# SCALE UP STUDY AND COMPARISON OF THREE DOWNSTREAM PROCESSES OF MONOCLONAL ANTIBODY PRODUCTION USING SUPERPRO DESIGNER

KAMARULNIZAL BIN AMRIN

UNIVERSITI MALAYSIA PAHANG

# UNIVERSITI MALAYSIA PAHANG

## BORANG PENGESAHAN STATUS TESIS♦

**JUDUL : SCALE UP STUDY AND COMPARISON OF THREE DOWNSTREAM PROCESSES OF MONOCLONAL ANTIBODY PRODUCTION USING SUPERPRO DESIGNER**

**SESI PENGAJIAN : 2009/2010**

Saya **KAMARULNIZAL BIN AMRIN**

**(HURUF BESAR)**

mengaku membenarkan tesis (PSM/~~Sarjana~~/~~Doktor Falsafah~~)* ini disimpan di Perpustakaan Universiti Malaysia Pahang dengan syarat-syarat kegunaan seperti berikut :

1. Tesis adalah hakmilik Universiti Malaysia Pahang
2. Perpustakaan Universiti Malaysia Pahang dibenarkan membuat salinan untuk tujuan pengajian sahaja.
3. Perpustakaan dibenarkan membuat salinan tesis ini sebagai bahan pertukaran antara institusi pengajian tinggi.
4. **Sila tandakan ( √ )

| | | |
|---|---|---|
| ☐ | SULIT | (Mengandungi maklumat yang berdarjah keselamatan atau kepentingan Malaysia seperti yang termaktub di dalam AKTA RAHSIA RASMI 1972) |
| ☐ | TERHAD | (Mengandungi maklumat TERHAD yang telah ditentukan oleh organisasi/badan di mana penyelidikan dijalankan) |
| ☑ | TIDAK TERHAD | |

Disahkan oleh

(TANDATANGAN PENULIS) (TANDATANGAN PENYELIA)

Alamat Tetap: **SEK. KEB. BATU 16 GUM-GUM, BEG BERKUNCI 05, 90500 SANDAKAN, SABAH.**

**PN. NURUL AINI BINTI MOHD AZMAN**

Nama Penyelia

Tarikh : **30 APRIL 2010** Tarikh: **30 APRIL 2010**

CATATAN :
- * Potong yang tidak berkenaan.
- ** Jika tesis ini **SULIT** atau **TERHAD**, sila lampirkan surat daripada pihak berkuasa/organisasiberkenaan dengan menyatakan sekali sebab dan tempoh tesis ini perlu dikelaskan sebagai **SULIT** atau **TERHAD**.
- ♦ Tesis dimaksudkan sebagai tesis bagi Ijazah Doktor Falsafah dan Sarjana secara penyelidikan, atau disertasi bagi pengajian secara kerja kursus dan penyelidikan, atau Laporan Projek Sarjana Muda (PSM).

"I hereby declare that I have read this thesis and in my opinion this thesis has fulfilled the qualities and requirements for the award of Degree of Bachelor of Chemical Engineering (Biotechnology)"

| | | |
|---|---|---|
| Signature | : | …… |
| Name of Supervisor | : | Pn. Nurul Aini binti Mohd Azman |
| Date | : | …… |

# SCALE UP STUDY AND COMPARISON OF THREE DOWNSTREAM PROCESSES OF MONOCLONAL ANTIBODY PRODUCTION USING SUPERPRO DESIGNER

**KAMARULNIZAL BIN AMRIN**

**A thesis submitted in partial fulfilment of the**
**requirements for the award of the degree of**
**Bachelor of Chemical Engineering (Biotechnology)**

**Faculty of Chemical & Natural Resources Engineering**
**Universiti Malaysia Pahang**

**APRIL 2010**

I declare that this thesis entitled “Scale up study and comparison of three downstream processes of monoclonal antibody production using SuperPro Designer“ is the result of my own research except as cited in the references. The thesis has not been accepted for any degree and is not concurrently submitted in candidature of any other degree.

Signature : .....

Name : Kamarulnizal bin Amrin

Date : .....

To my beloved father and mother

## ACKNOWLEGDEMENT

In preparing this thesis, I have been in contact with lecturers, researcher and friends. They have greatly contributed toward completion of this research. I would like to express my gratitude to my supervisor, Madam Nurul Aini binti Mohd Azman for her guidance and support for this report. I would like also to thank Madam Chua@ Yeo Gek Kee for providing data required and lot of information for my thesis. Not to forget, Miss Syarifah Fathiyah binti SY Mohamad, person that also help me a lot in completing my study and report. Without their support, guidance and motivation, this thesis will not be the same as presented here.

For my beloved mother and father, thank you for continuous support and prayer for my success all the time and I prayed to Allah to bless your life as your bless me since nurturing me from childhood. I would like also to thank all my friends especially my coursemate for their continuous cheering all the way. I am truly appreciated your moral support and wishing you all the best in the future.

My sincere appreciation also extends to all my colleagues and others who have provided assistance at various occasions. Their views and tips are useful indeed. Unfortunately, it is not possible to list all of them in this limited space. Simply, I am grateful to all of you that contributed in completing this thesis.

# ABSTRACT

The main purpose of this study is to simulate the large scale production of monoclonal antibody (mAb) by using SuperPro Designer (SPD). Since the first discovery of mAb at 1970s, this type of antibody had become the most rapidly growing class of pharmaceutical. Problem with mAb production is the high cost and low amount of production but high demand for this therapeutic. In order to overcome this problem, large scale production had become one of the top priorities for mAb production. Large scale simulation study by using SPD can minimize time and cost production by eliminate the high cost of trial-and-error steps, as well as to find and simulate the optimization process for mAb production. In order to achieve the objective of this research, study is conducted in two steps which are simulation on the upstream and downstream process. For the upstream process, the stoichiometric equation is constructed base on the laboratory data and used to simulate the large volume of fermenter. For a downstream process, three flow of downstream process from different source a built, simulated and compared to choose the best process for purification of mAb. The result shows that the upstream process for 20000 L fermenter produced 0.00510 kg/Batch of mAb with concentration of $3.8 \times 10^{-4}$ g/L (5.1103 g/Batch). Compared to the flow from SPD source and journal (S. Sommerfeld and J. Strube, 2005), the best downstream process was the flow from Inno Biologics Sdn.Bhd that yield 81% of mAb with a concentration of 4.14328 g/Batch. As a conclusion, the simulation of large scale production of mAb by using SPD are flow work for 20000 L of fermenter as upstream process and Inno Biologics Sdn. Bhd. flow work as downstream process.

# ABSTRAK

Objektif utama dalam kajian ini adalah untuk simulasi skala besar pengeluaran antibodi monoklonal (mAb) dengan menggunakan SuperPro Designer (SPD). Sejak pertama kali mAb di temui pada tahun 1970-an, jenis antibodi ini telah berkembang pesat untuk industri farmasi. Masalah yang dihadapi dalam pengeluaran mAb adalah kos yang tinggi dengan jumlah pengeluaran yang rendah tetapi mempunyai permintaan yang tinggi. Untuk mengatasi masalah ini, pengeluaran berskala besar telah menjadi salah satu keutamaan untuk pengeluaran mAb. Simulasi skala besar dengan menggunakan SPD boleh meminimumkan masa dan kos pengeluaran dengan mengurangkan kaedah cuba jaya yang ber kos tinggi, serta mencari proses optimum bagi pengeluaran mAb. Untuk mencapai objektif, kajian dilakukan dalam dua langkah iaitu simulasi untuk proses huluan dan hiliran. Untuk proses huluan, persamaan stoikiometri dibina berdasarkan data makmal dan digunakan untuk simulasi pada fermenter yang lebih besar. Untuk proses hiliran, tiga aliran proses hiliran dari sumber yang berbeza dibina, disimulasi dan dibandingkan untuk memilih proses yang terbaik untuk penulenan mAb. Proses huluan untuk 20000 L fermenter menghasilkan 0.00510 kg / Batch mAb dengan kepekatan $3{,}8 \times 10^{-4}$ g / L (5,1103 g / Batch). Jika sumber SPD dan jurnal (S. Sommerfeld dan J. Strube, 2005) dibandingkan dengan Inno Biologics Sdn. Bhd, proses hiliran terbaik adalah proses dari Inno Biologics Sdn. Bhd dengan 81% daripada mAb asal diperolehi dengan kepekatan 4.14328 g / Batch. Kesimpulan yang boleh dibuat dari kajian ini adalah simulasi pengeluaran berskala besar mAb dengan menggunakan SPD ialah proses fermentasi 20000 L fermenter sebagai proses huluan dan proses Inno Biologics Sdn. Bhd sebagai proses hiliran.

# TABLE OF CONTENTS

# LIST OF TABLES

# LIST OF FIGURES

## LIST OF ABBREVIATIONS

| | | |
|---|---|---|
| AEX | - | Anion-exchange chromatography |
| Amm | - | Ammonia |
| CAH | - | Congenital Adrenal Hyperplasia |
| CEX | - | Cation-exchange chromatography |
| Gln | - | Glutamine |
| Glu | - | Glucose |
| HIC | - | Hydrophobic chromatography |
| KCl | - | Potassium Chloride |
| $KH_2PO_4$ | - | Potassium Phosphate Anhydrous |
| Lac | - | Lactate |
| mAb | - | Monoclonal antibody |
| $Na_2HPO_4$ | - | Sodium Phosphate |
| RIPP | - | Recovery, Isolation, Purification, Polishing |
| SPD | - | SuperPro Designer |
| UF/DF | - | Ultrafiltration/Diafiltration |
| WFI | - | Water for Injection |

# LIST OF APPENDICES

# CHAPTER 1

# INTRODUCTION

## 1.1 Background of the Study

Monoclonal antibody (mAb or moAb) is a class of antibody that produced by a single type of immune cell that are all clones of a single parent cell that will recognizes a specific antigenic target. The term monoclonal implies antibody produced by a cell clone derived from a fusion of one antibody producing cell with one myeloma cell capable of growing indefinitely in culture (I. Y. Abdel-Ghany *et al*., 2009). Since from the first discovery of this technology at 1970s, advances in technology had produce high quality of mAb and led the development of excellent therapeutic agents that impact the human health. Up until 2009, there are four main type of mAb that is mouse (murine), humanized, chimeric and human (E. B. Rodrigues *et al*., 2009).

Since the production of mAb by hybridoma technology, mAb is currently used in many applications like the diagnosis and treatment of many diseases or standard binding protein for purification of substances (L. Legazpi *et al*., 2005). One of the applications of mAb is treatment for human intoxication like ciguatera seafood poisoning. Ciguatoxins are causative toxin for ciguatera is produced by the marine dinoflagellate *Gambierdiscus toxicus* and accumulated in various kinds of reef fish (T. Takeshi *et at*., 2009)

Hybridomas are hybrid between myeloma cell and mammalian cell that producing antibody (B-lymphocytes) (M. Butler, 2004). Myeloma is a tumor or cancerous cell that can replicate endlessly while B-lymphocytes are cell that can produce a single type of antibody (Prescott *et al*., 2005). When myeloma cells were fused with antibody-producing mammalian spleen cells, it was found that the resulting hybrid cells, or hybridomas, produced large amounts of mAb. This product of cell fusion combined the desired qualities of the two different types of cells: the ability to grow continually, and the ability to produce large amounts of pure antibody.

In the bioprocess, there is an upstream and downstream process for production of certain biological product like in this study, production of mAb. Upstream process is a first step in bioprocess which the biomolecules are grown, usually by bacteria or mammalian cell line in bioreactor (fermentation). It involved in cell line development, media optimization and cell culture optimization (Feng Li *et al*., 2005). When it reach the desired density, they are harvested and moved to downstream process of the bioprocess. In the downstream process, biological product will be purifying to meet purity and quality requirement. The downstream section can be divided into three parts: a capture section, a purification section and a polishing section (S. Sommerfeld and J. Strube, 2005).

Process simulators are offering opportunity to shorten or minimize the time and cost required for process development. Comparison of process alternatives on a consistent basis in simulation gave large number of process ideas to be synthesize and analyze interactively in a short time. SuperPro Designer (SPD) developed by Intelligent Inc. is software that suitable to simulate the bioprocess operation other than any simulation software like Aspen BPS that more towards chemical processes (S. A. Rouf *et al*., 2001). This package has the added advantage that it was specifically developed for simulation of biopharmaceutical process unit operations and processes and set up to capture the unique unit operational data requirements of biological processes (Ian Gosling, 2003).

## 1.2 Problem Statement

The production cost of mAb in industry is very high, it can achieve around US$ 100 to 1000 per gram of production cost (S. Sommerfeld and J. Strube, 2005). S.S Farid (2007) reported $660 to $1580/ft$^2$ and $1756 to $4220/L invested on antibody manufacturing site with total site capacities of 2000 L to 20000 L. Instead of that, low amount of mAb produced cannot afford the high demand of this rapidly growing therapeutics.

Because of the high production cost and low amount of product, the selling price becoming so high that ranges from US$5000 per gram for mAb to US$ 1 million for erythropoietin (S. K. W. Oh et al., 2004). To fulfill the high demand and lowering the cost mAb, large scale production of mAb had become one of the top priorities in biopharmaceutical industry.

Simulation can become one of the tools to minimize cost and time for production of mAb. Optimization can be made by firstly doing the simulation before undergo the large scale production and eliminate the trial-and-error step in biopharmaceutical process.

## 1.3 Objectives

The objective of this research is to study the upstream and downstream process of mAb from laboratory data base on SPD simulation. It is also to discover the potential for the large scale production of mAb by using the desired SPD.

## 1.4 Scope of the Study

Model for this study is hybridoma used to cultivate antibody towards Congenital Adrenal Hyperplasia (CAH), inherent disease that able to cause death within 14 days infant and abnormal sex. Scope in this study is to compare the simulation result with laboratory experiment data. The scope is also to build and simulate the large scale upstream process for mAb production by using the laboratory data. Other than that, the scope is to propose downstream process by build a several flow design for purification of mAb and analyze the best process base on yield and purity.

## 1.5 Rationale and Significance

This study has a potential to minimize cost and time for production of monoclonal antibody by doing the simulation first before furthering in clinical or large-scale production. Traditionally, process development of mammalian cell culture is based on trial-and-error experimentation. So, process simulation can overcome the trial-and error process development and facilitate rapid process improvement without extensive experimentation or disrupting existing operations.

# CHAPTER 2

# LITERATURE REVIEW

## 2.1 Upstream Process

In the bioreactor, cells survive, grow, die and produce mAb depending on their culture environment. Kinetic equation need to be developed to describe cell growth, nutrient consumption and product generation, the concentration of substrates and products is a factor for kinetics of hybridoma culture (L. Legazpi *et. al.*, 2005). In cell growth, glucose and glutamine are assumed to be the main substrate while ammonia and lactate produced from glucose and glutamine metabolism are assumed to be inhibitory to cell growth. Glutamine is also assumed as main limiting substrate for antibody production (J. D. Jae and J. P. Barford, 2000).

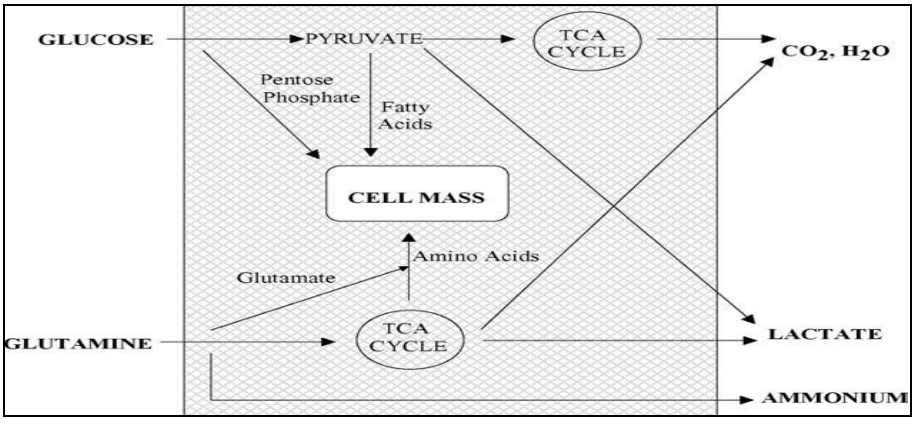


Figure 2.1: Main metabolic routes for hybridoma cells (L. Legazpi *et. al.*, 2005)

Specific of cell growth rate based on the concentration of key nutrient (glucose and glutamine) and the metabolites (lactate and ammonia) that follow monod kinetics. Cell death rate is based on the function of ammonia concentration that accumulates in the culture. For cell metabolism, concentration of nutrients and metabolites are compute by performing mass balance around bioreactor (C. Contoravdi *et. al*., 2007).

Model improvement over pre-existing model were developed by model development for batch and fed-batch operations were derived based on initial metabolic flux analysis(MFA) and the coefficient or parameters value for model equation were estimated by using quadratic programming(QP) and Metropolis-Hastings algorithm. Integrated model (combination between metabolites and biomass model) is capable of predicting concentration for substrates, extracellular metabolites, and viable and dead cell concentration (P. Dorka *et al*., 2009).

Many kinetic expressions and a large number of parameters are involved resulting in a complex identification problem. It is not possible to estimate simultaneously all parameters with the mathematical analysis, so strategy to narrow down the parameters involve should be taken by: firstly, estimate all parameters that could be analyzed independently, then the most insensitive parameters (mainly half-saturation constants-taken from literature), most sensitive parameters estimate by non-linear regression analysis, and finally improve model by tuning manually the most insensitive parameters (A. Teixeira *et al*., 2005).

From the study of A. C. Baughman *et al*. (2009) by taking the Gao *et al*. (2007) as case study, they state that half of generic metabolic reconstruction did not function significantly during major phase of the culture and that make the 32 reaction is reduced to 16 reactions. These 16 reactions then is further compacted by some technique which combine reactions that share common metabolites and reducing the reconstruction resulted in nine macro reaction.

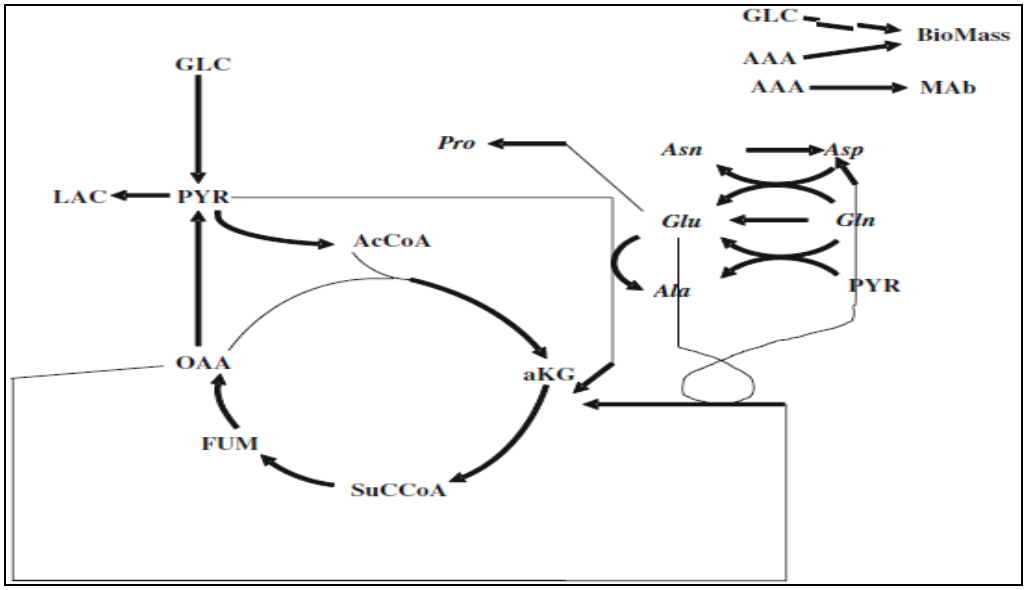


Figure 2.2: Reduced metabolic construction of hybridoma (A. C. Baughman *et al.*, 2009).

Table 2.1: The nine proposed macro-reactions (A. C. Baughman *et al.*, 2009).

| | |
|---|---|
| Reaction 1 : | GLC ⟶ 2LAC |
| Reaction 2 : | GLC + 2GLU ⟶ 2ALA + 2LAC |
| Reaction 3 : | GLC + 2GLU ⟶ 2ASP + 2LAC |
| Reaction 4 : | GLU ⟶ PRO |
| Reaction 5 : | ASN ⟶ ASP + $NH_3$ |
| Reaction 6 : | GLN + ASP ⟶ ASN + GLU |
| Reaction 7 : | 0.0508GLC + 0.0577GLN + 0.006ASN + 0.0201ASP + 0.0016GLU + 0.0133ALA + 0.0081PRO ⟶ BM |
| Reaction 8 : | 0.0104GLN + 0.0072ASN + 0.0082ASP + 0.0107GLU + 0.011ALA + 0.0148PRO ⟶ mAb |
| Reaction 9 : | GLN ⟶ GLU + $NH_3$ |

Balance for mammalian cell growth can be describes as an “equation” by considering the major “input” and “output” for the biomass formation. The composition of cells can be written as a “molecular formula” and an example for the equation for hybridoma growth is as Equation 2.1 (S. H. Wei, 2004).

$C_6H_{12}C_6$ (glucose) + $0.15C_{6.14}H_{12.36}N_{1.50}O_{2.08}$ (weighted average of amino acids) + $0.34C_5H_{10}N_2O_2$ (glutamine) + $1.39O_2 \longrightarrow 2.37CH_{1.97}N_{0.26}O_{0.49}$ (cell mass) + $0.0058CH_{1.83}N_{0.14}O_{2.06}$ (antibody) + $1.54CO_2 + 1.28H_2O + 1.44C_3H_6O_3$ (lactate) + $0.16NH_3$ (ammonia) + $0.13\ C_4H_7NO_2$ (alanine)

Equation 2.1: Stoichiometric equation for Hybridoma (S. H. Wei, 2004).

Y.H. Guan and R.B. Kemp (1999) had developed a stoichiometric equation for hybridoma growth reaction to represent the metabolic activity. In their study, it was shown that the set of stoichiometric coefficients constitute a validated growth equation has a one-to-one corresponding relationship to the metabolic activity of the average cell population.for both theoretically and experimentally. Table 2.2 is one of the growth reactions for discrete times under activated and triggered condition.

Table 2.2: Stoichiometric equation of hybridoma for discrete times under activated and triggered condition (Y.H. Guan and R.B. Kemp, 1999).

| **Metabolic condition** | | **Metabolic reaction** |
|---|---|---|
| Activated cells | Catabolism | $C_6H_{12}O_6 + 0.347C_5H_{10}N_2O_3 + 1.7960O_2 \longrightarrow 1.921C_3H_6O_3 + 1.969CO_2 + 0.693NH_3 + 0.929H_2O$ |
| | Anabolism | $0.078C_6H_{12}O_6 + 0.210C_5H_{10}N_2O_3 + 0.149CO_2 \longrightarrow 1.667CH_{1.821}O_{0.837}N_{0.252}$ |
| | Metabolism | $C_6H_{12}O_6 + 0.517C_5H_{10}N_2O_3 + 1.666O_2 + 0.149CO_2 \longrightarrow 1.782C_3H_6O_3 + 1.827CO_2 + 0.693NH_3 + 0.862H_2O + 1.546CH_{1.821}O_{0.837}N_{0.252}$ |
| Triggered cells | Catabolism | $C_6H_{12}O_6 + 0.317C_5H_{10}N_2O_3 + 3.836O_2 \longrightarrow 1.196C_3H_6O_3 + 3.994CO_2 + 0.633NH_3 + 3.044H_2O$ |
| | Anabolism | $0.184C_6H_{12}O_6 + 0.092C_5H_{10}N_2O_3 + 0.054CO_2 \longrightarrow 1.614CH_{1.938}O_{0.922}N_{0.114}$ |
| | Metabolism | $C_6H_{12}O_6 + 0.345C_5H_{10}N_2O_3 + 3.240O_2 \longrightarrow 1.010C_3H_6O_3 + 3.327CO_2 + 0.535NH_3 + 2.571H_2O + 1.363CH_{1.938}O_{0.922}N_{0.114}$ |

Commercial success of mAb had led to the need for large scale production in mammalian cell culture. Rapid expansion had increase the bioreactor size and optimization effort are improved since then for cell expression and process optimization like fed-batch cultures (J. R. Birch and A. J. Racher, 2006). K. H. Ting and K. A. McDonald (2009) had stated that bioreactor designs must provide an environment that is able to optimize the growth and productivity of the genetically engineered host cells and design of effective bioreactor should consider growth, nutrient uptake and production kinetics, oxygen and heat transfer, and fluid hydrodynamics.

Stirred tank bioreactor have been widely used for commercially antibody production by using cell lines like CHO, hybridoma and NSO and the agitation rates in these reactors are generally kept between 10 to 40 rpm depending upon the sensitivity of the cell lines being cultured (E. Jain and A. Kumar, 2008). L. Legazpi *et al*. (2005) had conducted a study of agitation effect to the hybridoma cell by using rocker set-up and shown high value of specific death rate provoked decreasing in number of viable cell.

Agitation and aeration are critical consideration in bioreactor for mammalian cell because it caused physical cell damage by induced hydrodynamic shear by agitation and bubble damage caused by mass transfer gas sparging (D. M. Marks, 2003).

## 2.2 Downstream Process

After the upstream section or the fermentation process, the production of mAb is further proceed to downstream section to be purified according to the product quality requirement and there are typical substances that need to be separated in downstream section. Cell culture medium like amino acid, inorganic salts and medium supplement like bovine serum and proteins need to be separated in downstream process. Other than that, substances like intact cells, cell debris, host cell protein and DNA are also separated in order to have pure desired product (S. Sommerfeld and J. Strube, 2005).

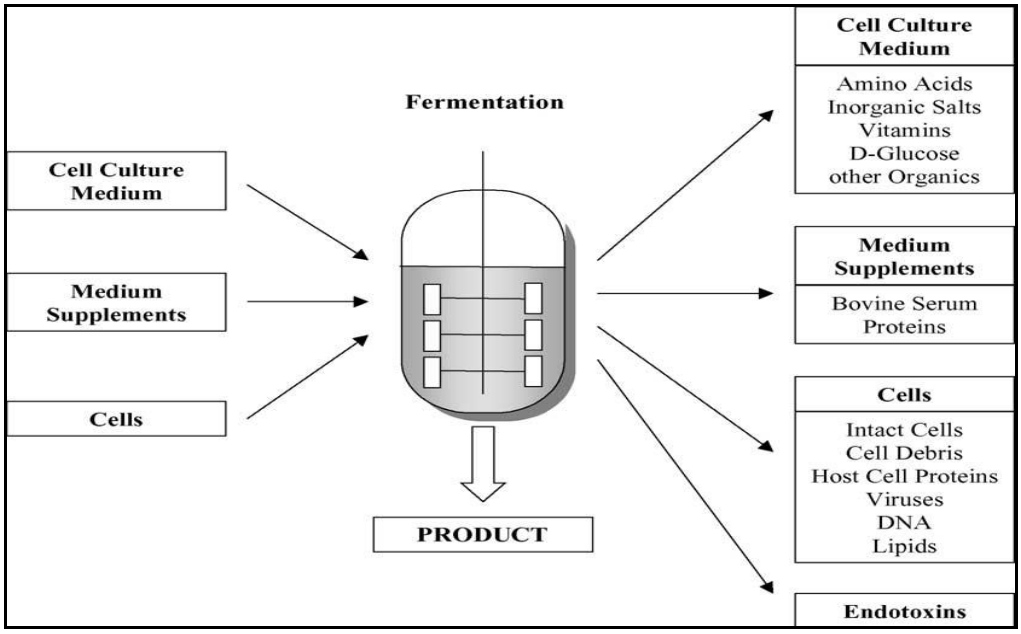


Figure 2.3: Downstream processing task (S. Sommerfeld and J. Strube, 2005).

As known today, mAb is used in many applications such as diagnosis and treatment of certain diseases like cancer therapy. Therapeutic treatment for cancer by using mAb produced by murine hybridomas cultured in a cell bioreactor in serum free conditions were harvested from supernatants and further purified by downstream process (A. L. Horenstein *et al.*, 2003).

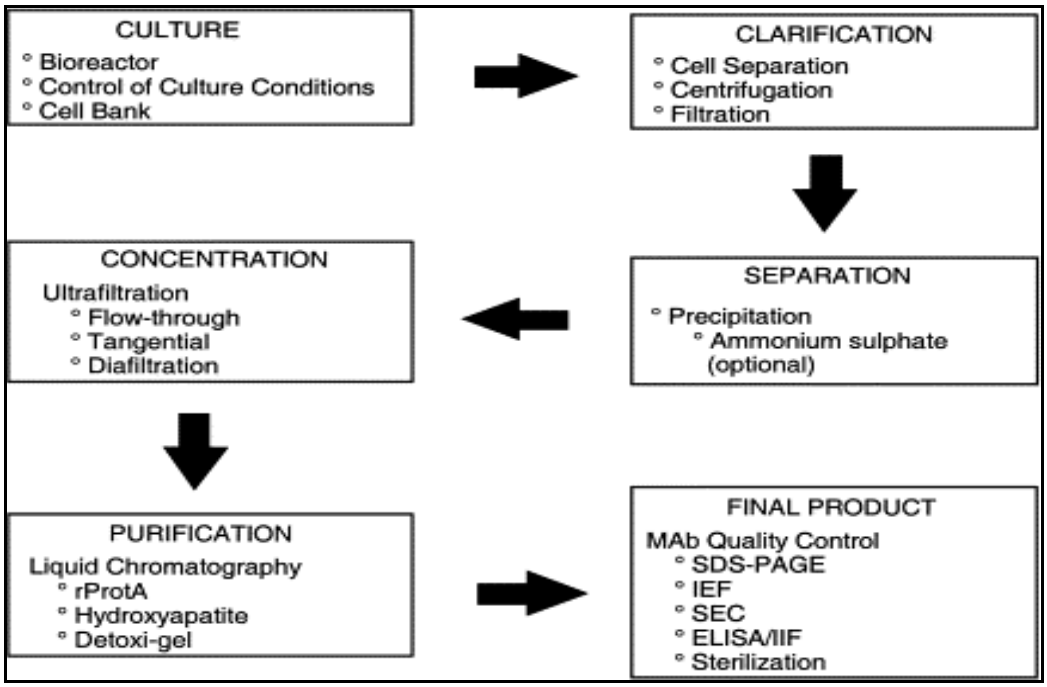


Figure 2.4: Major stages of the downstream processing of mAb for cancer therapy (A. L. Horenstein *et al*., 2003).

Limits imposed by the technology, equipment and facilities that are available had made downstream processes to take proportional response to the limits. As a result, manufacturers are intent to explore several ways or strategies of streamlining product recovery and purification like decreasing the number of steps, avoiding complex steps and reducing raw materials costs. In addition, alternative formats for recovery and purification unit operations are being reconsidered include expanded and simulated moving beds, membrane chromatography and non-chromatographic methods such as flocculation, precipitation, crystallization and aqueous two-phase systems (D. Low *et al*., 2006).

A. A. Shukla *et al*. (2006) had described a flexible, generic platform for mAb downstream processing that they develop at Amgen and applied for the production of over 20 molecules over a range of scales ranging from clinical production to commercial launch.

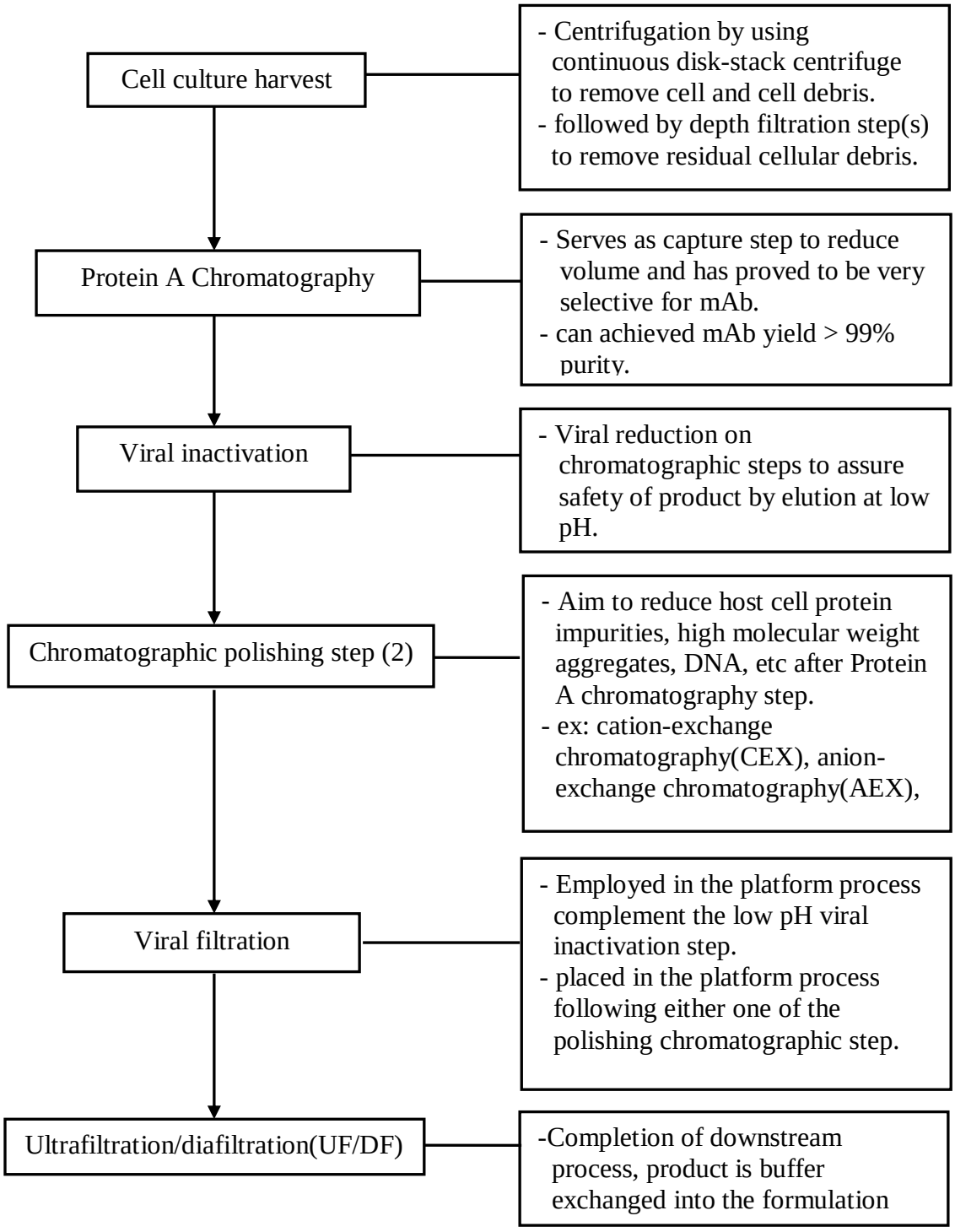


Figure 2.5: Generic Downstream process for mAb (A. A. Shukla *et al*., 2006)

### 2.2.1 RIPP Scheme

In a broader term, bioseparations engineering refers to the systematic study of the scientific and engineering principles utilized for the large scale purification of biological products. Bioseparation processes are based on multiple techniques and RIPP scheme is commonly used in bioseparation. RIPP stand for Recovery, Isolation, Purification and Polishing that involves use of low resolution techniques first for recovery and isolation followed by high resolution techniques for purification and polishing (R. Ghosh, 2006).

Table 2.3: RIPP scheme for downstream process (R. Ghosh, 2006).

| Stage | Objectives | Typical unit operations |
|---|---|---|
| Recovery (separation of insoluble) | - Remove or collect cells, cell debris<br>- Reduce volume | Filtration, sedimentation, extraction, adsorption, centrifugation |
| Isolation | - Remove materials having properties widely different from those target product<br>- Reduce volume | Extraction, adsorption, ultrafiltration, precipitation |
| Purification | - Remove remaining impurities, which typically are similar to those or target product | Chromatography, affinity methods, precipitation |
| Polishing | - Remove liquids<br>- Convert product to crystalline form (not always possible) | Drying, crystallization |

The objective of RIPP scheme is mainly to reduce volume from the fermentation process until get the pure biological product. The first step that is the recovery step is to remove or collect the cells and cell debris by some unit operations like filtration, sedimentation and centrifugation. Second step that is the isolation step is to remove the

materials that having widely different properties form the target product. This can be done by using extraction, adsorption, precipitation and ultrafiltration.

The next step is purification step that remove remaining impurities that had typical similar type of the target product. This step is very important for downstream process and can be done by using chromatography, affinity and precipitation. The final step is polishing where it is not always possible in downstream process. The objective of this step is to remove liquid and convert the target product into crystalline form by drying and crystallization process.

### 2.2.2 Centrifugation

Centrifuge is a device that separates particles from suspensions or even macromolecules from solutions according to the size, shape and density by subjecting these dispersed system to artificially induced gravitational fields. After the separation complete, the suspension cells will separate to supernatant and precipitate according to the density of substance in the centrifugation. A disc stack centrifuge is a special type of preparative centrifuge which is compact in design and gives better solid-liquid separation than the standard tubular bowl centrifuge (R. Ghosh, 2006).

For mAb production, centrifugation is the first unit operation that acts as cell removal at the capture step of mAb purification before further process in downstream section (S. Sommerfeld and J. Strube, 2005).

### 2.2.3 Filtration

Filtration is a separation process in which a solid liquid mixture called the feed is forced through the porous medium on which the solids are deposited or in which they are entrapped. Filtration can be classified in three categories that are cake filtration, clarification and cross-flow filtration and main mechanisms by which solids are retained by a filter is surface filtration and depth filtration. Filtration is driven by applying a pressure drop across the filter medium by applied the pressuring the feed side or by creating a vacuum in the filtrate side (R. Ghosh, 2006).

When centrifugation technology was less developed, microfiltration had become center effort for primary recovery where it result in better clarification and has advantage of requiring less capital. With the arrival of high cell densities and low shear centrifuges, a hybrid centrifugation–filtration system has become the industry standard for primary recovery. But, microfiltration can still be a valuable tool when low capital cost is important and or when processing is limited to small scales. In other way, depth filtration is used for secondary clarification for removing cell debris and smaller sized contaminant despite of low shear centrifuge design to prevent plugging in chromatography (D. Low *et al*., 2007).

In the E. Rosenberg *et al*. (2009) study, they come with the optimization method to minimize aggregation in the ultrafiltration for mAb solutions. Increase of large insoluble and structurally perturbed aggregates is directly correlated to the applied shear stress during the ultrafiltration concentration process on permeate flux and aggregation.

### 2.2.4 Chromatography

Chromatography is a solute fractionation technique which relies on the dynamic distribution of molecules to be separated between two phases: a stationary phase and a mobile phase. The different separation mechanisms used for chromatography are like ion exchange, reverse phase, hydrophobic interaction, affinity and size exclusion (R. Ghosh, 2006).

In a large scale production of mAb, affinity chromatography is use for purification step where it capture mAb more than 90% while ion exchange chromatography and hydrophobic chromatography are more towards polishing step where others impurities are capture to give a high purity of mAb (A. L. Horenstein *et al.*, 2003; S. Sommerfeld, and J. Strube, 2005; and D. Low *et al.*, 2006). There is several type of chromatography:

#### 2.2.4.1 Ion Exchange Chromatography

Ion exchange is based on electrostatic interactions between the molecule and the adsorbent. A cation exchange adsorbent it itself negatively charges and therefore bind positively charged molecules while anion exchange adsorbent is positively charged and can bind negatively charged molecules (R. Ghosh, 2006).

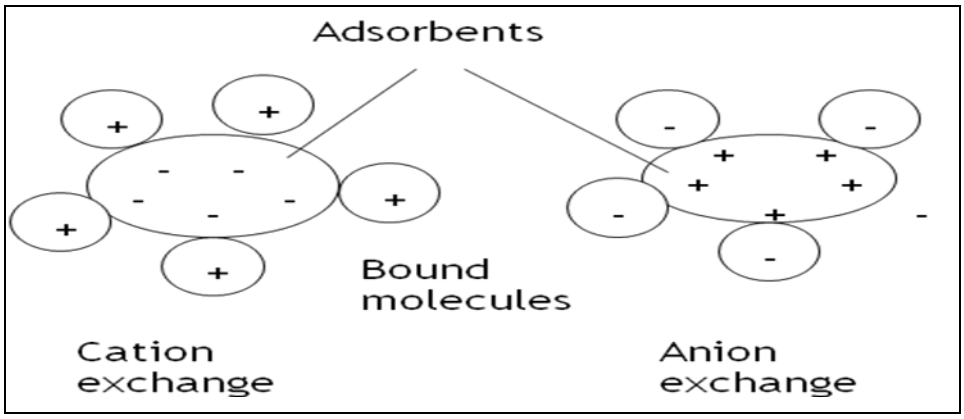


Figure 2.6: Ion exchange based adsorption (R. Ghosh, 2006).

### 2.2.4.2 Reverse Phase Chromatography

Reverse phase chromatography is based on the differences in the extent to which solutes partition into the non-polar stationary phase. They provide a non-polar environment into which non-polar molecules can partition favorably. Reverse phase is more widely used for binding low and intermediate molecular weight compounds but not suitable for macromolecules such as protein and nucleic acid since they do not partition very well into the hydrocarbon layer (R. Ghosh, 2006).

### 2.2.4.3 Hydrophobic Interaction Chromatography

This type of chromatography is based on the adsorption that relies on the interaction between the hydrophobic patches on molecules and those on the adsorbent. Hydrophobic interaction is mainly used for protein separation and the principle of this type of adsorption is shown in the Figure 2.7.

At first, the molecule and adsorbent are not interact because of shielded by water. High solute of anti-chaotropic salts like ammonium sulphate and sodium sulphate are able to remove the water and make the molecule interact with adsorbent. By lowering the salt concentration, desorption of molecule are occur (R. Ghosh, 2006).

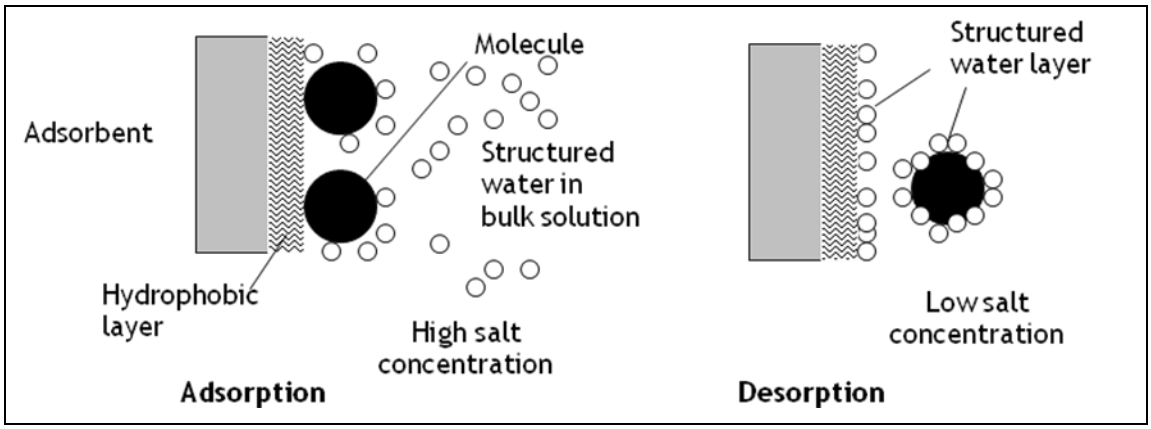


Figure 2.7: Hydrophobic interaction based adsorption (R. Ghosh, 2006).

### 2.2.4.4 Size Exclusion Chromatography

This type of chromatography which also frequently referred to as gel filtration chromatography is based on the use of inert porous particles as stationary phase and these separates solutes purely on the basis of size. During the solute journey in the chromatographic column, smaller solute molecules are easier to enter the pores while larger solutes are excluded and this make smaller solute spend longer time than large solute in the column. The size exclusion limit of gel filtration column specifies the molecular weight range that can be resolved by the column.

### 2.2.4.5 Affinity Chromatography

Affinity chromatography is based on the highly specific recognition and binding of target molecules to the ligands, the shapes of the ligand is complimentary to the shape of entire target molecules or at least a portion of the molecule. Affinity binding is so specific that it may pull out a particular solute from a mixture of hundreds or thousands of different solutes.

There are many types of affinity ligands exist but for purification of mAb, protein A affinity ligand is commonly used for capture step in the mAb downstream process. At early stage, the cell culture supernatant is introduced into a chromatography containing the protein A affinity absorbent. Protein A absorbent will recognize and bind with the mAbs leaving only the impurities in the supernatant. After the impurities have been moved, the mAbs are desorbed using low pH buffer that weaken the interaction mechanism involved.

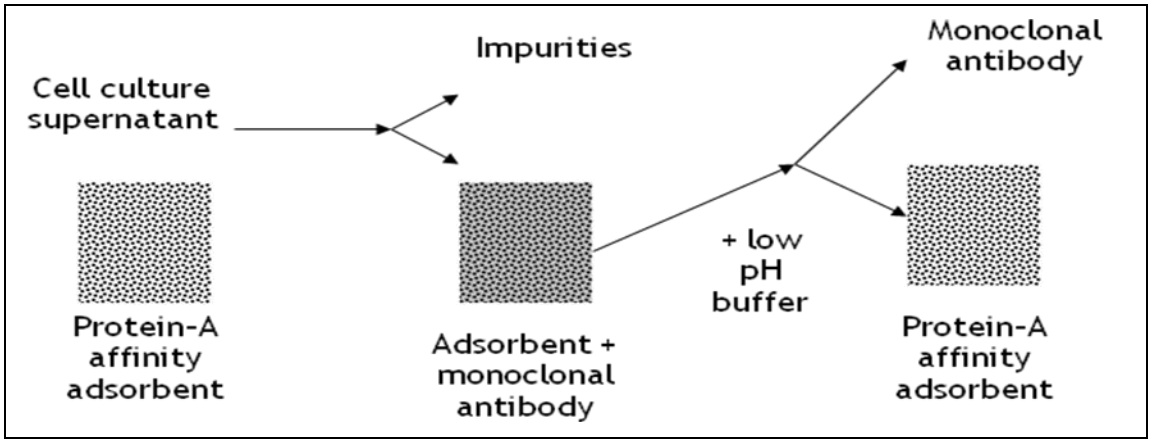


Figure 2.8: Affinity separation of mAb (R. Ghosh, 2006).

# CHAPTER 3

# RESEARCH METHODOLOGY

## 3.1 Stoichiometric Equation Derivation

Experimental data are provided by Chua @ Yeo Gek Kee, PhD student researching the hybridoma cell secreting mAb towards Congenital Adrenal Hyperplasia (CAH). The data provided are shown in the Tables 3.1 and 3.2 below:

Table 3.1: Bioreactor Batch Profile

| | |
|---|---|
| Medium | Dulbecco's Modified Eagle's Medium (DMEM) (D5648, Sigma) + 4mM L- Glutamine + 17% (v/v) antibiotic-antimycotic + 2% Fetal Bovine Serum (FBS) |
| Working volume | 1.25 L |
| Duration | 90 hours |
| Culture condition | Temperature: 37 $^{o}$C<br>pH: 7.4<br>Dissolved oxygen (DO): 60%<br>Stirring speed: 75 rpm<br>Sparging rate: 0.5 L/min<br>Pluronic F68: 0.1%<br>Sodium bicarbonate: 7.5% |

Table 3.2: Hybridoma growth profile

| (h) | Concentration (mmol/L) | | | | Viable cell(cells/ml) | mAb (µg/ml) |
|---|---|---|---|---|---|---|
| | Glc | Gln | Amm | Lac | | |
| 0 | 24.42 | 3.69 | 1.35 | 5.44 | 146250 | 0.057 |
| 18 | 22.10 | 3.58 | 2.28 | 7.86 | 264375 | 0.072 |
| 22 | 21.92 | 3.27 | 2.50 | 9.08 | 333750 | 0.076 |
| 41 | 17.81 | 1.52 | 3.43 | 16.06 | 405000 | 0.143 |
| 44 | 15.71 | 2.11 | 3.69 | 17.00 | 503750 | 0.141 |
| 48 | 13.01 | 1.92 | 3.80 | 17.81 | 515000 | 0.169 |
| 66 | 8.82 | 0.83 | 4.57 | 24.56 | 553125 | 0.298 |
| 70 | 8.11 | 0.56 | 5.06 | 24.92 | 538125 | 0.306 |
| 90 | 5.75 | 0.27 | 5.32 | 29.03 | 265000 | 0.442 |

From the laboratory data experiment, the stoichiometric equation can be derived by using the elemental balance method. Mole value for oxygen gas ($O_2$), carbon dioxide gas ($CO_2$), water ($H_2O$) and biomass which illustrated as bold capital letter (**a**, **b**, **c** and **d**) in Equation 3.1, is obtained by calculating the balance for carbon (C), oxygen (O), hydrogen (H) and nitrogen (N).

$24.42C_6H_{12}O_6$ (Glucose) + $3.69C_5H_{10}O_3N_2$ (Glutamine) + **a** $O_2$ (Oxygen)

$\longrightarrow$ **b** $CH_{1.8}O_{0.5}N_{0.2}$ (Biomass) + $9.07 \times 10^{-3}$ $CH_{1.83}O_{2.06}N_{0.14}$ (mAb) + **c** $CO_2$ (Carbon dioxide) + **d** $H_2O$ (water) + $5.32NH_3$ (Ammonia) + $29.03C_3H_6O_3$ (Lactate)

Equation 3.1: Unfinished stoichiometric equation for laboratory data.

## 3.2 Simulation of Laboratory Data

Framework of the laboratory data is simply done by inserting one unit of fermenter together with the two input streams, first stream for medium and water for

injection (WFI) and another stream for air supply. For the output stream of fermenter, two streams are connected that is for the gases emit and product produced. The stoichiometric equation derived and other operations data required are inserted in the fermenter operation data.

The amount of medium for fermenter is an initial amount of glucose and glutamine taken from the laboratory data. For WFI, the amount is same as the working volume for fermenter in the laboratory experiment that is 1.25 g/Batch. After all data for fermenter has been considered, the simulation is done and the results are compared with the laboratory data.

## 3.3 Simulation of Large Scale Production

Simulation of large scale production of mAb utilized the laboratory experiment data and consists of two parts that is for the upstream process and the downstream process. The upstream process is the fermentation process of mAb where the culture are growth to the desired value or volume and the downstream process is the purification process of mAb where the highest or desired purity and yield of the product will be obtained.

### 3.3.1 Upstream Process

Framework for fermentation process is built in the flow sheet of SPD for a 20000 L fermenter. First, 200 L fermenter was constructed by inserting one unit of fermenter. Considering the air requirements for fermentation, compressor and air filter are inserted at the drawing frame (window). The compressor and air filter are inserted at the air input stream and another one air filter at the output air stream. Then, with the

same arrangement of unit operations, 2000 L fermenter and 20000 L fermenter are constructed. The medium and WFI stream are the input for media blending tank and the output of this tank is distributed to the three different volume fermenter constructed before which is for 200 L, 2000 L and 20000 L fermenter.

The output medium has been sterilized using dead-end filtration. The inoculum is first inserted to the 200 L fermenter and then the output of this fermenter is transferred as the inoculum for the next fermenter and so on. After that, the specific operation data are inserted to the fermenter especially the stoichiometric equation that has been derived from the laboratory data. After the framework has been designed and the operation data have been inserted, the simulation is done to get the result or amount of product from the fermentation process.

### 3.3.2 Downstream Process

Three frameworks from different source have been built in the flow sheet of SPD and the input for each flow are imported from the product of the upstream process before. The frameworks for downstream process are built based on the SPD source, Inno Biologics Sdn. Bhd and the journal (S. Sommerfeld and J. Strube, 2005). The unit operations involved for the three flows are inserted at the drawing frame and connected by stream to complete the flow of downstream process. After that, the operation data for each equipment in the downstream process are specified and the simulations are done. The results obtained from the simulation of the three frameworks are analyzed and the best downstream process is determined according to the yield and concentration of mAb.

# CHAPTER 4

# RESULT AND DISCUSSION

## 4.1 Upstream Process

### 4.1.1 Stoichiometric Equation and Laboratory Data Comparison

Stoichiometric coeeficient and other process input that required for simulation were determined by only simple laboratory experiment. Then, the data obtained are assumed representing the large scale bioreactor. (S. K.W. Oh *et al*., 2004). In this case, the data was taken from the cell culture that has been grown in the 2 L fermenter with a 1.25 L of working volume. Table 3.2 shows the data that came from laboratory experiment that were needed to construct the equation. From the data, the stoichiometric coefficient was obtained by using the elemental balance method and the constructed model are as followed:

$C_6H_{12}O_6$ (Glucose) + 0.15 $C_5H_{10}O_3N_2$ (Glutamine) + 2.68 $O_2$ (Oxygen)

$\longrightarrow$ 0.4 $CH_{1.8}O_{0.5}N_{0.2}$ (Biomass) + 0.000371 $CH_{1.83}O_{2.06}N_{0.14}$ (mAb) + 2.78 $CO_2$ (Carbon dioxide) + 2.49 $H_2O$ (water) + 0.22 $NH_3$ (Ammonia) + 1.19 $C_3H_6O_3$ (Lactate)

Equation 4.1: Stoichiometric equation derived from laboratory data.

By using the stoichiometric equation that has been derived, the simulation was done for 1.25 L of working volume to mimic the laboratory experiment. Then, the result from simulation was compared with the data from laboratory experiment as shown in the Table 4.1.

Table 4.1: Data comparison for simulation result with laboratory data.

| | Initial (g/Batch) | | Final (g/Batch) | |
|---|---|---|---|---|
| | Laboratory data (calculation) | Simulation data | Laboratory data (calculation) | Simulation data |
| Glc | 4.3956 | 4.3956 | 1.0350 | 0.3212 |
| Gln | 0.5387 | 0.5387 | 0.0394 | 0.0394 |
| Amm | 0.4896 | - | 2.6127 | 2.4003 |
| Lac | 0.0230 | - | 0.0904 | 0.0838 |
| mAb | 0.000057 | - | 0.000442 | 0.00046 |

From the Table 4.1, percent error of 4 % resulted between the laboratory data and the simulation data. This might be due, in the SPD, the decimal point that can be inserted is only 2 decimal point for stoichiometric balance. This limitation causes a problem for mAb because the mole value is 0.000371 and only can be inserted as 0.00 in the simulation as illustrated in the Figure 4.1.

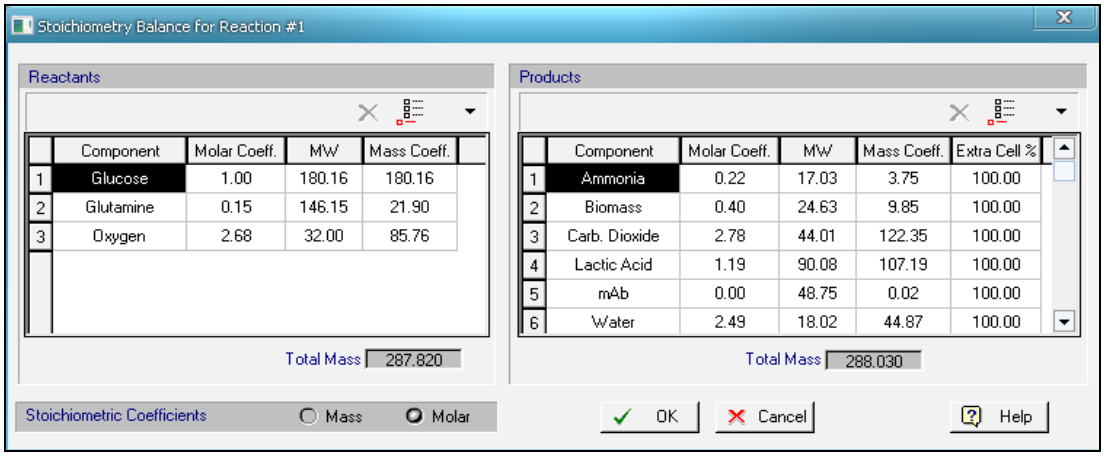


Figure 4.1: Window for stoichiometric balance data in the SPD

This problem can be overcome by providing one more information for mAb which is the mass coefficient of mAb by multiplying the molar coefficient with the molecular weight of mAb. Other than that, the values for other components were also increased because of the limitation in decimal point in SPD. This has cause the different between the two data. Since the error was too small, the equation can be used for the simulation of high volume fermenter.

### 4.1.2 Large Scale Production

For simulation of large scale fermentation, high volume of fermenter is needed. Before the 20000 L fermenter can be operated, the input or inoculum for this fermenter should be constructed and transferred by sequence. Start from 200 L of seed fermenter to the 2000 L fermenter and finally to the 20000 L fermenter, 10 % of the inoculum was assumed for each fermenter. After taking consideration all equipment for upstream process such as the fermenter, medium, air supply and other else, the frame work for upstream process are built. The results of the flow for upstream process are shown in the Figure.4.1.

After constructing the detail design and the operation data required, the upstream process was simulated to obtain the result or the amount of product of the fermenters. The amount of product in the fermenters from simulation was listed in Table 4.2. The input values for the 200 L fermenter was obtained by utilizing the laboratory experiment data, which is from 1.25 L of working volume. Total volume of the fermenter was 2 L and by assuming that the inoculum was 10 %, the input value for 200 L fermenter can be obtained by multiplying the data from 2 L fermenter (laboratory experiment) with 100.

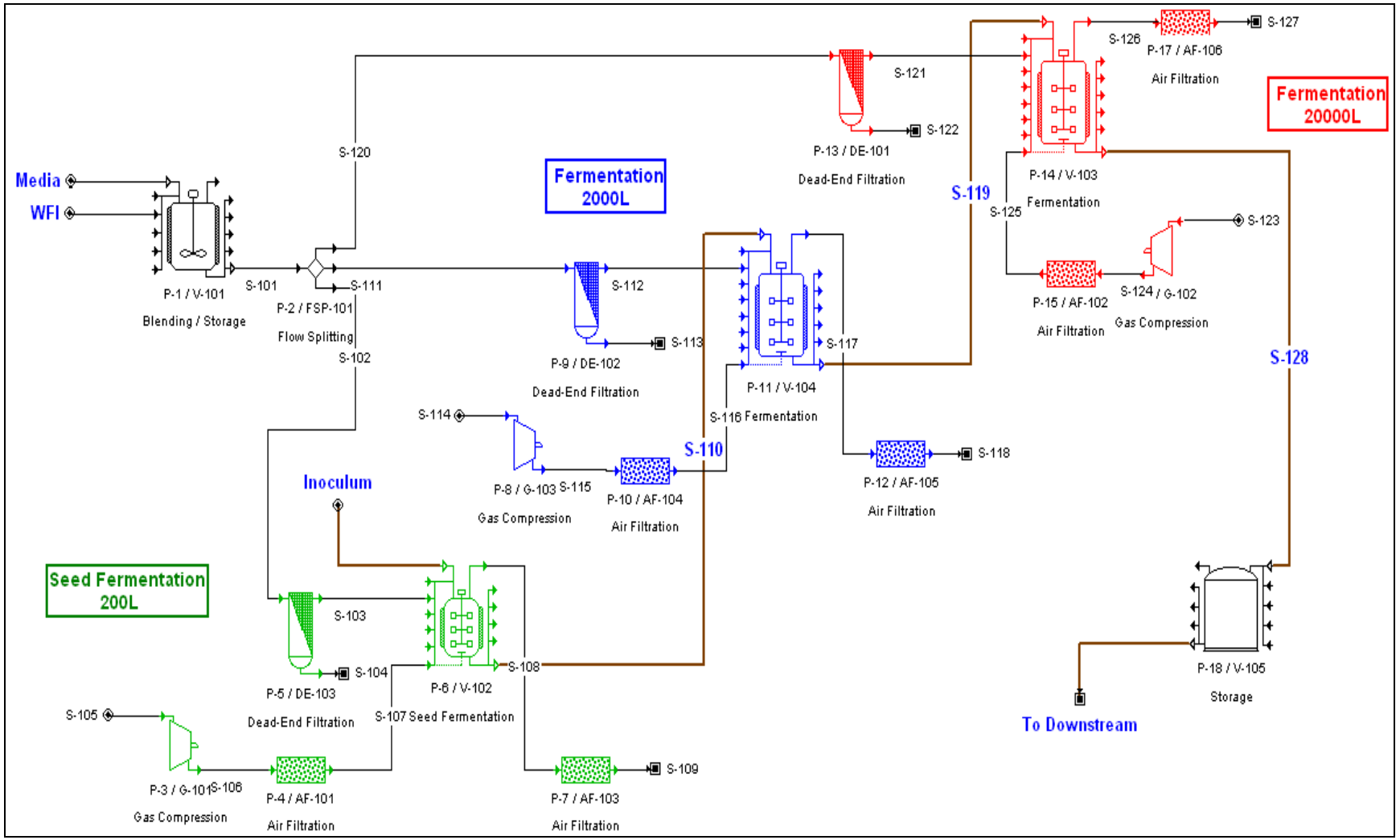


Figure 4.2: Flow work for upstream process

Table 4.2: Simulation result for upstream process.

| Component | 200 L (S-110) | | 2000 L (S-119) | | 20000 L (S-128) | |
|---|---|---|---|---|---|---|
| | Flow rate (kg/Batch) | Concentration (g/L or kg/$m^3$) | Flow rate (kg/Batch) | Concentration (g/L or kg/$m^3$) | Flow rate (kg/Batch) | Concentration (g/L or kg/$m^3$) |
| Amm | 0.00854 | 0.07102 | 0.09471 | 0.07144 | 0.95630 | 0.07148 |
| Biomass | 0.02247 | 0.18675 | 0.24904 | 0.18786 | 2.51466 | 0.18797 |
| Glc | 0.02866 | 0.23826 | 0.28557 | 0.21542 | 2.85176 | 0.21317 |
| Gln | 0.00395 | 0.03281 | 0.03980 | 0.03003 | 0.39800 | 0.02975 |
| Lac | 0.24446 | 2.01396 | 2.70967 | 2.04403 | 27.36051 | 2.04522 |
| mAb | 0.00005 | 0.00038 | 0.00051 | 0.00038 | 0.00510 | 0.00038 |
| Water | 0.10233 | 0.85055 | 1.13423 | 0.85560 | 11.45272 | 0.85610 |
| WFI | 106.14375 | 882.2708 | 1168.7606 | 881.6526 | `11793.75 | 881.5917 |

Referring to the simulation result, the final amount of the mAb was 0.00510 kg/Batch with $3.8 \times 10^{-4}$ g/L of concentration for 20000 L fermenter. The amount of mAb was increased from 200 L fermenter to 20000 L fermenter but with the same concentration. The stoichiometric equation that has been derived from the laboratory data are used for each fermenter. Since the equation used was similar, increasing amount of mAb from 200 L to 20000 L are ratio to the factor of ten from the previous fermenter. Then, the concentration of mAb goes the same way because of the volume was also increased by factor ten from fermenter to fermenter. The result data from 20000 L fermenter were transferred to downstream process to recover the high purity of mAb.

By using the flow of upstream process that has been design and the stoichiometric equation from the laboratory data, the simulation can be done for any volume of fermenter that wants to be study. Thus, amount of mAb can be known for higher volume of fermenter such as 40000 L or 200000 L. Instead of that, costing and scheduling can also be known, but problems usually appear for a large volume of fermenter. SPD can be used to optimize the product of fermenter as well as to identify possible constraint and production bottlenecks.

## 4.2 Downstream Process

### 4.2.1 Process Selection and Consideration

For the simulation of downstream process, three framework has been selected to compare the most efficient and high purity of mAb. The framework was obtained from SPD source, Inno Biologics Sdn. Bhd. and journal written by S. Sommerfeld and J. Strube (2005). These three frameworks are chosen because the product produced was

similar which is mAb. The choice of downstream process from the Intelligent Inc. (SPD source) and Inno Biologics Sdn. Bhd. were already known to produce and sell their product by using the process flow.

S. Sommerfeld and J. Strube (2005) in their journal, has reviewed and compared several mAb production processes and proposed it framework by taking consideration some equipment for better mAb production. The mAb that has been reviewed are already in the market, like Herceptin$^{TM}$, Rituxan, MabCampath$^{TM}$, Synagis$^{TM}$, Remicade$^{TM}$, and Simulect$^{TM}$.

Figure 4.3, 4.4 and 4.5 shows the framework for three generic downstream processes of mAb production. For these three flows, the process were just slightly different from one another in type of equipment but shares same downstream step or stage that is recovery, purification and polishing stage as illustrated in the Table 4.3.

Recovery step was more on product isolation, removal of cell biomass and concentrated before it was further process. Purification step was a step where bulk impurities are removed and the final purity was achieved at the polishing step where the remaining impurities are removed to get high purity of product.

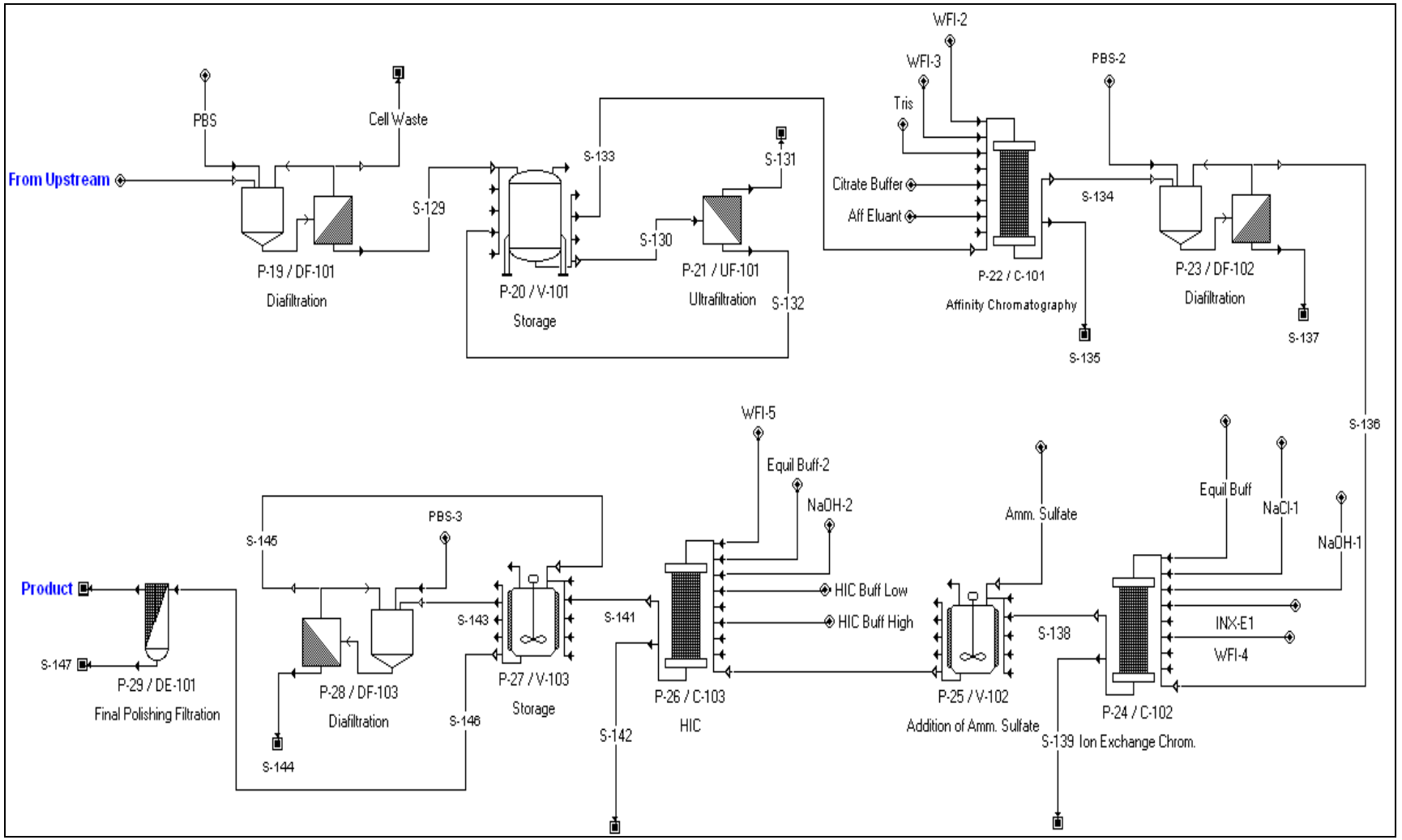


Figure 4.3: Downstream process for SPD source

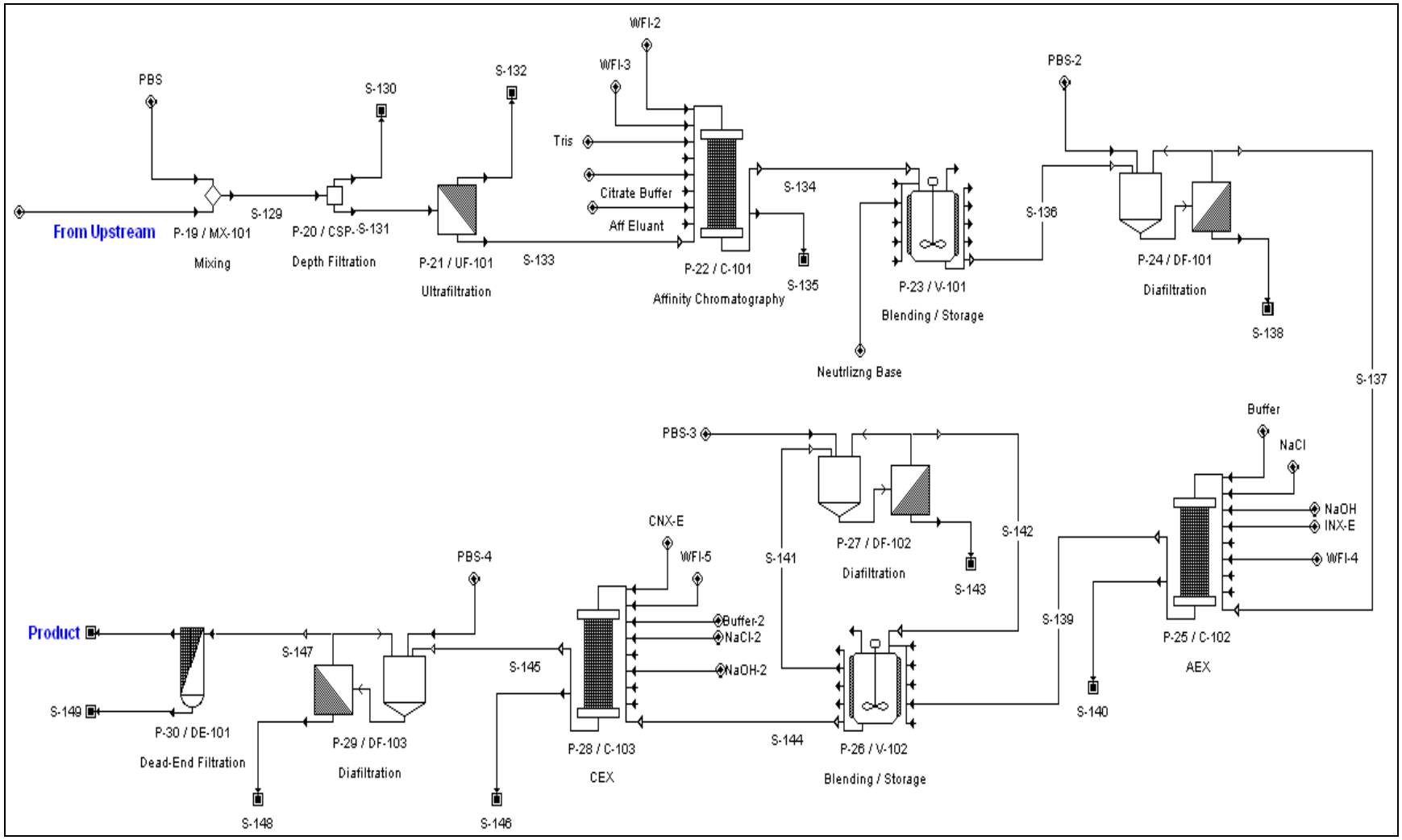


Figure 4.4: Downstream process for Inno Biologics Sdn. Bhd.

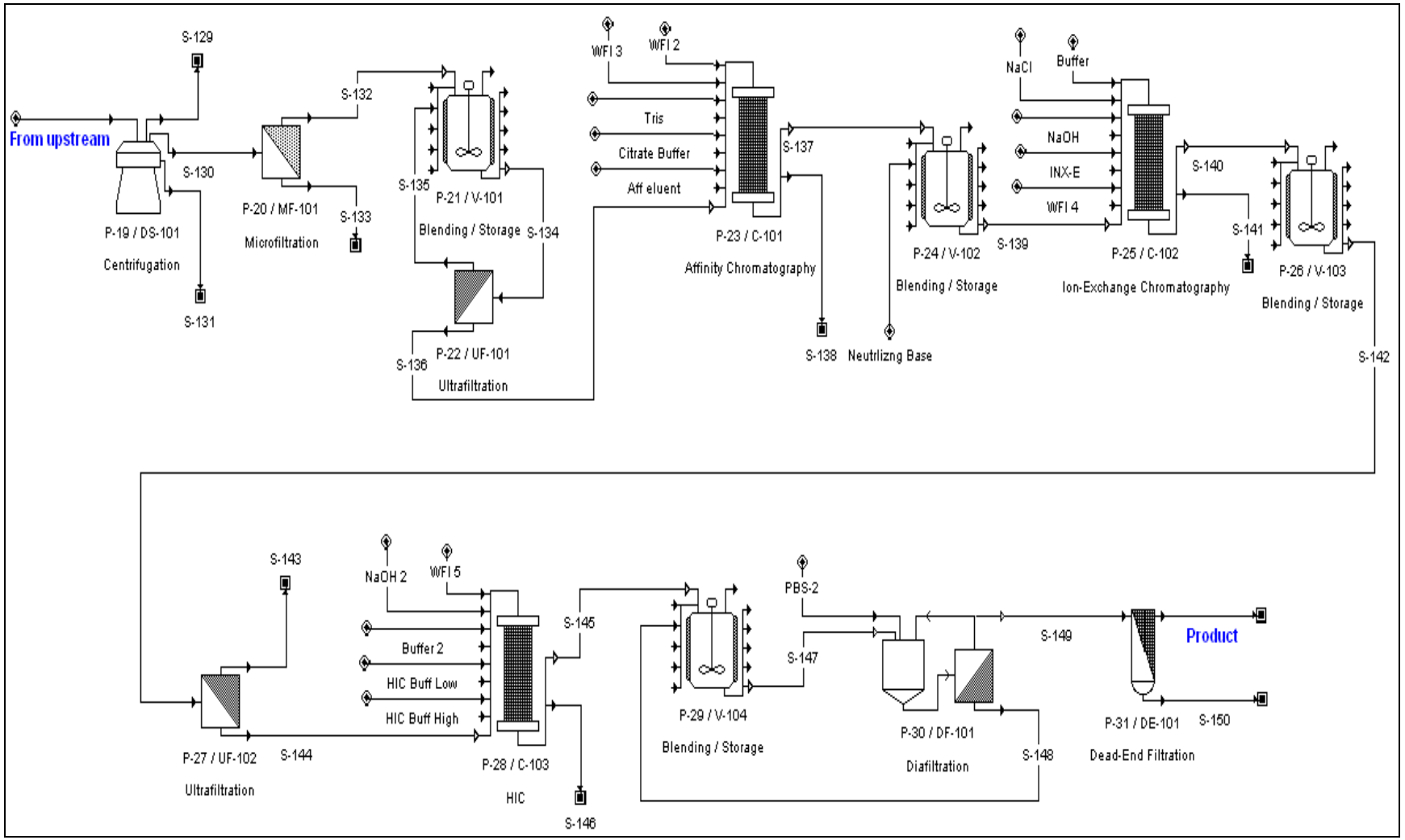


Figure 4.5: Downstream process for Journal (S. Sommerfeld and J. Strube, 2005)

Table 4.3: Stages for the three downstream processes involved.

| Stage | Unit Operations | | |
|---|---|---|---|
| | SPD source | Inno Biologics Sdn. Bhd. | Journal (S. Sommerfeld and J. Strube, 2005) |
| Recovery | - Disc-Stake Centrifuge<br>- Microfiltration | - Depth Filtration<br>- Ultrafiltration | - Diafiltration |
| Purification | - Affinity Chromatography | - Affinity Chromatography | - Affinity Chromatography |
| Polishing | - Anion-Exchange Chromatography<br>- Hydrophobic Chromatography<br>- Dead-end Filtration | - Anion-Exchange Chromatography<br>- Cation-exchange Chromatography<br>- Dead-end Filtration | - Anion-Exchange Chromatography<br>- Hydrophobic Chromatography<br>- Dead-end Filtration |

### 4.2.2 Simulation of the Three Downstream Processes Selected.

After the flow was built, the operational data and the input for downstream processes are inserted which was taken from the upstream process. Table 4.4 shows the simulation results for the three downstream processes. Some properties of the same equipments are assumed similar which are chromatography unit, 95% yield of mAb for affinity, 90% yield for ion exchange and 95% for HIC. For filtration, it was assumed to permeate 100% of mAb. These assumptions were made because some data and details for certain process equipment are not available. The framework for Inno Biologics Sdn. Bhd. and the journal were only the flow but do not have the operational data. So, the basis of the operational data was taken from the SPD source.

Table 4.4: Simulation result for product of three downstream processes involved.

| Composition | SPD source | | | Inno Biologics Sdn. Bhd. | | | Journal (S. Sommerfeld and J. Strube, 2005) | | |
|---|---|---|---|---|---|---|---|---|---|
| | Flowrate (kg/Batch) | Mass Comp. (%) | Concentration (g/L) | Flowrate (kg/Batch) | Mass Comp. (%) | Concentration (g/L) | Flowrate (kg/Batch) | Mass Comp. (%) | Concentration (g/L) |
| Amm. Sulfate | 0.00019 | 0.00060 | 0.00611 | - | - | - | 0.00000 | 0.00060 | 0.00604 |
| KCl | 0.00000 | 0.00000 | 0.00000 | - | - | - | - | - | - |
| $KH_2PO_4$ | 0.00000 | 0.00000 | 0.00000 | - | - | - | - | - | - |
| mAb | 0.00400 | 0.01300 | 0.12968 | 0.00414 | 0.28700 | 2.85350 | 0.00347 | 0.49100 | 4.88260 |
| $Na_2HPO_4$ | 0.00003 | 0.00010 | 0.00089 | 0.00000 | 0.00010 | 0.00051 | 0.00000 | 0.00010 | 0.00113 |
| Sodium Chloride | 0.00006 | 0.00020 | 0.00185 | 0.00001 | 0.00040 | 0.00367 | 0.00000 | 0.00040 | 0.00367 |
| WFI | 30.69179 | 99.98610 | 994.56974 | 1.43943 | 99.7126 | 991.56132 | 0.70315 | 99.5079 | 989.52728 |
| | Total flowrate:<br>Mass flow = 30.696 kg/Batch<br>Volumetric flow = 30.859 L/Batch | | | Total flowrate:<br>Mass flow = 1.444 kg/Batch<br>Volumetric flow = 1.452 L/Batch | | | Total flowrate:<br>Mass flow = 0.707 kg/Batch<br>Volumetric flow = 0.711 L/Batch | | |

### 4.2.3 Selection for the Best Downstream Process.

Table 4.5 shows that the best downstream process for large scale mAb production from the laboratory data was the work flow from Inno Biologics Sdn. Bhd. This flow had recovered high mAb with 81% yield and 4.14328 g/Batch of concentration. Because the outlets for each downstream process are different, the concentration of mAb was calculated by multiplying the total volumetric flow (in L/Batch) of outlet with the concentration of mAb (in g/L). Yield of mAb was calculated by dividing the outlet flow rate of mAb with inlet flow rate of mAb and multiply by 100%.

Table 4.5: Comparison between three downstream process for the amount of mAb.

| Framework | Flowrate (kg/Batch) | Concentration (g/Batch) | Yield (%) |
|---|---|---|---|
| SPD source | 0.00400 | 4.00180 | 78 |
| Inno Biologics Sdn. Bhd. | 0.00414 | 4.14328 | 81 |
| Journal (S. Sommerfeld and J. Strube, 2005.) | 0.00347 | 3.47153 | 68 |

The three framework of downstream process differ in terms of yield, mass flow and concentration of mAb because some equipment give differ yield of product. In this case, a factor that contributes to this different was in the recovery step. Even though the data inserted for microfiltration is 100% permeated for mAb, but the simulation by using SPD had automatically decreased the amount of mAb compared to the others flow.

Another differences for the three flow was the total volumetric flow for product output for each downstream process. As can be seen in the Table 4.4, the volumetric

flow rate for SPD source was higher and had more composition of chemical compared to the other process. This could be explained by observing the flow process of the three downstream processes in Figure 4.2, 4.3 and 4.4. The final polishing step consists of diaflitration and dead-end filtration. For SPD source flow, the input stream for dead-end filtration was the output stream of storage tank that has been concentrated by diafiltration with recycling process.

Compared to the journal (S. Sommerfeld and J. Strube, 2005.) flow, the input stream for dead-end filtration was directly come from the output stream of diaflitration that had lower volume and less composition of chemical. For Inno Biologics Sdn. Bhd., the input stream for dead-end filtration was the output of diafiltration with no recycling process and the volume was already lowered from the cation-exchange chromatography. Because of this reason, that is why the volumetric flow rate for SPD source was higher and had more composition of chemical compared to the other process.

The result of simulation can become the milestone for real large scale production in industry. Pilot scale experiment was based on the trial and error and known to be high cost and time consuming. This problem can be minimized by using the SPD to do the simulation first, and then construct the large scale production plant after taking consideration all factors for the plant optimization. After constructed the upstream process, built and choose the suitable downstream process for large scale production of mAb from laboratory data, further process can be done to facilitate the planning process through systematic and comprehensive consideration.

By making modification to this model or flow, different approach may be simulated to study their impact on production and profitability. In this case, fermentation with 20000 L of fermenter and purification process by Inno Biologics Sdn. Bhd. was the large scale production for mAb from laboratory data and the basic model for further process of plant wide simulation.

# CHAPTER 5

# CONCLUSION AND RECOMMENDATION

## 5.1 Conclusion

Simulation that mimic the laboratory experiment data from Mdm Chua @ Yeo Gek Kee had been successfully design by using SPD and resulting in 4% error when compared for both simulation and laboratory data. Upstream process for large scale mAb production had been successfully built at the SPD. From the framework that been built for upstream process, the flow is successful to be simulated. The value of simulation for mAb from a fermentation of 20000 L fermenter is 0.00510 kg/Batch with concentration of $3.8 \times 10^{-4}$ g/L (5.1103 g/Batch). For downstream process, three downstream process had been successfully built and simulated at the SPD. Comparing the three frameworks, the best downstream process was the framework from Inno Biologics Sdn. Bhd. that yield 81% of mAb with a concentration of 4.14328 g/Batch.

As a conclusion, the objective of this research had been achieved, that is to study the upstream and downstream process of mAb from laboratory data and to discover the potential for the large scale production of mAb by using the desired SPD.

## 5.2 Recommendation

Simulation study should be done by using the latest version of SPD that is SPD v7.5. This version of SPD has more added advantages and features compare to version used for this study. The study can be done with more accurate and give a better result for the large scale production of mAb.

With some additional information, SPD can be use as a platform for optimization process of laboratory experiment and large scale production. Parameters like temperature, agitation rate and aeration rate are possible to be optimize by using simulation of SPD.

The study should be expand or include the costing and process scheduling for large scale production of mAb. Simulation by using SPD is capable of estimate the fixed capital investment, cash flow analysis and other cost analysis. Other than that, include scheduling can make SPD has an ability to identify bottlenecks and formulate strategies to increase throughput by debottlenecking.

## REFERENCES


Abhinav A. Shukla, Brian Hubbard, Tim Tressel, Sam Guhan, Duncan Low (2007). Downstream processing of monoclonal antibodies - Application of platform approaches. *Journal of Chromatography B*. 848: 28–39

Adam C. Baughman, Xinqun Huang, Susan T. Sharfstein, Lealon L. Martin (2009). On the dynamic modeling of mammalian cell metabolism and mAb production. *Computers and Chemical Engineering.* 34: 210–242

Alberto L. Horenstein, Federico Crivellin, Ada Funaro,Marcela Said, Fabio Malavasi (2003). Design and scaleup of downstream processing of monoclonal antibodies for cancer therapy: from research to clinical proof of principle. *Journal of Immunological Method.* 275: 99– 112

A. Teixeira, A.E. Cunha, J.J. Clemente, J.L. Moreira, H.J. Cruz, P.M. Alves, M.J.T. Carrondo, R. Oliveira (2005). Modelling and optimization of a recombinant BHK-21 cultivation process using hybrid grey-box systems. *Journal of Biotechnology*. 118: 290–303

Cleo Kontoravdi, Steven P. Asprey, Efstrains N. Pistikopoulos, Athanasios Mantalaris (2007). Development of a dynamic model of monoclonalantibody production and glycosylation for product quality monitoring. *Computers and Chemical Engineering*. 31: 392 – 400

David M. Marks (2003). Equipment design considerations for large scale cell culture. *Cytotechnology* 42: 21–33

Duncan Low, Rhona O'Leary, Narahari S. Pujar (2007). Future of antibody purification. *Journal of Chromatography B*. 848: 48–63

Eduardo B. Rodrigues, Michel E. Farah, Maurı´cio Maia, Fernando M. Penha, Caio Regatieri, Gustavo B. Melo, Marcelo M. Pinheiro, Carlos R. Zanetti. (2009). Therapeutic monoclonal antibodies in ophthalmology. *Progress in Retinal and Eye Research*. 28: 117–144

Era Jain, Ashok Kumar (2008). Upstream processes in antibody production: Evaluation of critical parameters. *Biotechnology Advances* 26: 46–72

E. Rosenberg, S. Hepbildikler, W. Kuhne, G.Winter (2009). Ultrafiltration concentration of monoclonal antibody solutions: Development of an optimized method minimizing aggregation. *Journal of Membrane Science*. 342 : 50–59

Feng Li, Joe X. Zhou, Xiaoming Yang, Tim Tressel, Brian Lee (2005). Current Therapeutic Antibody Production and Process Optimization. *Bioprocessing Journal.* 4: 1 – 8

Ian Gosling, (2003, September). Process Simulation and Modeling Strategies for the Biotechnology Industry- Optimizing Productivity in Multiproduct Batch Facilities. *Genetic Engineering News*.

I. Y. Abdel-Ghany, A. S. El-Bayoumy, N. R. A. Elmouhty, H. M. Shafik (2009). Production and evaluation of monoclonal antibodies for alphafetoprotein. *Journal of Radioanalytical and Nuclear Chemistry*. 280: 651–655

Jae Deog Jang, John P. Barford (2000). An unstructured kinetic model of macromolecular metabolism in batch and fed-batch cultures of hybridoma cells producing monoclonal antibody. *Biochemical Engineering Journal*. 4: 153–168

John R. Birch, Andrew J. Racher (2006). Antibody production. *Advanced Drug Delivery Review.* 58: 671 – 685

Loreo Legazpi, Jaime Diaz, Adriana Laca, Mario Diaz (2005). Kinetic analysis of hybridoma cell culture in a protein-free medium: Substrate and agitation effects. *Biochemical Engineering Journal.*26: 122-130

M. Butler. *Animal Cell Culture and Technology.* 2nd edition. West 35th Street, N.Y: Garland Science/BIOS Scientific. 2004

Penny Dorka, Christian Fischer, Hector Budman, Jeno M. Scharer (2009). Metabolic flux-based modeling of mAb production during batch and fed-batch operations. *Bioprocess Biosyst Eng.* 32:183–196

Prescott *et al*. *Microbiology*. 6th edition. New York: McGraw-Hill. 2007

Raja Ghosh. *Principles of Bioseparation Engineering*. Singapore: World Scientific Publishing Company. 2006

S.A. Rouf, P.L. Douglas, M. Moo-Young, J.M. Scharer (2001). Computer simulation for large scale bioprocess design. *Biochemical Engineering Journal*. 8: 229–234

Steve K.W. Oh, K.H. Kuek, Victor V.T. Wong (2004). Design, Simulation, and Optimization of a Large-Scale Monoclonal Antibody Production Plant: Upstream Design. *Pharmaceutical engineering*. 24: 42–60

Suzanne S. Farid (2007). Process economics of industrial monoclonal antibody manufacture. *Journal of Chromatography B.* 848(1): 8-18

Sven Sommerfeld , Jochen Strube (2005). Challenges in biotechnology production – generic processes and process optimization for monoclonal antibodies. *Chemical Engineering and Processing* 44: 1123–1137

Takeshi Tsumuraya, Ikuo Fujii, Masahiro Hirama (2009). Production of monoclonal antibodies for sandwich immunoassay detection of Pacific ciguatoxins. *Toxicon*. Article in Press: 1–7

Ting-Kuo Huang, Karen A. McDonald (2009). Bioreactor engineering for recombinant protein production in plant cell suspension cultures. *Biochemical Engineering Journal*. 45: 168–184

Wei-Shou Hu (2004). Stoichiometry and kinetics of cell growth and product formation. *Cellular Bioprocess Technology.* 1–18

Y.H. Guan, R.B. Kemp (1999). Detection of the changing substrate requirements of cultured animal cells by stoichiometric growth equations validated by enthalphy balances. *Journal of Biotechnology.* 69: 95–114

# APPENDIX A

## Calculation for stoichiometric coefficient

$C_6H_{12}O_6$ (Glucose) + $C_5H_{10}O_3N_2$ (Glutamine) + $O_2$ (Oxygen) ⟶

$CH_{1.8}O_{0.5}N_{0.2}$ (Biomass) + $CH_{1.83}O_{2.06}N_{0.14}$ (mAb) + $CO_2$ (Carbon dioxide) + $H_2O$ (water) + $NH_3$ (Ammonia) + $C_3H_6O_3$ (Lactate)

By using the laboratory data,

Initial conc. for i) Glucose = 24.42 mmol/L and

ii) Glutamine = 3.69 mmol/L

Final conc. for i) Lactate = 29.03 mmol/L

ii) Ammonia = 5.32 mmol/L

iii) mAb = $9.07 \times 10^{-3}$ mmol/L

Elemental Balance:

$24.42C_6H_{12}O_6$ (Glucose) + $3.69C_5H_{10}O_3N_2$ (Glutamine) + **a** $O_2$ (Oxygen) ⟶

**b** $CH_{1.8}O_{0.5}N_{0.2}$ (Biomass) + $9.07 \times 10^{-3}$ $CH_{1.83}O_{2.06}N_{0.14}$ (mAb) + **c** $CO_2$ (Carbon dioxide) + **d** $H_2O$ (water) + $5.32NH_3$ (Ammonia) + $29.03C_3H_6O_3$ (Lactate)

C balance:

$6 + 0.75 = \mathbf{b}/24.42 + 0.000371 + \mathbf{c}/24.42 + 3.57$

H balance:

$12 + 1.5 = 0.074\mathbf{b} + 6.7893 \times 10^{-4} + 0.082\mathbf{d} + 0.66 + 7.14$

O balance:

$6 + 0.45 + 0.082\mathbf{a} = 0.02\mathbf{b} + 7.6426 \times 10^{-4} + 0.082\mathbf{c} + d/24.42$

N Balance:

$0.3 = 8.19 \times 10^{-3}\ \mathbf{b} + 5.194 \times 10^{-5} + 0.22$

**a** = 65.45

**b** = 9.76

**c** = 67.87

**d** = 60.7

Stoichiometric equation:

$C_6H_{12}O_6$ (Glucose) + **0.15**$C_5H_{10}O_3N_2$ (Glutamine) + **2.68**$O_2$ (Oxygen) ⟶

**0.4**$CH_{1.8}O_{0.5}N_{0.2}$ (Biomass) + **0.000371**$CH_{1.83}O_{2.06}N_{0.14}$ (mAb) + **2.78**$CO_2$ (Carbon dioxide) + **2.49**$H_2O$ (water) + **0.22**$NH_3$ (Ammonia) + **1.19**$C_3H_6O_3$ (Lactate)

# APPENDIX B

## Stream details of Upstream Process in SPD report

| Stream Name | S-123 | S-124 | S-125 | S-114 |
|---|---|---|---|---|
| Source | INPUT | P-16 | P-15 | INPUT |
| Destination | P-16 | P-15 | P-14 | P-8 |
| Stream Properties | | | | |
| Activity (U/ml) | 0.00 | 0.00 | 0.00 | 0.00 |
| Temperature (°C) | 25.00 | 40.00 | 40.00 | 25.00 |
| Pressure (bar) | 1.01 | 6.01 | 6.01 | 1.01 |
| Density (g/L) | 1.18 | 6.66 | 6.66 | 1.18 |
| Component Flowrates (kg/batch) | | | | |
| Nitrogen | 29,084.437 | 29,084.437 | 29,084.437 | 2,882.181 |
| Oxygen | 8,829.472 | 8,829.472 | 8,829.472 | 874.974 |
| TOTAL (kg/batch) | 37,913.908 | 37,913.908 | 37,913.908 | 3,757.155 |
| TOTAL (L/batch) | 32,153,016.314 | 5,689,283.360 | 5,689,283.360 | 3,186,267.955 |

| Stream Name | S-115 | S-116 | S-105 | S-106 |
|---|---|---|---|---|
| Source | P-8 | P-10 | INPUT | P-3 |
| Destination | P-10 | P-11 | P-3 | P-4 |
| Stream Properties | | | | |
| Activity (U/ml) | 0.00 | 0.00 | 0.00 | 0.00 |
| Temperature (°C) | 40.00 | 40.00 | 25.00 | 40.00 |
| Pressure (bar) | 6.01 | 6.01 | 1.01 | 6.01 |
| Density (g/L) | 6.66 | 6.66 | 1.18 | 6.66 |
| Component Flowrates (kg/batch) | | | | |
| Nitrogen | 2,882.181 | 2,882.181 | 261.664 | 261.664 |
| Oxygen | 874.974 | 874.974 | 79.436 | 79.436 |
| TOTAL (kg/batch) | 3,757.155 | 3,757.155 | 341.101 | 341.101 |
| TOTAL (L/batch) | 563,791.001 | 563,791.001 | 289,271.435 | 51,184.845 |

| Stream Name | S-107 | Media | WFI | S-101 |
|---|---|---|---|---|
| Source | P-4 | INPUT | INPUT | P-1 |
| Destination | P-6 | P-1 | P-1 | P-2 |
| Stream Properties | | | | |
| Activity (U/ml) | 0.00 | 0.00 | 0.00 | 0.00 |
| Temperature (°C) | 40.00 | 25.00 | 25.00 | 25.00 |
| Pressure (bar) | 6.01 | 1.01 | 1.01 | 1.01 |
| Density (g/L) | 6.66 | 1,151.02 | 994.70 | 995.33 |
| Component Flowrates (kg/batch) | | | | |
| Glucose | 0.000 | 48.836 | 0.000 | 48.836 |
| Glutamine | 0.000 | 5.994 | 0.000 | 5.994 |
| Nitrogen | 261.664 | 0.000 | 0.000 | 0.000 |
| Oxygen | 79.436 | 0.000 | 0.000 | 0.000 |
| WFI | 0.000 | 0.000 | 11,793.750 | 11,793.750 |
| TOTAL (kg/batch) | 341.101 | 54.830 | 11,793.750 | 11,848.580 |
| TOTAL (L/batch) | 51,184.845 | 47.636 | 11,856.538 | 11,904.174 |

| Stream Name | S-120 | S-111 | S-102 | S-103 |
|---|---|---|---|---|
| Source | P-2 | P-2 | P-2 | P-5 |
| Destination | P-13 | P-9 | P-5 | P-6 |
| Stream Properties | | | | |
| Activity (U/ml) | 0.00 | 0.00 | 0.00 | 0.00 |
| Temperature (°C) | 25.00 | 25.00 | 25.00 | 25.00 |
| Pressure (bar) | 1.01 | 1.01 | 1.01 | 1.01 |
| Density (g/L) | 995.33 | 995.33 | 995.33 | 995.33 |
| Component Flowrates (kg/batch) | | | | |
| Glucose | 43.997 | 4.400 | 0.440 | 0.440 |
| Glutamine | 5.400 | 0.540 | 0.054 | 0.054 |
| WFI | 10,624.989 | 1,062.617 | 106.144 | 106.144 |
| TOTAL (kg/batch) | 10,674.386 | 1,067.557 | 106.637 | 106.637 |
| TOTAL (L/batch) | 10,724.471 | 1,072.566 | 107.138 | 107.138 |

| Stream Name | S-108 | S-110 | S-109 | S-112 |
|---|---|---|---|---|
| Source | P-6 | P-6 | P-7 | P-9 |
| Destination | P-7 | P-11 | OUTPUT | P-11 |
| Stream Properties | | | | |
| Activity (U/ml) | 0.00 | 0.00 | 0.00 | 0.00 |
| Temperature (°C) | 20.00 | 37.00 | 20.00 | 25.00 |
| Pressure (bar) | 1.01 | 1.01 | 1.01 | 1.01 |
| Density (g/L) | 1.20 | 885.68 | 1.20 | 995.33 |
| Component Flowrates (kg/batch) | | | | |
| Ammonia | 0.000 | 0.009 | 0.000 | 0.000 |
| Biomass | 0.000 | 0.022 | 0.000 | 0.000 |
| Carb. Dioxide | 0.279 | 0.000 | 0.279 | 0.000 |
| Glucose | 0.000 | 0.029 | 0.000 | 4.400 |
| Glutamine | 0.000 | 0.004 | 0.000 | 0.540 |
| Lactic Acid | 0.000 | 0.244 | 0.000 | 0.000 |
| mAb | 0.000 | 0.000 | 0.000 | 0.000 |
| Nitrogen | 261.664 | 0.000 | 261.664 | 0.000 |
| Oxygen | 79.241 | 0.000 | 79.241 | 0.000 |
| Water | 0.000 | 0.102 | 0.000 | 0.000 |
| WFI | 0.000 | 106.144 | 0.000 | 1,062.617 |
| TOTAL (kg/batch) | 341.184 | 106.554 | 341.184 | 1,067.557 |
| TOTAL (L/batch) | 284,425.816 | 120.307 | 284,425.816 | 1,072.566 |

| Stream Name | S-117 | S-119 | S-118 | S-121 |
|---|---|---|---|---|
| Source | P-11 | P-11 | P-12 | P-13 |
| Destination | P-12 | P-14 | OUTPUT | P-14 |
| Stream Properties | | | | |
| Activity (U/ml) | 0.00 | 0.00 | 0.00 | 0.00 |
| Temperature (°C) | 20.00 | 37.00 | 20.00 | 25.00 |
| Pressure (bar) | 1.01 | 1.01 | 1.01 | 1.01 |
| Density (g/L) | 1.20 | 885.06 | 1.20 | 995.33 |
| Component Flowrates (kg/batch) | | | | |
| Ammonia | 0.000 | 0.095 | 0.000 | 0.000 |
| Biomass | 0.000 | 0.249 | 0.000 | 0.000 |
| Carb. Dioxide | 2.814 | 0.000 | 2.814 | 0.000 |
| Glucose | 0.000 | 0.286 | 0.000 | 43.997 |
| Glutamine | 0.000 | 0.040 | 0.000 | 5.400 |
| Lactic Acid | 0.000 | 2.710 | 0.000 | 0.000 |
| mAb | 0.000 | 0.001 | 0.000 | 0.000 |
| Nitrogen | 2,882.181 | 0.000 | 2,882.181 | 0.000 |
| Oxygen | 873.002 | 0.000 | 873.002 | 0.000 |
| Water | 0.000 | 1.134 | 0.000 | 0.000 |
| WFI | 0.000 | 1,168.761 | 0.000 | 10,624.989 |
| TOTAL (kg/batch) | 3,757.997 | 1,173.274 | 3,757.997 | 10,674.386 |
| TOTAL (L/batch) | 3,132,889.314 | 1,325.648 | 3,132,889.314 | 10,724.471 |

| Stream Name | S-126 | S-128 | To Downstream | S-127 |
|---|---|---|---|---|
| Source | P-14 | P-14 | P-18 | P-17 |
| Destination | P-17 | P-18 | OUTPUT | OUTPUT |
| Stream Properties | | | | |
| Activity (U/ml) | 0.00 | 0.00 | 0.00 | 0.00 |
| Temperature (°C) | 20.00 | 37.00 | 36.98 | 20.00 |
| Pressure (bar) | 1.01 | 1.01 | 1.01 | 1.01 |
| Density (g/L) | 1.20 | 885.00 | 885.01 | 1.20 |
| Component Flowrates (kg/batch) | | | | |
| Ammonia | 0.000 | 0.956 | 0.956 | 0.000 |
| Biomass | 0.000 | 2.515 | 2.515 | 0.000 |
| Carb. Dioxide | 28.136 | 0.000 | 0.000 | 28.136 |
| Glucose | 0.000 | 2.852 | 2.852 | 0.000 |
| Glutamine | 0.000 | 0.398 | 0.398 | 0.000 |
| Lactic Acid | 0.000 | 27.361 | 27.361 | 0.000 |
| mAb | 0.000 | 0.005 | 0.005 | 0.000 |
| Nitrogen | 29,084.437 | 0.000 | 0.000 | 29,084.437 |
| Oxygen | 8,809.750 | 0.000 | 0.000 | 8,809.750 |
| Water | 0.000 | 11.453 | 11.453 | 0.000 |
| WFI | 0.000 | 11,793.750 | 11,793.750 | 0.000 |
| TOTAL (kg/batch) | 37,922.322 | 11,839.289 | 11,839.289 | 37,922.322 |
| TOTAL (L/batch) | 31,614,360.905 | 13,377.791 | 13,377.648 | 31,614,360.905 |

# APPENDIX C

## Stream details of Downstream Process for SPD source in SPD report

| Stream Name | PBS | From Upstream | Cell Waste | S-129 |
|---|---|---|---|---|
| Source | INPUT | INPUT | P-19 | P-19 |
| Destination | P-19 | P-19 | OUTPUT | P-20 |
| Stream Properties | | | | |
| Activity (U/ml) | 0.00 | 0.00 | 0.00 | 0.00 |
| Temperature (°C) | 25.00 | 25.00 | 25.00 | 25.00 |
| Pressure (bar) | 1.01 | 1.01 | 1.01 | 1.01 |
| Density (g/L) | 1,000.00 | 891.94 | 994.01 | 920.91 |
| Component Flowrates (kg/batch) | | | | |
| Ammonia | 0.000 | 0.959 | 0.001 | 0.958 |
| Biomass | 0.000 | 2.510 | 2.510 | 0.000 |
| Glucose | 0.000 | 2.853 | 0.002 | 2.851 |
| Glutamine | 0.000 | 0.403 | 0.000 | 0.402 |
| KCl | 0.012 | 0.000 | 0.000 | 0.012 |
| KH2PO4 | 0.012 | 0.000 | 0.000 | 0.012 |
| Lactic Acid | 0.000 | 27.361 | 0.018 | 27.342 |
| mAb | 0.000 | 0.005 | 0.000 | 0.005 |
| Na2HPO4 | 6.543 | 0.000 | 0.004 | 6.538 |
| Sodium Chloride | 47.583 | 0.000 | 0.032 | 47.551 |
| Water | 0.000 | 11.449 | 0.008 | 11.441 |
| WFI | 5,893.695 | 11,793.750 | 1,092.239 | 16,595.207 |
| TOTAL (kg/batch) | 5,947.845 | 11,839.289 | 1,094.814 | 16,692.320 |
| TOTAL (L/batch) | 5,947.845 | 13,273.698 | 1,101.416 | 18,125.854 |

| Stream Name | S-130 | S-131 | S-132 | S-133 |
|---|---|---|---|---|
| Source | P-20 | P-21 | P-21 | P-20 |
| Destination | P-21 | OUTPUT | P-20 | P-22 |
| Stream Properties | | | | |
| Activity (U/ml) | 0.00 | 0.00 | 0.00 | 0.00 |
| Temperature (°C) | 25.00 | 25.00 | 25.00 | 25.00 |
| Pressure (bar) | 1.01 | 1.01 | 1.01 | 1.01 |
| Density (g/L) | 920.91 | 920.54 | 926.12 | 926.12 |
| Component Flowrates (kg/batch) | | | | |
| Ammonia | 0.958 | 0.899 | 0.059 | 0.059 |
| Glucose | 2.851 | 2.676 | 0.176 | 0.176 |
| Glutamine | 0.402 | 0.377 | 0.025 | 0.025 |
| KCl | 0.012 | 0.011 | 0.001 | 0.001 |
| KH2PO4 | 0.012 | 0.011 | 0.001 | 0.001 |
| Lactic Acid | 27.342 | 25.658 | 1.684 | 1.684 |
| mAb | 0.005 | 0.000 | 0.005 | 0.005 |
| Na2HPO4 | 6.538 | 6.135 | 0.403 | 0.403 |
| Sodium Chloride | 47.551 | 44.622 | 2.929 | 2.929 |
| Water | 11.441 | 10.736 | 0.705 | 0.705 |
| WFI | 16,595.207 | 15,488.536 | 1,106.671 | 1,106.671 |
| TOTAL (kg/batch) | 16,692.320 | 15,579.662 | 1,112.658 | 1,112.658 |
| TOTAL (L/batch) | 18,125.854 | 16,924.439 | 1,201.415 | 1,201.415 |

| Stream Name | WFI-2 | WFI-3 | Tris | Citrate Buffer |
|---|---|---|---|---|
| Source | INPUT | INPUT | INPUT | INPUT |
| Destination | P-22 | P-22 | P-22 | P-22 |
| Stream Properties | | | | |
| Activity (U/ml) | 0.00 | 0.00 | 0.00 | 0.00 |
| Temperature (°C) | 25.00 | 25.00 | 25.00 | 25.00 |
| Pressure (bar) | 1.01 | 1.01 | 1.01 | 1.01 |
| Density (g/L) | 994.70 | 994.70 | 1,075.55 | 1,000.00 |
| Component Flowrates (kg/batch) | | | | |
| Sodium Chloride | 0.000 | 0.000 | 0.272 | 0.000 |
| Sodium Citrate | 0.000 | 0.000 | 0.000 | 0.004 |
| TRIS HCl | 0.000 | 0.000 | 0.009 | 0.000 |
| WFI | 1.014 | 2.704 | 1.546 | 2.035 |
| TOTAL (kg/batch) | 1.014 | 2.704 | 1.827 | 2.039 |
| TOTAL (L/batch) | 1.019 | 2.718 | 1.699 | 2.039 |

| Stream Name | Aff Eluant | S-134 | S-135 | PBS-2 |
|---|---|---|---|---|
| Source | INPUT | P-22 | P-22 | INPUT |
| Destination | P-22 | P-23 | OUTPUT | P-23 |
| Stream Properties | | | | |
| Activity (U/ml) | 0.00 | 0.00 | 0.00 | 0.00 |
| Temperature (°C) | 25.00 | 25.00 | 25.00 | 25.00 |
| Pressure (bar) | 1.01 | 1.01 | 1.01 | 1.01 |
| Density (g/L) | 1,000.00 | 995.53 | 926.78 | 1,000.00 |
| Component Flowrates (kg/batch) | | | | |
| Ammonia | 0.000 | 0.000 | 0.059 | 0.000 |
| Glucose | 0.000 | 0.000 | 0.176 | 0.000 |
| Glutamine | 0.000 | 0.000 | 0.025 | 0.000 |
| KCl | 0.000 | 0.000 | 0.001 | 0.000 |
| KH2PO4 | 0.000 | 0.000 | 0.001 | 0.000 |
| Lactic Acid | 0.000 | 0.000 | 1.684 | 0.000 |
| mAb | 0.000 | 0.005 | 0.000 | 0.000 |
| Na2HPO4 | 0.000 | 0.000 | 0.403 | 0.023 |
| Sodium Chloride | 0.000 | 0.000 | 3.201 | 0.164 |
| Sodium Citrate | 0.007 | 0.004 | 0.007 | 0.000 |
| TRIS HCl | 0.000 | 0.000 | 0.009 | 0.000 |
| Water | 0.000 | 0.000 | 0.705 | 0.000 |
| WFI | 4.070 | 2.035 | 1,116.004 | 20.339 |
| TOTAL (kg/batch) | 4.077 | 2.043 | 1,122.275 | 20.526 |
| TOTAL (L/batch) | 4.077 | 2.053 | 1,210.942 | 20.526 |

| Stream Name | S-136 | S-137 | Equil Buff | NaCl-1 |
|---|---|---|---|---|
| Source | P-23 | P-23 | INPUT | INPUT |
| Destination | P-24 | OUTPUT | P-24 | P-24 |
| Stream Properties | | | | |
| Activity (U/ml) | 0.00 | 0.00 | 0.00 | 0.00 |
| Temperature (°C) | 25.00 | 25.00 | 25.00 | 25.00 |
| Pressure (bar) | 1.01 | 1.01 | 1.01 | 1.01 |
| Density (g/L) | 994.70 | 998.91 | 999.74 | 1,030.00 |
| Component Flowrates (kg/batch) | | | | |
| KCl | 0.000 | 0.000 | 0.000 | 0.000 |
| KH2PO4 | 0.000 | 0.000 | 0.000 | 0.000 |
| mAb | 0.005 | 0.000 | 0.000 | 0.000 |
| Na2HPO4 | 0.000 | 0.023 | 1.555 | 0.000 |
| Sodium Chloride | 0.000 | 0.164 | 0.162 | 8.256 |
| Sodium Citrate | 0.000 | 0.004 | 0.000 | 0.000 |
| Water | 0.000 | 0.000 | 0.000 | 137.357 |
| WFI | 1.012 | 21.362 | 139.618 | 0.000 |
| TOTAL (kg/batch) | 1.016 | 21.553 | 141.336 | 145.613 |
| TOTAL (L/batch) | 1.022 | 21.576 | 141.372 | 141.372 |

| Stream Name | NaOH-1 | INX-E1 | WFI-4 | S-138 |
|---|---|---|---|---|
| Source | INPUT | INPUT | INPUT | P-24 |
| Destination | P-24 | P-24 | P-24 | P-25 |
| Stream Properties | | | | |
| Activity (U/ml) | 0.00 | 0.00 | 0.00 | 0.00 |
| Temperature (°C) | 25.00 | 25.00 | 25.00 | 25.00 |
| Pressure (bar) | 1.01 | 1.01 | 1.01 | 1.01 |
| Density (g/L) | 1,021.00 | 1,028.93 | 994.70 | 1,011.74 |
| Component Flowrates (kg/batch) | | | | |
| mAb | 0.000 | 0.000 | 0.000 | 0.004 |
| NaH2PO4 | 0.000 | 0.979 | 0.000 | 0.489 |
| Sodium Chloride | 0.000 | 5.164 | 0.000 | 2.582 |
| Sodium Hydroxid | 2.122 | 0.000 | 0.000 | 0.000 |
| Water | 106.134 | 0.000 | 0.000 | 0.000 |
| WFI | 0.000 | 84.387 | 88.260 | 86.323 |
| TOTAL (kg/batch) | 108.255 | 90.530 | 88.260 | 89.399 |
| TOTAL (L/batch) | 106.029 | 87.985 | 88.730 | 88.362 |

| Stream Name | S-139 | Amm. Sulfate | S-140 | WFI-5 |
|---|---|---|---|---|
| Source | P-24 | INPUT | P-25 | INPUT |
| Destination | OUTPUT | P-25 | P-26 | P-26 |
| Stream Properties | | | | |
| Activity (U/ml) | 0.00 | 0.00 | 0.00 | 0.00 |
| Temperature (°C) | 25.00 | 25.00 | 25.00 | 25.00 |
| Pressure (bar) | 1.01 | 1.01 | 1.01 | 1.01 |
| Density (g/L) | 1,009.78 | 1,769.00 | 1,124.75 | 994.70 |
| Component Flowrates (kg/batch) | | | | |
| Amm. Sulfate | 0.000 | 27.419 | 27.419 | 0.000 |
| mAb | 0.000 | 0.000 | 0.004 | 0.000 |
| Na2HPO4 | 1.555 | 0.000 | 0.000 | 0.000 |
| NaH2PO4 | 0.489 | 0.000 | 0.489 | 0.000 |
| Sodium Chloride | 11.001 | 0.000 | 2.582 | 0.000 |
| Sodium Hydroxid | 2.122 | 0.000 | 0.000 | 0.000 |
| Water | 243.490 | 0.000 | 0.000 | 0.000 |
| WFI | 226.953 | 0.000 | 86.323 | 156.248 |
| TOTAL (kg/batch) | 485.611 | 27.419 | 116.819 | 156.248 |
| TOTAL (L/batch) | 480.908 | 15.500 | 103.862 | 157.080 |

| Stream Name | Equil Buff-2 | NaOH-2 | HIC Buff Low | HIC Buff High |
|---|---|---|---|---|
| Source | INPUT | INPUT | INPUT | INPUT |
| Destination | P-26 | P-26 | P-26 | P-26 |
| Stream Properties | | | | |
| Activity (U/ml) | 0.00 | 0.00 | 0.00 | 0.00 |
| Temperature (°C) | 25.00 | 25.00 | 25.00 | 25.00 |
| Pressure (bar) | 1.01 | 1.01 | 1.01 | 1.01 |
| Density (g/L) | 1,081.89 | 1,013.45 | 1,000.38 | 1,153.55 |
| Component Flowrates (kg/batch) | | | | |
| Amm. Sulfate | 23.561 | 0.000 | 0.000 | 16.604 |
| Na2HPO4 | 1.577 | 0.000 | 1.091 | 0.630 |
| Sodium Hydroxid | 0.000 | 4.906 | 0.000 | 0.000 |
| WFI | 110.816 | 122.448 | 77.234 | 37.409 |
| TOTAL (kg/batch) | 135.954 | 127.354 | 78.324 | 54.643 |
| TOTAL (L/batch) | 125.664 | 125.664 | 78.294 | 47.369 |

| Stream Name | S-141 | S-142 | PBS-3 | S-143 |
|---|---|---|---|---|
| Source | P-26 | P-26 | INPUT | P-27 |
| Destination | P-27 | OUTPUT | P-28 | P-28 |
| Stream Properties | | | | |
| Activity (U/ml) | 0.00 | 0.00 | 0.00 | 0.00 |
| Temperature (°C) | 25.00 | 25.00 | 25.00 | 25.00 |
| Pressure (bar) | 1.01 | 1.01 | 1.01 | 1.01 |
| Density (g/L) | 1,058.11 | 1,048.27 | 1,000.00 | 1,058.11 |
| Component Flowrates (kg/batch) | | | | |
| Amm. Sulfate | 8.302 | 59.282 | 0.000 | 8.302 |
| KCl | 0.000 | 0.000 | 0.001 | 0.000 |
| KH2PO4 | 0.000 | 0.000 | 0.001 | 0.000 |
| mAb | 0.004 | 0.000 | 0.000 | 0.004 |
| Na2HPO4 | 0.860 | 2.438 | 0.346 | 0.860 |
| NaH2PO4 | 0.000 | 0.489 | 0.000 | 0.000 |
| Sodium Chloride | 0.000 | 2.582 | 2.513 | 0.000 |
| Sodium Hydroxid | 0.000 | 4.906 | 0.000 | 0.000 |
| WFI | 57.321 | 533.156 | 311.320 | 57.321 |
| TOTAL (kg/batch) | 66.488 | 602.853 | 314.180 | 66.488 |
| TOTAL (L/batch) | 62.836 | 575.096 | 314.180 | 62.836 |

| Stream Name | S-145 | S-144 | S-146 | Product |
|---|---|---|---|---|
| Source | P-28 | P-28 | P-27 | P-29 |
| Destination | P-27 | OUTPUT | P-29 | OUTPUT |
| Stream Properties | | | | |
| Activity (U/ml) | 0.00 | 0.00 | 0.00 | 0.00 |
| Temperature (°C) | 25.00 | 25.00 | 25.00 | 25.00 |
| Pressure (bar) | 1.01 | 1.01 | 1.01 | 1.01 |
| Density (g/L) | 994.71 | 1,010.14 | 994.71 | 994.71 |
| Component Flowrates (kg/batch) | | | | |
| Amm. Sulfate | 0.000 | 8.302 | 0.000 | 0.000 |
| KCl | 0.000 | 0.001 | 0.000 | 0.000 |
| KH2PO4 | 0.000 | 0.001 | 0.000 | 0.000 |
| mAb | 0.004 | 0.000 | 0.004 | 0.004 |
| Na2HPO4 | 0.000 | 1.206 | 0.000 | 0.000 |
| Sodium Chloride | 0.000 | 2.513 | 0.000 | 0.000 |
| WFI | 30.692 | 337.949 | 30.692 | 30.692 |
| TOTAL (kg/batch) | 30.696 | 349.972 | 30.696 | 30.696 |
| TOTAL (L/batch) | 30.859 | 346.459 | 30.859 | 30.859 |

# APPENDIX D

## Stream details of Downstream Process for Inno Biologics Sdn. Bhd. in SPD report

| Stream Name | PBS | From Upstream | S-129 | S-130 |
|---|---|---|---|---|
| Source | INPUT | INPUT | P-19 | P-20 |
| Destination | P-19 | P-19 | P-20 | OUTPUT |
| Stream Properties | | | | |
| Activity (U/ml) | 0.00 | 0.00 | 0.00 | 0.00 |
| Temperature (°C) | 25.00 | 25.00 | 25.00 | 25.00 |
| Pressure (bar) | 1.01 | 1.01 | 1.01 | 1.01 |
| Density (g/L) | 1,000.00 | 891.94 | 894.85 | 1,050.00 |
| Component Flowrates (kg/batch) | | | | |
| Ammonia | 0.000 | 0.959 | 0.959 | 0.000 |
| Biomass | 0.000 | 2.510 | 2.510 | 2.510 |
| Glucose | 0.000 | 2.853 | 2.853 | 0.000 |
| Glutamine | 0.000 | 0.403 | 0.403 | 0.000 |
| KCl | 0.001 | 0.000 | 0.001 | 0.000 |
| KH2PO4 | 0.001 | 0.000 | 0.001 | 0.000 |
| Lactic Acid | 0.000 | 27.361 | 27.361 | 0.000 |
| mAb | 0.000 | 0.005 | 0.005 | 0.000 |
| Na2HPO4 | 0.408 | 0.000 | 0.408 | 0.000 |
| Sodium Chloride | 2.965 | 0.000 | 2.965 | 0.000 |
| Water | 0.000 | 11.449 | 11.449 | 0.000 |
| WFI | 367.242 | 11,793.750 | 12,160.992 | 0.000 |
| TOTAL (kg/batch) | 370.616 | 11,839.289 | 12,209.905 | 2.510 |
| TOTAL (L/batch) | 370.616 | 13,273.698 | 13,644.671 | 2.390 |

| Stream Name | S-131 | S-132 | S-133 | WFI-2 |
|---|---|---|---|---|
| Source | P-20 | P-21 | P-21 | INPUT |
| Destination | P-21 | OUTPUT | P-22 | P-22 |
| Stream Properties | | | | |
| Activity (U/ml) | 0.00 | 0.00 | 0.00 | 0.00 |
| Temperature (°C) | 25.00 | 25.69 | 25.69 | 25.00 |
| Pressure (bar) | 1.01 | 1.01 | 1.01 | 1.01 |
| Density (g/L) | 894.82 | 892.14 | 903.58 | 994.70 |
| Component Flowrates (kg/batch) | | | | |
| Ammonia | 0.959 | 0.787 | 0.172 | 0.000 |
| Glucose | 2.853 | 2.340 | 0.513 | 0.000 |
| Glutamine | 0.403 | 0.330 | 0.072 | 0.000 |
| KCl | 0.001 | 0.001 | 0.000 | 0.000 |
| KH2PO4 | 0.001 | 0.001 | 0.000 | 0.000 |
| Lactic Acid | 27.361 | 22.441 | 4.919 | 0.000 |
| mAb | 0.005 | 0.000 | 0.005 | 0.000 |
| Na2HPO4 | 0.408 | 0.334 | 0.073 | 0.000 |
| Sodium Chloride | 2.965 | 2.432 | 0.533 | 0.000 |
| Water | 11.449 | 9.390 | 2.058 | 0.000 |
| WFI | 12,160.992 | 9,728.023 | 2,432.969 | 1.015 |
| TOTAL (kg/batch) | 12,207.395 | 9,766.079 | 2,441.316 | 1.015 |
| TOTAL (L/batch) | 13,642.281 | 10,946.794 | 2,701.819 | 1.020 |

| Stream Name | WFI-3 | Tris | Citrate Buffer | Aff Eluant |
|---|---|---|---|---|
| Source | INPUT | INPUT | INPUT | INPUT |
| Destination | P-22 | P-22 | P-22 | P-22 |
| Stream Properties | | | | |
| Activity (U/ml) | 0.00 | 0.00 | 0.00 | 0.00 |
| Temperature (°C) | 25.00 | 25.00 | 25.00 | 25.00 |
| Pressure (bar) | 1.01 | 1.01 | 1.01 | 1.01 |
| Density (g/L) | 994.70 | 1,075.55 | 1,000.00 | 1,000.00 |
| Component Flowrates (kg/batch) | | | | |
| Sodium Chloride | 0.000 | 0.272 | 0.000 | 0.000 |
| Sodium Citrate | 0.000 | 0.000 | 0.004 | 0.007 |
| TRIS HCl | 0.000 | 0.009 | 0.000 | 0.000 |
| WFI | 2.706 | 1.547 | 2.036 | 4.073 |
| TOTAL (kg/batch) | 2.706 | 1.828 | 2.040 | 4.080 |
| TOTAL (L/batch) | 2.720 | 1.700 | 2.040 | 4.080 |

| Stream Name | S-134 | S-135 | S-136 | PBS-2 |
|---|---|---|---|---|
| Source | P-22 | P-22 | P-23 | INPUT |
| Destination | P-23 | OUTPUT | P-24 | P-24 |
| Stream Properties | | | | |
| Activity (U/ml) | 0.00 | 0.00 | 0.00 | 0.00 |
| Temperature (°C) | 25.00 | 25.69 | 25.00 | 25.00 |
| Pressure (bar) | 1.01 | 1.01 | 1.01 | 1.01 |
| Density (g/L) | 995.53 | 903.96 | 995.53 | 1,000.00 |
| Component Flowrates (kg/batch) | | | | |
| Ammonia | 0.000 | 0.172 | 0.000 | 0.000 |
| Glucose | 0.000 | 0.513 | 0.000 | 0.000 |
| Glutamine | 0.000 | 0.072 | 0.000 | 0.000 |
| KCl | 0.000 | 0.000 | 0.000 | 0.000 |
| KH2PO4 | 0.000 | 0.000 | 0.000 | 0.000 |
| Lactic Acid | 0.000 | 4.919 | 0.000 | 0.000 |
| mAb | 0.005 | 0.000 | 0.005 | 0.000 |
| Na2HPO4 | 0.000 | 0.073 | 0.000 | 0.045 |
| Sodium Chloride | 0.000 | 0.805 | 0.000 | 0.329 |
| Sodium Citrate | 0.004 | 0.007 | 0.004 | 0.000 |
| TRIS HCl | 0.000 | 0.009 | 0.000 | 0.000 |
| Water | 0.000 | 2.058 | 0.000 | 0.000 |
| WFI | 2.036 | 2,442.308 | 2.036 | 40.705 |
| TOTAL (kg/batch) | 2.045 | 2,450.940 | 2.045 | 41.079 |
| TOTAL (L/batch) | 2.054 | 2,711.350 | 2.054 | 41.079 |

| Stream Name | S-137 | S-138 | Buffer | NaCl |
|---|---|---|---|---|
| Source | P-24 | P-24 | INPUT | INPUT |
| Destination | P-25 | OUTPUT | P-25 | P-25 |
| Stream Properties | | | | |
| Activity (U/ml) | 0.00 | 0.00 | 0.00 | 0.00 |
| Temperature (°C) | 25.83 | 25.83 | 25.00 | 25.00 |
| Pressure (bar) | 1.01 | 1.01 | 1.01 | 1.01 |
| Density (g/L) | 994.40 | 998.78 | 999.74 | 1,030.00 |
| Component Flowrates (kg/batch) | | | | |
| KCl | 0.000 | 0.000 | 0.000 | 0.000 |
| KH2PO4 | 0.000 | 0.000 | 0.000 | 0.000 |
| mAb | 0.005 | 0.000 | 0.000 | 0.000 |
| Na2HPO4 | 0.000 | 0.045 | 0.028 | 0.000 |
| Sodium Chloride | 0.000 | 0.329 | 0.003 | 0.151 |
| Sodium Citrate | 0.000 | 0.004 | 0.000 | 0.000 |
| Water | 0.000 | 0.000 | 0.000 | 2.511 |
| WFI | 2.029 | 40.713 | 2.552 | 0.000 |
| TOTAL (kg/batch) | 2.034 | 41.090 | 2.583 | 2.661 |
| TOTAL (L/batch) | 2.045 | 41.141 | 2.584 | 2.584 |

| Stream Name | NaOH | INX-E | WFI-4 | S-139 |
|---|---|---|---|---|
| Source | INPUT | INPUT | INPUT | P-25 |
| Destination | P-25 | P-25 | P-25 | P-26 |
| Stream Properties | | | | |
| Activity (U/ml) | 0.00 | 0.00 | 0.00 | 0.00 |
| Temperature (°C) | 25.00 | 25.00 | 25.00 | 25.00 |
| Pressure (bar) | 1.01 | 1.01 | 1.01 | 1.01 |
| Density (g/L) | 1,021.00 | 1,027.72 | 994.70 | 994.99 |
| Component Flowrates (kg/batch) | | | | |
| mAb | 0.000 | 0.000 | 0.000 | 0.004 |
| Na2HPO4 | 0.000 | 0.000 | 0.000 | 0.000 |
| Sodium Chloride | 0.000 | 0.002 | 0.000 | 0.001 |
| Sodium Hydroxid | 0.039 | 0.000 | 0.000 | 0.000 |
| Water | 1.940 | 0.000 | 0.000 | 0.000 |
| WFI | 0.000 | 0.026 | 3.185 | 1.606 |
| TOTAL (kg/batch) | 1.979 | 0.028 | 3.185 | 1.611 |
| TOTAL (L/batch) | 1.938 | 0.028 | 3.202 | 1.619 |

| Stream Name | S-140 | PBS-3 | S-141 | S-142 |
|---|---|---|---|---|
| Source | P-25 | INPUT | P-26 | P-27 |
| Destination | OUTPUT | P-27 | P-27 | P-26 |
| Stream Properties | | | | |
| Activity (U/ml) | 0.00 | 0.00 | 0.00 | 0.00 |
| Temperature (°C) | 25.15 | 25.00 | 25.00 | 25.83 |
| Pressure (bar) | 1.01 | 1.01 | 1.01 | 1.01 |
| Density (g/L) | 1,004.40 | 1,000.00 | 994.99 | 994.40 |
| Component Flowrates (kg/batch) | | | | |
| KCl | 0.000 | 0.000 | 0.000 | 0.000 |
| KH2PO4 | 0.000 | 0.000 | 0.000 | 0.000 |
| mAb | 0.000 | 0.000 | 0.004 | 0.004 |
| Na2HPO4 | 0.029 | 0.036 | 0.000 | 0.000 |
| Sodium Chloride | 0.155 | 0.259 | 0.001 | 0.000 |
| Sodium Hydroxid | 0.039 | 0.000 | 0.000 | 0.000 |
| Water | 4.450 | 0.000 | 0.000 | 0.000 |
| WFI | 6.187 | 32.092 | 1.606 | 1.599 |
| TOTAL (kg/batch) | 10.860 | 32.387 | 1.611 | 1.603 |
| TOTAL (L/batch) | 10.812 | 32.387 | 1.619 | 1.612 |

| Stream Name | S-143 | S-144 | CNX-E | WFI-5 |
|---|---|---|---|---|
| Source | P-27 | P-26 | INPUT | INPUT |
| Destination | OUTPUT | P-28 | P-28 | P-28 |
| Stream Properties | | | | |
| Activity (U/ml) | 0.00 | 0.00 | 0.00 | 0.00 |
| Temperature (°C) | 25.83 | 25.82 | 25.00 | 25.00 |
| Pressure (bar) | 1.01 | 1.01 | 1.01 | 1.01 |
| Density (g/L) | 998.75 | 994.40 | 1,027.72 | 994.70 |
| Component Flowrates (kg/batch) | | | | |
| KCl | 0.000 | 0.000 | 0.000 | 0.000 |
| KH2PO4 | 0.000 | 0.000 | 0.000 | 0.000 |
| mAb | 0.000 | 0.004 | 0.000 | 0.000 |
| Na2HPO4 | 0.036 | 0.000 | 0.000 | 0.000 |
| Sodium Chloride | 0.260 | 0.000 | 0.001 | 0.000 |
| WFI | 32.099 | 1.599 | 0.024 | 2.867 |
| TOTAL (kg/batch) | 32.395 | 1.603 | 0.025 | 2.867 |
| TOTAL (L/batch) | 32.435 | 1.612 | 0.025 | 2.882 |

| Stream Name | Buffer-2 | NaCl-2 | NaOH-2 | S-145 |
|---|---|---|---|---|
| Source | INPUT | INPUT | INPUT | P-28 |
| Destination | P-28 | P-28 | P-28 | P-29 |
| Stream Properties | | | | |
| Activity (U/ml) | 0.00 | 0.00 | 0.00 | 0.00 |
| Temperature (°C) | 25.00 | 25.00 | 25.00 | 25.00 |
| Pressure (bar) | 1.01 | 1.01 | 1.01 | 1.01 |
| Density (g/L) | 999.74 | 1,030.00 | 1,021.00 | 994.99 |
| Component Flowrates (kg/batch) | | | | |
| mAb | 0.000 | 0.000 | 0.000 | 0.004 |
| Na2HPO4 | 0.026 | 0.000 | 0.000 | 0.000 |
| Sodium Chloride | 0.003 | 0.136 | 0.000 | 0.001 |
| Sodium Hydroxid | 0.000 | 0.000 | 0.035 | 0.000 |
| Water | 0.000 | 2.259 | 1.746 | 0.000 |
| WFI | 2.297 | 0.000 | 0.000 | 1.445 |
| TOTAL (kg/batch) | 2.325 | 2.395 | 1.781 | 1.450 |
| TOTAL (L/batch) | 2.326 | 2.326 | 1.744 | 1.458 |

| Stream Name | S-146 | PBS-4 | S-147 | S-148 |
|---|---|---|---|---|
| Source | P-28 | INPUT | P-29 | P-29 |
| Destination | OUTPUT | P-29 | P-30 | OUTPUT |
| Stream Properties | | | | |
| Activity (U/ml) | 0.00 | 0.00 | 0.00 | 0.00 |
| Temperature (°C) | 25.14 | 25.00 | 25.79 | 25.79 |
| Pressure (bar) | 1.01 | 1.01 | 1.01 | 1.01 |
| Density (g/L) | 1,004.64 | 1,000.00 | 994.42 | 998.78 |
| Component Flowrates (kg/batch) | | | | |
| KCl | 0.000 | 0.000 | 0.000 | 0.000 |
| KH2PO4 | 0.000 | 0.000 | 0.000 | 0.000 |
| mAb | 0.000 | 0.000 | 0.004 | 0.000 |
| Na2HPO4 | 0.026 | 0.016 | 0.000 | 0.016 |
| Sodium Chloride | 0.139 | 0.117 | 0.000 | 0.117 |
| Sodium Hydroxid | 0.035 | 0.000 | 0.000 | 0.000 |
| Water | 4.005 | 0.000 | 0.000 | 0.000 |
| WFI | 5.341 | 14.444 | 1.439 | 14.449 |
| TOTAL (kg/batch) | 9.546 | 14.576 | 1.444 | 14.583 |
| TOTAL (L/batch) | 9.502 | 14.576 | 1.452 | 14.601 |

| Stream Name | Product |
|---|---|
| Source | P-30 |
| Destination | OUTPUT |
| Stream Properties | |
| Activity (U/ml) | 0.00 |
| Temperature (°C) | 25.79 |
| Pressure (bar) | 1.01 |
| Density (g/L) | 994.42 |
| Component Flowrates (kg/batch) | |
| mAb | 0.004 |
| Na2HPO4 | 0.000 |
| Sodium Chloride | 0.000 |
| WFI | 1.439 |
| TOTAL (kg/batch) | 1.444 |
| TOTAL (L/batch) | 1.452 |

# APPENDIX E

## Stream details of Downstream Process for Journal (S. Sommerfeld and J. Strube, 2005) in SPD report

| Stream Name | From Upstream | S-129 | S-130 | S-131 |
|---|---|---|---|---|
| Source | INPUT | P-19 | P-19 | P-20 |
| Destination | P-19 | P-20 | OUTPUT | P-21 |
| Stream Properties | | | | |
| Activity (U/ml) | 0.00 | 0.00 | 0.00 | 0.00 |
| Temperature (°C) | 25.00 | 25.69 | 25.69 | 25.69 |
| Pressure (bar) | 1.01 | 1.01 | 1.01 | 1.01 |
| Density (g/L) | 891.94 | 889.10 | 901.31 | 889.10 |
| Component Flowrates (kg/batch) | | | | |
| Ammonia | 0.959 | 0.787 | 0.172 | 0.787 |
| Biomass | 2.510 | 0.000 | 2.510 | 0.000 |
| Glucose | 2.853 | 2.342 | 0.511 | 2.342 |
| Glutamine | 0.403 | 0.330 | 0.072 | 0.330 |
| Lactic Acid | 27.361 | 22.461 | 4.900 | 22.461 |
| mAb | 0.005 | 0.004 | 0.001 | 0.004 |
| Water | 11.449 | 9.398 | 2.050 | 9.398 |
| WFI | 11,793.750 | 9,436.138 | 2,357.612 | 9,436.138 |
| TOTAL (kg/batch) | 11,839.289 | 9,471.461 | 2,367.828 | 9,471.461 |
| TOTAL (L/batch) | 13,273.698 | 10,652.832 | 2,627.104 | 10,652.826 |

| Stream Name | S-132 | S-133 | WFI 2 | WFI 3 |
|---|---|---|---|---|
| Source | P-21 | P-21 | INPUT | INPUT |
| Destination | P-20 | P-22 | P-22 | P-22 |
| Stream Properties | | | | |
| Activity (U/ml) | 0.00 | 0.00 | 0.00 | 0.00 |
| Temperature (°C) | 26.39 | 26.39 | 25.00 | 25.00 |
| Pressure (bar) | 1.01 | 1.01 | 1.01 | 1.01 |
| Density (g/L) | 886.18 | 898.83 | 994.70 | 994.70 |
| Component Flowrates (kg/batch) | | | | |
| Ammonia | 0.647 | 0.141 | 0.000 | 0.000 |
| Glucose | 1.924 | 0.419 | 0.000 | 0.000 |
| Glutamine | 0.271 | 0.059 | 0.000 | 0.000 |
| Lactic Acid | 18.447 | 4.014 | 0.000 | 0.000 |
| mAb | 0.000 | 0.004 | 0.000 | 0.000 |
| Water | 7.719 | 1.680 | 0.000 | 0.000 |
| WFI | 7,548.274 | 1,887.863 | 1.666 | 4.442 |
| TOTAL (kg/batch) | 7,577.281 | 1,894.179 | 1.666 | 4.442 |
| TOTAL (L/batch) | 8,550.502 | 2,107.384 | 1.675 | 4.466 |

| Stream Name | Tris | Citrate Buffer | Aff eluent | S-134 |
|---|---|---|---|---|
| Source | INPUT | INPUT | INPUT | P-22 |
| Destination | P-22 | P-22 | P-22 | P-23 |
| Stream Properties | | | | |
| Activity (U/ml) | 0.00 | 0.00 | 0.00 | 0.00 |
| Temperature (°C) | 25.00 | 25.00 | 25.00 | 25.00 |
| Pressure (bar) | 1.01 | 1.01 | 1.01 | 1.01 |
| Density (g/L) | 1,075.55 | 1,000.00 | 1,000.00 | 995.53 |
| Component Flowrates (kg/batch) | | | | |
| mAb | 0.000 | 0.000 | 0.000 | 0.004 |
| Sodium Chloride | 0.447 | 0.000 | 0.000 | 0.000 |
| Sodium Citrate | 0.000 | 0.006 | 0.006 | 0.003 |
| TRIS HCl | 0.015 | 0.000 | 0.000 | 0.000 |
| WFI | 2.539 | 3.343 | 3.343 | 1.672 |
| TOTAL (kg/batch) | 3.002 | 3.349 | 3.349 | 1.679 |
| TOTAL (L/batch) | 2.791 | 3.349 | 3.349 | 1.686 |

| Stream Name | S-135 | S-136 | Buffer | NaCl |
|---|---|---|---|---|
| Source | P-22 | P-23 | INPUT | INPUT |
| Destination | OUTPUT | P-24 | P-24 | P-24 |
| Stream Properties | | | | |
| Activity (U/ml) | 0.00 | 0.00 | 0.00 | 0.00 |
| Temperature (°C) | 26.38 | 25.00 | 25.00 | 25.00 |
| Pressure (bar) | 1.01 | 1.01 | 1.01 | 1.01 |
| Density (g/L) | 899.57 | 995.53 | 999.74 | 1,030.00 |
| Component Flowrates (kg/batch) | | | | |
| Ammonia | 0.141 | 0.000 | 0.000 | 0.000 |
| Glucose | 0.419 | 0.000 | 0.000 | 0.000 |
| Glutamine | 0.059 | 0.000 | 0.000 | 0.000 |
| Lactic Acid | 4.014 | 0.000 | 0.000 | 0.000 |
| mAb | 0.000 | 0.004 | 0.000 | 0.000 |
| Na2HPO4 | 0.000 | 0.000 | 0.035 | 0.000 |
| Sodium Chloride | 0.447 | 0.000 | 0.004 | 0.186 |
| Sodium Citrate | 0.009 | 0.003 | 0.000 | 0.000 |
| TRIS HCl | 0.015 | 0.000 | 0.000 | 0.000 |
| Water | 1.680 | 0.000 | 0.000 | 3.091 |
| WFI | 1,901.525 | 1.672 | 3.142 | 0.000 |
| TOTAL (kg/batch) | 1,908.309 | 1.679 | 3.181 | 3.277 |
| TOTAL (L/batch) | 2,121.351 | 1.686 | 3.182 | 3.182 |

| Stream Name | NaOH | INX-E | WFI 4 | S-137 |
|---|---|---|---|---|
| Source | INPUT | INPUT | INPUT | P-24 |
| Destination | P-24 | P-24 | P-24 | P-25 |
| Stream Properties | | | | |
| Activity (U/ml) | 0.00 | 0.00 | 0.00 | 0.00 |
| Temperature (°C) | 25.00 | 25.00 | 25.00 | 25.00 |
| Pressure (bar) | 1.01 | 1.01 | 1.01 | 1.01 |
| Density (g/L) | 1,021.00 | 1,028.93 | 994.70 | 1,011.68 |
| Component Flowrates (kg/batch) | | | | |
| mAb | 0.000 | 0.000 | 0.000 | 0.004 |
| NaH2PO4 | 0.000 | 0.011 | 0.000 | 0.006 |
| Sodium Chloride | 0.000 | 0.058 | 0.000 | 0.029 |
| Sodium Hydroxid | 0.048 | 0.000 | 0.000 | 0.000 |
| Water | 2.389 | 0.000 | 0.000 | 0.000 |
| WFI | 0.000 | 0.950 | 0.993 | 0.971 |
| TOTAL (kg/batch) | 2.436 | 1.019 | 0.993 | 1.010 |
| TOTAL (L/batch) | 2.386 | 0.990 | 0.999 | 0.998 |

| Stream Name | S-138 | S-139 | PBS | S-140 |
|---|---|---|---|---|
| Source | P-24 | P-25 | INPUT | P-26 |
| Destination | OUTPUT | P-26 | P-26 | P-27 |
| Stream Properties | | | | |
| Activity (U/ml) | 0.00 | 0.00 | 0.00 | 0.00 |
| Temperature (°C) | 25.00 | 25.00 | 25.00 | 25.83 |
| Pressure (bar) | 1.01 | 1.01 | 1.01 | 1.01 |
| Density (g/L) | 1,007.55 | 1,011.68 | 1,000.00 | 994.40 |
| Component Flowrates (kg/batch) | | | | |
| KCl | 0.000 | 0.000 | 0.000 | 0.000 |
| KH2PO4 | 0.000 | 0.000 | 0.000 | 0.000 |
| mAb | 0.000 | 0.004 | 0.000 | 0.004 |
| Na2HPO4 | 0.035 | 0.000 | 0.022 | 0.000 |
| NaH2PO4 | 0.006 | 0.006 | 0.000 | 0.000 |
| Sodium Chloride | 0.219 | 0.029 | 0.160 | 0.000 |
| Sodium Citrate | 0.003 | 0.000 | 0.000 | 0.000 |
| Sodium Hydroxid | 0.048 | 0.000 | 0.000 | 0.000 |
| Water | 5.480 | 0.000 | 0.000 | 0.000 |
| WFI | 5.785 | 0.971 | 19.777 | 0.984 |
| TOTAL (kg/batch) | 11.576 | 1.010 | 19.959 | 0.987 |
| TOTAL (L/batch) | 11.489 | 0.998 | 19.959 | 0.993 |

| Stream Name | S-141 | WFI 5 | NaOH 2 | Buffer 2 |
|---|---|---|---|---|
| Source | P-26 | INPUT | INPUT | INPUT |
| Destination | OUTPUT | P-27 | P-27 | P-27 |
| Stream Properties | | | | |
| Activity (U/ml) | 0.00 | 0.00 | 0.00 | 0.00 |
| Temperature (°C) | 25.83 | 25.00 | 25.00 | 25.00 |
| Pressure (bar) | 1.01 | 1.01 | 1.01 | 1.01 |
| Density (g/L) | 999.58 | 994.70 | 1,013.45 | 1,081.89 |
| Component Flowrates (kg/batch) | | | | |
| Amm. Sulfate | 0.000 | 0.000 | 0.000 | 0.537 |
| KCl | 0.000 | 0.000 | 0.000 | 0.000 |
| KH2PO4 | 0.000 | 0.000 | 0.000 | 0.000 |
| Na2HPO4 | 0.022 | 0.000 | 0.000 | 0.036 |
| NaH2PO4 | 0.006 | 0.000 | 0.000 | 0.000 |
| Sodium Chloride | 0.189 | 0.000 | 0.000 | 0.000 |
| Sodium Hydroxid | 0.000 | 0.000 | 0.112 | 0.000 |
| WFI | 19.765 | 3.561 | 2.790 | 2.525 |
| TOTAL (kg/batch) | 19.981 | 3.561 | 2.902 | 3.098 |
| TOTAL (L/batch) | 19.989 | 3.580 | 2.864 | 2.864 |

| Stream Name | HIC Buff Low | HIC Buff High | S-142 | S-143 |
|---|---|---|---|---|
| Source | INPUT | INPUT | P-27 | P-27 |
| Destination | P-27 | P-27 | P-28 | OUTPUT |
| Stream Properties | | | | |
| Activity (U/ml) | 0.00 | 0.00 | 0.00 | 0.00 |
| Temperature (°C) | 25.00 | 25.00 | 25.00 | 25.07 |
| Pressure (bar) | 1.01 | 1.01 | 1.01 | 1.01 |
| Density (g/L) | 1,000.38 | 1,153.55 | 1,057.81 | 1,026.35 |
| Component Flowrates (kg/batch) | | | | |
| Amm. Sulfate | 0.000 | 0.189 | 0.095 | 0.632 |
| mAb | 0.000 | 0.000 | 0.003 | 0.000 |
| Na2HPO4 | 0.012 | 0.007 | 0.010 | 0.046 |
| Sodium Hydroxid | 0.000 | 0.000 | 0.000 | 0.112 |
| WFI | 0.880 | 0.426 | 0.653 | 10.513 |
| TOTAL (kg/batch) | 0.892 | 0.623 | 0.761 | 11.302 |
| TOTAL (L/batch) | 0.892 | 0.540 | 0.719 | 11.012 |

| Stream Name | PBS-2 | S-144 | S-146 | S-145 |
|---|---|---|---|---|
| Source | INPUT | P-28 | P-29 | P-29 |
| Destination | P-29 | P-29 | P-30 | P-28 |
| Stream Properties | | | | |
| Activity (U/ml) | 0.00 | 0.00 | 0.00 | 0.00 |
| Temperature (°C) | 25.00 | 25.00 | 25.79 | 25.79 |
| Pressure (bar) | 1.01 | 1.01 | 1.01 | 1.01 |
| Density (g/L) | 1,000.00 | 1,057.81 | 994.42 | 1,005.04 |
| Component Flowrates (kg/batch) | | | | |
| Amm. Sulfate | 0.000 | 0.095 | 0.000 | 0.095 |
| KCl | 0.000 | 0.000 | 0.000 | 0.000 |
| KH2PO4 | 0.000 | 0.000 | 0.000 | 0.000 |
| mAb | 0.000 | 0.003 | 0.003 | 0.000 |
| Na2HPO4 | 0.008 | 0.010 | 0.000 | 0.018 |
| Sodium Chloride | 0.058 | 0.000 | 0.000 | 0.058 |
| WFI | 7.129 | 0.653 | 0.704 | 7.078 |
| TOTAL (kg/batch) | 7.194 | 0.761 | 0.707 | 7.248 |
| TOTAL (L/batch) | 7.194 | 0.719 | 0.711 | 7.212 |

| Stream Name | Product |
|---|---|
| Source | P-30 |
| Destination | OUTPUT |
| Stream Properties | |
| Activity (U/ml) | 0.00 |
| Temperature (°C) | 25.79 |
| Pressure (bar) | 1.01 |
| Density (g/L) | 994.42 |
| Component Flowrates (kg/batch) | |
| Amm. Sulfate | 0.000 |
| mAb | 0.003 |
| Na2HPO4 | 0.000 |
| Sodium Chloride | 0.000 |
| WFI | 0.704 |
| TOTAL (kg/batch) | 0.707 |
| TOTAL (L/batch) | 0.711 |