Novel Design of the RTS,S Malaria Vaccine Process Train Employing Single Use Systems

A Technical Report
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Bachelor of Science in Chemical Engineering

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In partial fulfillment of the requirement for the degree of Bachelor in Science in

Chemical Engineering, we present the design of a biopharmaceutical plant located in Belgium

for the production of RTS,S antigen, the virus like particle (VLP) needed for the malaria vaccine.

The facility has a production capacity of 392 g of purified RTS,S antigen per year, with a

minimum annual production goal of 350 g.

The overall process consists of fermentation of *Saccharomyces cerevisiae* (yeast) cells

containing the RTS,S antigen. This design implements single-use bioreactors of 50 and 1,000 L

capacity, after which RTS,S is physically and chemically extracted from the yeast cells and

subject to a series of purification steps: centrifugation, cell disruption, silica

adsorption/desorption, ultrafiltration, diafiltration, anion exchange chromatography, hydrophic

interaction chromatography, and sterile filtration, before it is filled into vials and lyophilized for

later use.

Since there has not been significant progress toward malaria eradication in sub-Saharan

Africa using insecticidal nets or indoor residual sprays, the implementation of a malaria vaccine

seems necessary in this region. We believe that access to vaccinations is important regardless of

socioeconomic status or poverty rate. Underdeveloped countries in sub-Saharan Africa are some

of the most impoverished in the world, but are as deserving of healthcare as more developed

countries. Thus, the purpose of designing an RTS,S antigen production facility that implements

single-use systems is to offer a more cost effective product that is accessible for the target population.

The design of this process involves several chemical engineering skills that the group has learned over their time at UVA. Starting with basic mass balances and moving forward to fermenter design, complex bioseparations, as well as the use of realistic economic analyses, this design project represents the culmination of all of the skills learned over the course of the group's chemical engineering education. The completion of the project represents a mastery of these skills and a preparedness to enter the workforce in a biochemical engineering role.

Regards,

RTS,S Vaccine Team

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II.   Summary

The manufacture of GlaxoSmithKline's Mosquirix vaccine, which provides immunity to the malaria disease, using single use systems represents another step in the biopharmaceutical industry to transition all manufacturing processes towards a cheaper, faster production model. Many companies within the biopharmaceutical industry are making the transition to single use systems, as they eliminate the need for cleaning and in-house sterilization, while also reducing validation costs. The Mosquirix manufacturing process described in this report uses disposable conical flasks as well as 50 L and 1000 L disposable bioreactor bags to grow *Saccharomyces cerevisiae* yeast cells. These cells are genetically modified to overexpress the RTS,S antigen, the protein responsible for granting immunity. Since *S. Cerevisiae* is Gram-negative, and expresses the antigen through inclusion bodies, centrifugation and homogenization are both used to separate host cell proteins from cellular debris. The protein solution is then sent through an aerosil column and filtered twice through a 300 kD nominal weight cutoff filter to remove much of the host cell protein. Anion exchange chromatography is then used as a capture step to further purify the RTS,S antigen, letting host cell proteins and other impurities pass through. Hydrophobic interaction chromatography further purifies the RTS,S antigen, capturing most of the other impurities present while allowing the RTS,S antigen to pass through. The final purification step is sterile filtration, which removes any viral debris from the product, and serves as an extra purification step. The purified RTS,S antigen solution is then filled into vials and lyophilized. Throughout the process, ultrafiltration and diafiltration are used to change feed concentrations and exchange buffers. Assuming the plant completes seven batch campaigns per year, its production capacity is 350 g of antigen per year, enough to vaccinate 3.5 million babies annually.

The process in this report also includes adjuvant manufacture. The adjuvant used with this vaccine, AS01E, is a saponin liposome adjuvant, and is produced in a six-step operator-intensive process. First, the lipid components are dissolved in ethanol in a large boiling flask. Next, the ethanol is evaporated to form a lipid cake at the bottom of the boiling flask. Phosphate buffer is then used to dissolve the lipid cake, forming large multilamellar vesicles. Next, the solution is microfluidized to transform the multilamellar vesicles into small unilamellar vesicles. Finally, the saponin component is added, and the solution is sterile filtered and filled into vials. Enough adjuvant is manufactured yearly to match the production of RTS,S antigen.

Capital costs for this process add to $39.3 million, with annual operating costs of $17.5 million. The annual revenue was calculated to be $70 million. After taxes, the net profit is calculated to be $39.4 million per year, with an internal rate of return of 79% and a net present value of $178.7 million, making this project very appealing for investment. In a worst-case scenario of only selling half the product made, the anticipated annual rate of return is 12%, with a net present value of $53.7 million. Therefore, further pursuit of the Mosquirix vaccine manufacturing plant employing single use systems is highly recommended.

III.   Body of Report

A.   Introduction

According to the 2018 World Malaria Report, malaria affected 219 million people around the world in 2017 (World Health Organization, 2019). Although the number of global malaria cases dropped by 20 million between 2010 and 2017, the World Health Organization (WHO) claims that there has not been significant recent progress in global malaria eradication. The WHO reported that while the number of malaria cases decreased in Rwanda, India, Ethiopia, and Pakistan between 2016 and 2017, countries such as Nigeria, Madagascar, and the Democratic Republic of the Congo each experienced at least 500,000 more cases in 2017 than in 2016 (World Health Organization, 2019). The unbalanced global malaria burden suggests an uneven application of resources towards implementation of preventative measures, such as diagnostic testing, insecticidal nets, and indoor residual spraying (World Health Organization, 2019).

Malaria is caused by the transmission of one of five different parasitic plasmodium (*P. falciparum*, *P. malariae*, *P. vivax*, *P. ovale*, and *P. knowlesi*), which are transferred from infected mosquitoes to humans during a blood meal (World Health Organization, 2018). Since *P. falciparum* is one of the more infectious and potentially fatal parasites, it is the main focus of vaccination efforts. The *P. falciparum* parasites enter the bloodstream and travel to the liver of a human, where they enter the pre-erythrocytic stage (Foquet et al., 2014). In this stage, which lasts 6.5 days, the parasites invade liver cells (hepatocytes) and begin to mature. After maturing into schizonts, the parasitic cells rupture and release merozoites. The merozoites then infect erythrocytes (red blood cells), and mature into erythrocytic schizonts. The rupture of these schizonts lead to an immune response and the patient becomes symptomatic. To prevent infection and disease proliferation, GSK designed a vaccine using the RTS,S antigen. This

antigen triggers an immune response, which helps prevent the initial infection of hepatocytes (GlaxoSmithKline, 2016).

The RTS,S antigen is a combination of proteins designated R, T, and S. The "R" portion is based on the last 16-18 NANP amino acid repeats of the *P. falciparum* circumsporozoite (CSP) (strain NF54). The carboxy-terminal region of CSP consists of T-cell epitopes, which make up the "T" region of RTS,S. The fusion of "R" and "T" with the hepatitis B surface antigen, "S", generates the RTS protein. When the RTS and additional "S" proteins are expressed in *Saccharomyces cerevisiae* yeast cells, they spontaneously assemble into virus-like particles (VLPs) (Cohen et al., 2010). Upon vaccination, RTS,S induces the production of IgG antibody and T cells; high concentrations of IgG result at the asparagine-alanine-asparagine-proline (NANP) repeat region of CSP and the T cells prevent infection of hepatocytes (Foquet et al., 2014).

To reduce the malaria burden in sub-Saharan Africa, the pharmaceutical company GlaxoSmithKline (GSK) recently brought the RTS,S antimalarial vaccine, or Mosquirix, to market. It was approved by the European Medicines Agency (EMA) after three phases of clinical trials (European Medicines Agency, 2015). These rigorous trials determined its safety and efficacy in children from sub-Saharan Africa ranging from 6 weeks to 17 months in age after the administration of three or four doses. The combination of the dosage requirement for Mosquirix and the 41% average poverty rate for regions in sub-Saharan Africa contribute to the difficulty of distributing the vaccine in the areas bearing the majority of the global malaria burden (Patel, 2018).

The goals of our project are to design a manufacturing plant for the RTS,S malaria vaccine that will produce the vaccine at a reduced cost in order to better contribute to malaria

eradication in sub-Saharan Africa, as well as to design a water for injection (WFI) plant to supplement the vaccine production. We aim to lower the cost of the drug while also ensuring profitability by implementing a process that incorporates Single Use Systems (SUS). The use of single use systems will remove Sterilize-In-Place (SIP) and Clean-In-Place (CIP) steps that would otherwise be necessary. With fewer SIP and CIP steps in the process, chemical costs will decrease, hold times will decrease, and batch turnover will be more efficient.

1.   Drug Product Explanation

The final products of the manufacturing process will be a lyophilized cake containing the RTS,S antigen, and the AS01E adjuvant system. The cake will be contained in a 1-milliliter clear glass vial with a rubber stopper, aluminum crimp-cap, and plastic cap. The product that will be injected into patients consists of two parts: the lyophilized cake to be manufactured in this process, and a liquid suspension containing the AS01E adjuvant system. The reconstitution of one vial of each part will be used for one infant dose of vaccine. The vaccine product is intended to be administered on infants between the ages of 6 weeks and 17 months.

The lyophilized cake will consist of the RTS,S antigen at an RTS and S protein purity of at least 96%, dimer at a concentration no more than 5%, no high molecular weight aggregates, less than 10 EU/mg endotoxin, and no detectable proteolytic clipping (Allen et al., 2015). The cake will also contain trace amounts of polysorbate 80 (TWEEN® 80), disodium phosphate dihydrate, and sodium dihydrogen phosphate dihydrate. Potential impurities may include those from the yeast cells such as yeast DNA, endotoxin, host cell proteins (HCP), or impurities from substances used during fermentation or purification.

The AS01E adjuvant system to be reconstituted with the lyophilized cake will consist of two immunoenhancers (25 µg of 3D-Monophosphoryl Lipid A (MPL) and 25 µg of QS-21),

along with 125 µg of cholesterol and 500 µg of 1,2-Dioleoyl-sn-Glycero-3-Phosphocholine

(DOPC) in a phosphate NaCl buffer system with a volume of 0.5 mL. The buffer system will

consist of sodium chloride, anhydrous disodium phosphate, potassium dihydrogen phosphate,

and water for injection (WFI). Potential impurities may arise from the materials that the product

contacts during the manufacturing processes, including the plastics used in single use materials

(polyvinyl acetate and polypropylene) and polytetrafluoroethylene (PTFE).

2.   Business Scale and Location

According to the World Health Organization (WHO), 6.97 million babies are born each

year in the countries targeted by the Mosquirix pilot program. A 50% product penetration rate

will be assumed to account for potential lack of access, personal belief exemptions, and personal

medical exemptions to vaccination. Each person who is vaccinated with Mosquirix will have a

four-dose schedule to complete to be fully vaccinated. Each dose will contain 25 micrograms of

RTS,S antigen. Using these metrics, the annual RTS,S antigen production requirement can be

estimated:

$$\frac{25 \cdot 10^{-6} \; g}{dose} \times \frac{4 \; doses}{baby} \times \frac{7 \cdot 10^{6} \; babies}{year} \times 0.5 = \frac{350 \; g}{year} \qquad \text{(B2-1)}$$

The target price of each dose, set by GlaxoSmithKline (Ness, 2016) is $5. Using this

information, the annual revenue earned by the vaccine can be estimated:

$$\frac{7 \cdot 10^{6} \; babies}{year} \times 0.5 \times \frac{4 \; doses}{baby} \times \frac{\$5}{dose} = \$70{,}000{,}000 \qquad \text{(B2-2)}$$

The Mosquirix pilot plant will be built in Belgium, attached to the GlaxoSmithKline

Biologicals SA manufacturing plant. The pilot plant outlined in this report is too small to warrant

building a completely new facility, and building onto an already completed facility will save on

some capital costs, as new infrastructure such as WFI systems and steam generation will not have to be built from scratch.

3.   Regulations

The countries in which the RTS,S vaccine will be distributed have National Medicines Regulatory Authorities (NMRAs), but 90% of them have minimal to no functional capacity. Therefore, the regulations upheld by the European Medicines Agency (EMA) will be followed instead. The EMA provides guidance and support to medicine developers on the manufacture, characterization, and control of antigen vaccines and adjuvants in vaccines for human use, detailed in Volume 1 of the EudraLex. We will use these guidelines to ensure the compliance and quality of Mosquirix as it is manufactured. We will also uphold the EMA's Good Manufacturing Practice guidelines, detailed in Volume 4 of the Eudralex.

B.   Previous Work

In countries where malaria is a prevalent disease, pesticides and mosquito nets are widely used to prevent both malaria infections and mosquito bites in general. Although somewhat effective, mosquitos are adapting to pesticides and learning to circumvent mosquito nets. For this reason, an anti-malarial vaccine is needed to keep malaria infections at a minimum.

GSK is currently the only company licensed to produce a malaria vaccine (either for P. falciparum or any of the other plasmodia). There is no production process for Mosquirix on a large scale beyond the scaleup of the process described in the patent cited for this work. There are a few undergraduate theses that address the scaleup of this process. However, none of these have been implemented, and none have suggested the use of single-use technology for savings. Additionally, this project aims to treat only a fraction of those affected by malaria based on the limits of poverty and accessibility in sub-Saharan Africa.

C.   **Discussion of RTS,S Production Train**

The overall RTS,S production train makes 56 grams of RTS,S antigen per batch, requiring that 7 batches be produced annually to meet the yearly production goal of 350 g of RTS,S antigen. Each batch comprises RTS,S antigen, polysorbate 80 (TWEEN® 80), disodium phosphate dihydrate, and sodium dihydrogen phosphate dihydrate. All other components of the batch besides the RTS,S antigen are introduced to the batch during various steps in the production train, and are present in trace amounts. They do not affect the efficacy of the vaccine, and are safe to be injected.

1.   **General Upstream Design**

The overall process design for upstream growth of RTS,S antigen in *S. cerevisiae* yeast cells includes laboratory-scale cell growth in Petri dishes and conical flasks followed by growth in 50 L and 1000 L reactors. The reactors and associated single use components are manufactured by Sartorius, a brand chosen for its transparency in reactor specifications and its focus on scaleup.

2.   **Laboratory Scale Cell Growth**

The first step in upstream processing is to increase cell counts from a master seed vial. This is done by transferring the master seed, stored at -70 °C, to four Petri dishes. (Master seed production is out of the scope of this process, but more detail can be found in the United States Patent # 6,169,171 (De Wilde et al., 2001).) After incubating for 63 hours at 30°C, the cells from the Petri dishes will be suspended in 1.6 L of HB4 media and equally distributed to four 2 L conical flasks. To bring the initial substrate concentration to 20 g/L for cell growth, 32 grams of dextrose will be added to the media/cell solution. An initial yeast cell concentration of 1 g/L was assumed for the batch (constant volume) fermentation of the conical flasks on a rotary shaker.

Monod kinetics were used to determine the fermentation time in the conical flasks that led to optimal cell growth and consumed most, but not all, of the substrate. Cell growth rate was modeled by the equation:

$$\mu = \frac{\mu_{max} \cdot S}{K_S + S} \tag{C2-1}$$

The variables μ and μ_{max} represent growth rates in h^{-1}, K_S is the substrate affinity constant (g/L), and S is the instantaneous substrate concentration (g/L) (Kovárová-Kovar & Egli, 1998). Multiple values for μ_{max} and K_S were observed in the literature, so the most conservative values were used for calculations to avoid overestimating cell concentrations and yields. Since a higher K_S value corresponds to a lower substrate affinity, a value of 0.034 g/L was chosen over 0.025 g/L (Doran, 2012; Papagianni et al., 2007). Similarly, a lower maximum growth rate (μ_{max}) leads to more conservative calculations, so a value of 0.44 h^{-1} was chosen (Papagianni et al., 2007).

Differential equations C2-2 and C2-3 are also relevant to cell growth.

$$\frac{dX}{dt} = \mu X \tag{C2-2}$$

$$\frac{dS}{dt} = -\frac{1}{Y_{X/S}} \mu X \tag{C2-3}$$

Equation C2-2 represents the change in cell concentration with respect to time in terms of growth rate (μ, in units of h^{-1}) and instantaneous cell concentration (X, in units of g/L). Equation C2-3 represents the change in substrate concentration with respect to time and is dependent on growth rate and instantaneous cell concentration as well as the biomass yield ($Y_{X/S}$, in units of g biomass per g substrate). The biomass yield was found in the literature and was set equal to 0.5 g/g for this step in the process (Doran, 2012).

Simultaneous solution of the above differential equations led to a functional incubation time of 5 hours. Cell and substrate concentrations over time were plotted in MATLAB to yield Figure C2-1.
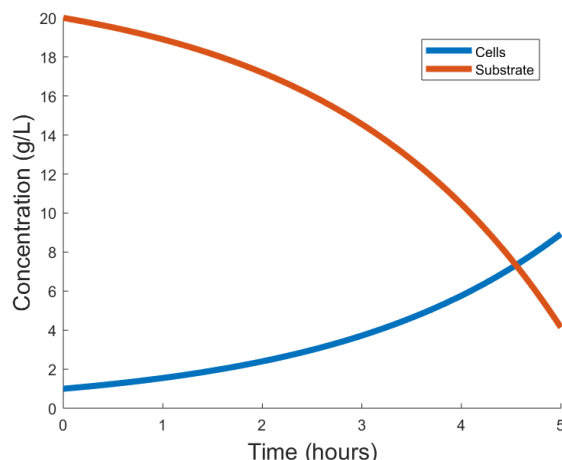
Figure C2-1: Concentrations Over Time in Conical Flasks

The substrate and cell concentrations after 5 hours of incubation were 4.14 g/L and 8.93 g/L, respectively. Although the incubation could have been carried out for more than 5 hours, we chose to stop the process before substrate was depleted and before the cells entered either a stationary or death phase.

3.   First Dilution and 50 L Reactor Growth

The 50 L Sartorius BIOSTAT STR reactor has a minimum working volume of 12.5 liters. Since the volume after 5 hours on the rotary shaker is only 1.6 L, a dilution must take place before the solution can be transferred to the 50 L reactor. To accomplish this, 10.9 liters of HB4 media will be added to the existing 1.6 L. After dilution, the 12.5 total liters will be pumped into the 50 L reactor and the fed-batch process will be initiated.

The kinetics for cell growth in the 50 L reactor can be modelled using the same differential equations that were relevant for the conical flask incubation, C2-1, C2-2, and C2-3, with two major differences: a lower biomass yield and an additional differential equation for

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volume change. The value for biomass yield ($Y_{X/S}$) was decreased from 0.5 g/g to 0.2 g/g in accordance with some values found in the literature – the literature indicates that biomass yields decrease with scaleup, so 0.2 g/g was chosen as a moderately conservative value (Vieira et al., 2013). To account for volume change in the reactor during the fed-batch process, equation C3-1 was added to the existing system of equations. It is dependent only on the feed flow rate of the media (L/h).

$$\frac{dV}{dt} = F \tag{C3-1}$$

To simultaneously solve these equations, initial guesses were provided for fermentation time and feed flow rate and iteratively modified to reach but not exceed a 50 L volume in the reactor, to use as must substrate as possible without total depletion, and to avoid the stationary and death phases of cell growth.

Solutions to the differential equations for cell concentration, substrate concentration, and reactor volume over time were plotted in MATLAB (Figure C3-1).
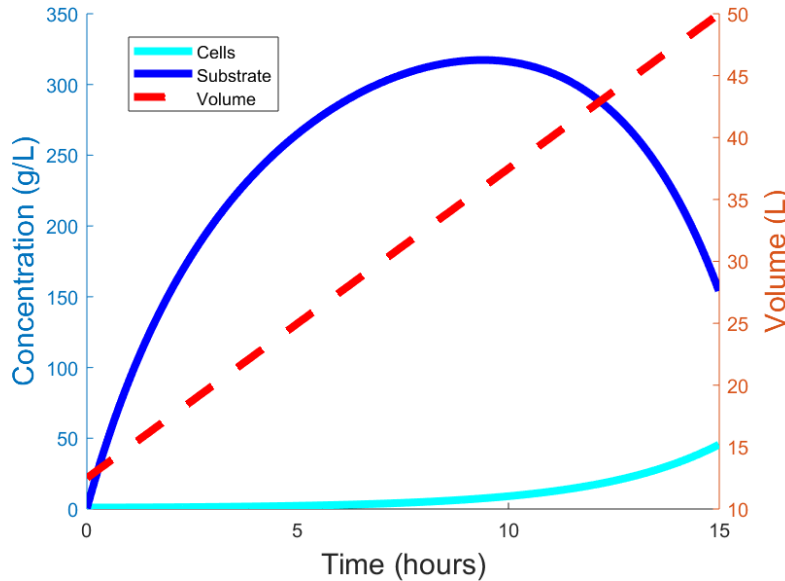
Figure C3-1: Concentrations and Volume Over Time in 50 L Reactor

The required fermentation time was 15 hours with a feed flow rate of 2.5 L/h. The feed should consist of a 5:4 ratio of HB4 media to 80% dextrose. This ratio was chosen to align with United States Patent #6,169,171 (De Wilde et al., 2001). This led to a final volume of 50 L, a final substrate concentration of 153.33 g/L, and a final cell concentration of 45.67 g/L.

In addition to substrate addition requirements modelled by growth kinetics, oxygenation conditions must also be met to promote cell growth and viability. The rate of oxygen supplied to the reactor must equal or exceed the rate required by the cells for cell growth to be better than a "worst case scenario". The target volumetric mass transfer coefficient ($k_L a$) is expressed via the following equation, where Q_{O2} is the oxygen uptake rate, X is the required cell density, C_{O2}^* is the oxygen solubility in the media, and $C_{O2,crit}$ is the critical dissolved oxygen required by the cells.

$$k_L a = \frac{(Q_{O2}X)_{max}}{(C_{O2}^* - C_{O2,crit})}$$
(C3-2)

All of the variables in the above equation are either known process variables or are quantities that can be found in the literature. The literature indicated an oxygen uptake rate of 0.112 h^{-1}, an oxygen solubility of 0.0075 g O_2/L, and a critical dissolved oxygen level of $1.5 \cdot 10^{-4}$ g O_2/L (Hagman et al., 2014, *Dissolved Oxygen*, n.d., Prpich, 2019). The maximum expected cell density in the 50 L reactor was kinetically determined to be 45.67 g/L, leading to a target $k_L a$ of 696 h^{-1}.

Calculating the experimental mass transfer coefficient is an iterative process. Typically, there would be numerous reactor variables to iterate through and optimize, but because this is a pharmaceutical process and the associated federal regulations can be strict, it was impractical for the team to design our own reactor. Instead, we selected the Sartorius BIOSTAT STR single use reactors and accompanying Flexafe STR bags. Since all of the reactor dimensions were given in

a comprehensive brochure provided by Sartorius (Sartorius Stedim, 2018), the only variable to optimize was the gas flow rate.

The first step in calculating the experimental mass transfer coefficient was to guess an aeration rate in vvm. Units of vvm are commonly used for bioreactor design and represent the volume of air fed to the reactor (L) per volume of media in the reactor (L) per unit time (min). Because this is a fed-batch process with changing volume, a constant aeration rate would *not* represent a constant gas flow rate. A variable gas flow rate is difficult to regulate, so our team opted to reinterpret vvm as volume of air fed to the reactor per *tank volume* per minute to maintain a constant gas flow rate. With a guess for aeration rate, the gas flow rate (Q_g [m^3/s]) was calculated using equation C3-3, where AR is the aeration rate in vvm and V is the tank volume in liters.

$$Q_g = \frac{AR \cdot V}{60} \tag{C3-3}$$

Using known or estimated quantities for gas flow rate (Q_g [m^3/s]), impeller speed (N [rpm]), impeller diameter (D_i [m]), media density (ρ [kg/m^3]), and media viscosity (μ [kg/m·s]), the Reynold's Number (Re) and dimensionless Aeration Number (N_a) were calculated.

$$Re = \frac{N}{60} \frac{D_i^2 \rho}{\mu} \tag{C3-4}$$

$$N_a = \frac{Q_g}{\left(\frac{N}{60}\right) D_i^3} \tag{C3-5}$$

The impeller speed and diameter were taken from the Sartorius Brochure (Sartorius Stedim, 2018). The chosen impeller speed was the maximum possible for the 50 L reactor: 240 rpm. The media density was approximated as an aqueous solution with a mass percent of between 30 and 40% dextrose (Engineering Toolbox, 2017). Lastly, it was found that the viscosity of yeast slurries was significantly higher than the viscosity of dextrose solutions, so the

reactor contents were approximated as a 15% yeast slurry to yield a viscosity of 0.18 kg/m·s

(Global Pumps, n.d.).

After reasonably approximating the Reynold's Number, Rushton curves were used to

determine the dimensionless power number N_p (Prpich, 2019). Since N_p also depends on impeller

type, it was important to choose an impeller with a higher power number so that greater mass

transfer could be achieved. The Sartorius reactor bags could be purchased with two six-blade

Rushton impellers, two three-blade segment impellers, or a combination of the two. Because

Rushton impellers are associated with higher power numbers and yeast is robust to high shear,

the team selected reactor bags with two Rushton impellers. The resulting power number was 3.5.

The dimensionless aeration number (N_a) from equation C3-5 can be correlated with a

ratio of gassed power to ungassed power (P_g/P) (Moreira et al., 2014). Using a P_g/P of 0.6, the

number of impellers (n_i), the power number (N_p), media density (ρ), impeller speed (N), and

impeller diameter (D_i), the gassed power requirement in watts was calculated via equation C3-6.

$$P_g = \left(\frac{P_g}{P}\right) n_i N_p \rho \left(\frac{N}{60}\right)^3 D_i^5 \qquad \text{(C3-6)}$$

Finally, the known or calculated quantities for tank diameter, gassed power, and tank

volume were combined in the following equation

$$k_L a = \frac{0.0333}{D_t^4} \left(\frac{P_g}{V}\right)^{0.541} Q_g^{0.541/\sqrt{D_t}} \qquad \text{(C3-7)}$$

Although there were many $k_L a$ correlations to choose from, equation C3-7 was chosen because it

has no volume restrictions and explicitly takes scale into account (Prpich, 2019).

By altering aeration rate, and all subsequently affected equations, the value of $k_L a$ in

equation C3-7 was matched with the value obtained by equation C3-2. This was achieved with

an aeration rate of 3 tank volumes of air per minute, or 150 L of air per minute.

As a final step, two additional quantities were calculated to ensure operation within some common guidelines. If the gas flow rate (Q_g) was less than the quantity calculated using equation C3-8, gas flooding was avoided. Additionally, if superficial velocity, calculated using equation C3-9, was less than 125 m/h or 2.083 m/s, slugging was assumed to be prevented.

$$Q_g \leq 0.6 \frac{D_i^5 (\frac{N}{60})^2}{D_t^{1.5}} \qquad (C3\text{-}8)$$

$$v_s = \frac{Q_g}{\pi D_t^2 / 4} \qquad (C3\text{-}9)$$

4.   Second Dilution and 1000 L Reactor Growth

Following the 50 L fermentation, the total reactor volume is 50 L. For fermentation to be effective in the 1000 L reactor, a working volume of 250 L is required. With an addition of 200 L HB4 media, the required volume of 250 L is achieved. Kinetic information for the 1000 L reactor was determined using equations C2-1, C2-2, C2-3, and C3-1 – the same equations used for calculating kinetics of the 50 L reactor. Similarly, the method used for calculating the target and experimental $k_L a$ values for the 50 L reactor was applied here. Although tank geometries and other operating conditions differed, the method remained the same.

Growth in the 1000 L reactor was again modelled using MATLAB, based on the continuation of cell growth from the 50 L reactor (Figure C2-1). The resulting plot can be found in Figure C4-1.
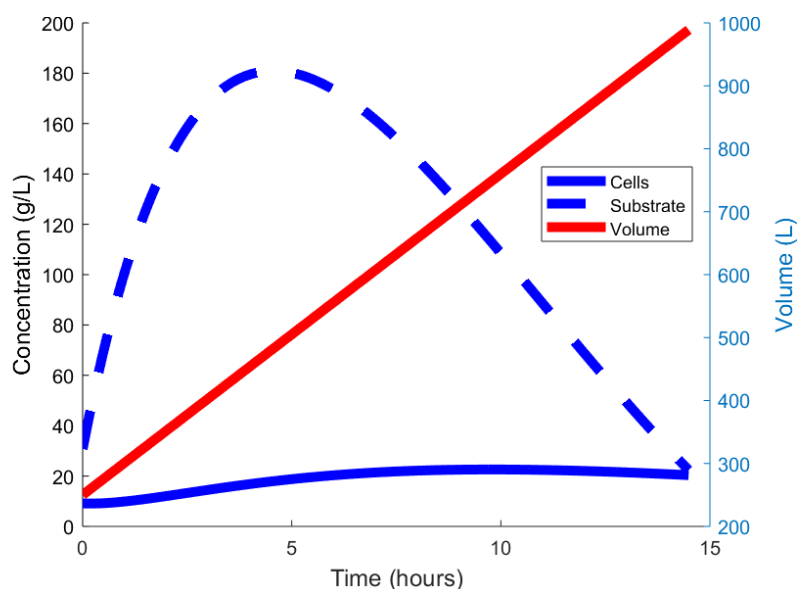
Figure C4-1: Concentrations and Volume Over Time in 1000 L Reactor

Substrate concentration increased dramatically from the outset, which indicated that the yeast cells were in a lag phase and adapting to growth conditions (Rolfe et al., 2012). After a few hours in the 1000 L fermenter, the substrate concentration peaked and began to decline, which suggests significant uptake of substrate and exponential cell growth. Note, however, that the plot of cell concentration over time does not appear functionally exponential. This can be attributed to continuous dilution as a result of the fed-batch process; although the cells continue to multiply, the overall concentration remains approximately constant.

Following 14.5 hours of fermentation, the final cell concentration reached 20.35 g/L, the final substrate concentration was 22.18 g/L, and the final volume was 989.5 L. This was accomplished with a media flow rate of 51 L/h (5:4 ratio of HB4 media to 80% dextrose) and an aeration rate of 2.6 reactor volumes of pure oxygen per minute.

Since yeast cells are not adherent cells, harvesting after the end of fermentation simply involves pumping out of the reactor with a peristaltic pump and into the continuous centrifuge.

5.   First Centrifugation

The first step in the extraction process is continuous centrifugation that separates the *S. cerevisiae* cells from the fermentation broth. From the previous fermentation step, the working volume was 1000 L with a yeast concentration of 20.0 g/L. It was assumed that 84.6% of the growth media was removed in the overflow (Chungcharoen et al., 2017). The equipment chosen was the Sartorius Stedim kSep® 6000S, a single use centrifuge with a throughput capacity of 720 L/h and yield of 97% (Sartorius Stedim, 2019). A low feed flow rate of 600 L/h was chosen to facilitate better separation. The operating parameter to be calculated is the rotational speed (RPM). First, the terminal settling velocity (v_g) of yeast cells is needed, found using the following equation:

$$v_g = \frac{4r_p^2(\rho_p - \rho_f)g}{18\eta} \tag{C5-1}$$

Where r_p is the radius of the cell (m), ρ_p and ρ_f are the densities of the particle (cell) and fluid, respectively (kg/m^3), and is the viscosity of the solution (Pa·s). The sigma factor (Σ_T) can correlate the feed flow rate (Q) and terminal settling velocity to the geometric factors of the centrifuge. For a bowl centrifuge:

$$Q = v_g \cdot \Sigma_T \tag{C5-2}$$

$$\Sigma_T = \frac{\omega^2\pi(R_o^2 - R_i^2)L}{g \cdot \ln\left(\frac{R_o}{R_i}\right)} \tag{C5-3}$$

Where is the rotational speed to be determined (rev/s), R_o and R_i are the outer and inner radii of rotation, respectively (m), and *L* is the length of the centrifuge (m). The sigma factor was found by dividing the feed flow rate of 600 L/h, or 2.8·10^{-5} m^3/s, by v_g, which resulted in a value of 20.4 m^2. With the centrifuge dimensions provided by Sartorius, the sigma factor was used to solve for the rotational speed:

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$$\omega = \sqrt{\frac{\Sigma_T \cdot g \cdot \ln\left(\frac{R_o}{R_i}\right)}{\pi\left(R_o^2 - R_i^2\right)L}}$$ (C5-4)

A rotational speed of 1500 was determined, where most of the fermentation broth was removed in the overflow, while the yeast cells suspended in the remnant growth media were recovered in the underflow.

6.   Homogenization

Following cell harvesting, cells must be lysed to release the inclusion bodies within the yeast cells, which can be done using a high pressure homogenizer (HPH). HPH exposes the cells to high turbulence and shear force to rupture the cell walls. The DeBEE 2000 Pilot Plant Homogenizer will be employed to do this. The product stream from the first centrifugation step must be diluted with 154 mL of WFI so that the feed stream is at an appropriate concentration for HPH as specified by DeBEE. From empirical data of yeast cell homogenization, it was observed that 80% cell lysis occurs at the operating parameters of 150 mPa applied pressure and 1.7 L/min flow rate for two passes (Spiden et al., 2013). The average weight of a yeast cell is $7.9 \cdot 10^{-11}$ grams, a yeast cell is about 35% dry matter, the ratio of overexpressed protein to total protein is 15% for yeast cells, and the total amount of protein in a yeast cell is about $4 \cdot 10^{-12}$ grams. Using these values, it was found that of the yeast cells, a maximum of 2.17 weight percent of the dry cell weight is the desired RTS,S antigen and 14.5 weight percent is other proteins such as HCP or cellular DNA. For conservative estimates, 1 weight percent RTS,S was assumed.

7.   Second Centrifugation

After cell disruption to release the inclusion bodies, a centrifugation step is necessary to separate them from remaining whole cells and large cell debris. The terminal settling velocity again must be calculated, but for inclusion bodies. Using a protein inclusion body density of

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1300 kg/m^3 and radius of 415 nm (Margreiter et al., 2008), a value of 1.13·10^{-7} m/s was found for v$_g$. With the same kSep® 6000S centrifuge as the initial centrifugation, a lower feed flow rate of 60 L/h to account for the reduction in volume, and again 84.6% removal of waste, a rotational speed of 1650 RPM was determined for separation. The waste overflow consists of growth media, unlysed yeast cells, and cell debris, while the underflow contains the inclusion bodies and small amounts of cellular material.

8.   Depth Filtration

To remove remaining macromolecular contaminants, namely whole yeast cells and cell debris, depth filtration is employed. Depth filtration utilizes a porous medium made of fibrous media, which creates a tortuous path due to the random arrangement of the fiber. The nature of the fibrous media typically traps molecules of larger sizes. A cellulose based depth filter was specified by the patent for RTS,S (De Wilde et al., 2001). Cellulose media offers the advantage of being cost effective and having high yields for separating small molecules, such as proteins (Buyel et al., 2015). Depth filtration is necessary before further purification steps to prevent fouling in later equipment due to the presence of large molecule contaminants.

For this step, the Millistak+ Pod Depth Filter by Millipore will be used. This model offers disposable filters that have a surface area of 1.4 m^2. For the clarification of yeast cell lysates post centrifuge, recommended operating parameters were offered by Millipore. For a 90% yield, the suggested flux is 2000 L/m^{2}h, a pressure drop across the filter of 15 psi, and a flow rate of 37 L/min (Millipore, 2009).

9.   Aerosil Adsorption/Desorption

The media containing the desired RTS,S protein and contaminating intracellular protein are subsequently adsorbed to colloidal silica at 4˚C. Adsorption to silica particles can be

attributed to hydrogen, hydrophobic, or van der Waals forces. The Aerosil 380 resin is composed of colloidal silica, which is then packed into a 2 L column. Molecules with larger surface areas, in this case the large contaminating proteins, form more bonds with the silica which are usually irreversible. Components that bind weakly to the resin, such as the RTS,S, can be desorbed with a buffer of differing pH or salt concentration. With an increasing pH, the proteins carry less of a positive charge, and past the isoelectric point of the protein, it becomes negatively charged. Molecules that are more strongly positive can easily adhere to the silica surface, and so an increasing pH aids in desorbing the protein from the resin. Additionally, increasing salt concentration influences the solubility of the protein. The buffer to be used for elution is 10 mM pyrophosphate buffer containing 1% TWEEN 20 (pH 9.5), specified by the patent (De Wilde et al., 2001).

To determine the amount of Aerosil 380 needed for this process, it was found that 40 g silica is needed per liter of lysate (Langley et al., 1993). For the 47.4 working volume, 1.9 kg of silica is required. Furthermore, a residence time of five minutes was specified, resulting in a flow rate of 0.4 L/min and a total process time of 1.98 hours. The patent specified that this step removes 15% of contaminating proteins (De Wilde et al., 2001).

10. Ultrafiltration 1

To prepare the media for Anion Exchange Chromatography (AEX), batch ultrafiltration is needed to concentrate the protein. The filter chosen for all ultrafiltration and diafiltration steps is the single use, polyethersulfone Sartocon® Self Contained Filter Loop by Sartorius. The filter has a nominal molecular weight cutoff (NWCO) of 300 kD, and since the RTSS has a molecular weight of 2700 kD, it was assumed that most of the protein would be retained in the retentate (Collins et al., 2017). Furthermore, it was assumed that the buffer would completely flow

through the filter as the molecules are significantly smaller than the cut off of the membrane. The membrane area of the Sartocon® filter is 1.4 m², and the recommended operating condition for a similarly sized protein were a transmembrane pressure (TMP) of 2.8 barg and flux of 64 L/m²·h. The permeate flow was determined to be 89.6 L/h by multiplying the flux and membrane area.

Following the Aerosil step, the protein has a concentration of 2.77 g/L, and must be concentrated to 19.8 g/L for AEX (Tosoh Bioscience, 2015a). Using this information, a concentration factor (CF) can be determined:

$$\frac{C}{C_o} = (CF)^\sigma \qquad \text{(C10-1)}$$

Where C_o and C are the initial and final concentrations of RTS,S antigen, respectively, (g/L), and σ is the protein rejection coefficient. Although all RTS,S antigen should be retained due to the difference in the NWCO, a conservative value of 0.95 was used for the rejection coefficient.

The concentration factor can be used to determine the final volume of media in the retentate:

$$CF = \frac{V_o}{V} \qquad \text{(C10-2)}$$

Where V_o and V are the initial and final volumes of the media (L). It was assumed that there was no gel-layer buildup of protein on the membrane surface and so the given flux could be taken as the average for the process. The process time for the ultrafiltration step is calculated as:

$$t = \frac{V_o - V}{A \cdot J_{avg}} \qquad \text{(C10-3)}$$

Where t is the process time (h), A is the membrane area (m²), and J_{avg} is the average flux across the membrane (L/m²·h). The process time was found to be 0.46 hours.

11.   Diafiltration 1

Additionally, the buffer that the protein is suspended in must be exchanged to 10 mM Tris-HCl before AEX. Using the same filter, a feed flow rate of the new buffer will be equivalent

to that of the permeate flow rate calculated for ultrafiltration of 89.6 L/h to maintain a constant volume process. To deduce the total volume of Tris-HCl that must be added for diafiltration, the following equation was employed:

$$\left(\frac{C}{C_o}\right)_{buffer} = \exp\left(-\frac{V_W}{Vo} \cdot \left(1 - \sigma_{buffer}\right)\right) \tag{C11-1}$$

Where C_o and C are the initial and final concentrations of buffer to be removed, respectively, (g/L), V_w is the total volume of new buffer to be added (L), V_o is the volume to be diafiltered (L), and σ_{buffer} is the rejection coefficient of the buffer, assumed to be 0 due to its low molecular weight. Assuming 95% removal of the previous buffer species, 17.9 L of Tris-HCl to would be needed for a constant media volume of 6.0 L. The processing time can be calculated as:

$$t = \frac{V_W}{A \cdot J_{avg}} \tag{C11-2}$$

The processing time was found to take 0.20 hours.

12.   Anion Exchange Chromatography (AEX)

Anion exchange chromatography (AEX) is used as a capture step. This purification method employs a column packed with positively charged ligands on the resin as the stationary phase. The mobile phase is maintained at a pH that is higher than the isoelectric point of the RTS,S VLP in order for the protein to be negatively charged. The RTS,S antigen will then bind to the resin particles and be captured in the column, while any positively charged impurities flow through the chromatography column without binding. Once the impurities are washed out of the interstitial space, the RTS,S protein is eluted from the column with a high salt buffer. The feed concentration for AEX is 19.4 g/L and the feed volume will be about 6 L.

The resin to be used is the TOYOPEARL DEAE-650M as specified by the patent (De Wilde et al., 2001). The mobile phase is 10 mM Tris-HCl (pH 8.1), with an increasing salt

gradient for elution. The temperature for the AEX step will be 4˚C to ensure stability of the protein.

The productivity (P_r) can be calculated for a chromatography column and is defined as the ratio between recovered protein and the total cycle time:

$$P_r = \frac{Amount\ of\ Protein\ Recovered}{Total\ Cycle\ Time \cdot Column\ Volume} = \frac{\eta_E \cdot DBC \cdot CV}{t_{cycle} \cdot CV} \tag{C12-1}$$

Where P_r is the productivity of the column (g/L·min), η_E is the recovery yield fraction, DBC is the dynamic binding capacity (g/L) and was provided by the resin supplier, t_{cycle} is the total cycle time (min), and CV is the calculated column volume (L). A residence time for the solution in the column must be one which maximizes productivity. For AEX, the residence time was found to be approximately 2 minutes.

Cycle Time

The equation for productivity requires the total cycle time. The cycle time is the sum of all processing times: equilibration, load, wash, elution, and clean-in-place (CIP).

$$t_{cycle} = t_{equil} + t_{load} + t_{wash} + t_{elution} + t_{CIP} \tag{C12-2}$$

The first step is to equilibrate the stationary phase (resin) to the desired start conditions. Equilibration results in the exchangeable binding of counterions from the equilibration buffer to the resin ligands. This is necessary so that during sample application, proteins of interest can displace the counterions that occupy the ligands while impurities do not bind. The equilibration buffer chosen is 10 mM Tris-HCl for two column volumes (CV_{equil}). The time required for equilibration is:

$$t_{equil} = CV_{equil} \cdot \frac{L}{u} \tag{C12-3}$$

Where L/u is the specified residence time (min). During sample application, the media is loaded onto the column and the RTS,S antigen is captured by the resin, while unbound impurities occupy the interstitial space. The total time needed for loading can be calculated as:

$$t_{load} = \frac{DBC}{C_f} \cdot \frac{L}{u} \qquad (C12\text{-}4)$$

Where C_f is the concentration of the feed to the column (g/L). It was assumed that all subsequent steps would operate at the same linear velocity as the loading step.

Once the solution has been loaded, the weakly bound impurities are evacuated from the interstitial space by washing the column. The patent indicates that the washing step takes place for five total column volumes (CV_{wash}), two CV using 10 mM Tris-HCl and three CV using 10 mM Tris-HCl + 40 mM NaCl. The time taken for washing can be calculated as:

$$t_{wash} = CV_{wash} \cdot \frac{L}{u} \qquad (C12\text{-}5)$$

After the column has been washed, the desired bound protein can be eluted with a high salt buffer, specifically 10 mM Tris-HCl + 150 mM NaCl. To maximize recovery of protein, five column volumes were assigned for the elution step ($CV_{elution}$). The time taken for elution is:

$$t_{elution} = CV_{elution} \cdot \frac{L}{u} \qquad (C12\text{-}6)$$

Following the recovery of the RTS,S antigen, the column is cleaned according to the procedure detailed by the resin supplier to minimize resin fouling. The clean-in-place (CIP) step rids the column of remaining bound or unbound molecules by applying a high ionic strength buffer (1 M NaOH). CIP was chosen to last for 45 minutes. Using the above equations, the total cycle time was determined to be 71 minutes. Assuming a modest RTS,S antigen recovery yield from AEX to be 0.6, the productivity from AEX was 0.17 g/L·min.

Column Length and Column Diameter

The column length could be solved for by employing the Karmen-Cozeny equation:

$$L \cdot u = \Delta P \cdot \frac{d_p^2 \varepsilon^3}{150(1-\varepsilon)^2 \cdot \eta} \tag{C12-7}$$

Where L is the column length (m), u is the linear flow rate (m/s), P is the pressure drop across the column (operating pressure assumed to be 100000 Pa, which is below the 3 bar maximum specified by the resin vendor) (Pa), d_p is the particle diameter (m), is the extra-particle porosity (assumed to be the typical value of 0.35), and η is the feed viscosity (Pa·s). The column length is found by multiplying the product of the Karmen-Cozeny equation by the specified residence time (L/u), and the linear flow rate is found using the calculated column length:

$$L = \sqrt{(L \cdot u) \cdot \left(\frac{L}{u}\right)} \tag{C12-8}$$

$$u = \frac{L \cdot u}{L} \tag{C12-9}$$

Yielding a column length of 2.3 cm and a linear flow rate of 0.69 m/min, respectively. The column diameter (d_c) can be calculated by relating feed volume, load time, and linear velocity:

$$d_c = \sqrt{\frac{4 \cdot V_f}{\pi \cdot u \cdot t_{load}}} \tag{C12-10}$$

Where V_f is the feed volume (m³), resulting in a column diameter of 7.3 cm. Finally, the column volume is found to be 97 mL using the following equation:

$$CV = \pi \left(\frac{d_c}{2}\right)^2 \cdot L \tag{C12-11}$$

A summary of the operating assumptions and process specifications are shown below in Table C12-1 and Table C12-2:

Table C12-1: AEX Design Assumptions and Column Operating Conditions

Parameter	Value	Units
Temperature (T)	277	K
Feed Volume (V_f)	5.99	L
Feed Concentration of Impurities (C_f)	19.4	g/L
Dynamic Binding Capacity (DBC)	20.0	g/L
Particle Diameter (d_p)	65	m
Extra-particle Porosity (ε)	0.35	--
Operating Pressure Drop (ΔP)	100000	Pa
% Recovery of RTS,S (E)	60	%
Feed Viscosity (η)	1.06	mPa·s
# of Column Volumes for Equilibration (CV_{equil})	2	--
# of Column Volumes for Washing (CV_{wash})	5	--
# of Column Volumes for Regeneration (CV_{regen})	5	--
Clean-in-Place (CIP) Time (t_{CIP})	45	min

Table C12-2: AEX Process Specifications

Specification	Value	Units
Residence Time (L/u)	2.0	min
Karmen-Cozeny Product (L·u)	160.8	cm^2/min
Column Length (L)	2.32	cm
Linear Velocity (u)	0.69	m/min
Equilibration Time (t_{equil})	4.00	min
Loading Time (t_{load})	2.06	min
Washing Time (t_{wash})	10.0	min
Elution Time ($t_{elution}$)	10.0	min
Cycle Time (t_{cycle})	71.1	min
Productivity (P_r)	0.17	g/(L·min)
Column Diameter (d_c)	7.29	cm
Column Volume (CV)	96.8	mL

13.   Hydrophobic Interaction Chromatography (HIC)

Following the AEX step, the concentration of contaminating proteins is 11.6 g/L but must be around 1 g/L before the next chromatography step (Tosoh Bioscience, 2015b). An agitated holding tank will be placed between AEX and hydrophobic interaction chromatography (HIC) that adds 5 L of 10 mM Tris-HCl, as well as enough NaCl to bring the total salt concentration of the solution to 650 mM.

After the anion-exchange capture chromatography step, a hydrophobic interaction chromatography step is used to further purify the RTS,S antigen. Residual negatively charged impurities may have been collected with the desired protein in AEX and require an additional step to remove them. HIC acts as another step to separate the RTS,S antigen from intracellular contaminants, such as HCP and cellular DNA.

For flow-through operation, the hydrophobic surface of the HIC medium binds with the surface hydrophobicities of the undesired proteins and allows the RTS,S antigen to flow through the column. The equilibrium for which impurities are adsorbed onto the chromatography resin can be influenced by the salt concentration of the buffer. At high salt concentrations, the interaction between hydrophobic molecules and the resin is strong. This is because the structure of a protein varies with the solvent in which it is submerged. In a hydrophilic solvent such as water, the protein folds so that hydrophobic portions are buried in the interior of the protein. However, increased salt concentration allows for the hydrophobic patches to surface on the protein and bind to the hydrophobic ligands on the resin (Tosoh Bioscience, 2015b). High salt concentration promotes the adsorption of proteins to the HIC media, which is why the salt concentration of the mobile phase was increased to 650 mM NaCl to ensure binding of the impurities.

The feed concentration of contaminating proteins for HIC is 1 g/L and the feed volume will be about 5.6 L. The resin indicated by the GSK patent was the BUTYL-TSK 650M by TOYOPEARL (De Wilde et al., 2001). The mobile phase is 10 mM Tris-HCl (pH 8.1) + 650 mM NaCl. The temperature for the HIC step will remain at 4˚C for protein stability.

Similar to AEX, the residence time for this chromatography step needed to be specified before proceeding with additional calculations. Because this is operating as flow-through, a short residence time is acceptable for operation. The residence time chosen for HIC that optimizes productivity was 0.5 minutes. Recovery of RTS,S antigen for flow-through conditions was assumed to be 90% to account for losses from binding of the desired protein to the hydrophobic media.

The same calculations using equations C12-1 to C12-11 from AEX were carried out for HIC. The steps for hydrophobic interaction chromatography are the same as those outlined for AEX: equilibration, loading, washing, regeneration, and clean-in-place (CIP). The column volume for each (except loading, which has a time specified by Equation C12-4) was: five column volumes for equilibration and regeneration, two hundred column volumes for wash (to prevent precipitation of the protein) and 30 minutes for CIP. It was assumed that all steps operate in the same linear flow rate, and so the total cycle time was 151.1 minutes. The buffers used for each were: 20 mM Tris-HCl + 600 mM NaCl for equilibration, 10 mM Tris-HCl + 650 mM NaCl for wash, 10 mM Tris-HCl for regeneration, and 1 M NaOH for CIP.

A summary of the operating conditions and process specifications are shown below in Table C13-1 and Table C13-2.

Table C13-1: HIC Design Assumptions and Column Operating Conditions

Parameter	Value	Units
Temperature (T)	277	K
Feed Volume (V_f)	5.63	L
Feed Concentration of Impurities (C_f)	1	g/L
Dynamic Binding Capacity (DBC)	32.2	g/L
Particle Diameter (d_p)	65	m
Extra-particle Porosity (ε)	0.35	--
Operating Pressure Drop (ΔP)	100000	Pa
% Recovery of RTS,S (E)	90	%
Feed Viscosity (η)	1	mPa·s
# of Column Volumes for Equilibration (CV_{equil})	5	--
# of Column Volumes for Washing (CV_{wash})	200	--
# of Column Volumes for Regeneration (CV_{regen})	5	--
Clean-in-Place (CIP) Time (t_{CIP})	30	min

Table C13-2: HIC Process Specifications

Specification	Value	Units
Residence Time (L/u)	0.5	min
Karmen-Cozeny Product (L·u)	171.5	cm^2/min
Column Length (L)	1.19	cm
Linear Velocity (u)	1.43	m/min
Equilibration Time (t_{equil})	2.5	min
Loading Time (t_{load})	16.1	min
Washing Time (t_{wash})	100	min
Elution Time ($t_{elution}$)	2.5	min
Cycle Time (t_{cycle})	151.1	min
Productivity (P_r)	0.21	g/(L·min)
Column Diameter (d_c)	1.76	cm
Column Volume (CV)	2.92	mL

14.   Diafiltration 2

After HIC, the working volume is about 0.60 L at a concentration of 104.2 g/L. Before sterile filtration, the drug product must be exchanged from the chromatography buffer to water for injection (WFI) and diluted to 0.53 g/L. Before the diafiltration step for buffer exchange, the HIC product is diluted with about 14 L of WFI in an agitated holding tank to bring the concentration to 0.53 g/L.

After the dilution, another constant volume diafiltration step is employed using the same single use Sartocon® Self Contained Filter Loop by Sartorius. The total amount of WFI needed is 448.7 L. The permeate flow rate is 89.6 L/h and the total process time is 5.0 hours.

15.   Sterile Filtration

The last step of product purification is sterile filtration. Sterile filtration is a very specific type of microfiltration used to ensure viral inactivation of the drug product. This step removes any viral debris from the product, and provides an extra purification step, ensuring that all previous filtration steps performed as intended. Several other methods could have been used to ensure viral inactivation of the drug product, namely heat and low pH. However, sterile filtration was better suited for this process. Heat was not used for viral inactivation as it could potentially denature the VLP. Viral inactivation by low pH is traditionally done in a chromatography column that requires a buffer with a low pH. Since neither of the chromatography columns used in this process require a low pH, this viral inactivation step is unnecessary. Also, since the chromatography columns appear earlier in the downstream purification process, sterilization at that point in the process would require that all subsequent steps in the purification process also be declared sterile steps. Therefore, it is more economical and appropriate to implement a sterile

filtration step at the end of the downstream purification process rather than another viral inactivation steps.

Industrial viral inactivation filters normally have a pore size of either 0.20 μm or 0.22 μm. For this process, a filter with a pore size of 0.20 μm will be used to ensure complete removal of viruses and other components. Although the expected RTS,S antigen yield for this processing step is 100% since the RTS,S antigen is significantly smaller than the pore size, some of the antigen could potentially adsorb onto the filter material. Therefore, the expected yield will be estimated at 95%. This process step uses the Sartobran® P 0.20 μm Size 8 MidiCaps with a filtration area of 0.1 m². These filters allow large throughput with low product adsorbance, allowing for the fastest and most efficient filtration possible. The Sartobran® P filters will undergo filter integrity testing after each use. The filters are also non-fiber releasing, nontoxic, and operate within the desired pH range.

The Size 8 MidiCaps can be used with a maximum differential pressure of 5 bar, which is well above the operating differential pressure of our system, 0.75 bar. The approximate recommended product flow rate through the filter for our differential pressure is 450 L/h, and since the volume needing to be filtered at this point in the process is 114.6 L, a processing time of 0.26 hours is required for this process step. After each use the filter will be disposed of appropriately.

16.   Vial Filling

Vial filling is the first of two post-purification processing steps. It is used to divide the bulk drug product into the desired quantities to be administered by doctors and nurses at the dose-level. During vial filling, small amounts of drug product are transferred into dose-sized vials, and lightly stoppered to maintain the sterility introduced during the sterile filtration step.

Throughout the vial filling step, drug product sterility should be maintained. Since the vial filling step inherently causes the drug product to come into contact with open air, the step should occur in Grade A Space. At the end of the vial filling step, each vial will contain 0.05 mL of drug product consisting of 25 μg of RTS,S antigen. An alternative filling method using syringes was considered. This would mean nurses do not need to perform the extra step of drawing the vaccine from a vial into a syringe before injecting it into a patient. However, this filling method was quickly eliminated from consideration since the drug product is meant to be separate from its adjuvant until injection (European Medicines Agency, 2015).

The industrial vial filler used for this process was the Chase-Logeman Corporation Model 2FS-12H. This vial filler was chosen specifically for its high vial filling rate, about 240 vials filled per minute. At that fill rate, the processing time required to fill a batch of product into vials is about 160 hours. Although this may seem like an unreasonable amount of time, this process is making only 6 batches per year, so this processing time would not interfere with other batches in the process of being made. No RTS,S antigen is expected to be lost during this process step. All parts of the vial filler that contact the drug product are stainless steel.

17.   Lyophilization

Lyophilization is the second of two post-purification processing steps. It is used to prevent the rapid degradation of the RTS,S antigen, thus greatly extending the expiry date of the vaccine. During lyophilization, the RTS,S antigen and WFI are freeze dried, evaporating all of the WFI from the vial, leaving only the RTS,S antigen and any trace buffer solutes left from other purification steps. The industrial lyophilizer chosen for this process step is the Millrock Technology Quanta-S Steam Sterilizable Freeze Dryer. The chosen model is able to freeze dry 150,000 vials per pass, so with a total vial requirement of 2,300,000 vials per batch, and a freeze

dry time of 24 hours per pass, this process step will take approximately 16 days per batch. This may seem like an unreasonable amount of time, but with 7 batches of RTS,S antigen product to be produced per year, the annual requirement, the annual requirement will be met even with this very long process step.

D.   Discussion of Adjuvant Process Train

Adjuvants are one of two components of vaccines, with the other component being the

molecule which is used to build immunity, such as an antigen, virus, or VLP. The adjuvant's

purpose is to stimulate the immune system to respond against the second component of the

vaccines. Most adjuvants are mixtures of aluminum compounds, as aluminum is known to have

immunostimulatory properties. However, the adjuvant used in the RTS,S vaccine,

AS01E, comprises liposomes and a saponin in a buffer solution, and does not contain any

aluminum. Liposomes are small vesicles composed of phospholipid bilayers, similar to that of a

cell membrane. The liposomes in AS01E are composed of three lipids: 1,2-Dioleoyl-sn-glycero-

3-phosphocholine (DOPC), cholesterol, and monophosphoryl lipid A (MPL). In order for the

adjuvant to have maximum immunostimulatory effects, the liposomes must be less than 100 nm

in diameter. Saponins are plant-based compounds that contain water-soluble triterpene

glycosides, which are also known to have immunostimulatory effects. The raw ingredients of the

adjuvant are the powdered forms of its lipid components, and the aqueous form of the buffer

solution.

A total of 7,000 L per year of adjuvant are required to match the amount of RTS,S

antigen produced. Each dose of adjuvant contains 500 µg DOPC, 125 µg cholesterol, 25 µg

MPL, and 25 µg QS21. The liposomes and saponin component are suspended in phosphate

buffer saline. A total of 120 batches of adjuvant will be manufactured per year. Therefore, each

batch will have a volume of 58.4 liters, and contain 58.4 grams of DOPC, 14.2 grams of

cholesterol, 2.92 grams of MPL, and 2.92 grams of QS-21. 15 liters of phosphate buffer saline is

also added to the adjuvant, with the remaining liquid volume being WFI. The buffer comprises

of 9 mM disodium phosphate, 41 mM monopotassium phosphate, and 100 mM sodium chloride.

1.   **Lipid Dissolution**

The first step of adjuvant manufacture is the dissolution of the lipid components of the adjuvant in ethanol. Ethanol is the chosen solvent because both ends of the lipid, the hydrophilic and hydrophobic ends, are soluble in ethanol. Typically, 1 mL of organic solvent is used for every 10-20 mg of lipids that are to be dissolved (*Liposome Preparation—Avanti® Polar Lipids*, n.d.). Therefore, around 3.5 – 7 L of ethanol are needed per batch. The 78.44 g of powdered lipids will be charged to a 20 L flat bottom boiling flask, followed by 4 L of ethanol. The flask will be stirred by the Magnetic Stirrer by BT Lab Systems for 1 hour to ensure complete lipid dissolution. The magnetic stirrer can stir up to 20 L of solution and can reach stirring speeds of up to 1200 RPM. The 4 L of solution will be stirred for 1 hour at 1000 RPM to ensure complete dissolution.

2.   **Lipid Cake Formation**

After the powdered lipids are fully dissolved into the ethanol, the ethanol is evaporated to form thin lipid film at the bottom of the boiling flask. The evaporation of the ethanol prompts the lipids to assemble into bilayers, where each end of the lipids is oriented towards the same end of the other lipids. The ethanol is evaporated using the HBX Industrial 20 L Rotary Evaporator by Heidolph. The lipid cake at the bottom of the boiling flask will appear to be dry after 24 hours of evaporation.

3.   **Lipid Cake Hydration**

After the lipid cake has been fully expelled of ethanol, it is then hydrated using phosphate buffer. Hydrating the dry lipid cake forms very large multilamellar vesicles that must be transformed into small unilamellar vesicles later. The vesicle layers are composed of lipid bilayers: two layers of lipids. Since phosphate buffer is an aqueous solution, the hydrophilic side

of the lipids in solution will face away from the center of the bilayers, while the hydrophobic side will face towards the center of the bilayers. The exact time needed to fully hydrate the lipid cake is unknown, but generally, a hydration time of 1 hour with vigorous stirring, mixing, or agitation is recommended. It is also recommended that allowing the vesicle suspension to age overnight prior to microfluidization makes it easier to break up the vesicles into a smaller size.

15 L of phosphate buffer saline, comprising 9 mM disodium phosphate, 41 mM monopotassium phosphate, and 100 mM sodium chloride, is added to the 20 L boiling flask after the lipid cake has been fully purged of ethanol. The contents of the 20 L boiling flask are then vigorously stirred by an operator using a long stainless steel rod for five minutes, and subsequently stirred with another magnetic stir bar for 2 hours, and then aged overnight. Aging the lipids overnight is believed to make the microfluidization process easier, and improves the homogeneity of the vesicle size distribution (*Liposome Preparation—Avanti® Polar Lipids*, n.d.).

4.   Microfluidization of Suspension

In order to transform the large multilamellar vesicles into unilamellar vesicles, the liposome suspension must be microfluidized. For this process step, the M815 Microfluidizer by Microfluidics was chosen. This piece of equipment was chosen in particular because it is marketed specifically for pilot plants and small production facilities, and its recommended uses include liposome production. The maximum flow rate through the microfluidizer is 1.2 L/min. The 15 L of multilamellar solution is fed from the 20 L boiling task into a small holding tank connected to the microfluidizer. The solution is passed through the microfluidizer 9 times at a process time of 16 minutes per pass for a total process time of 144 minutes at a final homogenization rate of 99 % (Spiden et al., 2013). After the liposome solution is microfluidized

into a unilamellar vesicle solution, it is transferred into a 100 L mixing tank. 43.3 L of room temperature WFI at a concentration of 0.0674 g/L of QS-21 is charged into the mixing tank and allowed to fully mix with the liposome solution.

5.   Sterile Filtration

This filtration is extremely similar to the filtration described in section C15. However, the volume processed through this sterile filter is different than the volume processed by the other sterile filtration step. Since the liquid volume being processed is about 58.4 L, and the approximate recommended product flow rate through the filter for our differential pressure is 450 L/h, a processing time of 0.13 hours is required for this process step.

6.   Filling of Suspension

The industrial vial filler used for this process is the Chase-Logeman Corporation Model 2FS-12H. This vial filler was chosen specifically for its high vial filling rate, about 240 vials filled per minute. At that fill rate, the processing time required to fill a batch of product into vials is about 8.1 hours. No adjuvant product is expected to be lost during this process step. All parts of the vial filler that contact the drug product are stainless steel.

E.   Ancillary Equipment

Ancillary pumps and tanks will be necessary for transferring and storing process materials and chemicals. These pieces of equipment are necessary for supporting the main process stages and represent a significant energy and capital investment. The exact designs of these units directly impact product quality and integrity, and thus demand attention.

1.   Tanks

Tanks are essential for the storage and mixing of product and chemicals such a buffers, CIP chemicals, and fermentation media and substrate. All tanks used are agitated cylindrical tanks constructed with stainless steel. This agitation allows for reliable mixing of chemicals and maintains homogeneity of solutions, ensuring product integrity. Tank sizes were determined by calculating the per-batch volume of their contents and rounding up to the nearest 10, 20, or 100 liter increment depending on the size. The purchase costs for these tanks were determined by comparing some prices found online with correlations in literature (Petrides, 2015) Because many tanks were smaller than even the minimum size shown on this chart, the power scaling rule from Turton was used as a costing rule as well. These tanks of course require power for agitation, and this power requirement was determined by equation C3-6 for fermenters as fermenters are agitated tanks that are functionally similar.

2.   Pumps

Pumps are necessary for transporting fluids from one major unit operation to the next, as well as introducing buffers at certain points in the process. For all processes, peristaltic pumps were chosen. Peristaltic pumps are desirable in pharmaceutical applications for several reasons. They are capable of transporting high viscosity fluids with low shear, which is ideal for higher

viscosity fermentation broth and protein solutions that are sensitive to high shear conditions. Additionally, peristaltic pumps constructed with silicone tubing are preferable to metal turbines and other pumps that wear over time, thus slightly contaminating the product. Peristaltic lines can be replaced and disposed of, instead of necessitating repair. Moreover, they are adjustable and have a wide range of flow rates across for a given model, allowing for parts interchange between identical models used for different flow rates, increasing efficiency of the plant operation.

In order to determine the power requirement of each pump, the necessary volumetric flowrate was multiplied by the pressure difference applied to each stream by the pump.

$$P_p = \Delta P \times Q \qquad\qquad \text{(E2-1)}$$

All pumps were estimated to operate at a pressure differential of 1.0 bar, as this minimizes potential unwanted damage to cells and protein, but is sufficient for the relatively low flow rates of the process and within the operating parameters of the pumps researched for costing. Piping and tubing between unit operations contributes about 0.5 bar of frictional losses, so the pressure difference must be higher than 0.5. The combined efficiency of the pump motor and actual transfer to the fluid was assumed to be 50%. Many steps have specific flowrates that need to be met precisely. Because peristaltic pumps can be used for these very specific flows, the volumetric flow rate used in power calculations was exactly what was suggested by manufacturers of the unit operations they support, such as chromatograph columns and high pressure homogenizers. Prices found online for MasterFlex pumps with a range of 0.0001 to 42 liters per minute were used to predict the cost of each pump based on their specified flow rate.

F.   CIP and SIP

Clean-In-Place (CIP) and Steam-In-Place (SIP) procedures are used to clean and sterilize product contacting equipment without disassembly. Although most of this process uses SUS, the adjuvant processing equipment, the downstream homogenizer, and all three downstream chromatography columns do not use SUS, and therefore need to use CIP and SIP procedures. However, the AEX and HIC columns have their own CIP procedure, which is not within the scope of this section. The CIP and SIP procedures comprise three steps: mechanical wash, chemical wash, and sterilization. The mechanical wash step removes debris from product contacting surfaces using high turbulence WFI. The chemical wash step removes lingering debris using a caustic solution, usually 1 M NaOH. The sterilization step kills any organisms in the equipment that survived the caustic wash.

The mechanical wash step of the CIP procedure requires flushing turbulent hot WFI through the product contacting equipment. Typically, this step is labelled as the "pre-rinse" step of the overall CIP process. The pre-rinse step usually lasts for about 10-20 minutes. The next step in the CIP process is the caustic wash. Usually a solution which contains NaOH at concentrations of about 1-2 weight percent is used(Broadhurst, n.d.). The caustic wash detergent has several requirements, such as non-foaming, non-corrosive, rinseable, and controllable. For the equipment in this process, 1 M NaOH is used as the caustic detergent.

After each caustic wash step, a rinse step is executed to remove any residual soil and caustic detergent. In many pharmaceutical applications, multiple caustic wash and rinse steps are executed. Depending on the size and use of the equipment being cleaned, the caustic wash and rinse steps can last anywhere from 30 minutes to 4 hours. For this process, the caustic wash and rinse steps shall last 2 hours each, to ensure complete removal of soil and cleaning solution.

The CIP system for this process consists of pipework and tanks to hold the caustic detergent. An autoclave chamber was considered to CIP and SIP the flasks used in the adjuvant process, but we determined that using a new flask for each adjuvant batch would be cheaper and more practical than installing a large autoclave chamber. Our CIP procedure consists of a pre-rinse, caustic detergent, and final rinse tank. The rinse and CIP solution flow rates depend on the process pipe diameter, as shown in Table F1 below (Broadhurst, n.d.):

Table F-1: Clean in Place Solution Flow Rates

Process Pipe Diameter (mm)	Minimum CIP Flow Rate (m^3/h)
25	2.1
38	5.2
50	10
65	16
75	24
100	42
125	70
150	100

The amount of caustic detergent needed for each piece of equipment depends on the size of the equipment, CIP procedure run time, and the detergent flow rate. Based on estimated run times, and assuming a process pipe diameter of 25 mm, rinse and detergent amounts were calculated for each piece of equipment as shown in Table F2:

Table F-2: Process Equipment CIP Summary

Step	Pre-Rinse Time (min)	Caustic Wash Time (min)	Final Rinse Time (min)	CIP Volume (L)
Homogenizer	20	60	40	2100
Aerosil Column	10	30	20	1050

After the homogenizer and aerosil column are each cleaned in place, they need to be steamed in place for sterilization. Sterilization involves flowing hot steam at high pressures

through each piece of equipment. Typically, the steam used is at 121°C, and the sterilization time

for all pieces of equipment is 30 minutes at a minimum, with the timer starting after the

equipment reaches 121°C.

G.   Water For Injection System

Water for Injection, or WFI, is self-sterilizing pure water, completely free of any chemical or biological contaminants, such as salts, viruses, or bacteria. WFI can be used both as a product ingredient, or as a CIP component. It is sterilized by distillation, reverse osmosis, or another comparable purification process, and maintains its sterility by being kept at least 80 degrees Celsius. Since this process will be added to an existing GlaxoSmithKline vaccine manufacturing plant in Belgium that is already equipped with its own WFI production system, we do not have to design an entirely new system. However, we still find it necessary to technically describe a WFI production system that suits this plant's production needs, should the WFI production load be too much for the WFI production system in the Belgium plant to handle.

The WFI specifications are the same across Europe, the US, Japan, and China. The conductivity must be less than 1.3 mS/cm at 25 C, the bacterial levels must be less than 10 cfu/100 mL, and the endotoxin levels must be less than 0.25 (IU)/mL. Total organic carbon must be less than 0.5 mg/L (Mattile & Parkka, n.d.).

Traditionally, WFI has been produced in pharmaceutical manufacturing plants through multiple-effect distillation (MED) and vapor compression distillation (VC) in stainless steel equipment. This method entails boiling impure water, and allowing the steam to travel through a column. Water containing impurities has a higher boiling point than pure water, so as the steam travels up the column, impure water cools and falls back into the distillation container, while the pure steam travels further up the column, and is condensed when it travels far enough up the column to meet all of the WFI specifications. This process is an effective method for removing microbial contaminants because it involves boiling the water. However, the water must be

treated to remove any compounds that can damage the stainless-steel equipment at high

temperatures, such as chlorine.

A newer technology for producing WFI is reverse osmosis (RO). Although RO has been

used commercially in the United States for over 100 years, its use for WFI production has been

allowed for only 30 years. In 2002, the European monograph introduced Highly Purified Water

(HPW), which has all of the same specifications as WFI, but could only be produced through

RO. In 2020, WFI and HPW will be merged under the WFI name. The RO method entails

passing water tangentially across a membrane that rejects heavy ions and allows water

containing few ions to pass. The impure water to be purified using RO must be treated for

chlorine and ammonia, just as with MED and VC, so that the membrane is not damaged. Most

RO systems only involve two passes, followed by electrodeionization and ultrafiltration. RO is

executed at ambient temperature and pressure, allowing for potential microbial growth (Mattile

& Parkka, n.d.). Therefore, WFI storage systems and piping must be subject to sanitization, such

as ozonation with UV light exposure.

We believe RO is superior to MED and VC for WFI production. MED WFI stills can cost

upwards of $600,000, not factoring in the treatment requirements. Also, the MED and VC

equipment takes up a very large amount of facility space, and consumes a large amount of

energy for steam heating and cooling. RO systems do not require steam or cooling. The RO

systems can be sanitized using an electric heater or steam. Table G1 below lists the WFI

requirement for each process step, showing the total WFI requirement of 8,824 L per batch.

Including a safety cushion, in the case of increased WFI demand, 15,000 L of WFI should be

ready for use for each batch.

Table G-1: Overall Process WFI Requirements

Process Step	WFI Requirement (L)
Fermentation	1,000
Aerosil Column	47
Diafiltration 1	18
Anion Exchange Chromatography	4
Hydrophobic Interaction Chromatography	6
Diafiltration 2	449
CIP and SIP	6,300
Adjuvant Process (Per RTS,S batch)	1,000
Total	8,824

In an RO process, water moves from a high concentration of solute to a low concentration of solute. These areas are separated by a membrane that is permeable to water while rejecting other solutes. Under osmostic conditions, the water would diffuse from the low concentration area to the high concentration area until the concentrations are the same. The pressure gradient required to prevent osmotic flow across the membrane is called the osmotic pressure. To create reverse osmotic conditions, a pressure gradient that opposes the concentration gradient is imposed on the system. Since the Mosquirix plant we are designing will be attached to a plant that is already running in Belgium, we assumed that the WFI production already in that facility will be equipped to handle the additional WFI required for this process. Therefore, the cost and power requirements of a WFI production system are out of the scope of this report. However, if this Mosquirix plant were standalone, and required a new WFI production system, we would use the EPRO 1200 RO system, made by Newterra. This model has a maximum flow rate of 4500 L/day, a membrane area of 645 cm^2, a maximum operating pressure of 180 psi, a purification rate of 99%, and a nominal recovery rate of 40%. Assuming the water being filtered into WFI is the same quality as tap water, the operating pressure at which the RO system will operate most

efficiently is 60 psi, according to literature (*How Long Does it Take to Fill a Reverse Osmosis Storage Tank?*, n.d.).

After the WFI passes through the RO system, it must pass through an electrodeionization system to remove any remaining ionic species. Electrodeionization (EDI) uses electricity, ion exchange, and resin to remove ions from water. Impurities are moved into a concentrate water stream, enabling the resins to have a long lifespan. The EDI system we would use, if our plant were not attached to an already running facility, is the EDI-1XL200 by Applied Membranes Inc. It has a system capacity of 2880-9640 L/min, which will operate well with the RO system. The final step in the WFI production process is a pass through an ultrafiltration system. This step ensures that the endotoxin specification is achieved. A 6 kD molecular weight cutoff filter is recommended. For WFI storage and distribution, ozonation coupled with UV light exposure is usually used to ensure the sterility of the WFI is maintained.

H. Final Recommended Design

This section of the report summarizes the equipment and stream compositions, quantities, and locations. The upstream, downstream, and adjuvant process flow diagrams are presented, followed by the respective stream tables.

1. Equipment Table and Process Flow Diagram
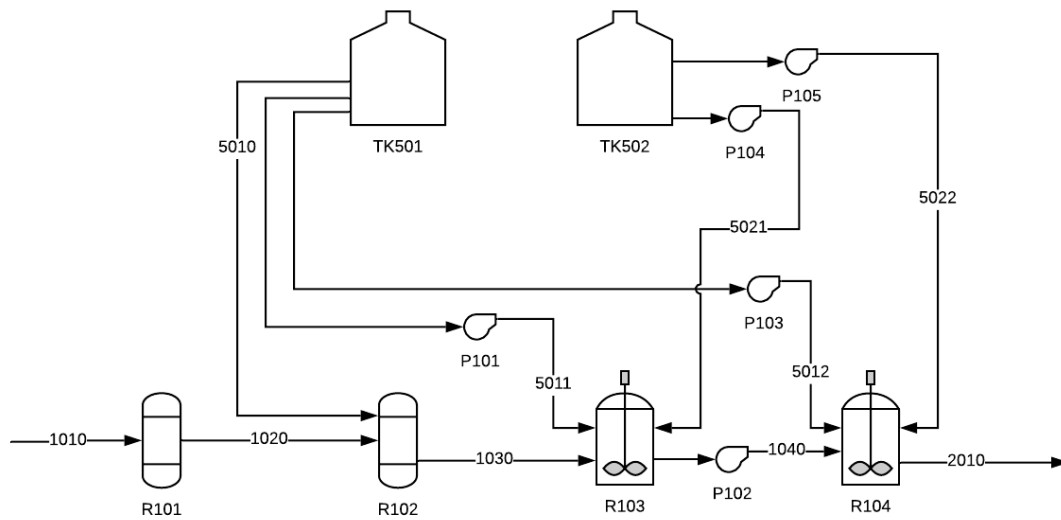
Figure H1-1: Upstream Process Flow Diagram
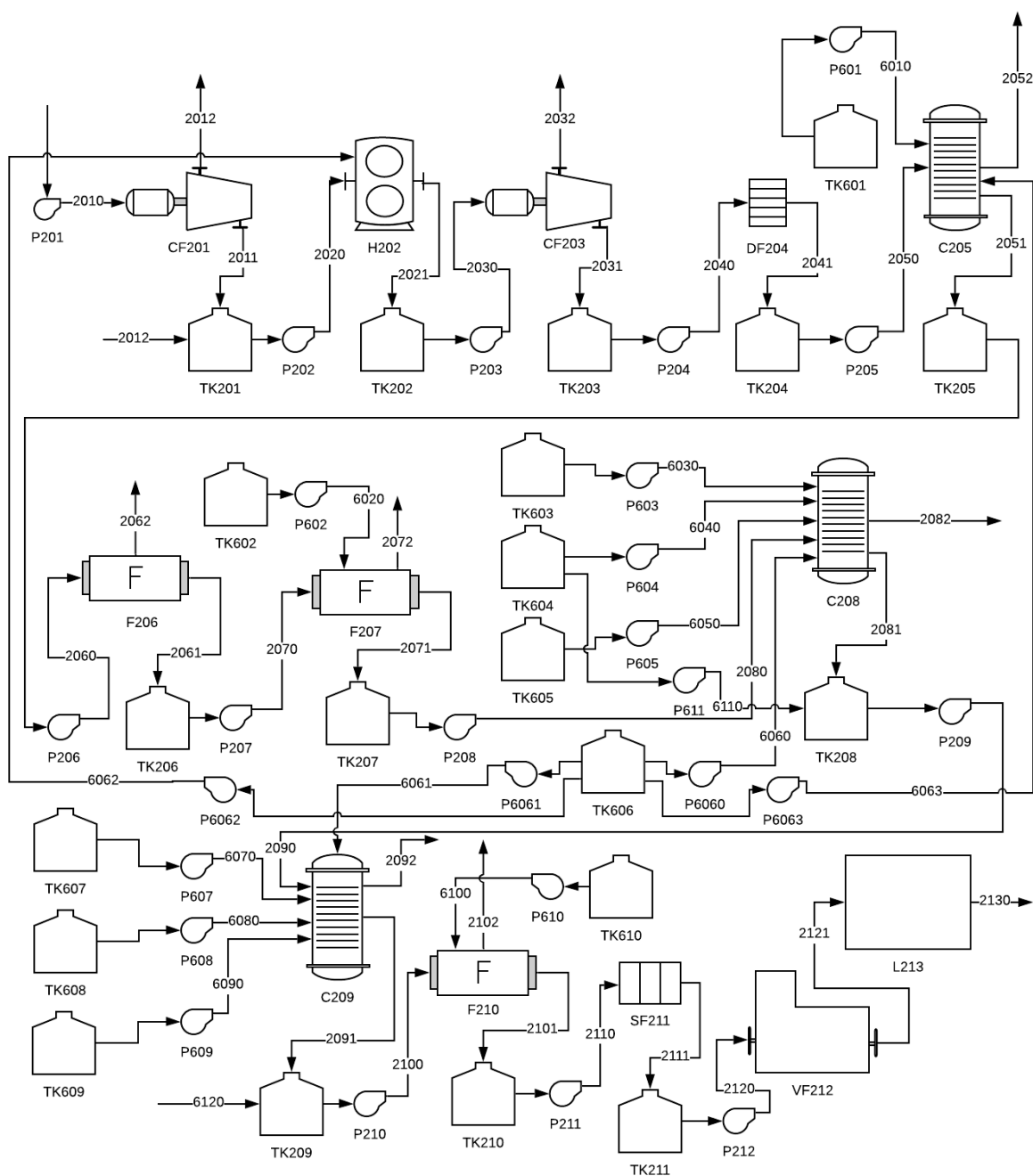
Figure H1-2: Downstream Process Flow Diagram

3022

3010 → S301 → 3020 → RE302 → 3030 → S303 → 3040 → H304 → 3041 → SF305 → 3050 → 3051 → 3060 → VF212 → 3061 →

7010 7020 7030

TK701 P701 TK702 P702 TK703 P703 TK304 P305 TK305 P306
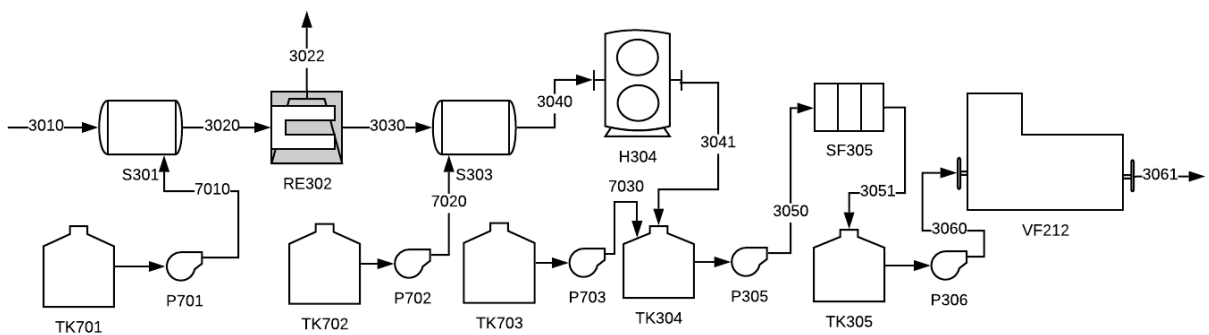
Figure H1-3: Adjuvant Process Flow Diagram

Table H1-1: Equipment Table for RTS,S Process with Yields

Equipment Tag	Description	Step Yield (%)	Overall Yield (%)
R101	Petri Dishes	100	100
R102	Conical Flasks	100	100
R103	50 L Fermenter	100	100
R104	1000 L Fermenter	100	100
CF201	First Centrifuge	97	97
H202	HPH	80	77.6
CF203	Second Centrifuge	97	75.3
DF204	Depth Filtration	90	67.7
C205	Aerosil Column	97	65.7
F206	Ultrafiltration 1	90	59.1
F207	Diafiltration 1	95	56.2
C208	AEX	60	33.7
C209	HIC	90	30.3
F210	Diafiltration 2	95	28.8
SF211	Sterile Filtration	100	28.8
VF212	Vial Filling	100	28.8
L213	Lyophilization	100	28.8

: Equipment Table for AS01E Adjuvant Process with Yields

Equipment Tag	Description	Step Yield (%)	Overall Yield (%)
S301	Lipid Ethanol Mixer	100	100
RE302	Rotary Evaporator	100	100
S303	Lipid Hydrate Mixer	100	100
H304	Microfluidizer	100	100
SF305	Sterile Filter	100	100
VF212	Vial Filler	100	100

2. Stream Tables

Table H2-1: Equipment Stream Inlet and Outlet Materials for Upstream RTS,S Process

Stream	Description	Material	Amount
1010	Petri Dish Feed	Master Seed	1 mL
1020	Conical Flask Feed	Yeast Cells	1.6 g
		Media	1.6 L
		Pure Dextrose	32 g
5010	Pre-50 L Reactor Dilution	Media	10.9 L
1030	50 L Reactor Feed	Yeast Cells	14.29 g
		Media	12.5 L
5011	50 L Reactor Substrate Addition	Media + 80% Dextrose	37.5 L
5021	50 L Reactor pH Adjustment	Ammonium Hydroxide	Variable
1040	1000 L Reactor Feed	Yeast Cells	2.28 kg
		Media	50 L
5012	Pre-1000 L Reactor Dilution	Media	200 L
5012	1000 L Reactor Substrate Addition	Media + 80% Dextrose	739.5 L
5022	1000 L Reactor pH Adjustment	Ammonium Hydroxide	Variable

Table H2-2: Equipment Stream Inlet and Outlet Materials for Downstream RTS,S Process

Stream	Description	Material	Amount (kg/batch)
2010	First Centrifuge Feed	Yeast Cells	20
		Media	1000
2012	First Centrifuge Overflow (Waste)	Yeast Cells	0.6
		Media	846
2011	First Centrifuge Underflow (Product)	Yeast Cells	19.4

Stream	Description	Material	Amount (kg/batch)
		Media	154
2012	Post First Centrifuge Dilution	WFI	154
2020	HPH Feed	Yeast Cells	19.4
		Media	308
2021	HPH Outlet	RTS,S	0.16
		Intracellular Protein	2.25
		Cell Debris	13.1
		Unlysed Yeast Cells	3.88
		Media	308
6063	HPH CIP	WFI	2100
		NaOH	83.8
2030	Second Centrifuge Feed	RTS,S	0.16
		Intracellular Protein	2.25
		Cell Debris	13.1
		Unlysed Yeast Cells	3.88
		Media	308
2032	Second Centrifuge Overflow (Waste)	RTS,S	0.005
		Intracellular Protein	0.068
		Cell Debris	11
		Unlysed Yeast Cells	3.28
		Media	261
2031	Second Centrifuge Underflow (Product)	RTS,S	0.15
		Intracellular Protein	2.18
		Cell Debris	2.02
		Unlysed Yeast Cells	0.6
		Media	47.4
2040	Depth Filtration Feed	RTS,S	0.15
		Intracellular Protein	2.18
		Cell Debris	2.02
		Unlysed Yeast Cells	0.6
		Media	47.4
2041	Depth Filtration Outlet	RTS,S	0.13
		Intracellular Protein	1.96
		Media	47.4
2050	Aerosil Column Feed	RTS,S	0.13

Stream	Description	Material	Amount (kg/batch)
		Intracellular Protein	1.96
		Media	47.4
6010	Aerosil Column Buffer	Pyrophosphate	$8.20 \cdot 10^{-5}$
		TWEEN 20	$4.70 \cdot 10^{-4}$
		WFI	47.4
2052	Aerosil Column Waste	RTS,S	0.002
		Intracellular Protein	0.29
		Media	47.4
2051	Aerosil Column Outlet	RTS,S	0.13
		Intracellular Protein	1.67
		Aerosil Buffer	47.4
6062	Aerosil Column CIP	WFI	1050
		NaOH	42.1
2060	Ultrafiltration 1 Feed	RTS,S	0.13
		Intracellular Protein	1.67
		Aerosil Buffer	47.4
2062	Ultrafiltration 1 Waste	RTS,S	0.013
		Intracellular Protein	0.42
		Aerosil Buffer	41.4
2061	Ultrafiltration 1 Outlet	RTS,S	0.12
		Intracellular Protein	1.25
		Aerosil Buffer	5.98
2070	Diafiltration 1 Feed	RTS,S	0.12
		Intracellular Protein	1.25
		Aerosil Buffer	5.98
6020	Diafiltration 1 Buffer	Tris	$2.17 \cdot 10^{-2}$
		HCl	$6.52 \cdot 10^{-3}$
		WFI	17.9
2072	Diafiltration 1 Waste	RTS,S	0.006
		Intracellular Protein	0.13
		Aerosil Buffer	5.69
		Diafiltration 1 Buffer	11.9
2071	Diafiltration 1 Outlet	RTS,S	0.11
		Intracellular Protein	1.13
		Aerosil Buffer	$3.00 \cdot 10^{-4}$

Stream	Description	Material	Amount (kg/batch)
		Diafiltration 1 Buffer	5.98
2080	AEX Feed	RTS,S	0.11
		Intracellular Protein	1.13
		Aerosil Buffer	$3.00 \cdot 10^{-4}$
		Diafiltration 1 Buffer	5.98
6030	AEX Equilibration	Tris	$2.30 \cdot 10^{-4}$
		HCl	$7.00 \cdot 10^{-5}$
		WFI	0.19
6040	AEX Wash	Tris	$5.90 \cdot 10^{-4}$
		HCl	$1.80 \cdot 10^{-4}$
		NaCl	$6.80 \cdot 10^{-4}$
		WFI	0.48
6050	AEX Elution	Tris	$5.90 \cdot 10^{-4}$
		HCl	$1.80 \cdot 10^{-4}$
		NaCl	$4.24 \cdot 10^{-3}$
		WFI	0.48
6060	AEX CIP	NaOH	0.087
		WFI	2.17
2082	AEX Waste	RTS,S	0.05
		Intracellular Protein	1.12
		Tris	$2.25 \cdot 10^{-2}$
		HCl	$6.77 \cdot 10^{-3}$
		NaCl	$6.80 \cdot 10^{-4}$
		WFI	8.82
2081	AEX Product	RTS,S	0.067
		Intracellular Protein	0.006
		Tris	$5.90 \cdot 10^{-4}$
		HCl	$1.76 \cdot 10^{-4}$
		NaCl	$4.24 \cdot 10^{-3}$
		WFI	0.48
6110	Post AEX Dilution	Tris	$6.20 \cdot 10^{-3}$
		HCl	$1.80 \cdot 10^{-3}$
		NaCl	0.21
		WFI	5.16
2090	HIC Feed	RTS,S	0.067

Stream	Description	Material	Amount (kg/batch)
		Intracellular Protein	0.006
		Tris	$6.80 \cdot 10^{-3}$
		HCl	$2.00 \cdot 10^{-3}$
		NaCl	0.21
		WFI	5.64
6070	HIC Equilibration	Tris	$3.50 \cdot 10^{-5}$
		HCl	$1.10 \cdot 10^{-5}$
		NaCl	$5.10 \cdot 10^{-4}$
		WFI	0.015
6080	HIC Wash	Tris	$7.00 \cdot 10^{-4}$
		HCl	$2.10 \cdot 10^{-4}$
		NaCl	$2.20 \cdot 10^{-2}$
		WFI	0.583
6090	HIC Regeneration	Tris	$1.80 \cdot 10^{-5}$
		HCl	$5.00 \cdot 10^{-6}$
		WFI	0.015
2090	HIC CIP	NaOH	$7.00 \cdot 10^{-3}$
		WFI	0.18
2092	HIC Waste	RTS,S	0.007
		Intracellular Protein	0.006
		Tris	$1.37 \cdot 10^{-2}$
		HCl	$2.01 \cdot 10^{-3}$
		NaCl	0.22
		WFI	5.85
2091	HIC Product	RTS,S	0.06
		Intracellular Protein	$5.64 \cdot 10^{-5}$
		Tris	$7.00 \cdot 10^{-4}$
		HCl	$2.10 \cdot 10^{-4}$
		NaCl	$2.20 \cdot 10^{-2}$
		WFI	0.583
6120	HIC Dilution	WFI	115
2100	Diafiltration 2 Feed	RTS,S	0.06
		Intracellular Protein	$5.64 \cdot 10^{-5}$
		Tris	$1.80 \cdot 10^{-5}$
		HCl	$5.00 \cdot 10^{-6}$

Stream	Description	Material	Amount (kg/batch)
		NaCl	$5.50 \cdot 10^{-4}$
		WFI	115
6100	Diafiltration 2 Buffer	WFI	449
2102	Diafiltration 2 Waste	RTS,S	0.003
		Intracellular Protein	$5.63 \cdot 10^{-6}$
		Tris	$1.76 \cdot 10^{-5}$
		HCl	$4.90 \cdot 10^{-6}$
		NaCl	$5.40 \cdot 10^{-4}$
		WFI	334
2101	Diafiltration 2 Outlet	RTS,S	0.058
		Intracellular Protein	$5.07 \cdot 10^{-6}$
		Tris	$4.00 \cdot 10^{-6}$
		HCl	$1.00 \cdot 10^{-7}$
		NaCl	$1.00 \cdot 10^{-5}$
		WFI	115
2110	Sterile Filtration Feed	RTS,S	0.058
		Intracellular Protein	$5.07 \cdot 10^{-6}$
		WFI	115
2111	Sterile Filtration Outlet	RTS,S	0.056
		Intracellular Protein	$4.82 \cdot 10^{-6}$
		WFI	115
2120	Vial Filling Feed	RTS,S	0.056
		Intracellular Protein	$4.82 \cdot 10^{-6}$
		WFI	115
2121	Vial Filling Outlet	RTS,S	0.056
		Intracellular Protein	$4.82 \cdot 10^{-6}$
		WFI	115
2121	Lyophilization Feed	RTS,S	0.056
		Intracellular Protein	$4.82 \cdot 10^{-6}$
		WFI	115
2130	Lyophilization Outlet	RTS,S	0.056
		Intracellular Protein	$4.82 \cdot 10^{-6}$
		WFI	115

Table H2-3: Equipment Stream Inlet and Outlet Materials for AS01E Adjuvant Process

Stream	Description	Material	Amount (kg/batch)
3010	Lipid Ethanol Mixer Feed	Cholesterol	0.0142
		DOPC	0.0584
		MPL	0.00292
7010	Ethanol Feed	Ethanol	3.16
3020	Rotary Evaporator Feed	Cholesterol	0.0142
		DOPC	0.0584
		MPL	0.00292
		Ethanol	3.16
3022	Rotary Evaporator Waste	Ethanol	3.16
3030	Lipid Hydrate Mixer Feed	Cholesterol	0.0142
		DOPC	0.0584
		MPL	0.00292
7020	Phosphate Buffer Saline Feed	Disodium Phosphate	0.019
		Potassium Phosphate	0.084
		Sodium Chloride	0.088
		WFI	14.96
3040	Microfluidizer Feed	Cholesterol	0.0142
		DOPC	0.0584
		MPL	0.00292
		Disodium Phosphate	0.019
		Potassium Phosphate	0.084
		Sodium Chloride	0.088
		WFI	14.96
3041	Microfluidizer Product	Cholesterol	0.0142
		DOPC	0.0584
		MPL	0.00292
		Disodium Phosphate	0.019
		Potassium Phosphate	0.084
		Sodium Chloride	0.088
		WFI	14.96
7030	QS-21 Addition Feed	QS-21	0.00292

Stream	Description	Material	Amount (kg/batch)
		WFI	43.2
3050	Sterile Filter Feed	Cholesterol	0.0142
		DOPC	0.0584
		MPL	0.00292
		Disodium Phosphate	0.019
		Potassium Phosphate	0.084
		Sodium Chloride	0.088
		WFI	58.16
3051	Sterile Filter Product	Cholesterol	0.0142
		DOPC	0.0584
		MPL	0.00292
		Disodium Phosphate	0.019
		Potassium Phosphate	0.084
		Sodium Chloride	0.088
		WFI	58.16
3060	Vial Filler Feed	Cholesterol	0.0142
		DOPC	0.0584
		MPL	0.00292
		Disodium Phosphate	0.019
		Potassium Phosphate	0.084
		Sodium Chloride	0.088
		WFI	58.16
3061	Vial Filler Product	Cholesterol	0.0142
		DOPC	0.0584
		MPL	0.00292
		Disodium Phosphate	0.019
		Potassium Phosphate	0.084
		Sodium Chloride	0.088
		WFI	58.16

3.   RTS,S Antigen Process Parameters and Operation

a.   Petri Dishes and Conical flasks

Petri dishes should be streaked with the contents of one master seed vial (total volume of 1 mL) and incubated at 30°C for 63 hours. The surface growth from the Petri dishes will then be suspended in 1.6 liters of HB4 media, supplemented with 32 grams of pure dextrose, and distributed to four 2 L conical flasks. After 5 hours of incubation on a rotary shaker, the cell concentration is 8.93 g/L and the substrate concentration is 4.14 g/L. No volume change occurs during this incubation because the conical flask growth is a batch process. The 1.6 L from the conical flasks is then diluted to 12.5 L with HB4 media and pumped into the 50 L reactor.

b.   50 L Fermenter

All relevant reactor dimensions and kinetic quantities for the 50 L Sartorius BIOSTAT STR Reactor can be found in Table H3-1.

Table H3-1: Reactor Dimensions and Kinetic Quantities for 50 L Reactor

Parameter	Value
Tank Height*	0.667 m
Tank Diameter*	0.370 m
Impeller Diameter*	0.143 m
Maximum Impeller Speed*	240 rpm
Working Volume*	12.5 – 50 L
Gassed Power Requirement	18.49 W
Aeration Rate	3 vvm (air)
Initial Cell Concentration	1.14 g/L
Initial Substrate Concentration	0.53 g/L
Initial Volume	12.5 L
Final Cell Concentration	45.67 g/L
Final Substrate Concentration	153.33 g/L
Final Volume	50 L
Fermentation Time	15 h
Feed Flow Rate	2.5 L/h
* Quantities given by Sartorius (Sartorius Stedim, 2018)	

c.   1000 L Fermenter

All relevant reactor dimensions and kinetic quantities for the 1000 L Sartorius BIOSTAT

STR Reactor can be found in Table H3-2.

Table H3-2: Reactor Dimensions and Kinetic Quantities for 1000 L Reactor

Parameter	Value
Tank Height*	1.795 m
Tank Diameter*	0.997 m
Impeller Diameter*	0.38 m
Maximum Impeller Speed*	90 rpm
Working Volume*	250 – 1000 L
Gassed Power Requirement	92.27 W
Aeration Rate	2.6 vvm (O_2)
Initial Cell Concentration	9.13 g/L
Initial Substrate Concentration	30.67 g/L
Initial Volume	250 L
Final Cell Concentration	20.35 g/L
Final Substrate Concentration	22.18 g/L
Final Volume	989.5 L
Fermentation Time	14.5 h
Feed Flow Rate	51 L/h
* Quantities given by Sartorius (Sartorius Stedim, 2018)	

d.   First Centrifugation

Following fermentation, the yeast cells must be separated from the fermentation broth.

The equipment chosen was the Sartorius Stedim kSep® 6000S, a single use centrifugation

system. The product overflow consists of higher density cells and excess growth media. The feed

flow rate is 600 L/h with a rotational speed of 1500 RPM. The processing time for this step is 1.7

hours. Since the centrifuges used in this process are single use, no CIP time is allocated for this

step, but the replacement of all product contacting surfaces within the centrifuge should take

about 0.5 hours. However, this should not affect the processing time. The underflow containing

the yeast cells are directed to cell lysis after it has been diluted with WFI in the agitated holding tank and the overflow is sent to waste.

e.   Homogenization

Following cell harvesting, the DeBEE 2000 Pilot Plant Homogenizer will be employed to rupture cell walls. The operating pressure will be 150 mPa and the capacity will be 1.7 L/min. The high pressure homogenizer will release the desired RTS,S protein from within the cells while minimizing denaturing of the product. Each homogenization pass takes 3.0 hours. Since each batch must pass through the homogenizer 2 times, the total processing time for the homogenization step is 6.0 hours. The homogenization step is one of the few steps in this process that require CIP and SIP procedures. Both of these steps will take about a combined 3 hours, but this will not affect the process time or the batch schedule.

f.   Second Centrifugation

Following cell lysis, another centrifugation step will occur to separate the majority of inclusion bodies in the underflow from macromolecular contaminants such as unlysed yeast cells, cell debris, and growth media in the overflow. The same Sartorius Stedim kSep® 6000S centrifuge will run at a feed flow rate of 60 L/h and rotational speed of 1650 RPM. The processing time for this step is 5.13 hours. Since the centrifuges used in this process are single use, no CIP time is allocated for this step, but the replacement of all product contacting surfaces within the centrifuge should take about 0.5 hours. However, this should not affect the processing time.

g.   Depth Filtration

Following the second centrifugation step, the media will flow through a depth filter to remove remaining macromolecular contaminants that become trapped in the disposable cellulose

filter mats. The depth filter chosen is the Millistak+ Pod Depth Filter by Millipore with a flux of 2000 L/m^2·h, a pressure drop across the filter of 1 barg, and a flow rate of 37 L/min. The processing time for this step is 0.02 hours, or 1.28 minutes. Since the depth filter used in this process is single use, no CIP time is allocated for this step, but the replacement of all product contacting surfaces within the depth filter should take about 0.5 hours. However, this should not affect the processing time.

h.   Aerosil Adsorption/Desorption Column

Following depth filtration, the media will adsorb onto a 2 L column packed with 1.9 kg of Aerosil 380 colloidal fumed silica resin. The RTS,S antigen is desorbed with a 10 mM pyrophosphate buffer containing 1% TWEEN 20. This step removes 15% of contaminants with an RTS,S antigen recovery of 97%. A residence time of 5.0 minutes was specified, resulting in a flow rate of 0.4 L/min and a total process time of 1.98 hours. The aerosil column will undergo CIP and SIP procedures, but these will not affect the processing time or the batch schedule.

i.   Ultrafiltration 1

Following the colloidal silica adsorption column, batch ultrafiltration is necessary to concentrate the protein for the first chromatography step. The filter to be employed for all ultrafiltration and diafiltration steps is the single use, polyethersulfone Sartocon® Self Contained Filter Loop by Sartorius. The protein solution is concentrated from 2.77 g/L to 19.8 g/L with a permeate flow rate of 89.6 L/h and transmembrane pressure of 2.8 barg. The process time for this step is 0.46 hours. Since the ultrafilters used in this process are single use, no CIP time is allocated for this step, but the replacement of the sterile filter should take about 0.5 hours. However, this should not affect the processing time.

j.   Diafiltration 1

In addition to concentrating the product through ultrafiltration, the buffer of the protein must be exchanged to 10 mM Tris-HCl for anion exchange chromatography. A constant volume process is maintained, thus the diafiltration feed and permeate flow rates are 89.6 L/h. The amount of 10 mM Tris HCl needed is 17.9 L.

k.   Anion Exchange Chromatography (AEX)

The anion exchange chromatography (AEX) step is the capture step to purify the RTS,S antigen. It is expected that the RTS,S antigen binds to the positively charged resin while contaminating proteins such as host-cell proteins and DNA flow through. The AEX column is packed with the TOYOPEARL DEAE-650M resin, with a column diameter of 7.29 cm and length of 2.32 cm. The buffers for equilibration, wash, elution, and CIP are 10 mM Tris-HCl, 10 mM Tris-HCl + 40 mM NaCl, 10 mM Tris-HCl + 150 mM NaCl, and 1 M NaOH, respectively. The chosen residence time is 2.0 minutes and the interstitial velocity was determined to be 0.69 m/min, yielding a total cycle time of 71 minutes per batch, including CIP. Each run loads 6 L of 19.4 g/L RTS,S solution into the column with an assumed recovery of 60%. The column is equilibrated with 2 column volumes (CV), loaded for 2.06 minutes, washed in 5 CV, and eluted in 5 CV, with a following 45 minutes CIP cycle.

l.   Hydrophobic Interaction Chromatography (HIC)

The hydrophobic interaction chromatography (HIC) step is employed to remove trace amounts of residual contaminating proteins. Unlike AEX, the RTS,S antigen will flow through the HIC column while contaminants bind to the resin. For HIC, the resin to be used is the BUTYL-TSK 650M by TOYOPEARL. The feed concentration of contaminating proteins for HIC is 1 g/L for a feed volume of 5.6 L, with a recovery of 90%. Buffer requirements include 20

mM Tris-HCl + 600 mM NaCl for equilibration, 10 mM Tris-HCl + 650 mM NaCl for wash, 10 mM Tris-HCl for regeneration, and 1 M NaOH for CIP. Five column volumes are required for equilibration and regeneration chromatography steps, two hundred column volumes are needed for the wash step, and 30 minutes is dedicated for CIP, yielding a total cycle time of 151.1 minutes. The column diameter is 1.76 cm, and length is 1.19 cm, with a linear velocity of 1.43 m/min.

m.   Diafiltration 2

Following the chromatography steps, the buffer must be exchanged for water for injection (WFI) before the final purifying process, sterile filtration. To replace 98% of the previous buffer, 448 L of WFI is required. With the same operating parameters as previous diafiltration steps, the process time is 5.0 hours.

n.   Sterile Filtration

Following diafiltration, sterile filtration will ensure viral inactivation of the drug product. This process step will use the Sartobran® P 0.20 µm Size 8 MidiCaps with a filtration area of 0.1 m^2, with an expected yield of 95%. The differential pressure across the sterile filter is 0.75 bar. The approximate product flow rate is 450 L/h with a processing time of 0.256 hours.

o.   Vial Filling

The drug product is divided into the appropriate amounts for administration during vial filling. Each vial will contain 0.05 mL of drug product consisting of 25 µg of RTS,S antigen in WFI. The Chase-Logeman Corporation Model 2FS-12H vial filler will be used for its high vial filling rate of 240 vials filled per minute. The processing time required to fill a batch of product into vials is about 160 hours.

p.   Lyophilization

Before the drug product is stored, the RTS,S antigen and WFI are freeze dried,

evaporating all of the WFI from the vial. The industrial lyophilizer to be used is the Millrock

Technology Quanta-S Steam Sterilizable Freeze Dryer which will freeze dry 150,000 vials per

pass with a freeze dry time of 24 hours per pass. This process step will take approximately 16

days per batch.

4.   Adjuvant AS01E Process Parameters and Operation

The process to manufacture the AS01E Adjuvant System consists of six main steps: lipid

dissolution, lipid cake formation, lipid cake hydration, microfluidization, sterile filtration, and

vial filling. None of the steps required any calculations or discussion, as the adjuvant

manufacturing process is a recipe to be followed strictly. As such, please refer to the process

description for more details concerning the purpose of each step in the process. Process times

and equipment names are below in Table H4-1.

Table H4-1: Adjuvant Process Times and Equipment

| Process Step	Process Time (h)	Equipment Name
Lipid Dissolution	1	Magnetic Stirrer - BT Lab Systems
Lipid Cake Formation	24	Industrial 20 L Rotary Evaporator - HBX
Lipid Cake Hydration	14	Magnetic Stirrer - BT Lab Systems
Microfluidization	2.5	M815 Microfluidizer by Microfluidics
Sterile Filtration	0.13	Sartobran® P 0.20 µm Size 8 MidiCaps - Sartorius
Vial Filling	8.1	Model 2FS-12H - Chase-Logeman Corporation

5.   Batch Production Schedule
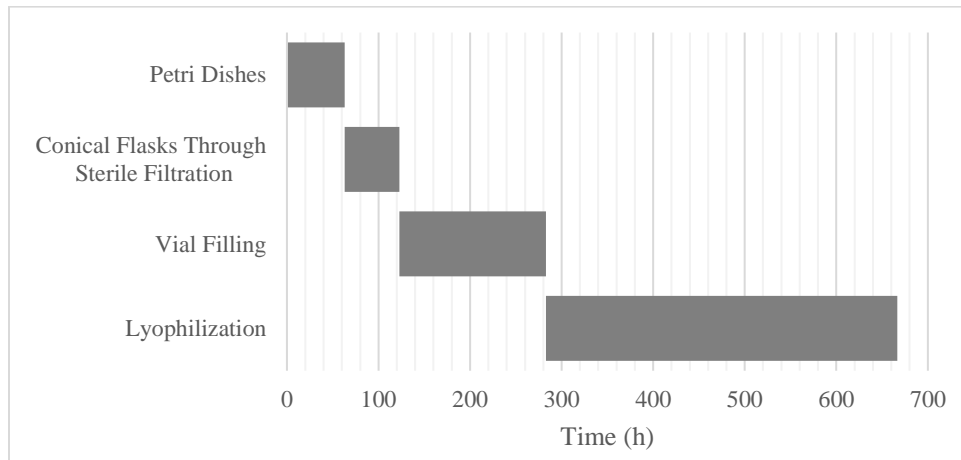
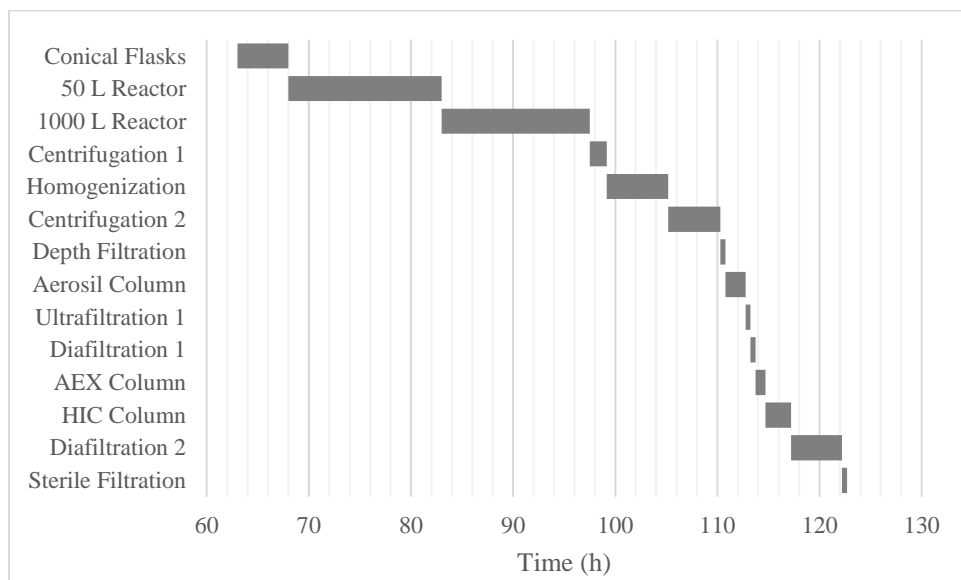*Figure H5-1*: RTS,S Overall Process Train Schedule

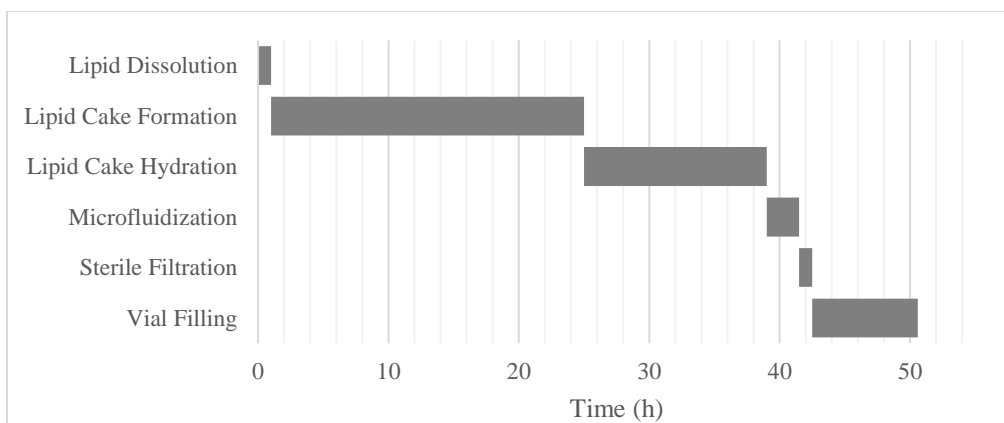*Figure H5-2*: RTS,S Downstream Process Train Schedule

Figure H5-3: Adjuvant Process Train Schedule

As shown in Table H2-2, each processed batch of RTS,S antigen yields 56 g of RTS,S antigen. Therefore, 7 batches of RTS,S antigen must be produced annually to meet the 350 g annual production goal. Since each batch of RTS,S antigen takes just short of 28 days to be processed, and 16 of those days are for lyophilization, producing the annual required amount of RTS,S antigen is very feasible, even assuming 300 days of operation per year.

As mentioned previously in the discussion of the adjuvant process train (Section D), 120 batches of adjuvant will be produced per year. Since each batch of adjuvant takes just over 50 hours to produce, manufacturing the annual required amount of the adjuvant is very feasible, even assuming 300 days of operation per year.

6. Financial Analysis

Capital Costs

Fixed capital costs were determined using several different resources including direct contact with manufacturers as well as correlations from literature as a method for checking (Anonymous, 2020; Green & Southard, 2019; Petrides, 2015; Shaeiwitz & Turton, 2017). Table H6-1 below details all of the equipment purchase costs for each unit operating and ancillary equipment.

Table H6-1: Capital Costs of Equipment with Installation Adjustment

	Equipment Tag	Purchase Price	Wroth Factor	Installed Cost
Upstream				
Main Equipment				
Sartorius Biostat 20/50	R103	$24,000	2.5	$63,000
Sartorius Biostat 1000	R104	$145,000	2.5	$380,625
Ancillaries				
Pre 50L Reactor Media/Substrate Pump	P101	$2,000	4	$8,400
Pre 1000L Reactor Culture Pump	P102	$2,000	4	$8,400
Pre 1000L Reactor Media/Substrate Pump	P103	$2,000	4	$8,400
Pre 50L Ammonium Hydroxide Pump	P104	$2,000	4	$8,400
Pre 1000L Ammonium Hydroxide Pump	P105	$2,000	4	$8,400
Pre 50L Reactor Media/Substrate Tank	TK501	$30,000	4	$126,000
Pre 50L Ammonium Hydroxide Tank	TK502	$30,000	4	$126,000
Downstream				
Main Equipment				
Sartorius Stedim kSep 6000S	CF201	$100,000	2	$210,000
DeBEE 2000-25-100 HPH	H202	$150,000	2.5	$393,750
Sartorius Stedim kSep 6000S	CF203	$100,000	2	$210,000
Millistak+® Pod Disposable Depth Filter	DF204	$42,000	2.8	$123,480
Aerosil Ad/Desorption Column	C205	$10,000	4	$42,000
Sartorius Filter Loop	F206	$90,000	2.8	$264,600
Sartorius Filter Loop	F207	$90,000	2.8	$264,600
AEX Column	C208	$10,000	4	$42,000
HIC Column	C209	$10,000	4	$42,000
Sartorius Filter Loop	F210	$90,000	2.8	$264,600
Model 2FS-12H	VF212	$370,000	1	$388,500
QuantaS Steam Sterilizable Freeze Dryer	L213	$213,000	1	$224,000
Ancillaries				
Pre First Centrifugation Protein Pump	P201	$7,000	4	$29,400
Pre HPH Protein Pump	P202	$2,000	4	$8,400
Pre Second Centrifugation Protein Pump	P203	$7,000	4	$29,400
Pre Depth Filtration Protein Pump	P204	$7,000	4	$29,400
Pre Silica Column Protein Pump	P205	$2,000	4	$8,400

	Equipment Tag	Purchase Price	Wroth Factor	Installed Cost
Pre Ultrafiltration 1 Protein Pump	P206	$2,000	4	$8,400
Pre Diafiltration 1 Protein Pump	P207	$2,000	4	$8,400
AEX Protein Pump - Load	P208	$2,000	4	$8,400
HIC Protein Pump - Load	P209	$1,000	4	$4,200
Pre Diafiltration 2 Protein Pump	P210	$2,000	4	$8,400
Pre Sterile Filtration Protein Pump	P211	$2,000	4	$8,400
Pre Vial Filling Protein Pump	P212	$2,000	4	$8,400
Pre Silica Column Buffer Pump	P601	$2,000	4	$8,400
Pre Diafiltration 1 Buffer Pump	P602	$2,000	4	$8,400
AEX Buffer Pump - Equilibrate	P603	$2,000	4	$8,400
AEX Buffer Pump - Wash	P604	$2,000	4	$8,400
AEX Buffer Pump - Elute	P605	$2,000	4	$8,400
AEX Buffer Pump - CIP	P6060	$2,000	4	$8,400
HIC Buffer Pump - CIP	P6061	$1,000	4	$4,200
HPH Buffer Pump - CIP	P6062	$2,000	4	$8,400
Silica Column Buffer Pump - CIP	P6063	$2,000	4	$8,400
HIC Buffer Pump - Equilibrate	P607	$1,000	4	$4,200
HIC Buffer Pump - Wash	P608	$1,000	4	$4,200
HIC Buffer Pump - Regen	P609	$1,000	4	$4,200
Diafiltration 2 WFI Pump	P610	$2,000	4	$8,400
Post AEX Dilution Pump	P611	$2,000	4	$8,400
Post First Centrifugation Protein Tank	TK201	$60,000	4	$252,000
Post HPH Protein Tank	TK202	$60,000	4	$252,000
Post Second Centrifugation Protein Tank	TK203	$30,000	4	$126,00
Post Depth Filtration Protein Tank	TK204	$30,000	4	$126,000
Post Silica Column Protein Tank	TK205	$30,000	4	$126,000
Post Ultrafiltration 1 Protein Tank	TK206	$15,000	4	$63,000
Post Diafiltration 1 Protein Tank	TK207	$15,000	4	$63,000
Post AEX Protein Tank	TK208	$15,000	4	$63,000
Post HIC Protein Tank	TK209	$60,000	4	$252,000
Post Diafiltration 2 Protein Tank	TK210	$60,000	4	$252,000
Post Sterile Filtration Protein Tank	TK211	$60,000	4	$252,000
Pre Silica Column Buffer Tank	TK601	$20,000	4	$84,000
Pre Diafiltration 1 Buffer Tank	TK602	$20,000	4	$84,000
AEX Buffer Tank - Equilibrate	TK603	$20,000	4	$84,000

	Equipment Tag	Purchase Price	Wroth Factor	Installed Cost
AEX Buffer Tank - Wash	TK604	$20,000	4	$84,000
AEX Buffer Tank - Elute	TK605	$20,000	4	$84,000
CIP Tank	TK606	$30,000	4	$126,000
HIC Buffer Tank - Equilibrate	TK607	$15,000	4	$63,000
HIC Buffer Tank - Wash	TK608	$15,000	4	$63,000
HIC Buffer Tank - Regen	TK609	$15,000	4	$63,000
Pre Diafiltration 2 WFI Tank	TK610	$60,000	4	$252,000
Adjuvant				
Main Equipment				
20 L Rotary Evaporator - Heidolph	RE302	$85,000	2.5	$223,125
M815 Microfluidizer - Microfluidics	H304	$272,000	2.5	$714,000
Ancillaries				
Pre Sterile Filtration Pump	P305	$2,000	4	$8,400
Pre Vial Filling Pump	P306	$2,000	4	$8,400
Pre Dissolution Ethanol Pump	P701	$2,000	4	$8,400
Pre Lipid Cake Hydration Phosphate Pump	P702	$2,000	4	$8,400
Post Microfluidization WFI/QS21 Pump	P703	$2,000	4	$8,400
WFI/QS21/Adjuvant Mixing Tank	TK304	$30,000	4	$126,000
Post Sterile Filtration Tank	TK305	$30,000	4	$126,000
Ethanol Tank	TK701	$20,000	4	$84,000
Pre Lipid Cake Hydration Phosphate Tank	TK702	$20,000	4	$84,000
Post Microfluidization WFI/QS21 Tank	TK703	$2,000	4	$8,400
Total Cost		**$2,715,000**		**$7,563,000**

In order to scale the purchase cost of the equipment to accurately reflect the cost of installing equipment and making necessary developments to support the plant, Wroth factors and multiplicative factors were used (Green, Southard, 2020). Wroth factors represent the cost of purchasing and fully installing specific unit equipment, while general multiplicative factors capture the supporting work to build a plant. Wroth factors are listed above in Table H6-3 with an additional 5% cushion. The result is a scaled cost of purchase and installation of all necessary

process equipment. Next, using multiplicative factors, the true cost of a working plant was

determined, as shown below in Table H6-2.

Table H6-2: Total Capital Costs Adjusted for Additional Multiplicative Factors

Category	Multiplicative Factor	Cost
Installed Equipment cost	1	$7,563,000
Piping	0.4	$3,025,000
Electrical/Instrumentation	0.35	$2,647,000
Buildings	0.45	$3,403,000
Yard Improvements	0.2	$1,513,000
Service Facilities	0.65	$4,916,000
Contingency	0.2	$1,513,000
Total Direct Costs	-	**$24,579,000**
Contracting	0.35	$8,603,000
Engineering	0.25	$6,145,000
Total fixed capital		**$39,326,000**

Operating costs

The operating costs of the process are comprised of the raw materials and single-use

items used to complete each batch, utility costs like electricity, waste disposal, and labor costs.

The most significant operating costs are disposable items, chemicals, and labor.

Table H6-3: Disposable Material and Equipment Costs

Materials	Annual Quantity	Annual Cost
Upstream		
Media & Substrate	N/A	$65,000
Reactors		
Conical flasks	14 flasks	$400
50/250L Fermenter bags	7 bags	$3,900
1000L Fermenter bags	7 bags	$7,700
Adjuvant		
Conical flasks	120 flasks	$8,800
Vials	28,000,000 vials	$1,400,000
Downstream		
Filter Cartridges		
DF	14 cartridges	$1,400
UF	7 cartridges	$700
Depth Filter	7 cartridges	$700
Column Resins		
Silica	13.3 kg	$100
AEX	7 L	$900
HIC	210 mL	$100
Vials	28,000,000 vials	$1,400,000
Total Disposables Costs		$2,890,000

The chemicals used for media, substrate, buffer, and CIP represent a significant operating cost. The most expensive disposable items are the vials for storing the final product, with flasks and reactor bags also representing an appreciable cost. Chemical costs were determined through speaking with EDM Millipore sales reps for bulk pricing information.

It was determined that the optimal number of operators per step was four, with a supervisor at each step. In this context, a "step" refers to a major process step such as a fermentation or filtration step and all of the ancillary equipment used for it. This allows for teamwork on tasks with assigned oversight. Operators will be paid $60,000 per year, with supervisors being paid $74,000 a year, based on mean salaries from the US Bureau of Labor Statistics (*Chemical Plant and System Operators*, n.d.). These costs, along with utilities, are summarized in Table H6-4 to reflect the annual operating costs of the plant.

Table H6-4: Overall Operating Costs

Category	Cost
Disposable Equipment	$ 2,846,000
Substrate/Media	$ 65,000
Adjuvant Materials	$ 6,134,000
Buffer/CIP Chemicals	$ 380,000
Electricity	$ 134,000
Water (WFI)	$ 22,000
Disposal Costs	$ 186,000
Labor costs	$ 7,726,000
Annual Operating Costs (COGs)	**$ 17,493,000**

One important aspect of running the process is that it specifies no recycling of any fluids, resins, or disposable equipment. All liquid and solid wastes are disposed of offsite with disposal service paid for. Because of the relatively low volume of the process, these costs are very manageable.

Financial Feasibility and Accounting

The final, functional facility will produce 14 million vials of adjuvant and 14 million vials of RTS,S antigen, sold in pairs for $5 per dose. This is expected to be fully sold annually, as it aims to serve only half of the population that could use it. The construction of the plant is expected to take one year, with full production activities in the second year and the nine years following. This means that after the second year, the capital costs of the plant will begin being recouped. In fact, the after-tax cash flow and profits exceed the initial investment, resulting in profitability after just one year of operation and an impressive return on investment (ROI), internal rate of return (IRR), and net present value (NPV), summarized by tables H6-5 through Table H6-7. The discount rate was set at 11% based on a risk-free rate of 1.58%, overall S&P

500 return of 8% and a beta value of 1.47 for the pharmaceutical industry. Depreciation was set

at 10-years with a straight line.

Table H6-5: Expected Income Statement for 10 Years of Operation

Year	0	1-9
Capital Investment	($39,326,000)	0
Revenue	-	$70,000,000
COGs	-	$17,493,000
Gross Profit	($39,326,000)	$52,507,000
Depreciation	-	$3,932,600
Taxes	-	$13,126,750
Net Cash Flow	**($39,326,000)**	**$35,447,650**
Net Profit	**($39,326,000)**	**$39,380,250**

Table H6-6 summarizes the cash flow over 10 years after the project is initiated, with 9

years of operation. Due to a fairly low initial investment and strong profit margin, the process is

highly profitable and offers attractive returns. The ROI for the process was calculated as the net

present value of the investment (NPV) divided by the initial cost of investment (C_i). NPV is

equal to the cumulative (summed) discounted cash flow 10 years after the initial investment is

made.

$$ROI = \frac{NPV}{C_i} \tag{H6-1}$$

$$Discounted\ Cash\ Flow = \frac{Annual\ Cash\ Flow}{(1+Discount\ Rate)^t} \tag{H6-2}$$

Where t represents the number of years since the initial investment.

The IRR of the investment was calculated using equation H6-3:

$$0 = NPV = \sum_{t=1}^{T} \frac{C_t}{(1+IRR)^t} - C_i \tag{H6-3}$$

Where C_t represents the net cash inflow from period t, and T represents the number of
periods.

Table H6-6: Discounted Cash Flow over 10 years of Operation

Year	Cash Flow	Discounted Cash flow	Cum. Discounted Cash Flow
0	($39,326,000)	($39,326,000)	($39,326,000)
1	$39,380,250	$35,477,703	($3,848,297)
2	$39,380,250	$31,961,894	$28,113,597
3	$39,380,250	$28,794,499	$56,908,096
4	$39,380,250	$25,940,990	$82,849,087
5	$39,380,250	$23,370,262	$106,219,349
6	$39,380,250	$21,054,290	$127,273,638
7	$39,380,250	$18,967,829	$146,241,467
8	$39,380,250	$17,088,134	$163,329,601
9	$39,380,250	$15,394,715	$178,724,316

Table H6-7: ROI, IRR, and NPV Summary

10 Year ROI	10 Year IRR	Net Present Value
711%	79%	$178,724,000
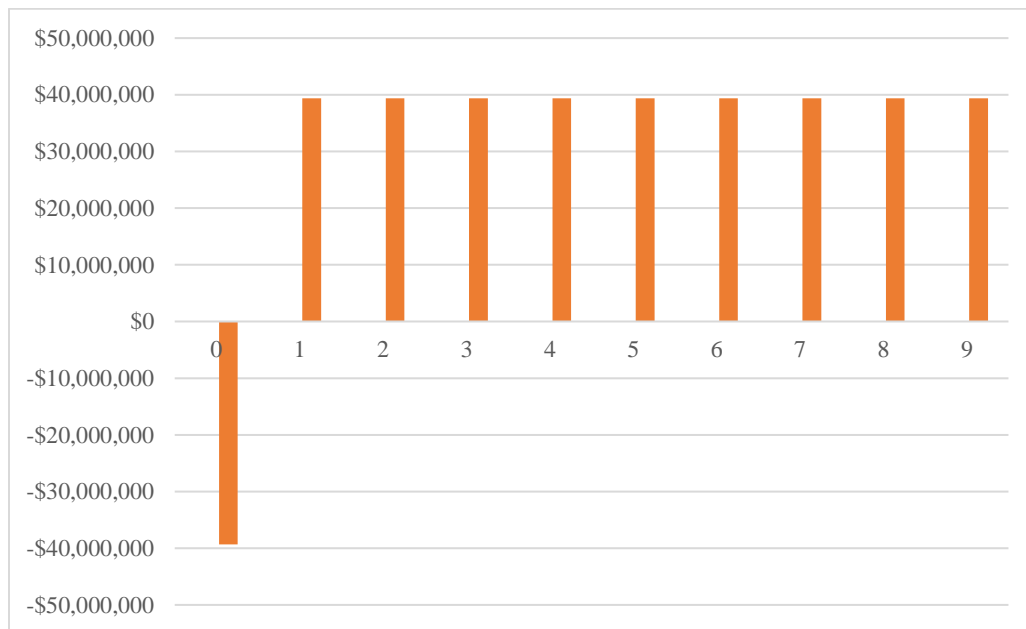
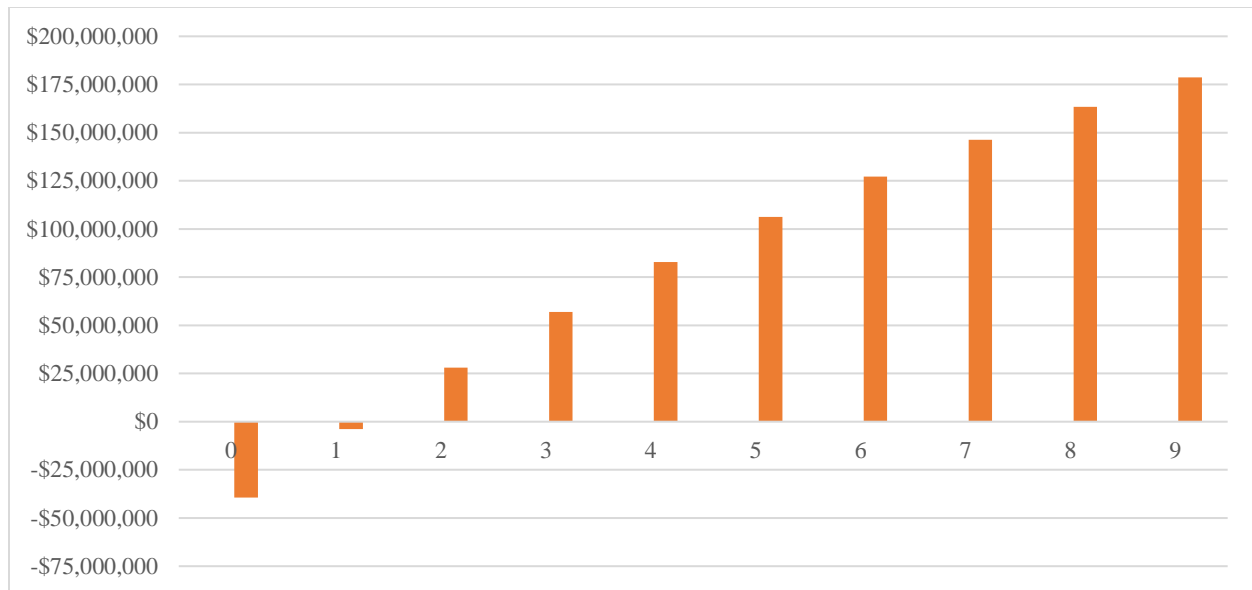*Figure H6-1*: Discrete Cash Flow Over 10 Years of Operation

Figure H6-2: Cumulative Cash Flow Over 10 Years of Operation

If, for whatever reason, only half of the product can be sold, the project will still be profitable. It is assumed that after 3 years of half-sales, the plant would adjust to only produce half of its designed capacity (14,000,000 down to 7,000,000 doses per year). While the process is still profitable with only half sales, COGs could be reduced by only producing half of the planned annual batches, improving profits and cash flow in years 4-9. In this scenario, returns would first be seen at the end of year 4 as opposed to the end of year 2. The overall returns can be seen in Table H6-8 with cumulative discounted cash flows shown in Figure H6-3.

Table H6-8: ROI, IRR, and NPV Summary

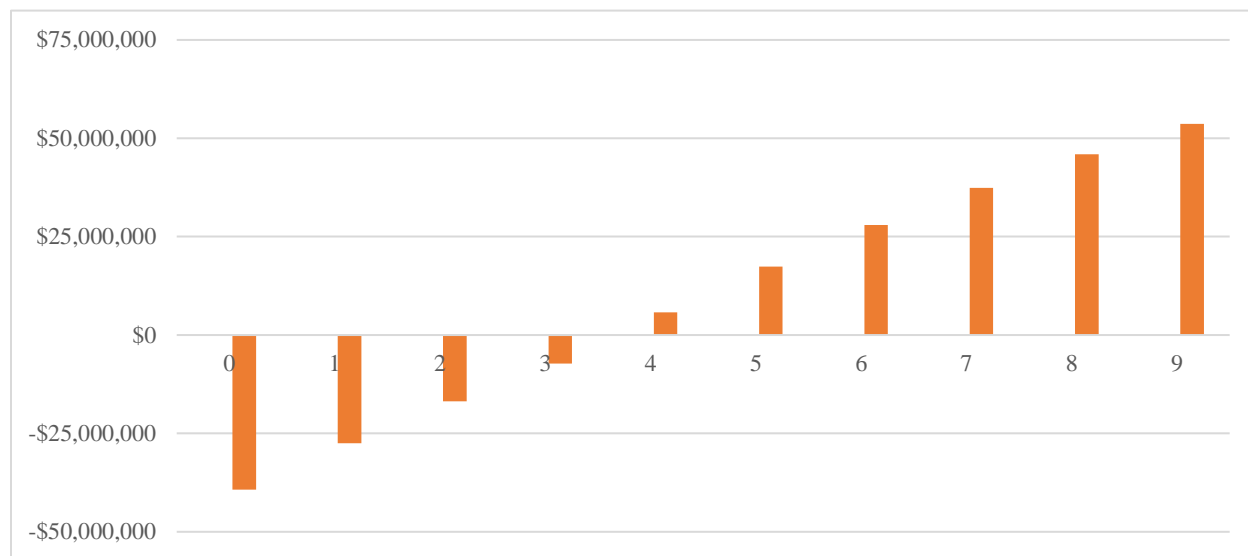| 10 Year ROI	10 Year IRR	Net Present Value
208%	12%	$53,669,000

Figure H6-3: Cumulative Cash Flow with Half Sales

While these returns are not as attractive as the full-sales scenario, they are still certainly

profitable. Additionally, they represent a low-end estimate of sales, given this process is

designed to serve just half of the major market for the RTS,S vaccine, so this estimate is serving

only a quarter of the market.

7.   Safety, Environmental, and Social Concerns

Safety in pharmaceutical manufacturing combines safe practices in a laboratory setting

with safe industrial practices. Through the use of personal protective equipment (PPE), including

safety glasses, gloves, and lab coats, employees can reduce risks to both themselves and the

product. Pharmaceutical manufacturing is especially strict in its sterility requirements because

the final products are administered to the human body via either parenteral or nonparenteral

routes. Sterility is ensured through the use of HEPA filters, additional PPE (for example:

additional pairs of gloves, shoe covers, face masks, coveralls), airlocks, pressure gradients,

quality control, and microbial plate readers. As much as possible, automation will be used to

limit operator exposure to yeast cells, media, and buffers. This will help maintain aseptic processing and will keep operators safe from the product and the product safe from operators.

Because some equipment used in the process can be dangerous (high operating pressures, high temperatures, rotating parts), operators will be thoroughly trained before being assigned responsibilities for independent operation of equipment. Operators will wear appropriate PPE in accordance with EMA regulations for classified spaces.

To ensure that the final product meets EMA purity specifications, Quality Assurance (QA) employees will test and approve product purity throughout the process. Most steps have a broad range of acceptable purities, since the final product is able to be sterile filtered. If, at any point in the process, a batch does not meet purity specifications, it will be discarded and a financial loss will be suffered.

Waste streams for the process primarily include cell debris, buffer solutions, and single-use plastic components. Liquid waste streams from centrifugation, filtration, and chromatography (buffers and small concentrations of cell debris) will be sent to a wastewater treatment facility located onsite. Since this process is to be added to an existing GSK manufacturing site, the wastewater treatment facility should already exist and be functional. The only concern is that the existing facility can manage the additional waste generated from the RTS,S production process, but such calculations are out of the scope of this project.

Polyethylene bags from upstream fermentation will be the largest source of plastic waste. This single-use plastic equipment will be outsourced and incinerated; the cost associated with this will be roughly estimated based on volume of waste generated and distance to nearest incineration facility. Incineration is a widely accepted alternative to landfill disposal, especially when considering the bags' contact with toxic chemicals which could leach into groundwater.

Although incineration leads to a release of pollutants, a benefit is that the heat generated can boil water into steam. The steam can then be used in other parts of the manufacturing plant for heating or electricity generation.

There are numerous social and ethical problems related to the pharmaceutical industry. In the present day, there is a stigma toward the pharmaceutical industry as a result of drug pricing. For this reason, one of the primary aims of this project is to produce the RTS,S antigen at the *lowest possible cost per dose*. With the help of the Bill and Melinda Gates Foundation, the cost of the vaccine may be reduced even further. We suspect that by incorporating single-use equipment into the manufacturing process, operating costs as well as chemical costs can be decreased. With fewer costs associated with the process, the market value can be decreased and still maintain a stable profit margin.

Although incredibly unlikely, there can be severe side effects to vaccinations. However, there are fewer adverse effects to intramuscular vaccines, like Mosquirix, than there are for intravenous vaccines. Regardless, the manufacturing process is designed to achieve the highest possible purity to limit adverse effects.

I.   Conclusions and Recommendations

The technical research and design of the plant production capacity demonstrated that 392 g of purified RTS,S antigen can be produced per year. This exceeds the initial goal of 350 g per year. The overall yield for all unit operations is 28.8%. From the production capacity and vaccine administration schedule, the plan will be able to support 3.5 million people per year.

Economic analysis has indicated that the project is profitable, and thus the construction of the facility should move forward. The internal rate of return is 79% and the net present value is $178,724,000. Assuming the $70,000,000 in revenue and $17,493,000 cost of goods sold can be consistently met, this process is highly profitable. The internal rate of return suggests that the initial investment would be recouped after just two years of operation, with an impressive ROI after nine years of operation. Therefore, further pursuit of this Mosquirix vaccine manufacturing plant is highly recommended.

There are various areas of improvement or exploration for future designs involving the RTS,S antigen. It is possible that after running the fermentation for several batches, tweaks in the fermentation process (specifically the timing of substrate and media feeds) could lead to faster fermentation and therefore lowered costs. Additionally, vial filling is projected to take up significant process time, but it is likely that this time could be reduced after running many batches, as often occurs as a pharmaceutical process is completed many times.

Furthermore, design alternatives could also be considered. Data regarding unit operations and yields for isolating the RTS,S antigen is rather limited at this point. With more experimental values, aspects of the process train that are inefficient or result in significant loss of the desirable product can be re-examined, specifically alternatives for the silica adsorption/desorption column.

Additionally, the AEX and HIC columns are small enough where replacing the resin rather than performing CIP/SIP could be an option, and thus an economic analysis could be explored.

      This RTS,S production facility is intended to be an addition to an existing GSK manufacturing plant in Belgium. This location is optimal due to its proximity to regions with high malaria incidence (i.e. Africa and southern Asia), which helps to lower distribution costs. Although other GSK plants exist that may be closer to the relevant regions of sub-Saharan Africa, the Belgium plant is the global headquarters for the vaccines division of GSK. As a larger and more developed GSK plant, the Belgium site seems the most capable of undertaking this project.

J.   Acknowledgements

K.   Table of Nomenclature

Symbol	Meaning	Units
μ	Cell growth rate	h^{-1}
μ_{max}	Maximum cell growth rate	h^{-1}
S	Substrate concentration	g/L
K_s	Substrate affinity constant	g/L
dX/dt	Derivative of cell concentration with respect to time	g/L·h
X	Cell concentration	g/L
dS/dt	Derivative of substrate concentration with respect to time	g/L·h
$Y_{X/S}$	Biomass yield	g biomass / g substrate
dV/dt	Derivative of volume with respect to time	L/h
F	Media feed flow rate	L/h
$k_L a$	Volumetric mass transfer coefficient	s^{-1} or h^{-1}
Q_{O2}	Oxygen uptake rate	g O_2 / L·h
C_{O2}^*	Oxygen solubility in media	g O_2 / L
$C_{O2, \, crit}$	Critical dissolved oxygen	g O_2 / L
Q_g	Gas flow rate	m^3/s
AR	Aeration rate	vvm
V	Tank volume	m^3
Re	Reynold's number	unitless
N	Impeller speed	rpm
D_i	Impeller diameter	m
ρ	Media density	kg/m^3
μ	Media viscosity	kg/m·s
N_a	Aeration number	unitless
N_p	Power number	unitless
n_i	Number of impellers	unitless
P_g	Gassed power	W
P_p	Pump Power	W
D_t	Tank diameter	m
v_s	Superficial velocity	m/s
v_g	Terminal Settling Velocity	m/s
r_p	Cell Radius	m

Symbol	Meaning	Units
ρ	Density of Particle	kg/m^3
f	Density of Fluid	kg/m^3
g	Gravitational Acceleration	m/s^2
η	Viscosity	Pa·s
Σ_T	Sigma Factor	m^2
Q	Centrifugation Feed Flow Rate	m^3/s
ω	Rotational Speed	rev/s
R_o	Outer Radius	m
R_i	Inner Radius	m
L	Centrifuge Length	m
C_o	Initial Concentration	g/L
C	Final Concentration	g/L
CF	Concentration Factor	unitless
σ	Protein Rejection Coefficient	unitless
V_o	Initial Volume	L
t	Process Time	h
A	Membrane Area	m^2
J_{avg}	Average Flux	$L/(m^2 \cdot h)$
P_{avg}	Productivity	$g/(L \cdot min)$
E	Recovery Yield Fraction	unitless
DBC	Dynamic Binding Capacity	g/L
t_{cycle}	Cycle Time	min
CV	Column Volume	L
t_{equil}	Equilibration Time	min
t_{load}	Load Time	min
t_{wash}	Washing Time	min
$t_{elution}$	Elution Time	min
t_{CIP}	CIP Time	min
L/u	Residence Time	min
C_f	Feed Concentration	g/L
L	Column Length	m
u	Linear Flow Rate	m/s
ΔP	Pressure Drop	Pa

Symbol	Meaning	Units
d_p	Particle Diameter	m
ε	Extra-particle Porosity	unitless
d_c	Column Diameter	m
V_f	Feed Volume	m^3
C_t	Cash Flow in Period t	$
C_i	Initial Capital Investment	$
T	Total Periods	years
t	Period of focus	years

L.   References

Allen, J., Cantin, G., & Haverstock, R. (2015). *Process for purifying recombinant Plasmodium falciparum circumsporozoite protein* (United States Patent No. US9169304B2). https://patents.google.com/patent/US9169304B2/en?oq=WO+93%2f10152

Anonymous. (2020). Economic Indicators. *Chemical Engineering; New York*, *127*(2), 56.

Broadhurst, G. (n.d.). *Principles and Practice of Cleaning in Place*. 41.

Buyel, J. F., Gruchow, H. M., & Fischer, R. (2015). Depth Filters Containing Diatomite Achieve More Efficient Particle Retention than Filters Solely Containing Cellulose Fibers. *Frontiers in Plant Science*, *6*. https://doi.org/10.3389/fpls.2015.01134

Chemical Plant and System Operators. (n.d.). Retrieved March 21, 2020, from https://www.bls.gov/oes/2017/may/oes518091.htm

Chungcharoen, T., Netjaibun, K., Pratabkong, T., Suwannasam, P., & Limmun, W. (2017). Effects of inner angle of bowl, flow rate and speed on the efficiency of glycerol separation from the raw biodiesel using cylindrical bowl centrifuge. *Energy Procedia*, *138*, 405–410. https://doi.org/10.1016/j.egypro.2017.10.184

Cohen, J., Nussenzweig, V., Vekemans, J., & Leach, A. (2010). From the circumsporozoite protein to the RTS,S/AS candidate vaccine. *Human Vaccines*, *6*(1), 90–96. https://doi.org/10.4161/hv.6.1.9677

Collins, K. A., Snaith, R., Cottingham, M. G., Gilbert, S. C., & Hill, A. V. S. (2017). Enhancing protective immunity to malaria with a highly immunogenic virus-like particle vaccine. *Scientific Reports*, *7*. https://doi.org/10.1038/srep46621

De Wilde, M., Cohen, J., Cohen, J., & BE. (2001). *United States Patent: 6169171 - Hybrid protein between CS from plasmodium and HBSAG* (Patent No. 6169171).

http://patft.uspto.gov/netacgi/nph-

Parser?Sect1=PTO2&Sect2=HITOFF&p=1&u=%2Fnetahtml%2FPTO%2Fsearch-

bool.html&r=4&f=G&l=50&co1=AND&d=PTXT&s1=6%2C169%2C171&OS=6%2C1

69%2C171&RS=6%2C169%2C171

Dissolved Oxygen. (n.d.). Retrieved March 18, 2020, from

http://www.fao.org/3/AC183E/AC183E04.htm

Doran, P. (2012). *Bioprocess Engineering Principles—2nd Edition*. Academic Press.

https://www.elsevier.com/books/bioprocess-engineering-principles/doran/978-0-08-

091770-2

Engineering Toolbox. (2017). *Density of aqueous solutions of organic substances as sugars and*

alcohols. https://www.engineeringtoolbox.com/density-aqueous-solution-organic-sugar-

alcohol-concentration-d_1954.html

European Medicines Agency. (2015, July 23). *Mosquirix*.

https://www.ema.europa.eu/en/documents/medicine-outside-eu/mosquirix-public-

assessment-report_en.pdf

Foquet, L., Hermsen, C. C., van Gemert, G.-J., Van Braeckel, E., Weening, K. E., Sauerwein, R.,

Meuleman, P., & Leroux-Roels, G. (2014). Vaccine-induced monoclonal antibodies

targeting circumsporozoite protein prevent Plasmodium falciparum infection. *The*

Journal of Clinical Investigation, *124*(1), 140–144. https://doi.org/10.1172/JCI70349

GlaxoSmithKline. (2016, February). *Fact Sheet: RTS,S malaria vaccine candidate (Mosquirix)*.

https://www.malariavaccine.org/files/content/page/files/RTSS%20vaccine%20candidate

%20Factsheet_FINAL.pdf

Global Pumps. (n.d.). *List of typical viscosities*. Retrieved March 20, 2020, from

    https://www.globalpumps.com.au/list-of-typical-viscosities

Green, D. D. W., & Southard, D. M. Z. (2019). *Perry's Chemical Engineers' Handbook, 9th*

    Edition. McGraw-Hill Education: New York, Chicago, San Francisco, Athens, London,

    Madrid, Mexico City, Milan, New Delhi, Singapore, Sydney, Toronto.

    /content/book/9780071834087

Hagman, A., Säll, T., & Piškur, J. (2014). Analysis of the yeast short-term Crabtree effect and its

    origin. *The FEBS Journal*, *281*(21), 4805–4814. https://doi.org/10.1111/febs.13019

How Long Does it Take to Fill a Reverse Osmosis Storage Tank? (n.d.).

    ESPWaterProducts.Com. Retrieved April 16, 2020, from

    https://www.espwaterproducts.com/blog/how-long-does-it-take-to-fill-a-reverse-osmosis-

    storage-tank/

Kovárová-Kovar, K., & Egli, T. (1998). Growth kinetics of suspended microbial cells: From

    single-substrate-controlled growth to mixed-substrate kinetics. *Microbiology and*

    Molecular Biology Reviews, *62*(3), 646–666.

Langley, K. E., Bitter, G. A., Sachdev, R. K., & Fieschko, J. C. (1993). *A hepatitis b vaccine*

    formulation incorporating a bile acid salt (World Intellectual Property Organization

    Patent No. WO1993005811A1). https://patents.google.com/patent/WO1993005811A1/en

Liposome Preparation—Avanti® Polar Lipids. (n.d.). Sigma-Aldrich. Retrieved March 18, 2020,

    from https://www.sigmaaldrich.com/technical-documents/articles/biology/liposome-

    preparation.html

Margreiter, G., Messner, P., Caldwell, K. D., & Bayer, K. (2008). Size characterization of

inclusion bodies by sedimentation field-flow fractionation. *Journal of Biotechnology*,

138(0), 67–73. https://doi.org/10.1016/j.jbiotec.2008.07.1995

Mattile, J., & Parkka, M. (n.d.). *Design Considerations for WFI Distillation Systems Part 1*.

ISPE | International Society for Pharmaceutical Engineering. Retrieved December 10,

2019, from https://ispe.org/pharmaceutical-engineering/ispeak/design-considerations-wfi-

distillation-systems-part-1

Millipore, E. (2009). *Millipore Millistak+ Pod Disposable Depth Filtration System Data Sheet*.

https://beta-

static.fishersci.com/content/dam/fishersci/en_US/documents/programs/scientific/technica

l-documents/data-sheets/emd-millipore-millistak-pod-disposable-data-sheet.pdf

Moreira, V. S. F. D., Alhadeff, E. M., Gimenes, M. A. P., & Pereira, N. (2014). *Evaluation of the*

agitation effective power in aerated and non-aerated systems for yeast and filamentous

fungus suspensions. https://www.semanticscholar.org/paper/Evaluation-of-the-Agitation-

Effective-Power-in-and-Moreira-

Alhadeff/3f859b1c41d25299de4c977382cd9d4a28733206/figure/7

Ness, T. E. (2016). *Should the malaria vaccine (Mosquirix) be implemented as a routine*

immunization for children in Nigeria? 6.

Papagianni, M., Boonpooh, Y., Mattey, M., & Kristiansen, B. (2007). Substrate inhibition

kinetics of Saccharomyces cerevisiae in fed-batch cultures operated at constant glucose

and maltose concentration levels. *Journal of Industrial Microbiology & Biotechnology*,

34(4), 301–309. https://doi.org/10.1007/s10295-006-0198-9

Patel, N. (2018, November 21). Figure of the week: Understanding poverty in Africa. *Brookings*.

    https://www.brookings.edu/blog/africa-in-focus/2018/11/21/figure-of-the-week-

    understanding-poverty-in-africa/

Petrides, D. (2015). *BioProcess Design and Economics, 2nd Edition*.

    https://www.researchgate.net/publication/306253307_BioProcess_Design_and_Economi

    cs_2nd_Edition

Prpich, G. (2019). *Oxygen Transfer, Lecture 17*.

Rolfe, M. D., Rice, C. J., Lucchini, S., Pin, C., Thompson, A., Cameron, A. D. S., Alston, M.,

    Stringer, M. F., Betts, R. P., Baranyi, J., Peck, M. W., & Hinton, J. C. D. (2012). Lag

    phase is a distinct growth phase that prepares bacteria for exponential growth and

    involves transient metal accumulation. *Journal of Bacteriology*, *194*(3), 686–701.

    https://doi.org/10.1128/JB.06112-11

Sartorius Stedim. (2018). *BIOSTAT STR®—Stirred Tank Single-Use Bioreactor*. Sartorius.

    https://www.sartorius.com/en/products/fermentation-bioreactors/single-use-

    bioreactors/biostat-str-flexsafe-str

Sartorius Stedim. (2019). *KSep® 6000S System | Sartorius*.

    https://www.sartorius.com/shop/us/en/usd/bioprocess-products-and-services-filtration-

    and-purification-technologies-harvesting-centrifugation-systems/ksep%C2%AE-6000s-

    system/p/KSEP6000S

Shaeiwitz, J. A., & Turton, R. (2017). *Chemical Process Equipment Design*. Prentice Hall.

Spiden, E. M., Scales, P. J., Kentish, S. E., & Martin, G. J. O. (2013). Critical analysis of

    quantitative indicators of cell disruption applied to Saccharomyces cerevisiae processed

with an industrial high pressure homogenizer. *Biochemical Engineering Journal*, *70*,

120–126. https://doi.org/10.1016/j.bej.2012.10.008

Tosoh Bioscience. (2015a). *Ion Exchange Chromatography*.

https://www.separations.asia.tosohbioscience.com/File%20Library/TBJS/Lit_EN/Catalog

/Medias/Cat2_IEX.pdf

Tosoh Bioscience. (2015b). *TOYOPEARL Butyl-650*.

https://www.separations.eu.tosohbioscience.com/solutions/process-media-products/by-

mode/hic/toyopearl-butyl-650

Vieira, É. D., da Graça Stupiello Andrietta, M., & Andrietta, S. R. (2013). Yeast biomass

production: A new approach in glucose-limited feeding strategy. *Brazilian Journal of*

Microbiology, *44*(2), 551–558. https://doi.org/10.1590/S1517-83822013000200035

World Health Organization. (2018). *Malaria*. WHO; World Health Organization.

https://www.who.int/ith/diseases/malaria/en/

World Health Organization. (2019). *Fact sheet about Malaria*. https://www.who.int/news-

room/fact-sheets/detail/malaria

IV.   Appendix

A.   Sample Calculations

1.   50 L Fermentation

50 L Reactor Target Mass Transfer Coefficient

$$k_L a = \frac{(Q_{O2}X)_{max}}{(C_{O2}^* - C_{O2,crit})} \tag{C3-2}$$

$$k_L a = \frac{\left(0.112 \ h^{-1} \cdot 45.67 \frac{g}{L}\right)}{\left(0.0075 \frac{g \ O_2}{L} - 0.00015 \frac{g \ O_2}{L}\right)}$$

$$k_L a = 659.9 \ h^{-1}$$

Air Flow Rate

$$Q_g = \frac{AR \cdot V}{60} \tag{C3-3}$$

$$Q_g = \frac{3 \ vvm \ (air) \cdot 0.05 \ m^3}{60 \frac{s}{min}}$$

$$Q_g = 0.0025 \frac{m^3}{s}$$

Reynold's Number

$$Re = \frac{N}{60} \frac{D_i^2 \rho}{\mu} \tag{C3-4}$$

$$Re = \left(\frac{240 \ rpm}{60 \frac{s}{min}}\right) \cdot \left(\frac{(0.143 \ m)^2 \cdot 1150 \frac{kg}{m^3}}{0.18 \frac{kg}{m \cdot s}}\right)$$

$$Re = 522.69$$

Aeration Number

$$N_a = \frac{Q_g}{\left(\frac{N}{60}\right)D_i^3} \tag{C3-5}$$

$$N_a = \frac{0.0025 \frac{m^3}{s}}{\left(\frac{240 \ rpm}{60 \frac{s}{min}}\right) \cdot (0.143 \ m)^3}$$

$$N_a = 0.214$$

Gassed Power Requirement

$$P_g = \left(\frac{P_g}{P}\right) n_i N_p \rho \left(\frac{N}{60}\right)^3 D_i^5 \tag{C3-6}$$

$$P_g = 0.6 \cdot 2 \cdot 3.5 \cdot 1150 \ \frac{kg}{m^3} \cdot \left(\frac{240 \ rpm}{60 \frac{s}{min}}\right)^3 \cdot (0.143 \ m)^5$$

$$P_g = 18.487 \ W$$

Experimental Mass Transfer Coefficient

$$k_L a = \frac{0.0333}{D_t^4} \left(\frac{P_g}{V}\right)^{0.541} Q_g^{\frac{0.541}{\sqrt{D_t}}} \tag{C3-7}$$

$$k_L a = \frac{0.0333}{(0.37 \ m)^4} \cdot \left(\frac{18.487 \ W}{0.05 \ m^3}\right)^{0.541} \cdot \left(0.0025 \ \frac{m^3}{s}\right)^{\frac{0.541}{\sqrt{0.37 \ m}}}$$

$$k_L a = 0.211 \ s^{-1} = 760.16 \ h^{-1}$$

Gas Flooding Check

$$Q_{g,max} = 0.6 \cdot \frac{D_i^5 \left(\frac{N}{60}\right)^2}{D_t^{1.5}} \tag{C3-8}$$

$$Q_{g,max} = 0.6 \cdot \frac{(0.143 \ m)^5 \cdot \left(\frac{240 \ rpm}{60 \frac{s}{min}}\right)^2}{(0.37 \ m)^{1.5}}$$

$$Q_{g,max} = 0.002551 \ \frac{m^3}{s}$$

$$\rightarrow Q_g \le Q_{g,max}$$

$$\rightarrow 0.0025 \ \frac{m^3}{s} < 0.002551 \ \frac{m^3}{s} \quad \checkmark$$

Slugging Check

$$v_s = \frac{Q_g}{\frac{\pi D_t^2}{4}} \tag{C3-9}$$

$$v_s = \frac{0.0025 \frac{m^3}{s}}{\pi \cdot \frac{(0.37\ m)^2}{4}}$$

$$v_s = 0.0233 \frac{m}{s}$$

$$\rightarrow 0.0233 \frac{m}{s} < 2.083 \frac{m}{s} \ \checkmark$$

2.   1000 L Fermentation

1000 L Reactor Target Mass Transfer Coefficient

$$k_L a = \frac{(Q_{O2}X)_{max}}{(C_{O2}^* - C_{O2,crit})} \tag{C3-2}$$

$$k_L a = \frac{\left(0.112\ h^{-1} \cdot 39.3 \frac{g}{L}\right)}{\left(0.0075 \frac{g\ O_2}{L} - 0.00015 \frac{g\ O_2}{L}\right)}$$

$$k_L a = 603.0\ h^{-1}$$

Air Flow Rate

$$Q_g = \frac{AR \cdot V}{60} \tag{C3-3}$$

$$Q_g = \frac{2.6\ vvm\ (O_2) \cdot 1\ m^3}{60 \frac{s}{min}}$$

$$Q_g = 0.0433 \frac{m^3}{s}$$

Reynold's Number

$$Re = \frac{N}{60} \frac{D_i^2 \rho}{\mu} \tag{C3-4}$$

$$Re = \left(\frac{90\ rpm}{60 \frac{s}{min}}\right) \cdot \left(\frac{(0.38\ m)^2 \cdot 1150 \frac{kg}{m^3}}{0.18 \frac{kg}{m \cdot s}}\right)$$

$$Re = 1383.83$$

Aeration Number

$$N_a = \frac{Q_g}{\left(\frac{N}{60}\right) D_i^3} \tag{C3-5}$$

$$N_a = \frac{0.0433 \frac{m^3}{s}}{\left(\frac{90 \, rpm}{60 \frac{s}{min}}\right) \cdot (0.38 \, m)^3}$$

$$N_a = 0.526$$

Gassed Power Requirement

$$P_g = \left(\frac{P_g}{P}\right) n_i N_p \rho \left(\frac{N}{60}\right)^3 D_i^5 \tag{C3-6}$$

$$P_g = 0.6 \cdot 2 \cdot 2.5 \cdot 1150 \frac{kg}{m^3} \cdot \left(\frac{90 \, rpm}{60 \frac{s}{min}}\right)^3 \cdot (0.38 \, m)^5$$

$$P_g = 92.274 \, W$$

Experimental Mass Transfer Coefficient

$$k_L a = \frac{0.0333}{D_t^4} \left(\frac{P_g}{V}\right)^{0.541} Q_g^{\frac{0.541}{\sqrt{D_t}}} \tag{C3-7}$$

$$k_L a = \frac{0.0333}{(0.997 \, m)^4} \cdot \left(\frac{92.274 \, W}{1 \, m^3}\right)^{0.541} \cdot \left(0.0433 \frac{m^3}{s}\right)^{\frac{0.541}{\sqrt{0.997 \, m}}}$$

$$k_L a = 0.171 \, s^{-1} = 612.63 \, h^{-1}$$

Gas Flooding Check

$$Q_{g,max} = 0.6 \cdot \frac{D_i^5 \left(\frac{N}{60}\right)^2}{D_t^{1.5}} \tag{C3-8}$$

$$Q_{g,max} = 0.6 \cdot \frac{(0.38 \, m)^5 \cdot \left(\frac{90 \, rpm}{60 \frac{s}{min}}\right)^2}{(0.997 \, m)^{1.5}}$$

$$Q_{g,max} = 0.011 \frac{m^3}{s}$$

$$\rightarrow Q_g \leq Q_{g,max}$$

$$\rightarrow 0.0433 \frac{m^3}{s} < 0.011 \frac{m^3}{s}$$

Slugging Check

$$v_s = \frac{Q_g}{\frac{\pi D_t^2}{4}} \tag{C3-9}$$

$$v_s = \frac{0.0433 \frac{m^3}{s}}{\pi \cdot \frac{(0.997 \ m)^2}{4}}$$

$$v_s = 0.278 \frac{m}{s}$$

$$\rightarrow 0.278 \frac{m}{s} < 2.083 \frac{m}{s} \ \checkmark$$

3.   Centrifugation

Terminal Velocity of Yeast Cells

$$v_g = \frac{4r_p^2(\rho_p - \rho_f)g}{18\eta} \tag{C5-1}$$

$$v_g = \frac{4(2.5 \cdot 10^{-6} \ m)^2 \left(1100 \frac{kg}{m^3} - 1000 \frac{kg}{m^3}\right)(9.8 \frac{m}{s^2})}{18(0.001 \ Pa \cdot s)}$$

$$v_g = 1.36 \cdot 10^{-6} \ m/s$$

Rotational Speed

$$\omega = \sqrt{\frac{\Sigma_T \cdot g \cdot \ln\left(\frac{R_o}{R_i}\right)}{\pi(R_o^2 - R_i^2)L}} \tag{C5-4}$$

$$\omega = \sqrt{\frac{(20.41 \ m^2) \cdot (9.8 \frac{m}{s^2}) \cdot \ln\left(\frac{0.1208 \ m}{0.0508 \ m}\right)}{\pi((0.1208m \ )^2 - (0.0508m \ )^2) \cdot (0.19 \ m)}}$$

$$\omega = 155 \frac{rad}{s} = 1484 \ RPM$$

4.   Ultrafiltration 1

Concentration Factor

$$\frac{C}{C_0} = (CF)^\sigma \tag{C10-1}$$

$$\frac{19.8 \ g/L}{2.77 \ g/L} = (CF)^{0.95}$$

$$CF = 7.93$$

Retentate Volume

$$CF = \frac{V_0}{V} \tag{C10-2}$$

$$7.93 = \frac{47.4 \ L}{V}$$

$$V = 5.98 \ L$$

Process Time

$$t = \frac{V_0 - V}{A \cdot J_{avg}} \tag{C10-3}$$

$$t = \frac{47.4 \ L - 5.98 \ L}{(1.4 \ m^2) \cdot (64 \frac{L}{m^2 h})}$$

$$t = 0.46 \ h$$

5.   Diafiltration 1

Total Buffer Added

$$\left(\frac{C}{C_0}\right)_{buffer} = \exp\left(\left(\frac{-V_W}{V_0}\right)(1 - \sigma_{buffer})\right) \tag{C11-1}$$

$$(0.95) = \exp\left(\left(\frac{-V_W}{5.98 \ L}\right)(1 - 0)\right)$$

$$V_W = 17.9 \ L$$

Process Time

$$t = \frac{V_W}{A \cdot J_{avg}} \tag{C11-2}$$

$$t = \frac{17.9 \ L}{(1.4 \ m^2) \cdot (64 \frac{L}{m^2 h})}$$

$$t = 0.20 \ h$$

6.   Anion Exchange Chromatography

Equilibration Time

$$t_{equil} = CV_{equil} \cdot L/u \tag{C12-3}$$

$$t_{equil} = (2\ CV) \cdot (2.0\ \text{min})$$

$$t_{equil} = 4\ min$$

Loading Time

$$t_{load} = \frac{DBC}{C_F} \cdot L/u \tag{C12-4}$$

$$t_{load} = \frac{20\frac{g}{L}}{19.4\frac{g}{L}} \cdot (2.0\ \text{min})$$

$$t_{load} = 2.06\ min$$

Washing Time

$$t_{wash} = CV_{wash} \cdot L/u \tag{C12-5}$$

$$t_{wash} = (5\ CV) \cdot (2.0\ \text{min})$$

$$t_{wash} = 10\ min$$

Elution Time

$$t_{elution} = CV_{elution} \cdot L/u \tag{C12-6}$$

$$t_{elution} = (5\ CV) \cdot (2.0\ \text{min})$$

$$t_{elution} = 10\ min$$

Productivity

$$P_r = \frac{\eta_E \cdot DBC}{t_{cycle}} \tag{C12-1}$$

$$P_r = \frac{(0.60) \cdot (20\frac{g}{L})}{71.1\ min}$$

$$P_r = 0.17 \frac{g}{L \ min}$$

Karmen-Cozeny Product

$$L \cdot u = \Delta P \cdot \frac{d_p^2 \cdot \varepsilon^3}{150(1-\varepsilon)^2 \cdot \eta} \qquad \text{(C12-7)}$$

$$L \cdot u = (100{,}000 \ Pa) \cdot \frac{(6.5 \cdot 10^{-5} \ m)^2 \cdot (0.35)^3}{150(1 - 0.35)^2 \cdot (0.00106 \ Pa \cdot s)}$$

$$L \cdot u = 2.68 \cdot 10^{-4} \frac{m^2}{s} = 160.9 \frac{cm^2}{min}$$

Column Length

$$L = \sqrt{(L \cdot u) \cdot (L/u)} \qquad \text{(C12-8)}$$

$$L = \sqrt{(160.9 \ \frac{cm^2}{min}) \cdot (2.0 \ min)}$$

$$L = 2.32 \ cm$$

Linear Velocity

$$u = \frac{L \cdot u}{L} \qquad \text{(C12-9)}$$

$$u = \frac{160.9 \ \frac{cm^2}{min}}{2.32 \ cm}$$

$$u = 69.5 \frac{cm}{min} = 0.695 \frac{m}{min}$$

Column Diameter

$$d_c = \sqrt{\frac{4 \cdot V_f}{\pi \cdot u \cdot t_{load}}} \qquad \text{(C12-10)}$$

$$d_c = \sqrt{\frac{4 \cdot (0.00598 \ m^3)}{\pi \cdot (0.695 \ \frac{m}{min}) \cdot (2.06 \ min)}}$$

$$d_c = 0.0729 \ m = 7.29 \ cm$$

Column Volume

$$CV = \pi(\frac{d_c}{2})^2 \cdot L \tag{C12-11}$$

$$CV = \pi(\frac{0.0729\ m}{2})^2 \cdot 0.0232\ m$$

$$CV = 9.68 \cdot 10^{-5}\ m^3 = 96.8\ mL$$

7. Return on Investment

$$ROI = \frac{NPV}{C_i} \tag{H6-1}$$

$$ROI = \frac{\$53,052,181}{\$39,326,000} = 208\%$$

8. Discounted Cash Flow

$$DCF = \frac{Annual\ Cash\ Flow}{(1+Discount\ Rate)^t} \tag{H6-2}$$

$$DCF = \frac{\$12,975,750}{(1+.11)^1} = \ \$11,689,865$$