
	Custom 7300 L Stainless Steel Tank	2	$324,000	$648,000
	Custom 10900 L Stainless Steel Tank	2	$1,080,000	$2,160,000
High-Pressure Homogenization	Ariete NS3030	2	$69,500	$139,000
Diafiltration	UniFlux 120	14	$200,000	$2,800,000
Ultrafiltration	UniFlux 120	12	$200,000	$2,400,000
Incubation	Custom 20,000 L Stainless Steel Tank	98	$440,000	$43,120,000
CEX Chromatography	AKTA pcc	8	$250,000	$2,000,000
HPLC	Kromasil C18 HPLC Column (250x4.6 mm)	4	$989	$3,956
Prep-HPLC	Xselect Peptide CSH C18 Prep Column (250x4.6 mm)	8	$1,430	$11,440
Lyophilization	GEA LYOVAC FCM 800	2	$2,500,000	$5,000,000
Total Main Equipment Cost				**$67,528,096**

Table 17.1.b

Ancillary Equipment Costs

Equipment	Model	Quantity	Cost per Unit	Total Cost
Pump	Alfa Laval SX2WLD	22	$4,250	$93,500
1 L Holding Tank	Custom Stainless Steel Tank	1	$30	$30
50 L Holding Tank	Custom Stainless Steel Tank	1	$1,500	$1,500
1500 L Holding Tank	Custom Stainless Steel Tank	1	$45,000	$45,000
2000 L Holding Tank	Custom Stainless Steel Tank	1	$60,000	$60,000
4000 L Holding Tank	Custom Stainless Steel Tank	1	$120,000	$120,000
5000 L Holding Tank	Custom Stainless Steel Tank	153	$145,000	$22,200,000
6000 L Holding Tank	Custom Stainless Steel Tank	40	$180,000	$7,200,000
7300 L Holding Tank	Custom Stainless Steel Tank	2	$324,000	$648,000
11000 L Holding Tank	Custom Stainless Steel Tank	1	$1,080,000	$1,080,000
Total Ancillary Equipment Cost				**$31,448,030**

Once the cost of purchasing all equipment was determined. The other capital costs were scaled according to the equipment cost. The total capital cost was calculated using a lang factor according to Equation 17.1.a (Turton et al., 2012).

$$Capital\ Cost\ =\ Lang\ Factor\ *\ Sum\ of\ all\ major\ purchased\ equipment \quad (17.1.a)$$

4.74 was chosen for the lang factor because our plant is fluid processing (Turton et al., 2012). Peters and Timmerhaus provide a recommended range of the percent of capital cost for each capital expenditure (1991). These ranges are seen in Table 17.1.c. The percentages we used in our calculations were chosen based on previous capstone projects and estimation based on our project (Wilson et al., 2015; Bloom et al., 2022). A breakdown of the capital cost is shown in Table 17.1.c. The total capital cost for our project is about $469 million.

Table 17.1.c

Capital Cost

Component	Recommended Range (%)	Actual %	Cost
Direct Costs			
Purchased Equipment	15-40	21	$98,976,126
Purchased Equipment Installation	6-14	8	$37,062,600
Instrumentation and Controls (installed)	2-12	6	$28,148,810
Piping (installed)	4-17	6	$28,148,810
Electrical (installed)	2-10	3	$14,074,405
Buildings (including services)	2-18	11	$51,606,152
Yard Improvements	2-5	2	$9,382,937
Service Facilities (installed)	8-30	10	$46,914,684
Land	1-2	2	$9,382,937
Indirect Costs			
Engineering and Supervision	4-20	15	$70,372,026
Construction Expense	4-17	5	$23,457,342
Contractor's Fee	1-3	1	$4,691,469
Contingency	5-15	10	$46,914,684
Total Fixed Capital Investment			**$469,146,837**

ii. Plant Operating Cost

The plant operating cost includes costs associated with running the process and operating such as raw materials, utilities, and labor. These expenses are continuous throughout the life of the plant. The first step in calculating the operating cost was to determine the raw materials and utilities costs. The cost for raw materials was found from various vendors. While the plant would purchase bulk quantities of all raw materials, bulk prices were not always available. Thus, the operating cost will most likely be an overestimate of the actual operating cost. The prices for the raw materials are found in Table 17.2.a. The total cost of raw materials is about $1.1 billion per year.

Table 17.2.a

Raw Materials Cost

Category	Material	Amount /batch	Unit	Amount /year	Unit Price	Total Price	Percent of Total
Fermentation	LB Medium	500	kg	457000	$162.92	$74,455,354	6.87%
	Ampicillin	1.00	kg	914	$9,120.00	$8,335,680	0.77%
	glucose	4,444	kg	4062182	$14.96	$60,770,236	5.60%
Washing	sucrose	712.13	kg	650887	$22.03	$14,339,036	1.32%
	Tris	86.27	kg	78851	$115.84	$9,134,074	0.84%
	EDTA	104.06	kg	95111	$134.00	$12,744,852	1.18%
	NaCl	83.23	kg	76072	$14.84	$1,128,911	0.10%
	Tris	25.62	kg	23417	$115.84	$2,712,588	0.25%
	EDTA	3.09	kg	2824	$134.00	$378,450	0.03%
	Lysozyme	2.86	kg	2614	$12,056.00	$31,514,866	2.91%
	Triton X-100	80.96	L	96662	$10.04	$970,342	0.09%
	Urea	317.58	kg	290268	$17.60	$5,108,718	0.47%
Refolding	Urea	9662.19	kg	8831242	$17.60	$155,429,853	14.34%
	Glycine	98.06	kg	89627	$31.70	$2,840,815	0.26%
	β-mercaptoethanol	6.54	L	7806	$3,556.00	$27,758,328	2.56%
	HCl	43.97	kg	40189	$7.44	$298,883	0.03%
Conversion	Boric Acid	1.69	kg	1545	$2.67	$4,120	0.00%
	Sodium Tetraborate	10.43	kg	9533	$2.07	$19,755	0.00%
	Citraconic Anhydride	194.14	kg	177444	$346.00	$61,395,610	5.66%
	Trypsin	0.16	kg	146	$822.00	$120,209	0.01%
	NaOH	5.92	kg	5411	$2.25	$12,166	0.00%
	HCl	5.71	kg	5219	$7.44	$38,813	0.00%
	ZnCl	8.25	kg	7541	$45.12	$340,227	0.03%
Resolve	Urea	569.05	kg	520112	$17.60	$9,153,965	0.84%
	Acetic Acid	14.81	L	17688	$12.48	$220,784	0.02%
Filtration	Filter	-	-	$22.00	$10,500.00	$231,000	0.02%

CEX	Urea	13117.1	kg	11989029	$17.60	$211,006,917	19.46%
	Packing	600	L	1200	$582,000.00	$1,164,000	0.11%
	Acetic Acid	1523.56	L	1819130	$12.48	$22,706,609	2.09%
	NaCl	294.54	kg	269210	$0.77	$207,623	0.02%
Prep-HPLC	Acetic Acid	682.97	L	815471	$12.48	$10,178,810	0.94%
	ACN	11520	L	10529280	$20.29	$213,622,026	19.70%
	Packing	100	L	200	$101,020.41	$20,204,081	1.86%
Sterilization	NaOH	293.69	kg	268430	$2.25	$603,563	0.06%
Neutralize Waste	Sulfuric Acid	340	kg	310760	$4.90	$1,523,714	0.14%
Packaging	Glass Jars	185199	jars	169271886	$0.73	$123,568,476	11.40%
Total Cost of Raw Materials						**$1,084,243,470**	**$/year**
						$908,076	**$/batch**

To determine the cost of waste treatment, the incineration price for waste is estimated to be around $108 per tonnes based on the price in European countries, as the price in Singapore should be similar to this (Anderson, Personal conversion, 2023; European Commision, 2006). The total amount of waste to be incinerated per batch is around 69,600 L and around 906 batches are produced per year; the total cost of waste treatment is around $6.9 million per year.

The cost of utilities was then determined. Water for injection, steam, and oxygen are all assumed to be utilities as we will purchase them from a supplier. Utilities associated with the plant and not the process such as lighting, air conditioning, and general water usage were considered out of the scope of this project and not considered in this economic analysis. The cost of electricity was determined by the power needed to maintain the main and ancillary equipment. The power requirements for each piece of equipment are seen in Table 17.2.b.

Table 17.2.b

Power Consumption

Equipment Tag	Description	Power Consumption (kW)	Process Time (h)	CIP/SIP Time (h)	Energy Usage (kWh)
F-101	2L Fermenter	0.23	13.20	0.00	3.03
F-102	20L Fermenter	1.06	5.42	0.00	5.75
F-103	200L Fermenter	4.50	5.42	0.00	24.39
F-104	2,000L Fermenter	21.00	5.42	0.00	113.82
F-105	20,000L Fermenter	43.00	5.42	0.00	233.06
C-101	Centrifuge	16.00	0.98	1.00	31.65
T-101	7300 L Tank	1.02	0.01	1.56	1.61
T-102	10900 L Tank	1.99	0.01	1.26	2.54
T-103	1500 L Tank	0.07	0.01	0.65	0.05
H-101	High-Pressure Homogenizer	30.00	11.50	1.00	375.00
C-102	Centrifuge	16.00	0.37	1.00	21.86
D-101	Diafiltration	0.00	44.70	1.00	0.00
U-101	Ultrafiltration	0.00	0.97	1.00	0.00
D-102	Diafiltration	0.00	9.13	1.00	55.54
I-101	Incubator	5.48	48.00	9.40	314.72
C-103	Centrifuge	16.00	5.95	1.00	111.20
U-102	Ultrafiltration	0.00	11.20	1.78	0.00
I-102	Incubator	5.48	28.00	1.77	163.23
C-104	Centrifuge	16.00	2.58	1.00	57.28
U-103	Ultrafiltration	0.00	0.12	1.00	0.00
E-101	Cation Exchange Chromatography	1.00	9.14	1.00	10.14
M-101	HPLC	0.00	0.00	1.00	0.00
L-101	Prep-HPLC	1.40	35.95	1.00	51.73
M-102	HPLC	0.00	0.00	1.00	0.00
LY-101	Lyophilizer	210.00	1.50	1.00	525.00

P-101	Pump	1.32	0.00	0.00	0.00
P-102	Pump	1.32	0.00	0.10	0.14
P-103	Pump	1.32	0.04	0.23	0.35
P-104	Pump	1.32	0.40	0.56	1.26
P-105	Pump	1.32	4.00	1.83	7.69
P-106	Pump	1.32	0.07	4.09	5.49
P-107	Pump	1.32	1.50	4.09	7.37
P-108	Pump	1.32	1.50	0.13	2.15
P-109	Pump	1.32	0.07	4.09	5.49
P-110	Pump	1.32	2.19	0.48	3.52
P-111	Pump	1.32	2.12	1.35	4.57
P-112	Pump	1.32	0.81	1.35	2.84
P-113	Pump	1.32	0.37	1.35	2.27
P-114	Pump	1.32	24.32	5.89	39.88
P-115	Pump	1.32	24.32	4.09	37.50
P-116	Pump	1.32	9.31	1.35	14.07
P-117	Pump	1.32	10.56	0.85	15.06
P-118	Pump	1.32	0.90	4.09	6.59
P-119	Pump	1.32	0.28	0.11	0.51
P-120	Pump	1.32	0.11	1.35	1.92
P-121	Pump	1.32	1.44	0.00	1.90
P-122	Pump	1.32	2.88	0.00	3.80
Total		**419.28**	-	-	**2265.96**

Ethylene glycol is used throughout our process as a cooling agent. The price of the utility was calculated as the cost of energy to remove heat from the units. Finally, steam is used during sterilization. The price of steam was calculated as the cost to heat the volume of steam needed to 121°C, the temperature necessary for sterilization. The cost estimate for utilities is shown in Table 17.2.c. The total cost of utilities is about $282 million per year.

Table 17.2.c.

Utility Cost

Material	Amount /batch	Unit	Amount /year	Unit Price	Total Price	Percent of Total
Water for Injection	436255	L	398736878	$0.70	$279,115,814	98.82%
Electricity	2266	kWh	2071084	$0.23	$476,349	0.17%
Ethylene Glycol	7,575	kWh	6923742	$0.23	$1,592,460	0.56%
Oxygen	1388	kg	1268808	$0.99	$1,253,143	0.44%
Steam	45	kWh	41083	$0.23	$9,449	0.00%
Total Cost of Utilities					**$282,447,217**	**$/year**
					$236,555	**$/batch**

The cost of operating labor was determined using Equation 17.2.a where N_{OL} is the number of operators on shift, P is the number of processes that need operators, and N_{np} is the number of remaining processes (Turton et al., 2012).

$$N_{OL} = (6.29 + 31.7P^2 + 0.23N_{np})^{0.5} \quad (17.2.a)$$

Five of the 25 steps in our process involve operators, so we will need 28 operators per process. Because we have two full upstream and downstream processes, we will need 56 operators for the entire plant. The operators will work 40 hours a week, and the plant will be running 24 hours a day and 7 days a week. We will need 4.5 times the number of operators on shift staffed to cover the total shifts per year. Thus, we will have 254 operators staffed. In Singapore, the average salary of an operator is $33,336, so our total operating labor cost is about $8.5 million per year (*How much does an Operator make in Singapore, Singapore?*, 2023).

The cost of manufacturing, COM, was calculated using Equation 17.2.b where FCI is the fixed capital investment described in the previous section (Turton et al., 2012). C_{rm} is the cost of raw materials, C_{wt} is the cost of waste treatment, and C_{ut} is the cost of utilities.

$$COM = 0.28FCI * 2.73C_{OL} + 1.23(C_{rm} + C_{wt} + C_{ut}) \quad (17.2.b)$$

Other costs associated with operation were scaled using factors from Turton et al. (2012). The various operating costs are shown in Table 17.2.d. The total cost of operation is about $1.8 billion per year.

Table 17.2.d

Operating Cost

Direct Cost		
Raw materials	Crm	$1,084,243,470
Waste treatment	Cwt	$6,867,175
Utilities	Cut	$282,447,218
Operating labor	Col	$8,466,699
Direct and supervisory and clerical labor	0.18*Col	$1,524,006
Maintenance and repairs	0.06*FCI	$28,148,810
Operating supplies	0.009*FCI	$28,148,810
Laboratory charges	0.15*Col	$1,270,005
Fixed Costs		
Depreciation	0.1*FCI	$46,914,684
Local taxes and insurance	0.032*FCI	$15,012,699
Plant overhead costs	0.708*Col + 0.036*FCI	$22,883,709
General Cost		
Administration	0.177*Col+0.009*FCI	$5,720,927
Distribution and selling costs	0.11*COM	$202,834,651
Research and development	0.05*COM	$92,197,569
Total Operating Cost		**$1,826,680,431**

iii. Discounted Cash Flow Analysis

A discounted cash flow analysis was conducted to determine the value of investing in this process. Because of the large amount of equipment needed in our process, the construction time was estimated to be two years. Once construction is complete our plant will be operational for 20 years. The capital cost will be accrued over the two-year construction period and paid back over the first 10 years of operation. A 10-year straight-line depreciation was assumed to account for tax credits from the capital investment. Our plant is located in Singapore where the corporate income tax is a flat 17%.

Our current competitors include Sanofi with Lantus and Eli Lilly with Basaglar. Basaglar is a biosimilar to Lantus and is priced lower than the original therapeutic. Lantus costs $0.36 per unit which is $1,135 per month for someone who weighs 75 kg (SingleCare Team, 2022). Basaglar, the cheaper of the two, still costs $0.28 per unit or $890 per month for someone who weighs 75 kg (SingleCare Team, 2022). We intend to price our insulin glargine at $0.0075 per unit. Thus, our product will cost someone who weighs 75 kg $43.88 a month, a much less expensive price for patients. Our process does not include formulation, we will sell our API to another company at $0.0065 per unit to formulate. With this price, our yearly revenue will be about $4.8 billion.

To determine the discounted cash flow, a discount rate of 20% was assumed because insulin glargine is already on the market and produced by other companies (Stasior et al., 2018). The discounted cash flow was determined using Equation 17.3.a where TCF is the post-tax cash flow, DCF is the discounted cash flow, i is the discount rate, and t is the year.

$$TCF_t = DCF_t(1 + i)^t \quad (17.3.a)$$

Table 17.3.a

Cash Flow Analysis

Year	TCF	DCF	Cumulative DCF
-2	-$234,573,419	-$337,785,723	-$337,785,723
-1	-$234,573,419	-$281,488,102	-$619,273,825
0	$2,514,855,263	$2,514,855,263	$1,895,581,438
1	$2,514,855,263	$2,095,712,720	$3,991,294,158
2	$2,514,855,263	$1,746,427,266	$5,737,721,424
3	$2,514,855,263	$1,455,356,055	$7,193,077,479
4	$2,514,855,263	$1,212,796,713	$8,405,874,192
5	$2,514,855,263	$1,010,663,927	$9,416,538,119
6	$2,514,855,263	$842,219,939	$10,258,758,059
7	$2,514,855,263	$701,849,949	$10,960,608,008
8	$2,514,855,263	$584,874,958	$11,545,482,966
9	$2,514,855,263	$487,395,798	$12,032,878,764
10	$2,467,940,580	$398,586,182	$12,431,464,946
11	$2,467,940,580	$332,155,152	$12,763,620,098
12	$2,467,940,580	$276,795,960	$13,040,416,057
13	$2,467,940,580	$230,663,300	$13,271,079,357
14	$2,467,940,580	$192,219,416	$13,463,298,773
15	$2,467,940,580	$160,182,847	$13,623,481,620
16	$2,467,940,580	$133,485,706	$13,756,967,326
17	$2,467,940,580	$111,238,088	$13,868,205,414
18	$2,467,940,580	$92,698,407	$13,960,903,821
19	$2,467,940,580	$77,248,672	$14,038,152,494

Figure 17.3.a

Discounted Cash Flow Over the Plant Lifetime

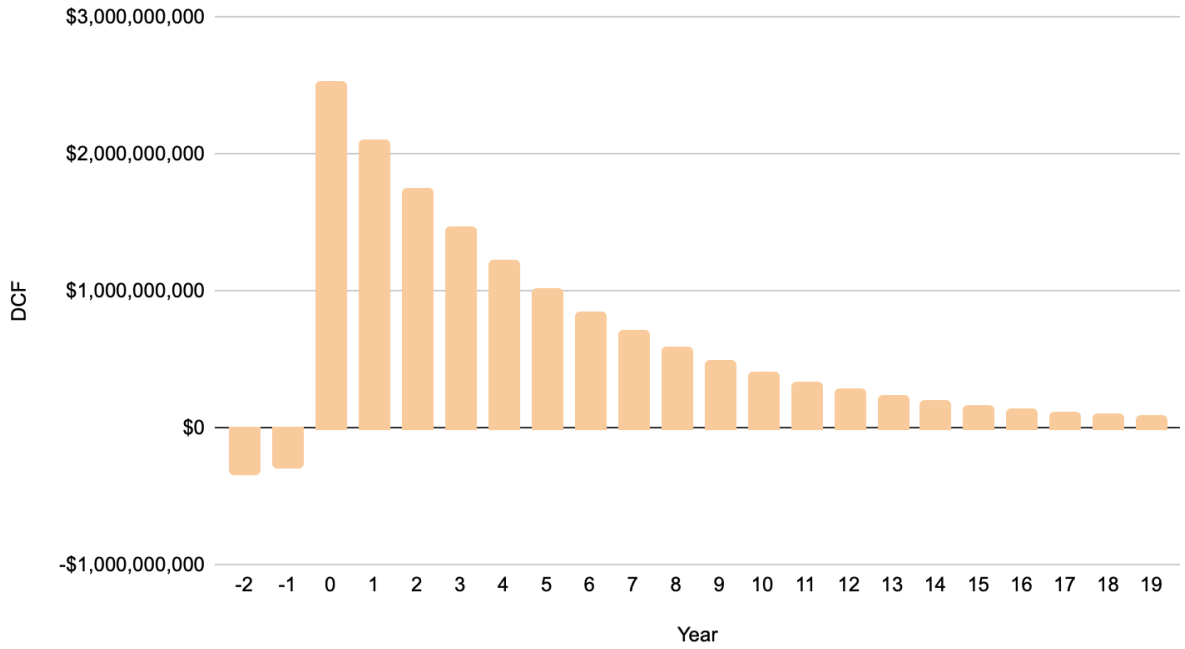
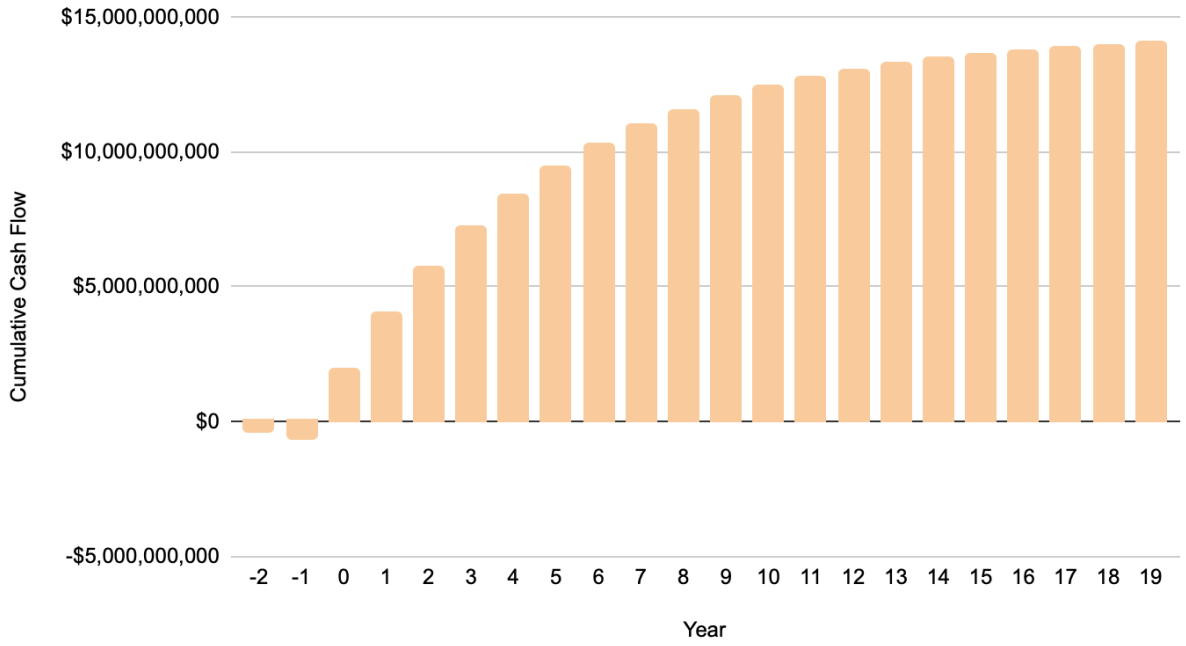


Figure 17.3.b

Cumulative Discounted Cash Flow Over Plant Lifetime



Net present value, NPV, is similar to DCF except that NPV accounts for the initial investment. In our analysis, NPV is equivalent to the cumulative cash flow. NPV is used to determine the value of the project taking into account the initial investment. The NPV of our process is over $14 billion. The internal rate of return, IRR, is the discount rate necessary to make the NPV zero. The IRR of our process is 242% meaning our process is highly profitable.

iv. Risk Analysis

The economic analysis provided here is only an estimate and many factors could change when executing this project. To be thorough with our economic calculations, a risk analysis was conducted to determine the profitability of the process if various situations occurred which could lower the profit. Two situations were modeled. The first of which is a doubled discount rate. In this scenario, a discount rate of 40% was used instead of 20%. The same calculations were conducted, and the following models were created.

Figure 17.4.a

Discounted Cash Flow Over Plant Lifetime

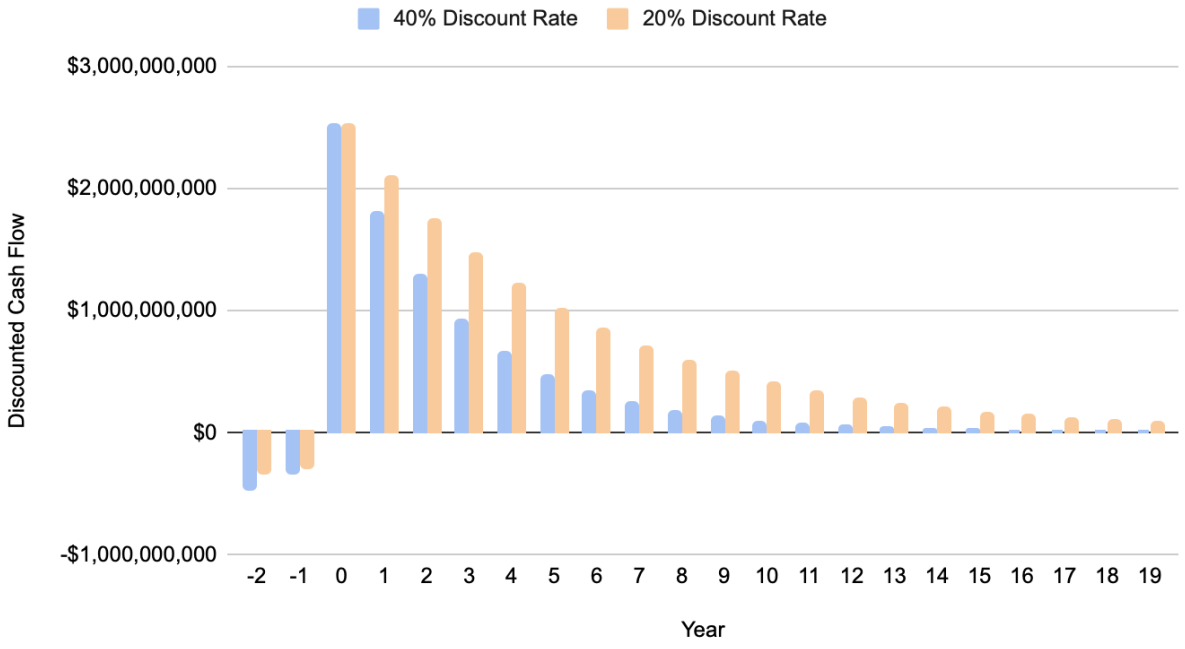
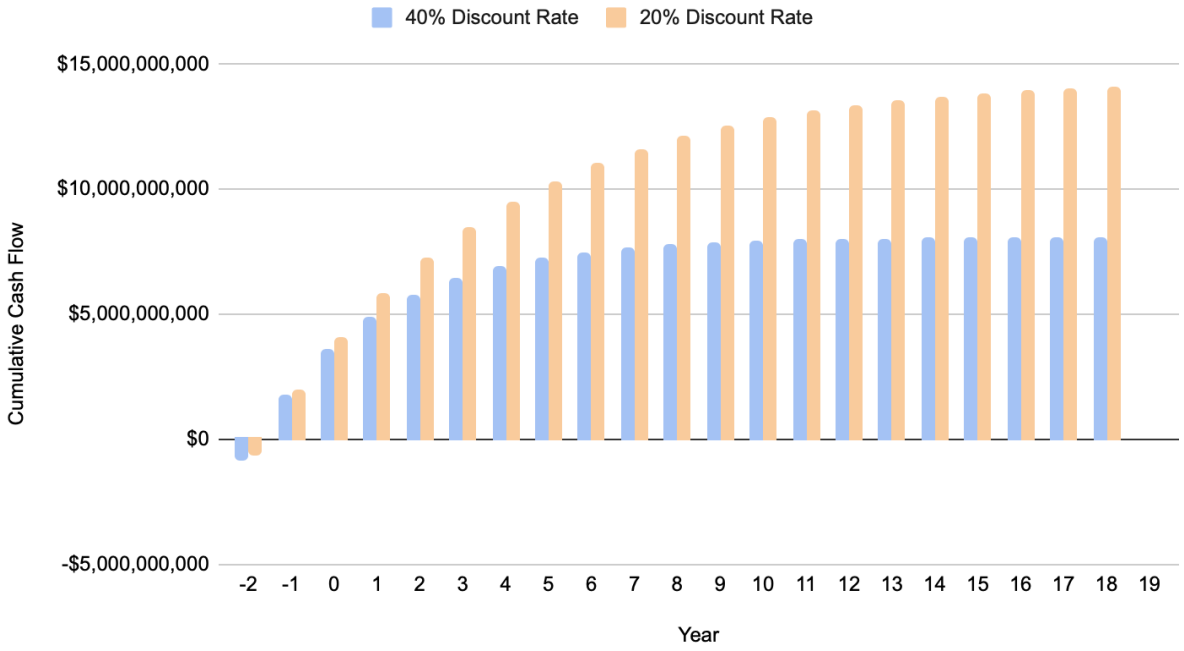


Figure 17.4.b

Cumulative Discounted Cash Flow Over Plant Lifetime



In this situation, both the DCF and the cumulative cash flow will be greatly reduced by the larger discount rate. Where there is a lowered profitability, the NPV of the process is still almost $8 billion, and the IRR remains the same at 242%. The execution of the project can continue if the discount rate is increased up to 40%.

The second modeled situation is a delay in startup. Delays can occur for a variety of reasons. The profitability of the project would decrease with a delay, as a year of production would be lost. The following models were created for this situation.

Figure 17.4.c

Discounted Cash Flow Over Plant Lifetime

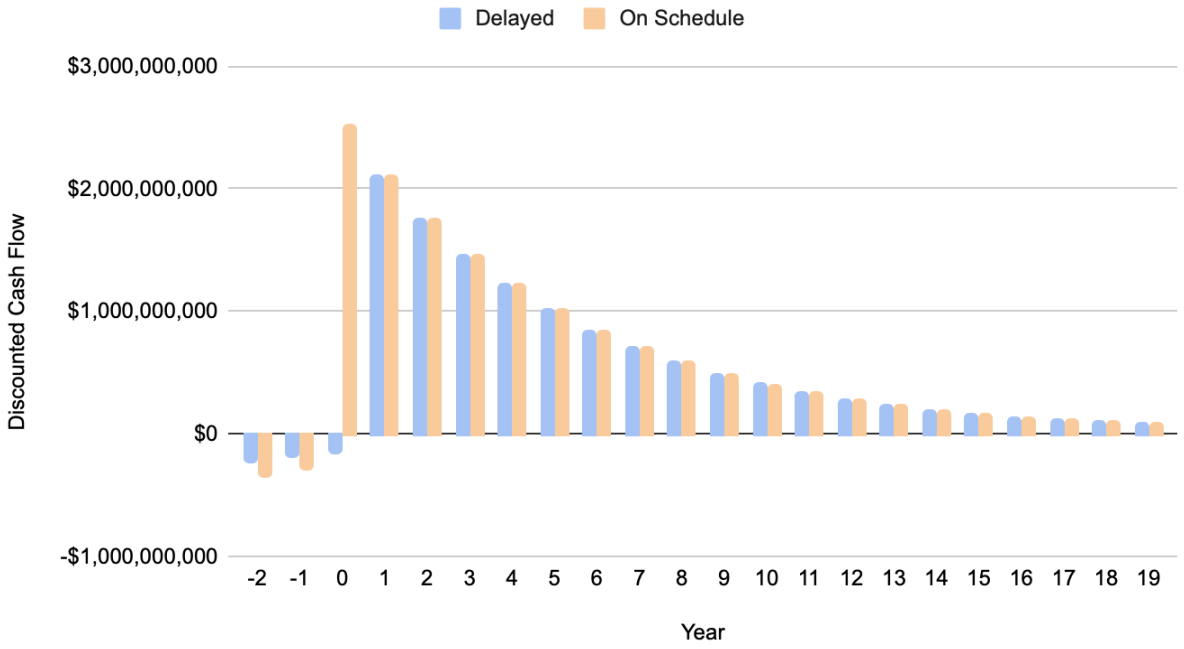
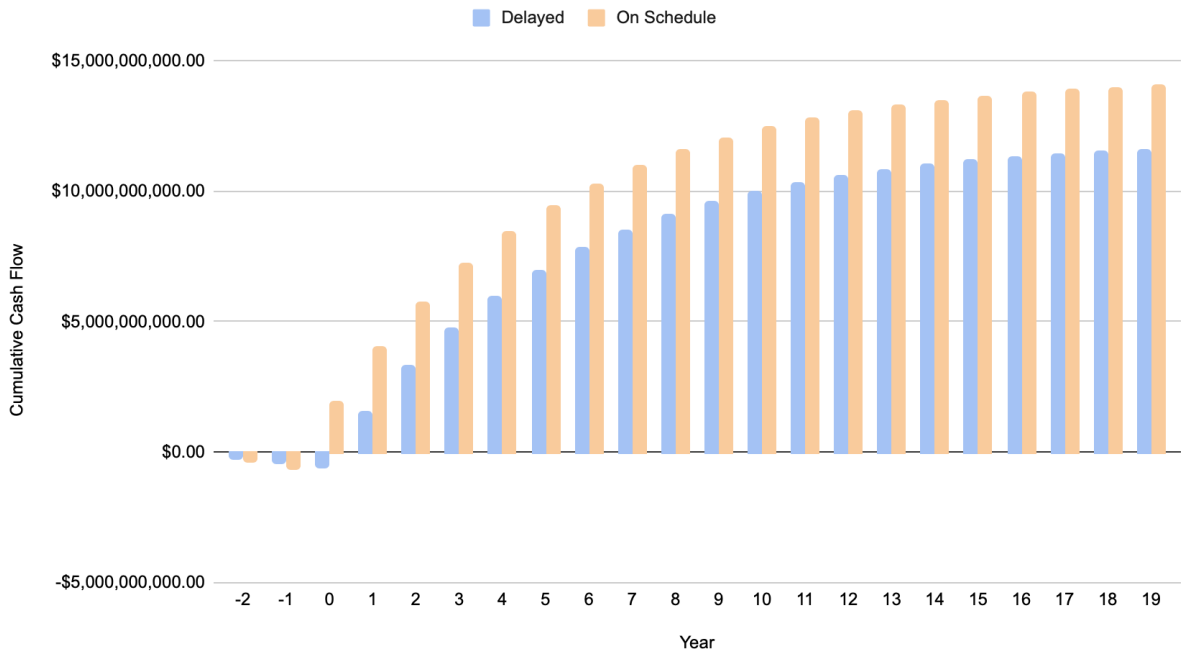


Figure 17.4.c

Cumulative Discounted Cash Flow Over Plant Lifetime



The DCF remains the same over the lifetime of the plant because the discount rate is the same, 20%, but a large sum of profit is lost in the first year of operation if the production of the insulin glargine is delayed. Due to the lost profit, the cumulative cash flow is greatly reduced in this scenario. However, the NPV is still $11.6 billion, and the IRR is 158%. Thus, in this scenario as well, the project remains profitable.

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

i. Safety and Health Considerations

The first safety consideration in this project is the safety of the workers at the site. This facility will have a multitude of workers present at all times including operators, engineers, administration, or any of the other employees who support the production of our insulin glargine product. The main hazards associated with this facility are associated with the chemicals and equipment used. Hazardous chemicals in our process such as acetic acid, sulfuric acid, hydrochloric acid, sodium hydroxide, citraconic anhydride, and acetonitrile present a danger to workers in the facility if used improperly. The dangers associated with these chemicals include but are not limited to corrosivity, toxicity, and flammability which pose a threat to employees at the site. Potential dangers associated with the necessary materials and equipment will be studied to mitigate risk and points of failure. On top of this, ample employee training and education will be conducted to further reduce risk to the health and safety of workers at the site.

The second safety consideration in this project is the safety of the patients who use our insulin glargine product. Many measures have been incorporated into the proposed process design such as filtration, sterilization, and chromatography to ensure the safety of the API. Further, our site will follow regulations set forth by the Food and Drug Administration (FDA) in the US as well as the Health Sciences Authority (HSA) in Singapore to ensure our site complies with current good manufacturing practices. Our site will also conduct regular validation testing to ensure our product is safe for patients.

ii. Environmental Concerns

The majority of the environmental concerns come from the waste generated through the process. Some waste streams contain the *E.coli* cells from equipment operations such as centrifuges. This waste has the potential to contaminate the environment if the facility fails to sterilize these gene-modified cells. Other waste streams contain hazardous chemical waste, such as ACN, which would enter the local environment and cause environmental issues if the facility sends it to the sewer system directly. Therefore, these waste streams will be sent to the Singapore government-authorized factory for proper treatment including incineration. Additionally, waste treatment costs are accounted for in the operating cost and considered in the project economic analysis. Other environmental concerns come from the materials used to produce insulin glargine. Issues like coolant leakage in the cooling jacket or other chemical releases could cause environmental concerns. These releases will be avoided as much as possible through employee training and regular equipment maintenance.

iii. Social and Ethical Concerns

There are several ethical and social issues associated with this project, including the indigent population of our target market, hesitancy toward American pharmaceuticals in the region, and the supporting employees associated with our plant. Although our plant is located in Singapore, which is considered a very stable and wealthy country, our intended consumers are those in impoverished regions in the Asian-Pacific area. The price for our insulin glargine product should be manageable by those in the targeted lower economic groups. The goal of this project was to increase the accessibility of long-acting insulin glargine in Asia where there is currently a diabetes epidemic. The proposed project will accomplish this goal, as we designed our insulin glargine product to sell for a lower price than our competitors.

We must also consider the human response to bringing an American pharmaceutical company to Asia. Some Asian countries have a distrust of American companies, and we need to alleviate this distrust. We must be respectful and become educated about the culture of the region as much as possible to ensure the project does not negatively impact the community. We must also be completely transparent in educating the developing Asian-Pacific countries about the diabetes epidemic and how our product will help the community by supplying affordable health care. Demonstrating how our plant will create jobs and how our product will help those suffering from diabetes is important in gaining trust in the area.

Finally, we must ensure our facility provides sufficient support to employees. Our plant will pay all employees working at the site standard Singaporean wages and benefits. Our employees will also undergo safety and ethical training programs to ensure that everyone is safe within the workplace.

6. Conclusion and Final Recommendations

The purpose of this project is to produce insulin glargine in an efficient and affordable manner. In order to reach 4% of the diabetic population in the Asia-Pacific region, it is proposed that an insulin glargine manufacturing plant be built in Singapore. As detailed in this report, we have designed an upstream and downstream process that will effectively, efficiently, and affordably reach the goal of producing 7.46×10^{11} units of insulin glargine per year meeting our target market.

Initially, the project was set to target 10% of the diabetic population in the Asia-Pacific region or about 23 million people. With our batch and scale design, we realized that we would have to produce about 2000 batches a year. Ultimately, we decided that servicing 4% of the target market, or about 9 million people, would be much more reasonable. With this design, production is estimated to take 49.5 hours per batch upstream and 554 hours per batch downstream. With 2 production trains, the facility is capable of producing 906 batches in a year. 906 batches a year yields $7.46*10^{11}$ units/year, suitable for 9 million patients (or 4% of the target market). The project is thus concluded to be successful in reaching the target market. Considering capital costs, operating costs, and selling each unit of our API for $0.0065, the plant will create $4.8 billion in annual revenue, with an IRR of 242% over 20 years of operation. The project is thus concluded to be economically feasible.

Due to the nature of this project being completed over the course of less than a year, some technical and logistical assumptions were made. In order to fuel additional research in the field, recommendations can be made to increase the accuracy of the design. The group has multiple recommendations for optimizing the process for sustainability. First, an in-depth analysis of using a CIP/SIP system versus single-use equipment should be conducted. Due to the

short time period that this project was conducted over, we chose to use a CIP/SIP system for cleaning our equipment due to the nature of it causing shorter time and delay than single use. However, there has been research to prove that the waste created by CIP/SIP systems is more detrimental to the environment than that of single-use equipment. In order to finally determine this tradeoff, an analysis of the pros and cons of CIP/SIP versus single-use equipment should be conducted, and the cleaning system that causes the least amount of damage to the environment should be used. Second, we have done preliminary research into the reuse of caustic and WFI cleansing cycles; however, the results were inconclusive. Further testing is required in order to determine the effectiveness of reused WFI and caustic, but the possible reuse of these materials could have extreme environmental benefits. Additionally, the results from this testing could be used in the analysis of using CIP/SIP versus single-use equipment.

Another assumption that the group had to make was in regard to operating costs. Specifically, many of the prices for raw materials were based on the price of small amounts of said materials (as these were the only quotes available). Using these figures fails to consider the bulk price of these materials, and the price relief that may come along with buying such large quantities. Additionally, the amount of pH adjusters was estimated based on pH balances. In reality, the amount of pH adjusters added would need to be determined based on monitoring the system. When estimating these costs, we erred on the side of overestimating; obtaining more accurate numbers would potentially drive down annual operating costs.

The last recommendation that our group makes is in regard to the formulation process of our insulin product. As a four-person group, it was out of the scope of our project to design the formulation process. We estimated that we would sell our API to a formulation company for $0.0065/unit, and the final product would sell to patients for $0.0075/unit. Without the ability to

fully analyze the cost of a formulation process, this was an estimate and may not be economically viable. We recommend that the formulation process is researched and designed in order to explore if it would be economically viable to include it at the same manufacturing price as our project or if selling the API to a formulation company for $0.0065/unit would be reasonable.

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