DESIGN OF A MANUFACTURING TRAIN FOR THE PRODUCTION OF RECOMBINANT THERAPEUTIC PROTEINS AND MONOCLONAL ANTIBODIES USING MAMMALIAN EXPRESSION SYSTEMS

A DISSERTATION

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

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CHEMICAL ENGINEERING

(With Specialization in Computer Aided Process Plant Design)

By

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CANDIDATE'S DECLARATION

I hereby certify that the work which is being presented in this dissertatic entitled "**Designing of a Manufacturing Train for the Production of Recombina Therapeutic Proteins and Monoclonal Antibodies using Mammalian Expressic Systems**" in the partial fulfillment of the requirements for the award of the degree **Master of Technology** in **Chemical Engineering** with specialization in **Comput Aided Process Plant Design (C.A.P.P.D)**, submitted in the Department of Chemic Engineering, Indian Institute of Technology Roorkee, Roorkee, is an authentic reco of my own work carried out under the esteemed guidance of **Dr. Arnab Kapa** Director, Reliance School of Life Sciences, Navi Mumbai and **Dr. Surendra Kuma** Professor, Department of Chemical Engineering, Indian Institute of Technolo Roorkee, Roorkee.

The matter embodied in this dissertation work has not been submitted by n for the award of any other degree of this or any other Institute/University.

Place: IIT Roorkee
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CERTIFICATE

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

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ABSTRACT

Pharmaceuticals used for the treatment of various diseases in human hav been usually based on the natural or synthetic organic molecules produced b microbes or synthesized in vitro by organic chemists. However, the post genomic-er projects a remarkable change in the therapeutics market, with the realization tha proteins have many potential therapeutic advantages for preventing and curin diseases and disorders. Among all the therapeutic proteins currentl biopharmaceutical market is led by monoclonal antibodies. The clinical an commercial success of monoclonal antibodies has led to the need for very large-scal production in mammalian cell culture.

The production process for monoclonal antibodies must be designed in such way that it meets the highest requirements with regard to consistency an reproducibility. These therapeutics usually require high doses and therefore vas manufacturing capacities. On the basis of the projected market demand in this repo the plant capacity is determined to be 2500 L for meeting a total protein requiremen of 2250 grams per year.

Technology to manufacture high-dose therapeutic monoclonal antibodie (MAbs) at large scale has evolved in several ways over the last two decades. Majo cost reductions have been reported through the use of platform technologies both i upstream and downstream. The platform approach is illustrated in the present wor for the downstream processing of monoclonal antibodies. On the basis of the mas balance major equipments (bioreactor, chromatography column, crossflow syster CIP system) that are used for the production of monoclonal antibodies are sized an designed as per the standards. Also the control loops for controlling the variou parameters in equipments are discussed.

cGMP (Current Good Manufacturing Practices) compliance is the foundatio of the pharmaceutical industry and has become the benchmark for success of an enterprise involved in the development, manufacture or testing of human and anima drug products. In the present report a designing philosophy (premises design, produc

and material flow, personnel flow and area classification) is proposed for biopharmaceutical facility which is in compliance with the cGMP requirements.

With the advancement in the level of automation available for process equipment there is a tremendous increase in the product yields and product quali On the basis of the S88 standards the design philosophy for software, equipment, a procedures is proposed for automating the production train.

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NOMENCLATURE

ΔP_r	Return Pressure Loss
ΔP_T	Total tube side Pressure drop
A	Heat transfer area or Base area of the Chromatography column
AIC	pH Indicator and Controller
a'_t	Flow area per tube
a_s	Shell side flow area
a_t	Tube side flow area
B	Baffle spacing
C'	Baffle cut
CFU	Colony Forming Unit
CIP	Clean in Place
CM	Control Module
c_P	Water Specific heat
CS	Conductivity Sensor
D	Internal diameter of the column
D_a	Impeller Diameter
DCS	Distributed Control System
D_e	Equivalent diameter
DF	Diafiltration
D_i	Internal Diameter of Vessel or Tube internal Diameter
DIC	Dissolved Oxygen Indicator and Controller
DP cell	Differential Pressure sensor
D_o	Outer Diameter of Vessel
d_p	Particle diameter
DV	Dish Volume
E	Weld Joint Efficiency
EM	Equipment Module

EV	Expansion Vessel
F	Molar flow rate of gas
FIC	Flow Indicator and Controller
F_{in}	Delivery of the pump
FM	Flow Meter
FS	Flow Sensor
F_T	Temperature difference factor
f_t	Tube side friction factor
f_s	Shell side friction factor
G or RCF	Relative Centrifugal Force
GPLF	GPM per Linear Foot of Vessel Circumference
G_s	Tube side Mass velocity
G_t	Shell side Mass velocity
h	Working Level Height
H	Tan to Tan height of the vessel
h_d	Depth of Dish excluding thickness
HEPA	High Efficiency Particulate Air Filter
h_i	Tube side heat transfer coefficient
h_{io}	Heat transfer coefficient (based on outside)
h_s	Shell side heat transfer coefficient
L	Length of tubes
LA	Foam Sensor
L_{fg}	Latent Heat of Steam
LMTD	Log Mean Temperature Difference
m_c	Cooling water flow rate
MH	Manhole
n	Number of disks
N	Impeller speed
N+1	Number of Crosses
N_i	Number of Impellers
N_p	Impeller power number

N_q	Flow number for propellers
N_{Re}	Reynolds Number
OTR	Oxygen Transfer Rate
P&IDs	Piping and Instrumentation Diagrams
P_a	Maximum allowable external working pressure
PFDs	Process Flow Diagrams
P_g	Gassed power
pHS	pH Sensor
P_i	Shell Internal design pressure
PIT	Pressure Indicator and Transmitter
PLC	Programmable Logic Controller
P_o	Shell External design pressure or Un-gassed power
PS	Pressure Sensor
P_T	Tube pitch
PV	Process Value
PWM	Pulse Width Modulator
Q	Overall heat transfer rate or Feed flow rate
Q_{tot}	Total heat generated during growth
r	Radial position of the particle
R	Radius of the shell
RD	Rupture Disk
r_1	Minimum radius of disk
r_2	Maximum radius of disk
R_C	Crown Radius
R_d	Dirt Factor
Re_s	Shell side Reynolds number
Re_t	Tube side Reynolds number
R_J	Jacket inner Radius
R_K	Knuckle Radius
R_o	Outer crown radius
s	Fluid Specific gravity

S	Max. Allowable Stress for SS316 L
SIC	Speed Indicator and Controller
SIP	Sterilization in place
SG/LG	Sight Glass and Light Glass
SP	Set Point
SV	Set Value
T	Thickness of vessel shell
T_a	Approach temperature
$t_{c,in}$	Cold fluid inlet Temperature
$t_{c,out}$	Cold fluid outlet Temperature
T_{ci}	Cooling water inlet Temperature
T_{co}	Cooling water outlet temperature
TFF	Tangential Flow Filtration
$T_{h,in}$	Hot Fluid inlet Temperature
$T_{h,out}$	Hot fluid outlet Temperature
TIC	Temperature Indicator and Controller
T_o	Operating temperature of the bioreactor
TOC	Total Organic Carbon
TS	Temperature Sensor
U_c	Clean Overall Coefficient heat transfer coefficient
U_D	Dirty Overall Heat Transfer Coefficient
UF	Ultrafiltration
UP	Unit Procedure
UVS	Ultra Violet Sensor
V	Velocity through tubes
VFD	Variable Frequency Drive
v_g	Settling velocity
V_L	Volume of liquid
X	Cell concentration
Y	Mole fraction of oxygen
$Y_{x/o}$	Cell yield coefficient on oxygen

Greek Letters

ρ_p	Particle density
μ	Cell specific growth rate or broth viscosity
ϵ	Oxygen transfer efficiency
α	Half cone angle of the disk
ρ	Broth density
Σ	Sigma factor
φ	Viscosity correction factor
ω	Angular velocity of the rotor

Chapter I

INTRODUCTION

The pharmaceutical industry has undergone incredible changes with the discovery and introduction of biotech drugs. Biotech drugs are medicines that are therapeutic proteins which are produced by living organisms to fight disease. They are usually produced through microbial fermentation or by mammalian cell culture.

Among Biopharmaceuticals, therapeutic proteins represent a growing market segment expected to reach an approximate 60$ billion by 2010 [1]. As indicated by the type of products in the current development pipelines, the diversity of therapeutic proteins is anticipated to continue to increase in future. In addition, the completion of the human genome sequencing combined with high-throughput post-genomic technologies is likely to speed up disease target identification while also boosting understanding of cell regulations. This milieu will in turn enhance the discovery rate of novel potential therapeutic proteins. This diversity should also be driven by other factors such as developments of new orphan drugs, the need for cheaper drugs and also potentially by the growing market for generic therapeutic proteins.

Among all the therapeutic proteins currently biopharmaceutical market is led by monoclonal antibodies with about 28% value share. Monoclonal antibodies have played a role in several of the important advances in pharmacotherapy; agents such as Herceptin™, Enbel™, ReoPro™, and Remicade™ have contributed to the treatment of infectious diseases, cancer and autoimmune diseases [2]. However, they are amongst the most expensive drugs where annual cost per patient can reach $35,000 for the antibodies treating cancer conditions. These high prices are a reflection of the fact that antibodies are now marketed for chronic conditions and of their relatively low potency which results in the need for high cumulative doses. Consequently expensive large-scale manufacturing capacity is required to fulfill market demand and produce 10-100s Kg/year [3].

Majority of monoclonal antibodies are produced in batch/fed-batch culture using mammalian cell followed by purification steps that rely primarily on chromatography with intermediate filtration and viral inactivation/clearance

operations. With increasing titers in mammalian cell cultures, there are also pressures to improve downstream technology.

For starting a manufacturing facility multiple options are available, such as:

- Single product v/s multi-product
- Single purpose v/s multi-purpose
- Single production train v/s multiple trains
- Pilot plant v/s commercial scale plant
- Built as per local regulatory standard v/s USFDA
- Build v/s acquired

Considering all these alternatives makes the decision making for starting a manufacturing facility a very complex job. Taking into account the current trend of market demand for large quantity of variety of drugs generally, manufacturers opt for a multi-product, multi-purpose, multiple trains and commercial scale facility.

The companies that are involved in pharmaceutical production to follow GMPs (Good Manufacturing Practices) is essential to get new products approved by regulatory agencies and for producing drugs that have the required safety, identity, potency, purity and quality. Now-a-days automation is one of the imperative element of manufacturing facility. It is implemented on the various equipments present in the plant in order to enhance the operation of the facility by improving the overall reliability, safety and quality of complex operations as well as reducing the cycle time for certain processes. All computer aided control systems are implemented using GAMP (Good Automation Manufacturing Practices) methodologies.

Technology can provide a way out of the cost-speed-quality dilemma. The ideal biomanufacturing facility would be inexpensive to build, rapidly expandable and reconfigurable to handle new processes and make product quickly- all the while maintaining or improving on the high level of quality needed for cGMP manufacturing. Current facilities make extensive use of stainless steel vessels that must be connected by stainless steel piping to other unit operations, to media and

buffer supplies, to water, and clean-in-place and steam-in-place systems. Proces: equipments and the utilities that support them are expensive and may involve lon lead times for fabrication and installation. The resulting facility can be difficult t reconfigure and expand for new process. To address all these issues, a ne technology to manufacture products in disposable (single-use) components i rapidly emerging. Single use technologies have gained increasing acceptance b the industry as a means of achieving safe, compliant, and efficient processes. A the industry awakens to the technologies benefits, there is a demand for furthe integration of disposables into processes.

Chapter II

LITERATURE REVIEW

2.1 Therapeutic Proteins

Proteins that are engineered in the laboratory for pharmaceutical use are known as therapeutic proteins. The advent of sophisticated genomics and proteomics based functional identification has unraveled large numbers of proteins with a potential for therapeutic intervention. The majority of drugs marketed to date are recombinant therapeutic proteins.

Today recombinant therapeutic proteins are used to relieve patients suffering from many conditions, including: Various cancers (Monoclonal antibodies, Interferons), Heart attacks, strokes, cystic fibrosis, Gaucher's disease (Enzymes, Blood factors), Diabetes (Insulin), Anaemia (Erythropoietin) and Haemophilia (Blood clotting factors) [4].

Multiple sources are available for production of therapeutic proteins [5]. Each source has different characteristics. Bioengineering allow production of therapeutic proteins in: Microorganisms (*E. coli, S. cerevisiae*), Animal cell lines (Chinese Hamster Ovary, Baby Hamster Kidney), Natural Sources (Blood Plasma), Transgenic Plants (Tobacco, Potato, Aquatic Plants) [6] and Transgenic Animals (Sheep, Cattle). Microbial and mammalian cell fermentation are the most common expression systems utilized. Many efforts continue to be directed at transgenic expression system development with improved characteristics. All of these systems have their own advantages and disadvantages.[7].

2.2 Monoclonal Antibodies: "the Magic Bullets"

Monoclonal antibodies (mAbs) are antibodies that are identical because they were produced by one type of immune cell and are all clones of a single parent cell. Given (almost) any substance, it is possible to create monoclonal antibodies that specifically bind to that substance; they can then serve to detect or purify that substance. By contrast with polyclonal antibodies, mAbs are monospecific and homogeneous which makes them effective tools in the development of therapies and diagnostics.

The therapeutic potential of mAbs was realized after the Hybridoma Technology allowed their development. About a quarter of all biotech drugs in

development are mAb, and several products are in use or being investigatec Licensed products are available for inhibition of alloimmune, autoimmun reactivity and antiviral therapy. mAbs can be used in several diagnostic tests an in radioimmunodetection and radioimmunotherapy of cancer [8]. They are als very useful in immunohistochemistry which detect antigen in fixed tissu sections. MABs are also used to purify substances such as in affinity chromatography. A diversity of engineered antibody forms have been created t improve their efficacy, including enhancing the effector functions of full lengt antibodies, delivering toxins to kill cells or cytokines in order to stimulate the immune system, and biospecific antibodies to target multiple receptors.

2.3 Technologies For Biopharmaceutical Production

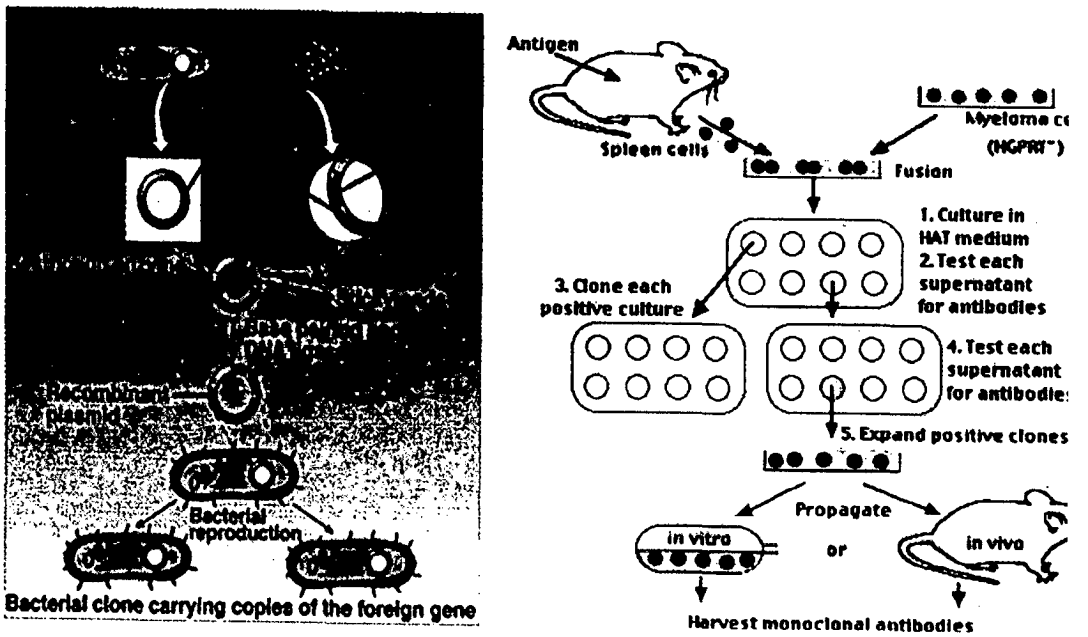
Over the last 20 years, there has been extraordinary growth in the biopharma industry based on the development of Recombinant DNA and Hybridoma technologies. Currently, there are more than 18 monoclonal antibodies approved for therapeutic uses out of these most of them are produced by recombinant DNA technology and only few by Hybridoma Technology.

2.3.1 Recombinant DNA Technology

Recombinant refers to DNA (or the protein resulting from such DNA) that has been genetically engineered to contain genetic material from another organism. Genetically altered microorganisms are usually referred to as recombinant, whereas plants and animals so modified are called *transgenic*.

An over view of the strategy typically employed in recombinant DNA technology is given in Figure 1A. The first step is to select a piece of DNA to be inserted into a vector. The second step is to cut that piece of DNA with a restriction enzyme and then ligate the DNA insert into the vector with DNA Ligase. The insert contains a selectable marker which allows for identification of recombinant molecules. An antibiotic marker is often used so a host cell without a vector dies when exposed to a certain antibiotic, and the host with the vector will live because it is resistant. The vector is inserted into a host cell, in a process

called transformation. The host cells must be specially prepared to take up the foreign DNA.



A B

Figure 1: A) Recombinant DNA Technology
B) Hybridoma Technology

2.3.2 Hybridoma Technology

To produce monoclonal antibodies, B-cells are removed from the spleen of a mouse that has been immunized with the antigen of interest (Figure 1B). These B-cells are then fused with myeloma tumor cells and that is deficient in an enzyme HGPRT (hypoxanthine-guanine phospho ribosyl transferase). Fused cells are incubated in the *HAT medium* (Hypoxanthine Aminopterin Thymidine). The fused hybrid cells being cancer cells, will multiply indefinitely thus, producing large amounts of antibodies. MABs can be produced in cell culture or in live animals. When the hybridoma cells are injected in mice they produce tumors containing an antibody-rich fluid called ascites fluid. Fermentors have been used to produce antibodies on a larger scale.

2.4 Manufacturing Process for Production of Monoclonal Antibodies

In today's biopharmaceutical pipeline, mAbs are a predominant modality for a wide range of clinical indications. The commercial and clinical success of mAbs has led to the need for large scale production. This has resulted in rapid expansion of global manufacturing capacity of up to 2,00,000 L and a greatly increased effort to improve process efficiency with concomitant manufacturing cost reduction [3].

Most antibody therapies require high doses over a long period of time, which require large amounts of purified product per patient therefore, careful considerations need to be given with respect to selection of the host cell line, culture medium, process scale-up, culture vessel design and so forth. Two major areas of commercial antibody process developments are:

A. Upstream Process Development: includes cell line development, media optimization and cell culture process optimization.

B. Downstream Process Development: comprises cell harvest, antibody capture, viral inactivation and polishing steps.

2.4.1 Expression Systems

The most important factors to take into consideration when choosing an optimal expression system are: productivity, process economics, product quality and safety, lead times, scalability and regulatory acceptance. The ability of a cell line to achieve high volumetric productivities depends on various characteristics:

- An appropriately designed expression vector is required for efficient transcription of antibody genes.
- Cell line selected should be capable of efficiently translating antibody mRNAs, assembling and modifying the antibody at high rates with minimal accumulation of in correctly processed polypeptides.
- Cell line must be capable of achieving high viable cell concentration within an acceptable time also it must produce antibody with desired product quality characteristics such as glycosylation.

Expression vectors system most frequently used for production o therapeutic MAbs are Glutamine synthetase (GS) gene expression system anc those based on Dihydrofolate reductase (DHFR) genes, an enzyme involved ir nucleotide metabolism (hypoxanthine and thymidine) [9]. DHFR$^-$ cell lines are transfected with an expression vector containing of DHFR gene and gene o interest and are placed in a medium containing MTX (Methotrexate). MTX inhibits DHFR production which is essential for the survival of the cell. Only cells that overproduce this enzyme will survive. Often more DNA than just target gene is amplified therefore when transfected genes are amplified, other tightly linkec sequences including immunoglobulin genes on the vector are co-amplified.

GS catalyses the synthesis of glutamine from glutamate and ammonia. As glutamine is an essential amino acid, transfection of cells that lack endogenous GS, with GS vector confers the ability to grow in glutamine free media. In GS expression system, which uses GS as a selectable marker, a weak promoter is usually used for the expression of GS enzyme while a strong promoter is used to drive the product gene expression. This enables the selection of high producing transfectants where the gene of interest has integrated into transcriptionally active sites within the host cell genome. The transfectants that can survive using the weakly transcribed GS gene in glutamine free media containing a GS inhibitor, methionine sulphoximine (MSX), should produce relatively high level of desired protein due to strong promoter. Some advantages of GS expression system are that it requires fewer copies of the gene per cell to obtain an efficient production level than DHFR production system (which requires several 100 copies of the gene), it mediates a low level of toxic and inhibitory ammonia accumulation during cultivation. It is also interesting to note that cell growth using glutamate in glutamine free medium reduces the accumulation of inhibitory lactate in culture as result of reduced specific glucose consumption rate.

2.4.2 Cell Lines

The key issues affecting the choice of a cell line for use in a manufacturing process are the capability to produce high antibody concentration

in chosen production system the ability to consistently produce a product o uniform characteristics and the speed with which a high yielding cell line can b obtained. Chadd et al [7] described that evaluation of various expression system depends on two factors: molecular fidelity and the cost of goods. They conclude that mammalian cell culture and transgenic organisms show the greatest promis for the expression of full-length, recombinant human antibodies, and bacteria fermentation are most favorable for the expression of antibody fragments.

2.4.3 Mammalian Cell Culture

Mammalian cells are the preferred production vehicle for man therapeutic proteins due to the requisite post-translational processing, in particula appropriate glycosylation and proper folding of the proteins produced (require for efficacy, pharmacokinetics tolerability or stability). These cell lines are ofte chosen as host for recombinant protein production. These are generally adapted t grow in suspension cultures which offer process scale up advantage over adheren cell cultures [10]. The most commonly used cell lines for antibody production ar Chinese hamster ovary (CHO) cell lines and murine lymphoid cell lines (SP2/0 Ag14 and NSO). Therapeutic proteins produced in CHO cells include tissu plasma activator, erythropoietin, coagulation factor IX, interferon β-1a, an several monoclonal antibodies (Herceptin™, and Rituxan™).

CHO cells are widely used to produce recombinant antibodies using bot DHFR and GS expression system. CHO cell lines have been used primarily in th commercial production of biopharmaceuticals and are readily accepted b regulatory authorities because of their safety profile (non antigenic potentia related to carbohydrates), precise post translation modification and correc folding. Unlike other cell types NSO cells are obligate glutamine auxotroph glutamine independence can be conferred upon NSO cells following transfectio with a functional GS gene. There are reports of other types of cell lines being use to produce mAbs including Hamster line BHK21 and the human retina derive PER.C6 cell line (with human glycosylation patterns, but with risk of human viru contamination) [11].

2.4.3.1 Cell Culture Media and Raw Materials

Mammalian cells require a comprehensive mixture of nutrients in order to survive and grow in vitro. Many basal media containing essential components such as carbohydrates, amino acids, vitamins and various salts are available commercially. However, supplementation of animal serum to these media is often required for cell culture viability, productivity and protein expression. But there is an increasing emphasis to remove animal serum and other animal derived components from culture media as they carry a potential risk of adventitious agent contamination. These concerns have been heightened with the identification of prions as the causative agent of transmissible spongiform encephalopathy (e.g. mad cow disease) also viruses and mycoplasma. In addition, animal sera increase the raw material cost in cell culture and cost of downstream protein purification in large scale manufacturing and batch to batch variation.

More recently, serum free media have been developed and successfully employed in large scale biological manufacturing [12, 13]. These media are more complex and typically include additives such as growth factors (e.g. insulin), iron transporting reagents (e.g. transferrin), reducing reagents (e.g. glutathione), shear protecting reagents (e.g. Pluronic F-68), trace metals (e.g. Selenium and Zn), lipids or lipid precursors and carriers (e.g. linoleic acid and lipoic acid) and protein hydrolysates (e.g. peptone) and hormones (account for cell proliferation). Defined media are inherently less expensive and make downstream processing straight forward as there are fewer contaminants to monitor and remove. Process optimization is also easier in a defined environment.

The most common approach for developing a feed medium uses concentrated basal media without salts (to avoid high osmolality). Using the traditional one-factor at a time approach to develop the serum free medium is a very time consuming and laborious process. However, the introduction of statistical design significantly reduces the labors of composition screening and concentration optimization. Liu et al [14] adopted pre-weaning strategy combined with experimental design to develop serum-free media of rCHO cells for production of Microphage colony stimulating factor (M-CSF), also Liu et al [15]

used the steepest ascent method to find optimal concentration of stimulatory ingredients for CHO cells.

2.4.4 Reactor Systems For Large Scale Antibody Production

Two types of culture system are used for large scale manufacturing are fed batch and perfusion culture (Figure 2). In perfusion system, cells are retained in the reactor and a variety of retention devices can be used which may be internal or external to the reactor [16]. Though productivity in perfusion bioreactors are greater than fed batch it has some shortcomings which makes it less attractive, such as additional time and complexity is involved in developing the process.

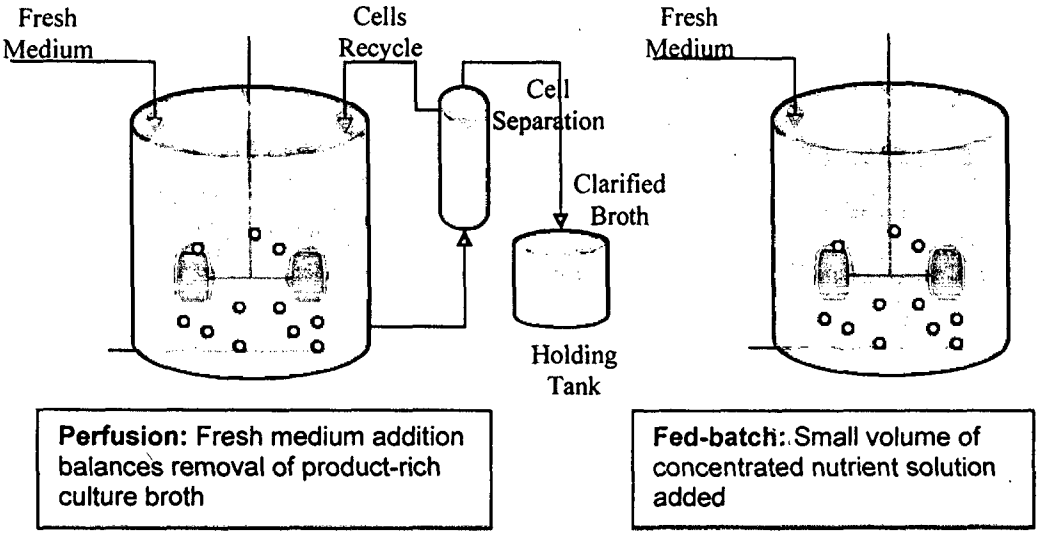


Figure 2: Schematic Diagram of Perfusion and Fed Batch Processes.

Whitford [17] suggested various operating mode choice factors to choos Batch, Fed batch or Perfusion. The prevalence of fed batch over other modes i due to many practical factors including reliability, ease of scalability an application latitude.

In fed batch optimization of feed strategies has been a major facto contribution to improvement in growth and productivity. To optimize a feedin strategy consideration should be given to nutrient consumption, by product accumulation and balance between growth and production. However frequen

feeding is less desirable for large scale production, in manufacturing due to its complexity, stepwise bolus addition of the feed solution to the production bioreactor is widely used in industry due to its simplicity and scalability. During fermentation high titer, high cell density, sustained cell viability, short process time, process robustness and high success rates are to be addressed.

2.4.5 Downstream Processing

Efficient recovery and purification of mAbs from cell culture media is a critical part of the production process. Primary considerations during downstream processing are productivity, economics, product quality, safety, dead times scalability, reliability, robustness and regulatory acceptance. Important product purity attributes include product related impurities like dimmers, aggregates and isoforms resulted from amidation and oxidation and process related impurities like host cell proteins, nucleic acids, leached protein A, and potential viral contamination. All these impurities are cleared according to FDA guidelines. Generally, industries follow platform approach for downstream processing of mAbs [18]. Schematic of a platform downstream process for mAbs is shown in Figure 3.

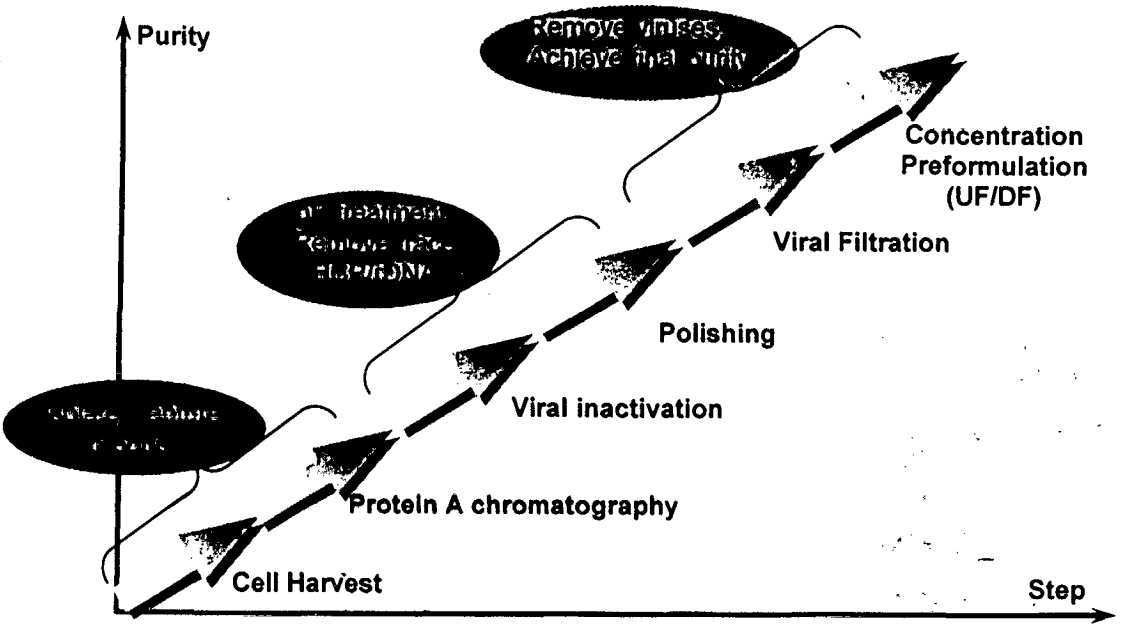


Figure 3: Platform Downstream Process for Monoclonal Antibodies.

In downstream processing, development must focus on limited number o process steps, product quality and high overall productivity. The advantage o using a platform approach include saving in time and effort and harmonization o practices and information across different functions and sites within biotecl organization [19].

2.4.5.1 Cell Harvest

The harvest process separates the product released in the culture brot from the cells and cell debris. At commercial scale centrifugation is preferre over other harvesting technologies (e.g. microfiltration) due to its scalabilit better clarification and economical operations for large volumes. With the adver of high cell densities and low shear centrifuges a hybrid centrifuge filter syster can also be used. The supernatant is further clarified through depth filtration t remove residual cellular debris.

2.4.5.2 Protein A chromatography

Affinity chromatography separates proteins on the basis of a reversibl interaction between a protein (or group of proteins) and a specific ligand couple to a chromatographic matrix. The technique is ideal for a capture or intermediat step and can be used whenever a suitable ligand is available for the protein(s) c interest.

Protein A Chromatography is the most efficient purification step fc antibodies it can purify product to more than 99% purity and remove most of th process impurities [20], still it suffers from many short comings such as high cos of resin, it adds another impurity into the process in the form of leached Protein / ligand and the need to carry out product elution at low pH (this can lead t formation of soluble high molecular weight aggregates and insoluble precipitate which results in reduction in product yield and added burden on polishing steps).

As Protein A resins are unstable to alkaline cleaning agents ne engineered ligands (like MAb Select SuRe®) resins are being designed which ar alkaline, stable and permits the use of sodium hydroxide for column regeneratio

[21]. Various other alternative to Protein A capture such as bioaffinity ligands tags, etc. are reviewed by Low et al [22]. Also membrane chromatography adsorbers can be used in place of protein A as process time is less [23 & 24].

Arunakumari et al [25] developed a non affinity purification platform with efficient cation exchange capture chromatography. By optimizing the ion exchange they achieved purity >97 % and recovery of 82% for various HuMAbs In order to improve process time economics and validation activities they substituted conventional polishing steps by disposable chromatography.

2.4.5.3 Low pH Viral Inactivation

In a typical mAb purification process low pH treatment and viral filtration (0.2 μm) are specific steps designed for viral inactivation and removal. Since Protein A column eluate is at low pH, it is relatively easy to include a low pH incubation step to inactivate viruses. The choice of pH level largely depends on the stability profile of the antibody product and buffer components. Other inactivation methods like gamma irradiation or high temperature short time may gain more relevance if and when non chromatography process becomes realistic.

2.4.5.4 Polishing

Two or three polishing steps are usually require to remove impurities such as high molecular weight aggregates, trace amounts of Host cell protein, isomers of product, rDNA , leached protein A and viral contaminants.

Cation exchange chromatography (CEX), Hydrophobic interaction Chromatography (HIC), Anion exchange chromatography (AEX), Hydroxyapatite (HA) is commonly used for polishing. Table 1 shows the modes of chromatography employed as polishing steps in mAb processes for clearing specific kinds of contaminants.

Ion Exchange Chromatography (IEX) separates proteins on the basis of differences in charge to give a very high resolution separation with high sample loading capacity. The separation is based on the reversible interaction between a charged protein and an oppositely charged chromatographic medium. Proteins

bind when they are loaded into a column. Conditions are then altered so that bound substances are eluted differentially. Elution is usually performed by increasing salt concentration or by changing pH. Changes are made stepwise or with a continuous gradient.

Table 1: Modes of Chromatography

IMPURITY	MODE
High molecular aggregates	HIC, CEX
Host cell protein	AEX, HIC, CEX
Leached Protein A	HA, HIC, CEX
Viral clearance	AEX, CEX, HIC, HA

HIC separates proteins on the basis of differences in hydrophobicity. The technique is ideal for the capture or intermediate steps in purification [26] Separation is based on the reversible interaction between a protein and the hydrophobic surface of a chromatographic medium. This interaction is enhanced by high ionic strength buffer which makes HIC an ideal 'next step' after precipitation with ammonium sulphate or elution in high salt during IEX. Elution is usually performed by decreasing in salt concentration. Changes are made stepwise or with a continuous decreasing salt gradient.

Gel Filtration (or Molecular Exclusion Chromatography) separates proteins on the basis of differences in molecular size. The technique is ideal for the final polishing steps in purification when sample volumes have been reduced (sample volume significantly influences speed and resolution in gel filtration) Samples are eluted isocratically (single buffer, no gradient).

2.4.5.5 Viral Filtration

Viral filtration is employed to compliment the low pH inactivation step Viral filters can be classified on the basis of their pore sizes into retro viral (<50nm) and parvoviral (<20nm) grade filters [27]. The placement and size of filter vary from product to product.

2.4.5.6 Concentration (Ultrafiltration/Diafiltration)

After the completion of downstream processes, the product is buffe exchanged into the formulation using Diafiltration setup. Filter sizing can var depending on the volume being filtered and the extent of particulates in the fee stream. Ultrafiltration (UF) membranes are used extensively in the downstrean purification of recombinant monoclonal antibodies. However, the fouling o membranes after a unit operation—especially when recombinant proteins o monoclonal antibodies are highly concentrated—is a common problem. Typically normalized water permeability (NWP) of a membrane can be reduced to about 2(percent of its original permeability at the end of an UF-DF operation. Wu et a [28] described an effective, safe, and economic approach for cleaning a PES (Pol Ether Sulfone) membrane at ambient temperature, using a scale-down simulatio of the antibody UF-DF process with a cleaning solution of 250 ppm sodium hypochlorite in 0.5 M NaOH. The results showed that the cleaning solution of a room temperature was effective in cleaning the membrane, following execution o an UF/DF step in a monoclonal antibody process.

2.5 cGMP Manufacturing

For companies involved in pharmaceutical production following GMPs (Good Manufacturing Practices) are essential to get new products approved by regulatory agencies. They are the requirements that manufacturers of regulated health care products must follow so that the products they make have the safety, identity, strength, purity and quality (SISPQ) that they purport or are represented to have. If the product does not possess the above characteristics or is not produced or tested according to GMP, the product is considered adulterated and not fit for use. The FDA (Food and Drug Administration) does state that its "current Good Manufacturing Practices" or cGMPs are the minimum requirements and that what defines a manufacturing practice as current and good is being "feasible and valuable in assuring drug quality". Vesper [29] summarized GMP into 7 elements: Protect the product from contamination; Prevent mix ups; Know what you are to do before you do it; Document what really occurred; Strive

for consistency and control; Have an independent group make the final decisions Solve problems, learn from mistakes, monitor and continually improve. GMP has evolved gradually, however the recent scientific risk based frame work and the Process Analytical Technology (PAT) [30] initiatives, developed by regulatory authorities to support innovation and efficiency in a cGMP environment, suggests a new way of thinking for the 21st century.

GMP principle as applied to manufacturing facilities states that premises and equipment must be located, designed, constructed, adapted and maintained to suit the operation 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 the product. Various design issues for a cGMP manufacturing facility can be categorized as: process related issues, layout issues, automation strategy issues, flow issues, regulatory issues and validation strategy issues. It should be ensured that there is absence of material crossovers or reverse flows. Also movement of personnel and equipment from "dirty" to clean and back to "gray" spaces should be challenged to ensure that contamination control guidelines for each space would not be compromised.

Lias et al [31] demonstrated a case study of a cGMP facility which has flexible design that can be expanded as conditions warrant. To manufacture multiple products simultaneously a manufacturing scheme comprises of several parallel cleanroom suites, separated by airlocks, accessed through clean corridors and served by dedicated HVAC (heating, ventilation and air conditioning) units. Area classification is done according to the criticality of an area. Generally a processing suites are designed as Class 10,000 areas accessed from inner/ white corridor. Laminar flow hoods/ biosafety cabinets are Class 100 while buffer preparation, glass washing and support areas are Class 100,000. Exterior/ gray corridor is provided for exiting the suites. The facility is usually designed for unidirectional flow i.e. personnel entering a processing suite can not re-enter the white corridor; they must exit through the gray corridor. This provision is to

prevent cross contamination. Table 2 shows the comparison among the International clean room classifications.

Table 2: Comparison among the International Clean Room Classifications

Particles / m³ ≥0.5µm	US 209D non-metric	US 209E 1992 metric	EC cGMP Annex 1 1997	Germany VDI 2083 1990	UK BS 5295 1989	Japan JIS B 9920 1989	ISO 14644-1
3.5				0		2	2
10		M 1					
35	1	M 1.5		1		3	3
100		M 2					
353	10	M 2.5		2		4	4
1,000		M 3					
3,530	100	M 3.5	A= uni-directional B = turbulent	3	E or F	5	5
10,000		M 4					
35,300	1,000	M 4.5		4	G or H	6	6
100,000		M 5					
353,000	10,000	M 5.5	C	5	J	7	7
1,000,000		M 6					
3,530,000	100,000	M 6.5	D	6	K	8	8
10,000,000		M 7					

Processing areas are served by Air Handling Units (AHUs) and controlled by Building Automation System. The air flow is also unidirectional. All classified areas have terminal High Efficiency Particulate Air (HEPA) filters with low wall returns. Walls are epoxy painted with coving at ceiling, floor and corners to prevent accumulation of dust. Facility is served by USP compliant PW system and WFI system. Materials are transferred into and out of the manufacturing area through separate vestibules.

2.6 Facility Design Aspects

An efficient manufacturing plant should accommodate growth, different products, and different phases of development. It can make large quantities of product quickly and will be inexpensive to build and operate [32]. The design

should give attention to overall security of the site and the building, including safety within the labs and GMP suites, so a person can work alone safely. It also needs to allow for easy future expansion [33]. Following are the GMP requirements for a biopharma facility:

2.6.1 Area Requirements

The facility should allow multiple products to be handled cocurrently and should be able to simultaneously process mammalian and microbial technologies. Each suite should contain separate upstream and downstream processing areas, including separate inoculum and media preparation areas, and bulk filling areas.

2.6.2 Process Development Requirements

The process development labs are critical to the overall facility, handling the upstream and downstream development of biopharmaceutical process including comprehensive cell biology studies, process derivation, design optimization, scale-up, and modeling. In addition, they should produce pilot-scale preclinical materials to Good Laboratory Practices (GLP) quality standards.

2.6.3 Analytical and Quality Control Requirements

The analytical and QC labs provide a valuable product testing facility for products generated and developed in compliance with GMP and process development areas, as well as providing QC release testing and facility monitoring. Areas for wash, preparation, stores, cold storage, fine balances and booking support the QC labs.

2.6.4 Regulatory Requirements

Two main regulatory requirements relate to product quality and operator safety. Relevant parts of the facility must comply with the current good manufacturing practices (cGMPs) of the United States and the European Union for the production of biopharmaceutical medicines. In live areas, operators must

be protected according to guidelines on the handling of genetically modifie organisms and dangerous pathogens.

2.6.5 Support Area Requirements

As in all biopharmaceutical facilities, the labs, offices, and GMP suite need support in the form of utilities, wastewater treatment, storage, media an buffer preparation, cleaning and sterilization, and maintenance and worksho facilities. These should all be designed to complement the main operations Special attention should be given to cleaning and sterilization because th performance and verification of cleaning, disinfection, and sterilization of th facility and equipment will be main contributor to down time. The followin requirements need to be designed in:

- Minimization of cleaning and sterilization validation.
- Ability to perform turnaround of upstream and downstream suit independently of one another.
- Robust and rapid cleaning and sterilization methods.
- Ability to demonstrate successful cleaning between campaigns.

2.6.6 Facility Design Process

The facility design process follows definite prescribed pattern. The process is divided into five phases: Conceptual design, Design development, Construction documents, Bidding and Construction administration. During conceptual design phase, the design professional assimilates the program statements and develops concepts to solve the stated problem. The process is then advanced to the design development stage. The interior arrangement and the building mass are studied, to accommodate finer details of individual spaces within the building, and arrangement of the building on the site. Once the arrangement has been defined, construction documents are started. These documents are used to describe the building in sufficient detail so competitive bids can be obtained and construction completed. The construction phase

concludes with the physical building being completed and turned over the owne for production to begin.

2.7 Disposable Components (Single-Use) Technology

Much of the expenses in biopharmaceutical manufacturing come from th large capital investment required to build and operate a manufacturing facility This poses a strategic problem for biopharmaceutical companies. Also th difficulty of accurately predicting capacity requirements, development timelines dosages, market sizes and even clinical success and regulatory requirements lead to uncertainties in process development. Considering all these factors Hodge [34 discussed various emerging technologies (Single use disposable manufacturin components, electronic documentation and automation and isolators) that can b integrated to create a manufacturing platform (FlexMax) significantly differer from traditional approaches and that offer economics, efficiency and qualit improvements. They evaluated that a fully functional FlexMax facility can b installed for <50 of capital cost of an equivalent (100L scale train) scal traditional facility. Disposables such as tubing, filter capsules and bioprocess bag can be used in place of expensive stainless steel (SS316L) vessels and piping. Us of these components minimize capital equipment costs, reduce cleaning an turnaround time, make more efficient use of space, or ease the burden on SI (Steam-In-Place) and CIP (Cleaning-In-Place) system and the utilites that suppo them.

Sinclair et al [35 & 36] compared the leading edge design for a concep facility that exploits fully the benefits of single use disposable technologies wit traditional facility based on reusable equipments. They designed a facility c 1000L capacity using disposable material and found that it enabled fast produc change over, enhances product security, cost effective manufacturing, reduce labor, utilities and capital.

2.8 Plant Automation

The level of automation available in processing equipment has advanced rapidly over the past decade. Increased yields and product quality are two of the most obvious advantages obtained through deployment of automated process. Automation is viewed as a fixed and highly regulated process designed to eliminate human interaction. An equipment operation can be implemented through automated equipment, such as Programmable Logic Controller (PLC) or Distributed Control System (DCS) control, or it may be performed manually. In practice, process technology, an automation system, apart from the field instrumentation (sensors/actuators), is dominated by process control and instrumentation technology. These tools for automation are fitted into a basic structure of the automation system, which is universally accepted as a means of reference. This basic configuration comprises the typical components process control console, switch room and field level (Figure 4).

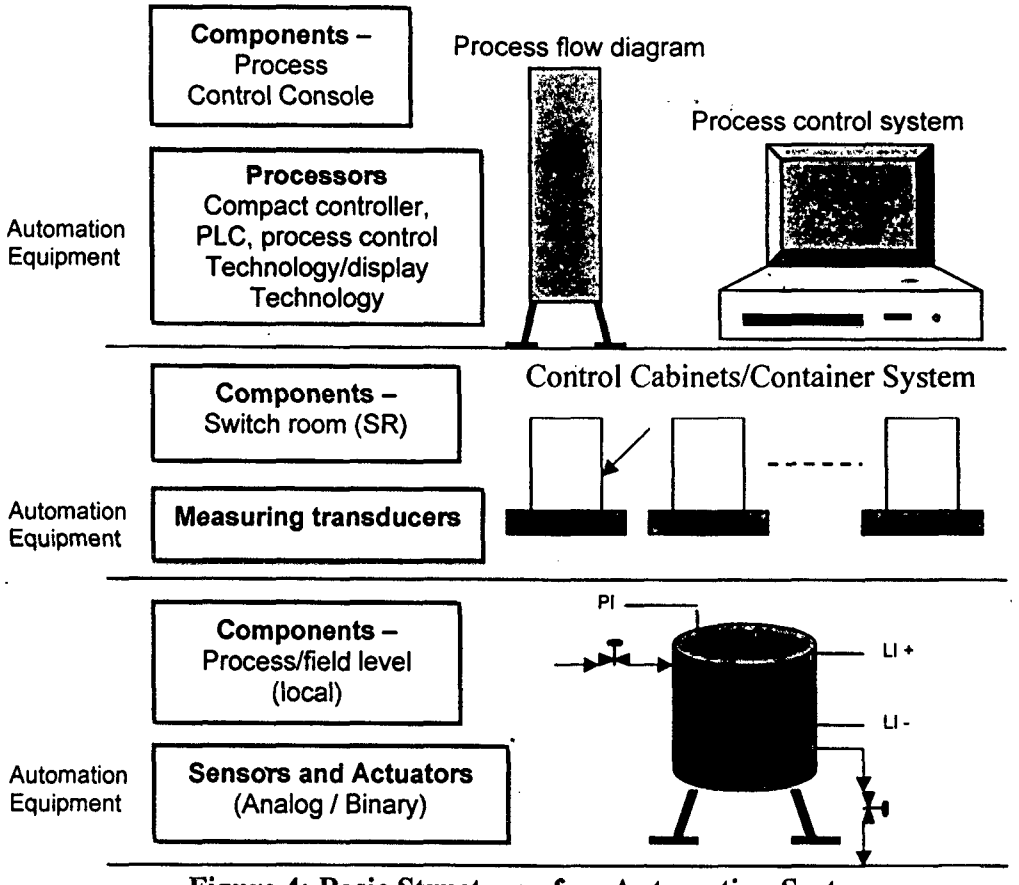


Figure 4: Basic Structure of an Automation System

2.8.1 Control Loop Strategy

In order to consistently manufacture a product that meets appropriate targets for attributes, a robust process with appropriate control should be developed. The basic element of a control system is the control loop. In Figure 5 the various components that make up the control loop are summarized.

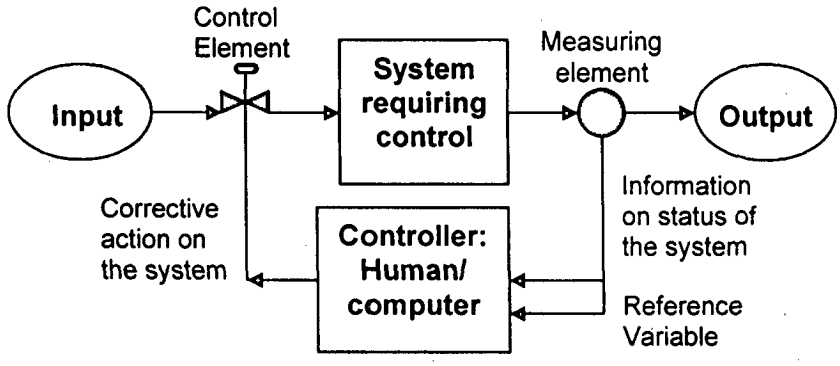


Figure 5: Control Loop Components

There are two fundamental types of controls: Sequence control and loop control. Sequence control is that part of control which permits automation of the equipment operation such as sterilization and other valve automation sequences i.e. for the most part providing an ordered array of digital (on/off) signal control Loop control deals with combinations of digital and analog control signals Whatever type of controller is selected for a process control, effective action will depend on the response of the controller. This response is determined by the nature of the control algorithm programmed into the system. The types of control algorithm most frequently encountered are 3-term or PID controllers. The PID controller is made up of three elements: P stands for proportional, I for integral and D for derivative control; the purpose of these functions is to provide a fast acting response to process deviation and scale the response to the output to achieve smooth control action. The characteristics of PID control are:

- Proportional control provides an output the magnitude of which is proportional to the deviation between the measured variable and set point
- Integral control tends to reduce the effect of proportional control alone helping to bring the measured variable back to set point faster by minimizing the integral of control error.

- Derivative action also tends to reduce the effect of P-control alone, by eliminating the slope of measured variable with time and maximizing the slope of the measured variable compared with the set point.

Now a days adaptive automation is used to anticipate changes under active control of a developer while maintaining precise control of all background variables not currently of interest. Kay [23] addressed various areas in which adaptive automation can be used to accelerate the process development activities.

2.8.2 Distributed Control System Architecture

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. It 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 (i.e. field) and output modules transmit instructions to the output instruments in the field. Computer buses or electrical buses connect the processor and modules through multiplexers/demultiplexers. Buses also connect the distributed controllers with the central controller and finally to the Human-Machine Interface (HMI) or control consoles. A typical DCS consists of functionally and/or geographically distributed digital controllers capable of executing from 1 to 256 or more regulatory control loops in one control box. The input/output devices (I/O) can be integral with the controller or located remotely via a field network. Today's controllers have extensive computational capabilities and, in addition to PID control, can generally perform logic and sequential control. DCSs may employ one or several workstations and can be configured at the workstation or by an off-line personal computer. Local communication is handled by a control network with transmission over twisted pair, coaxial, or fiber

optic cable. A server and/or applications processor may be included in the systen for extra computational, data collection, and reporting capability.

2.8.2.1 Elements of Distributed control system

Following are the primary elements of a Distributed control system:

- **Operator Console**: All the commands, sequences and functions ar shown in the operator console. Here the operator issues commands to fiel instruments and also acknowledge operator prompts.
- **Engineering Station**: These are stations for engineers to configure th system and also to implement control algorithms.
- **History Module:** History module stores the configurations of the DCS a well as the configurations of all the points in the plant. They also store th graphic files that are shown in the console and in most systems these da they are able to store some plant operating data.
- **Data Historian**: These are usually extra pieces of software that a dedicated to store process variables, set points and output values. They a usually of higher scanning rates than that available in the history module.
- **Control Modules**: These are customized to do control functions like PI control, ratio control, simple arithmetic and dynamic compensation
- **I/Os**: These manage the input and output (digital or analog) of the DCS Digital I/Os are those like on/off, start/stop signals.

S88 is a standard addressing batch control and provides a desi philosophy for software, equipment and procedures. Following S88 on a proce means: Defining the physical mode, procedures and recipes. Deitz et al [3 discussed the steps required to apply S88 standard to a process. They illustrat these steps by modularizing control of a buffer preparation vessel.

Industry standards such as the FDA's 21 CFR Part 11 (which provid criteria for electronic records, electronic signatures and handwritten signatu wherever computer systems are used for regulated activities) and the newer P/ initiative [39, 40] demand increased automation capabilities in t biopharmaceutical field.

OBJECTIVES

The overall objective of this study is to design a manufacturing train for the production of recombinant therapeutic protein and monoclonal antibodies using mammalian cell culture which is cGMP complaint. The facility is designed for an annual production of 2250 grams of purified product.

The study is divided into four major parts:

1. **Plant Design**: In this section overall mass balance is applied on the process and Process Flow Diagram is generated. On the basis of mass balance, size (i.e. capacity) of various equipments is defined.
2. **Equipment Design**: All the major equipments are designed as per the standards.
3. **Facility Design**: In this section a manufacturing facility design is proposed which is cGMP compliant.
4. **Computer System Design**: DCS architecture as per S88 standards is discussed and control strategy of various processes is studied on the equipments.

Chapter IV

METHODOLGY AND ANALYSIS FOR PLANT DESIGN

4.1 Market Demand

MAbs represent the fastest growing pharmaceutical market segment (43% compounded annual growth rate). Even with the conservative assumptions about growing attrition rates, substitution pressure and margin squeezes, mAbs sales will probably reach a stable plateau of $ 20 Billion by 2010. Currently there are 20 therapeutic antibodies and fusion proteins on the market targeting different diseases and 150 more in various stages of clinical trials [41 and 42]. While the commercialization of mAbs is gathering momentum, the sector is facing a worldwide shortfall of available biomanufacturing capacity that is becoming a critical strategic limitation. Figure 6 shows the increasing annual sales and demands of the mAbs [43].

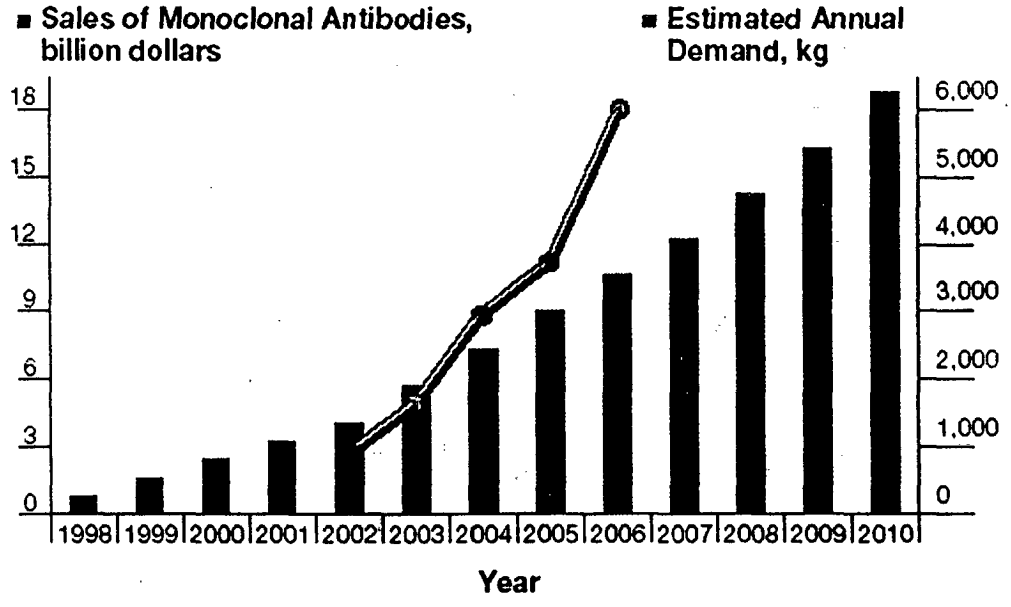


Figure 6: Annual Sales and Manufacturing Demands for Marketed MAbs.

The production process for mAbs must be designed in such a way that i meets the highest requirements with regard to consistency and reproducibility These therapeutics usually require high doses and therefore vast manufacturin capacities. The typical dose for a mAb therapeutic is assumed to be 2-5 g pe patient per year. Assuming an average of 80,000-1,00,000 patients, there is requirement of 500 kg of product per year worldwide.

4.2 Plant Capacity Calculation

The plant capacity depends upon previous planning estimates and calculation, which determine the quantity of raw material necessary to meet the demands of production per year. These estimates take into consideration the product titer, yields through the purification process, and losses during final fill operations or during sampling required for quality control and in process monitoring. This information will provide the total number of liter of cell culture medium required. Based on the mode of operation, the batch size and batches per year can be established.

In this report the design of the biotechnology facility is driven by requirements of Monoclonal Antibodies in India. From market demand we require around 90,000 vials (each of 1ml capacity) of lyophilized monoclonal antibodies expressed in mammalian cell culture to meet the dosage requirement of 25 mg per year. Therefore, total protein requirement is 2250 gm per year. The estimated product titer in bioreactor is 1.0 g/L. Considering an overall recovery efficiency of 50%, we achieve a purified product titer of 0.5 g/L. If in each run 1200 grams of product is obtained from the bioreactor so, required working volume of Bioreactor is 2400 L. After considering losses in sampling etc. reactor working volume is taken as 2500 L. In one run 1200 grams of product is produced so, in order to meet the requirement of 2250 grams of product per year two production runs are entailed. Table 3 shows the calculation summary.

Table 3: Calculation Summary

Demand (no. of vials)	Dosage		Total protein reqt. (gms/yr)	Bioreactor runs		
	Qty mg	Nature		Size (L)	Gms/run	No. of runs
90,000	25	Lyophilized	2,250	2500	1,200	1.9

4.3 Process Mass Balance

Tables 4, 5 and 6 provide a summary of the overall material balances per batch. The quantities are in kilograms per batch. The duration of a single batch is 648 hours. The overall recovery yield of the product is taken as 50% (1224 g of product is recovered out of the 2500 g that is present in the cell culture broth).

Table 4: Cell Culture Operations

Bioreactor Size	3,333 L
Working %	75
Bioreactor Maximum Working Volume	2,500 L
Fed-Batch	
Product Titer in bioreactor-crude	1 gms/L
Recovery/Purification overall Yield	50 %
Net product Titer per bioreactor-Purified	0.5 gms/L
Media/or/Harvest Volume / Batch Vol/Day	0.11 V/V/D
Harvest Volume/ Day	275 L/Day
Growth Cycle	3 Days
Production Cycle	9 Days
Turn-around time (CIP/SIP + QA +Other)	15 Days
Total batch cycle	27 Days
Operating days / Year	300 Days/Year
Batch cycles / Year	11 Cycles/Year
Productivity / Day	136 gms/Day
Batch Poductivity / Cycle	1224 gms/Batch cycle
Annual Production	13464 gms/Year

Table 5: Recovery and Purification Operations

			Recovery	Feed Volume	Product Volume	Protein Amount	Protein Conc.	Solutions Volume	Waste Volume
			(%)	(L)	(L)	(gms)	(g/L)	(L)	(L)
Harvest									
Collect harvest batch for:	1	Days							
Number of harvest batches per Run:	1								
Harvest Batch			100	2,500	2,500	2500	1		
Centrifugation									
Wash Buffers	0.5 * Feed							1250	
Clarified Harvest			90	2,500	2250	2250	1		1,500
Ultrafiltration	Conc. by	20							
Retentate			90	2250	113	2025	18		
Permeate									2,137
Chromatography-1 (Affinity)	Col. Vol.(L)	405							
Protein Load/column	5	(g/L)							
Eluate	2	Col. Vol.	90	113	810	1823	2		
Buffer Solutions (L)	15	Col. Vol.						6075	
Waste Fractions (L)									5,378
Diafiltration-1									
Buffer Solutions (L)	3 * Feed			810	810	1823	2	2430	2,430
Chromatography-2 (Ion Exchange)	Col. Vol.(L)	182.3							
Protein Load/column	10	(g/L)							
Eluate	2	Col. Vol.	80	810	364.6	1458	4		
Buffer Solutions (L)	20	Col. Vol.						3646	
Waste Fractions (L)									4,091

			Recovery	Feed Volume	Product Volume	Protein Amount	Protein Conc.	Solutions Volume	Waste Volume
			(%)	(L)	(L)	(gms)	(g/L)	(L)	(L)
Nanofilter-Viral									
Wash Buffers	1.0 * Feed							365	
Post viral product stream per wash			98	364.6	729.6	1429	2		
Ultrafiltration-2	Conc. by	10							
Retentate			95	729.6	73	1358	19		
Permeate									657
Chromatography-3 (GPC)	Col. Vol. (L)	729							
Feed as % of bed	10	%							
Eluate	30	%Col. Vol.	95	72.9	218.7	1290	6		
Buffer Solutions (L)	10	Col. Vol.						7290	
Waste Fractions (L)									7,144
Ultrafiltration-3/Diafiltration	Conc. by	4							
Concentrate: Retentate			95	218.7	55	1226	22		
Permeate									164
Diafilter: Buffer	3*Concentrate							165	
Permeate									165
Total								21,221	23,666

Table 6: Overall Mass Balance

Per Batch (L)	In		Out
Buffer Solutions (L)	21,221	**Waste (L)**	23,666
Harvest Batch (L)	2,500	**Purified Product (L)**	55
Total	23,721		23,721

4.4 Critical analysis of the Mass Balance

The maximum working volume of the bioreactor is taken to be 2500 L i order to meet the annual product requirement of 2250 gms. The working volum of a bioreactor is generally taken as 70 – 85 % of the geometric volume [44]. I order to provide space for the foam a head space of 25 % is given. Therefore, th geometric volume of the bioreactor is 3333 L (~ 3400 L). A fed-batch process i selected in which media is added intermittently. A total of 2500 L of media i added, half of which is added at the start and remaining half at the end of th growth phase. The volume of harvest is taken as the volume of the media addec A total of 27 days, batch cycle runs in which 3 days are for growth of cells, 9 day for the production cycle and the remaining 15 days are dedicated to Clean i Place/Sterilization in Place and other Quality Assurance/Quality Contrc activities. A total of 300 operating days are used for the production while th remaining days are used for maintenance activities. For a total of 11 batches pe cycle, annual productivity of the plant is 13464 grams/year.

For the recovery and purification operations a "Platform Approach" i followed. The unit operations are selected on the basis of the literature reviewe for the use of platform approach for downstream processing of monoclon antibody. For harvesting a low shear centrifuge is preferred over microfiltratio due to its scalability, better clarification and economical operations for larg scale. If viscosity increases there is a decrease in the separation efficiency. I most of the cases, a 25-50% dilution of the feed may lead to a 2 to 5 fol reduction in viscosity. Therefore, in this case feed is diluted with a buffer quantit half of the feed volume thus increasing the separation efficiency of the centrifug The product recovery yield of this step is 90%. Before going to Protein chromatography the product stream from the centrifuge is ultrafiltered t

concentrate the protein by 20-fold with a recovery yield of 90 %. Affinity chromatography offers high selectivity, hence high resolution, and usually high capacity for the protein(s) of interest. Protein A Chromatography is the most efficient purification step for antibodies with a recovery yield of more than 90% For a protein load per column of 5g/l, a 405 L bed volume is opted. The key stages in affinity purification are shown in Figure 7 [45]. On the basis of this graph a total of 15 column volumes of buffer is taken.

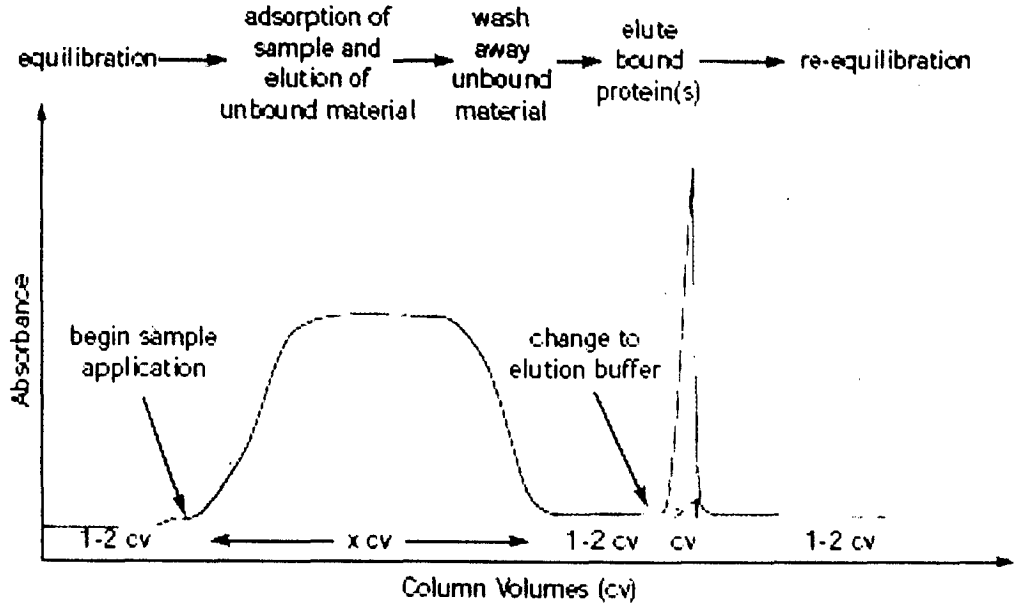


Figure 7: Typical Affinity Purification.

After chromatography the product is diafiltered with buffer solution (3 times the feed volume) in order to remove salts. Ion exchange Chromatography is used for the removal of high molecular aggregates, host cell proteins, leached Protein A and viral clearance with a recovery yield of more than 80%. For a protein load per column of 10g/l, a 182.3 L bed volume is required. The key stages in Ion exchange chromatography are shown in Figure 8 [45]. On the basis of this graph a total of around 20 column volumes of buffer solution is taken.

After ion exchange chromatography the product is sent through a nano filter (with a recovery of 98 % in order to remove viruses) to ultrafiltration unit to increase the concentration of the product by 10-fold.

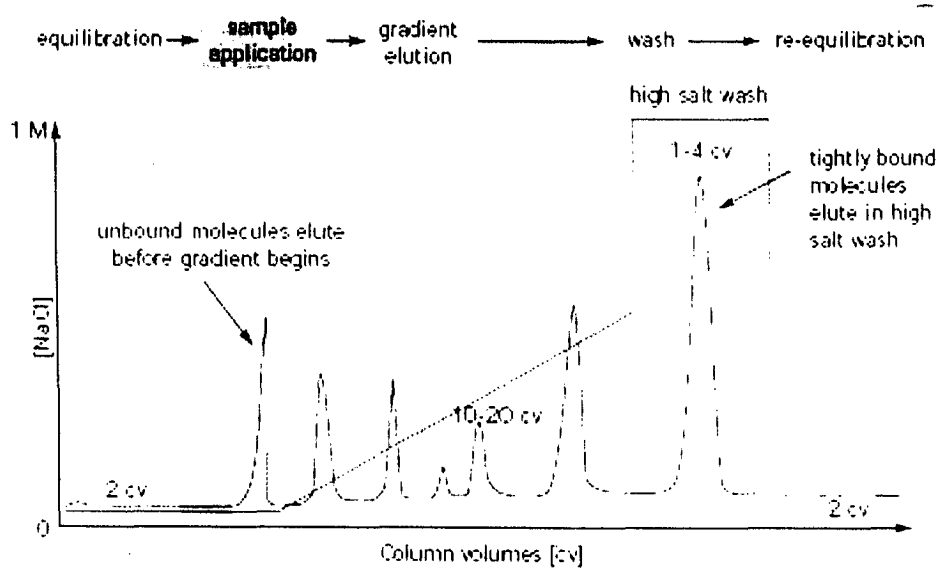


Figure 8: Typical IEX Gradient Elution.

Gel Filtration chromatography is an ideal operation for the final polishin steps in purification when sample volumes have been reduced. For a feed of 10 % of bed volume a 729 liters of column volume is selected. Finally, after th completion of downstream processes, the product is concentrated (by 4-fold) an buffer exchanged into the formulation using Ultrafiltration / Diafiltration setup.

4.5 Equipment Sizing

On the basis of above calculations all the major equipments are sized.

4.5.1 Bioreactor Sizing

As discussed in the plant capacity calculation section in order to obtai 2250 grams of product annually a 2500 L bioreactor is required. For mammalia cell culture the bioreactor height and diameter ratio lies in the range of 1:1 to 2:1 Here we select height and diameter ratio of 1.5:1.

4.5.2 Chromatography column sizing

As shown in the mass balance three chromatography columns are require with a bed volume of 405 L, 182 L, and 728 L. Taking a bed height to diamete ratio of 1.2:1, column diameter and height are calculated (Table 7).

Table 7: Chromatography Column Sizes

Chromatography Columns Bed Volumes (L)			Column Bed Dimensions H/D = 1.2:1			
Col-1	Col-2	Col-3		Col-1	Col-2	Col-3
			Diameter	75	58	92
405	182	728	Height	90	70	110

The commercially available columns bed volume and dimensions are selected from the vendors' catalogue (Table 8).

Table 8: Commercially Available Chromatography Column Sizes

Chromatography Columns Bed Volumes (L)			Column Bed Dimensions			
Col-1	Col-2	Col-3		Col-1	Col-2	Col-3
			Diameter	44.6	44.6	50
130	91	251	Height	83	58	128
			H/D	1.9	1.3	2.6

Therefore, the numbers of cycles required by the columns are: Column 1 ≈ 3, Column 2 ≈ 2 and Column 3 ≈ 3.

4.5.3 TFF System Sizing

For the first ultrafiltration step a processing time of 8 hours is estimated while for other two 5 hours of processing are sufficient as they process less amount of product volume. Generally in industrial practices for concentration a flux of 20-30 $L/m^2.h$ is specified [44]. Referring to the mass balance calculations the amount of permeate coming out at the end of the ultrafiltraion operation is used to calculate the membrane area (Table 9).

Table 9: TFF System Sizes

System	Permeate (L)	Processing time (Hr)	Flux (LMH)	Membrane Area (sq. mt)
UF-1	2138	8	20	13.4
UF-2	656	5	20	6.6
UF-3	164	5	20	1.6

4.5.4 Buffer Preparation/Supply Tank Sizing

As the chromatography operations require a large amount of buffer as compared to the other unit operations such as UF/DF, the buffer tank sizing is done on the basis of the requirement by the chromatography operations. For chromatography different types and amounts of buffers are used for the various functions performed on it, summary of which is given in Tables 10, 11 and 12.

a. **Column-1:** Bed Volume is 130 L

Table 10: Buffer Requirements for Column-1

Steps	Function	Buffers Name	Type	CVs	1 Cycle (L)	3 Cycles (L)
1	Wash	A1	PBS-1	3	390	1170
2	Equilibration	B	PBS-2	3	390	1170
3	Loading		Product		0	0
4	Wash	B	PBS-2	2	260	780
5	Elution-1	C	Tris+NaCl	1	130	390
	Elution-2	D	Tris+NaCl	1	130	390
6	Wash	B	PBS-2	3	390	1170
7	Sanitize	S1	NaOH	3	390	1170
8	Wash	B	PBS-2	3	390	1170
			Subtotals / Run:	19	2470	7410
9	Storage-Short	S2	NaOH	1	130	390
10	Storage-Long	S3	EtOH	1	130	N.A.

Total Buffer per Run = 8,190 L

b. **Column-2:** Bed Volume is 90 L

Table 11: Buffer Requirements for Column-2

Steps	Function	Buffers Name	Type	CVs	1 Cycle (L)	2 Cycles (L)
1	Wash	A2	Citrate	3	270	540
2	Equilibration	B	PBS-2	3	270	540
3	Loading		Product		0	0
4	Wash	B	PBS-2	2	180	360
5	Elution	A2	Citrate	2	180	360
6	Wash	A2	Citrate	3	270	540
7	Sanitize	E	B+Thimersol	2	180	360
			Subtotals / Run:	15	1350	2700
8	Storage-Short	S2	NaOH	1	90	180
9	Storage-Long	S3	EtOH	1	90	N.A.

Total Buffer per Run = 2,880 L

c. **Column-3:** Bed Volume is 250 L

Table 12: Buffer Requirements for Column-3

Steps	Function	Buffers Name	Type	CVs	1 Cycle (L)	3 Cycles (L)
1	Wash	A1	PBS-1	2	500	1500
2	Equilibration	A1	PBS-1	1	250	750
3	Loading		Product		0	0
4	Elution	A1	PBS-1	2	500	1500
5	Sanitize	S1	NaOH	2	500	1500
			Subtotals / Run:	7	1750	5250
6	Storage-Short	S2	NaOH	3	750	2250
7	Storage-Long	S3	A1+Preservative	1	250	N.A.

Total Buffer per Run = 7,500 L

Therefore, the total amount of Buffer solutions required in all the three columns for different functions is 18,570 L.

4.5.4.1 Totals by Buffer Type for Buffer Preparation/Supply Tank Sizing

On the basis of the amount of the various buffers required by the chromatography columns the buffer preparation or supply tank sizing is done. It is estimated that total amount of buffer prepared is three times of the concentrate preparation. Also it is assumed that the buffer in the tank is filled to 75 % of its total volume. Therefore, the buffer tank volume required to prepare different types of buffer is summarized in Table 13. This table shows that a total of around two 2400 L, two 1300 L, one 700 L and three 200 L buffer supply tanks are required to fulfill the buffer requirements in the facility. In order to prepare buffer, single buffer preparation tank of each above stated capacity can be used.

Table 13: Buffer Tank Volume Required

Buffers Name	Type	Column Buffer Requirement			Totals/ Buffer (L)	Buffer Tank Volume (L
		Col-1	Col-2	Col-3		
A1	PBS-1	1170	0	3750	4920	2187
A2	Citrate	0	1440	0	1440	640
B	PBS-2	4290	900	0	5190	2307
C	Tris+NaCl	390	0	0	390	173
D	Tris+NaCl	390	0	0	390	173
E	B+Thimersol	0	360	0	360	160
S1	NaOH	1170	0	1500	2670	1187
S2	NaOH	390	180	2250	2820	1253
	Subtotals:	7800	2880	7500	18180	

4.6 WFI Requirements for Media and Buffer Solutions

US Pharmacopoeia based WFI (Water-For-Injection) is required for med preparation, buffer preparation and for clean-in-place operations. A summary (the total WFI needed by chromatography columns. media preparation and buff preparation operations (excluding the CIP requirements) are given along with t peak WFI requirements by Process (Table 14).

Table 14: WFI Requirement for Buffer Solution of Chromatography

	Chromatography Columns		
	Bed Volumes (L)		
	Col-1	Col-2	Col-3
Preliminary Design Basis	405	182	728
Selected Design Basis	130	91	251
No. of cycles / column	3	2	3
Column volume per column	20	15	7
Preliminary Average Buffer Volume	8100	2730	5096

Total WFI needed by columns = 15,926 L

Media Solution required per run = 2,500 L

Buffer Solution required per run = 21,222 L

Total WFI per run = 23,722 L

Peak WFI requirements by "Process" only

- 2,500 L Fed-Batch cell culture use 23,722 L WFI. Three days on an average it will use 7,907 L/Day of WFI.
- Column-1 uses the maximum amount of 7,800 L WFI. One day on an average it will use 7,800 L/day of WFI.
- Average peak WFI requirements for the process (without CIP) are 7,854 L/Day.

4.7 Process Description

The entire process for manufacturing monoclonal antibodies is shown in a simplified flow diagram in Figure 9.

4.7.1 Upstream section

The serum-free and low-protein-content media powder is dissolved in WFI in a stainless steel tank, and the solution is sterilized using a 0.1 mm dead-end polishing filter. A stirred-tank bioreactor is used to grow the mammalian cells that express the therapeutic monoclonal antibody. The bioreactor operates in fed batch mode. A cycle time of 648 hours (288 hours for fermentation and 360 hours for turnaround) was assumed for the bioreactor. The volume of broth generated per bioreactor batch is around 2,500 liters containing 2500 g of product (the product titer is 1 g/L). The total volume of the bioreactor vessel is 3,400 liters.

4.7.2 Downstream section

The generated biomass and other suspended compounds are removed using a centrifuge. If viscosity increases there is a decrease in the separation efficiency. In most of the cases, a 25-50% dilution of the feed may lead to a 2 to 5 fold reduction in viscosity. Therefore, in this case feed is diluted with a buffer quantity half of the feed volume thus increasing the separation efficiency of the centrifuge. The product recovery yield of this step is 90%. This filtration step takes 5.1 h and requires a membrane area of around 30 m^2. The clarified solution is concentrated 20-fold using a 5,000 Dalton MW cut-off ultrafilter (UF-1). The

recovery yield of this step is 90%. This step takes 8 h and requires a membrane area of 13.4 m^2. The bulk of the contaminant proteins are removed using a protein A affinity chromatography column (C-1). The following operating assumptions were made: (1) Protein load per column volume is taken as 5 g/l; (2) the product is recovered in 2 CV's of eluant buffer with a recovery yield of 90%, and the pH is maintained near neutral to ensure product stability; and (3) the total volume of the solutions for column equilibration, wash, elution and regeneration is 15 CV's. This step requires a column volume of around 405 liters. The protein A elution buffer is exchanged with another buffer using Diafiltration. The purification proceeds using an ion-exchange chromatography column (C-2). The following operating assumptions were made: (1) Protein load per column volume is taken as 10 g/l; (2) the product is recovered in 2 CV's of eluant buffer with a recovery yield of 80%; and (3) the total volume of the solutions for column equilibration, wash, elution and regeneration is 20 CV's. This step requires a column volume of 182 liters. The clarified solution is concentrated 10-fold using a 30,000 Dalton MW cut-off ultrafilter (UF-2). The recovery yield of this step is 95%. This step takes 5 h and requires a membrane area of 6.6 m^2. The purification proceeds using a Gel Permeation chromatography column (C-3). The following operating assumptions were made for the GPC step: (1) Feed is fed in the column as 10% of the bed volume; (2) the product is recovered in 30% CV of eluant buffer with recovery yield of 95%; and (4) the total volume of the solutions for column equilibration, wash, elution and regeneration is 10 CV's. This step requires column volume of 729 liters. The purified product solution is concentrated four fold and elution buffer is exchanged with formulation buffer. The product concentration in the final solution is around 22 g/liter. Finally, the purified product is stored in disposable bags and is sent to Fill Finish area where it is bulk manufactured and sterile filtered to vials and finally they are lyophilized.

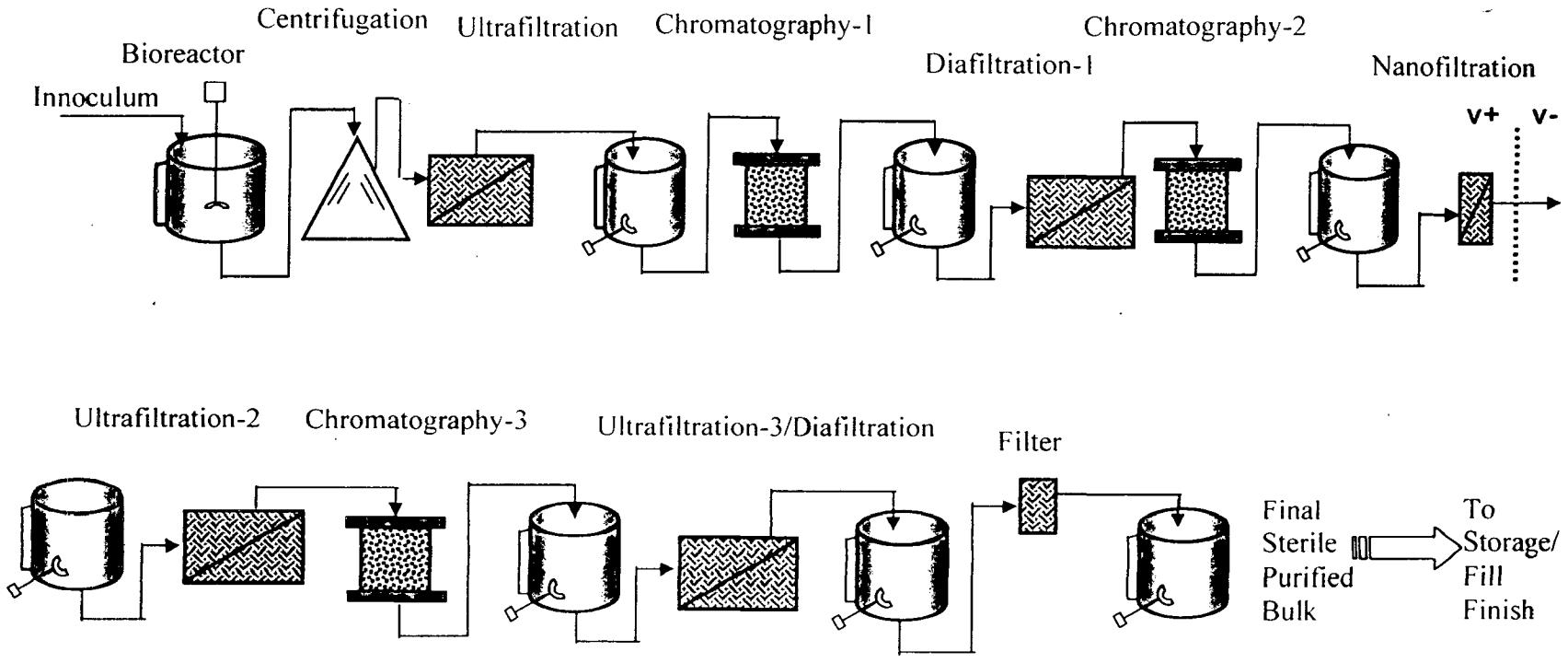


Figure 9: A Simplified Flow Diagram for Monoclonal Antibody Production

Chapter V

EQUIPMENT DESIGN

5.1 Design of Production Bioreactor

The most striking characteristic of animal cells, which makes their large scale culture fundamentally different from conventional microbial fermentation, is their fragility. These cells are easily damaged by mechanical stress, and cannot be cultured under conditions of high aeration and agitation that are the hallmarks of turbulent microbial fermentation. Animal cells are considerably larger than their microbial counterparts, and do not possess a rigid, highly resistant cell wall to protect them from the shear generating environment of a rapidly stirred and aerated tank.

Animal cells that are capable of being propagated in suspension culture have been the most attractive to industry for a variety of reasons, with monoclonal antibody production receiving most attention. Stirred tanks are perceived as being reliable, well understood, and easily scaled up. Stirred suspension cultures are amenable to various kinds of operations, including batch culture, fed-batch, semi-continuous, or continuous perfusion. Indeed, stirred tanks can support the growth both of suspension cells and of anchorage-dependent cells when used in conjunction with micro carriers.

The design of a fully functional bioreactor requires perfect integration of several components to ensure that cultures will finish at the desired productivity. These components include: a jacketed stainless steel vessel, temperature control, agitation, sparge gas control, surface gas control, pH and dissolved oxygen control, a sampling mechanism, weight indication and control, cleaning hardware, sterilization hardware, piping and tubing to deliver all services, add media, pH control solutions, and gases, feed and addition pumps, and a supervisory control and a data-acquisition system.

5.1.1 Characteristics and Design of Bioreactor

Various aspects of the mechanical design of a bioreactor are discussed as following:

- **Safety codes:** The single most important factor in vessel designing is that the vessel should be safe to operate at design conditions. For safe

operation, the vessel must be built and tested in accordance with th ASME Code for Unfired Pressure Vessels.

- **Materials:** The choice of materials for vessel construction should b based on the compatibility with the organism, corrosiveness of th fermentation broth, cost and equipment life time and material testing Most vessels and process lines designed for aseptic operation are buil from SS316L as it is less corrosive and does not shed particles. For jacke and black utility supply lines we can go with SS304 or SS304L. Th primary differences among these are concentrations of carbon and alloyin materials, such as molybdenum, which affect primarily corrosio resistance and welding characteristics.
- **Welds**: The quality of vessel welds is extremely important, not only fo code purposes, but also to insure maximum smoothness and cleanability and to minimize corrosion problems. All vessels welding for asepti bioreactors should be done under inert gas shielding, so as to minimiz oxidation and flux residue, and to yield smoother, pit-free welds Therefore, orbital welding and TIG welding are preferred.
- **Finish**: The smoother a surface is, the easier it is to clean. Surfac roughness inside the vessel should be less than 0.3 microns and outsid should be less than 0.8 micron. A vessel should be electropolished fror inside to minimize the surface roughness. For outer surface mechanica polishing suffice for the purpose. During fabrication and polishing there i some destruction of the surface film that protects stainless steel fror corrosion. For this we need passivation of the internal surface of th vessel. A typical passivation process involves cleaning with NaOH an citric acid followed by nitric acid and a complete water rinse.
- **Cleanability**: The Bioreactor must be designed to insure that th Cleaning-In-Place (CIP) system is not compromised and that all interic surfaces can be cleaned. There should not be any accumulation of solids smooth and free-draining, and dead legs should be within permitted limit i.e. not more than 1 to 3 times of the pipe diameter.

- **Nozzles**: Nozzles should be aseptically connected to the external piping Above 4" size nozzle can be selected which are flanged with O-rings. Th nozzles should be mounted at an angle of 5-15 °C off the horizontal fo free draining thus minimizing the trapping of material in the nozzle or J type nozzles can be mounted on the vessel head. Nozzle cavity should b kept as small as possible for easy cleaning and sterilization. It shoul slightly protrude from the surface in order to avoid added liquids dribblin down the surface.

5.1.1.1 Size and Geometry

Stirred cell culture tanks are almost always cylindrical vessels with a rati of height and diameter lying in the range of 1:1 to 2:1. In this case a height t diameter ratio of 1.5:1 is taken. A flat head is not considered appropriate, due t the possible presence of stagnant areas that may accumulate solid particulates an require more forceful mixing than is desirable. The working volume of bioreactor is 70-85 % of its geometric volume. Leaving a head space of 25 % w get a geometric volume of around 3400 L.

Considering H:D=1.5:1, and torispherical head

Total volume of Reactor = Cylindrical volume + 2 * head volume.

$V = ((\pi/4)*D_i^2* H) + 2 * 0.0809*D_i^3$ (for Torispheric head, Table 10-65 [46])

$D_i = 1363$ mm

Tan to Tan height of the vessel, H = 1.5 * 1363 = 2044 mm.

Dish Volume (DV) = $0.0809 * D^3$

$= 0.2048\ m^3$

Working Level Height, h = $[(WV-DV) * 4] / (\pi * D^2)$

$= 1.574$ m

Crown Radius, $R_C = fD$ (where $f > 0.5$)

Knuckle Radius $R_K = kD$ (where $0<k<0.5$)

Considering depth for 6% Torispherical Dish, k = 0.06 and f = 1.

Crown Radius = 1.363 m

Knuckle Radius = 0.082 m

Depth of Dish excluding thickness, h_d

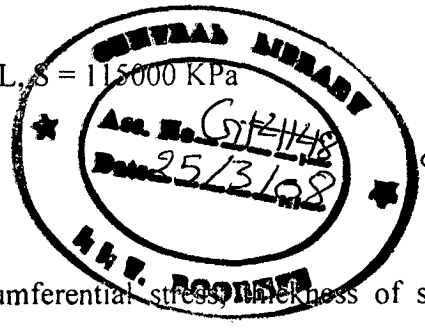
$$h_d = R_C - SQRT\{[(R_C - R_K)\ ^2]-[(R_C/2) - R_K]^2\}$$

$$= 0.231 \text{ m}$$

5.1.1.1.1 Vessel Shell Thickness Design

Design parameters (Input parameters)

- Shell inner operating pressure = 3.1 Bar (= 310 KPa)
- Shell operating temperature = 126 °C
- Shell Internal design pressure, P_i = 4.1 Bar (= 410 KPa)
- Shell External design pressure, P_o = 9.6 Bar (= 960 KPa)
- Shell design temperature = 150 °C
- Weld Joint Efficiency, E = 0.85
- Max. Allowable Stress for SS316 L, S = 115000 KPa
- Shell inner radius, R = 681.5 mm



Output Parameters

For cylindrical shell under circumferential stress, thickness of shell i calculated by following equation [47].

a) Thickness for Internal Pressure

Thickness, t = P*R/ (S*E-0.6*P)

Where,

t = Thickness of vessel shell (mm)

P = Internal Design Pressure (KPa)

R = Radius of the shell (mm)

S = Maximum allowable circumferential stress of SS316L Materia

E = Weld joint efficiency for shell seam joint

Therefore, inner shell thickness = 2.87 mm

b) Thickness for External Pressure

For cylindrical shells and tubes (seamless or with longitudinal butt joints)

Distance from top tan line to jacket closure, Dl = 0.0 mm

Total length, L = H – Dl = 2044.5 mm

Assumed initial thickness, t = 20 mm

Hence shell outer diameter, $D_o = D_i + (2*t) = 1403$ mm

$D_o/t = 70.2$

$L/ D_o = 1.46$

Factor A = 0.0015 (Figure. HA-3 [47])

Factor B = 55 (at 150 °C from Figure. HA-4 [47])

Maximum allowable external working pressure, P_a

$$P_a = 4*B/ (3*(D_o/t)) = 1.045 \text{ MPa}$$

As maximum allowable external working pressure is greater than the external design pressure therefore, thickness assumed is safe to use.

Hence, Vessel shell thickness for external pressure = 20 mm

5.1.1.1.2 Vessel Dish Thickness Design

a) Vessel dish thickness for Internal Pressure

For top dish weld joint efficiency is taken as 1 and External design pressure, P_o as 110 KPa

Hence, Thickness, $t = P_i*R/ (S*E-0.1*P_i) = 2.43$ mm

b) Vessel dish thickness for 1.67 times of External Pressure

For 1.67 times the external design pressure, $P_{ii} = 1.67 * P_o = 183.7$ KPa

Hence, Thickness, $t = P_{ii}*R/ (S*E-0.1*P_{ii}) = 1.09$ mm

c) Thickness for External Pressure

Assumed initial thickness, t = 8 mm

Hence outer crown radius, $R_o = Rc + t = 1371$ mm

$R_o/t = 171.4$

Factor $A = 0.125 / (R_o / t) = 0.00073$

Factor B = 46 (at 150 °C from Figure. HA-4 [47])

Maximum allowable external working pressure, $P_a = B/(R_o/t) = 0.268$ MPa

As maximum allowable external working pressure is greater than th external design pressure therefore, thickness assumed is safe to use.

Hence, Vessel dish thickness provided = 8 mm

5.1.1.1.3 Vessel Jacket Shell Thickness Design

Design parameters (Input parameters)

- Jacket inner operating pressure = 8.6 Bar (= 860 KPa)
- Jacket operating temperature = 126 °C
- Jacket Internal design pressure, P_i = 9.6 Bar (= 960 KPa)
- Jacket External design pressure, P_o = 1.1 Bar (= 110 KPa)
- Jacket design temperature = 150 °C
- Weld Joint Efficiency, E = 0.85
- Max. Allowable Stress for SS316 L, S = 115000 KPa
- Max. Allowable Stress for SS304 = 115000 KPa
- Jacket spacing = 20 mm
- Vessel shell thickness = 20 mm

Calculations (Output Parameters)

a) Vessel jacket thickness for Internal Pressure

Jacket inner Radius, R_J = 721.5 mm

Hence, Thickness, $t = P_i*R/ (S*E-0.6*P_i)$ = 7 mm

b) Thickness for External Pressure

For cylindrical shells and tubes (seamless or with longitudinal butt joints)

Distance from top tan line to jacket closure, Dl = 0.0 mm

Total length, L = H – Dl = 2044.5 mm

Assumed initial thickness, t = 6 mm

Hence jacket outer diameter, $D_o = R_J * 2 + (2 * t)$ = 1455 mm

D_o/t = 242.5

L/ D_o = 1.41

Factor A = 0.00026

Factor B = 23

Maximum allowable external working pressure, P_a

$P_a = 4*B/ (3*(D_o/t)) = 0.126$ MPa

As maximum allowable external working pressure is greater than the external design pressure therefore, thickness assumed is safe to use.

Vessel shell thickness for external pressure = 6 mm

Hence, Vessel shell thickness provided = 6 mm

5.1.1.2 Aeration System Design

In standard batch culture conditions, animal cells grow to relatively low cell concentrations compared to high biomass seen in microbial fermentations. In addition animal cells have dramatically lower metabolic oxygen requirements than those of microbial systems. Increasing oxygen mass transfer through high agitation rates and liberal sparging is often not possible in animal cell cultures, due to their sensitivity to mechanical damage and shear. These effects need to be minimized by reducing the impeller tip speed and the power input per unit volume as much as is feasible. Along with the sparger air, overlay air is also send to the overhead space of the bioreactor in order to remove the gases accumulated in the top head via a vent filter.

Oxygen Transfer rate $OTR = (\mu/Y_{x/o})*X$

Where,

μ is cell specific growth rate

$Y_{x/o}$ is cell yield coefficient on oxygen

X is cell concentration

For constant dissolved oxygen concentration, oxygen material balance gives,

$OTR = F_i*Y_i - F_o*Y_o$

Where,

F is molar flow rate of gas

Y is mole fraction of oxygen

For mammalian cell culture we need low gas flowrates, considering air flowrate to be 0.25 VVM,

Flow rate, Vs = 0.25 * 2500 = 625 SLPM

Molar flowrate of gas, F = Vs/22.4 = 27.90 mmol/min

Therefore,

$$OTR = (60*1000*Y*F*\epsilon)/V_L = 21 \text{ mmol /L hr}$$

Where,

Y is mole fraction of oxygen in air (= 0.21)

ϵ is oxygen transfer efficiency (= 15%, [48])

V_L is Volume of liquid (= 2500L)

5.1.1.3 Agitation system design

The agitator is required to achieve a number of mixing objectives, e.g bulk fluid and gas-phase mixing, air dispersion, oxygen transfer, heat transfer suspension of solid particles and maintaining a uniform environment throughou the vessel contents. Bulk mixing and micromixing broth are influenced strongl by impeller type, broth rheology, and tank geometry and internals.

Agitation and aeration for mammalian cell culture are very different fron those for microbial culture. OTR requirements are much lower, but the cells ar much more easily damaged by the fluid mechanical forces generated by impeller or collapsing gas bubbles. Axial flow impellers (such as marine propellers provide good top-to-bottom mixing, but draw relatively little power and therefor do not contribute much to good oxygen transfer. The impeller called Elephant ea (2-segmented propeller) has been shown in commercial applications to scale u reliably and to provide adequate mixing and OTR. Swirling and vortexin problems can be overcome by mounting the shaft at an angle of 15°.

Here, a 2-segmented pitched blade propeller or paddle impeller (calle Elephant ear) is selected with pitch 1:1 and angle 15°.

- No. of Impellers = H * Broth specific gravity/ D_i = 1.57 ≈ 2

Where,

H is height of the bioreactor, m

D_i is diameter of the bioreactor, m

Broth specific gravity = 1.05

- Impeller Diameter, $D_a = 0.5 * D_i = 682$ mm

  Spacing between impeller is generally 1.0 to 1.5 times diameter [49] Therefore, spacing between two impellers = 682 mm

- Tip Speed and Impeller speed

  Tip Speed for microbial fermentation should be in the range of 300-50(m/min and for mammalian cell culture it should be in the range of 3-4 m/sec Considering tip speed to be 3 m/sec.

  Tip speed = $\pi * Da * N = 3$ m/sec

  Therefore,

  Impeller speed, N = 84 rpm

- Power Requirement

  Power is delivered to the fluid through two mechanisms: mechanical powei from the impeller and power generated by gas expansion. Which is greatei depends primarily on whether the gas flow or the impeller controls flow in the vessel.

Un-gassed power, $P_o = N_p * N_i * N^3 * \rho * D_a^5$

Where,

N_p is impeller power number

N_i is No. of Impellers

N is Impeller Speed, m/sec

D_a is Impeller Diameter, m

Power number is a function of impeller geometry, tank geometry, and impeller Reynolds number.

$$N_{Re} = \frac{\rho * N * Da^2}{\mu} = 6.9 * E05$$

Where,

μ is the broth viscosity (= 1 cp)

ρ is the broth density (= 1050 Kg/m^3)

From N_{Re} vs N_p graph for propeller without baffles and of pitch 1:1 (Figure 18-17 [46]) Power number is determined as 0.9

Therfore,

Ungassed power, P_o = 0.7 KW = 1 hp

Ratio of gassed power to ungassed power depends on aeration number o flow number [48].

Flow number for propellers, Nq = 0.5

The ratio of gassed power to ungassed power for flow number 0.5 is

$$\frac{P_g}{P_o} = 0.45$$

Therefore, Gassed power, P_g = 0.45 hp

- Heat Load

  Total heat generated during growth, Q_{tot}, is approximately equal to the sum o metabolic heat generated, and the heat generated by the agitation required t provide adequate oxygen transfer and mixing [49].

  $Q_{tot} = 0.45 * OTR * V/Y_{x/O2} + 2545\ P_g$

  Q_{tot} = 0.017*E06 BTU/hr

Operating temperature of the bioreactor, To = 37 °C

Cooling water In, T_{ci} = 25 °C

Assumed approach temperature, T_a = 4 °C

Cooling water out, T_{co} = 33 °C

ΔT_c = 8 °C

Log Mean Temperature Difference is defined as

$$LMTD = \frac{(T_{h,out} - t_{c,in}) - (T_{h,in} - t_{c,out})}{\ln\left(\frac{T_{h,out} - t_{c,in}}{T_{h,in} - t_{c,out}}\right)}$$

Where,

$T_{h,in}$ is hot fluid inlet Temperature

$T_{h,out}$ is hot fluid outlet Temperature

$t_{c,in}$ is cold fluid inlet Temperature

$t_{c,out}$ is cold fluid outlet Temperature

c_P is specific heat of water (= 1 BTU/lb.°F)

Therefore, LMTD = 7 °C

Generally, Overall heat transfer coefficient for fermentation broth is taken as 15(BTU/hr.ft^2.°F

Overall heat transfer rate, Q = U*A*LMTD

Therefore, required heat transfer area, A = 0.24 m^2

Required Cooling water flow rate, $m_c = Q_{tot}/c_P * \Delta T_c$ = 166 Kg/hr

5.1.2 Control Strategy

The principal bioreactor control requirement is population growth of the microorganism of interest. The role of the control strategy is to provide, by control of environmental effectors such as temperature, aeration, pH and dissolved oxygen, the optimum conditions for growth and colonization. Putting al the components together for the bioreactor Figure 10 shows the Schematics of a bioreactor.

5.1.2.1 Sensors

The problem with monitoring the bioreactor system is maintaining the sterile integrity of the bioreactor hence any sensor has to be capable o sterilization either in situ during steam sterilization or remotely and ther aseptically introduced to the bioreactor. Typical sensors that are placed or bioreactors for monitoring and control are:

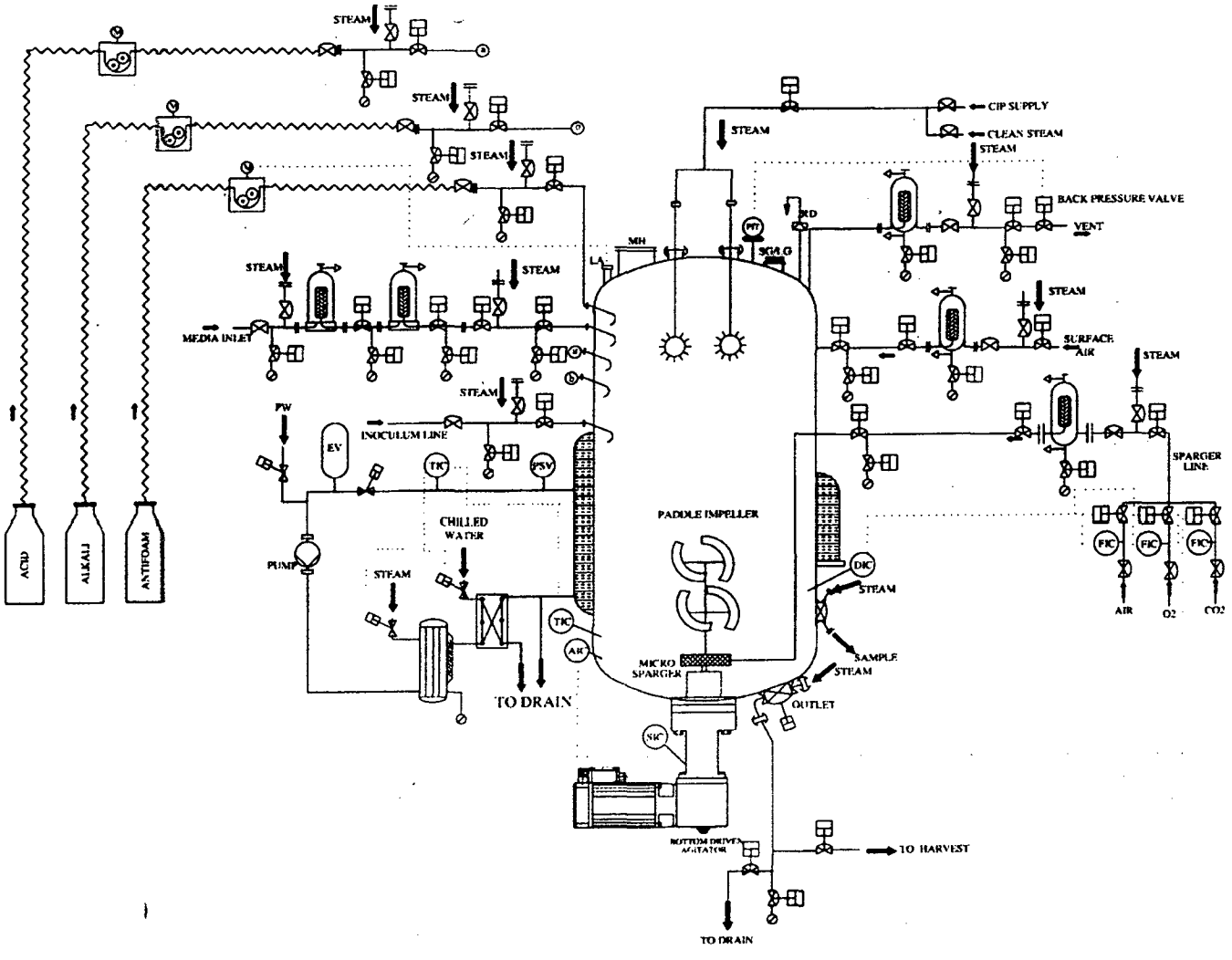


Figure 10: Schematics of a Bioreactor.

- **Temperature:** measured using a platinum resistance thermometer where the increase in temperature is proportional to increase in electrical resistance in the probe.
- **Air Flow rate:** measured using a standard pressure drop device such as variable area flow meters or thermal mass flow controllers.
- **Vessel pressure:** measured using diaphragm protected bourdon gauges or strain gauge pressure transducers.
- **pH:** Measured using steam sterilizable combined glass electrodes.
- **Dissolved oxygen:** measured using polarographic type probes. here galvanic voltages on membrane-covered oxygen reducing cathode induce a current proportional to the amount of oxygen diffusing through the membrane.
- **Foam:** detected by conductance or capacitance probes completing an electric circuit when foam is contacted.

5.1.2.2 Control Loops

5.1.2.2.1 Temperature Control Loop

Temperature needs to be controlled within narrow limits generally of 37 °C for mammalian cells. Regulation of the temperature should be within 0.5 °C of the set point. Mammalian cells can not withstand temperature above 40 °C Temperature will be controlled by introducing cooling water into jacket and circulating the water in a closed loop using a pump. There will be two heat exchangers one Plate type for cooling and one Shell and Tube for heating. If temperature decreases plant steam will be introduced to respective heat exchanger and if temperature increases steam is shut off and chilled water is supplied to cooling heat exchanger. The temperature controller is implemented as a cascade control loop with a master controller for the vessel temperature and a slave controller for the jacket temperature. So there should be a primary temperature controller which measures the vessel temperature and gives set point to secondary temperature controller in the jacket this will in turn control by controlling chilled water and steam inlet valves (Figure 11).

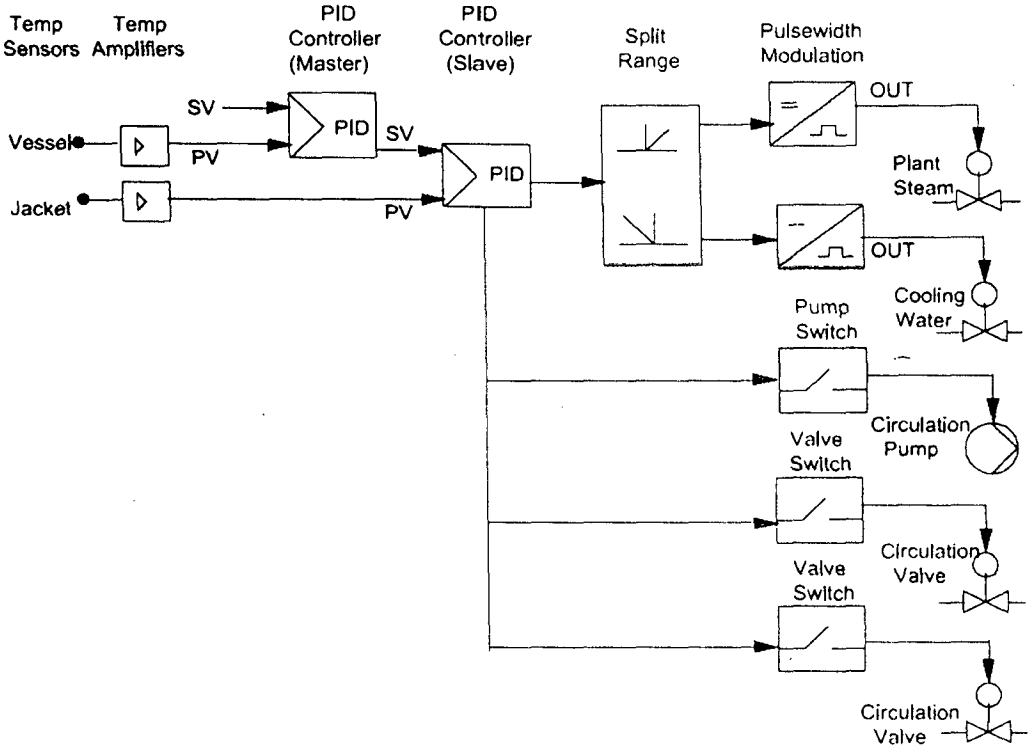


Figure 11: Temperature Control Loop

5.1.2.2.2 pH Control Loop

The pH is one of the most important parameters that affect the well-bein of cultured cells, and its control needs to be closely attended. In most o traditional cell culture media, it is controlled by bicarbonate-carbon dioxide buffe system. Bicarbonate ions and carbon dioxide are also used by cells fo biosynthesis to some extent. Carbonic acid equilibrates with carbon dioxide i both the liquid and the gaseous phases, and it is necessary to maintain a increased concentration of carbon dioxide in the gaseous phase to maintain the pH in appropriate range (Figure 12).

Two separate mechanisms can be used to control the pH in a bioreactor:

- pH can be controlled by controlling the acid , alkali addition pump flov rates with Pulse width modulation of On/Off of these pumps within a tim interval which helps in stabilization of pH by allowing time to mix th acid or alkali in the reactor.

- pH for mammalian cell culture is generally controlled by controlling the Carbon dioxide flowrate into the bioreactor.

The pH Control Loop is built from a PID controller with split range output:

– If Output is 50% i.e. neutral position, both actuators will be OFF.

– If Output is 50% to 100%, then it will operate the base pump for increasing the pH value

– If Output 50% to 0% will operate the CO2 mass flow controller or acid pump for decreasing the pH value.

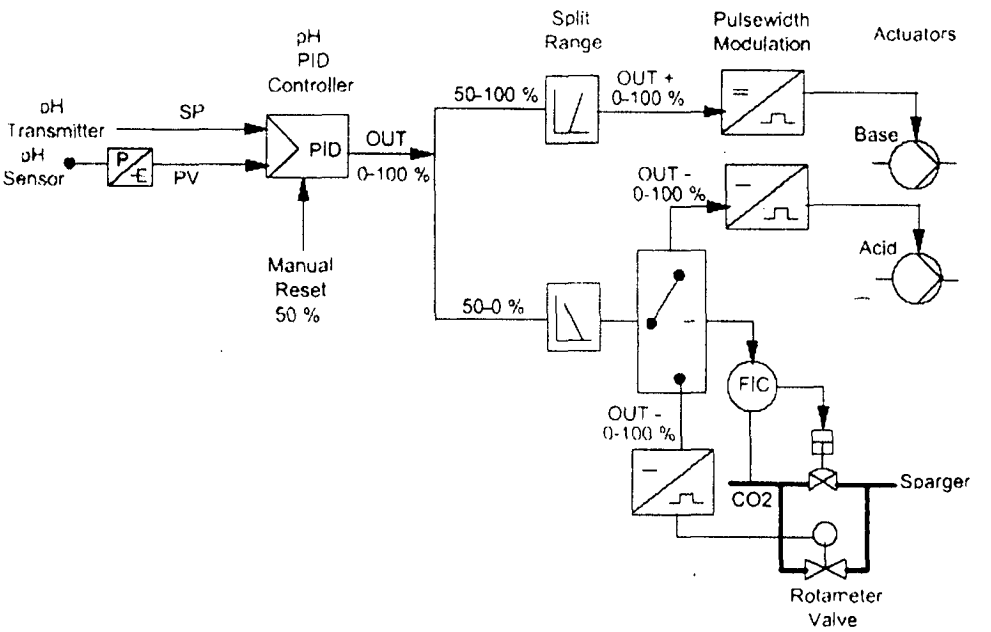


Figure 12: pH Control Loop

5.1.2.2.3 Pressure Control Loop

Pressure control loop is used to maintain the head pressure in the bioreactor. This can be achieved by using a back pressure control valve in the exhaust line. Percentage of opening of this valve will depend on the pressure inside the vessel measured by pressure indicator and transmitter mounted on the vessel top (Figure 13).

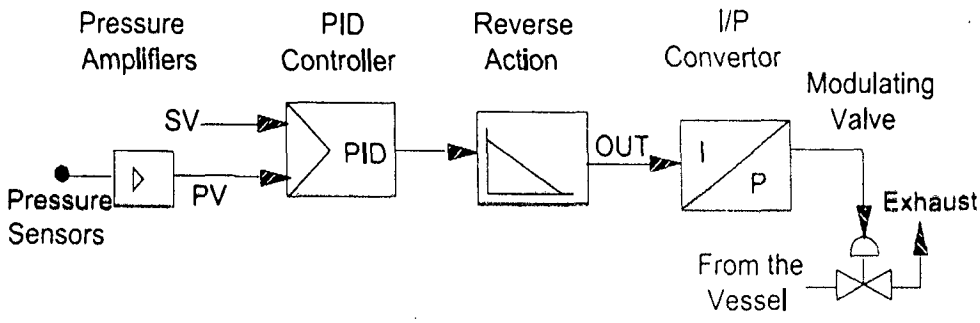


Figure 13: Pressure Control Loop

5.1.2.2.4 Agitator Control Loop

The agitator control loop adjusts the speed of the agitator motor via variable frequency drive. The agitator controller is a set point controller wit continuous output, which is the set point signal for the external VFD (Figure 14).

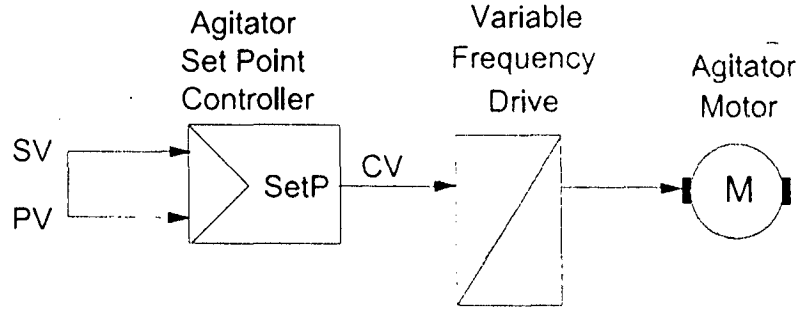


Figure 14: Agitator Control Loop

5.1.2.2.5 Dissolved Oxygen Control Loop

Dissolved oxygen control is achieved by either increasing the air flow rat or by introducing fresh oxygen into the reactor. The control strategy is DO_2 senso gives an input to the total mass flow controller which is combination of both ai and oxygen mass flow controllers. First of all air flow rate will be increased if w are unable to achieve the set point then oxygen flow rate is increased. The DO control loop maintains the DO_2 in the culture broth by modulating oxygen flo through the sparger. The DO_2 Control Loop which operates O_2 mass flo controllers is part of a Total Gas flow Control Strategy with a separate Total flo Controller which adds air to sparger aeration (Figure 15).

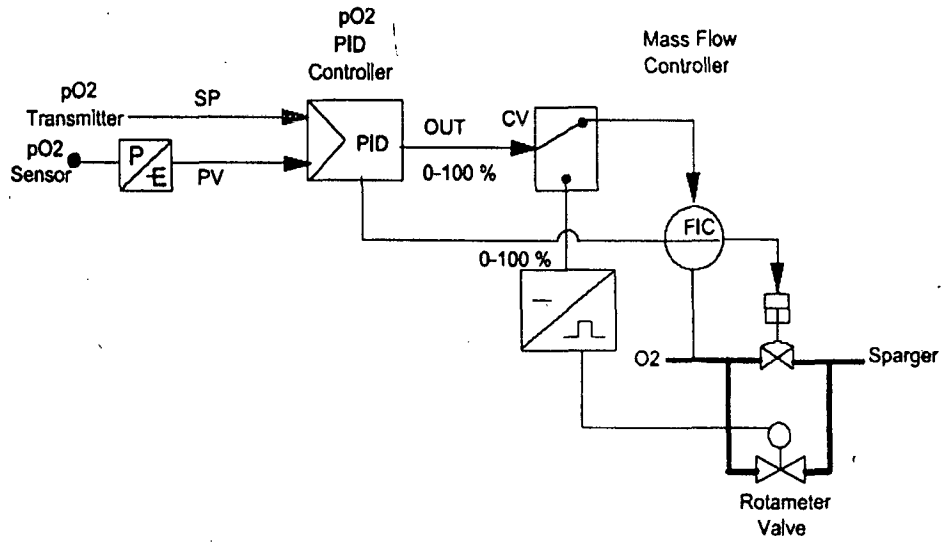


Figure 15: Dissolve Oxygen Control Loop

5.2 Design of a Continuous Centrifuge

Micro-organisms and other similar sized particles can be removed from a broth by using a centrifuge when filtration is not a satisfactory separation method. Although a centrifuge may be expensive when compared with a filter it may be essential when filtration is slow and difficult and continuous separation to a high standard of hygiene is required.

Various issues considered for selecting a type of centrifuge are: feed solids content, biomass particle size, nature or texture of solids, product quality desired, and operating environment [50]. In this case a disk stack centrifuge is selected on the basis of % solids, supernatant, solids type, and solid liquid boundary. Although their installation and operating costs are higher than those of other types of centrifuges available, the Disk stack centrifuges are often preferred because they give a better quality effluent.

According to Stoke's law, the rate of sedimentation of spherical particles suspended in a fluid of Newtonian viscosity characteristics is proportional to the square of the diameter of the particles, thus the rate of sedimentation of a particle under gravitational force is:

Rate of sedimentation or Settling velocity, $v_g = \frac{d_p^2(\rho_p - \rho)g}{18\mu}$ .....(1)

Where:

μ is viscosity of liquid (kg/m/s)

d_p is particle diameter (m)

v_g is settling velocity (m/s)

ρ_p is particle density (kg/m^3)

ρ is liquid density (kg/m^3)

This equation is modified for sedimentation in a centrifuge as:

$$v_c = \frac{d_p \varpi^2 r(\rho_p - \rho)}{18\mu} \quad \text{.....(2)}$$

Where,

ω is angular velocity of the rotor (s^{-1})

r is radial position of the particle (m)

Dividing equation (2) by (1) yields

$$G = \frac{\varpi^2 r}{g}$$

Where,

G or RCF is Relative Centrifugal Force

RCF is a measure of the separating power of a centrifuge compared t gravity settling. G value for a disk stack centrifuge is 4000-13,000. The disks ar generally spaced about 0.5-2 mm apart.

Considering, d_p = 12 μm (Mammalian cells diameter), μ = 0.001 kg/m/s, ρ_p = 1060 kg/m^3, and ρ = 1000 kg/m^3

Therefore, Sedimentation Rate, v_g = 4.7E-06 m/s

In the processing scheme, the centrifuge will be continuously fed with 2500-L solution of mammalian cells over 2.5 hours; hence centrifuge to b selected should have a throughput of 16.7 L/min.

The most used quantity to characterize centrifuge is sigma "Σ" concept. It is the calculated equivalent surface area of a static settling tank with the same theoretical performance.

For Disk Stack centrifuges, Σ factor is

$$\Sigma = \frac{2}{3} \cdot \frac{\pi \varpi^2}{g} \cdot n \cdot \left(r_2^3 - r_1^3\right) \cdot \cot \alpha$$

where,

r_2 is maximum radius of disk

r_1 is minimum radius of disk

n is number of disk

α is half cone angle of the disk

Feed flow rate, $Q = v_g * \Sigma = 16.7$ L/min

Therefore, $\Sigma = 59.2\ m^2 (= 0.1 * 10^{-4}\ ft^2)$

From Table 18-13 [46], the smallest sigma factor nearest to calculated value is $1.1 * 10^{-4}\ ft^2$ for this speed of a disk stack centrifuge is 10,000 rpm with as disk Diameter of 4.1" and number of disks = 33.

For a speed of around 10,000 rpm from Table 18-12 [46] bowl diameter of 7" is selected. Therefore, required G-force = 9938. This is a satisfactory design for an industrial disk stack centrifuge as it lies in the specified range.

5.3 TFF System Design

As the process is carried out in batches, a batch TFF system is selected. With batch systems, the process flux is initially high and decreases as the retained species are concentrated. Batch systems provide the most efficient separation, while requiring minimum membrane area, being simpler and less expensive. This system requires a feed tank, pump, pressure gauges and filter. The tank serves as a reservoir for the solution being processed; as the material is filtered, the volume in the tank is reduced. The pump provides both pressure to push the fluid through the membrane and the fluid velocity to keep the retained components from

settling on and plugging the membrane. The pressure gauges are used fc monitoring the recirculation rate (Figure 16).

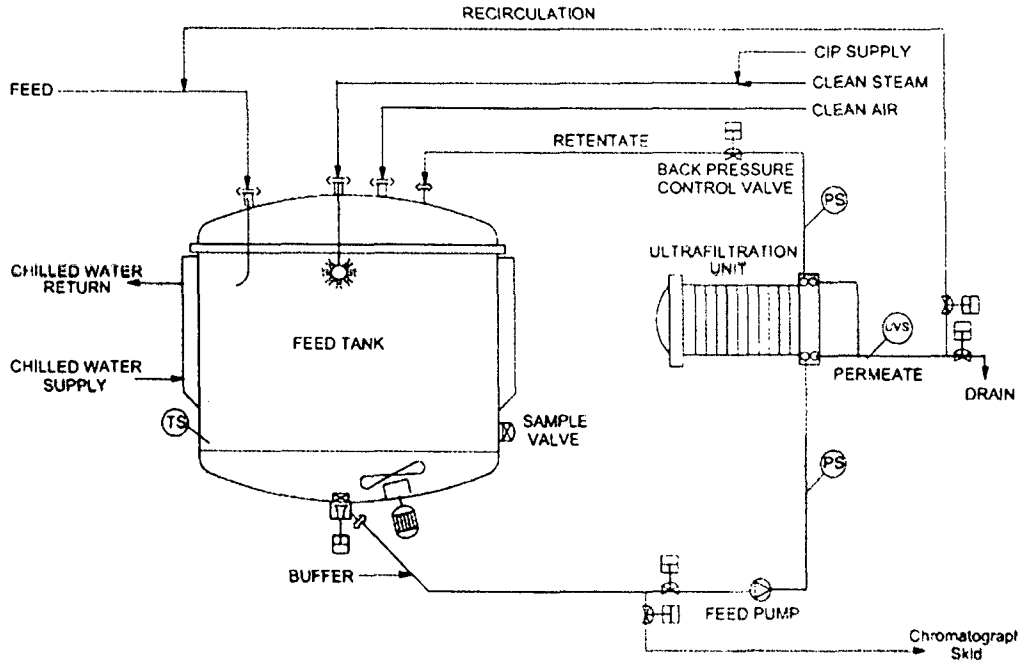


Figure 16: Schematics of Crossflow filtration system with Feed Tank.

The concentration and diafiltration of the clarified product after chromatography step also involve the handling of small volumes of expensiv product. The membranes used in these applications need to be highly retentive c the product, and must allow for the exchange of appropriate buffer solutions. Th membrane cutoff employed is generally one-third to one-fifth of the molecul weight cutoff of the target protein. For concentration and diafiltration tran membrane pressure is kept at 20 - 40 psi (1.4 – 2.8 bar) with flux in the range c 10-100 L/h.m^2 (Table 4.2 [44]). Also for cleaning of cassettes we require aroun 300-400 L/hr per cassette of cleaning solution.

5.3.1 Ultrafiltration (UF) System Design

i. **UF-1**: In UF-1 there is a 20 fold concentration of the product from 2250 to 113 L. Processing conditions for proteins require a temperature of 8-10 °C

and TMP of 20-40 psi. Protein molecular weight is 150000 Dalton, we use a membrane of 5000 Dalton molecular weight cutoff.

Process Parameters:

- Concentration: Initial = 1g/L, final = 18 g/L
- Process Temperature: 8 °C
- Process Time: 8 Hr
- Flux: 20 L/h.m^2 (generally in the range of 10-100 L/h.m^2 for mammalian cell culture protein for concentration)

Design Calculations

a) Membrane area required to concentrate batch for 8 hrs:

- Design Rate = (initial vol. – final vol.)/process time = 267 L/h
- Membrane area = design rate/flux = 13.4 m^2

  The device comes in 0.685 m^2 increments, so we use 20 cassettes, of 13.7 m^2.
- Actual rate = Flux *area = 274 L/h

b) Pump Requirements: Assuming a maximum of 400 L/hr per cassette flow rate requirement for cleaning of cassettes. Therefore, for 20 cassettes required pump capacity is 400 L/hr per cassette * 20 Cassettes = 8000 l/hr (= 8 m^3/h). Maximum pressure which membranes can withstand is 40 psi (≈ 3 bar). Therefore, presuming a worst case scenario a pump of capacity 8 m^3/h at 4 barg pressure is selected. From pump performance curve maximum speed of the pump comes out to be 780 rpm and maximum motor power is 3 KW (≈ 4 hp).

c) According to the heat exchange rule of thumb [44], 1 ft^2 (≈ 0.1 m^2) of heat exchange area is required per motor hp, so the approximate heat exchange area is 0.4 m^2.

Design Considerations

a) **Pump Selection:** A rotary lope pump or peristaltic pump is the best choice for this application because of low internal pump volume and low flow

rate. Rotary lobe pump is preferred because it offers maximum mechanic integrity. Centrifugal pump is not selected because of the flood volum and high speed operation they entail.

b) **System tankage and piping:** For the 8 m^3/hr (≈ 35 gpm) flow rate achieve suitable cleaning velocity requires 1.5" sanitary piping.

ii. UF-2: In UF-2 there is a 10 fold concentration of the product from 729.6 to 73 L. Processing conditions for proteins require a temperature of 8-10 °C and TMP of 20-40 psi. Protein molecular weight is 150000 Dalton, we use membrane of 30,000 Dalton molecular weight cutoff which is 1/5rd of t molecular weight cutoff of the product of interest.

Process Parameters

- Concentration: Initial = 2g/L, final = 19 g/L
- Process Temperature: 8 °C
- Process Time: 5 Hr
- Flux: 20 L/h.m^2

Design Calculations

a) Membrane area required to concentrate batch for 5 hrs:
   - Design Rate = (initial vol. – final vol.)/process time = 131.3 L/h
   - Membrane area = design rate/flux = 6.6 m^2

     The device comes in 0.79 m^2 increments, so we use 10 cassettes, of m^2.
   - Actual rate = Flux *area = 158 L/h

b) Pump Requirements: Assuming a maximum of 400 L/hr per cassette fl rate requirement for cleaning of cassettes. Therefore, for 10 casset required pump capacity is 400 L/hr per cassette * 10 Cassettes = 4000 L (= 4 m^3/h). Maximum pressure which membranes can withstand is 40 (≈ 3 bar). Therefore, presuming a worst case scenario a pump of capac 4m^3/h at 4 bar pressure is selected. From pump performance cu

maximum speed of the pump comes out to be 550 rpm and maximum motor power is 2.3 KW (≈ 3 hp).

c) According to the heat exchange rule of thumb, 1 ft^2 (≈ 0.1 m^2) of heat exchange area is required per motor hp, so the approximate heat exchange area is 0.3 m^2.

Design Considerations

a) **Pump Selection:** A rotary lope pump or peristaltic pump is the best choice for this application.

b) **System tankage and piping:** For the 4 m^3/hr (≈ 17.6 gpm) flow rate to achieve suitable cleaning velocity requires 1.5" sanitary piping.

iii. UF-3: In UF-3 there is a 4 fold concentration of the product from 218.7 L to 55 L. Processing conditions for proteins require a temperature of 8-10 °C and TMP of 20-40 psi. Protein molecular weight is 150000 Dalton, we use a membrane of 30,000 Dalton molecular weight cutoff which is 1/5rd of the molecular weight cutoff of the product of interest.

Process Parameters

- Concentration: Initial = 6g/L, final = 22 g/L
- Process Temperature: 8 °C
- Process Time: 5 Hr
- Flux: 20 L/h.m^2

Design Calculations

a) Membrane area required to concentrate batch for 8 hrs:
   - Design Rate = (initial vol. – final vol.)/process time = 32.7 L/h
   - Membrane area = design rate/flux = 1.6 m^2

     The device comes in 0.53 m^2 increments, so we use 3 cassettes, of 1.6 m^2.

b) Pump Requirements: Assuming a maximum of 400 L/hr per cassette flow rate requirement for cleaning of cassettes. Therefore, for 3 cassettes

required pump capacity is 400 L/hr per cassette * 20 Cassettes = 1200 l/hr (= 1.2 m^3/h). Maximum pressure which membranes can withstand is 40 psi (≈ 3 bar). Therefore, presuming a worst case scenario a pump of capacity 4 m^3/h at 4 bar pressure is selected. From pump performance curve maximum speed of the pump comes out to be 350 rpm and maximum motor power is 1.5 KW (≈ 2hp).

c) According to the heat exchange rule of thumb, 1 ft^2 (≈ 0.1 m^2) of heat exchange area is required per motor hp, so the approximate heat exchange area is 0.2 m^2.

Design Considerations

a) **Pump Selection:** A rotary lope pump or peristaltic pump is the best choice for this application.

b) **System tankage and piping:** For the 4 m^3/hr (≈ 17.6 gpm) flow rate to achieve suitable cleaning velocity requires 1.5" sanitary piping.

5.3.2 Control Loop Strategy

a. Temperature control: The temperature can be maintained by using chilled water in the jacket of the UF feed tank. Temperature sensor mounted on the vessel control the actuated valve in the chilled water supply or return line.

b. Feed Pressure Control: 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 DCS. If the Feed Line pressure controller is switched off, separate discrete output signal will be activated which stops the Feed pump via the start/stop input of the VFD (Figure 17).

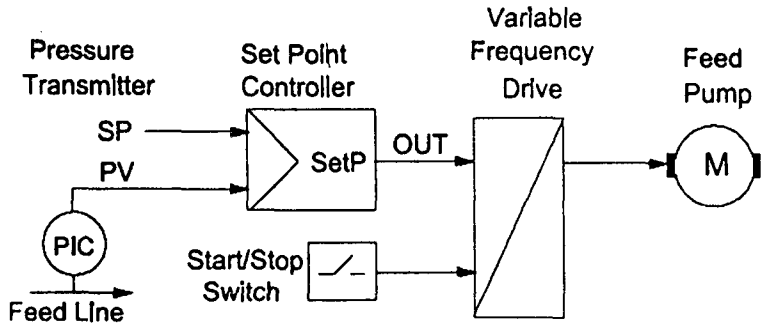


Figure 17: Feed Pressure Control Loop

c. Retentate pressure control

The Retentate Line Pressure Control Loop maintains the pressure via proportional control valve in the Retentate Line. The Retentate Pressure Control Loop is implemented as a PID Controller with Continuous output, which is the control signal for the external proportional control valve. The measured value output of the Retentate line pressure Transmitter is indicated as Process value for the DCS (Figure 18).

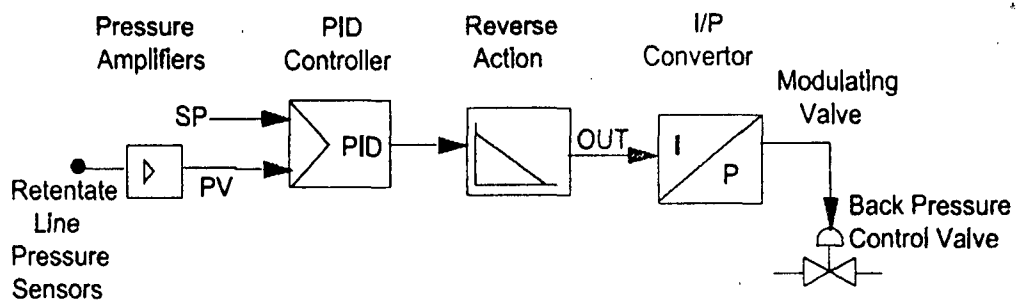


Figure 18: Retentate Pressure Control Loop

5.4 Chromatography Design

For larger-scale chromatography processes, purification becomes more challenging. Yet, the introduction of additional purification steps, such as membrane separations, often translates into higher product losses and lower overall product yield. Through the careful selection of the medium and buffer for individual chromatography steps, and the employment of these steps in the proper sequence, one can eliminate or reduce certain membrane separation stages Chromatography is one of the few techniques that can achieve both the high

resolution and the high capacity needed to generate large quantities of highly purified product. Furthermore, it is relatively easy to increase the scale o operation economically over several orders of magnitude, and to ensure tha extraneous substances are not introduced into the process stream. Othe techniques, such as aqueous two phase partitioning and electrophoretic separatio methods, do not have all of these attributes and consequently these techniques ar not in widespread use for the purification of therapeutic-grade proteins. I general, a relatively small number of chromatographic steps, each utilizing different aspect of the product's chemistry (such as molecular charge, size, c hydrophobicity) can be sufficient to meet the purity required for pharmaceutica applications in place of using a single high resolution chromatography.

5.4.1 Chromatography Columns Design

The one requirement that a chromatographic process must meet, regardles of scale, is achieving a uniform flow distribution across the cross-sectional area c the column, from the inlet to the outlet ports, with minimal mixing and dilution i the flow-distribution chambers at either end of the column. The column itse must be able to withstand the operating pressure without flexing. The end plate need to be carefully designed and constructed to produce uniform laminar flo across the entire width of the gel bed. The volume of the flow distributio chamber within the end plate should be as small as possible in order to reduc zone spreading. Where low pressures are used (≈ 3 bar), transparent borosilicat glass columns have many advantages. For low-pressure chromatography, glass c acrylic columns are frequently specified. In high pressure applications, column are generally constructed of 316L stainless steel. In most cases, Type 316 stainless steel, glass (borosilicate), and certain polymers and elastomers (such a EPDM or Kalrez) are used to ensure good material compatibility and stability However, buffers containing chloride ions may not be compatible with stainles steels. Metallic components that are in contact with buffers containing high level of chloride ions may be made of Hastelloy C or titanium.

Typical process design procedure for chromatography is as following:

1. Choose the best selective resins from batch binding experiments under pre-decided conditions.
2. Determine isotherms/equilibrium capacities under chosen conditions.
3. Perform breakthrough experiments with pure solute and mixture at different velocities. Estimate HETP vs velocity relation.
4. Elution at different conditions (isocratic, step gradient and linear gradient) and determine elution plate height and resolution parameters.
5. From 3 and 4 establish optimum operating conditions, so that the adsorbent is optimally used, and the elution resolves the solutes.
6. Determine the effect of scale-up on performance indicators of column e.g dynamic capacities and resolution.

5.4.2 Pumps

Pumps selected for feeding the column are diaphragm pumps. The key requirements are: the set flow rate should be maintained irrespective of back pressure and the pulsations should be minimized, to avoid disturbance of the chromatography bed. Assuming a maximum linear velocity of 3 cm/min we can find out the delivery rate of the pump (Table 15).

Linear flow rate, $u = \frac{F_{in}}{6A} = \frac{2 \cdot F_{in}}{3 \cdot d^2 \cdot \pi}$

Where,

F_{in} = Delivery of the pump, in m^3/min

A = Base area of the column in m^2

D = Internal diameter of the column, in m.

Table 15: Pump Delivery Rate of all the Columns

Column		Col-1	Col-2	Col-3
Diameter	m	0.446	0.446	0.5
Linear Flow Rate	m/min	0.03	0.03	0.03
Pump Delivery Rate	m^3/min	0.0281	0.0281	0.0353
	m^3/hr	1.6864	1.6864	2.1195

5.4.3 Automated Chromatography Systems

The simplest chromatographic system consists of a pump, a column, detector, fraction collector, and interconnecting tubing. The additiona components incorporated into production scale systems are used to ensure that ai does not get into the system (air sensors and bubble traps), that it operates in a sterile a manner as possible (in line filters). Further components are then added t control the purification process and to improve the convenience of operatio (controller, automatic valves, CIP facilities etc.). An air sensor is frequently use on the sample inlet line so as to allow loading of the entire sample volume ont the column. Only after the air sensor has detected air in the sample inlet line, th next step in the purification cycle is initiated. A 0.2 micron sterile filter is ofte incorporated into the system before the column. If the sample itself is prefiltere off-line, this filter may be bypassed during the loading operation and used only t filter the buffer solutions. A bubble trap located immediately prior to the colum serves not only to eliminate any air that may have entered the system, but als serves as a pulse damper, which may be necessary, depending on the pump desig (Figure 19).

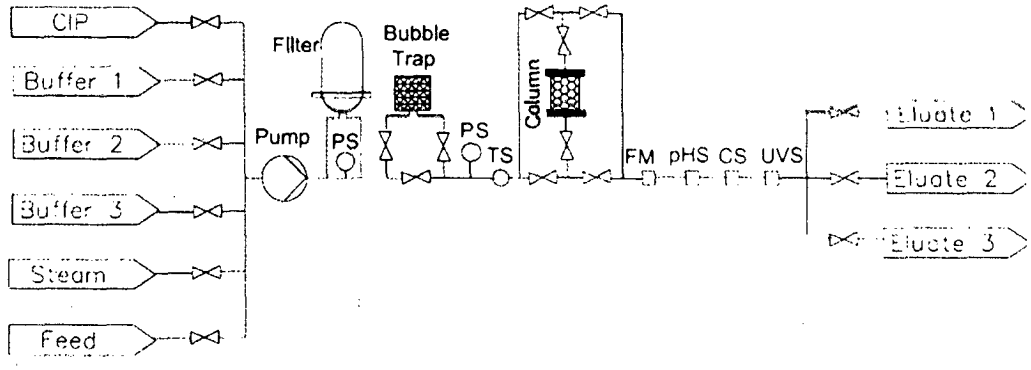


Figure 19: Schematics of Chromatography System.

5.5 Nano Filter Design

In downstream processing, viruses are de-activated by using a buffe solution followed by nanofiltration for virus removal using hydrophilic membran pleated filters rated at 0.04 μm or less is applicable to clarified mammalian cel culture harvested fluids. The recommended position in the purification scheme i

immediately after initial clarification and 0.2 or 0.1μm filtration, or after subsequent concentration by ultrafiltration. Performing virus reduction filtration when protein concentration of the process feed is relatively high reduces loss of product by adsorption on the viral reduction membrane. Microporous membrane cartridge filtration is preferred as it freely passes proteins while providing a sieving mechanism for virus removal. Therefore, a hydrophilic membrane of absolute rating 0.04 μm is selected for viral reduction and is placed after DF-1. In harvest line a 0.5 μm absolute-rated filter for secondary cell and cell debris removal from harvested fluid and 0.2μm sterilizing grade filters are also selected as they act as prefilters to 0.04 μm filters.

Typical water flow rates per 10" module is 1-2 gpm [44]. Amount of feed passing through the filter is 728 L. Assuming a processing time of 1 hr. in a depth filter thus, the feed flow rate is 12.1 L/min (≈ 3 gpm). Therefore, for 3 gpm flow rate we require a filter module of 30".

5.6 Vessel Design

Here we have considered the design of the vessel which is integrated with the first ultrafiltration system. As shown in the mass balance calculation the final concentrated product quantity in the vessel integrated to UF-1 is 113 L, therefore a tank of working volume 120 L is selected for retentate collection. Figure 20 shows the dimensional parameters of a Vessel.

Considering H:D =1.5:1, and torispherical head

Total volume of Reactor = Cylindrical volume + 2 * head volume.

$$V = ((\pi/4)*D_i^2* H) + 2 * 0.0809*D_i^3$$ (for torispherical head Table 10-65 [46])

$$D_i = 472 \text{ mm}$$

Tan to Tan height of the vessel H= 1.5 * 472 = 708 mm.

Dish Volume (DV) = $0.0809 * D^3 = 0.0085 \text{ m}^3$

Working Level Height, $h = [(WV-DV) * 4] / (\pi * D^2) = 0.638$ m

Crown Radius, R_C= fD (where f >0.5)

Knuckle Radius R_K= kD (where 0<k<0.5)

Considering depth for 6% Torispherical Dish, k = 0.06 and f = 1.

Crown Radius = 0.472 m

Knuckle Radius = 0.028 m

Depth of Dish excluding thickness, h_d

$$h_d = R_C - SQRT\{[(R_C - R_K)\ ^2]-[(R_C/2) - R_K]^2\} = 0.08\ m$$

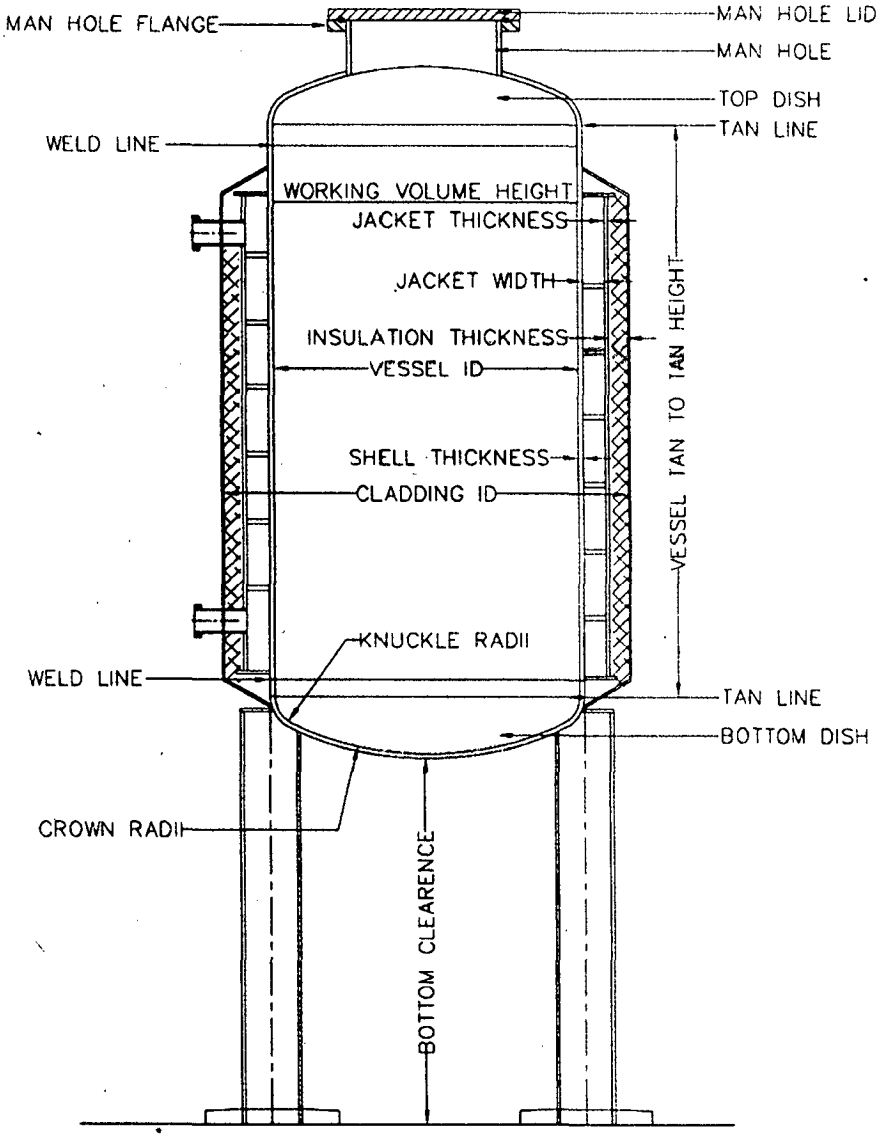


Figure 20: Dimensional Parameters of a Vessel

5.6.1 Vessel Shell Thickness Design

Design parameters (Input parameters)

- Shell inner operating pressure = 3.1 Bar (= 310 KPa)
- Shell operating temperature = 126 °C
- Shell Internal design pressure, P_i = 4.1 Bar (= 410 KPa)
- Shell External design pressure, P_o = 9.6 Bar (= 960 KPa)
- Shell design temperature = 150 °C
- Weld Joint Efficiency, E = 0.85
- Max. Allowable Stress for SS316 L, S = 115000 KPa
- Shell inner radius, R = 0.503/2 = 0.251 m

Output Parameters

For cylindrical shell under circumferential stress thickness of shell is calculated by following equation [47].

a. Thickness for Internal Pressure

Thickness, t = P*R/ (S*E-0.6*P)

Where,

t = Thickness of vessel shell (mm)

P = Internal Design Pressure (KPa)

R = Radius of the shell (mm)

S = Maximum allowable circumferential stress of SS316L Materia

E = Weld joint efficiency for shell seam joint

Therefore, inner shell thickness = 0.992 mm

b. Thickness for External Pressure

For cylindrical shells and tubes (seamless or with longitudinal butt joints)

Distance from top tan line to jacket closure, Dl = 0.0 mm

Total length, L = H – Dl = 708 mm

Assumed initial thickness, t = 7 mm

Hence shell outer diameter, $D_o = D_i + (2*t)$ = 486 mm

D_o/t = 69.4

$L/D_o = 1.46$

Factor A = 0.0016 (From Figure. H3-A [47])

Factor B = 55 (at 150 °C from Figure. H3-4 [47])

Maximum allowable external working pressure, P_a

$$P_a = 4*B/(3*(D_o/t)) = 1.056 \text{ MPa}$$

As maximum allowable external working pressure is greater than the external design pressure therefore, thickness assumed is safe to use.

Vessel shell thickness for external pressure = 7 mm

Hence, Vessel shell thickness provided = 8 mm

5.6.2 Vessel Dish Thickness Design

a. Vessel dish thickness for Internal Pressure

For top dish weld joint efficiency is taken as 1 and Shell External design pressure, P_o as 110 KPa

Hence, Thickness, $t = P_i*R/(S*E-0.1*P_i) = 0.841$ mm

b. Vessel dish thickness for 1.67 times of External Pressure

For 1.67 times the external design pressure, $P_{ii} = 1.67 * P_o = 183.7$ KPa

Hence, Thickness, $t = P_{ii}*R/(S*E-0.1*P_{ii}) = 0.377$ mm

c. Thickness for External Pressure Assumed initial thickness, t = 8 mm

Hence outer crown radius, $R_o = R + t = 488$ mm

$R_o/t = 60$

Factor $A = 0.125 / (R_o / t) = 0.0021$

Factor B = 57

Maximum allowable external working pressure, $P_a = B/(R_o/t) = 0.95$ MPa

As maximum allowable external working pressure is greater than the external design pressure therefore, thickness assumed is safe to use.

Hence, Vessel shell thickness provided = 8 mm

5.6.3 Vessel jacket shell Thickness Design

Design parameters (Input parameters)

- Jacket inner operating pressure = 8.6 Bar (= 860 KPa)

- Jacket operating temperature = 150 °C
- Jacket Internal design pressure, P_i = 9.6 Bar (= 960 KPa)
- Jacket External design pressure, P_o = 1.1 Bar (= 110 KPa)
- Jacket design temperature = 150 °C
- Weld Joint Efficiency, E = 0.85
- Max. Allowable Stress for SS316 L, S = 115000 KPa
- Max. Allowable Stress for SS304 = 115000 KPa
- Jacket spacing = 19 mm
- Vessel shell thickness = 8 mm

Calculations (Output Parameters)

a. Vessel jacket thickness for Internal Pressure

Jacket inner Radius = 251+19+8 = 263 mm

Hence, Thickness, $t = P_i*R/ (S*E-0.6*P_i)$ = 2.60 mm

b. Thickness for External Pressure

For cylindrical shells and tubes (seamless or with longitudinal butt joints)

Distance from top tan line to jacket closure, Dl = 0.0 mm

Total length, L = H – Dl = 708 mm

Assumed initial thickness, t = 3 mm

Hence shell outer diameter, D_o = 263*2 + (2*3) = 532 mm

D_o/t = 177.3

L/ D_o = 1.33

Factor A = 0.00038

Factor B = 33

Maximum allowable external working pressure, P_a

$P_a = 4*B/ (3*(D_o/t))$ = 0.248 MPa

As maximum allowable external working pressure is greater than the external design pressure therefore, thickness assumed is safe to use.

Vessel shell thickness for external pressure = 3 mm

Hence, Vessel shell thickness provided = 3 mm

5.7 CIP System

A mobile CIP System is selected for cleaning of various pieces of proces: equipment, including tanks of various sizes, as well as associated piping. A typical CIP system comprises of a tank with level probes and water inlet, a centrifugal pump for distributing the cleaning reagents around a CIP feed anc returns, a heat exchanger for heating the reagents and CIP additives are injecte by diaphragm metering pumps. The operation of the CIP system requires th control of several conditions, i.e., the fluid flow rates and velocities, temperatures cleaning times and the concentrations of the cleaning chemicals (detergents caustic soda). The schematics of a CIP system are shown in the Figure 21.

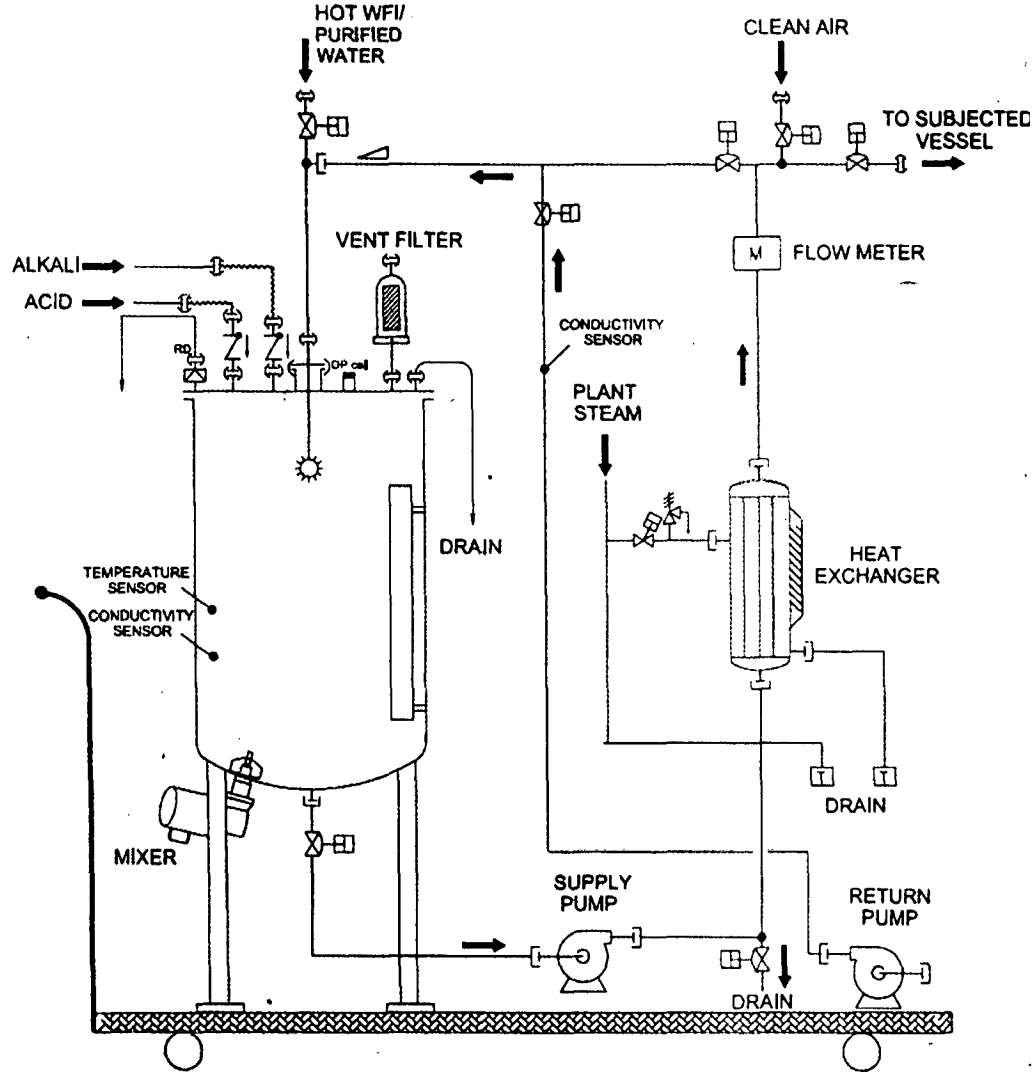


Figure 21: Schematics of a CIP System.

Figure 22 shows the typical CIP phases along with the purpose of each phase. Fluid velocities in the process piping are approximately 5 ft/s. In the case of tanks, flow rates of either water or cleaning solution are largely determined by the size of the tank, as well as the number and the properties of the spray devices. These flow rates usually range from 10 to 160 gallons per minute (~ 2–36 m^3/hr). The temperature of the CIP process may vary from 135 to 175°F (~ 60 to 80°C) and control is usually critical. The necessary heat transfer demands are met either by incorporating heat exchangers into the CIP system, or by direct injection of steam. Concentrations of the cleaning solutions are monitored and controlled by the measurement of the pH or electrical conductivity. The CIP process involves a sequence of cycles that includes an initial and final drain step, a pre-rinse, wash and post-rinse. The duration of the rinse and wash cycles vary from 5 minutes to 1 hour. An integrated design will clean the process tanks and transfer lines as one circuit, potentially reducing the number of cleaning circuits by a factor of two.

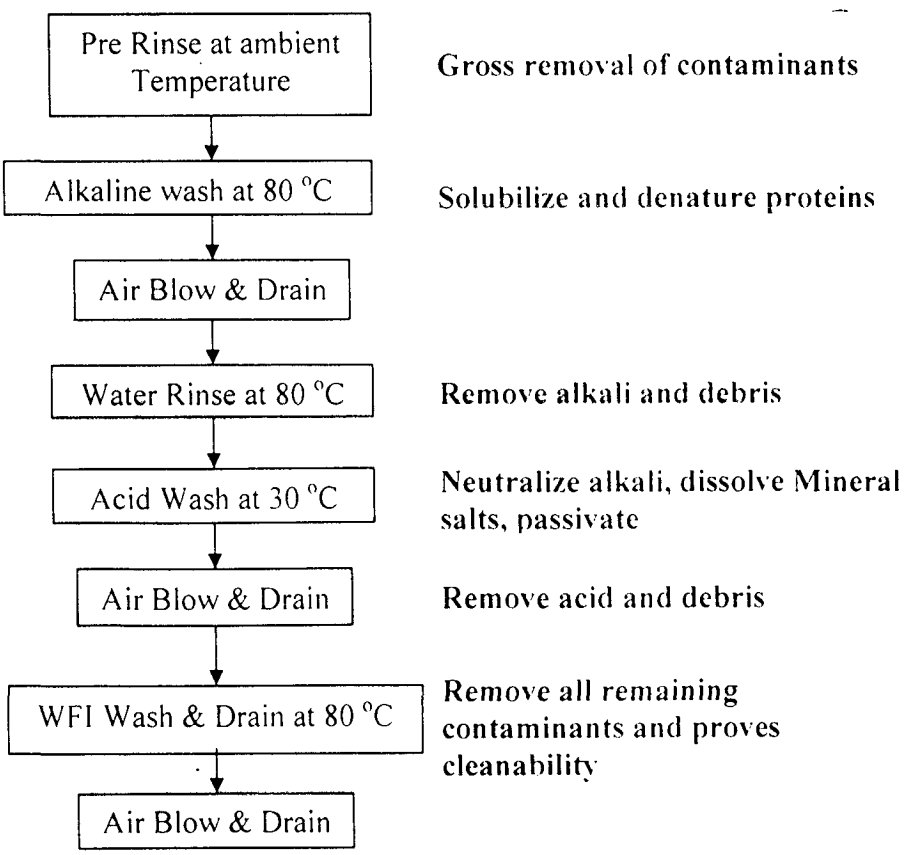


Figure 22: Typical CIP Phases.

5.7.1 Cleaning Cycle Strategy

The four most significant factors that affect the efficiency of the cleaning process are cleaning solution temperature, cleaning agent concentration, cleaning solution contact time, and the external energy put into the cleaning solution in the form of velocity and pressure, resulting in momentum and turbulence. The effective specification and control of all these factors results in efficacious, repeatable, and reliable cleaning. The highest concentration of cleaner should be used with the worst-case residues. Offline analysis of the process residues helps in determining the wash chemical concentrations. Cleaning-agent concentrations usually fall within these ranges (measured as supply conductivity) [51]:

- Alkaline cleaning agents: 20 to 25 mS/cm (1500-10,000 ppm) for bioreactors and harvest systems (highest concentration of residues), 10 to 12 mS/cm (500-3000 ppm) for media preparation (moderate concentrations of residues) and 8 to 10 mS/cm for purification systems (lower concentrations of residues)
- Acid cleaning agents: 4 to 6 mS/cm
- Buffer vessels and lines are often only water rinsed.

Alkaline wash temperatures are typically 60 to 70°C. However, if silicon based antifoam agents are present, alkaline wash temperatures are 70 to 80°C. Acid wash temperatures are typically 25 to 35°C as this is adequate for neutralization and de-mineralization, the purpose of the acid wash. A temperature just above ambient gives little cleaning benefit but helps ensure better temperature control. The alkaline wash should remove most product residues in 5 to 15 min (3 to 10 iterations through all sub-circuits). Difficult residue areas may receive 15 to 25 min of alkaline exposure. Easier residue areas may receive 5 to 10 min of alkaline exposure. Acid washes should remove alkaline and mineral residues in to 10 min (recirculate 4 to 6 circuit volumes).

Spray devices are used for distribution of cleaning solutions over equipment surfaces. Generally, fixed spray devices are preferred over rotating spray devices. Fixed spray balls are used for cleaning vertical cylindrical vessels with domed head. Generally, more than one spray ball is placed in the vessel c

capacity more than 1000 Liters. Cleaning solution flow rate requirement for th subjected vessel is 2.5 GPLF (GPM per Linear Foot of Vessel Circumference) Therefore, for a 2500 L bioreactor two spray balls are placed in the vessel hea with a cleaning solution of 35.1 GPM (the circumference of a 1363 diamete vessel is 4279.8 mm (~14.04 ft)).

Typical acceptance criteria for a clean vessel are:

a. **Visual:** no residue present, no pooling or standing WFI and vessel i visibly clean and dry.

b. **Rinse Water Samples:** Rinse Water Conductivity is USP Limit (Stage or Stage 2), Rinse Water TOC is <1.0 ppm, Bioburden is < 10 CFU/100mL t 100 CFU/100mL and Endotoxin level is <0.25 EU/mL.

c. **Surface Samples:** Surface Swabs <1.0 ppm TOC to 10 ppm TOC in 4 mL Diluent.

5.7.2 CIP SKID DESIGN

5.7.2.1 Vessel Design

For cleaning the equipments and their auxiliary lines a movable CIP ski is chosen. The volume of CIP solution required for cleaning a vessel is about 1/3 of the vessel volume. Maximum volume in the whole manufacturing train is of th production bioreactor (2500 L). Therefore, a CIP tank volume required is aroun 833 L. As a movable CIP skid is selected we have space constraint, therefore, 250 L CIP tank will suffice the purpose by filling it four times to meet th cleaning requirements of the bioreactor.

Considering working volume of CIP tank as 83% of the geometric volum therefore, geometric volume of the tank is 300 L.

Considering H:D ratio =1.5:1, with flat head and torispherical bottom.

Total volume of Reactor = Cylindrical volume + dish volume.

$$GV = [(\pi/4)*D_i^2* H] + 0.0809*D_i^3$$

$$D_i^3 = GV/\{(0.0809) + [(\pi/4) * (H/D \text{ ratio})]\}$$

$$D_i = 620 \text{ mm}$$

Tan to Tan height of the vessel H = 1.5 * 620 = 930 mm.

Dish Volume (DV) = $0.0809 * D^3 = 0.019\ m^3$

Working Level Height, h = $[(WV-DV) * 4] / (\pi * D^2) = 0.765$ m

Crown Radius, R_C= fD (where f >0.5)

Knuckle Radius R_K= kD (where 0<k<0.5)

Considering depth for 6% Torispherical Dish, k = 0.06 and f = 1.

Crown Radius = 0.62 m

Knuckle Radius = 0.0372 m

Depth of Bottom Dish excluding thickness, h_D

$$h_D = R_C - SQRT\{[(R_C - R_K)\ ^2]-[(R_C/2) - R_K]^2\} = 0.105\ m$$

Design parameters (Input parameters)

Shell inner operating pressure = 1.5 Bar (= 150 KPa)

Shell operating temperature = 80 °C

Shell Internal design pressure, P_i = 2.5 Bar (= 250 KPa)

Shell External design pressure, P_o = 1.1 Bar (= 110 KPa)

Shell design temperature = 100 °C

Weld Joint Efficiency, E = 0.85

Max. Allowable Stress for SS316 L, S = 115000 KPa

Shell inner radius, R = 0.62/2 = 0.31 m

Output Parameters

For cylindrical shell under circumferential stress thickness of shell is calculated by following equation [47].

- **Vessel Shell Thickness Design**

  a. Thickness for Internal Pressure

  Thickness, t = P*R/ (S*E-0.6*P)

Where,

t = Thickness of vessel shell (mm)

P = Internal Design Pressure (KPa)

R = Radius of the shell (mm)

S = Maximum allowable circumferential stress of SS316L Material

E = Weld joint efficiency for shell seam joint

Therefore, inner shell thickness = 0.794 mm

b. Thickness for External Pressure

For cylindrical shells and tubes (seamless or with longitudinal butt joints)

Distance from top tan line to jacket closure, Dl = 0.0 mm

Total length, L = H – Dl = 930 mm

Assumed initial thickness, t = 9 mm

Hence shell outer diameter, $D_o = D_i + (2*t) = 638$ mm

$D_o/t = 71$

$L/ D_o = 1.46$

Factor A = 0.0015

Factor B = 60

Maximum allowable external working pressure, $P_a = 4*B/ (3*(D_o/t))$

= 1.128 MPa

As maximum allowable external working pressure is greater than the external design pressure therefore, thickness assumed is safe to use.

Vessel shell thickness for external pressure = 9 mm

Hence, Vessel shell thickness provided = 9 mm

- **Vessel Bottom Dish Thickness Design**

a. Vessel dish thickness for Internal Pressure

For bottom dish weld joint efficiency is taken as 1 and Shell External design pressure, P_o as 110 KPa

Hence, Thickness, $t = P_i*R/ (S*E-0.1*P_i) = 1.106$ mm

b. Vessel dish thickness for 1.67 times of External Pressure

For 1.67 times the external design pressure, $P_{ii} = 1.67 * P_o = 183.7$ KPa

Hence, Thickness, $t = P_{ii}*R/ (S*E-0.1*P_{ii}) = 0.495$ mm

c. Thickness for External Pressure

Assumed initial thickness, t = 8 mm

Hence outer crown radius, $R_o = Rc + t = 628$ mm

$R_o/t = 79.5$

Factor A = $0.125 / (R_o / t) = 0.0016$

Factor B = 56

Maximum allowable external working pressure, $P_a = B/ (R_o/t) = 0.71$ MPa

As maximum allowable external working pressure is greater than the external design pressure therefore, thickness assumed is safe to use.

Hence, Vessel dish thickness provided = 8 mm

5.7.2.2 Heat Exchanger Design for CIP System

A shell and tube heat exchanger is designed for heating 4000 Kg/h (m^3/hr) water coming from CIP tank at a temperature of 25 °C to 85 °C using pla steam at 143.6 °C.

Given process conditions are

$T_{h,in}$ is Steam inlet Temperature = 143.6 °C (= 290.5 °F)

$T_{h,out}$ is Steam outlet Temperature =143.6 °C (= 290.5 °F)

$T_{c,in}$ is Water inlet Temperature = 25 °C (= 77 °F)

$t_{c,out}$ is Water outlet Temperature = 85 °C (= 185 °F)

Mass Flow rate of water, w = 4000 Kg/hr (= 8820 lb/hr)

Average temperature of water at which all the properties are calculated is t_{avg} = 5 °C (= 131 °F)

Water Specific heat, c_P (at 55 °C) = 1 Btu/lb °F (= 1 cal/g °C)

a. Heat Balance

For water, amount of heat transfer, $Q = w*c_P*\Delta t_c$ = 952560 Btu/hr (= 2 KW)

From Table-7 [24], Latent Heat of Steam, L_{fg} = 916 Btu/lb (= 2130 KJ/Kg)

For steam, amount of heat transfer, $Q = w * L_{fg}$ = 952560 Btu/hr

Therefore, mass flow rate of steam = 1039.9 lb/hr (= 471.7 Kg/hr)

b. True temperature difference, Δt

Log Mean Temperature Difference (LMTD)

$$R = \frac{T_{h,in} - T_{h,out}}{t_{c,out} - t_{c,in}} = 0$$

$$S = \frac{t_{c,out} - t_{c,in}}{T_{h,in} - t_{c,in}}$$

As R = 0 therefore, there is a true countercurrent flow (i.e. Temperature difference factor, $F_T = 1$)

$$LMTD = \frac{(T_{h,out} - t_{c,in}) - (T_{h,in} - t_{c,out})}{\ln\left(\frac{T_{h,out} - t_{c,in}}{T_{h,in} - t_{c,out}}\right)} = 153\ ^{\circ}F\ (= 67.2\ ^{\circ}C)$$

$\Delta t = LMTD * F_T = 153\ ^{\circ}F\ (= 67.2\ ^{\circ}C)$

c. Caloric Temperature

As the viscosity of both water and steam is less than 1 cP, therefore in place of caloric temperature for evaluating physical properties we use average temperature.

i) With the aid of Table 8 [52] which specifies the Dirty Overall Heat Transfer Coefficient for Steam-Water system between 200-700 Btu/ft^2.hr.$^{\circ}$F, we assumed a value of U_D = 350 Btu/ft^2.hr.$^{\circ}$F. Also for this system a dirt factor of 0.0005 is specified in Table 11-3 [46].

ii) Shell and Tube dimensions

As the flow rate of water is larger than steam, water is placed in the tube side and steam in the shell side of the heat exchanger.

Tube side

Length of tubes, L = 3 ft (This much small length is taken as we have space constraints for movable CIP system)

OD, BWG, ID, pitch = ¾", 10 BWG, 0.482",

P_T = 1" triangular pitch

Passes = 2

Shell side

Baffle spacing, B = 2 in

Baffle cut, C' = 25 %

Passes = 1

Heat transfer area, $A = \frac{Q}{U_D \cdot \Delta t} = 17.79\ ft^2$

From table 10 for ¾" OD tubes surface area per linear ft, a" = 0.1963 ft^2

Therefore, number of tubes = $\frac{A}{L * a''} = 30$

From Table 9 [52] for ¾" OD tubes on 1-in trangular pitch for 2 passes number of tubes, $N_t = 30$ in a 8" shell internal diameter, are selected.

iii) Corrected coefficient, U_D

New heat transfer area, $A = N_t * L * a" = 17.67\ ft^2$

Therefore, $U_D = \frac{Q}{A \cdot \Delta t} = 352.34$ Btu/hr.ft^2.°F

Cold fluid: tube side, water

d. Flow area per tube (Table 10, [52]), $a'_t = 0.182\ in^2$

Flow area, $a_t = \frac{N_t \cdot a'_t}{144 \cdot n} = 0.02\ ft^2$

e. Mass velocity, $G_t = \frac{w}{a_t} = 441000$ lb/hr.ft^2

f. Tube side Reynolds number, $Re_t = \frac{D \cdot G_t}{\mu}$

Tube internal diameter, Di = 0.04 ft

Viscosity of water (at 131 °F, from Fig.14 [52]), μ = 0.5315 cp (= 1.3 lb/ft.hr)

$Re_t = 13466$

Velocity through tubes, $V = \frac{G_t}{3600 \cdot \rho} = 2$ ft/s

From Fig. 25 [52] for V = 2 ft/s, tube side heat transfer coefficient, $h_i = 68$ Btu/hr.ft^2. °F. From Fig. 25 the correction factor corresponding to the inside diameter of the tube is 1.4.

Therefore, $h_i = 680 * 1.4 = 986$ Btu/hr.ft^2.°F

Heat transfer coefficient (based on outside), $h_{io} = \frac{h_i \cdot D_i}{D_o} = 634$ Btu/hr.ft^2.°F

g. **Pressure Drop, ΔP_T**

From Fig. 26 [52] for Re_t = 13466, tube side friction factor, f = 0.00025 ft^2/in^2

$$\Delta P_t = \frac{f \cdot G_t^2 \cdot L \cdot n}{5.22 * 10^{10} \cdot D_i \cdot s \cdot \phi_t}$$

Where, s is Fluid Specific gravity

Viscosity correction factor, $\phi_t = \left(\frac{\mu}{\mu}\right)^{0.14}$ is unity for less viscous fluids.

Therefore, ΔP_t = 0.14 psi (= 0.009 Bar)

Return Pressure Loss, $\Delta P_r = \frac{4 \cdot n}{s} \cdot \frac{V^2}{2g'}$

From Fig. 27 [24] One velocity head at s=1 (water),

$$\frac{V^2}{2g'} = 0.025 \text{ psi } (= 0.002 \text{ Bar})$$

Therefore, Tube side return pressure loss, ΔP_r = 0.2 psi (= 0.01 Bar)

Total Pressure drop, $\Delta P_T = \Delta P_t + \Delta P_r$ = 0.34 psi (= 0.02 Bar)

This value is within the allowable pressure drop of 10 psi (= 0.69 Bar).

Hot fluid: shell side, steam

h. For shell without baffles Flow area, a_s

$$a_s = \frac{1}{144}\left(\frac{D_i \cdot C' \cdot B}{P_T}\right) = 0.028 \text{ ft}^2$$

i. Mass velocity, $G_s = \frac{w}{a_s} = 37139.7$ lb/hr.ft^2

j. Shell side Reynolds number, $Re_s = \frac{D_e \cdot G_s}{\mu}$

Equivalent diameter, $D_e = 4.a_s$ / (wetted perimeter)

$$D_e = \frac{4 \cdot a_s}{N_t \cdot \pi \cdot (D_i/12)} = 0.061 \text{ ft (from Fig. 28 for ¾" O.D. tube and 1" triangular pitch is 0.73 in)}$$

Viscosity of steam (at 290.5 °F, from Fig.15 [52]), μ = 0.016 and cp (= 0.039 lb/ft.hr). Therefore, Re_s = 700207

For Steam heat transfer coefficient is generally taken as, h = 1500 Btu/hr.ft^2 °F [52].

k. Pressure Drop, Δ P_s

Number of Crosses, (N+1) = 12*L/B = 18

From Fig. 29 [52] for shell side friction factor, f = 0.00095 ft^2/in^2

$$\Delta P_s = \frac{f \cdot G_s^2 \cdot D_S \cdot (N+1)}{5.22 * 10^{10} \cdot D_e \cdot s \cdot \phi_s}$$

Where, Viscosity correction factor, $\phi_s = \left(\frac{\mu}{\mu}\right)^{0.14}$ is unity.

Therefore, Δ P_s = 2.22 psi (= 0.15 Bar)

This value is within the allowable pressure drop of 10 psi (= 0.69 Bar).

l. Clean Overall Coefficient, $U_c = \frac{h_{io} \cdot h_0}{h_{io} + h_0}$ = 445.48 Btu/hr.ft^2. °F

m. Design overall coefficient U_D = 352.34 Btu/hr.ft^2. °F

n. Dirt Factor, $R_d = \frac{U_c - U_D}{U_c \cdot U_D}$ = 0.0006 hr.ft^2. °F/Btu

As $R_{d,calculated}$ is near to $R_{d,reqired}$ value and also shell and tube side pressu drop are within specified limit, the heat exchanger is satisfactory.

5.8 Sterilization of Process Equipment

The importance of proper sterilization of bioprocess equipment a solutions has long been known. In mammalian cell cultures, presence of even o contaminating micro-organism can be disastrous. At present, by far the m common technique for sterilization of large-scale process equipment is h sterilization using steam. Heat sterilization has to kill both live microorganis and their spores. Of the two, the spores have a thermal resistance to moist h several thousand times greater than that of live microorganisms.

The primary challenge with *Sterilization in place* (SIP) is that it must be designed into the equipment. For successful steam sterilizing of process equipment, the time and temperature of the sterilization process are very important. The usual temperature/time combination chosen for SIP is heating at 121°C for 15 min. In practice these values are taken as minimum and the time period of steaming is often extended to ensure a good margin of safety time may go upto 30 min. Steam supplied should be saturated at 1.5 barg. In practice this means that it is adequately trapped and drained.

For mammalian cell culture, the clean steam supplied and distributed along stainless steam pipe work. Plant steam is not used as it has many impurities, which may either contaminate the product or inhibit growth of the organism being cultured.

In order to design equipment for steam sterilization the following things are taken into consideration:

(a) All the parts of equipment should be able to withstand sterilizing temperature of upto 130°C.

(b) Welded connections are preferred as a joint is a potential weakness.

(c) Wherever joints are necessary, sanitary fittings must be used.

(d) Dead spaces, crevices must be avoided as much as possible.

(e) Sterile and nonsterile areas must be separated by more than one valve where ever possible.

(f) Only valves that are easy to clean, maintain, and sterilize can be used therefore, diaphragm valves are generally used for sterile service.

(g) Equipment must be designed in such a way that it can be sterilized in sections, and so that each part of the section has a unique direction of steaming.

(h) The steam must be dry and saturated and free from particles and gases.

(i) Steam should be introduced at highest point and condensate should be removed at lowest point.

(j) Lines must be designed for complete drainage, without pockets where condensate can accumulate.

5.8.1 Sterilization of Bioreactor

Taking into consideration the aforementioned points a strategy fo sterilization of bioreactor is developed. Inlet ports and filter should have provision of being sterilized separately and part of that can be sterilized alon with the vessel. Typical sterilization scheme for a reactor is shown in the Figur 23 A and B. Before starting sterilization the vessel jacket should be drained (if nc done the vessel takes longer time to heat up and may have cold spots). The vesse must be pressure tested before use, to detect minor leaks or pinholes. Sterilizatio is divided into various steps:

1. **Set up Equipment:** The bioreactor tank is set for sterilization by checking th availability of utilities (steam and compressed air).
2. **Condensate drain:** In this step steam is injected into the vessel through spra balls, all the drain valves are opened to remove the condensate collected at th vessel bottom.
3. **Heating:** In this step the vessel is heated till its temperature reaches aroun 100 °C.
4. **Evacuation:** In order to remove air pockets vent line valves are opened ar steam supply is shut off.
5. **Sterilization:** In this step vent valves are closed and steam is supplied to th vessel till vessel temperature reaches 121 °C. A stabilization time of 2 mins provided to stabilize the temperature. This temperature is maintained for 3 mins.
6. **Cooling and pressurization**: At the end of sterilization, all the drain valv should be closed. When the pressure inside the vessel has dropped to 1 bar sterile air at a pressure of 1 barg should be introduced through the air inl filter. This ensures that as the vessel cools it does not develop vacuum. It al allows a slow replacement of the condensing steam with sterile air at a high than ambient pressure as the vessel cools. In this way the risk of pc sterilization contamination is greatly reduced.

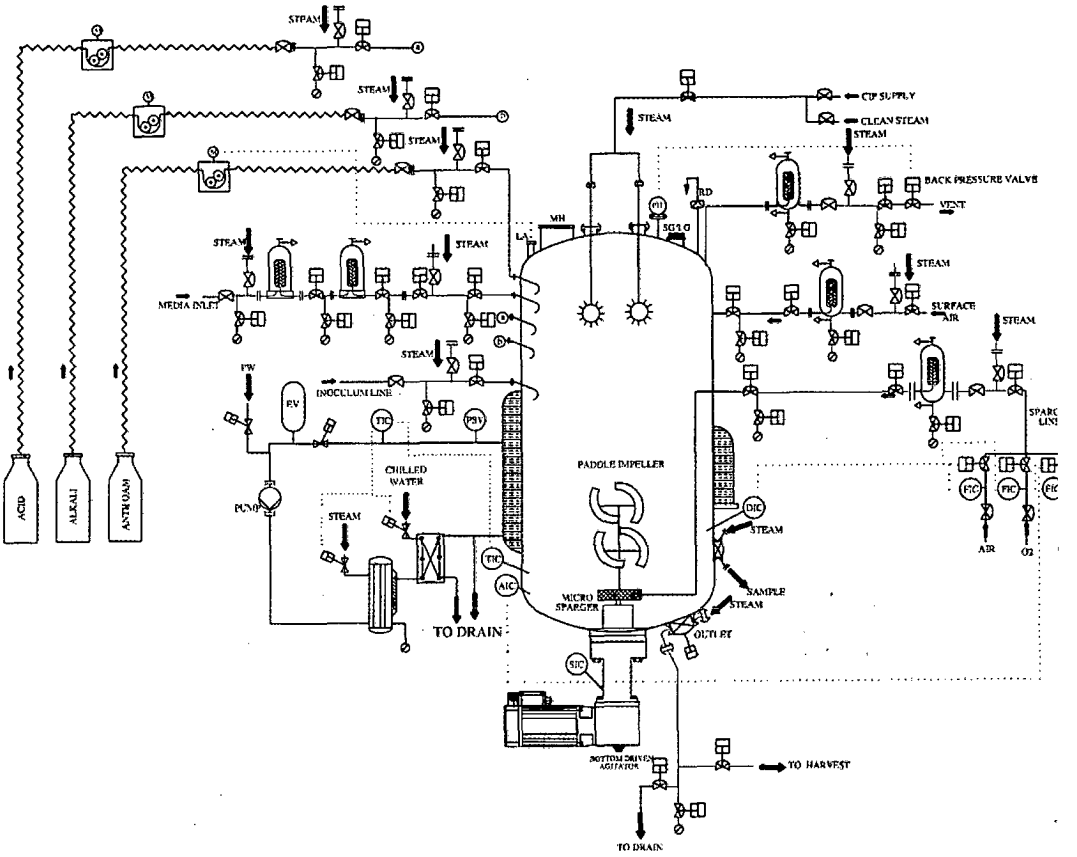


Figure 23 A: Sterilization Cycle of Transfer lines

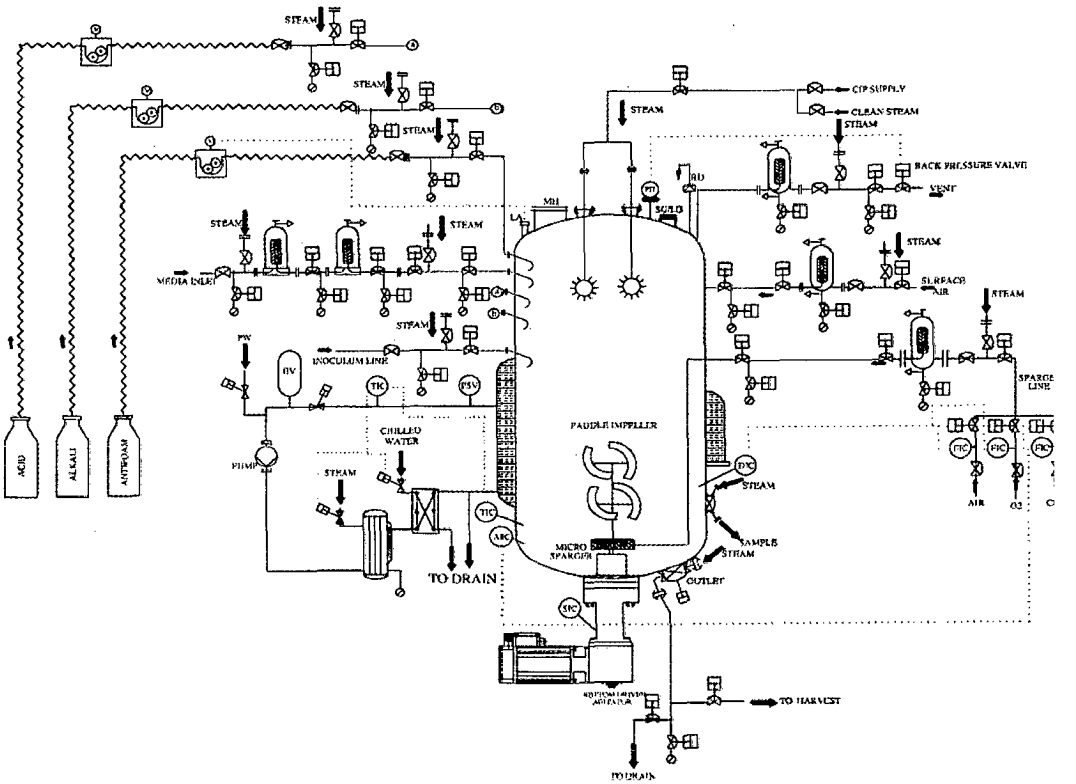


Figure 23 B: Sterilization Cycle of Vessel

Chapter VI

FACILITY DESIGN

6.1 Design Philosophy

The scope of a multipurpose development and bulk manufacturing facili should include: process development, preparation of material to meet researc needs, preparation of material to support phase I, II and III clinical trials, and tl preparation of material used to launch or support a licensed product. To me these needs the facility is divided into five general areas: laboratories to suppo scale-up, clinical and manufacturing efforts, utility areas for the production ar distribution of purified waters and other utilities, receiving and shipping areas warehouse raw materials and the finished product, and administrative offices. Tl sterile product manufacturing area is further classified into several areas: See preparation Area, Media and Buffer preparation area, Bioreactor area, Harve area, Downstream Processing area (V+ purification and V- Purification area) an Formulation area. The production suites, utility, and receiving/shipping area should be designed to comply with current FDA guidelines [53].

A significant portion of the expense in constructing and operating a GM facility is in providing the necessary validated utilities: deionized water, water fc injection, clean air, plant steam, chilled water and clean steam. These systen require both validation and routine monitoring of the fluid quality. An area withi the facility is designated for preparing the raw materials such as growth medi nutrients and buffer for use in processing. The preparation area require equipment for weighing and dispensing solid and liquid raw materials, meterin water and tanks for batching the combined raw materials. A battery of bioreactor ranging in size are provided in the bioreactor area where small ones can be ski mounted and large ones can be fixed. In Harvest area centrifuge and harvest tank can be accommodated. In purification areas skid mounted chromatography an ultrafiltration system can be placed along with the feed tanks. Transfer panels c nodes are well suited for providing the required flexibility: several transfer line can meet at a node, and jumper connections are made from one line to another. A a portable CIP system is used suitable utility stations are required to provide th utilities for the CIP system as well as to allow the connection of CIP supply an return lines to the processing equipment. Decontaminating small volumes of soli

and liquid waste can be handled manually in an autoclave whereas large quantiti of waste require a separate decontamination (i.e. kill tank type) system which confined to a specific location in the facility.

6.1.1 Premises

Areas that are related functionally should also be physically adjacent one another. Maintaining the required adjacencies improves the efficiency of t facility operation and reduces the amount of circulation space between relat areas. Therefore, the facility should be designed in such a way that the entry to processing areas should be through airlocks. For personnel, these airloc generally take the form of changing rooms that have a variable number interconnecting rooms, depending on the grade of area. Ideally, separate airloc should be provided for the entry of materials into the area. Airlocks should flushed with filtered air. Unnecessary entry to all processing areas should avoided. False ceilings should be sealed so that no dirt can fall from the vo above. This should permit access to light fittings from above allowi maintenance without stopping production. Sinks and drains should be avoided possible and must not be installed in aseptic areas. Drains should have cleanab traps and air breaks to prevent back flow.

6.1.2 Isolation of Clean Areas

Clean areas must be isolated with clean corridor and return corrid concept. With this concept bioreactor, cell culture, and purification areas a organized as separate suites, and may be accessed only through entry air loc from the clean corridor. Once having entered any suite from the clean corridor, a personnel and equipment are considered to be in contact with biologically acti material, and they must leave through an exit air lock leading to a return corrid Control may be established by a standard operating procedure to explain the rul governing access to and exit from biological suites, or control may be maintain by interlocks integrated with door hardware and card readers or other devic which permit access in only one direction. In order to avoid cross contaminatic

and maintain clean environment HVAC (Heating Ventilation and / Conditioning) system is employed. The close temperature tolerances used biologics processing require the use of specialty environmental clean rooms. / entering a production area is filtered using terminal HEPA filter mounted in near the ceiling and the air is exhausted from the room through ducts near t floor. Consequently, the rate of air flow into the room directly affects t cleanliness of the room. As the product proceeds from the fermentation throu purification, the environment should be made progressively cleaner to protect t integrity of the product. The clean areas are classified according to requir characteristics of air. Laminar air flow is used to obtain the requir characteristics. Entries to clean areas are through air locks for personnel and/ goods. The inoculum room is designed as Class 10,000 area accessed from white corridor. Bioreactor room is Class 100,000. Hoods throughout the facili are Class 100. Buffer preparation, glass washing, and support areas are Cla 100,000. A gray corridor is provided for visitor access and for exiting the Cla 10,000 areas. Both V+ and V- purification rooms are Class 1000. A small Cla 1000 filling suite is also included. For various classified rooms Table 16 show the Air Change rate and percentage ceiling coverage for HEPA filters. Exhaust a can be either vented to the atmosphere or partially recirculated. To avoid cros contamination, the air from different processing areas should not be mixe recirculated air should only be returned to the room from which it was exhausted

Table 16: HVAC - Classified Areas and Air Changes

Classified Areas	Class	Air Change Rate	% Ceiling Coverage for HEPA
Inoculum Prep.	10,000	50	10%
Laminar Hood	100	180	50%
Bioreactor	100,000	20	NA
Harvest	100,000	20	NA
V+ Purification	1000	90	25%
V- Purification	1000	90	25%
Media Prep.	100,000	20	NA
Buffer Prep.	100,000	20	NA
Bulk Filling	1000	90	25%

Rooms of higher classification should have a positive pressure differential relative to adjacent lower classified areas. Therefore, inoculum and bioreactor area is kept at negative air pressure levels; however purification area is maintained at positive pressures. Room pressure is maintained at 0.05 inch of water (~ 12.74 Pa) in order to minimize ingress of contamination when doors are opened. Clean rooms temperature is generally maintained at 23+/- 2 deg C. In controlled environment area, Relative Humidity should be maintained at 50 +/-5 % and in non controlled area it should be maintained at 70%.

6.1.3 Product flow

The shipping, receiving and warehouse areas of the facility need to be designed to comply with cGMP requirements. The primary concern of a biotechnology facility is integrity of the product, from raw material delivery to final manufactured product shipment. Well-defined flow paths enhance the ability to maintain the product integrity. Minimizing crossing and circuitous paths also reduces the potential of contamination and mix-up of materials and products. The flow of raw materials and product through the facility should follow the production process. Raw materials classified in several distinct categories exis within the facility at the same time. At the time of receiving, the raw material need to be kept separate from other materials and product. After testing the quality of the raw materials, the materials should be segregated according to whether they are released for manufacture or rejected. During processing operation, the product could be classified as suitable for or rejected from further processing. After the final processing step, the product is quarantined until it i verified that it is suitable for release or rejected.

6.1.4 Personnel flow

Personnel flow is unlikely parallel to product flow. Product flow is locate in the production area, with a prescribed path. Personnel flow occurs throughou the facility with differing intensities and paths. People cross the boundaries of th different areas, going from production to administration, to warehouse, t

research, and to other areas within the facility. There should be unidirectio personnel and product flow. The more contact personnel have with the prodı the greater the chance of contamination. If the circulation paths are separated a properly placed, the risk of product contamination is reduced. To maint cleanliness, the manufacturing staff is required to enter the control environment of the process areas through a plant locker and wash-up spa Personnel required changing into appropriate garments, and then they p through an air lock to access controlled clean areas such as the bioreactor, c culture, and purification areas. Clothing should be such that it should not cat any possible contamination of the product or containers.

6.1.5 Process Flow

Process flow begins when raw materials are released for productic Because the primary objective of the GMP is to guarantee the quality and pur of the end product, the process flow is critical. As much as possible the proce flow must be separated from the personnel flow and material flow. The prodı may go through four separate classifications during production: in proce released, rejected and quarantined. In process product is any product that currently in a production state. Released product is any product that h completed a production stage and has been checked and verified as acceptable f the next production stage. Rejected product is any product that at any time h been found unacceptable for further production. Quarantined product is produ that has completed the production process and awaiting final release f distribution. At all times during processing, measures should be taken to ensu that contamination of product, material and components, is minimized. Th processing of preparations containing live micro-organisms is not allowed in th same facility. During processing in sterile areas, it is important that the activitie taken place in these areas are less. The temperature and humidity in the area should be controlled to ensure that the integrity of materials is maintained. Th microbial contamination load or bio-burden for starting materials prior t sterilization should be kept to a minimum.

DCS DESIGN

7.1 Design Standards

S88, a standard addressing batch control, is well known in the proces automation world. It is a design philosophy for software, equipment, ar procedures. S88 provides a consistent set of standards and terminology for batc control. The first step of a process-automation is to define the requirement Typically, the main deliverable of this effort is a functional specification. I regulated industries, a written and approved functional specification is require for computer system validation. The S88 standard facilitates object oriente class-based designs. Class-based equipment and procedures can save time ar money because the software is reusable.

7.1.1 Functional Specification (FS)

A functional specification "defines what the system should do, and wh functions and facilities are to be provided. It provides a list of design objective for the system. According to GAMP, the functional specification is based on use requirement specification, which identifies the system requirements with regard t data, interfaces, environment and constraints. The functional specification define the process-automation requirements and becomes bases for design specification. Inputs to the FS include the process description, Piping and Instrumentatio Diagrams (P&IDs), Process Flow Diagrams (PFDs), and Instrument List.

7.1.2 S88 Models

The S88 standard provides models and terminology that can be used t specify automation requirements in a modular fashion. It classifies the system int two models: physical and procedural model (Figure 24).

a. Physical Model

The physical model defines the hierarchy of equipment used in the batcl processes. This model provides a means to organize and define the equipmen used to control the processes. The levels are:

- **Enterprise** defines the company that owns the facility (plant).

- **Site** defines the location of the facility. These first two levels provide link to business systems, regulatory compliance requirements and possil engineering standards.
- **Area** defines a specific section of the Site, such as a building name. T area may contain one or many Process cells.
- **Process Cell** contains all of the production and supporting equipme (Units, Equipment modules, and control modules). A process cell is t collection of all equipment that is required for the production of batch A process cell is made up of all of the units that operate on the batch.
- **Unit** is a major piece of equipment that performs a specific task within t batch process. A unit consists of equipment modules and control module
- **Equipment module** is a group of equipments that can carry out mir processing activities. Typically, an equipment Module consists of Cont Modules and does not directly interface to Plant I/O (input/output).
- **Control Module** is a single entity that performs state-oriented regulatory control. A control module directly interfaces to plant I/O.

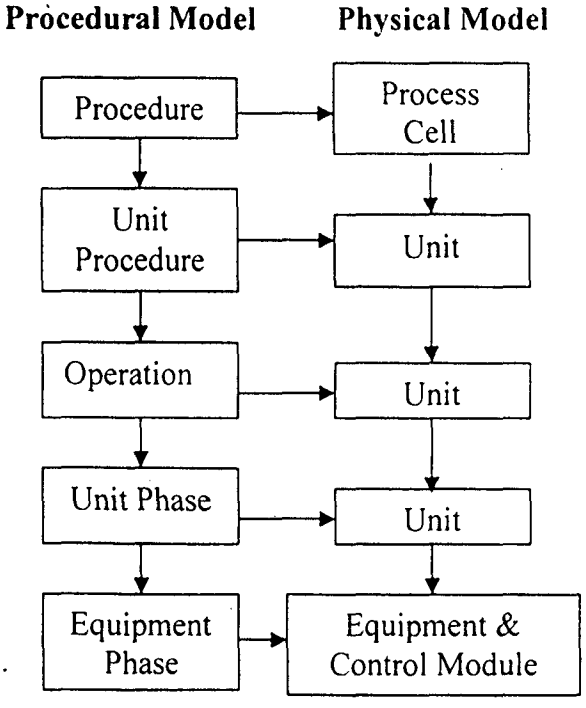


Figure 24: Hierarchy of Physical and Procedural Model

b. Procedural Model

The procedural model defines the control that enables the equipment in physical model to perform a process task. The levels are:

- **Procedure** is the sequence of Unit procedures required to make a batch orchestrates the control of equipments in the process cell.
- **Unit procedure** is a sequence of operations. It controls the function o single unit. A Unit may have more than one Unit procedure, but only o Unit procedure may control the unit at a time.
- **Operation** is a sequence of phases. Typically, an operation controls portion of the unit function.
- **Unit phase** performs a unique or independent process function on a Un It coordinates the control of Equipment Modules and control modules.
- **Equipment phase** performs a simple process function on an Equipme module. It coordinates the control of control modules.

7.1.1 States and Commands

Procedural states can either be transient or quiescent. Transient state typically contain a sequence of actions that must be completed in order to proceed to a corresponding quiescent state (e.g. Running and Complete). Procedura commands cause the sate of procedural element to change (e.g. Start or Stop). An operator or another procedural element can issue these commands. The procedura states can be defined as follows:

- Idle: Waits for a start command to transition to Running.
- Running: Begins when the start command is received. Normal operation actions are executed.
- Holding: Equipment is placed in Safe State. The Running State is disrupted and placed in Holding when an exception to normal Operation is detected or the operator issues the Hold command.
- Restarting: The restart command has been issued by the operator when the state is Held. Action is taken to return equipment to normal operation. Once the Restarting finishes, it transitions to running state.

- Complete: The Running State has completed.
- Stopping: Equipment is placed in a safe state. The current state disrupted when the operator issues the Stop command.
- Stopped: Stopping State has completed. At this point, the recipe can r be restarted.
- Aborting: Equipment is placed in safe state. Current state is disrupt when the operator issues the Abort command.

7.1.2 Application of S88 to the Process

All the above definitions and strategies are considered to design a cont system for monoclonal antibody manufacturing plant as per Good Automati Practices. First of all one should focus on individual units present in the facili Here we have considered "Bioreactor Area" in which one bioreactor system a as a Unit. Figure 25 shows the various sequences taking place on a bioreactor.

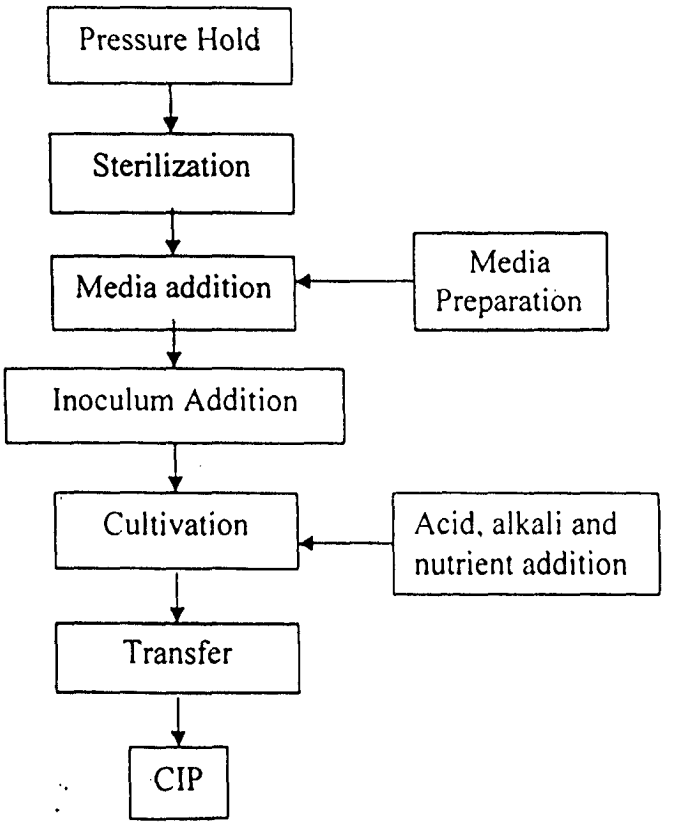


Figure 25: Hierarchy of sequences running on bioreactor.

All the sequences need to be automated completely, from medi preparation and pressure hold, media addition, cultivation, and CIP. Each of thes sequences represents Phase classes. For one bioreactor unit we may require mor than one phases running together. So combination of phases forms a Un operation. Then combination of these unit operations forms a Unit procedur Unit procedures are developed for each piece of equipment and then integrate into an over all production recipe.

7.1.2.1 Defining System Boundary, Control and Equipment Modules

Boundary of the bioreactor unit needs to be defined which includes a inlet line, Utility line, product transfer line valves present in the system. Th Bioreactor is used to cultivate microorganisms using media and required nutrier and transfer the product for further processing.

All the state-oriented (ON/OFF), regulatory control entities should b identified before defining the equipment modules. For bioreactor, the contrc modules includes all the individual valves, agitator motor, all the digital an analogy indicators such as temperature, pH, DO, pressure, Mass flow controllers.

The equipment module has multiple control sequences that can b commanded to accomplish specific tasks. For the bioreactor, the equipmen modules and control modules work together to perform a function. Boundaries ar drawn in Figure 26 to show system boundary, control modules and differen equipment modules. For example Exhaust Equipment Module comprises of all th valves, instruments including CIPS, Steam supply which are present in th exhaust lines are grouped into this equipment module. These entities are initiate according to the scheme. Similarly all the following equipment modules such a Sparger EM, Overlay EM, Spray ball EM, Addition EM, Media Filter EM, an Jacket EM are designed. Once these equipment modules are identified, phas classes are designed which runs on the particular unit to perform the given task This needs a Class based Approach.

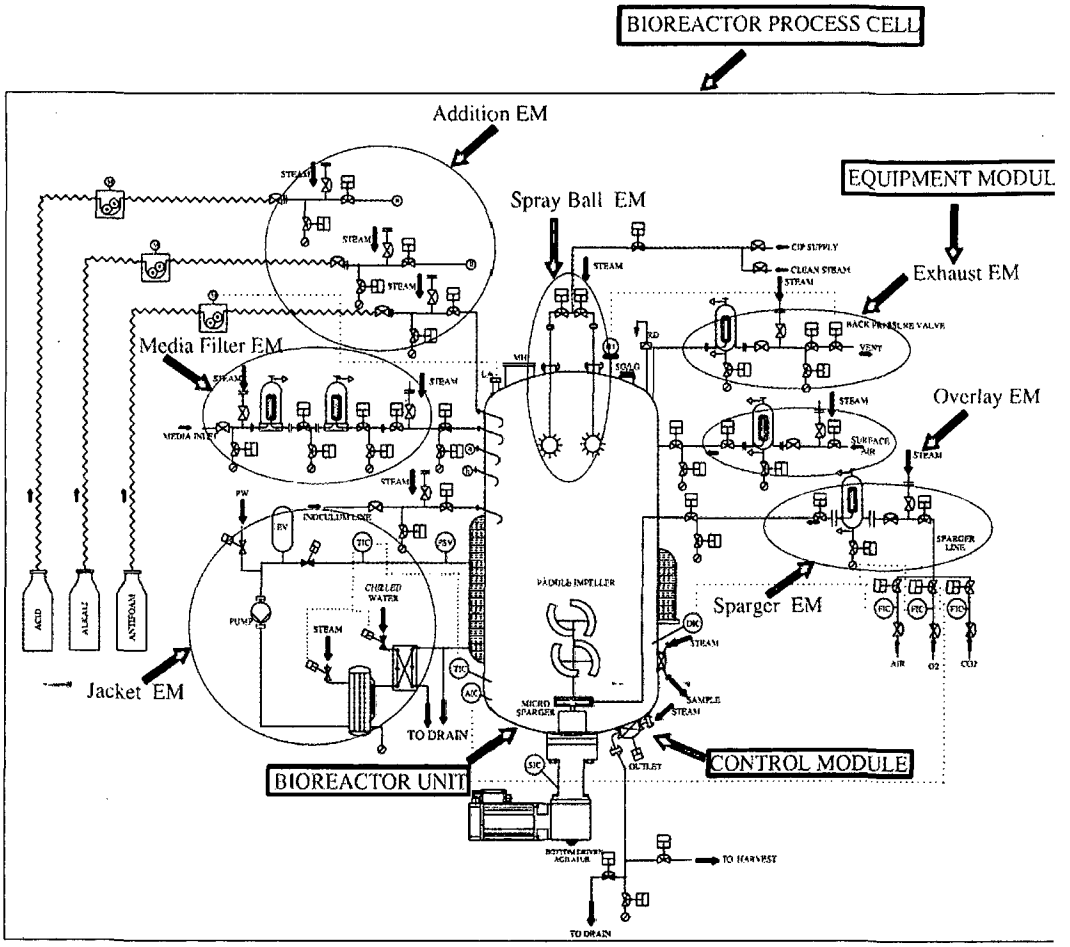


Figure 26: Hierarchy of Physical Model Defining System Boundary Unit, Equipment Module and Control Modules of a Bioreactor.

7.1.2.2 Class based Approach

Class based approach reduces the repetition of code. For example, multiple reactors in a single area are performing the similar tasks such as fe transfer, cultivation, CIP, SIP etc. Rather than creating a reactor unit each time, reactor unit class with properties and methods could be created and instanc whenever a new reactor is needed. This approach allows unintentional deviatio from one unit to other. A reactor system may contain product transfer in, med transfer in, cultivation, CIP, SIP, addition of nutrient etc., phase classes. The phase classes drive the equipment modules into specific scheme in whi opening/ closing of different control modules is defined. Starting from the lo

level of hierarchy, first phase class needs to be designed. Then putting togeth these phase classes a unit operation is formed, and then a Unit procedure.

7.1.2.3 Designing Cultivation Phase Class

This phase class is used to perform the Mammalian Cell Cultivati process under controlled conditions of the required process parameters, li temperature, agitation speed, pressure, pH etc. The main purpose of this pha class is to activate all control loops with the set points as set up with t parameters, and to open all valves in the process line that are required for runni the control loops. The logic algorithm for cultivation phase class is shown in t Figure 27. First, all the equipment modules and control modules required for th sequence to run should be acquired by the reactor unit on which this phase cla runs. Once these modules acquired, they should be driven to required schen Input parameters, instrument set points are defined at the beginning or taken fr higher hierarchical levels such as unit procedure.

7.1.2.4 Designing Cultivation Operation

This operation will be used for running all the phases required f cultivation in the following sequence. Firstly, Media and Inoculum are received the Bioreactor, then Cultivation starts at specified conditions, then SIP of Trans Out Line is done to sterilize the product outlet line and finally, product transferred from the bioreactor to the harvest vessel. The logic algorithm cultivation operation comprising all these phase classes is shown in Figure 28. A these phase classes need to be designed separately and then triggered one by o in the unit operation. All the input parameters and set-points are passed to t respective phase classes before starting the operation.

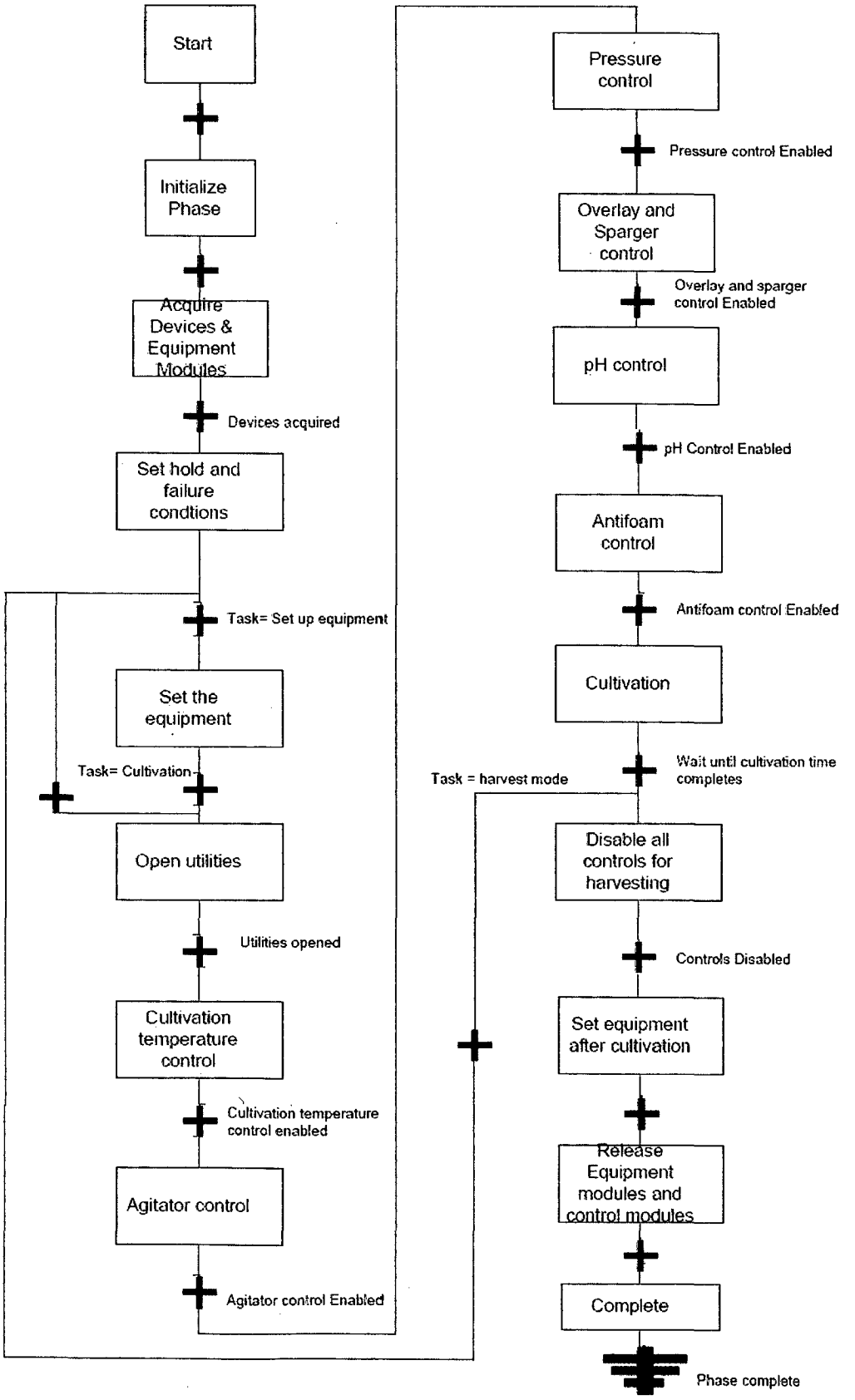


Figure 27: Logic Algorithm for Cultivation Phase Class

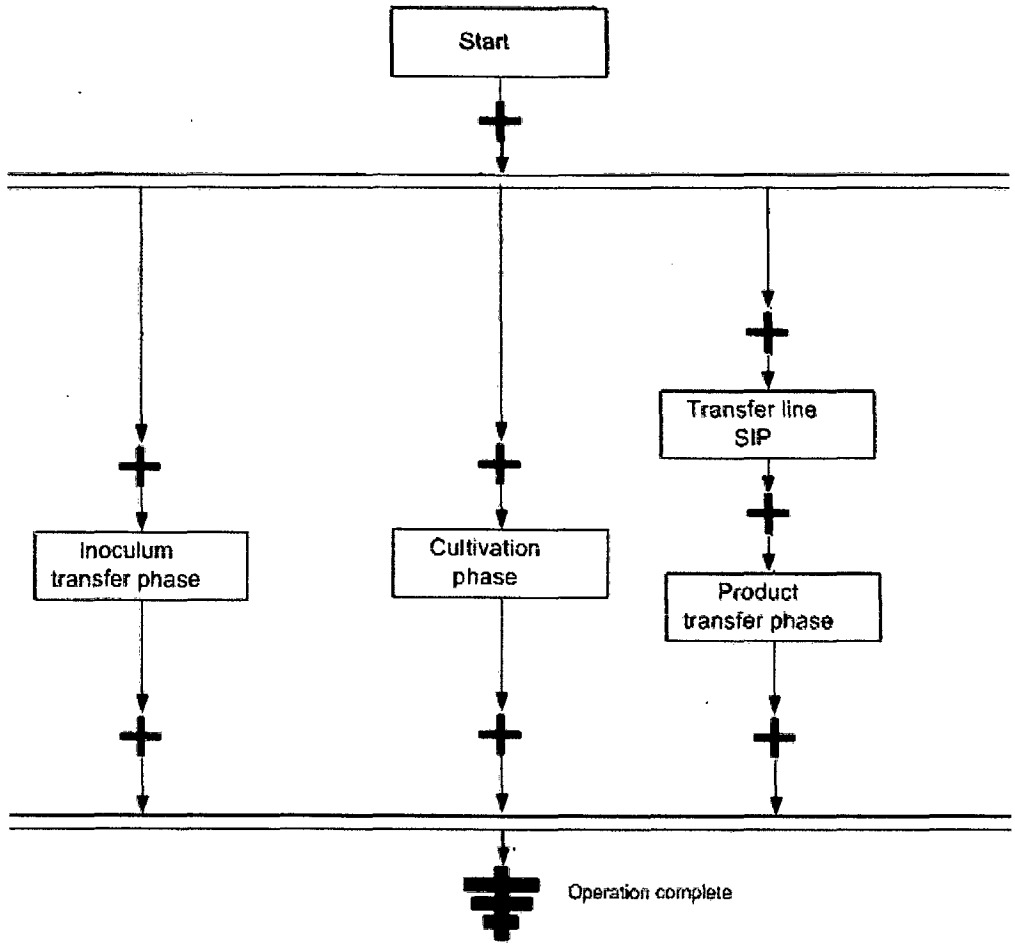


Figure 28: Logic Algorithm for Cultivation Operation.

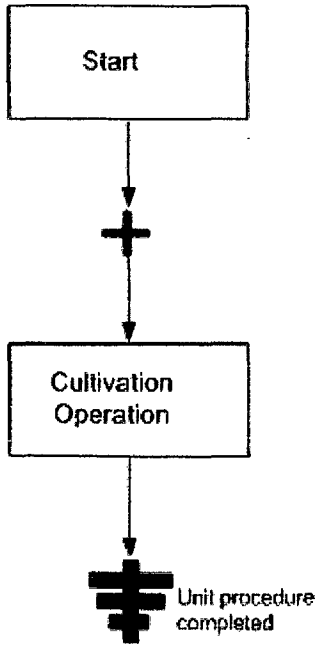


Figure 29: Logic Algorithm for Cultivation Unit Procedure.

7.1.2.5 Designing Cultivation Unit Procedure

This Unit procedure is used to perform cultivation task. Only one u procedure can run on one reactor Unit. All the input parameters and set points a passed on to operation and then to phase classes. The logic algorithm cultivation unit procedure is shown in Figure 29. Similarly, other unit procedu are Media Addition UP, Innoculum Addition UP, Media filter SIP UP, Mec filter CIP UP, SIP of filters, CIP of filters, SIP of Addition lines, CIP of additi lines, Transfer out, Bioreactor SIP and CIP UP. Once these UPs are in place decision is made which procedures needs to be run in parallel for example wh cultivation is going on if filters get chocked then there should be a provision SIP those filters without disturbing the cultivation, for this phases are interlock accordingly. These Unit procedures are applicable to Bioreactor Unit class, whi includes all the bioreactors present in the Bioreactor area of the facility.

7.1.2.6 Defining Process Cells and Areas

The complete facility is divided into different process cells; they a Bioreactor Process Cell, Harvest Process Cell, TFF Skid Process Cell, TFF Fe Tanks Process Cell, Chromatography Process Cell, Chromatography Feed Tan Process Cell Etc. The Areas are defined as, Upstream (includes bioreactor proce cell), Harvest, Purification (includes TFF, chromatography process cells), Utili media and buffer area.

7.2 Master Recipes

Individual recipes are prepared to operate different equipment in parall such as bioreactor, media vessels, and CIP skid needs synchronization. When operator schedules and starts the recipe, known as master recipe, the syste creates the control recipe (a working version of Master recipe), acquires all t equipment, sterilizes each, puts in production, and monitors it through t completion. As each unit procedure completes, transfer lines, tanks, bioreactc and skids are cleaned and get ready for next cycle. Figure 30 shows the mas recipe for the bioreactor.

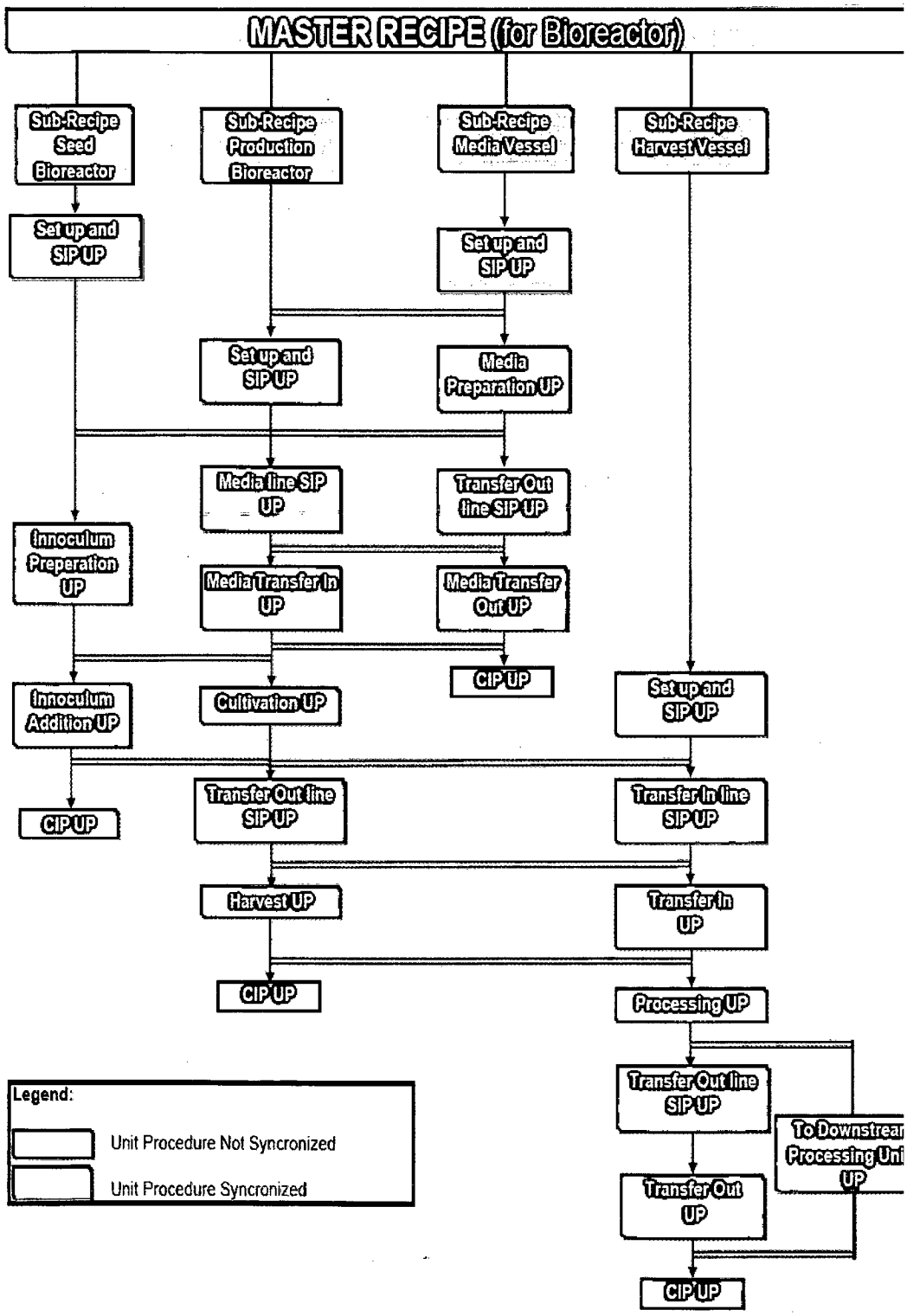


Figure 30: Master Recipe for the Bioreactor.

CONCLUSION

In this study, a method is proposed for the development of generic proce for the production of therapeutic proteins. As a first step, literature and intern sources have been screened for relevant process details. The demand f monoclonal antibodies seems set to increase for the foreseeable future. This likely to be matched by increased global capacity for mammalian cell culture the one hand and by technological progress on the other. For this study a pla capacity of 2500 L is used for producing 2250 grams of product per year. It likely that mammalian cell culture will be dominant for the immediate future ar those improvements will continue to be made in process efficiency. This w result from improvements to the inherent productivity of cell lines which in th future will be driven to a greater extent by a better understanding of th fundamental biology of the cell. It is generally believed that such improvemer combined with further progress in media and feed development will lead antibody concentrations of at least 10 g/L.

As concentrations increase, downstream processing will become a muc more significant component of cost and this will be a driver to develop mo efficient processes, potentially using radically different approaches to those use now. In this case fundamental steps in downstream processing of monoclon antibodies have been identified. As a result, generic flowsheet has been develope based on the platform approach. Urgent needed optimization potential in scale ar cost to solve the gap between therapeutic demands and bottlenecks of healthca systems could be identified in downstream processing.

Increasingly stringent regulatory requirements in the pharmaceutical ar biotechnological industries are fundamentally changing the criteria fo pharmaceutical plant design and construction. A facility design philosophy suggested which is in compliance with the cGMP requirements. To meet thes needs the facility is divided into five general areas: laboratories to support scal up, clinical and manufacturing efforts, utility areas for the production an distribution of purified waters and other utilities, receiving and shipping areas t warehouse raw materials and the finished product, and administrative offices. Th sterile product manufacturing area is further classified into several areas: See

preparation Area, Media and Buffer preparation area, Bioreactor area, Har area, Downstream Processing area (V+ purification and V- Purification area) Formulation area. The inoculum room is designed as Class 10,000 area acces from a white corridor. Bioreactor room is Class 100,000. Hoods throughout facility are Class 100. Buffer preparation, glass washing, and support areas Class 100,000. A gray corridor is provided for visitor access and for exiting Class 10,000 areas. Both V+ and V- purification rooms are Class 1000. A sn Class 1000 filling suite is also included. The facility is designed for one-v flow, meaning that personnel entering a processing suite cannot reenter the wh corridor; they must exit through the gray corridor. This provision is to prev cross-contamination.

In order to consistently manufacture a product that meets appropri targets for attributes, a robust process with appropriate control is develop Various control loop strategies for the critical equipments in the plant a proposed. S88 standard is used to describe the design philosophy for softwa equipment and procedures. Various steps are discussed which are required apply S88 standard to a process. These steps are illustrated by modularizi control of Production Bioreactor. Finally, a Master Recipe is developed f operating the whole monoclonal antibody production scenario, which shows th which equipment is synchronized with other equipment for executing a ur procedure.

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