DESIGN OF A MANUFACTURING TRAIN FOR THE PRODUCTION OF RECOMBINANT THERAPEUTICS EXPRESSED IN MICROBES

A DISSERTATION

Submitted in partial fulfillment of the requirements for the award of the degree of

MASTER OF TECHNOLOGY

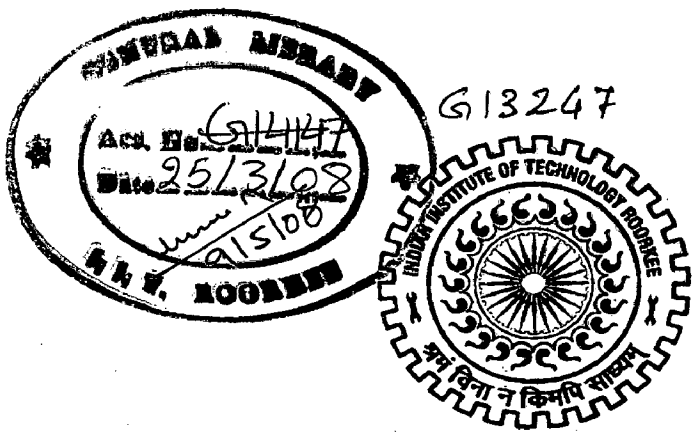
in

CHEMICAL ENGINEERING

(With Specialization in Computer Aided Process Plant Design)

By

GANGADHARARAJU GUMMA



DEPARTMENT OF CHEMICAL ENGINEERING
INDIAN INSTITUTE OF TECHNOLOGY ROORKEE
ROORKEE - 247 667 (INDIA)
JUNE, 2007

CANDIDATE'S DECLARATION

I hereby declare that the work, which is being presented in this dissertation entitled **"Design of a Manufacturing Train for Production of Recombinant Therapeutics Expressed in Microbes "** in the partial fulfillment of the requirement for the award of the degree of **Master of Technology in Chemical Engineering** with specialization in **Computer Aided Process Plant Design,** submitted in the **Department of Chemical Engineering, Indian Institute of Technology Roorkee,** Roorkee, is an authentic record of my own work carried out during July 2006 to June 2007 under the supervision of **Dr. Arnab Kapat,** Director, Reliance School of Life Sciences, Navi Mumbai, and **Dr. I.M.Mishra,** Professor, Department of Chemical Engineering, Indian Institute of Technology Roorkee, Roorkee.

I have not submitted the matter embodied in this dissertation for award of any other degree.

Gangadhara Raju Gumma

Date: 28 June 2007 **(Gangadhara Raju Gumma)**

Place: Roorkee

CERTIFICATE

This is to certify that the above statement made by the candidate is true to the best of my knowledge.

Arnab Kapat

Dr. Arnab Kapat
Director,
Reliance School of Life Sciences,
Dhirubhai Ambani Life Sciences Center,
Navi Mumbai-400701.
India

Dr.I.M.Mishra
Professor,
Department of Chemical Engineering,
Indian Institute of Technology Roorkee,
Roorkee – 247667.
India

ACKNOWLEDGEMENTS

I express my foremost and deepest gratitude to **Dr.I.M.Mishra**, Professor, Department of Chemical Engineering, Indian Institute of Technology Roorkee, Roorkee for his valuable guidance, support and motivation throughout this work. I have deep sense of admiration for his innate goodness and inexhaustible enthusiasm. The valuable hours of discussion and suggestions that I had with him have undoubtedly helped in supplementing my thoughts in the right direction for attaining the desired objective.

My sincere thanks to **Dr. Arnab Kapat,** Director, Reliance School of Life Sciences, Navi Mumbai for his constant encouragement, caring words, constructive criticism and suggestions towards the successful completion of this work. I consider myself extremely fortunate for having got the opportunity to learn and work under his able supervision over the entire period of my association with him.

I do acknowledge with immense gratitude the timely help and support, which I received from my friends in the "Reliance Life Sciences Pvt.Ltd." I am also thankful to the Projects Department of "Reliance Life Sciences Pvt.Ltd." for their kind cooperation.

(GANGADHARARAJU GUMMA)

ABSTRACT

Biopharmaceutical plays a major role over convectional pharmaceutical products in curing diseases. Recombinant DNA technology has made a revolutionary impact in the area of human healthcare by enabling mass production of safe, pure and effective rDNA expression products. The main objective of the work described in this thesis was design of a manufacturing train for the production of recombinant therapeutics expressed in microbes.

Emphasis has been given on design, sizing and control strategies for the key steps in the process. In case of fermentation, sizing calculations, finding out mass transfer coefficient, power consumption and jacket designing have been dealt with. For centrifuge calculation of settling velocity, g-force and throughput analysis are highlighted and for homogenizer, design features and extent of disruption have been discussed.

Additionally, focus has been on discussing main issues in purification steps viz. chromatography and TFF. Sizing calculations, pressure drop, dimensions of bed, HETP and asymmetry have been discussed for chromatography and for TFF system membrane area calculation and pump selection strategies have been illustrated. Apart from these pump sizing calculations for CIP pump, pressure vessel design and Transfer panel design aspects have also been considered.

Basic control strategies for plant automation have been described. In addition, various plant optimization softwares have also been described.

CONTENTS

LIST OF TABLES

LIST OF FIGURES

CHAPTER 1
INTRODUCTION

In contrast to the conventional drugs biopharmaceuticals are complex drugs. One of the driving forces behind the biotech industry today is the need for new drugs, for the treatment of numerous diseases that are not treatable by means of conventional molecules and for which therapeutic proteins offer the best promise of treatment. According to Biotechnology "*Biopharmaceuticals are defined as pharmaceuticals manufactured by biotechnology methods, with the products obviously having biological sources, usually involving live organisms or their active components*" Unlike orally delivered small molecule drugs that underpin the traditional pharmaceutical industry, biopharmaceuticals are usually administered by subcutaneous, intravenous, or intramuscular injection. Biopharmaceutical's greatest potential lies in genetic engineering, which has revolutionized medicine by its ability to mass produce safe and more effective versions of proteins and enzymes created naturally by the human body.

Biopharmaceuticals are complex macromolecules derived from recombinant DNA technology, cell fusion, or processes involving genetic manipulation. They include recombinant proteins, genetically engineered vaccines; therapeutic monoclonal antibodies; and nucleic acid based therapeutics, including gene therapy vectors. Recombinant DNA technology has made a revolutionary impact in the area of human healthcare by enabling mass production of safe, pure and effective rDNA expression products. Recombinant DNA technology is an important tool in scientific research, the diagnosis and treatment of diseases and genetic disorders in many areas of medicine.

For manufacturing of biopharmaceuticals, facility requirements are challenging because they are heat sensitive and many require processing and purification to take place within 2-8°C [1]. These molecules originate from living organisms that can mutate over time leading to instability and unwanted byproducts. Biopharmaceuticals are proteins that can support microbial, or microscopic bacterial growth. This results in the generation of unknown contaminants and product degradation. Therefore, design of manufacturing facility is driven by special considerations particular to Biopharma products. Facility design must comply with "current Good Manufacturing Practices" and viewed from the perspective of validation. The biopharmaceutical manufacturing plant also requires high degree of automation to reduce the batch variability, to improve the quality and to improve the yield.

1.1 Objectives

The objective of this study is to design a manufacturing train for the production of recombinant therapeutics expressed in microbial systems with emphasis on

- Design and sizing calculations for fermentation and primary recovery equipment.
- Design and sizing calculations for purification equipment.
- Pump sizing calculations for CIP solution.
- Pressure vessel and transfer panel design.
- Basic control strategies for plant automation.

CHAPTER 2
LITERATURE REVIEW

2.1 Recombinant DNA Technology

It is the Procedure used to join together DNA segments in a cell-free system (an environment outside a cell or organism). Under appropriate conditions, a recombinant DNA molecule can enter a cell and replicate there, either autonomously or after it has become integrated into a cellular chromosome [2].

Now days the most of the biopharmaceutical industries are producing the medicines based on recombinant DNA technology and monoclonal antibody technology. Recombinant DNA technology has enabled many advances, like

- Identification of mutations
- Isolation of large quantities of pure protein
- Diagnosis of affected and carrier states for hereditary diseases
- Transferring of genes from one organism to another
- Mapping of human genes on chromosomes

The advent of recombinant DNA (rDNA) technology and its application in the pharmaceutical industry has brought about a rapid growth of biotechnology companies and a number of therapeutic rDNA products are available for human use.

2.1.1 Steps to recombination

1. First identify the gene that codes for the production of the protein of interest. One way is to work backwards from the amino acid sequence of the desired protein to the nucleotide sequence of the gene.
2. The gene must be isolated. Restriction enzymes or endonucleases from bacterial cells are key in this step. They are isolated from bacteria that use them to destroy, by cleaving or cutting out, any foreign DNA that gets into the bacterial cell. The use of Restriction enzymes makes them so useful is that each enzyme recognises and cleaves only a very specific sequence of DNA [3]. The staggered cuts yield "sticky" ends, single strands of nucleotide bases capable of binding with complementary sticky ends.
3. Once the gene of interest is obtained it will get into the host cell. This transfer step is accomplished by plasmids, small ring shaped pieces of DNA found naturally in most

bacteria. Plasmids have a region called the replication origin that enables them to be replicated. Plasmids perform one or two other functions, such as antibiotic resistance. Multiple copies of the plasmids used in recombinant DNA technology exist normally within a bacterial cell.

4. After removing a plasmid from a bacterial cell, cleave the plasmid using the same enzyme which is used to clip out the gene. This way the sticky ends of the plasmid will match those of the gene.
5. The cleaved gene and the cleaved plasmid are then mixed together; the sticky ends of the gene and the plasmid come together, their complementary base pairs joined by hydrogen bonds. A DNA ligase enzyme is added which creates a phosphodiester linkage and completes the bond.
6. Finally the plasmids are mixed with the host bacteria. To check that the plasmid has been taken up, they test the bacteria for an innate characteristic of the plasmid. . Only bacteria containing plasmids with antibiotic resistance and the replication origin will survive.
7. Inside the bacteria, the plasmids are replicated so that they exist in multiple copies. The gene becomes active and the bacteria begin producing protein.

2.1.2 Expression system

Both prokaryotic and eukaryotic, are used as hosts for recombinant protein production. Primarily, the choice of host is based on the properties of the target protein and its future purpose. The host systems differ mainly concerning production cost, ease of use, expression levels and achieved final yield. As endogenous proteins are the main contaminations, downstream processing is also dependent on the host. Moreover, the quality of the protein and its proper folding, including post-translational modifications to assure biological activity, are determined by the available processes in the host cell. Prokaryotes are often attractive as hosts for recombinant production because of their cost efficiency and suitability for large-scale cultivation [4]. The most widely used prokaryotic host is the gram-negative enterobacteria *E. coli*. This is because of the detailed understanding of its genetics and physiology which makes *E. coli* easy to manipulate and work with. Since the bacteria grows rapidly in simple media, high

densities of protein producing cells can be obtain in a short period of time. However, being a gram-negative bacterium, the *E. coli* cell is encapsulated by two cell membranes separated by the cell wall and a periplasmic space, which complicates downstream processing of the protein product.

Gram-positive bacteria, such as *Bacillus substilus* (*B. substilus*) (and *Staphylococcus aureus* (*S. aureus*) lacks the outer membrane which simplifies the secretion pathway and makes them suitable for expression of recombinant proteins secreted into the cell media Suitable production vectors are now available although genetic manipulations are not as easy as with *E. coli*. However, *B. subtilis* produces large amounts of extra cellular proteases, which often leads to severe degradation of the protein of interest and thus decreased yields of the full-length gene product.Yeast such as *Saccharomyces cerevisiae*, *Hansenulla polymorpha* and *Pichia pastoris* are among the simplest eukaryotic organisms. They grow relatively quickly and are highly adaptable to large-scale production. These organisms do not produce endotoxin.

2.2 Therapeutic applications of rDNA products

- Hormones of therapeutic interest
- Haemopoietic growth factors
- Blood coagulation factors
- Thrombolytic agents
- Anticoagulants
- Human interferons
- Human interleukins
- Therapeutic enzymes

2.3 Bioprocesses

The bioprocess generally associated with cultivation in appropriate bioreactors (fermentors).

The types of Bio Process are

- Microbial Cell Process
- Animal Cell Process
- Other Cell Process

2.3.1 Microbial Processes

Microbial processes have been implemented in industry for more than 100 years with a minimum of sensor systems. Microbial processes in industry are mostly conducted as fed-batch processes to reach a high volume to time yield .Currently many processes are based on the production of target proteins using *E. coli* as a heterologous host system [5]. The process performance here is mostly based on mineral salt medium processes where yeast extract or other amino acid substrates can be added. Generally the processes are performed as glucose limited fed-batch or directly as a fed-batch and induction is performed by either a chemical inducer or temperature the fed-batch phase. The products are either accumulated as cytoplasm accumulation or inclusion body or mostly at low temperature for products with slow folding rates or if transport of the product to the periplasmic is needed. A challenge to controlling targets during recombinant protein production with *E.Coli* systems is the proper addition of the carbon substrate after induction. Under these conditions severe disturbances of carbon flows in the cell can often occur and the feed rate then has to be adjusted according to the maximum uptake rate of the carbon substrate to avoid high accumulation of acetate.

2.3.2 Animal cell Processes

The increased market for recombinant proteins, and the limited capabilities of microbial cells to express more complicated proteins correctly, has resulted in the development of a multitude of animal cell expression systems. almost all recombinant proteins accepted for human therapeutic use are manufactured either utilizing the microbial hosts *E. coli* and *S. cerevisiae*, or the animal cell hosts CHO (Chinese hamster ovary cell), BHK (baby hamster kidney cell) and hybridoma cells. In most animal cell-based host systems the recombinant protein is secreted out of the cell. The growth media used in animal cell

cultures are very complex and thus purifying the product protein from the complex background of other proteins can be very tedious. The biggest technical differences between the animal cell Stirred Tank Reactors and the Stirred Tank Reactors s used in microbial fermentations are the gassing system in order to avoid bubble formation in animal cell cultures a semi-permeable silicone tube with a valve on exit, which results in higher internal pressure and agitation i.e. slowly rotating blades compared to Rushton-type impellers [5].

2.3.3 Other Cell Processes

Besides microbes and animal cell lines, plants and plant cells, and whole insects and animals, are also used for manufacture of biologics mainly recombinant proteins. Plant cell and tissues are cultured both submerged and on solid surfaces. For submerged cultures range of different reactor like Stirred Tank Reactor, airlift, bubble column, and temporary immersion system bioreactors. Plant cells, like many animal cell lines, are very sensitive to shear stress and mechanical mixing is not an option. The oxygen transfer rates required by plant cells and tissues are much lower than in microbial fermentations and thus aeration is sufficient for mixing. Sedimentation and clumping can be a problem and should be avoided. Wave type and mist reactors have also been studied for plant cell cultures i.e. the former is a closed, disposable plastic bag on a shaker and in the latter the medium is sprayed on the culture.

2.4 Plant Design

The design and build of a biopharmaceutical plant is a complex process requiring multidisciplinary input throughout. A cost effective and fit-for-purpose facility, delivered on time, is the overall objective. This requires that the best practices with respect to engineering, operations, QA, QC, health, safety and environment are adequately addressed during the entire process. The major challenges are that the scope of the project is defined as early as possible, with the input from multi disciplined teams from the operating company and consultants/contractors, with reference to appropriate recognized

guidelines and legislation, and then to ensure that changes are minimized as the project proceeds into the procurement and construction phases.

The development of a design project always starts with an initial idea or plan. This initial idea must be stated as clearly and concisely as possible in order to define the scope of the project, the manufacturing function must ensure that the correct information is available to enable the project to progress. Process information is required to identify the major process steps, along with an initial mass balance (to size equipment and the outline footprint of the facility), raw materials, the finished product form (sterile injectable, freeze dried and so on) and the standard and critical utility requirements. The regulatory status of the product needs to be reviewed to identify the inspection requirements of the facility and which cGMPs are applicable. The cGMP requirements have a significant impact on the design and operation and it is important that they are identified early.

Before any detailed work is done on the design the technical and economic factors of the proposed process should be examined. The various reactions and physical processes involved must be considered, along with the existing and potential market conditions for the particular product. A preliminary survey of gives and indication of the probable success of the project and also shows what additional information is necessary to make a complete evaluation.

2.4.1 Types of Designs

The methods for carrying out a design project may be divided in to the following classifications depending on the accuracy and detail required [6].

- Preliminary or quick-estimated designs
- Detailed-estimate designs
- Firm process designs or detailed designs

Preliminary designs are ordinarily used as a basis for determining whether further work should be done on the proposed process. The design is based on approximate process methods, and rough cost estimate are prepared. Few details are included, and the time spent on calculations kept at a minimum. If the results of the preliminary design show that further work is justified a *detailed –estimate design* may be developed. In this

type of design the cost and profit potential of an established process is determined by detailed analyses and caluculations.The exact specifications are not given for the equipment and drafting room work is minimized. When the detailed-estimate design indicates that the proposed project should be a commercial success the final step before developing construction plans for the plant is the preparation of a *Firm process design.* Complete specifications are presented for all components of the plant, and accurate costs based on quoted prices are obtained. The Firm process design includes blue prints and sufficient information to permit immediate development of the final plans constructing the plant. The need for various types of design estimates during the lifecycle of product development and commercialization has been illustrated in figure 1 [7]. The trapezoidal shape of the graph represents the drastic reduction in product candidates as we move from feasibility studies to commercialization.

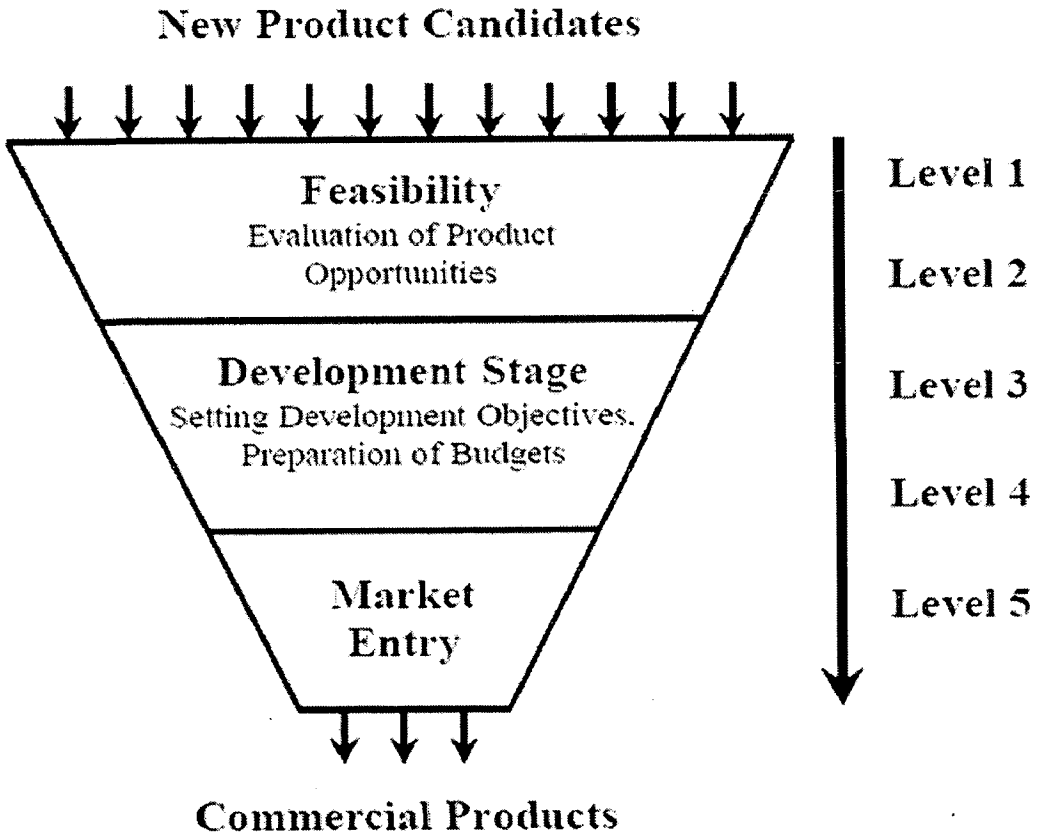


Fig1.Types of design estimates during the lifecycle of a product

The main objective of level 2 and 3 studies is to evaluate alternatives and pinpoint the most cost-sensitive areas the economic "hot-spots" of a complex process. The results

of such analyses are used to plan future research and development and to generate project budgets. Level 4 and 5 studies are usually performed by engineering and construction companies that are hired to build new plants for promising new products that are at an advanced stage of development the opportunities for creative process design work are usually limited to preliminary studies. By the time detailed engineering work is initiated, a process is more than 80% fixed. Furthermore, the vast majority of important decisions for capital expenditures and product commercialization are based on results of preliminary process design and cost analysis [8]. Environmental impact assessment is an activity closely related to process design and cost estimation. Biochemical plants generate a wide range of liquid, solid, and gaseous waste streams that require treatment prior to discharge. The cost associated with waste treatment and disposal has skyrocketed in recent years due to increasingly stricter environmental regulations. This cost can be reduced through minimization of waste generation at the source.

2.4.2 Guidance

ISPE (International Society of Pharmaceutical Engineers) has developed a number of 'Base Line Guides' which have been developed by the pharmaceutical industry in conjunction with the FDA. These guides are invaluable at all stages of the design process and whilst they are specifically directed to the requirements of FDA they have a general applicability. These baseline guides can be used very effectively during the front end design phase and give excellent guidance on aspects of the facility design. They have been developed with input from the FDA, but users must ensure that the design complies with legal requirements and cGMPs. There are also other sources of guidance that should be referenced, in particular the relevant cGMPs, health, safety and environment legislation and best environmental practices and so on.

To contain costs and make products available to the market in a timely manner, many biotech companies are now electing to develop multiuse manufacturing facilities where two or more products can be processed within the same, or shared, manufacturing space. Multiuse facilities can effectively design and operate these facilities to comply with GMP [9]. Multiuse facilities may be considered for biological manufacturing steps such as culture preparation, fermentation, initial and final purification, formulation,

filling and lyophilization. Activities that are found in multiuse facilities include the manufacture of a single product in multiple lots at various stages of production within a common area. The manufacture of multiple products at similar stages of production in a common area, or the use of a facility for different products on a campaign basis.

The important issues in designing and operating multiuse manufacturing facilities are the engineering design, procedural and/or temporal separation of production activities, use of validated closed systems and the implementation of a validated cleaning/changeover program.

2.4.3 Engineering Design

The key elements for the proper design and operation of multiuse facilities are

- Good facility design including proper separation of manufacturing and testing activities, proper material, product and personnel flows, adequate space and layout for manufacturing and support operations.
- Good plant systems for HVAC, water, sterilization and waste treatment.
- A good validation program that covers all equipment, systems, procedures and testing activities.
- Good personnel training in the areas of GMP compliance and work skills.
- Good environmental monitoring including air and water quality as well as personnel.

2.4.4 Facility Design

The first key to successful multiuse facility design is to know the products will be produced in the facility. Whether the product is in development, early clinical stages or full-scale production, it is important to evaluate the potential impact of each product on all operations of the facility. This evaluation should include the host system(s), the potential for cross-contamination, biocontainment requirements, biological effects, toxic or infectious agents and cleaning validations.

2.4.5 Layout

Good facility design should provide for the movement of equipment, personnel, raw and waste materials and product through the facility while minimizing the interaction between staff and process streams from different stages of the process [10]. Because biopharmaceuticals are derived from living organisms, it is important to identify areas where physical containment of the manufacturing process is required, the area where containment is not required and the flow and control of personnel. There are three primary reasons for containment, first is personnel protection, second is product protection and third is Environmental protection. Of the three, protection of the product in a multiuse facility the most important.

In general, the flow should be unidirectional where feasible so that materials and equipment enter contained areas by means of an airlock and leave through either a double-door autoclave or by an airlock. Clean equipment and supplies should not cross the path of "dirty" equipment and supplies. "Dirty" in this context refers to equipment and/or supplies that are not only possibly contaminated, but to components that have been used in the process and have not gone through a validated cleaning procedure.

Since people are the major sources of contamination within any biotech facility, the facility layout must take into consideration the flow of personnel from the time a person enters the facility until they reach their destination of work. In multiuse facilities, it is important that the layout of the facility allows for segregated flow of components that are leaving process areas to be cleaned." Dirty" components should not be transported through clean corridors where contamination potential is greater unless they are isolated, contained or the movement of items is strictly controlled through the implementation of SOPs (Standard Operating Procedures) that segregate the scheduling of this movement.

It is important that adequate space is provided for each operation and for the storage of supplies and equipment that are necessary for execution of the process, but may or may not be used for each separate product. If it is necessary to use multiple sets of components that will be dedicated to only one product, those components, once cleaned, should not be stored in the room where a different product is being processed.

2.4.6 HVAC Systems

In biopharmaceutical manufacturing facility, it is important to control both viable and nonviable airborne particulates. This fact is critical for multiuse facilities, where the particulates that are a concern as physical contaminants can also become vehicles for introducing microorganisms from different products or product lots. The quality of the environment must ensure that the individual product stream is protected from microbial and particulate contamination during any "open" processing steps, such as fermenter inoculation, centrifugation or column chromatography.

To minimize the risk of cross contamination between different processing operations is through isolation. This isolation is best accomplished by using separate air handling systems to establish isolated manufacturing areas within the facility. Operations such as fermentation, purification and product filling should have separate air handling systems that supply HEPA (High Efficiency Particulate Arrester) filtered air only to the particular room or suite where the operation is performed.

Effective contamination control also requires air pressure differentials between different manufacturing areas. The areas with the highest allowable particulate load (class 100,000) will be maintained at a lower pressure than areas with less allowable particulate loads (class 10,000) [10]. Generally, as the purity of the product increases through the process, the flow of the product moves through series of rooms or suites that have increasing pressure differentials and increasing air quality.

Contamination control also depends on the control of particulates found on personnel and equipment that must continually pass through the various areas of any multiuse facility. The use of air- locks for equipment and personnel, and pass throughs for materials and components can prevent airborne cross-contamination of products and raw materials between operating areas. Separate autoclaves for sterilization and decontamination should be provided to ensure that these flows remain distinct.

2.4.7 Clean Space Specifications

Clean Space Specifications are defined in terms of maximum number of particles per unit volume of air. Typical particle sizes used are 0.1 micron through 5 μm. USA

Pharmaceutical/Biopharmaceutical industry utilizes Class 100through 100,000 clean areas based on 0.5 micron or larger size particles per cubic foot and for "in-operation" conditions. A space to meet a classification of Class 100, it can contain no more than 100 particles per cubic foot or 3500 particles per m^3 [11]. Table 1 "Airborne particulate cleanliness classes" lists in both metric and English units, the class numbers typically used in biopharmaceutical facilities.

Table 1. Airborne particulate cleanliness classes for clean room given by experts from Fed SVd.209 E

Class Name		Class Limits			
		0.5 μm		5 μm	
		Volume Units		Volume Units	
S1	English	(m^3)	(ft^3)	(m^3)	(ft^3)
M 3.5	100	3,530	100	-	-
M 4.5	1,000	35,300	1,000	247	7
M 5.5	10,000	353,000	10,000	2,470	70
M 6.5	100,000	3,530,000	100,000	24,700	700

ISO 14644-1"Classification of Air Borne Cleanliness." defines clean spaces in terms of class numbers from 1 through 9 based on acceptable number of particles per m^3. Table 2 below shows the classes typically used in pharmaceutical and biopharmaceutical facilities. To define a class, three items must be specified: Class number (1 through 9), occupancy state (as-built, at rest, or in operation) and particle size (0.1 through 5 μm) including count (number of particles)

Table 2. Airborne particulate cleanliness classes for clean room given by experts from ISO 14T44-1

ISO Classification Number (N)	Maximum number of particles/m^3 Equal to or larger for sizes shown below	
	0.5 μm	5 μm
ISO Class 5	3,520	29
ISO Class 6	35,200	293
ISO Class 7	352,000	2,930
ISO Class 8	3,520,000	29,300
ISO Class 9	35,200,000	293,000

2.5 Plant Optimization

An optimum design is based on the best or most favorable conditions. In almost every case, these optimum conditions can ultimately be reduced to a consideration of costs or profits. Thus an optimum economic design could be based on conditions giving the least cost per unit of time or the maximum profit per unit of the production in the tightly regulated field of biopharmaceuticals the primary concern is production throughput and optimum use of limited facilities. The demand arise from the increasing use of flexible multi-product facilities and contract manufacturing the methods are required to effectively and economically allocate resources and utilities for competing parallel processes. Applying process simulation to maximize the utilization of resource-constrained multi-product facilities can increase the yield profound economic benefits for biotechnology companies. The largest supplier of simulation software to the biotech and pharmaceutical industry is Microsoft [12] which offers several basic software tools that can be used for simulation:

1. Visio® - Constructs flow sheets with a drawing package
2. Excel® - Process calculations are performed with spreadsheets and reported graphically
3. Project® - Constructs process schedules and labor assignments

The disadvantage of these tools is that they are not designed for bioprocess simulation. The popularity of these tools is due to their low cost and familiarity to users. Using these tools for simulation of multi-product resource constrained facilities can become extremely time-consuming. There are several process simulation software packages available commercially. Of these, only a few are directly applicable to Bioprocessing. Development of simulators specific to biochemical processes began in the mid 1980's. Bioprocess Simulator (BPS) (from Aspen Technology, Inc.) was the first tool of this type. For a given flow sheet, BPS used to carry out material and energy balances, estimate the size and cost of equipment, and perform economic evaluation. BPS has had limited commercial success because it was designed as an extension of Aspen Plus, an inherently steady-state simulator, and could not satisfactorily represent batch biochemical processes, which normally operate in batch mode.

Aspen Technology originally created a dedicated Bioprocess Simulator (BPS), which was eventually phased out and replaced by Batch Plus, a recipe driven modeling environment for batch processes. Batch Plus can perform complex simulations on multiple batches in a manufacturing plant to determine the total demand on shared resources. Batch Plus is just one product in a suite of engineering software products offered by Aspen Technology. Aspen Batch+ which consisted of software and services to manage batch information.

SuperProDesigner from Intelligen combines the drawing, calculation and scheduling features. SuperProDesigner also offers a database feature to log equipment and utility capacities. This package has the added advantage that it was specifically developed for simulation of biopharmaceutical unit operations and processes. It is user-friendly and set up to capture the unique unit operational data requirements of biological processes.

2.5.1 Statistical analysis

Statistical approaches to process simulation are gaining popularity as biotech and Pharmaceutical companies search for ways of characterizing processes with many variables. For processes where the mechanistic models are not sufficiently developed, a statistical treatment is a valid alternative approach. An advanced statistical technique is to construct a neural network model of the process. Gensym offer a customizable neural network framework NeuronLine, for applying Monte Carlo based statistical simulation; the tool of choice is Crystal Ball® from Decisoneering. As a fully integrated Excel add-in program with its own toolbar and menus, Crystal Ball performs Monte Carlo analysis on data in a spreadsheet format. DesignofExperiments (DOE) is another technique used to statistically characterize the process to produce a mathematical representation.

2.5.2 K-TOPS® – Total Optimum Plant Simulation

K-TOPS® is event-based plant simulation software tool specially developed by Alfa Laval Biokinetics Inc. for the biopharmaceutical industry [13]. It is used in the planning, analysis and optimization of batch processes and facilities at any stage of a project.

K-TOPS is focused on efficient facility design, debottlenecking and optimization. It employs modern, dynamic computer simulation techniques rather than traditional trial-and-error methods to provide rapid, effective solutions to a wide range of design and planning challenges. The modular approach used in K-TOPS makes it possible to identify problems and develop solutions rapidly based on varying levels of information. As more information becomes available, modifications can be made and more details added to refine the simulation results. This software provides an unbeatable process solution to

- Maximize capacity
- Balance capital versus operating cost quickly evaluates economics and identifies savings
- Analyze multiple "what if" scenarios
- Determine impact on critical resources

2.5.3 Scheduling

The manufacturing facility can optimize the use of its own facilities, thus increasing the throughput and an increase in profits, possibly deferring the expensive costs and delays of expansion. To optimize productivity in a multi-product, contract-manufacturing facility where processes are run in parallel requires a large degree of overlap between batches, and effective utilization of shared resources and equipment. In fact the complex batch, multiprocess, constrained optimization problem is becoming one of the most important problems in design and optimization of production facilities in biotechnology.

Scheduling tools are now being added to process simulation offerings specifically for the biotechnology industry. Intelligen, the makers of SuperPro Designer (SPD), has recently released a scheduling software package, SchedulePro [14]. Although SchedulePro is a standalone application, independent of SPD, models can be developed first in SPD and then exported to SchedulePro. Aspen Technology expanded its original simulator and now includes a complete Manufacturing Execution System (MES) [15]. This solution includes site planning and scheduling, work order management, execution and analysis. Based on the process and utility information provided to it, SchedulePro

generated equipment occupancy profiles for the overall production schedule of a production campaign.

2.6 cGMP for Biopharmaceutical facility

cGMP means "current Good Manufacturing Practices". WHO defines Good Manufacturing Practices (GMP) as "*that part of quality assurance which ensures that products are consistently produced and controlled to the quality standards appropriate to their intended use and as required by the marketing authorization*" GMP covers all aspects of the manufacturing process like defined manufacturing process, validated critical manufacturing steps, suitable premises, storage, transport, qualified and trained production and quality control personnel, adequate laboratory facilities, approved written procedures and instructions.

The guiding principle of GMP is that quality is built into a product, and not just tested into a finished product [16]. Therefore, the assurance is that the product not only meets the final specifications, but that it has been made by the same procedures under the same conditions each and every time it is made. There are many ways this is controlled - controlling the quality of the facility and its systems, controlling the quality of the starting materials, controlling the quality of production at all stages, controlling the quality of the testing of the product, controlling the identity of materials by adequate labeling and segregation, controlling the quality of materials and product by adequate storage, etc.

2.6.1 Personnel responsibility

The establishment and maintenance of a satisfactory system of quality assurance and the correct manufacture of medicinal products relies upon people. For this reason there must be sufficient qualified personnel to carry out all the tasks which are the responsibility of the manufacturer. Individual responsibilities should be clearly understood by the individuals and recorded. All personnel should be aware of the principles of Good Manufacturing Practice that affect them and receive initial and continuing training, including hygiene instructions, relevant to their needs.

- The manufacturer should have an adequate number of personnel with the necessary qualifications and practical experience. The responsibilities placed on any one individual should not be so extensive as to present any risk to quality.
- The manufacturer must have an organization chart. People in responsible positions should have specific duties recorded in written job descriptions and adequate authority to carry out their responsibilities. Their duties may be delegated to designated deputies of a satisfactory qualification level. There should be no gaps or unexplained overlaps in the responsibilities of those personnel concerned with the application of Good Manufacturing Practice.

2.6.2 Premises and Equipment

Premises and equipment must be located, designed, constructed, adapted and maintained to suit the operations to be carried out. Their layout and design must aim to minimize the risk of errors and permit effective cleaning and maintenance in order to avoid cross contamination, build up of dust or dirt and, in general, any adverse effect on the quality of products.

2.6.2.1 Premises

Premises should be situated in an environment considered together with measures to protect the manufacture, presents minimal risk of causing contamination of materials or products Premises should be carefully maintained, ensuring that repair and maintenance operations do not present any hazard to the quality of products. Premises should be cleaned and, disinfected according to detailed written procedures. Lighting, temperature, humidity and ventilation should be appropriate and such that Premises do not adversely affect, directly or indirectly, either the medicinal products during their manufacture and storage, or the accurate functioning of equipment. Premises should be designed and equipped so as to afford maximum protection against the entry of insects or other animals. Steps should be taken in order to prevent the entry of unauthorized people. Production, storage and quality control areas should not be used as a right of way by personnel who don't work in them.

2.6.2.2 Production Area

Contamination of a starting material or of a product by another material or product must be avoided. The risk of accidental cross-contamination arises from the uncontrolled release of dust, gases, vapors, sprays or organisms from materials and products in process, from residues on equipment, and from operators' clothing. The significance of the risk varies with the type of contaminant and of product being contaminated. In order to minimize the risk of a serious medical hazard due to cross-contamination, dedicated and self contained facilities must be available for the production of particular medicinal products, such as highly sensitizing materials or biological preparations. The production of additional products, such as antibiotics, hormones, cytotoxics highly active drugs and non-medicinal products should not be conducted in the same facilities. For those products, in exceptional cases, the principle of campaign working in the same facilities can be accepted provided that specific precautions are taken and the necessary validations are made. Cross-contamination should be avoided by appropriate technical or organizational measures

- Production in segregated areas
- Providing appropriate air-locks and air extraction;
- Minimizing the risk of contamination caused by recirculation or re-entry of untreated or insufficiently treated air;
- Keeping protective clothing inside areas where products with special risk of cross contamination are processed;
- Using cleaning and decontamination procedures of known effectiveness.
- Cleaning of equipment is a common source of cross-contamination;
- Using "closed systems" of production;
- Testing for residues and use of cleaning status labels on equipment.

2.6.2.3 Storage Areas

Storage areas should be of sufficient capacity to allow orderly storage of the various categories of materials and products starting and packaging materials, intermediate, bulk and finished products, products in quarantine, released, rejected, returned or recalled.

Storage areas should be designed or adapted to ensure good storage conditions. In particular, they should be clean and dry and maintained within acceptable temperature limits where special storage conditions are required temperature, humidity should be provided, checked and monitored. Receiving and dispatch bays should protect materials and products from the weather.

2.6.2.4 Quality Control Areas

Normally, Quality Control laboratories should be separated from production areas. This is particularly important for laboratories for the control of biologicals, and microbiologicals, which should also be separated from each other. Control laboratories should be designed to suit the operations to be carried out. Sufficient space should be given to avoid mix-ups and cross-contamination. There should be adequate suitable storage space for samples and records. Separate rooms may be necessary to protect sensitive instruments from vibration, electrical interference, humidity, etc.

2.6.2.5 Ancillary Areas

Rest and refreshment rooms should be separate from other areas. Facilities for changing clothes and for washing and toilet purposes should be easily accessible and appropriate for the number of users. Toilets should not directly communicate with production or storage areas. Maintenance workshops should as far as possible is separated from production areas. The parts and tools are stored in the production area; they should be kept in rooms or lockers reserved for that use. Animal houses should be well isolated from other areas, with separate entrance and air handling facilities.

2.6.3 Equipment

Manufacturing equipment should be designed, located and maintained to suit its intended purpose. Repair and maintenance operations should not present any hazard to the quality of the products. Manufacturing equipment should be designed so that it can be easily and thoroughly cleaned. It should be cleaned according to detailed and written procedures and stored only in a clean and dry condition. Washing and cleaning equipment should be

chosen and used in order not to be a source of contamination. Equipment should be installed in such a way as to prevent any risk of error or of contamination. Production equipment should not present any hazard to the products.

2.7 Validation

Validation is the most important in biopharmaceutical plants. Validation is defined as the "establishing of documented evidence which provides a high degree of assurance that a planned process will consistently perform according to the intended specified outcomes". Validation studies are performed for analytical tests, equipment, facility systems such as air, water, steam, and for processes such as the manufacturing processes, cleaning, sterilization, sterile filling, lyophilization, etc. Every step of the process of manufacture of a drug product must be shown to perform as intended. Validation studies verify the system under test under the extremes expected during the process to prove that the system remains in control. Once the system or process has been validated, it is expected that it remains in control, until no changes are made. In the process modifications are made, or problems occur, or equipment is replaced or relocated, revalidation is performed. Critical equipment and processes are routinely revalidated at appropriate intervals to demonstrate that the process remains in control. The validity of systems, equipment, tests, processes can be established by prospective, concurrent or retrospective studies.

If a new process or system is implemented, a Design Qualification (DQ) may be necessary. Guidelines for such cases should be included in the Master Validation Plan. The order in which each part of the facility is validated must be addressed in the Master Validation Plan. The IQ (Installation Qualification), OQ (Operational Qualification) and PQ (Performance Qualification) must be performed in order [17]. The master validation plan should indicate how to deal with any deviations from these qualifications, and state the time interval permitted between each validation

2.8 Automation

Automation is considered as an important means to protect the investment in more advanced and expensive process equipment. By using Automation to optimize manufacturing processes, the drug manufacturers can achieve better resource utilization, improved quality and proactive regulatory compliance. By applying small, sophisticated controllers, the automation of even small processes is economical with benefits including reduced variability, improved quality and increased yield. Automation systems can reduce operator exposure to hazardous conditions such as toxic and corrosion materials, high temperature equipment and media rotating equipment.

2.8.1 Control

The main goals in applying control methods to microbiological systems are to improve operational stability and production efficiency and profit, and to handle dynamic changes during start-up and shutdown. The number of ways to control a biotechnological process and the number of reports about such control is vast. Control loop consists of three parts:

1. Measurement by a sensor connected to the process or the "plant"
2. Decision in a controller element,
3. Action through an output device "actuator" such as a control valve

2.8.1.1 Feedback control

A classical automatic control system in the form of feedback control is shown in figure 2.

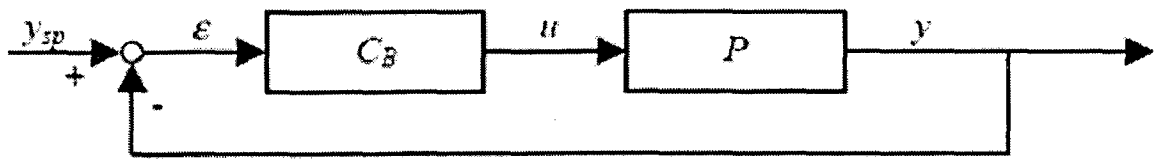


Fig 2: Feed Back control Loop

The controller generates an error signal, ε by subtracting the measured process output (y) the controlled variable from a desired value (y_{SP} set-point), and calculates the control signal u, by applying a certain algorithm to the error signal. This control signal then

manipulates the process input to reduce the error. The most common controller is called a PID controller and uses proportional, integral, and derivative actions.

Advantage:

- Does not require a good model
- Insensitive to modeling errors
- Insensitive to parameter changes

Disadvantage:

- Waits until the effect of a disturbance is felt
- Unsatisfactory for slow systems
- Might create instability

2.8.1.2 Feed-Forward Control

In most situations simple feedback control is adequate. There are situations in which simple feedback control is inadequate, and more advanced control is required. For instance with feed-forward control where, unlike with normal feedback control, one does not wait until a disturbance actually affects the output. Instead the disturbance is measured and corrective control is applied in anticipation of the expected effect. This is a better control for eliminating the effect of disturbance. A classical automatic control system in the form of feed forward control is shown in figure 3.

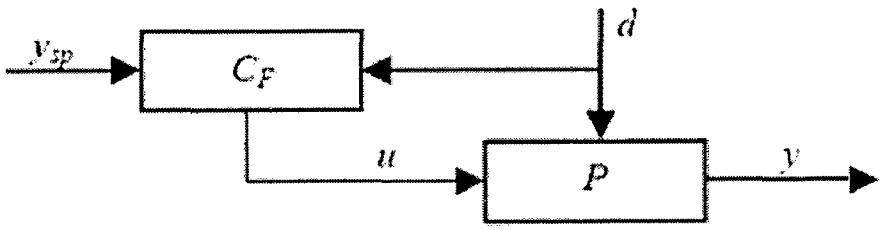


Fig 3: Feed Forward control Loop

The Feed Forward control basically involves a multiple input process in which inputs can be measured. It makes use of the input measurements and the relationship between the inputs and outputs to adjust the process to minimize or eliminate the effects of input disturbance on the process output.

Advantages:

- Acts before the effect of a possible disturbance
- Good for slow systems
- Does not introduce instability

Disadvantages:

- Requires an accurate process model
- Cannot cope with unmeasured disturbances
- Sensitive to process parameter variation

2.8.1.3 Feed-forward-Feedback Control

A feed-forward control scheme is rarely fulfill all the control requirements. Generally it is combined with feedback control to correct errors caused by imperfect knowledge in predicting the effect of disturbances on the output. A combination of feedback and feed-forward control is shown in below figure. This permits the major effect of the disturbance to be corrected by the feed forward controller leaving the fine trimming to the feedback loop. A classical automatic control system in the form of feedback and feed forward control loop is shown in figure 4 [18].

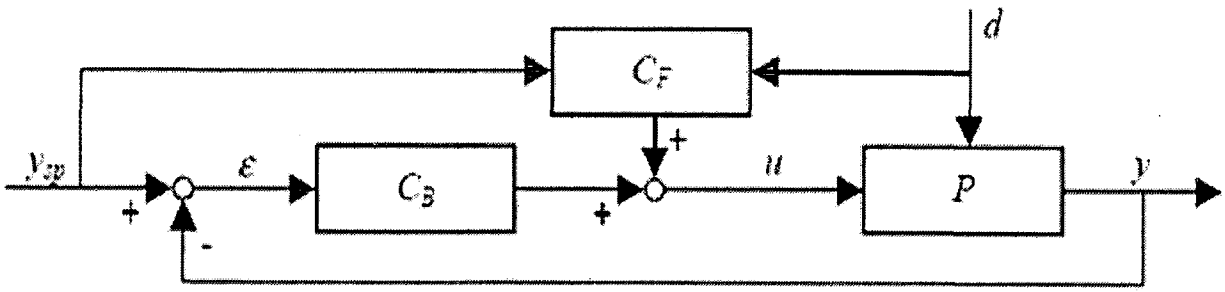


Fig 4: Feed Back and Feed Forward control Loop

The controller used in most of the Control loops is the PID controller. Because it has the three actions named as Proportional, Integral and Derivative.

2.8.2 Types of Process control system:

There are two types of process control systems named as embedded control system and Standalone process control system.

Embedded systems are microprocessor based systems such as programmed Integrated Circuits, Programmable Logic Controller or PC with the sole purpose of controlling or monitoring a piece of manufacturing or analytical equipment which is usually delivered as an integrated part of the equipment.

Standalone systems are either custom or configured, self contained systems that are components of an automated manufacturing application. They are delivered as free standing computer systems, separate to plant equipment, for connection to the field instrumentation or regulate devices and to each other. These systems may be integrated vertically with high level data management systems.

Examples of standalone system include

- Multi loop controllers or PLC s controlling part of a process
- Supervisory Control and Data Acquisition system
- Distributed Control system controlling the process plant
- Building Management System

2.8.2.1 Controller Selection

When selecting a control solution for an automated system, four options that merit consideration are single-loop controllers, Personal Computer-based control system (PC), Programmable Logic Controllers (PLC) and Distributed Control Systems (DCS). PLC and Distributed Control System (DCS) are the two major process control systems used in process plants [19]. Programmable Logic Controllers are well suited to a spectrum of application in pharmaceutical manufacturing. The PLCs available today range from behemoths capable of controlling entire facilities to units smaller than paperback novels.

2.8.2.2 Programmable Logic Controllers

PLCs were developed in 1968 as an alternative to inflexible hardwired relay control systems. Changing an existing control scheme required shutting down the system, rewiring the relay panel and adding or removing hardware. Today, PLCs offer similar programmable relay operation for discrete control as well as analog input analog output (I/O) Control. Program changes are made without physical changes and in many

controllers, without interrupting a system's operation. Many high level functions improve control of vital process parameters. For example, closed loop Proportional, Integral, Derivative Control of feed material temperature can improve the consistency of final product by minimizing operator, equipment and utility system variances.

A. Characteristics of PLCs

The inherent reliability of PLCs is a result of hardware and software design. The PLC is built to operate in industrial environments .It functions reliably in wide ranges of temperature, humidity and vibration. The concise and compact operating systems used in PLCs offer fewer modes failure compared to other control architectures and are noteworthy for their stability. The PLC is a special purpose device optimized to its task. Training, troubleshooting and maintenance costs are typically lower for PLC – based control systems than for PCs. In-depth and specialized training are not needed for the routine operation of a PLC control system.

The executive area contains the operating system as well as the scratchpad memory. The operating system supervises all system activities and communication with the I/O modules. The scratchpad area is used by the operating system for internal calculations. The application area is divided into data table (input, output and registers) and control program memory. The control program memory holds the control logic, or user program, for the physical system.

The application memory (data cable) is area of focus in PLC communications. PLC communication consists of reading from or writing to the data table of a remote station, whether it is a PLC, PC or operator terminal. The operating system handles communication overhead and handshaking.

B. Advantages of PLC

1. Excellent for sequential control.
2. A good fit for small to medium batch applications.
3. Rugged, fast & low cost I/O.
4. Cost effective entry solution.

5. Ladder logic understood by many people.

6. PLC's are sold by Distributors and engineered by System Integrators

2.8.2.3 Distributed control system

A distributed control system (DCS) refers to a control system usually of a manufacturing system, process or any kind of dynamic system, in which the controller elements are not central in location but are distributed throughout the system with each component sub-system controlled by one or more controllers. The entire system may be networked for communication and monitoring Distributed control systems (DCSs) are used in industrial, electrical, computer and civil engineering applications to monitor and control distributed equipment with or without remote human intervention Distributed Control Systems (DCSs) are dedicated systems used to control manufacturing processes that are continuous or batch-oriented, such as oil refining, petrochemicals, central station power generation, pharmaceuticals, food & beverage manufacturing, cement production, steelmaking, and papermaking.

A DCS typically uses computers as controllers and use both proprietary interconnections and protocols for communication. Input & output modules form component parts of the DCS. The processor receives information from input modules and sends information to output modules. The input modules receive information from input instruments in the process and output modules transmit instructions to the output instruments in the field. Computer buses or electrical buses connect the processor and modules through multiplexers or demultiplexers. Buses also connect the distributed controllers with the central controller and finally to the Human-Machine Interface or control consoles DCSs are connected to sensors and actuators and use set point control to control the flow of material through the plant. The most common example is a set point control loop consisting of a pressure sensor, controller, and control valve. Pressure or flow measurements are transmitted to the controller, usually through the aid of a signal conditioning Input or Output device. When the measured variable reaches a certain point, the controller instructs a valve or actuation device to open or close until the fluidic flow process reaches the desired set point. Large oil refineries have many thousands of I/O points and employ very large DCSs.

Advantages of DCS:

1. Excellent for regulatory control applications
2. A good fit for large or complex batch applications.
3. Pre-packaged integrated system of hardware and software and hence low engineering time.
4. Robust multitasking system to handle large global common databases.
5. Easy to interface with Enterprise applications.
6. Implementation of Dynamic simulation and Advanced Process application packages.
7. Future expansions i.e. scalability.
8. Migration for legacy systems.
9. DCS is application and solution driven plus industry aligned.
10. DCS systems are sold directly by the supplier and also Engineering of the DCS is done by the Supplier

Distributive controllers can be linked to centralized control systems that can implement both batch and continuous control using a remote set point approach. For a multipurpose facility, individual centralized control systems can be implemented in each processing module. These systems can then be linked to each other and to an analogous laboratory research and development system using a conventional local area network.

CHAPTER 3
METHODOLOGIES

3.1 Market Demand and Assumptions

The global market for biopharmaceuticals, which is currently valued at US$54 billion, has been growing at an impressive compound annual growth rate of 19% over the previous five years. With over one third of all pipe-line products in active development are biopharmaceuticals, this segment is set to outperform the total pharmaceutical market and could easily reach US$100 billion by the end of the decade.

The leading names comprising the elite group with combined revenue of over US$38 billion are: Epogen / Aranesp, Eprex / Procrit / Erypo, Remicade, Neupogen / Neulasta, MabThera / Rituxan, Avonex, Enbrel, Pegasys / Roferon A, Peg-Intron / Intron A, Betaferon / Betaseron, Humulin / Humalog, Rebif, Synagis and Herceptin.

Erythropoietin with a market size of over US$11 billion and rising, while that of human growth hormone can be $12,000-$18,000 per patient per year [20]. Monoclonal Antibodies with a market size approaching US$10.2 billion and a stratospheric growth rate, Human Insulin and analogs, Interferon and Colony Stimulating Factors are some of the blockbuster names. Table 3 gives the clear picture about the global market for biotech healthcare products.

Highlighting upon the Indian biotechnology industry, it is advancing into new heights in tandem with the growth and progression observed globally. The past performance of the industry indicates that it has surpassed the growth rate of many other industries. India is counted among the top 12 global biotech hotspots and is the 3rd largest in Asia Pacific (in terms of number of biotech companies). The global biotech industry size is currently US$54 billion, whereas the Indian biotech industry just crossed the US$1.07 billion (Rs 4,745 crore) mark in 2004-05 [21]. Bio Pharma remained the largest contributor to the sector with a value contribution of Rs. 3,570 crore accounting for a 75% contribution. Diagnostics, vaccines and recombinant therapeutic proteins are the three main segments in biopharma industry.

Table 3. Global Market for Biotech Healthcare Products, 2000-2002 ($ Millions) (source: BCC Inc. 2007)

	2000	**2001**	**2002**	**Annual average growth rate% 2000-2002**
Biologics	15,702	19,330	20,382	13.9
New Chemical Entities	2,425	3,705	3755	24.4
Total	18,127	23,035	24,137	15.4

In spite of the encouraging picture there are a series of challenges that this budding industry faces:

Issues & Challenges

- Increasing productivity & profitability
- Lack of skilled manpower in the area of bio-pharma
- Daunting regulatory system
- A confusing patent Law for India
- Ethical issues
- FDA regulations
- Environmental factors
- Bioequivalence issue in bio-generics
- Comparative low R&D expenditures & price wars

Assumption for Plant Design

The current trends indicate a bright tomorrow for the biotech industry in our country. But a lot needs to be done to prepare the ground for consistent and long-term growth of this industry. At present, most of the players are injecting a lot of funds into the development of generic drugs. This may be required for providing initial impetus to the industry but in the long run only novel products obtained through innovative research and development will confer sustainability to this field. One who has the largest number of viable products

in the pipeline will be ahead in the game. The requirement of recombinant protein of interest in the market is about 125 g/year and which can be produced by 100 L fermentation with twice a year of duration.

3.2 Steps in manufacturing of recombinant protein

The process flow sheet (schematic) is shown in Fig 5. The process flow is divided in to two parts.

- Up stream Process
- Down stream Process

Up stream process involves fermentation step where product will get produced and down stream process consist of

- Cell Harvesting
- Cell Disruption
- Protein solubilization and Renaturation
- Protein Purification

3.2.1 Process description

The process description for the manufacture of recombinant therapeutic protein expressed in microbes is as follows. This process is shown as process flow diagram in figure 5.

3.2.1.1 Fermentation

Protein of interest is produced as non-soluble inclusion bodies in 100 L *E.coli* fermentation. The batch time is optimized to 24 h with 1% inoculum and the fermentation temperature is 37°C.

3.2.1.2 Primary recovery (Cell harvesting and cell disruption)

After the end of the fermentation, the broth is transferred into a harvest tank which isolates the upstream from the down stream section of the plant. 100 L fermented broth is

subjected to centrifugation to separate *E.coli* cells containing non-soluble inclusion bodies. About 30 L of cells slurry separated from centrifuge will get mixed with 170 L of lysis buffer. 200 L of solution will pass through the high pressure homogenizer to disrupt and break the cells. Inclusion bodies will get separated from the cell debris through centrifugation.

3.2.1.3 Protein solubilization and Renaturation

Inactive protein in the form of inclusion bodies will get solubilized in 80 L urea solution and get active nature in 2000 L refolding buffer.

3.2.1.4 Protein Purification

Because of high capacity, high selectivity and concentrating effect, ion exchange chromatography will be selected as the first step to separate ionic impurities from 2100 L protein solution. From 10 L bed volume having binding capacity of 30 g/L about 20 L of purified protein will get eluted and passed through Hydrophobic Interaction Chromatography. 80 L purified protein will get eluted from 40 L bed volume Hydrophobic Interaction Chromatography having binding capacity of 5 g/L. Eluted protein solution after 5 times concentration in ultra filtration is passed through Gel Filtration Chromatography for further purification. 48 L protein eluted from 160 L bed volume Gel Filtration Chromatography will get subjected to buffer exchange in Diafiltration. Final purified 48 L protein solution is passed to bulk filling.

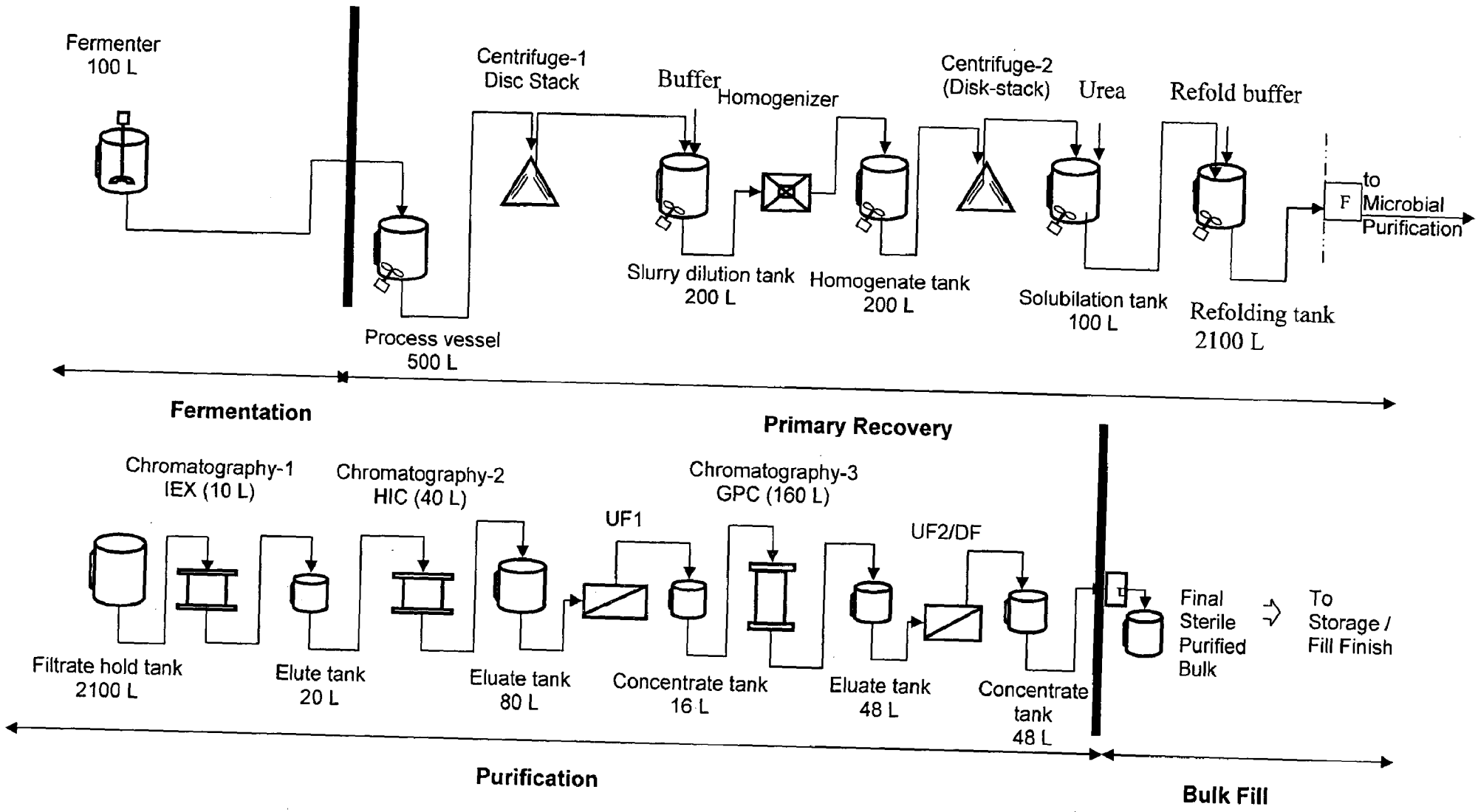


Fig 5. Process Flow Diagram

3.3 Mass Balance

Microorganism used in fermentation is *E.coli.* The product type is intracellular product. The mass balance for the entire process is shown in Table 4. The volume of the fermenter for producing the protein of interest is 100 L. The titer is 4.0 g/L

Table 4. Mass Balance for the entire process

	Volume (L)	Protein (g/L)	Amount of Protein (g)	Recovery Step (%)	Yields (%) Overall	Solutions (L)	Waste (L)
Fermenter Harvest	100	4.0	400				
Cell Recovery- Centrifuge Cell slurry-30% of feed Wash buffer	 30 70	 12.7	 380	 95	 95	 70	 70
Slurry Dilution Dilute Slurry: 2x Ferment. Harvest	200	12.7	380			170	
Homogenate (after 3 passes)	200	1.9	372	98	93		
IB recovery- sharples IB slurry-10% of feed Centrate-waste	 20 180	 15.8	 317	 85	 79	 	 180
IB solubilization/ Urea (8 M) at 4-10^{0}C ,12 hrs Target Protien - 3 g/L Total Solution Urea solution	 100 80	 3.0	 301	 95	 75	 80	

Refolding step Buffer – 20xfeed Refolding solution	2005 2105	0.14	286	95	75	2005	
Chromatography -1, 10 L bed volume, Ion Exchange							
Protein load/column (30 g/L) Buffer solution (20 col.vol) Eluate (2 col.vol)	19	10.5	200	70	50	190	2276
Chromatography -2, 40 L bed volume, Hydrophobic							
Protein load/column (5 g/L) Buffer solution (20 col.vol) Eluate (2 col.vol)	80	1.8	140	100	35	800	739
Ultra filtration-1 concentrated by 5 times Retentate Permeate	16	8.8	140	100	35		64
Chromatography -3,160 L bed volume, Gel filtration							
Feed as % of bed (10%) Buffer solution (20 col.vol) Eluate (2 col.vol	48	2.6	126	90	31	1600	1568
Diafiltration Buffer solution (3xfeed)	48	2.6	126	90	31	144	144
Final purified bulk	48	2.6	126	90	31		

Input:

Harvest amount = 100 L

Solutions added = 5059 L

Total solution = 5159 L

Output:

Waste solutions = 5111 L

Product solution = 48 L

Total solution = 5159 L

3.3.1 Critical analysis of calculations:

- For producing the recombinant therapeutic protein in microbes, *E.coli* is the best host system among all other microbes because of its simple structure and well known genome [Blattner et al., 1997].
- For *E.coli* cells with optimized conditions in fermentation, the protein expressed is generally 4.0 g/L.
- Based on the market demand, titer and overall process yield will lead the selection of the fermenter volume to get the protein of interest. Generally the overall process yields with *E.coli* cells are in the range of 30-35% and the requirement of particular recombinant protein of interest considered is 125 g/year. With these conditions for high cell density system like *E.coli* it is better to go for 100 L working volume fermenter.
- The separation effiencicy of disc stack centrifuge on wet basis is 70%. The solid content will be around 10% on dry basis for *E.Coli* cells in fermentation broth. So the solid slurry coming from the centrifuge on wet basis is 30%. Based on the solid content the bowl volume is fixed to 3 L.
- The solid slurry which is coming from the centrifuge behaves as Bingham plastic fluid; and is very difficult to flow (yield stress is to be surmounted); to make it as Newtonian fluid for achieving the free flow through out the homogenizer it is diluted to 2 fermenter harvest volumes (making to nearly density of water) with lysis buffer which will smoothen the cell wall surfaces.
- Three passes are required through the homogenizer to break the cells effectively, because multiple passes are required if the product forms as inclusion bodies. This allows the inclusion bodies to be released, and also breaks the cell debris into very small particles, which facilitates the separation of inclusion bodies from cell debris further downstream.
- The inclusion bodies are recovered in heavy phase of centrifuge, while the most cell debris particles remain in the light phase which will go for biowaste. The IB s getting from the centrifuge on wet basis is 10%.

- Volume, concentration and temperature of solubilizing urea are optimized to 80 L to get maximum yield of active protein.
- Depending upon the protein of interest coming from the homogenization and sub sequent refolding process and the optimized parameters like sample load based on binding capacity and linear velocity kept in mind the bed volume can be calculated as 10 L. Based on the binding capacity of the resin, the sample load is optimized to 30 g/L. The buffer solution will be taken on the basis of amount of buffer required for equilibration, washing, elution and regeneration of the resin.
- With proper packing and proper elution conditions, the protein can be eluate in 2 column volumes.
- Based on the resin binding capacity and optimized sample load (5 g/L) and the protein quantity getting from the ion exchange chromatography, the bed volume required for hydrophobic interaction chromatography is 40 L.
- The protein solution is concentrated by 5 times in ultra filtration step because too much concentration of protein solution will result in loss of protein in permeate side, where as the lesser time concentration will lead to affect in next processing step. The processing step after the ultra filtration is the Gel filtration. In gel filtration generally the loading is 10% of column volume. If the feeding solution is high it results in increase of bed height and column height which will result in an increase in cost and loss of product.
- The protein solution fed to the gel filtration chromatography is 16 L. Based on the sample loading (generally 10% of column volume) the bed volume for gel filtration is calculated as 160 L.
- In diafiltration step buffer exchange will takes place. To exchange the buffer with the protein solution coming from the gel filtration chromatography, the dia-filtration volume is optimized to three times the feed.

CHAPTER 4

DESIGN OF MANUFACTURING TRAIN

4.1 Equipment sizing and Design

The amount of protein requirement is 125 g/year which can be produced by 100 L working volume fermentation.

4.1.1 Fermenter sizing and Design

The criteria used for design of fermentation processes depend on the product. Depending upon the market value of the product it is to be decided whether large fermentation production is needed or small fermentation production is needed, along with the market value the three most important design parameters are

- Yield of product on the substrate
- Productivity
- Final titer

The steps in fermenter design are translation of process demands to oxygen transfer (mass transfer coefficient and gas flow rate), heat transfer, bulk mixing, and power requirements. These parameters are also called the transport processes parameters for the Fermenter. The nature of each transport process parameter and the relationships among them vary with the process requirements. The transport process also varies significantly with the basic nature of the organism and the culture broth. The major factors here are the rheological nature of the broth and the sensitivity of the organism to fluid mechanical forces. Single-cell organisms i.e. most bacteria and yeasts tend to tolerate fluid forces very well and tend to have low-viscosity and behaves like Newtonian broths [22]. Newtonian viscosity depends only on composition and temperature.Mass transfer (oxygen), heat transfer, and bulk fluid motion all depend strongly on rheological characteristics. Generally, all these transport parameters are poorer in non-Newtonian than in Newtonian broths.

Assumptions:

- Homogeneity of broth through out the reactor.
- The medium broth containing *E.coli* cells behave like Newtonian fluids, because most of the bacteria tend to tolerate fluid forces very well and tend to have low viscosity.

- The height to diameter ratio (H/D) is 2, because in a microbial process, the agitator speed can maintain higher speed since microbes can withstand more shear.
- The type of impeller is Rushton turbine with multiple impellers (radial flow); to deliver the high power required to enhance micro mixing, mass transfer and to avoid bulk mixing problems.
- The ratio of impeller diameter to tank diameter is 0.33 to avoid the risk of vortexing.
- The impeller spacing of Rushton turbines 1-1.5 times the impeller diameter apart in Newtonian broths.
- The width of the baffle is equal to 10% of the tank diameter, to minimize fluid swirling and vortex formation.
- For radial flow, high mass transfer type suitable for high cell density microbial processes with oxygen transfer rates up to 300 mMol/L/h.

In microbial fermentation as mentioned above the Height to Diameter ratio (H/D_t) is 2, the working volume of Fermenter is 100 L. Generally 80% of the geometric volume is taken as the working volume.

The batch size is 100 L and the process temperature is maintained at 37°C and pressure is 1.2 bar. The batch time is 24 h.

The geometric volume of the fermenter is 125L

$$125 = (\pi / 4) \times D_t^2 \times H \qquad (1)$$

$$125 = (\pi / 4) \times D_t^2 \times 2D_t \qquad (H = 2D_t)$$

$D_t =$ 430 mm

$H =$ 860 mm

The impeller diameter is (D_a) = 142 mm

The baffle width = 43 mm

With the impeller spacing 1-1.5 times the impeller size 3 impellers can maintain in the 100 L working volume fermenter.

4.1.1.1 Gas Flow and Linear Velocity.

The oxygen material balance is

$$OTR = F(y_{in} - y_{out})/V_L \tag{2}$$

The material balance also can be expressed as

$$F = OTR \times V_L /(\varepsilon \times y_{in}) \tag{3}$$

The value of ε will be between 0.15 and 0.35 [22] for Newtonian broths. With the passage of time, the viscosity of broth will increase. It is usually adequate to use average ε.

$$F = (300x100)/ (1000x60x0.22x0.188)$$
$$= 12.089 \text{ mol/min}$$

The material balance provides information about the gas volumetric flow rate, which often is expressed as the standard flow $v_{std,,}$ with units of standard L per min (SLPM).

$$v_{std} = 22.4 \times F \tag{4}$$
$$= 22.4x12.089$$
$$v_{std} = 270.79 \text{ L/min}$$

This is useful because most flow meters are calibrated in terms of standard flow; the actual flow is more useful for fermenter design purposes. For calculating the actual flow average pressure of the tank needs to be calculated because the gas increases from the bottom to the top of the broth because of pressure change. As a practical matter, it is usually adequate to use the average pressure in the fermenter.

$$v_{act} = v_{std} \times \left(1/P_{avg}\right) \times \left(T_f/298\right) \tag{5}$$

Generally microbial processes operate at a maximum of 1-1.5 atm and 37°C temperature.

$$v_{act} = 270.79 \times \left(1/25\right) \times \left(303/298\right)$$
$$v_{act} = 220.266 L/\min$$

The linear gas velocity

$$V_S = \left(\frac{v_{act}}{\pi/4 \times D_t^2}\right) \tag{6}$$

$$V_S = \left(\frac{220.266}{\pi / 4 \times 43^2} \right)$$

$$= 151.67 \text{ cm/min}$$

As a rule of thumb V_S should be held below 200 cm/min to avoid problems associated with gas holdup, foaming and aerosol formation.

4.1.1.2 Power Calculations

Power delivered to the broth is used for micro mixing and gas dispersion, which are related to mass transfer, and macro mixing, which provides overall homogeneity. Calculation of power input relies on empirical correlations. The power consumption calculations for stirring is normally expressed as a function of the dimensionless power number, defined as [23]

$$N_P = \left(\frac{P}{\rho_l N^3 D_a^2} \right) \tag{7}$$

The power number normally can be expressed as a function of Reynolds number, Re_S [23]

$$\mathrm{Re}_S = \left(\frac{\rho_l N D_a^2}{\eta} \right) \tag{8}$$

The density and viscosity of the medium containing *E.coli* is more or less the same as water; $\rho_l = 1000 kg/m^3 : \eta = 1cp \cdot$ The maximum speed of the agitator is 750 rpm; generally at operating conditions the speed of the agitator will take as 50- 80% of the maximum speed. The speed of the agitator at operating condition is therefore taken as 400 rpm.

$$\mathrm{Re}_S = \left(\frac{1000 \times 6.66 \times 0.142^2}{10^{-3}} \right)$$

$$= 134413.224$$

In a baffled reactor, only the inertial forces are important for a turbulent flow and power number N_P has a constant value and is independent of Re_S .The power number will also

depend on impeller type. Rushton turbine type impeller equipped with baffles, the power number for fully turbulent flow is [22]

$$N_P = 5.2$$

For a known power number, the power consumption [23] can be calculated from

$$P = N_P \rho_l N^3 D_a^5 \quad (9)$$

$$P = 5.2 \text{x} 1000 \text{x} 6.66^3 \text{x} 0.142^5$$

$$= 88.68 \text{ W}$$

For multiple impellers the power input can be calculated by just multiplying the power consumption for a single impeller with the number of impellers. The power required for three impellers is 266 W

The power consumption calculation, when aeration is present, is given by Aeration number (N_A)

$$\text{Aeration number, } N_A = v / ND_a^3 \quad (10)$$

$$N_A = 220.266 \text{x} 10^{-3} / (320 \text{x} 0.142^3)$$

$$= 0.24$$

The ratio of gassed power to ungassed power can be expressed as empirical function of N_A

$$P_g / P = Func(N_A) \quad (11)$$

For multi impeller Rushton turbine type impeller at an aeration number of 0.24, the value of (P_g / P) is 0.4 [22] .The power consumption of impeller when aeration is present is

$$P_g = 0.4 \times P$$

$$P_g = 0.4 \times 266.04$$

$$P_g = 106.416W$$

The power input per unit volume of the medium broth, (P_g / V_L) is 1.064 W/L

4.1.1.3 Mass Transfer Calculations

The empirical correlation for volumetric mass transfer coefficient proposed by Moo-Young and Blanch [23] is given as

$$k_l a_d = k u_s^{a} \left(\frac{P_g}{V_L} \right)^{\beta} \tag{12}$$

The parameters k,a,β will depend on impeller type and medium broth. For Rushton turbine with Non-coalescing medium the values are k=0.0018; a=0.3; β=0.7; u_s is the superficial gas velocity which is the ratio of gas volumetric flow rate and cross sectional area of the tank.

$$u_s = 220.266/(60 \times \pi/4 \times 0.43^2)$$
$$u_s = 0.025 m/\sec$$
$$k_l a_d = 0.0018 \times 0.025^{0.3} \times 1064^{0.7}$$
$$k_l a_d = 0.0782 \sec^{-1}$$

The Van't Reit correlation for calculating volumetric mass transfer coefficient in stirred vessel with non coalescing medium is [24]

$$k_l a = 2.0 \times 10^{-3} \left(\frac{P_g}{V_L} \right)^{0.7} u_{gs}^{0.2} \tag{13}$$

u_{gs} is the superficial gas velocity which is equal to the gas feed volumetric flow rate divided by the vessel cross section area times the gas hold up. The gas hold up can be calculated by [22]

$$HU = 1.8 \left(\frac{P_g}{V_L} \right)^{0.14} V_S^{0.75}$$
$$HU = 1.8 \times 1064^{0.14} \times 0.025^{0.75} \tag{14}$$
$$HU = 0.3$$
$$k_l a = 2.0 \times 10^{-3} \times 1064^{0.7} \times 0.0075^{0.2}$$
$$k_l a = 0.09 \sec^{-1}$$

At maximum speed of the agitator 750 rpm from the above correlations

Reynolds Number (Re_S)	=	252050
The unaerated power requirement (for 3 impellers)	=	1759 W
The power requirement in aeration condition	=	703.2 W
The gas hold up (HU)	=	0.39
The mass transfer coefficient by Moo-Young & Blanch correlation	=	0.29 sec^{-1}
The mass transfer coefficient by Van't Reit correlation	=	0.39 sec^{-1}

4.1.1.4 Vessel Jacket Design

Design Input data:

1. Vessel Geometric Volume : 125 L
2. Vessel Working Volume (V_L) : 100 L
3. Inside Diameter of the tank (D_t) : 430 mm
4. TL to TL Height of the tank : 860 mm
5. Height of agitated liquid (consider V_w) : 688 mm
6. Material : SA 240 TP 316L
7. Design Pressure : 8.6 bar (g)
8. Design Temperature : 150°C
9. Allowable stress of material (S) : 115000 KPa
10. Max. Speed of Agitator (N) : 750 rpm
11. Oxygen Transfer rate (OTR) : 300 mMol/L/ h
12. Power delivered at rated rpm (P_g) : 703 W
13. Yield of cells on oxygen (Yx/o) : 0.3
14. Process fluid in the jacket : Chilled water
15. Process fluid in the vessel : Protein solution
16. Inlet temperature of jacket side fluide : 7°C
17. Temperature difference between inlet and outlet Of jacket side fluid ($\Delta T = Tci - Tco$) : 0.15°C
18. Design code : ASME SEC.VIII DIV.1, BPE 2005
19. Kinematic Viscosity (υ) : 0.657 X 10^{-6} m^2/sec
20. Impeller Diameter (Da) : 140 mm
21. Viscosity Ratio (μ/μ_w) : 1
22. Thermal conductivity of SS 316L (K_{metal}) : 19.1 W/m K
23. Wall thickness of vessel (t) : 4 mm
24. Fermentation temperature (T_f) : 37°C
25. Outside diameter of vessel (D_o) : 434 mm

Calculations:

1. Heat loads

$$Q_{tot} = Q_{metab} + Q_{mech} \quad (15)$$

$$Q_{tot} = 0.48 \times (OTR)\left(\frac{V_L}{Y_{X/O}}\right) + 2545\ P_g \quad (16)$$

$$Q_{tot} = 0.48\text{x } 300 \text{ x } \frac{100}{0.4} + 2545\text{x}0.956$$

$$Q_{tot} = 38443.02 \text{ Btu/h} = 11260.68 \text{ W}$$

2. Volumetric flow rate of the liquid in the jacket

Specific heat of the fluid in the jacket (C_P) = 4.178 KJ/kg K

Temperature difference between inlet and outlet (ΔT) = 0.15°C

Volumetric flow rate of fluid in the jacket (V_j) = $\left(\frac{Qtot}{Cp * \Delta T}\right)$ = 17.96 L/s ≡ 20 L/s

3. Process side Heat transfer Coefficient [25]

$$N_{Nu} = \frac{h_i \times D_t}{K} = 0.85\ N_{Re}^{0.66} N_{pr}^{0.33} (Z/Dt)^{0.56} (Da/D_t)^{0.13} (\mu/\mu_w)^{0.14} \quad (17)$$

$$N_{Re} = \frac{\rho \times N \times Da^2}{\mu} = \frac{N \times Da^2}{\upsilon} = 3.72 \text{ x } 10^5$$

$$N_{Pr} = \frac{\mu \times Cp}{K} = 4.37$$

Substituting all the values in the Eq. (17)

Process side Heat transfer coefficient (h_i) = 10734.27 W/m^{2}K

4. Jacket side Heat transfer Coefficient [25]

$$N_{Nu} = h_o \text{ (Jacket)} \frac{D_t}{K} = 0.74 \left(\frac{\rho \times N \times D_a^2}{\mu} \right)^{0.67} \left(\frac{\mu \times C_p}{K} \right)^{0.33} \left(\frac{\mu}{\mu_w} \right)^{0.14} \quad (18)$$

On substituting the values of different parameters in the Eq. (18), the Jacket side heat transfer coefficient (h_o) = 8538.39 W/m^2 K

5. Overall Heat transfer Coefficient

Considering the broth fouling factor and jacket fluid fouling factor is negligible [26], we get

$$U_o = (1/h_i + 1/h_o + t/K_{metal})^{-1} \quad (19)$$

Where t= tank wall thickness

Substituting the values in the Eq. (19)

U_o = 2383.24 W/m^{2}K

6. Jacket Heat transfer surface area

$$\text{Log mean temperature } \Delta T_{LM} = \left(\frac{T_{co} - T_{ci}}{\ln\left(\frac{T_f - T_{ci}}{T_f - T_{co}} \right)} \right) = 30.92^{\circ}\text{C}$$

$$\text{Heat transfer surface area } A = \frac{Q_{tot}}{U_0 \times \Delta T_{LM}} = 0.1528 \text{ m}^2$$

7. Inside diameter of the jacket

$$D_j = D_o + \frac{A}{L_j \times \pi} \quad (20)$$

Inside diameter of the jacket D_j = 504.69 mm

The mechanical design of vessel, material of construction, sanitary piping, non-sanitary piping, vessel surface finish and valving should strictly follow ASME BPE standards.

4.1.1.5 Sparger

A sparger may be defined as a device for introducing air in to the liquid in a fermenter. Three basic types of sparger have been used; they are porous sparger, the orifice sparger, and nozzle sparger (an open or partially closed pipe). Ring sparger is used in microbial process to get good aeration. The ring sparger is designed in such a way that it gives good aeration in a baffled vessel when the agitator is operated at a range of rpm.The ring sparger is arranged below the impeller at approximately three-quarters of the impeller diameter. In most of ring sparger designs the air holes are drilled on the under surfaces of the tubes making up the ring. It is recommended that in production vessels, sparger holes should be of at least 6 mm diameter, because of the tendency of smaller holes to get blocked as also to minimize the pressure drop [27].

The success of fermentation depends upon the existence of defined environmental conditions for biomass and product formation, to achieve good productivity in fermentation process. Therefore, the optimal operating conditions like temperature, pH, degree of agitation and oxygen concentration in the medium need to be kept constant during the process. The provision of such conditions requires careful attention on the fermentation so that any deviation from the specified optimum might be corrected by a control system. The control system should be designed for these parameters to keep them optimum.

4.1.1.6 Control loops

A. Temperature Control

The temperature in a reactor can be controlled via jacket. At the time of fermentation to keep the optimum temperature 37°C inside the reactor, chilled water will be sent through the heat exchanger to the jacket. The temperature will increase as time passes in fermentation, because microbial processes are exothermic in nature. If temperature will increase the controller will give the signal to open the chilled water inlet valve until the temperature is adjusted to 37°C. If temperature will fall below the optimum temperature the controller will open the steam valve to the exchanger to heat the chilled water and

increase the temperature to 37°C. For sterilization the temperature controller at the bottom drain of the vessel will help. At the time of sterilization, send the steam through the spray balls in to the vessel in empty vessel sterilization and the temperature indicator at the bottom vessel drain line measures the temperature and it compares with the controller set point which is 121°C and whenever the error in the controller will become zero the controller will automatically stop the steam valves which are supplying the steam to the vessel. After the sterilization, the vessel needs to be cooled by sending the chilled water in to the jacket. The temperature controller in the jacket circulation loop can maintain the vessel to ambient temperature by measuring the vessel temperature through temperature measurement and compare this value with the set point of jacket temperature controller. If the error is zero, the chilled water supply will shut off, otherwise the chilled water supply is on until error gets zero. At the time of full vessel sterilization the jacket temperature controller loop is at 121°C and steam will be sent through the jacket and supply of steam flow will be maintained through the jacket until the controller error gets zero.

B. Flow Controller

Flow controller is to be designed to give the gas flow rate of 220 L/min. Generally mass flow controller is used for controlling the gas flow rate. The mass flow controller works on the principles of measuring a temperature difference across a heating device placed in the path of the gas flow. Temperature probes such as thermistors are placed upstream and downstream of the heat source, which may be inside or outside the piping.

C. Pressure Measurement and Control

Pressure is one of the crucial measurements that must be measured when the fermentation processes is going on inside the reactor. Pressure measurements may be needed for several reasons, the most important being the safety. Different working pressures are required in different parts of a fermentation plant. During normal operation, a positive head pressure of 1.2 atmospheres absolute is maintained in a fermenter to assist in the maintenance of aseptic conditions. This pressure will obviously be raised during a steam-

sterilization cycle. The correct pressure in different components should be maintained by regulatory valves controlled by associated pressure gauges.

D. Antifoam sensor

The formation of foam in microbial fermentation is often creates serious problems, if not controlled. It is common practice to add antifoam to a fermenter when the culture starts foaming above a certain predetermined level. In a foam sensing and control unit, a probe is inserted through the top plate of the fermenter. Normally the probe is a stainless-steel rod, which is insulated except at the tip and set at a defined level above the broth surface. When the foam rises and touches the probe tip, a current is passed through the circuit of the probe with the foam acting as an electrolyte and the vessel acting as an earth. The current actuates a pump or valve and antifoam is released into the fermenter for a few seconds.

D. Weight Indicator Controller

A load cell offers a method of determining the weight of a fermenter. This is done by placing compression load cells in or at the foot of the vessel supports.

E. Measurement and Control of Dissolved Oxygen

In aerobic fermentations it is essential to ensure that the dissolved oxygen concentration does not fall below a specified minimal level. The actual reading is normally expressed as percentage saturation with air at atmospheric pressure. The DO controller needs to be designed in such a way it actuate the sparger filter valve whenever the DO level goes beyond the set point. It is necessary to increase the dissolved oxygen concentration in a medium this may be achieved by increasing the air flow rate or the rpm of the impeller or a combination of both processes.

F. pH Measurement and Control

In batch culture the pH of an actively growing culture will not remain constant for very long. There is a need for pH measurement and control during the fermentation if

maximum yield of a product is to be obtained. pH measurement is carried out using a combined glass reference electrode that will withstand repeated sterilization at temperature of 121°C and pressures of 138 kN/m^2. Generally for *E.coli* fermentation the pH is maintained as 6.5-7. The controller needs to be designed for this pH. Whenever the pH goes below the set point the controller actuates the alkali pump to recover to set point and pH goes above the set point the controller actuates the acid pump to recover to set point.

When the fermentation process is completed, the fermentation broth is fed to centrifuge to recover the cells from cell broth.

4.1.2 Centrifuge Sizing and Design

For liquid-solid separation, centrifugal processing provides a number of unique advantages compared with other methods which are using for separating liquid solid separation.

4.1.2.1 Disc stack Centrifuge

The disc bowl type centrifuge operates at speeds of 12000 times gravity, providing a continuous clarification system that is suitable for materials with a solids content of 10%. It is designed to separate solid and liquid, suspension on a continuous basis. In disk centrifuge feed is admitted to the center of the bowl near its floor and rises to the stack of sheet metal disks arranged in truncated cone spaced 0.4 and 3 mm apart. The half angle made by the disks with the vertical is typically between 42°. Each disk caries several holes 6-12 mm in diameter which form when disks are assembled in place in the bowl, several channels through which the liquid rises. The purpose of the disks is primarily to reduce the sedimentation distance since a solid particle must travel only short distance before it reaches the underside of one of the disc.

The feed input to the centrifuge is 100L which is coming from the fermentation. Based on process design time cycle centrifugation time is fixed on basis of 400 L/h. The separation effiencie y of disc stack centrifuge on wet basis is 70%. The solid content will be around 10% on dry basis for *E.Coli* cells in fermentation broth. Based on particle size

and distribution the speed of the centrifuge bowl is optimized to 10000 rpm. Based on solid content the bowl volume is fixed to 3 L, if it is too low the process time will increase and the product may get dilute, if it is too high maintenance cost will increase and capital cost also.

The assumptions are

- The flow around the particle is laminar since creeping flow conditions are usually satisfied in sedimentation, Strokes law applies.
- The flow in disc bowls between the discs is laminar and symmetrical.
- The liquid rotates as the same speed as the bowl.
- The particle concentration is low (no hindered settling).
- The particle at all times move with its final settling velocity.
- The settling velocity is proportional to the g-force.

Design input data:

The bowl inner diameter (D)	= 254 mm
The inner radius of disc (r1)	= 80 mm
The Outer radius of disc (r_2)	= 200 mm
Number of discs (N)	= 100
Conical Angle (θ)	= 42°
The diameter of the bacterial particle (d_p)	= 1 μm
The density of the bacterial cell (ρ_p)	= 1.1 x 10^3 kg/m^3
Viscosity of bacterial broth (μ)	= 1 cp
The density of surrounding medium (ρ)	= 1000 kg/m^3

4.1.2.2 Settling Velocity and Throughput

The settling velocity of the particles or droplets is a fundamental property of an emulsion or suspension and determines the flow rate through or size of the centrifuge together with the residence time and settling length. In most centrifugations, the settling velocity is so small that the Strokes law applies [28].

$$u_t = \left(\frac{(\rho_p - \rho) d_p^2}{18\eta} r\omega^2 \right) \tag{21}$$

$$u_t = 7.74 \times 10^{-6} \text{ cm/s}$$

The angular velocity which is related to the bowl speed is

$$\omega = 2\pi n / 60 \tag{22}$$

$$\omega = 1047.19 \text{ rad/s}$$

The Reynolds number can be calculated by

$$\text{Re} = \left(\frac{d_p u_t \rho}{\mu} \right)$$

$$\text{Re} = 7.74 \times 10^{-4}$$

The G-force corresponding to the speed of the bowl can be calculated by [29]

$$G = \omega^2 r / g \tag{23}$$

$$G = 14200$$

The most used quantity to characterize centrifuges, the $\sum$ concept. It is the calculated equivalent surface area of a static settling tank with the same theoretical performance the throughput depends on the separating ability and the sediment space volume of the bowl relative to the concentration, particle size, density and viscosity of the process liquid. A better separation result can often be achieved by reducing the throughput, i.e. by increasing settling time. The through put from the centrifuge can be calculated by [29]

$$Q = u_g \Sigma \tag{24}$$

$\sum$ can be calculated by

$$\Sigma = \left[\frac{2N\pi\omega^2 (r_2^3 - r_1^3) \cot\theta}{3g} \right]$$

$$\Sigma = 24353 m^2$$

$$u_g = \left(\frac{(\rho_p - \rho) d_p^2 g}{18\eta} \right)$$

$$u_g = 5.45 \times 10^{-6} cm/\text{sec}$$

Therefore, through put, $Q = u_g \Sigma = 79.635$ L/min

4.1.2.3 Sediment discharge interval

The appropriate time to be chosen between sediment discharges depends on local conditions because many factors influence accumulation and hardening of sediment between discharges. Keep the discharge interval within the min. and max time Long intervals between sediment discharges can cause accumulation and compacting of sediment. The sediment may then break-up unevenly on discharge and cause the bowl to become unbalanced. If such unbalance is too large, there is risk of serious separator damage and injury to personnel Unbalance due to improper discharge of solids may lead to solids accumulation and unbalance which may lead to contact between rotating and non-rotating parts.

The parameters like bowl speed, flow, temperature and turbidity needs to be maintained throughout the running of centrifuge.

4.1.2.4 Control loops

A. Bowl speed controller

The control of bowl speed in centrifuge is very essential; the control system uses the parameter for the bowl speed to be reached to calculate a control value which is transmitted to the VFD. The bowl speed is then supervised via various functions controlled by parameters.

B. Flow Control

The feed flow can be controlled via flow controller. The actual set point is set to the controller and the flow through the inlet line is measured and transmitted to the controller and it compares with the set point ,depending on the error generated in the controller the controller regulate the flow by closing and opening the valves.

C. Temperature controller

During sterilization, cooling and production sequence temperature is the major parameter. The actual set point is set to the controller and the temperature is measured and transmitted to the controller and it compares with the set point ,depending on the error generated in the controller the controller regulate the flow of steam and cooling water .

D. Turbidity monitor

The turbidity monitor is a concentration measuring system utilizing the principle of light absorption in liquids that contain dissolved or suspended solids. The system consists of a turbidity transmitter (QE) in the line, and a turbidity converter. The separator control equipment receives an analogue turbidity signal and, in case of system failure, an alarm signal from the turbidity converter. The turbidity system with sensor in the centrifugate outlet is used for discharge initiation.

From the centrifuge we will get the cells as solid slurry form. The protein of interest is intracellular so the cells needs to be break to get product out which can be done by high pressure homogenization.

4.1.3 Homogenizer Sizing and Design

4.1.3.1 High pressure cell homogenizers

The high pressure cell homogenizer is among the most popular homogenizers in biotechnology operations, very high pressure homogenizer (VHPH) consists of a horizontal single acting reciprocating multi plunger pump with a homogenizing valve installed on the high-pressure outlet manifold. The homogenizer pump operates at a near constant flow rate regardless of the homogenizing valve set-pressure (back-pressure to the pump). The homogenizing valve consists of three main components. They are the impact head, impact ring and passage head. Homogenizing pressure is increased when pressure is applied by the pneumatic actuator to the valve shaft, closing the adjustable gap or flow area between the impact head and the passage head. The homogenizing effect is caused by the product entering the valve inlet at pressure, as it passes through the

minute gap the velocity rapidly increases while the pressure rapidly decreases to atmospheric. The product is subject to shear or impingement as it is forced through the valve then impact as it hits the impact ring at high velocity, the intense energy change also causes turbulence and cavitation. The homogenizing efficiency is due to a combination of the pressure applied and the geometry of the valve. There are different valve geometries available depending on the application, whether you are trying to obtain a stable emulsion, particle size reduction of a suspension or cell rupture.

For biological cell lysing, the VHPH system offers distinct advantages over chemical lysing and impinging flow type technologies. Whereas chemical lysing require additional costly purification and recovery process steps to yield the desired product, the VHPH system requires no additional processing steps, achieving yields of better than 90% on a single pass [30]. Unlike cell lysing with impinging flow type equipment, the VHPH systems can be easily scaled up from the lab and pilot systems through to the commercial size systems.

Assumptions:

- The number of passes through the homogenizer is 3 passes for getting enough breakage of cells.
- The pressure used for breaking the cells is 1000 bar.

The inlet feed flow rate is determined on the basis of total production time required for homogenizer and amount of feed to process. The feed flow rate for the protein of interest is 1 L/min. The positive displacement plunger pump is to be sized to give 1000 bar discharge pressure. After N passes through the valve the extent of disruption is based on the basis of the amount of soluble protein released. The extent of disruption usually depends on a power function of the applied pressure and on the number of passes [31]. For *E.coli* bacteria, the extent of disruption for high pressure homogenizer could be correlated to the number of passes and pressure as follows [Harrison, R.G et al].

$$\ln(1-R) = -kN^{b}P^{a} \qquad (25)$$

For *E.coli* cells the value of b is 0.6, the value of k is 0.1 Mpa^{-a} and the value of 'a' is 0.9.

The number of passes = 3

The pressure applied = 1000 bar

$$\ln(1-R) = -(0.1\times10^{6})^{-0.9}\times3^{0.6}\times(10^{8})^{0.9}$$

The disruption rate (R) = 1

The homogenizer system options must include

- IQ (Installation Qualification), OQ (Operational Qualification) and PQ (Performance Qualification) support.
- SIP (Sterilization In Place) including necessary instrumentation and steam traps.
- Systems meeting hazardous area classifications including Class 1.
- Dual redundant PLC or DCS based controls.
- WFI (Water For Injection) break tank for aseptic plunger flush.
- WFI (Water For Injection) aseptic plunger flush cooler.
- Product pre-cooler.

Very High Pressure homogenizer design features have to consider following points

- Up to continuous operating pressure.
- Minimum batch volume has to deliver.
- VHPH units available for product flow ranges from minimum to maximum flow rate.
- High efficiency ceramic sharp profile dynamic homogenizing valve with pneumatic pressure control which includes dampening to maintain a constant homogenizing pressure.
- Aseptic flush double plunger seal arrangement with FDA compliant.
- Multi block homogenizer compression head which eliminates stress concentration points and minimizes dead-legs allowing optimal drain-ability, designed for both CIP and SIP.
- The homogenizer compression head has non shedding components and parts due to material selection and design.
- Product contact materials are FDA compliant and are AISI 316L or better.
- Meets the ASME BPE requirements for cell disruptors.

4.1.3.2 Control loops

The design criteria for control loops are the sterilization temperature 121°C for sterilization control loop, the product temperature after the homogenizing valve 8-10°C for process temperature control loop, the WFI break tank level 10 L for the WFI water flush control sequence and the pressure 1000 bar for the pressure control sequence.

A. Sterilization temperature control loop

The Sterilization Temperature control loop is used for maintaining the temperature during Sterilization of the system. For temperature control a clean steam inlet valves is installed on the utility manifold. The Sterilization temperature Control loop is implemented as a PID Controller with pulse width modulated output for the clean steam valve.

B. Process Temperature Control Loop

The Process Temperature control loop is used for maintaining the temperature of the process after the homogenization valve. For temperature control the homogenizer is equipped with the heat exchanger. The heat exchanger on is equipped with a chilled glycol inlet and outlet valves. The temperature control loop maintains the temperature of the process solution by chilled glycol outlet valve. The Process Temperature Controller is implemented as a PID controller with pulse width modulated output for the chilled glycol return valve. The Process Temperature Controller has reversed action, as an increase of the set point will decrease the output.

C. WFI flush water control sequence

Fill the WFI break tank with the WFI up to the maximum level and open the outlet valve of the break tank and close the inlet valve. When ever the level decreases the inlet valve will open and fills the tank up to maximum level.

D. Pressure Control:

It is used to maintain the pressure coming from the positive displacement pump. The pressure is controlled through a PID loop control from Pressure indicator transmitter near the pump.

After breakage of cells through the homogenizer the cell debris needs to be removed from the protein of interest which can be accomplished by centrifugation. After removing the cell debris from protein solution urea and refolding buffer is added for solubilization and renature of product. Once protein gets renatured the protein solution will go for combination of chromatography and ultra filtration steps where protein solution will go for further purification.

4.1.4 Chromatography Sizing and Design

The high capacity, high selectivity and concentrating effect of Ion exchange chromatography makes it selected as the first step in purification sequence. In ion exchange chromatography solutes bind to the gel at low ionic strength and are eluted from the column at a higher ionic strength. The converse situation occurs in hydrophobic interaction chromatography. Thus if these two techniques are to be used in a separation scheme it is logical to have them adjacent to each other following with gel filtration which gives high resolution in separation [32]. For capturing step Ion exchange step can be used, for intermediate purification hydrophobic interaction chromatography can be used and for polishing step gel filtration chromatography can be used.

4.1.4.1 Ion Exchange Chromatography:

- The sample load is 30 g/L.
- The linear velocity through the column is 75 cm/h.
- The inlet batch size is 2100 L and final batch size is 19 L.
- Concentration of protein: initial is 0.14 g/L and final is 10 g/L.
- The bed volume is 10 L.
- The process temperature is 37°C.

Assumptions:

- Considering the particle as spherical shape and with pore size.
- Newtonian fluid.
- Uniform packing through out the column.
- The height to diameter ratio of chromatography bed is 1.2 to maintain the good balance between the pressure drop across the column and residence time.
- The curve behaves like Gaussian curve [33].

The volume of the bed = 10 L

$$\pi/4 \times D^2 \times H = 10$$
$$\pi/4 \times D^2 \times 1.2D = 10$$

Diameter of the bed (D) = 22 cm

Height of the bed (H) = 26.4 cm

The pressure drop across the bed with that optimized linear velocity is as follows

4.1.4.1.1 Pressure drop across the bed:

With optimized bed packing condition and linear velocity, the pressure drop across the bed can be calculated with Kozney's Karman equation.

Pore size (mean) (D) = 1000 A°

Particle size (mean) (D_p) = 65 μm

Porosity (φ) = void volume of particle/volume of particle

$$(\varphi) = \left(\frac{\pi/4D^2h}{\pi/6D_p^3}\right) = 0.99$$

Void fraction (ε) = volume of voids in bed/total volume of bed

= 0.85

Area available on particle = 13239×10^{-6} m^2

Specific surface of particle (a_v) = S_p/V_p

= $6/D_p(1-\varphi)$

= 2307.7 m^{-1}

Total surface area in bed/ total volume of bed (a) = $a_v(1-\varepsilon)$

$= 13860.01\ m^{-1}$

Average interstitial velocity in bed (V) = 0.0002083(m/s) = 75 cm/h

Superficial velocity (m/s) ($V' = \varepsilon V$) = 17.71×10^{-5} m/s

r_H= cross sectional area available for flow/ wetted perimeter

r_H= volume of voids/volume of bed/wetted surface/volume of bed = $\varepsilon/a = 6.132 \times 10^{-5}$ m

$D_{eq} = 4r_H = 2.453 \times 10^{-4}$ m

$$N_{Re,P} = D_{eq} V \rho / (1-\varepsilon)\mu$$

$$N_{RE,P} = 2.453 \times 10^{-4} \times 20.83 \times 10^{-5} \times 10^{3} / (0.001 \times 0.15)$$

$$N_{Re,P} = 0.076$$

The $N_{Re,P}$ is less than 10, so the flow is laminar. In laminar flow the pressure drop per unit length is given by

$$\left(\frac{\Delta P}{\Delta L}\right) = \left[\frac{150\mu V'(1-\varepsilon)^2}{\varepsilon^3 D_{eq}^2}\right]$$

$$\left(\frac{\Delta P}{\Delta L}\right) = \left[\frac{150 \times 10^{-3} \times 17.71 \times 10^{-5} \times 0.15^2}{0.85^3 \times 2.45 \times 10^{-4}}\right] \qquad (26)$$

$$\left(\frac{\Delta P}{\Delta L}\right) = 16.21 N/m^3$$

The pressure drop per unit length across the bed is 16.21 N/m^3.

4.1.4.1.2 Height Equivalent to Theoretical Plate (HETP)

The height equivalent to theoretical plate (HETP) can be calculated by

$$HETP = L/N \qquad (27)$$

Where L is the length of the column and N is the number of plates. The number of plates can be calculated by experimental. Pack the column with the resin properly and pass the acetone solution through the column and note down the values of eluate volume and corresponding optical density (OD). Plot the graph between the eluate volumes and corresponding OD s as shown in figure 6 and calculated the number of plates by using the correlation.

$$N = 5.54\left(V_e / W_b\right)^2 \qquad (28)$$

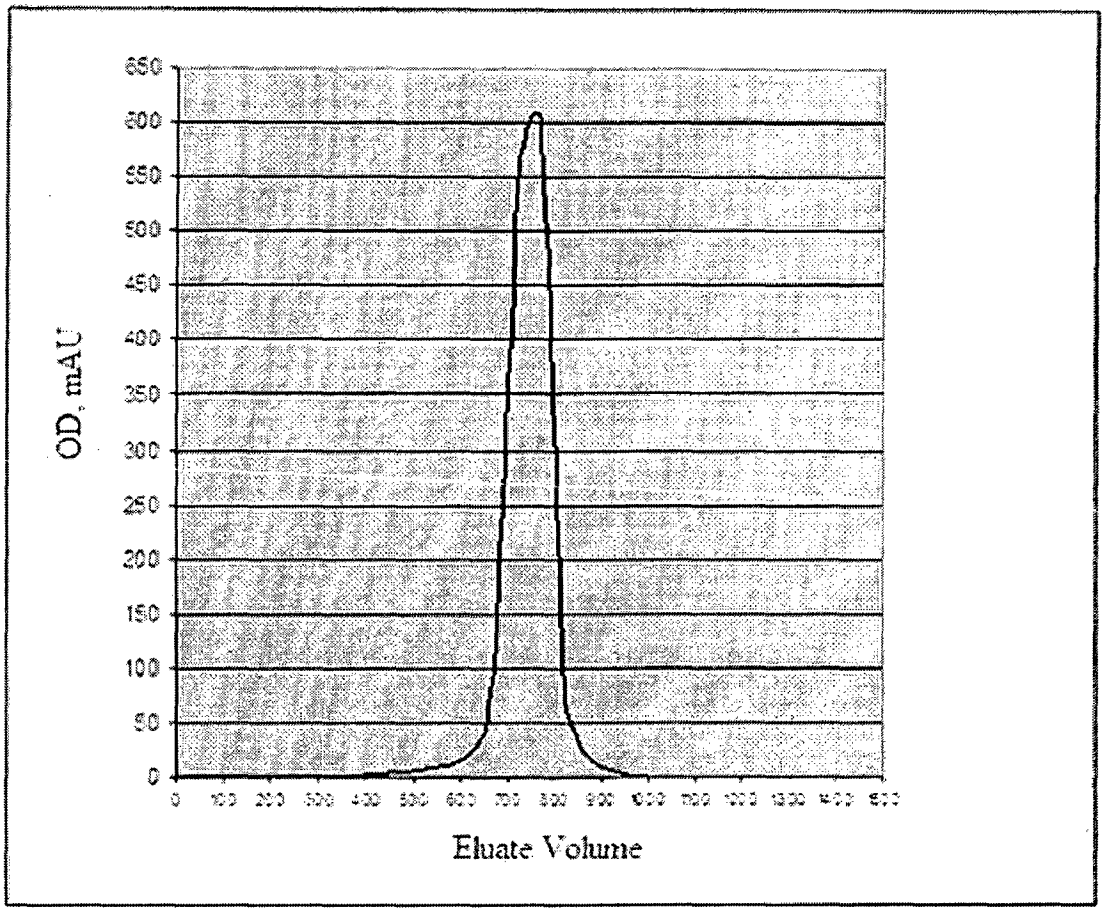


Fig 6. The graph between the eluate volumes and corresponding ODs

From graph:

Elution volume (V_e) = 450 ml

Width at half of the peak height (W_b) = 110 ml

Elution volume at left side for 10% peak height (A) = 100 ml

Elution volume at right side for 10% peak height (B) = 90 ml

Numbers of theoretical plates are calculated by the following formulae [34]

$$N = 5.54\left(V_e / W_b\right)^2$$

$$N = 5.54\left(\frac{450}{110}\right)^2$$

$$N = 92.714$$

The HETP can be calculated by

$$HETP = L/N$$

$$HETP = 26.4/92.714$$

$$HETP = 0.28 \text{ cm}$$

The Height Equivalent to Theoretical Plate is 0.28 cm.

4.1.4.1.3 Asymmetry

Different measures to demonstrate the symmetry of peaks called asymmetry or skewness. For ideal peaks the asymmetry is 1 [35], the peak asymmetry will decrease dramatically when using eluent pre conditioning.

The asymmetry for the above peak is B/A i.e. 0.9.

4.1.4.2 Hydrophobic Interaction Chromatography

- The sample load is 5 g/L.
- The inlet batch size is 19L and final batch size is 80L.
- Concentration of protein: initial is 10 g/L and final is 1.8 g/L.
- The bed volume is 40 L.
- The process temperature is 37°C.

The height to diameter ratio of chromatography bed is 1.2

The volume of bed is 40 L

$$\pi/4 \times D^2 \times H = 40$$

$$\pi/4 \times D^2 \times 1.2D = 40$$

Diameter of the bed (D) = 34.9 cm

Height of the bed (H) = 41.9 cm

4.1.4.3 Gel Filtration Chromatography

For GFC column the bed volume can be taken as 10 times greater than the feed which is coming to this column. The amount of the protein coming from the ultra filtration is 16 L.

The bed volume is 160 L. The height to diameter ratio of chromatography bed is 1.2. The process temperature is 37°C.

$$\pi / 4 \times D^2 \times H = 160$$
$$\pi / 4 \times D^2 \times 1.2D = 160$$

Diameter of the bed (D) = 55.4cm

Height of the bed (H) = 66.4 cm

4.1.4.4 Elution

Elution is the most important step in chromatographic column. There are different desorption principles can be applied to achieve the objectives of any particular chromatographic step in the most optimal way.

- Stepwise elution
- Gradient elution
- Isocratic elution

Stepwise elution is often preferred in large scale applications since it is technically simpler than elution with continuous gradients. This type of elution is usually applied in initial chromatographic steps (capture) where the purpose is to remove bulk impurities and substances differing greatly from the product. In later purification steps however, applying feed material that has been partly purified and using chromatography media with higher resolving power, it will be easier to resolve closely related substances by applying multi-step or gradient elution techniques. In final purification steps (polishing), where the main focus will be to reach the predefined purity of the molecule of interest, resolution is maximized by applying shallow gradients or even isocratic elution using high resolution media with small bead size.

4.1.4.5 Aspects of column design

- Flow distribution system
- Material resistance and durability
- Sanitary design

- Pressure vessel safety
- Regulatory support

4.1.4.6. Control loops

A. Flow controller

The flow rate measurement and flow controller present after the column and after the feed pumps. The flow measurement after the column measures the total flow flowing through the column and it can be used as set point for flow rate regulation in to the column and volume calculations. The flow measurement after the feed pump for diluting the buffers online. The flow rate of the buffer flowing through pump can be measured and it gives its value to the flow rate totalizer and flow rate totalizer decides the how much water has to pump.

B. Pressure measurement

The pressure measurement in chromatography skid is present before the column and pressure measurement system between inlet and outlet of the prefilter. The pressure measuring system before the column is used for secures the column in case of high pressure. Between the filter inlet and outlet is to secure the filter incase of the filter is plugged.

C. pH measurement

In the chromatography skid the pH sensor is installed after and before the column. A pH measurement system after the column is used during the chromatography process to end a purification step. A pH measurement system before the column is used during the chromatography process to control the buffer quality during purification sequence.

D. UV measurement

Two UV sensors with different wavelengths like 254 nm and 280 nm can be used in chromatography. The UV sensor with 254 nm used to check buffer quality during the

load and elution steps of the purification sequences. The UV sensor with 280 nm is used during the chromatography process to end a purification step. Usually used to switch solution flow between waste container and fraction collection container.

E. Conductivity measurement

Conductivity measurement can be used after and before the column. The conductivity measurement has to taken only when the solution is passing through the column during wash, equilibration, load, and elution steps of purification process. Conductivity meter before the column is to control the buffer quality during purification sequence. Conductivity meter after the column used during the chromatography process to end the purification step.

F. Temperature measurement

Temperature measurement after the column is used for information on screen of the buffer temperature used during a purification sequence. Temperature measurement before used to control the buffer quality during purification sequence.

4.1.5 TFF System Design

4.1.5.1 Ultra filtration Step

Ultra filtration is the first step used for concentrating the product in this process. In this ultra filtration there is a 5 fold concentration of the product from 80 L to 16 L. The molecular weight of recombinant protein of interest is near about 21 KD. The NMWL of the membrane selected for processing protein of interest is 5 KD, because generally the membrane's NMWL rating is one third to one fifth of the molecular weight of the product that is to be retained [36]. Processing conditions require a temperature of 8-10°C and Trans Membrane Pressure (TMP) of 1.3-2.3 bar.

Input parameters:

- Concentration of protein: intial 1.8 g/L and final 8.8 g/L.

- Intial batch size is 80 L and final batch size is 16 L.
- Process time is 0.8 h.
- Flux is 20 L/h m^2; generally flux is in the range of 10-30 L/h $m^2$for microbial cell process.

4.1.5.1.1 Membrane Area

Membrane area= Filtrate volume/ (filtrate flux x process time)

In the above equation the filtrate flux is the average flux, because in initial time flux will be more as the time is preceding the flux will decrease, so it is better to take average flux for calculating membrane area.

The inlet feed volume coming to the UF	= 80 L
The retentate volume	= 16 L
The filtrate volume	= 64 L
The average flux	= 20 L/m^2h
The area required for separation	= 64/ (20x0.8)
	= 4 m^2

The cassette area available in 0.5 m^2 increments, so the no of cassette required for equivalent to 4 m^2 area are 8.

4.1.5.1.2 Pump Selection

The rotary lobe pump or peristaltic pump can be used for pumping the protein solution. The filtrate flow rate through the membrane is 80 L/h. Before starting the process the clean water flux need to be checked to establish base line flow and pressure. Generally the clean water flux through the 0.5 $m^2$is 100 L/m^2h. So the water flux through 4 m^2 area is 800 L/m^2h. When clean water flux is conducting 80% of feed will get in retentate line. The total flow rate need to pump is 4 m^3/h. For doing clean in place the solution need to be pump at 4 m^3/h. The pump need to be selected which will give discharge flow rate 4 m^3/h at 4 bar pressure.

4.1.5.2 Diafiltration Step

Diafiltration is used after the gel filtration chromatography to exchange the buffer solution. The same membrane used for ultra filtration can be used for the Diafiltration since we are processing the same protein solution of molecular weight. In this Diafiltration step 3 times the feed solution buffer is taken to exchange with protein solution.

Input parameters:

- Concentration of protein: intial is 2.6 g/L and final is 2.6 g/L.
- Intial batch size is 48 L and final batch size is 48 L.
- Process time is 1.2 h.
- Flux is 30 L/h-m^2, generally flux is in the range of 10-30 L/h-$m^2$for microbial cell process.
- The buffer solution for exchange is 144 L (3 times the feed solution).

4.1.5.2.1 Membrane area

The filtrate volume	=144 L
The average flux	= 30 L/m^2h
The area required for separation	= 144/ (30x1.2)
	= 4 m^2

The same pump can be used for pumping the protein solution used in ultra filtration step. The key design parameters for ultra filtration and Diafiltration steps are cross flow rate, Trans membrane pressure, filtrate control and membrane area.

The typical sequences of steps in an ultrafiltration/diafiltration process are as follows.

- Set up and Pre use cleaning
- Integrity and Permeability testing
- Pre use equilibration and protein processing
- Product Recovery:
- Post-Use Cleaning and Testing
- Storage of Cassettes

4.1.5.3 Control loops

A. Process Temperature Control Loop

The Process Temperature control loop is used for maintaining the temperature of the process solution during Concentration and Diafiltration sequences. For temperature control the vessel is equipped with a double jacket with chilled water inlet and outlet valves. The temperature control loop maintains the temperature of the process solution by chilled water outlet valve. The Process Temperature Controller is implemented as a PID controller with pulse width modulated output for the chilled water return valve. The Process Temperature Controller has reversed action, as an increase of the set point will decrease the output.

B. Sterilization Temperature Control Loop

The Sterilization Temperature control loop is used for maintaining the temperature (121°C) during Sterilization of the system with / without cassettes. For temperature control a clean steam inlet valves is installed on the utility manifold. The Sterilization temperature Control loop is implemented as a PID Controller with pulse width modulated output for the clean steam valve.

C. Feed Pressure Control Loop

The Feed Line pressure Control Loop adjusts the speed of the Feed pump via a Variable Frequency Drive (VFD) unit to maintain the set pressure in the feed line. The Feed Line pressure controller is a set point controller with continuous output, which is the set point signal for the external VFD unit. The measured value output of the feed line Pressure transmitter is indicated as process value for the PLC. If the Feed Line pressure controller is switched to off, a separate discrete output signals will be activated which stops the Feed pump via the start/stop input of the VFD unit.

D. UV Interlock

During Concentration and Diafiltration sequences the UV controller is used for diverting the permeate flow back to the vessel in order to avoid loss of the product.

4.1.6 Clean In Place

In biopharmaceutical operations, post-production residues are primarily removed by chemical, rather than physical means. Depending upon the tank size and spray ball type will designed to cover the all vessel contacting surfaces. The cleaning solution needs to send at 4 m^3/h and 3.5 bar for proper cleaning. The pump is designed for 4 m^3/hr at 3.5 bar. The table 5 shows the design input data needs for CIP pump.

Table 5. Input data for CIP pump

Fluid to be pumped		CIP Solution
Head req. ($H = Hs + H_d$)		35 m
Diameter of the Pipe		0.0254 m
Length of the pipe ($L = Ls + L_d$)		9 m
Overall efficiency of the pump (η_o)		70%
Pipe Material		SS 316 L
Discharge		2.2 – 11.28 m^3/h

4.1.6.1 Pump sizing calculations

The flow rate is 4 m^3/h through the pump.

A. Velocity of the fluid in the pipe (V):

$$V = \text{Discharge} / \text{Area} = 1.11\times10^{-3} /(\pi/4\times0.025^2)$$
$$= 2.22 \text{ m/sec}$$

B. Reynolds Number (Re):

$$\text{Re} = \left(\frac{VD}{\nu}\right)$$

$$= \left(\frac{2.22 \times 0.0254}{0.01 \times 10^{-4}}\right)$$

$$= 5.63 \times 10^{4}$$

C. Coefficient of friction (f):

It is a function of Reynolds Number.

$$f = \left(\frac{0.079}{\text{Re}^{1/4}}\right) \tag{29}$$

$$= 5.128 \times 10^{-3}$$

D. Loss of Head due to friction (h_f)

$$h_f = \left(\frac{4fLV^2}{2g_cD}\right) \tag{30}$$

$$= \left(\frac{4 \times 5.128 \times 10^{-3} \times 9 \times 2.22^2}{9.81 \times 2 \times 0.0254}\right)$$

$$= 1.8 \text{ m}$$

E. Manometric Head (Hm):

$$\text{Hm} = \text{H} + h_f + \frac{V_d^2}{2g_c} \tag{31}$$

$$= 35 + 1.8 + \frac{2.22^2}{2 \times 9.81}$$

$$= 37.05\text{m}$$

F. Shaft Power (S.P):

$$\text{S.P} = \frac{\rho \times g_c \times Q \times H_m}{1000 \times \eta_o} \tag{32}$$

$$= \frac{1000 \times 9.81 \times 4 \times 37.05}{1000 \times 3600 \times 0.7}$$

$$= 0.57 \text{ KW}$$

At different flow rates of CIP solution the shaft power required is tabulated in

Table 6. Shaft Power at different flow rates

S.No	Flow in m^3/hr	Velocity m/sec	Shaft power KW
1	2.2	1.2	0.317
2	4	2.2	0.57
3	8	4.38	1.79
4	11.28	6.18	2.15

Motor power required by considering the 80% efficiency of the motor is **2.68 KW** The amount of CIP solution send to the subjected vessel is generally 1/3 times the working volume of the tank for getting proper clean [37].

4.1.6.2 CIP cycle

Equipment CIP cycle [38] used in biopharmaceutical facility may consists of following steps

Phase 1: It consists of pre-rinses and intermittent drains of the system. Ambient temperature water (potable or purified) is used to rinse water soluble residues from process equipment and its interconnecting piping. The water is passed once through the CIP circuit and sent to a drain. The pre-rinse removes water-soluble post-production residues, minimizing the residue load in the subsequent alkaline wash.

Phase 2: It is the alkaline wash. Water is circulated within the circuit, and cleaning agents are added to a predetermined concentration. Then the cleaning solution is heated to a chosen temperature by a heat exchanger. The circuit-return temperature is monitored and used as an interlock to ensure that all inline piping and equipment is washed at the specified temperature for a specified time. The alkaline wash removes water resistant residues.

Phase 3: It consists of air blowing and draining the circuit. Air blowing clears the alkaline solution from the CIP supply lines and draining clears the CIP return lines. This reduces the rinsing time and the amount of water required to clear the alkaline chemical from the circuit during subsequent rinses and drains.

Phase 4: It consists of rinsing with ambient-temperature or hot water and intermittent draining. The rinses pass once through the circuit and to the drain, removing the bulk of the alkaline cleaning agent.

Phase 5: It is the acid wash. Similar to the alkaline wash, water is circulated within the circuit with acidic cleaning agents, and the cleaning solution is heated to a chosen temperature by a heat exchanger. The acid wash is used to neutralize any residual alkaline cleaning agent, demineralize the surfaces of process equipment, and provide some Passivation.

Phase 6: It consists of air blowing and draining to clear the acid solution from the circuit. This reduces rinsing time and water consumption.

Phase 7: It is the final rinse step, using WFI that is passed once through the circuit in bursts with intermittent drains. The WFI rinse removes the acid cleaning agent and all other residues prior to cycle completion. Return conductivity is used to determine the end point of the rinse.

Phase 8: It is a final air-blowing and draining, leaving the system "drained and dry."

4.1.6.3 Control system

A correctly designed and installed control system may eliminate the problem of hydraulic shock, leading to lower maintenance costs and longer component life.

A. High temperature control loop

The high temperature control loop is used for heating the CIP solution and maintaining the temperature at high temperature set points. Heating is performed by steam injection in to the heat exchanger. The high temperature controller is implemented as a PID controller with pulse width modulated output for the steam valve.

B. Conductivity control loop

Two conductivity control loop can be operated in CIP skid. One controller which is used to prepare cleaning solutions precisely to process requirement. Filling is performed via the dosing metering pump for the acid and alkali. The conductivity control loop is implemented as PID controller with pulse width modulated output for the dosing pumps. And another conductivity control loop is used to control the valve for WFI during WFI rinse.

4.1.7 Pressure Vessel Design

Pressure vessels are used as storage tanks or collection tanks between the process steps. A major concern in the design is to make the walls of vessel are sufficiently to thick to permit safe usage under all operating condition. The design input data for pressure vessel is tabulated in table 7.

4.1.7.1 Vessel Design Calculations

Table 7. Design Data

Geometric Volume	2500 L (As per Process requirement)
Working Volume	2100 L
Vessel ID	1260 mm
Vessel Tan to Tan Height	1880 mm
H / D Ratio	1.5 : 1 (As per BPE guide lines)
Design Shell Internal Pressure	310 Kpa / Full Vacuum (As per Process requirement)
Shell Design Temperature	150° C (As per process requirement)
Hydraulic Test Pressure for Shell	403 Kpa
Vessel MOC	SA240TP 316 L (As per BPE guide lines)
Insulation MOC	160mm thick polisocyanurate
Gasket	PTFE ENCAPS EPDM
Process Fluid	Protein solution
D'ends	Torispherical (For moderate pressures)
Design code	ASME SEC.VIII DIV.1, BPE-2005
Joint efficiency	0.85

Shell Design Parameters (Input parameters)

Shell Inner Operating Pressure	2 bar (200 Kpa)
Shell Operating Temperature	130 C
Shell Internal Design Pressure, P_i	3.1 bar (310 Kpa)
Shell Design Temperature	150° C
Shell External Design Pressure, P_o	1.1 bar (110 Kpa)
Shell Inner Diameter, D	1260 mm
Shell Tan to Tan Height, H	1880 mm
Weld Joint Efficiency, E	0.85
Max. Allowable Stress For SA240TP316 L, S	115000 Kpa

4.1.7.2 Vessel Shell Thickness Calculations

Calculations (Output parameters):

1. **Thickness For Internal Pressure (UG-27)**

For Cylindrical Shell under circumferential Stress (Longitudinal Joints)

Ref. ASME Section VIII, Page-18

Shell Inner Radius, R	1260/2 = 630 mm
Thickness, $t = P_i \times R/(SxE - 0.6 \times P_i)$	(310 x 630)/(115000 x 0.85 – 0.6 x 310) = 2.002 mm
Hence Shell Thickness for Internal Pressure	2.002 mm

2. **Thickness for External Pressure (UG-28)**

For Cylindrical Shells and Tubes (Seamless or With Longitudinal butt Joints)

Ref. ASME Section VIII, DIV.1 Page-18

Dish Height, D_h	220.12 mm
$D_h/3$	220.12/3 = 73.4 mm
Total Length, L = H-DI+(2 x $D_h/3$)	1880-0+2 x 73 = 2026.7493 mm
Assumed initial thickness, t	6.00 mm
Hence Shell Outer Diameter, D_o	1260+6+6 = 1272 mm
D_o/t	1272/6 = 212.00
L/D_o	2026.75/1272 = 1.59
Factor A, determined from the applicable chart in sub part 3 of section II, part D	0.00029

Ref. ASME Section II D, Part 3

Factor A falls on right of the end of the material / temperature line

Factor B, determined from the applicable chart in sub part 3 of section II, part D, Fig HA 4,page 713	26

Ref. ASME Section II D, Part 3

Max. Allowable External Working Pressure, P_a = 4 x B/(3x(D_o/t))	4 x 26/(3 x 212) = 0.164 Mpa = 163.5 Kpa

If P_a Max. allowable external Working Pressure > P_o External Design Pressure then thickness assumed is safe.

Vessel Shell Thickness for External Pressure = 6 mm

Hence Vessel Shell Thickness Provided = 12 mm

Remarks

1.P_o = 1.1 bar as jacket is not present.

2.As limpet coil is attached to shell extra thickness is considered.

4.1.7.3 Vessel Shell Hydro test

Reference code

1. ASME, SECTION VIII, DIV. 1 -2004, PART UG 27, UG 28 and UG-99.
2. ASME, SECTION II, PART D -2004.

Design Parameters (Input parameters)

Selected thickness of the Shell	12 mm
Hydro test pressure, P_h	1.3 x Design pressure = 1.3 x 3.1 = 4.03 bar (403 Kpa)
Radius of the vessel = R	630 mm
So allowable Stress for the thickness considered = ((P_h x R/12)+0.6 x P_h)/E	[(403 x630/12)+0.6x403)]/0.85 = 25175.64 Kpa

So from the above result the thickness selected is safe under Hydro test condition.

4.1.7.4 Depth of Torispherical Dish

ID of Vessel (D)	1260.00 mm
Crown Radius (CR) = ID of Vessel (D)	1260.00 mm
Knuckle Radius (KR)= 6% of ID of Vessel	1260 x 0.06 = 76.86 mm
Depth of dish excluding shell thickness & SF = (CR – SQRT(((CR – KR)^2) – ((CR/2) – KR)^2))	(1260 – SQRT(((1260 – 76.86)^2) – ((1260/2) – 76.86)^2)) =214.124 mm
Thickness of Shell	6.00 mm
Straight face	45.00 mm
Total depth (including thickness)	220.124 mm
Total depth (including SF)	214.124+6+45= 265.12 mm

4.1.7.5 Vessel Top Dish Thickness

Reference code:

3. ASME, SECTION VIII, DIV. 1 -2004, PART UG 32 AND UG 33.
4. ASME, SECTION II, PART D -2004.

Calculations (Output Parameters)

1. Shell Dish for Internal Pressure(UG 32)

For Torispherical Head

Ref. ASME Section VIII, DIV.1 Page 28

Thickness, $t = 0.885xP_ixD/(SxE - 0.1xP_i)$	0.885x 310 x 1260/(115000 x 1 – 0.1 x 310) = 3.007 mm
Hence Shell Dish Thickness for Internal Pressure	3.007 mm

2. Shell Dish for 1.67 times external pressure (UG 32 and UG 33)

Ref. ASME Section VIII, Page 28

1.67 times External Design Pressure, P_{ii} = 1.67 x P_o	183.70 Kpa
Thickness, $t = 0.885\ xP_{ii}xD/(SxE - 0.1xP_{ii})$	0.885 x 183.7 x 1260/(115000 x 1 – 0.1 x 183.7) = 1.782 mm
Hence Shell Dish Thickness for 1.67 times External Pressure	1.782 mm

3. Thickness for External Pressure (UG-33)

Ref. ASME Section VIII, Page 29

Assumed Initial Thickness, t	4 mm
Outer Crown Radius, R_o	1260+4+4 = 1268.00 mm
R_o/t	1268/4 = 317.00
Factor A = 0.125 / (R_o/t)	0.125/317 = 0.000394 mm

Factor A falls on right of the end of the material / temperature line

Factor B, determined from the applicable chart in sub part 3 of section II, part D	35.2

Ref. ASME Section II D, Sub part 3

Max. Allowable External Working Pressure, $P_a = B / (R_o/t)$	35.2/317 = 0.111041 Mpa = 111.0410 Kpa

If P_a Max. allowable external working pressure > P_o External Design Pressure then thickness assumed is safe

Vessel Dish Thickness for External Pressure = 4 mm

Hence Vessel Top Dish Thickness provided = 10 mm Min.

4.1.7.6 Vessel Bottom Dish Thickness

Reference code:

5. ASME, SECTION VIII, DIV. 1 -2004, PART UG 32 AND UG 33.
6. ASME, SECTION II, PART D -2004.

Calculations (Output Parameters)

1. Shell Dish for Internal Pressure (UG 32)

For Torispherical Head

Ref. ASME Section VIII, DIV.1 Page 28

Thickness, $t = 0.885xP_ixD/(SxE - 0.1xP_i)$	0.885 x 310 x 1260/(115000 x 1 – 0.1 x 310) = 3.007 mm
Hence Shell Dish Thickness for Internal Pressure	3.007 mm

2. Shell Dish for 1.67 times external pressure (UG 32 and UG 33)

Ref. ASME Section VIII, DIV.1 Page 28

1.67 times External Design Pressure, P_{ii} = 1.67 x P_o	183.70 Kpa
Thickness, $t = 0.885xP_{ii}xD/(SxE - 0.1xP_{ii})$	0.885 x 183.7 x 1260/(115000 x 1 – 0.1 x 183.7) = 1.782 mm
Hence Shell Dish Thickness for 1.67 times External Pressure	1.782 mm

3. Thickness for External Pressure (UG-33)

Ref. ASME Section VIII, DIV.1 Page 29

Assumed Initial Thickness, t	4 mm
Outer Crown Radius, R_o	1260+4+4 = 1268.00 mm
R_o/t	1268/4 = 317.00
Factor A = 0.125 / (R_o/t)	0.125/317 = 0.000394 mm

Factor A falls on right of the end of the material / temperature line

Factor B, determined from the applicable chart in sub part 3 of section II, part D	35.2

Ref. ASME Section II D, Sub part 3

Max. Allowable External Working Pressure, $P_a = B / (R_o/t)$	35.2/317 = 0.111041 Mpa = 111.0410 Kpa

If P_a Max. allowable external working pressure > P_o External Design Pressure then thickness assumed is safe

Vessel Dish Thickness for External Pressure = 4 mm

Hence Vessel Bottom Dish Thickness provided = 10 mm Min.

Remarks

P_o = 1.1 bar as Jacket is not Considered.

4.1.7.7 Vessel Dish Hydro test

Reference code:

7. ASME, SECTION VIII, DIV. 1 -2004, PART UG 27, UG 28 And UG99.
8. ASME, SECTION II, PART D -2004.

Top Dish

Selected thickness of the Dish	10 mm
Design Pressure	310 Kpa
Hydro test pressure, P_h	1.3xDesign pressure = 1.3x3.1 = 4.03 bar (403 Kpa)
So allowable Stress for the thickness considered = $((0.885xP_hxD/10)+0.1xP_h)/E$	((0.885x403x1260/10)+0.1x403)/1.00 = 44979 Kpa

So from the above result The Bottom Dish Thickness selected is safe under Hydro test condition and The Top Dish Thickness Selected is Safe under Hydro test condition. The summary of pressure vessel dimensions calculated is shown in table 8.

Table 8. Summary of pressure vessels dimensions calculated.

S.NO	Description	Dimension
1	Vessel Shell thickness	12 mm
2	Vessel Top Dish thickness	10 mm
3	Vessel Bottom Dish thickness	10 mm

4.1.8 Transfer Panel Design

Transfer panels are used for transferring of protein solution between the vessels and equipments. Transfer panels are composed of a series of nozzles or ports attached to a plate, usually wall mounted. The nozzles are connected by hard piping to the inlets and outlets of process vessels or other process functions.

Detailed Design

Process contact surfaces and components need to match the system-wide specifications. Material of construction and surface finishes must be chosen to be compatible with

contacted solutions and cleaning requirements. Panels themselves are generally heavy gauge 316L SS plate and all welded construction.

1. Transfer Line Ports: These are the "plug-in" parts of the "switchboard," constructed of sanitary tubing with tri-clamp ends. For operation with U-bends, precise alignment of ports during construction is critical. Usually, these are welded into the transfer panel.
2. U-Bends: These are the "wiring" in the switchboard, jumpering transfer ports. Generally, one or almost two sizes of U-bends are preferred. J-Bends may be useful where more than one supply or return line is needed to service multiple users.
3. Behind- the –Panel Jumpers: These connect transfer ports behind the panel. They are an integral part of the transfer panel and are not reconfigured for different transfer operations.
4. Proximity switches: These are used to provide positive confirmation of process connections. They play a major role in the automation of the transfer panel design [39].
5. Drains: For capturing residual liquids from the transfer lines when panel connections are broken may be either a trough attached to the transfer panel or a separate floor pit at the base of the transfer panel.
6. Low point automated drain valves: These are employed as an option to ensure full transfer line drainage, particularly between CIP steps, without disconnecting the U-bends. Valves located behind the panel are automated.
7. Steam traps: It may be incorporated into the transfer panel as part of integrated SIP processes. In operation, a sanitary ball valve would be attached to the port for isolation. In such applications, high point clean steam supplies also need to be included in the design, through not physically part of the panel.
8. Filters of sanitary design for filtration of buffer and media are readily incorporated into the transfer panel. If filters are incorporated, the design must include appropriate low point drains and steam traps for them as well.
9. Other process utilities, such as WFI may be incorporated into the panel with automatic valves behind the panel.

CHAPTER 5
CONCLUSION

Proper designing of a cGMP manufacturing train needs consideration of a number of critical factors in different steps in the train. Apart from mechanical aspects, there are more complex issues like optimal execution of process i.e., optimum production, maintenance of sterility, proper integration of clean in place, sterilization in place procedures and personnel and material flow. The long list of cGMP guidelines that has to be followed and executed practically makes the task even more complex. The core issues that need to be addressed are:

- Transfer of sterile fluid through different parts of the facility.
- Validation of each and every step in the facility.
- Compliance to cGMP guidelines.
- Cross contamination issue in multi product facility.

In the coming years the manufacturing facilities will witness greater use of disposable technology which can not only address the critical issues of contamination but can also reduce cost effectively in the long run. Another dimension which is already gaining importance is complete automation of the plant. An array of technologies are available to achieve success. Usage of disposable technology and the usage of high end automation will definitely reduce the operational difficulties, but these may impact higher initial investment for the manufacturing plant. A pragmatic approach needs to be taken considering various parameters on cost saving with out compromising the quality of the product.

NOMENCLATURE & ABBREVIATIONS

η	Viscosity	(kg/m-sec)
γ	Shear rate	
H	Height of the fermenter	(m)
D_t	Diameter of the fermenter tank	(m)
OTR	Oxygen Transfer Rate	(mMol/L-hr)
F	Gas flow rate	(mol/min)
y	mole fraction of the oxygen in gas	
V_L	Liquid volume	(L)
ε	The oxygen transfer efficiency	
v_{std}	Standard gas volumetric flow rate	(SLPM)
v_{act}	Actual gas volumetric flow rate	(SLPM)
P_{avg}	Average pressure	(atm)
T_f	Fermentation temperature	(K)
V_S	Gas linear velocity	(cm/min)
N_P	Power Number	
Re_S	Reynolds Number	
ρ_l	Density of medium broth	(kg/m^3)
N	Speed of the agitator	(rpm)
D_a	Diameter of the impeller	(m)
P	Power consumption	(W)
D_i	Jacket inside diameter	(m)
N_A	Aeration Number	
P_g	Power consumption of agitator when aeration is present	(W)
a_d	Specific surface area	(m^2/m^3)
k_l	Liquid mass transfer coefficient	(sec^{-1})
u_s	Superficial gas velocity	(cm/sec)
HU	Gas holdup	
WFI	Water For Injection	
RA	Roughness average	
VVM	Volumetric flow rate of the gas/volume of the reactor	

Q_{tot}	Total Heat load	(Btu/hr)
Q_{metab}	Metabolic heat transfer rate	(Btu/hr)
Q_{mech}	Mechanical heat transfer	(Btu/hr)
U	Over all heat transfer coefficient	(Btu/hr-ft^2-$^\circ$F)
ΔT_{LM}	Log mean temperature difference	($^\circ$C)
T_f	Fermentation Temperature	($^\circ$C)
T_{ci}	Inlet temperature of jacket side fluid	($^\circ$C)
T_{co}	Outlet temperature of jacket side fluid	($^\circ$C)
h_i	Process side heat transfer coefficient	(Btu/hr-ft^2-$^\circ$F)
h_o	Jacket side heat transfer coefficient	(Btu/hr-ft^2-$^\circ$F)
t	Vessel thick ness	(mm)
K	Thermal conductivity	(Btu/hr-ft-$^\circ$F)
Z	Height of the liquid level in the tank	(m)
N_{Nu}	Nusselt Number	
N_{Pr}	Prandtl Number	
$Y_{X/O}$	Yield of cells on oxygen	
u_t	terminal settling velocity	(cm/min)
ρ_p	Density of the particle	(kg/m^3)
d_p	Diameter of the particle	(mm)
r	Radius of the bowl	(mm)
ω	Angular velocity	(radians/min)
n	Speed of the bowl	(rpm)
g	Acceleration due to gravity	(m/sec^2)
Q	Through put	(L/min)
u_g	Settling velocity in gravitational field	(cm/min)
N	Number of disks	
r_1	Inner radius of disk	
r_2	Outer radius of disk	
θ	Conical angle	
R	Disruption rate	
N	Number of passes through the homogenizer	

NMWL	Nominal Molecular Weight Limit	
TMP	Trans Membrane Pressure	(bar)
TFF	Tangential Flow Filtration	
φ	Porosity	
ε	Void fraction	
D_{eq}	Equivalent diameter of the particle	(mm)
$\Delta P/\Delta L$	Pressure drop per unit length of the bed	(N/m^3)
H	Height Equivalent to theoretical plate	
L	Length of the bed	(cm)
N	Number of theoretical plates	
H	Suction Head	(m)
H_d	Discharge head	(m)
L	Length of pipe	(m)
h_{fs}	Loss of Head due to friction on suction side	
h_{fd}	Loss of Head due to friction on discharge side	
h_f	Loss of Head due to friction	
Q	Pump discharge	(m^3/hr)
D	Diameter of pump	(m)
f	Coefficient of friction	
η_o	Overall efficiency of the pump	
V	Velocity of the fluid	
V_S	Suction side velocity	(m/sec)
V_d	Discharge velocity	(m/sec)
L_S	Suction side length of the pipe	(m)
L_d	Discharge side length of the pipe	(m)
υ	Kinematic viscosity	(cm^2/sec)
ASME	American Society for Mechanical Engineers	
BPE	Bio processing Equipment	
OD	Optical Density	
cGMP	current Good Manufacturing Practice	

REFERENCES

1. **Manning, M. C.** (1989). Stability of protein pharmaceuticals. Pharm. Res. 6, 903-918.
2. **Bhopale, G. M. and Nanda, R. K.** (2005) Recombinant DNA expression products for human therapeutic use. Current sci. 89 (4).
3. **Walsh, G.** (1998). Biopharmaceuticals: Biochemistry and Biotechnology. J. Wiley & Sons Ltd. Chichester, U.K.
4. **Hedhammar, M.** (2005) Strategies for Facilitated Protein Recovery after Recombinant Production in *Escherichia coli*. School of Biotechnology, Royal Institute of Technology, Stockholm, Sweden. ISBN 91-7178-176-5.
5. **Markku, K., Marika, K., Niklas, V.W., Pentti, N. and Peter, N.** (2006) Process analytical technology (PAT) needs and applications in the bioprocess industry. VTT Technical Research Centre of Finland. ISBN 951.38.6612.2.
6. **Peters, M.S. and Timmerhaus, K.D.** (1991) Plant design and economics for chemical engineers. 4th ed. McGraw-Hill Che. Eng. Ser. ISBN 0-07-049613-7.
7. **Petrides, D.** (2001) Bioprocess Design. Intelligen, Inc. 2326 Morse Avenue Scotch Plains, NJ 07076
8. **Freestone,R.** (2005) The challenges in designing and building a modern biomanufacturing facility. European BioPharmaceutical Review Winter '05 issue.
9. **Balsman, W., Doshi, D., Lang, J. and Thanos, H.** Injecting life into Biopharmaceutical design. The official journal of ISPE, 15 (5) Sep/Oct 1995.
10. **Odum, J.** (1995) Fundamental guidelines for biotech multiuse facilities. The official journal of ISPE, 15 (5) Sep/Oct 1995.
11. **Del valle, M.A.** USA/EC/ISO Regulatory considerations for designing aseptic processing facilities. The official journal of ISPE, 24 (4) (2004).
12. **Walker,T. H.** Introducing superpro designer software to bioprocessing courses. Department of Agricultural and Biological Engineering. Clemson University. Clemson, South Carolina USA
13. **www.alfalaval.com** (28 Dec 2006)

14. **Ficenec, D., Osborne, M., Pradines, J., Richards, D., Felciano, R., Cho, R.J. and Chen, R.O.** (2003) Computational knowledge integration in biopharmaceutical research. Briefings in bioinformatics. 4 (3) 260–278.
15. **Lavin, D., Himabindu.G. and Notwick, N.** (2006) The role of process simulation in a renovated biologics facility. The official journal of ISPE, 26 (5).
16. **Larsson, C.G. and Anderson, R.** (1997) A WHO guide to good manufacturing practice (GMP) requirements. World Health Organization, Geneva.
17. **21 CFR PARTS 210 & 211 (1997) Guidelines from US FDA**
18. **Neeleman, R.** Biomass Performance: Monitoring and Control in Bio-pharmaceutical Production Wageningen University.-With ref.-With summary in Dutch ISBN 90-5808-733-6
19. **Harry, J., Browne, PE and Olsson, K.I.** (1998) Discussion of Control systems in pharmaceutical manufacturing. The official journal of ISPE, 18 (4).
20. **www.globalbusinessinsights.com** (11 Feb 2007)
21. **www.ibef.org** (30 Mar 2007)
22. **Charles, M. and Wilson, J.** Fermenter design. Lehigh University Bethlehem, Pennsylvania. 1157-1183
23. **Nielsen, J.H., Villadsen, J. and Liden.G.** Bioreaction engineering principles. 2nd ed. Kluwer Academic/ Plenum, New York. (2003) ISBN 0-306-47349-6.
24. **Bailey, James E. and Ollis, David.F.** Biochemical engineering fundamentals. 2nd ed. McGraw-Hill chemical engineering series. (1986) ISBN 0-07-003212-2.
25. **Oldshue, James Y.** Fluid mixing technology. McGraw-Hill Che. Eng. Ser. (1986) ISBN 0-07-047685-3.
26. **McCabe, W.L., Smith, J.C. and Harriot, P.** Unit operations of chemical engineering. 5th ed. McGraw-Hill Che.Eng.Ser. (1993) ISBN 0-07-044844-2.
27. **Perry, R.H. and Green, D.W.** Perry's chemical engineering hand book. 7th ed. McGraw-Hill Che. Eng. Ser. (1997) ISBN 0-07-049841-5.
28. **Axelsson, H. and Madsen, B.** Centrifuges, Sedimenting. Alfa Laval separation AB, Thumba, Sweden.
29. **Harrison, R.G., Todd, P., Rudge, Scott R. and Petrides, D.P.** Bioseparations sciences and engineering.

30. **Kleinig, A.R.,and Middelberg, A.P.J.** (1996) The correlation of cell disruption with homogenizer valve pressure gradient determined by computational fluid dynamics. *Chemical Engg. Sci.* 51 (23) 5103- 5110.
31. **www.niroinc.com** (4 Apr 2007)
32. Protien Purification Handbook. (1999) Amersham Pharmacia Biotech AB, Uppsala Sweden.
33. Ion Exchange Chromatography Principles and Methods. Amersham Pharmacia Biotech AB, Uppsala Sweden. ISBN 91 970490-3-4.
34. **Dream, Robert F.** (2000) Process design considerations or large scale chromatography of biologics.The official journal of ISPE, 20 (4).
35. **Gromping, A.M., Hahn, G.M., Maio, G. and Arnold, F.** (2002) Optimization of Chromatography efficiency at high temperatures using eluent preheating. Dionex Softron, Germany
36. **www.millipore.com** (25 Apr 2007)
37. **Forder, S. and Hyde, J.M.** (2005) Increasing plant efficiency through CIP. Biopharm. In
38. **Shnayder, L** **ıa, M.** (2005) Equipment cleaning in place in modern biopharmace s: Engineering concepts and challenges. The official journal of ISI
39. **Louie, Ed. And Williams, B.** (2000) Transfer panel design: Aseptic solution handling in biopharmaceutical facilities. The official journal of ISPE, 20 (4).