

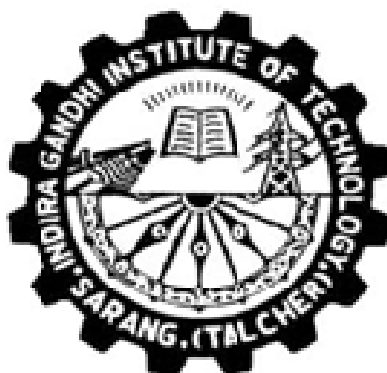


Fundamentals of Biochemical Engineering

Subject code-PCCH 4402

Chemical Engineering Department

7th Semester



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SYLLABUS

Module I

Overview of microbiology, Aerobic & Anaerobic fermentation processes, fermenter design, sterilization of microbial medium, kinetics of microbial growth, enzymes and its kinetics, immobilization of enzymes, chemostats.

Module II

Transport phenomena in Biochemical Engineering, Heat and Mass transfer in Bioprocessing, oxygen transfer in fermenter, monitoring and control of fermentation process.

Module III

Downstream processing: - Recovery and Purification of products, allied unit operation for product recovery, production of biogas and ethanol, Effluent treatment by biological method

Text book

1. Bailey JB and oillis OR, Biochemical Engineering Fundamentals
2. Aiba S, Biochemical Engineering, Academic press
3. Rao D G, Introduction to Biochemical Engineering, Tata Mc Grow Hill
4. Michael L. Shuler/ Fikret Kargi, Bio Process Engineering , Pearson Education

LESSON PLAN

Class No.	Brief description of the Topic
1.	Introduction of FBE
2.	Overview of microbiology
3.	Application of Microbiology
4.	Aerobic & Anaerobic fermentation processes
5.	SSF and SmF and applications
6.	Fermentation Design
7.	Microbial sterilization
8.	kinetics of microbial growth
9.	Kinetics of microbial growth
10.	Problem discussion
11.	Problem discussion
12.	Enzyme and its characteristics
13.	Enzyme kinetics
14.	Problem discussion
15.	Immobilization of enzymes & applications
16.	Chemostats
17.	Question Discussion
18.	Heat transfer in bioprocessing
19.	Mass transfer in bioprocessing
20.	Oxygen transfer in fermenter
21.	Monitoring and control of fermentation process.

22	Question discussion
23	Unit operation for product recovery
24	Effluent Treatment Physical methods
25	Effluent Treatment Chemical methods
26	Effluent Treatment Biological methods
27	Production of biogas
28	Production of ethanol
29	Question discussions
30	Semester question discussion

Module-I

INTRODUCTION

Biotechnology is the art and science of converting reactants (substrate) into useful products by the action of microorganisms or enzymes. Microorganisms have been honestly serving the mankind. Thus any process in which microbes or living organisms play a vital role in getting transformation of the feed into useful products is termed as **BIOPROCESSING**. For example the way of converting milk into curds, or fruit juice into wines, or sugar into alcohol.

BIOCHEMICAL ENGINEERING is of more recent origin, since the biological industries did not recognize the importance of engineering inputs until the experience of penicillin manufacture.

Microbiology

- (Micro-small, bios-life) is the study of microscopic organism, which are defined as any living organism i.e. either a single cell(unicellular), a cell cluster or has no cell at all(a cellular). This includes eukaryotes, such as fungi and protista and prokaryotes.
- Microbiology is a broad term which includes virology, mycology, parasitology etc.
- It is the study of microorganisms which are not only microscopic and exist as single cells, but also that ultramicroscopic organism which are not cellular and hence cannot exist independently. E.g. viruses
- Microbiology deals with study and functioning of cells, their interaction with environment, other living organism and man.
- It is studied with respect to two major aspects.

a) As basic biological science

b) As applied biological science

Basic biological science:

It provides a system to understand the nature of life processes, the principle behind it and the genetics which is involved in the heritage of traits to next generation.it has several sub streams such as

i) Medical microbiology: Study of pathogenic microorganism, the causes of diseases and way to eliminate them.

ii) Agricultural microbiology: Study of plant diseases, understanding various beneficial interactions with plant system like soil fertility, crop-protection and increasing field.

iii) Environmental microbiology: Study of relationship of microorganisms with its habitat, pollution effect and its impact on environment from the stand point of ecology balance and health.

iv) Food and dairy microbiology: Study of microorganisms that produce various food and dairy products.

Applied biological science:

It deals with study of useful microorganisms as well as that of pathogenic organism.

i) In dairy and food industry: Food microbiology not only includes the study of those microbes which provides food to their high protein content but also includes other those microbes which use our food supply as a source of nutrients for their growth and result in deterioration of the food by increasing their number, utilizing nutrients, contributing of flavours by means of breakdown of food.

ii) Medical microbiology: Microbes causes infections resulting in diseases among human and animals. On other side they help in creating a “disease free world”, where people are from pain by this disease.

Application of microbiology

- Microbes in food and dairy industries
- Microbes in production of industrial products
- Microbes in genetic engineering and biotechnology
- Microbes in environmental microbiology
- Microbes in medical microbiology
- Microbes in agriculture
- Microbes in bioterrorism

a. Food and dairy industry:

- ❖ Provides food due to high protein content.
- ❖ Food nutrient for their growth (deterioration of food), enzymatic changes, contributing flavour.
- ❖ Certain moulds used for manufacturing of food and ingredients of food.
- ❖ Some moulds used in production of oriental food, soya sauce etc.

- ❖ Used for enzyme making like amylase.
- ❖ **Yeast:** These are used in manufacturing of foods such as bread, beer, wines, vinegar, surface ripened, and cheese. Some yeasts are grown for enzymes and food.
- ❖ **Bacteria:** Some pigmented bacteria cause changes in colour on the surfaces of liquid food. Acetobacteria oxidises ethyl alcohol to acetic acid. Some bacteria causes ropiness in milk and slimy growth cottage cheese.

b. Microbes in production of industrial products:

- ❖ Enzymes amino acid, vitamins, antibiotics, organic acid and alcohol are commercially produced by microorganism.
- ❖ **Primary microbial product:** These products are used by microorganism for their growth. E.g. amino acids, enzymes, vitamins
- ❖ **Secondary microbial products:** Not used by cell for their growth. E.g. alcohol, antibiotics, organics acids.
- ❖ Microbes produce some important amino acids such as glutamic acid, lysine, and methionine.

c. Microbes in Genetic Engineering and Biotechnology:

- ❖ Microbes used for mammalian proteins such as insulin and human growth factor.
- ❖ Making vaccine for microbial and viral genes and induce a new strain of microbes.
- ❖ Vaccine and diagnostic kits also depend on the improved strains of microorganism.
- ❖ Lactic acid as food preservative.
- ❖ Acetic acid plays a major role in tanning and textile industry.
- ❖ Interferons are produced in animal cell if included by viral infection. These are used in testing interleukins (which stimulate T-lymphocytes).
- ❖ Production of viral, bacterial or protozoan, antigen for protecting against dysentery, typhoid, bacteria etc.
- ❖ N₂ fixing bacteria reduce nitrogen gas to ammonia required plant growth.

d. Environmental Microbiology:

- ❖ Plays an important role in recycling of biological elements such as oxygen, carbon, N₂, sulphur, and phosphorous.
- ❖ **Microbes in biochemical cycle:** Photosynthesis and chemosynthesis microorganism convert CO₂ into organic carbon. Methane is generated anaerobically from CO₂ and H₂ by methanogenic archaea. The organic forms of N₂ are interconverted by metabolic activities of microorganism which maintain the natural N₂ balance.
- ❖ **Microbes in pollution microbiology:** Biological sewage treatment and self-purification. Both results in mineralisation of organic, pollutants and in utilisation of dissolved oxygen.

e. Medical Microbiology:

- ❖ **Vaccination:** Small pox, diphtheria, whooping cough.

f. Computer application:

- ❖ **Optimisation via computer:** Computers are used on scale of, to store, evaluate effects of individual parameters on metabolic behaviour of culture.
- ❖ **Control via computer:** Control fermentation process.

g. Microbes in agriculture:

- ❖ During compost formation by the crop residue like wheat straw, rice straw, sugar cane bagasse are very difficult to degrade due to presence of highly resistant lignified tissues. So, breakdown of these complex organic materials can be done by microbes by a short span.
- ❖ Biogas production through anaerobic fermentation is must relevant to fulfil their energy demand in rural population.
- ❖ The productivity of leguminous crop largely depend on efficient and suitable management of the ecosystem by specific rhizobial association.
- ❖ Some bacterial helps in killing a wide range of insects like beetles, mosquitoes, flies, ants, termites which is very useful for agricultural industries

h. Microbes in Bioterrorism: It has been defined as deliberate release of disease causing germs, microorganism with the intent of killing large number of people. Accordingly microorganisms are used as weapons of mass destruction of people and causes small pox, plague, cholera and also anthrax.

Mode of transmission:

- Air droplets and dust
- Food fruits and vegetables
- Drinking water

FERMENTATION

Fermentation is the word derived from the Latin verb *FERVERE* (to boil), which describe the evolution of carbon dioxide bubble in anaerobic conditions by the action of yeast on fruit juice.

General Requirements of Fermentation Process:-

Since fermentation is a biochemical process brought about by the intervention of living organisms, it is essential that any fermentation process should have:

- ✓ A microorganism for carrying out the bioconversion.
- ✓ A substrate to get converted into useful products.
- ✓ Maintenance of fermentation conditions.
- ✓ Effluent treatment section.
- ✓ A provision for recovery and purification of the products.
- ✓ Facilities for packaging and marketing.

Fermentation carried out in the presence of air / O_2 is known as aerobic fermentation, where as in absence of air is called anaerobic fermentation.

Anaerobic Fermentation:-

- Yeast fermentation process to produce alcohol requires a small amount of aeration for the cells to multiply. After word no air is required.
- On the other hand air is detrimental for the process which will otherwise oxidise the substrate.
- Most of the anaerobic fermentation produces carbon dioxide gas.
- Many times gas covers the surface and acts as a blanket to prevent the effect of O_2 .
- The evolved CO_2 will also help in better mixing conditions, which is more evident in large industrial tanks because of longer pathways for the gas bubbles to go before they leave the fermenter.

Aerobic Fermentation:-

Sparaging air / O_2 is very common phenomenon in fermentation process to supply O_2 for cells to meet their specific O_2 demand .Such fermentation process which are associated with the bubbling of O_2 are termed as aerobic fermentation.

Solid state and submerged fermentation and their Applications

Solid state fermentation:-

SSF is a method of growing microorganisms in an environment of limited moisture without having free flowing water. The microorganisms grow on a solid surface which is moistened and which has also got free access to air.

It is also known as “*Koji*” fermentation, for the production of soya products such as tempeh, soya sauce etc. certain metabolites. It has some advantages that lower manufacturing cost because of the use of crude solid agro wastes like wheat bran. The solid surface directly comes in contact with the air and hence the aeration costs are avoided. The other economic **advantages** are

- Low capita investment and recurring expenditures.
- Low water utilization and hence negligible outflow of water.
- Low energy requirements for the fermentation process because of absence of agitation.
- Absence of foam formation because of absence of excess water.
- High reproducibility of the result.
- Simple fermentation media.
- Less fermentation space, and less complex plant and machinery.
- Absence of rigorous control techniques.
- Any level of scale of operation.
- Absence of elaborate aeration requirements.
- Ease in controlling bacterial contamination.
- Facilities of using wet and dry fermented solids directly.
- Ease in induction and suppression of spores.
- Lower costs of downstream processing.

Submerged Fermentation:-

In case of submerged fermentation (SmF) the microorganisms and the substrate are present in the submerged state in the liquid medium, where a large quantity of solvent is present. This has many **advantages** over SSF.

- Since the contents are in submerged state in the liquid medium, the transfer of heat and mass is more efficient, and is amenable for modelling the process.
- The scaling-up the process is very easy.

Differences between SSF and SmF

<i>Characteristics feature</i>	<i>SSF</i>	<i>SmF</i>
Condition of microorganisms and substrate	static	Agitated
Status of the substrate	Crude	Refined
Nature of the microorganism	Fungal system	_____
Availability of water	Limited	High
Supply of oxygen	By diffusion	By bubbling/ sparging
Contact with oxygen	Direct	Dissolved O ₂
Requirement of fermentation medium	Small	Huge
Energy requirement	Low	High
Study of kinetics	Complex	Easy
Temp and concentration gradient	Steep	Smooth
Controlling of reaction	Difficult	Easy
Chances of bacterial contamination	Negligible	High
Quantity of liquids to be disposed	Low	High
Pollution problems	Low	high

Applications:-

- Citric acid can be produced by both SSF and SmF. Generally, the later technique is used in industrially. SSF method has not yet become commercial success because of its labour intensity.
- Soya-based oriental foods like tempeh and soya sauce are produced by SSF alone.
- Production of Roquefort cheese from sheep milk is a classical example of SSF.
- Mushroom cultivation is another example of the growth of fungus on solid medium like paddy straw.
- Fish and meat production are preserved in the form of sausages as fermented foods.

FERMENTER DESIGN

A fermenter is a type of bioreactor for containing and controlling microorganisms during a fermentation process.

BASIC FUNCTIONS OF FERMENTERS

The main function of a fermenter is to provide a controlled environment for growth of a microorganism, or a defined mixture of microorganism, to obtain a desired product.

(Bioreactors refer to production units of mammalian and plant cell culture)

CRITERIA USED IN DESIGNING AND CONSTRUCTING A FERMENTER-

- Vessel should be capable of being operated aseptically and should be reliable for long term operation
- Interplay of the transport parameters
- Supply of adequate quantity of oxygen so that cells do not suffer from inadequacy of oxygen supply
- Adequate aeration and agitation to meet the metabolic requirements of the microbes
- Adequate amount of mixing should be ensured without causing damage to the cells
- Vessel geometry should be such that it should facilitate scale-up
- Flexibility in operation of the fermenter for various purposes, so that the vessel should be suitable for a range of processes
- Low power consumption
- Temperature and pH control
- Low evaporation losses
- Minimal use of labour in operation, harvesting, cleansing and maintenance
- Proper sampling facility
- Cheapest and best materials should be used
- Adequate service provisions must be available for individual plants.

TYPES OF FERMENTER-

Based on shape it can be classified as-

- (i) Tabular &
- (ii) Stirred tank

(Cooling coils are provided to maintain constant temperature inside the bioreactor. It can be operated aseptically for many days and simple in construction.

Disadvantages-

- high power requirement
- shearing on the organisms caused by vigorous agitation and inhibition exercised by the product)

- (i) **Fluidized Bed Bioreactor:** - It is more popular in chemical industry rather new to biochemical industry. These are mostly used in conjunction with immobilized cells or enzyme system and are operated continuously.
- (ii) **Loop or Air Lift Bioreactor:** - In the conventional bioreactor, oxygen is supplied by vigorous agitation of the bioreactor content. The heat is generated which is a problem in conventional type. In this cooling becomes simpler due to the position of inner or outer loop.
- (iii) **Membrane Bioreactor:** - These consist of a semipermeable membrane made up of cellulose acetate or other polymeric materials. The primary purpose of the membrane is to retain the cells within the bioreactor, thus increasing their density, while at same time allowing metabolic products to pass through the membrane.
- (iv) **Pulsed Column Bioreactor:** - It has a column bioreactor generator connected to the bottom of the column. It can be utilised as an aerobic bioreactor, enzyme bioreactor or as separation unit.
- (v) **Bubble Column Bioreactor:** - Multistage bubble column bioreactor are suitable in the equivalent batch process. In this it is possible to provide different environmental conditions in various stage. It is not suitable for fungal fermentation due to oxygen demanding system.
- (vi) **Photo Bioreactor:** - For the growth and production of photosynthetic organisms, a light source is required. In this, there is an important 'reactant', the photons which must be absorbed in order to react and produce products. The design of the light source is critical in the

performance of this type of bioreactor. Example- Annular Reactor. In this source of radiation is a cylinder with a annular section, which enclose the lamp completely. The nutrient passing from the product is removed from the top. It is used for *Spirulina*(SCP) and other algal protein production.

- (vii) **Packed tower Bioreactor:** - It consists of cylindrical column packed with an inert material like wood shavings, twigs, cake, polythene or sand. Initially, both medium and cells are fed into the top of the packed bed. Once the cells adhered to the support and were growing well as a thin film fresh medium is added at the top of the packed bed and the fermented medium removed from the bottom of the column. It is used for vinegar production, sewage effluent treatment and enzymes conversion of penicillin to 6-amino penicillanic acid.

The design of fermenter involves the co-operation between experts in microbiology, biochemistry, mechanical engineering and economics.

CONSTRUCTION OF FERMENTERS

The criteria considered before selecting materials for construction of a fermenter are:

- (a) The material that have no effect on sterilisation
- (b) Its smooth internal finish-discouraging lodging of contamination.
- (c) Internal surface should be corrosion resistant.

There are two types of such materials:

- (i) Stainless Steel, and
- (ii) Glass.

The construction of the fermenter depends upon the following-

- (i) **Control of Temperature.** Since heat is produced by Microbial Activity and the mechanical agitation, thus it is sometimes necessary to remove it. On the other hand, in certain processes extra heat is produced by using thermostatically controlled water bath or by using internal heating coil or jacket meant for water circulation.

- (ii) **Aeration and Agitation.** The main purpose is to provide oxygen require to the metabolism of microorganisms. The agitation should ensure a uniform suspension of microbial cells suspended in nutrient medium. There are following necessary requirements for this purpose:
- The agitator (impeller) for mixing:** The size and position of the impeller in the vessel depends upon the size of the fermenter. More than one impeller is needed if adequate aeration agitation is to be obtained. Ideally, the impeller should be $1/3$ or $1/2$ of the vessel diameter (D) above the base of the vessel. The number of impeller may vary from size to size to the vessel.
 - Stirrer glands and bearings meant for aseptic sealing:** Four basic types of seals assembly have been used-
 - The packed gland seal
 - The simple bush seal
 - The mechanical seal and
 - The magnetic drive.
 - Baffles for checking the vortex resulting into foaming:** The baffles are incorporated into the agitated into the agitated vessels to prevent a vortex ant to improve aeration efficiency. They are metal strips roughly one-tenth of the vessel diameter and attached radially to the walls.

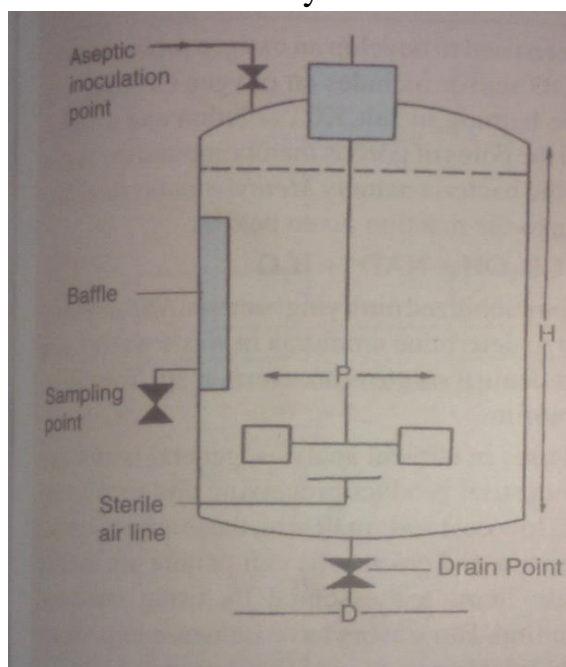


Fig. 20.1 : Different parts of fermenter (diagrammatic).

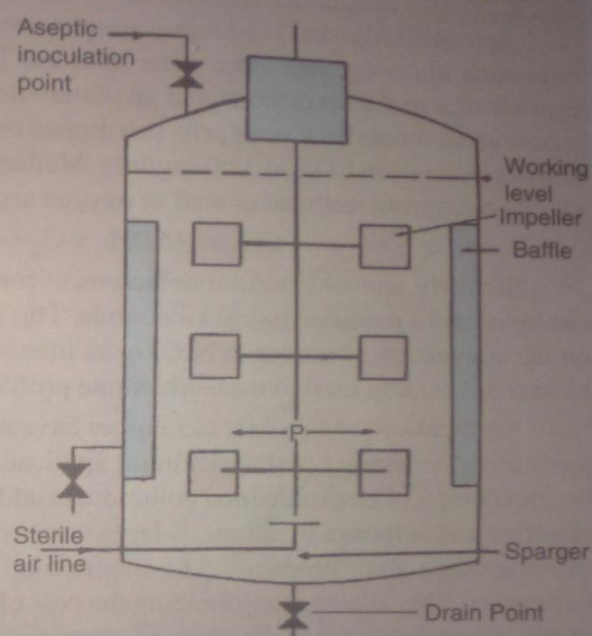


Fig. 20.2 : Diagram of a fermenter with multi-bladed impeller.

(d) **The sparger (aeration) meant for introducing air into liquid:** A sparger is a device for introducing air into the liquid into a fermenter. It is important to know whether the sparger is to be used on its own or with mechanical agitation as it can influence equipment design to determine initial bubble size.

Three basic types of sparger are:

- (i) Porous sparger
- (ii) Orifice sparger
- (iii) Nozzle sparger

(e) **Microbial sensors:** It consists of a microorganism immobilized on a membrane and an electrode. The principle of working is the change in respiration or the amount of metabolites produced as a result of the assimilation of substrate by the microorganism. A wide range of thermophilic microbes have been used for the manufacturing of microbial sensors as mentioned in the table below.

Immobilised yeast, *Trichosporoncutaneum* has been used to develop an oxygen probe for BOD estimation in sewage and other water samples. The BOD sensor includes an oxygen electrode that consists of a platinum cathode and an aluminium anode bathing in salt KCl solution and a Teflon membrane. Immobilised yeast cells are crapped between the pores of a porous membrane and the Teflon sensor can measure BOD at 3-60/mg/litre. Methanotrophic bacteria is used in measuring methane as well as oxygen. Similarly, ammonia and nitrate biosensors consist of immobilised nitrifying bacteria. This is used to determine ammonia in waste water based on the conversion of nitrate to N_2O by an immobilised denitrifying *Agrobacterium sp.* The nitrate biosensor has been used to measure nitrate profiles in biofilm.

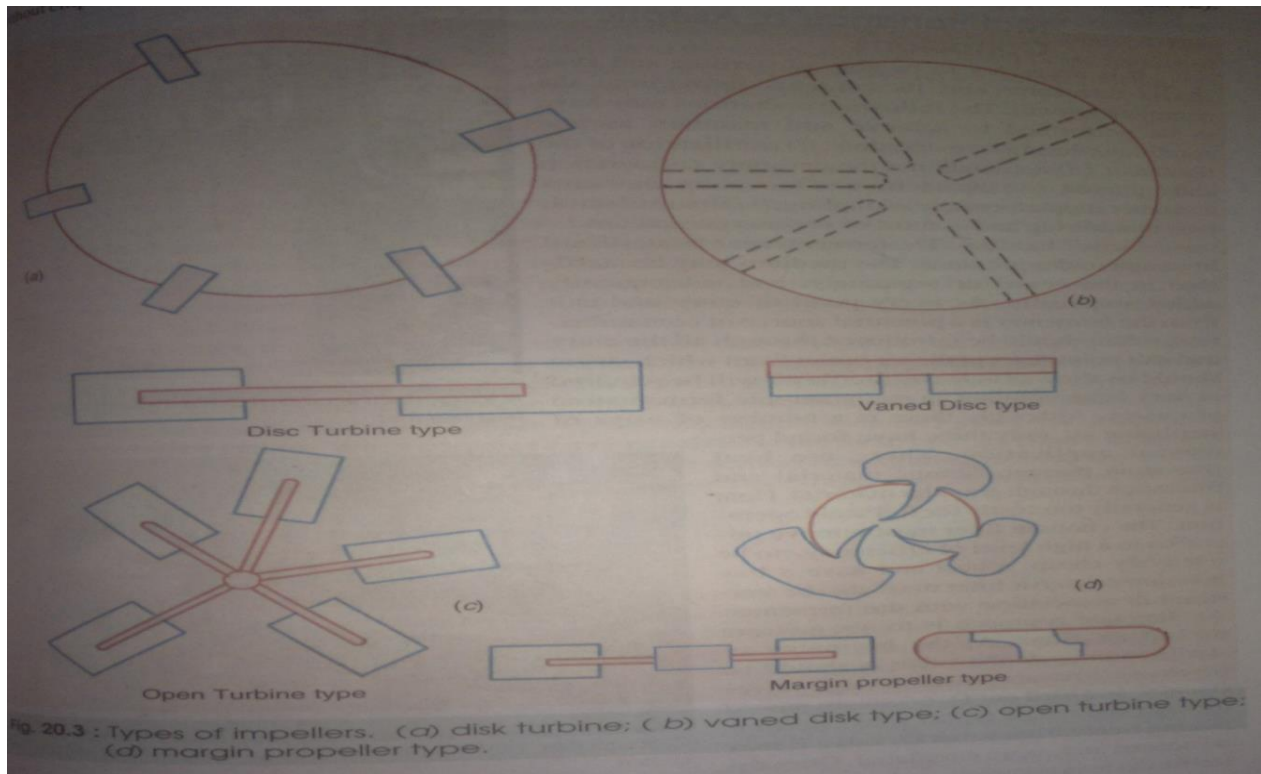
APPLICATIONS: Microbial biosensors have several uses in:

- clinical analysis,
- general health care monitoring,
- veterinary and agricultural applications,
- industrial product processing,
- monitoring and control of environment pollution and
- in military and defence for detection of chemical and biological species used in weapons.

DESIGN AND OPERATION

The basic purpose of design of a fermenter or bioreactor is to visualise the size of the unit to deliver the product both qualitatively and quantitatively. After the size is designed, the next task is to achieve the transport properties i.e;

- Fluid mechanics
- Heat transfer
- Mass transfer effects.



- Fermenters are designed to provide support to best possible growth and biosynthesis for industrially important cultures and to allow ease of manipulation for all operations associated with the use of fermenters.
- These vessels must be strong enough to resist the pressure of large volume of agitating medium.
- The product should not corrode the material nor contribute toxicity to the growth medium. This involves a meticulous design of every aspect of the vessel parts and other openings, accessories in contact, etc.
- In fermentations, provisions should be made for the control of contaminating organisms, for rapid incorporation of sterile air into the medium in such a way that the oxygen of air is dissolved in the medium and therefore, readily available to the microorganisms and CO₂ produced from microbial metabolism is flushed from the medium.

- Some stirring devices should be available for mixing the organisms through the medium so as to avail the nutrients and the oxygen.
- The fermenter has a possibility for the intermittent addition of antifoam agent.
- Some form of temperature control efficient heat transfer system is also there for maintaining a constant predetermined temperature in the fermenter during the growth of organism.
- The pH should be detected.
- Other accessories in the fermenter consist of additional inoculum tank or seed tank in which inoculum is produced and then added directly to the fermenter.

Media Design

Any fermentation process proceeds through the action of microorganisms which perform in the presence of a medium. Hence, proper design of the medium is an essential component in the design of a fermentation process. Thus, detailed investigations are needed to identify the most suitable medium for any fermentation process to proceed.

Medium Requirements

Since the medium is desired to support the functioning of microorganisms, the requirements of the medium are decided by those of the microorganisms. They are:

- carbon
- nitrogen
- energy source
- minerals
- other nutrients like vitamins, etc.
- oxygen/air for aerobic processes.
- water.

The medium used in a laboratory-scale process, or for that matter even in the pilot plant-scale level, can be reasonably composed of pure components; but such a luxury is not affordable in the case of commercial production, where the cost of production rules the economic viability, and hence the commercial viability of the process. Thus, for large-scale productions, we look for a medium, which has the following attributes:

- It should be cheap, and easily available at a consistent cost and quality.

- It should have higher productivity, i.e. it should produce maximum amount of product per unit of the substrate consumed.
- The rate of formation of the product should be high.
- It should minimize the formation of undesired products.
- Carbon Source

The carbon requirement for the medium is normally provided by molasses, starches, some cereal grains like maize, tubers like potatoes and cassava, sucrose, glucose, and lactose. The carbon source also provides the energy requirements for the medium. By and large, one of the cheapest sources of carbohydrates which provide the carbon requirement for the medium is molasses, a byproduct of the sugar industry. Another important source is corn starch. Malt extract, which is an aqueous extract of the malted barley, is also an excellent substrate for many fungi, yeast and actinomycetes. The selection of the carbon source plays a major role in the economics of the processes, particularly in those casts where the raw material constitutes 60-75% of the cost of production of the product.

Nitrogen Source

Microorganisms can utilize the nitrogen from organic or inorganic sources. Ammonia and salts of ammonia (like ammonium sulphate or nitrate) are common inorganic sources of nitrogen salts which provide both acidic and basic environments, depending upon the type of the salt. When once the ammonium ion is consumed, ammonium sulphate creates acidic environment, whereas ammonium nitrate provides basic environment. Amino acids, proteins or urea serve as organic sources of nitrogen. Microorganisms are able to grow faster in the presence of organic nitrogen, and some of them will have an absolute need for amino acids. Using pure amino acids is often expensive. We may prefer to use some precursor to the amino acid. For example, methionine and threonine are obtained from soyabcan hydrolysate, which are in turn used in the production of lysine. The following are a few nitrogen sources through proteins or amino acids:

- corn steep liquor
- soya meal
- soya beans
- groundnut meal
- cotton seed meal

- fish meal
- casein hydrolysate
- slaughter house wastes
- yeast extract, etc

Synthetic media

A synthetic medium is one in which all constituents are specifically defined and added more or less in pure form. Their composition and concentrations are pre-defined. Thus, a typical synthetic medium containing inorganic salts, water, purified sugar and magnesium sulphate, etc. is given in Table 5.5, which is used for the production of thermostable α -amylase in the laboratory. Composition of various other laboratory media are available in any manual on microbiology

Minimal medium composition for production of α -amylase in laboratory

• Component	Amount
• Soluble starch (g/l)	20.0
• Glucose(g/l)	5.0
• Peptone (g/l)	5.0
• Yeast extract (g/l)	2.5
• K_2HPO_4 (g/l)	2.0
• $MgSO_4 \cdot 7H_2O$ (g/l)	1.0
• pH	7.0
• Distilled water (ml)	100.0

Synthetic medium has some specific advantages. Since the chemical structure and composition of various components of the medium are known, their effect on the product yield can be assessed by specifically altering one of their compositions. Thus, the composition of one or several components can be varied to assess the product yield. This will help in re-designing the medium for maximum product yield. These studies also help in finding out the metabolic process route leading to the formation of the desired product. Radioactive isotopes can be incorporated into one of the components of the medium, which help us to study the effect of it on the product yield by identifying whether the radioactive isotopic component is present in the product or not, and if so, to what extent. Based on these studies, the medium can be designed. The synthetic media have got various other advantages given as follows:

- It will help in reproducibility of the results from time to time, because the composition of the medium is well-defined.
- The problem of foaming is almost nil since the foaming agents like proteins and high molecular weight peptides are absent.
- The recovery and purification of the fermentation products are much easier, since the medium can be designed by avoiding the interfering components.

Sometimes, all the above advantages will be counter-balanced by the single disadvantage of higher cost of the medium, which destabilizes the economic viability of the process. However, the synthetic media are generally used in the developmental stage, i.e. at the R&D stage.

crude medium

Which does not have any of the advantages of the synthetic medium. Their compositions are not defined. They are available as they are, e.g. soya bean meal, molasses, corn steep liquor, ammonium sulphate, etc. The main feature of the crude medium is its economic advantage. It also contains the sources of carbon and nitrogen in a form that can be easily consumed by the microorganisms. The crude media are generally used in the industrial scale of production after perfecting the composition of the medium at the R&D stage.

STERILIZATION

General Introduction:

Sterilization is probably one of the most important operations which differentiate a biochemical process from a chemical process. The role of an engineer (or more precisely, biochemical engineer) in the overall process is probably to provide a 'contamination free' environment.

Earlier, most of the processes which have fared well in terms of technical feasibility at laboratory scale processing faced the formidable problem of contamination when the scale of operation increased. One of the most classical examples is penicillin fermentation. The technical and operational problem faced by the engineers in penicillin fermentation was to design and operate a deep aerobic fermenter under absolutely contamination free environment with pure culture, since the environment in the fermenter was also conducive for the contaminants to grow. In fact, this has ushered in the new era of biochemical engineering as a distinct discipline in the field of engineering and technology. As has already been mentioned in Chapter 1, even the mechanical fabrication of the fermenter should preferably be kept in the hands of engineers who are well-versed with design and fabrication of fermenters, since otherwise a mechanical engineer, who is versatile in fabrication of chemical equipment, is likely to overlook the subtle point of asepsis, which is so vital for biochemical engineering operations, and not so important for a typical chemical equipment.

Fermentation is a biochemical process of producing metabolic products by the action of a microorganism or a group of microorganisms on a substrate, in the presence of nutrients in the medium. Obviously, fermentation proceeds with the involvement of

- A microorganism
- A medium
- A fermenter
- Nutrients/other additives
- Air in the case of aerobic processes

Thus, a sterile environment is needed for all the above. Otherwise, the contaminants would dominate the whole scene, and virtually no product will form, or a contaminated product forms with toxicants. Invasion by a foreign (or contaminant) organism may result in the following ills of fermentation:

- The foreign organism may contaminate the final product.
- The medium would be consumed unnecessarily to support the growth of contaminating organisms.
- The contaminated product may outweigh the desired product, it is more so in the case of continuous fermentation processes.

- The contaminated product may interfere with the recovery of the desired product, and may render it inaccessible.
- Unsterile air in aerobic fermentations (antibiotic fermentations) may result in the spoilage of the fermentation product.

To overcome the above difficulties, the only alternative is to have a contamination-free environment which can be achieved by sterilizing all the involved parameters as shown below.

METHODS TO AVOID CONTAMINATION IN A FERMENTATION PROCESS

- Sterilization of the medium
- Employing as pure an inoculum as possible
- Sterilization of the fermenter
- Sterilizing the pipes, valves, bends, etc., which come in contact with the fermentation process
- Sterilization of all materials to be added to the fermenter
- Sterilization of air
- Disinfecting the fermenter and contact parts with a non-toxic disinfectant
- Maintaining aseptic conditions in the fermenters during fermentation
- Maintaining optimum or desired P^H which discourages the growth of certain contaminants or undesired organisms or undesired products

The sterilization of a biochemical process can be achieved by the application of some lethal agent, viz.

- Heat in the form of steam
- Radiation
- A chemical
- Or by restricting the undesired organisms or spores by some physical unit operation such as filtration.

Sterilization of Medium:

Media used in industrial fermentations need to be sterilized before being used, to avoid the contamination of microorganisms which may

- Use the nutrients in the medium
- Change the chemical structure of the nutrients
- Change the P^H
- Create more foam in the fermentation which affects the aeration
- Produce metabolic products which affect the growth of fermentation microorganism

- Alter the oxidation-reduction potential of the medium
- Further convert, degrade or destroy the desired fermentation product

Sterilization is normally done by

- Boiling in water
- Passing live stream
- Autoclaving (subjecting the medium to steam under pressure in a pressure cooker)

All the above methods are based on thermal treatment. Various techniques were developed to achieve thermal sterilization of the medium. It should be a batch or a continuous process or a HTST (High Temperature, Short Time) process. Sometimes, the cost of sterilization would be prohibitive and destabilize the process economics. In such situations, the industrial fermentation are carried out by employing other means, viz.

- Low P^H
- Contamination inhibitors (such as Lactic acid)

Synthetic medium do not require much sterilization efforts as compared to crude media. The synthetic media may require a small amount of heating for sterilization. The crude media are likely to contain much of the heat-resistant bacterial spores, and require prolonged heating. However, care must be exercised to see that the medium does not get cooked due to over-heating, but still adequate amount of heating must be given to see that the medium is thoroughly sterilized. This requires a bit of experience. Thermal death studies need to be made 'a priori' to evaluate the sterilization time. Excessive heating may denature the proteins or caramelize the sugars or encourage thermal degradation of the components in the nutrient medium by inter-actions (like sugars reacting with phosphates). If P^H is a critical factor during sterilization, it is advisable to adjust the P^H to neutrality, sterilize the medium and then bring the P^H of the medium to the original value after the sterilization by addition of pre-sterilized acid or alkali. If some of the enzymes or nutrients like vitamins are sensitive to heat sterilization, they are separated initially by passing through a bacteriological filter; sterilization is carried out, and the separated (filtered) enzymes/nutrients are added back to the medium. Similarly the volatile components in the medium may be separated by bacteriological filtration to avoid/reduce their loss due to thermal sterilization.

Thus, various types of methods/alternatives need to be devised to see that the active components/nutrients are not lost during heat sterilization, but at the same time, the containing organisms are destroyed to the maximum possible extent.

Batch Sterilization:

Having understood the principles of sterilization, let us see the two distinct methods of thermal sterilization. They are:-

1. Batch sterilization
2. Continuous sterilization

As has already been mentioned for batch processes, batch sterilization process is one in which all the contents are loaded into the sterilizer, steam is injected to see that the sterilization takes place as per the programme, and later the contents are discharged for further processing or transferred directly into the fermenter. The batch sterilization process has some inherent disadvantages. This procedure is less successful in avoiding the risk of destruction of nutrients while destroying the contaminants as compared to the continuous sterilization. However, it has the following advantages:

- The initial costs of investment are lower.
- The chances of contamination after the sterilization is over are less because the sterilization can be done in the same vessel or in the fermenter itself.
- Manual control is normally done, and hence the chances of mechanical failure are less. Media containing high proportion of solids can be handled easily.

Continuous Sterilization:

In the continuous sterilization process, there will be continuous inflow and outflow of material. Thus it is best suited if the capacity of the operation is high. The continuous sterilization has many advantages vis-à-vis the batch sterilization.

- I. The throughputs (capacity) can be higher.
- II. The medium quality can be maintained better.
- III. The system can be automated for control; hence it avoids human error during operation
- IV. The running costs are less.
- V. The sterilization times can be shorter.
- VI. This is the only option left out for HTST operation.
- VII. The holding capacities of the stream can be less.
- VIII. Ease in scaling-up of the process.

The major disadvantage with the continuous operation is the initial cost investment. It necessitates the use of aseptic transfer system for the sterile broth to be transported to the sterile broth.

However, if the medium is to be exposed to high temperatures for a short time (HTST Process) to avoid denaturation of proteins or to avoid destruction of some of the enzymes, etc., it is not possible in commercial scale operations in batch sterilization to quickly heat large volumes of the medium in a short time and to cool it also in short time. HTST is possible only in continuous sterilization.

Basically there are two methods of continuous sterilization.

1. **Continuous plate heat exchange sterilization.**
2. **Continuous steam injection and flash cooling sterilization.**

In a plate heat exchanger, the incoming unsterile medium is preheated by heat exchange with the outgoing sterile medium. Subsequently it is heated with steam in a heat exchanger. It is then passed through the holding section. The residence time in the holding section is decided by the holding time requirement, flow rate of the heated medium and length of the holding section. Heating time and cooling time are considerable, before and after the holding time.

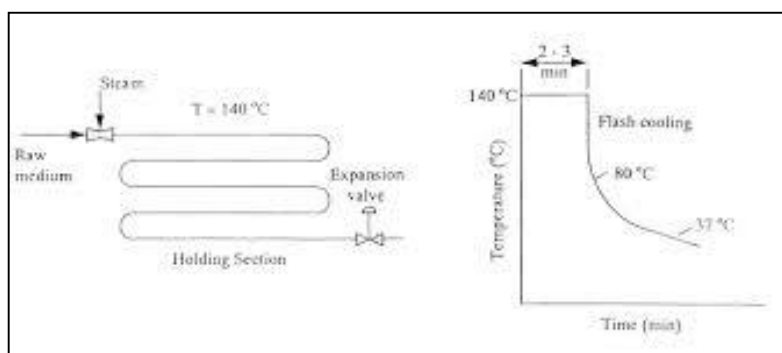


Fig. Steam injection flash cooling continuous sterilization

In case of steam jet sterilization, steam is directly injected along with the medium continuously. Hence, the heating time and the heating section are negligible. The holding time is based on the length of the holding pipe. Sterilization takes place during the holding period. The steam and sterilized medium under pressure are passed through the expansion valve into the vacuum chamber. Steam is removed out under vacuum. The sterile medium passes into the preheat exchange zone and gives out some heat to the incoming sterile medium through the heat exchanger. Finally, the sterile medium passes through the cooling zone. Hence, it will have a considerable cooling zone; but the temperature is much below 100°C and hence it may be neglected for the purpose of thermal death kinetics. The advantages and disadvantages of the sterile injection system is summarised as:

Advantages	Disadvantages
<ul style="list-style-type: none"> Media containing solids can be sterilized better. 	<ul style="list-style-type: none"> Direct contact of the steam with the medium makes it necessary that the steam should be clean and free of any anti-corrosive agents.
<ul style="list-style-type: none"> Capital investment is low. 	<ul style="list-style-type: none"> Foaming may occur during both heating and cooling.
<ul style="list-style-type: none"> It is easy to clean and maintain the system. 	
<ul style="list-style-type: none"> Heating and cooling periods are shorter. 	
<ul style="list-style-type: none"> Steam efficiency is very high as live steam is directly injected into the medium. 	

Sterilization of Air:

For aerobic fermentation processes on industrial scale, a huge quantity of air is required, and it is needless to say that the air should be clean and sterile. Achieving sterility for air is a stupendous task for biochemical engineers.

There is an excellent coverage on air sterilization (Aiba et al., 1965 (c)) which mentions various species and numbers of air-borne microbes. A cubic meter of air contains approximately 4×10^3 to 20×10^3 particles with a maximum possibility of 12×10^3 . However, on an average, we can assume that air contains 10^3 to 10^4 particles/m³. The average size of the dust particles is approximately 0.6 microns.

Methods of Air Sterilization:

There are various possible ways to sterilize the air:

- Sterilization by heating
- Use of ultraviolet rays and other electromagnetic waves
- Use of germicidal sprays
- Sterilization by filtration

Even though it is possible to heat the air and sterilize it, normally it is not economical to heat the air because of its poor thermophysical properties resulting in lower heat transfer coefficient. Ultraviolet rays in the wavelength range 2265-3287 Å are effective in killing air-borne microbes. This is one of the

oldest known techniques. Hospital rooms, food factories and fermentation rooms are equipped with lamps emitting UV rays. However, their reliability to sterilize large volumes of air in factories is doubtful, even though they are good to sterilize the air to a reasonable extent in rooms or in small containers.

There will be a significant reduction in air-borne bacteria by spraying of small amount of germicides, viz. phenol, ethylene oxide or formalin (formaldehyde). This is also an effective method of disinfecting the air in a room to a good extent. This method also cannot ensure 100% sterility of air. The disinfectants are sprayed into the room along with the air recirculation systems like air-conditions or pedestal fans, etc.

Thus, it can be seen that amongst the various options given above, none of them was very effective or economical or practicable. Hence, normally air is sterilized in the process industries by passing it through a filter bed which separates the spores present in the air. The air filters used for the removal of microorganisms or spores from the air are of two types:

- I. Those filters whose pores are smaller than the size of the microorganisms to be removed.
- II. Those in which the pore size is bigger than the size of the microorganisms.

In the former category, virtually all the organisms are filtered off and hence they are called as absolute filters. In the latter category of filters, fibrous materials such as cotton, glass-wool, slag or steel-wood are used to make filter beds with bigger gaps or pore openings. They are called as fibrous-type air filters. Obviously, they cannot assure 100% sterilization of air, no matter whatever be the length of the filter bed. Comparison of the two filter beds is shown below:

Absolute air filter	Fibrous-type air filter
<ul style="list-style-type: none"> • The pore size is smaller than the particle size. 	<ul style="list-style-type: none"> • The gaps between the fibrous materials are bigger than the particle size.
<ul style="list-style-type: none"> • Sterilization efficiency is 100%. 	<ul style="list-style-type: none"> • Sterilization cannot be 100%.
<ul style="list-style-type: none"> • Pressure drops are high. 	<ul style="list-style-type: none"> • Pressure drops are not that high.
<ul style="list-style-type: none"> • Filter thickness is small. 	<ul style="list-style-type: none"> • Filter thickness is more.
<ul style="list-style-type: none"> • Cost of initial investment is high. 	<ul style="list-style-type: none"> • Initial investments are low.
<ul style="list-style-type: none"> • Choking/clogging takes place very fast. 	<ul style="list-style-type: none"> • Choking and clogging do not take place that fast.
<ul style="list-style-type: none"> • Cleaning and replacement are difficult. 	<ul style="list-style-type: none"> • Cleaning and replacement are easy.

Kinetics of microbial growth

Kinetics is the study that deals with the speed. The kinetic of reaction, in general parlance means how fast a reaction is progressing.

Microbial growth:-

Microbial growth is considered for the observation of the living cell activities. It is important to monitor cell growth and biological and biocatalytic activities in cell metabolism. A variety of methods are available to predict cell growth by direct or indirect measurements. Cell dry weight, cell optical density, cell turbidity, cell respiration, metabolic rate and metabolites are quite suitable for analysing cell growth, substrate utilisation and product formation. The rate of cell growth is described in this chapter. Various bioprocesses are modelled for substrate utilisation and product formation.

Microbial growth is usually studied as a population not an individual. Individual cells divide in a process called **binary fission** where two daughter cells arise from a single cell. The daughter cells are identical except for the occasional mutation.

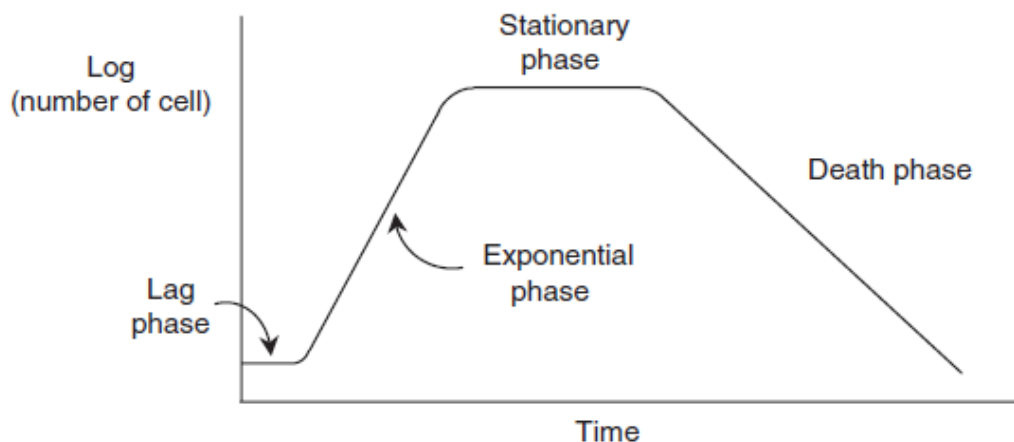
Binary fission requires:

- Cell mass to increase
- chromosome to replicate
- cell wall to be synthesized
- cell to divide into two cells

Cell Growth in Batch Culture

Batch culture is a closed system without any inlet or outlet streams, as nutrients are prepared in a fixed volume of liquid media. The inocula are transferred and then the microorganisms gradually grow and replicate. As the cell propagates, the nutrients are depleted and end products are formed. The microbial growth is determined by cell dry weight (g.l^{-1}) and cell optical density (absorbance at a defined wavelength). A growth curve can be divided into four phases, as shown in following Figure. As inocula are transferred to the fermentation media, cell growth starts rapidly in the media. The lag phase shows almost no apparent cell growth. This is the duration of time represented for adaptation of microorganisms to the new environment, without much cell replication and with no sign of growth. The length of the lag phase depends on the size of the inocula. It is also results from the shock to the environment when there is no acclimation period. Even high concentrations of nutrients can cause a

long lag phase. It has been observed that growth stimulants and trace metals can sharply reduce the lag phase.



(Typical batch growth curve of a microbial culture)

Growth Phases

Once there is an appreciable amount of cells and they are growing very rapidly, the cell number exponentially increases. The optical cell density of a culture can then be easily detected; that phase is known as the exponential growth phase. The rate of cell synthesis sharply increases; the linear increase is shown in the semi-log graph with a constant slope representing a constant rate of cell population. At this stage carbon sources are utilised and products are formed. Finally, rapid utilisation of substrate and accumulation of products may lead to stationary phase where the cell density remains constant. In this phase, cell may start to die as the cell growth rate balances the death rate. It is well known that the biocatalytic activities of the cell may gradually decrease as they age, and finally autolysis may take place. The dead cells and cell metabolites in the fermentation broth may create toxicity, so deactivating remaining cells. At this stage, a death phase develops while the cell density drastically drops if the toxic secondary metabolites are present. The death phase shows an exponential decrease in the number of living cells in the media while nutrients are depleted. In fact the changes are detected by monitoring the pH of the media.

Kinetics of Batch Culture

The batch culture is a simple, well-controlled vessel in which the concentration of nutrients, cells and products vary with time as the growth of the microorganism proceeds. Material balance in the reactor may assist in following the biochemical reactions occurring in the media. In batch fermentation, living cells propagate and many parameters of the media go

through sequential changes with time as the cells grow. The following parameters are monitored while the batch process continues:

- Cells and cell by-product
- Concentration of nutrients
- Desirable and undesirable products
- Inhibition
- pH, temperature, substrate concentration

The objective of a good process design is to minimise the lag phase period and maximise the length of exponential growth phase. The substrate balance in a batch culture for component i in the culture volume of V_R and change of molar concentration of C_i is equal to the rate of formation of product:

$$\frac{d}{dt}(V_R \cdot C_i) = V_R \cdot r_{fi}$$

Where V_R is the culture volume, assumed to be constant while no liquid media is added or removed, C_i is the molar concentration of component i and r_{fi} is the rate of product formation. Then the above equation is reduced to:

$$\frac{dC_i}{dt} = r_{fi}$$

The rate of product formation, r_{fi} , depends upon the state of the cell population, environmental condition, temperature, pH, media composition and morphology with cell age distribution of the microorganism.^{2,3} A similar balance can be formulated for microbial biomass and cell concentration. The exponential phase of the microbial growth in a batch culture is defined by:

$$\frac{dX}{dt} = \mu X$$

There is no cell removal from the batch vessel and the cell propagation rate is proportional to specific growth rate, μ (h^{-1}), using the differential growth equation the cell concentration with respect to the time is:

$$X(t) = X_0 e^{\mu t}$$

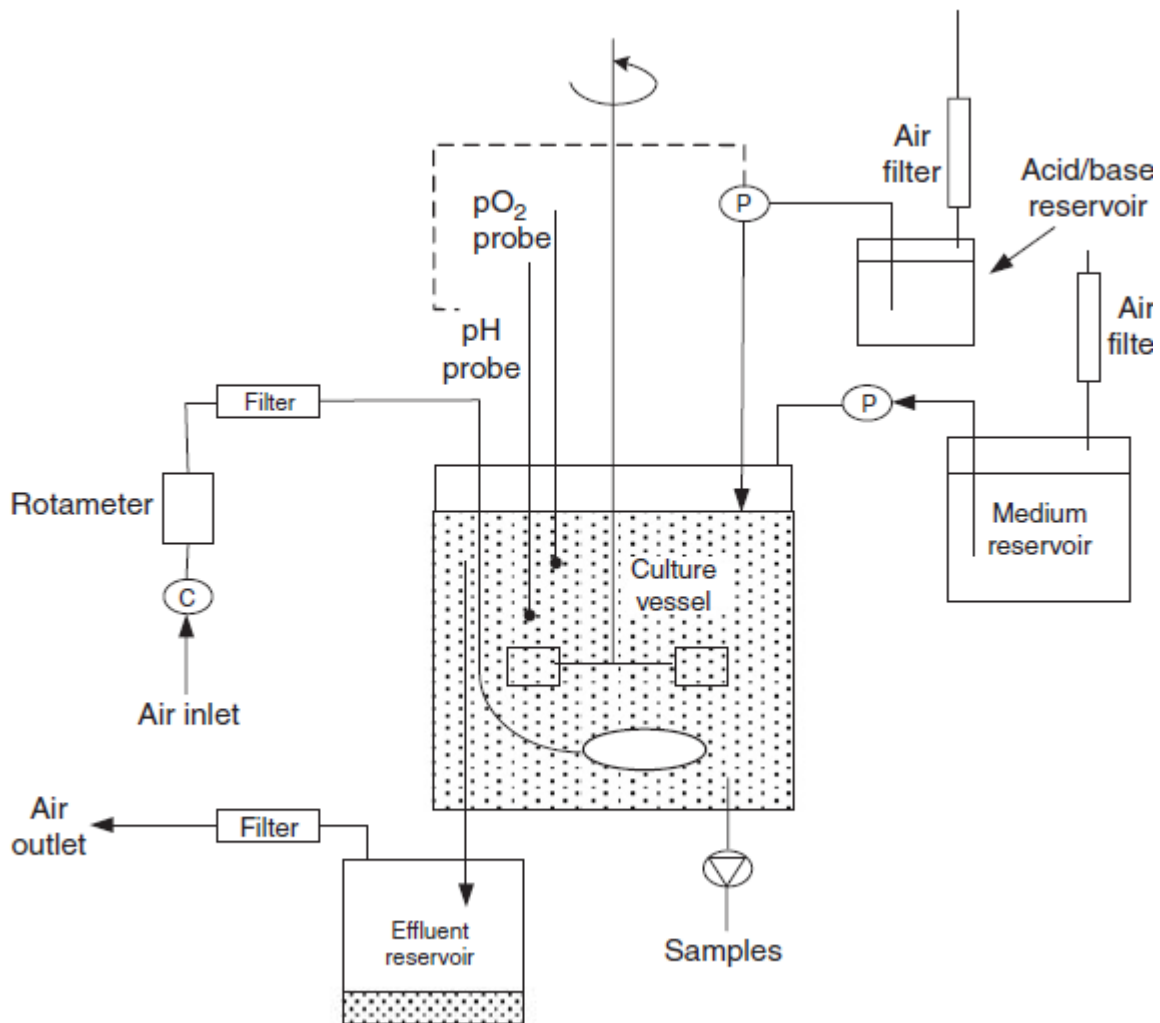
GROWTH KINETICS FOR CONTINUOUS CULTURE

The fermentation system can be conducted in a closed system as batch culture. The batch system growth kinetics and growth curve were explained in the above sections. The growth curve is the best representation of a batch system. Disadvantages exist in the batch system such as substrate depletion with limited nutrients or product inhibition growth curve. The growth environment in the batch system has to follow all the phases projected in the growth curve. Besides nutrient depletion, toxic by-products accumulate. Even the composition of media with exponential growth is continuously changing; therefore it will never be able to maintain any steady-state condition. The existing limitation and toxic product inhibition can be removed if the system is an open system and the growing culture is in a continuous mode of operation. In engineering, such a system is known as an open system. There would be an inlet medium as fresh medium is pumped into the culture vessel and the excess cells are washed out by the effluents, leaving the continuous culture from the fermentation vessel. The advantages of continuous culture are that the cell density, substrate and product concentrations remain constant while the culture is diluted with fresh media. The fresh media is sterilised or filtered and there are no cells in the inlet stream. If the flow rate of the fresh media gradually increases, the dilution rate also increases while the retention time decreases. At high flow rate, the culture is diluted and the cell population decreases; with the maximum flow rate when all the cells are washed out, the composition of the inlet and outlet conditions remain about the same. In this condition a washout phenomenon takes place. In continuous culture, the flow rate is adjusted in such a way that the growth rate and the cell density remain constant. There are two types of are given below.

- (i) Chemostat (growth rate controlled by dilution rate, D , h^{-1})
- (ii) Turbidostat (constant cell density that is controlled by the fresh medium)

Chemostat

The nutrients are supplied at a constant flow rate and the cell density is adjusted with the supplied essential nutrients for growth. In a chemostat, growth rate is determined by the utilisation of substrates like carbon, nitrogen and phosphorus. A simple chemostat with feed pump, oxygen probe, aeration and the pH controlling units is shown in Figure below. The system is equipped with a gas flow meter. Agitation and aeration provided suitable mass transfer. The liquid level is controlled with an outlet pump.

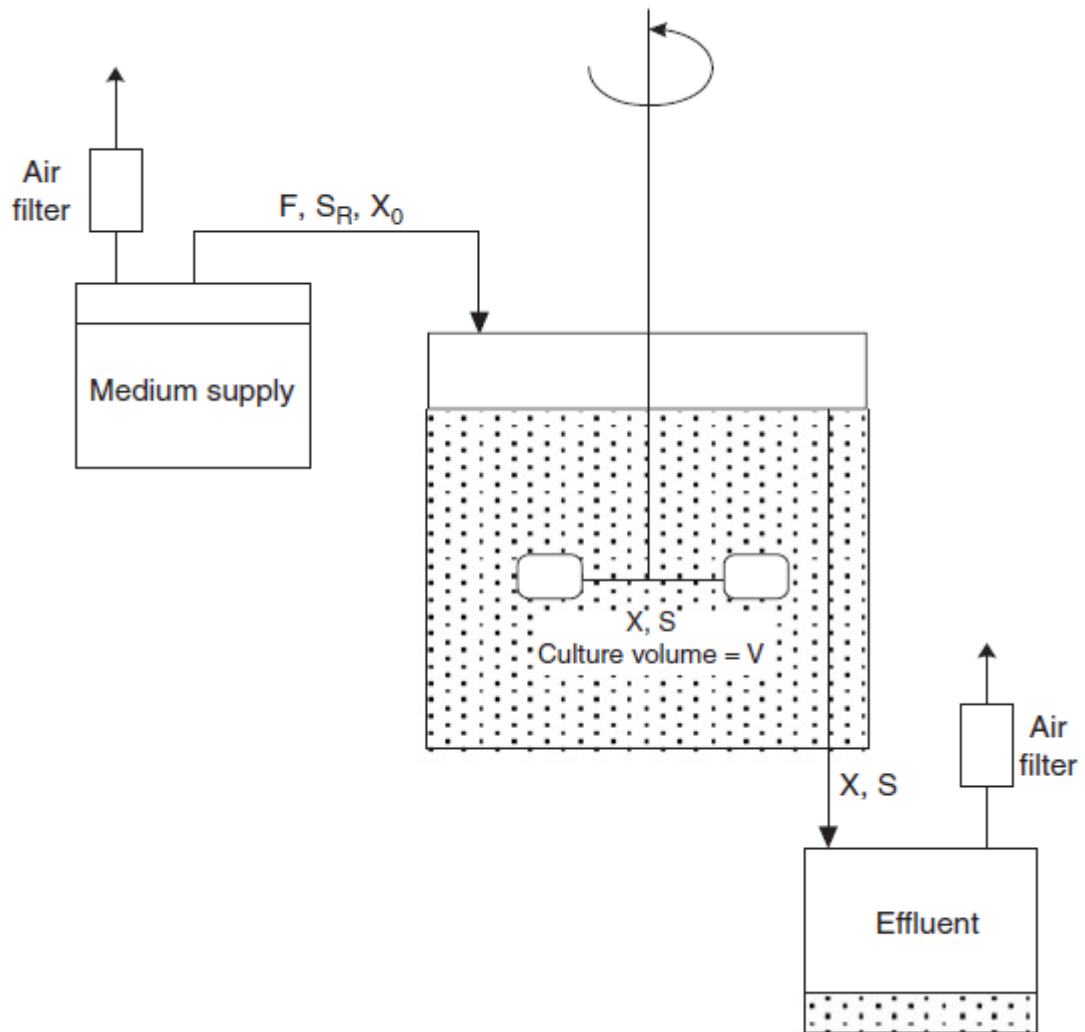


| Schematic diagram of continuous culture with control units in a constant volume chemostat.

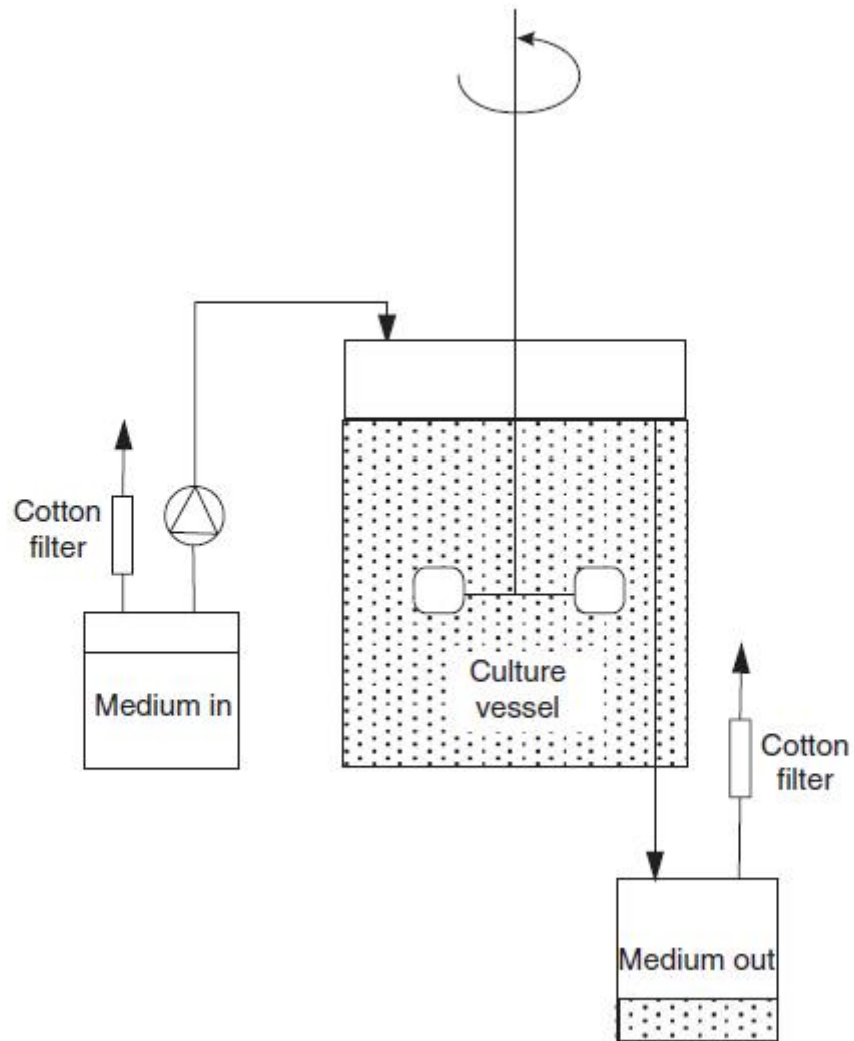
Fresh medium is pumped into the culture vessel. The liquid level is controlled as the overflow is drained to a product reservoir.

For constant volume of the fermentation vessel, a liquid level controller is used. The system is also designed with an outlet overflow to keep the liquid level constant. The below figures show various mechanisms for constant-volume bioreactors. An outlet pump is customarily used to maintain a constant flow rate. Complex systems are designed to control the mass of the generated cells; photocells or biosensors are used to monitor the optical density of the cells. Cell concentration is controlled by the supplied nutrients and the flow rate of fresh media. The substrate concentration and the retention time in the fermentation vessel may dictate the cell density. Besides the nutrients and the controlling dilution rate, there are several physiological and process variables involved in the kinetics and the design of a bioreactor. These parameters are temperature, pH, redox (reduction and oxidation) potentials, dissolved oxygen, substrate concentration and many process variables. In a chemostat, cell growth rate is determined by an expression that is based on substrate utilisation, mainly

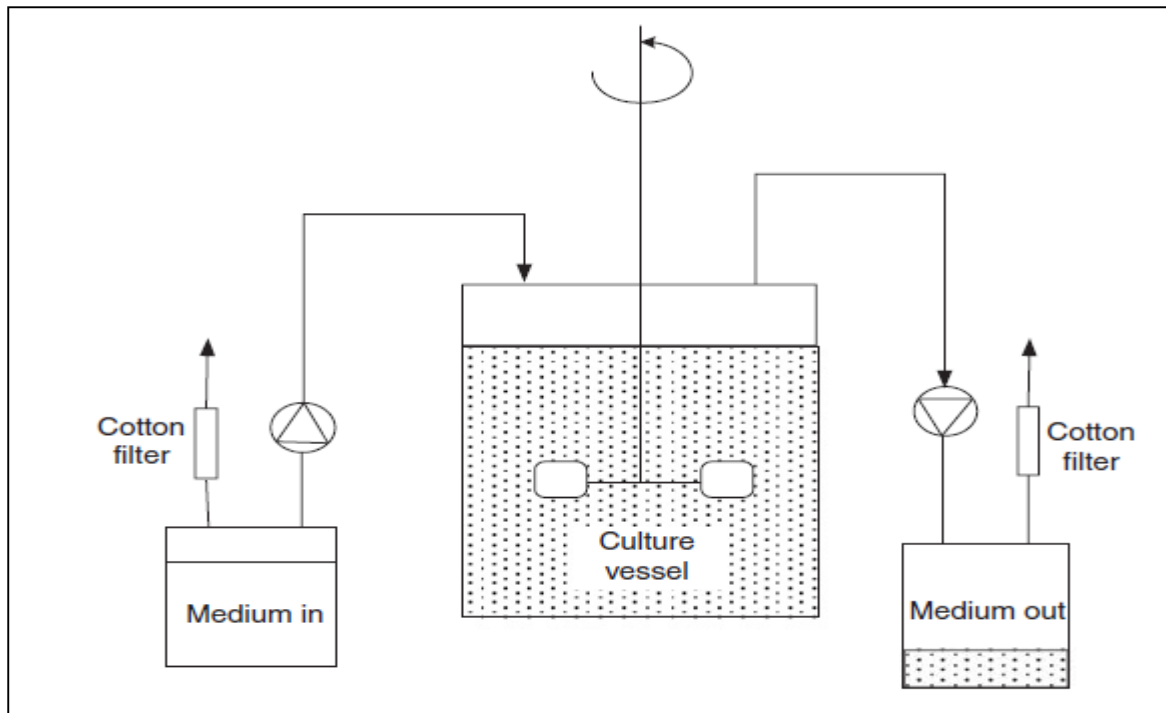
C, N and P with trace amounts of metals and vitamins. The advantages of continuous culture are that the essential nutrients can be adjusted for maximum growth rate and to maintain steady-state conditions. There is a determined relation between cell concentration and dilution rate. At steady state, cell concentration is maximised with optimum dilution rate. There is also a critical dilution rate where all the cells are washed out and there is no chance for the microorganisms to replicate; this is known as the maximum dilution rate.



(Chemostat without pumps maintained at constant level).



(Chemostat with feed pump overflow drainage maintained at constant level)



(Chemostat using single medium inlet feed and outlet pumps)

Disadvantages of Batch Culture

There are several disadvantages of batch culture. The nutrient in the working volume becomes depleted; the other major problem is the limitation and depletion of the substrate. Since there is no flow stream to take effluent out, as the system is closed, toxins form there. A disadvantage related to substrate depletion is that the growth pattern may reach the death phase quickly in an old culture. The long duration of the batch system for slow growth results in exhaustion of essential nutrients and an accumulation of metabolites as by-products. Exhaustion of nutrients and substrate may cause the system to become retarded. The technical problem resulting in changes to media composition may directly affect the microbial exponential growth phase. Inhibition is another factor affecting the bioprocess, which causes the reaction rate shift. As a result, inhibition may slow down bio-catalytic activities. Product inhibition may block enzyme activities, and the cells become poisoned by the by-product. One common disadvantage of the batch process is that one has to carry out a cycle for production: the product should be sent for downstream processing, then the system has to be cleaned and recharged with fresh feed, so the process is highly labour intensive for downtime and cleaning.

Advantages of Continuous Culture

There are several advantages to continuous culture, where all the problems associated with the batch culture are solved. Firstly, the growth rate is controlled and the cells are well maintained, since fresh media is replaced by old culture while the dilution is taking place. As a result, the effect of physical and chemical parameters on growth and product formation can easily be examined. The biomass concentration in the cultured broth is well maintained at a constant dilution rate. The continuous process results in substrate-limited growth and cell-growth-limiting nutrients. The composition of the medium can be optimised for maximum productivity; in addition secondary metabolite production can also be controlled. The growth kinetics and kinetic constants are accurately determined. The process leads to reproducible results and reliable data. High productivity per unit volume is achieved. The continuous culture is less labour-intensive, and less downtime is needed. Finally, steady state growth can be achieved, even if mixed cultures are implemented.

ENZYMES

Biological reactions occur rapidly due to the presence of natural catalyst called enzymes. They are biocatalysts present in living matter. Pure enzyme catalyses the transformation of 10000 to 1000000 moles of substrate per min/mole of enzyme. Enzymes are biological catalysts used to increase the rate of biochemical reaction, taking place with living systems, without themselves undergoing any overall changes.

- **Cofactor:** the non protein component of an enzyme
- **Coenzyme:** an enzyme with organic molecule as its cofactor
- **Holo enzyme :**an active enzyme in cofactor
- **Apo enzyme:** the in active portion of protein

CLASSIFICATION OF ENZYMES:

1. Nomenclature are usually named in terms of the reactions those are catalysed. The suffix represents the name of the enzyme “ase”.
2. The enzyme that attacks urea-urease , argenin-arginase. Enzymes are also named by chemical reactors proleuse, lipase, oxidase etc.
3. All the enzymes are classified into two groups: hydrolysing enzyme or hydrolase, other enzyme or desmolases.

ENZYME SPECIFICITY:

Enzymes as biological catalysts are particularly credited for their specificity in action. Each enzyme usually catalyses only one particular type of reactions by selectively lowering the activation energy of only one of the several possible chemical or biochemical reactions.

TYPES OF SPECIFICITY:

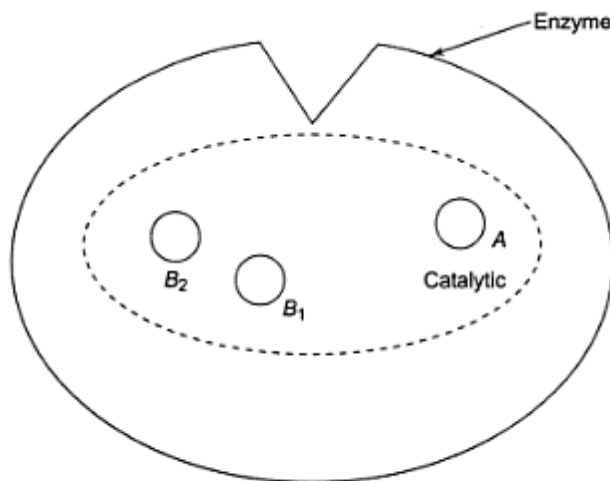
- ❖ Depending upon the reaction conditions and specific natures of enzyme; the enzymatic catalytic processes exhibit different kind of specificity.
 1. Absolute specificity
 2. Group specificity
 3. Stereochemical specificity
 4. Product specificity
 5. Substrate specificity

- ❖ Alcohol dehydrogenase is a group specific enzyme, which catalyses the oxidation of variety of alcohols. Those enzymes which act on a particular substrate are known as absolute specific enzymes. Some enzymes even exhibit stereo specificity i.e. if a substrate exists in two stereo chemical forms but chemically identical, the enzymes will specially catalyse one of the isomers. A typical example is oxidation of L-amino acid to oxo-acid which can take place by enzymatic catalytic action of L-amino acid oxidase.

ACTIVE SITE:-

An enzyme possesses two different kinds of site,

- Binding site
- Catalytic site
 - The active site or active centre of the enzyme is the region which contains the binding and catalytic sites which are only a small fraction of the total volume of the enzyme.
 - This is situated at or near the surface of the enzyme and is thus easily accessible for substrate molecule to get attached.



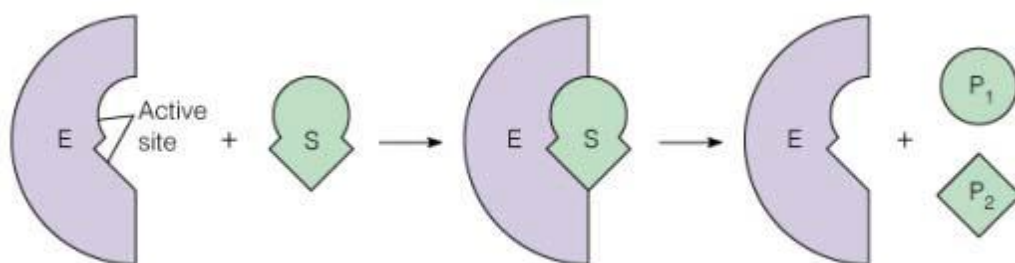
B₁ and B₂ are binding sites. A is the catalytic site

Active and catalytic sites in enzyme

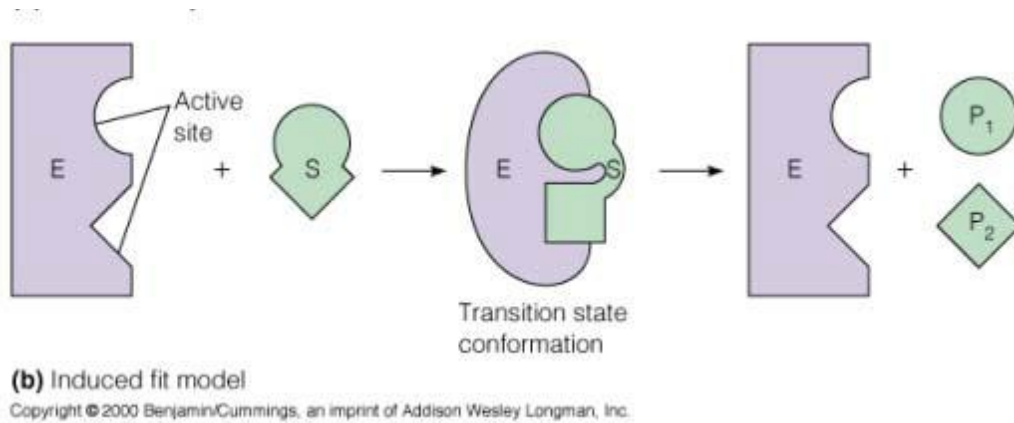
ENZYME SPECIFICITY HYPOTHESIS:-

- Several hypotheses are proposed to explain the enzyme specificity in catalytic activity and its ability to interact with the substrate.
 - Fischer lock and key hypothesis
 - Koshland induced fit hypothesis

- Hypotheses involving strain and transition state stabilisation.
- b) Fischer lock and key hypothesis is probably the first theory gives the concept of “complementary structural features” between enzyme and the substrate.
 - c) The catalytic process is brought about because the substrate fits into the complementary site on the enzyme just as key fits into the lock, thus the reaction group of the substrate gets struck with the catalytic site of the enzyme.
 - d) Similarly the binding groups attach to the binding sites in the enzyme.
 - e) The lock-and-key theory has been successful in explaining many features of enzyme specificity, but one serious drawback with this hypothesis is that it could not explain some of the conformational changes taking place in the enzyme when they come in contact with substrate.
 - f) An enzyme may not be having as an exactly complementary feature that is compatible to the substrate, but still there are instances when reactions have taken places; particularly protein which are known for flexibility.
 - g) X-ray diffraction analysis and some spectroscopic analysis have been shown differences in structures of the free enzyme and substrate bound enzyme. This states that structure of the substrate may not be complementary to the enzyme in its native form, but it is complementary to the active site in the substrate enzyme-complex.
 - h) When substrate or enzyme brought together, the binding of the substrate brings in structural changes in the enzyme so that the catalytic sites in the enzymes and reacting group in the substrate match each other, like hand and glove example.
 - i) The substrate like hand which is rigid and does not change its shape and enzyme is like a woollen glove which can be wrapped around the hand.



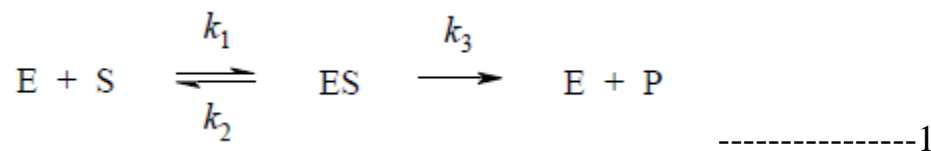
(a) Lock-and-key model



ENZYME KINETICS

The Michaelis-Menten equation is an important equation in biochemistry and as such it is imperative that you understand the derivation of this equation. By understanding the derivation, you will have insight into the assumptions that went into this model, and therefore you will have a better appreciation for the proper use of this equation as well as the limitations of this model. In the following sections you will see two different derivations of the Michaelis-Menten equation. When one is learning a subject for the first time, it often helps to have the same or similar information presented from alternative perspectives. One way might be clearer to you whereas the other way might be clearer to someone else. That is ok! You should familiarize yourself with both approaches, and then settle on the one that you prefer.

First Derivation. We start with the kinetic mechanism shown in equation (eq) 1:



In eq 1, E is enzyme, S is substrate, ES is the enzyme-substrate complex, and P is product. This equation includes the assumption that during the early stages of the reaction so little product is formed that the reverse reaction (product combining with enzyme and re-forming substrate) can be ignored (hence the unidirectional arrow under k_3). Another assumption is that the concentration of substrate is much greater than that of total enzyme ($[S] \gg [Et]$), so it can essentially be treated as a constant.

From General Chemistry we can equate the rate of this process ($k_3[ES]$) to the change in product concentration as a function of time ($d[P]/dt$), or, equivalently, we can designate the rate with an italicized v (v) as follows in eq 2:

$$\frac{d[P]}{dt} = v = k_3[ES] \text{-----}2$$

Because the concentration of the enzyme•substrate complex ($[ES]$) cannot be measured experimentally, we need an alternative expression for this term. Because the enzyme that we add to the reaction will either be unbound (E) or bound (ES) we can express the fraction of bound enzyme as follows in eq 3 :

$$\frac{[ES]}{[E_t]} = \frac{[ES]}{[ES] + [E]} \text{-----}3$$

In eq 3 E_t is the concentration of total enzyme, and the other variables are as defined above. If we multiply both sides of eq 3 by E_t we arrive at eq 4:

$$[ES] = \frac{[E_t][ES]}{[ES] + [E]} \text{-----}4$$

If we multiply the numerator and denominator of the right-hand side of eq 4 by $1/[ES]$, we are, in effect, multiplying by one and we do not change the value of this expression. When we do this we obtain eq 5:

$$[ES] = \frac{[E_t]}{1 + \frac{[E]}{[ES]}} \text{-----}5$$

We have almost achieved our goal of isolating $[ES]$. Next, we need to come up with an alternative expression for the ratio $[E]/[ES]$. We do this by recalling that a major assumption in enzyme kinetics is the steady-state assumption. Basically, it says the rate of change of $[ES]$ as a function of time is zero: $d[ES]/dt = 0$. Another way to express the steady-state assumption is that the rate of formation of ES equals the rate of breakdown of ES . We can express this latter statement mathematically as in eq 6:

$$k_1[E][S] = k_2[ES] + k_3[ES] = (k_2 + k_3)[ES] \text{-----}6$$

The left-hand side of eq 6 expresses the rate of formation of ES (according to eq 1), and the right-hand side expresses the two ways that ES can break down (also according to eq1). We can rearrange eq 6 to isolate the ratio $[E]/[ES]$. When we do we get eq 7:

$$\frac{[E]}{[ES]} = \frac{(k_2 + k_3)}{k_1[S]} \quad \text{-----7}$$

We now define a new constant, the Michaelis constant (K_m), as follows in eq 8:

$$K_m = \frac{(k_2 + k_3)}{k_1} \quad \text{-----8}$$

If we substitute K_m back into eq 7 we obtain eq 9:

$$\frac{[E]}{[ES]} = \frac{K_m}{[S]} \quad \text{-----9}$$

We now substitute the ratio $K_m/[S]$ from eq 9 in place of the ratio $[E]/[ES]$ in eq 5 and we obtain eq 10:

$$[ES] = \frac{[E_t]}{1 + \frac{K_m}{[S]}} \quad \text{-----10}$$

If we multiply the numerator and denominator of the right-hand side of eq 10 by $[S]$, we are, in effect, multiplying by one and we do not change the value of this expression. When we do this we obtain eq 11:

$$[ES] = \frac{[E_t][S]}{[S] + K_m} = \frac{[E_t][S]}{K_m + [S]} \quad \text{-----11}$$

Now we have achieved our goal of isolating $[ES]$ and we can substitute this alternative expression of $[ES]$ into eq 2 and obtain eq 12:

$$v = \frac{k_3[E_t][S]}{K_m + [S]} \quad \text{-----12}$$

Next, we imagine what happens to eq 12 when $[S] \gg K_m$ as follows in eq 13:

$$v \approx \frac{k_3[E_t][S]}{[S]} = k_3[E_t] = k_{cat}[E_t] \quad \text{-----13}$$

The constant k_{cat} in the right-hand most term of eq 13 is used to signify that k_3 is considered the catalytic constant. Under such conditions, when $[S]$ is said to

be saturating, the enzyme is functioning as fast as it can and we define $k_3[\text{Et}]$ (or $k_{\text{cat}}[\text{Et}]$) to be equal to V_{max} , the maximum velocity that can be obtained. Therefore, eq 12 can be rewritten into the familiar form of the Michaelis-Menten equation (eq 14):

$$v = \frac{V_{\text{max}}[\text{S}]}{K_m + [\text{S}]} \quad \text{-----14}$$

Next, we imagine what happens when $K_m \gg [\text{S}]$ as follows in eq 15:

$$v \approx \frac{V_{\text{max}}[\text{S}]}{K_m} = k'[\text{S}] \quad \text{-----15}$$

Since $k' = V_{\text{max}}/K_m$ in eq 15, we refer to V_{max}/K_m as an apparent (or pseudo) first order rate constant. Another way to look at a similar, related concept is to rewrite eq 14 as follows:

$$v = \frac{k_{\text{cat}}[\text{E}_t][\text{S}]}{K_m + [\text{S}]} \quad \text{-----16}$$

Since we are imagining the case where $K_m \gg [\text{S}]$ we neglect $[\text{S}]$ in the denominator and include the assumption that $[\text{E}_t] \approx [\text{E}]$ since at very low $[\text{S}]$ relatively little $[\text{ES}]$ should form:

$$v \approx \frac{k_{\text{cat}}[\text{E}][\text{S}]}{K_m} \approx k''[\text{E}][\text{S}] \quad \text{-----17}$$

Once again, since $k'' = k_{\text{cat}}/K_m$ in eq 17, we refer to k_{cat}/K_m as an apparent second order rate constant. Because k_{cat}/K_m is a measure of the rate of the reaction divided by the term that reflects the steady-state affinity of the enzyme for the substrate, it is considered an indicator of the catalytic efficiency of the enzyme and sometimes is called the specificity constant. It also is more relevant to the physiological situation because in cells, $[\text{S}]$ generally is equal to or less than K_m . Is there an upper limit to the value that k_{cat}/K_m can approach? Yes, there is and the following shows how we can determine this limit. To illustrate this limit we first need to rewrite k_{cat}/K_m as follows:

$$\frac{k_{\text{cat}}}{K_m} = \frac{k_3}{\frac{k_2 + k_3}{k_1}} \quad \text{-----18}$$

Next, we imagine the case where $k_3 \gg k_2$:

$$\frac{k_{\text{cat}}}{K_m} \approx \frac{k_3}{k_3} \approx k_1 \quad \text{-----19}$$

So we see that k_{cat}/K_m can approach k_1 as a limiting value, and k_1 is the second-order rate constant for the productive collision of enzyme and substrate and as such it is limited by diffusion to about 10^8 to 10^9 M⁻¹ s⁻¹. Thus, if we see an enzyme that has a k_{cat}/K_m value in the neighborhood of 10^8 to 10^9 M⁻¹ s⁻¹ we say that the enzyme has attained “catalytic perfection”. You will see later in the class that a number of enzymes that catalyze “nearequilibrium” reactions in metabolic pathways are catalytically perfect. Next, we return to eq 16 and consider what happens when $v = \frac{1}{2} V_{\text{max}}$

Next, we return to eq 16 and consider what happens when $v = \frac{1}{2} V_{\text{max}}$:

$$\frac{V_{\text{max}}}{2} = \frac{V_{\text{max}}[S]}{K_m + [S]} \quad \text{-----20}$$

When we simplify eq 20 we find that $K_m = [S]$ (under the above conditions; i.e., $v = \frac{1}{2}V_{\text{max}}$). So, in other words, K_m is formally defined as a collection of rate constants (eq. 8), but it is also equal to the substrate concentration that gives half-maximal velocity of the enzyme-catalysed reaction.

Before we discuss the second derivation, we will consider what happens when we take the reciprocal of both sides of eq 14. When we do this we obtain eq 21:

$$\frac{1}{v} = \frac{K_m}{V_{\text{max}}} \cdot \frac{1}{[S]} + \frac{1}{V_{\text{max}}} \quad \text{-----21}$$

Eq 21 is in the form of an equation for a straight line (i.e., $y = mx + b$, with $y = 1/v$; $m = \frac{K_m}{V_{\text{max}}}$; $x = \frac{1}{[S]}$; and $b = \frac{1}{V_{\text{max}}}$). When experimental data are plotted using this transformation the resulting plots are called double-reciprocal plots or Lineweaver-Burk plots in honor of the researchers who pioneered this method.

The authors of many textbooks extol the virtues of using Lineweaver-Burk plots to obtain estimates of V_{max} and K_m . I disagree strongly with this practice because initial velocity data determined at low substrate concentrations (where there is inherently more uncertainty since $[S] \cdot K_m$) end up being the points in a Lineweaver-Burk plot that have too much sway in determining the best-fit line through the data.

IMMOBILIZATION OF ENZYMES

Immobilization of Enzymes:

- The movement of enzymes in a fixed location is restricted by technique known as immobilization.
- Immobilized enzymes are attached to an insoluble support medium or enclosed by the support medium which is also known as a “carrier”.
- In some cases the enzyme molecules are cross linked to each other so that their movement is restricted but their catalytic activities are retained .Thus immobilization restricts only the movement of enzymes either completely or to some region.
- Since enzymes are proteins and they are soluble in water, it is difficult to separate them once the reaction is over for reuse in a batch process.
- To overcome this difficulty enzymes are immobilized on the carrier material or inside an insoluble matrix by various physical or chemical method

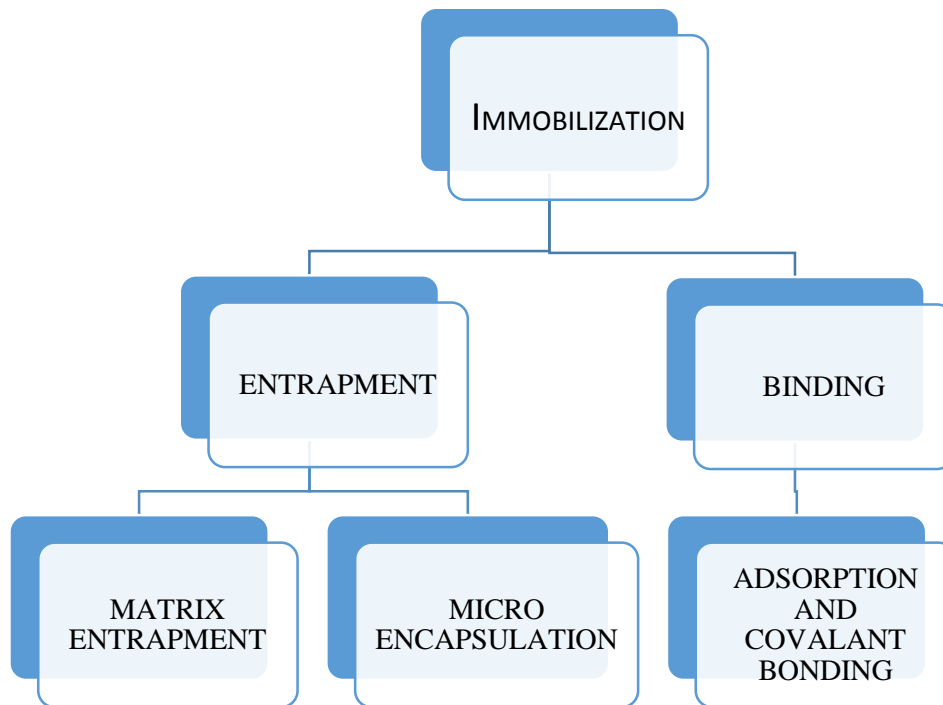
Immobilization has the following advantages:

- Component enzymes can be reused.
- The cell density or enzyme density in a location can be increased.
- Continuous operation is possible.
- Enzyme stability is improved considerably.
- Product is enzyme free and hence is particularly useful with intra cellular particles.
- Since this process is continuous, it is easy to incorporate control process.
- Catalytic process route can be controlled more accurately.
- Allows development of multi enzyme reaction system.
- Effluent disposal problems can be considerably reduced.

In view of the above advantages, immobilized enzymes have become a potential source for industrial and medical use.

Immobilization Methods:

Immobilization techniques can be divided into four main groups; the division is based on whether the enzymes are entrapped in a limited space or bound to a carried material.



1. Entrapment or Occlusion:

It is a simple physical process of immobilization. The active enzyme is entrapped in a matrix of a polymerised gel. Polyacrylamide is generally the most preferred gel. This method is very simple and can be utilised for most of the enzymes.

The advantages of this process are:

- Enzymes are not chemically modified.
- Enzyme properties are not altered.

There are certain disadvantages with this method:

- The deactivation of enzymes may take place during gel formation.
- Enzyme leakage may take place continuously depending upon the pore size of the gel.
- Diffusional limitation may pose reduced accessibility for the substrate.

2. Micro Encapsulation:

- In this method the enzyme is entrapped within semipermeable membrane in the form of microscopic hollow spheres.

- The entrapping methods do not affect the activity of enzymes. However the diffusional limitations may restrict the movement of the substance to the activity site.
- Thus this method of immobilization may not suitable for proteolytic enzymes or for macro molecule substrate.
- The best advantage of his method is that each enzyme is in much close contact with the substrate in the surrounding solution.

3. Covalent Attachment:

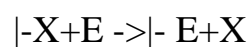
In this method, the enzyme is attached to the surface by the covalent bond formation via certain functional groups. The most commonly used functional groupings of the support material are:

- Amino groups
- Carboxyl groups
- Hydroxyl groups
- Sulphydryl groups

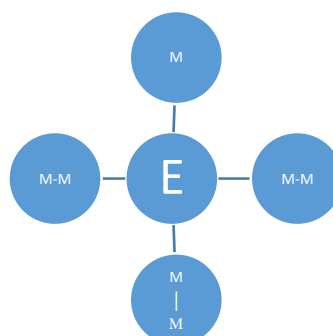
The only restriction imposed by this method is that these functional groups should not be in the active sites of the enzymes. Certain chemical reagents are used for activating the functional groupings. They are;

- Cyanogens bromides
- Carbodiimide and
- Glutaraldehyde.

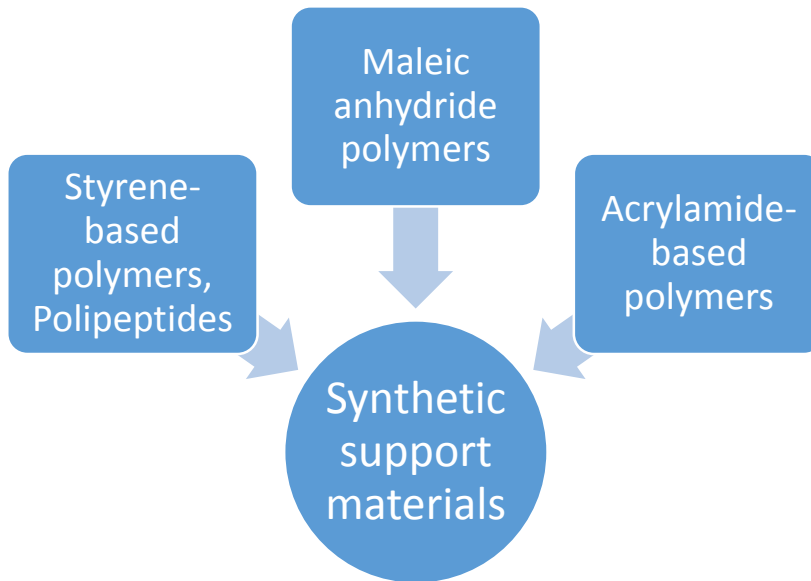
The covalent attachments of enzyme molecules can be simply described as follows:



The copolymerisation of enzymes of a reacting monomer of the support material is also followed for some immobilisation by the covalent attachments.

$$nM+E \rightarrow M_nE$$


Some of the water-insoluble support materials are:



Covalent bonding method provides more permanent linkage between the enzyme and the support material. Covalent bonds can be formed under mild conditions, and the active site of enzyme must remain free from covalent attachments. There is still some possibility for loss of activity of the enzyme during bond formation mainly because of chemical reaction.

4. Adsorption:

- One of the simplest methods for enzyme immobilisation is by adsorption. Enzymes can be adsorbed physically on a surface-active adsorbent by weak physical forces such as Vander Waals' forces or dispersion forces.
- Commonly used adsorbents are: alumina, clay, silica, anion-exchange resins, CaCO_3 , C, cation exchange resins, collagen and glass plates.
- For effective immobilisation the surfaces of these support materials may have to be physically or chemically pretreated.
- Ion-exchange resins DEAE sephadex and carboxy methyl cellulose (CMC) can also be used as support media.

Drawbacks:

- Since adsorption is non-specific process. Many other substances may also be attached to the carrier in addition to the immobilised enzyme.
- This method is the loading of enzyme on a unit amount of surface is always very low and the bonding strength is very weak.

Advantages:

- The immobilisation procedure is easy and simple
- The adsorption process is reversible
- Enzymes are not deactivated by adsorption

Properties of Immobilised Enzymes:

- Enzymes are usually immobilised in particle or pellet form; but enzymes may be attached to, or entrapped within carriers in the form of membranes, tubes or fibres, based on the requirements of a given application.
- In view of this, an immobilised enzyme may have different properties as compared to the same enzyme in a free solution form.
- The method of immobilization and nature of insoluble carrier may have influence on the enzyme properties.
- The specific activity may reduce in the immobilised enzyme, mainly because of the new microenvironment as compared to the pure enzyme the internal and external mass transfer limitations are imposed on the immobilised systems because of diffusional limitations.
- They will reduce biochemical reaction rate considerably making the effectiveness factor less than one.

Reactors for Immobilized Enzyme System:

As has been mentioned earlier, the immobilised enzyme systems are particularly credited for their

1. Amenability to use in continuous system
2. Ease in separation of the immobilised pellets for reuse or recycling.
3. Separation of the enzyme free or cells free products at the end of reactions.

Thus, immobilised enzyme systems are ideal for use in continuously operated processes, and hence continuously operated reactors can be used with advantage. Some of the continuously operated flow reactors in chemical/biochemical systems are:

- A. Continuous stirred tank reactors
- B. Plug-flow reactors
- C. Packed-bed reactors
- D. Fluidised-bed reactors

- All the above reactors have got their own advantages and operational features. The continuous stirred tank reactors (CSTR) have the advantage of providing fully mixed conditions so that every time the enzyme pellet can have an opportunity to have a new environment of the substrate concentration.
- Except for the disadvantage of the shearing, which may be detrimental to the shear-sensitive particles, these reactors are very ideal.
- The packed-beds and plug flow reactors almost operated under similar flow conditions. In either case, there is no movement of the bed, and hence are ideal for any systems except those in which heat generation and dissipation are a problem to reckon with.
- The fluidised bed bio-reactors incorporate some of the beneficial features of both stirred tank and packed bed reactors.
- In this reactor, the upward flow system is ideally suitable for processes in which the substrate solution is highly viscous.

Enzyme Applications:

There are major 4 broadly classifications.

- A. Food and beverage industry including baking and brewery.
 - B. Pharmaceuticals.
 - C. Medical applications.
 - D. Analytical purpose
- Rennet enzymes used in cheese making.
 - α -amylase and protease are used in baking industry converts starch to dextrin and sugar.
 - Also hydrolysis of gluten which is a major flour protein.

Clarification of Fruit-Juice:

Pectolytic enzyme is used to clarify the fruit juice and wine to remove pectin and which causes haze to the juice.

- Some fruit pulp like banana, guava, and need proteolytic enzyme to break pectin to release the free flowing fruit juice from the fibrous material.

Chill Proofing of Beer:

Beer brewed from cereal grains contain a certain amount of haze (cloudiness) which needs to be removed. It is done by using enzyme papain.

Tenderization of Meat:

Meat obtained from aged animals need to be tenderised to improve the texture. It is done by pectolic enzymes like papain or bromelin.

- Lactase is used for hydrolysis of lactose present in skin, milk and whey.
- Also enzymes used in food industries for analytic purpose to measure the sterility of food.
- Enzymes also used in medicines utilised for diagnosis, therapy and treatment purposes.
- Basically chirally pure compounds from isomers are used in pharmaceutical industries.
- In anti-biotic industries penicillin acylase is used for conversion of penicillin-G to 6-amino penicillin acid.
- Enzyme in immobilised form used in bio-sensors.
- It can sense some of the biological materials either quantitatively and qualitatively or both. E.g.: glucose oxidase and urease are used.
- Also enzymes can be used in washing powder, bacterial protease used in washing powder. It is used for removal of blood and other protein stains.

Module-II

HEAT TRANSFER IN BIOPROCESSING

Heat transfer plays an important role in bioprocessing, both for fermentation and for enzymatic reactions. The temperature effect on biochemical reaction rates is conveniently represented by the Arrhenius equation. The fermentation broths are either heated to the desired temperatures or maintained at a desired lower temperature by heating or cooling the fermenters, depending upon whether the biochemical reaction is endothermic or exothermic. Here, we use the term heat transfer which means it is the process of transfer of heat; it could be for the purpose of heating or vaporizing or cooling or freezing. The heat transfer mechanism is both same in heating and cooling. The direction of heat transfer could be; either from the heat source to the body or from the body to the cooling medium.

Heat-transfer applications in biotechnology:-

HT Operation	Remarks
Heating of fermentation broth reactors	There are many examples; hydrolysis of starch to glucose, etc. The reactor is maintained at 95 ⁰ c.
Cooling of fermentation broths	There are many examples. Fermentation of molasses to alcohol. The fermentation is maintained at 35-37 ⁰ C.
Sterilisation of media	---
Lyophilisation	Preservation of isolated strains by freezing.
Desolventisation	Desolventisation is done to recover the extracted product.
Simultaneous heat and mass transfer operations	Drying , crystallization , distillation, solvent extraction, etc.
Concentrating the substrates product	Evaporation, crystallization as in the case of production of citric acid, etc.

First, let us look into the principles of heat-transfer. Heat moves from a body at a higher temperature to a body at a lower temperature. Any transfer process is always proportional to the

- Driving force
- Contact area

In heat-transfer, the driving force is the temperature difference between the source and sink. Thus,

$$\begin{aligned} \text{the rate of heat transfer} &\propto (\Delta T) \\ &\propto (A) \end{aligned}$$

Where ΔT is the temperature difference, and

A is the contact area.

The above equation can also be written as

$$\begin{aligned} \text{Rate process} &\propto \text{driving force} \\ &\propto 1/\text{resistance} \end{aligned}$$

The constant of proportionality is known as the **heat-transfer coefficient**, which we shall discuss later. The heat-transfer process also depends upon whether the materials are in direct contact or otherwise.

Depending upon whether the material is in direct contact with the heating source or otherwise, heat is conceived to transfer by any of the following three fundamental heat-transfer methods;

- Conduction
- Convection
- Radiation

Heat Transfer By Conduction:-

Conduction is the method of heat-transfer in which the heat is transferred from one body to another with/without direct contact and without the movement of either the source or the sink. This kind of heat-transfer takes place in solids. For example, heating of a metallic rod by putting one end in a hot flame and holding the other end. Initially, the other end is cold. Slowly it gets heated; i.e., the heat travels in the rod from one end to the other without the movement of the rod. How fast the transfer depends upon the nature of the material. Some materials conduct heat very fast, and are known as conductors; whereas other materials conduct very slowly or virtually do not conduct any heat, are known as **insulators or non-conductors**.

Fourier's Law of Heat Conduction:-

It states that rate of heat conduction through a body is directly proportional to the temperature difference and the area which is perpendicular to the body and it is inversely proportional to the thickness of the body.

The rate of heat transfer per unit area is given by

$$q/A \propto (\Delta T)$$

$$\propto 1/B$$

or $q/A = k/B(\Delta T) = (\Delta T)/(B/k) = \Delta T/R$

in which q is the rate of heat transfer in kJ/s or kW

k is the thermal conductivity (W/m⁰C)

ΔT is the temperature difference (⁰C)

B is the total thickness of the slab(m)

R is known as the resistance for heat transfer and is equal to (B/k)

$$R = B/k$$

Conduction of Slabs in Series:-

Heat insulating walls are usually made by having the insulating materials in series. Usually, the furnace walls are made up of a series of

- Metallic wall inside
- Silo-cel bricks or asbestos
- Glass-wood or asbestos

The overall temperature difference ΔT is equal to the sum of the individual temperature differences. Thus,

$$\Delta T = \Delta T_A + \Delta T_B + \Delta T_C$$

Since the same amount of heat has to transfer through all the slabs,

$$q_A = q_B = q_C = q$$

also

$$R = R_A + R_B + R_C$$

Heat Transfer By Convection:-

Heat transfer in fluids takes place by convection. In convective heat-transfer, there will be virtually physical movement of layers of the fluid. If we heat water in a vessel or in a beaker, the temperature at the bottom layers of the fluid will rise resulting in decreasing its density. This kind of heat transfer is known as convective heat transfer. If the fluid layers move by means of temperature difference alone, this kind of convection is known as natural or free convection. On the contrary, if the movement of layers is facilitated by means of an external pump, convection is known as forced convection.

The convective heat-transfer rate is also proportional to the temperature difference and contact area. Thus,

$$q \propto \Delta T$$

$$\propto A$$

Combining the above two expression, we can write

$$q = hA(\Delta T)$$

in which h is known as the convective heat-transfer coefficient and has the units of $\text{kJ}/(\text{s m}^2\text{ }^\circ\text{C})$

Overall Heat Transfer Coefficient:-

The overall heat-transfer coefficient are also represented by inside overall heat-transfer coefficient represented by U_i and outside overall heat-transfer coefficient represented by U_o . Thus ,

$$dq / dA = U(\Delta T)$$

$$U_o = 1 / ((d_o/d_i) (1/h_i) + x_w/k (d_o/d_i) + 1/h_o)$$

Design of Heat Exchanger Equipment:-

Design of heat exchanger equipment mainly involves in finding out the heat-transfer area for affecting a desired amount of heat to be transferred or for achieving a desired amount of heat to be transferred which we can evaluate based on the

- Quantity of fluid / fluid flow rate
- Heat capacity (C_p)
- Temperature rise to be achieved

The *heat* transfer rate calculated by the above equation is equated to

$$q = mc_p(\Delta T)$$

$$q = hA(\Delta T)$$

Heat Transfer By Radiation:-

Radiation heat transfer is defined as “the transfer of energy across a system boundary by means of an electromagnetic mechanism which is caused solely by a temperature difference”. Radiation heat transfer does not require any medium. Radiation exchange, occurs most effectively in vacuum.

The total radiation thus intercepted will be distributed into reflected, absorbed and emitted radiations which depends on reflectivity, absorptivity and transmissivity of the material respectively.

Emissivity:-

The ratio of the total emissive power of a body to that of a black body is called emissivity.

Kirchhoff's law:-

This law states that at temperature equilibrium, the ratio of the total radiating power of a body to its absorptivity is a constant and depends upon the temperature of the body. Considering any two bodies in temperature equilibrium with common surroundings, according to the Kirchhoff's law-

$$E_1/\alpha_1 = E_2/\alpha_2$$

Radiant heat transfer:-

Expression for radiant heat transfer is given as-

$$Q = \sigma F (T_1^4 - T_2^4)$$

σ = Stefan-Boltzmann constant

F = view factor

Mass Transfer in Bioprocessing

There are many situations in bioprocessing where concentrations of compounds are not uniform; we rely on mechanisms of mass transfer to transport material from regions of high concentration to regions where the concentration is low. An example is the supply of oxygen in fermenters for aerobic culture. Concentration of oxygen at the surface of air bubbles is high compared with the rest of the fluid; this concentration gradient promotes oxygen transfer from the bubbles into the medium.

Another example of mass transfer is extraction of penicillin from fermentation liquor using organic solvents such as butyl acetate. When solvent is added to the broth, the relatively low concentration of penicillin in the organic phase causes mass transfer of penicillin into the solvent. Solvent extraction is an efficient downstream-processing technique as it selectively removes the desired product from the rest of the fermentation fluid.

Mass transfer plays a vital role in many reaction systems. As distance between the reactants and site of reaction becomes greater, rate of mass transfer is more likely to influence or control the conversion rate. Taking again the example of oxygen in aerobic culture, if mass transfer of oxygen from the bubbles is slow, the rate of cell metabolism will become dependent on the rate of oxygen supply from the gas phase. Because oxygen is a critical component of aerobic fermentations and is so sparingly soluble in aqueous solutions, much of our interest in mass transfer lies with the transfer of oxygen across gas-liquid interfaces. However, liquid-solid mass transfer can also be important in systems containing clumps, pellets, flocs or films of cells or enzymes; in these cases, nutrients in the liquid phase must be transported into the solid before they can be utilized in reaction. Unless mass transfer is rapid, supply of nutrients will limit the rate of biological conversion.

In a solid or quiescent fluid, mass transfer occurs as a result of molecular diffusion. However, most mass-transfer systems contain moving fluid; in turbulent flow, mass transfer by molecular motion is supplemented by convective transfer. There is an enormous variety of circumstances in which convective mass transfer takes place. In this chapter, we will consider the theory of mass transfer with applications relevant to the bioprocessing industry.

Molecular Diffusion

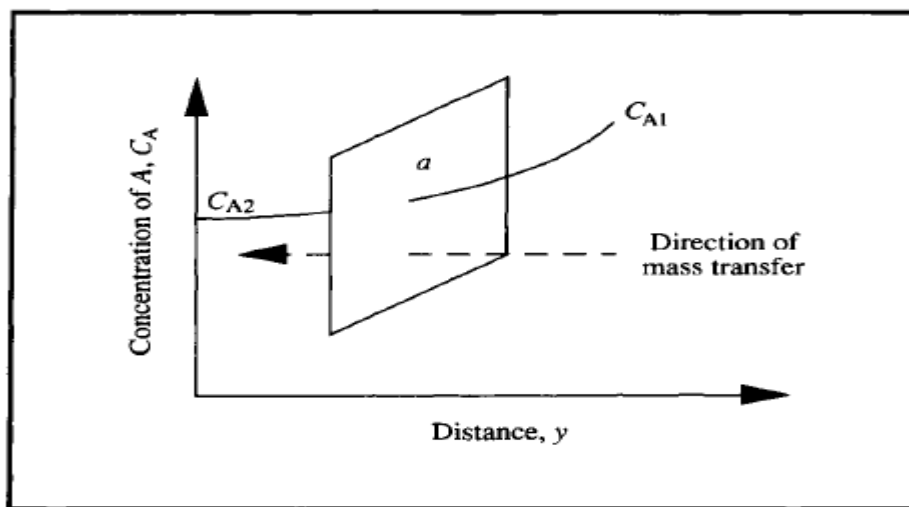
Molecular diffusion is the movement of component molecules in a mixture under the influence of a concentration difference in the system. Diffusion of molecules occurs in the direction required to destroy the concentration gradient. If the gradient is maintained by constantly supplying material to the region of high concentration and removing it from the region of low concentration,

diffusion will be continuous. This situation is often exploited in mass-transfer operations and reaction systems.

Diffusion Theory

In this text, we confine our discussion of diffusion to *binary mixtures*, i.e. mixtures or solutions containing only two components. Consider a system containing molecular components A and B. Initially, the concentration of A in the system is not uniform; as indicated in Figure below, concentration C_A varies from C_{A1} to C_{A2} as a function of distance y . In response to this concentration gradient, molecules of A will diffuse away from the region of high concentration until eventually the whole system acquires uniform composition. If there is no large-scale fluid motion in the system, e.g. due to stirring, mixing occurs solely by random molecular movement.

Assume that mass transfer of A occurs across area a perpendicular to the direction of diffusion. In single-phase systems, the rate of mass transfer due to molecular diffusion is given by *Fick's law of diffusion*, which states that mass flux is proportional to the concentration gradient:



(Concentration gradient of component A inducing mass transfer across area a)

Role of Diffusion in Bioprocessing

Fluid mixing is carried out in most industrial processes where mass transfer takes place. Bulk fluid motion causes more rapid large-scale mixing than molecular diffusion; why then is diffusive transport still important? Areas of bioprocessing in which diffusion plays a major role are described below.

(i) **Scale of mixing.** Turbulence in fluids produces bulk mixing on a scale equal to the smallest eddy size. Within the smallest eddies, flow is largely streamline so that further mixing must occur by diffusion of fluid components. Mixing on a molecular scale therefore relies on diffusion as the final step in the mixing process.

(ii) **Solid-phase reaction.** In biological systems, reactions are sometimes mediated by catalysts in solid form, e.g. clumps, flocs and films of cells and immobilised-enzyme and -cell particles. When cells or enzyme molecules are clumped together into a solid particle, substrates must be transported into the solid before reaction can take place. Mass transfer within solid particles is usually unassisted by bulk fluid convection; the only mechanism for intraparticle mass transfer is molecular diffusion. As the reaction proceeds, diffusion is also responsible for removal of product molecules away from the site of reaction.

(iii) **Mass transfer across a phase boundary.** Mass transfer between phases occurs often in bioprocessing. Oxygen transfer from gas bubbles to fermentation broth, penicillin recovery from aqueous to organic liquid, and glucose transfer from liquid medium into mould pellets are typical examples. When different phases come into contact, fluid velocity near the phase interface is significantly decreased and diffusion becomes crucial for mass transfer across the phase interface. This is discussed further in the next section.

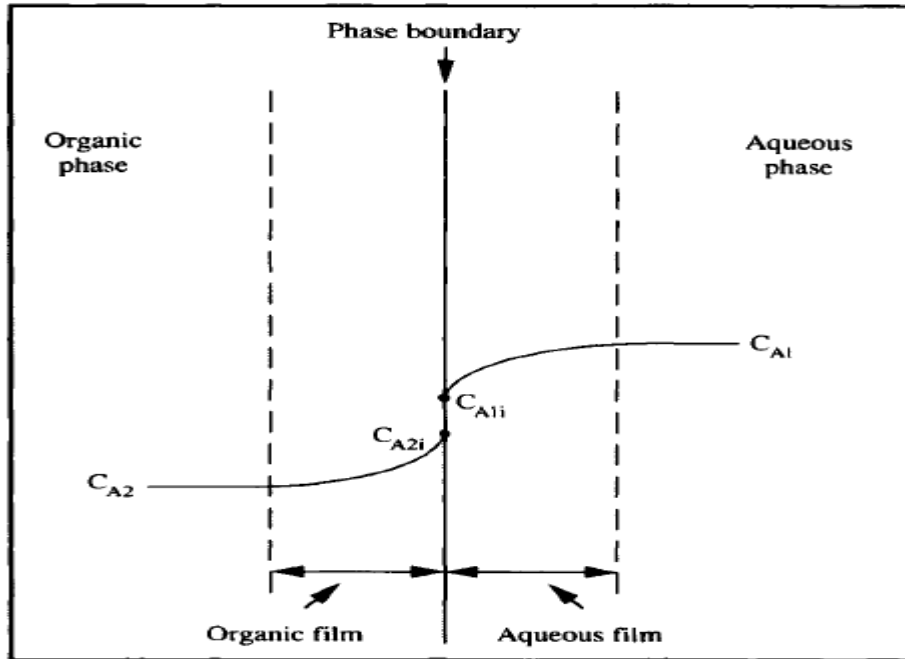
Film Theory

The *two-film theory* is a useful model for mass transfer between phases. Mass transfer of solute from one phase to another involves transport from the bulk of one phase to the *phase boundary* or *interface*, and then from the interface to the bulk of the second phase. The film theory is based on the idea that a fluid film or *mass-transfer boundary layer* forms wherever there is contact between two phases.

Let us consider mass transfer of component A across the phase interface represented in Figure below. Assume that the phases are two immiscible liquids such as water and chloroform, and that A is initially at higher concentration in the aqueous phase than in the organic phase. Each phase is well mixed and in turbulent flow. The concentration of A in the bulk aqueous phase is C_{A1} ; the concentration of A in the bulk organic phase is C_{A2} .

According to the film theory, turbulence in each fluid dies out at the phase boundary. A thin film of relatively stagnant fluid exists on either side of the interface; mass transfer through this film is effected solely by molecular diffusion. The concentration of A changes near the interface as indicated in Figure. C_{A1i} is the interfacial concentration of A in the aqueous phase; C_{A2i} is the interfacial concentration of A in the organic phase. Most of the resistance to

mass transfer resides in the liquid films rather than in the bulk liquid. For practical purposes it is generally assumed that there is negligible resistance to transport at the interface itself; this is equivalent to assuming that the phases are in equilibrium at the plane of contact.



Film resistance to mass transfer between two immiscible liquids.

The difference between C_{A1i} and C_{A2i} at the interface accounts for the possibility that, at equilibrium, A may be more soluble in one phase than in the other. For example, if A were acetic acid in contact at the interface with both water and chloroform, the equilibrium concentration in water would be greater than that in chloroform by a factor of between 5 and 10. C_{A1i} would then be significantly higher than C_{A2i} .

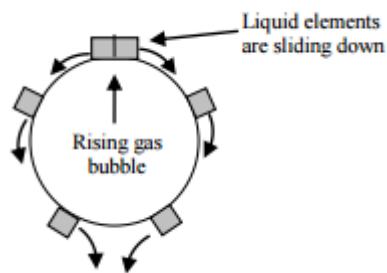
Even though the bulk liquids in Figure above may be well mixed, diffusion of component A is crucial in effecting mass transfer because the local fluid velocities approach zero at the interface. The film theory as described above is applied extensively in analysis of mass transfer, although it is a greatly simplified representation. There are other models of mass transfer in fluids which lead to more realistic mathematical outcomes than the film theory. Nevertheless, irrespective of how mass transfer is visualised, diffusion is always an important mechanism of mass transfer close to the interface between fluids.

Penetration theory

Most of the industrial processes of mass transfer is unsteady state process. In such cases, the contact time between phases is too short to achieve a stationary state. This non stationary phenomenon is not generally taken into account by the film model. In the absorption of gases from bubbles or absorption by wetted-wall columns, the mass transfer surface is formed instantaneously and transient diffusion of the material takes place. Figure demonstrates the schematic of penetration model.

Basic assumptions of the penetration theory are as follows:

- 1) Unsteady state mass transfer occurs to a liquid element so long it is in contact with the bubbles or other phase
- 2) Equilibrium exists at gas-liquid interface
- 3) Each of liquid elements stays in contact with the gas for same period of



Schematic of penetration model.

Under these circumstances, the convective terms in the diffusion can be neglected and the unsteady state mass transfer of gas (penetration) to the liquid element can be written as:

$$\frac{\partial c}{\partial t} = D_{AB} \frac{\partial^2 c}{\partial Z^2} \quad \text{-----} 1$$

The boundary conditions are: $t = 0, Z > 0 : c = c_{Ab}$ and $t > 0, Z = 0 : c = c_{Ai}$. The term c_{Ab} is the concentration of solute at infinite distance from the surface and c_{Ai} is the concentration of solute at the surface. The solution of the partial differential equation for the above boundary conditions is given by the following equation:

$$\frac{c_{Ai} - c}{c_{Ai} - c_{Ab}} = \operatorname{erf}\left(\frac{Z}{2\sqrt{D_{AB}t}}\right)$$

Where $\operatorname{erf}(x)$ is the error function defined by

$$\operatorname{erf}(x) = \frac{2}{\sqrt{\pi}} \int_0^x \exp(-Z^2) dZ \quad \text{-----2}$$

If the process of mass transfer is a unidirectional diffusion and the surface concentration is very low ($c_{Ab} \sim 0$), the mass flux of component A, N_A [$\text{kg m}^{-2} \text{s}^{-1}$], can be estimated by the following equation:

$$N_A = \frac{-\rho D_{AB}}{1 - c_{Ab}} \left(\frac{\partial c}{\partial Z}\right)_{Z=0} \approx -\rho \left(\frac{\partial c}{\partial Z}\right)_{Z=0} \quad \text{-----3}$$

Substituting Equation (2) into Equation (3), the rate of mass transfer at time t is given by the following equation:

$$N_A(t) = \rho \sqrt{\frac{D_{AB}}{\pi t}} (c_{Ai} - c_{Ab}) \quad \text{-----4}$$

Then the mass transfer coefficient is given by

$$k_L(t) = \sqrt{\frac{D_{AB}}{\pi t}} \quad \text{-----5}$$

The average mass transfer coefficient during a time interval t_c is then obtained by integrating Equation (2) as

$$\bar{k}_{L,av} = \frac{1}{t_c} \int_0^{t_c} k(t) dt = 2 \sqrt{\frac{D_{AB}}{\pi t_c}} \quad \text{-----6}$$

So from the above equation, the mass transfer coefficient is proportional to the square root of the diffusivity. This was first proposed by R. Higbie in 1935 and the theory is called Higbie's penetration theory.

Surface Renewal Theory

For the mass transfer in liquid phase, Danckwert (1951) modified the Higbie's penetration theory. He stated that a portion of the mass transfer surface is replaced with a new surface by the motion of eddies near the surface and proposed the following assumptions:

- 1) The liquid elements at the interface are being randomly swapped by fresh elements from bulk
- 2) At any moment, each of the liquid elements at the surface has the same probability of being substituted by fresh element
- 3) Unsteady state mass transfer takes place to an element during its stay at the interface.

Hence, average molar flux, $N_{A,av}$

$$N_{A,av} = (C_{Ai} - C_{Ab})\sqrt{s \times D_{AB}} \text{-----7}$$

Comparing the equations

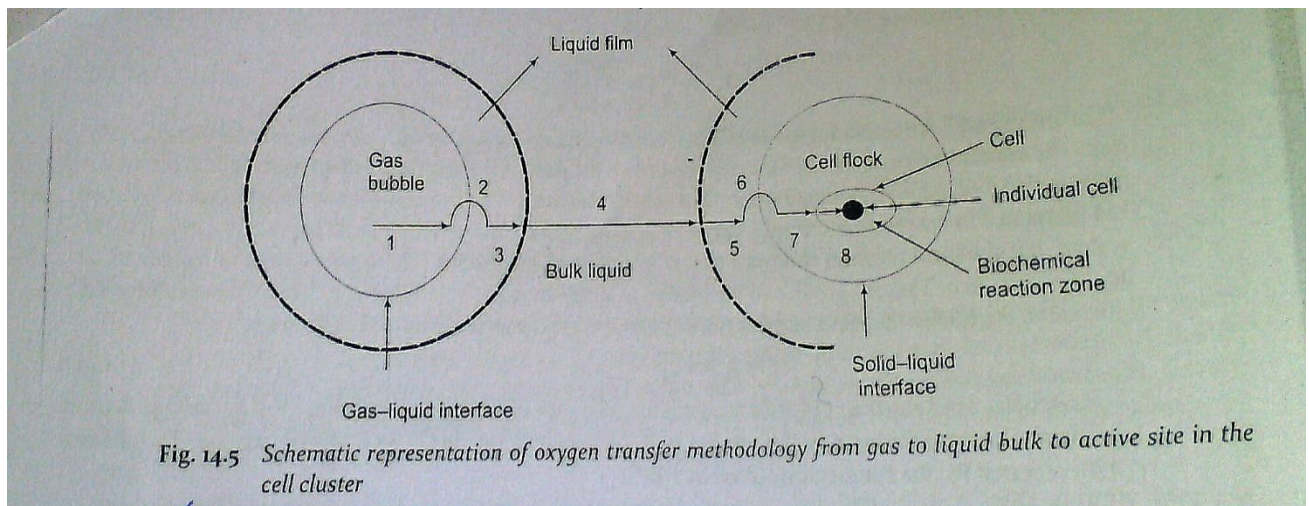
$$k_{L,av} = \sqrt{s \times D_{AB}} \text{-----8}$$

where s is fraction of the surface renewed in unit time, i.e., the rate of surface renewal [s⁻¹].

OXYGEN TRANSFER METHODOLOGY IN FERMENTERS

The transfer of gas from a bubble to a liquid medium in which the gas is dispersed in the form of bubbles is a typical case of gas-liquid mass transfer, and has been dealt extensively in chemical engineering literature. In fermenters, the subject is more or less restricted to oxygen transfer into fermentation broths. In typical chemical reacting systems, the transferred oxygen is consumed in the chemical reaction, and hence the mass transfer rate is enhanced due to chemical reaction, which we call as a classical case of **mass transfer with chemical reaction**. Similarly, in aerobic fermentation, the oxygen is consumed by the microorganisms, and thus the oxygen transfer rate is enhanced.

In this section, we consider the oxygen transfer methodology from air bubbles in a fermentation liquid broth. It is shown schematically in fig. 14.5. There are about eight steps involved. The gaseous component has to overcome these barriers before it reaches the active site in the cell or in the cell cluster for biochemical reaction to take place. Some of them are limiting and some are not. They are shown in the table below.



Various steps involved in the transport of gas from gas bubbles to the bulk liquid and to the active site in the cluster.

Step	Transfer operation	Remarks
1	Transfer of the gaseous component from the bulk gas (air) to the gas- liquid interface.	This step is relatively fast, and no resistance is involved in it
2	Crossing the gas-liquid interface.	Resistance is negligible.
3	Diffusion of gaseous component through the relatively stagnant liquid film into the well mixed bulk liquid	The liquid film offers resistance for gas diffusion, and hence may be rate limiting.
4	Transport of the gaseous component through the relatively well mixed bulk liquid.	Resistance is negligible.
5	Diffusion of gaseous component through the relatively stagnant liquid film surrounding the Liquid- solid interface.	Resistance is negligible if the liquid-solid interface surrounds a single cell. In case of lumps of cells, the resistance is considerable
6	Transport of the gaseous component through the liquid-solid interface.	Generally the resistance is negligible
7	Diffusion of gaseous component within the internals of the cell cluster	Internal mass transfer resistance is significant and hence may be rate limiting.
8	Transport within the cell to reach the active site (cytoplasm) for the biochemical reaction to take place.	Since the cell is very small in diameter, distance travelled are less, and hence the resistance is negligible.

FACTORS AFFECTING OXYGEN TRANSFER RATE:

Various operating parameters and the physic-chemical properties of the broth affect the mass transfer rate which we quantify in terms of $K_L a$. As has been mentioned, the mass transfer rate can be increased by increasing the mass transfer coefficient (K_L) or interfacial area (a) or the driving force ($C_{AL}^* - C_{AL}$).

The possibility to increase $C_{AL}^* - C_{AL}$ is very less, because C_{AL}^* itself is very small (of the order of 10 ppm or less). Hence, the option left out is to increase the K_L or a . Interestingly, the factors which increase a have a negative effect on K_L , which is caused by decreasing the bubble size or bubble diameter. The effect of the following on the mass transfer rate will be discussed in the section.

- | | |
|-------------------------------|------------------------------|
| (i) Bubble size | (ii) gas hold up |
| (iii) Gas velocity | (iv) type of gas sparger |
| (v) Type of agitation | (vi) power input to agitator |
| (vii) Temperature | (viii) pressure |
| (ix) Antifoaming agents | (x) presence of cells |
| (xi) Surface – active solutes | |

Bubble size:

The bubble size has a significant effect on the mass transfer rate. As the gas is sparged discretely in the fermentation broth, in the form of small bubbles, each will be of 2.1 mm^3 volume, and will have a radius of 0.795 mm and surface area of 7.91 mm^2 . Thus, fragmentation of the bubble into smaller sizes will be increasing the interfacial area. However, the smaller bubbles will have a negative effect on the mass transfer coefficient. They contain a smaller quantity of oxygen, which would be transferred quickly. Subsequently, what is left out is only nitrogen, which does not contribute for mass transfer. The smaller bubbles will have a rigid surface. They behave like a rigid sphere with less internal recirculation, so that the fresh batch of oxygen gas within the bubbles does not reach the surface. If the bubble diameter is less, the bubble rise velocity will also be less. Thus, the swirling of the bubbles will also be less, with the results that the chances for new surface area to come into contact with the bulk liquid will also be less. This results in low mass transfer coefficients. Similarly, bigger bubbles will have higher rise velocity, and hence their stay in the broth is less; with the result that adequate time is not given for the entire oxygen to transfer to the liquid. Table 14.2 shows these effects, even though the results are not quantified. Bubbles are normally of size 2-3 mm in diameter. Bubbles with diameter greater than 3mm are called as large bubbles, and those with diameters less than 2 mm are classified as small bubbles.

Gas Hold- up:

Gas Hold-up is the volume fraction of the gas held up in the total volume comprising the liquid and the held-up gas together, i.e.

$$\epsilon = \frac{V_G}{V_G + V_L} + \frac{V_G}{V}$$

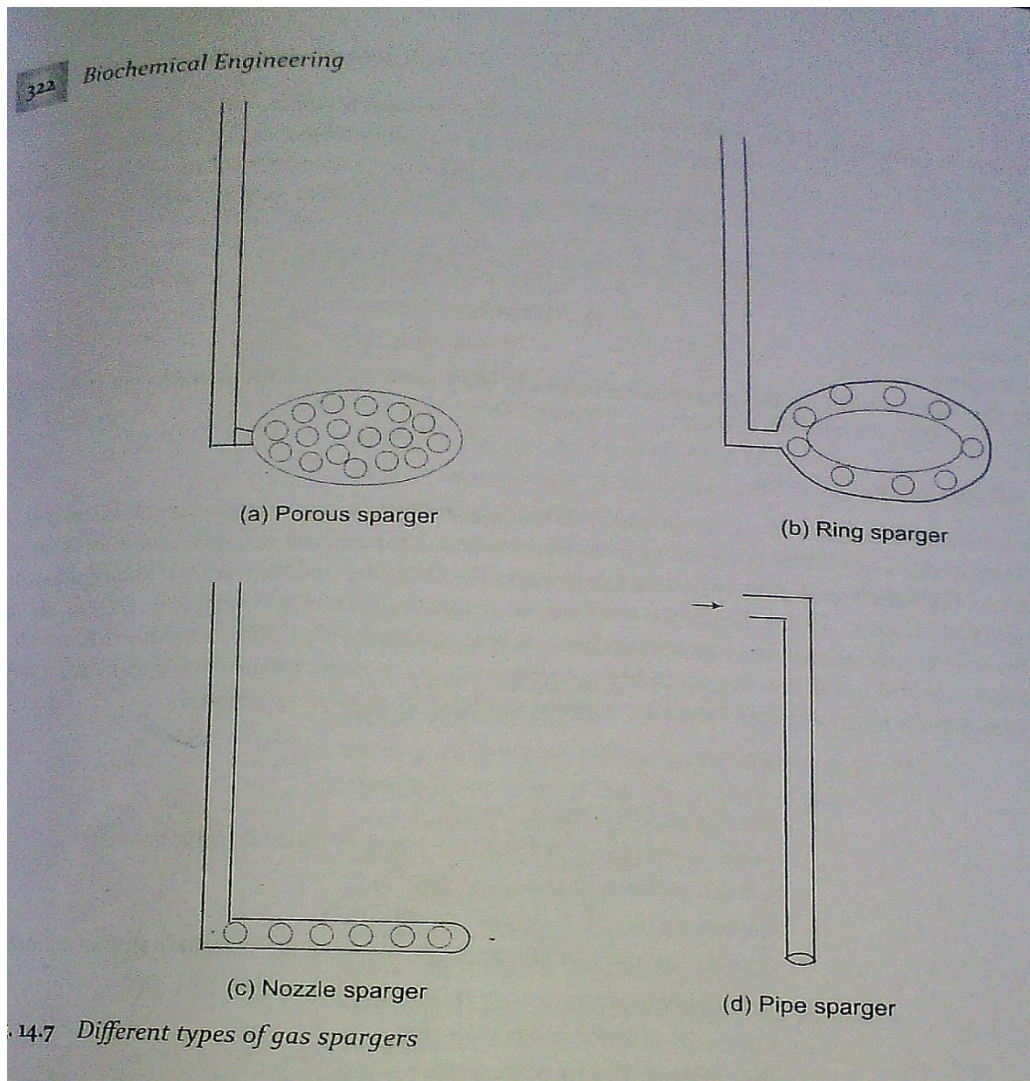
Thus, higher values of ϵ indicates higher amount of gas held up in the system. Even though ϵ does not speak of the bubble size, generally smaller bubbles stay for longer times in the liquid broth and contribute for higher ϵ . Thus high ϵ results in higher interfacial area, and hence higher mass transfer rates. Normally, bioreactors. For example, very small air bubbles will transfer the oxygen content in them to the liquid broth quickly, and what is remaining is only nitrogen which does not contribute anything for the performance of to the mass transfer rates. One of the main reasons for it is that the K_L values decrease with decreasing bubble diameters below 2 mm.

Gas velocity:

The superficial gas velocity (u_G) is the linear velocity of the gas obtained by dividing the volumetric flow rate of the gas with the cross-sectional area of the vessel. The mass transfer rate increases with u_G . If the gas flow rate is very high, it may not allow the solute to dissolve in the liquid, and hence may escape in the outlet. In agitated vessels, the effect is compounded by the agitator speed also. Different gas flow regimes develop. If the gas flow rate is high, and the stirrer speed is low, the gas does not get dispersed properly. The gas bubbles rise up just like in a bubble column. The contacting pattern is poor, resulting in interfacial area less, and most of the gas escape unutilized. The situation is called **impeller flooding**. i.e. the gas is being supplied to the system at a rate which the impeller is not able to disperse. To overcome this, either the gas flow rate should be reduced or the agitator speed is to be increased. If the flow is too low compared to the agitator speed, then the gas bubbles will be simply recirculating, and hence the gas hold-up will increase, but mass transfer coefficient will reduce, with the result that the mass transfer rate will come down.

Type of gas sparger:

The effect of spargers on mass transfer has not been studied extensively. Hassan and Robinson reported that sparger design did not have much effect on gas-liquid dispersion in aerated aqueous phases. However, they felt that it may have effect in large-scale industrial tanks in which a ring-type sparger would yield more uniform distribution of the gas as compare to other types of spargers. The most commonly used variety is a porous sparger (Fig 14.7(a)). it is made out of a metal or glass or ceramic material with a small amount of projection. The diameter of the sparger is almost equal to that of the impeller, and as a rule of thumb, will be one-third the diameter of the tank, and is positioned below the agitator. The number of holes and their matrix one-third the diameter of the tank, and its seldom disclosed as a design secret. The cells may grow along the holes, particularly during off-operation period. They may block the holes and may



cause nuisance. Another variety is a ring sparger (Fig 14.7(b)). It is also known as **orifice sparger**. A number of holes is made to a pipe, and it is made into a

ring. The ring diameter is again comparable to that of the agitator and is kept below it. This kind of sparger is used in single-cell protein production and in waste-water treatment. Figure 14.7(c) shows nozzle sparger with the holes along the length of the sparger. Sometimes the holes are made in the bottom of the sparger pipe, particularly when the flow rates are low, to effect better mixing and also to avoid that the gas bubbles directly come under the impeller. Pipe sparger Figure 14.7(d) is simply a pipe through which gas is sparged. It also can be used when the gas flow rate are very slow.

Type of Agitator:

Type of agitator and agitator design for effective mixing have been a subject of a lot of discussion in the chemical engineering and biochemical engineering literature. Various type of agitation have been designed for mixing of **gas-liquid, liquid-liquid, liquid-solid, gas-liquid-solid** systems.

The subject received a lot of attention from the standpoint of gas-liquid reaction systems, different types of agitator used and the power input requirements, correlation for power input for gassed and ungassed systems, etc. Here we restrict our discussion to various types of agitation used. They can be broadly classified as:

- propeller
- turbine
- Paddle
- vaned discs
- Anchor
- helical screw etc.

Vaned disc or turbine impellers are used for better gas-liquid contacting. Anchor-type agitators are used for highly viscous liquid and slurries. Helical and ribbon-type agitators are used for highly viscous semi-solid masses.

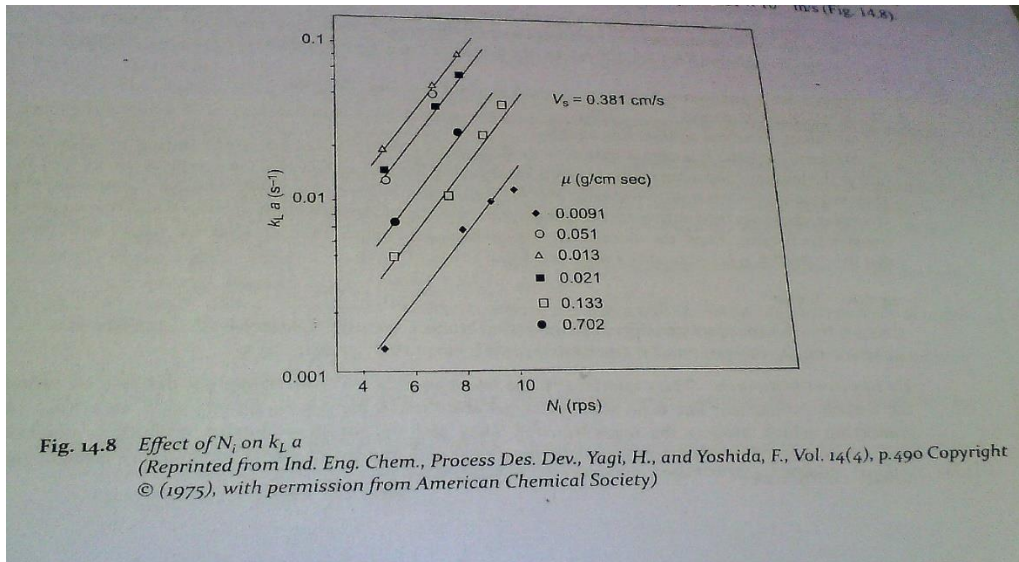
Power input to Agitator:

The effect of power input on was shown. The interfacial area varies as 0.4 power of the power input per unit volume (P/V). The power input is also related to agitator speed through the power number (N_p), where

$$N_p = \frac{P}{\rho N_i^3 d_i^5}$$

$$\text{Or} \quad P = N_p \rho N_i^3 d_i^5$$

The dependence of $K_L a$ on N_i was demonstrated by Yagi and Yoshida (1975) for gas absorption in agitated vessels by Newtonian and non-Newtonian fluids with a superficial gas velocity of 3.81×10^{-3} m/s (Fig. 14.8).



Temperature:

The temperature has both the effects on mass transfer. It increases the diffusivity of the gas into the liquid and hence increase the value of K_L . However, increase in temperature decreases the solubility of the gas, and hence reduces C_{AL}^* , which in turn reduces the driving force $C_{AL}^* - C_{AL}$ for the mass transfer. Hence, it is reported that in the temperature is above 40°C , the mass transfer rate decreases.

Pressure:

Pressure affects the mass transfer by increasing the solubility of the gas in the liquid phase, which is given by Henry's law:

$$P_{AG} = H \times C_{AL}^*$$

The partial pressure and total pressure of the system are related by

$$P_{AG} = P_T \times Y_A$$

Thus, as total pressure P_T increases, P_{AG} increases, and hence C_{AL}^* increases, which in turn increase the driving force $C_{AL}^* - C_{AL}$. Generally, we do not come across high-pressure system in fermentation processes.

Antifoaming Agents:

Most of the fermentation broths contain proteins which cause foaming. Thus, foaming is an uninevitable nuisance in fermentation broths, and should be avoided. Otherwise,

- It may choke the pipelines
- It may choke the measuring instrument lines
- It may harbor unnecessary microorganism to thrive and thrash the fermentation subsequently
- The choked pipe lines are difficult to be cleaned and may be a potential source for development of toxins.

Hence, ways and means have been devised to avoid foaming. They are

- Mechanical means
- Adding chemical antifoaming agents.

Of them, the latter is a very common method. Some of the silicon-based antifoam agents are added, which affect the surface chemistry of the bubbles by reducing the surface tension. One positive effect of it is that they reduce coalescence of the smaller bubbles into bigger bubbles. Thus they increase the interfacial area. However, these surface active agents reduce the mobility of the gas-liquid interface and reduce the mass transfer coefficient. Thus, the increase in a is countered by reduction of K_L , which is larger, with the result that the overall $K_L a$ reduced

Cells:

Oxygen transfer (or mass transfer) in fermentation broths is greatly influenced by the presence of cells. Their influence on the oxygen transfer can be described by the following two ways.

(i) Physical influence: They interfere in the break-up and make-up of the gas bubbles by influencing the surface properties. The cells sometimes get absorbed at the gas-liquid interface, and cause surface blanketing which hinders the mass transfer. They also don't allow smaller bubbles to coalesce into bigger bubbles. This effect increases the interfacial area. However, surface-blanketing reduces the mass transfer coefficient.

(ii) Quantitative influence: The cells absorb oxygen during the process which increase the driving force term $C_{AL}^* - C_{AL}$. As the oxygen is absorbed, C_{AL} will be becoming smaller, and the term $C_{AL}^* - C_{AL}$ will be higher. This results in

higher N_A . The effect is similar to enhancement of mass transfer due to chemical reaction, which is frequently described in chemical reaction engineering literature.

The influence of cells in enhancing the mass transfer rate depend upon

- the type of cells
- morphology of cells
- concentration of cells

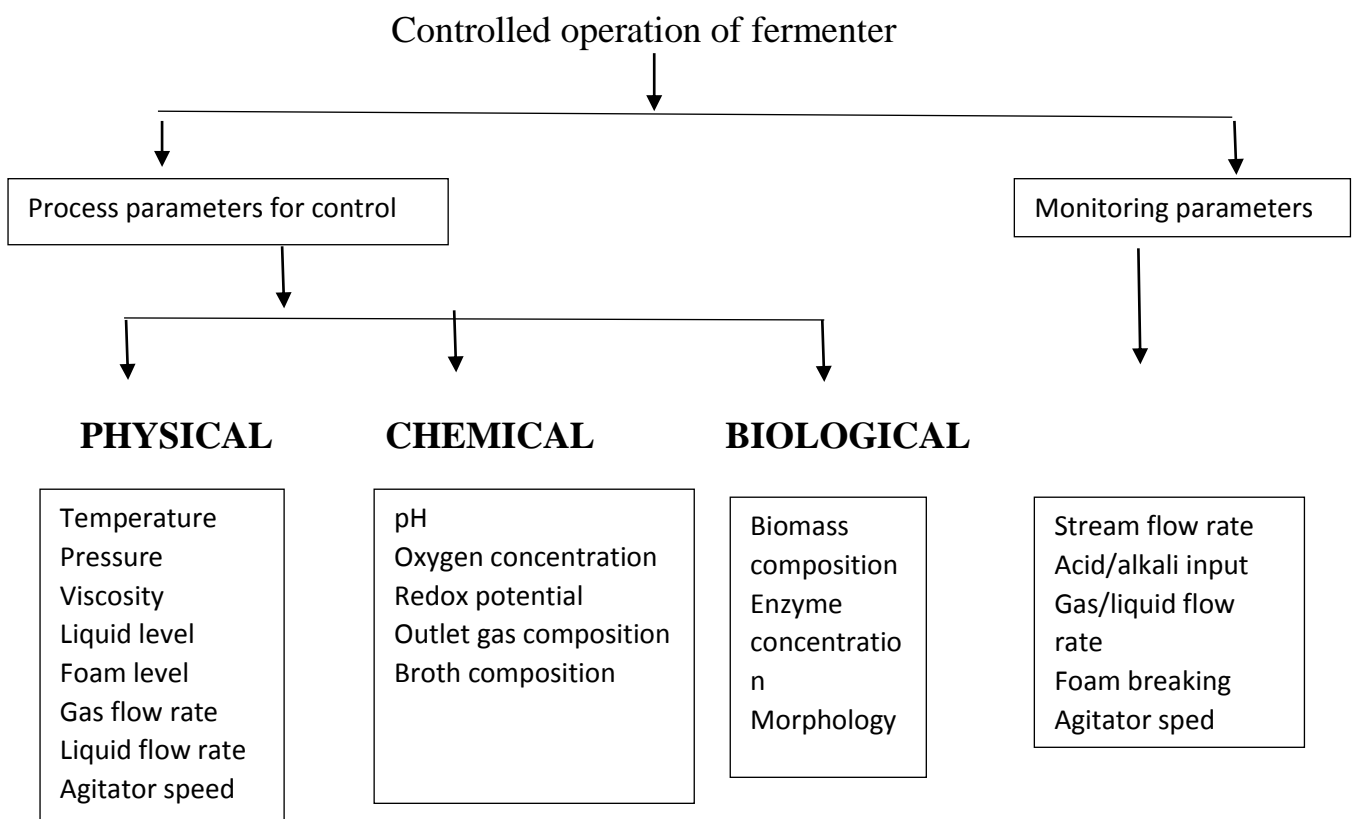
However, the information on the above parameters is only qualitative. The quantitative influence is System-specific.

Surface-active solutes:

The surface active solute, which are hydrophilic in nature, will alter the surface characteristic of the gas-liquid interface, and do not allow the gas bubble to coalesce. This results in increased interfacial area. The concentration of the solute could be very low, but its effect in increasing the surface area could be large. Since the concentration of solute is very low, they do not affect the interfacial tension to any appreciable or measurable extent. Calderbank explained this with a solute like alcohol in water did not allow the air bubbles to coalesce in the aqueous media. The bubbles form stable raft of bubbles which maintain their identity without coalescing until they burst.

MONITORING AND CONTROL OF FERMENTATION PROCESSES

Controlling of process parameters in fermentation operation is one of the most crucial steps in successful operation of the fermenter. Since living organisms are at job in the fermenter, their ability in terms of their growth, multiplication, proliferation and performance to bring out a certain biochemical conversion is dependent upon the environment in the bioreactor. The environment in the bioreactor is dictated by physical, chemical and biochemical parameters (Doran, 1995), and is detailed in following diagram.



Monitoring & control of fermenter

On-line & Off-line Control

- The measurement & control of the parameters can be done on-line or off-line. On-line measurement is the method of measuring the process parameters within the proximity while the fermenter is working. For example, the temperature in a fermenter can be measured with a thermocouple on a digital indicator while the fermentation process is going on.
- The observed value will be compared with the set/desired value. Accordingly, the corrective action can be initiated. If the temperature is rising, the steam value will be adjusted to decrease the flow rate, or electrical input will be reduced to control the heating.
 - Similarly, the dissolved oxygen (DO) concentration can be measured with a DO analyser & will be compared with the desired value. Based on the set point & concentration, oxygen concentration will be monitored either by adjusting the oxygen flow rate or the agitator speed.
- Thus, the on-line measurement reduces the time-gap between the measurement & control, hence is desired.
- However, all the process parameters cannot be measured on-line. Then they will be measured off-line, in which case, the sample is collected from the fermenter & analysed separately, may be in a separate room. The results of analysis can be made available after 2-24 hours only.
- For this purpose, the samples will be collected once in every 4-8 hours & sent for analysis. Since the results of the analysis are available only after sometime, the corrective measures cannot be implemented immediately.
- There is a time-lag between the measurement & control, i.e. the monitoring is delayed. Sometime, by the time the corrective measure is implemented, the environment in the fermenter may also change & need a different type control. Thus, the off-line measurement is not desired.
- For example, the pH of broth can be measured on-line with a pH measuring electrode, accordingly acid or alkali can be added to broth to control the pH to the desired level by operating a solenoid valve connected to either the alkali tank or alkalinity by titration with a time-lag or delay in monitoring.

Controlling System

The measurement, monitoring & controlling steps involve the following four basic components, irrespective of whether it is on-line or off-line controlling operation:

- The process parameter
 - The measuring element
 - Comparing with the set point
 - Corrective measure.
- For example, the cell concentration is needed to be measured. If the cell concentration is correlated to the viscosity of the broth, the process parameter is viscosity. The viscosity of the broth will be measured off-line by usual techniques (by using a viscometer).
- The measured viscosity is compared to the set or desired value. If the value is higher, it is an indication that the cell concentration is on the higher side & vice-versa. One method of controlling the cell population is by adjusting the oxygen flow rate to the broth.
- This can be done either by adjusting the air flow rate or by adjusting the agitator speed. Usually, the air flow rate is adjusted. This will control the cell population, which can be gauged by the changes in viscosity of the broth.

The controlling can be done in the following ways:

- 1) Manual control
- 2) Automatic control
- 3) Computer control

1) **Manual control:**

- In the case of manual control, the process parameter, after its measurement, is compared with the desired value (set point). Depending upon whether the measured value is higher or lower than the set point, the corrective action is initiated until the measured value matches with the set value.
- For example, in the case of measurement & control of temperature of the fermentation broth, initially the temperature of the broth is measured with a thermometer or a temperature indicator.
- The measured value is compared with the set value. If the temperature is higher, the steam valve supplying steam is adjusted to reduce/stop the steam supply manually, until the set point is reached.

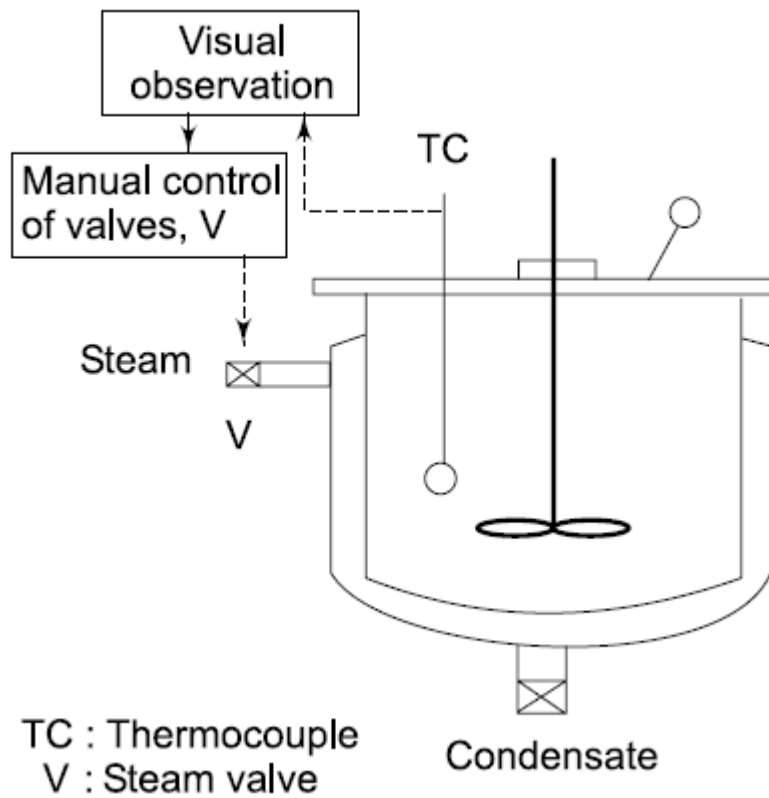
- Thus, the manual control is subject to errors & delay in operation. It also depends upon the skill of the operator. The running costs are higher for it, but the initial capital investment is negligible.

2) **Automatic Control:**

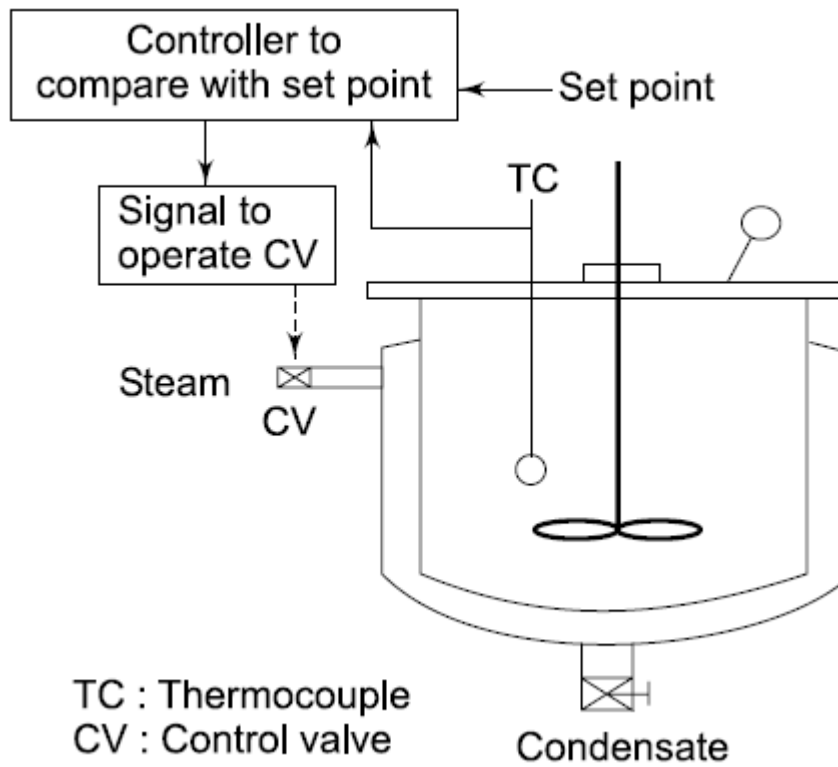
- The automatic controlling systems are better than the manual control systems, since they avoid human-biased error & skill. The controlling is done by an instrument. For this purpose, the measuring device should produce a signal or impulse.
- This will be compared with the set value in a loop. Accordingly an output signal is generated which implements the controlling actions. Now considering the earlier example of temperature control, the temperature is measured with a thermocouple which gives impulse in the form of 'emf' (electro motive force).
- The thermocouple is connected to a temperature controller, which in turn generates some output which will operate the solenoid valve connected in the stream line to increase/reduce the temperature depending upon the set value.

The automatic control systems can be of the following four types, depending upon how the controlling is done:

- On/Off controller
- Proportional controller
- Integral controller
- Derivative controller



(a) Manual control

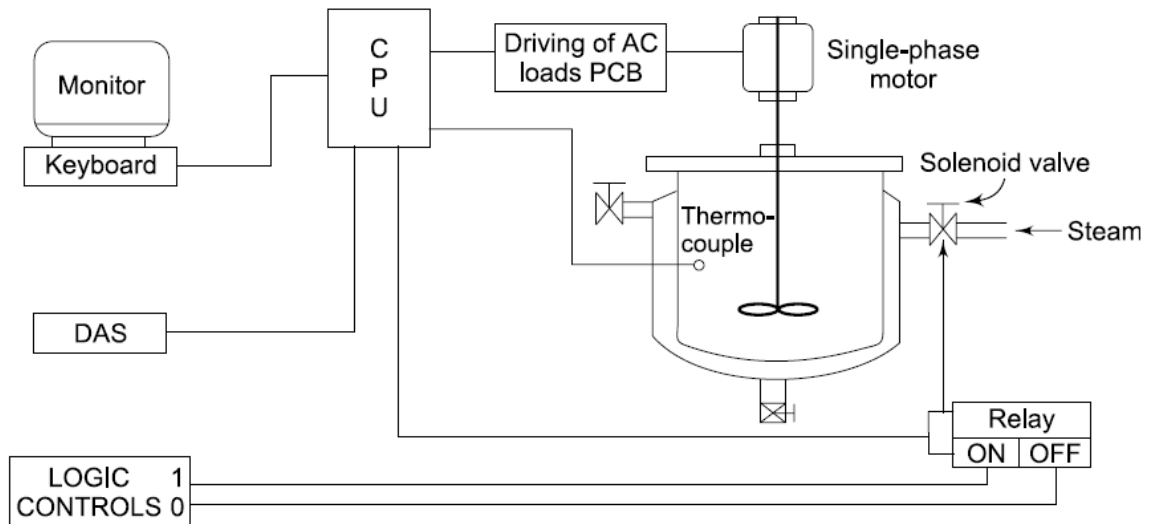


(b) Simple automatic control

Computer Control of Fermentation Systems:

- In the last two-three decades, computers have changed the methodology of controlling process parameters in fermenters. The speed of operations in the on-line control systems has considerably improved.
- The computer with its huge memory capacity (compared to pneumatic & electronic control systems) has become the nerve centre of the control systems. A large number of process parameters can be controlled from a signal control room which is remotely placed, & connected to the fermenter through softwiring. (Obviously, the fermenter has a larger number of process parameters to be controlled). Then the computer is interfaced to the fermenter with an analogous-to-digital (A/D) converter.
- All the physiological changes in the fermenter are manifested in the form of some physical or chemical environmental parameters in on-line experiments (Hampel, 1979). For example, the changes in cell growth will result in increase/decrease of dissolved oxygen concentration.
- The DO analyser senses the oxygen concentration in the broth & gives the form of millivolts, will be compared to the stimulus from the system by the CPU (central processing unit), & instructions will be issued to the actuator. In the present case, if the dissolved oxygen concentration is less, the air /oxygen inlet valve is activated to counter/ correct the error, i.e. the solenoid valve admitting air/oxygen will be opened more to admit more gas. Thus, the interfacing can be classified to meet the following objectives:

Operator-computer	: instruction
Sensor-computer	: input
Computer-operator	: information
Computer-actuator	: manipulation
Computer-output devices	: communication



Block diagram for PC-controlled heating system for fermenter

Biosensor for Fermentation Control

So far, various techniques have described for on-line control of the fermentation processes. Generally, spectrophotometric or chromatographic techniques are used the on-line measurements of concentration of the components. These methods take relatively longer times for responding to the process change. To overcome these difficulties, biosensors are one useful alternative. Biosensors are developed as a result of wedding of enzymes/microbial activity or reactivity with microelectronics

Thus, a biosensor consists of:

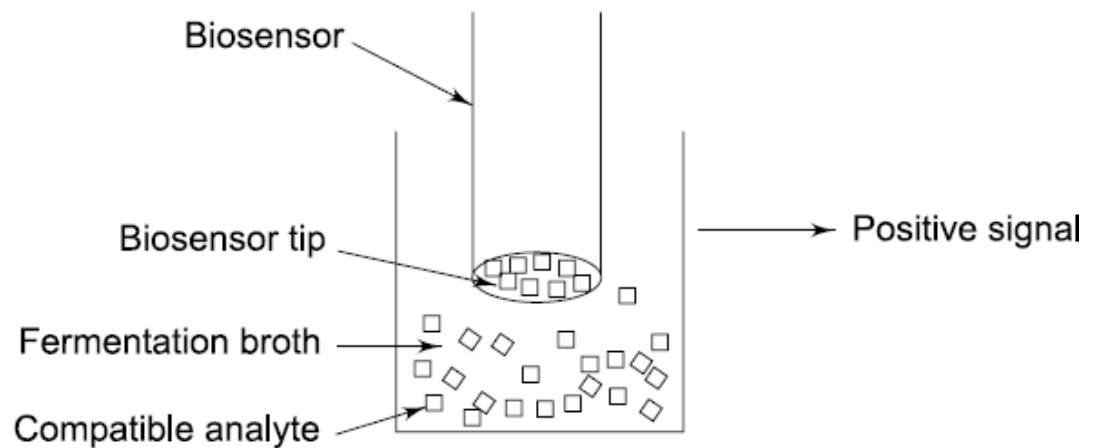
- A biological sensing element which is chemically receptive
 - A signal transducer which gives the output in the form of a measurable signal
- The biological sensing element is usually an enzyme or a microorganism or an antibody. To give physical strength & rigidity to the sensor, the biological molecule is immobilized on a probe.
 - In view of its industrial importance, a good amount of studies was made on the biosensors. Even textbooks were published on the subject (Hall, 1991). However, we restrict our discussion for the present to describe only the basic principles of biosensors.
 - The tip of the biosensor is provided with a provided with a biologically sensitive material like an enzyme. If it comes across a compatible analyte it would give the output signal indicating positive response.

- The quantity of the signal also depends upon the number of the analytes, which in turn is proportional to the concentration of the analyte. An incompatible analyte is not detected or sensed by the tip, hence does not give any response in the form of a signal output. An incompatible analyte is not detected or sensed by the tip, and hence does not give any response in the form of a signal output.
- Since the enzymes are generally expensive & are also unstable to be used as sensing elements in bioprocess control, some microorganisms, which are known for the specificity to certain molecules can as well be employed for recognizing or sensing the biomolecules or analytes.
- Such sensors are termed as microbial sensors. For example, a cell membrane from the acetic bacterium **Gluconobacter suboxydans** can be used as an alcohol sensor. Karube & Sode (1989) have described the use of biosensor for sensing the concentration of:
 - Sugars (glucose, fructose & sucrose)
 - Alcohol
 - Glutamic Acid
 - Carbon dioxide

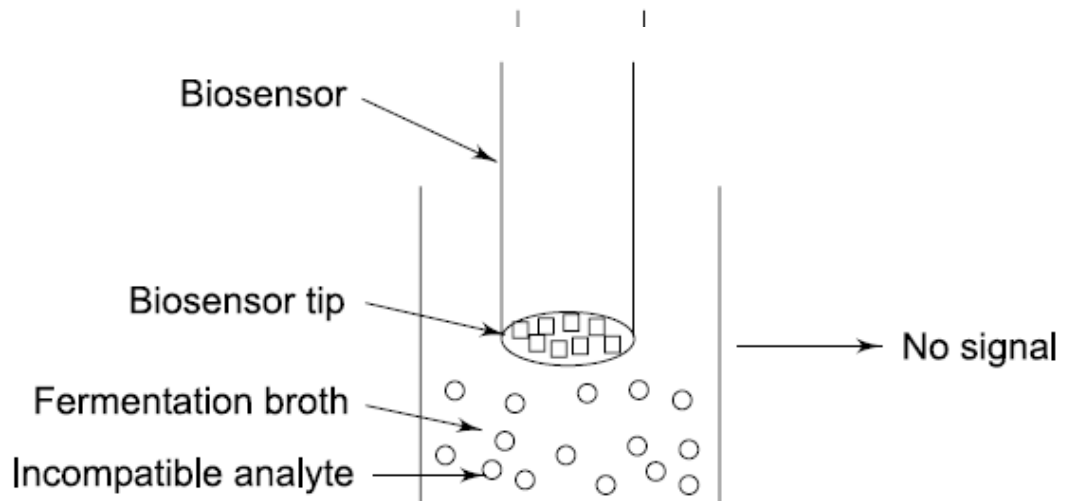
Schuger (2000) observes that a good number of sensors was developed in this area in the last two decades, but their applications are restricted to mostly laboratory investigations.

A short analysis time (response time) is a prerequisite for process control purposes; particularly the response times of the order of few minutes will suit the purpose of on-line monitoring.

Biosensors can be used for both qualitative & quantitative measurement. However, their use is more restricted to qualitative testing than quantitative estimation. Their prohibitive costs restrict their use in industry. They are being more used for medical or diagnostic purposes. A continuous research going on in this area for their industrial application would make them highly selective, sensitive & reliable, in addition to making them cost-effective, this would make them suitable as disposable-types sensors.



(a) Biosensor responding to compatible analytes



(b) Biosensor not responding to incompatible analytes

Schematic representation of the working of biosensor

Parameters for Control

- Various parameters for monitoring & control have been described in the above Section. Since the fermentation process proceeds with the help of living cells (microorganisms) or enzymes, it is very important how closely the environment in the fermenter is controlled/monitored so that the living organisms find congenial atmosphere for bioconversion of the organic feed or biotransformation .
- So, it is imperative that the process parameters, like temperature, pH, flow rates, DO concentration, should be controlled as closely as possible. Deindoerfer (1960), almost four decades back, observed “There is no reason whatsoever

why batch fermentation should be run at constant temperature, constant pH, etc., when very likely a controlled variation of these influencing factors might have improved yields”.

- This observation puts us to rethink our concept of fermenter controlling/monitoring. This means, it is obvious that some process variables are critical & some are non-critical, which to be controlled or otherwise.

Critical Parameters

The critical parameters are those which have a direct bearing on the progress of fermentation. It could be by the way of

- Cell growth, or
- Conversion of substrate.

Some process parameters which are important are:

- Temperature
- pH
- DO concentration
- Substrate flow rate
- Air flow rate
- Agitator speed
- Redox potential

Generally, the critical parameters are controlled online to avoid time delay by automatic methods or by computer-controlled techniques. The time delay in their control is critical & makes the process run away from control.

Non-critical Parameters

Non-critical parameters are those which need to be controlled, but the time delay in their controlling does not affect the progress of the fermentation process drastically.

They are:

- Pressure
- Viscosity of the broth
- Liquid level
- Foam control
- Outlet gas composition.

- They are the process parameters which are not directly involved with the fermentation process.
- They are the outcome of some changes in the process. For example, the viscosity of the broth increases with the increase in mycelium production, or the liquid level in the fermenter alters due to some failure in the inlet feed systems.
- The pressure control does not play much important role in the biochemical processes, as the pressure fluctuations do not normally occur. The pressure effects in terms of oxygen (or gas) solubility.

Module-III**PRODUCT RECOVERY**

Product recovery in bioprocessing plays a vital role. Sometimes the product recovery costs may even decide the economic viability of the process. They vary anywhere between 20-60% of the product cost. In some extreme cases, the costs may go up to 90% ,as in the case of recombination DNA fermentation products .

The product recovery operations are also known as downstream processing steps. They include all the steps we take up after the fermentation step is completed in the bioreactor. They play a very crucial role because the products that come out of the bioreactor may consist of the following, in addition to the metabolic product:

- Microorganisms
- Whole cells
- Cell debris/fragments
- Soluble and insoluble medium products
- Pellets of aggregated proteins
- Undissolved nutrient components, etc.

In view of large number of unnecessary products being present in the fermented broth, downstream processing steps are important .They may be broadly classified into

- Initial isolation
- Product recovery
- Purification and concentration

The type of the downstream processing step to be adopted for a particular fermentation process depends upon the product, its value and the use for which it is meant. A very costly product to be used for medicinal purposes in small quantities will be purified and concentrated using very sophisticated downstream processing steps; Whereas those meant for industrial use in bulk quantities, may be separated by simple sedimentation initial isolation steps. If the solids are present in suspension, they will be separated by simple sedimentation initial isolation steps. If the solids are present in suspension, they will be separated by simple sedimentation or filtration or configuration. It also depends upon the location of the product in the presence of cells, viz. extracellular or intracellular, i.e. whether the product is independently existing

of the cells or the product is trapped inside the cells .In the latter case , We may have to go for some of the steps for disrupting cells .

The cell distribution techniques may be classified as:

- Physical methods
- Chemical methods

Various unit operations involved in product isolation and purification are highlighted as step 6 in figure below.

The specific choice of the recovery of the product may be summarized as follows:

- The location of the product , viz. intracellular or extracellular, and the heat labile nature of the product
- The quantity of the product in the fermentation broth, I.e. whether it is available in a concentrated form or in a very dilute form
- The cost of the product
- The minimal acceptable standards of the product
- The impurities present in the fermentation product, and their nature of interference with the product recovery steps.

Table.1 shows various unit operations in bioprocessing for separation of different phases based on the fact in which phase the product is available and in which form (whether soluble or suspended).

REMOVAL OF SUSPENDED SOLIDS

The suspended solids could be microbial cells or some kind of undesirable solids. In certain cases , like in the case of baker's yeast, the biomass (the suspended solids) is the desired product and needs to be separated in a pure dry form. The following unit operations are used for the separation of solids;

- Filtration
- Sedimentation
- Centrifugation
- Foam separation
- Precipitation

In this section .we describe foam separation and precipitation

Table.1 Various unit operations in bioprocessing for separation of different phases

SYSTEM	TYPE	WITH PHASE CHANGE	WITHOUT PHASE CHANGE
Solid-Liquid	Soluble	Drying Evaporation Crystallization Adsorption	Ultra filtration reverse osmosis
	insoluble	drying	Filtration Sedimentation Centrifugation
Liquid-Liquid	Soluble	Distillation extraction	Chromatography
	insoluble		Sedimentation cetrifugation
Solid-solid	Miscible	Cell distribution(highpressure4 homogenisation,grinding with abrasives)	
	immiscible	Selective solubilisation	Air aspiration sieving screening , winnowing
Liquid-Liquid-solid	Miscible	Adsorption	
	Immiscible		Centrifugation Sedimentation decanting

FOAM SEPARATION

Foaming is a surface phenomenon and is based on the difference in the surface activity. It is applicable to such products which have a tendency to form foams or to those which attach themselves with the foam. Air/gas is usually blown through the liquid medium containing some kind of foaming agents, viz. fatty acids or quaternary ammonium salts such as:

- Lauric acid
 - Stearic amine
 - T-octyl amine
 - Ethyl hexadecyl dimethyl ammonium bromide.
- The suspended solids adhere to the foam and rise to the upper layer along with the foam. Later the foam is separated and discarded.
 - If the foam contains some active or useful solids, usually the foam is broken by applying some mechanical foam-breaking techniques.
 - Generally, foaming is used for the removal of impurities.

PRECIPITATION

- It is also a very useful method for recovery. By adding the precipitating agent, the compounds form the corresponding salts or the suspending solids which would settle down to the bottom.
- This method is extensively used for product recovery of protein isolates or protein concentrates from oil seeds.
- In one process of making the groundnut (peanut) protein isolate, the groundnut seeds are treated with an alkali to extract the protein. Later the extract is neutralised with an acid to precipitate the groundnut protein.
- It is later isolated, dried and packed for ready to use as a protein source in baby foods, etc.

SEDIMENTATION

- Sedimentation is a method of separation of solids from liquids or liquid globules from immiscible liquids.
- The only force acting downwards on a solid particle suspended in a fluid is the gravitational force. This is opposite in direction to the buoyancy forces which act upwards.
- The settling velocity is dictated by the difference in the densities of the particle and fluid ($\rho_p - \rho_f$) and the viscosity of the fluid. In view of the small values of ($\rho_p - \rho_f$), the settling velocities are usually very small.
- Thus, sedimentation takes a lot of time for the suspensions to settle, probably of the order of a few days.
- Hence, normally, sedimentation is not used as an effective tool for product recovery.

- It is generally used in the effluent treatment for separation of the solid suspensions in the slurries, before the clear liquid is disposed of to the environment.

Theory of sedimentation:

- A particle falling from rest in a fluid, under gravity, will be having increasing velocities.
- A stage comes where the drag force on the particle acting in the upward direction will be equal to gravitational pull.
- Afterwards, the particle attains a maximum constant velocity, which is known as **terminal velocity**.
- The terminal velocity depends upon the :
 1. Size and shape of the particle
 2. Density of the particle
 3. Density and viscosity of the fluid
 4. The gravitational pull which is usually constant.

The forces acting on a sedimenting particle are shown in fig.S

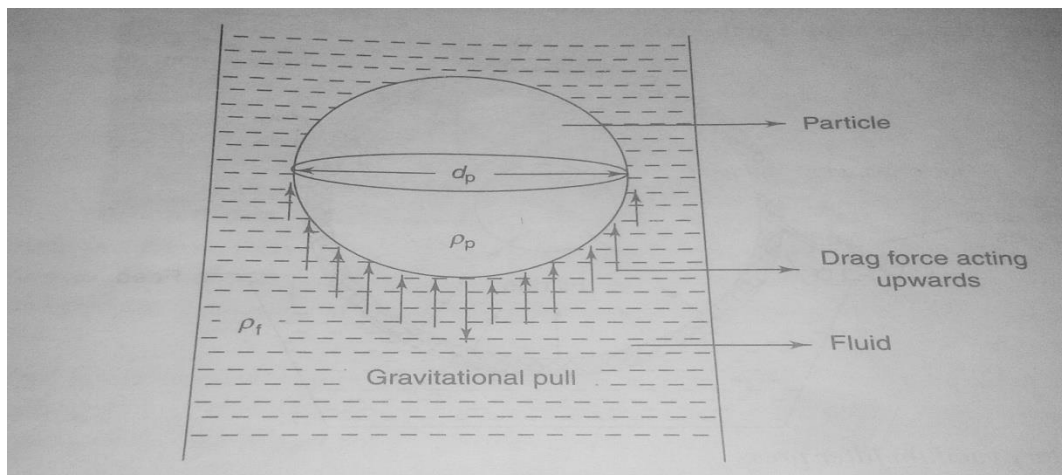


Fig.S1 various forces acting upon a sedimenting particle

The net force acting on the particle is given by (Earle, 1966):

$$F_s = V_s (g/g_c) (\rho_p - \rho_f) \text{ -----i}$$

And the drag force F_d is given by

$$F_d = C_D \rho_f u^2 A_p / 2g_c \text{ -----ii}$$

here C_D is the drag coefficient which is obtained by

Stoke's law in the streamline flow:

$$C_D = 24 / N_{Re,p}$$

In the above equation

$$V_p = \pi/6 d_p^3$$

$$A_p = \pi/4 d_p^2$$

$$N_{Re,p} = \rho d_p u / \mu$$

- When the particle velocity approaches the terminal velocity or settling velocity, the net gravitational force and drag force will be equal.
- Equating eqs.(i) and (ii) and substituting for various quantities, we get an expression for the terminal velocity of the particle.

$$u_t = d_p^2 g (\rho_p - \rho_f) / 18\mu$$

The above equation is useful in process design to find out the minimum surface area of the thickener or the sedimentation tank.

- If the slurry is held in a cylindrical tank, it exhibits various zones. Initially the suspension is uniform as shown in fig.S2(a)
- As time progresses, the solids particles start setting down resulting in clear supernatant liquid on the top. In the intermediate stage (fig.S2 (b)), almost four zones will be existing. The topmost layer is the clear liquid. The bottommost layer is the thick solids. Over the thick solids, the slurry will have a composition which is richer in solids and poorer in liquid. Above this, there will be a layer which has the composition similar to that of the feed slurry. Slowly these zones vanish, and ultimately we find only two zones (fig.S2(c)), the upper layer is a clear liquid and the bottom layer is thick solids which may be removed.

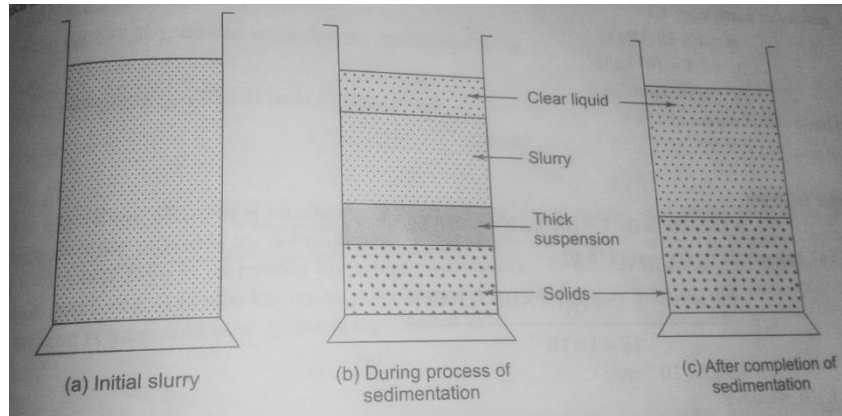


Fig.S2 Settling of solids in a batch sedimentation process

- The design equations are arrived at to find the minimum area of a continuous thickener or sedimentation tank, so that the solids (or the sludge) are withdrawn from the bottom continuously and the clear liquid can be decanted from the top. For a continuous thickener, the rate of sedimentation can be equated to the counter-flow velocity of the rising fluid (Earle, 1996).

$$u_u = [(F - L) r_s] / A \rho_f \text{ -----iii}$$

Where u_u is the upward velocity of flow of liquid, which is also equal to the settling velocity of the particle (u_t); F is the ratio of liquid to the solids in the feed and L is that in that in the exit stream; and r_s is the mass feed rate of the slurry.

Equation (iii) is obtained by simple mass balance. It can be used to calculate the minimum area (A) of the sedimentation tank (or thickener).

$$A = [(F - L) r_s] / u_t \rho_f \text{ -----iv}$$

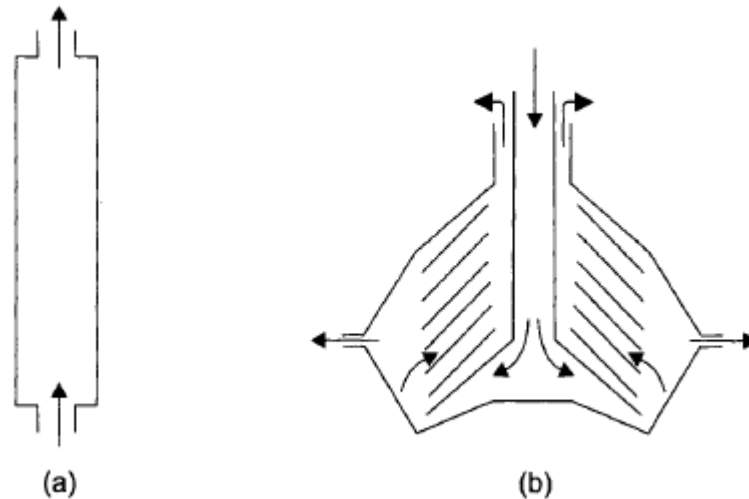
The above equation can be used to evaluate the minimum cross-sectional area of the thickener, provided we know the average particle size d_p , which is the major drawback. The sedimentation slurry may not have all particles of same diameter, In which case, we may have to find out the mean particle size by averaging out various particle sizes and their mass fractions, which is also a difficult task. Mostly, the sedimentation operations are highly time-consuming. It takes a lot of time for the solids to settle.

CENTRIFUGATION

Centrifugation is an alternative method when the filtration is ineffective, such as in the case of small particles. Centrifugation requires more expensive equipment than filtration and typically cannot be scaled to the same capacity as filtration equipment.

Two basic types of large-scale centrifuges are the tubular and the disk centrifuge as shown schematically in Figure below. The *tubular centrifuge* consists of a hollow cylindrical rotating element in a stationary casing. The suspension is usually fed through the bottom and clarified liquid is removed from the top leaving the solid deposit on the bowl's wall. The accumulated solids are recovered manually from the bowl. A typical tubular centrifuge has a bowl of 2 to 5 in. in diameter and 9 to 30 in. in height with maximum rotating speed of 15,000 to 50,000 rpm.

The *disk centrifuge* is the type of centrifuge used most often for bioseparations. It has the advantage of continuous operation. It consists of a short, wide bowl 8 to 20 in. in diameter that turns on a vertical axis. The closely spaced cone-shaped discs in the bowl decrease the distance that a suspended particle has to be moved to be captured on the surface and increases the collection efficiencies. In operation, feed liquid enters the bowl at the bottom, flows into the channels and upward past the disks. Solid particles are thrown outward and the clear liquid flows toward the centre of the bowl and is discharged through an annular slit. The collected solids can be removed intermittently or continuously.



Two basic types of centrifuges: (a) tubular and (b) disk.

When a suspension is allowed to stand, the particles will settle slowly under the influence of gravity due to the density difference between the solid and surrounding fluid, a process known as sedimentation. The velocity of a particle increases as it falls and reaches a constant velocity (known as terminal velocity) at which

$$\text{Weight force} - \text{Buoyancy force} = \text{Drag force}$$

The expression for the terminal velocity can be derived from the balance of the forces acting on a particle as,

$$v_t = \frac{d_p^2 (\rho_s - \rho) a}{18\mu} \quad \text{----- v}$$

Which is applicable when the Reynolds number is less than 1. Which is always the case for biological solutes. In the case of a sedimentation process, the acceleration term in Eq. (v) is equal to the acceleration due to gravity. Due to the small difference in density between the cells and the broth, simple settling can take a long time unless cells are large or the cells form a large aggregate.

Under the centrifugal force, the acceleration term in Eq. (v) becomes

$$a = \omega^2 r$$

Where ω the angular velocity and r is the radial distance from the centre of a centrifuge to a particle. Therefore, the increase in acceleration by the centrifugal force speeds up the settling process.

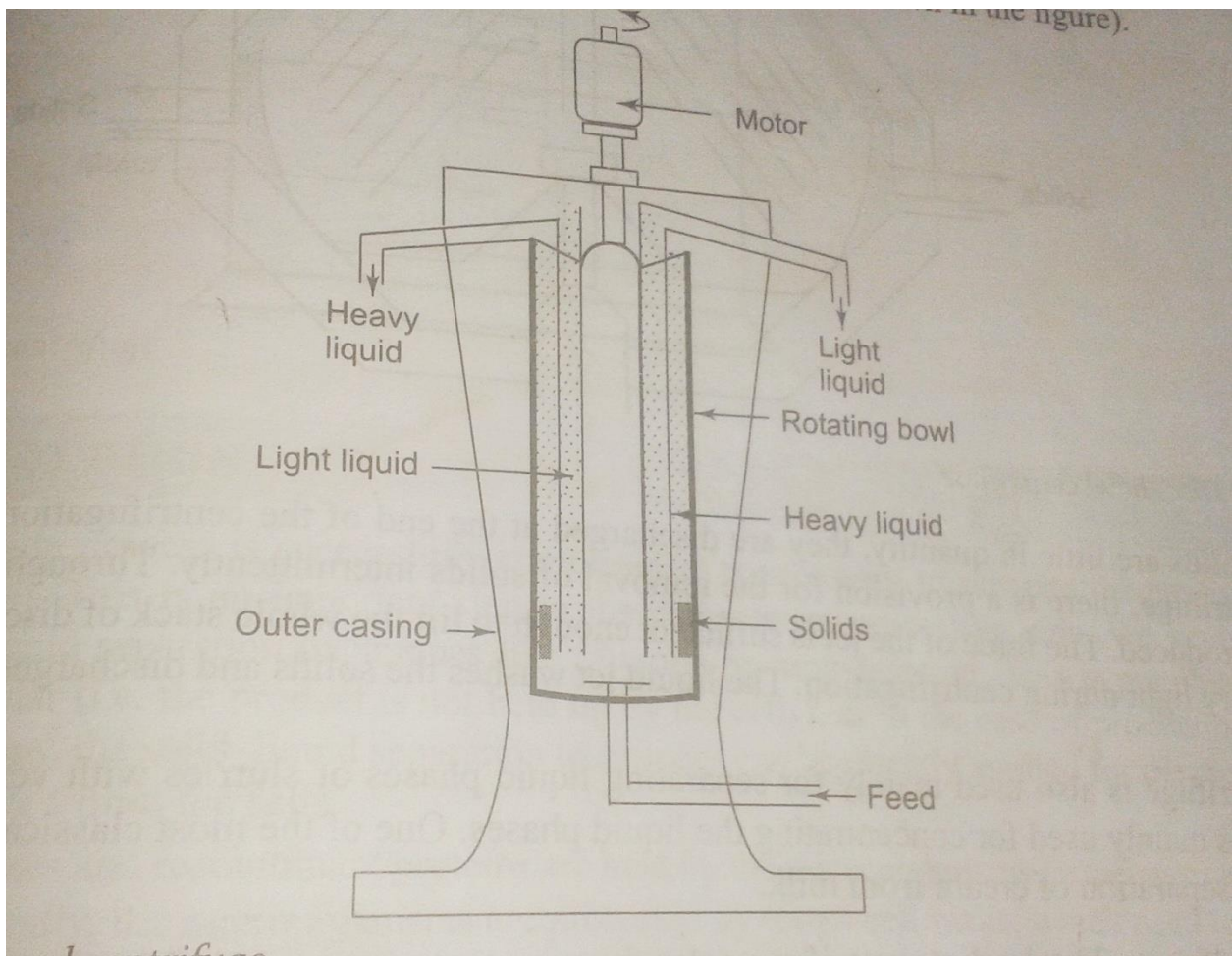
After having seen the theory of centrifugation, we will describe some of the common centrifuges used in bioprocessing.

Tubular bowl centrifuge:-

Tubular bowl centrifuge, also popular known as tubular centrifuge, is commonly used in biochemical operation for the separation of immiscible phase of

1. Heavy liquid-light liquid
 2. Solids-liquid
 3. Heavy liquid-light liquid-solids
- According to the type of operation, some adjustments are made in the internal arrangement for the separation of two liquid phase or liquid-solid phase.
 - Generally, it is used for separation of two liquid phases which are immiscible and have relative differences in densities.
 - The two liquid phases are known as light-liquid phase and heavy-liquid phase.
 - This centrifuge can be used for slurries containing up to 10% solids of 0.1-200 microns size.
 - It consists of a tall and narrow bowl of 10-15 cm diameters, rotating at a speed of 15,000 rpm.

- The laboratory centrifuges (with a bowl capacity of 200 cm³) can operate up to 50,000rpm with an air turbine to produce 62,000 g.
- The feed enters through a nozzle at the bottom into the tubular bowl, which rotates at very high speed.
- The feed liquid mixture is subjected to centrifugal force.
- Accordingly, the two phases slowly separate out.
- Heavy liquid is thrown away from the centre and the lighter liquid concentrates at the centre of the rotating bowl.
- The two phases slowly rise up in the bowl.
- An adjustable ring at the top separates both the phases, which discharge from the outlets provided at the top.
- If the solids are present, they will be settling at the bottom and slowly rise up away from the centre.
- The solids are removed intermittently by flushing water through a nozzle provided for the purpose.

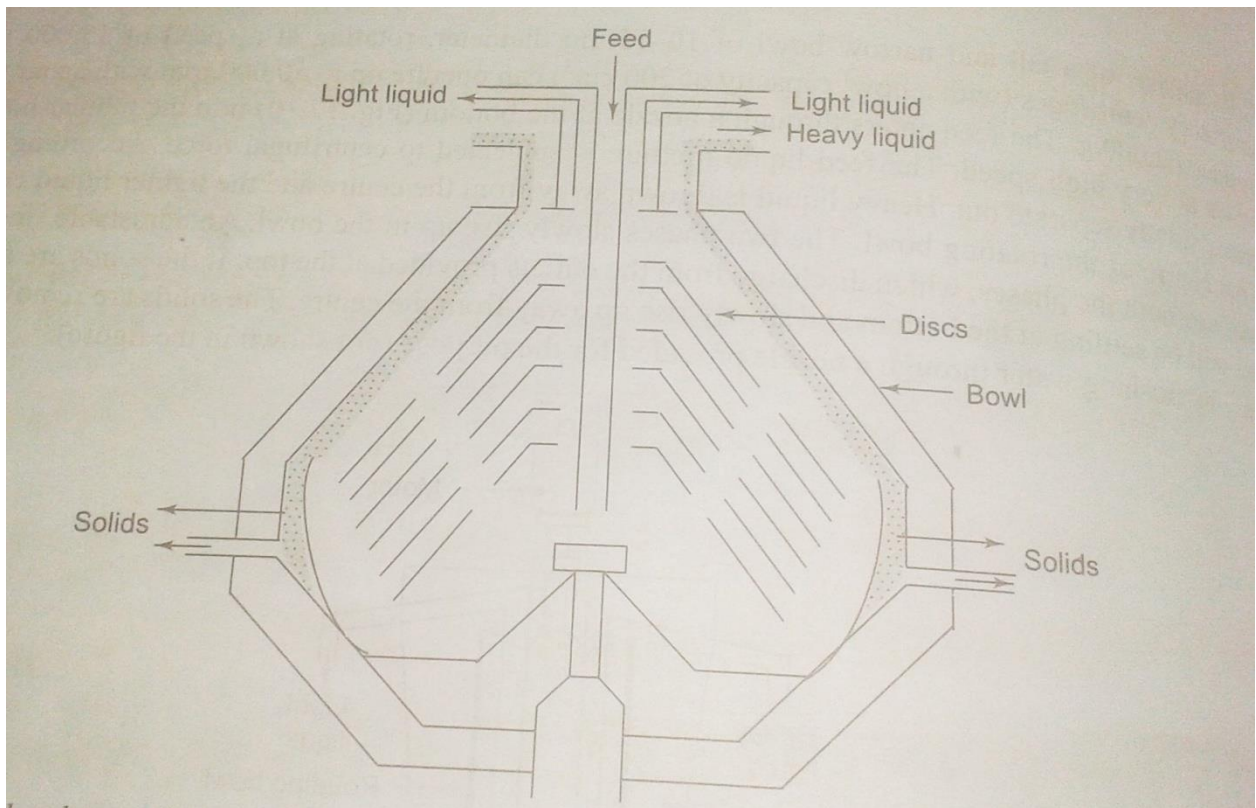


Tubular bowl centrifuge

- One of the major disadvantages with the tubular blow centrifuge is that it is not efficient for solid separation or for heavy loads of solids.
- Particularly, it does not work effectively with three phases (solid-liquid-liquid).

DISC-BLOW CENTRIFUGE:-

- The disc-blow centrifuge (also known as disk centrifuge) consists of an outer bowl in which a large number of discs are stacked in series one over the other, with a small clearance of 3 mm.
- In fact, they are not discs; they are actually thin cones made up of a metallic sheet and having two holes on either side almost at the central portions.
- The conical discs are so placed that the holes on both sides come in the same position.
- The bowl is usually of 20-50 cm in diameter and rotates at high speed.
- The slurry is fed from the centre through a feed nozzle which is stationary.
- The feed comes and falls at the centre of the discs which are rotating at high speed along with the bowl.
- Due to centrifugal force, the heavier liquid separates from the lighter liquid and flows away to the periphery through the holes in the discs.
- The solids, if any, will be collected at the bottom along the periphery of the bowl.
- The lighter liquid reaches to the centre and rises up.
- This is discharged through the outlet.
- The heavy liquid also rises to the top along the periphery of the bowl and discharges through the outlet.



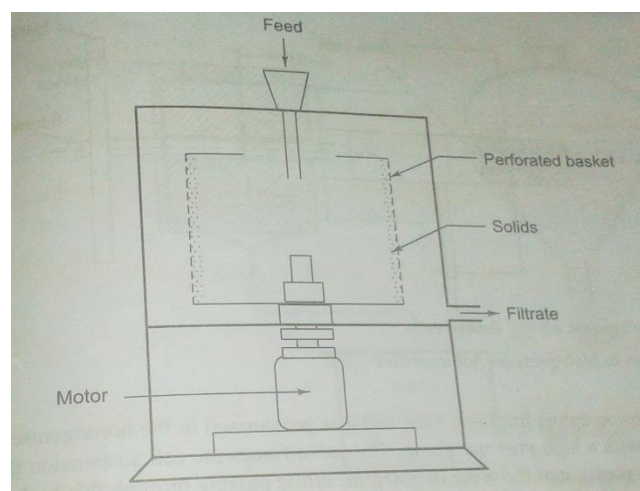
Disc bowl centrifuge

- If the solids are little in quantity, they are discharged at end of the centrifugation.
- In the nozzle discharge centrifuge, there is a provision for the removal of solids intermittently.
- Through the nozzle, a jet of liquid is introduced.
- The force of the jet is sufficient enough to lift the whole stack of discs.
- The stack of discs becomes very light during centrifugation.
- The liquid jet washes the solids and discharges through the bottom outlet.
- This centrifuge is also used mainly for separating the liquid phases.
- One of the most classical applications of disc centrifuge is separation of cream from milk.

BASKET CENTRIFUGE:-

- The basket centrifuge (also known as top suspended basket centrifuge) is generally used for separating solids of good concentration of liquids.
- The baskets are of different diameter (75-120 cm) and depths (45-75 cm).
- The basket has a large number of perforations.
- It rotates at speeds up to 4000 rpm.

- The perforated basket is held in position and rotated by a motor from top (in which case it is called as suspended basket centrifuge) or from the bottom.
- The latter case and the feed is fed from the top.
- The moment the feed falls at the centre of the centrifuge from the top into the rotating bowl, it gets centrifuged.
- The solids which are denser will move towards the walls of the centrifuge, the liquid collects at the centre at the bottom, from particles are smaller in size, sometimes a filter medium is used.
- The filter medium could be simply a filter cloth.
- An adjustable loader knife is used sometimes to scrape the solids from the filter medium.
- The scraped solids are washed with water or solvent through the wash inlet.
- The wash inlet sprinkles water on the cake, which serves two purpose
 - (i) To wash off the cake from the traces of the fermentation fluid
 - (ii) To discharge the cake from the centrifuge.
- The solids cake is discharge intermittently at bottom along with the wash water.
- If the desired product is in the form of solids, as biomass in the case of production of baker's yeast, we may only wash the solids, but we do not scrape intermittently.
- We shall collect the washed cake only after the centrifuge is stopped after complete centrifugation.

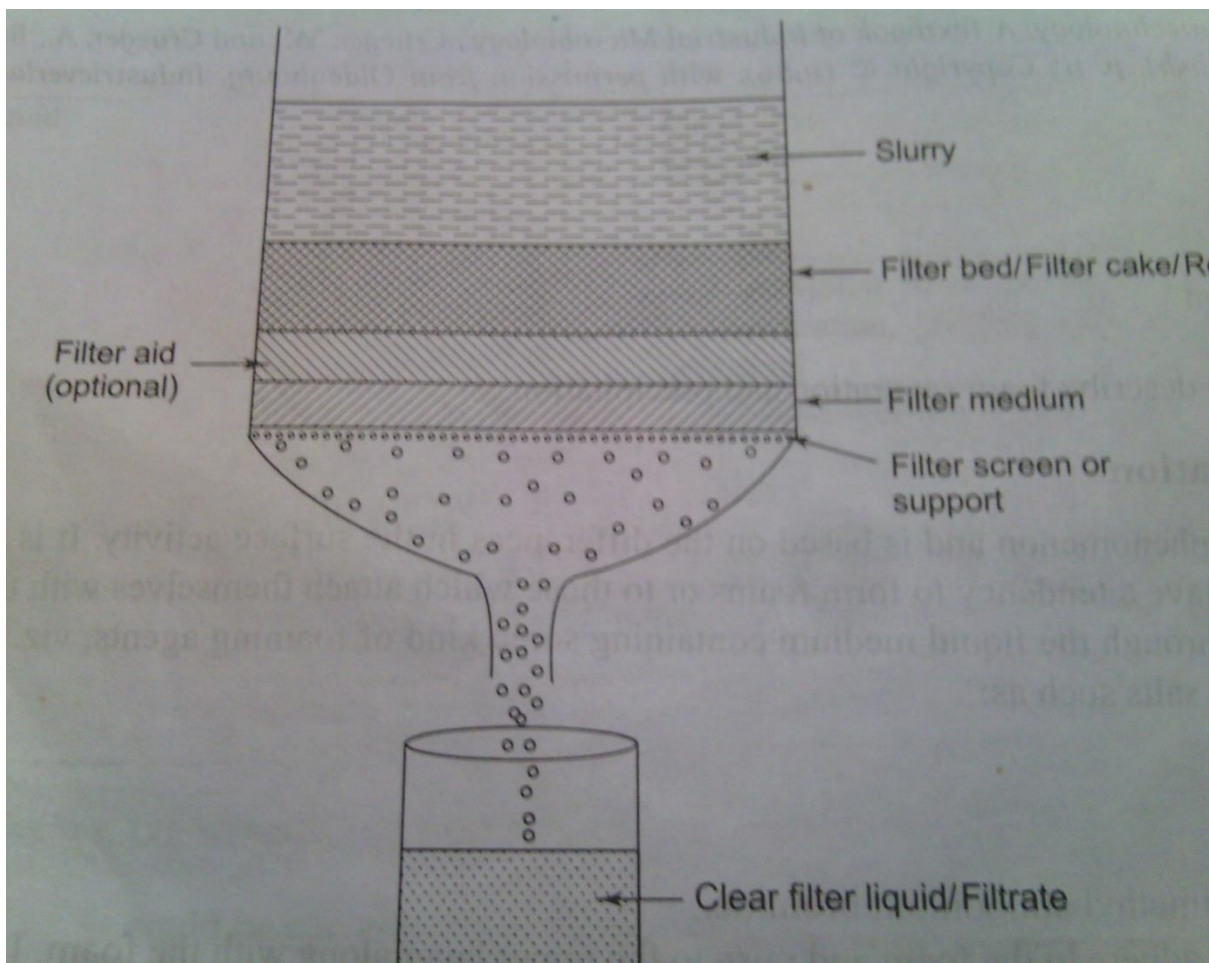


Basket centrifuge

FILTRATION

Filtration is a physical method of separation of suspended particles of any size from the liquid medium. Here, the only criterion is that the particle size should be larger than the molecular size of the liquid, which is always true. It is a very effective and convenient method of separating the suspended solids from the slurries. In this method of separation, either the solids or the liquid or both could be the desired products.

The liquid along with the suspended solids will be transferred on to a filter medium. The filter medium will retain all the particles which are bigger than the size of its aperture, and will pass on all the liquid medium along with some of the very fine suspended solid particles. All the materials remaining on the filter medium is known as residue or filter cake, and all the material passing through (the filter medium) is known as filtrate. The filter medium, usually known as filter cloth, is kept on a perforated screen to provide mechanical support to the filter aid. As the filtration process continues, more and more solids build up, the filtration efficiency will also improve, that is to say that still finer particles can be retained in the residue and the filtrate can be more clear of the solids. Thus, filtration efficiency will improve, but the filtration rate will reduce.



Initially, when there is no thickness of the filter cake, the filtration efficiency is the least. To overcome this, we add initially some filter aid which does not interfere or react with the filtering slurry. It could be cellulose or diatomaceous earth or any inert chemical (such as supercel). However, it is to be noted that the addition of filter aid is purely optional. It is normally added when the residue is not the desired component. If the residue is the desired product and if we wish that the residue should not be contaminated with the filter aid, we may allow initially the filtration to proceed for sometime until a good amount of filter bed builds up, and the filtrate that has passed through during this period will be recycled. If the filtrate is the desired product, then the filter aid can be safely used.

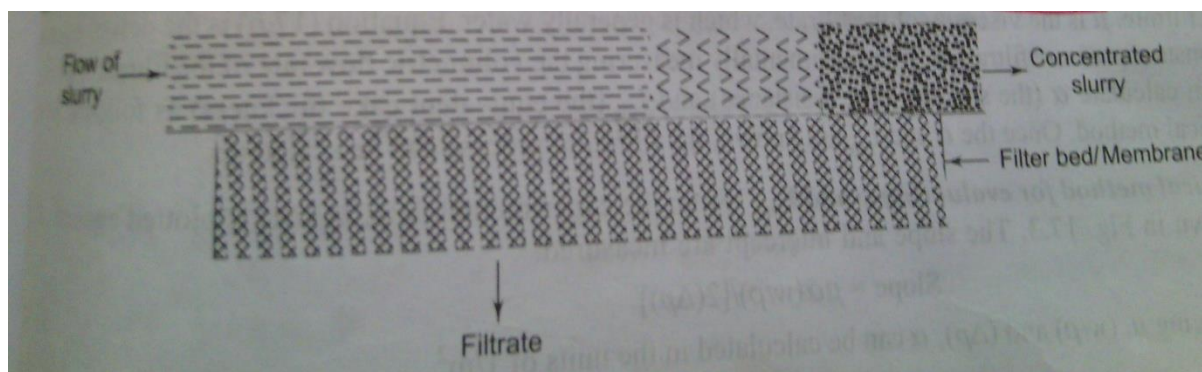
Since a large number of industrial products are to be separated by filtration method, a good number of variety of industrial filters has been developed. In all these cases, the fluid passes through the filter bed and the filter medium by means of pressure differential across the medium. The pressure differential could be achieved by applying pressure on the upstream side of the filter medium or by applying vacuum on the downstream side. Based on the industrial need, they could be operated on continuous or semi-continuous manner. In a semi-batch (semi-continuous) manner, the slurry flows continuously, the filtrate comes out continuously, but the residue can be taken only immediately. In a continuous operation, the slurry is fed continuously, both the residue and the filtrate are discharged continuously. It will be stopped only once in a while to clean the filter medium, when the filter medium pores are blocked.

Filters are divided into three main categories as follows:

- Cake filters
- Clarifying filters
- Cross-flow filters.

Slurries with high solid concentration are operated in cake filters. The cake build-up is more, and offers the desired resistance for the flow and thereby allows the solids to be retained on the filter medium as filter cake. Very dilute liquids are passed through the clarifying filters. They are more used for clarification purposes like clarified fruit juices, etc. The cross flow filters are used for concentrating the solutions by using filtration techniques. They are so named because the slurry flows on the filter medium in a cross flow direction. In this type of filtration, a clear demarcation is not made as residue and filtrate. The filtration process is essentially meant for concentrating a slurry, i.e. the feed

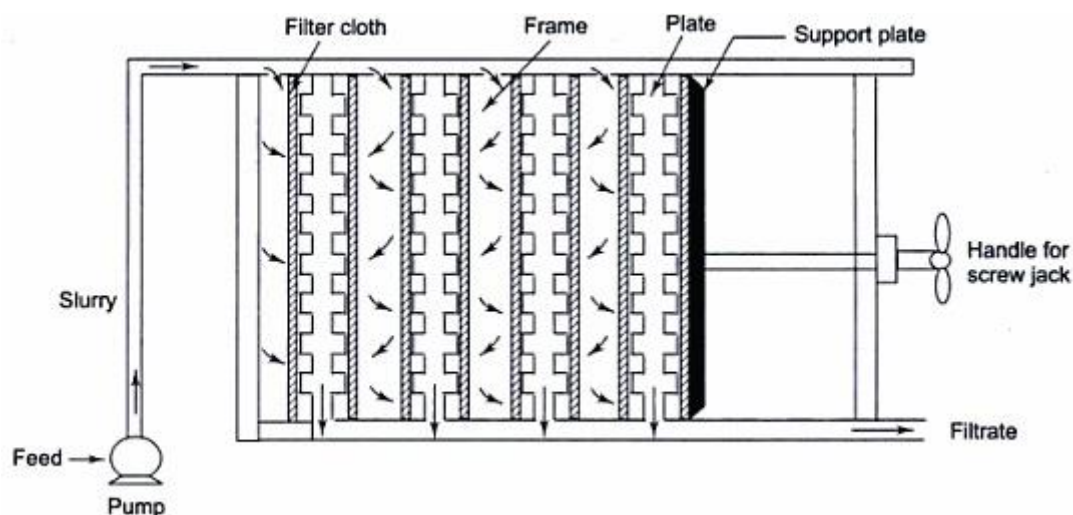
contains dilute solids, and after filtration, the feed leaves the filtration unit in the form of concentrated slurry, as in the case of ultrafiltration.



FILTRATION EQUIPMENT

Plate-and-frame filter press

This is one of the most widely used filter presses in the industry in view of its versatility and ease of operation. It is normally operated under pressure. The operation of it is similar to the one described schematically in Fig. 17.1, where we have a filter medium on which the slurry with the suspended solids is fed to the filter press. The filtration rate is proportional to the filter area and is restricted. Instead of having only one filter medium, we can have more of them in a consecutive manner, so that the filtration area increases many times, and accordingly the filtration rate also increases. Such an arrangement is shown in Fig. below.

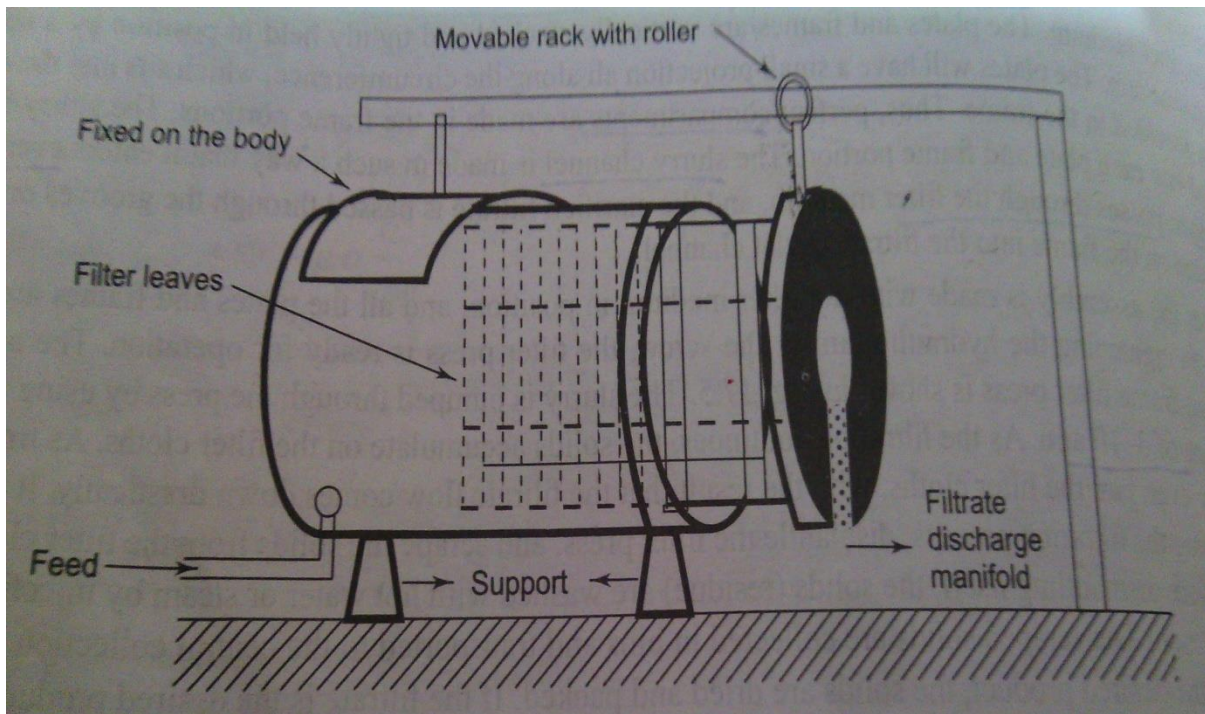


The plate-and-frame filter press consists of a set of square plates separated by hollow frames to make compartments. The plates are 6-50 mm thick and the frames are 6-200 mm thick. The plates are covered with filter medium. The plates and frames are vertically stacked and tightly held in position by a screw or hydraulic ram. The plates will have a small projection all along the

circumference, which sits into the depression provided in the frame. Thus, perfect compartments are made in the frame portions. The slurry is introduced into each plate and frame portion. The slurry channel is made in such a way that it enters every frame chamber, passes through the filter medium, and the clarified filtrate is passed through the grooves or cavities provided on the frame into the filtrate outlet channel. After the assembly is made with the filter medium in position, and all the plates and frames are stacked tightly by tightening the hydraulic ram or the screw, the filter press is ready for operation. The assembled plate-and-frame filter press is shown in Fig. The slurry is pumped through the press by using a pump at a pressure of 3-10 atm. As the filtration continues, the solids accumulate on the filter cloths. As time passes, they may even jam the filter cloths, with the result that the filtrate flow comes down drastically. It is the time for stopping the filtration process. Dismantle the filter press, and scrape the solids from the filter cloth. Sometimes, before dismantling itself, the solids (residue) are washed with hot water or steam by injecting it in the other direction. The scraped solids are collected in bins and transported to the central collection zone. If the residue is the desired product, the solids are dried and packed. If the filtrate is the desired product and solids are not required, the residue is discarded.

Pressure leaf filter press:

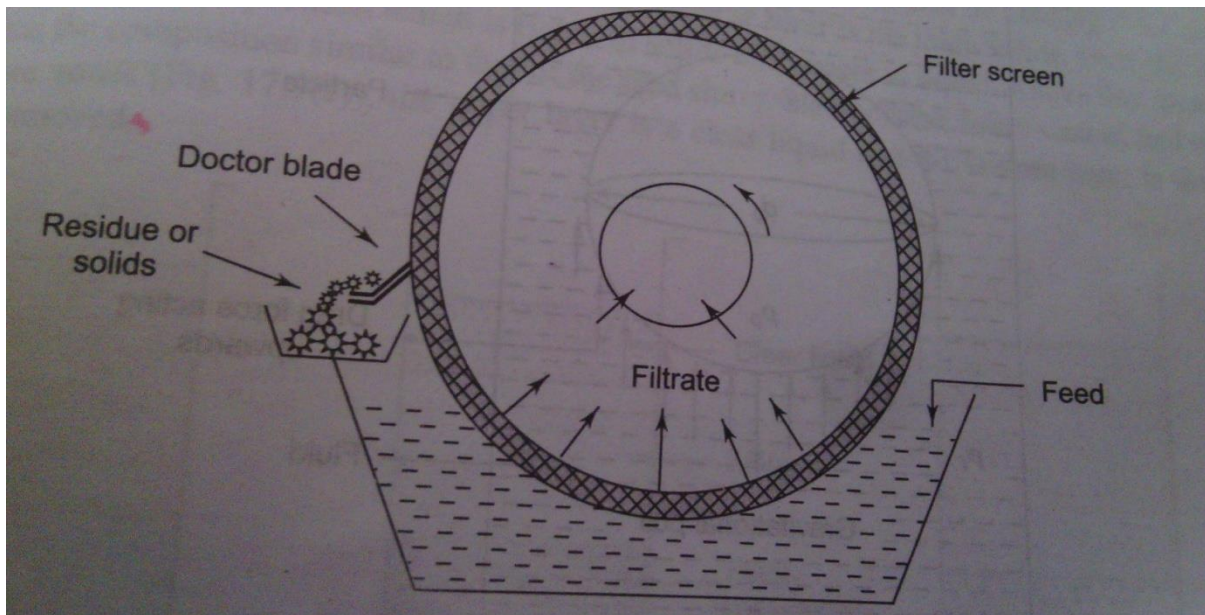
It is also similar to the plate-and-frame filter press, with an additional advantage of operating it under high pressures. It consists of a number of filter leaves stacked horizontally (or vertically) in a retractable rack. The filter leaves assembly is put in a horizontal cylindrical drum, into which the slurry is pumped under high pressure. The slurry passes through the filter leaves and discharges out through a filtrate discharge manifold.



Continuous rotary vacuum filter press:

It is a continuous filtration unit in which both the residue and filtrate will be obtained continuously. Problems associated with the choking of the filter bed are not present in this filter. It operates under vacuum unlike the other fibres. Obviously, the mechanical problems associated with the maintaining vacuum cannot be avoided.

It consists of a large circular hollow drum of about 50-100cm width and having a diameter of 2-3m. The slurry flows continuously into which a segment of the filter press is dipped. The drum is provided with the filter medium all along the rim, and rotates continuously at 0.1-2rpm speed with vacuum inside. When the drum dips into the slurry in the trough, the slurry is sucked because of the vacuum, and is filtered. The solids remain on the surface of the drum, while the filtrate is sucked into the drum which is separately discharged through a valve at the axis.



There can also be a provision for washing of the cake when it comes on to the top position. The solids are collected by scraping with a doctor blade into a tray from which the contents are discharged.

Some filters will have provision for consecutively applying vacuum to some portion of the drum, later it is blown with dry air from inside. This will help dry the solids. The screen slightly bloats, which helps crack the residue cake, and can be easily scraped for collecting the solids. During the process of pressuring and blowing from inside, there will not be any vacuum applied to the drum and hence there will not be any filtration during this period.

The drum is normally submerged into the trough to an extent of 30%, and in some cases, when washing of the cake is not desired, it may go up to 60-70%. The cake thickness in the industrial filters varies from 3 to 40 mm, depending upon the solid content and nature of solids in the feed slurry.

CELL DISRUPTION

- After the fermentation process is successfully completed, it is necessary to separate the product from solids (usually cell).
- This is an important step in the downstream processing side.
- We generally resort to classical solid-liquid separation techniques like filtration or centrifugation.
- This is possible only if the product is extracellular (i.e. the product is not held up by the cell), as in the case of production of alcohol or citric acid, etc.

- Thus, the solid-liquid separation techniques can be directly applied for the recovery of the product, if it is excreted from the cell.
- Some of the enzymes and recombinant proteins are held by the microorganisms (which we call as intracellular product).
- Generally, the microorganisms are protected by tough cell walls, which need to be disruption techniques described in detail in the literature .
- There is a large number of such method available, but only a very few can be practiced at industrial scale.

MECHANICAL METHODS:-

- (i) high-speed agitation
- (ii) grinding with abrasives
- (iii) high pressure pumping

NON-MECHANICAL METHODS:-

- (i) osmotic shock
- (ii) treatment with solvent and detergents
- (iii) freezing and thawing
- (iv) Enzymatic digestion of cell walls.

MECHANICAL METHODS OF CELL DISRUPTION:-

- High-pressure homogenization is one of the most widely used techniques in mechanical methods at industrial scale in view of the availability of equipment.
- The feed as a cell suspension enters from one side, and it is pressurized in the homogenizer with a pressure value which is operated with a high-pressure pump.
- The value forces the cell suspension through an orifice which develops very high pressure of the order of 550 atm.
- While passing through this high –pressure system, the cell get disrupted and release the product.
- The released products are isolated from the cell debris by using usual solid-liquid separation techniques.

NON-MECHANICAL METHODS OF CELL DISRUPTION:-

- The non-mechanical methods of cell disruption include chemical and biological methods.
- Osmotic shock treatment is caused by sudden change in salt concentration.
- This will cause the cell disruption.
- This technique was used for extraction of *luciferase* from *photobacterium fischeri*.
- Detergents are also used for disrupting the cells.
- The chemical detergents like quaternary ammonium compounds and sodium lauryl sulphates will damage the lipoproteins of the cell walls (membrane) and release the intracellular product.
- Sudden freezing and thawing will also cause cell disruption, because the freezing causes the ice crystals to form, which melt on thawing, and break the cell walls.
- Some enzymes are also used to hydrolyse specific bonds in cell walls.
- Lysozyme and enzyme extracts from leucocytes have such enzymatic activity.

CHROMATOGRAPHY

Chromatography is a method of separation of components based on their relative adsorption and desorption capacities on an adsorbent. Obviously, the system calls for a suitable

- Adsorbent
- Eluant

By and large, the absorption-desorption can be characterised by their molecular weights. The low molecular weight compounds elute faster than the high molecular weight compounds. The process is similar to separation using air aspiration technique. Suppose there is a mixture of materials of different densities. If they are all suspended freely and a breeze of air is over them, materials of low density will move to farther distances as compared to the materials of high density. This is how the solids can be separated.

In a chromatography separation process, we use a polar adsorbent, viz.

- a silica gel
- alumina

- diatomaceous earth
- Charcoal, etc.

The adsorbent is taken in a column and is suitably wetted with a solvent. Later the mixture to be separated is added at the top of the column, and is loosely plugged with cotton at both the top and bottom. The arrangement is shown in fig. A suitable solvent, known as the **eluant**, is added at the top, either continuously or until filling up to the top of the column. The eluant starts moving through the column. The mixture is adsorbed on the silica gel. Slowly the eluant starts desorbing the compounds from the adsorbent. The least desorbed or low molecular weight compound C will come down along with the solvent (eluant) and get collected separately along with the eluant. Now the eluant is removed by distillation, and the pure compounds A, B and C can be separately collected. This method is popularly known as column chromatography.

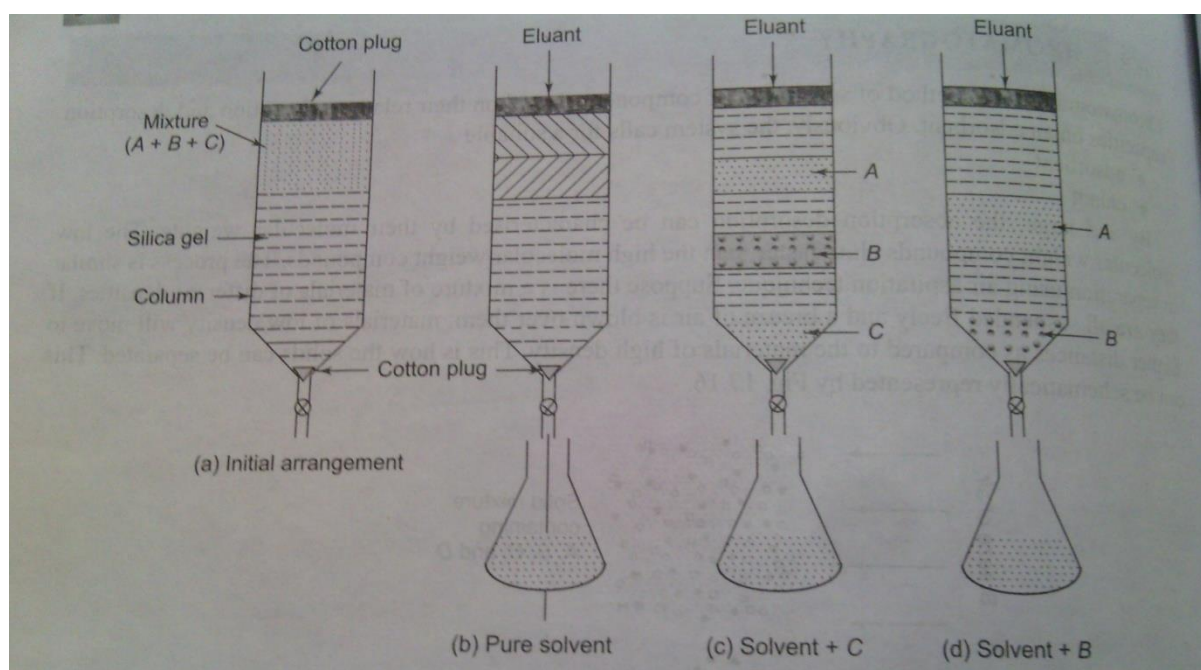


Figure-A

This chromatographic separation technique is highly involved and time-consuming. This is a high resolution technique, and hence is selectively used for:

- recovery of high-purity pharmaceuticals and therapeutics
- purification of proteins
- purification of peptides
- purification of amino acids

- purification of nucleic acids
- purification of alkaloids
- purification of vitamins
- purification of steroids, etc.

One of the classical applications of chromatography is gas chromatography (GC), which is used as an analytical equipment of identifying the compounds rather than as a separation technique. In gas chromatography, a suitable adsorbent is taken in a thin (capillary) long column and maintained at high temperature. The eluant is a neutral gas. The mixture to be identified is injected in microliter quantities into the hot column. Separation takes place in the column, and the separated compounds come to the outlet, where a suitable detector is installed. The detector detects the compounds and gives separate peaks for different compounds. The output, in the form of peaks, is known as **chromatograms**.

For the separation of mixtures into their individual components, the following types of chromatographic techniques are available:

- adsorption chromatography
- ion-exchange chromatography
- gel filtration chromatography
- affinity chromatography.

PRODUCTION OF BIOGAS

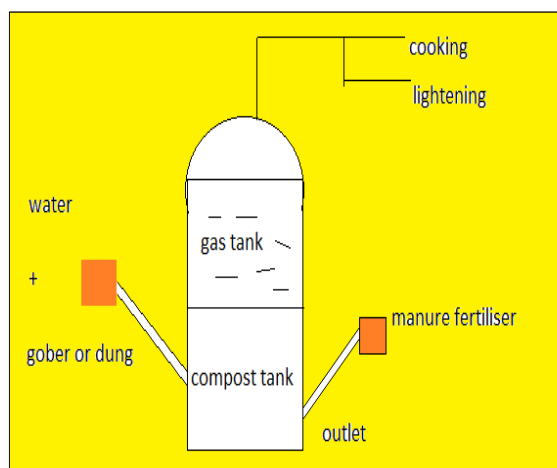
Many developing countries are encouraging for the installation of biogas plant to out the demand of fuel. India is one of the pioneer countries in biogas technology. It is estimated that five cattle generate dung to produce 2m³ plant to meet the demand of cooking and lighting of a family of 4-5 people.

Benefits of Biogas Plant

Biogas is mainly use for cooking and lighting purpose. Also it is used in internal combustion engines to power pumps and electric generators. Sludge is used as fertilizer .The most economical benefits are minimising environmental pollution and meeting the demand of energy for various purpose.

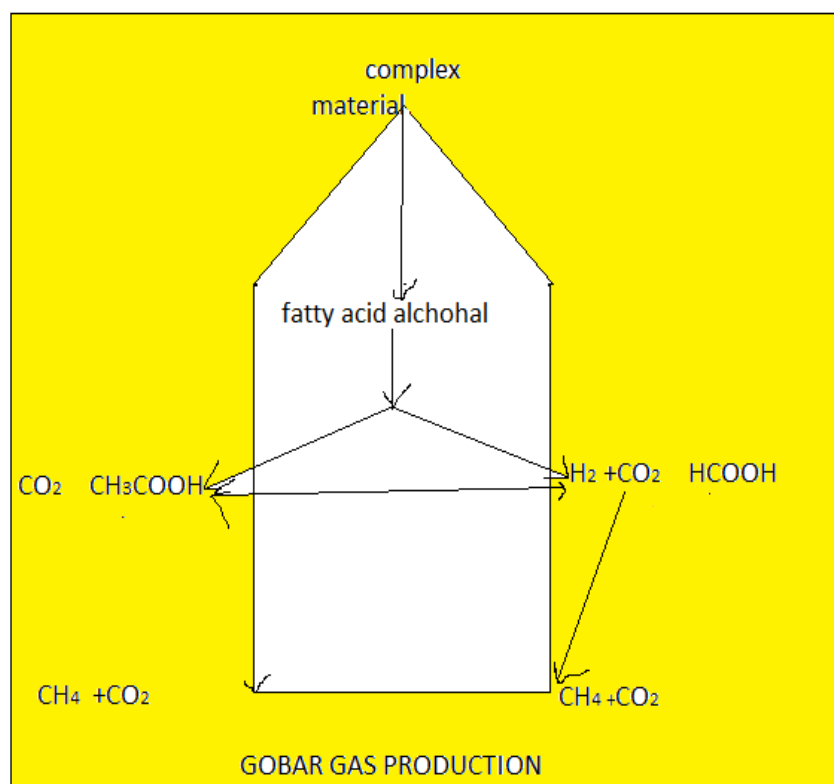
Feed Stock Material

- There are two sources of biomass that is from animal and plant.
- Biomass obtained from plant is aquatic and terrestrial in origin.
- Biomass obtained from animals include cattle dung, fishery waste etc.
- Besides these, agricultural waste like dumped wheat grain provides good source of biomass production.



Mechanism

- Anaerobic digestion is carried out in an air-tight cylinder tank which is called digester.
- It is made up of concrete, cement, steel.
- The digester has a side opening into which material for digestion are incorporated. Above the digester a cylindrical container lies to collect the gas.
- For first stage of gobar gas formation it takes 50 days to produce sufficient amount of gas which is used for house hold.
- Basically digesters are built inside the soil to provide insulation to the digester.
- In cold climate, digester can be heated.
- Anaerobic digestion is accomplished in three stages
 - 1) Solubilisation
 - 2) acidogenesis
 - 3) methonogenesis



Solubilisation

- It is the initial stage, when feed stock is solubilised by water and enzymes.
- Complex polymers are hydrolysed in organic acid and alcohol is hydrolysed by hydrolytic methanogenic bacteria which is mostly anaerobic in nature.

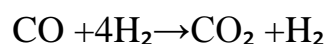
Acidogenesis

- In this stage, two types of bacteria are used.
 - 1) Facultative anaerobic
 - 2) Hydrogen producing bacteria
- These two bacterial convert the simple organic matter via oxidation and reduction to acetate, hydrogen, carbon dioxide.
- These substances are served as food for microorganism up to large extent.
- By obligate hydrogen producing acidogenic bacteria, fatty acid is converted into acetate, hydrogen, carbon dioxide.
- Another group of acidogenic bacteria produces acetate from hydrogen and carbon dioxide via acidogenic hydrolysis.

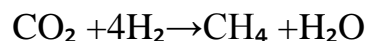
Methanogenic

- Last stage of anaerobic digestion, where acetate and hydrogen, carbon dioxide, water, other substance.

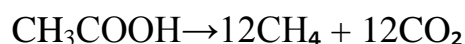
Primary reaction:



Secondary reaction:



Methane formation:



Factor Affecting Methane formation

Following are the factors affecting methane formation.

- (i) Slurry: - proper solubilisation of organic materials (the ratio between the solid and water) should be 1:1 when it is house hold type.
- (ii) Seeding: - In the beginning, seeding of slurry with small amount of sludge of another digester activates methane evolution sludge contains acetogenic and methanogenic bacteria.
- (iii) PH:- For production of sufficient amount of methane , optimum PH of digester should be maintained between 6-8, as acidic medium lowers down the methane formation.
- (iv) Temperature:- reduction in temperature reduces methane formation , because of inhibition in growth of methanogens. In case of mesophilic digestion, temperature should be between 50 to 60°C.
- (v) Carbon-Nitrogen ratio: - Improper C: N ratio lowers methane formation. Maximum digestion occurs when C: N ratio is 30: 1.
- (vi) Creation of anaerobic conditions: - It is obvious that methane formation takes place in strictly anaerobic conditions, therefore, the digesters are buried in soil.
- (vii) Addition of algae: - On addition of algae, zygonium species, doubles the rate of formation of biogas from cow dung.

ETHANOL PRODUCTION

- Yeast is preferred organism for production of ethanol in industrial scale.
- Different species can be utilized depending on the composition of raw materials used.

Ex: *s. cerevisiae* for hexose ,

Kluyveromyces fragilis for lactose

candida species for pentose.

- Then other micro-organism *Zymomonas mobilis* and *pachysolen* species used in ethanol production but in industrial application it is not used.
- There are some thermophilic microorganisms that help in ethanol production but including ethanol they produce some by-products.

RAW MATERIALS:

- It consists up to 70% of the cost of production of ethanol production. So selection of raw material plays an important role in process economy.
- The selected raw material should be readily available in fermentation plant.
- For this purpose, different countries use different raw material for ethanol production. Ex: corn is used in USA, Brazil-sugar cane.
- Along with the raw material sugar compounds in the form of sweet serum, juices sweet molasses are used.
- Other raw materials used are grain, fruit, vegetable wood, biomass which should be hydrolysed before fermentation process.



BATCH PROCESS:

- Convection ethanol fermentation operates in batch mode under aseptic condition.
- Mechanically agitated stainless steel reactor is used for this purpose.
- A reactor is filled with nutrient medium up to 70% of its total volume of the total volume.
- The required temp and PH is maintained after this the total system is allowed for sterilisation.

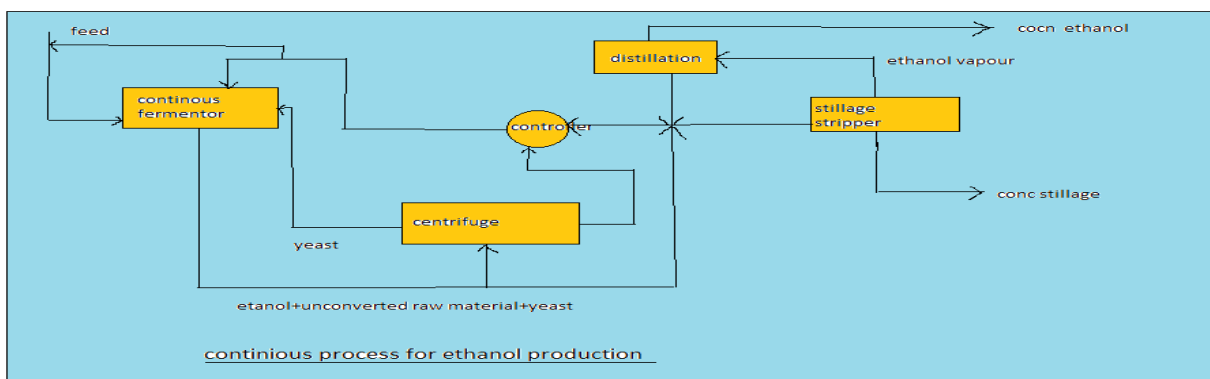
- After the sterilization it is cooled to required fermentation condition.
- Redox potential should be maintained at -100 MV by reducing agent like sodium sulphate.
- Sterile yeast culture is prepared and used for inoculation of reactor.
- Batch fermentation requires 30-40 hours for its completion.
- At the end of batch fermentation reactor contents are separated and these are go for filtration or centrifugation.
- Further liquid broth is allowed to separation of ethanol by distillation.

OPTIMUM CONDITION:

- Temp: (30-50)⁰c & (50-60)⁰c for thermophilic organisms
PH : 4-6
- Oxygen content for anaerobic condition: (.05-.1)mm Hg
- Traces elements: NH₄Cl, MgSO₄, CaCl₂ etc.

CONTINIOUS PROCESS:

- A mechanical agitated stainless steel fermenter is used for this purpose.
- After fermentation is completed the effluent is centrifuged for yeast



separation.

- A part of separated yeast is recycled back to fermenter.
- The liquid medium containing more amount of medium is fed back to fermenter.
- The stream coming from the bottom of distillation column contains maximum amount of ethanol feed to the stillage stripper.
- Ethanol coming from stillage stripper recycled back to distillation column.

ADVANTAGE OF CONTINUOUS PROCESS:

- 90% of the sugar can be converted to alcohol.
- Time required is 21 hours.
- Under optimum condition, 95% of conversion can be done in 10 hours.
- Sterilise and aseptic condition is maintained to avoid contamination.

Effluent treatment by biological method

Most of the organic effluents are treated biologically using microorganisms to utilise the organic matter in the effluent. In the process, the microorganisms produce some useful products/gases which can be used as fuels. The biological methods are broadly classified as:

- Anaerobic process
- Aerobic process

The type of fermentation depends upon the fermentable solute load. If the fermentable solute load is between 1 and 3 %, anaerobic fermentation process economical and is frequently used. If the solute load is below 1%, one of the aerobic fermentation methods can be applied and if the load is above 3%, it may be better to go for natural slow drying of the solid in yards/farms, and dried solids can be used as cattle feed-poultry-feed. Alternatively, the effluent waste solid material can be digested using blue- green algae for production of biomass, which in turn can be used as fish feed.

Anaerobic Fermentation

The anaerobic fermentation method is one of the classical and economical methods to digest the process effluents to produce methane gas using methano bacterium. This popularity known as biogas generation unit or biogas plants.

This process includes

- Biogas generation
- Bioconversion using micro algae

- Suspended solids separation techniques
 1. Settling tank
 2. Clarifier.

Settling tank:

These are used especially when the suspended solid concentration is more than 5000mg/l during rainy seasons in the natural river waters. Here the settling tanks are used within surface loading of 50-80m³/m² per day with detention time of 1h. As the concentration of suspended solids is generally high, 90% of the suspended solids are removed in this process.

Clarifier:

This clarifies the water from suspended solids in primary treatment of water and waste waters from approx 1000mg/l. The efficiency of this process depends on the addition of the coagulant and pH adjustments with preferably 5% dilute lime solution. The surface loading for sizing of the unit in water treatment is in the range 24-40m³/m² per day. However when the same unit is used as clarifier in sewage treatment or in the waste water treatment, it is called primary clarifier.

QUESTIONS

FUNDAMENTALS OF BIOCHEMICAL ENGINEERING

Q1. What are the basic differences between upstream and downstream processing?

Answer: The fermentation process is divided into two stages- upstream and downstream.

The upstream process deals with the following-

- i) Inoculum preparation which involves screening and selection of production strain, genetic modification if required and preparation of pure culture at lab scale.
- ii) Media development and optimisation of growth parameters at lab scale
- iii) Scale up of entire process i.e both inoculum and media preparation
- iv) Inoculation

The downstream processing deals with the following:

Post harvest product recovery- clarification, concentration, purification, Polishing and formulation till packaging of the desired product.

Q2. How solid state fermentation is different from submerged fermentation?

Answer: Submerged Culture Method - In this process, the organism is grown in a liquid medium which is vigorously aerated and agitated in large tanks called fermentors. The fermentor could be either an open tank or a closed tank and may be a batch type or a continuous type and are generally made of non-corrosive type of metal or glass lined or of wood.

Solid State Fermentation - In such fermentations, microbial growth and product formation occur at the surface of solid substrates. Examples of such fermentations are mushroom cultivation, mold ripened cheeses, starter cultures, etc. According to the physical state, solid state fermentations are divided into two groups:

- i) low moisture solids fermented without or with occasional/continuous agitation,
- ii) suspended solids fermented in packed columns, through which liquid is circulated. The fungi used for solid state fermentations are usually obligate aerobes.

Solid state fermentations on large scale use stationary or rotary trays. Temperature and humidity controlled air is circulated through the stacked solids. Less frequently, rotary drum type fermenters have been used.

3Q. What are the basic strategies of down stream processing?

Answer: The basic strategy for downstream processing is as follows:

Separation of insolubles (cells, debris, precipitates etc)

Extraction (removal of molecules that are dissimilar to product , removal of water

Purification (removal of molecules similar to product)

Polishing (packing in a form easy to transport, handle and give stability to product.

Q4.What are the different techniques one can adopt for separation of insoluble after fermentation?

Answer: The first step in DSP is the separation of solids, usually cells, from the liquid medium. This is generally achieved as follows:

Filtration. It is used for the separation of filamentous fungi and filamentous bacteria, e.g., streptomycetes, and often for yeast flocks. The various techniques of filtration employed are, surface filtration, depth filtration, centrifugal filtration, cross flow filtration, and rotary drum vacuum filtration.

Centrifugation. It may be used to separate bacteria and usually protein precipitates. But difficulties arise due to small differences in the densities of the particles and the medium. In addition, equipment cost, power consumption, temperature, etc. are the other disadvantages.

Flocculation and Floatation. Flocculation, i.e., sticking together of cells, can be induced by inorganic salts, mineral hydrocolloids or organic polyelectrolytes. Since sedimentation rate of a particle increases with size, flocculated cells can be recovered by centrifugation.

In cases, where flocculation is not effective, very fine gas bubbles can be created by sparging. The gas bubbles adsorb to and surround the cells, raising them to the surface of medium in form of foam (floatation); long chain fatty acids or amines promote stable foam formation.

The cells collected in the foam are readily recovered. Flocculation and floatation are used for the most efficient recovery of microbial biomass in some single cell protein production systems.

Q.5. Differentiate between ion exchange and gel filtration chromatography?

Answer: Ion exchange chromatography works on the principle of separation of molecule on the basis of charge or the ionic interaction while in gel filtration chromatography, separate the targeted molecule on molecular size basis, the solid phase matrix have a defined porosity due to which large molecule are unable to enter the pores and eluted out while smaller ones are retained.

Questions

1. Describe how enzymes are classified.
2. What are various chemical theories that describe the functioning of enzymes as biocatalysts?
3. What is meant by enzyme specificity? What are various types of enzyme specificity?
4. Describe the Fischer lock-and-key hypothesis for enzyme specificity.
5. Describe the Koshland induced-fit hypothesis for enzyme specificity.
6. What is meant by immobilization of enzymes? Describe different methods of immobilization.
7. What type of bioreactors do you suggest for immobilized enzyme systems?
8. What are various industrial applications of enzymes?
9. What are the applications of enzymes in food and beverage industries?
10. What are the medical applications of enzymes?

Model-1

1.		Answer the following questions :	2 x 10
	(a)	What is enzyme specificity? What are various types of enzyme specificity?	
	(b)	What are the various applications of heat transfer in bioprocessing?	
	(c)	Why sterilization is required for bioprocessing?	
	(d)	What are the different methods of cell disruption?	
	(e)	What do you mean by critical and non-critical parameters for a fermentation process?	
	(f)	What are the general requirements of fermentation process?	
	(g)	Describe the effects of gas velocity on mass transfer rate in fermentation broths?	
	(h)	Compare the absolute air filter and fibrous type air filter for sterilization of air?	
	(i)	Find the g- number of a centrifuge with an effective radius of 10cm and rotating at a speed of 30rps.	
	(j)	Describe the growth associated and non-growth associated product	

		formation in fermentation process.	
2.	(a)	Briefly explain different methods of continuous sterilization?	06
	(b)	What are the advantages and disadvantages of continuous sterilization?	04
3.	(a)	Derived Michaelis- Menten equation for enzyme kinetics from first principle?	05
	(b)	What is Lineweaver –Burk plot and Languir plot and how it can be used to calculate Michaelis-Menten contant?	05
4.	(a)	What are the different methods of controlling fermentation process condition? Describe them briefly.	07
	(b)	Write some applications of mass transfer in bioprocessing?	03
5.	(a)	What is solid state and submerged fermentation and give some applications of both.	06
	(b)	Describe about synthetic medium and crude medium.	04
6.	(a)	Describe the process of oxygen transfer methodology from the air bubble to the cell or cluster of cells in fermentation broths.	06
	(b)	What are the various factors affecting oxygen transfer rate in fermentation process.	04
7.		What are the various effluent treatment methods? Describe them briefly.	10
8.		Write short notes on any TWO :	5 x 2
	(a)	Activated sludge treatment	
	(b)	Immobilization of enzyme	

	(c)	Plate-and-frame filter press	
	(d)	Chromatography	

Model-2

1.		Answer the following questions :	2 x 10
	(a)	Define the term conversion, yield and selectivity for an ideal bioreactor.	
	(b)	Describe substrate-inhibited and product-inhibited cell growth.	
	(c)	What are the different methods of air sterilization?	
	(d)	What do you mean by Ostwald ripening?	
	(e)	What is an enzyme? Give some industrial applications of enzyme.	
	(f)	Define Σ factor for a centrifuge.	
	(g)	Define the term flocculation and coagulation.	
	(h)	Mention the complexities that found in kinetic study of a biochemical reaction?	
	(i)	In case of penetration theory and surface renewal theory, what is the relation between mass transfer coefficient and diffusivity?	
	(j)	Define the term reflectivity and transmissivity.	
2.	(a)	Briefly explain the modern applications of biotechnology.	05
	(b)	Describe the five kingdoms classification of microorganism proposed by Whittaker.	05

3.	(a)	What are the various parameters that can be control for the successful operation of a fermentor?	06																											
	(b)	Briefly explain what are factors affecting oxygen transfer rate in fermentation process.	04																											
4.		Explain in details the production of biogas and what are the factors affecting methane formation.	10																											
5.		<p>The following data have been obtained for two different initial enzyme concentrations for an enzyme-catalyzed reaction.</p> <table border="1"> <tr> <td>$v([E_0]=0.015\text{g/l})$ (g/l-min)</td> <td>1.1 4</td> <td>0.8 7</td> <td>0.70</td> <td>0.59</td> <td>0.50</td> <td>0.44</td> <td>0.39</td> <td>0.35</td> </tr> <tr> <td>[S] (g/l)</td> <td>20. 0</td> <td>10. 0</td> <td>6.7</td> <td>5.0</td> <td>4.0</td> <td>3.3</td> <td>2.9</td> <td>2.5</td> </tr> <tr> <td>$v([E_0]=0.00875\text{g/l})$ (g/l-min)</td> <td>0.6 7</td> <td>0.5 1</td> <td>0.41</td> <td>0.34</td> <td>0.29</td> <td></td> <td></td> <td></td> </tr> </table> <p>i) Find K_m</p>	$v([E_0]=0.015\text{g/l})$ (g/l-min)	1.1 4	0.8 7	0.70	0.59	0.50	0.44	0.39	0.35	[S] (g/l)	20. 0	10. 0	6.7	5.0	4.0	3.3	2.9	2.5	$v([E_0]=0.00875\text{g/l})$ (g/l-min)	0.6 7	0.5 1	0.41	0.34	0.29				
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$v([E_0]=0.00875\text{g/l})$ (g/l-min)	0.6 7	0.5 1	0.41	0.34	0.29																									

		ii) Find V_m for $[E_0]=0.015$ g/l and $[E_0]=0.00875$ g/l iii) Find K_2	10
6.	(a)	What are the general requirements of a fermentation process?	03
	(b)	Explain in details the design and construction of a fermentor.	07
7.	(a)	Briefly explain the enzyme specificity hypothesis.	05
	(b)	Explain different methods of air sterilization.	05
8.		Write short notes on any TWO :	5 x 2
	(a)	Tubular bowl centrifuge	
	(b)	Trickling filter	
	(c)	vaccines	
	(d)	Chromatography	

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