Letter of Transmittal

Dear Dr. Mallette,

We, the AIChEs and Pains, completed the design for a facility capable of producing 1000 kg of a monoclonal antibody proteins annually. Per our calculations, our design will require a direct fixed capital cost of $38 million to complete construction of the plant. The annual operating cost for the plant will be $58 million. Competitor antibody manufacturers have a selling price point of around $5 million per kilogram of antibodies. Using this figure, the annual revenue for the plant will be around $4.9 billion. We believe further verification is required to determine the accuracy of this costing calculation. We recommend proceeding with a more detailed design after the costing calculations are verified and more accurately determined.

Best regards,

AIChEs and Pains Design Team

AIChE Design Competition

Nikita Rozanov, Max Morrow, Adam Marion, and Benjamin Avery

Table of Contents

Abstract

Early clinical data suggests the potential for multiple future applications for newly developed humanized monoclonal antibodies (mAbs). Thus, the preliminary design for a manufacturing facility capable producing one ton of MAB proteins annually has been requested, with flexibility to produce product at the current reported titers of 1 to 2 g/L as well as the projected 5 to 10 g/L titers. As titers increase, excess capacity should be available for contract manufacturing, considering two-thirds of biopharmaceuticals stem from companies without the cash reserves to build their own facilities. The facility designed is animal free and ensures sterile conditions for the entire process.

The process can be broken into cell and protein production followed by protein isolation and storage. Recommended unit operations have been developed. Cell growth was modeled using Monod kinetics, assuming a doubling time of 36 hours. The commercial media BalanCD was used to determine the remaining growth and inhibition parameters. Sequentially small batch reactors of increasing size made up a seed train for rapidly growing cells, resulting in a 100-liter broth to be used for inoculating the final bioreactor. Fourteen bioreactors of 5000-liters are operated fed-batch for 660 hours each run, resulting in 7.5 kg MAB each batch at a titer of 1.5 g/L. To reach annual demands, a new batch will be started every three days. Only one downstream process is required to keep up with batch demands. A centrifuge (sigma factor of 12,300 m^2) and a depth filter (25 m^2 of equivalent area) are used to remove insoluble particles. A 116-liter MabSelect SuRe protein A chromatographic column selectively removes 3 kg of MAB proteins per cycle (resulting in three cycles per batch) and releases them into a low pH buffer. The buffer solution is stored for the first step of viral inactivation, followed by a detergent wash and an orthogonal method of microfiltration. Polishing (the final step before storage for purification) consists of a cation exchange column followed by an anion exchange. These use the POROS 50 HS and Q Sepharose FF resins and will have volumes of 72 L and 85 L, respectively. A diafiltration/ultrafiltration step between the two polishing columns will exchange the buffer and concentrate the mAb process stream. The final product is deposited into metal mini-tanks of 5 mL that are mostly submerged in a –20 °C methanol-water mixture for 45 minutes such that it is completely frozen, then these will be stored at –10 °C for up to one year.

The projected cost of the facility neglects infrastructure requirements, considering an existing facility has been proposed for use. The MABs are expected to sell at $5 per milligram, resulting in an annual revenue of $5 billion despite $32 million in capital costs and $58 million in annual utilities. Detailed design is recommended, although it is warranted to estimate research and development costs to ensure viability.

It is also recommended to perform lab and pilot scale experimentation for better cell growth kinetic models, media optimization for the current cell line, and broth characteristics such as viscosity and response to shear. Fouling mechanisms for the filters and membranes as well as resin capacity should also be investigated.

Introduction

Background

Antibodies, or immunoglobulin, are forked proteins produced by plasma cells whose purpose is to fight infection. On the edge of each fork on an antibody is a binding site. These binding sights, called fragment antigen-binding (Fab) variable regions, attach to the fitting portion of the infections, called the antigen (Janeway et al.). This binding process can cause one of two outcomes. The direct outcome is the antibody neutralizes the infection by inhibiting the methods by which the infection is spreading. Alternatively, the antibody can stick and simply become a beacon to alert more effective parts of the immune system, such as macrophages to respond (Lee and Hatzimanikatis). Monoclonal Antibodies (mAbs) are a group of antibodies all made by identical parent cells, in our case a specific mutation of Chinese hamster ovaries (CHO) and therefore they all have identical structures and binding sites. The first time this concept was widely recognized then used was in 1908 when Paul Ehrlich and Élie Metchnikoff received the Nobel Prize in Physiology and Medicine, leading to the 1910 treatment for syphilis (Tansey and Catterall). Now, mAbs are being looked to as a potential "magic bullet" therapy mode for cancer. The therapy has already demonstrated effectiveness in treating breast cancer and some varieties of lymphoma, and they show promise against blood vessel generation by tumors (Rader). The mAb therapy technique takes advantage of the selective binding utilizing the effect of once the mAb is connected to the infection or tumor, it cannot be easily removed. Since it cannot be removed, along with the bound mAb, there will be an active ingredient such as a radioisotope, for example Yttrium-90 which emits a 933.7 keV electron directly into the tumor when attached with little to no damage to surrounding tissue.

The type of cell that produces the mAb that we are concerned with is Chinese hamster ovary cell or CHO. CHOs are the cell of choice for protein production processes because of their high production rate in suspension ("CHO Cell Transfection: Reagents, Methods, Protocols"). CHO cells are the most frequently used cells in the therapeutic protein industry as they can produce up to 10 g/L of cell culture (Wurm and Hacker).

Objective and Design Considerations

The objective of this project is to generate a preliminary design for a large scale and flexible monoclonal antibody production plant. For the purposes of analyzing the final product at this stage we will be treating ad though it is Avastin and must have a final titer of 1 – 2 g/L and be flexible to operate at a higher purity achieving 5 – 10 g/L. When considering the economics of this project we can omit facility surroundings set up cost as this plant will be built at a preexisting facility with all necessary infrastructure. Good manufacturing (GMP) practices and European regulatory guidelines will both be used as a foundation for the plant design.

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Good manufacturing (GMP) practices and European regulatory guidelines will both be used as a foundation for the plant design.

The design is constrained by several factors at each phase in the plant. The initial cell seed entering the seed train will be 1 mL of 10^6 cells in a vial, and for modelling purposes we will use 36 hours for the doubling time. After the seed train is the bioreactor step which is where the titers of 1 – 2 g/L enters as the most important design constraint. In the downstream section of the reactor we start with the centrifugal and filtering section, or primary harvest, where the biomass is removed via the simple filtration methods before moving to protein A chromatography (PAC). In PAC, will yield a single step purification of 99.5% mAb in the solution, however, since the resin is expensive a regeneration sub-step is involved requiring a regenerant solution and storage for both the regenerant and the buffer. After PAC, there is a viral inactivation step whose only constraint is to not use heat induced viral inactivation.

Process Flow Diagrams and Material Balances

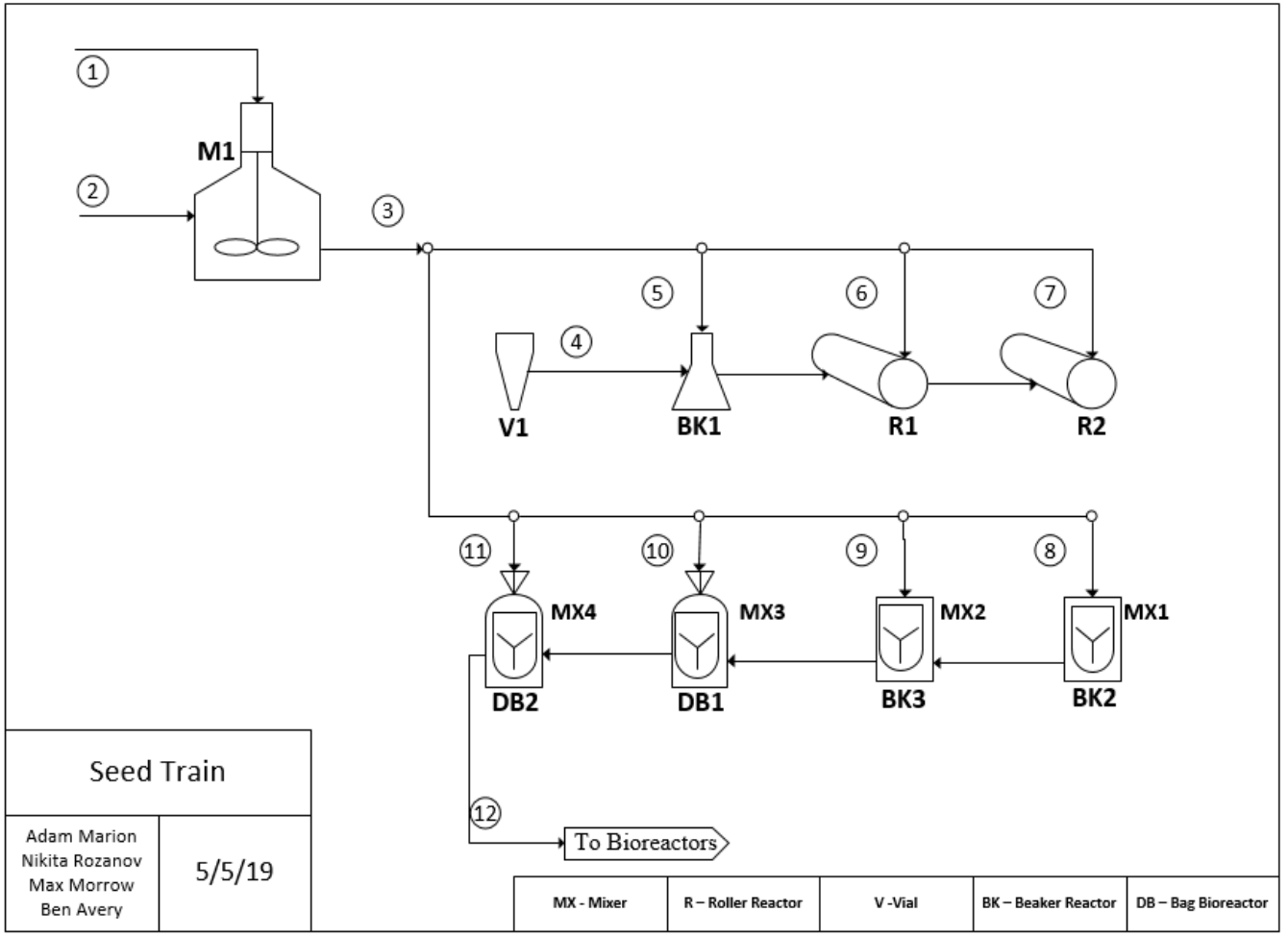


Figure 1. Process Flow Diagram of Seed Train Section.

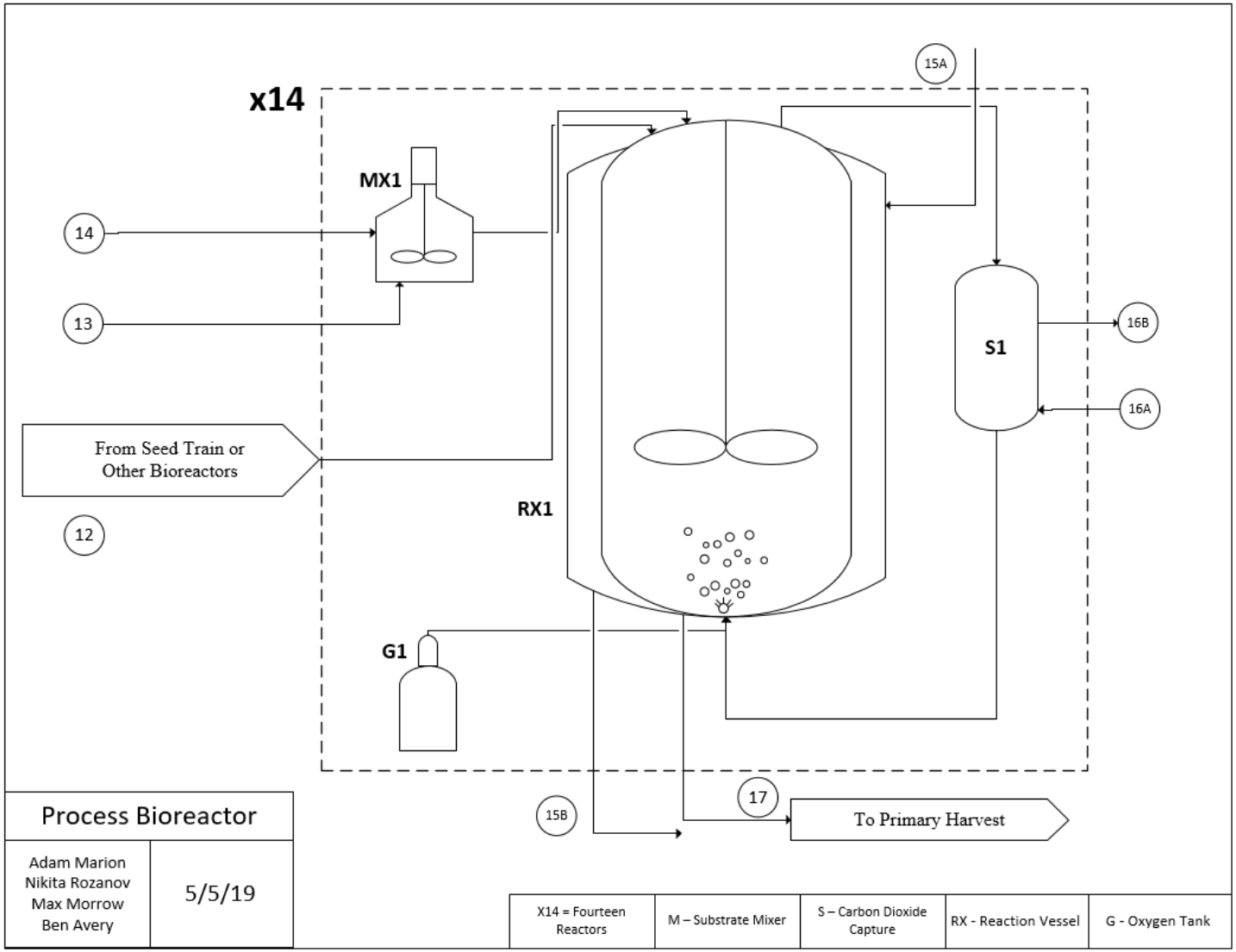


Figure 2. Process Flow Diagram of Bioreactor Section.

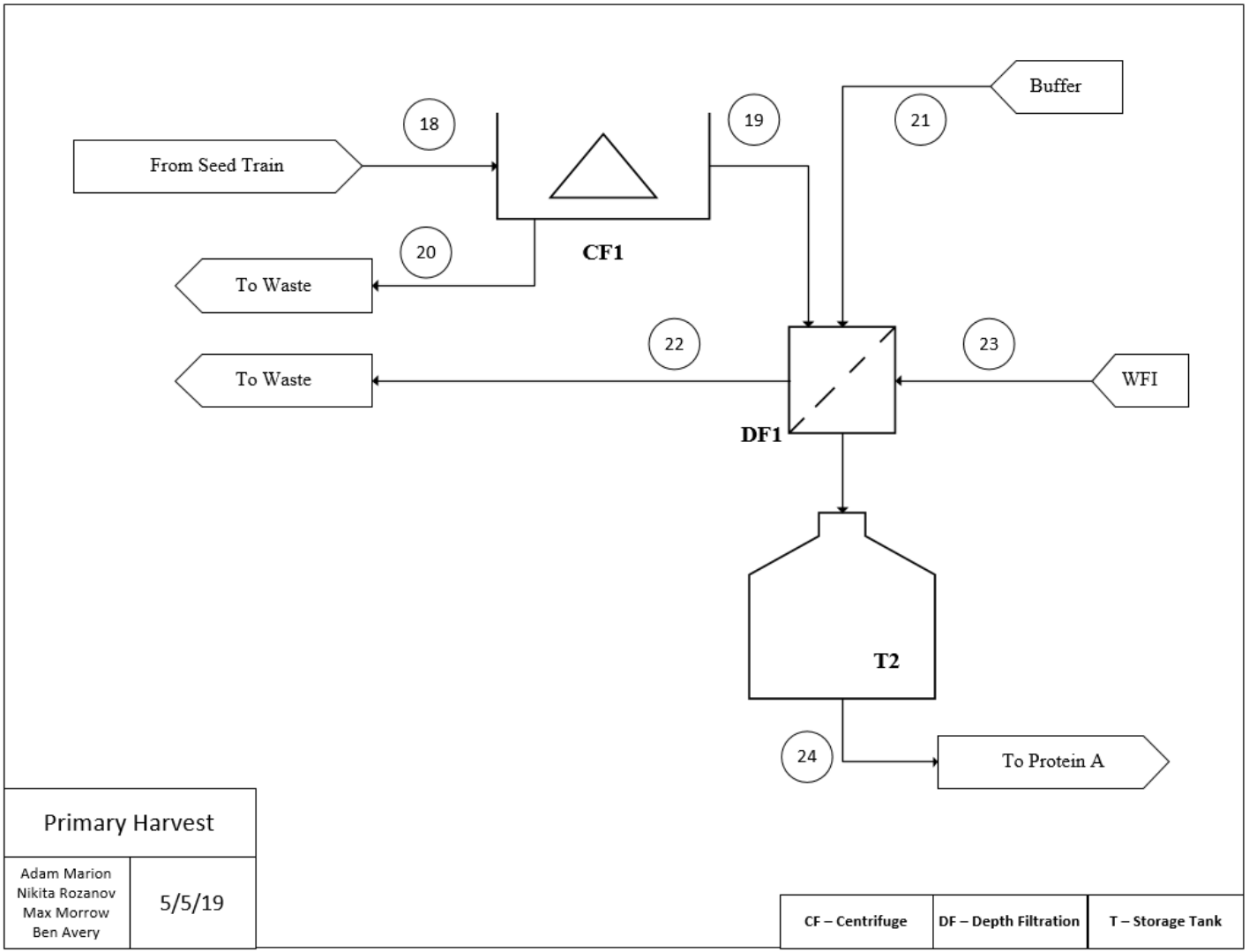


Figure 3. Process Flow Diagram of Primary Harvest Section.

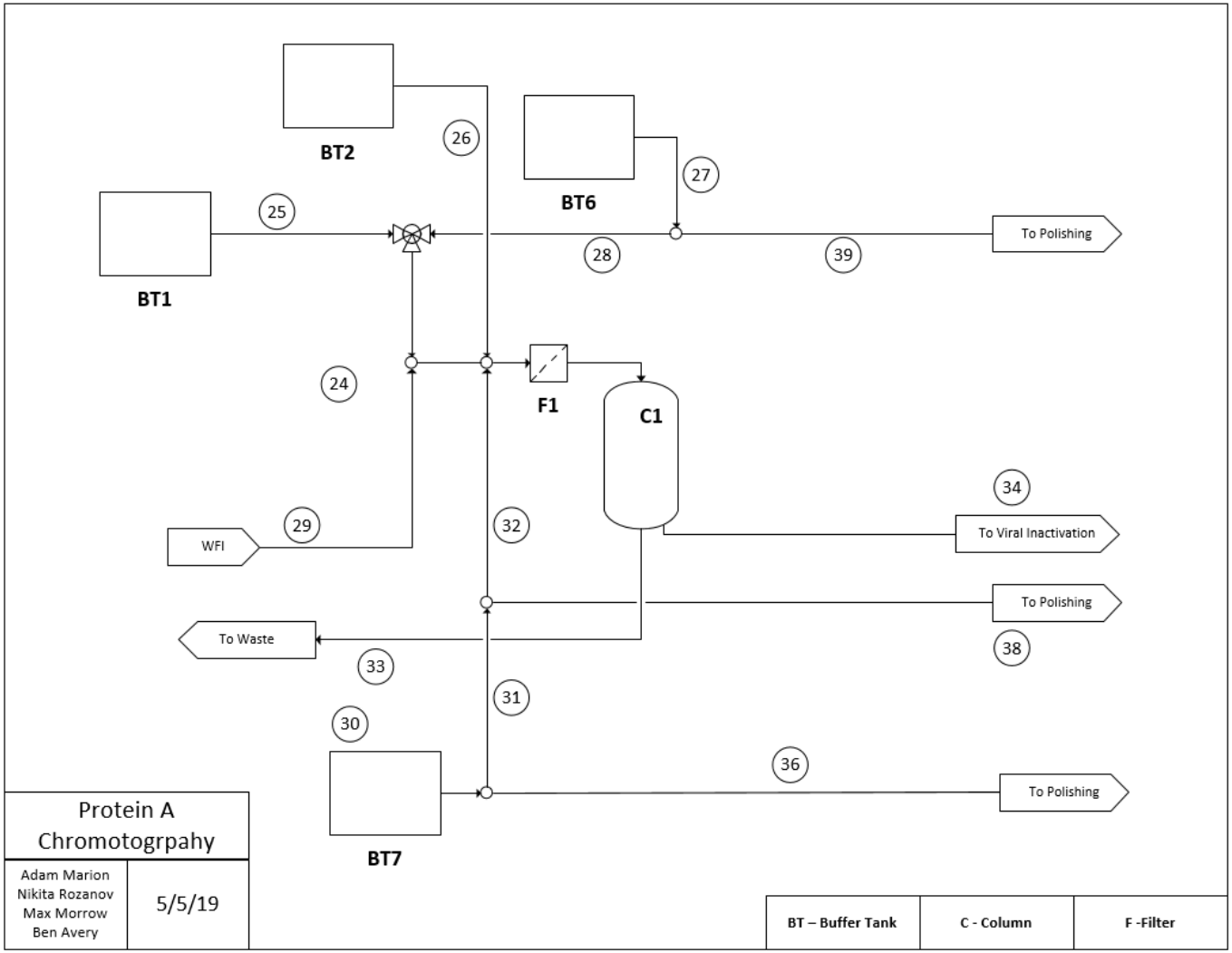


Figure 4. Process Flow Diagram of Protein A Chromatography Section.

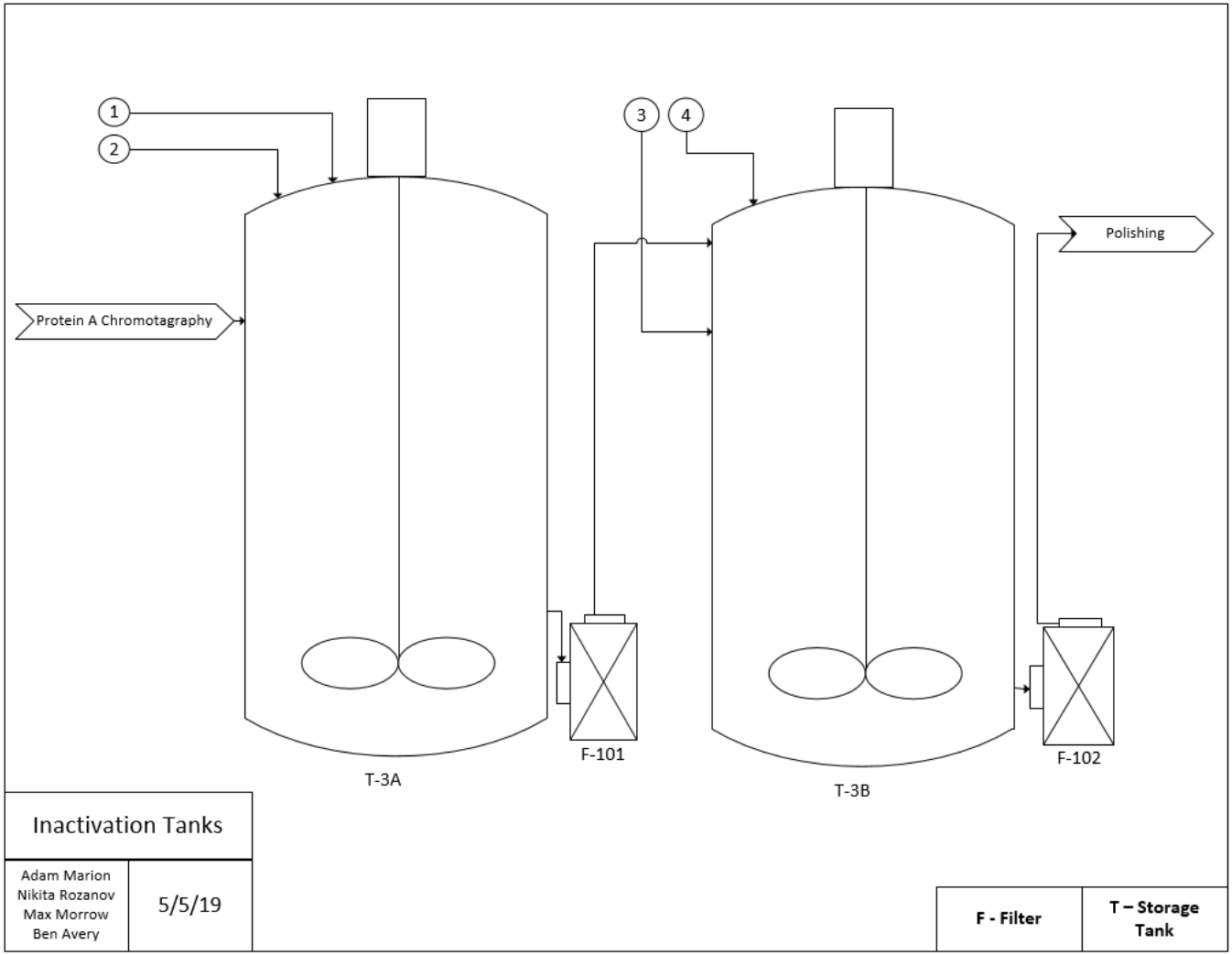


Figure 5. Process Flow Diagram of Inactivation Tanks Section.

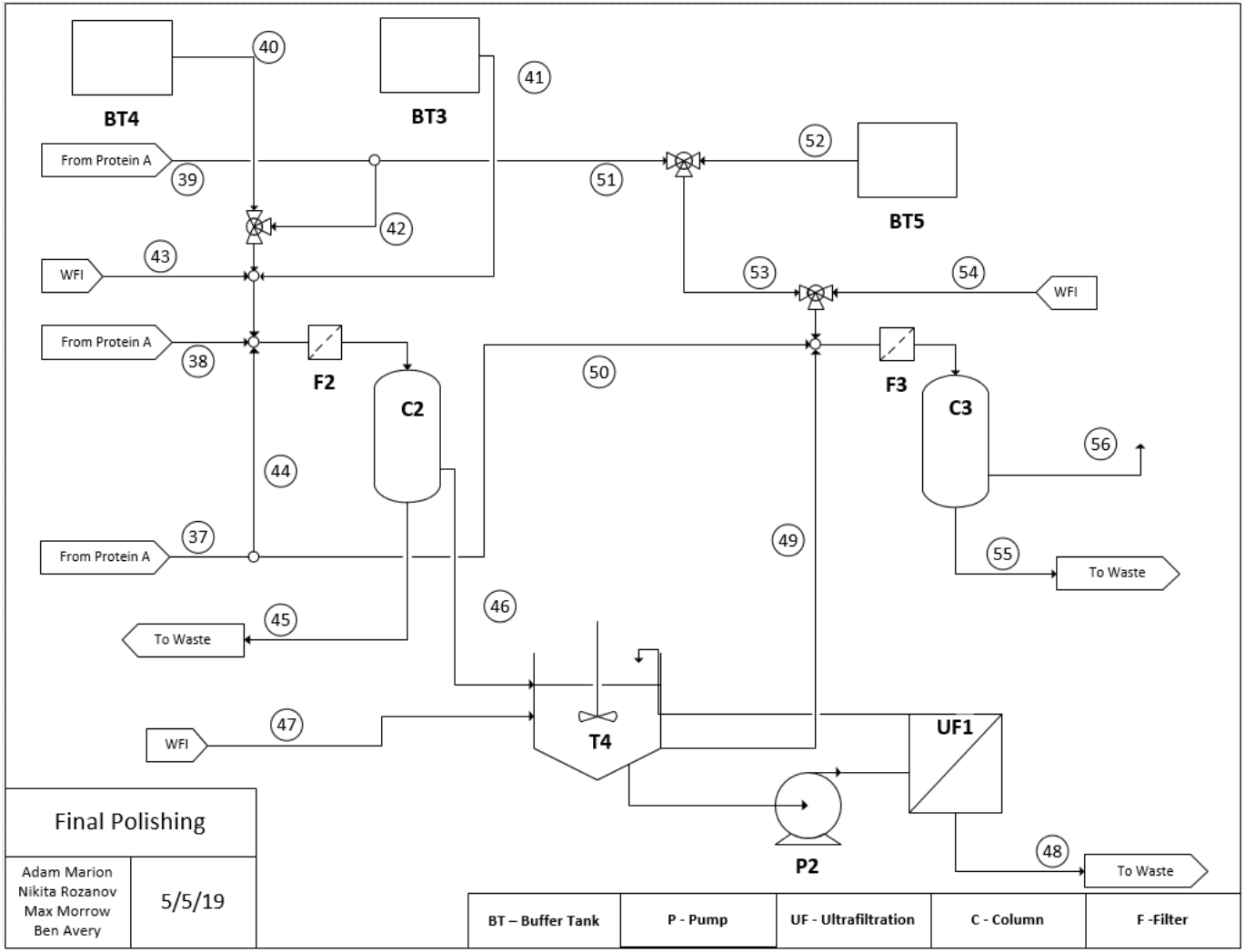


Figure 6. Process Flow Diagram of Final Polishing Section.

Per Batch												
Stream	1	2	3	4	5	6	7	8	9	10	11	12
Temperature (ºC)	21	21	37	37	37	37	37	37	37	37	37	37
Mass Flow (kg)	79.2	20.8	100	0.001	0.004	0.03	0.165	0.8	4	20	75	100
Component Mass Flows												
CHO Broth	0	0	0	0	0	0	0	0	0	0	0	100
Powdered Growth Media	0	20.8	20.8	0	0.0008	0.006	0.034	0.166	0.83	4.16	15.6	0
WFI	79.2	0	79.2	0	0.0032	0.024	0.131	0.634	3.17	15.84	59.4	0
Inoculum	0	0	0	0.001	0	0	0	0	0	0	0	0

Table 1. Material Balances for Seed Train Reference. See Figure 1.

Per Batch						
Stream	12	13	14	15	16	17
Temperature (ºC)	37	21	21	21	37	37
Mass Flow (kg)	100	4900	411	345	514	5130
Component Mass Flows						
Oxygen	0	0	0	345	514	0.4
Cells	0.0001	0	0	0	0	102.4
Media Powder	0	0	113.9	0	0	2.2
Media	*0*	*0*	*103.2*	*0*	*0*	*2.0*
CHNaCO3	*0*	*0*	*9.6*	*0*	*0*	*0.2*
Misc	*0*	*0*	*1.1*	*0*	*0*	*0.02*
Pure Glucose	0	0	183.4	0	0	9.6
MAB	0	0	0	0	0	7.5
Ammonia	0	0	0	0	0	6.1
Water	100	4900	0	0	0	5000

Table 2. Material Balances for Bioreactor Section. See Figure 2.

Per Batch							
Stream	18	19	20	21	22	23	24
Temperature (ºC)	37	37	37	21	21	21	37
Mass Flow (kg)	5127.8	4928.0	199.8	416.7	2928.5	2500.0	4916.2
Component Mass Flows							
Biomass	114.2	11.4	102.8	0	11.4	0	0
Ammonia	6.1	6.1	0	0	0	0	6.1
Water	5000	4903	97	0	2500	0	4903
MAB	7.5	7.5	0	0	0.4	0	7.1
WFI	0	0	0	0	0	2500	0
Buffer	0	0	0	416.7	416.7	0	0

Table 3. Material Balances for Primary Harvest Section. See Figure 3.

Per Cycle															
Stream	25	26	27	28	29	30	31	32	33	34	35	36	37	38	39
Mass Flow (kg)	14.7	7.7	107.6	73.1	2232	48.5	47.3	15.6	4763.15	582.05	582.05	1.2	582.05	31.7	34.5
Component Mass Flows															
Monoclonal Antibodies	3.00	0	0	0	0	0	0	0	0.15	2.85	2.85	0	2.85	0	0
WFI	6.5	5.5	82.8	56.2	2232	31.7	31	8.7	4734	577	577	0.7	577	22.3	26.6
Phosphate	5.2	0	0	0	0	0	0	0	5.2	0	0	0	0	0	0
Citrate	0	2.2	0	0	0	0	0	0	0	2.2	2.2	0	2.2	0	0
NaCl	0	0	24.8	16.9	0	0	0	0	16.9	0	0	0	0	0	7.9
NaOH	0	0	0	0	0	16.8	16.3	6.9	6.9	0	0	0.5	0	9.4	0

Table 4. Material Balances of Protein A Chromatography Section. See Figure 4.

Per Batch															
Stream	25	26	27	28	29	30	31	32	33	34	35	36	37	38	39
Mass Flow (kg)	43	23	323	219	6696	146	142	47	14289	1745	1745	4	1745	95	104
Component Mass Flows															
Monoclonal Antibodies	7.4	0	0	0	0	0	0	0	0.37	7.03	7.03	0	7.03	0	0
WFI	19.5	16.5	248	169	6696	95	93	26	14202	1731	1731	2.1	1731	67	80
Phosphate	15.6	0	0	0	0	0	0	0	15.6	0	0	0	0	0	0
Citrate	0	6.6	0	0	0	0	0	0	0	6.6	6.6	0	6.6	0	0
NaCl	0	0	74	51	0	0	0	0	51	0	0	0	0	0	23.7
NaOH	0	0	0	0	0	50	49	21	21	0	0	1.5	0	28.2	0

Table 5. Material Balances of Inactivation Tanks Section. See Figure 5.

Per Cycle																				
Stream	37	38	39	40	41	42	43	44	45	46	47	48	49	50	51	52	53	54	55	56
Mass Flow (kg)	582.1	31.7	34.5	26.9	5.6	27.0	1530	9.6	1543	509.0	1250	1125.1	128	22.1	7.5	5.9	13.4	652.0	686.7	134.4
Component																				
Monoclonal Antibodies	2.85	0	0	0	0	0	0	0	0.14	2.71	0	0.08	2.63	0	0	0	0	0	0.13	2.49
WFI	577	22.3	26.6	22.4	4.5	20.8	1530	5.3	1532	499	1250	1125	125	17	5.8	4.2	10	652	679	131
Citrate	2.2	0	0	0	0	0	0	0	2.3	0	0	0	0	0	0	0	0	0	0	0
MES	0	0	0	4.5	0	0	0	0	2.2	2.2	0	0	0	0	0	0	0	0	0	0
MOPS	0	0	0	0	1.1	0	0	0	1.1	0	0	0	0	0	0	0	0	0	0	0
Tris	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1.7	1.7	0	1.3	0.4
NaCl	0	0	7.9	0	0	6.2	0	0	1.1	5.1	0	0	0	0	1.7	0	1.7	0	1.2	0.5
NaOH	0	9.4	0	0	0	0	0	4.3	4.3	0	0	0	0	5.1	0	0	0	0	5.1	0

Table 6. Material Balances of Final Polishing Section. See Figure 6.

Process Description

Medium Preparation

Cells need a tailored environment to thrive and achieve maximal growth and production rates. The primary goal of cell media is to provide this environment. Media success is assessed through multiple criteria (Epstein et al.):

- limitation of cell damaging processes such as shear force
- low inhibitor production (species such as lactic acid that hinder efficient glucose use)
- high viable cell densities and percentages (contributing cell count per unit volume of media and total cell population)
- high titers (protein mass produced per volume of media)
- high specific productivities (protein mass produced per cell per unit time)

The optimum media composition can vary greatly between cell lines (Epstein et al.), thus it is difficult to sufficiently pick a cell media, or optimize an in-house formula, before the cell line is available for testing. For initial design modeling, the Chemically Defined Medium BalanCD will be used due to its superior performance (in terms of the above criteria) reported in comparison to eight other commercially available (Reinhart et al.). CHO cell culture medias used for antibody production. This selection provides a specific productivity and inhibitor production rate for the seed train and bioreactor, as well as some media preparation procedures that will be discussed later in this section.

However, there is great potential for future performance improvement by tailoring an in-house developed Chemically Defined Medium to each cell line brought into major production. A consistent parent medium that works well with most cell lines is also desirable to allow for a starting point for each new development as well as a generally applicable medium that may be applied to contract production of small batches that are not worth the extra resources. In the past, much of the nutritional needs for the culture of mammalian cells was provided by variable sources such as serum, the supernatant of blood. However, this makes room for batch to batch variation, risk of contamination (which can result in end-product corruption on top of the possibility of cell disease), and introduction of unwanted contents that hinder growth (Butler and Burgener). Because these sources are typically animal based, they are also at risk of shortages and market fluctuation.

Medium Development

Being animal free, this facility is using Chemically Defined Media, which also gives more freedom to tailor to cell lines for maximal production efficiency. The following

discussion reviews the major components of Chemically Defined Media from *Medium Development* by Butler and Burgener.

An important starting point is the energy source. While glucose is the primary carbohydrate for energy, it metabolizes to lactate, which builds up in the medium and inhibits growth. It is also used rapidly, so it can be a limiting factor. For the fed-batch bioreactor, pure glucose will be used to supplement the base media to maintain growth. Glutamine is another energy source in media. It often must be added in media preparation separately from the base powder to extend shelf life, but this will not be an issue for this facility because each bioreactor will have media powder preparation performed for each batch (in the *Mixers* subsection). Glutamine is also consumed rapidly, but instead of culminating in lactate, it causes a buildup of ammonia, which causes growth inhibition for certain cell lines. To avoid inhibition for these cell lines, glutamate or glutamine dipeptides are often substituted.

Amino acids are broken up as non-essential (produced by the cells) and essential (must be provided for optimal cell growth). Whether an amino acid is non-essential or essential depends on the cell line. Limitations in amino acids reduce the achievable growth rate and maximal cell density but must be balanced with glucose to avoid high lactate and ammonia production rates by promoting alternative metabolic pathways. Glutamine, for example, is an amino acid that can reroute production to ammonia rather than lactate.

Trace elements and vitamins often appear in medium formulas. Selenium, the most common, promotes cell growth and has antioxidative properties. Ferrous salts can act as an iron carrier, and calcium control can be used to optimize cell clumping and division. Certain lipids (fatty acids, phospholipids, lecithin, and cholesterol) have found their way into Chemically Defined Media, too, buffing cell growth rates and dampening death rates.

Meanwhile, a few types of ingredients control general medium environment. Buffers such as sodium bicarbonate are used to maintain pH. Salts are vital for maintaining isotonic conditions. The standard osmolality is about 300 milli-osmoles per kilogram. Osmotic balance ensures that cells do not swell or get crushed if the concentration of salt is higher or lower outside the cell than inside it. Lastly, the splicing during development of mAb cell lines can include the addition of drug resistance. This allows for productive cell selectivity by the addition of an antibiotic cocktail that eradicates unwanted cell growth.

The Plackett-Burman statistical approach to medium development uses a factorial design of experiments to home in on useful compositions for many components. Simply put, two concentrations are established for high (+) and low (-) for each component and a matrix of combinations is put together for media trials. Variances are looked at to determine which single factors and interactions are significant. A general range of components for eukaryotic cells can be found in Table 7 (Epstein et al.).

	Concentration (mg/L)			Concentration (mg/L)	
Component	**Min**	**Max**	**Component**	**Min**	**Max**
Anhydrous $CaCl_2$	5	200	L-histidine HCL H_2O	100	500
Anhydrous $MgCl_2$	15	50	L-isoleucine	50	1000
Anhydrous $MgSO_4$	20	80	L-leucine	50	1000
$FeSO_4$ $7H_2O$	0.05	0.5	L-lysine HCl	100	1000
$Fe(SO_3)_3$ $9H_2O$	0.01	0.08	L-methionine	50	500
$ZnSO_4$ $7H_2O$	0.4	1.2	L-ornithine HCl	0	100
Ferric Ammonium Citrate	0.04	200	L-phenylalanine	25	1000
KCl	280	500	L-proline	0	1000
NaCl	5000	7500	L-serine	50	500
NaH_2PO_4 H_2O	30	100	L-taurine	0	1000
Na_2HPO_4	30	100	L-threonine	50	600
$CuSO_4$ $5H_2O$	0.001	0.005	L-tryptophan	2	500
$CoCl_2$ $6H_2O$	0.001	0.1	L-tyrosine 2Na $2H_2O$	25	250
$(NH_4)_6MO_7O_{24}$ $4H_2O$	0.001	0.005	L-valine	100	1000
$MnSO_4$ H_2O	0.00007	0.008	d-biotin	0.04	1
$NiSO_4$ $6H_2O$	0.000025	0.0005	D-calcium Pantothenate	0.1	5
Na_2SeO_3	0.004	0.07	Choline Chloride	1	100
Na_2SiO_3 $9H_2O$	0.02	0.4	Folic Acid	1	10
$SnCl_2$ $2H_2O$	0.000025	0.0005	i-Inositol	10	1000
NH_4VO_3	0.0001	0.0025	Nicotinamide	0.5	30
D-Glucose	500	6000	p-aminobenzoic acid	0.1	20
Sodium Pyruvate	0	1000	Riboflavin	0.05	5
Sodium Hypoxanthine	0	20	Thiamine HCl	0.5	20
Glycine	0	150	Thymidine	0	3
L-alanine	0	150	Vitamin B12	0.05	5
L-arginine HCl	200	5000	Linoleic Acid	0.01	2
L-asparagine H_2O	40	150	DL-α-lipoic Acid	0.03	1
L-aspartic acid	20	1000	Pyridoxine HCl	0.5	30
L-cysteine HCl H_2O	25	250	Putrescine 2HCl	0.025	0.25
L-cystine 2HCl	15	150	Ethanolamine HCl	2	100
L-glutamic Acid	0	1000	L-histidine HCL H_2O	100	500

Table 7. A patented general range for beginning medium development for eukaryotic cells

Mixers

Media powder is mixed with water at 20.8 g/L. This was taken as the sum of the average of the high and low concentrations in Table 1, which compares well to the recommended BalanCD input of 23.72 g/L (Irvine Scientific). Four and a half grams per liter of this powder is glucose. Sodium bicarbonate will be added proportionally to the recommended BalanCD input, resulting in 1.9 g/L. Osmolality and pH must be monitored and controlled with sodium chloride and sodium hydroxide, respectively, as needed. Bioreactor fed-batch input is supplemented with enough pure glucose to meet required demands for growth. To avoid abnormalities due to clumping, a sterile filtration is performed on the liquid media mixture through a 0.2-micron filter membrane before addition to the seed train or bioreactor. By mixing immediately before input, cryopreservation (2-8°C) can be avoided, which reduces energy costs associated with cooling, maintaining, and heating the liquid media for introduction to the cells.

Seed Train

For calculations involving the seed train some important assumptions were made. The first step in the process is the seed train (ST), or inoculation step. The ST consists of a series of vessels with fresh media, produced in the media preparation step. Cells are transferred through in series to increase the population in preparation for the bioreactor. The initial 5 mL beaker contains 1 mL of inert fluid with 10^6 cells, and 4 mL of the media, warmed to 38 °C. The first vessel, T-1 is continuously stirred for 30 seconds. The 5 mL culture is then transferred to a 35 mL roller flask with 30 mL of media at 38 °C, T-2, which is used for 2.5 hrs and contained. This was repeated for vessels T-3, T-4, T-5, T-6, T-7 with volumes 200 mL, 1 L, 5 L, 25 L, and 100 L, operation times were 13.5, 42.7, 70.5, 83.8, and 87.2 hrs respectively. Encoded in the calculations is a transition time with each vessel size correlating to a different amount of time, 30 minutes. All vessels except T-7 be run at 38°C for selectivity of cell growth and cultivation over protein production. Cell T-8 will be started at 38-°C and ramped down in temperature to 33°C dependent on need to limit cell growth and production while maintaining viability. This will be used as an alternative to having a storage vessel in between the seed train and the bioreactor removing equipment costs for both the storage vessel and storage heaters. The cell cultivation was modelled in MATLAB (See App. D) and Monod constants for glucose and glutamine uptake were taken from *Biotechnical Products and Process Engineering* (Reinhart et al.). Fundamental differential equations for the process included cell growth rate (μ) and nutrient uptake rate (q_{gc}/q_{gn}) each as a function of glutamine and glucose concentrations, both present within the media (Kern et al.). Then a simple Newton's numeric method was used utilizing the calculated differential equations to linear extrapolate over a time step of 10^{-3} hrs once the glucose concentration in the vessel converged past 10^{-4}x the initial glucose concentration (near-depletion).

To produce the necessary number of cells based on bioreactor calculations there will need to be five independent seed trains with each one starting three days into the last one, this means T-1, T-2, T-3, and T-4 are run and then 14 hrs into T-5 a new seed trains is began. T-1 through T-5 (5 mL through 5 L) will be entirely disposable vessels. T-6 and T-7 (25

L and 100 L) will have cylindrical metal tanks and electric stirrers with disposable bags lining the inside as to reduce costs involved in cleaning.

Bioreactor

Following growth of initial cells in the seed train, cells are transferred from the seed train into a bioreactor. The purpose of the bioreactor is to keep the CHO cells continuously growing within the growth phase and avoid growth inhibition caused by lack of available substrate. A 5000 L fed-batch reactor was chosen for the process given longstanding usage through the biopharmaceutical industry Source the reactor can operate continuously as a profusion reactor and in single batches. The bioreactor was modeled within MATLAB assuming growth can be approximated with Monod kinetics. The primary indicator of substrate concentrations within the medium is Glucose. A system of differential equations was used to model cell growth in the fed-batch bioreactor assuming the primary inhibitors for cell growth will be glucose concentrations and ammonia concentrations. Lactose concentrations were also found to inhibit cell growth; however, their influence is due primarily to increasing the overall osmolality of the system.Typical osmolality values for CHO cells are in the range of 260-320 mOsm/kg according to Goyal et al *pg. xxviii*, so the reactor is designed to add salt or water to maintain osmolality. The reactor consists of one 5000 L main vessel which is a stirred tank. The overhead air within the tank is recirculated through a CO_2 absorber to keep carbon dioxide concentrations low within the reactor, and pure oxygen is bubbled through the reactor. Substrate and water are premixed and sparged with O_2 before entering the reactor. The sparger and reactor are operated at atmospheric pressures. For a single run, the bioreactor is estimated to take 660 hrs, it will require a total Oxygen mass of 480 kg.

Primary Harvest

Insoluble particulate (i.e. cells, cell debris, and colloids) removal is conducted on the broth immediately downstream of the bioreactor. This step, primary harvest, is performed in bioprocesses for one of two purposes: to recover intact cells or to clarify broth. This facility is designed to capture monoclonal antibodies from purified broth through chromatography. Thus, clarifying the broth to reduce interference with chromatography is the focus of this primary harvest, so design is motivated by broth purification, not dry cake quality.

There are many techniques for biomass removal that depend on scale and goals. For example, microfiltration membranes are a popular lab scale method, but are sensitive to changes in bioreactor broth (Roush and Lu). Hence, it would be impractical for this manufacturing scale facility due to the potential broth variability if titers are increased and if future excess capacity is contracted out for use.

Depth filters are more generally applicable to varying broths and typically have the highest capacity for normal flow filters; their anisotropic character allows for a higher solid capacity (Butler and Burgener). These filters typically consist of a series of cartridges with decreasing pore sizes. This protects subsequent cartridges from larger particle blockage, further extending the capacity and lifetime of the stack. Depth filters

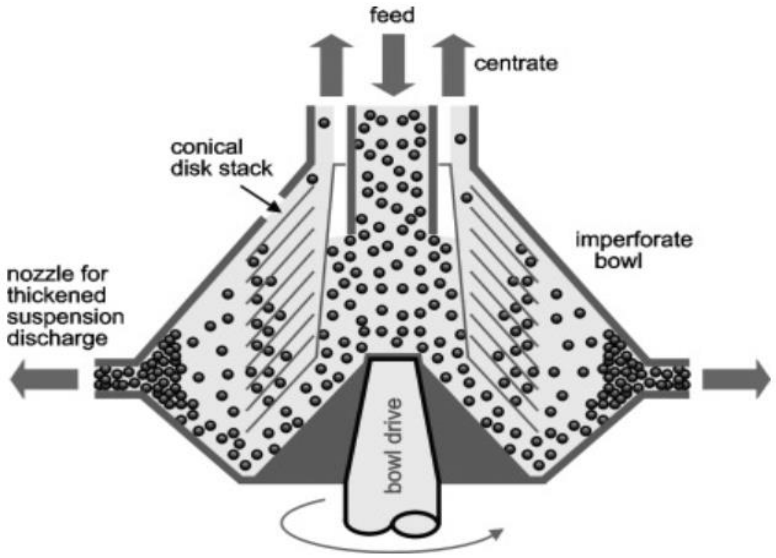


Figure 7: A simple flow diagram of a nozzle disc stack centrifuge displays the path liquid and solids take to separate. The outward centrifugal force pushes particles down the slanted disks to be discharged out of the bowl nozzles, while the lower density liquid phase leaves back out the top of the centrifuge (Tarleton and Wakeman).

are most frequently made up of diatomaceous earth, perlite, cellulose fibers, and a positively charged resin binder, which allow for separation by adsorption as well as size exclusion (Roush and Lu). The biomass captured by depth filters cannot be recovered from the filter matrix, which is most commonly disposable.

A less expensive separation method is coupled with depth filtration to share its load because once manufacturing scale reaches liquid volumes above 1000-3000 liters, the cost of disposable filters becomes limiting (Butler and Burgener). Tangential flow filtration is sometimes used to supplement or even replace depth filtration. Broth flow is run parallel to a membrane to reduce clogging and fouling. Low shear is applied to cells, so flow can be increased without cell disruption (cell lysis results in smaller particles that are more difficult to remove from the protein of interest). The drawbacks for tangential flow filtration are that it requires long residence times, has limited control of separation performance, and can also require substantial cleaning or membrane replacement costs (Butler and Burgener).

Thus, the other supplemental technique, centrifugation, is used. To expedite the separation of different density species, higher gravitational force is applied by increasing rotational velocity in a bowl or cylinder. The result is a solid paste and a clarified liquid broth, which is sufficient for the purposes of this facility. Centrifuges have dramatically lower operating costs than filters, although they do have high energy consumption. There are also multiple configurations to choose from. Tubular bowl centrifuges can achieve very high centrifugal forces and good dewatering but must be dismantled and cleaned. Decanter centrifuges create quality dry cakes, but allow more solids into the liquid

product (Butler and Burgener). As mentioned, cell paste quality is not a concern, so the drawbacks of these two options outweigh their advantages.

The configuration of choice is the disc stack centrifuge, which consists of a cylindrical bowl containing a stack of conical discs (Figure 7). Broth enters the center of the stack and as the liquid phase rises to the top of the centrifuge, particles are pushed outward on the underside of the discs. The liquid product exits the top of the centrifuge and the biomass exits nozzles at the edge of the bowl. This configuration allows for high liquid throughputs and centrifugal forces while not requiring the system to stop for cleaning.

Disc stack centrifuges are ideally suited for separating particles between 3 to 30 microns, but can operate as low as about a tenth of a micron (Milledge and Heaven). Average CHO size ranges between 13 and 15 microns, with cell debris <5 microns, dead cells 5 - 10 microns, single viable cells between 10 – 15 microns, and clumped cell masses >15 microns (Hewitt et al.; Han et al.). Hence, a disc stack centrifuge will be placed upstream of a depth filtration system to most efficiently remove insoluble particles. The liquid output sent to depth filtration must be sufficiently clarified to reduce fouling by minimizing small cell debris. To avoid the production of small particles through cell lysis, shear forces in the centrifuge must be eliminated. The largest particles that can be captured must be greater than the proteins of interest. Avastin molecules reach a maximum size around 30 nm (Khalili et al.). Most breakage occurs in the centrifugal feed zone and computational fluid dynamics can be used to map flow fields to determine the impact of shear for centrifugal design, but ultra-scale down centrifugation is often performed to determine the effects on the broth being used (Boychyn et al.).

Design of centrifuges involves the introduction of the sigma factor, which represents an equivalent area for which the centrifuge must provide for the desired separation (Ambler). The model used in this design for a disc stack centrifuge neglects acceleration and deceleration and assumes both laminar and symmetrical flow through the disks. Typical geometry ranges for disc stack centrifuges include 50 to 150 discs, 35 to 50° disc angle, and an outer disc radius between 0.15 and 1.0 meters (Dunn). Assumptions for modeling are that all particles with diameter of one-tenth of a micron or greater are removed; liquid properties are those of water; the density of the particles is 1060 kg/m^3; the outer and inner disc radii are 0.3 and 0.05 meters, respectively; and it takes one hour

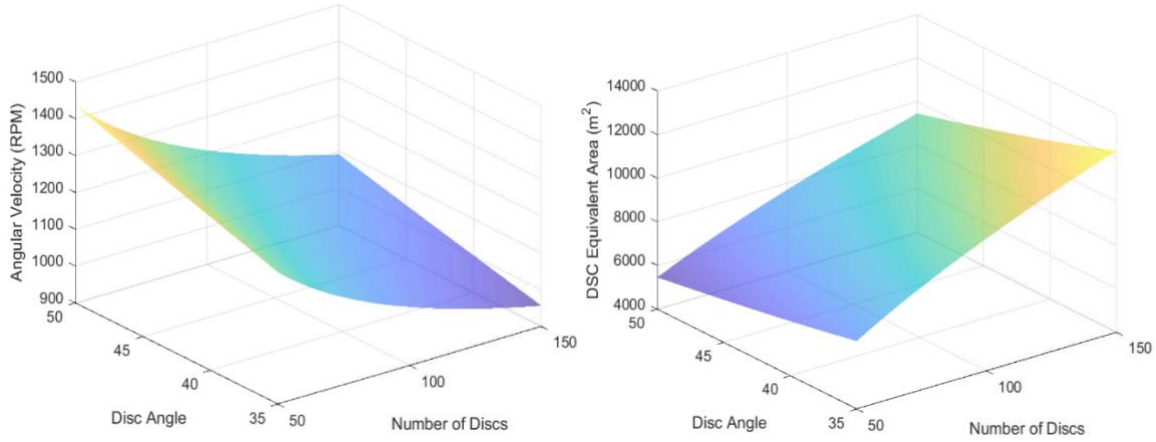


Figure 8: The ranges for angular velocity and equivalent area (sigma factor) are plotted using the ranges of disc count and angle. This gives an operating range for many values: a smaller angular velocity corresponds to a safer g-force and a moderate settling velocity, but requires more equivalent area, thus a larger capital cost.

to clarify each 5000 L batch of broth. The ranges of discs and disc angles are used to get an operating range for the angular velocity and disc stack centrifuge (DSC) equivalent area (Figure 8), which is a useful metric for costing.

From this model, the centrifugal acceleration was found to range from 300-700 times gravitational acceleration, which is well within the range of capability for DSCs (Dunn). The settling velocity range, which is the ratio of flow rate to the sigma factor and represents the speed in which the particles are pushed to the bowl, is between 1.1 and 2.5 $\times 10^{-7}$ meters per second. This is high enough that negligible temperature increase is expected, and no cooling jacket will be required for the DSC (Wojciechowski et al.).

Approximately 90% of the bulk biomass is expected to be removed by centrifugation (Wojciechowski et al.), with a paste made up of 50 vol.% extracellular liquid (Petrides 2003). The rest of the biomass particles are assumed to be removed by depth filtration; negligible insoluble particulates are expected to move passed primary harvest once final design has been polished. Ninety-five percent of the protein is expected to proceed through to chromatography .

A Millipore Pod system is used for the depth filtration system (Millipore, *Processing Economics of the Millipore Pod System Versus Millistak+® HC Lenticular Stacks*). It is recommended to have a filter tailored for the CHO broth to maximize capacity usage. For small particle filters, the dominant fouling mechanisms in modeling are cake filtration and complete blockage (Sampath et al.). Scaled down depth filters can thus be modelled to predict large scale capacity, frequency of replacement, and the required storage capacity of depth filters at the facility. Until such data can be acquired (if desired for optimization), the X0HC grade Millistak+ Depth Filter Media will be used as it is designed for post-centrifugation loading for secondary clarification to protect chromatography columns below one-tenth micron particle size from the centrifuge (Millipore, *Depth Filters at a Glance*).

The liquid throughput for depth filtration is the same as for centrifugation to avoid having to clean, validate, and allocate space for an intermediate holding tank in primary harvest. Thus, the flow rate through primary harvest will be a constant 5000 L/hr to a holding tank for the variable flow of chromatography. This flow was chosen because it allows the settling velocity to be high enough to reasonably minimize shear, temperature changes, and sizing for the centrifuge. The resultant required filtration area for the XOHC filter is 25 m^2 with a maximum differential pressure of 34 psid. This size range (5.5 to 33 m^2) of POD filter operates as a stack of rows of disposable filters that do not require any housing, only structural support for alignment (Millipore, *Millistak + ® Pod Carbon Depth Filter Media System High Adsorption Capacity in the Innovative Pod Format*). As a result, no CIP or SIP is required for upkeep. Only a filter buffer and WFI prewash are used to saturate the filter media in preparation for the next batch. The sizing performed also ensures that the filters will reach or will be close to reaching capacity at the end of each 5000 L batch, so they can be replaced between batches. This removes the requirement to stop flow to replace filters mid-batch as well as the requirement to dispose of contaminated filter stacks that have not reached capacity by the end of a batch.

For future step yield increases, a post wash of the depth filter with recirculating WFI is recommended (Millipore, *Millistak + ® Pod Disposable Depth Filter Performance Guide Innovative , High-Performance Pod Filters Ideal for Primary and Secondary Clarification at Lab , Pilot*). Flushing the filter can recover held up protein. However, single pass flushing adds a substantial amount of water to be processed in chromatography and reduces the protein concentration in the filtrate. Recirculation can allow for a reduction of water gain (and use) while recovering this amount of water. The increased load on the chromatography columns must be weighed against the alternative increase in bioreactor time to achieve the same total yield.

Protein A

Purification of mAbs from depth filtered cell culture is achieved using affinity chromatography. A column packed with protein A resin can bind mAbs to separate them from impurities. This process is standard across industrial production of mAbs due to the high purity (99.5%) and recovery (95%) achieved(Ghose et al.; Liu et al.). The resin consists of protein A ligands attached to ~100 μm diameter glass or polymer beads(Ghose et al.). These are packed into a chromatography column. mAbs bind to the ligands during the sample loading step of the separation.

Protein A resin selection is dependent on the particular pharmaceutical manufactured, and it is typically chosen early in the clinical development process(Ghose et al.). The dynamic binding capacity, the lifetime, yield, productivity, and the flow characteristics are important considerations for resin selection. The MabSelect SuRe resin was selected because it is the most established resin within the commonly used MabSelect series of protein A resins. It has a dynamic binding capacity of 35 g mAb per L of resin and lifetime of around 40 cycles(GE Healthcare, “Process-Economy Simulation of MAb Capture Step with MabSelect PrismA Protein A Chromatography Resin”; GE Healthcare, “MabSelect SuRe”). Newer resins can achieve up to a 66 g/L dynamic binding capacity

and lifetimes of over 100 cycles(GE Healthcare, “Process-Economy Simulation of MAb Capture Step with MabSelect PrismA Protein A Chromatography Resin”). The size of the protein A column, as well as the polishing stages, was based on a 3000 g mAb load at 1 g/L. The system is flexible because it can iterate through several cycles to process a single batch. For a typical batch consisting of 5000 L of mAb solution at 1.5 g/L, three cycles are required. If future titers reach up to 5-10 g/L, a resin with a higher capacity may be chosen and the column may be operated for additional cycles to accommodate the load.

A 116 L column is used with a 30 cm height and a 65 cm inner diameter. A flowrate of 300 cm/hr is chosen based on manufacturer flow recommendations(GE Healthcare, “MabSelect SuRe”). A 0.22 μm dead-end filtration unit with a 0.12 m^2 surface area is placed before the column. The protocol for operating the column is provided by the resin manufacturer(GE Healthcare, “A Flexible Antibody Purification Process Based on ReadyToProcess Products”). The column is first equilibrated with a phosphate buffer. The depth filtration effluent is then loaded at 30 g mAb per L of resin onto the column. The column is washed with a phosphate buffer, and a citrate buffer elutes the mAbs bound to the column. A sodium hydroxide solution sanitizes the column between cycles. Column temperature is set to 6-8℃. The process yields 1740 L of purified mAb solution sent to the viral inactivation unit and 14,200 L sent to waste per bioreactor batch. Detailed information about the protocol is available in the appendix.

Viral Inactivation

All biological processes are required to demonstrate a capability of reducing vires by a factor of 10^5. Pasteurization has been a historically effective in destroying viruses(source), however there is risk of protein damage. Virial inactivation will be carried out through three rigorous processes: low pH storage, detergent treatment, and filtration. Following elution from protein A chromatography the pH is low enough to kill enveloped viruses. The effluent from chromatography is stored in a 5000 L tank at a pH of 2- 5 for 1 hr to achieve an LVR of 4. Following low pH storage, the tank is brought to a pH of 4 and filtered through a rack of Millipore® filters. The filters removal larger viruses before the effluent is then transferred to a detergent tank and should achieve LRV of 4-6. The broth is then transfer and mixed in a storage tank with tri-n-butyl phosphate and Tiron® X-100 until mass fractions of 0.3 and 1 % are achieved. The effluent is then stored for one hour before transfer to final polishing. The three initial steps following chromatography will achieve a LVR of 12-18, so a precautionary step in final polishing will be using ultrafiltration of the broth before final storage. Pelican Ultrafiltration Cassettes will be used for final viral separation, and should push the LVR beyond 16 to be FDA compliant.

Polishing

Effluent from the viral inactivation section is still not purified to acceptable pharmaceutical standards. A polishing step consists of additional chromatography to remove host cell proteins, leached protein A, aggregates, and host cell DNA (Fahrner et al.; Gagnon). Several chromatography methods are available, including cation exchange

(CEX), anion exchange (AEX), hydrophobic interaction, and ceramic hydroxyapatite (Gagnon; Liu et al.). We reviewed current recommendations for chromatography methods in industrial mAb production and determined that CEX and AEX are the most recommended methods(Liu et al.). Furthermore, data disclosed in U.S. Pat. No. 7,323,553 indicates that the sequence of CEX followed by AEX achieves the optimal removal of host cell proteins when compared to other two-column processes (Fahrner et al.). We also considered a one-step polishing process, but determined that it may not remove all categories of impurities to the necessary degree (Gagnon).

The CEX step uses the process for purifying bevacizumab (Avastin) described in U.S. Pat. No. 2018/0118781 (Lebrenton et al.). The POROS 50 HS resin is used, which has a capacity of 57 to 75 g mAb per L of resin (Thermo Fisher Scientific Inc.). The column is designed to handle 3000 g of mAb per cycle. This capacity can be doubled by using a newer resin, such as the POROS 50 XS (Thermo Fisher Scientific Inc.). A column with a 30 cm height and 55 cm inner diameter is chosen (71 L volume) to achieve a 50 g/L loading. The flowrate is set to 100 cm/hr (Lebrenton et al.). A 0.22 μm dead end filter with a 0.3 m^2 area is used to remove particles from fluid entering the column. The column is equilibrated prior to loading. Two wash steps remove impurities bound to the column, and the elution step regenerates the resin. The column is sanitized using a sodium hydroxide solution between cycles. MOPS and MES buffers are used in the equilibration, wash, and elution buffers. 1,500 L of eluent proceeds to the DF/UF step and 4,590 L to waste per batch.

A Diafiltration/Ultrafiltration (DF/UF) step is required between CEX and AEX because the elution buffer for the CEX is not suitable for loading on the AEX. The buffer must be exchanged with one that can accommodate the required pH for the AEX. DF/UF uses a semipermeable membrane to remove solute molecules from the incoming process stream. The process circulates the stream between a holding tank and a membrane. Solute molecules pass through the membrane while the larger antibodies are retained. Water is periodically added to the tank to compensate for the water that is removed in the filtrate. The volume of the retained solution can be reduced while performing buffer exchange. The design of the DF/UF system is based on U.S. Pat. No. 2013/0195888 (Wang et al.). The 1,500 L of load per batch from the CEX is concentrated fourfold to 375 L before it proceeds to the next stage. Five diavolumes totaling 3,750 L of WFI is added to the holding tank during filtration and 4,870 L of filtrate exits as waste per batch. A tangential flow filtration (TFF) configuration is chosen. A membrane area of 3.6 m^2 is required to achieve a 9 hr time per cycle.

The AEX step is designed based on information provided in U.S. Pat. No. 7,323,553 (Fahrner et al.) and 7,863,426(Wan et al.). This step is operated in flow-through mode, which differs from the bind-and elute operation of the previous two columns. The AEX resin binds remaining impurities in the system while the purified protein does not. Only equilibration, loading, and cleaning steps are necessary, in that order. Tris buffer is used for the first two steps, and a sodium hydroxide solution cleans the column between cycles. A 150 cm/hr flowrate and a 40 g/L loading was used for the column(Wan et al.). The column is 30 cm high, 60 cm in diameter, and 85 L in volume. A dead-end filter with

a 0.5 m^2 area cleans the incoming column stream. 390 L of mAb exits the column per batch while 2040 L of fluid exits as waste. The yield for the entire downstream processing section, including the primary harvest, protein A chromatography, and polishing, is 80%.

Final Product Storage

The final product is to be stored based on the conclusions made in *Frozen-State Storage Stability of a Monoclonal Antibody: Aggregation is Impacted by Freezing Rate and Solute Distribution* (Miller et al.). The final product will be injected into 50 mL electropolished stainless steel (SS316L) minitanks. These will have 5 cm inner diameter and 7.5 mm wall thickness with a 6 mm thick lid bolted on with an O-ring guarantee proper seal. The sealed minitanks will be immersed in a 35 wt% ethanol in water solution at –20°C until frozen after 45 minutes. Then the minitanks will be moved to utility freezer maintained at –10°C. The two major options were an energy light freezing method and an energy intensive holding method or an energy intensive freezing method and an energy light holding method. We recommend the latter as the holding time is relatively long. The study this method is based on concluded that there will be negligible protein freezing aggregation, concentration gradients formed due to solubility of protein in liquid being more energetically favorable than trapped in a lattice structure, when quick freezing techniques even if the holding temperature is not particularly low. The former method would require a holding temperature of around –80ºC to maintain the integrity of the product. The chosen method is not only less energy intensive but should simplify the melting procedure for potential buyers as well, however that is out of the scope of this design.

SIP and CIP Procedure

Clean in Place (CIP) and Steam in Place (SIP) make up a cost efficient, repeatable cleaning cycle whose adoption at this facility will provide ease of validation with FDA requirements as well as a fully automatic scheduled cleaning for all non-disposable equipment and piping. Another perk is the minimization of equipment dismantling, which decreases worker hazard (both introduced by manual cleaning methods and potentially toxic bio-contaminants) and decreases overall time required for cleaning. A general CIP protocol has been developed based on the 1996 Chemical Engineering Journal article by Stewart and Seiberling, *The Secret's Out: Clean in Place* (Stewart and Seiberling, Dale)." Only one vessel can be cleaned at a time with CIP or SIP, with air operated valves and transfer panels that connect vessel inlets and outlets to cleaning solution inputs and drains. Vessels must also be corrosion resistant for the cleaning solutions, be water tight to prevent spills, must drain completely, have no corners (only rounded edges of a minimum radius of one inch), all piping and ducts should be pitched towards drain ports, and all valves must be cleanable (such as butterfly and ball valves).

A typical CIP cleaning cycle appears as the following. First, a warm pre-rinse with water removes loose soil. Second, a longer alkaline wash with recirculated solution between 55-80°C is run through the vessels. A typical alkaline cleaner is up to 0.5% sodium hydroxide solution depending on the mature system. The third step is a post-rinse with

ambient temperature water to remove the alkaline cleaner. Fourth is an ambient temperature acidic rinse with recirculated water intended to neutralize residual alkaline cleaner as well as remove mineral deposits. The last step is a final post-rinse with recirculated water. The required contact times for the rinses, especially the alkaline rinse, necessitates recirculation of the solutions. The recirculation system consists of an educator to return drained rinsing fluid, a strainer that automatically removes the bulk solids, a recirculation tank (~60 gallon capacity), a heat exchanger to heat solution, and a chemical feed system from drums to supply cleaning chemicals.

CIP rinses are administered with built in sprays for vessels. Assuming equal diameter and height for the cylindrical 5000-liter vessels at this facility (about 7 ft), the typical rinse input flow should be about 60 gal/min. Turbulence is an important factor for complete cleaning; thus, the optimum velocity in pipes to be cleaned is 5 ft/s. For unit operations without otherwise defined rinse formulas or durations, this procedure is currently planned to be run for two hours for each vessel.

SIP supplements CIP for cleaning between batches. Pure steam (WFI fed) at 1.2 barg and 121°C is used pumped through the each system, the standard at which *Bacillus Stereothermophilis* spores are destroyed (Bennet and Cole). SIP typically follows CIP, as it can only sterilize the surface, not remove mass. The duration of SIP is determined by covering the system being cleaned with thermocouples, then timing when the coldest spot reaches the necessary sterilization temperature. For preliminary estimation, this is expected to be take an average of one hour for each piece of equipment. The equipment to be used for SIP is a boiler with a fresh WFI input that can will drain spent water to the kill tanks for disposal into the sewer.

Costing is generally estimated by assuming WFI costing for fresh fluid, with an equivalent fluid requirement of about 50% of each bioreactor volume per batch. This results in $315,000 per year in extra utility costs.

Kill Tank

Waste inactivation for the biological facility before disposal into the sewage is necessary for the safety of the environment and general public. Inactivation can be chemical, thermal, or both. CHO cells typically live in around a 37°C environment. Heat exposure to 80°C for 1 min is enough for decontamination of CHO waste so it may be safely disposed of as sewage (Gregoriades et al.). Each batch, all the waste from the primary harvest and chromatography steps will be collected in a single 25 m^2 tank ($127,800 with a pressure rating of 50 psi) where they will be heated to 80°C for five minutes then cooled to ambient conditions (additional time will not negatively impact performance). The yearly energy using a 24,410 kg cumulative amount of waste and assuming the heat capacity of water is 3.68×10^5 kWh, which translates to an additional $18,400 in energy costs per year. Minor additional costs may be associated with this tank to neutralize the liquid before it enters the sewage. An alternative configuration would be to have a continuous flow heat exchanger that cools waste as it is produced, which would require more automation, but less space.

Additional Systems

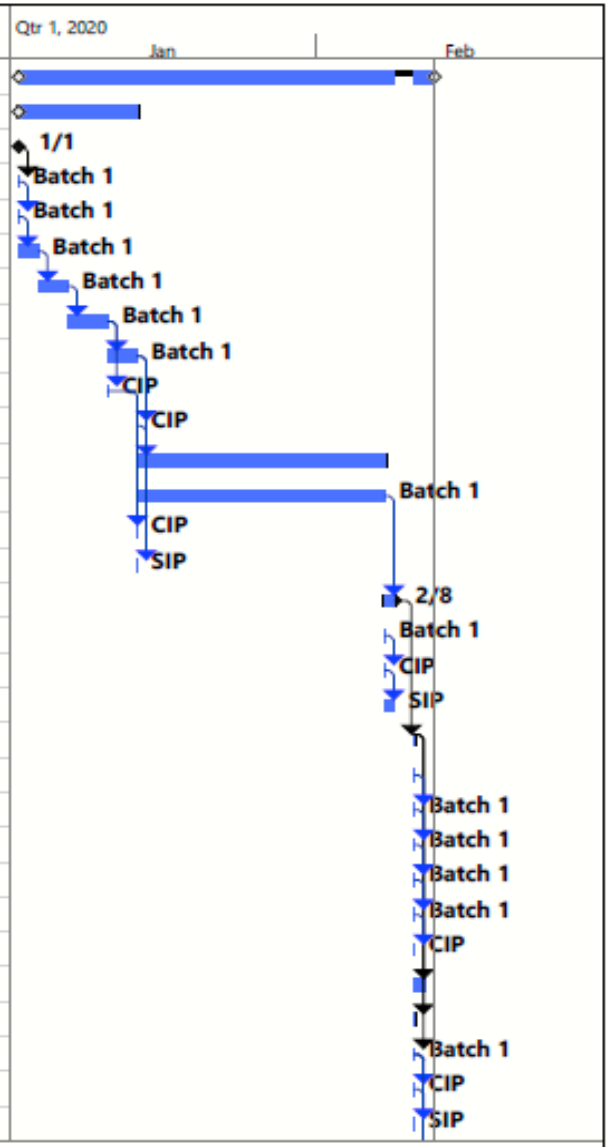
In accordance with the FDA regulations in 21CFR211 several additional systems must be considered. We must establish a workstation for incoming materials such as the cell growth media, the water for injection, clean air, and regeneration solvent for Protein A can be tested for quality assurance purposes, thus satisfying the 21CFR211.22. The air used in the process will be air from the surrounding area however there will be a filter attached to the inlet and the facility will have air quality and humidity controls in accordance with 21CFR211.42c10ii - iv. There will be an established workstation for product quality and stability testing, likely in combination with the incoming product quality station, in accordance to 21CFR211.165a-b and 21CFR211.166a. Once the product is tested if it is determined to low of quality it will be determined whether reprocessing through the downstream system is possible or if the product simply needs to be disposed.

Schedule

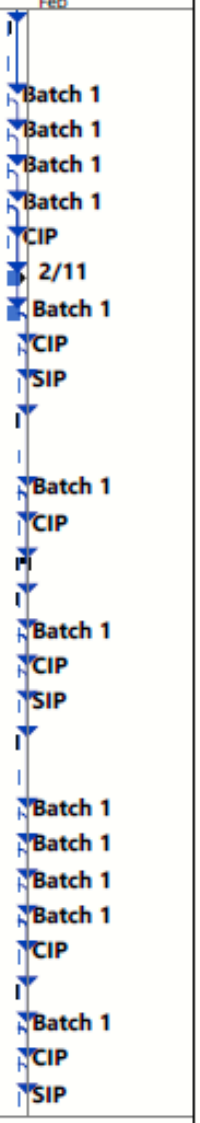
The plant will run in batches constrained by a bioreactor size of 5000 L and a total product requirement of 1000 kg/yr at 1.5 g/L MAb concentration. Therefore, we will need to run 134 batches in a year and this is done by staggering the batches every three days. As a result, 14 bioreactors and 14 associated seed train lines are used. Pictured below is the schedule of events that are occurred such that we only require one downstream (centrifuge through final storage) system and a single CIP and SIP system.

Task Name	Start	Finish
Batch 1	Wed 1/1/20	Wed 2/12/20
Batch 2	Sat 1/4/20	Thu 2/13/20
Batch 3	Tue 1/7/20	Mon 2/17/20
Batch 4	Fri 1/10/20	Wed 2/19/20
Batch 5	Mon 1/13/20	Mon 2/24/20
Batch 6	Thu 1/16/20	Wed 2/26/20
Batch 7	Sun 1/19/20	Fri 2/28/20
Batch 8	Wed 1/22/20	Mon 3/2/20
Batch 9	Sat 1/25/20	Thu 3/5/20
Batch 10	Tue 1/28/20	Mon 3/9/20
Batch 11	Fri 1/31/20	Wed 3/11/20
Batch 12	Mon 2/3/20	Mon 3/16/20
Batch 13	Thu 2/6/20	Wed 3/18/20
Batch 14	Sun 2/9/20	Fri 3/20/20

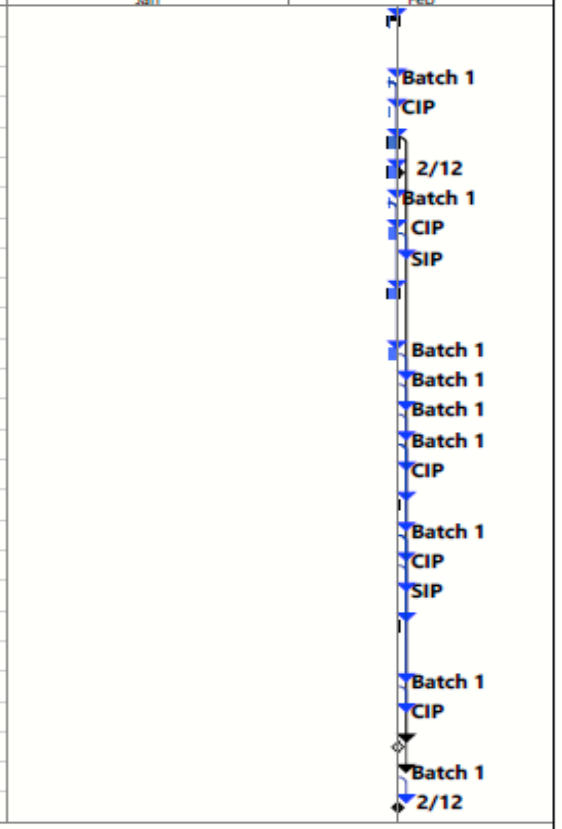
ID	Task Mode	Task Name	Start	Finish
1		**Batch 1**	**Wed 1/1/20**	**Wed 2/12/20**
2		**Seed Train**	**Wed 1/1/20**	**Mon 1/13/20**
3		BK1	Wed 1/1/20	Wed 1/1/20
4		R1	Wed 1/1/20	Wed 1/1/20
5		R2	Wed 1/1/20	Thu 1/2/20
6		BK2	Thu 1/2/20	Fri 1/3/20
7		BK3	Fri 1/3/20	Mon 1/6/20
8		DB1	Mon 1/6/20	Fri 1/10/20
9		DB2	Fri 1/10/20	Mon 1/13/20
10		DB1 CIP	Fri 1/10/20	Fri 1/10/20
11		DB2 CIP	Mon 1/13/20	Mon 1/13/20
12		**Bioreactor**	**Mon 1/13/20**	**Fri 2/7/20**
13		Bioreactor Batch	Mon 1/13/20	Fri 2/7/20
14		Biorector CIP	Mon 1/13/20	Mon 1/13/20
15		Bioreactor SIP	Mon 1/13/20	Mon 1/13/20
16		**Primary Harvesting**	**Fri 2/7/20**	**Sat 2/8/20**
17		Primary Harvesting Batch	Fri 2/7/20	Fri 2/7/20
18		Primary Harvesting CIP	Fri 2/7/20	Fri 2/7/20
19		Primary Harvesting SIP	Fri 2/7/20	Sat 2/8/20
20		**Protein A**	**Mon 2/10/20**	**Mon 2/10/20**
21		Protein A Equilibration	Mon 2/10/20	Mon 2/10/20
22		Protein A Loading	Mon 2/10/20	Mon 2/10/20
23		Protein A Wash 1	Mon 2/10/20	Mon 2/10/20
24		Protein A Wash 2	Mon 2/10/20	Mon 2/10/20
25		Protein A Elution	Mon 2/10/20	Mon 2/10/20
26		Protein A CIP	Mon 2/10/20	Mon 2/10/20
27		**Downstream 1**	**Mon 2/10/20**	**Tue 2/11/20**
28		**Viral Inactivation**	**Mon 2/10/20**	**Mon 2/10/20**
29		Viral Inactivation Batch	Mon 2/10/20	Mon 2/10/20
30		Viral Inactivation CIP	Mon 2/10/20	Mon 2/10/20
31		Viral Inactivation SIP	Mon 2/10/20	Mon 2/10/20



ID	Task Mode	Task Name	Start	Finish
32		**Cation Exchange Chromatography**	**Mon 2/10/20**	**Mon 2/10/20**
33		CEX Equilibration	Mon 2/10/20	Mon 2/10/20
34		CEX Loading	Mon 2/10/20	Mon 2/10/20
35		CEX Wash 1	Mon 2/10/20	Mon 2/10/20
36		CEX Wash 2	Mon 2/10/20	Mon 2/10/20
37		CEX Elution	Mon 2/10/20	Mon 2/10/20
38		CEX CIP	Mon 2/10/20	Mon 2/10/20
39		**Ultra Filtration / Diafiltration**	**Mon 2/10/20**	**Tue 2/11/20**
40		UF/DF Batch	Mon 2/10/20	Tue 2/11/20
41		UF / DF CIP	Tue 2/11/20	Tue 2/11/20
42		UF / DF SIP	Tue 2/11/20	Tue 2/11/20
43		**Anion Exchange Column**	**Tue 2/11/20**	**Tue 2/11/20**
44		AEX Equilibration	Tue 2/11/20	Tue 2/11/20
45		AEX Loading	Tue 2/11/20	Tue 2/11/20
46		AEX CIP	Tue 2/11/20	Tue 2/11/20
47		**Downstream 2**	**Tue 2/11/20**	**Wed 2/12/20**
48		**Viral Inactivation**	**Tue 2/11/20**	**Tue 2/11/20**
49		Viral Inactivation Batch	Tue 2/11/20	Tue 2/11/20
50		Viral Inactivation CIP	Tue 2/11/20	Tue 2/11/20
51		Viral Inactivation SIP	Tue 2/11/20	Tue 2/11/20
52		**Cation Exchange Chromatography**	**Tue 2/11/20**	**Tue 2/11/20**
53		CEX Equilibration	Tue 2/11/20	Tue 2/11/20
54		CEX Loading	Tue 2/11/20	Tue 2/11/20
55		CEX Wash 1	Tue 2/11/20	Tue 2/11/20
56		CEX Wash 2	Tue 2/11/20	Tue 2/11/20
57		CEX Elution	Tue 2/11/20	Tue 2/11/20
58		CEX CIP	Tue 2/11/20	Tue 2/11/20
59		**Ultra Filtration / Diafiltration**	**Tue 2/11/20**	**Tue 2/11/20**
60		UF/DF Batch	Tue 2/11/20	Tue 2/11/20
61		UF / DF CIP	Tue 2/11/20	Tue 2/11/20
62		UF / DF SIP	Tue 2/11/20	Tue 2/11/20



ID	Task Mode	Task Name	Start	Finish
63		**Anion Exchange Column**	**Tue 2/11/20**	**Wed 2/12/20**
64		AEX Equilibration	Wed 2/12/20	Wed 2/12/20
65		AEX Loading	Tue 2/11/20	Tue 2/11/20
66		AEX CIP	Tue 2/11/20	Tue 2/11/20
67		**Downstream 3**	**Tue 2/11/20**	**Wed 2/12/20**
68		**Viral Inactivation**	**Tue 2/11/20**	**Wed 2/12/20**
69		Viral Inactivation Batch	Tue 2/11/20	Tue 2/11/20
70		Viral Inactivation CIP	Tue 2/11/20	Wed 2/12/20
71		Viral Inactivation SIP	Wed 2/12/20	Wed 2/12/20
72		**Cation Exchange Chromatography**	**Tue 2/11/20**	**Wed 2/12/20**
73		CEX Equilibration	Wed 2/12/20	Wed 2/12/20
74		CEX Loading	Tue 2/11/20	Wed 2/12/20
75		CEX Wash 1	Wed 2/12/20	Wed 2/12/20
76		CEX Wash 2	Wed 2/12/20	Wed 2/12/20
77		CEX Elution	Wed 2/12/20	Wed 2/12/20
78		CEX CIP	Wed 2/12/20	Wed 2/12/20
79		**Ultra Filtration / Diafiltration**	**Wed 2/12/20**	**Wed 2/12/20**
80		UF/DF Batch	Wed 2/12/20	Wed 2/12/20
81		UF / DF CIP	Wed 2/12/20	Wed 2/12/20
82		UF / DF SIP	Wed 2/12/20	Wed 2/12/20
83		**Anion Exchange Column**	**Wed 2/12/20**	**Wed 2/12/20**
84		AEX Equilibration	Wed 2/12/20	Wed 2/12/20
85		AEX Loading	Wed 2/12/20	Wed 2/12/20
86		AEX CIP	Wed 2/12/20	Wed 2/12/20
87		**Final Storage**	**Wed 2/12/20**	**Wed 2/12/20**
88		Final Storage Freezing	Wed 2/12/20	Wed 2/12/20
89		Final Storage Refridgeration	Wed 2/12/20	Wed 2/12/20



Page 3

Figure 9. Gant charts representing all the steps within a single batch. Over 140 batches are run per year.

Equipment Tables

Equipment List and Unit Descriptions

Unit Operation	Step	ID	Short Description
Media Preparation	1.01	T1	Media Preperation Tank
Media Preparation	1.02	MX1	316L stainless steel removable mixer for T1
Seed Train	2.01	V1	Cell Vial
Seed Train	2.02	BK1	5 mL polystyrene vial with push cap
Seed Train	2.03	R1	35 mL Polystyrene Roller Flask
Seed Train	2.04	R2	200 mL Polystyrene Roller Flask
Seed Train	2.05	BK2	1 L Polystyrene Beaker
Seed Train	2.06	BK3	5 L Polystyrene Beaker
Seed Train	2.07	DB1	25 L 316L stainless steel tank with bag
Seed Train	2.08	DB2	100 L 316L stainless steel tank with bag
Seed Train	2.09	MX2	Magnetic Stir Bar for BK2
Seed Train	2.1	MX3	Magnetic Stir Bar for BK3
Seed Train	2.11	MX4	316L Stainless Steel Removable Mixer for DB1
Seed Train	2.12	MX5	316L Stainless Steel Removable Mixer for DB2
Seed Train	2.13	HX1	Hotplate and Magnetic Mixer Mount for all vessels
Protein A Chromotagrpahy	5.01	C1	Protein A Column
Polishing	5.02	C2	Cation Exchange Column

Polishing	5.03	C3	Anion Exchange Column
Polishing	5.04	BX1	Buffer Exchange (UF/DF)
Protein A Chromotagrpahy	5.05	F1	Dead End Filtration
Polishing	5.06	F2	Dead End Filtration
Polishing	5.07	F3	Dead End Filtration
Polishing	5.08	BT1-6	Buffer Tanks
Bioreactor	3.01	RTX1	Oxygen Sparger
Bioreactor	3.02	S1	CO2 Separator
Bioreactor	3.03	RX1	Reactor
Viral Inactivation	6.01	T3A	5000 L Storage Tank
Viral Inactivation	6.02	T3B	5000 L Storage Tank
Viral Inactivation	6.03	F101	Mircofilters
Viral Inactivation	6.04	F102	Mircofilters
Viral Inactivation	6.05	UF1	Ultrafiltration Membrane
Primary Harvest	4.01	CF1	Centrifuge

Table 8. Table of equipment and description sorted by unit operation and chronological point in the batch process.

Equipment Specification Sheets

Operation:	Seed Train, V1
Description/Functionality:	Cell Vial containing the initial seed cells given by the associated lab.
Key Dimensions:	1 mL $1*10^6$ cells
Construction Material:	Glass
Operating Conditions:	Temperature: 20 °C Pressure: 1 atm

Operation:	Seed Train, BK1
Description/Functionality:	5 mL beaker, containing 1 mL of cells from V1 and 4 mL of prepared media used as the first step of the seed train.
Key Dimensions:	7.5 mL 5 mL active volume
Construction Material:	Polystyrene (Disposable)
Operating Conditions:	Temperature: 38 °C Pressure: 1 atm

Operation:	Seed Train, R1
Description/Functionality:	35 mL roller flask, contain 5 mL of cells from BK1 and 30 mL of prepared media used as the second step of the seed train.
Key Dimensions:	40 mL 35 mL active volume
Construction Material:	Polystyrene (Disposable)
Operating Conditions:	Temperature: 38 °C Pressure: 1 atm

Operation:	Seed Train, R2
Description/Functionality:	200 mL roller flask, containing 35 mL of cells from R1 and 165 mL of prepared media used as the third step of the seed train.
Key Dimensions:	210 mL 200 mL active volume
Construction Material:	Polystyrene (Disposable)
Operating Conditions:	Temperature: 38 °C Pressure: 1 atm

Operation:	Seed Train, BK2
Description/Functionality:	1 L beaker, containing 200 mL of cells from R2 and 800 mL of prepared media used as the fourth step of the seed train. Stirred using MX2.
Key Dimensions:	1.2 L 1 L active volume
Construction Material:	Polystyrene (Disposable)
Operating Conditions:	Temperature: 38 °C Pressure: 1 atm

Operation:	Seed Train, BK3
Description/Functionality:	5 L beaker, containing 1 L of cells from BK2 and 4 L of prepared media used as fifth step of the seed train. Stirred using MX2.
Key Dimensions:	5.4 L 5 L active area
Construction Material:	Polystyrene (Disposable)
Operating Conditions:	Temperature: 38 °C Pressure: 1 atm

Operation:	Seed Train, DB1
Description/Functionality:	25 L tank with a bag inserted to prevent need for CIP. Contains 5 L if cells from BK3 and 20 L of prepared media, used as the sixth step of the seed train. Stirred using MX4.
Key Dimensions:	30 L tank 28 L bag 25 L active area
Construction Material:	SS 316L for Tank. Polystyrene (disposable) for bag.
Operating Conditions:	Temperature: 38 °C Pressure: 1 atm.

Operation:	Seed Trian, DB2
Description/Functionality:	100 L tank with a bag inserted to prevent need for CIP. Contains 25 L if cells from DB1 and 75 L of prepared media, used as the sixth step of the seed train. Stirred using MX4.
Key Dimensions:	120 L tank 120 L bag 100 L active area
Construction Material:	SS 316L for tank. Polystyrene (Disposable) for bag.
Operating Conditions:	Temperature: 32 °C - 38 °C Pressure: 1 atm.

Operation:	Seed Train, MX2
Description/Functionality:	Magnetic stir bar used to stir BK2 and BK3 via the HX1s magnetic stir function.
Key Dimensions:	5 cm's in length 0 – 500 rpm
Construction Material:	Iron coated in polystyrene
Operating Conditions:	Temperature: 38 °C Pressure: 1 atm

Operation:	Seed Train, MX4

Description/Functionality:	Large industrial mixer for mixing DB1 and DB2.
Key Dimensions:	Max Stirring Quantity 100 L 0 – 6000 rpm
Construction Material:	SS 316L
Operating Conditions:	Temperature: 32 °C – 38 °C Pressure: 1 atm

Operation:	Seed Train, HX1
Description/Functionality:	Hotplate and magnetic mixer for seed train, used to maintain seed train steps at specified temperature between 38 °C
Key Dimensions:	150 L stirring cap. 0 – 600 rpm 500 mm x 500 mm 80 W
Construction Material:	Steel casing
Operating Conditions:	Temperature: 32 °C – 38 °C Pressure: 1 atm

Operation:	Disc Stack Centrifugation
Description/Functionality:	The bulk (~90%) of the insoluble particulates are removed in a paste that is approximately 50/50 v/v liquid to solid. The cheaper operating costs allow for offsetting primary harvest costs.
Key Dimensions:	Sigma Factor: 12,300 m^2 Outer Disc Radius: 0.3 m Inner Disc Radius: 0.05 m
Construction Material:	Stainless Steel 316
Operating Conditions:	Temperature: ~37°C Pressure: 1 atm

Operation:	Depth Filtration
Description/Functionality:	Supplemental insoluble particle removal designed for holdup based on size exclusion as well as adsorption.
Key Dimensions:	Filtration Area: 25 m^2
Construction Material:	Animal free: Cellulose, Inorganic Filter
Operating Conditions:	Temperature: ~37°C Maximum Differential Pressure: 34 psi

Operation:	Primary Harvest to Chromatography Intermediate Storage
Description/Functionality:	Primary recovery is rapid by comparison to chromatography, while requires multiple batches. Hence, an intermediate storage container is necessary.
Key Dimensions:	5 m^3
Construction Material:	Stainless Steel 316
Operating Conditions:	Temperature: 20-37°C Pressure: 1 atm

Operation:	Bioreactor, RX1
Description/Functionality:	Fed batch bioreactor is used to produce monoclonal antibodies at a faster rate than the seed train
Key Dimensions:	Reactor Volume = 5000 L Reaction Time = 660 hr Feed Flowrate = 7.5 Lhr^{-1}
Construction Material:	316L SS
Operating Conditions:	Pressure: 1 atm Temperature: 38 °C

Operation:	Substrate Mixer, MX1
Description/Functionality:	Premixes concentrated gluclose substrate with water to feed the reactor.
Key Dimentsions:	Maximum Substrate Concentration: 106 gL^{-1}
Construction Material:	316L SS
Operating Conditions:	Pressure: 1 atm Temperature: 22 °C

Operation:	Carbon Dioxide Removal, S1
Description/Functionality:	Removes carbon dioxide to reduce reactor acidification and carbon dioxide poisoning.
Key Dimentsions:	Max CO_2 Removal Rate: 5.8 $kg\text{-}hr^{-1}$
Construction Material:	316L SS
Operating Conditions:	Pressure: 1 atm Temperature: 38 °C

Operation:	C1
Description/Functionality:	Protein A affinity column
Key Dimensions:	Height: 30 cm Inner Diameter: 70 cm Volume: 116 L Loading: 30 g mAb/L resin Operating Flowrate: 300 cm/hr
Construction Material:	Polyethylene
Operating Conditions:	Temperature: 6-8℃ Pressure: 0.05 MPa

Operation:	C2
Description/Functionality:	Cation exchange column
Key Dimensions:	Height: 30 cm Inner Diameter: 55 cm Volume: 71 L Loading: 50 g mAb/L resin Operating Flowrate: 100 cm/hr
Construction Material:	Polyethylene
Operating Conditions:	Temperature: 6-8℃ Pressure: 0.2 MPa

Operation:	C3
Description/Functionality:	Anion exchange column
Key Dimensions:	Height: 30 cm Inner Diameter: 55 cm Volume: 85 L Loading: 40 g mAb/L resin Operating Flowrate: 150 cm/hr
Construction Material:	Polyethylene
Operating Conditions:	Temperature : 6-8℃ Pressure: 1 bar

Operation:	BT1-6
Description/Functionality:	Buffer Storage Tanks
Key Dimensions:	Size: 100 L
Construction Material:	Disposable Plastic
Operating Conditions:	Temperature : 22℃ Pressure: 1 bar

Operation:	UF1
Description/Functionality:	Tangential Flow Filtration (TFF) membrane
Key Dimensions:	MWCO: 30 kDa Membrane Area: 3.6 m^2
Construction Material:	
Operating Conditions:	Transmembrane Flux: 50 $L/m^2/hr$ Transmembrane Pressure: 10 psig

Operation:	P2
Description/Functionality:	Buffer exchange pump
Key Dimensions:	Flowrate: 18 L/min
Construction Material:	
Operating Conditions:	

Operation:	T4
Description/Functionality:	Buffer exchange holding tank
Key Dimensions:	Capacity: 600 L
Construction Material:	316L Stainless Steel
Operating Conditions:	Temperature : 22℃ Pressure: 1 bar

Operation:	F1
Description/Functionality:	Dead-end filtration unit
Key Dimensions:	Membrane Area: 0.12 m^2 Pore size: 0.22 μm
Construction Material:	
Operating Conditions:	Transmembrane Flux: 10,000 $L/m^2/hr$

Operation:	F2
Description/Functionality:	Dead-end filtration unit
Key Dimensions:	Membrane Area: 0.3 m^2 Pore size: 0.22 μm
Construction Material:	
Operating Conditions:	Transmembrane Flux: 10,000 $L/m^2/hr$

Operation:	F3
Description/Functionality:	Dead-end filtration unit
Key Dimensions:	Membrane Area: 0.5 m^2 Pore size: 0.22 μm
Construction Material:	
Operating Conditions:	Transmembrane Flux: 10,000 $L/m^2/hr$

Equipment Cost Summary

Key	ID	Equipment Description	Cost/Batch	Initial Cost	Costing Parameter	Source
1	T1	Media Preparation Tank	$600	$70	NA	1
1	MX1	316L stainless steel removable mixer for T1	$ -	$6,211	NA	2
2	V1	Cell Vial	$ -	$-	NA	NA
2	BK1	5 mL polystyrene vial with push cap	$ -	$-	NA	3
2	R1	35 mL Polystyrene Roller Flask	$16	$2,414	NA	4
2	R2	200 mL Polystyrene Roller Flask	$16	$-	NA	4
2	BK2	1 L Polystyrene Beaker	$8	$-	NA	4
2	BK3	5 L Polystyrene Beaker	$25	$-	NA	1
2	DB1	25 L 316L stainless steel tank with bag	$600	$60	NA	1
2	DB2	100 L 316L stainless steel tank with bag	$600	$70	NA	5
2	MX2	Magnetic Stir Bar for BK2	$ -	$10	NA	NA
2	MX3	Magnetic Stir Bar for BK3	$ -	$10	NA	6
2	MX4	316L Stainless Steel Removable Mixer for DB1	$ -	$6,211	NA	6
2	MX5	316L Stainless Steel Removable Mixer for DB2	$ -	$-	NA	NA
2	HX1	Hotplate and Magnetic Mixer Mount for all vessels	$ -	$4,377	NA	7
3	O1	Oxygen Sparger	$ -	$14,000	NA	8
3	S1	Carbon Dioxide Separator	$ -	$38,000	NA	9
3	RX1	Bioreactor Vessel	$ -	$202,000	Vessel Mass	10
3	T1	Media Preparation Tank	$ -	$1,200	Vessel Mass	10
4	CF1	Primary Cetrifuge	$ -	$124,000	Centrifuge Flow	11
4	DF1	Depth Filter	$8,750	$ -	Surface Area	12
5	C1	Protein A Column	$ -	$200,000	Column Volume	13
5	F1	Dead End Filtration	$ -	$1,000	Surface Area	10

6	T3A	Low pH Storage Tank	$ -	$96,000	Vessel Mass	10
6	T3B	Detergent Treatment Storage Tank	$ -	$96,000	Vessel Mass	10
6	F4	Microfilters	$920	$ -	Surface Area	12
6	F5	Microfilters	$917	$ -	Surface Area	12
6	F6	Ultrafiltration Membrane	$617	$ -	Surface Area	12
7	C2	Cation Exchange Column	$ -	$170,000	Column Volume	13
7	C3	Anion Exchange Column	$ -	$180,000	Column Volume	13
7	BX1	Buffer Exchange for ultrafiltration and diafiltration	$ -	$140,000	Column Volume	13
7	F2	Dead End Filtration	$ -	$1,000	Surface Area	12
7	F3	Dead End Filtration	$ -	$1,000	Surface Area	12
7	BT1-6	Buffer Tanks	$1,080	$ -	Vessel Mass	10
8	T6	Kill Tank	$ -	$127,800	Vessel Mass	10
9	T7	Final Storage Tank	$ -	$32,000	Vessel Mass	10
		Total	$1,881,817	$5,016,233		

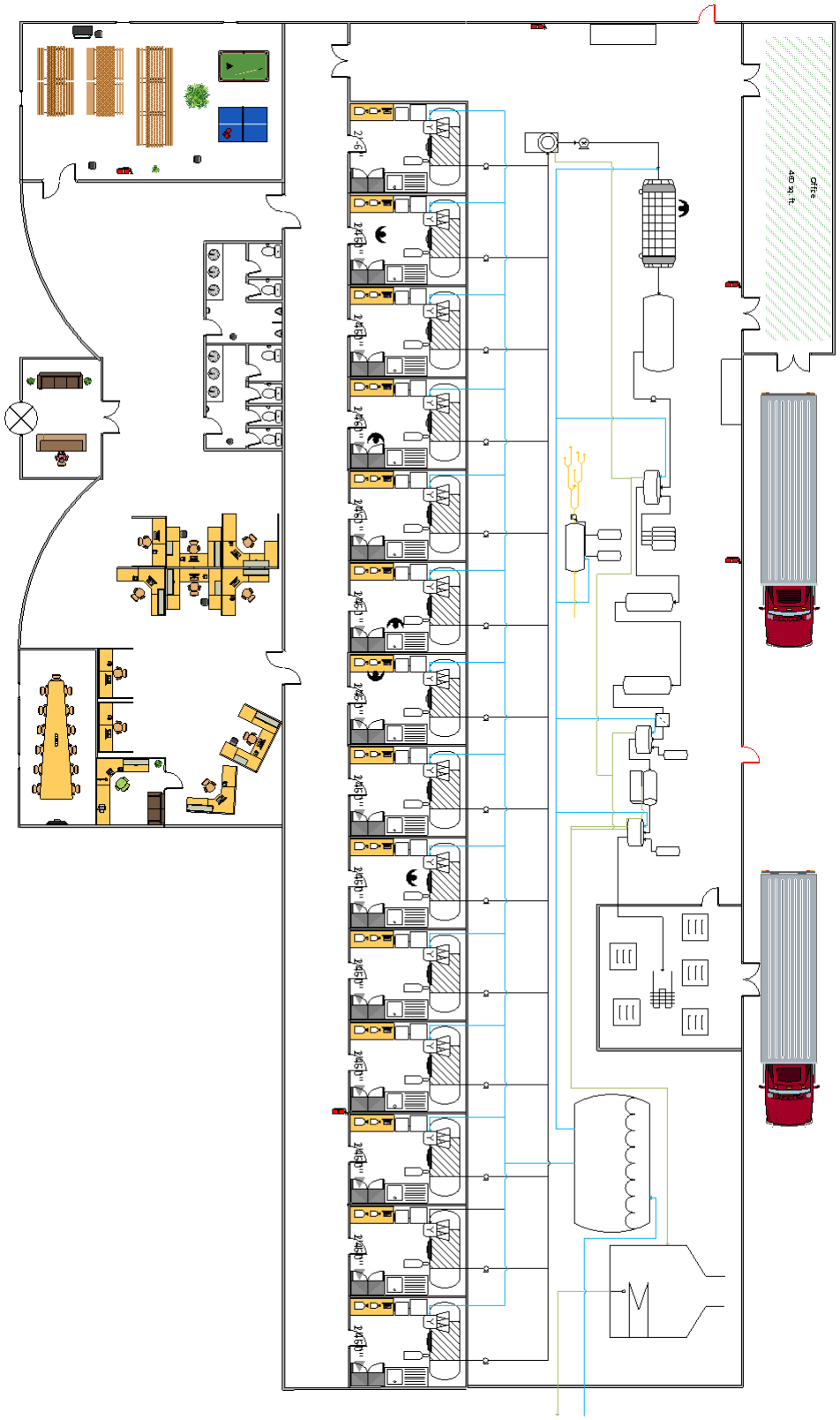
Table 9. A summary of capital equipment costs for major pieces of equipment, which are disposable and non-disposable

1Media Preparation2Seed Train3Bioreactor4Primary Harvest5Protein A chromatography6Viral Inactivation7Final Polishing8Waste Disposal9Final StorageTable 10. Key for which numbers represent each system unit operation.

Safety, Health, and Environmental Considerations

When approaching safety considerations, we first use the following: substitute, to swap hazardous chemicals for less hazardous chemicals; minimize, to reduce the size of equipment and the amount of raw materials that are stored at any given time, particularly stored in hazardous forms such as liquids; moderate, to design in such a way that parameters stay away from the extremes such as using vacuum to reduce boiling points; and simplify, to reduce complexity saving maintenance requirements and preventing confusion which leads to risk. One potential candidate for substitution would be the media, since we opted to make our own that added a step and significantly more chemicals we have chosen an inherently less safe route, however we have determined that in this case the economic saving outweighs the potential safety risk, as the additional chemicals are highly inert and unlikely to have significant risk associated. Minimization was heavily considered, as our equipment size is small forcing us to run significantly more batches. This was done in the interest of flexibility for future production however, it also introduces a safer design as defective equipment is easily and cheaply replaced rather than repaired in ways that may compromise future work. In addition, the total amount of chemical stored at any time is relatively low as the seed train was designed to not require a storage step but rather adjust the speed of the final step using temperature controls such that storage is unnecessary. Finally, powdered media is mixed per requirement rather than mixing upon arrival which would require storage in a liquid form at low temperatures. For moderation, we chose to have the final storage at –10 °C rather than –80 °C, as a less extreme temperature is inherently safer.

Siting and Layout of Processes and Equipment



Other Important Considerations

The most important additional considerations for this project are those required by regulatory agencies, specifically the food and drug administration. We are required to have a quality control unit consisting of at least three operators whose purpose is to test, approve and reject all incoming materials (21CFR211.22). These designated operators will also oversee quality testing the product batch every before and after storage in the freezer for quality (21CFR211.165a-b) and stability (21CFR211.166a). We will be required to provide protective apparel for all employees (21CFR211.28a). The facility itself must be must be aseptic including temperature and humidity controls and an air supply filtered through high-efficiency particulate air filters under positive pressure (21CFR211.42c10ii - iv.), however since we are building within a pre-existing facility, who already adheres to FDA guidelines this should already be accomplished. All nondisposable materials were chosen to be Stainless Steel 316L and all disposable materials were chosen to be polystyrene to adhere to the nonreactive or absorptive materials clause (21CFR211.65a). In the future we should note maximum deviations for phase time (21CFR211.111) however that is impossible to pinpoint at this time in a preliminary design. We also must have a reprocessing system must be in place for product outside of specification (21CFR211.115a) if we choose in the future to not dispose of poor product, however at this time the plan is to dispose of the any product graded below high quality.

Economic Analysis

Manufacturing Costs (exclusive of Capital Requirements)

Raw Materials Costing				
Operation	**Material**	**kg per batch**	**Cost per kg**	**Annual Cost**
Bioreactor	Media	115	$ 1,724.50	$ 26,376,227.50
Bioreactor	Gluclose	183.4	$ 10.00	$ 243,922.00
Bioreactor	WFI	5000	$ 1.00	$ 665,000.00
Bioreactor	Purified Oxygen	345	$ 4.00	$ 183,540.00
Depth Filtration	Buffer	417	$ 1.00	$ 55,461.00
Depth Filtration	WFI	2500	$ 1.00	$ 332,500.00
Viral Inactivation	Triton X 100	50	$ 107.30	$ 713,545.00
Viral Inactivation	Tri-n-butyl Phosphate	15	$ 34.60	$ 69,027.00
Protein A Chromotagraphy	phosphate	15.6	$ 75.00	$ 155,610.00
Protein A Chromotagraphy	citrate	6.6	$ 40.00	$ 35,112.00
Protein A Chromotagraphy	NaCl	50.7	$ 10.00	$ 67,431.00
Protein A Chromotagraphy	NaOH	20.7	$ 30.00	$ 82,593.00
Protein A Chromotagraphy	WFI	6927	$ 1.00	$ 921,291.00
Protein A Chromotagraphy	Waste	14202	$ 0.00	$ 2,455.53
Protein A Chromotagraphy	Resin	9.9	$ 15,000.00	$ 19,750,500.00
Viral Inactivation	NaOH	1.5	$ 30.00	$ 5,985.00
Viral Inactivation	WFI	5.1	$ 1.00	$ 678.30
Cation Exchange	MES	13.5	$ 270.00	$ 484,785.00
Cation Exchange	MOPS	3.3	$ 390.00	$ 171,171.00
Cation Exchange	NaCl	18.6	$ 10.00	$ 24,738.00
Cation Exchange	NaOH	12.9	$ 30.00	$ 51,471.00
Cation Exchange	WFI	4275	$ 1.00	$ 568,575.00
Cation Exchange	Waste	4596	$ 0.00	$ 794.65
Cation Exchange	Resin	5.4	$ 2,500.00	$ 1,795,500.00
Buffer Exchange	WFI	3750	$ 1.00	$ 498,750.00
Buffer Exchange	Waste	3375	$ 0.00	$ 583.54
Anion Exchange	Tris	5.1	$ 100.00	$ 67,830.00
Anion Exchange	NaCl	5.1	$ 10.00	$ 6,783.00
Anion Exchange	NaOH	15.3	$ 30.00	$ 61,047.00
Anion Exchange	WFI	2037	$ 1.00	$ 270,921.00
Anion Exchange	Waste	2037	$ 0.00	$ 352.20
Anion Exchange	Resin	6.3	$ 2,500.00	$ 2,094,750.00
Buffer Exchange	Buffer	0	$ -	$ -
			Total	$ 55,758,929.71

Table 11. Raw Materials Cost Organized by batches and multiplied by total number of batches per year to achieve yearly cost.

Utilities Costing				
Operation	**Utility**	**Cost Per Batch**	**kW Per Batch**	**Annual Cost**
Depth Filtration	Filter Replacement	8750	NA	$1,102,500.00
Bioreactor	Heating Jacket	NA	0.144	$1,024.83
Bioreactor	Agitator	NA	0.01	$1,226.50
Centrifugation	Separation	NA	1.4	$44.10
				$1,104,795.43

Economic Discussion

Total plant cost (TPC) and Direct Fixed Cost (DFC) is shown in the table below. The TPC is $28.2 million and DFC is $32.5 million, as calculated using the purchase cost and the Lang factors shown.

		Multiplier		Amount (million $)
Total Plant Direct Cost (TPDC)				**17.7**
	Equipment Purchase Cost (PC)			**5.0**
	Installation	0.50	x PC	2.5
	Process Piping	0.40	x PC	2.0
	Instrumentation	0.35	x PC	1.8
	Insulation	0.03	x PC	0.2
	Electrical	0.15	x PC	0.8
	Buildings	0.45	x PC	2.3
	Yard Improvement	0.15	x PC	0.8
	Auxiliary Facilities	0.50	x PC	2.5
Total Plant Indirect Cost				**10.6**
	Engineering	0.25	x TPDC	4.4
	Construction	0.35	x TPDC	6.2
Total Plant Cost (TPC)				**28.2**
	Contractor's Fee	0.05	x TPC	1.4
	Contingency	0.10	x TPC	2.8
Direct Fixed Capital (DFC)				**32.5**

Table 12. Direct Fixed Capital by Direct Cost, Indirect Cost, and Other Considerations

The ten-year operation of the plant is shown below assuming a price of $5 million per kilogram for mAbs. The yearly expenditures is $58 million and a revenue of $5,000 million. This yields a significant profit margin that needs additional support and verification before proceeding to a detailed design. In comparison to similar plants, the capital requirements and annual operating costs are significantly smaller for the designed plant.

Interest	12%							
Year	Expenditures	Revenue	Gross Profit	Depreciation	Taxable Income	Taxes Paid	CF	PV
0	$ (33)	$ -	$ (33)	$ -	$ -		$ 33	$ 33
1	$ (58)	$ 5,000	$ 4,942	$ (7)	$ 4,949	$ -	$ 4,942	$ 4,413
2	$ (58)	$ 5,000	$ 4,942	$ (10)	$ 4,952	$ 1,039	$ 3,903	$ 3,111
3	$ (58)	$ 5,000	$ 4,942	$ (6)	$ 4,948	$ 1,040	$ 3,902	$ 2,777
4	$ (58)	$ 5,000	$ 4,942	$ (4)	$ 4,946	$ 1,039	$ 3,903	$ 2,480
5	$ (58)	$ 5,000	$ 4,942	$ (374)	$ 5,316	$ 1,039	$ 3,903	$ 2,215
6	$ (58)	$ 5,000	$ 4,942	$ (2)	$ 4,944	$ 1,116	$ 3,826	$ 1,938
7	$ (58)	$ 5,000	$ 4,942	$ -	$ 4,942	$ 1,038	$ 3,904	$ 1,766
8	$ (58)	$ 5,000	$ 4,942	$ -	$ 4,942	$ 1,038	$ 3,904	$ 1,577
9	$ (58)	$ 5,000	$ 4,942	$ -	$ 4,942	$ 1,038	$ 3,904	$ 1,408
10	$ (58)	$ 5,000	$ 4,942	$ -	$ 4,942	$ 1,038	$ 3,904	$ 1,257
								$ 22,975

Table 13. Net Present Value Calculation Table Using MACRS 10-year depreciation.

Conclusions and Recommendations

The recommended plant design consists of cell and protein production followed by protein purification, curing, and storage, and final process flow and sizing is summarized here. For each reactor batch, cells are systematically grown using a seed train. The seed train batches consists of seven steps of 0.005, 0.035, 0.200, 1.0, 5.0, 25, and 100 L, where each batch contains the contents from the prior step and the remainder fresh media. The first batch step is 30 seconds, followed respectively by 2.5, 13.5, 42.7, 70.5, 83.8, and 87.2 hours. Each step is consistently stirred and maintained at 38°C and atmospheric pressure excluding the last step, which should be run between 32 °C and 38 °C depending on the amount of time until the next bioreactor is ready.

Once the final seed step has been transferred to the bioreactor, fed-batch protein production will be performed for 660 hours using a water for injection feed flow of 7.5 L/hr containing media and glucose at controlled and constrained concentrations. The media to be used is modeled here as BalanCD due to its performance relative to other commercially marketed chemically-defined animal-free media, although it is recommended to tailor media compositions for each cell line starting with the proposed components. The excess glucose required for fed batch operation will be initially supplied using pure glucose. Conveyors will add dry powder media and glucose to water for injection in a small mixer before the reactor. Control systems will be used to moderate mixing speed, injection rate, pH level, pressure, and temperature. The bioreactor must run at 37°C, and carbon dioxide replacing the oxygen bubbling through the reactor must be vented consistently. Use of a sampling port will ensure adequate monitoring of conditions. The reactor is designed using Monod kinetics resulting in a final titer of 1.5 g/L, corresponding to 7.5 kg per batch. To provide one ton of monoclonal antibody proteins annually, 14 batch reactors are required. Only one downstream system is necessary, which means batches are staggered by about three days.

The first purification step is primary harvest, in which insoluble particles are separated to avoid interactions downstream. A stainless-steel disc stack centrifuge removes the bulk of particles (dead cells, cell debris, aggregates, etc.) in the form of a paste. The sigma factor (the sizing metric) for the centrifuge is 12,300 cubic meters, and the centrifuge does not require a cooling jacket, although lab or pilot scale broth centrifugation data is required to avoid detrimental shear stress occurrence. The remaining particles are removed by a 25 m^2 depth filter stack, which is designed to use cartridges that will reach capacity and be disposed for each reactor batch, removing the need for cleaning. Although the depth filter is modelled using an XOHC Millipore Pod system, filter media should be adjusted for better adsorption of free small charged particles. Primary harvest is performed at 5000 L/hr and is stored in a tank for use in chromatography.

Protein A chromatography involves the selective binding of the monoclonal antibody proteins to resins followed by a buffer wash to separate the proteins from most of the reactor broth. The protein A chromatography step uses a 116 L MabSelect SuRe column to purify 3000 g of mAb per cycle. This means three cycles must be run per reactor batch. Viral inactivation is done after protein A chromatography. Two storages tanks are used

for detergent treatment and low pH treatment to drastically reduce the number of enveloped virus. Between both tanks, Millipore microfilters are used to reduce the number of non-enveloped viruses between the tanks. There are two microfiltration steps, the first is done between the first and second tanks, and the second is done after detergent treatment. The last step in virial filtration revolves around ultrafiltration with the final polishing stage. Polishing (the final step before storage for purification) consists of a cation exchange column followed by an anion exchange. These use the POROS 50 HS and Q Sepharose FF resins and will have volumes of 72 L and 85 L, respectively. A diafiltration/ultrafiltration step between the two polishing columns will exchange the buffer and concentrate the mAb process stream. The final product is deposited into metal mini-tanks of 5 mL that are mostly submerged in a –20 °C methanol-water mixture for 45 minutes such that it is completely frozen, then these will be stored at –10 °C for up to one year.

The projected cost of the facility neglects infrastructure requirements, considering an existing facility has been proposed for use. The MABs are expected to sell at $5 per milligram, resulting in an annual revenue of $5 billion despite $32 million in capital costs and $58 million in annual utilities. Detailed design is recommended, although it is warranted to estimate research and development costs to ensure viability.

It is also recommended to perform lab and pilot scale experimentation for better cell growth kinetic models, media optimization for the current cell line, and broth characteristics such as viscosity and response to shear. Fouling mechanisms for the filters and membranes as well as resin capacity should also be investigated.

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Appendices

Appendix A: HAZOP Study

To develop the detailed design and process and instrumentation diagram (P&ID) first a hazard and operability (HAZOP) analysis was performed. Four deviations each focusing on a different parameter in the bioreactor section: temperature, pressure, acidity, and flow rate. Guide words were chosen as the worst-case scenario for each of these parameters, which in most cases was “more” except in the case of flow rate was “none”.

Deviation	Cause	Consequence	Safeguard	Action
No water flow to the media mixer (M-120)	Pump failure (L-123) or shortage of water for injection (WFI)	Solids Buildup in Mixer	V-117 fail to closed to prevent solids from moving to reactor	Add Conveyor Emergency Shutdown
		Solids Buildup in Reactor	Excess storage of WFI to ensure enough prior to each batch initiation	V-101 Emergency Shutdown
		Cell Death		Dump reactor through V-111
	Solids buildup restricting flow	Clogging: V-101, Mixer, P-6, Impeller	Scheduled and Preventative Maintenance	Unscheduled CIP and SIP to clean moving parts and clean vessels to remove build up and clogging
			Pump Check Valve after the water for injection inlet pump	
			Clean in Place (CIP) and Steam in Place (SIP)	
			Feedback of WFI inlet flow rate to solids conveyor speed	
		Instrument Failure	Add non-zero values for instrument pneumatic signals for failure detection	Timely sterile replacement of dysfunctional instrumentation

Table 14. Hazard and Operability Analysis of the deviation "no water flow to the media mixer (M-120)". Causes and consequences were considered from one degree of separation away to limit scope of HAZOP directly to bioreactor section, V-101 and V-111 were added to the P&ID as a result of this analysis.

Deviation	Cause	Consequence	Safeguard	Action
More Reactor Acidity	L-122 or L-114 Fail Open, or L-121 or L-115 Fail Close (or too rapid input)	Accelerated Corrosion	Secondary pH check through sampling port	Dump reactor contents to kill tank
	pH Controller Malfunction	Downstream Failure	Scheduled and Preventative Maintenance	Perform unscheduled SIP/CIP
		Cell Death	Proper pump sizing (low capacity) to ensure small enough increments of pH adjustment are made.	Check for corrosion and replace components that have been eroded
		Pressure Buildup from Vaporization of Water Due to Acids.	Add caustic solutions to center of vessel	
		Toxic Fumes	Slow pH control to avoid local acidity/alkalinity and overcorrection	
	Venting Failure	CO_2 buildup resulting in dissolved acid	Pump check valve	
			Vent valve fail open	

Table 15. Hazard and Operability Analysis of the deviation "more reactor acidity". Causes and consequences were considered from one degree of separation away to limit scope of HAZOP directly to bioreactor section. The focus of the acidity was on the pH control system of the bioreactor and media mixer, specifically the positive displacement pumps that are to control basic and acidic solution injection. However, acidity can also be caused by a CO_2 buildup in the headspace creating more carbonic acid.

Deviation	Cause	Consequence	Safeguard	Action
More Headspace Pressure	Regulator Failure	Vessel Overpressure resulting in reactor explosion, reverse flow of other inlet streams, and induced cracking or leaking.	Scheduled and Preventative Maintenance	Broken or damaged components must be replaced.
	Blocked Exit Valve		Fail to Open Vent	
	Vent Valve Fails Closed		Fail to Close Regulator	
	Accelerated CO2 production from Cells		Fail to Close Inlet Valve	
	Sparger Failure		Heating Water Fail to Close	
	Sensor Failure		High Level Pressure Alarm	
			Reactor PSV	
			Reactor sufficiently thick	

Table 16. Hazard and Operability Analysis of the deviation "more headspace pressure". Causes and consequences were considered from one degree of separation away to limit scope of HAZOP directly to bioreactor section. The pressure analysis was found to be largely mitigated by the already existing safeguards.

Deviation	Cause	Consequence	Safeguard	Action
More Reactor Temperature	L-117 Overload	Vessel Overpressure	Preventative and Scheduled Maintenance	Dumping in Water
	Neutralization Reaction	Reactor Explosion	Pressure Relief Valve	Emergency Cooling Water
		Pressure Buildup; Reverse Flow	Heating Water is Fail to Close	
		System Cracking	Water Pump Alarm Dump	
	Impeller Speed Overload	Reactor Warping		
		Impeller Warping		
	External Fire	Vessel Damage		
		Cell Death		

Table 17. Hazard and Operability Analysis of the deviation "more reactor temperature". Causes and consequences were considered from one degree of separation away to limit scope of HAZOP directly to bioreactor section. The temperature analysis yielded the addition of an emergency cooling water system, although the temperatures are incapable of exceeding operation temperature by a significant enough degree to be dangerous for operators, there is enough thermal energy to cause damage to the cells and the vessels.

Appendix B: Bioreactor Detailed Design:

General ceiling height for building is approximately 14 feet and working at heights can be especially dangerous for maintenance personal. To minimize hazards and make the reactors small enough to place indoors, a height of 8' was chosen so that there was ample space between the ceiling and the top of the reactor, and to minimize exposure to working at heights. A headspace of 45% was chosen to allow for overflow protection and additional sparging volume. Vessel thickness was based on an operating pressure of 5.5 bar. Pressures higher than atmosphere were chosen to increase the mass transfer driving force within the reactor. A mixer and baffles were designed based on manufacturer recommendations to reduce potential vortexing and shear stress[5].

Vessel Sizing Calculations:

Vessel Height Calculations:

$$V = \pi r^2 h \quad (1.)$$

a.
$$\sqrt{\frac{5\ m^2}{\pi * 2.3\ m}} = \text{r}$$

b.
$$\sqrt{\frac{5\ m^2}{\pi * 2.3\ m}} = 0.83\ \text{m}$$

To calculating the radius for 45% Headspace:

$$1.5 = \frac{r_1^2}{r_2^2} \quad (2.)$$

a.
$$\sqrt{1.45 r_2^2} = r_1$$

b.
$$\sqrt{1.45 * 0.83^2} = r_1 = 9.994\ m$$

round to one

Calculating the additional headspace:

$$V = \pi r^2 h \quad (3.)$$

a.
$$V = \ \pi * 1^2 * 2.3$$

$$V = \pi * 1^2 * 2.3 = 7.22\ m^3$$ b.

$$5 = \pi r^2 h_l$$ c.

$$h_l = \sqrt{\frac{5}{\pi r^2}} = 1.26\ m$$ d.

$$h_t = 2.3\ m$$ e.

$$headspace = \frac{2.3\ m - 1.26\ m}{2.3\ m} * 100 = 45\ \%$$

Calculation of the additional hydrostatic head:

$$P_{max} = P_{headspace} + \rho g h = 5\ bar + \frac{1000 * 9.81 * 2.3}{10^5}\ bar$$ 4.

$$P_{max} = 5.25\ bar$$

Hydrostatic head even at full capacity was below headspace pressure, so the tank was designed assuming an operating pressure of 5.5 bar and that at overpressure the forces will be ½ the yield strength of the material (316L SS). Calculations were done with equations in Towler[8].

$$t = \frac{P_i D_i}{2SE - 1.2P_i}$$ 5.

$$P_{max} = 10.0\ bar$$ a.

$$t = \frac{10^6\ Pa * 2\ m}{2 * 1.034 * 10^8 * 0.85\ Pa - 1.2 * 10^6\ Pa}$$ b.

$$t = 0.0113\ m = 12\ mm$$ c.

Baffles are within the reactor are to prevent vertexing Dynamix recommended four baffles in the orientation shown in *Figure 1* and baffle dimensions were also calculated from recommendations by Dynamix Eqns 7-10.

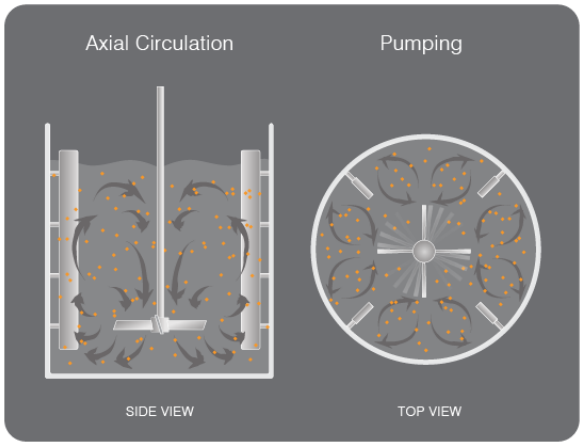

Figure 10. Display of expected baffle orientation of the tank.

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$$W = \frac{1}{12}D = \frac{1}{6}\,m \sim 18\,cm \quad (6)$$

$$L = h_l - 2 * 0.1524\,m = 0.96\,m \quad (7)$$

$$K = \frac{1}{6} * \frac{1}{6} \sim 3\,cm \quad (8)$$

$$Baffle\ \# = 4 \quad (9)$$

To size the mixer, a Reynolds number near 1000000 was chosen so that the system was just beyond a turbulent Reynolds number within the tank[1]. This was to minimize shear stress on cells while having more efficient mixing. Values were found through iterative guess and check given the agitator power curve in *Figure 11*.

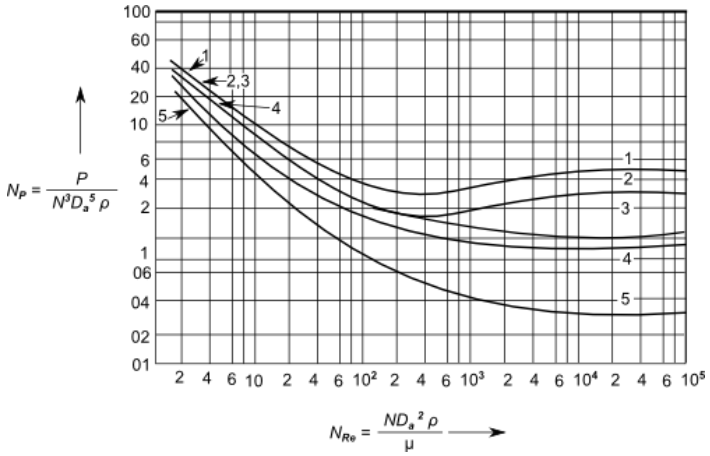

Figure 11. An agitator power curve relating a dimensionless power number to the mixing Reynolds number.

An online program[1] was used to find the RPM, Power, Mixer Diameter, and Mixing Reynolds Number with the power curve shown in *Figure 2.*

$$RPM = 125$$
$$P = 2.0\ kW$$
$$D = 0.6\ m$$
$$Re = 692000$$

The sparger was sized according to the greatest oxygen consumption rate within the reactor *Figure 3*. Mass transfer coefficients were based on literature and the henrys law constant was pulled from data provided by the National Institute of Standards[7,8,4]. An average bubble diameter of 5.0 mm was assumed, and average DO concentrations are assumed based on literature of typical concentrations in cell broths[8,4].

$$\dot{W} = JA \quad (10)$$

$$MaxOxygen\ Consumption\colon\ 3.9\ kg/hr \quad \text{a.}$$

$$K_L = 0.4\ m/hr \quad \text{b.}$$

$$J = 0.4\frac{m}{hr}\left(0.0053\frac{mol}{L} - 0.00065\frac{mol}{L}\right) * 10^3\frac{L}{m^3} \quad \text{c.}$$

$$3.9\ k\,g/hr = 0.2\frac{m}{hr}\left(0.0053\frac{mol}{L} - 0.00065\frac{mol}{L}\right) * 10^3\frac{L}{m^3} * \frac{0.0032\ kg}{mol} \quad \text{d.}$$

$$A = 1400\ m^2 \quad \text{e.}$$

$$Bubbles\ = \frac{1400\ m^2}{4\pi * (0.0025)^2} = 1.78 * 10^7 \quad \text{f.}$$

$$V = \frac{4}{3}\pi(0.0025)^3 * n = 1.16\ m^3 \quad \text{g.}$$

$$h_b = 0.34\ m \quad \text{h.}$$

$$h_{max} = h_b + h_l = 0.34\ m + 1.26\ m = 1.6\ m \quad \text{i.}$$

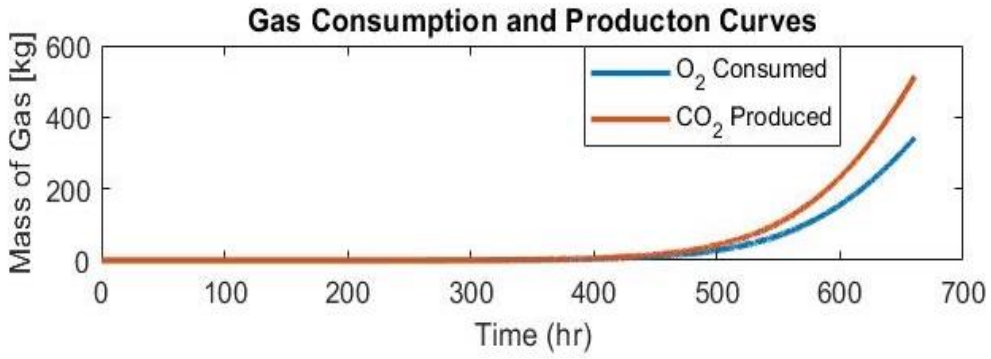

Figure 12. Gas consumption and production curves within the reactor. The highest consumption rate occurs at the very end of the reactor cycle.

PRV Calculations are based on flows into the reactor and equations are sourced from Crowl and Tipler[9].

$$A = \frac{W}{K_d P_1}\sqrt{\frac{R_g T}{\gamma g_c M}\left(\frac{\gamma+1}{2}\right)^{(\gamma+1)(\gamma-1)}}$$

$W = mass\ flowrate\ [kg/s]$ $\qquad$ $R_g = Ideal\ gas\ constant\ [J/mol \cdot K]$

$K_d = discharge\ coefficient\ [\ ]$ $\qquad$ $T = Absolute\ temperature\ [K]$

$P_1 = upstream\ relieving\ pressure\ [Paa]$ $\qquad$ $M = molecular\ weight\ [kg/mol]$

$\gamma = heat\ capacity\ ratio\ [\ ]$ $g_c = gravitational\ acceleration\ [m^2/s]$

For preliminary sizing purposes, a discharge coefficient, K_d, of 0.975 is used. Meanwhile, the bioreactor pressure is 5 bar upon requiring relief. The bioreactor is maintained at 37°C and the molecular weight and heat capacity ratio are assumed to be those of air at that temperature (0.029 kg/mol and 1.400, respectively). The mass flow rate is taken as the sum of the inflows per batch.

$$W = \left(\frac{4900\ kg}{660\ hr} + \frac{411\ kg}{660\ hr} + \frac{514\ kg}{660\ hr}\right)\left(\frac{hr}{3600\ s}\right) = 5.96 \times 10^{-4}\frac{kg}{s}$$

$$\therefore A = \frac{5.96 \times 10^{-4}\frac{kg}{s}}{0.975\left(5\ bar \times 10^5 \frac{kg}{bar \cdot m \cdot s^2}\right)}\sqrt{\frac{\left(8.314\frac{kg \cdot m^2}{mol \cdot K \cdot s^2}\right)(310.15K)}{(1.4)\left(9.81\frac{m^2}{s}\right)\left(0.029\frac{kg}{mol}\right)}\left(\frac{1.4+1}{2}\right)^{(1.4+1)(1.4-1)}} = 1.07 \times 10^{-7}\ m^2$$

$$A = 1.66 \times 10^{-4} in^2$$

Orifice Design D

The following tables are the results of the detailed design:

Vessel Dimensions	
Height	2.3 m
Diameter	2 m
Liquid Height	1.26 m
Wall Thickness	12 mm
Design Pressure	5.5 bar
Max Pressure	10 bar
Headspace	1.04 m

Sparger Dimensions	
Bubble Diameter	5.0 mm
Bubble Volume	0.6 m
Agitator Power	2 kW
Agitator Blade Size	0.6 m
Agitator Speed	125 RPM
Blade Angle	20°
Blades	6

Baffle Dimensions	
Baffles	4
Baffled Width	18 cm
Baffle Length	1.3 m
Baffle Mount	3.0 cm

Pressure Relief Device
Orifice D

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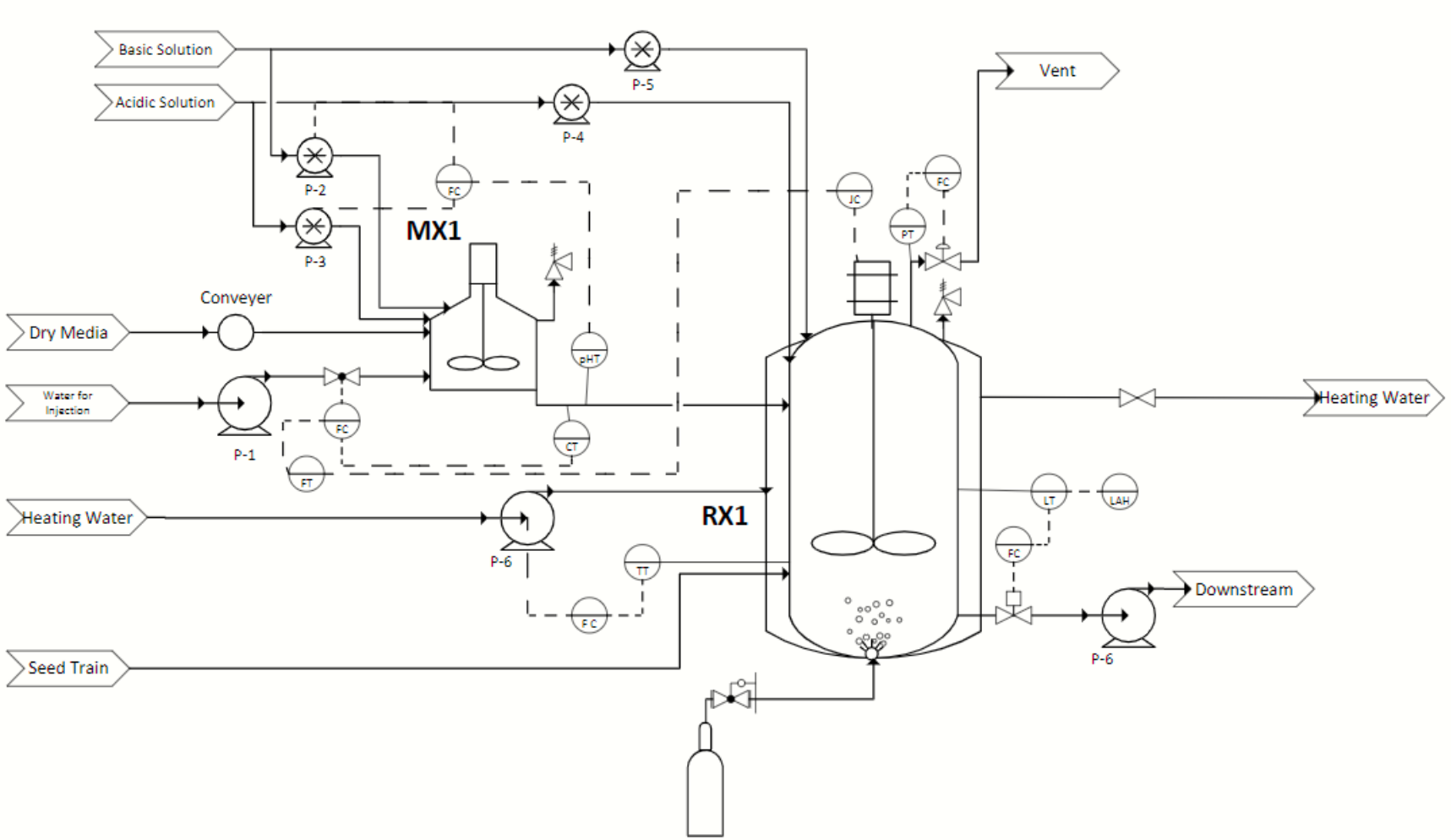
Appendix C: Bioreactor Detailed Process and Instrumentation Diagram

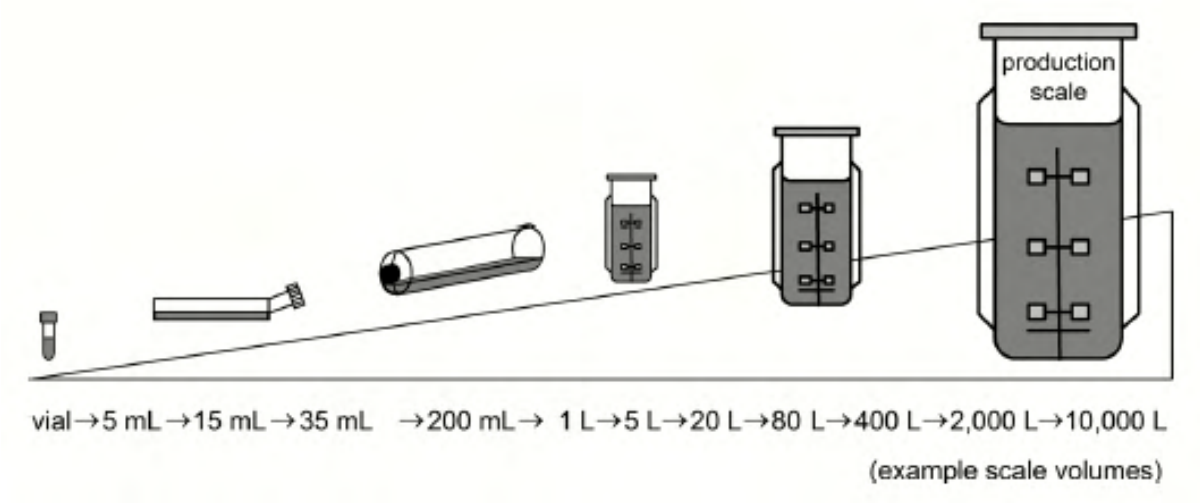


Appendix D: General Design

Seed Train

Material Balances

Material balances were performed in the MATLAB using numerical methods of solving systems of differential equations. The chosen final volume was 100 L and the initial volume was given as 1 mL with 10^6 cells. The rate of volume scaling was approximated to the example given *Model-based strategy for cell culture seed train layout verified at lab scale* by Kern et. al pictured below.



The fundamental material balance equation was used at each batch stage as:

$$Accumulation = Generation - Consumption$$

Where nutrients only have the consumption term and the cells only have a generation term as we assume at the current operation cell death is negligible. Lag time was able to be neglected as well because the volume scaling rate was consistently low enough that we expect to see little to no lag time which is caused by nutrient concentration shock.

The Kern paper also provided the fundamental differential equations required to solve this system, in the biophase we used:

$$\frac{dX}{dt} = \mu X$$

Where X is the cell concentration and μ is a growth constant it is a simplified version of the one given as it neglects death rate. μ is calculated by

$$\mu = \mu_{max} * (C_{glc} * C_{gln})/((C_{glc} + K_{glc}) * (C_{gln} - K_{gln}))$$

Where C is concentration, K is the monod constant and glc and gln represent glucose and glutamine respectively. C of glc and gln are also dependent back on the cell concentration as:

$$\frac{dC_x}{dt} = -q_x X$$

Where q is the consumption and is calculated via:

$$q_x = q_{max,x} * \frac{C_x}{C_x + k_x}$$

Where k_x, not to be confused with K_x, is the consumption monod constant.

The constants were determined from *Benchmarking of commercially available CHO cell culture media* by Reinhart et. al and using constant associated with a monod constant most similar to our doubling time.

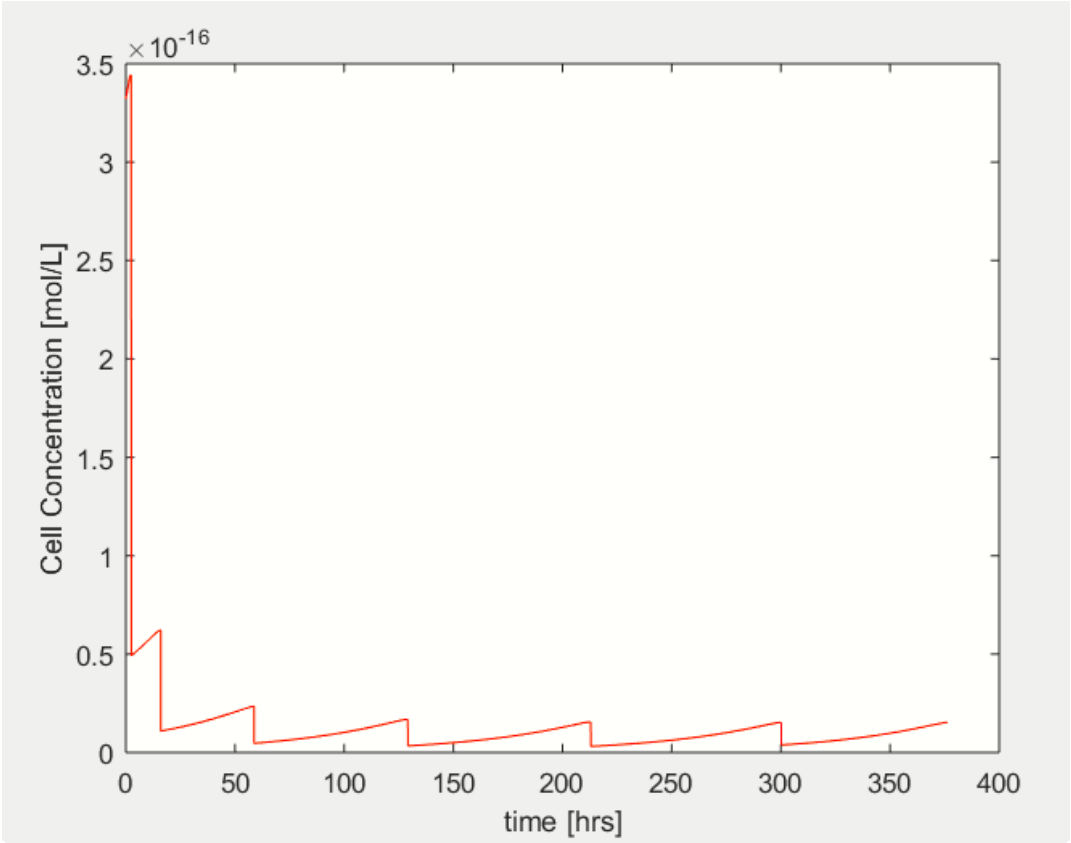
Matlab Code

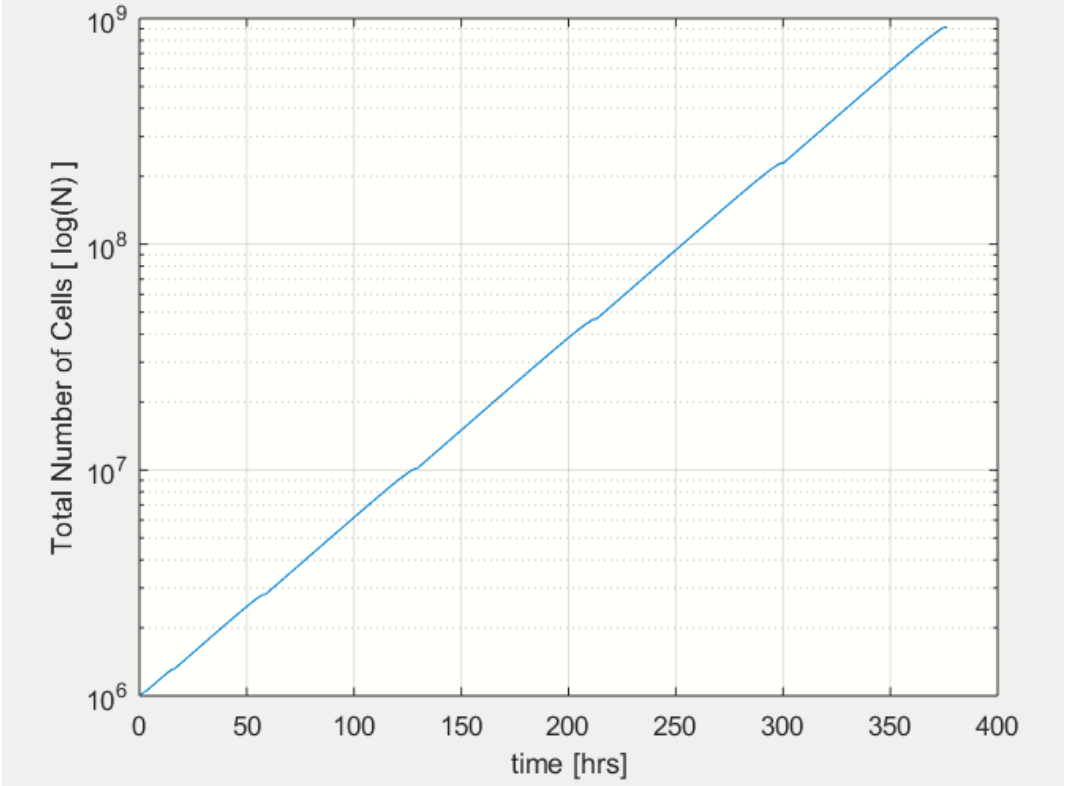
```
%%  Seed Train Codes.
clc; clear all
%%  Process Parameters
Nc(1) = 10^6;          % Given Number of Cells
t2x = 36;              % Doubling time
V = [1e-3,5e-3,35e-3,200e-3,1,5,25,100];
                       % Volume in liters of a common seed train [1]
t = 0;                 % Initial time
dt = 1e-3;             % Numerical methods step size
mu = log(2)/t2x;       % Cell growth rate defined by doubling time, hr^-1
Cc = Nc/(V(1)*6.022e23);% Concentration of cells in given vial
Qgc = 385.25e-12*6.022e23*24/180.156;
                       %Consumption rate of glucose in mol/molc*hr [2]
Qgn = 91.625e-12*6.022e23*24/147.13;
                       %Consumption rate of glutamine in mol/molc*hr [2]
Cgcm = 4.5/180.156;    %Initial glucose concentration mol/L
Cgnm = 0.73/147.13;    %Initial glutamine concentration mol/L
Cgc = 0;               %No glucose in the initial vial
Cgn = 0;               %No glutamine in the initial vial
Kgc = 0.03e-3;         %Glucose monod kinetic constant mol/L [2]
Kgn = 0.03e-3;         %Glutamine monod kinetic constant mol/L [2]
kgc = 0.0605e-3;       %Glucose monod uptake constant mol/L [2]
kgn = 0.4966e-3;       %Glutamine monod kinetic constant mol/L [2]
T(1) = 0; T(2:length(V)) = 0.5;
i=2;
tm=0;
```

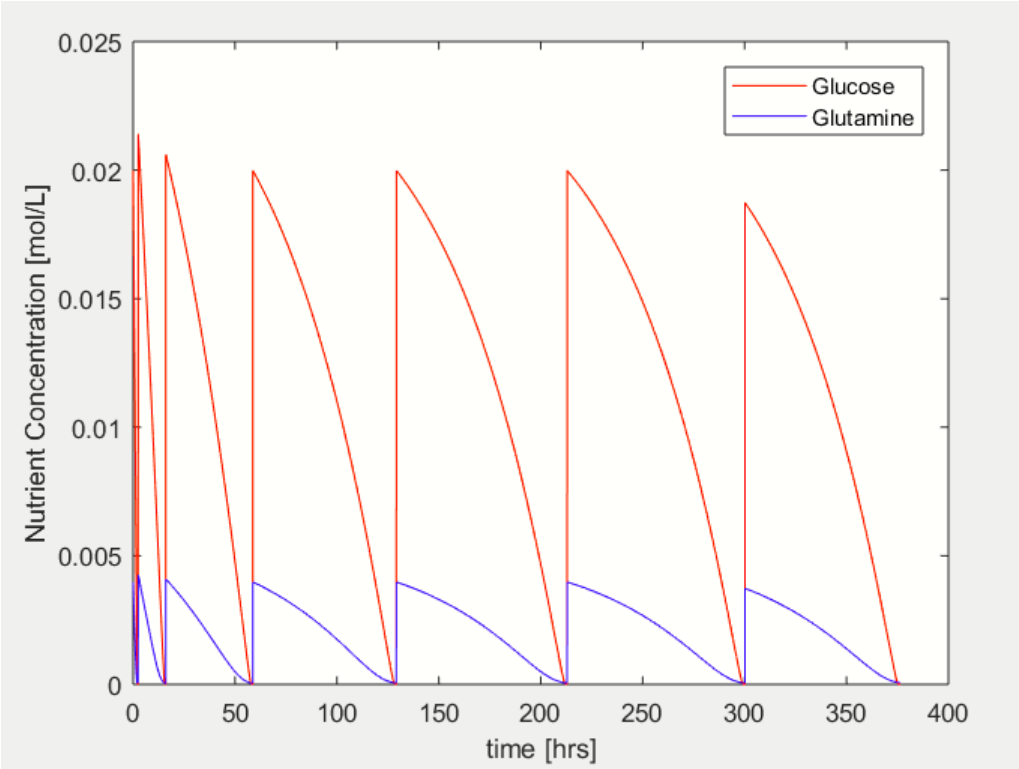
```
for v=2:length(V)
    m=i-1;
    %% Loop Primers
    % Starting growth rate is maximum, because fresh feeds
    Cc(m) = V(v-1)*Cc(m)/V(v);  % New concentration in diluted
    Cgc(m) = (Cgc(m)*V(v-1) + Cgcm*(V(v) - V(v-1)))/V(v);
    % Fresh glucose in new vessel is diluted by the depressed
    % concentration in the previous vessel
    Cgn(m) = (Cgn(m)*V(v-1) + Cgnm*(V(v) - V(v-1)))/V(v);
    % Fresh glucose in new vessel is diluted by the depressed
    % concentration in the previous vessel
    Qgc(m) = Qgc(1);    % Starting glucose uptake is maximized in new feeds
    Qgn(m) = Qgn(1);    % Starting glucose uptake is maximized in new feeds
    %% Seed Train Vessel
    dCc(1) = 0;         % Loop initializers
    while Cgc(i-1) > Cgc(1)/1e4 % Keep glucose concentration above
        % glutamine to stop glutamine accumulation
      mu(i) = mu(1)*(Cgc(i-1).*Cgn(i-1)) ./ ((Cgc(i-1) + Kgc).*(Cgn(i-1) +
Kgn));
        % Equation 7 for growth death kinetics [2]
      Qgc(i) = Qgc(m)*(Cgc(i-1)/(Cgc(i-1)+kgc))*((mu(i-1)/(mu(i-
1)+mu(1))+0.5));
      Qgn(i) = Qgn(m)*(Cgn(i-1)/(Cgn(i-1)+kgn));
        % Equations 9 and 10 in substrate uptake [2]
      Cc(i) = Cc(i-1) + dt*mu(i-1)*Cc(i-1);
        % Stepping to the next time steps for cell concentrations
        % (t+dt) using equation 1 biophase derivative equations [2]
      Cgc(i) = Cgc(i-1) - dt*Qgc(i-1)*Cc(i);
      Cgn(i) = Cgn(i-1) - dt*Qgn(i-1)*Cc(i);
        % Stepping to the next time steps for glucose and glutamine
        % concentrations (t+dt) using equations 3 and 4 liquid
        % phase equations. [2]
      dCc(i) = (Cc(i)-Cc(i-1))/dt;
        % Numerical methods for determine instantaenous derivative
        % with respect to time, used for determining when to step
        % to next vessel.
      N(i) = Cc(i)*V(v);
        % The total number of cells for plotting later
      t(i) = t(i-1) + dt;
      i=i+1;
        % Proceeding to the next time step.
    end
```

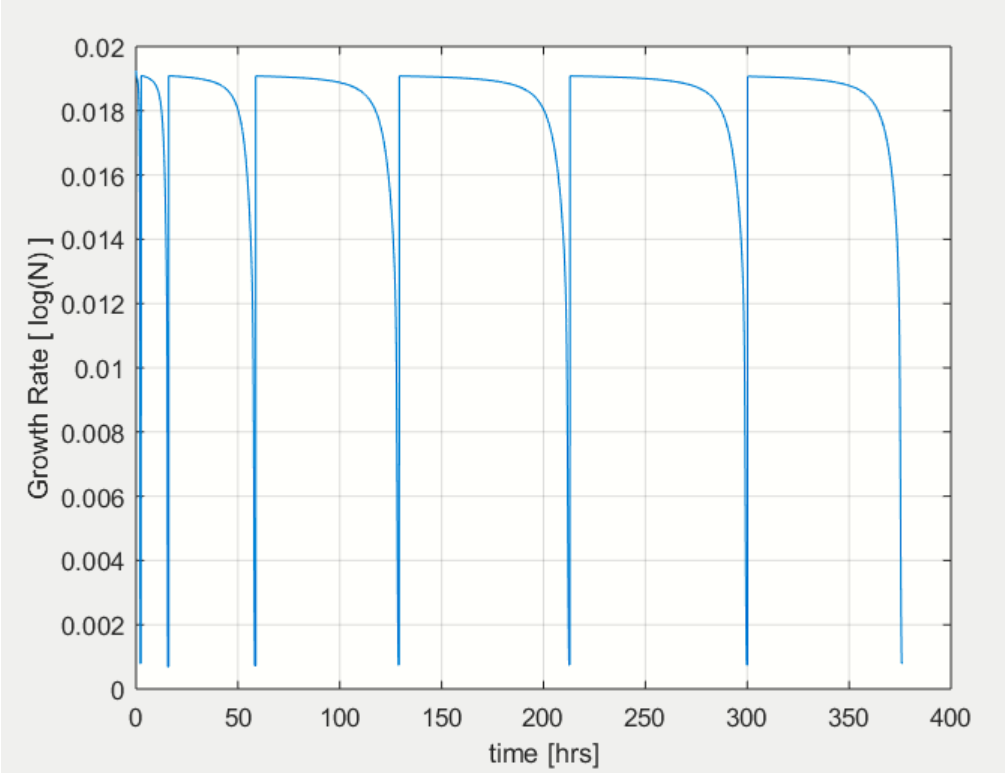
```
        tm(v) = m;
        p = round(T(v)/dt);
        Cc(i:i+p)=Cc(i-1);
        Cgc(i:i+p)=Cgc(i-1);
        Cgn(i:i+p)=Cgn(i-1);
        N(i:i+p)=N(i-1);
        mu(i:i+p)=mu(i-1);
        t(i-1:i+p-1)=t(i-1):dt:t(i-1)+p*dt;
        i=i+p;
            % This block represents the time delay required to

end
t(length(t)+1)=t(length(t))+dt;
figure
plot(t,Cc,'r'); xlabel("time [hrs]");
ylabel("Cell Concentration [mol/L]")
%%
figure
plot(t,Cgc,'r',t,Cgn,'b'); xlabel("time [hrs]"); ylabel("Nutrient Concentration
[mol/L]")
legend("Glucose","Glutamine")
%%
figure
semilogy(t,N*6.022e23);
xlabel("time [hrs]"); ylabel("Total Number of Cells [ log(N) ]")
grid on
%%
figure
plot(t,mu);
xlabel("time [hrs]"); ylabel("Growth Rate [ log(N) ]")
grid on
```


×10^-16
3.5
3
2.5
2
1.5
1
0.5
0
Cell Concentration [mol/L]
0
50
100
150
200
250
300
350
400
time [hrs]


10^9
10^8
10^7
10^6
Total Number of Cells [log(N)]
0
50
100
150
200
250
300
350
400
time [hrs]


Glucose
Glutamine
Nutrient Concentration [mol/L]
time [hrs]
0.025
0.02
0.015
0.01
0.005
0
0
50
100
150
200
250
300
350
400


Growth Rate [log(N)]
time [hrs]
0.02
0.018
0.016
0.014
0.012
0.01
0.008
0.006
0.004
0.002
0
0
50
100
150
200
250
300
350
400

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2. D. Reinhart (*):L. Damjanovic:R. KunertVienna Institute of BioTechnology, Department of Biotechnology,University of Natural Resources and Life Sciences, Vienna, Muthgasse 11, 1190 Vienna, Austria. April 7, 2015.

Bioreactor

Material Balance

Bioreactor material balances were solved in MatLab using ODE 15. Bioreactor kinetics were assumed to behave according to a modified Monod model [Equations 1-8], and glucose was assumed to be a primary indicator of substrate concentration. The final reactor had overall inputs and outputs listed in table _. Total reaction time was 660 [hrs]. Fed batch time is initiated as soon as the reactor is loaded with cells. The feed flowrate is 7.5 [L-hr^{-1}] and the inlet feed concentration is a variable and is displayed in *Figure 3*. The reactor is loaded with an initial volume of 100 [L], which contains 10^7 [cells-L^{-1}]. The final reactor volume is 5000 [L] with a final drug concentration of 1.5 [g-L^{-1}] and the reactor produces 7.5 [kg] of antibodies per batch. The reactor sparger must provide oxygen at a maximum rate of 39 [kg-hr^{-1}] and carbon dioxide must be removed at a rate of 60 [kg-hr^{-1}]. Oxygen and carbon dioxide loading curves are shown in *Figure 4*. Mixing of the reactor and sparger design is cell dependent and will be up to the detailed design team to find the appropriate mixing speeds and bubble sizes to avoid high shear stress on cell populations.

Kurano et al have reported inhibition parameters and growth parameters. The following equation represents the specific growth rate of cells within the reactor proposed by Kurano et al.

$$\mu = \mu_{max} \frac{K_a}{K_a + W} \frac{K_g}{K_g + S} \tag{1}$$

Cell concentration is derived from an unsteady-state mass balance around the bioreactor.

$$input - output + generation - consumption = accumulation \tag{2.a}$$

$input = 0$ $accumulation = \frac{dXV}{dt}$ generation = XVμ-FXμ consumption = 0 (2.b)

$$\frac{dXV}{dt} = \text{XV}\mu - \text{FXV}\mu \tag{2.c}$$

$$\frac{dX}{dt} = \left[\mu - \frac{F}{V}\right] X \tag{2}$$

Substrate feed concentration is going to be equal to the total cell consumption, and all the proceeding equations are of similar structure with a production coefficient associated with cell concentration.

$$FS = XY_{dss} \tag{3}$$

Volume change:

$$\frac{dV}{dt} = F \tag{4}$$

Drug Production:

$$\frac{dD}{dt} = XY_d \tag{5}$$

Waste Production:

$$\frac{dD}{dt} = XY_W \tag{6}$$

Oxygen Consumption:

$$\frac{dO_2}{dt} = XY_{O_2} \tag{7}$$

Carbon Dioxide Production:

$$\frac{dCO_2}{dt} = XY_{CO_2} \tag{8}$$

Reactor Design Plots:

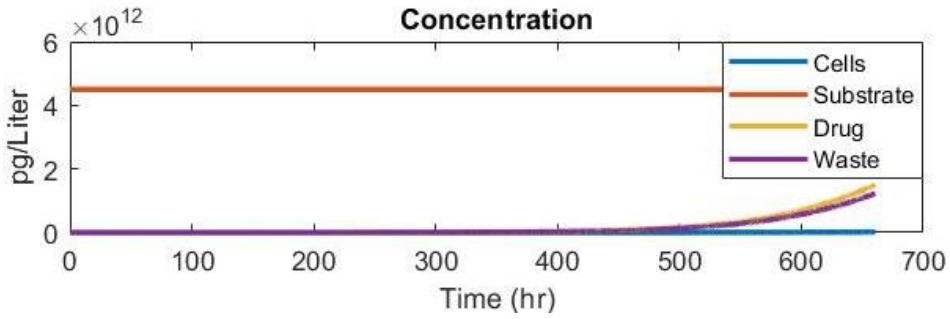

Figure 13. Concentrations for various species within the reactor. The largest growth phase occurs in the last 160 hours of production due to the exponential growth of the CHO cells.

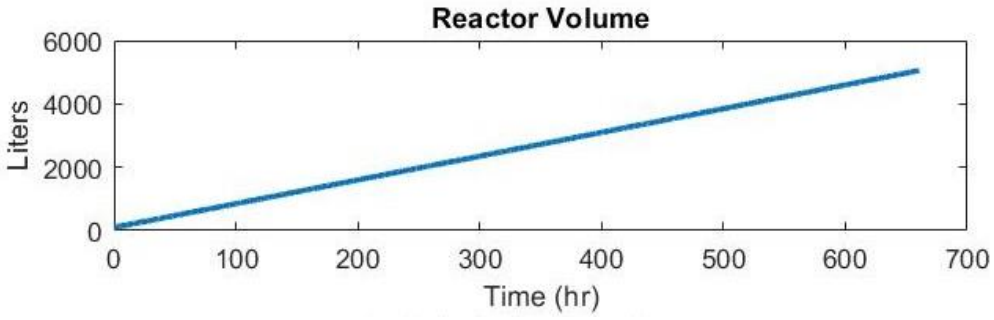

Figure 14. Operational volume of the reactor over time.

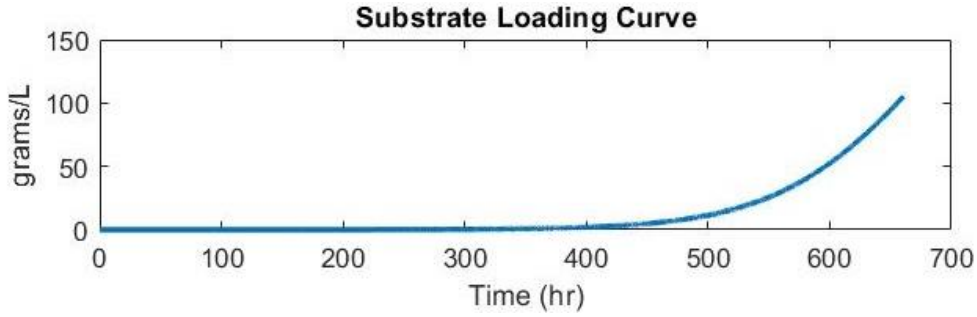

Figure 15. Inlet substrate loading curve for the fed-batch reactor. Inlet substrate must increase exponentially at the end of reactor operation.

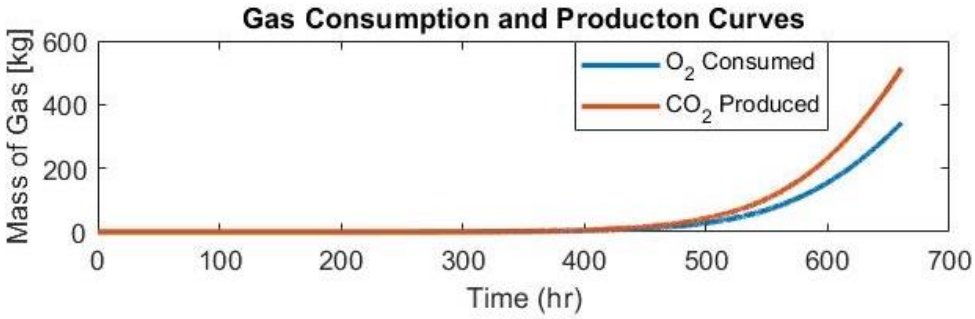

Figure 16. Consumption curves for oxygen and the production curve of carbon dioxide in the reactor over time. Large amounts of oxygen are consumed in the final growth phase of the reactor.

MATLAB Code

```
% Fed Batch Reactor Code for AiChE design Competition
%Housekeeping

close all; clear all; clc;


   %Initializing Variables
   InitialVolume = 100; %L
   ReactionTime = 10000 ; %hr
   InitialCells = 10.0*10^6; % cells/L
   InitialDrug = 0; %g/L
   InitialWaste = 0; %g/L
   InitialSubstrate = 4.5*10^12 ; % pg Gluclose/L
   TimePartition = ReactionTime/100000;
   tspan = [0:TimePartition:ReactionTime];

   Fed_Substrate = 20*10^12; % pg/L

      %Feeding inital conditions to a component vector for the coupled set of
      %ordinary differential equations
      InitialComposition = [InitialVolume; %V
                  InitialCells; %X
                  InitialSubstrate; %S
                  InitialDrug; %g/L
                  InitialWaste %g/L]
                  ];
      %Pass initial composition to a solver (ODE15) to solve the coupled ordinary
      %differential equations based on the rate equation

      options = odeset('Events',@DrugLimit);
```

```
[t,components] = ode15s(@(t,components) fedbatch(t,components,Fed_Substrate),tspan,InitialComposition,options);

%System Plots

% subplot for concentration
subplot(4,1,1)                %3x1 subplot, first axis
plot(t,components(:,2),'linewidth',2) % X curve
hold on
plot(t,components(:,3),'linewidth',2) % S curve
plot(t,components(:,4),'linewidth',2) % D curve
plot(t,components(:,5),'linewidth',2) % W curve
title('Concentration')
xlabel('Time (hr)')
ylabel('pg/Liter')
legend('Cells','Substrate','Drug','Waste')

% subplot for reactor volume
subplot(4,1,2)                % 3x1 subplot, second axis
plot(t,components(:,1),'linewidth',2) % V curve
title('Reactor Volume')
xlabel('Time (hr)')
ylabel('Liters')

% subplot for Substrate Loading
subplot(4,1,3)    % 3x1 subplot, third axis
SubstrateConcentration = components(:,1).*components(:,2).*12.5/10*10^-12;
TotalSubstrate = SubstrateConcentration.*t/1000;
AverageConcentration = mean(SubstrateConcentration);

%Plot the substrate loading curve
plot(t,SubstrateConcentration,'linewidth',2)
title('Substrate Loading Curve')
xlabel('Time (hr)')
ylabel('grams/L')

%subplot for oxygen loading and carbon dioxide production
subplot(4,1,4)
OxygenConsumption = components(:,1).*components(:,2).*t*(6.16*10^(-12))/1000;
CarbonDioxideProduction = components(:,1).*components(:,2).*t*(9.24*10^-12)/1000;
plot(t,OxygenConsumption,'linewidth',2);
hold on
plot(t,CarbonDioxideProduction,'linewidth',2);
title('Gas Consumption and Producton Curves')
xlabel('Time (hr)')
```

```
ylabel('Mass of Gas [kg]')
legend('O_2 Consumed','CO_2 Produced')
%calulate the maximum production and consumption rates of oxygen and carbon
%dioxide
MaxOxygenConsumtpion                                                    =
components(length(components),1)*components(length(components),2)*(6.16*8^-
12)/1000; % kg/hr
MaxCarbonDioxideProduction                                              =
components(length(components),1)*components(length(components),2)*(9.24*10^-
12)/1000; %kg/hr

%-------------------------------------------------------
%-------------------------------------------------------

%Creating an event function for when the drug concentration reaches 1.5 g/L


function d_dt = fedbatch(t,components,Fed_Substrate)

% unpack components for readable variable names
V = components(1);
X = components(2);
S = components(3);
D = components(4);
W = components(5);

% define constants
S_0 = Fed_Substrate ; % (pg/L) feed substrate concentration
mu_max = 0.035;   % (1/hr) maximum specific growth rate [Tatsuay , Glacken et al.]
Ka = 1.39*10^12;     % (pg/L)  ammonia inhibition constant [Kurano et al.]
Kg = 5.03*10^12 ;    % (pg/L) gluclose inhibition constant [Kurano et al.]
Y_xs = 12.5 ;   % (pg Gluclose/Cell-hr)  substrate constumption rate [Goudar et al.]
Y_ws = 0.851 ;      % (pg ammonia/Cell-hr) [Kurano et al.]
Y_ds = 1.04 ;   % (pg Drug/cell-hr) drug production rate [AiChE Design Packet]
Y_o2 = 6.16 ;    % (pg-cell-hr) Oxygen Consumption Rate [Goudar]
Y_co2 = 9.24 ;   % (pg-cell-hr) Carbon Dioxide Production Rate [Goudar]
F = 7.5 ;         % (L/hr) feed flowrate


% calculate mu
mu = mu_max*Ka/(Ka+W)*Kg/(S+Kg);

% dV/dt
d_dt(1) = F;
```

```
% dX/dt
d_dt(2) = (mu-F/V)*X;

% dS/dt
d_dt(3) = 5;

% dD/dt
d_dt(4) = X*Y_ds;

% dW/dt
d_dt(5) = X*Y_ws;

% transpose to column
d_dt = d_dt';

end

function [value, isterminal,direction] = DrugLimit(t,components)
value = components(4)-1.5*10^12;
isterminal=1;
direction = 0;
end
```

References

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2.) Goyal, A. et al. Effects of Elevated pCO2 and Osmolality on Growth of CHO Cells and Production of Antibody-Fusion Protein B1: A Case Study. Biotechnol. Prog. 21, 70–77 (2005).

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4.) Ogawa, T., Kamihira, M., Yoshida, H., Iijima, S. & Kobayashi, T. Effect of dissolved oxygen concentration on monoclonal antibody production in hybridoma cell cultures. J. Ferment. Bioeng. 74, 372–378 (1992).

Primary Harvest

Disc-Stack Centrifuge Mathematical Modeling

The effective centrifugal force (F_g) on a particle in a centrifuge is

$$F_g = (m_s - m_L)r\omega^2 \qquad (1)$$

where m_s and m_L are the particle mass and displaced fluid mass, respectively; r is distance the particle travelled from the axis of rotation; and ω is the angular velocity of the centrifuge. Assuming spherical particles of diameter d:

$$F_g = \frac{\pi}{6} d^3 \Delta\rho r\omega^2 \qquad (2)$$

where $\Delta\rho$ is the density difference between the particle and the liquid. Assuming the particle is small, the drag imposed upon it can be represented using Stokes' law

$$F_D = 3\pi\mu d v_s \qquad (3)$$

where F_D is the drag force, μ is the liquid viscosity, and v_s is the particle settling velocity through the liquid. While the particle is moving at the settling velocity, these forces are in equilibrium:

$$\frac{\pi}{6} d^3 \Delta\rho r\omega^2 = 3\pi\mu d v_s$$

$$\therefore v_s = \frac{d^2 \Delta\rho r\omega^2}{18\mu} \qquad (4)$$

The equivalent area of the disc-stack centrifuge, referred to as the sigma factor (Σ), is the quotient of the flow rate over the settling velocity:

$$\Sigma_{\text{DSC}} = \frac{Q}{v_s} \qquad (5)$$

This is the sizing requirement of a disc-stack centrifuge to completely remove all particles with at least diameter d. Charles M. Ambler developed a geometrical model for the equivalent area by taking a ratio of the particle velocity vertically between two discs to the velocity parallel to the discs (Ambler). This sigma factor is more general in that it is not limited to complete removal.

$$\Sigma_{\text{DSC}} = \frac{2\pi n\omega^2 (r_o^3 - r_i^3)}{3gC\tan\theta} \qquad (6)$$

where n is the number of discs, r_o is the outer disc radius, r_i is the inner disc radius, g is the gravitational constant, and θ is the angle of the disc up from the vertical axis. C is the

average value of all particles of diameter d that are sedimented against the upper disc. Assuming laminar and symmetrical flow between two discs, then C is unity when all particles of diameter d are centrifuged out of the liquid product. This model also neglects acceleration and deceleration of batch centrifuges, which can be account for with a correction factor (Maybury et al.). Combining equations (4), (5), and (6), the rotational velocity required to completely remove all particles with at least diameter d can be determined given disc stack geometry:

$$\omega = \left(\frac{27\mu Q g tan\theta}{\pi n d^2 \Delta\rho r_o (r_o^3 - r_i^3)}\right)^{\frac{1}{4}} \quad (7)$$

Combining equations (4) and (5) allows for the calculation of the sigma factor based on operating conditions, which is used for costing:

Sample Calculation

$\mu = 8.9 \times 10^{-4} \frac{kg}{m\,s}$ $\quad$ $g = 9.81 \frac{m}{s^2}$

$Q = 5000 \frac{L}{hr} = 0.0014 \frac{m^3}{s}$ $\quad$ $\Delta\rho = 60 \frac{kg}{m^3}$

$n = 50\ discs$ $\quad$ $r_o = 0.3\ m$

$d = 0.1\ micron = 10^{-7} m$ $\quad$ $r_i = 0.05\ m$

$\theta = 35°$

$$\omega = \left[\frac{27\left(8.9 \times 10^{-4} \frac{kg}{\cancel{m}\,s}\right)\left(0.0014 \frac{\cancel{m^3}}{s}\right)\left(9.81 \frac{\cancel{m}}{s^2}\right)(\tan(35))}{\pi(50)\left(60 \frac{kg}{\cancel{m^3}}\right)(10^{-7}\cancel{m})^2(0.3\,\cancel{m})((0.3\,\cancel{m})^3 - (0.05\,\cancel{m})^3)}\right]^{\frac{1}{4}} = (1.01 * 10^8\ s^{-4})^{\frac{1}{4}}$$

$$= 100 \frac{1}{s}$$

$$\therefore \Sigma_{DSC} = \frac{18\mu Q}{d^2 \Delta\rho r \omega^2} = \frac{18\left(8.9 \times 10^{-4} \frac{kg}{m\,\cancel{s}}\right)\left(0.0014 \frac{m^3}{\cancel{s}}\right)}{(10^{-7}\cancel{m})^2\left(60 \frac{kg}{\cancel{m^3}}\right)(0.3\,\cancel{m})\left(100 \frac{1}{\cancel{s}}\right)^2} = 12378\ m^2$$

$$\therefore v_s = \frac{0.0014 \frac{m^3}{s}}{12378\, m^2} = 1.13 \times 10^{-7} \frac{m}{s}$$

Disc-Stack Centrifuge MATLAB Modeling

```
% Centrifuge Model

mu=8.9*10^(-4); % Viscosity assumed to be of water [kg/m s]
Q=(5000/1); % Flow rate [L/hr]
Q=Q/(1000*3600); % Flow rate [m^3/s]
g=9.81; % Gravitational constant [m/s^2]
theta=35:0.15:50; % Disc angle [degrees]
n = 50:150; % Number of discs [  ]
d=10^(-7); % Smallest completely removed particle diameter (m)
ps=1060; % Particle density [kg/m^3]
pl=1000; % Liquid density [kg/m^3]
ro=0.3; % Disc outer radius (m)
ri=0.05; % Disc inner radius (m)

tang=transpose(tan(theta*2*pi/360)); % Tangent of theta in radians
rn=n.^(-1); % Reciprical of n

% Angular velocity [radians/second]
w=(27*mu*Q*g*tang*rn/(pi*(d^2)*ro*((ro^3)-(ri^3))*(ps-pl))).^(1/4);

% Revolulations per minute
RPM=w*60/(2*pi);

% Centrifugal acceleration [m/s^2]
gc=ro*(w.^2);

% Sigma Factor [m^2]
Sigma1=18*mu*Q*(gc.^(-1))/((d^2)*(ps-pl));
Sigma2=2*pi*((tang*rn).^(-1)).*(w.^2)*((ro^3)-(ri^3))/(3*g);

% 3D plot of RPMs as a function of number of discs and their angle
figure
s=surf(n,theta,RPM,'FaceAlpha',0.6);
s.EdgeColor='none';
xlabel('Number of Discs');
ylabel('Disc Angle');
zlabel('Angular Velocity (RPM)');
```

```
% 3D plot of Sigma factor vs number of discs and disc angle
figure
s=surf(n,theta,Sigma1,'FaceAlpha',0.6);
s.EdgeColor='none';
xlabel('Number of Discs');
ylabel('Disc Angle');
zlabel('DSC Equivalent Area (m^2)');
```

XOHC Depth Filter Sizing

The depth filtration used runs at constant flow rate to synergize with centrifugation. Constant flow rate corresponds to a maximal pressure drop across the filter before it reaches its loading capacity (volume of incoming liquid filterable per unit area of filter). Resistance, the ratio of differential pressure to feed flux, normalizes this pressure drop for use in scaling. In the figure below, the 200 L/m^2 loading capacity was selected to provide a process scale filter area between 5.5 and 33 m^2 (Millipore, *Millistak + ® Pod Carbon Depth Filter Media System High Adsorption Capacity in the Innovative Pod Format*): $\frac{5000\frac{L}{batch}}{200\frac{L}{m^2}} = 25\frac{m^2}{batch}$

By constraining this data point, the resistance can be extrapolated at about 0.17 psid/LMH. The flux of the depth filter can be determined by dividing the flow rate by the filter area, which can in turn be multiplied by the resistance to determine the necessary pressure differential:

$$\frac{5000\frac{L}{hr}}{25\ m^2} \times 0.17\frac{psid}{LMH} = 34\ psid$$

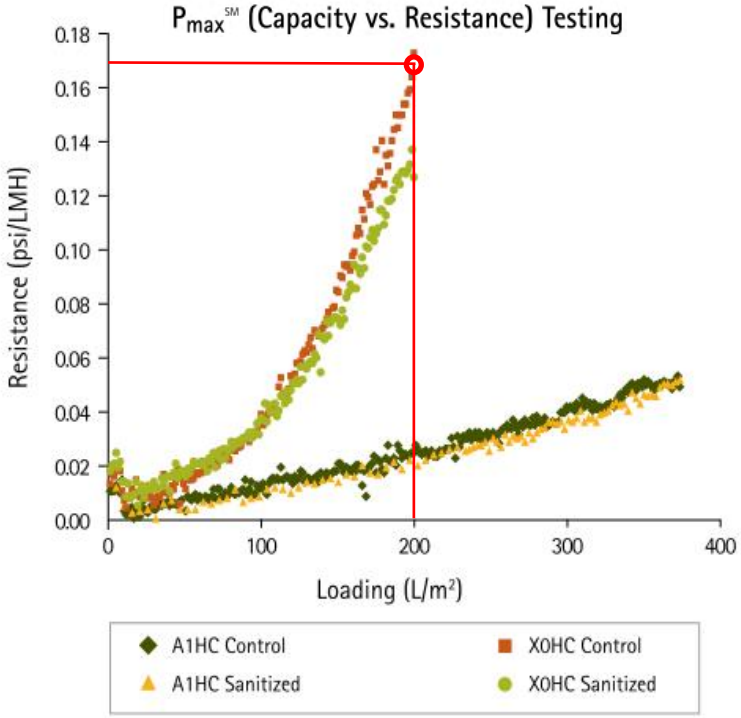

Figure 17: Resistance (differential pressure [psid] to feed flux [L/min/hr] ratio) for XOHC control versus loading capacity [L/m^2]. In this instance, differential pressure is the maximum produced pressure across the depth filter if filtration is performed at a constant flux. Loading capacity is the volume of incoming fluid that can be filtered per unit area of filter. The graph shows that as the maximum differential pressure increases, the loading capacity does as well, but with diminishing return. The operating differential pressure chosen (red) was chosen because it appears at the limit of diminishing return as the capacity asymptotes with resistance (Millipore, *Millistak + ® Pod Disposable Depth Filter Performance Guide Innovative , High-Performance Pod Filters Ideal for Primary and Secondary Clarification at Lab , Pilot*).

Primary Harvest Work Cited

Ambler, C. (1952). The evaluation of centrifuge performance. *Chemical Engineering Progress*, *48*, 150–158.

Maybury, J., Hoare, M., & Dunnill, P. (2000). The use of laboratory centrifugation studies to predict performance of industrial machines: Studies of shear-insensitive and shear-sensitive materials. *Biotechnology and Bioengineering*, *67*, 265–270.

Millipore. (2013). *Millistak + ® Pod Disposable Depth Filter Performance Guide Innovative , high-performance Pod filters ideal for primary and secondary clarification at lab , pilot*.

Protein A Chromatography

Sizing

The protein A column is sized such that it can process 3000 g of mAbs per cycle. The column will require two cycles to process a 1 g/L titer from a 5000 L bioreactor, a three cycles for a 2 g/L titer. The MabSelect SuRe™ resin is chosen for the process, which has a dynamic binding capacity of 35 g mAb per liter of resin at a mobile phase velocity of 500 cm/h and 10% breakthrough(GE Healthcare, "MabSelect SuRe"). The column is loaded at 30 g mAb per liter of resin to allow for a safety factor in the calculations. Using this loading, the minimum volume of resin required is

$$V_{\text{min}} = \frac{3000 \text{ g mAb}}{30 \text{ g mAb/L resin}} = 100 \text{ L}.$$

The height of the column should is recommended to be between 20 and 30 cm(Ghose et al.), and a 30 cm column is chosen to maximize its volume. A 300 cm/hr operating velocity is chosen based on the recommended operating window provided for the resin(GE Healthcare, "MabSelect SuRe"). The required diameter of the column is calculated using the volume of a cylinder

$$\begin{aligned} V_{\text{min}} &= \frac{\pi D_{\text{min}}^2}{4} H \\ 10{,}000 \text{ mL} &= \frac{\pi D_{\text{min}}^2}{4} \times 30 \text{ cm} \\ D_{\text{min}} &= 65 \text{ cm}. \end{aligned}$$

A diameter of $D = 70$ cm is chosen to allow for a factor of safety. Under these conditions, the actual volume of the column is

$$\begin{aligned} V &= \frac{\pi D^2}{4} H \\ V &= \frac{\pi \times (70 \text{ cm})^2}{4} \times 30 \text{ cm} \\ V &= 116 \text{ L}. \end{aligned}$$

Timing and Protocol

The protocol used for the chromatography column is shown in the table below. The steps, column volumes (CV), conditioned pH's, and buffers are supplied by the resin manufacturer. The required buffer volume in liters is calculated by multiplying its required CV by the dry column volume(GE Healthcare, "MabSelect SuRe"). For the equilibration step, this will yield a required buffer volume of

Step	Volume (CV)	Volume (L)	Flowrate (cm/hr)	Time (hr)	pH	Buffer
Equilibration	6	693	300	0.6	7.2	20 mM phosphate
Loading	26	3002	300	2.6		
Wash 1	5	577	300	0.5	7.2	35 mM phosphate, 500 mM NaCl
Wash 2	1	115	300	0.1	7.2	20 mM phosphate
Elution	5	577	300	0.5	3.6	20 mM citrate
Clean In Place	3	346	300	0.3		500 mM NaOH
Total	**46**	**5311**		**4.6**		

$$6\ \text{CV} \times \frac{116\ \text{L}}{1\ \text{CV}} = 693\ \text{L}.$$

The required time for this step is determined by multiplying the CV by the residence time. The residence time is the duration for one CV to flow through, and is calculated as the column height divided by the operating velocity. For the equilibration step, the required time is

$$6\ \text{CV} \times \frac{30\ \text{cm}}{300\ \text{cm/hr}} = 0.6\ \text{hr}.$$

The required times and buffer volumes are calculated using a spreadsheet.

Material Balances

The amount of source material required for the operation of the column for a single cycle is given in the next table. Buffer compositions are tabulated. The mass of phosphate is taken to be 142 g/mol, which is the molar mass of Na_2HPO_4(Harris). This mass is chosen because it most closely matches the primary species of phosphate present in a solution at pH 7.2. The citrate mass is taken to be 192 g/mol, the molar mass of citric acid(Harris), because it is the dominant species at pH 3.6. The molar masses for sodium chloride and sodium hydroxide is also taken from Harris (Harris). Total mass for each buffer component is calculated by multiplying the buffer concentration by the volume used, summing over each column step, and multiplying by the molar mass. For example, the amount of phosphate required is

$$m = M \sum_i n_i V_i$$

$$m = \frac{142\ \text{g}}{\text{mol}} \times (20\ \text{mM} \times 693\ \text{L} + 0\ \text{mM} \times 3002\ \text{L} + 35\ \text{mM} \times 577\ \text{L} + 20\ \text{mM} \times 115\ \text{L} + 0\ \text{mM} \times 577\ \text{L} + 0\ \text{mM} \times 346\ \text{L}) \times \frac{1\ \text{mol/L}}{1000\ \text{mM}}$$

$$m = 5.2\ \text{kg}.$$

Step	phosphate (mM)	citrate (mM)	NaCl (mM)	NaOH (mM)	WFI (kg)
Equilibration	20	0	0	0	
Loading	0	0	0	0	
Wash 1	35	0	500	0	
Wash 2	20	0	0	0	
Elution	0	20	0	0	
Clean In Place	0	0	0	500	
Molar Mass (g/mol)	142.0	192.1	58.4	40.0	
Total Amount (kg)	5.2	2.2	16.9	6.9	5311
Amount Supplied (kg)	5.2	2.2	16.9	6.9	2309
Amount Eluted (kg)	0.0	2.2	0.0	0.0	577
Amount in Waste (kg)	5.2	0.0	16.9	6.9	4734
Concentration of Stock (% w/w)	0.8	0.4	0.3	0.8	
Water from stock (kg)	6.5	5.5	56.2	8.7	76.9
Water from WFI Stream					2232

Some of this buffer may be supplied by the loading stage, while the rest is provided by buffer storage tanks. For material balance and costing purposes, it is important to determine the amount of material that must be supplied by the buffer tanks rather than the loading step. This is done by subtracting the amount supplied by the loading step from the total amount. For phosphate, the amount supplied is

$$5.2\ \text{kg} - \frac{142\ \text{g}}{\text{mol}} \times (0\ \text{mM} \times 3002\ \text{L}) \times \frac{1\ \text{mol/L}}{1000\ \text{mM}} = 5.2\ \text{kg}.$$

Part of the material entering the column, the eluent, will proceed to the viral inactivation step, while the rest will be discarded as waste. The amount of buffer material eluted is given by multiplying the volume eluted by the concentration and the molar mass of the material. The amount of phosphate eluted is

$$\frac{142\ \text{g}}{\text{mol}} \times (0\ \text{mM} \times 577\ \text{L}) \times \frac{1\ \text{mol/L}}{1000\ \text{mM}} = 0.0\ \text{kg}.$$

The amount going to the waste stream is the total amount of material minus the amount eluted. The amount of phosphate is the waste stream is

$$5.2\ \text{kg} - 0.0\ \text{kg} = 5.2\ \text{kg}.$$

A similar approach is used for calculating the amount of water for injection (WFI) needed, except the total volume supplied to the column is used to determine the mass needed. This calculation assumes that the density of water is 1 kg/L and that the volume change due to buffer components is negligible. The WFI needed is the total volume supplied to the column, 5311 kg. The amount supplied is

$$5311\ \text{kg} - 3002\ \text{kg} = 2309\ \text{kg}.$$

The amount eluted is 577 kg and the amount in waste is

$$5311\ \text{kg} - 577\ \text{kg} = 4734\ \text{kg}.$$

Stock Solutions

Buffers are stored in concentrated form to minimize the size required for tanks and storage. The concentration of stock solution is the limited by the maximum solubility of the buffer components in water. Maximum solubility is accessed by using literature values for a model constituent component, are given. Solubility for citric acid(National Center for Biotechnology Information, *PubChem Database. CID=311*) and Tris(National Center for Biotechnology Information, *PubChem Database. CID=6503*) are obtained from the PubChem database. MOPS(Sigma-Aldrich, *MOPS Sodium*) and MES(Sigma-Aldrich, *MES Hydrate*) solubility are obtained from Sigma-Aldrich. The remaining sodium hydroxide, sodium chloride, and disodium hydrogen phosphate solubility are obtained from the CRC Handbook of Chemistry and Physics(Rumble). Concentrations of stock solutions are chosen to be near the solubility limit to allow for minimal storage requirements, but they are lower than the solubility limit to prevent precipitation.

Buffer	Model Constituent	Maximum Solubility (g/100 g H_2O)	Concentration of Stock (g/100 g H_2O)
phosphate	Disodium hydrogen phosphate	95	80
citrate	Citric acid	59	40
MOPS	MOPS Sodium	33	25
MES	MES Hydrate	25	20
Tris	Tris	50	40
NaCl	NaCl	36	30
NaOH	NaOH	100	80

The amount of water from the stock solution is given by dividing the amount of buffer component supplied by the concentration. For example, the amount of WFI in the phosphate buffer for the protein A chromatography is

$$\frac{5.2\ \text{kg phosphate}}{0.80\ \text{kg phosphate/kg WFI}} = 6.5\ \text{kg}.$$

The concentrated buffers are diluted using an inline dilution system to achieve the desired concentrations entering the column. The amount of WFI required for this purpose is calculated by subtracting the amount buffer stock solution water from the WFI that needs to be supplied. WFI needed for inline dilution is

$$2309\ \text{kg} - 0.8\ \text{kg} - 0.4\ \text{kg} - 0.3\ \text{kg} - 0.8\ \text{kg} = 2232\ \text{kg}.$$

To obtain the cost per batch, all process streams are multiplied by three because three cycles are required to process a 5000 L bioreactor with a 1.5 g/L titer. Each batch processes 7.5 kg of mAbs.

Viral Inactivation

Filtration Calculations

Virial inactivation is highly dependent upon the type of virus strain and the virial composition. Therefore, historically proven methods were used to determine process parameters for virial inactivation. Blank et al recommended a pH range of 2-4 for acidic inactivation of viruses at a storage time of 1 hour and found LVR rates to exceed four. Therefore, the low pH inactivation is based on their conclusions. Since the effluent from the Protein A Chromatography column is more tolerant to acidity, the chromatography effluent will be transferred to a 5000 [L] storage vessel for low pH inactivation. Following low pH inactivation, the tank solution will be neutralized and then pumped through a virial micro-filter. Filter area and flow are based on flux values provided by Millipore® for their standard micro filters (Source). The effluent is then transferred to a second tank for detergent treatment. Detergent treatment is based on experimental data provided by Roberts. Roberts recommended that two detergents are added to the tank, tri-n-butyl phosphate and Triton X-100, until their respective mass percent were 0.3% and 1% of the total solution mass. For one 5000 [L] tank, this corresponds to approximately 15 [kg] of tri-n-butyl phosphate and approximately 50 [kg] of Triton X-100. The tank solution is held with the detergent for 1 [hr], and then transferred to final polishing. After final polishing, the process fluid undergoes an ultrafiltration step. Ultrafiltration membrane sizes are based on Millipore's recommended flows for their Pelican® Single-Pass cassettes. Ultrafiltration serves two purposes, viral separation, and purification for protein.

Calculating Microfiltration Area:

Millipore recommendations for microfiltration area:

$$LMH = 16\ \frac{L}{m^2 - hr - psi} \tag{1}$$

$$Q_{Max} = 440\ \frac{L}{hr} \tag{2}$$

$$A = \frac{Q_{max}}{LMH * P} = \frac{440}{16 * 10} = 2.75\ m^2 \tag{3}$$

Millipore's Magnus 1.3 unit provides 0.22 m^3 of surface area per unit:

$$Units = \frac{A}{0.22} = 14 \tag{4}$$

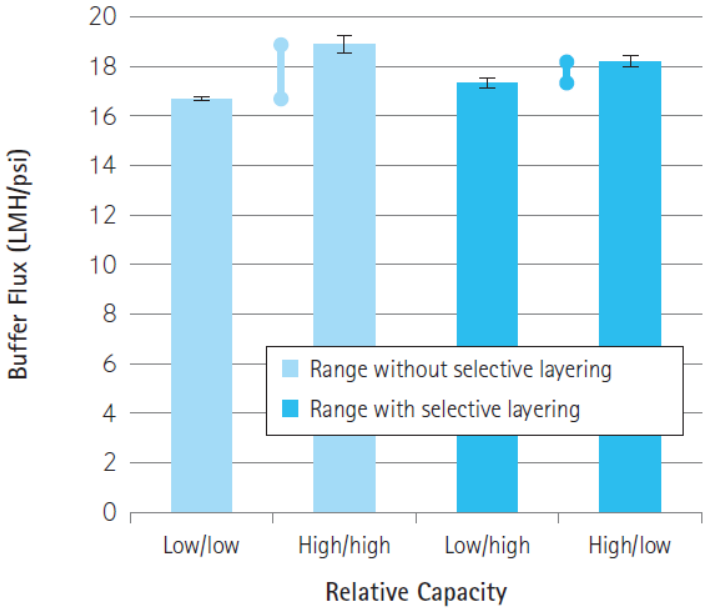

Figure 2.
Flux of devices with layering of two membranes and the effect of selective layering

Figure 18. Millipore data for flux through Virosolve© Pro deices with specific buffers. LMH is [L-m^{-2}-hr^{-1}-psi^{-1}] and relative capacity is based on pre-filter type and filter type.

Ultrafiltration Calculations:

Manufacturers Data for Pellicon® 3 Cassettes used for ultrafiltration:

$$\text{LMM} = 4\,\frac{\text{L}}{m^2 - min} \tag{1}$$

Max flow out of the chromatography columns:

$$Q_{max} = 440\,\frac{L}{hr} \tag{2}$$

Required Surface Area:

$$A = \frac{Q_{max}}{LMM} = \frac{440\ \frac{L}{hr}}{240\ \frac{L}{m^2 - hr}} = 1.83\ m^2 \tag{3}$$

Operating Conditions

	Pellicon® 3 Cassettes with Ultracel Membrane	Pellicon® 3 Cassettes with Biomax Membrane
Recommended Feed Flow Rate	4 – 8 L/m²/min	4 – 6 L/m²/min
Pressure/Temperature	40 psi (2.7 bar) @ 50° C	80 psi (5.5 bar) @ 40° C
Maximum Reverse Pressure	30 psi (2 bar)	30 psi (2 bar) @ 25° C
Maximum Caustic Concentration	0.5N NaOH up to 50 hours (Contact Millipore for exposure parameters)	1.0N NaOH up to 200 hours
Operating pH Range	2 – 13	1 – 14

Figure 19. Product data for Millipore ultrafiltration membranes. Specifically for Pellicon® cassestes.

Material Balance

Following the viral inactivation filtration step, the process stream must be adjusted from pH 3.6 to 8.5. This is accomplished by adding sodium hydroxide solution. The speciation of the 20 mM citrate solution at pH 3.6 is determined using the Henderson-Hasselbalch equation

$$\mathrm{pH} = \mathrm{pK_a} + \log\frac{[\mathrm{A}]}{[\mathrm{HA}]},$$

where $\mathrm{pK_a}$ is the acid dissociation constant, [HA] and [A] are the molar concentrations of the acid and its conjugate base, respectively. Using constants provided in Harris(Harris),

$$3.6 = 3.13 + \log\frac{[\mathrm{H_2A^-}]}{[\mathrm{H_3A}]} \quad (\mathrm{pK_{a1}})$$
$$3.6 = 4.76 + \log\frac{[\mathrm{HA^{2-}}]}{[\mathrm{H_2A^-}]} \quad (\mathrm{pK_{a2}})$$

The material balance for the acid is

$$[\mathrm{H_3A}] + [\mathrm{H_2A^-}] + [\mathrm{HA^{2-}}] = 20\ \mathrm{mM}.$$

This yields $[\mathrm{H_3A}] = 4.18\ \mathrm{mM}$, $[\mathrm{H_2A^-}] = 14.21\ \mathrm{mM}$, $[\mathrm{HA^{2-}}] = 0.98\ \mathrm{mM}$. The initial concentration of sodium ions at pH 3.6, $[\mathrm{Na^+}]_i$, is given by a charge balance on the solution, as follows

$$[\mathrm{Na^+}]_i = 2[\mathrm{HA^{2-}}] + [\mathrm{H_2A^-}] = 2 \times 0.98\ \mathrm{mM} + 14.21\ \mathrm{mM} = 16.17\ \mathrm{mM}.$$

A similar approach is used to determine the composition of the solution as pH 8.5.

$$8.5 = 4.76 + \log\frac{[\mathrm{HA}^{2-}]}{[\mathrm{H_2A}^{-}]} \quad (\mathrm{pK_{a2}})$$
$$8.5 = 6.40 + \log\frac{[\mathrm{A}^{3-}]}{[\mathrm{HA}^{2-}]} \quad (\mathrm{pK_{a3}})$$

The material balance is

$$[\mathrm{H_2A}^{-}] + [\mathrm{HA}^{2-}] + [\mathrm{A}^{3-}] = 20\ \mathrm{mM}.$$

This gives $[\mathrm{H_2A}^{-}] = 2.78\ \mathrm{mM}$, $[\mathrm{HA}^{2-}] = 15.29\ \mathrm{mM}$, $[\mathrm{A}^{3-}] = 1.93\ \mathrm{mM}$. The final concentration of sodium ions, $[\mathrm{Na}^{+}]_f$, is

$$[\mathrm{Na}^{+}]_f = 3[\mathrm{A}^{3-}] + 2[\mathrm{HA}^{2-}] + [\mathrm{H_2A}^{-}] = 3 \times 2.78\ \mathrm{mM} + 2 \times 15.29\ \mathrm{mM} + 1.93\ \mathrm{mM} = 39.15\ \mathrm{mM}.$$

The amount of sodium hydroxide added is

$$n_{\mathrm{NaOH}} = ([\mathrm{Na}^{+}]_f - [\mathrm{Na}^{+}]_i)V = (39.15\ \mathrm{mM} - 16.17\ \mathrm{mM}) \times 577\ \mathrm{L} = 13.3\ \mathrm{mol}$$

The mass of NaOH required is $0.040\ \mathrm{kg/mol} \times 13.3\ \mathrm{mol} = 0.53\ \mathrm{kg}$. The amount of WFI that will be part of the incoming buffer stream is $0.53\ \mathrm{kg} / (0.3\ \mathrm{kg/kg}) = 1.7\ \mathrm{kg}$.

Cation Exchange Chromatography

The cation exchange column is sized using the same procedures used for the protein A column. The POROS® 50 HS resin was used for the design, which has a dynamic binding capacity of 57 to 75 g mAb per L of resin(Thermo Fisher Scientific Inc.). A 50 g/L loading is chosen for design calculations, and a 3000 g capacity is assumed. Under these conditions, the minimum required volume is 60 L. A chosen height of 30 cm yields a minimum diameter of 50.5 cm. The working diameter is chosen to be 55 cm, which gives a volume of 71 L for the column.

Column operation is shown in the table below, which is based on U.S. Pat. No. 2018/011878 (Lebrenton et al.). The volume of buffer required and elution times are calculated the same as with the protein A column.

Step	Volume (CV)	Volume (L)	Flowrate (cm/hr)	Time (hr)	pH	Buffer
Equilibration	4	285	100	1.2	5.5	23 mM MES, 60 mM NaCl
Loading	8.5	606	100	2.6	5.5	20 mM citrate
Wash 1	3	214	100	0.9	7.0	25 mM MOPS
Wash 2	3	214	100	0.9	5.5	23 mM MES, 10 mM NaCl
Elution	7	499	100	2.1	5.5	23 mM MES, 175 mM NaCl
CIP	3	214	100	0.9		500 mM NaOH
Total	**28.5**	**2031**		**8.6**		

Material balances for the column's operation are also tabulated.

Step	MES (mM)	MOPS (mM)	citrate (mM)	NaCl (mM)	NaOH (mM)	WFI (kg)
Equilibration	23	0	0	60	0	
Loading	0	0	20	0	0	
Wash 1	0	25	0	0	0	
Wash 2	23	0	0	10	0	
Elution	23	0	0	175	0	
Clean In Place	0	0	0	0	500	
Molar Mass (g/mol)	195.2	209.26	192.123	58.443	39.9971	
Total Amount (kg)	4.5	1.1	2.3	6.2	4.3	2031
Amount Supplied (kg)	4.5	1.1	0.0	6.2	4.3	1425
Amount Eluted (kg)	2.2	0.0	0.0	5.1	0.0	499
Amount in Waste (kg)	2.2	1.1	2.3	1.1	4.3	1532
Concentration of Stock (% w/w)	0.2	0.25	0.4	0.3	0.8	
Water from stock (kg)	22.4	4.5	0.0	20.8	5.3	53.0
Water from WFI Stream						1373

Ultrafiltration/Diafiltration

The MES buffer used for eluting the cation exchange column is not suitable for the anion exchanger because the operating pH of the column is outside its buffer capacity(AppliChem). An ultrafiltration/diafiltration (UF/DF) steps is included to exchange the buffer and concentrate the solution. The process used is based on U.S. Pat. No. 2013/0195888(Wang et al.). The volume of the solution is first reduced to one diavolume (250 L). Five diavolumes are fed into the UF/DF system to remove the citrate buffer. The volume of the retentate is further reduced to 125 L before proceeding to the anion exchange column. Process time t was constrained to 9 hours, and a transmembrane flux of $J = 50$ L/hr/m^2 was assumed(Schwartz and Seeley). For calculating the required area, the volume that is transmitted through the membrane is the total volume supplied minus the amount exiting as retentate, $V = 499\text{ L} + 5 \times 250\text{ L} - 125\text{ L} = 1624\text{ L}$. The required area A is calculated as(Schwartz and Seeley; He)

$$A = \frac{V}{J \times t} = \frac{1624\text{ L}}{50\text{ L/hr/m}^2 \times 9\text{ hr}} = 3.6\text{ m}^2.$$

Anion Exchange Chromatography

The sizing and material balances for the anion exchange column is done using the procedure described previously. The anion exchange column is operated in flow-through mode, unlike the previous two columns operated in bind and elute mode. The sequencing of buffers is taken from U.S. Pat. No. 7,323,553 (Fahrner et al.), while the 150 cm/hr flowrate and 40 g/L loading capacity is adapted from U.S. Pat. No. 7,863,426 (Table 2) (Wan et al.). The Q Sepharose FF resin is used. The sequencing of steps for the column is shown, and the material balance is given.

Step	Volume (CV)	Volume (L)	Flowrate (cm/hr)	Time (hr)	pH	Buffer
Equilibration	5	424	150	1.0	8.0	25 mM Tris, 50 mM NaCl
Loading	1.55	131	150	0.3	8.0	25 mM Tris, 65 mM NaCl
CIP	3	254	150	0.6		500 mM NaOH
Total	**9.6**	**810**		**1.9**		

Step	**Tris (mM)**	**NaCl (mM)**	**NaOH (mM)**	**WFI (kg)**
Equilibration	25	50	0	
Loading	25	65	0	
CIP	0	0	500	
Molar Mass (g/mol)	121.1	58.443	39.9971	
Total Amount (kg)	1.7	1.7	5.1	810
Amount Supplied (kg)	1.7	1.7	5.1	679
Amount Eluted (kg)	0.4	0.5	0.0	131
Amount in Waste (kg)	1.3	1.2	5.1	679
Concentration of Stock (% w/w)	0.4	0.3	0.3	
Water from stock (kg)	4.2	5.8	17.0	27.0
Water from WFI Stream				652

Dead-End Filtration

Dead-end filtration units with a $0.22\,\mu m$ pore size are required before each chromatography column to reduce interference from particulates (Davies and Smith). A transmembrane flux of $J = 10{,}000\ \text{L/hr/m}^2$ is assumed (Davies and Smith). The required area is calculated the same as with UF/DF. For example, the filter area for the protein A column should be

$$A = \frac{V}{J \times t} = \frac{5311\ \text{L}}{10{,}000\ \text{L/hr/m}^2 \times 4.6\ \text{hr}} = 0.12\ \text{m}^2.$$

Similarly, the filter areas for the cation exchange and anion exchange steps should be $0.3\ \text{m}^2$ and $0.5\ \text{m}^2$, respectively.

Downstream Efficiency

Downstream efficiency for the process is near 80%. The yield of mAbs for the depth filtration and centrifugation step is 95%. Each column is capable of recovering 95% of the product(Brian Kelley; GE Healthcare, "Affinity Chromatography: MabSelect SuRe"). The recovery for the depth filtration step is taken to be 97%(Wang et al.). Complete recovery is assumed for viral inactivation and dead-end filtration steps.

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