Control and validation of quality

An internship Report submitted

for the partial fulfilment of the Degree of Master of Science
By

JANKI JETHWA

210621022

[M.Sc. biotechnology]

Under the supervision of
Tejash hathi

Designation of the Guide

DEPARTMENT OF BIOTECHNOLOGY
ATMIYA UNIVERSITY
'YOGIDHAM GURUKUL' KALAWAD ROAD
RAJKOT (GUJARAT) – 360005
2022-23

(On letterhead of the Department)

C E R T I F I C A T E

This is to certify that this training report entitled "**control and validation of quality**" was successfully carried out by Miss **JANKI JETHWA** towards the partial fulfilment of requirements for the degree of Master of Science in Biotechnology of Atmiya University, Rajkot. It is an authentic record of her own work, carried out by her under the guidance of **TEJASH HATHI** of Supervisor for a period of 3 months during the academic year of 2022-23. The content of this report, in full or in parts, has not been submitted for the award of any other degree or certificate in this or any other University.

Signature

Name of the Head of the Department

Signature

Name of the Supervisor

D E C L A R A T I O N

I hereby declare that the work incorporated in the present dissertation report entitled “CONTROL AND VALIDATION OF QUALITY” is my own work and is original. This work (in part or in full) has not been submitted to any University for the award of any Degree or a Diploma.

Date (Name and signature of Student)

Company information

Aptus Pharma Pvt. Ltd. established in 2010. They started with nine products& two headquarters in 2011 and in Very short period of time they enlarged our company with Number of products and many headquarters at state level. They strictly follow all parameters of exports, their all plants and associate plants are W.H.O and G.M.P certified, further company has special pharmacist team, from procurement of raw material to finish product they take care of all parameters, company has tie up with world class lab and batch to batch analysis protocol. They strictly follow good distribution practice at all level. Company always try to follow path of excellence and as a result they have been awarded also in 2017 for "Quality Excellence Award" in MSME segment.by India 5000 MSME & "Best co. In quality control" By India's Most prestigious publication CIMS MEDICA & IDR Group. Product associated with company is dolotus gel, kalrol tablet, leebond syrup, vomitus tablet and many more.

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1. Introduction

Cells in animals exist in an organised tissue matrix which require for their controlled growth and differentiation. These cells from intact organisms may be isolated, maintained and grown *in vitro* in culture media aseptically containing a suitable mixture of nutrients and growth factors. This process is called as animal cell culture.

1.1. Primary and Secondary Cell Cultures

A primary cell culture is prepared by inoculating cells directly from tissues of an organism into culture media, that is, without cell proliferation *in vitro.* With the exception of some cells derived from tumours, most primary cell cultures have a limited life span. After a certain number of divisions, cells undergo the process of senescence and stop dividing. In these cells, the limited proliferation capacity reflects a progressive shortening of the cell's telomeres, the repetitive DNA sequences and associated proteins that cap the ends of each chromosome.

The primary cell culture is of two types depending on the kind of cells in culture- attachment culture and suspension culture. Attachment culture involves the adherent or anchorage dependent cells. To survive and grow, most cells require a surface to which they can attach, thus they are anchorage dependent. These adherent cells are usually derived from tissues of organs, such as- kidney, where they are immobile and embedded in connective tissue. Suspension culture involves non-adherent or anchorage independent cells which do not require attachment for growth or do not attach to the surface of the culture vessels. Lymphocytes are anchorage independent cells commonly grown in culture.

A secondary culture is prepared by subculturing a primary culture. Subculture or passage refers to the transfer of cells from one culture vessel to another [1].

1.2. Cell Line

When a primary cell culture is subcultured it becomes a cell line. The cell lines may be finite cell line or infinite cell line. A finite cell line is a line of cells that will undergo only a finite number of divisions in cell culture and has a limited number of possible subcultures or passages. Normal mammalian cells generally have a finite life span in culture. These cell lines exhibit the property of contact inhibition, density limitation and anchorage dependence. A cell line that has the potential to be subcultured indefinitely is termed infinite (immortal or continuous) cell line. Tumor cells or normal cells that have undergone transformation

induced by chemical carcinogens or viruses can be propagated indefinitely in tissue culture, thus have unlimited number of possible subcultures. Infinite cell lines are also known as transformed cell lines due to altered growth properties of immortalised cells. Transformed cell lines do not exhibit the property of contact inhibition, density-dependent inhibition of proliferation and anchorage dependence. They have a reduced requirement for serum or growth factors for optimal growth.

1.3. CHO cell lines

Chinese hamster ovary cells are derived from ovary of Chinese hamster and are epithelial cell lines which are more often used for medical and biological research. They are robust cell lines found wide application in genetic studies, gene expression studies, toxicity studies and production of recombinant therapeutic protein in industries. Epidermal Growth factor receptor (EGFR) is not expressed in CHO cells, hence they are used in investigating for various EGFR mutations.

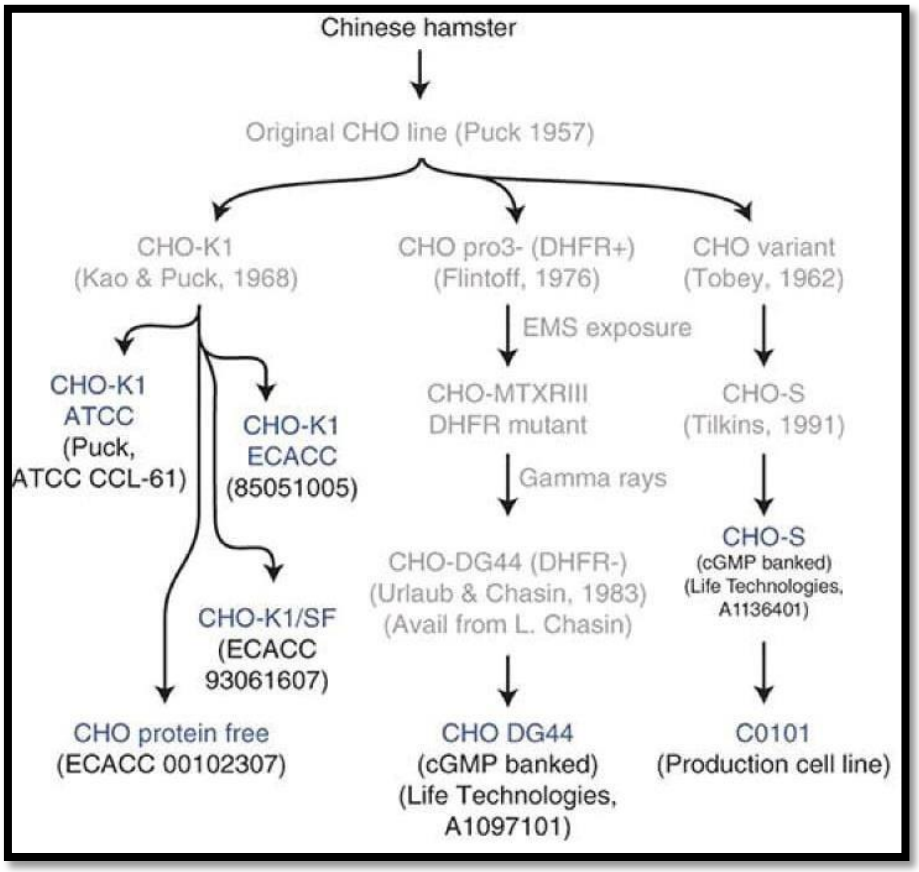

Fig 1: Evolution of CHO cell lines

1.3.1. Variants of CHO cell lines

The original CHO cell lines was described in the year 1956, since then many variants of CHO cell lines were developed for various purpose. In 1957, CHO-K1 cells was cloned by Kao, Punk and co-workers and distributed to collaborators. CHO-K1 cells requires support for their growth whereas CHO-S cells are capable in growing in liquid media without support (suspension culture), hence they have wide application in industries for large scale production of therapeutic protein. Using ethyl methanesulfonate CHO-K1 cells was mutagenized to generated new cell lines called CHO-DXB11 which lack dihydrofolate reductase (DHFR) activity. CHO-DXB11 cells can revert back to DHFR activity, hence their application is limited and it becomes difficult to screen them. In 1983, the gamma radiation was used to completely eliminate the allele which is capable of producing DHFR and termed the cells as CHO-DG44. The DHFR deficient cells requires glycine, hypoxanthine, and thymidine for their growth.

2. Methods

2.1. Sterile Handling:

- Always wipe your hands and your work area with 70% v/v ethanol / 70% v/v IPA.
- Wipe the outside of the containers, flasks, plates, and dishes with 70% ethanol/ 70% v/v IPA before placing them in the cell culture hood.
- Avoid direct transfer of media and reagent from flasks or bottles.
- Use sterile glass or disposable plastic pipettes and a pipette dispenser to work with liquids, and use each pipette only once to avoid cross contamination. Do not unwrap sterile pipettes until they are to be used. Keep your pipettes at your work area.
- Always the bottles and flasks cap should be closed after use and seal it with parafilm to prevent the entry of microorganisms and airborne contaminants.
- Never uncover a sterile flask, bottle, petridish, etc. until the instant you are ready to use it and never leave it open to the environment. Return the cover as soon as you are finished.
- Place the cap with facing down on the work surface while removing a cap or cover.
- Use only sterile glasswares and other equipments.
- Be careful not to sing, whistle, or talk when sterile procedures are being carried out.
- Perform your experiments as rapidly as possible to minimize contamination.

2.2. Cell Culture Equipments:

All cell culture laboratories have the common requirement of being free from pathogenic microorganisms and share some of the basic equipment that is essential for culturing cells.

2.2.1. Basic Equipments

- Cell culture hood (i.e. laminar-flow hood or bio safety cabinet)
- Incubator (humid CO_2 incubator)
- Water bath
- Centrifuge
- Refrigerator and freezer (–20°C)
- Cell counter (e.g. Countess® Automated Cell Counter or haemocytometer)
- Inverted microscope
- Liquid nitrogen (N_2) freezer or cryo storage container
- Sterilizer (i.e. autoclave)

2.2.2. Expanded Equipments

- Aspiration pump (peristaltic or vacuum)
- pH meter
- Phase contrast microscope
- Haemocytometer

2.2.3. Additional Supplies

- Cell culture vessels (e.g., flasks, Petri dishes, roller bottles, multi-well plates)
- Pipettes and pipette dispenser
- Syringes and needles
- Waste containers
- Media, serum and reagents
- Cells

2.3. Bio safety Cabinet (BSC):

A bio safety cabinet (BSC) also called a biological safety cabinet or microbiological safety cabinet is an enclosed, ventilated laboratory workspace for safely working with materials

contaminated with pathogens requiring a defined bio safety level. There are three types of bio safety cabinets: Class I, Class II and Class III.

2.3.1. Class II Bio Safety Cabinet:

The Class II vertical laminar-flow biological cabinet is an open-front, ventilated cabinet. This cabinet provides a HEPA-filtered, recirculated mass air flow within the work space. The exhaust air from the cabinet is also filtered by HEPA filters. Thus, the Class II bio safety cabinet will provide personnel, environmental and product protection. While HEPA filters are effective for trapping particulates and infectious agents, these filters will not capture volatile chemicals or gases. This type of cabinet is widely used in clinical, hospital, life science, research and pharmaceutical laboratories [2].

There are different types of Class II bio safety cabinets: -

Type A1, Type A2, Type B1 and Type B2.

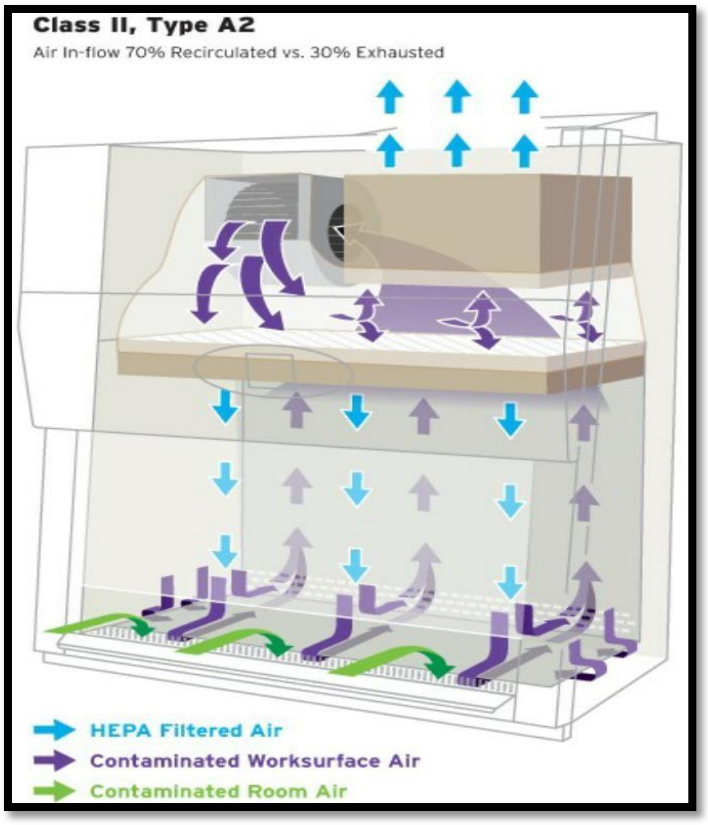

Fig 2: Class II Bio Safety Cabinet

The bio safety cabinet we used in animal cell culture laboratory is **Class II Type A2** bio safety cabinet.

- The class II Type A2 cabinet recirculates approximately 70% of the air through a supply filter, and exhausts approximately 30% of the air through an exhaust filter to the room.
- HEPA-filtered air descends through the work zone in a vertical, unidirectional flow.
- At the approximate centre of the work area, the air splits as it flows to the base of the cabinet. A portion flows through a perforated front grille and the balance flows through the perforated rear grille.
- Room air entering through the front access opening is drawn into the perforated front grille. It does not enter the work area.
- At the cabinet base, the total volume of room and recirculated air is moved by the blower through the rear and the side airflow ducts to the top of the cabinet.
- At the top, approximately 30% of the air is moved through the exhaust filter to either re-enter the room, or exhausted to a facility exhaust system. When exhaust is untreated, personnel and product are protected from vapours and gases. When exhaust is treated, the environment is also protected from vapours and gases.
- The remaining air is moved through the supply filter and recirculated to the work area as clean, vertical flowing air.

2.4. Bioreactor

Bioreactor is a device which is capable of providing biologically active environment for growth and proliferation of animal cells or microbial cells. The process can be either aerobic or anaerobic and the volume of the reactor varies from 250 mL to 2000 L. Basically the material of construction is Borosilicate glass and stainless steel. The basic bioreactor consists of following parts and they are.

a) Reactor
b) Media charge bottle
c) Antifoam
d) Seed bottle
e) Acid and Base charge
f) Bioreactor controller system
g) Sparger
h) Impeller
i) pH probe
j) DO probe

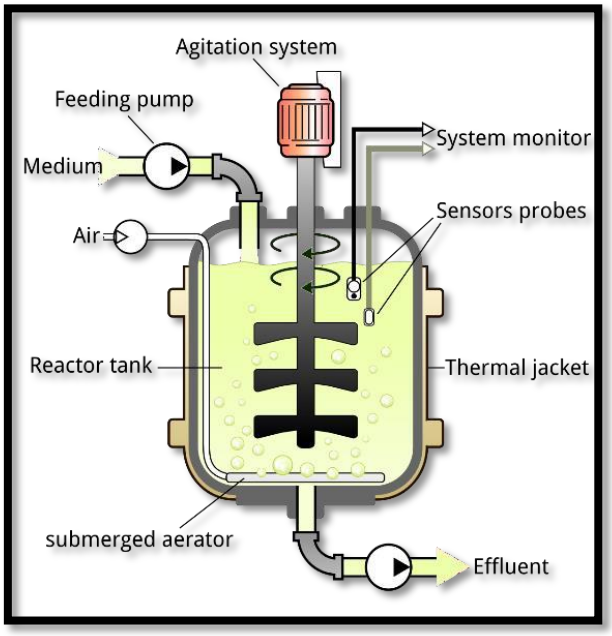

Fig 3: Schematic diagram of Bioreactor

Types of stir tank bioreactors are as follows

1. Batch Reactor
2. Fed Batch Reactor
3. Perfusion Reactors

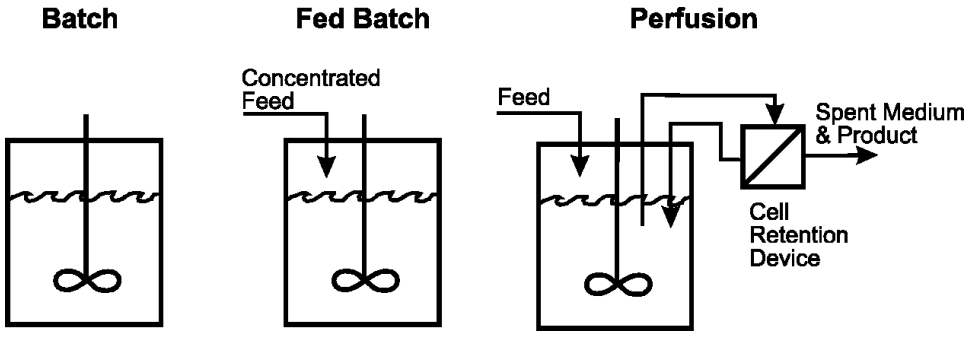

Fig 4: Types of Stir tank Reactor

2.4.1. Batch Reactor

A batch reactor is a basic reactor which usually contains a storage tank with ports for acid, base, antifoam, inoculum, sparger and an agitator. The reactor is integrated with heating and cooling system and size of the vessel varies from 1 litre to more than 15,000 litres. The material of construction will be basically borosilicate glass and the head plate with be made up of stainless steel. The advantage of batch reactors is: they are simple, robust, versatile and simple to handle.

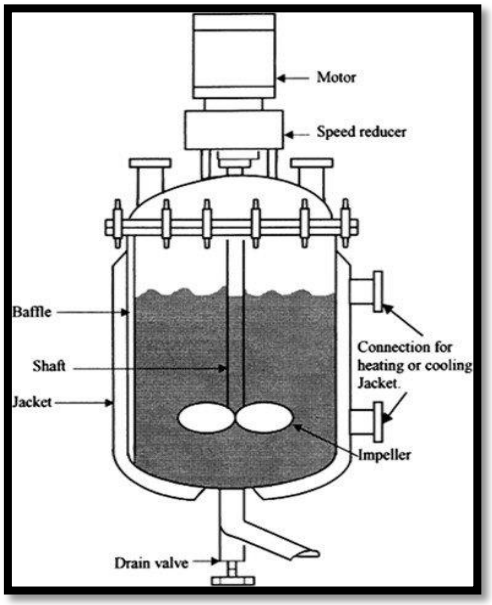

Fig 5: Batch Reactor

Batch culture system is a closed system in which the limited amount of nutrients is added to the reactor. Once the cells are inoculated in the reactor the cells enter lag phase during which synthesis of DNA and protein takes place and cells increase in size. After lag phase they enter exponential phase during which the cells divide and increase in number. After utilizing the entire nutrients in the media the cells will enter stationary phase during which the number of cell replicating will be equal to the number of cell death. Final the cells start dyeing during death phase and viability reduces drastically.

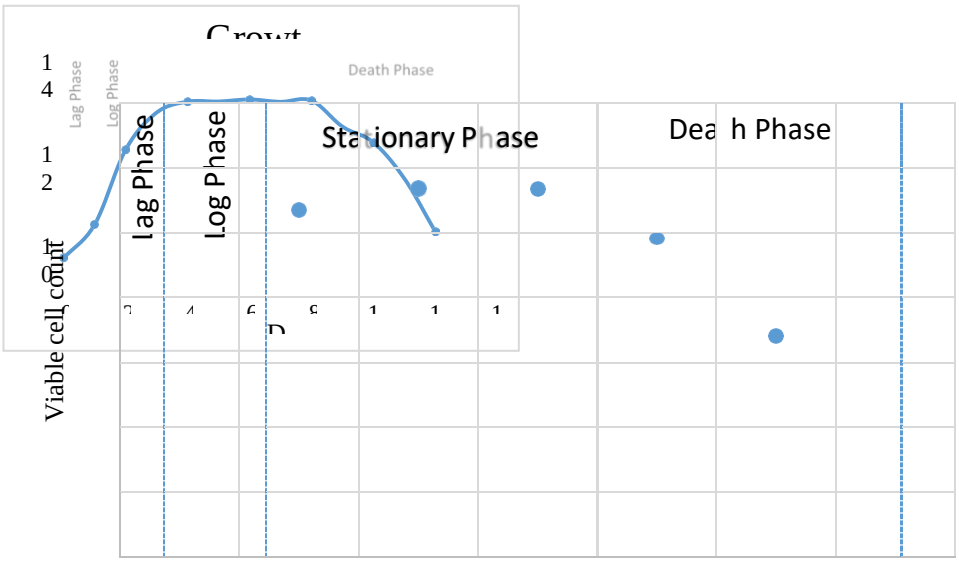

Fig 6: Growth Curve

2.4.2. Fed batch Reactor

Fed batch reactors are a semi batch process in which the nutrient media is added to the reactor at once and the process continues for few days.

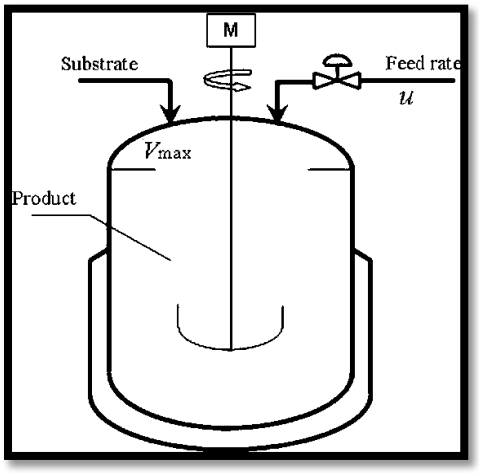

Fig 7: Fed batch reactor

As the day pass by the nutrients in the reactor start depleting and the feed is provided in the regular interval the volume of the reactor increase as the day pass by and the product remain in the reactor until the end of the process.

2.4.3. Perfusion reactor

Perfusion system in upstream process helps in retaining cells within the reactor with the help of ATF system. In this system the waste products and metabolites are continuously removed from the reactor due to which productivity of the therapeutic protein increases. The volume with in the reactor remains constant as the input and output remains constant [3].

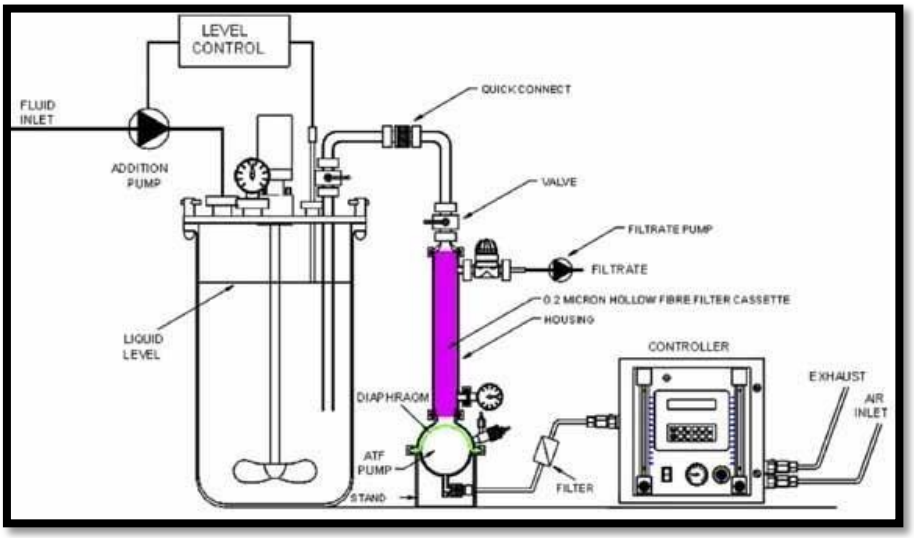

Fig 8: Perfusion Reactor

2.5. ATF system

ATF system is used for pilot plant perfusion which is used to separate cells from product. The tangential flow filtration happens which constantly cleans the fibres from getting clogged. The backflow action takes place every five to ten second which clears the ATF filters every time. The diaphragm moving up and down controls the flow of suspension culture through filter, ensures rapid exchange of fluid, concentrated cells suspension is pumped back to the reactor and minimizes the residency of cells outside the reactor. The choice of filter mainly depends on the elements as to be retained, volume of suspension and on some other factors.

Table 1: Types of ATF system

System	Effective surface area (m^2)	Approximate ATF volume exchanged (L)	Suspension culture volume
ATF 2	0.13	0.1	1 – 10
ATF 4	0.77	0.4	7 – 25
ATF 6	2.5	1.2	20 – 200
ATF 10	11.0	6.0	200 - 1000

Fig 9: ATF systems

2.6. Types of Impeller

In stirred tank reactor growing microbes or animal cell culture, it is very critical to choose best impeller type for the process. Choosing the wrong impeller can lead to some serious damage of cells on the other hand choosing a right impeller can let increasing in productivity

and reduce cell damage. Radial flow of culture suspension occurs when fluid is pushed away from the impeller axis towards the reactor wall. Axial flow of fluid occurs when the cell suspension is pushed up and down along the impeller axis. Depending on the direction and orientation of impeller blade the direction of the fluid is determined. The right handed impeller will push the cell suspension in upward direction and the agitation occur in the clockwise direction. Whereas the left handed impeller will push the fluid in the downward direction alone the axis in anticlockwise direction.

2.6.1. Rushton Impellers / flat blade turbine for Fermentation

Flat blade turbine was termed by J.H. Rushton and hence it is also known as Rushton turbine. The blades are flat and placed vertically to the agitation shaft. Radial flow occurs by using these kind of impellers and should not be considered when the cell lines are shear sensitive. They are ideal for microbial fermentation process, including bacteria, yeast and some fungi.

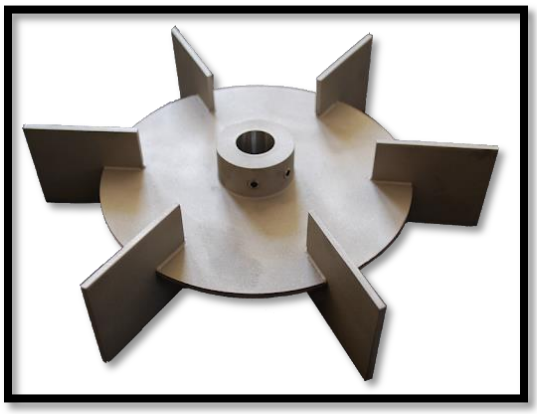

Fig 10: Flat blade turbine

2.6.2. Pitched-Blade Impellers

The blades in pitched blade impellers are set at 45-degree angle to the agitator shaft and hence both radial and axial flow occurs. The combination of both axial and radial flow the proper mixing of the sample occurs and oxygen mass transfer rate (K_La) increases. These kinds of impellers are used for shear sensitive cells such as mammalian, insect and few fungi cell culture.

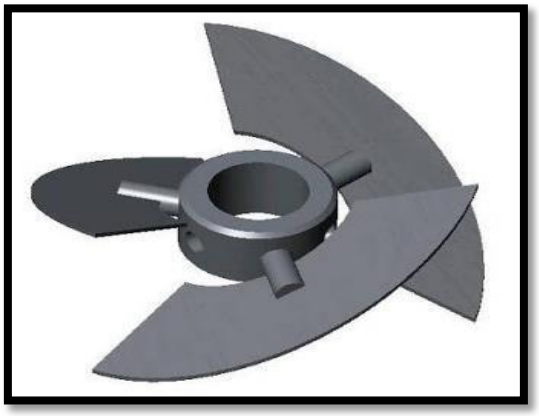

Fig 11: Pitched blade impeller

2.7. Scale up

Development of any process requires scale up studies. Initially studies are done at small scale because the main advantage of initial studies at low volume is that; handling of small volumes is easy, controlling the process is easy, operate at low resource, low cost and man power and less duration is required. One such study is done using Ambr 250 bioreactors. The total volume of Ambr reactors is 250 mL and hence the working volume will be around 180 mL. The data obtained by small reactors can be used and can be implemented on large volume reactors. Once the initial studies are done the studies is carried on to next level such 2L bioreactor or 5L bioreactor. Some process shows good result at low scale but at larger scale will fail drastically. Hence it is necessary to run few batches at 2 or 5L scale. Once the process works good at this scale level they are shifted to next scale which 50L scale. The 50L scale operates at high volume and all the studies are carried out at this scale will be replicated at higher scale in manufacturing level [4].

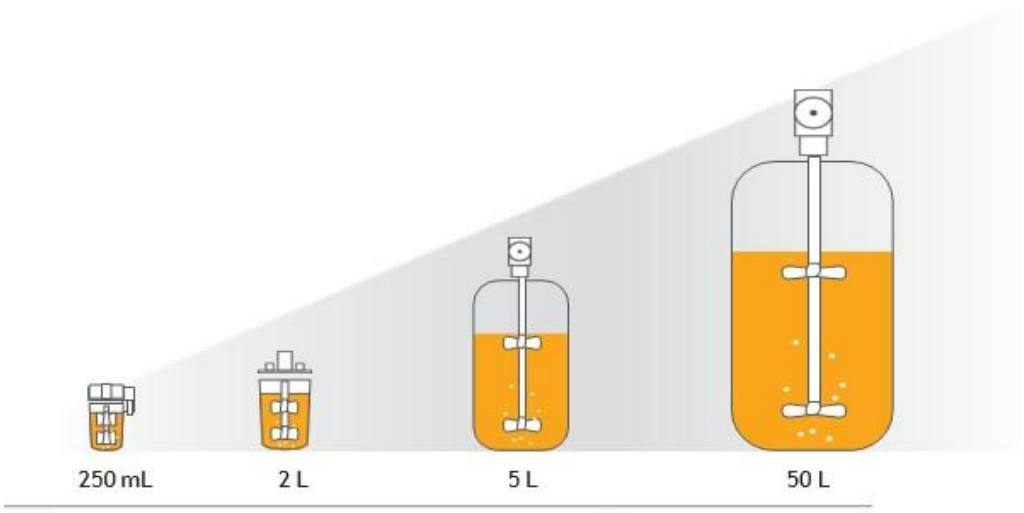

Fig 12: Scale up studies

2.8. Ambr 250 high throughput

Ambr 250 system is fully automated system used to study culture condition of fermentation or animal cell culture. It is observed in Ambr 250 high throughput system the productivity increase dramatically and it also enables the Design of experiments to be performed at much lower cost when compared to traditional bench top reactors. It also provides parallel system to develop process for new strains or clones in industries. The geometry of Ambr bioreactor is similar to large bioreactor, hence all process on the system empirically correlate to larger reactors.

Fig 13: Ambr 250 high throughput system.

The data obtained by the Ambr reactors can implemented on the large scale reactor for optimum scalability. Ambr 250 high throughput system is a fully automated system which is handled with the help of fully automated liquid handler, hence the user no need to spend long hours in front of the reactor for manipulating, setting up, cleaning and feeding the reactor.

Fig 14: Ambr 250 mL Bioreactor

2.9. Procedure to setup the Reactor:

2.9.1. Setting up a Bioreactor:

a. The jacket was evacuated and the bioreactor was initially rinsed with WFI.
b. 1000mL WFI was added to the bioreactor vessel.
c. The head plate of the bioreactor was properly closed by tightening the opposite screws simultaneously.
d. All the air line ports including the surface air, sparger, and the exhaust line were connected using hose pipes and filters were connected at the distal ends.
e. The pH probe was calibrated using pH 4 and pH7 standard buffers and inserted to the bioreactor.
f. The acid, base and antifoam lines were connected to the 4-way port on the top plate and the tubings were clamped properly to avoid loss of water from the bioreactor.
g. The exhaust line was connected to the condenser and outlet of condenser was connected to a bottle with filter for the exhaust.
h. The D.O. probe was cleaned and fresh electrolyte was added to it and placed in its respective port.
i. The temperature probe was also inserted.
j. The tubing of the sampling line was connected with silicon tubing.
k. Inoculation bottle was sterilized along with the bioreactor.
l. Each tubing was clamped and the leakage test was performed.

2.9.2. Pressure Leak Test:

a. The leakage in the vessel or connections was checked by passing a minimal quantity of air into the vessel through surface line, with all outlets and ports closed, except the exhaust line.
b. The open end of the exhaust line was dipped into a beaker filled with water for immediate visualizing of the air bubbles. In case of any leakage, the bubbling in the beaker would commense after some time or will not occur at all. If there is no leakage, bubbles will rise instantly.

2.9.3. Sterilization of bioreactor:

a. the bioreactor along with clamped piping, filters, bottles, probes, and condenser was autoclaved at 121^{o} C for 45 minutes.

b. After autoclaving, the bioreactor was brought back to the lab and all the connections were reconnected.
c. The surface air was switched on to set up a positive back pressure in order to avoid any contamination.
d. The bioreactor was allowed to cool down slowly.
e. After cooling, the agitator pump and sparger air were switched on.

2.9.4. Media and Inoculum transfer:

a. The bottles containing filter sterilized media, antifoam and alkali were connected, aseptically to the reactor in a biosafety cabinet.
b. Sterile media is transferred to the reactor using peristaltic pump through capsule filter and the allow it to stand in reactor for 24 hours for sterility check.
c. Inoculum is transferred to the reactor using peristaltic pump and usually the seeding density will be around 0.5 million cells/mL.
d. The aeration through sparger line was maintained and was controlled by control system.
e. The agitator speed was set at 200 rpm and the temperature was maintained at 37^{o} C.
f. The culture was allowed to grow in the bioreactor and regular sampling was done for cell counts at specific intervals.
g. At the end of the batch, the culture was harvested through the sampling line by pressurizing the vessel by surface air line.
h. Then, vessel was cleaned and made ready for use.

2.10. Cell Culture Media

The media which contains serum is poorly defined as the composition of the serum varies drastically and consistency will never remain constant from one batch to other. Even though consistency of the serum as improved over the years physiological variance is quite high and low reproducibility.

On the other hand, the serum free media the components are precisely defined. Hence the variance is very low and high reproducibility.

The advantage of serum free media is

Low risk of infectious agents: Some of the infectious agents can be avoided such as mycoplasma, virus and bacteria etc. which is very important in large scale production of therapeutic protein.

Lower risk of interfering components: Serum in the media sometimes may interact with components present in the media which will influence the productivity. On the other hand, the serum free media has fewer interfering factors and hence it can be controlled easily.

Less contaminants: The concentration of serum protein is generally higher when compared to the recombinant protein by mammalian cells. Serum protein are some of the major contaminants which hiders the downstream process. Were as in serum free media the protein concentration is very low which basically reduce the downstream process step with high recovery.

Ethical issues: Since serum is taken from animal source which rises ethical issue, so it is better to avoid it and take an alternative such as serum free media. Usually the components of serum free media are given below.

Table 2: Components of Serum free media

Components
Calcium Chloride (anhydrous)
Ferric Nitrate.$9H_2O$
Magnesium Sulphate (anhydrous)
Potassium Chloride
Sodium Chloride
Sodium Phosphate Monobasic (anhydrous)
L-Arginine. HCl
L-Cysteine. 2HCl
L-Glutamine
L-Histidine.HCl.H_2O
L-Isoleucine
L-Leucine
L-Lysine.HCl
L-Methionine
L-Phenylalanine
L-Threonine
L-Tryptophan
L-Tyrosine.2Na.$2H_2O$
L-Valine
Choline Chloride
Folic acid
Myo-Inositol

Niacinamide
D-pentothenic acid (Hemicalcium)
Pyridoxal.HCl
Riboflavin
Thiamine.HCl
D-Glucose

2.11. Filtration

Filtration is a physical, chemical or biological process in which the solid particles are separated from the fluid mixture. The solid particles which don't pass through filters are known as oversize, whereas the particles with pass through the filter are called as filtrate. In cell culture lab or upstream lab, the media which is prepared as to be filtered to remove bacteria and other unwanted microorganism.

2.12. Types of filters

Paper filtrations: Filter paper is semi-permeable barrier which is placed perpendicular to the liquid flow and are used to filter some reagents. This kind of filters is used when sterility is not an issue. There are different grades of paper filters.

Fig 15: Filter Paper

Table 3: Grades of filter paper

Categories	Pore size
Grade 1	11 µm
Grade 2	8 µm
Grade 3	6 µm
Grade 4	20 – 25 µm
Grade 602 h	2 µm

Capsule Filters: Capsule filters are available with filter surface area of range 200 cm^2 to 3.5 m^2 per capsule. These are designed to remove bacteria and other unwanted microorganism. There are ready to use filters and can be used to filter media and no need of dissemble, clean and reuse the filter assembly.

Fig 16: Capsule filters

Depth filters: This is types of filters in which particles are retained throughout the media of the filter rather than just on the surface. These filters are used to filter fluid containing high load of particle and can filter high volume before getting clogged.

Fig 17: Depth Filters

2.13. Vi-CELL XR Cell Viability Analyzer

Aim: To find the viable cell count and percentage viability present in the given sample.

Principle: The number of viable cells present in the sample can be determined by the help of trypan blue exclusion method. The basic principle of this method is that the live cells having intact cell membrane will exclude dyes such as propidium, eosin and trypan blue, whereas the

dead cells will intake the dye. Vi-CELL XR cell viability analyser is used to find percentage viable cells present in the given sample. It is an automatic device which performs Trypan blue dye exclusion method and 12 samples can be loaded at one run. The device as an inbuilt software which can be customized to our requirements easily.

Materials required:

- Vi-CELL XR cell viability analyser
- Samples to be analysed
- Vi CELL XL reagent Pack
- Sample cups

Procedure:

1. Inside the biosafety cabinet approximately 700 µL of sample is taken to the sample cups.
2. The sample cup containing the samples are place in Vi-CELL XR cell viability analyser at a particular position.
3. The particular sample id is given and run the program.
4. The machine runs automatically and capture 50 images and provide the result by taking the average reading.

(a)

(b)

Fig 18: Vi-CELL XR cell viability analyser (a) and Vi CELL XL reagent Pack (b)

Reagent pack mainly consist of four components and they are: a) Tryphan Blue, b) Disinfectant, c) PBS buffer, d) Cleaning Agent

Observation:

The live cells appear brighter when compared to dead cells which can be clearly observed in figure 19. Based on the several such image the software will compile and show the average viable cell count.

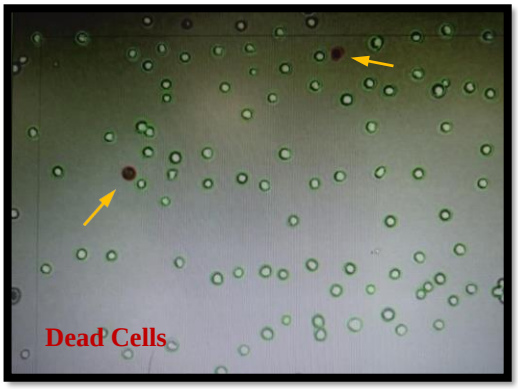

Fig 19: Vi CELL image of CHO cell lines

Result: The viable cell count and percentage viability present in the given sample was determined.

2.14. Osmometer

Aim: To find the osmatic strength of the given sample.

Principle: The osmometer works based on the principle of freezing point depression. It is very crucial to find the osmolality of the bioreactor samples. The osmolality of the solution can be determined using the following formula.

$$\frac{1000\ mOsm\ particles}{-186\ ºC} = \frac{\text{X}\ mOsm\ particles}{measured\ freezing\ point\ of\ sample}$$

Materials required:

- Osmometer
- Samples
- Sample cups
- Pipette and tips

Procedure

1. The sample of about 250 µL is taken in osmometer sample cups and is place in the slot provided in the instrument.
2. Proper sample id was given and the analysis was started.
3. It works on the principle of freezing point depression and the final osmolality is displayed on the digital display.

Fig 20: Osmometer

2.15. Metabolite analysis using Cedex Bio

Metabolite analysis of bioreactor samples is one of the crucial steps in understanding the process and it is done by using cedex bio instrument.

Fig 21: Cedex Bio instrument

2.15.1. Glucose analysis

Aim: Quantitative determination of glucose in the given sample by using Cedex Bio instrument.

Principle: Glucose present in the sample is phosphorylated by ATP in the presence of enzyme called hexokinase to produce glucose – 6 – phosphate. The glucose – 6 – phosphate is oxidized by $NADP^+$ in the presence of glucose – 6 – phosphate dehydrogenase to produce NADPH and gluconate. The rate of NADPH produced is spectrometrically analyzed and is directly proportional to glucose concentration.

$$D - Glucose + ATP \xrightarrow{hexokinase} D\ Glucose\ 6\ Phosphate + ADP$$

$$D\ Glucose\ 6\ Phosphate + \beta\ NADP \xrightarrow{G\ 6\ P\ DEHFDR0GENASE} 6\ Phospho\ D\ Gluconate + NADPH$$

$$WST - 1 \xrightarrow{NADPH \rightarrow NADP} WST - 1\ Formazan$$

Materials Required

- Cedex Bio instrument
- Sample
- WFI
- Glucose Bio kit
- Calibrator A Bio
- Control A Level 1
- Control A Level 2
- Control A Level 3

Protocol

1. Cedex Bio instrument was calibrated using Calibrator A Bio kit.
2. The sample which as to be analysed is placed in the sample cuvettes and sample id is provided to each samples.
3. Select the glucose test in the software and press start button.

Measuring Range

Glucose concentration can be determined in following ranges:

- GLC2B: 0.02 - 7.50 g/L (0.111 - 41.6 mmol/L)
- GLC2D: 0.2 - 75.0 g/L (1.11 - 416.0 mmol/L), up to maximal solubility with postdilution

2.15.2. Glutamine analysis

Aim: Quantitative determination of glutamine in the given sample by using Cedex Bio instrument.

Principle: L-Glutamine present in the sample is deaminated to L glutamate in the presence of enzyme glutaminase. L glutamate is oxidized to α ketoglutarate, hydrogen peroxide and ammonia in the presence of enzyme L-glutamate oxidase. Hydrogen peroxide generated produce quinone dye in the presence of peroxidase which can be measured photometrically and is directly proportional to glutamine concentration.

$$Glutamine \xrightarrow{GLNase} Glutamate + NH_3$$

$$Glutamate \xrightarrow{Glutamate\ oxidase} \alpha - Ketoglutarate + H_2O_2 + NH_3$$

$$H_2O_2 + 4 - AAP + TOOS \xrightarrow{Peroxidase} Quinone\ dye + H_2O$$

Materials Required

- Cedex Bio instrument
- Sample
- WFI
- Glutamine Bio kit
- Calibrator B Bio
- Control B Level 1
- Control B Level 2
- Control B Level 3

Protocol

1. Cedex Bio instrument was calibrated using Calibrator B Bio kit.
2. The sample which as to be analysed is placed in the sample cuvettes and sample id is provided to each samples.
3. Select the glutamine test in the software and press start button.

Measuring Range

Glutamine concentration can be determined in following ranges:

- GLN2B: 15 to 1500 mg/L (0.1 to 10 mmol/L)
- GLN2D: 75 to 7500 mg/L (0.5 to 50 mmol/L), up to maximal solubility with postdilution

2.15.3. Glutamate analysis

Aim: Quantitative determination of glutamate in the given sample by using Cedex Bio instrument.

L glutamate is oxidized to α ketoglutarate, hydrogen peroxide and ammonia in the presence of enzyme L-glutamate oxidase. Hydrogen peroxide generated produce quinone dye in the presence of peroxidase which can be measured photometrically and is directly proportional to glutamate concentration.

$$Glutamate \xrightarrow{Glutamate\ oxidase} \alpha - Ketoglutarate + H_2O_2 + NH_3$$

$$H_2O_2 + 4 - AAP + TOOS \xrightarrow{Peroxidase} Quinone\ dye + H_2O$$

Materials Required

- Cedex Bio instrument
- Sample
- WFI
- Glutamate Bio kit
- Calibrator A Bio
- Control A Level 1
- Control A Level 2
- Control A Level 3

Protocol

1. Cedex Bio instrument was calibrated using Calibrator A Bio kit.
2. The sample which as to be analysed is placed in the sample cuvettes and sample id is provided to each samples.
3. Select the glutamate test in the software and press start button.

Measuring Range

Glutamate concentration can be determined in following ranges:

- GLU2B: 0.015 to 1.50 g/L (0.1 to 10 mmol/L)
- GLU2D: 0.15 to 15.0 g/L (1.0 to 100 mmol/L), up to 150 g/L (1 mol/L) with postdilution

2.15.4. Ammonia analysis

Aim: Quantitative determination of ammonia in the given sample by using Cedex Bio instrument.

Ammonia reacts with 2-oxoglutarate and NADPH in the presence of enzyme glutamate dehydrogenase to produce glutamate, $NADP^+$ and water. The decrease in NADPH concentration is directly proportional to ammonia concentration and is measured photometrically at 340 nm.

$$NH_4^+ + 2-oxoglutarate + NADPH \xrightarrow{glutamate\ dehydrogenase} Glutamate + NADP^+ + H_2O$$

Materials Required

- Cedex Bio instrument
- Sample
- WFI
- Ammonia Bio kit
- Calibrator B Bio
- Control B Level 1
- Control B Level 2
- Control B Level 3

Protocol

1. Cedex Bio instrument was calibrated using Calibrator B Bio kit.
2. The sample which as to be analysed is placed in the sample cuvettes and sample id is provided to each samples.
3. Select the ammonia test in the software and press start button.

Measuring Range

NH3 concentration can be determined in following ranges.

- NH3B: 0.278 - 13.89 mmol/L (4.73 - 236 mg/L)
- NH3D: 5.56 - 278 mmol/L (95 - 4720 mg/L), up to 5.56 mol/L (95 g/L) with postdilution

2.15.5. Lactate analysis

Aim: Quantitative determination of lactate in the given sample by using Cedex Bio instrument.

L-lactate reacts with oxygen in the presence of lactate oxidase to produce pyruvate and hydrogen peroxide. H_2O_2 generated produce quinone dye in the presence of peroxidase which can be measured photometrically and is directly proportional to L-lactate concentration.

$$Lactate + O_2 \xrightarrow{Lactate\ oxidase} Pyruvate + H_2O_2$$

$$H_2O_2 + 4 - AAP + TOOS \xrightarrow{Peroxidase} Quinone\ dye + H_2O$$

Materials Required

- Cedex Bio instrument
- Sample
- WFI
- Lactate Bio kit
- Calibrator A Bio
- Control A Level 1
- Control A Level 2
- Control A Level 3

Protocol

1. Cedex Bio instrument was calibrated using Calibrator A Bio kit.
2. The sample which as to be analysed is placed in the sample cuvettes and sample id is provided to each samples.
3. Select the ammonia test in the software and press start button.

Measuring Range

Lactate concentration can be determined in following ranges:

- LAC2B: 0.018 - 1.40 g/L (0.20 - 15.5 mmol/L)

- LAC2D: 0.18 - 14.0 g/L (2.0 - 155.0 mmol/L), up to 140 g/L (1.55 mol/L) with postdilution

2.16. Radiometer

Aim: To determine the pH, pCO_2, pO_2 and some ion concentration using Radiometer.

Principle: Basically there are three measuring principle used in ABL-80 analyser. They are

Potentiometry: Using voltmeter the potential of the supply chain is measure and is related to the concentration of the sample using nerst equation.

Amperometry: The magnitude of electric current flowing through the sample is directly proportional to the substance concentration which as to oxidized or reduced at an electrode detected by the sensor.

Conductometry: The sample pass through two conducting electrode which is maintained at constant voltage and the impedance is measured which is directly proportional to the conductive properties of sample.

The following parameters are measured: pH, pCO2, pO2, cCa2+, cK+, cNa+ and cCl-.

Materials Required

- Sample
- Radiometer ABL80 Flex

Procedure

1. The Radiometer was calibrated by pressing the calibration button on the screen.
2. The sample is inserted into system using sampling port.
3. The proper sample id is provided to each sample and the analysis is done.
4. The result is noted which is displayed on the screen [5].

Observation

The instrument can measure the pH of range of about 4 – 10 pH and if the sample pH is out of this range it is unable to show the reading. It can detect partial pressure of oxygen and carbon di oxide in mmHg. The instrument can detect the ion concentration of sodium, potassium, calcium and chlorine in mmol/ L.

Fig 22: Radiometer ABL80 Flex instrument

3. Conclusion

The techniques which are mentioned above are crucial to understand the process involved in animal cell culture and handle bioreactor smoothly without any problem. The fate the cells mainly depends on handling and feeding strategy, so it is always better to understand the procedure and techniques involved in. Without any research and understanding if we start any process it would be very difficult to handle/ design any process which can be commercialized. Hence, it is very important to do initial studies at low volume reactors and the use the data generated from it to scale up the process. The type of reactor, impeller and media used will also play a crucial role for increasing the productivity and cell growth. Defined media is generally used in industrial research because of traceability and maintain consistency throughout the process. Currently the researchers are trying to inculcate new techniques and methods to increase the productivity, reduce cost, maximum profitability and produce new therapeutic protein.

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