A SIMULATION STUDY ON DOWNSTREAM PROCESSING OF MONOCLONAL ANTIBODY PRODUCTION USING SUPERPRO DESIGNER®

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UNIVERSITI MALAYSIA PAHANG

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BORANG PENGESAHAN STATUS TESIS♦

JUDUL: A SIMULATION STUDY ON DOWNSTREAM PROCESSING OF MONOCLONAL ANTIBODY PRODUCTION USING SUPERPRO DESIGNER®

SESI PENGAJIAN : 2008/2009

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Date : .....

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MUHAMAD NUR IMAN BIN AHMAD RAZALI

A thesis submitted in fulfillment
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 2009

I declare that this thesis entitled "Simulation study on downstream processing of monoclonal antibodies production using SuperPro Designer®" is the result of my own research except as cited in references. The thesis has not been accepted for any degree and is not concurrently submitted in candidature of any other degree."

Signature :……

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Date : 30th April 2009

To my beloved mother and father

ACKNOWLEDGEMENTS

In the name of the almighty ALLAH, the most gracious and merciful, with His gracing and blessing has led to success be upon this thesis and may peace upon on Muhammad S.A.W., our prophet, as the true teacher of humankind.

I would like to take this opportunity to express my sincere thanks and appreciation to my thesis supervisor, Miss Nurul Aini Binti Mohd Azman for encouragement, guidance, critics and insightful comment. This publication of this study would not be possible without her encouragements and advices. As for all the lessons, guidance and unparalleled knowledge shared will not be forgotten.

I am very thankful to Universiti Malaysia Pahang (UMP) for providing good facilities in the campus. To all the staff in Faculty of Chemical & Natural Resources Engineering, a very big thanks to all.

My sincere appreciation also extends to all my fellow friend and others who have provided assistance and guidance in preparing this thesis. Their views and tips are useful indeed. Thank you for the time sacrificed to accompany me. And last but not least, I am grateful to all my family members especially to my mother and father for their continued support for me to successfully accomplish my project.

ABSTRACT

The production of monoclonal antibodies and Fc fusion proteins form the largest and most rapidly expanding category of the biopharmaceuticals today with the annual sales exceeding USD$8 billion with the application across a wide range of diseases especially for the immunotherapy purpose. With the growth of this class of biomolecules, significant thought on the most efficient downstream processing steps for monoclonal antibodies has been taken into the highly consideration by the manufacturer in order to obtain the highest yield with the highest purity of the product which is why the simulation study are crucial in this development. The objectives of this study are to propose the most suitable framework of downstream processing for monoclonal antibodies production in order to achieve highest purities of mAbs and to stimulate the propose framework using SuperPro Designer® simulator. The process was initially done via the disk stack centrifuge and followed by microfiltration in order to harvest the protein. Three column of chromatography (Protein A affinity, anion exchange and hydrophobic interaction) were used to obtain the highest purity of the therapeutic. Viral inactivation also was employed to free the product from the endotoxins and pyrogens. The propose steps then, was introduced into the SuperPro Designer® simulator and the result obtained were 100% purity of IgG with 9.49345 g/L of concentration and 0.16687 kg/batch with 76.3% of recovery of the product.

ABSTRAK

Penghasilan antibodi monoklonal dan protein fc-penyatuan telah membentuk satu bidang biofarmasi yang paling maju and pantas berkembang dengan jualan tahunan melebihi USD$8 Bilion mencakupi aplikasi dalam merawat pelbagai penyakit terutamanya dalam bidang terapi imun. Dengan pertumbuhan kelas biokimia tersebut, proses hiliran untuk antibodi monoklonal telah dipandang dengan serius oleh pengilang bagi mendapatkan hasil dan ketulenan tertinggi dan disinilah simulasi telah dianggap sesuatu yang perlu dalam mencapai pembangunan tersebut. Tujuan kajian ini dilakukan adalah untuk mencadangkan satu langkah kerja proses hiliran untuk antibodi monoklonal bagi mendapatkan hasil ketulenan yang tinggi dan seterusnyer mensimulasikan langkah kerja tersebut menggunakan simulasi SuperPro Designer®. Proses tersebut dimulai dengan proses penuaian sel menggunakan ceper pengemparan dan disusuli pula dengan penapis mikro. Tiga kromatografi kolum (afiniti protein A, penukar anion, dan interaksi hidrofobia) digunakan untuk mencapai ketulenan tertinggi oleh terapeutik tersebut. Penyahaktifan virus juga dipakai untuk mendapatkan hasil yang bebas daripada endotoksin dan phirogen. Proses yang dicadangkan kemudian dimasukkan ke dalam simulasi SuperPro Designer® dan hasil yang diperolehi seperti ketulenan Immunoglobulin G mencapai peratusan 100% dengan kepekatan 9.49345 g/L dan penghasilan 0.16687 kg/kumpulan dengan peratusan 76.3%.

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LIST OF SYMBOLS/ABBREVIATIONS

AEC	Anion Exchange Chromatography
CIP	Cleaning In Place
g/L	grams per liters
GMP	Good Manufacturing Practice
h	Hour
HIC	Hydrophobic Interaction Chromatography
IgG	Immunoglobulin G
kDa	kiloDaltons
kg	Kilograms
L	Liters
L/Batch	Liters per Batch
mAbs	Monoclonal Antibodies
MOSTI	Ministry of Sciences, Technology and Innovation
nm	nanometer

pH		Hydrogen ion concentration point
p*I*		isoelectric point
RIPP Scheme		Recovery, Isolation, Purification an Polishing Scheme
SPD		SuperPro Designer®
TFF		Tangential flow filtration
USFDA		United State Food and Drug Administration
WFI		Water flow injection
˚C	-	Degree Celsius
%	-	Percentage

CHAPTER 1

INTRODUCTION

1.1 Background of Study

For the past ten years, biotechnology have been announced as one of five core technologies that will accelerate Malaysia`s transformation into a highly industrialized nation by 2020. Government of Malaysia, trough the established of National Biotechnology Division under Ministry of Science, Technology and Innovative (MOSTI), carries the mission to spearhead the biotechnology development for wealth creation and social well-being by establishing Malaysia as the biotechnology centre through.

In next ten years, antibody-based therapies will be a major source of new therapies however, it is major bottleneck for the biotechnology industry, in the processing the biopharmaceuticals antibodies to meet clinical demand (Alberto *et al.*, 2003). Monoclonal antibodies (mAbs) are the most widely used form of cancer immunotherapy. Monoclonal antibodies therapy uses antibodies outside the body which made in the lab, produce in large numbers rather than by a person's own immune. This type of treatment is considered as one of a form of passive immunotherapy. In biopharmaceutical industry, monoclonal antibodies have emerged as one of the most exciting therapeutic modalities (Abhinav *et al.*, 2007). In U.S and the European Union, there are nineteen monoclonal antibodies therapeutics has been approved by US food and Drug Administration (USFDA) for sale (Walsh, 2004) to treat cancer and transplant rejection and also use to combat autoimmune disease (Reichert, 2001).

A key segment of the production and marketing of any pharmaceutical product, whether it is an antibiotic, a peptide, or a complex protein, is the processing of the materials from its initial milieu (tissue, fermentation broth, etc.) to a pure form suitable for its intended use. This key segment, termed downstream processing can be, and often is, a complicated series of isolation and purification steps which usually quite costly (Spears, 1993). The recovery and purification steps of mAbs or downstream processing is aim to obtain a highly pure antibodies and to meet the USFDA specification which initially from mammalian cell culture supernatant. This process implies the use of a combination of a various bioseparation techniques by exploiting the physical and chemical properties of the antibodies. The purification of mAbs generally takes place in three phases which are a capture steps by which the mAbs present in hybridoma culture supernatant is separated from other sample components, an intermediate step by which the mAbs is isolated from contaminations similar in size or biochemical properties, and a cleaning step for the complete removal trace contaminant (Alberto *et al.,* 2003).

Product recovery methods which are effective, efficient, and well designed are essential in developing a downstream process which can deliver a marketable product to the public, and a financial return to the company. Due to this circumstances, a new developed technology called a simulation process are required. Process simulation have been realized, gives advantages to the development, evaluation and scale up of bioprocesses. The opportunity to shorten time required for the development by allowing comparison of process alternatives so that various ideas can be synthesized and analyzed interactively in a short time (Rouf *et al*., 2001). In this study, the commercial bioprocess simulator which is SuperPro Designer® (SPD) version 6.0 (Intelligen, 1999), was assessed for evaluation of production of a biopharmaceutical from a recombinant mammalian cell culture.

The study aim to stimulate a new set of workflow created using the bioseparation principles, in the simulator to get an exact data sheets. The study is the downstream processing of monoclonal antibodies production workflow, transforming into a framework of a simulator in the SuperPro Designer® (SPD) main frame thus stimulate to give an overall mass balance and so do for each stream.

1.2 Problem Statement

Biotechnology manufacturing concerns must be able to design and implement purification schemes that are repeatable, reliable and meet current good manufacturing practice (GMP) guidelines. The new generations of product from the biotechnology industries are more complex and are often quite labile. Thus, in manufacturing of monoclonal antibodies, similar processes are use by majority; A batch/fed batch culture using mammalian cell followed by purification steps, that rely primarily on chromatography method with intermediate filtration and viral clearance operation (Farid, 2007). Downstream processing must be improved as the increasing titrates in mammalian cell culture by different kind of variety and innovative steps in purification. The critical part of the production process and can be significant proportion of the total manufacturing costs is the efficiency of recovery and purification (Abhinav *et al.*, 2007).

The nature of bioseparation for every biological product face several difficulty in order to do the downstream processing such as present in very low concentrations in the starting material from which they are purified. In this study, mAbs are typically present in concentrations around 0.1 mg/ml in mammalian cell culture supernatants (Ghosh, 2006). Bioseparation also has to be very selective in nature due to the impurities and by-product present in the solution, have chemical and physical properties similar to the target product. The product also must meet the quality manufacturing criteria which is should be free from the endotoxin and pyrogens. Most dilemma faces is the denaturation and degradation of the biological product towards the extremes of physicochemical condition such as pH and ionic strength, hydrodynamic conditions such as high shear rates, therefore bioseparation technique have to be ‘gentle’ to avoid these conditions.

1.3 Scope of Research Work

The technique of downstream processing for mAbs production, which is used for initial recovery and isolation depends on the nature of the product, its own unique properties and characteristics, and the state of the product, as it leave the fermenter (Janson, 1993). The aim of a protein, in this case is mAbs, purification process is three fold; to remove unwanted contaminants, to concentrate the desired product and to transfer the mAbs to an environment where it stable. The analysis of the nature or fundamental properties of mAbs are relevant in the separation process such as size and for antibody molecular which is mAbs, possess much complex shape rather than the size of particulates matters such as cells, cell debris and macromolecular aggregates which far more simples and can be remove by separation technique easily. Other then the size of the mAbs, the molecular weight is often used to separates the mAbs with the impurities by the separation technique. After the physical properties been researched, the biochemistry properties also give the advantages in purification steps such as isoelectric point (p*I*) of a protein which is at certain pH value; the protein has the same amount of positive and negative charge which called as neutral in an overall sense. Above its p*I* a protein will has a net negative charge and vice versa (Ghosh, 2006).

These properties will be used and altered so that the objective of the downstream processing for the mAbs production is achieved. The objective will be achieve trough the simulation process in which the SuperPro Designer® will be use. The simulator will gives a result in term of mass and concentrations of the Immunoglobulin G (IgG) for each stream present in the framework of the downstream processing that are introduced to the main frame of SPD.

1.4 Objectives

The objectives of this study is to propose the most suitable framework of downstream processing for monoclonal antibodies production in order to achieve highest purities of mAbs with the highest recovery of the Immunoglobulin G at the end of the process. This study also aims to stimulate the propose framework using SuperPro Designer® simulator in order to determine whether the propose framework will achieve the purities and recovery required.

CHAPTER 2

LITERATURE REVIEW

2.1 Biotechnology

Biotechnology has undergone phenomenal growth in recent years. Cellular and molecular biology, biochemistry and biophysics are fundamental areas that are linked together in this new world of industry. Bioprocessing which deal with the manufacture of biochemical, biopharmaceuticals, foods, nutraceuticals and agrochemicals; is one of the major segments within biotechnology. A plethora of new biologically derived products have been developed, approved and licensed which include in this study, monoclonal antibodies used for the treatment of cancer and multiple sclerosis (Ghosh, 2006).

2.2 Monoclonal Antibodies

Monoclonal antibodies (mAb or moAb) are monospecific antibodies that are alike because they are produced by one type of immune cell that are all clones of a single parent cell. Given almost any substance, it is potential to create monoclonal antibodies that specifically bind to that substance; they can then serve to detect or purify that substance. This has become an important tool in biochemistry, molecular biology and medicine. When used as medications, the generic name ends in -mAbs ("Nomenclature of monoclonal antibodies").

Monoclonal antibodies are typically made by fusing the spleen cells from a mouse that has been immunized with the desired antigen with myeloma cells. However, recent advances have allowed the use of rabbit B-cells. Polyethylene glycol is used to fuse adjacent plasma membranes, but the success rate is low so a selective medium is used in which only fused cells can grow. This is because myeloma cells have lost the ability to synthesize hypoxanthine-guanine-phosphoribosyl transferase (HGPRT), an enzyme necessary for the salvage synthesis of nucleic acids. The figure shows the structure of a monoclonal antibody consist four regions which are V_H that is variable region with heavy chain, C_H for constant domain with heavy chain, V_L stands for variable region with light chain and C_L that is constant domain with light chain (A.A Shukla *et al.,* 2007).

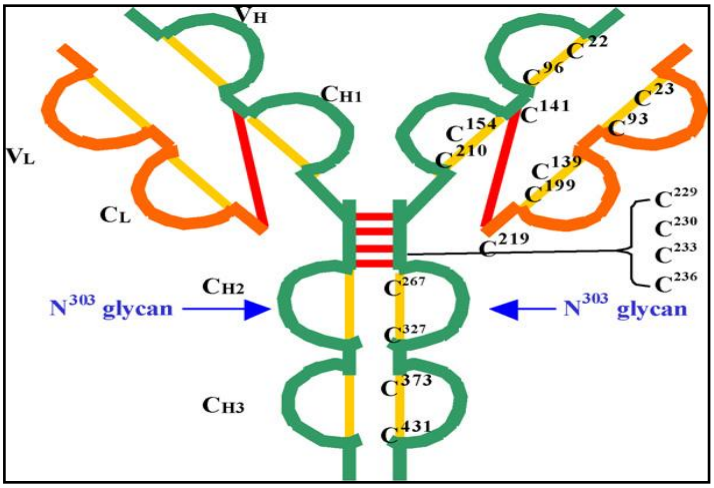

Figure 2.1 Structure of a monoclonal antibody.

Table 2.1 : Several of approval monoclonal antibodies by USFDA

Type	**Application**	**Mechanism**	**Mode**
infliximab	rheumatoid arthritis, Crohn's disease	inhibits TNF-α	chimeric
basiliximab	Acute rejection of kidney transplants	inhibits IL-2 on activated T cells	chimeric
abciximab	Prevent coagulation in coronary angioplasty	inhibits the receptor GpIIb/IIIa on platelets	chimeric
daclizumab	Acute rejection of kidney transplants	inhibits IL-2 on activated T cells	humanized
gemtuzumab	relapsed acute myeloid leukaemia	targets an antigen on leukemia cells	humanized
alemtuzumab	B cell leukemia	targets an antigen CD52 on T- and B-lymphocytes	humanized
rituximab	Non-Hodgkin's Lymphoma	targets phosphoprotein CD20 on B lymphocytes chimeric	chimeric

2.3 Bioseparation Engineering

The systematic study of the scientific and engineering principles utilized for the large scale purification of biological product is referred to bioseparation engineering. It is a broader term from the downstream processing which specifically referred to the separation and purification segment of a bioprocess which followed some form of biological reaction (Ghosh, 2006). Biological product can be categorized in different ways, such as based on chemical nature or application. The figure below, illustrating the basis of bioseparation and the unit operation involved based on the differences of physical properties of the materials.

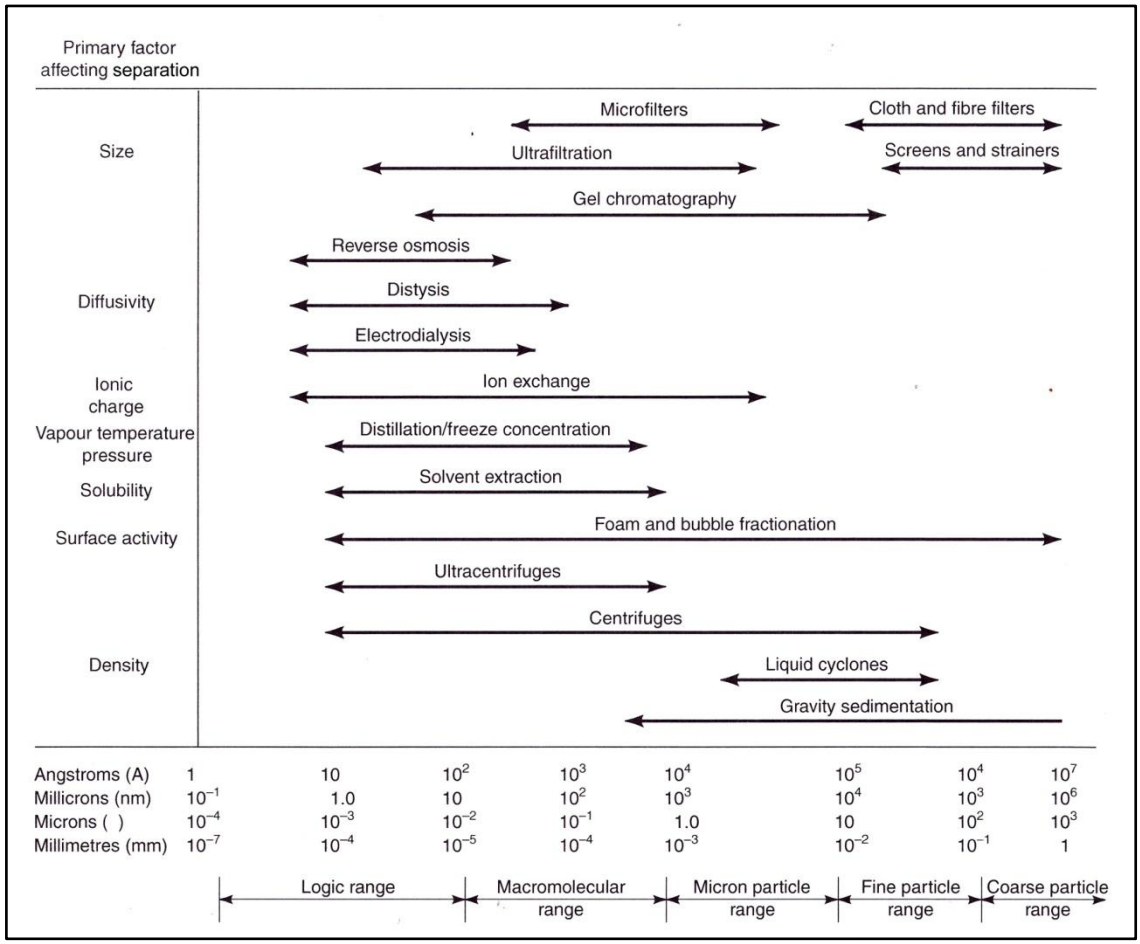

Figure 2.2 Basis of bioseparation and the unit operation involved.

2.3.1 RIPP Scheme

A bioseparation process must combine high selectivity (or resolution) with high throughput (productivity). To achieve these goals, a strategy has been developed, involving use of low resolution techniques first for recovery and isolation followed by high resolution techniques for purification and polishing (Gosh, 1999). The RIPP scheme is which stand for Recovery, Isolation, Purification and Polishing steps is use as a method in the bioseparation technique.

Table 2.2 : RIPP Scheme and typical unit operations categorized.

Stage	Objective(s)	Typical Unit Operations
Recovery (separation of insolubles)	• Remove or collect cells, cell debris • Reduce volume	Filtration, sedimentation, extraction, adsorption, centrifugation
Isolation	• Remove materials having properties widely different from those of target product • Reduce volume	Extraction, adsorption, ultrafiltration, precipitation
Purification	• Remove remaining impurities, which typically are similar to those of target product	Chromatography, affinity methods, precipitation
Polishing	• Remove liquids • Convert product to crystalline form (not always possible)	Drying, crystallization

The table above shows the stages, objectives and unit operations involved in the downstream process. The first step in the downstream process is the recovery stage, which remove or collect cells and cells debris. It also reduces the volume size of the supernatant by various unit operations such as filtration, sedimentation, extraction and also adsorption. The second stage is the isolation process which the objectives are to remove materials that have the widely different from the product. This can be done by using extraction, adsorption, ultrafiltration or precipitation. The next stage is the polishing step in which the impurities that are typically similar to those of target product in term of physical and biochemical properties. Chromatography, affinity method or precipitation are usually use in this stage. The polishing stage is the final stages in the schemes which the main objective is to remove the remaining liquid from the product in order to keep the product in crystalline form which however are impossible to majority of biological product. This objective can be accomplished by using drying and crystallization method.

2.4 Downstream Processing

There are variety steps of downstream processing technique for mAbs production, due to this issue, a guidance of the process is crucial in order to determine the accurate step and remove unnecessary step involved. The platform serves as a guidance document that defines the overall scheme of a process and brackets the operating conditions for individual unit operations, thus limiting the scope of experimentation required to reach a solution for a given molecule. The figure below shows the platform of the downstream processing for monoclonal antibodies production in which been use as guidance by the researcher worldwide.

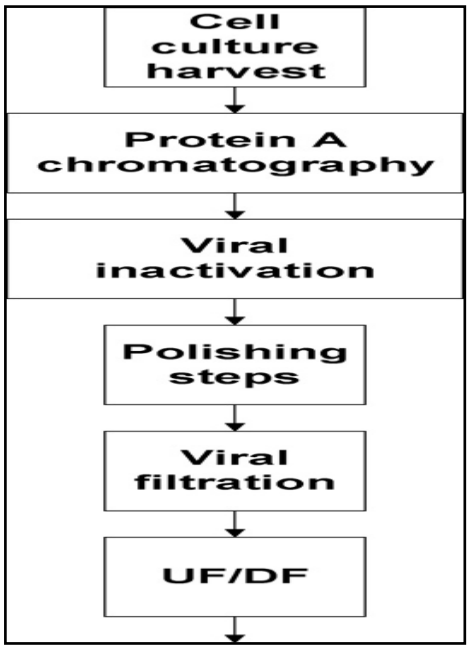

Figure 2.3 Platform downstream processes for mAbs production

2.4.1 Cell Culture Harvest

The platform begins with the cell culture harvest steps which accomplished by a combination of several unit operations. Maximizing product recovery, scalability, robustness, and clarification of the process stream are the factors that driven the process design while operating in a physical and chemical environment in which the product is stable. In this study, the mAbs is expressed extracellularly, then the first step in harvest is to remove cells via centrifugation using a continuous disk stack centrifuge. Centrifugation is preferred over other harvesting technologies such as cross-flow microfiltration due to its scalability and economical operation for large volumes (typically 2–15,000 L/batch) (Shukla *et al.*, 2007). Large-scale centrifugation acts as the primary harvesting step but cannot accomplish complete removal of cells and cell debris, which must be removed prior to chromatography.

2.4.2 Protein A Chromatography

The second stage in the platform is Protein A chromatography which are serves as the capture step. Protein A is a ~42 kDa protein consisting of a single polypeptide chain. The chain is made up of five homologous IgG binding domains followed by a C-terminal region necessary for cell wall to attachment (Gagnon, 1996). High selectivity and good physiochemical stability has made protein A the preferred generic ligand for affinity purification of antibodies and molecules tagged with an antibody Fc-region. This step has proved to be highly selective for mAbs and can in many cases yield >99% purity starting from the cell culture supernatant. This stage is also called the purification steps in which the affinity chromatography been used. Next stage in the platform is the viral in activation step.

2.4.3 Viral Inactivation

Since the Protein A column elute is at low pH and given that most mAbs are stable in solution under low pH conditions, it is relatively easy to include a low pH incubation step to inactivate viruses. Low pH treatment has been shown to successfully inactivate retroviruses for a variety of biotechnology products. The use of strong acids such as HCl is avoided despite the advantage of low volume addition due to the risk of product denaturation in the localized region where the solution is added (Shukla *et al*., 2007).

2.4.4 Polishing Step

The Polishing step is preferred after the viral inactivation step in the platform suggested. The subsequent chromatographic steps are aimed at reducing host cell protein impurities, high molecular weight aggregates, low molecular weight clipped species, DNA and leached Protein A that remain after the Protein A chromatographic step to acceptably low levels that assure safety of the product. Typically one of two polishing steps is operated in the flow through mode which impurities species are retained whereas the product does not bind to the column. Since generally monoclonal antibodies possess high p*I*s, Anion Exchange Chromatography (AEC) and Hydrophobic Interaction Chromatography (HIC) is often used (Shukla *et al.*, 2007).

2.4.5 Viral Filtration

After the polishing step, the next stage in the platform is viral filtration which employed to complement the low pH viral inactivation step. Viral filters can be classified on the basis of their pore sizes into retroviral (<50 nm) and parvoviral (<20 nm) grade filters. Viral filters are typically operated at constant pressure. Due to their relatively small pore sizes, viral filters (especially parvoviral grade filters) can clog relatively quickly in the presence of particulate aggregates**.**

2.4.6 Ultrafiltration and Diafiltration

The final stage in the platform is ultrafiltration and/or diafiltration in which the product is buffer exchanged into the formulation buffer and stored in the stable conditions. The type of membrane use is the transmembrane pressure employed, the cross-flow rate and the concentration at which diafiltration is carried out.

2.5 Recovery and Purification Units

In the downstream processing, there a various unit operation involved for the recovery and purification steps. These unit operations will operates based on the objectives and the platform order. For the recovery process, which is the cell harvest step in the platform are perform via Disk Stack Centrifugation and followed by microfiltration. Next is the Protein A Affinity Chromatography unit which is the purification stages and final stage is polishing step which required two different types of chromatography via flow trough method which using Anion Exchange Chromatography (AEX) and Hydrophobic Interaction Chromatography (HIC).

2.5.1 Disk Stack Centrifugation

Application of the solid-liquid separation theory can result in the prediction of clarification performance (Russell, 2003). The feed stream enters through the top of the centrifuge and process down through the stationary feed tube to the feed zone where it is accelerated to the bowl speed. Various improvements have been made to the method of feed delivery to feed zone including hydrohermetic, hermetic, and disk inlet in attempt to reduce the gas-liquid mixing that can cause significant shear damage to the cells. At the base of the bowl, the liquid reverses direction and flow up between a series of disk. In this moment, the light fluid continues up while the heavy solids are collected on the underside of a discs and then move down. The particles are forced to the underside of the disc by a net velocity vector, v_G, which has two components: one due to the centrifugal force acting on the particle, v_1, and the other due to the force of fluid flow, v_1. The net force drives the particles up to the disc underside at which point v_1 becomes the dominant force and the particles slide down the disc to the solids collection area (Russell *et al*., 2007).

2.5.2 Microfiltration

Microfiltration uses polymeric membranes to retain a biologic product while allowing low molecular weight solutes and water pass through the membrane. Microfiltration is widely used to concentrate or in order word dewatering the product and remove low molecular weight impurities. Microfiltration is typically operated in tangential flow filtration (TFF). TFF involves passing a permeate fluid through the membrane with a velocity component perpendicular, or normal to the membrane and passing fluid across the membrane surface with a velocity component tangent to the membrane (Lutz *et al.,* 2007). The fundamental phenomenon occurring in the microfiltration is polarization where retained solutes concentrate at the membrane surface.

2.5.3 Affinity Chromatography

Affinity method is used for separation methods based on more or less specific interactions between protein and variety of ligand molecules covalently attached to a solid phase (Janson *et al.,* 1993). Affinity binding is based on stereo-specific recognition by ligand which is the shape of the ligand is complimentary to the shape of the entire target molecule or at least a portion of the molecules (Ghosh, 2006). A combination of hydrogen bonding, hydrophobic interaction and van der Waals forces held together the ligand and the target molecule after the recognition. Antibodies are Y-shaped protein molecules which have the ability to recognize specific molecules called antigens. The affinity purification of a mAbs from mammalian cell culture supernatant using protein A based affinity absorbent is summarized in the figure below.

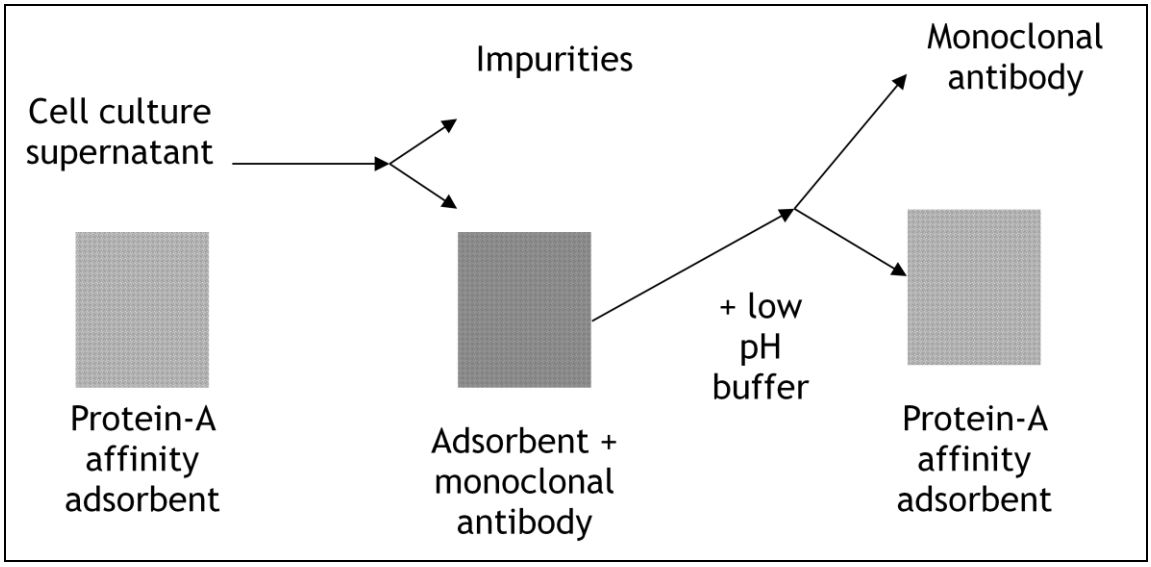

Figure 2.4 Actions of the affinity chromatography

The figure shows at early stage, the cell culture supernatant are 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.

2.5.4 Anion Exchange Chromatography

Anion exchange chromatography (AEX) have the ability to scavenge endotoxins that may heve entered the process via contaminated manufacturing materials or inappropriate sample handling and have been proven to reduce other key contaminant classes such as nucleotide, virus, and leached protein A with the ability to remove host cell protein (Gagnon, 2007). AEX is applied in a format referred to as flow-through mode. Based on the figure below, the absorbent is positively charged while buffer conditions are set so that the antibody pass through the column while strongly electronegative contaminants are captured.

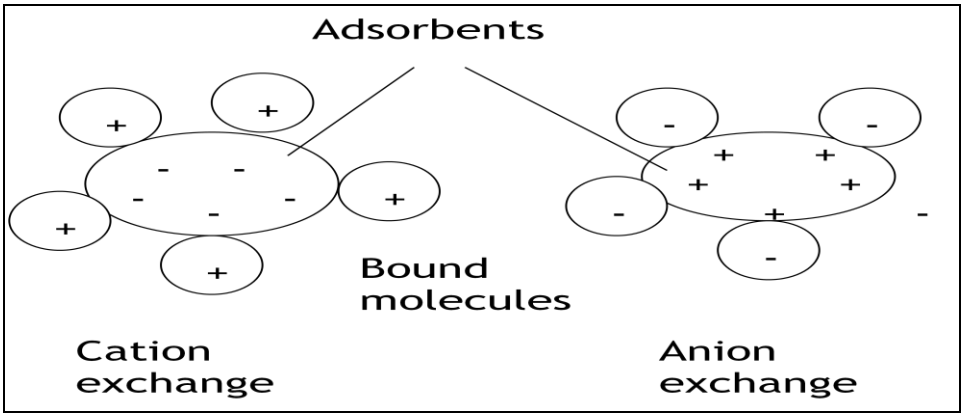

Figure 2.5 The action in anion exchange chromatography

2.5.5 Hydrophobic Interaction Chromatography

Hydrophobic interaction based adsorption relies on the interaction between the hydrophobic patches on the molecules and those on the absorbent. Proteins are made up of hydrophobic and hydrophilic amino acids. The hydrophobic amino acids remain shielded by a structured layer of water molecules in aqueous protein solutions. Furthermore, the hydrophobic patch on an absorbent is also shielded by a similar structured layer of water. The principle of hydrophobic interaction based adsorption is shown in the figure below.

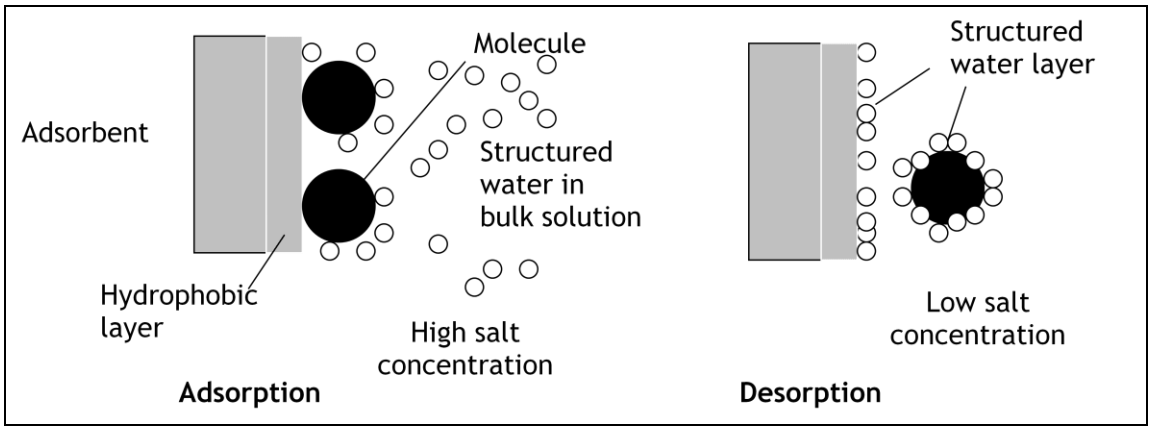

Figure 2.6 The action in hydrophobic interaction chromatography

However in high salt concentration situation, in which there are addition of anti-chaotropic salt to the protein solution, the water molecules making up the structured layers around the hydrophobic patches are removed and the hydrophobic patches on the absorbent surface are exposed.

2.6 SuperPro Designer® versions 6.0

In a wide range of industries such as Pharmaceutical, Biotechnology and etc, SuperPro Designer version 6.0 facilitates in modeling, evaluation and optimization of integrated processes. The user is enabling to concurrently design and evaluate manufacturing and end-of-pipe treatment processes and practice waste minimization via pollution prevention as well as pollution control with the combination of manufacturing and environmental operation models in the same package (Intelligen, 1999).

CHAPTER 3

METHODOLOGY

This chapter described on the procedure of framework designed and simulated. The framework was focusing on the concentration of Immunoglobulin G (IgG) which involving the removal of impurities in the stream. The flow chart below is the summaries of the methodology applied.

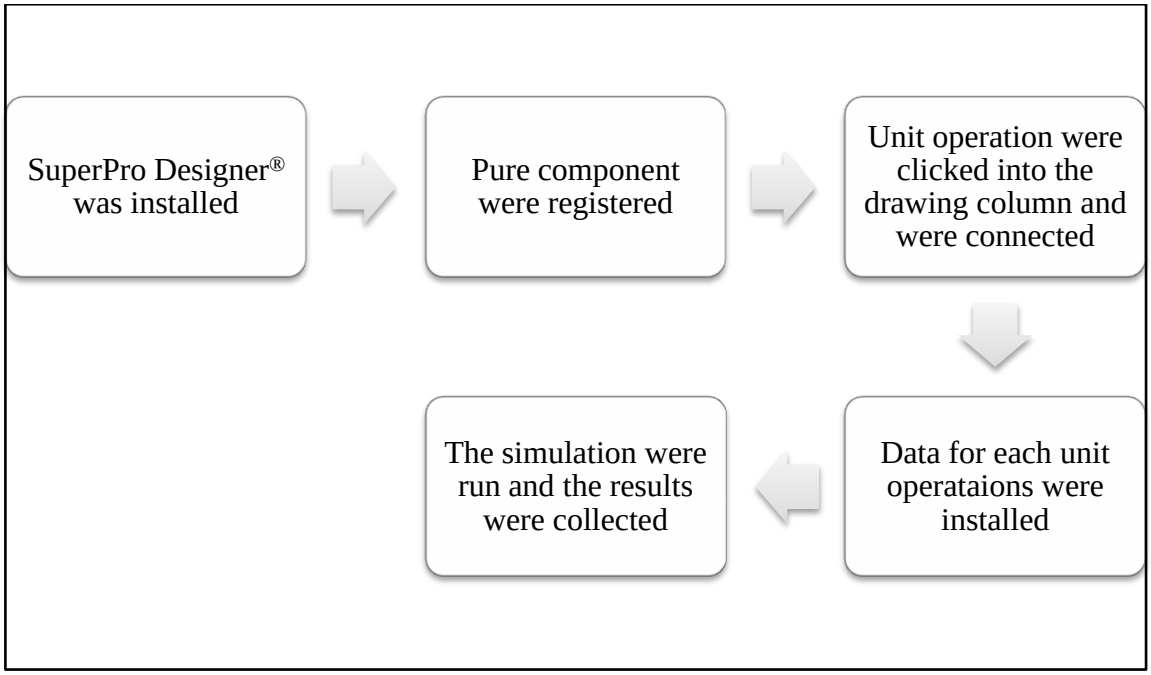

Figure 3.1 The flow chart of simulation conducted.

3.1 Installation of Simulator

The SuperPro Designer® version 6.0 for academic site software was installed from the CD`s software to the computer. The installed procedures required name and addresses typed in. By clicked on the SuperPro Designer® icon, the main window of the simulator was opened. The new flow sheet with the batch mode operation was chosen. The figure below shows the main window of SuperPro Designer® which has three main parts which are the quick access toolbar located on the top window, the icons, and the drawing window.

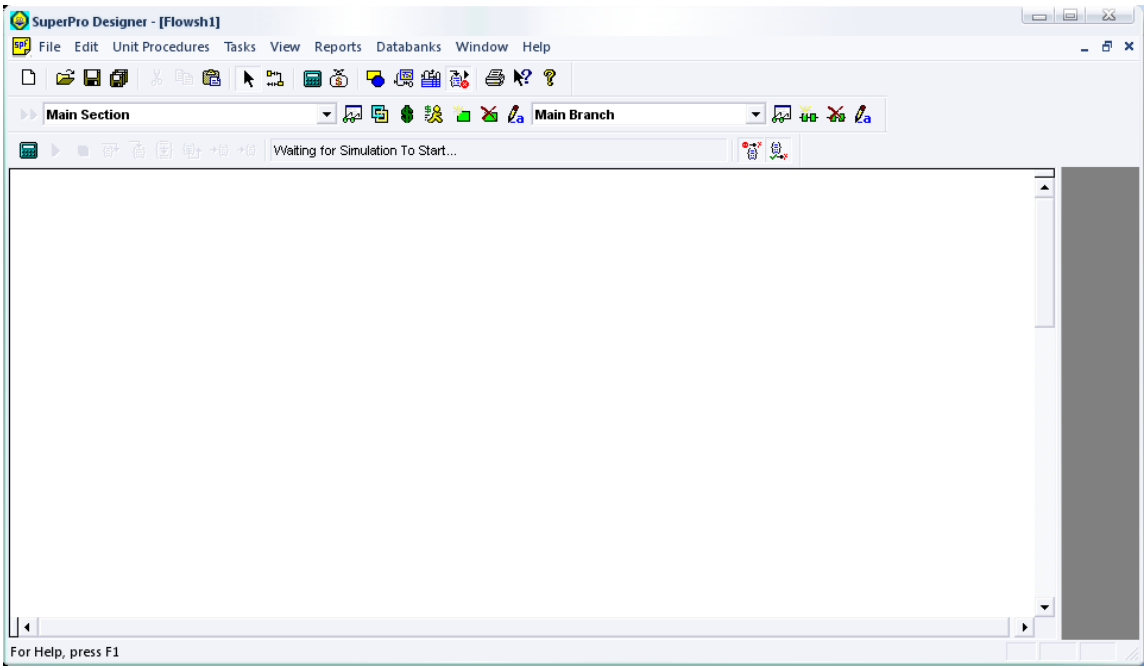

Figure 3.2 The SuperPro Designer® version 6 main window

3.2 Pure and Stock Mixture Components

The pure and stock mixture components involved in the simulation were registered by clicked on the [tasks] bar icon located at the quick access toolbar. The [edit pure component] or [edit stock mixture] was chosen and new register/edit pure component was opened. Some of the components involved were already existed in the designer simulator, the components register procedures was by select the component and then clicked on the register menu. However, for component that not exists, the component registered trough new component definition interface. Details such as name, physical and chemical properties were put in the registration window.

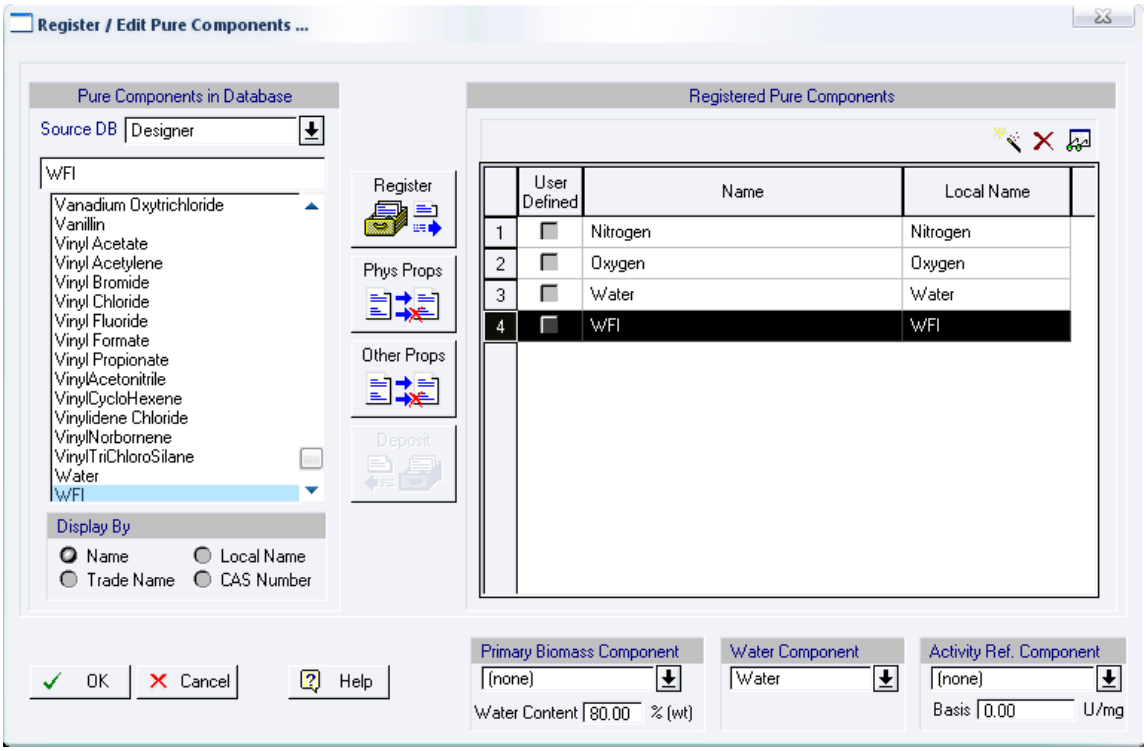

Figure 3.3 Register Pure Components window.

The figure above shows the register pure components window. The window consists of four main parts which are the list of existed pure components in database of the simulator designer, the registered pure components list, the icons task, and the properties of the process.

Table 3.1 : Lists of Chemical and Biological substances involved.

Pure components	**Stock mixtures**
Ammonium Sulfate	Air
Biomass	Methanol (50% w/w)
Glycine	Sodium Chloride (1M)
Immunoglobulin G (IgG)	Sodium Hydroxide (0.5M)
Potassium Chloride	Sodium Hydroxide (1M)
Potassium Di-hydrogen Phosphate	PBS
Media	
Methanol	
Sodium Hydro Phosphate	
Nitrogen	
Oxygen	
Phosphoric Acid	
Sodium Chloride	
Sodium Citrate	
Sodium Hydroxide	
Sodium Phosphate	
TRIS Base	
TRIC HCL	
Unreaction Media	
Urea	
Water	
Water flow Injection (WFI)	

The table above shows the required chemical and biological components of the downstream processing for monoclonal antibodies production to be registered in the simulator. There are twenty-two of pure components and six stock mixture involved in the process.

3.2 Unit Operations

The unit operations involved in the downstream processing of monoclonal antibodies was introduced into the drawing window by clicked on the [unit procedures] toolbar located at the quick access toolbar. The preferred unit operations were chosen from a list by their category and type. A Chain of unit operations involve was introduced and was well connected by a stream between each of the unit operations.

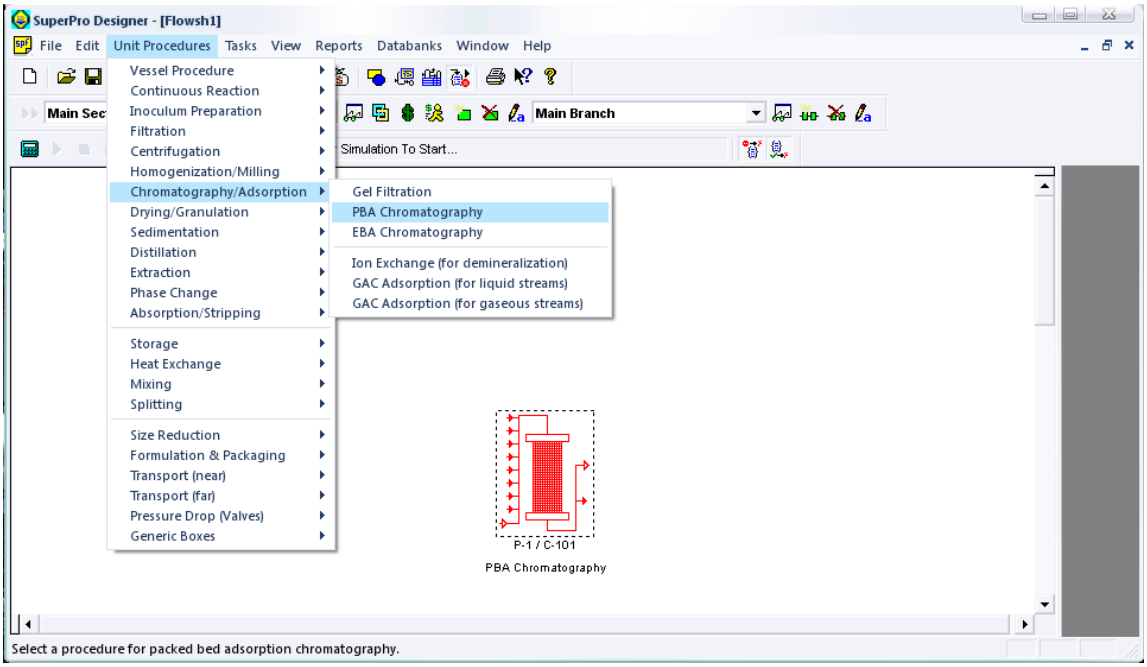

Figure 3.4 A unit operation was introduced to the drawing window.

3.3 Data of Unit Operations

Each of the unit operations have unique operation, based on their functions. The operation`s data were determined based on their functions and tasks. The operation`s data were introduced by left-clicked onto the unit operations. The figure below shows the steps to enter the sequence data interface.

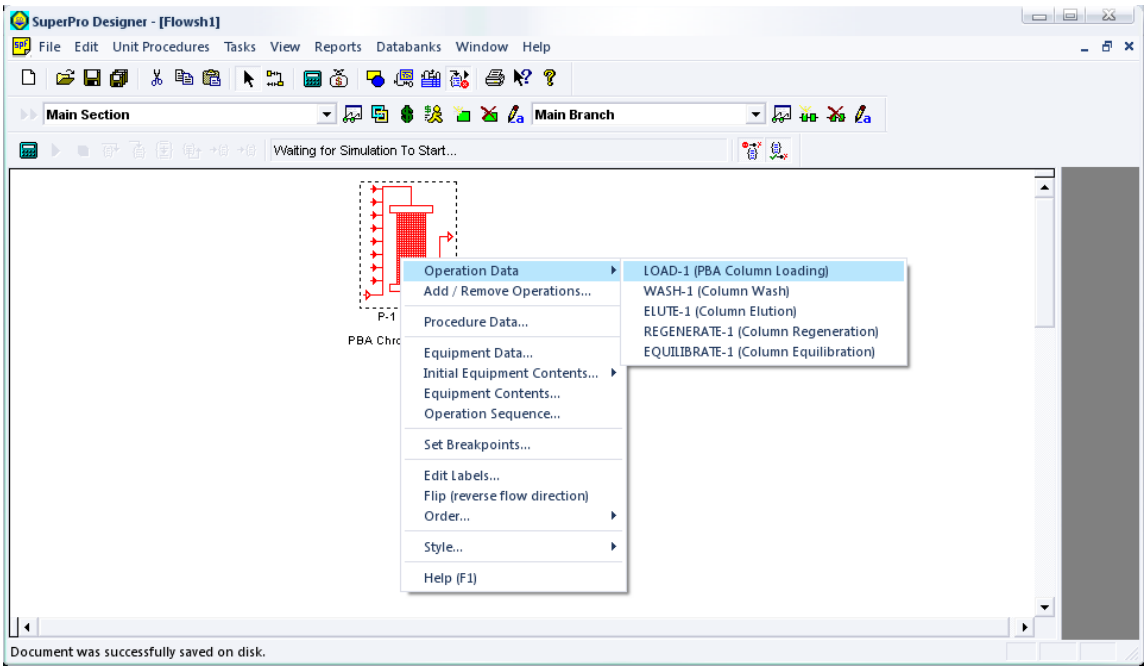

Figure 3.5 The operation data for the unit operation

Various operation data installed for each unit operations. This data installed determine the tasks of the unit operations. The table below shows the unit operations with the sequences operation data involved.

Table 3.2 : Types of operation data for unit operations.

Unit Operation	**Sequences Operation Data**
Storage Tank	Transfer In, Store, Transfer Out, Cleaning In Place (CIP)
Disk Stack Centrifuge	Centrifuge, CIP
Microfiltration	Filter, CIP
Affinity Chromatography	Load, Elute, Equilibrate, Wash, Regenerate
Anion Exchange Chromatography	Load, Elute, Equilibrate, Strip, Rinse
Hydrophobic Interaction Chromatography	Load, Elute, Equilibrate, Wash, Regenerate
Dead End Filtration	Filter, CIP

There are seven type unit operation reqiured to simulate the downstream processing for monoclonal antibodies production with every types, has it own unique sequences operation data.

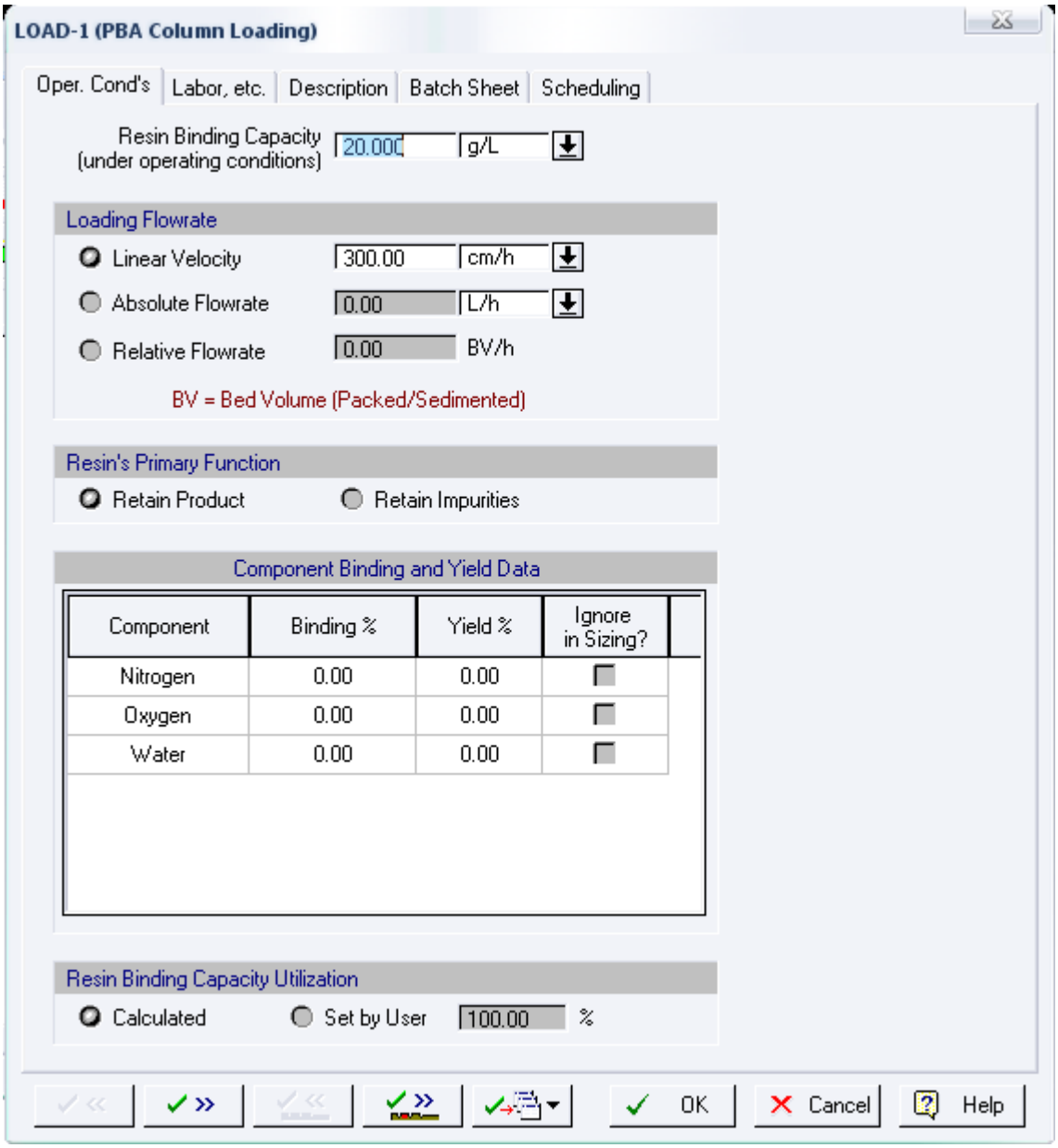

Figure 3.6 Example of sequence data interface.

3.4 Simulation

The simulation was performed by the clicked on the simulation icon. The simulation can maybe interrupt and lead to fail, if there are errors exists in the process such as unconnected stream, wrong data operation introduced or the stream are not be initialize yet. The simulation was considered successfully if the material and energy balance was successfully performed and can produce a report on the balance.

CHAPTER 4

RESULT AND DISCUSSION

In this chapter, the framework of downstream processing for monoclonal antibodies using SuperPro Designer® and the data obtained throughout the simulation are analyzed and discussed. The result of the simulation has shown a significant number of purity achieved by the Immunoglobulin G (IgG) using the framework suggested. Summaries of the results are presented in table and figures.

4.1 The Framework

This framework is designed using SuperPro Designer® version 6.0 which consists of eleven types of unit operation to carries the downstream process of monoclonal antibodies production. There are eleven unit operations involved in the framework which simulated the downstream processing that carries the recovery and purification of monoclonal antibodies. These units are categorized into three major segments, which are recovery, purification and polishing units. The summaries of the unit operation are listed in the table 4.1 and figure 4.1.

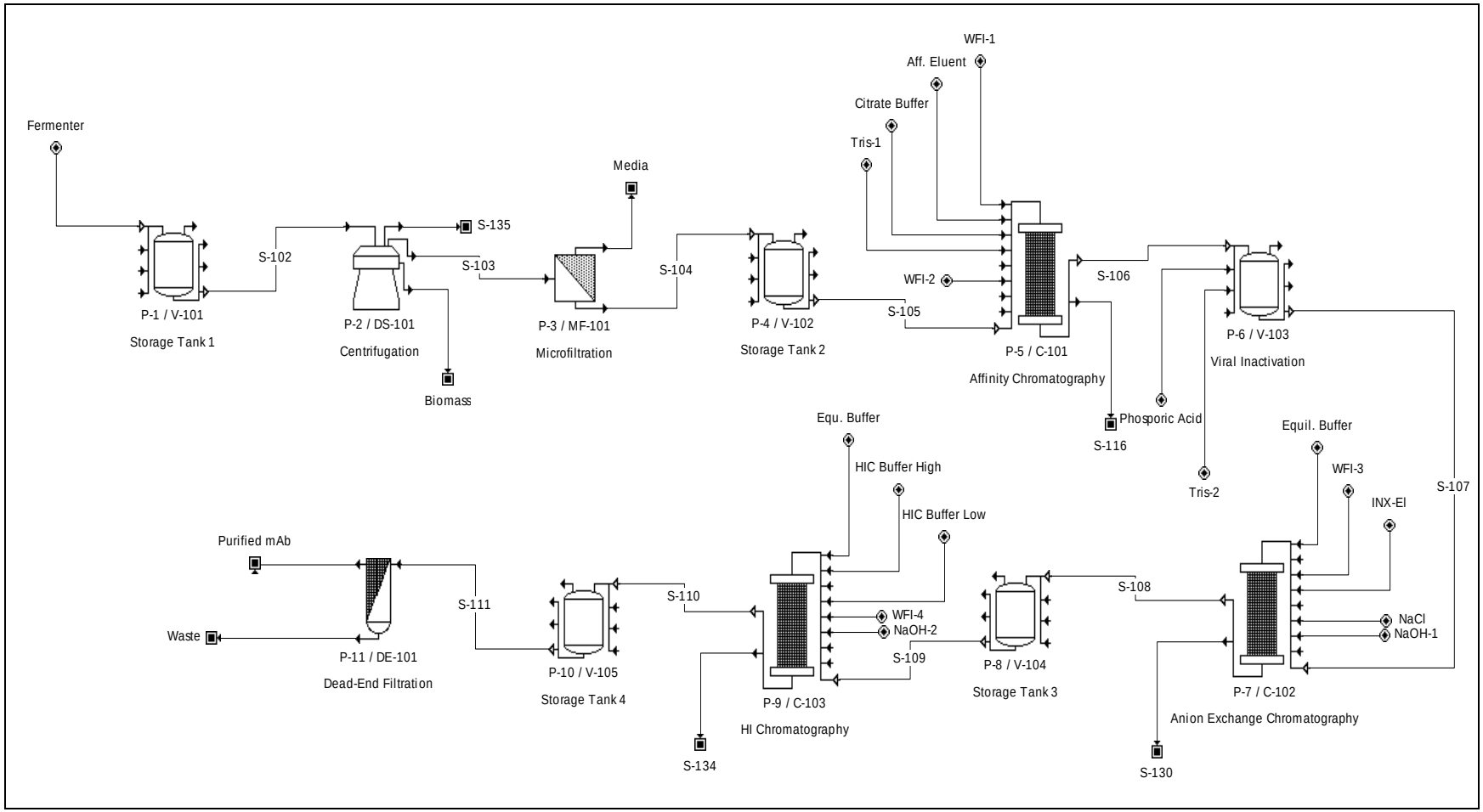

Figure 4.1 The framework of downstream processing for monoclonal production.

Table 4.1 : Unit operations preferred

Segment	Unit Operation
Recovery	Disk-stake centrifuge Microfiltration
Purification	Affinity Chromatography
Polishing	Anion Exchanged Chromatography Hydrophobic Interaction Chromatography Dead End Filtration

Figure 4.1 illustrate the proposed framework on downstream processing for monoclonal antibodies by using the SuperPro Designer® simulator. The process started with the storage tank 1 which received the components directly from a fermenter. Then proceed with disk stack centrifuge and microfiltration until the components stored in the storage tank 2. After the storage tank 2, the first chromatography, protein A affinity chromatography was used, then the components stored in the storage tank 3 before the process proceed with two more chromatography; Anion exchanged and hydrophobic interaction. The final product then filtered with dead end filtration.

The table 4.1 shows the techniques and unit operations involved in the proposed framework on the downstream processing for monoclonal antibodies production. These units were proposed because they gave the best result based on the recovery and purity of the mAbs. There are three stages includes recovery, purification and polishing techniques which consist of six unit operations; disk stack centrifuge and microfiltration for recovery, Protein A affinity chromatography for purification, and three unit operations for polishing technique which are anion exchanged chromatography, hydrophobic interaction chromatography and dead end filtration.

4.1.1 Storage Tank 1

The first unit operation is storage tank 1, which stored the chemical and biological substance directly from fermenter, in adiabatic condition. The purpose of this tank is to ensure that the next unit operation is in ready mode to accept the substances.. The volume of tank is 2500 L in order to fill the total volume of 2208.62 L of contents with the pressure condition of 1.013 bar. As the table below shows the contents of the tank, the mass of the contents of the transfer out shows some decreasing from the transfer in. This may be due to the accumulation of the contents in the tank.

Table 4.2 : Equipment contents of storage tank 1

		Start	Transfer in	Store	Transfer out
Duration	h		1.33	1	1.33
Time Line	h	0	1.33	2.33	3.67
Total Contents	kg	2.8937	2189.654	2189.654	2.8937
Nitrogen		2.2198	2.2198	2.2198	2.2198
Oxygen		0.6739	0.6739	0.6739	0.6739
Biomass		0	0.8747	0.8747	0.87470
IgG		0	0.2187	0.2187	0.21868
Media		0	19.1363	19.1363	19.13634
UnRxn Media		0	1.5307	1.5307	1.53073
WFI		0	2165	2165	2164.99955
Liq/Sol Phase	kg	0	2186.76	2186.76	
Biomass		0	0.8747	0.8747	0.87470
IgG		0	0.2187	0.2187	0.21868
Media		0	19.1363	19.1363	19.13634
UnRxn Media		0	1.5307	1.5307	1.53073
WFI		0	2165	2165	2164.99955
Vapor Phase	kg	2.894	2.894	2.894	2.894
Nitrogen		2.22	2.22	2.22	2.22
Oxygen		0.6739	0.6739	0.6739	0.6739
Total Mass	kg	2.8937	2189.654	2189.654	2.8937
Liq/Sol Mass	kg	0	2186.76	2186.76	2186.76
Liq/Sol Density	g/L	1000	990.36	990.36	990.36
Liq/Sol Volume	L	0	2208.049	2208.049	2208.049
Temperature	°C	25	36.98	36.98	36.98
Pressure	bar	1.013	1.013	1.013	1.013

4.1.2 Disk Stack Centrifuge

The second unit operation after storage tank 1 is disk stack centrifuge with limiting diameter of solid is 0.45 µm. This centrifuge removed cells and cells debris which majority of solid particles based on the density differences between two phases. However it also removed Immunoglobulin G (IgG), the target protein and Water for injection (WFI). The table below shows the flowrate (kg/batch) and concentration of components in the main stream. As it shows, the biomass was removed completely however others impurities such as media and unreacted media were retained in the stream with low reduction which is about lower than 0.5 %.

Table 4.3 : Components in disk stack centrifuge

Components	**Transfer In**		**Transfer Out**	
	Mass (kg)	Concentration (g/L)	Mass (kg)	Concentration (g/L)
IgG	0.21868	0.09904	0.21818	0.09853
Media	19.13634	8.66663	19.09300	8.62203
Unreacted Media	1.53073	0.69325	1.52727	0.68968
WFI	2164.99955	980.50356	2160.09684	975.25836
Biomass	0.87470	0.39614	0	0

The table above proved the theory by the output of biomass was turned down from exact 0.87470 kg to 0 kg, while the other components were still maintained the initial figures.

4.1.3 Microfiltration

Next, the unit operation is microfiltration which removes more solid particles that are passed through the centrifugation step. This unit operation is crucial since the cells and cells debris which is such as media, and unreaction media is not removed by the centrifugation. The table below shows the contents in the equipment. As the table shows, there was about 80% removal of media and unreacted media. Meanwhile, the IgG concentration increased due to the removal of these impurities and the WFI also reduced 80% of the contents made the stream more concentrated.

Table 4.4 : Components in the microfiltration

Components	**Transfer In**		**Transfer Out**	
	Flowrate (kg/batch)	Concentration (g/L)	Flowrate (kg/batch)	Concentration (g/L)
IgG	0.21868	0.09904	0.20727	0.46788
Media	19.13634	8.66663	3.81715	8.61652
Unreacted Media	1.53073	0.69325	0.30752	0.69417
WFI	2164.99955	980.50356	431.85512	974.83476

The table 4.4 shows the figures of two different components which were media and WFI were reduced significantly from exact 19.13634 kg of media to 3.81715 kg and exact 2164.99955 kg of WFI to 974.83476 kg. These shows that this stage was the stage of recovery which explained the removal of impurities which has widely differences with target IgG and also reduced the volume of components.

4.1.4 Storage Tank 2

After the microfiltration, the stream was introduced to storage tank 2 which the function same as the storage tank 1, that is to hold the substance before it can be continued by the next unit operation. The volume of tank is 2500 L in order to fill the total volume of 442.997 L of contents with the pressure condition of 1.013 bar. The tank is made of carbon steel. The table below is the contents of the equipment.

Table 4.5 : The equipment contents of storage tank 2

		Start	Transfer In	Store	Transfer Out
Duration	H	3.12	1.06	1	1.06
Time Line	H	0	1.06	2.06	3.12
Total Contents	Kg	0.5806	436.7676	436.7676	0.5806
Nitrogen		0.4454	0.4454	0.4454	0.4454
Oxygen		0.1352	0.1352	0.1352	0.1352
IgG		0	0.2073	0.2073	0.20727
Media		0	3.8171	3.8171	3.8171
UnRxn Media		0	0.3075	0.3075	0.3075
WFI		0	431.8551	431.8551	431.8551
Liq/Sol Phase	Kg	0	436.1871	436.1871	436.1871
IgG		0	0.2073	0.2073	0.2073
Media		0	3.8171	3.8171	3.8171
UnRxn Media		0	0.3075	0.3075	0.3075
WFI		0	431.8551	431.8551	431.8551
Vapor Phase	Kg	0.5806	0.5806	0.5806	0.5806
Nitrogen		0.4454	0.4454	0.4454	0.4454
Oxygen		0.1352	0.1352	0.1352	0.1352
Total Mass	Kg	0.5806	436.7676	436.7676	0.5806
Liq/Sol Mass	Kg	0	436.1871	436.1871	0
Liq/Sol Density	g/L	1000	984.63	984.63	1000
Liq/Sol Volume	L	0	442.9974	442.9974	0
Temperature	°C	25	52.65	52.65	52.65
Pressure	Bar	1.013	1.013	1.013	1.013

4.1.5 Affinity Chromatography

After the storage tank 2, the affinity chromatography was the unit operation installed, which the main objective to capture the Immunoglobulin G. This unit operation has several operation sequences which are load, elution, equilibration, and also wash. These sequences are crucial to determined whether it can perform efficiently. This chromatography use a protein A affinity absorbent which was the specific ligand, recognized and bind IgG from the stream while the impurities remain in the stream. The table below shows the equipment contents for each components and sequences. From the tables below, the data shows that the affinity chromatography has retained the IgG with a 94.723% of capture percentages. It also remove completely all impurities before which are media and unreacted media. However, this chromatography also adds others impurities which are buffer contain components such as Sodium Chloride (NaCl) and Tris (hydroxymethyl) aminomethane Cl^- (TRIS HCl) which were used in the operating procedures in the chromatography.

Table 4.6 : Equipment contents of Affinity Chromatography.

		Start	Load	Elute	Equilibrate	Wash -1	Regenerate	Wash-2	Transfer out
Duration	H	4.56	3.56	0.33	0.17	0.17	0.17	0.17	
Time Line	H	0	3.56	3.9	4.06	4.23	4.4	4.56	
Total Contents	Kg	0	436.1871	44.586	44.854	20.617	20.635	20.617	22.490
IgG		0	0.2073	0	0	0	0	0	0.19691
Media		0	3.8171	0	0	0	0	0	0
UnRxn Media		0	0.3075	0	0	0	0	0	0
WFI		0	431.8551	37.71536	0	20.61741	20.59752	20.61741	18.85768
Sodium Chloride		0	0	6.64192	0	0	0	0	3.32096
Sodium Citrate		0	0	0	0	0	0.03714	0	0
TRIS HCl		0	0	0.22895	44.85361	0	0	0	0.11448
Liq/Sol Phase	kg	0	436.1871	44.586	44.854	20.617	20.635	20.617	22.490
IgG		0	0.2073	0	0	0	0	0	0.19691
Media		0	3.8171	0	0	0	0	0	0
UnRxn Media		0	0.3075	0	0	0	0	0	0
WFI		0	431.8551	0	0	0	0	0	18.85768
Vapor Phase	kg	0	0	0	0	0	0	0	0
Total Mass	kg	0	436.1871	44.586	44.854	20.617	20.635	20.617	22.490
Liq/Sol Mass	kg	0	436.1871	44.586	44.854	20.617	20.635	20.617	22.490
Liq/Sol Density	g/L	1000	984.63	1000	1000	1000	1000	1000	984.63
Liq/Sol Volume	L	0	442.9974	41.454	20.727	20.727	20.727	20.727	20.925f
Temperature	°C	25	52.65	52.65	52.65	52.65	52.65	52.65	52.65
Pressure	bar	1.013	1.013	1.013	1.013	1.013	1.013	1.013	1.013

4.1.4 Viral Inactivation Tank

Next the affinity chromatography is viral inactivation tank which the function is to ensure the viral in the stream are in passive mode. This is done by adding phosphoric acid into the stream making the solution is in acidic condition. The tank also act as storage tank before continue to the second chromatography. The table below shows the equipment contents of the tank. In this tank, phosphoric acid and TRIS Base were added to lower the pH of the solution to 5.6.

Table 4.7 : The equipment contents of viral inactivation tank.

		Start	Transfer In	Charge 1	Charge 2	Store	Transfer Out
Duration	h	3.07	0.37	0.5	0.5	1	0.7
Time Line	h	0	0.37	0.87	1.37	2.37	3.07
Total Contents	kg	0.1581	22.6481	122.6481	222.6481	222.6481	222.490
Nitrogen		0.1213	0.1213	0.1213	0.1213	0.1213	0.1213
Oxygen		0.0368	0.0368	0.0368	0.0368	0.0368	0.0368
IgG		0	0.1969	0.1969	0.1969	0.1969	0.19691
Sodium Chloride		0	3.321	3.321	3.321	3.321	3.32096
TRIS HCl		0	0.1145	0.1145	0.1145	0.1145	0.11448
WFI		0	18.8577	18.8577	18.8577	18.8577	18.85768
Phosphoric Acid		0	0	100	100	100	100
TRIS Base		0	0	0	100	100	100
Liq/Sol Phase	kg	0	22.49	122.49	222.49	222.49	222.490
IgG		0	0.1969	0.1969	0.1969	0.1969	0.19691
Sodium Chloride		0	3.321	3.321	3.321	3.321	3.32096
TRIS HCl		0	0.1145	0.1145	0.1145	0.1145	0.11448
WFI		0	18.8577	18.8577	18.8577	18.8577	18.85768
Phosphoric Acid		0	0	100	100	100	100
TRIS Base		0	0	0	100	100	100
Vapor Phase	kg	0.1581	0.1581	0.1581	0.1581	0.1581	0.1581
Nitrogen		0.1213	0.1213	0.1213	0.1213	0.1213	0.1213

4.1.7 Anion Exchange Chromatography

After the viral inactivation tank, the second chromatography which is Anion Exchange Chromatography with several sequences such as load, elute, equilibrate, strip and rinse. This chromatography will be flow trough line with another chromatography which is next after this unit operation. The isoelectric point (p*I*) of IgG is around 7.0 and the impurities component`s p*I* is 5.6. Based on these differences of p*I*, these will be opposite charged which is the impurities was negatively charged while IgG was positively charged. Using Q-adsorbent, which is anion exchange absorbent, the impurities were bind with positively charged ligand while the IgG was not. The table below shows the equipment contents of the unit operation. The data from the table show that some impurities have been removed based on the charge of the components whether the components is cation or anion components. This chromatography removed completely phosphoric acid, TRIS Base and TRIS HCl. However a small fraction of IgG was also removed while some of Sodium Chloride still was in the stream.

Table 4.8 : Equipment contents in the Anion Exchange Chromatography.

		Start	Load	Elute	Equilibrate	Strip	Rinse	Transfer Out
Duration	h	1.85	1.02	0.33	0.17	0.17	0.17	
Time Line	h	0	1.02	1.35	1.52	1.69	1.85	
Total Contents	kg	0	222.49	0.345	19.686	20.282	20.104	19.769
IgG		0	0.1969	0	0	0	0	0.17722
Phosphoric Acid		0	100	0	0	0	0	0
Sodium Chloride		0	3.321	0.01969	0.02260	20.28154	0	0.00985
Sodium Hydroxide		0	0	0	0	0	20.10432	0
Sodium Hydro Phosphate		0	0	0.00373	0.21658	0	0	0.00187
TRIS Base		0	100	0	0	0	0	0
TRIS HCl		0	0.1145	0	0	0	0	0
WFI		0	18.8577	0	19.44661	0	0	19.58037
Liq/Sol Phase	kg	0	222.49	0	0	0	0	19.769
IgG		0	0.1969	0	0	0	0	0.17722
Phosphoric Acid		0	100	0	0	0	0	0
Sodium Chloride		0	3.321	0	0	0	0	0.00985
TRIS Base		0	100	0	0	0	0	0
TRIS HCl		0	0.1145	0	0	0	0	0
WFI		0	18.8577	0	0	0	0	0
Total Mass	kg	0	222.49	0	0	0	0	19.769
Liq/Sol Mass	kg	0	222.49	0	0	0	0	19.769
Liq/Sol Density	g/L	1000	1844.6	1000	1000	1000	1000	994.967
Liq/Sol Volume	L	0	120.6167	0.336	19.691	19.691	10.691	19.869

4.1.8 Storage Tank 3

The storage tank 3 is the next unit operation after the affinity chromatography. This unit is required as it is stored the substance between two units of chromatography. It also function same as previous storage tank. The volume of tank is 25 L in order to fill the total volume of 19.7953 L of contents with the pressure condition of 1.013 bar. The tank is made of carbon steel. The table below shows the equipment data from this unit.

Table 4.9 : Equipment contents for storage tank 3

		Start	Transfer In	Store	Transfer Out
Duration	h	1.73	0.37	1	0.37
Time Line	h	0	0.37	1.37	1.73
Total Contents	kg	0.026	19.7953	19.7953	19.769
Nitrogen		0.02	0.02	0.02	0.02
Oxygen		0.0061	0.0061	0.0061	0.0061
IgG		0	0.1772	0.1772	0.17722
Na2HPO4		0	0.0019	0.0019	0.0019
Sodium Chloride		0	0.0098	0.0098	0.0098
WFI		0	19.5804	19.5804	19.5804
Liq/Sol Phase	kg	0	19.7693	19.7693	19.7693
IgG		0	0.1772	0.1772	0.1772
Na2HPO4		0	0.0019	0.0019	0.0019
Sodium Chloride		0	0.0098	0.0098	0.0098
WFI		0	19.5804	19.5804	19.5804
Vapor Phase	kg	0.02604	0.02604	0.02604	0.02604
Nitrogen		0.01997	0.01997	0.01997	0.01997
Oxygen		0.006064	0.006064	0.006064	0.006064
Total Mass	kg	0.026	19.7953	19.7953	0.026
Liq/Sol Mass	kg	0	19.7693	19.7693	19.7693
Liq/Sol Density	g/L	1000	994.98	994.98	994.98
Liq/Sol Volume	L	0	19.869	19.869	19.869
Temperature	°C	25	25	25	25
Pressure	bar	1.013	1.013	1.013	1.013

4.1.9 Hydrophobic Interaction Chromatography

The next unit operation is Hydrophobic Interaction Chromatography. This unit must be present in the following of anion exchange chromatography as it is acts as the polishing steps while remove the unwanted impurities that are toxic to the stream in events such as protein A leach out and flow through the main stream. The table below shows the equipment contents of the chromatography.

Table 4.10 : Equipment contents of Hydrophobic Interaction Chromatography.

		Start	Load	Elute	Equilibrate	Wash	Regenerate	Transfer Out
Duration	h	1.02	0.19	0.33	0.17	0.17	0.17	
Time Line	h	0	0.19	0.52	0.69	0.85	1.02	
Total Contents	kg	0	19.7693	35.47	19.173	17.63	17.960	17.905
IgG		0	0.1772	0	0	0	0	0.16836
Amm. Sulfate				0.035	3.32266			0.01772
Na2HPO4		0	0.0019	0.493	0.22244	0	0	0.24682
Sodium Chloride		0	0.0098	0	0	0	0	0
Sodium Hydro.		0	0	0	0	0	0.69186	
WFI		0	19.5804	34.94	15.62778	17.63	17.26821	17.4717
Liq/Sol Phase	kg	0	19.7693	35.47	19.173	17.63	17.960	17.905
IgG		0	0.1772	0	0	0	0	0.16836
Na2HPO4		0	0.0019	0.035	3.32266	0	0	0.24682
Amm. Sulfate				0.493	0.22244			0.01772
Sodium Chloride		0	0.0098	0	0	0	0	0
WFI		0	19.5804	34.94	15.62778	17.63	17.26821	17.4717
Total Mass	kg	0	19.7693	35.47	19.173	17.63	17.960	17.905
Liq/Sol Mass	kg	0	19.7693	35.47	19.173	17.63	17.960	17.905
Liq/Sol Density	g/L	1000	994.98	994.9	994.98	994.9	994.98	994.98
Liq/Sol Volume	L	0	19.869	35.40	17.722	17.22	17.722	17.891
Temperature	°C	25	25	25	25	25	25	25
Pressure	bar	1.013	1.013	1.013	1.013	1.013	1.013	1.013

4.1.10 Storage Tank 4

The storage tank 4 is the unit operation after the HIC unit. This unit does the same function as the previous storage tank, which is to store the contents before it can be flow to the next unit operation. The volume of tank is 25 L in order to fill the total volume of 17.928 L of contents with the pressure condition of 1.013 bar. The tank is made of carbon steel. The table below shows the equipment data of this unit.

Table 4.11 : Equipment content of storage tank 4

		Start	Transfer In	Store	Transfer Out
Duration	h	1.73	0.36	1	0.36
Time Line	h	0	0.36	1.36	1.73
Total Contents	kg	0.0234	17.928	17.928	0.0234
Nitrogen		0.018	0.018	0.018	0.018
Oxygen		0.0055	0.0055	0.0055	0.0055
Amm. Sulfate		0	0.0177	0.0177	0.0177
IgG		0	0.1684	0.1684	0.1684
Na2HPO4		0	0.2468	0.2468	0.2468
WFI		0	17.4717	17.4717	17.4717
Liq/Sol Phase	kg	0	17.9046	17.9046	17.9046
Amm. Sulfate		0	0.0177	0.0177	0.0177
IgG		0	0.1684	0.1684	0.1684
Na2HPO4		0	0.2468	0.2468	0.2468
WFI		0	17.4717	17.4717	17.4717
Vapor Phase	kg	0.02345	0.02345	0.02345	0.02345
Nitrogen		0.01799	0.01799	0.01799	0.01799
Oxygen		0.00546	0.00546	0.00546	0.00546
Total Mass	kg	0.0234	17.928	17.928	17.928
Liq/Sol Mass	kg	0	17.9046	17.9046	17.9046
Liq/Sol Density	g/L	1000	1000.76	1000.76	1000.76
Liq/Sol Volume	L	0	17.891	17.891	17.891
Temperature	°C	25	25	25	25
Pressure	bar	1.013	1.013	1.013	1.013

4.1.11 Dead End Filtration

The final unit operation in the process is dead end filtration. This unit specialized in removal all impurities that are has a high range of differences with the Immunoglobulin G. The table below shows that the contents before and after this equipment. In the table, the impurities which are ammonium sulfate and sodium hydro phosphate were removed completely using 0.45 µm of filter membranes. The final product was 100% purity of IgG with 9.49345 g/L of concentration in a 985.21088 g/L of WFI solution.

Table 4.12 : The component flow trough Dead End Filtration.

Component	**Transfer In**		**Transfer Out**	
	Flowrate (kg/batch)	Concentration (g/L)	Flowrate (kg/batch)	Concentration (g/L)
Ammonium Sulfate	0.01772	0.99054	0	0
IgG	0.16836	9.41013	0.16687	9.49345
Sodium Hydro Phosphate	0.24682	13.79585	0	0
WFI	17.47170	976.56403	17.31699	985.21088

4.2 Concentration of Immunoglobulin G

The concentrations of Immunoglobulin G are observed throughout the simulation and at every each major stream. The initial concentration of IgG was 0.09904 g/L meanwhile the final concentration of IgG was 9.49345 g/L. This indicates that the simulator has achieved to increase the concentration of IgG at the final stream. The table below shows the concentration of IgG for each stream.

Table 4.13 : The table of stream number and concentration of IgG.

Stream	**Concentration of IgG (g/L)**
Stream 1	0.09904
Stream 2	0.09936
Stream 3	0.09853
Stream 4	0.46788
Stream 5	0.46789
Stream 6	9.41013
Stream 7	1.63252
Stream 8	8.91930
Stream 9	8.91930
Stream 10	9.41013
Stream 11	9.41013
Stream 12	9.49345

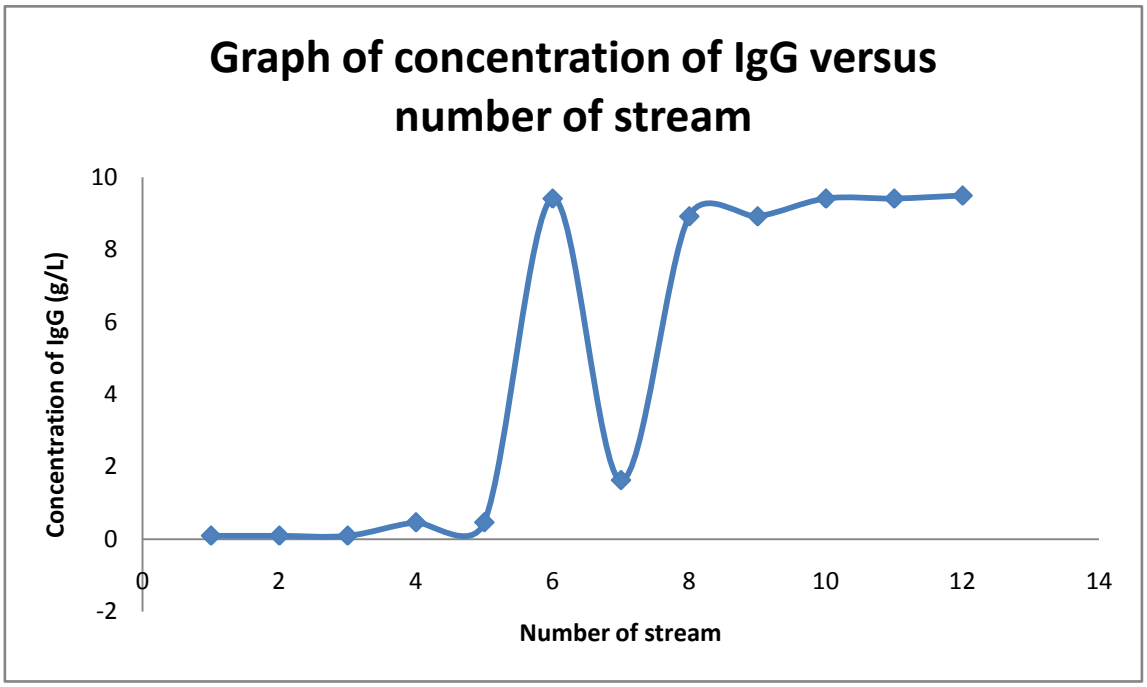

Figure 4.2 Simulation result based on IgG concentration for each stream.

Based on the graph above, the concentration of IgG is increasing against the number of stream. In the graph, there are three different stages which are the first, intermediate and the final stages. The first stage is from stream number 1 until 5, with low concentration of IgG range between 0 to 1 g/L. This is because at the first stage, there are recovery segment which consist of disk-stack centrifuge and microfiltration. At intermediate stage from stream 5 until 7, the concentration of IgG increases exponentially then decreases dramatically with the highest figure is 9.41013 and the lowest is 0.46789. This phenomenon cause by the unit operation involved which is affinity chromatography and then followed by viral inactivates tank. The affinity chromatography gives the highest concentration of IgG while the viral inactivates drop the concentration of IgG by adding impurities to the stream. The final stage is from stream number 8 until 12 giving high and stable readings of IgG concentration which in range of 8 until 10 g/L.

4.3 Recovery of Immunoglobulin G

The recovery of Immunoglobulin G is observed throughout the simulation and at every each major stream. The initial mass of IgG was 0.21868 kg/batch meanwhile the final mass of IgG was 0.16687 kg/batch. This indicates that the simulator has achieved to increase the concentration of IgG at the final stream. The table below shows the recovery of IgG for each stream.

Table 4.14 : The recovery of IgG for each stream

Stream	Mass of IgG (kg/batch)
Stream 1	0.21868
Stream 2	0.21868
Stream 3	0.21818
Stream 4	0.20727
Stream 5	0.20727
Stream 6	0.19691
Stream 7	0.19691
Stream 8	0.17722
Stream 9	0.17722
Stream 10	0.16836
Stream 11	0.16836
Stream 12	0.16687

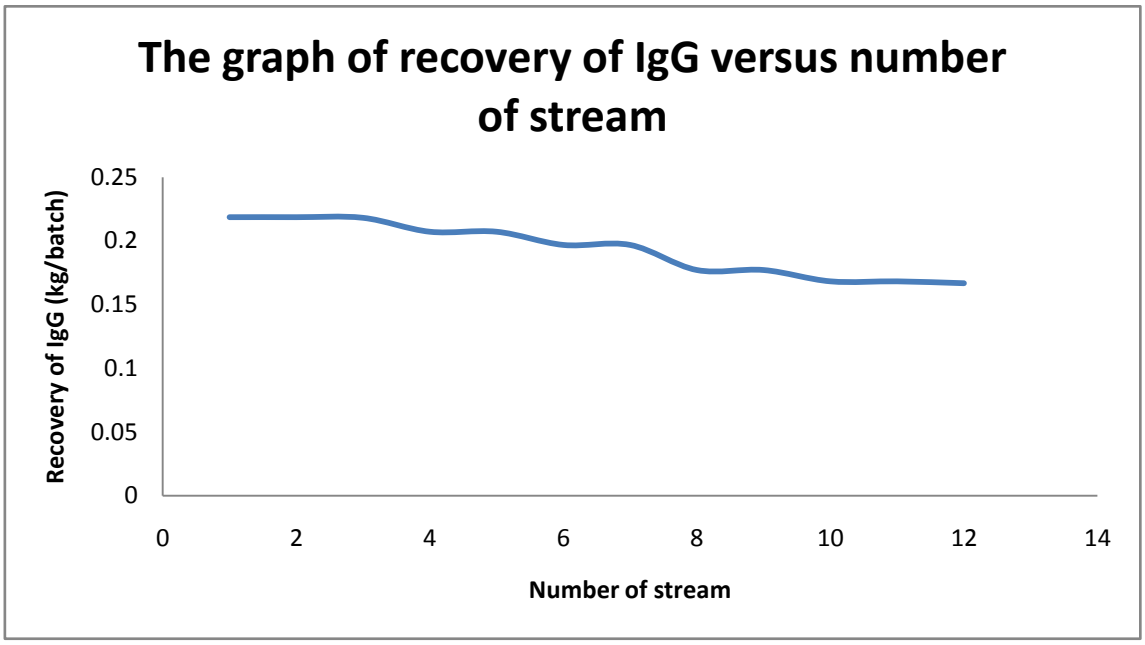

Figure 4.3 Simulation result based on recovery of IgG for each stream.

Based on the graph above, at the first stream which the IgG was initially exit from a fermenter, the mass of IgG for one batch was 0.21868 kg from a 2500 L of fermentation volume. As the numbers of stream increase, the recovery of IgG decreased within average percentages of reducing which is 0.3%. This was because due to unit operations involved, the remaining of IgG accumulated in the units and some were transferred out trough the impurities outlet stream. In the final stream, 0.16687 kg/batch of IgG were recovered throughout the whole process. This indicates the overall percentage of recovery of the downstream processing for mAbs production is 76%.

CHAPTER 5

CONCLUSION AND RECOMMENDATION

5.1 Conclusion

The steps of downstream processing for the monoclonal antibodies production have been identified. There were eleven unit operation involved and divided into three major sections which are recovery, purification and polishing steps. The recovery section consists of Disk Stack Centrifuge and Microfiltration. The Affinity Chromatography is in the purification step while the Anion Exchange Chromatography, Hydrophobic Interaction Chromatography and Dead End Filtration are in the polishing sections. The framework of these downstream processing for monoclonal antibodies production designed with eleven unit operations was introduced to the simulator of SuperPro Designer® version 6.0. The simulation runs successfully and the report of the material and energy balance was produced. The final product was 100% purity of IgG with 9.49345 g/L of concentration and 0.16687 kg/batch with 76.3% of recovery. The result indicates that the framework of downstream processing for monoclonal antibodies has potential to be developed and applied in the biopharmaceutical industry.

5.2 Recommendation

Result from this study will be useful to assist the researchers in a further study of the simulation on the downstream processing for monoclonal antibodies using SuperPro Designer®. Several recommendations proposed as stated below;

- Conduct an experimental study on the downstream processing of monoclonal antibodies to obtain the actual data in order to make a comparison of the between the experimental and simulation result.
- Conduct the study using new version of SuperPro Designer® in order to avoid several limitations in this version.
- Conduct the study using other simulator such as Aspen BPS™ in order to make a comparison and determine which simulators give more accurate result.

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Appendix B

Materials and Stream Report generated by SuperPro Designer®