GENERAL INDUSTRIAL TRAINING IN PRODUCTION DEPARTMENT

Internship Report

Submitted to the Postgraduate Department of Biotechnology, JSS College of Arts, Commerce and Science in partial fulfillment of the requirements for the award of Master of Science (M.Sc.,) degree in Biotechnology of University of Mysore

Submitted by

SANJAY RB

Register No: P01BE21S0556

IV Semester

Under the guidance of

Internal Guide

Dr.B.Y.Sathish kumar

Head of Postgraduate department of biotechnology

JSS College of Arts,Commerce and science, Ooty Road, Mysore

Postgraduate Department of Biotechnology

JSS COLLEGE OF ARTS, COMMERCE AND SCIENCE

(An Autonomous College of University of Mysore)

MYSORE –570025

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Postgraduate Department of Biotechnology

CERTIFICATE

Certified that project/internship report entitled '**GENERAL INDUSTRIAL TRAINING IN PRODUCTION DEPARTMENT** is a bonafide work carried out by **SANJAY RB** in partial fulfillment of the requirements for the award of Master of Science degree in Biotechnology of University of Mysore.

Internal Guide:

Dr. B.Y. Sathish Kumar
Head of the Department
PG Dept. of Bio-Technology

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Head of the Department
PG Dept. of Bio-Technology

Head of the Department
Postgraduate Department of Biotechnology
JSS College of Arts, Commerce and Science
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Ooty Road, MYSORE-570 025

Examiners:

1. 21/9/23
2. 21/9/23

Dr.S. Prathibha
Principal, JSSCACS
Ooty road, Mysuru - 25

Place: Mysore
Date: 21/09/2023

LAURUS Bio

Date: 05.09.2023

TO WHOMSOEVER IT MAY CONCERN:

This is to certify that **Mr. Sanjay R B** M.Sc. Biotechnology, has done his internship in Laurus Bio Pvt Ltd **From 14th June 2023 to 9th September 2023**

He has undergone training on ***"GENERAL INDUSTRIAL TRAINING ON BIOREACTORS FOR PRODUCTION OF FOOD GRADE PRODUCTS"***.

During the internship training he demonstrated good design skill with a self- motivated attitude to learn new things. His performance exceeded expectations and was able to complete the assigned tasks on time.

We wish him all the best for his future endeavours.

For Laurus Bio Pvt Ltd

Rakesh D
General Manager- Production

DECLARATION

I SANJAY RB here by declare that project entitled '**GENERAL INDUSTRIAL TRAINING IN PRODUCTION DEPARTMENT**' submitted to Postgraduate Department of Biotechnology JSS College of Arts ,Commerce and Science in partial fulfillment of the requirement for the award of Master of Science (M.Sc) degree in Biotechnology of University of Mysore .

This is an investigation carried out by me under the guidance of **Dr.B.Y Sathish Kumar Head of Biotechnology Department, JSS College, Ooty Road ,Mysore, Mr.Rakesh D (General Manager), Mr.Parthiban G (Senior Manager) And Mr.Balamurugan R (Manager Production) Laurus Bio pvt .limited** during IV semester of **M.Sc programme**.The results of this investigation have not been previously submitted by me elsewhere for the award of the any degree/diploma/associate ship/fellowship or other similar title.

Date : 21/09/2023

Place: MYSORE

Signature of the Student

SANJAY RB

Register No. P01BE21S0556

ACKNOWLEDGEMENT

First of all, I would like to express our gratitude to **Dr .B.Y Sathish Kumar, HOD** of Biotechnology department ,JSS College for valuable suggestion,encouragement and support throughout the academics.

My profound thanks **Dr Rekha N.D, Mr Vasanth patil, Dr Mallikarjun Chougalla, Dr Abhignan Gurukar, Mrs Bhavya** Assitant professor, JSS College of Arts ,Commerce and Science for the support throughout the academics and work .

The joy of successfully completing the industrial training would be incomplete without the support of above –mentioned professors who rendered help and guidance throughout. Hence before presenting the work, I wish to express my gratitude towards them. It is my privilege and pleasure to express gratitude and respectful thanks to , chief Executive officer, Laurus Bio Pharmaceuticals Pvt Ltd, for giving me an opportunity to carry out the internship training work in their industry.

I would also like to express deep gratification and sincere thanks to **Mr.Rakesh D (General Manager), Mr. Parthiban G (Senior manager) Mr.Balamurugan R (Manager-Production) Laurus Bio Pvt Ltd**, for their valuable guidance, committed supervision, suggestion, keen interest, timely help and motivation for making this intership work a success.

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Table No.1.1. List of Abbreviations:

Name	Abbreviation
GDP	Good Documentation Practices
GLP	Good Laboratory Practices
GMP	Good Manufacturing Practices
LAF	Laminar Air Flow
SOP	Standard Operating Procedure
STP	Standard Testing Procedure
UOM	Unit of Measurement
HMI	Human Machine Interface
ARN	Analytical reference number
PIS	Pre Inoculation Sample
POIS	Post Inoculation Sample
MF	Micro Filtration
CFG	Centrifugation
PUW	Purified water
MIN	Minutes
PCV	Packed cell volume
UF	Ultrafiltration
DF	Diafiltration
EOF	End of fermentation
SCADA	Supervisory Control and Data Acquisition

CHAPTERS

CHAPTER 1: INTRODUCTION.

1.1 General Introduction :

The pharmaceutical industry is one of the most enduring, largest and most critical knowledge-intensive sectors across the globe. For about one century following its emergence as a sizable economic force in the mid-19th century, pharmaceutical manufacturers essentially operated as a sub-sector of the chemicals industry. The pharmaceutical industry was distinctly shaped by advances in chemical engineering and their innovative application.

In the 1970s, developments under way since World War II ushered in the biotechnology revolution. Biotechnology is an outgrowth of interdisciplinary research in molecular biology, immunology and biochemistry, aided by new techniques such as X-ray structural analysis and computer-assisted drug synthesis.Due to the promise of therapeutic breakthroughs, many traditional pharmaceutical firms increased biotechnology research in their own laboratories leading to the production of biopharmaceutical,a combination of biotechnology and pharmaceuticals. The biopharmaceutical sector that has since emerged is one with extra ordinary research demands, together with phenomenal research promise.

Biopharmaceuticals are medical drugs produced using biotechnology. They are proteins(including antibodies), nucleic acids (DNA, RNA or anti-sense oligonucleotides) used for therapeutic or in vivo diagnostic purposes,and are produced by means other than direct extraction from a native(non- engineered)biological source. Biopharmaceutics independently and as a profession have important positions to play in positively impacting medication policy,medication use and results as well as other aspects of medical proper care.In many instances this will be through cooperation with other wellness care professionals at a community stage.

The following are the various actions that comprise the application of drug to individuals. If performed, in whole or in part, they will result in added value to medication

treatment by making a beneficial participation to the safe and affordable use of drugs, leading to beneficial results and improved medical proper care. Obtain and maintain medication records and relevant wellness details, if they do not already exist. This detail is essential to evaluate personalized medication treatment. Identify,evaluate and assess Medication related problems,Symptoms described patients,self-diagnosed conditions.The elements of drug intake,individual sufferers,taken together,explain comprehensive drug, proper care, the delivery of which requires an ongoing, conventional relationship between the pharmacologist and the affected person. Pharmacologist must use his clinical reasoning to determine the stage of drug proper care that is needed for each patient.

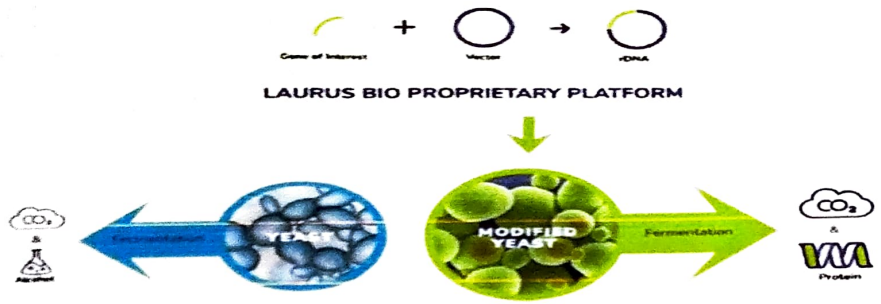
When a new biopharmaceutical is developed, the company will typically apply for a patent, which is a grant for exclusive manufacturing rights. This is the primary means by which the developer of the drug can recover the investment cost for development of the biopharmaceutical. The patent lawsin the United States and Europe differ somewhat on the requirements for a patent, with the European requirements perceived as more difficult to satisfy. The total number of patents granted for biopharmaceutical has risen significantly since the1970s.In1978 the total patents granted was 30.This had climbed to15,600 in1995,and by 2001 there were 34,527 patent applications. In 2012 the US had the highest IP (Intellectual Property) generation within the biopharmaceutical industry, generating 37 percent of the total number of granted patents worldwide; however, there is still a large margin for growth and innovation within the industry.

1.2 Introduction to Company :

LAURUS Bio (formerly Richcore Life sciences) is an integrated research-driven bio-manufacturing organization with deep expertise in precision fermentation and recombinant technology. We engineer microbes as "cell-factories" to manufacture novel and sustainable animal-free proteins and growth factors that replace animal-derived products in biopharma, food,health,nutrition,and personal care markets.

Laurus manufacture **animal-origin-free recombinant proteins, growth factors, and cell-culture media supplements** that are safe, virus-free, and sustainable. Our products and

solutions cater to the unique requirements of various industries such as Stem Cells and Regenerative Medicine, Vaccines and Biological Drugs, Cultured Meat and Cell-Culture



Media Manufacturing.

Fig.1 Laurus Bio Proprietary Platform

1.3 *Divisions / Department* :

Research& Development

- Manager
  R&D
- Research
  Scientist

Production

- General Manager
- Senior Manager
- Production Manager
- Deputy Manager
- Assistant Manager
- Shift In Charge

Quality Assurance/Quality Control

- Manager QA/QC
- QC Chemist
- QC Microbiologist

Engineering Department

Human Resource Department

- HR Administrator

Finance Department

- Accounting
- Taxation

CHAPTER 2: GLP (GOOD LABORATORY PRACTICE).

Good laboratory practice or GLP is a set of principles intended to assure the quality and integrity of nonclinical laboratory studies that are intended to support research or marketing permits for products regulated by government agencies. The term GLP is most commonly associated with the pharmaceutical industry and the required non-clinical animal testing that must be performed prior to approval of new drug products. However, GLP applies to many other non-pharmaceutical agents such as colour additives, food additives, food contamination limits, food packaging and medical devices.

Facilities and equipment:

The GLP principles emphasize that facilities and equipment must be sufficient and adequate to perform the studies.The facilities must be spacious enough to avoid problems such as over crowding,cross contamination or confusion between projects.For the proper

conduct of the study, appropriate equipment of adequate capacity must be available. All equipment, whether used to generate data or to maintain standard conditions should work to fixed specifications.

CHAPTER 3: GMP (GOOD MANUFACTURING PRACTICES).

GMP refers to the Good Manufacturing Practice Regulations promulgated by the US Food and Drug Administration under the authority of the Federal Food, Drug and Cosmetic Act (chapter IV for food and chapter V Sub chapters A, B, C, D and E for drugs and devices). These regulations which have the force of law, require that manufacturers, processors, and packagers of drugs, medical devices, some food, and blood take proactive steps to ensure that their products are safe,pure and effective.GMP regulations require a quality approach to manufacturing,enabling companies to minimize or eliminate instances of contamination, mix-ups and errors. This protects the consumer from purchasing a product which is not effective or even dangerous. Failure of firms to comply with GMP regulations can result in very serious consequences including recall,seizure, fines and jail time.

CHAPTER 4: GDP (GOOD DOCUMENTATION PRACTICES).

GDP can be defined as "good documentation practice is an essential part of the quality assurance and such,related to all aspects of GMP" this definition is based on WHO.

Clearly written documents prevent errors of various activities in pharma;each and every activity is written in specific documents such as SOP's and strictly followed.Spoken communications may be create errors so that all important documents such as Master formulae record, procedure and record must be free from errors and documented. It is difficult to make a list of required documents and totally depend upon Companies activity or environment.

- Good documentation is very essential to the organization and as such should exist for all aspects of GLP. Documentation ensures the availability of the data needed for validation, review and statistical analysis.

- It aims to define the specifications and procedures for all materials and methods of manufacture and control.
- To ensure that all personnel concerned with manufacture known what to do and when to do it.
- To ensure that authorized person have all the information necessary to decide whether or not to release a batch of a drug for sale.
- To ensure the existence of documented evidence,traceability and to provide records.

CHAPTER 5 : SOP (STANDARD OPERATING PROCEDURE).

A standard operating procedure, or SOP, is a set of step-by-step instructions compiled by an organization to help workers carry out complex routine operations. SOP s aim to achieve efficiency, quality output and uniformity of performance ,while reducing miscommunication and failure to comply with industry regulations.

SOP Overview

The SOP should include the following characteristics:

Comprehensive coverage of:

• All critical phases of study design, management, conduct ,monitoring and reporting,

• Scientific policies and procedures (e.g .formats, safety and hygiene ,security ,personnel management systems, etc.)

• Standard scientific techniques ,equipment ,etc.

Readability: The SOPs should follow a standard layout. The procedures should be written in the local language of the operational personnel and expressed with an appropriate vocabulary. All personnel should be encouraged to contribute to improving SOPs.

Usability and traceability: For reasons of traceability and easy use,at wo-tier system of

SOPs is often the preferred approach. For example, one tier reflects general policies and procedures (e.g. protocol writing ,review, approval ,distribution and modification, general rules for equipment use and maintenance, archives, etc.), the second represents technical methods (e.g. analytical methods, specific procedures for use and maintenance of equipment, etc.). It is advised to present the SOPs in binders or as SOP manuals with an up-to-date table of contents. All alterations to SOPs have to be made through formal revisions; notes and changes in hand-written margin comments are not acceptable.

Properly designed SOPs will bring the following benefits to the laboratory:

- Standardized, consistent procedures minimize person to person and test to test variability.
- An opportunity to optimize processes.
- Capture of technical and administrative improvements.
- Demonstration of management commitment to quality as part of the SOP approval process.
- Ease of documenting complicated techniques in study protocol sand reports (as imple reference to the procedure should often suffice).
- Continuity in case of personnel turnover.
- Using a straining manual.
- A means of study reconstruction after the event,even after a lapse of several years.
- A means of communication in case of audit,visits,technology transfer,etc.

CHAPTER 6 : STP (STANDARD TESTING PROCEDURE).

A Standard Testing procedure or STP is the validation practice demonstrates that an analytical method measures the correct substance, in the correct amount, and in the appropriate range for the intended samples. It allows the analyst to understand the conduct of the method and to establish the performance limits of the method. The validation of an analytical method demonstrates the scientific accuracy of the measurement or

characterization. It is mandatory for all regulatory submission processes.

CHAPTER 7 : INSTRUMENTS HANDLING.

7.1. Autoclave handling :

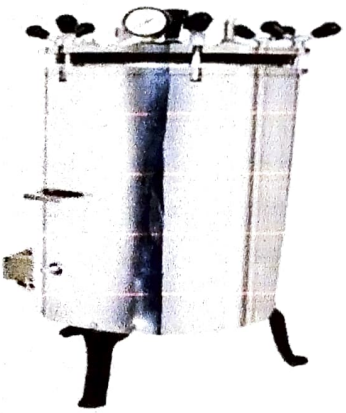


Fig.2 Autoclave

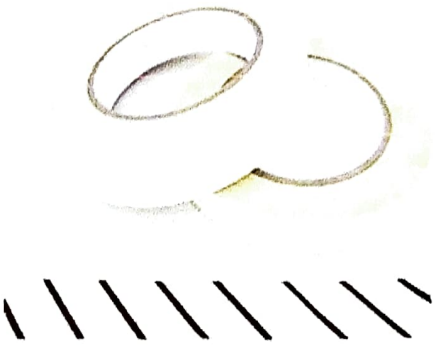


Fig.3 Sterilization indicator tape

Autoclave operates at high temperature and pressure in order to kill microorganisms and spores. Time ,steam ,temperature and pressure are four main parameters required for sterilization using an autoclave. Moist heat destroys microbes by the irreversible coagulation and denaturation of enzymes and structural proteins. Amount of time and temperature required for sterilization is 121^0C,16 to 20psi for 30minutes.

Steam sterilization indicator : Autoclave tape is an adhesive tape used to indicate whether a specific temperature is been reached. Autoclave tape works after changing colour after exposing to temperature commonly used in sterilization process, typically 121^0C in a steam autoclave.

7.1. Incubator :

In biology, an incubator is a device used to grow and maintain microbiological cultures or cell cultures. The incubator maintains optimal temperature, humidity and other conditions such as the carbon dioxide (CO2) and oxygen content of the atmosphere inside. Incubators are essential for a lot of experimental work in cell biology, microbiology and molecular biology and are used to culture both bacterial as well as eukaryotic cells.

The simplest incubators are insulated boxes with an adjustable heater, typically going up to 60 to 65 °C(140 to 150 °F), though some can go slightly higher (generally to no more than 100 °C). The most

commonly used temperature both for bacteria such as the frequently used *E.coli* as well as for mammalian cells is approximately 37 °C (99 °F), as these organisms grow well under such conditions. For other organisms used in biological experiments, such as the budding yeast ***Saccharomyces cerevisiae*** a growth temperature of 30 °C (86°F) is optimal.

Applications are:

- Growing cell cultures.
- Reproduction of germ colonies with subsequent germ count in food industries.
- Reproduction of germ colonies and subsequent determination of bio-chemical oxygen demand(wastewater monitoring).
- Reproduction of microorganisms such as bacteria ,fungi, yeast or viruses.
- Controlled sample storage.
- Growing of Protein Crystals

CHAPTER 8 : STERLIZATION.

Sterilization refers to any process that eliminates, removes, kills, or deactivates all forms of life and other biological agents(such as fungi, bacteria, viruses, spore forms, prions,

uni-cellular eukaryotic organisms such as ***Plasmodium***, etc.) present in a specified region, such as a surface, a volume of fluid, medication, or in a compound such as biological culture media. Sterilization can be achieved through various means ,including: heat ,chemicals ,irradiation ,high pressure and filtration. Sterilization is distinct from disinfection, sanitization, and pasteurization, in that sterilization kills, deactivates, or eliminates all forms of life and other biological agents which a represent.

8.1. Types of sterilization :

8.1.1. Steam Sterilization :

A widely used method for heat sterilization is the autoclave, sometimes called a converter or steam sterilizer. Autoclaves use steam heated to 121-134 °C under pressure. To achieve sterility, the article is placed in a chamber and heated by injected steam until the article reaches a time and temperature set point. Almost all the air is removed from the chamber, because air is undesired in the moist heat sterilization process (this is one trait that differ from a typical pressure cooker used for food cooking). The article is held at the temperature set point for a period of time which varies depending on what bio- burden is present on the article being sterilized and its resistance (D-value) to steam sterilization. A general cycle would be any where between 3 and 15 minutes, (depending on the generated heat) at 121 °C at 100 KPa, which is sufficient to provide a sterility assurance level of 10^{-4} for a product with a bio-burden of 106 and a D-value of 2.0 minutes. Proper autoclave treatment will inactivate all resistant

bacterial spores in addition to fungi, bacteria, and viruses, but is not expected to eliminate all prions, which vary in their resistance. For prion elimination, various recommendations state 121-132 °C for 60 minutes or 134 °C for at least 18minutes.For autoclaving, cleaning is critical .Extraneous biological matter or grime may shield organisms from steam penetration. Proper cleaning can be achieved through physical scrubbing, sonication, ultrasound, or pulsed air. Pressure cooking and canning is analogous to autoclaving, and when performed correctly renders food sterile. Moist heat causes the destruction of

microorganisms by denaturation of macro molecules, primarily proteins .This method is a faster process than dry heat sterilization.

8.1.2. Dry Heat Sterilization :

Fig.4 Autoclave

Dry heat was the first method of sterilization and is a longer process than moist heat sterilization. The destruction of microorganisms through the use of dry heat is a gradual phenomenon. With longer exposure to lethal temperatures, the number of killed microbes increases Forced ventilation of hot air that can be used to increase the rate at which heat is transferred to an organism and reduce the temperature and amount of time needed to achieve sterility. At higher temperatures, shorter exposure times are required to kill organisms. This can reduce heat-induced damage to food products.The standard setting for a hot air oven is at least two hours at 160°C.A rapid method heats air to 190°C for 6minutes for unwrapped objects and 12minutes for wrapped objects.

8.1.3.Flaming:

Flaming is done to loops and straight-wires in microbiology labs. Leaving the loop in the flame of a Bunsen burner or alcohol lamp until it glows red ensures that any infectious agent is inactivated. This is commonly used for small metal or glass objects, but not for large objects (sees incineration below). However, during the initial heating infectious material may be sprayed from the wire surface before it is killed, contaminating near by surfaces and objects.Therefore, special heaters have been developed that surround the inoculating loop with a heated cage, ensuring that such sprayed material does not further contaminate the area.

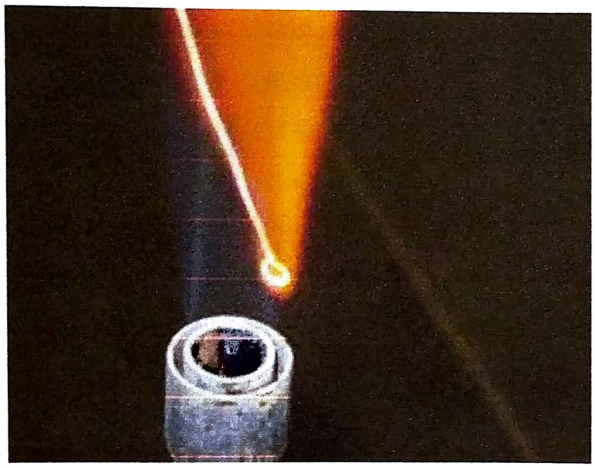


Fig.5 Flaming

8.1.4. Chemical sterilization :

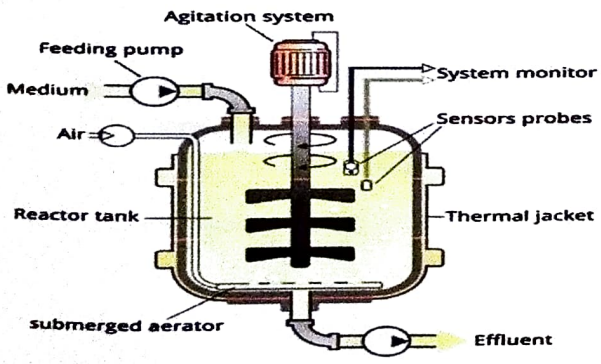
Chemicals are also used for sterilization. Heating provides are liable way to rid objects of all transmissible agents, but it is not always appropriate if it will damage heat-sensitive materials such as biological materials, fibre optics, electronics, and many plastics. In these situations, chemicals, either as gases or in liquid form,can be used as sterilant.While the use of gas and liquid chemical sterilant avoids the problem of heat damage, users must ensure that article to be sterilized is chemically compatible with the sterilant being used. In addition, the use of chemical sterilant poses new challenges for workplace safety, as the properties that make chemicals

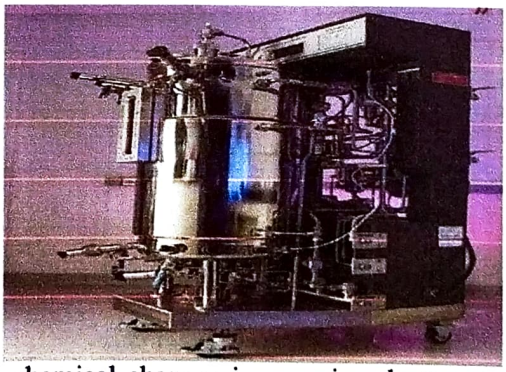
effective sterilant usually make them harmful to humans.

8.1.5.IPA (Isopropyl alcohol) :

Isopropyl alcohol is a compound with the chemical formula C3H8O. It is a colourless, flammable chemical compound with a strong odour. It is used as chemical sterilant. Only 70% solution of IPA acts as disinfectant killing all surface microbes. It kills the microbes by dissolving the plasma membrane of the cell wall. Plasma membrane of gram-negative bacteria consists of thin layer of peptidoglycan which can be easily dissolved by alcohol. Water plays a key role which is used to denature the proteins of cell membrane and acts as a catalyst in the reaction.70%IPA takes more time in evaporating from the surface ,increasing the contact time .100% IPA coagulates the protein instantly by creating a protein layer that protects the other protein from further coagulating ,due to which these microbes do not die but remain in dormant state. While 70% IPA penetrates in the cell wall at slower rate and coagulates the proteins of the cell wall hence killing the microorganism.

CHAPTER 9 : PRODUCTION OF RECOMBINANT PROTEIN FROM PICHIA PASTORIS (UPSTREAM PROCESSING).





Fermentation is a metabolic process that produces chemical changes in organic substrates through the action of enzymes. In biochemistry, it is narrowly defined as the extraction of energy from carbohydrates in the absence of oxygen. In food production, it may be more

broadly refer to any process in which the activity of microorganisms bring about a desirable changes to food stuff or beverages. The science of fermentation is known as zymology.

8.3 History of Fermenter :

De Beeze and Liebmann (1944) used the first large scale (above 20litre capacity) fermenter for the production of yeast. But it was during the first world war, a British scientist named Chain Weismann (1914-1918) developed a fermenter for the production of acetone. Since importance of aseptic conditions was recognized, hence steps were taken to design and construct piping, joints and valves in which sterile conditions could be achieved and manufactured when required. For the first time, large scale aerobic fermenters were used in central Europe in the year 1930's for the production of the compressed yeast. The fermenter consisted of a large cylindrical tank with the air introduced at the bottom of perforated pipes. The decision to use submerged culture technique for penicillin production, where aseptic conditions, good aeration and agitation were essential, was probably a very important factor in forcing the development of carefully designed and purpose-built fermentation vessels.In 1943, when the British Govt decided that surface culture was inadequate, none of the fermentation plants were immediately suitable for deep fermentation. The first pilot fermentor was erected in India at Hindustan Antibiotic Ltd., Pimpri, Pune in the year 1950.

8.4 Design of Fermenter :

All Fermenters deal with heterogeneous systems dealing with two or more phases ,e.g. , liquid ,gas ,solid. Therefore, optimal conditions for fermentation necessitate efficient transfer of mass, heat and momentum from one phase to the other. Chemical engineering principles are employed for design and operation of Fermenters.

A Fermenter should provide for the following :

(i) Agitation (for mixing of cells and medium),

(ii) Aeration (aerobic fermentors);for O2 supply,

(iii) Regulation of factors like temperature, pH, pressure, aeration, nutrient feeding,

liquid level etc.,

(iv) Sterilization and maintenance of sterility.

(v) Withdrawal of cells/medium (continuous fermentors).

Modern fermentors are usually integrated with computers for efficient process monitoring, data acquisition, etc. Generally, 20-25% of fermentor volume is left unfilled with medium as "**head space**" to allow for splashing, foaming and aeration. The fermentor design varies greatly depending on the type and the fermentation for which it is used. Bioreactors are so designed that they provide the best possible growth and biosynthesis for industrially important cultures and allow ease of manipulation for all operations.

8.5 Size of Fermenter :

The size of fermenters ranges from 1-2 litre laboratory fermenters to 5,00,000 litre or, occasionally ,even more, fermenters of up to 1.2 million liters have been used. The size of the fermentor used depends on the process and how it is operated. A summary of fermentor or size of fermentor (liters) industrial product sizes for some common microbial fermentations.

8.6 Construction of Fermenters :

Industrial fermenters can be divided into two major classes, anaerobic and aerobic. Anaerobic fermenters require little special equipment except for removal of heat generated during the fermentation process, whereas aerobic fermenters require much more elaborate equipment to ensure that mixing and adequate aeration are achieved.

8.7 Cooling jackets :

Large-scale industrial fermenters are almost always constructed of stainless steel.A fermentor is a large cylinder closed at the top and the bottom and various pipes and valves are fitted into it.The fermentor is fitted externally with a cooling jacket through which steam (for sterilization) or cooling water (for cooling)is been run.

Cooling jacket is necessary because sterilization of the nutrient medium and removal of the heat generated are obligatory for successful completion of the fermentation in the

fermentor. For very large fermenters, insufficient heat transfer takes place through the jacket and therefore, internal coils are provided. through which either steam or cooling water has been run.

8.8 Aeration System :

Aeration system is one of the most critical part of a fermenter. In a fermenter with a high microbial population density, there is a tremendous oxygen demand by the culture, but oxygen being poorly soluble in water hardly transfers rapidly throughout the growth medium.It is necessary, that elaborate precautions are taken using a good aeration system to ensure proper aeration an oxygen availability throughout the culture. However m,two separate aeration devices are used to ensure proper aeration in fermenter. These devices are sparger and impeller.The sparger is typically just a series of holes in a metal ring or a nozzle through which filter-sterilized air(or oxygen-enriched air) passes into the fermenter under high pressure.The air enters the fermentor as a series of tiny bubbles from which the oxygen passes by diffusion into the liquid culture medium.The impeller(also called agitator)is an agitating device necessary for stirring in the fermenter.

The stirring accomplishes two things :

(i) It mixes the gas bubbles through the liquid culture medium and

(ii) It mixes the microbial cells through the liquid culture medium. In this way, the stirring ensures uniform access of microbial cells to the nutrients.

The size and position of the impeller in the fermentor depends upon the size of the fermentor. In tall fermenters,more than one impeller is needed if adequate aeration and agitation is to be obtained. Ideally,the impeller should be 1/3 of the fermenters diameter fitted above the base of the fermentor. The number of impeller may vary from size to size to the fermenter.

8.9 Baffles :

The baffles are normally incorporated into fermenters of all sizes to prevent a vortex and to

improve aeration efficiency. They are metal strips roughly one-tenth of the fermenters diameter and attached radially to the walls.

9.0 Controlling Devices for Environmental Factors :

In any microbial fermentation, it is necessary not only to measure growth and product formation but also to control the process by altering environmental parameters as the process proceeds. For this purpose, various devices are used in a fermentor. Environmental factors that are frequently controlled includes temperature, oxygen concentration, pH, cell mass, levels of key nutrients, and product concentration.

- ***Use of Computer in Fermentor :***

Computer technology has produced a remarkable impact in fermentation work in recent years and the computers are used to model fermentation processes in industrial fermentors .Integration of computers into fermentation systems is based on the computers capacity for process monitoring, data acquisition ,data storage, and error-detection.Some typical ,on-line data analysis functions include the acquisition measurements, verification of data, filtering, unit conversion, calculations of indirect measurements, differential integration calculations of estimated variables, data reduction, tabulation of results, graphical presentation of results, process stimulation and storage of data.

CHAPTER 10 : MICROBIAL GROWTH.

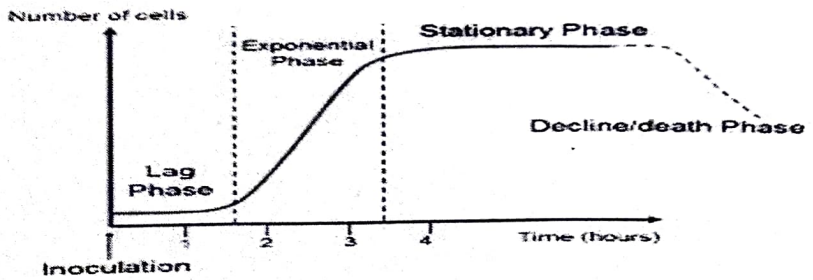


Fig.7 Microbial growth curve

CHAPTER 11 : DOWNSTREAM PROCESSING (RECOVERY OF BIOLOGICALS).

Different stages of Purification Process in Downstream Processing

- ***Centrifugation***
- ***Microfiltration***
- ***Ultra filtration***
- ***Types of filters***
- ***Filter press***
- ***Spray drying***

11.1 CENTRIFUGATION :

Centrifugation is a mechanical process which involves the use of the centrifugal force to separate particles from a solution according to their size, shape, density, medium viscosity and rotor speed. The more dense components of the mixture migrate away from the axis of the centrifuge, while the less dense components of the mixture migrate towards the axis. Chemists and biologists may increase the effective gravitational force of the test tube so that the precipitate (pellet) will travel quickly and fully to the bottom of the tube. The remaining liquid that lies above the precipitate is called a supernatant or supernatant

There is a correlation between the size and density of a particle and the rate that the particle separates from a heterogeneous mixture, when the only force applied is that of gravity. The larger the size and the larger the density of the particles, the faster they separate from the mixture. By applying a larger effective gravitational force to the mixture, like a centrifuge does, the separation of the particles is accelerated. This is ideal in industrial and lab settings because particles that would naturally separate over a long period of time can be separated in much less time. The rate of centrifugation is specified by the angular velocity usually expressed as revolutions perminute (RPM), or

acceleration expressed as g. The conversion factor between RPM and g depends on the radius of the centrifuge rotor. The particles' settling velocity in centrifugation is a function of their size and shape, centrifugal acceleration, the volume fraction of solids present, the density difference between the particle and the liquid,and the viscosity. The most common application is the separation of solid from highly concentrated suspensions, which is used in the treatment of sewage sludge ford-watering where less consistent sediment is produced.

The centrifugation method has a wide variety of industrial and laboratorial applications; not only is this process used to separate two miscible substances, but also to analyze the hydrodynamic properties of macromolecules. It is one of the most important and commonly used research methods in biochemistry, cell and molecular biology. In the chemical and food industries, special centrifuges can process a continuous stream of particle-laden liquid.

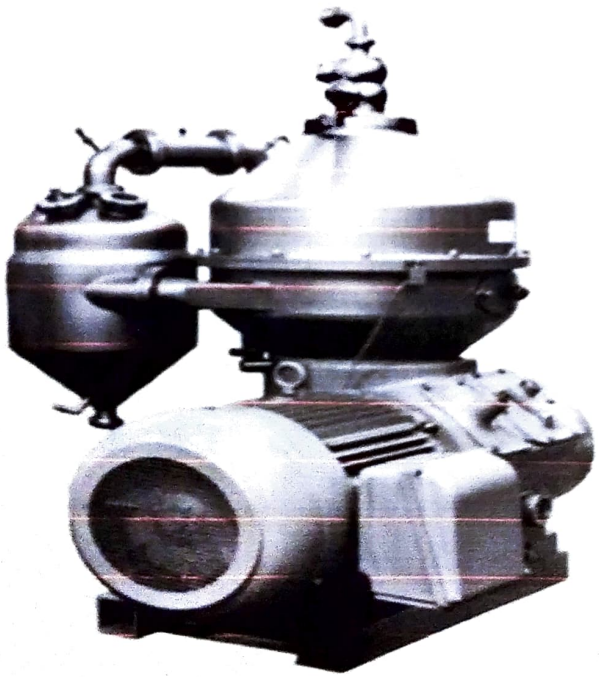


Fig.8 Continuous Centrifuge System (ALFA LAVAL MBUX-510)

11.2 MICRO FILTERATION :

Microfiltration usually serves as a pretreatment for other separation processes such as ultrafiltration and a post-treatment for granular media filtration. The typical particle size used for microfiltration ranges from about 0.1 to 10 μm. In terms of approximate molecular weight these membranes can separate macro molecules of molecular weights generally less than 100,000g/mol. The filters used in the micro filtration process are specially designed to prevent particles such as, sediment, algae, protozoa or large bacteria from passing through a specially designed filter. More microscopic, atomic or ionic materials such as water (H_2O), mono valent species such as Sodium (Na^+) or Chloride (Cl^-) ions, dissolved or natural organic matter, and small colloids and viruses will still be able to pass through the filter.

Fig. 9 Micro filtration Filter Membranes

The suspended liquid is passed through at a relatively high velocity of around 1–3 m/s and at low to moderate pressures (around 100-400 kPa) parallel or tangential to the semi-permeable membrane in a sheet or tubular form. A pump is commonly fitted onto the processing equipment to allow the liquid to pass through the membrane filter. There are also two pump configurations, either pressure driven or vacuum. A differential or regular pressure gauge is commonly attached to measure the pressure drop between the

outlet and inlet streams.

11.3 ULTRAFILTRATION :

Ultra filtration (UF) is a variety of membrane filtration in which forces like pressure or concentration gradients lead to a separation through a semipermeable membrane. Suspended solids and solutes of high molecular weight are retained in the so-called **retentate**, while water and low molecular weight solutes pass through the membrane in the **permeate** (filtrate). This separation process is used in industry and research for purifying and concentrating macro molecular(10^3-10^6Da)solutions especially protein solutions.

Ultra filtration is not fundamentally different from microfiltration. Both of these separate based on size exclusion or particle capture. It is fundamentally different from membrane gas separation, which separate based on different amounts of absorption and different rates of diffusion.Ultra filtration membranes are defined by the molecular weight cut-off (MWCO) of the membrane used. Ultra filtration is applied in cross-flow or dead-end mode.

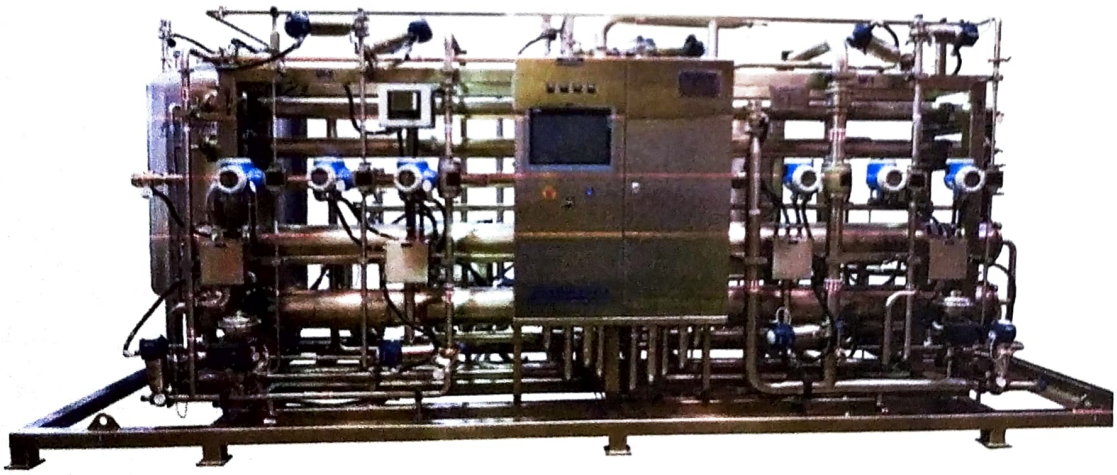


Fig.10 Ultra Filteration System (PERMIONICS)

11.3.1.1 Capsule filter :

A cartridge is encased within a housing or a casing and used to remove unwanted particles, pollutants, and chemicals from liquids. The cartridge is exposed to water, liquid or solvent that needs filtration ,a sit flows inside the housing and passes through the filter element. Capsule filters are compact, easy to use and specially designed to satisfy the highest standards of filtration reliability ,security and user convenience. Capsule filters combine a wide range of filter media, pore size rating and surface areas to satisfy numerous application.

Fig.11 Capsule Filters

11.3.1.2 Candle filter :

The candle filter is a filter that operates in a discontinuous manner .The candle-shaped filter elements are arranged vertically in a pressure vessel. The process steps typically performed with this unit are filtration, washing ,drying and discharge All these steps take place under pressure. The candle filter is used for the clarification of liquids with a low solids content .It is a compact unit ,operates fully automatically and enables high through ouput rates, dry cake discharge and a wide range of cake treatment options.

11.4 FILTER PRESS SYSTEM :

Filter press is a pressure filter in which the simplest form consists of plates and frame arranged alternatively. The plates are covered with filter cloths or filter pads .The plates and frame are assembled on a horizontal frame work and held together by means of hand screw or hydraulic ram so that there is no leakage between the plates and frames, which form a series of liquid – tight compartments. The slurry is fed to the filter through the continuous channel formed by the holes in the corners of the plates and frames. The filtrate passes through the filter cloth or pad, runs down grooves in the filter plates and is then discharged through outlet taps to a channel. Sometimes , if aseptic condition are required ,the outlets may lead directly into a pipe. The solids are retained within the frame and filtration is stopped when the frame are completely filled or when the flow of filtrate becomes uneconomically low. On an industrial scale, the plate and frame floor space, but it is cheapest filters per unit of filtering space and requires the least floor space, but it is intermittent in operation (a batch process) and there may be considerable wear of filter cloths as result of frequent dismantling. This type of filter is most suitable for fermentation broths with a low solids content and low resistance to filtration. It may also be used for collecting high value solids that would not justify the use of a continuous filter.

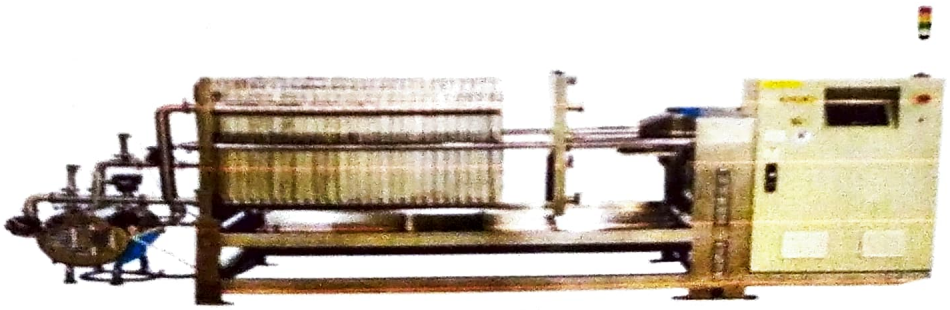


Fig.12 Filter Press System

11.5 SPRAY DRIER SYSTEM :

A spray dryer is the most widely used for drying of biological materials when the starting material is in the form of a liquid .The material to be dried does not come in contact with heat surfaces, instead ,it is atomized into small droplets through a nozzle or by contact with a rotating disc. The droplets then fall into a spiral stream of hot gas at 150° to 250°. The high surface volume Ratio of the droplets results in a few seconds, with drying rate produced by the atomizer. The evaporative cooling effect prevent the material from becoming overheated and damaged. The gas flow rate must be carefully regulated so that the gas has the capacity to contain at the cool-air exhaust temperature (75° to 100°). In most processes the recovery of very small particles from the exit gas must be conducted using cyclones or filters.

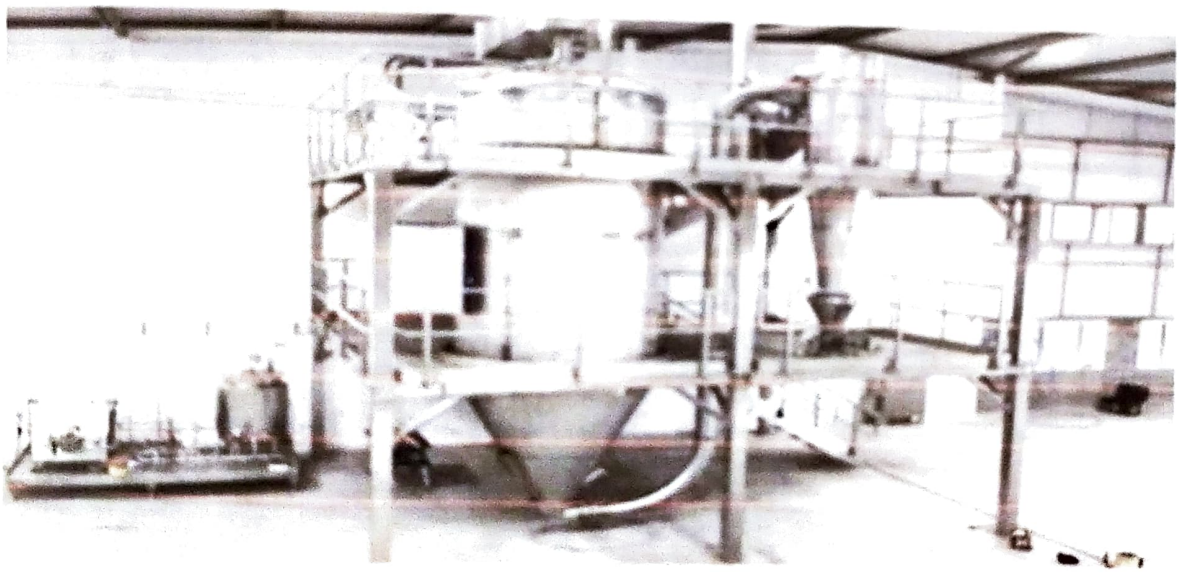


Fig.13 Spray Drier System

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