18BTU401 BIOPROCESS TECHNOLOGY 4H - 4C
Total hours/week: L:4 T:0 P:0 Marks: Internal: 40 External: 60 Total: 100

Course Objectives: This syllabus includes current technical procedures involved in isolation, screening and processing of industrially important products.

Course Outcomes: After completion of this course student will have overall knowledge of industrial biotechnology and application of microbes and their enzyme used in industries.

UNIT- I

Introduction to bioprocess technology. Range of bioprocess technology and its chronological development. Basic principle components of fermentation technology. Types of microbial culture and its growth kinetics– Batch, Fed batch and Continuous culture. Types of fermentation-submerged, solid state.

UNIT-II

Design of bioprocess vessels- Significance of Impeller, Baffles, Sparger; Types of culture/production vessels- Airlift; Cyclone Column; Packed Tower and their application in production processes. Principles of upstream processing – Media preparation, Inocula development and sterilization.

UNIT-III

Bioreactor control and monitoring, Introduction to oxygen requirement in bioprocess; mass transfer coefficient; factors affecting KLa. Bioprocess measurement and control system with special reference to computer aided process control.

UNIT-IV

Downstream processing: Filtration, Centrifugation, Cell disruption, Chromatography, liquid-liquid extraction, product recovery and purification. Effluent treatment- product recovery, sludge process, waste disposal.

UNIT-V

Application: Microbial production of ethanol, amylase, lactic acid, and Single Cell Proteins. Fermentation economics.

References

1. Patel, A.H. (2007). *Industrial Microbiology*. Macmillan India Ltd.
2. Stanbury, P.F., Whitaker, A. & S.J. Hall. (2007). *Principles of fermentation technology*. Elsevier Science Ltd.
3. Casida, LE. (1991). *Industrial Microbiology.* (1st ed.). Wiley Eastern Limited.
4. Crueger, W., & Crueger, A. (2000). *Biotechnology: A textbook of Industrial Microbiology* (2nd ed.). New Delhi: Panima Publishing Co.
5. Patel, A.H. (1996). *Industrial Microbiology*. (1st ed.). Macmillan India Limited.

KARPAGAM ACADEMY OF HIGHER EDUCATION

(Deemed to be University Established Under Section 3 of UGC Act 1956)

Coimbatore – 641 021

LECTURE PLAN

DEPARTMENT OF BIOTECHNOLOGY

STAFF NAME	**: Dr. SELVAKUMAR S**		
SUBJECT NAME	**: BIOPROCESS TECHNOLOGY**	SUB.CODE	**: 18BTU401**
SEMESTER	**: IV**	CLASS	**: II B.Sc. (BT)**

S.No.	Lecture Duration Period	Topics to be Covered	Support Material/Page Nos.
Unit - I			
1.	1	Introduction to bioprocess technology	T1: 1 -5
2.	1	Range of bioprocess technology and its chronological development	T2: 1 -7
3.	1	Basic principle components of fermentation technology.	T2: 9 & 10
4.	1	Types of microbial culture and its growth kinetics	T2: 13-15
5.	1	Batch, Fed batch culture.	T2: 13-15, 27 & 28
6.	1	Continuous culture.	T2: 16 & 17
7.	1	Types of fermentation- submerged, solid state.	W1
8.	1	Unit test	-
Total No of Hours Planned for Unit I			08 hr
Unit – II			
9.	1	Design of bioprocess vessels	T2: 167 & 168
10.	1	Significance of Impeller, Baffles, Sparger	T2: 178 -184
11.		Types of culture/production vessels	T2: 184 & 185
12.	1	Airlift; Cyclone Column; Packed Tower and their application in production processes.	T2: 200-205
13.	1	Principles of upstream processing	T2: 93-97
14.	1	Media preparation	T1: 43-45
15.	1	Inocula development and sterilization	T3: 136, T3: 128- 130
16.	1	Unit test	-
Total No of Hours Planned for Unit II			08 hr
Unit – III			
17.	1	Bioreactor control and monitoring	T2: 215-220
18.	1	Introduction to oxygen requirement in bioprocess	T2:243-246
19.	1	Mass transfer coefficient	T2: 247
20.	1	Factors affecting KLa	T2: 246-248, 254
21.	1	Bioprocess measurement and control system with special reference to computer aided process control.	T2: 228-238
22.	1	Continuation of topic V	T2: 228-238
23.	1	Continuation of topic V	T2: 228-238
24.	1	Unit test	-
Total No of Hours Planned for Unit III			08 hr
UNIT-IV			

25.	1	Downstream processing	T2: 277-280
26.	1	Filtration, Centrifugation	T2: 281 -292
27.	1	Cell disruption, Chromatography	T2: 292, 301 -304
28.	1	liquid-liquid extraction	T2: 296-300
29.	1	Product recovery and purification	T2: 277-279
30.	1	Effluent treatment- product recovery, sludge process	T2: 313-316
31.	1	Waste disposal	T2: 317-322
32.	1	Unit test	-
Total No of Hours Planned for Unit IV			08 hr
UNIT-V			
33.	1	Application: Microbial production of ethanol	T1: 145-150
34.	1	Application: Microbial production of amylase	T1: 141 -143
35.	1	Application: Microbial production of lactic acid	T1: 128-130
36.	1	Application: Microbial production of Single Cell Proteins	T1: 178-202
37.	1	Fermentation economics	T3 : 208-218
38.	1	Unit test	-
39.	1	Previous year ESE Question papers discussion	-
40.	1	Previous year ESE Question papers discussion	-
Total No of Hours Planned for Unit V			08 hr
Total Planned Hours			40 hr

REFERENCES

T1: Patel, A.H. (1996). Industrial Microbiology. (1st ed.). Macmillan India Limited.
T2: Stanbury, P.F., Whitaker, A. & S.J. Hall. (2007). Principles of fermentation technology. Elsevier Science Ltd.
T3: Casida, LE. (1991). Industrial Microbiology. (1st ed.). Wiley Eastern Limited.
W1: http://ecoursesonline.iasri.res.in/mod/page/view.php?id=5150

SYLLABUS

Introduction to bioprocess technology. Range of bioprocess technology and its chronological development. Basic principle components of fermentation technology. Types of microbial culture and its growth kinetics– Batch, Fed batch and Continuous culture. Types of fermentation-submerged, solid state.

INTRODUCTION TO BIOPROCESS TECHNOLOGY

The use of microbes to obtain a product or service of economic value constitutes industrial microbiology. Any process mediated by or involving microorganisms in which a product of economic value is obtained is called **fermentation**.

An apparatus, such as a large fermentation chamber, for growing organisms such as bacteria or yeast under controlled conditions. **Bioreactors** are used in the biotechnological production of substances such as pharmaceuticals, antibodies, or vaccines, or for the bioconversion of organic waste.

Bioreactor is a vessel in which a chemical process is carried out which involves organisms or biochemically active substances derived from such organisms. This process can either be aerobic or anaerobic.

The terms industrial microbiology and fermentation are virtually synonymous in their scope, objectives and activities.

The microbial product may be microbial cells (living or dead), microbial biomass (the total quantity or weight of organisms in a given area or volume), and components of microbial cells, intracellular or extracellular enzymes or chemicals produced by the microbes utilizing the medium constituents or the provided substrate.

The services generated by microorganisms range from the degradation of organic wastes, detoxification of industrial wastes and toxic compounds, to the degradation of petroleum to manage oil spills, etc. Industrial microbiology also encompasses activities like production of biocontrol agents, inoculants used as biofertilizers, etc.

The activities in industrial microbiology begin with the isolation of microorganisms from nature, their screening for product formation, improvement of product yields, maintenance of cultures, mass culture using bioreactors, and usually end with the recovery of products or metabolites and their purification.

The metabolism may be defined as the sum of the physical and chemical processes in an organism by which its substance is produced, maintained, and destroyed, and by which energy is made available.

RANGE OF BIOPROCESS TECHNOLOGY

Fermentation Processes can be classify into five different categories

1. Processes Producing Microbial Enzymes
2. Processes Producing Microbial Metabolites
3. Processes Producing Microbial Cells (Biomass) as the Products
4. Processes Producing Recombinant Products
5. Processes modifying Substrates (Transformation Process)

1. PROCESSES THAT PRODUCE MICROBIAL ENZYMES

Microbes, plants and animal are the major source of enzymes

Commercial production of many enzymes exploiting these sources have been achieved As being produced in large quantities by the fermentation processes, microbial enzymes have the enormous economic potential

Microbes are more prone to change in its genetics to enhance its productivity compared to plant or animal system

It is possible to produce enzymes of eukaryotes into the prokaryote systems with the help of recombinant DNA technology

It is possible to control and improve microbial enzyme production by introducing inducers and activators in the production medium

It is also possible to increase the copy number of gene coding for the a specific enzyme using principles of recombinant DNA technology

Industry	**Applications**	**Enzymes**	**Source**
Dairy	Curdling milk	Protease	Fungal/ Bacterial
	Production of whole milk concentrates, ice-cream & frozen desserts	Protease	Yeast
	Manufacture of protein hydrolysates	Protease	Fungal/ Bacterial
Fruit juices	Clarification	Pectinases	Fungal

Leather	Dehairing, Baiting (attract, temp)	Protease	Fungal
Pharmaceuticals	Digestive aids	Amylase, Protease	Bacterial
	Various clinical tests	Numerous	Fungal/ Bacterial
	Anti-blood clotting	Streptokinase	Bacterial
Textiles	Desizing (Reducing volume, Demassing) fabrics of	Amylase	Bacterial
Corn syrup	Manufacture of	Amylase	Fungal

	high maltose syrups		
	Manufacture of fructose syrup	Glucose isomerase	Bacterial
Brewing	Improvement of fine filtration	β-glucanases	Fungal/ Bacterial
	Chill proofing	Protease	Fungal/ Bacterial

2. MICROBIAL METABOLITES

- Metabolites which are essential to the growth of the cells like amino acids, nucleotides, proteins, nucleic acids, lipids, carbohydrates are produced during the log phase of the growth.
- Metabolites which are essential to the growth of the cells like amino acids, nucleotides, proteins, nucleic acids, lipids, carbohydrates are produced during the log phase of the growth
- The primary metabolites are also known as central metabolites
- Several primary metabolites are of economic importance and can be produced in large quantity by fermentation process
- The synthesis of primary metabolites by wild-type micro-organisms aims to meet the requirements of the organism
- During the stationary phases several microbial cultures produce certain compounds (these compunds are not produced during the “trophophase” and which do not appear to have any obvious function in cell metabolism). These compounds are called the secondary compounds of metabolism.
- The phase during which these compounds are produced (equivalent to the stationary

phase) as the "idiophase".

- The secondary metabolism is also known as "special metabolism"
- The products of secondary metabolism are not absolutely required for the survival of the organisms.
- All microorganisms do not undergo secondary metabolism. It is common amongst the filamentous bacteria and fungi and the spore forming bacteria
- The taxonomic distribution of secondary metabolism is different from that of primary metabolism
- The physiological role of secondary metabolism and hence secondary metabolites in the producer cells has been the subject of considerable debate
- The large scale production of secondary metabolites focus on the importance of these metabolites on organisms other than those that produce them
- Secondary metabolites play an important physiological role several ways. Many secondary metabolites possess antimicrobial activity, some acts as specific enzyme inhibitors and growth promoters and many have pharmacological properties Thus, due to a huge economic potential, the industrial production of these metabolites have formed the basis of a number of fermentation processes

3. PROCESSES THAT PRODUCE MICROBIAL CELLS (OR BIOMASS) AS THE PRODUCT

The commercial microbial biomass production can be divided into two major processes:

1. The production of yeast to be used in the baking industry and

2. The production of microbial cells which can be used as human and/or animal food (single-cell protein)

- ✓ Bakers' yeast has been produced on a large scale since the early 1900s and yeast was produced as human food in Germany during the First World War
- ✓ However, it was not until the 1960s that the production of microbial biomass as a source of

food protein was explored to any greatdepth

- ✓ A few large-scale continuous processes for animal feed production were established in the 1970s. These processes were based on hydrocarbon feedstocks which could not compete against other high protein animal feeds, resulting in their closure in the late 1980s.

4. RECOMBINANT PRODUCTS

- ❖ The advancement in the application of rDNA technology has made possible to produce a range of recombinant products by the fermentation process
- ❖ A wide range of microbial cells have been used as hosts for such systems including *Escherichia coli, Saccharomyces cerevisiae* and filamentous fungi
- ❖ Recombinant DNA is widely used in research, agriculture, medicine and biotechnology
- ❖ Several products that result from the use of rDNA technology are found in almost every pharmacy, medical testing laboratory, doctor's as well as and veterinarian's office, and biological research laboratory
- ❖ Following are the recombinant products that produced by genetically engineered organisms organism
- ❖ Human Growth Hormone (rHGH), Biosynthetic Human Insulin (BHI), Interferon (IF), Insulin-like growth factor 1 (IGF-1), Bovine somatotropin (bST), Porcine somatotropin, Bovine chymosin.

5. PROCESSES MODIFYING SUBSTRATES (TRANSFORMATION PROCESS)

I. Many microbial cells may be exploited to convert a compound into structurally related, financially more valuable compounds

II. As microbes can behave as catalysts with high positional specificity and stereo specificity, microbial processes are more specific than purely chemical ones

III. These microbial processes enable the removal, addition and/or modification of various functional groups at predefined specific sites on a complex molecule without

the use of chemical protection

IV. The reactions which may be catalyzed include Dehydrogenation, Oxidation, Hydroxylation, Dehydration and Condensation, Decarboxylation, Amination, Deamination and Isomerization

V. As microbial processes can be operated at a relatively low temperatures and pressures have the additional advantage over chemical processes which require high temperatures, more pressures and presence of heavy-metal catalysts-a potential environmental pollutant

VI. Production of vinegar is the most well-established microbial transformation process(conversion of ethanol to acetic acid)

VII. Many transformation processes have been rationalized by immobilizing either the whole cells, or the isolated enzymes on an inert support which catalyze the reactions

VIII. The immobilized cells or enzymes may be reused many times

CHRONOLOGICAL DEVELOPMENT OF BIOPROCESS TECHNOLOGY

In the late 1700s Lavoisier showed that in the process of transforming sugar to alcohol and carbon dioxide (as in wine), the weight of the former that was consumed in the process equaled the weight of the latter produced. In 1810 J.L. Guy-Lussac summarized the process with the famous equation $C_6H_{12}O_6$ yields $2CO_2$ + 2 C_2H_6O. The entire process was considered to be simply a chemical reaction and yeast (which was not yet even classified as a definite substance, much less a living organism instrumental to fermentation) was thought to play a physical rather than a chemical role, an idea dating back to the time of George Stahl in 1697. It was held that either the catalytic action at the yeast cell or the molecular vibrations from the decomposing organic matter arising from the death of the cells, sparked the chemical changes resulting in fermentation. Putrefaction, spoilage, and fermentation were all considered to be processes of death, not life.

The early 1800s saw a great increase of interest in microbiology in Europe. The scientific period began with great advances in botany, increased interest in microscopy, and willingness to

investigate individual organisms. The two major problems that would challenge the greatest researchers in the new field of microbiology concerned the basic nature of the fermentation process and the basic nature of enzymes. The scientific breakthroughs that would lead to the unraveling of the mysteries of fermentation starting in the 1830s were made primarily by French and German chemists.

The debate was finally brought to an end by the great French chemist Louis Pasteur (1822-1895) who, during the 1850s and 1860s, in a series of classic investigations, proved conclusively that fermentation was initiated by living organisms. In 1857 Pasteur showed that lactic acid fermentation is caused by living organisms. In 1860 he demonstrated that bacteria cause souring in milk, a process formerly thought to be merely a chemical change, and his work in identifying the role of microorganisms in food spoilage led to the process of pasteurization.

In 1877, working to improve the French brewing industry, Pasteur published his famous paper on fermentation, *Etudes sur la Biere* , which was translated into English in 1879 as *Studies on Fermentation* . He defined fermentation (incorrectly) as "Life without air," but correctly showed specific types of microorganisms cause specific types of fermentations and specific end products.

Many scientists, including Pasteur, had attempted unsuccessfully to extract the fermentation enzyme from yeast. Success came finally in 1897 when the German chemist Eduard Buechner ground up yeast, extracted a juice from them, then found to his amazement that this "dead" liquid would ferment a sugar solution, forming carbon dioxide and alcohol, just like living yeasts. Clearly the so-called "unorganized ferments" behaved just the organized ones. From that time on the term "enzyme" came to be applied to all ferments.

The term "ferment" dropped out of the scientific vocabulary altogether and the vitalist position collapsed, never to recover. Thereafter it was agreed that only one set of laws applied to all things, both animate and inanimate and that there was no special vital force which characterized living things and acted under different laws. And it was finally understood that fermentation is caused by

enzymes which are produced by microorganisms.

In 1907, a German chemist named Eduard Buchner received the Nobel Prize for showing that enzymes in yeast cells cause fermentation. About two decades, two other scientists determined exactly how enzymes cause fermentation. Their names are Arthur Harden and Hans Euler-Chelpin, and they won the Nobel Prize for their work in 1929. By the 1940s, technology was developed to use fermentation to produce antibiotics.

The sciences of microbiology, biochemistry, fermentation technology, mycology, and bacteriology all shared a deep interest in the nature and working of enzymes. In 1905 Harden and Young discovered coenzymes, agents necessary for the action of enzymes. In 1926 the American biochemist J.B. Sumner first purified and crystallized an enzyme (urease) and showed that it was a protein, more precisely a protein catalyst. Eventually enzymes came to be seen as the key catalysts in all the life processes, each highly specialized in its catalytic action and generally responsible for only one small step in complex, multi-step biochemical reactions.

Advances in microbiology and fermentation technology have continued steadily up until the present. For example, in the late 1930s it was discovered that microorganisms could be mutated with physical and chemical treatments to be higher yielding, faster growing, tolerant of less oxygen, and able to use a more concentrated medium. Strain selection and hybridization developed as well, affecting most modern food fermentations.

Since ancient times the Koji making process has been unique to East Asia, where it has been used in the preparation of fermented foods such as miso, soy sauce, soy nuggets, sake, shochu (spirits), and rice vinegar (*yonezu*). Some of the scientist suggested that molds are widely used since they grow well in areas having a humid climate and long rainy season during the warm months. In the West mold fermented foods are limited primarily to a number of cheeses characterized by their strong flavors and aromas: Camembert, Blue, Brie, and related types. Because of the widespread use of mold-fermented foods in East Asia, the word "mold" there has a rather positive connotation,

something like "yeast" in the West. Most Westerners still have a deep- seated prejudice against moldy products, and they generally associate the word "mold" with food spoilage, as in "moldy bread". The Chinese had distinct names for two types of molds used in fermented soyfoods; what we now call *Aspergillus* was then called "yellow robe" and *Rhizopus* was called "white robe." These cultures were carefully distinguished and propagated from year to year. By the 10th century a koji starter or inoculum was deliberately being used in the preparation of koji for fermented foods.

During the 20th century, Japanese microbiologists have made many important contributions to the development of applied and industrial microbiology, including the manufacture of fermented soyfoods, as well summarized by Tamiya (1958) and Sakaguchi (1972). Until quite recently, their strength was more in the area of application of scientific knowledge than in pioneering basic scientific and microbiological breakthroughs. From the early 1900s, important studies on the koji mold and its enzymes were done by Japanese scientists. Important advances in enzymology, with much of the work done on koji molds, began in the 1920s. In 1928 Miyazaki developed the combined Amylo-Koji process. By the 1950s Japanese scientists had isolated various protease and amylase enzymes, induced mutations, and used them commercially. They also developed the technology for the microbial production of L- glutamic acid and monosodium glutamate (MSG), lysine and other amino acids, flavor enhancing nucleotides such as inosinic acid, and organic acids. They used the koji mold *Aspergillus oryzae* in the commercial production of enzymes including proteases, amylases, amyloglucosidase, and lipase. They made microbial rennet and numerous other products. Indeed in the period following World War II, Japan became the world leader in the field of industrial fermentations. Wang and Hesseltine (1979) have suggested that this may have been "in large part due to the food fermentation base from which it launched its industrialization of micoorganisms."

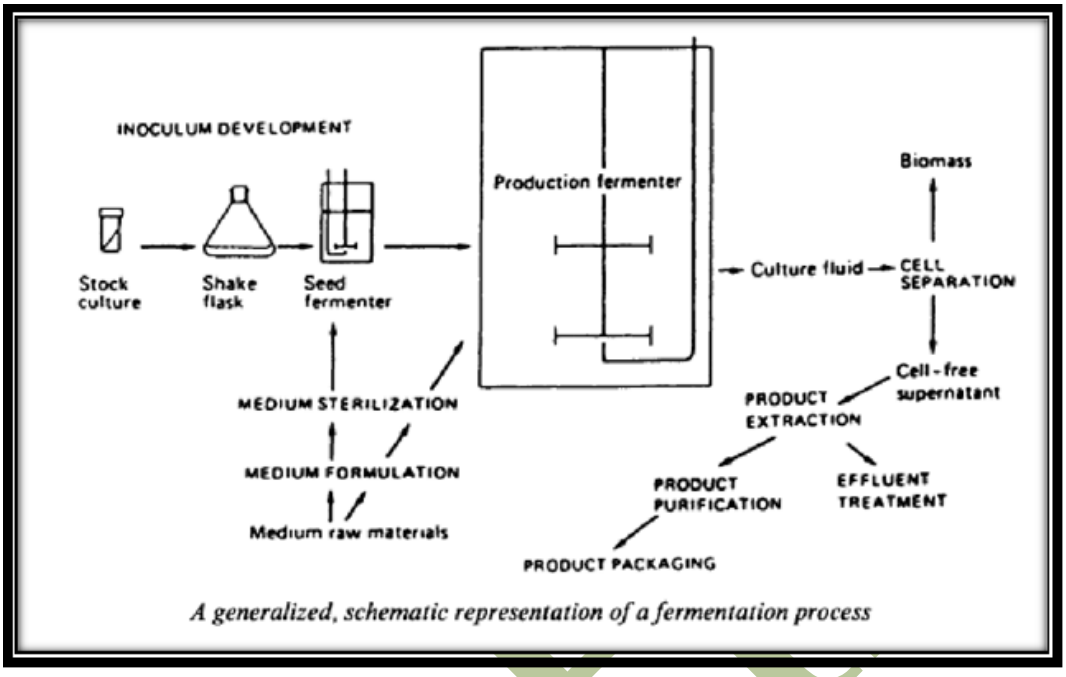
According to Tamiya, in 1958 food and drinks produced with koji retailed for $1,000 million a year, and the taxes from these foods amounted to more than $500 million, which was as much as 20% of the Japanese national budget! In 1970 in Japan, foods made from koji molds accounted for

1.5% of the nation's Gross National Product, or about $205,000 million. Prominent among these were miso and shoyu (Sakaguchi 1972). Production of fermented soyfoods continues to be the most important of the fermented food industries of East Asia.

Starting in about the 1960s and increasing rapidly after the mid-1970s, East Asian fermented soyfoods (especially soy sauce or shoyu, miso, and tempeh, in that order), began to be widely used in the West. Reasons for this include the growing general interest in soyfoods, the cultural and religious movement toward meatless and vegetarian diets, the increasing interest in nutritious foods with less animal fats, the awareness these foods as a good vegetarian source of vitamin B-12, the growing worldwide travel stimulating interest in foreign foods, the increase of East Asian refugees to the West, and the increased interest in microbiology and enhanced image of fermented foods.

BASIC PRINCIPLE COMPONENTS OF FERMENTATION TECHNOLOGY

The central component of the system is obviously the fermenter itself, in which the organism is grown under conditions optimum for product formation, one must not lose sight of operations upstream and downstream of the fermenter. Before the fermentation is started the medium must be formulated and sterilized, the fermenter sterilized, and a starter culture must be available in sufficient quantity and in the correct physiological state to inoculate the production fermenter. Downstream of the fermenter the product has to be purified and further processed and the effluents produced by the process have to be treated.



A generalized, schematic representation of a fermentation process

TYPES OF MICROBIAL CULTURE AND ITS GROWTH KINETICS

Various types of fermentation based on the culturing microbes

1. Batch fermentation
2. Fed-batch fermentation
3. Continuous fermentation

1. **Batch fermentation**

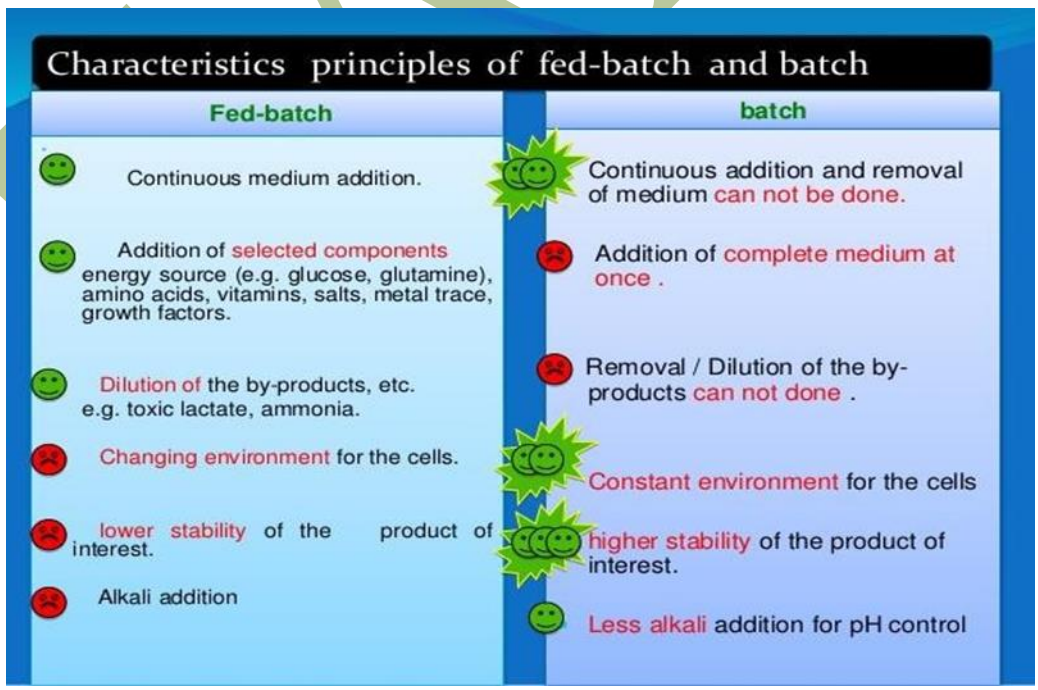
Batch fermentation can be considered to be a closed system i.e. Fermentation conducted from start to end in a single vessel. At time t=0 the sterilized nutrient solution in the fermenter is inoculated with microorganisms and incubation is allowed to proceed. In the course of the entire microorganisms), an antifoam agent, and acid or base to control the pH. The composition of the culture medium, the biomass concentration, and the metabolite concentration generally change constantly as a result of the metabolism of the cells.

2. **Fed-batch fermentation**

In the conventional batch process just described, all of the substrate is added at the beginning of the fermentation. An enhancement of the closed batch process is the fed batch fermentation. In the fed-batch process, substrate is added in increments as the fermentation progresses. In the fed-batch method the critical elements of the nutrient solution are added in small concentrations at the beginning of the fermentation and these substances continue to be added in small doses during the production phase.

3. **Continuous fermentation**

In continuous fermentation, an open system is set up. Sterile nutrient solution is added to the bioreactor continuously and an equivalent amount of converted nutrient solution with microorganisms is simultaneously taken out of the system. In the case of a homogeneously mixed bioreactor we refer to a chemostat or a turbidistat. In the chemostat in the steady state, cell growth is controlled by adjusting the concentration of one substrate. In the turbidistat, cell growth is kept constant by using turbidity to monitor the biomass concentration and the rate of feed of nutrient solution is appropriately adjusted.



BATCH CULTIVATION	CONTINUOUS CULTIVATION
1. The bacteria are inoculated into the bioreactor (always stirred tank bioreactor). 2. Then, under certain conditions (temperature, pH, aeration, etc.) the bacteria go through all the growth phases (lag, exponential, stationary).	1. The fresh medium flows into the fermentor continuously, and part of the medium in the reactor is withdrawn from the fermenter at the same flow rate of the inlet flow. 2. The bacteria is grown under certain conditions (temperature, pH, aeration)
Advantages: • can be used for diff reactions every day. • Safe: can be properly sterilized. • Little risk of infection or strain mutation • Complete conversion of substrate is possible	Advantages: • Works all the time: low labor cost, good utilization of reactor • Often efficient: due to the autocatalytic nature of microbial reactions,. • the productivity can be high. • Automation may be very appealing. • Constant product quality
Dis-advantages: •High labor cost •Much idle time – Sterilization, growth, cleaning •Safety – filling emptying, cleaning.	Dis-advantages: •promised continuous production for months fails due to a. infection. b. spontaneous mutation of microorganisms to non producing strain

MICROBIAL GROWTH AND ITS KINETICS

Lag phase

Physicochemical equilibration between microorganism and the environment following inoculation with very little growth.

Log phase

By the end of the lag phase cells have adapted to the new conditions of growth. Growth of the cell mass can now be described quantitatively as a doubling of cell number per unit time for bacteria and yeast's, or a doubling of biomass per unit time for filamentous organisms as fungi.

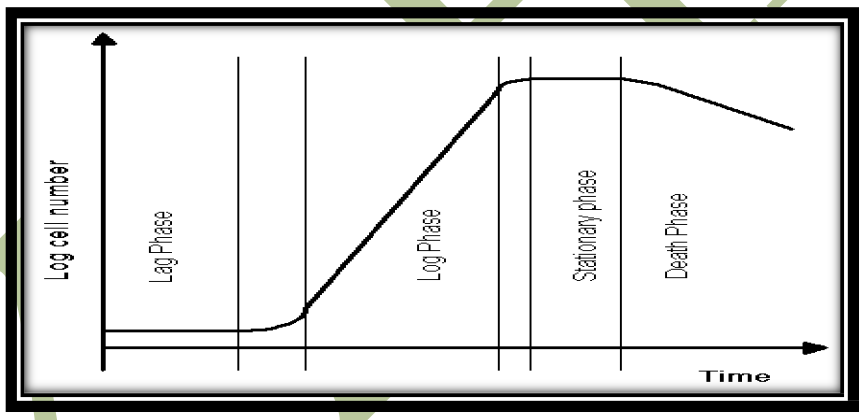
Stationary phase

As soon as the substrate is metabolized or toxic substances have been formed, growth slows down or is

completely stopped. The biomass increases only gradually or remains constant during this stationary phase, although the composition of the cells may change. Due to lysis, new substrates are released which then may serve as energy sources for the slow growth of survivors. The various metabolites formed in the stationary phase are often of great biotechnological interest.

Death phase

In this phase the energy reserves of the cells are exhausted. A straight line may be obtained when a semi logarithmic plot is made of survivors versus time, indicating that the cells are dying at an exponential rate. The length of time between the stationary phase and the death phase is dependent on the microorganism and the process used. The fermentation is usually interrupted at the end of the log phase or before the death phase begins.



Growth Kinetics of Microorganisms

The different types of fermentation processes- batch, fed-batch, semi-continuous and continuous are described above. The kinetics of microbial growth with special reference to log phase of batch fermentation are briefly discussed here.

After completion of lag phase, the cell enters log phase which is characterized by exponential growth. If the initial number of cells is N_0, then

After 1st generation, the cell number will be $N_0 \times 2^1$. After 2nd generation, the cell number will be $N_0 \times 2^2$.

After 3rd generation, $N_0 \times 2^3$ and so on. Thus, the number of cells after a given time (Nt) will be as follows:

$Nt = N_0 \times 2^n$

where n is the number of generations.

The term doubling time (td) or mean generation time (MGT) refers to the time taken for doubling the cell number or biomass. The specific growth rate constant expressed by μ, is the direct measure of rate of growth of the organism. If N is the number of cells at a given time, then the increase in the number of cells (growth rate) with time is given by the formula.

$dN/dt = \mu N$ (1)

If X is the biomass concentration at a given time, then the increase in the biomass (growth rate) with time is given by.

$dX/dt = \mu X$ (2)

In general, the specific growth rate (n) is a function of the concentration of limiting substrate (S), the maximum specific growth rate (μ_{max}) and a substrate specific constant (K_s). Their relationship was expressed by Monond by the following equation

$\mu = \mu_{max} S / K_s + S$ (3)

Both S and K_s are expressed as concentrations e.g., in moles or grams per liter.

The growth rate (μ) of an organism is not fixed but it is variable depending on the environmental conditions such as concentration of substrate and temperature. At a low concentration, the substrate

is the limiting factor for growth.

In batch culture, the substrate is initially present at a higher concentration i.e. (S) > K_s, hence the equation (3) is approximately 1.

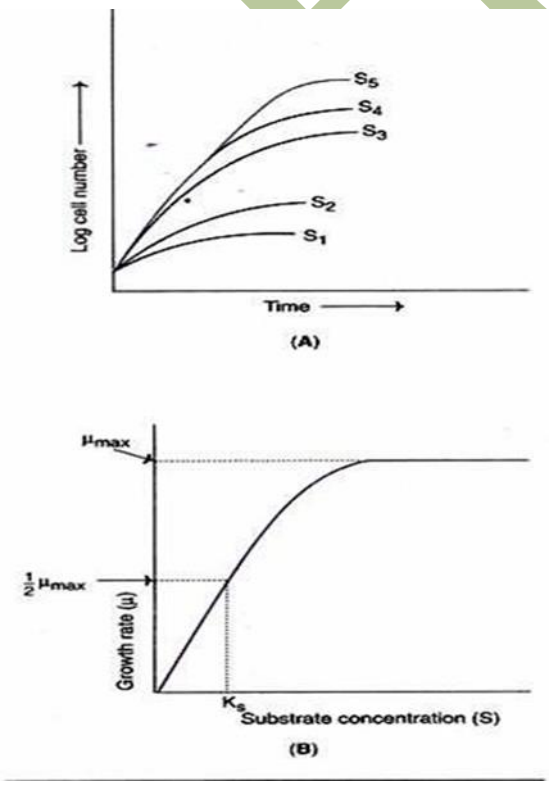
$S/ K_s + S = 1$

Thus, $\mu = \mu_{max}$.

When the substrate concentration is low, as usually occurs at the end of growth phase, then,

$S/ K_s + S < 1$

Hence $\mu < \mu_{max}$.



TYPES OF FERMENTATION

- Solid-State Fermentation (SSF)
- Submerged Fermentation (SmF)/Liquid Fermentation (LF)

SUBMERGED FERMENTATION (SMF)/LIQUID FERMENTATION (LF)

SmF utilizes free flowing liquid substrates, such as molasses and broths. The bioactive compounds are secreted into the fermentation broth. The substrates are utilized quite rapidly; hence need to be constantly replaced/supplemented with nutrients. This fermentation technique is best suited for microorganisms such as bacteria that require high moisture Substrates content. An additional advantage of this technique is that purification of products is easier. SmF is primarily used in the extraction of secondary metabolites that need to be used in liquid form.

- The raw materials used in fermentation constitute the substrates for the fermentation process. The substrates could be seen as either the raw materials that will be ultimately transformed into the desired fermentation products or they could be regarded as the source of nutrients for the fermentationmicroorganisms.
- The substrates form the bulk of the fermentation broth and often considered as one of the most important component in the cost of the fermentation products. Thus in order to lower the costs of production the search for the most cheapest and economical source of fermentation substrate will be the top most agenda in any proposed fermentation industry.
- The search for the most suitable substrate is not only dictated by costs and availability of the substrate but by other factors such as complexity of unwanted reactions that affect not only upstream, midstream but downstream activities. This would also mean the problem of treating its effluent from polluting the environment
- Agro-industrial residues are generally considered the best substrates for the Solid state fermentation (SSF) processes, and use of SSF for the production of enzymes is no exception to that. A number of such substrates have been employed for the cultivation of microorganisms to

produce host of enzymes.

- Some of the substrates that have been used included sugar cane bagasse, wheat bran, rice bran, maize bran, gram bran, wheat straw, rice straw, rice husk, soyhull, sago hampas, grapevine trimmings dust, saw dust, corncobs, coconut coir pith, banana waste, tea waste, cassava waste, palm oil mill waste, aspen pulp, sugar beet pulp, sweet sorghum pulp, apple pomace, peanut meal, rapeseed cake, coconut oil cake, mustard oil cake, cassava flour, wheat flour, corn flour, steamed rice, steam pre-treated willow, starch, etc.
- Wheat bran however holds the key, and has most commonly been used, in various processes.
- The selection of a substrate for enzyme production in a SSF process depends upon several factors, mainly related with cost and availability of the substrate, and thus may involve screening of several agro-industrial residues.
- In a SSF process, the solid substrate not only supplies the nutrients to the microbial culture growing in it but also serves as an anchorage for the cells.
- The substrate that provides all the needed nutrients to the microorganisms growing in it should be considered as the ideal substrate.
- However, some of the nutrients may be available in sub-optimal concentrations, or even absent in the substrates. In such cases, it would become necessary to supplement them externally with these.
- It has also been a practice to pre-treat (chemically or mechanically) some of the substrates before using in SSF processes (e.g. ligno-cellulose), thereby making them more easily accessible for microbial growth.
- Among the several factors that are important for microbial growth and enzyme production using a particular substrate, particle size and moisture level/water activity are the most critical.
- Generally, smaller substrate particles provide larger surface area for microbial attack and, thus, are a desirable factor. However, too small a substrate particle may result in substrate agumulation,

which may interfere with microbial respiration/ aeration, and therefore result in poor growth.

- In contrast, larger particles provide better respiration/aeration efficiency (due to increased inter-particle space), but provide limited surface for microbial attack. This necessitates a compromised particle size for aparticular process.

SOLID-STATE FERMENTATION (SSF)

Solid state fermentation involves the growth of microorganisms on moist solid particles in situation in which the spaces between the particles contains a continuous gas phase and a minimum of visible water. Although droplets of water may be present between the particles and there may be thin films of water at the particle surface, the inter-particle water phase is discontinuous and most of the inter-particle space is filled by the gas phase. The majority of the water in the system is absorbed within the moist solid – particles.

SSF utilizes solid substrates, like bran, bagasse, and paper pulp. The main advantage of using these substrates is that nutrient-rich waste materials can be easily recycled as substrates. In this fermentation technique, the substrates are utilized very slowly and steadily, so the same substrate can be used for long fermentation periods.

Hence, this technique supports controlled release of nutrients. SSF is best suited for fermentation techniques involving fungi and microorganisms that require less moisture content. However, it cannot be used in fermentation processes involving organisms that require high aw (water activity), such as bacteria.

POSSIBLE QUESTIONS

1. Write any three unique features of microbial enzymes and their applications.
2. Define lag phage.
3. What are types of fermentation?
4. State the advantages of batch fermentation.
5. Name some microbial metabolites.

6. What is fermentation?
7. Define microbial growth.
8. Mention about the phase of microbial growth curve?
9. Comment on solid fermentation.
10. List out the microbial enzymes.
11. What are primary metabolites?

EIGHT MARK QUESTIONS

1. Explain the details about range of bioprocess technology.
2. Describe the chronological development of bioprocess technology.
3. What is fermentation? Explain in detail about the types of fermentation.
4. What are the difference between Batch and Continuous culture?
5. Give a short note on Basic principle components of fermentation technology.
6. Comment on i) Fed batch culture ii) Microbial culture growth kinetic

SYLLABUS

Design of bioprocess vessels- Significance of Impeller, Baffles, Sparger; Types of culture/production vessels- Airlift; Cyclone Column; Packed Tower and their application in production processes. Principles of upstream processing – Media preparation, Inocula development and sterilization.

DESIGN OF BIOPROCESS VESSELS

Bioreactor

A bioreactor is a manufactured or engineered device system for carrying out biochemical processes which employ microbes, fungus, plant cells or mammalian cells systems for production of biological products and it is provide a controlled environment for the production of metabolites which can help to achieve the optimal growth of microbes.

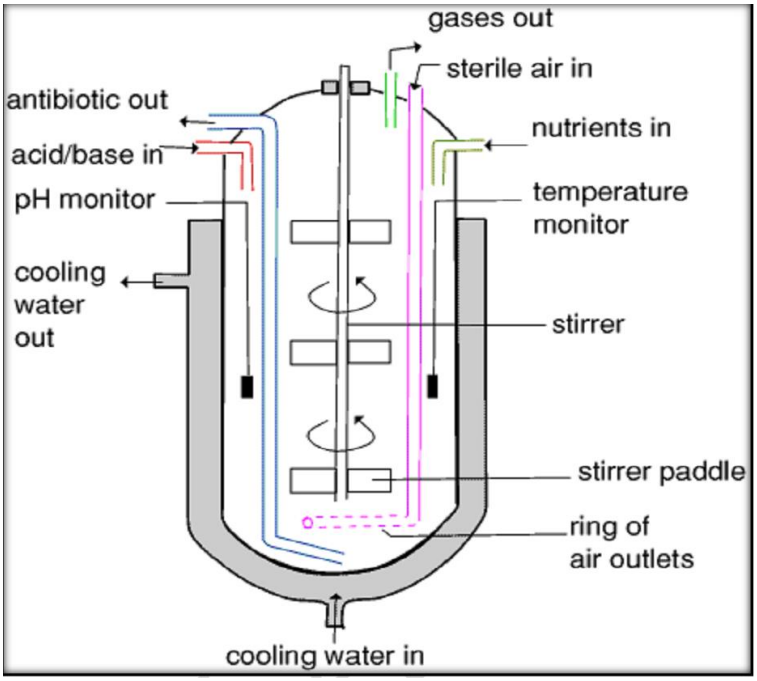
This process can either be aerobic or anaerobic. These bioreactors are commonly cylindrical, ranging in size from litres to cubic metres and are often made of stainless steel. The term fermentor is used as synonym to bioreactor.

Components of Bioreactors

- Vessel : is a container which holds the media and the cells
- Agitator : is required to mix the contents in the vessel to ensure a homogeneous environment and it consist of a shaft and impellers
- Sparger : is an apparatus used to introduce gasses into the vessel.
- Baffles are obstructions on the side of the vessel that generate turbulence in the flow of the culture. Baffles are made out of stainless steel and are welded to the inside of the vessel. It help to mix the culture by creating a more turbulent flow.
- Probes to monitor the culture in the vessel of following.
  - ❖ Temperature
  - ❖ Dissolved Oxygen
  - ❖ pH

- ❖ Pressure Gauge

- Cooling Jacket : to maintain a constant temperature in the reactor, the vessel is covered by a cooling jacket
- Ports for input and output of material
- Condenser
- Back Pressure Valve
- Inlet Filters and Exhaust Filters
- Valves
- Load Cell

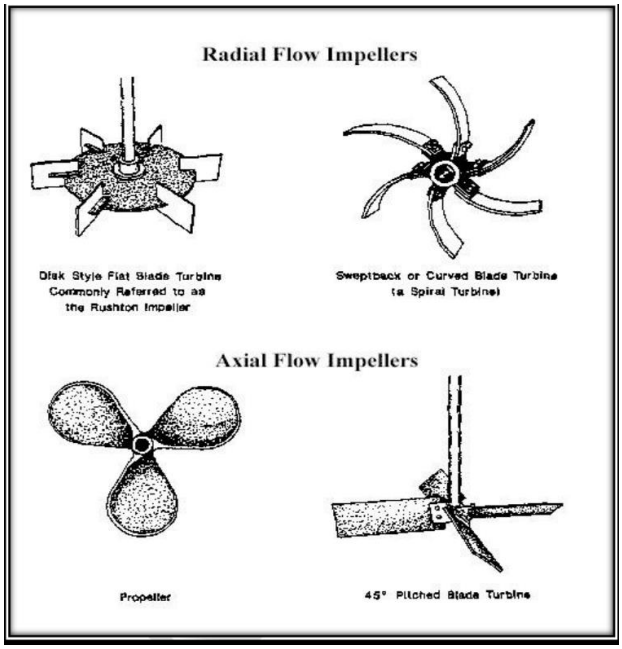


SIGNIFICANCE OF IMPELLER, BAFFLES, SPARGER

THE AGITATOR (IMPELLER)

The agitator is required to achieve a number of mixing objectives, e.g. bulk fluid and gas-phase mixing, air dispersion, oxygen transfer, heat transfer, suspension of solid particles and maintaining a uniform environment throughout the vessel contents. It should be possible to design a fermenter to achieve these conditions; this will require knowledge of the most

appropriate agitator, air sparger, baffles, the best positions for nutrient feeds, acid or alkali for pH control and antifoam addition.



BAFFLES

- Four baffles are normally incorporated into agitated vessels of all sizes to prevent a vortex and to improve aeration efficiency. In vessels over 3-dm3 diameter six or eight baffles may be used (Scragg, 1991).
- Baffles are metal strips roughly one-tenth of the vessel diameter and attached radially to the wall. The agitation effect is only slightly increased with wider baffles, but drops sharply with narrower baffles (Winkler, 1990).
- Walker and Holdsworth (1958) recommended that baffles should be installed so that a gap existed between them and the vessel wall, so that there was a scouring action around and behind the baffles thus minimizing microbial growth on the baffles and the fermenter walls. Extra cooling coils may be attached to baffles to improve the cooling capacity of a fermenter without unduly affecting the geometry.

SPARGER (THE AERATION SYSTEM)

A sparger may be defined as a device for introducing air into the liquid in a fermenter. Three basic types of sparger have been used and may be described as the porous sparger, the orifice sparger (a perforated pipe) and the nozzle sparger (an open or partially closed pipe).

POROUS SPARGER

The porous sparger of sintered glass, ceramics or metal, has been used primarily on a laboratory scale in n on-agitated vessels. The bubble size produced from such spargers is always 10 to 100 times larger than the pore size of the aerator block. The throughput of air is low because of the pressure drop across the sparger and there is also the problem of the fine holes becoming blocked by growth of the microbial culture.

ORIFICE SPARGER

Various arrangements of perforated pipes have been tried in different types of fermentation vessel with or without impellers. In small stirred fermenters the perforated pipes were arranged below the impeller in the form of crosses or rings (ring sparger), approximately three-quarters of the impeller diameter. In most designs the air holes were drilled on the under surfaces of the tubes making up the ring or cross. Walker and Holdsworth (1958) commented that in production vessels, sparger holes should be at least 6 mm (1/4 inch) diameter because of the tendency of smaller holes to block and to minimize the pressure drop.

TYPES OF CULTURE/PRODUCTION VESSELS

AIR LIFT BIOREACTOR

- Air-lift bioreactors are similar to bubble column reactors, but differ by the fact that they contain a draft tube. The draft tube is always an inner tube (this kind of airlift bioreactor is called "air-lift bioreactor with an internal loop) or an external tube (this kind of air-lift bioreactor is called "air-lift bioreactor with an external loop) which improves circulation and oxygen transfer and equalizes shear forces in the reactor. The figure below illustrates the basic structure of an air-lift bioreactor with an internal loop.

- The stirred tank bioreactors lack well defined flow of air. In these, air is pumped from below. This creates the bubbles in the medium which rises up through the draught tube by buoyancy and drags the surrounding fluid up. The air that is used to lift up is sufficient to stir up the content.

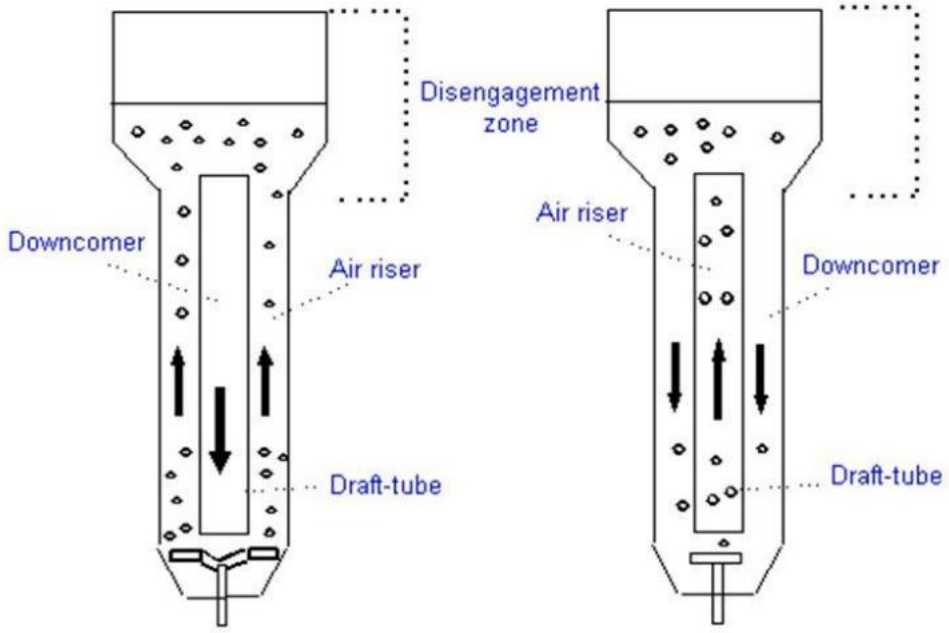
Advantages

- Low friction
- Less energy requirement
- The mechanical parts are easy to construct. There is no need of special aseptic seals.
- Scaling up is easier
- Metabolic performance does not drastically reduce on scale up.

Disadvantage

- Capital needed is more
- Difficulty of sterilization

Airlift bioreactor



CYCLONE COLUMN

- Dawson (1974) developed the cyclone column, particularly for the growth of filamentous cultures. The culture liquid was pumped from the bottom to the top of the cyclone column through a closed loop.
- The descending liquid ran down the walls of the column in a relatively thin film. Nutrients and air were fed in near the base of the column whilst the exhaust gases left at the top of the column. Good gas exchange, lack of foaming and limited wall growth has been claimed with this fermenter.
- Dawson (1974, 1988) has listed a number of potential bacterial, fungal and yeast applications including the batch production of a vaccine for scours in calves with the vessel being operated as batch, continuous or fed-batch.

PACKED TOWER

- The packed tower is a well established application of immobilized cells. A vertical cylindrical column is packed with pieces of some relatively inert material, e.g. wood shavings, twigs, coke, an aggregate or polythene.
- Initially both medium and cells are fed into the top of the packed bed. Once the cells have adhered to the support and are growing well as a thin film, fresh medium is added at the top of the column and the fermented medium is removed from the bottom of the column.
- The best known example is the vinegar generator, in which ethanol was oxidized to acetic acid by strains of *Acetobacter* supported on beech shavings; the first recorded use was in 1670. More recently, packed towers have been used for sewage and effluent treatment.

❖ In treatment of gas liquor, a column was packed to a height 7.9 m with 'Dowpac', a polystyrene derivative. The main advantages compared with other methods of effluent treatment being its simplicity of operation and a saving in land because of the increased surface areas within the column. Other possible applications with immobilized cells are now being investigated.

PRINCIPLES OF UPSTREAM PROCESSING – MEDIA PREPARATION, INOCULA DEVELOPMENT AND STERILIZATION

MEDIA FORMULATION

Most fermentations require liquid media, often referred to as broth, although some solid-substrate fermentations are operated. Fermentation media must satisfy all the nutritional requirements of the microorganism and fulfill the technical objectives of the process. The nutrients should be formulated to promote the synthesis of the target product, either cell biomass or a specific metabolite. Formulation process expressed as Carbon energy sources + Nitrogen source + O2 + other requirements Biomass + Product + CO2 + H2O + heat **Role of Media in Fermentation** In most industrial fermentation processes there are several stages where media are required. They may include several inoculum (starter culture) propaga-tion steps, pilot-scale fermentations and the main production fermentation. The

technical objectives of inoculum propagation and the main fermentation are often very different, which may be reflected in differences in their media formulations. Where biomass or primary metabolites are the target product, the objective is to provide a production medium that allows optimal growth of the microorganism. For secondary metabolite production,. such as antibiotics, their biosyn-thesis is not growth related. Consequently, for this purpose, media are designed to provide an initial period of cell growth, followed by conditions optimized for secondary metabolite production. At this point the supply of one or more nutrients (carbon, phosphorus or nitrogen source) may be limited and rapid growth ceases. Media used in the cultivation of microorganisms must contain all elements in a form suitable for the synthesis of cell substance and for the production of metabolic products. In laboratory research with microorganisms, pure defined chemicals may be used in the production of culture media, but in industrial fermentations, complex, almost undefinable (interms of composition) substrates are frequently used for economic reasons. Depending on the particular process, from 25 to 70% of the total cost of the fermentation may be due to the carbohydrate source. In many cases, media ingredients are byproducts of other industries and are extremely varied in composition. Considerations made when formulating media for fermentation.

- An optimally balanced culture medium is mandatory for maximal production. A supplement of critical elements must be used if necessary.
- The composition of culture media must constantly- be adapted to the fermentation process. New batches of substrate have to be carefully evaluated in trial fermentations be-fore they can be used in production.
- In addition to product yield, product recovery must be examined in trial fermentations.
- If catabolism repression or phosphate repression cannot be eliminated by optimization of the nutrient medium or suitable fermentation management (e.g. feeding), deregulated mutants must be used as production strains.
- Besides material cost and product yield, it must be considered whether materials used are readily available in sufficient supply without high transportation costs, and whether impurities will hinder product recovery or increase cost of product recovery

Media Requirements and Media formulations

Most fermentations, except those involving solid substrates, require large quantities of water in which the medium is formulated.

- General media requirements in-clude a carbon source, which in virtually all industrial fermentations provides both energy and carbon units for biosynthesis, and sources of nitrogen, phosphorus and sulphur.
- Other minor and trace elements must also be supplied, and some microorganisms require added vitamins, such as biotin and riboflavin.
- Usually, media incorporate_buffers, or the pH is controlled by acid and alkali additions.
- Antifoam agents may be required. The initial step in media formulation is the, examination of the overall process based on the stoichiometry for growth and product formation. This primarily involves consideration of the input of the carbon and nitrogen sources, minerals and oxygen and their conversion to cell biomass, metabolic products, carbon dioxide, water and heat. From this information it should be possible to calculate the minimum quantities of each element required to produce a certain quantity of biomass or metabolite. Typically, the main elemental formula of microbial cells is approximately C4H7O2N, which on the basis of dry weight is 48% C, 7% H, 32%O and 14% N. Ideally, a knowledge of the complete elemental composition of the specific industrial microorganism allows further media refinement. This ensures that no element is limiting, unless this is desired for a specific purpose. Once the elemental requirements of a microorganism have been established, suitable nutrient sources can be incorporated into the media.

Factor for media formulation

Compounds that are rapidly metabolized may repress product formation. To overcome this, intermittent or maintain a relatively-low concentration that is not repressive. Certain media nutrients or environmental conditions may affect the physiology, biochemistry, and morphology of the microorganism. In some yeasts the single cells may develop into pseudo-mycelium or flocculate, and filamentous fungi may form pellets. This is not desirable as it

affects the product yield The media adopted also depend on the scale of the fermentation. For small-scale laboratory fermentations pure chemical are often used in well defined media. However, this is not possible for most industrial – scale fermentation processes, simply due to cost, as media components may account for up to 60-80% of process expenditure. Industrial-scale fermentations primarily use cost-effective complex substrates, where many car-bon and nitrogen sources are almost un-definable. Most are derived from natural plant and animal materials, often byproducts of other industries, with varied and variable composition. Small-scale trials are usually performed with each new batch of substrate, particularly to examine the impact on product yield and product recovery.

The main factors that affect the final choice of individual raw materials are as follows.

1. Cost and availability: ideally, materials should be inexpensive, and of consistent quality and year round availability.
2. Ease of handling in solid or liquid forms, along with associated transport and storage costs, e.g. requirements for temperature control.
3. Sterilization requirements and any potential denaturation problems.
4. Formulation, mixing, complexing and viscosity characteristics that may influence agitation, aeration and foaming during fermentation and downstream processing stages
5. The concentration of target product attained, its rate of formation and yield per gram of substrate utilized
6. The levels and range of impurities, and the potential for generating further undesired products during the process.
7. Overall health and safety implications

ENERGY SOURCES

SUBSTRATES USED AS CARBON SOURCES

Carbohydrates are traditional energy sources in the fermentation industry. Pure glucose or sucrose can seldom be used as the sole carbon source, except in processes, which demand exact fermentation control as they are very expensive media.

Sugar beet and Cane molasses

Molasses, a byproduct of sugar production, is one of the cheapest sources of carbohydrate. Besides a large amount of sugar, molasses contains nitrogenous substances, vitamins, and trace elements. How-ever, the composition of molasses varies de-pending on the raw material used for sugar production. Table below shows a comparison of the analysis of sugar beet and sugar cane molasses

Malt Extract

Malt extract, an aqueous extract of malted barley, is an excellent substrate for many fungi, yeasts, and actinomycetes. Dry malt extract consists of about 90-92% carbohydrates, and is composed of hexoses (glucose, fructose), disaccrides (maltose, sucrose), trisaccharides (maltotriose), and dextrins, as shown in table below. Nitrogenous substances present in malt extract include proteins, peptides, amino acid, purines, pyrimidines, and vitamins. The amino acids composition of different malt extracts varies according to the grain used, but praline always makes up about 50% of the total amino acids present. Culture media containing malt extract must be carefully sterilized. When overheating occurs, the Maillard reaction results, due to the low pH value and the high proportion of reducing sugar. In this conversion, the amino groups of amines, amino acid (especially lysine), or proteins react with the carbonyl groups of reducing sugars, aldehydes or ketones, which results in the formation of brown condensation products. These reaction products are not suitable substrates for microorganisms. The Maillard reaction is one of the main causes of damage to culture media during heat sterilization, resulting in considerably reduced yields

Typical composition of malt extract

- Composition (%)
- Maltose 52.2

- Hexoses (glucose, fructose) 19.1
- Sucrose 1.8
- Dextrin1 5.0
- Other carbohydrates 3.8
- Nitrogenous materials 4.6
- Ash 1.5
- Water content 2.0

Starch and dextrins can be directly metabolized as carbon sources by amylaseproducing organisms. In addition to glucose syrup, which is frequently used as a fermentation substrate, starch has become more important as a substrate for ethanol fermentation.

Sulfite waste liquors, sugar-containing waste products of the paper industry, which have a dry weight of 9-13%, are primarily used in the cultivation of yeasts. Sulfite liquors from coniferous trees have a total sugar content of 2-3%, and 80% of the sugars are hexoses (glucose, man-nose galactose), the others being pentoses (xylose arabinose). Sulfite liquors from deciduous contain mainly pentose sugars.

- Because of its wide availability and low cost, cellulose is being extensively studied as a substrate for conversion to sugar or alcohol. It is usually not possible to use cellulose directly as a carbon source, so it must first be hydrolysed chemically or enzymatically.
- The sugar syrup formed from cellulose hydrolysis has been used for ethanol fermentation, and the fermentative production of butanol, acetone, and isopropanol is also being considered. Work is in progress to develop one-step processes for direct conversion of cellulose to ethanol, using fermentative organisms which produce cellulases.
- Whey, a byproduct of the dairy industry, is produced annually on a world-wide basis to the amount of 74 million tons (containing 1.2 million tons of lactose and 0.2 million tons of milk pro-tein). Only about 56% of this product is used for human or animal feed.

- The lactose is used primarily for the production of ethanol or single-cell protein, but also in the production of xanthan gum, vitamin B12, 2,3-butandiol, lactic acid, and gibberellic acid. Because of storage and transportation costs, whey is often not economical as a substrate. Animal fats such as lard and animal and plant oils are readily utilized by some microorganisms, but are generally added as supplemental substrates rather than as the sole fermentable carbon source. For instance, in certain antibiotic fermentations, soy, palm, and olive oils are used. With respect to its carbon content, methanol is the cheapest fermentation substrate, but it can be metabolized by only a few bacteria and yeasts. Methanol has commonly been used as a substrate for single cell protein production. Research has been carried out on processes for the production of glutamic acid, serine, and vitamin B12 using methanol as the sole carbon .source or as a co-substrate.

SUBSTRATES USED AS NITROGEN SOURCES

Many large-scale processes utilize ammonium salts, urea, or gaseous ammonia as nitrogen source. A nitrogen source, which is efficiently metabolized, is corn steep liquor, which is formed during starch production from corn.

The concentrated extract (about 4% nitrogen) contains nu-merous amino acids, such as alanine, arginine, glutamic acid, isoleucine, threonine, valine, phenylalanine, methionine, and cystine. The sugar present in corn steep liquor becomes largely converted to lactic acid (9-20%) by lactic acid bacteria.

Yeast extracts are excellent substrates for many microorganisms. They are produced from baker's yeast through autolysis at 50-55%C through plasmolysis in the presence of high concentrations of NaCl.

Yeast extract contains amino acids and peptides, water-soluble vitamin, and carbohydrates.

The composition of yeast extract varies partly because the substrates used for yeast cultivation affect the quality of the yeast extract.

Peptones (protein hydrolysates) can be utilized by many microorganisms but they are relatively expensive for industrial application. Sources of peptones include meat, casein, gelatin, keratin, peanut seeds, soy meal, cotton seeds, and sunflower seeds.

Peptone composition varies depending upon its origin. For instance, peptone from gelatin is rich in proline and hydroxyproline, but has almost no sulfur-containing amino acids. On the other hand, peptone from keratin has a large proportion of proline and cystine, but lacks lysine.

Peptones of plant origin (soy peptone, cottonseed peptone) have large proportions of carbohydrates. The end product is also influenced by the type of hydrolysis, whether acid or enzymatic, especially in regard to its tryptophan content.

Soy meal, the residue from soybeans after the extraction of soybean oil, is a complex substrate. Analysis shows a protein content of 50%, a carbohydrate content of 30% (sucrose, stachyose-, raffinose, arabinoglucan, arabinan, and acidic polysaccharides), 1% residual fat, and 1.8% lecithin.

Soy meal is frequently used in, antibiotic fermentations; catabolite regulation does not occur because of the slow catabolism of this complex mixture

Water sources

- Water is the major components of all fermentation media preparation
- When assessing the stability of water supply it is important to consider pH, dissolved salts, and effluent contaminations
- The mineral content of the water is very important is brewing and mashing process of fermentation.
- The reuse or efficient use of water normally high priority

Oils and fats

- Mainly the oil and fats used as anti-foam agents in fermentation process

- Vegetative oils will be used as oil sources i.e. olive oil, maize oil, cotton seed oil, lin seed oil, etc.,
- These oils contains fatty acids, oleic acid, linoleic acid, linolenic acid etc., these acids some time used as carbon sources.

Hydrocarbons and their derivatives

- n-alkanes – for the production of organic acid, amino acid, vitamins, nucleic acid, antibiotics, enzymes and proteins
- Methane

Minerals

- Microorganism requires certain mineral element for growth and metabolism i.e. magnesium, phosphorus, potassium, calcium, sulphur and chlorine are essential elements
- Others such as cobalt, copper, iron, manganese, molybdenum and zinc are used as elemental sources for microorganism.

INOCULA DEVELOPMENT

It is essential that the culture used to inoculate a fermentation satisfies the following criteria:

1. It must be in a healthy, active state thus minimizing the length of the lag phase in the subsequent fermentation.
2. It must be available in sufficiently large volumes to provide an inoculum of optimum size.
3. It must be in a suitable morphological form.
4. It must be free of contamination.
5. It must retain its product-forming capabilities.

❖ The preparation of inocula for fermentations employing mycelial (filamentous) organisms is more involved than that for unicellular bacterial and yeast processes. The majority of industrially important fungi and streptomycetes are capable of asexual

sporulation, so it is common practice to use a spore suspension as seed during an inoculum development programme.

- A major advantage of a spore inoculum is that is contains far more 'propagules' than a vegetative culture. Three basic techniques have been developed to produce a high concentration of spores for use as an inoculum.
- The commercial production of bakers' yeast involves the development of an inoculum through a large number of aerobic stages. Although the production stages of the process may not be operated under strictly aseptic conditions a pure culture is used for the initial inoculum, thereby keeping contamination to a minimum in the early stages of growth.
- Reed and Nagodawithana (1991b) discussed the development of inoculums for the production of bakers' yeast and quoted a process involving eight stages, the first three being aseptic while the remaining stages were carried out in open vessels. The yeast may be pumped from one stage to the next or the seed cultures may be centrifuged and washed before transfer, which reduces the level of contamination.

MEDIA STERILIZATION

Sterilization

- It is processes that effectively kills or eliminate transmissible agents such as fungi, bacteria and virus from a surface of equipments, food materials, medications and biological culture medium.
- A process which removes all living things
- Therefore, sterilization is an essential requirement

Sterilizing types and agents

1. Wet heat up to 100ºC
2. Wet heat above 100ºC
3. Dry heat
4. Irradiation

5. Chemical Sterilization
6. Filtration

Wet heat above 100ºC

- In practice, nearly complete sterilization is needed
- So more severe methods are required
- Boiling point can be increased by heating water under pressure
- This need special equipment called "autoclave"
- With the knowledge of inactivation kinetics for heat resistant bacterial spores, suitable **autoclaving cycles** have been developed
- Killing action includes hydrolysis in addition to other factors.

Dry heat- Steam

- Steam should be at maximum water holding capacity, but with no water droplets
- Shouldn't be superheated/dry
- Non-condensable gases (air) in the steam can reduce the temperature at a given pressure
- With a 50% mixture of steam & gas the temperature at 15 psi will fell down to 112ºC, instead of 121ºC

Table 1 Standard autoclave cycles

Temperature (°C)	Time (min)	Pressure (bar)[a]	Survival[b]	Equivalent time[c] (min)
115	30	0.7	1 in 10^4	60
121	15	1.0	1 in 10^8	15
126	10	1.4	1 in 10^{17}	4.7
134	3	2.0	1 in 10^{32}	0.8

[a] 1 bar = 10^5 Pa.

[b] For a relatively heat-resistant bacterial endospore such as *Bacillus stearothermophilus*.

[c] Time required to give endospore survival of 1 in 10^8, i.e. time equivalent to 121 °C for 15 min.

Irradiation

a) Ultra-violet Light

- UV at 260 nm inactivates micro-organisms and viruses
- Acts on nucleic acids and cause
  - Strand breakage
  - Strand cross-linking
  - Pyrimidine dimmer formation

Uses Generally used as a supplementary option for

- Air sterilization
- Surface sterilization

Limitations

- Intensity of UV source decreases with time
- Poor penetration
- Bacterial and fungal spores are resistant
- DNA repair system in microbes may repair limited damage

Gamma Radiations

- Direct effect on nucleic acids
- Indirect damage by production of Free Radicals
- Very deep penetrations
- 2.5 M. rad is a standard for sterilization (Obtained from Cobalt 60)

Advantages

- Deep penetration
- Packed items can be sterilized
- Heat sensitive items e.g. plastic-ware and antibiotic can be sterilized

Limitations

- Not usable at laboratory scale
- Spores and some viruses are somewhat resistant
- Some viruses are extremely resistant

Chemical Sterilization

a) Fumigation

- Formaldehyde, ethylene oxide or ethylene glycol may be used
- Effective against all microbes including viruses
- Effective at higher temperatures and at high humidity i.e. (75-100%)
- Conditions reducing access of fumes to microbes reduce activity (e.g. wrapping in organic or inorganic matter)

Liquid Disinfectants

Applications

- Routine hygiene & disinfection (surfaces & equipment)
- Disinfection of cell culture items after use, and before washing and re-sterilization

- Treatment of used or contaminated cell culture media before disposal

Methods of Application

a) Cloth/cotton
b) Sprayers
c) Paper towels
d) Immersion

	Effectiveness[a]			
Type	**Fungi**	**Bacteria**	**Endospores**	**Viruses**
Aldehydes	+	+	+	+
Hypochlorites	+	+	+	+
Phenolics	+	+	–	+/v
Alcohol	–	+	–	+/v

[a] Effective (+) and non-effective (–). In the case of non-enveloped viruses, the effect may be variable or partial (v) depending on the particular virus. Examples of

Limitations/Factors to be considered

- Range/anti-microbial activity less than some other agents as heat
- Spores/some viruses may be resistant
- Some disinfectants are neutralized by organic matter
- Stability o working dilutions varies
- Required exposure dependent on nature of the agent
- Extent of toxicity be considered

Filtration

a) Fluid Filtration

1 For Bacteria and Fungi

- Depth filters developed in late 1800's

- Un-glazed porcelain, diatomaceous earth, asbestos used
- Largely replaced by 0.2μm membranes
- Act as sieves
- Used especially for solutions which can't be subjected to autoclaving e.g. sera, proteins, growth promoting substances

Types of Filters

- Cellulose acetate, cellulose nitrate and their mixtures
- Nylons or polysulfones
- Polyvenylidene difluoride (PVDF), low protein binding
- Polycarbonate by irradiation-etch technique

POSSIBLE QUESTIONS

TWO MARK QUESTIONS

1. What is sterilization?
2. Define Agitator.
3. Comment on components of Bioreactors
4. Draw the structure of fermenter.
5. What is the advantage of Baffles?
6. What is the role of impeller in fermenter?
7. What is the significance of sparger?
8. State the types of sparger.
9. What is Air-lift bioreactor?
10. Make short notes on cyclone column bioreactor.
11. Write any three advantages of Air-lift bioreactor.
12. Define Media formulation.
13. Mention about typical composition of media
14. What is inocula development?
15. Mention about the media sterilization.

EIGHT MARK QUESTIONS

1. Explain in detail about the components of bioreactors with diagram.
2. Write in detail: i) Significance of Impeller. ii) Application of Air-lift bioreactor.
3. Describe in detail about the methods of media sterilization in bioprocess technology.
4. Give a short note on principles of upstream processing of bioprocess technology.
5. Comment on: i) Cyclone Column bioreactor ii) Packed Tower bioreactor

SYLLABUS

Bioreactor control and monitoring, Introduction to oxygen requirement in bioprocess; mass transfer coefficient; factors affecting KLa. Bioprocess measurement and control system with special reference to computer aided process control.

BIOREACTOR CONTROL AND MONITORING

- The success of fermentation depends upon the existence of defined environmental conditions for biomass and product formation. To achieve this goal it is important to understand what is happening to a fermentation process and how to control it to obtain optimal operating conditions.
- Thus, temperature, pH, degree of agitation, oxygen concentration in the medium and other factors may have to be kept constant during the process. The provision of such conditions requires careful monitoring (data acquisition and analysis) of the fermentation so that any deviation from the specified optimum might be corrected by a control system.
- As well as aiding the maintenance of constant conditions, the monitoring of a process may provide information on the progress of the fermentation. Such information may indicate the optimum time to harvest or that the fermentation is progressing abnormally which may be indicative of contamination or strain degeneration. Thus, monitoring equipment produces information indicating fermentation progress as well as being linked to a suitable control system.
- In initial studies the number of functions which are to be controlled may be restricted in order to gain more knowledge about a particular fermentation. Thus, the pH may be measured and recorded but not maintained at a specified pH or the dissolved oxygen concentration may be determined but no attempt will be made to prevent oxygen depletion.
- Also, it is important to consider the need for a sensor and its associated control system to interface with a compute. This chapter will consider the general types of control

systems which are available, specific monitoring and control systems and the role of computers.

There are three main classes of sensor:

1. Sensors which penetrate into the interior of the fermenter, e.g. pH electrodes, dissolved-oxygen electrodes.
2. Sensors which operate on samples which are continuously withdrawn from the fermenter, e.g. exhaust-gas analysers.
3. Sensors which do not come into contact with the fermentation broth or gases, e.g. tachometers, load cells.

There are three main classes of sensor:

1. ***In-line sensor.*** The sensor is an integrated part of the fermentation equipment and the measured value obtained from it is used directly for process control.
2. ***On-line sensor.*** Although the sensor is an integral part of the fermentation equipment, the measured value cannot be used directly for control. An operator must enter measured values into the control system if the data is to be used in process control.
3. ***Off-line sensor.*** The sensor is not part of the fermentation equipment. The measured value cannot be used directly for process control. An operator is needed for the actual measurement (e.g, medium analysis or dry weight sample) and for entering the measured values into the control system for process control.

Temperature

The temperature in a vessel or pipe is one of the most important parameters to monitor and control in any process. It may be measured by mercury-in-glass thermometers, bimetallic thermometers, pressure bulb thermometers, thermocouples, metal-resistance thermometers or thermistors. Metal-resistance thermometers and thermistors are used in most fermentation applications. Accurate mercury-in-glass thermometers are used to check and calibrate the other forms temperature sensors, while cheaper thermometers still used with laboratory fermenters.

Mercury-In-Glass Thermometers

A mercury-in-glass thermometer may be used directly in small bench fermenters, but its fragility stricts its use. In larger fermenters it would be necessary to insert it into a thermometer pocket in to vessel, which introduces a time lag in registering the vessel temperature. This type of thermometer can used solely for indication, not for automatic control recording.

Electrical resistance thermometers

It is well known that the electrical resistance metals changes with temperature variation.

This property has been utilized in the design of resistant thermometers. The bulb of the instrument contains to resistance element, a mica framework (for very accurate measurement) or a ceramic framework (robust; but for less accurate measurement) around which the sensing element is wound. A platinum wire of 100Ω resistance is normally used.

Leads emerging from the bulb are connected to the measuring element. The reading is normally obtained by the use of a Wheat stone bridge circuit and is a measure of the average temperature of the sensing element. This type of thermometer does have a greater accuracy (± 0.25%) than some of the other measuring devices and is more sensitive to small temperature changes.

There is a fast response to detectable changes (1 to 10 seconds), and there is no restriction on distance between the very compact sensing point (30 X 5 mm) and the display point of reproducible readings. These thermometers are normally enclosed in stainless-steel sheaths if they are to be used in large vessels and ancillary equipment.

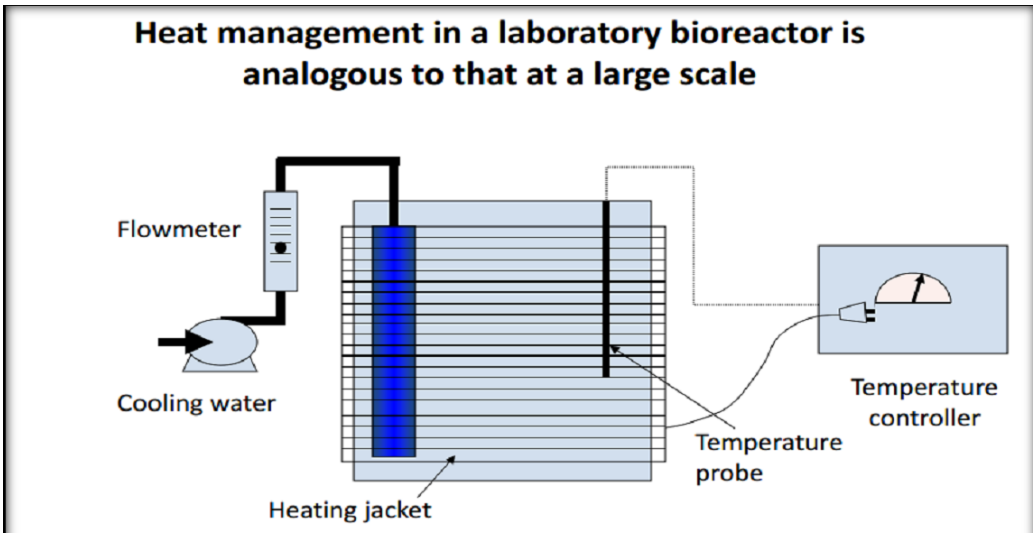
Thermistors

Thermistors are semiconductors made from specific mixtures' of pure oxides of iron, nickel and other metals. Their main characteristic is a large change in resistance with a small temperature change. The change in resistance is a function of absolute temperature. The

temperature reading is obtained with a Wheatstone bridge or a simpler or more complex circuit depending on the 'application. Thermistors are relatively cheap and have proved to be very stable, give reproducible readings, and can be sited remotely from the read out.

Temperature control

The use of water jackets or pipe coils within a vessel the temperature has been controlled In many small systems there is heating element, 300 to 400 W capacity being adjusted. The heating element should be as well as possible to reduce the size of the 'heat sink' resulting overshoot when heating is no longer required. In some cases it may be better to run the cooling water continuously at a steady rate and to have heating element only connected to the control unit. This can be an expensive mode of operation if the flows directly to waste.



pH control and monitoring

- Most bacteria can grow over a wide range of pH, although many enzymes upon which microbial growth depends function only within a narrower range of pH.
- The bacteria then must maintain their internal pH near a fixed optimal value.
- Bacteria (*E. col*i) that grow at neutral pH (6.0 Bacteria (*E. coli*) that grow at neutral pH (6.0-8.0) are called neutrophiles. 8.0) are called neutrophiles.
- Regardless of the external pH, the internal pH is maintained at ~7.6.

- pH is maintained by ion pumps on the membrane of the bacteria.
- Effort put into maintaining the pH will be at the expense of other cellular functions
- Bugs tend to grow more slowly when the pH is not at the optimum.
- Overfeeding substrate can cause the cells to produce organic acids, such as acetate – pH drops
- Lack of carbohydrate substrate causes the cells to consume protein in the media – producing NH3 → NH4OH – pH rises
- When producing a protein product, cells consume ammonia from the media from the cellular demand for more nitrogen – causes the release of a proton – pH drops.
- The main purpose of a pH sensor is to maintain a pH level during fermentation that can optimize the catalytic conversion to alcohol. A classic pH meter measures the acidity or alkalinity of a liquid.
- The pH meter involves the application of glass electrodes. Any pH meter will need to be calibrated before being used to test for the acid/alkalinity of a test solution. The pH meter does require regular calibration so that the glass electrode generates an accurate pH reading.
- A standard calibration process should be carried out with two buffer solutions that cover a spectrum of pH values. It is acceptable to use buffer solutions at a pH value of 4 and 10.
- It is important to set the pH meter to two control pH values that corresponds to the two pH buffer values. The pH meter is also adjusted to a control value for temperature. By calibrating the pH meter for all three control values, the meter can achieve linear accuracy.
- A standard pH meter involves glass electrodes and reference electrodes (such as Ag/AgCl [silver/silver chloride]) that are important for measuring the pH of a test liquid. Electrical circuits containing a glass electrode, a reference electrode, and an instrument to measure electrical potential between opposing electrical fields are the basic units to a pH meter.

- The main purpose of the glass electrode is to carry an electrical current via a wire that submerges into the test liquid. The wire and liquid are enclosed by a thin glass tube. It is the glass electrode that becomes the vital process in measuring the PH of a solution. The membrane is made up of oxygen atoms that carry an odd cluster of electrons and this is what creates a negative electrical potential.
- During a standard testing procedure, this electrode is exposed to an acidic solution which forces the positive ions from the acid solution to bind to the glass electrode. Whilst submerged, the electrodes need to maintain a neutral solution and so the electrons from the inner surface of the electrode move to the outer solution, but this will change the electrical potential of the testing solution.
- The purpose of the reference electrode is to carry the electrical current to a meter that can measure the difference in electrical potential.
- A graduated syringe is normally used to inject dilute sodium hydroxide (neutralizer) to the wine solution together with phenolphthalein. The indicator used changes the colour of the fermented media to a pink colour to ensure that the solution is no longer acidic.
- This is where the pH meter becomes useful – this device can work to provide a neutral end point on a digital monitor.

In Situ Transmissive pH Sensors

This type of pH sensor uses sol-gel solution combined with a colorimetric pH indicator dye which can reflect light through a read fibre that will then provide an estimation of colour change to a sample solution at a certain wavelength. pH sensors are typically susceptible to changes in salinity, though the In Situ Transmissive pH Sensor is built to avoid this problem.

This type of pH sensor works well with organic solvents including acetone, alcohols, and aromatic and so would be of benefit to the beverage brewing industry.

Non-Intrusive Reflective pH Sensors

These sensors are novel and have evolved pH sensor technology. The sensor uses an electroformed mesh material which adds a coating of metal on a non-metallic surface of the pH sensor and this creates a reflective ion permeable membrane allowing for pH measurement through a clear wall to a cavity containing reflective probes. Again, this type of sensor is applicable to the food and beverage processing industry.

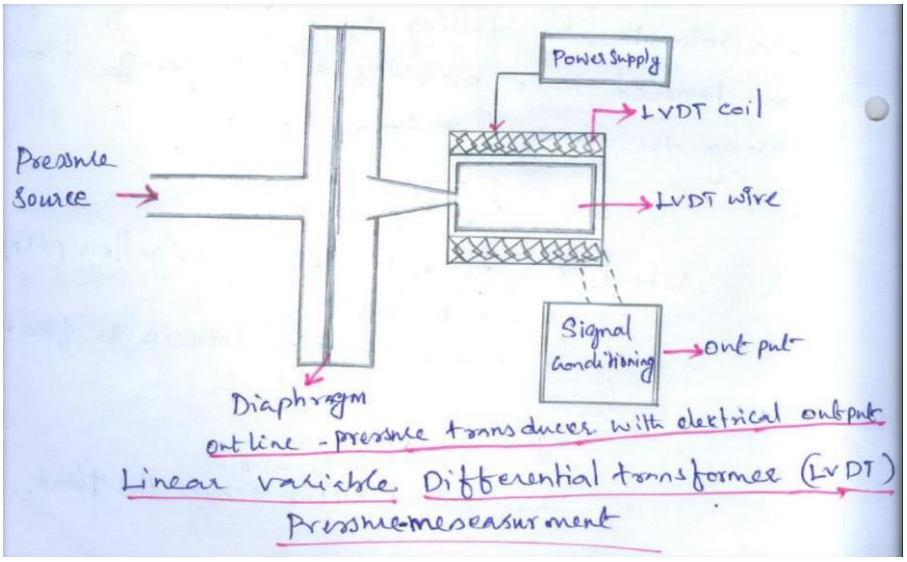
Pressure and control

Different working pressures are required in different parts of a fermentation plant. During normal operation a positive head pressure of atmospheres (161 kN-l) absolute is maintained in a fermenter to assist in the maintenance of aseptic conditions. This pressure will obviously be raised during a steam-sterilization cycle. The correct pressure in different components should be maintained by regulatory valves controlled by associated pressure gauges.

Pressure measurements may be made for several reasons, that most important of which is safety. Industrial and lab equipments is designed to withstand a specific working pressure a factor of safety.

Therefore the pressure indicating devices must fir in the equipments as a device. This device may be worked as sense indicate record and control the pressure.

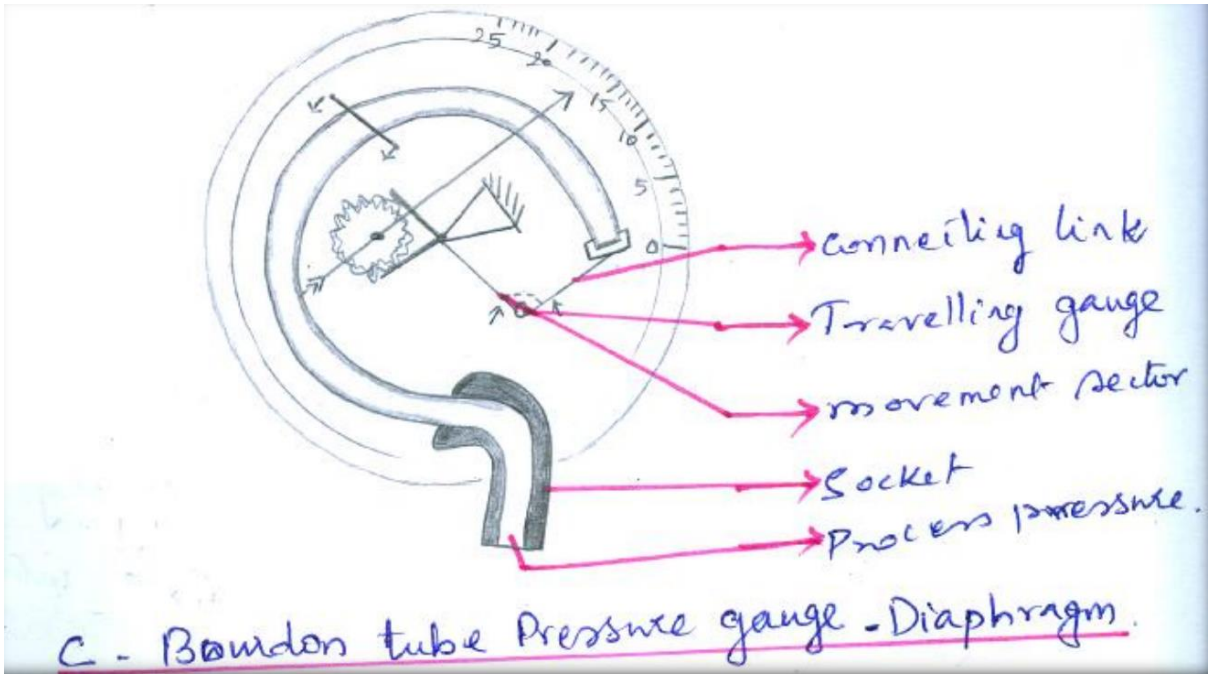
The measurement of pressure is important when the media sterilization and metabolic reactions in fermentation.



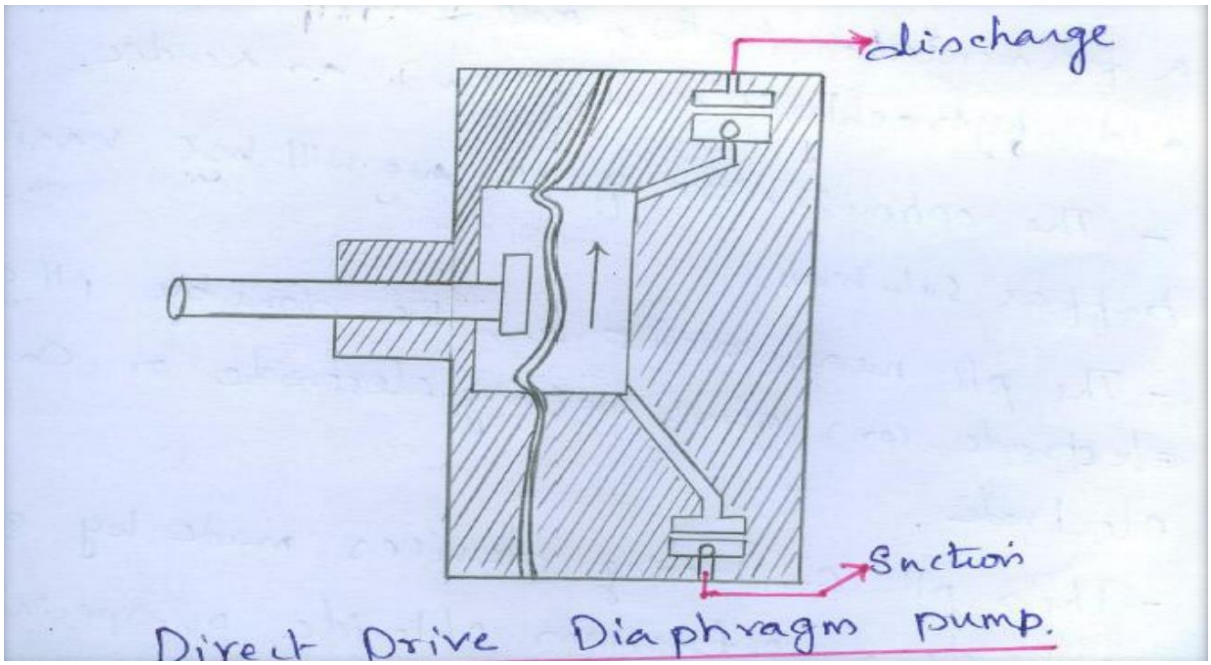
The high pressure will influence the solubility of gas and contribute to the maintenance of sterility.

This pressure can be measured by using C bourdon tube pressure gauge and it also contain direct indicating gauge.

This pressure will be maintained and controlled by using electrical output (linear variable differential transformer –LVDT). The LVDT containing primary and secondary coil, this coil movement a voltage change proportional to the displacement caused in the input pressure.



The pressure range of 3-15 to 0-3000 psi unit . The input pressure we can controlled using direct drive diaphragm pump.



On line analysis During fermentation, the chemical factors can influence growth and product formation and this to be continuously monitored the following techniques.

On line analysis

During fermentation, the chemical factors can influence growth and product formation and this to be continuously monitored the following techniques.

PID controller

The bioprocess control has different goals and objectives, function of bioprocess characteristics and imposed performances. In spite of high non-linearity linear control theory and basic controllers (on/off, PID) are still applied in most industrial applications.

- A proportional-integral-derivative controller (PID controller) is a generic control loop feedback mechanism (controller) widely used in industrial control systems.
- A PID controller calculates an "error" value as the difference between a measured process variable and a desired setpoint. The controller attempts to minimize the error by adjusting the process control inputs.
- The PID controller algorithm involves three separate constant parameters, and is accordingly sometimes called three-term control: the proportional, the integral and derivative values, denoted P, I, and D. Simply put, these values can be interpreted in terms of time: P depends on the present error, I on the accumulation of past errors, and D is a prediction of future errors, based on current rate of change.

INTRODUCTION TO OXYGEN REQUIREMENT IN BIOPROCESS

- The majority of fermentation processes is aerobic and, therefore, requires the provision of oxygen. If the stoichiometry of respiration is considered, then the oxidation of glucose may be represented as:
- C6H 120 6 + *602 = 6H 2O + 6CO2*
- The oxygen demand of an industrial fermentation process is normally satisfied by aerating and agitating the fermentation broth. However, the productivity of many fermentations is limited by oxygen availability and, therefore, it is important to consider the factors which affect a fermenter's efficiency in supplying microbial cells with oxygen**.**

OXYGEN SUPPLY

- Oxygen is normally supplied to microbial cultures in the form of air, this being the cheapest available source of the gas. The method for provision of a culture with a supply of air varies with the scale of the process:
- Laboratory-scale cultures may be aerated by means of the shake-flask technique where the culture (50 to 100 cm3) is grown in a conical flask (250 to 500 cm3) shaken on a platform contained in a controlled environment chamber.
- Pilot- and industrial-scale fermentations are normally carried out in stirred, aerated vessels, termed fermenters, However, it is often advantageous to culture relatively small volumes (1 dm^3) in a stirred, aerated vessel as this enables the cultural conditions to be better monitored and controlled, and facilitates the addition of supplements and the removal of samples.

MASS TRANSFER COEFFICIENT

Mass-Transfer Coefficient (k_L & k_G)

- Since the amount of solute transferred from the gas phase to the interface must equal that from the interface to the liquid phase,

$$N_G = N_L$$

$$\frac{C_G - C_{G_i}}{C_L - C_{L_i}} = -\frac{k_L}{k_G}$$

FACTORS AFFECTING KLa.

A number of factors have been demonstrated to affect the *KLa* value achieved in a fermentation vessel. Such factors include the air-flow rate employed, the degree of agitation,

the rheological properties of the culture broth and the presence of antifoam agents. If the scale of operation of a fermentation is increased (so-called 'scale-up') it is important that the optimum *KLa* found on the small scale is employed in the larger scale fermentation. The same *KLa* value may be achieved in different sized vessels by adjusting the operational conditions on the larger scale and measuring the *KLa* obtained. However, quantification of the relationship between operating variables and *KLa* should enable the prediction of conditions necessary to achieve a particular *KLa* value. Thus, such relationships should be of considerable value in scaling-up a fermentation and in fermenter design.

- The effect of air-flow rate on *K La*
- The relationship between *KLa* and power consumption
- The effect of medium and culture rheology on *K La*
- The effect of microbial biomass on *KLa*

BIOPROCESS MEASUREMENT AND CONTROL SYSTEM WITH SPECIAL REFERENCE TO COMPUTER AIDED PROCESS CONTROL

Computer in bioprocess

The bioprocess advancement is determined by the living cells capabilities and characteristics, the bioreactor performance as well as by the cultivation media composition and the main parameters evolution. The high metabolic network complexity inside the cells often determine very sophisticated, non-linear growth and product formation kinetics, with further consequences on the bioprocess behavior, but at the same time on the product quality and yield.

The key issue of this rather complicated situation is the use of modeling and further on of computer assisted control as a powerful tool for bioprocess improving. The process models, as relationships of the input, output and inner variables, though incomplete and simplified, can be effective to describe the phenomena and the influences of great importance for control, optimization and better theoretical knowledge.

UNIT-III

The function of any biological model is to describe the metabolic reactions rates and their stoichiometry on the basis of bioreactor conditions, with the main difficulties-the identification of principal factors affecting cellular growth and bioproduct formation, and the building up of a suitable model structure for the intracellular processes.

Moreover the scheduling, supervision and automatic control in modern bioprocessing is done by advanced process control systems, where all the functions are implemented in software. The main bioprocess control attributes are: handling of offline analyses; recipe and scheduling; high level overall control; state and parameters estimation; simulation; prediction; optimization.

For the industrial developments the central and manifold objective of the computer control is the realization of the economic interests in assuring high operational stability, process reproducibility and increased product yield together with the maintaining of rigorous safety and the implementation of the GMP or environmental regulations, important requests in modern biomanufacture imposed by the product quality improving needs.

The standard direct physical determinations are:

(1) temperature;
(2) pressure (over pressure);
(3) agitator shaft power and rate of stirring;
(4) foam;
(5) gas and liquid flow;
(6) weight.

Temperature determination is important for bioprocess evolution as well as other process operations (i.e. sterilization, concentration, and purification). The temperature measurement is made in the range +20oC to +130oC through mercury-in-glass thermometers, bimetallic thermometers, pressure bulb thermometers, thermocouples, metal-resistance thermometers or thermistors; all of them must be steam-sterilizable at 120oC. The most popular are the Pt100 resistance thermometers.

Pressure measurements may be needed for several reasons; the most important of them is the safety. Industrial and laboratory equipment is designed to withstand a specified working pressure plus a factor of safety. Also, the measurement of pressure is important in media sterilization. Moreover, the pressure will influence the solubility of gases and contribute to the maintenance of sterility, when a positive pressure is present. The standard measuring sensor is the membrane pressure gauge based on strain or capacitance measurements.

The **formation of foam** can create serious problems in no controlled situations: loss of broth, clogging of gas analyzers, infections, etc. It is a common practice to add an antifoam agent when the culture starts foaming above a certain predetermined level. A standard foam sensing consists in an electrical conductivity / capacitance / heat conductivity probe.

A number of mechanical antifoam devices have been made, including discs, propellers, brushes attached to the agitator shaft above the surface of the broth. Unfortunately, most of the mechanical devices have to be used in conjunction with an antifoam agent, without negative influence on the bioprocess behavior.

Computer in bioprocess control system

The computer aided fermentation control contains three distinct areas of computer function, that included as

- Logging of process data
- Data analysis
- Process control analysis

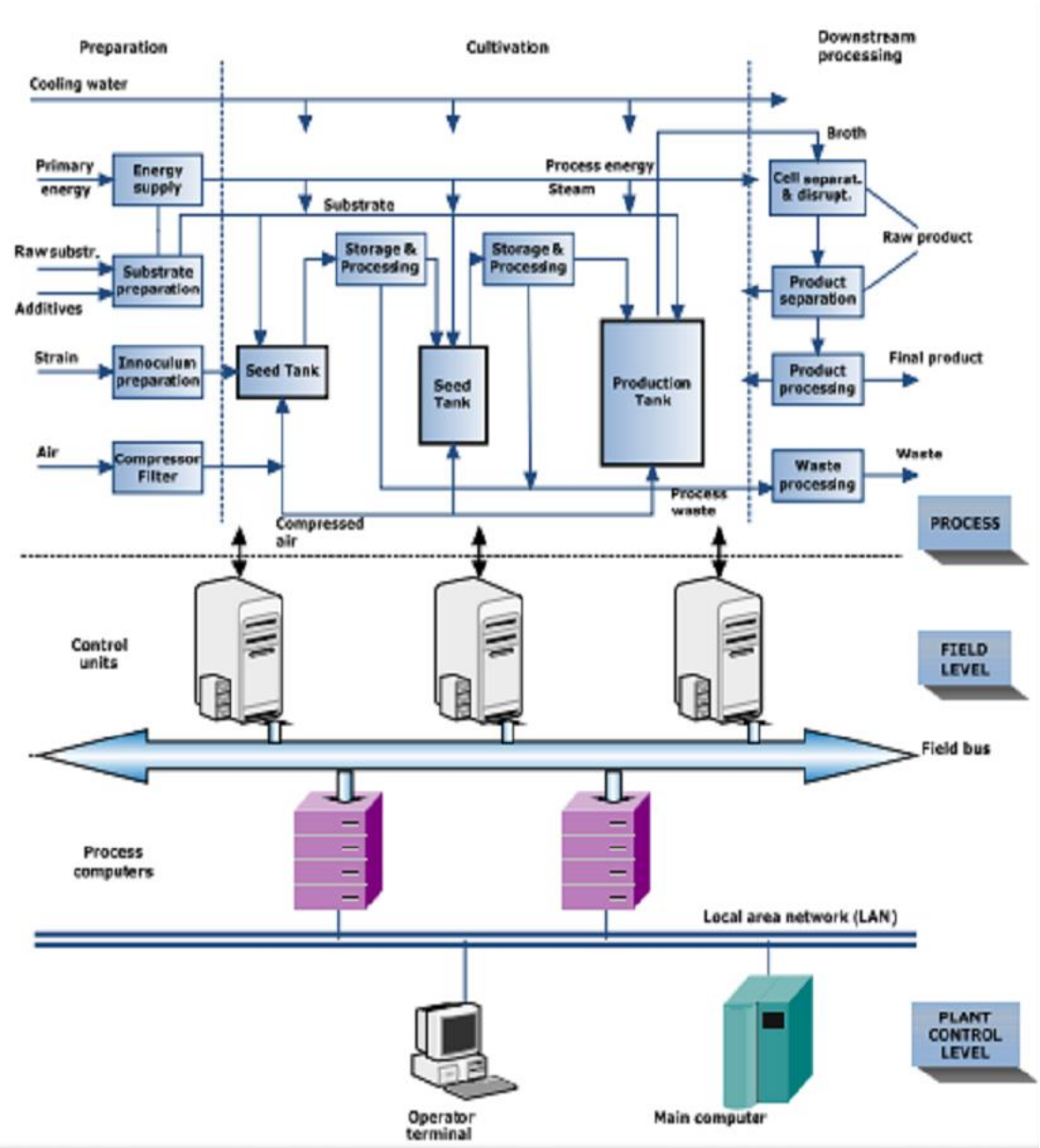
Logging of process data

Data logging is performed by the data acquisition system which has both hardware and software components. There is an interface between the sensor and the computer. The software should include the computer programme for sequencial scanning of the sensor signals and the procedure of data storage.

Data analysis Data reduction is performed by the data analysis system which is a computer programe based on a series of selected mathematical equation. The analysis information may be put on a print out, fed into a data bank or utilized for process control.

Process control

1. Process control is also performed using a computer programme. Signal from the computer are fed in to pumps , valves or switches via the interface. In addition the computer programme may contain instructions to display devices to include alarm.



2. At this poits it is necessary to be aware that there are two distinct fundamental approaches to computer control of fermenter.

The two distinct fundamental approaches are

Direct digital control (DDC)

Fermentor is under the direct control of the computer software

Supervisory set point control (SSC)

- It involves the use of independent controller to manage all control functions of a fermentor and the computer communications with the controller only to exchange information.
- All the controlling unit of fermenter such as temperature controller, pH, pressure, impeller speed, gas flow rates, liquid flow, dissolved O2 and Co2 controlling units with their sensor connected with software operation.
- After installation of the software an computer we can controlled all the controlling parameter of the instrument and fermentation process.

POSSIBLE QUESTIONS

TWO MARK QUESTIONS

1. What is In-line sensor?
2. Define Off-line sensor.
3. Comment on On-line sensor
4. How temperature is monitoring in fermenter?
5. What is the advantage of Thermistors?
6. What is the role of temperature controller in fermenter?
7. What is the significance of pH control?
8. State the Ion specific sensor.
9. Why oxygen required for bioprocess technology?
10. Make short notes on Mass spectrophotometer.
11. Write any three advantages of computer aided process control.
12. Define direct digital control.
13. Mention about supervisory set point control.
14. What is mass transfer coefficient?
15. Mention about the Mercury-In-Glass Thermometers.

EIGHT MARK QUESTIONS

1. Describe in detail about the control and monitoring of bioreactor using flow charts.
2. Explain in detail:
    i) Oxygen requirement for bioprocess technology.
    ii) Factors affecting the KLa.
3. Comment on:
    i) Types of sensor used in bioreactor
    ii) Role of electrical resistance thermometers in heat control of bioreactor
4. Explain in detail about the control of pressure in bioreactor.
5. Give a short note on control of bioreactor by computer aided process.

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SYLLABUS

Downstream processing: Filtration, Centrifugation, Cell disruption, Chromatography, liquid-liquid extraction, product recovery and purification. Effluent treatment- product recovery, sludge process, waste disposal.

DOWNSTREAM PROCESSING

- **Downstream processing** refers to the recovery and purification of biosynthetic products, particularly pharmaceuticals, from natural sources such as animal or plant tissue or fermentation broth, including the recycling of salvageable components and the proper treatment and disposal of waste.
- It is an essential step in the manufacture of pharmaceuticals such as antibiotics, hormones (e.g. insulin and human growth hormone), antibodies and vaccines; antibodies and enzymes used in diagnostics; industrial enzymes; and natural fragrance and flavor compounds.
- Downstream processing is usually considered a specialized field in biochemical engineering, itself a specialization within chemical engineering, though many of the key technologies were developed by chemists and biologists for laboratoryscale separation of biological products.
- Downstream processing and analytical bio-separation both refer to the separation or purification of biological products, but at different scales of operation and for different purposes. Downstream processing implies manufacture of a purified product fit for a specific use, generally in marketable quantities, while analytical bio-separation refers to purification for the sole purpose of measuring a component or components of a mixture, and may deal with sample sizes as small as a single cell.

FILTRATION

Filtration separates particles by forcing the fluid through a filtering medium on which solids are deposited. Filtration can be divided into several categories depending on the filtering medium used, the range of particle sizes removed, the pressure differences, and the principles

of the filtration, such as conventional filtration, microfiltration, ultra filtration, and reverse osmosis. In this section we limit our discussion on the conventional filtration which involves large particles. This technique is effective for dilute suspension of large and rigid particles.

Various factors will influence the choice of the most table type of equipment to meet the specified requirements at minimum overall cost, including:

I. The properties of the filtrate, particularly its viscosity and density.
II. The nature of the solid particles, particularly their size and shape, the size distribution and packing characteristics.
III. The solids: liquid ratio.
IV. The need for recovery of the solid or liquid fraction or both.
V. The scale of operation.
VI. The need for batch or continuous operation.
VII. The need for aseptic conditions.
VIII. The need for pressure or vacuum suction to ensure an adequate flow rate of the liquid.

The following filtration methods to be followed for filtering the content from fermentation broth.

Simple filter

Plate and frame filter

Pressure leaf filter

- vertical
- horizontal
- stacked disc filter Rotary vacuum filter

SIMPLE FILTER

A simple filtration consists of a support covered with a porous filter cloth. A filter cake gradually builds up as filtrate passes through the filter cloth. As the filter cake increases in thickness the resistance to flow will gradually increase. Thus, if the pressure applied to the surface of the slurry is kept constant the rate of flow will gradually diminish. Alternatively, if the flow rate is to be kept constant the pressure will gradually have to be increased. The flow rate may also be reduced by blocking of holes in the filter cloth and closure of voids between particles, if the particles are soft and compressible. When particles are compressible it may not be feasible to apply increased pressure.

Flow through a uniform and constant depth porous bed can be represented by the Darcy equation:

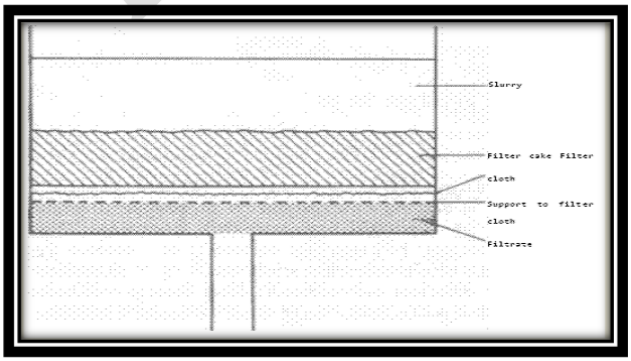


Plate and Frame Filters

A plate and frame filter is a pressure filter in which lie simplest form consists of plates and frames arranged alternately. The plates are covered with filter cloths or filter pads. The plates and frames it assembled on a horizontal framework and held ether by means of a hand screw or hydraulic ram so that there is no leakage between the plates and frames which form a series of liquid-tight compartments.

The slurry is fed to the filter frame through the continuous channel formed by the holes in the corners of the plates and frames. The filtrate passes through the filter doth or pad, runs down grooves in the filter plates and then discharged through outlet taps to a channel. Sometimes, if aseptic conditions are required, the out-lets may lead directly into a pipe.

The solids are reamed within the frame and filtration is stopped when lie frames are completely filled or when the flow of titrate becomes uneconomically low. On an industrial scale the plate and frame filter is one of the cheapest filters per unit of filtering space and requires the least floor space, but it is intermittent in operation (a batch process) and there may be considerable wear of filter cloths as a result of frequent dismantling.

This type of filter is most suitable for fermentation broths with a low solids content and low resistance to filtration. It is widely used as a 'polishing' device in breweries to filter out residual yeast cells following initial clarification by centrifugation or rotary vacuum filtration.

It may also be used for collecting high value solids that would not justify the use of a continuous filter. Because of high labour costs and the time involved in dismantling, cleaning and reassembly, these filters should not be used when removing large quantities of worthless solids from a broth.

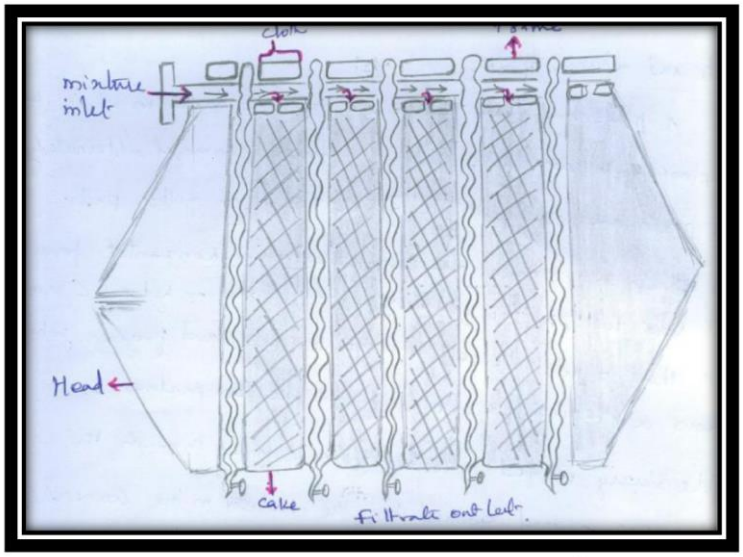


Plate and Frame Filters

Pressure Leaf Filters

- There are a number of intermittent batch filters usually called by their trade names. These filters incorporate a number of leaves, each consisting of a metal framework of

grooved plates which is covered with a fine wire mesh, or occasionally a filter cloth and often precoated with a layer of cellulose fibres.

- The process slurry is fed into the filter which is operated under pressure or by suction with a vacuum pump. Because the filters are totally enclosed it is possible to sterilize them with steam. This type of filter is particularly suitable for 'polishing' large volumes of liquids with low solids content or small batch filtrations of valuable solids.

(i) *Vertical metal-leaf filter*

- This filter consists of a number of vertical porous metal leaves mounted on a hollow shaft in a cylindrical pressure vessel. The solids from the slurry gradually build up on the surface of the leaves and the filtrate is removed from the plates via the horizontal hollow shaft.
- In some designs the hollow shaft can be slowly rotated during filtration. Solids are normally removed at the end of a cycle by blowing air through the shaft and into the filter leaves.

(ii) *Horizontal metal-leaf filter*

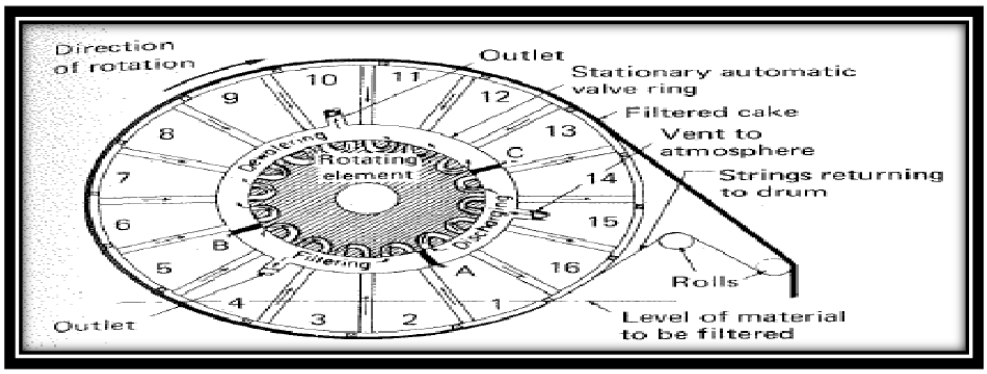
- In this filter the metal leaves are mounted on a vertical hollow shaft within a pressure vessel. Often, only the upper surfaces of the leaves are porous.
- Filtration is continued until the cake fills the space between the disc-shaped leaves or when the operational pressure has become excessive.
- At the end of a process cycle, the solid cake can be discharged by releasing the pressure and spinning the shaft with a drive motor.

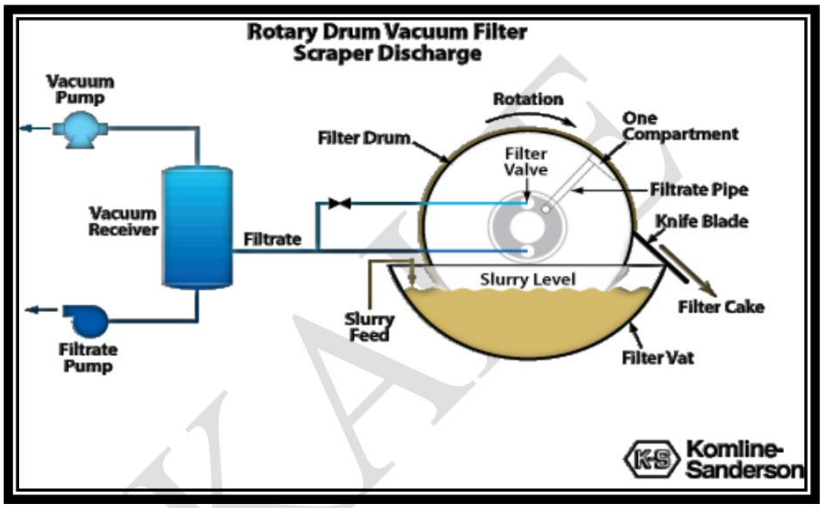
(iii) *Stacked-disc filter*

One kind of filter of this type is the Metafilter. This is a very robust device and because there is no filter cloth and the bed is easily replaced, labour costs are low. It consists of a number of precision-made rings which are stacked on a fluted rod. The rings

Rotary vacuum filter

Large rotary vacuum filter are commonly used by industries which produce large volumes of liquid which need continuous processing. The filter consists of a rotating hollow segmented drum covered with a fabric or metal filter whaich is partially immersed in a trough containing the broth to be filtered. The slurry is fed on to the outside of the revolving drum and vacuum pressure is applied internally so that the filtrate to a collecting vessels.





The rotary vacuum filter consists of three way of slurry discharge

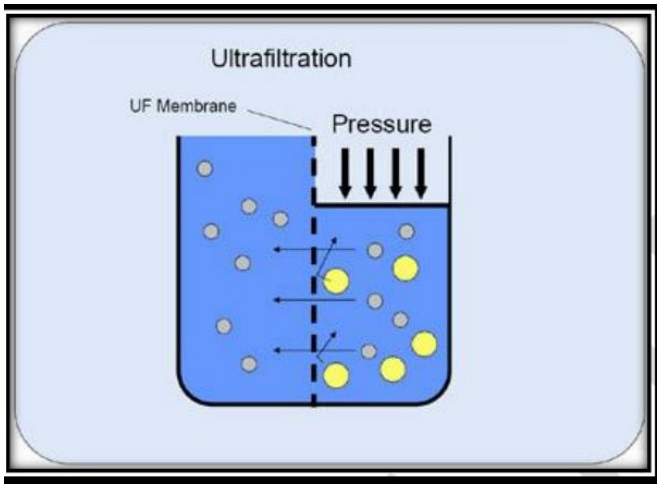
- String discharge
- Scarper discharge
- Scarper discharge with precoating of the drum.

Ultra Filtration

I. Ultrafiltration (UF) is a variety of membrane filtration in which forces like pressure or concentration gradients lead to a separation through a semipermeable membrane.

II. Suspended solids and solutes of high molecular weight are retained in the socalled retentate, while water and low molecular weight solutes pass through the membrane in the permeate.

III. This separation process is used in industry and research for purifying and concentrating macromolecular (103 - 106 Da) solutions, especially protein solutions.



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

CENTRIFUGATION

Microorganism and other similar sized particles can be removed from a broth by using a centrifuge when filtration is not a satisfactory separation method. Although a centrifuge may

be expensive when compared with a filter it may be essential when filtration is slow and difficult.

The cells or other suspended matter must be obtained free of filter aids. Continuous separation to a high standard of hygiene is required. Non- continuous centrifuges are of extremely limited capacity and therefore not suitable for large scale separation.

The centrifuges used in harvesting fermentation broths are all operated on a continuous or semi continuous basis.

Some centrifuge can be used for separating two immiscible liquids yielding a heavy phase and light phase liquid as well as a solids fraction.

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

$$V_g = \frac{d^2 g\ (\rho_P - \rho_L)}{18\mu}$$

where V_g = rate of sedimentation (m s^{-1})
d = particle diameter (m)
g = gravitational constant (m s^{-2})
ρ_P = particle density (kg m^{-3})
ρ_L = liquid density (kg m^{-3})
μ = viscosity (kg m^{-1} s^{-1})

This equation can then be modified for sedimentation in a centrifuge:

$$V_c = \frac{d\omega^2 r\ (\rho_P - \rho_L)}{18\mu}$$

When filtration is not a satisfactory separation method, a centrifuge can be used to remove microorganisms and other similar size particles, from a broth. It's more expensive, comparing with a filter, but may be essential when filtration is slow and difficult.

Centrifugation efficiency is favoured by:

- large particle diameter of cells

- large density difference between cell and liquid
- the liquid should have a low viscosity In practice, particles of biological material are often small and of low density, which fermentation broths is often viscous, and high density.

It requires:

- high angular velocity
- large radius(of centrifuge)
- large volume ϖ thin sedimentation layer

Centrifugation is a process that involves the use of the centrifugal force for the sedimentation of heterogeneous mixtures with a centrifuge, used in industry and in laboratory settings. This process is used to separate two immiscible liquids.

Centrifugation is used to separate materials of different density when a force greater than gravity is desired.

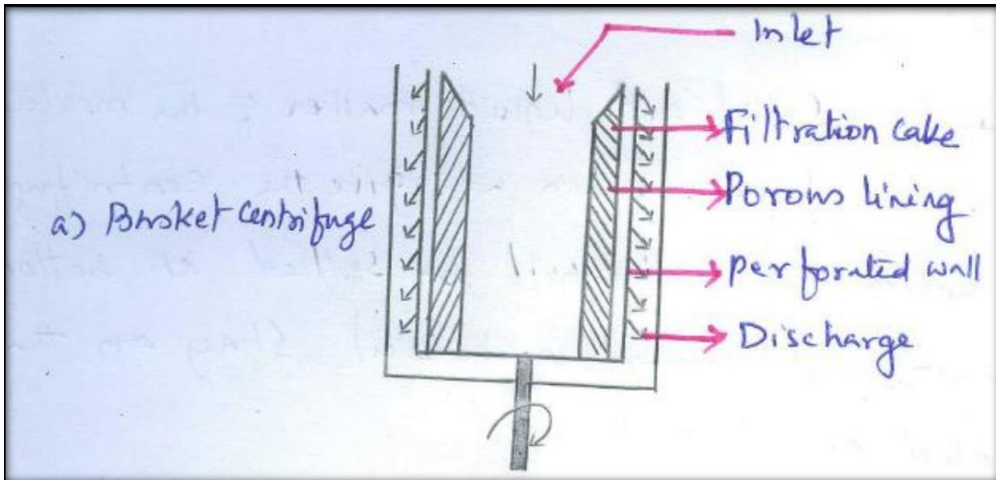
The type of industrial centrifugation unit:

Separate the solid and liquid matter of the mixture using centrifugal force. When we give the centrifugal force, the solid material will be settled down at the bottom of the container and liquid material stay on the above solid phase (pellet).

Basket centrifuge

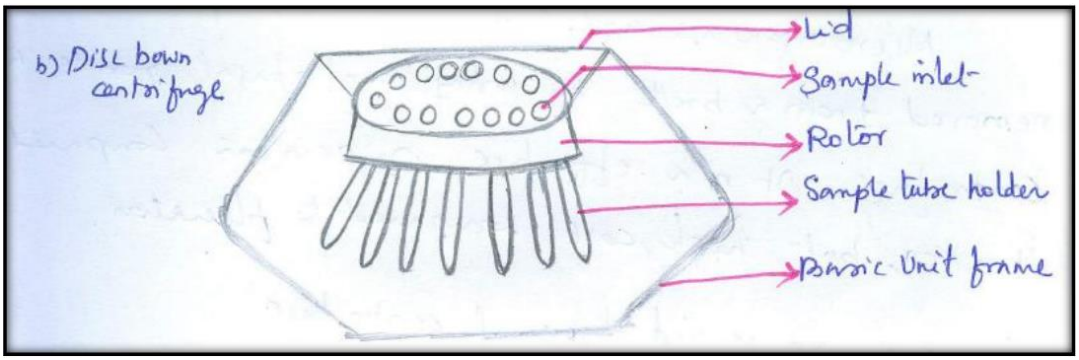
- Speed limit up to 4000 rpm
- Simple centrifugation process using this can remove the course particles from the fermented media.
- Mainly used for separation of mould mycelia and crystalline compounds.
- The centrifuge is most commonly used with a perforated bowl lined with a filter bag of nylon, cotton etc.,
- A continuous feed is used and when the basket is filled with the filter cake, is is possible to wash the cake before removing it.

- The basket centrifuge may be considered to be a centrifugal filter.



Disc bowl centrifuge Disc-stack bowl centrifuge.

This type is common in bioprocess. The developed forces is 5000-15000 G with minimal density difference between solid and liquid is 0.01 -0.03 kg/m3. The minimum particle diameter is 5 µm.



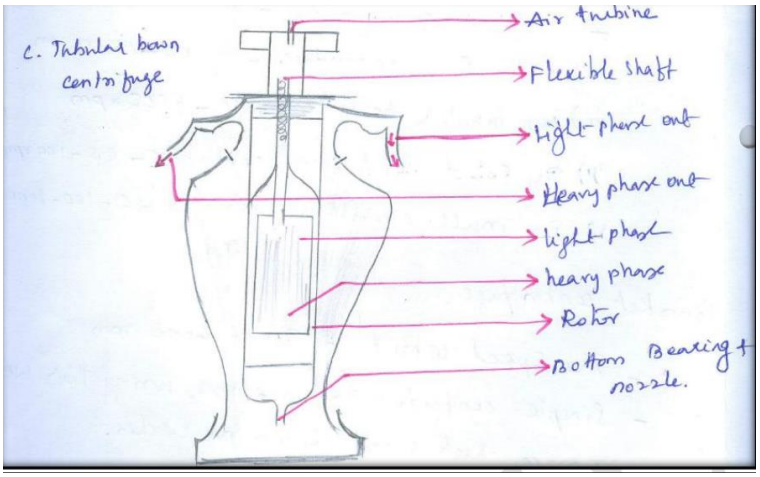
The tubular bowl centrifuge

Hazards to the enzyme are aeration and the consequent foaming of the clarified solution, which aerosol formation may be a hazard to the user. This occurs because of turbulence in the bowl.

Very high angular velocity ◊turbulence ◊foaming & aerosol

Tubular bowl centrifuge (Narrow tubular bowl centrifuge or ultracentrifuge, decanter centrifuge, etc). Simple and widely applied in food and pharmaceutical industry. Operates at 13000-16000 G, 105-106 G for ultracentrifuge

- This is a centrifuge to consider using for particle size ranges of 0.1 to 200µm and up to 10% solids in the ingoing slurry.
- The main components of the centrifuge is a cylindrical bowl (rotor) which may be a variable design depending on application suspended by a flexible shaft, driven by an overhead motor or air turbine
- The centrifuge may be altered
- Light phase / heavy phase and liquid phase separation.



The solid bowl scroll centrifuge

They used for continuously handling coarse material (such as sewage sludge. They're few hazards to enzymes.)

The multi chamber centrifuge They have large radius, low angular velocity, thus sedimentation occurs with high efficiency over large surface area. They're widely used for Baker's yeast. Some heating(because the bowl is located above the gearbox) represents a danger to the enzyme.

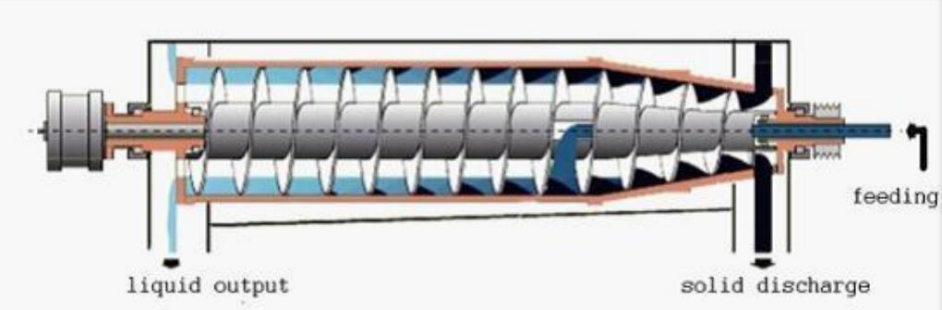
Wastewater Treatment Decanter Centrifuge

• Whole waste water treatment includes sludge feeding system, flocculent adding system, dewatering system, filter cake delivery system and PLC control system.

• The main equipment of sludge dewatering complete system is decanter centrifuge. There are basically two application types in the wastewater treatment industry for the decanter centrifuge: sludge thickening and sludge dewatering duties. Also it can be used for Sludge classification and Sludge clarification. With the decanter centrifuge, it is possible to separate the particles from suspending liquid with equivalent diameter bigger than 0.003mm,solid-liquid weight ratio less than 10% or volume ratio less than 70%,difference in specific gravity bigger than 0.05g/cm3

• Sludge dewatering complete system could be used in the following fields:

• Municipal sludge treatment, paper mill sludge, starch factory waste water treatment, steel mill sludge, printing and dyeing mill sludge, water works sludge, pharmaceutical waste sludge, PVC sludge, sewage de-sulfurization sludge, grain spillage, power plant ash sludge, dairy sludge, beer sludge, drilling fluid ,electroplating liquid, fermented liquid manure, oil refining sludge, soap sludge, leather sludge and etc.



CELL DISRUPTION

Cell distribution methods for intracellular products

The importance of microorganisms as a source of commercially useful chemicals, antibiotics and enzymes has been recognized for a very long time. Nearly all chemicals of microbial origin produced industrially today are of the extracellular type. That is, they are produced

within the microbial cell, but are then excreted into the surrounding environment. A much larger proportion of the potentially useful microbial products is retained within the cells. A vast majority of the enzymes known, for example, are intracellular. Even greater use of microbial products, many of which will be intracellular, can be expected from the predicted surge in biotechnology. The isolation of intracellular material requires that the cell either be genetically engineered so that what would normally be an intracellular product is excreted into the environment, or it must be disintegrated by physical, chemical, or enzymatic means to release its contents into the surrounding medium.

CELL DISRUPTION METHODS Microorganisms are more robust than is generally believed. The resistance to disruption of microorganisms has been referred to by Wimpenny. He points out that the internal pressure due to osmosis inside an organism such as *Micrococcus lysodeikticus or Sarcina lutea* is about 20 atmospheres and that the structures responsible for resisting this pressure are about as strong, weight for weight, as reinforced concrete. A variety of disruption methods are available to disintegrate these strong cellular walls and membranes and liberate the cell contents. A useful classification of the cell disruption methods were shown below. Only some, mainly mechanical, cell disruption methods have found industrial application.

Mechanical cell disruption

Both solid shear (e.g. bead mill) and liquid shear (e.g. high pressure homogenizer) based methods of cell disruption have proven successful on a large scale. The solid shear methods may involve either a grinding action as in a ball mill or may involve extrusion of frozen cells, either alone or as a cell-ice (or other abrasive) mixture, through narrow gaps or orifices under high pressure. Most of the ceil disruption equipment in current use was originally designed for the homogenization and size reduction of very different commercial products such as milk and paint.

Liquid shear methods

The high-pressure homogenizer. Among the liquid shear disruption devices, the highpressure Manton-Gaulin APV type homogenizer is probably the most widely used. The technical

feasibility of this system for cell disruption was demonstrated by Dunnill and Lilly and their coworkers and the use of this equipment has been further reported. The high-pressure homogenizer consists of a positive displacement piston pump with one or more plungers. The cell suspension is drawn through a check valve into the pump cylinder and, on the pressure stroke, is forced through an adjustable discharge valve with restricted orifice.

Ultrasonication

Ultrasonication is another liquid-shear method of disruption which has received some attention in the literature, Ultrasound, sound of frequency higher than 15-20 kHz which is inaudible to the human ear, is known to cause both inactivation and, at higher acoustic power inputs, disruption of microbial cells in suspension.

The bead mill

Cell disruption in bead mills is regarded as one of the most efficient techniques for physical cell disruption. Various designs of bead mills have been used for microbial cell disruption. These mills consist of either a vertical or a horizontal cylindrical chamber with a motor-driven central shaft supporting a collection of off centered discs or other agitating element. The chamber is filled to the desired level with steel or balloting glass beads which provide the grinding action. The charge of grinding beads is retained in the chamber by a sieve-plate covering the bottom inlet in vertical machines, while in horizontal units the fluid entry is above the level of the beads in the chamber and no retention mechanism is required. At the fluid exit port, three different types of bead retention systems have been employed: a sieve-plate, a disc rotating in very close proximity to a plate with a central exit port in its and a vibrating slot. The latter two types of bead retention devices are said to reduce fouling problems. The horizontal configuration of the mill is known to give a better efficiency of disruption relative to the vertical one. This is because the upward fluid flow in vertical machines tends to fluidize the grinding beads to some degree, thereby reducing grinding efficiency.

The freeze-press

Freeze-pressing of microbial cell suspensions can be used to disrupt the cells. Examples of the freeze-pressing equipment include the Hughes press in which a frozen paste of cells is forced through a narrow slit or orifice, either in the presence of an abrasive at temperatures just below zero or without the abrasive at temperatures of about -25°C. In the latter case, phase and consequent volume changes of ice contribute to disruption. In addition, solid shear due to crystalline ice is important. According to Wimpenny, cell breakage in the Hughes press yields cell wall membrane preparations that are relatively intact and may be a good method for isolation of membraneassociated enzymes. A semi continuous design of X-Press freeze-pressing equipment capable of handling about 10 kg material every hour has been studied. For a similar machine, a report suggests that the device can be scaled up almost unlimitedly. The thermodynamics of compression, and rheology in such devices have received theoretical treatment in a number of papers. We are not aware of any industrial freezepressing equipment commercially available or in use. The reader is referred to other papers for information on this technique.

Foam separation and control

- Foam separation is a chemical process which falls into a category of separation techniques called "Adsorptive bubble separation methods". It is further divided into froth flotation and foam fractionation.
- Foam is a type of colloidal dispersion where gas is dispersed throughout a liquid phase. The liquid phase is also called the continuous phase because it is an uninterrupted, unlike the gas phase.
- Foam is produced during most microbial fermentations. Foaming may occur either due to a medium component, e.g., protein present in the medium, or due to some compound produced by the microorganism. Proteins are present in cornsteep liquor, pharma media, peanut meal, soybean meal, etc.
- These proteins may denature at the air-broth interface and form a protein film that does not rupture readily. Foaming can cause removal of cells from the medium; such cell wills undergo autolysis and release more proteins into the medium.

This, in turn, will further stabilize the foam.

Five different patterns of foaming are recognized; these are listed below.

1. Foaming remains at a constant level throughout the fermentation. Initial foaming is due to the medium, but later microbial activity contributes to it.
2. Foaming declines steadily in the initial stages, but remains constant thereafter. This type of foaming is due to the medium.
3. The foaming increases after a slight initial fall', in this case, microbial activity is the major cause of foaming.
4. The foaming level increases with fermentation duration; such foaming pattern is solely due to microbial activity.
5. A complex foaming pattern that combines features of two or more of the above patterns.

Foaming may lead to several physical and biological problems.

Some examples of physical problems are as follows:

(1) The working volume of the fermenter may decrease due to a circulation of oxygendepleted gas bubbles in the system.
(2) The bubble size may also decrease, and
(3) The heat and mass transfer rates may also decline.
(4) Foaming may interfere with the functioning of sensing electrodes resulting in invalid process data, and incorrect monitoring and control of pH, temperature, etc.

The biological problems of foaming include

(1) deposition of cells in the upper parts of the fermenter,
(2) problems of sterile operation as the air filter exits of the fermenter become wet, and
(3) increased risk of contamination. In addition,
(4) there may be product loss due to siphoning of the culture broth.

Whenever excessive foaming occurs, the following approaches may be used to resolve the problem:

(1) A defined medium may be used to avoid foam formation. This may be combined with modifications in physical parameters like pH, temperature, aeration and agitation. This approach will be successful in such cases where medium is the main culprit, but will fail whenever microbial activity is the main contributor.

(2) Often the foam may be unavoidable; in such case, antifoam should be used. This is the most standard approach to combat foaming.

(3) A mechanical foam breaker may also be used. Antifoams are surface active agents; they reduce surface tension in the foams and destabilize protein film by the following effects:

   (a) hydrophobic bridges between two surfaces,

   (b) displacement of the absorbed protein, and

   (c) rapid spreading of the surface film.

- Several compounds meet and have been found to be suitable for different fermentation processes; these compounds are as follows: alcohols (stearyl and octyl decanol), esters, fatty acids and their derivatives (especially, triglycerides like cottonseed oil, linseed oil, soybean oil, sunflower oil, etc.), silicones, sulphonates, and miscellaneous compounds like oxaline, Alkaterge C, and polypropylene glycol.
- Many of the antifoams are of low solubility; therefore, they are added with a carrier like lard oil, liquid paraffin and castor oil. There carriers, however, may be metabolized, and they may affect the fermentation process. Further, many antifoams would reduce oxygen transfer by up to 50% when used at effective concentrations.
- Antifoams are generally added when foaming occurs during fermentation. But foam control in fermentation industry is still an empirical art. Therefore, the best method of foam control for a particular process in one factory is not necessarily the best for the same process in other factories. Further, the design and operating parameters of the fermenters may affect the properties and the foams produced during the fermentation process.

CHROMATOGRAPHY

In many fermentation processes, chromatographic techniques are used to isolate and purify relatively low concentrations of metabolic products. In this context, chromatography will be

concerned with the passage and separation of different solutes as liquid is passed through a column, i.e. *liquid chromatography.* Depending on the mechanism by which the solutes may be differentially held in a column, the techniques can be grouped as follows:

(a) Adsorption chromatography.
(b) Ion-exchange chromatography.
(c) Gel permeation chromatography.
(d) .Affinity chromatography.
(e) Reverse phase chromatography.
(f) High performance liquid chromatography.

Adsorption chromatography

- Adsorption chromatography involves binding of the solute to the solid phase primarily by weak Van de Waals forces. The materials used for this purpose to pack columns include inorganic adsorbants (active carbon, aluminium oxide, aluminium hydroxide, magnesium oxide, silica gel) and organic macro-porous resins.
- Adsorption and affinity chromatography are mechanistically identical, but are strategically different. In affinity systems selectivity is designed rationally whilst in adsorption selectivity must be determined empirically.
- Di-hydro-streptomycin can be extracted from filtrates using activated charcoal columns. It is then eluted with methanolic hydrochloric acid and purified in further stages. Some other applications for small-scale antibiotic purification are quoted by Weinstein and Wagman (1978). Active carbon may be used to remove pigments to clarify broths. Penicillin-containing solvents may be treated with 0.25 to 0.5% active carbon to remove pigments and other impurities.
- Macro-porous adsorbents have also been tested. The first synthetic organic macroporous adsorbents, the Amberlite XAD resins, were produced by Rohm and Haas in 1965. These resins have surface polarities which vary from non-polar to highly polar and do not possess any ionic functional groups.

Ion exchange

- Ion exchange can be defined as the reversible exchange of ions between a liquid phase and a solid phase (ion-exchange resin) which is not accompanied by any radical change in the solid structure. Cationic ion-exchange resins normally contain a suiphonic acid, carboxylic acid or phosphonic acid active group: Carboxy- methyl cellulose is a common cation exchange resin.
- Positively charged solutes (e.g. certain proteins) will bind to the resin, the strength of attachment de pending on the net charge of the solute at the pH of the column feed. After deposition solutes are sequentially washed off by the passage of buffers of increasing ionic strength or pH. Anionic ion-exchange resins normally contain a secondary amine, quaternary amine or quaternary ammonium active group.
- A common anion exchange resin, DEAE (diethylaminoethyl) cellulose is used in a similar manner to that described above for the separation of negatively charged solutes. Other functional groups may also be attached to the resin skeleton to provide more selective behaviour similar to that of affinity chromatography. ϖ The appropriate resin for a particular purpose will depend on various factors such as bead size, pore size, diffusion rate, resin capacity, range of reactive groups and the life of the resin before replacement is necessary. Weak-acid cation ion-exchange resins can be used in the isolation and purification of streptomycin, neomycin and similar antibiotics.

Gel permeation

This technique is also known as gel exclusion and gel filtration. Gel permeation separates molecules on the basis of their size. The smaller molecules diffuse into the gel more rapidly than the larger ones, and penetrate the pores of the gel to a greater degree. This means that once elution is started, the larger molecules which are still in the voids in the gel will be eluted first.

A wide range of gels are available, including cross-linked dextrans (Sephadex and Sephacryl) and cross-linked agarose (Sepharose) with various pore sizes depending on the fractionation range required.

One early industrial application, although on a relatively small scale, was the purification of vaccines. Tetanus and diphtheria broths for batches of up to 100,000 human doses are passed through a 13 dm3 column of G 100 followed by a 13 dm3 column of G 200. This technique yields a fairly pure fraction which is then concentrated ten-fold by pressure dialysis to remove the eluant buffer (Na2HP04).

Affinity chromatography

Affinity chromatography is a separation technique with many applications since it is possible to use it for separation and purification of most biological molecules on the basis of their function or chemical structure.

This technique depends on the highly specific interactions between pairs of biological materials such as enzyme-substrate, enzyme-inhibitor, antigen-antibody, etc.

The molecule to be purified is specifically adsorbed from, for example, a cell lysate applied to the affinity column by a binding substance (ligand) which is immobilized on an insoluble support (matrix). Eluent is then passed 'through the column to release the highly purified and concentrated molecule.

The ligand is attached to the matrix by physical absorption or chemically by a covalent bond. The pore size and ligand location must be carefully matched to the size of the product for effective separation. The latter method is preferred whenever possible.

Coupling procedures have been developed using cyanogen bromide, bisoxiranes, disaziridines and periodates, for matrixes of gels and beads. Four polymers which are often used for matrix materials are agarose, cellulose, dextrose and polyacrylamide. Agarose activated with cyanogen bromide is one of the most commonly used supports for the coupling of amino ligands.

Silica based solid phases have been shown to be an effective alternative to gel supports in affinity chromatography.

Purification may be several thousand-fold with good recovery of active material. The method can however be quite costly and time consuming, and alternative affinity methods such as affinity cross-flow filtration, affinity precipitation and affinity partitioning may offer some advantages.

Affinity chromatography was used initially in protein isolation and purification, particularly enzymes. Since then many other large-scale applications have been developed for enzyme inhibitors, antibodies, interferon and recombinant proteins and on a smaller scale for nucleic acids, cell organelles and whole cells. In the scale-up of affinity chromatographic processes bed height limits the superficial velocity of the liquid, thus scale-up requires.

Reverse phase chromatography (RPC)

This chromatographic method utilizes a solid phase (e.g. silica) which is modified so as to replace hydrophilic groups with hydrophobic alkyl chains. This allows the separation of proteins according to their hydrophobicity.

More-hydrophobic proteins bind most strongly to the stationary phase and are therefore eluted later than less-hydrophobic proteins.

The alkyl groupings are normally eight or eighteen carbons in length (C, and CIS)' RPC can also be combined with affinity techniques in the separation of, for example, proteins and peptides.

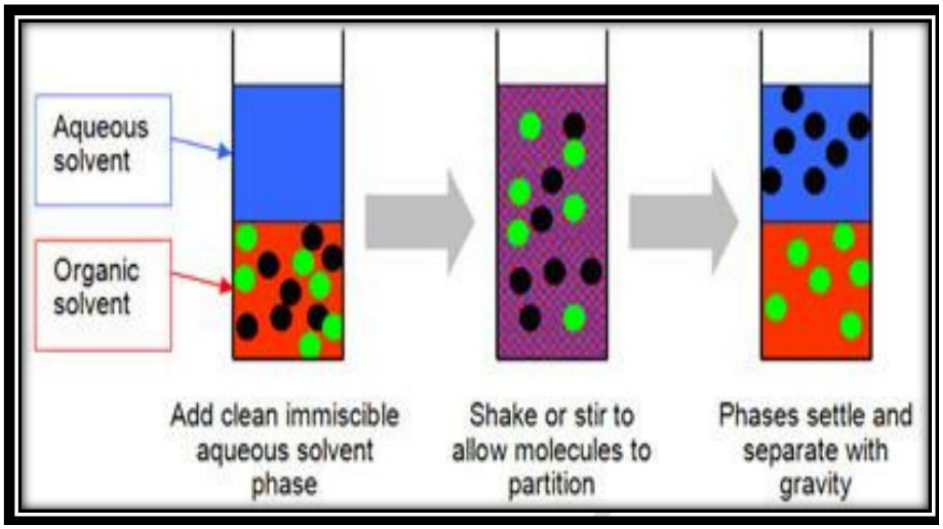
Chromatographic techniques are also used in the final stages of purification of a number of products. The scale-up of chromatographic processes can prove difficult, and there is much current interest in the use of mathematical models and computer programmes to translate data obtained from small-scale processes into operating conditions for larger scale applications.

LIQUID-LIQUID EXTRACTION

1. Liquid–liquid extraction (LLE) consists in transferring one (or more) solute(s) contained in a feed solution to another immiscible liquid (solvent). The solvent that is enriched in

solute(s) is called extract. The feed solution that is depleted in solute(s) is called raffinate.

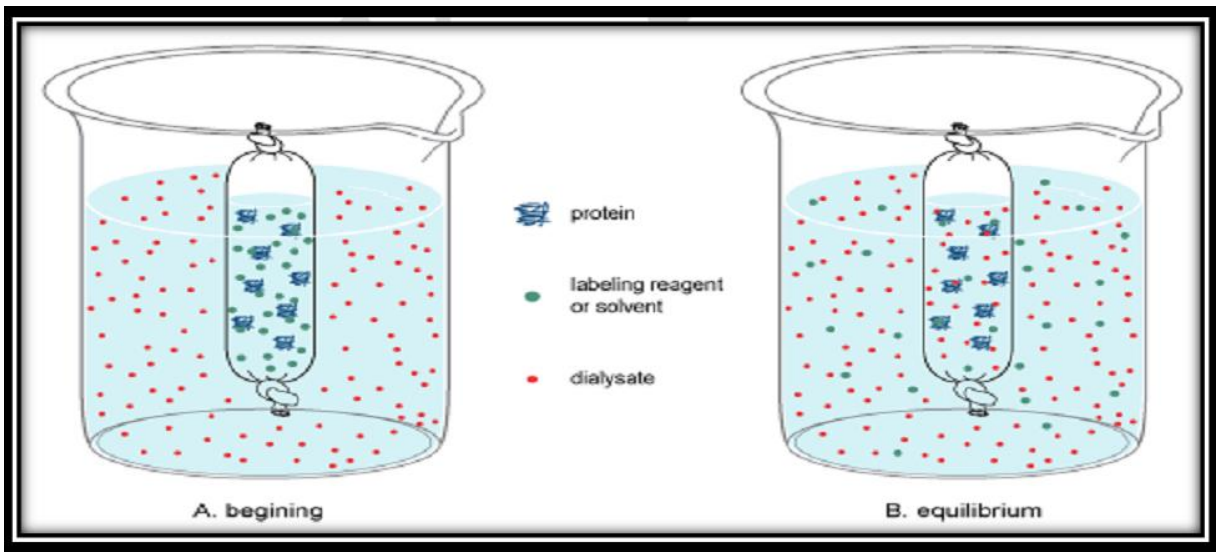
2. Liquid–liquid extraction also known as solvent extraction and partitioning, is a method to separate compounds based on their relative solubility in two different immiscible liquids, usually water and an organic solvent. It is an extraction of a substance from one liquid into another liquid phase.
3. Liquid–liquid extraction is a basic technique in chemical laboratories, where it is performed using a variety of apparatus, from separator funnels to countercurrent distribution equipment. This type of process is commonly performed after a chemical reaction as part of the work-up.
4. The term partitioning is commonly used to refer to the underlying chemical and physical processes involved in liquid–liquid extraction, but on another reading may be fully synonymous with it. The term solvent extraction can also refer to the separation of a substance from a mixture by preferentially dissolving that substance in a suitable solvent. In that case, a soluble compound is separated from an insoluble compound or a complex matrix.
5. Solvent extraction is used in nuclear reprocessing, ore processing, the production of fine organic compounds, the processing of perfumes, the production of vegetable oils and biodiesel, and other industries.
6. Liquid–liquid extraction is possible in non-aqueous systems: In a system consisting of a molten metal in contact with molten salts, metals can be extracted from one phase to the other. This is related to a mercury electrode where a metal can be reduced, the metal will often then dissolve in the mercury to form an amalgam that modifies its electrochemistry greatly.
7. For example, it is possible for sodium cations to be reduced at a mercury cathode to form sodium amalgam, while at an inert electrode (such as platinum) the sodium cations are not reduced. Instead, water is reduced to hydrogen. A detergent or fine solid can be used to stabilize an emulsion, or third phase.



PRODUCT RECOVERY AND PURIFICATION

Dialysis

In biochemistry, dialysis is the process of separating molecules in solution by the difference in their rates of diffusion through a semi permeable membrane, such as dialysis tubing.



The most common application of dialysis is for the removal of unwanted small molecules such as salts, reducing agents, or dyes from larger macromolecules such as proteins, DNA, or polysaccharides. Dialysis is also commonly used for buffer exchange and drug binding.

Equipment

Separating molecules in a solution by dialysis is a straightforward process. Other than the sample and dialysate buffer, all that is typically needed is:

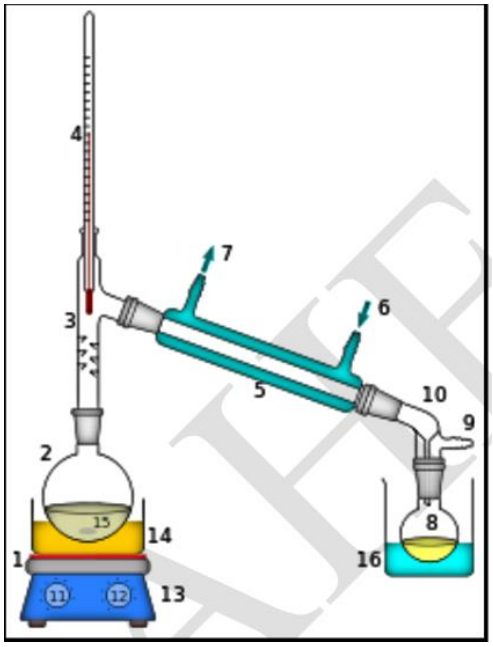
- Dialysis membrane in an appropriate format (e.g., tubing, cassette, etc.) and molecular weight cut-off (MWCO)
- A container to hold the dialysate buffer
- The ability to stir the solutions and control the temperature (optional) General Protocol

A typical dialysis procedure for protein samples is as follows:

- Prepare the membrane according to instructions
- Load the sample into dialysis tubing, cassette or device
- Place sample into an external chamber of dialysis buffer (with gentle stirring of the buffer)
- Dialyze for 2 hours (at room temperature or 4 °C)
- Change the dialysis buffer and dialyze for another 2 hours
- Change the dialysis buffer and dialyze for 2 hours or overnight

Distillation

Distillation is a process of separating the component substances from a liquid mixture by selective evaporation and condensation. Distillation may result in essentially complete separation (nearly pure components), or it may be a partial separation that increases the concentration of selected components of the mixture. In either case the process exploits differences in the volatility of mixture's components. In industrial chemistry, distillation is a unit operation of practically universal importance, but it is a physical separation process and not a chemical reaction.



Batch distillation

Heating an ideal mixture of two volatile substances A and B (with A having the higher volatility, or lower boiling point) in a batch distillation setup (such as in an apparatus depicted in the opening figure) until the mixture is boiling results in a vapor above the liquid which contains a mixture of A and B. The ratio between A and B in the vapor will be different from the ratio in the liquid: the ratio in the liquid will be determined by how the original mixture was prepared, while the ratio in the vapor will be enriched in the more volatile compound, A. The vapor goes through the condenser and is removed from the system. This in turn means that the ratio of compounds in the remaining liquid is now different from the initial ratio (i.e., more enriched in B than the starting liquid).

The result is that the ratio in the liquid mixture is changing, becoming richer in component B. This causes the boiling point of the mixture to rise, which in turn results in a rise in the temperature in the vapor, which results in a changing ratio of A : B in the gas phase (as distillation continues, there is an increasing proportion of B in the gas phase). This results in a slowly changing ratio A : B in the distillate.

If the difference in vapor pressure between the two components A and B is large (generally expressed as the difference in boiling points), the mixture in the beginning of the distillation is highly enriched in component A, and when component A has distilled off, the boiling liquid is enriched in component B.

Continuous distillation

Continuous distillation is an ongoing distillation in which a liquid mixture is continuously (without interruption) fed into the process and separated fractions are removed continuously as output streams occur over time during the operation.

ontinuous distillation produces a minimum of two output fractions, including at least one volatile distillate fraction, which has boiled and been separately captured as a vapor, and then condensed to a liquid. There is always a bottoms (or residue) fraction, which is the least volatile residue that has not been separately captured as a condensed vapor.

Continuous distillation differs from batch distillation in the respect that concentrations should not change over time. Continuous distillation can be run at a steady state for an arbitrary amount of time.

For any source material of specific composition, the main variables that affect the purity of products in continuous distillation are the reflux ratio and the number of theoretical equilibrium stages, in practice determined by the number of trays or the height of packing. Reflux is a flow from the condenser back to the column, which generates a recycle that allows a better separation with a given number of trays.

Equilibrium stages are ideal steps where compositions achieve vapor–liquid equilibrium, repeating the separation process and allowing better separation given a reflux ratio. A column with a high reflux ratio may have fewer stages, but it refluxes a large amount of liquid, giving a wide column with a large holdup.

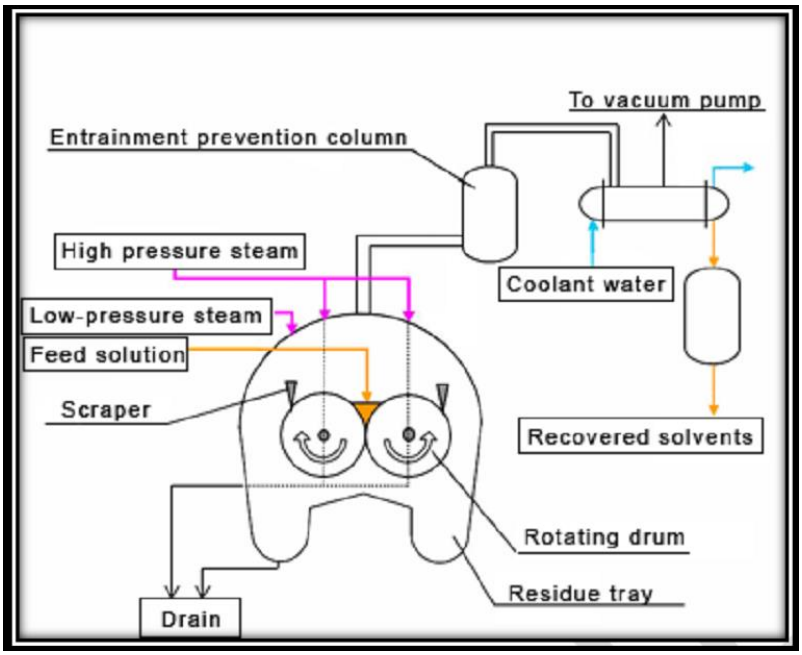
Conversely, a column with a low reflux ratio must have a large number of stages, thus requiring a taller column.

Drying

Drum drying

Drum drying is a method used for drying out liquids from raw materials with drying drum. In the drum-drying process, pureed raw ingredients are dried at relatively low temperatures over rotating, high-capacity drums that produce sheets of drum-dried product. This product is milled to a finished flake or powder form. Modern drum drying techniques results in dried ingredients which reconstitute immediately and retain much of their original flavor, color and nutritional value.

Some advantages of drum drying include the ability of drum dryers to dry viscous foods which cannot be easily dried with other methods. Drum dryers can be clean and hygienic and easy to operate and maintain.



EFFLUENT TREATMENT

- Utilization of medium or substrate in the fermentation process is responsible for the product formation. Based on type of the fermentation process and product formed and the medium used, the range of waste materials are produced.

- Type of the treatment to be applied for effluent depends upon various factors like physical state of the effluent, degree of hazard of pollutant, chemical complexity of the effluent and so on.
- The simple way of disposing the waste produced is just to dispose them no nearby water-bodies or to convenient open land. But, now it has become almost impossible to practice that measure due to increased population, increased industrial development, awareness on damages caused by pollution made industries in order to treat them prior to their disposal into environment.
- Concerned company need to install a effluent treatment plant in its premises, if company doesn't find a way to dispose the waste or if degree of composition is not suitable for the treatment by the nearby sewage treatment bodies.
- Effluents of fermentation do not contain toxic materials that affect the life around their disposal area. But unfortunately, effluents of fermentation now-a-days do contain many toxicants that affect the flora and fauna by reacting with the microbes and drastically decreasing the dissolved oxygen levels of that area.
- In various ways the effluent can be treated viz. by oxidative ponds (lagoons), spray irrigation, well disposal, and incineration etc. physical treatment process of sewage is done by filtration and sedimentation, chemical treatment process done by flocculation, coagulation, aggregation methods which are done by addition of various flocculants and coagulants like alum, ferric sulphide, calcium hydroxide etc. finally with biological treatment trickling filters, biologically aerated filters, rotating biological contractors, activated sludge process come with aerobic way and anaerobic digesters, anaerobic filters, up-flow anaerobic sludge blankets come with anaerobic treatment processes.

PRODUCT RECOVERY

- Recovery of extracellular proteins is from the clarified medium, whereas disrupted cell preparations are used both for intracellular proteins and those held within the periplasmic space.

- Some recombinant proteins expressed at high levels sometimes form inclusion bodies that are released by cell breakage. Following cell disruption, soluble proteins are separated from the cell debris by centrifugation.
- The resultant supernatant containing the proteins is then processed in a similar way to growth medium containing excreted proteins. Several methods are available for this process, viz., microfiltration and ultra filtration.

SLUDGE PROCESS AND WASTE DISPOSAL.

Sludge digestion involves the treatment of highly concentrated organic wastes in the absence of oxygen by anaerobic bacteria. The anaerobic treatment of organic wastes resulting in the production of carbon dioxide and methane, involves two distinct stages.

In the first stage, referred to as "***acid fermentation***", complex waste components, including fats, proteins, and polysaccharides are first hydrolyzed by a heterogeneous group of facultative and anaerobic bacteria. These bacteria then subject the products of hydrolysis to fermentations, b-oxidations, and other metabolic processes leading to the formation of simple organic compounds, mainly short-chain (volatile) acids and alcohols. However in the second stage, referred to as "***methane fermentation***", the end products of the first stage are converted to gases (mainly methane and carbon dioxide) by several different species of strictly anaerobic bacteria.

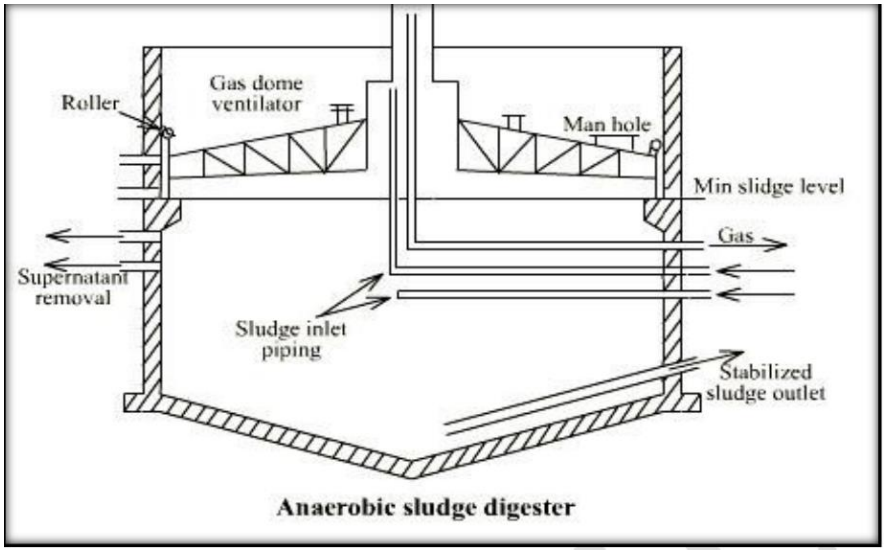
The bacteria responsible for acid fermentation are relatively tolerant to changes in pH and temperature and have a much higher rate of growth than the bacteria responsible for methane fermentation. If the pH drops below 6.0, methane formation essentially ceases, and more acid accumulates, thus bringing the digestion process to a standstill. As a result, methane fermentation is generally assumed to be the rate limiting step in anaerobic wastewater treatment. The methane bacteria are highly active in mesophilic (27-43°C) with digestion period of four weeks and thermophilic range (35- 40°C) with digestion period of 15-18 days. But thermophilic range is not practiced because of odour and operational difficulties.

Digestion Tanks or Digesters

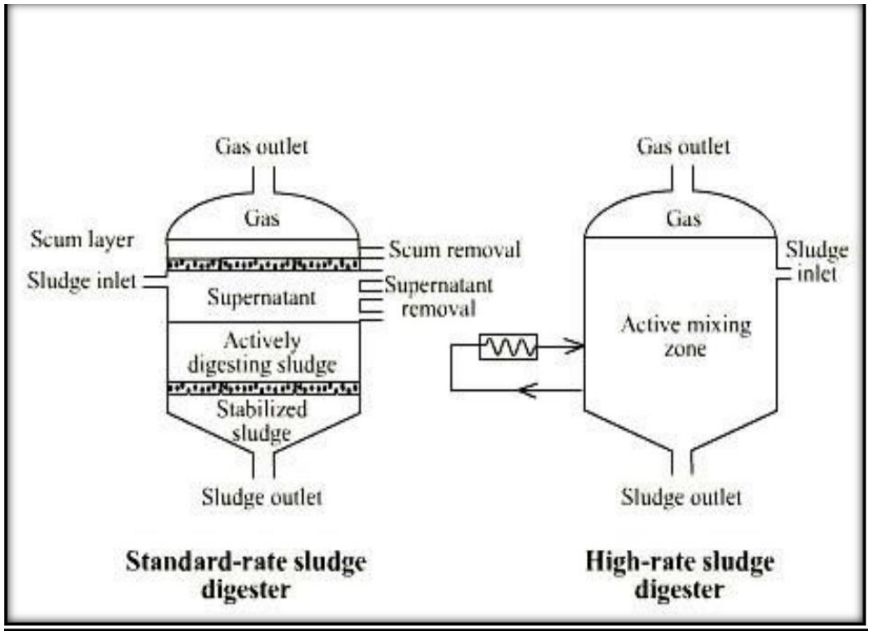
A sludge digestion tank is a RCC or steel tank of cylindrical shape with hopper bottom and is covered with fixed or floating type of roofs.

Types of Anaerobic Digesters

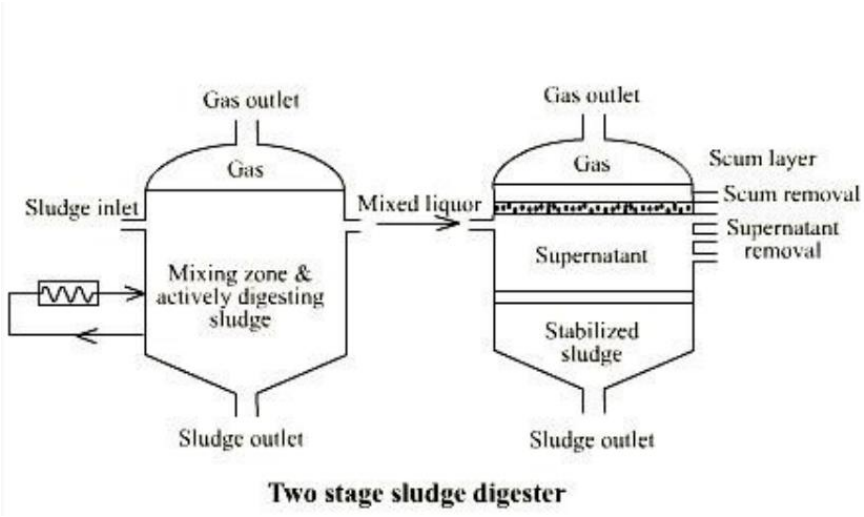
The anaerobic digesters are of two types: standard rate and high rate. In the standard rate digestion process, the digester contents are usually unheated and unmixed. The digestion period may vary from 30 to 60 d. In a high rate digestion process, the digester contents are heated and completely mixed. The required detention period is 10 to 20 d.



Anaerobic sludge digester



Often a combination of standard and high rate digestion is achieved in two-stage digestion. The second stage digester mainly separates the digested solids from the supernatant liquor: although additional digestion and gas recovery may also be achieved.



POSSIBLE QUESTIONS

TWO MARK QUESTIONS

1. What is downstream processing?
2. Define filtration.
3. Comment on types of filtration.
4. What are the factors affecting the filtration?
5. What is the advantage of ultra filtration?
6. What is the role of centrifugation in downstream process?
7. What are the methods used for purification of products?
8. State the types of centrifugation.
9. What are methods used in cell disruption?
10. Expand UF.
11. Write any three advantages of Ultrasonication.
12. Define Chromatography.
13. Mention about the types of chromatography.
14. What is Liquid–liquid extraction?
15. Mention about the effluent treatment.

EIGHT MARK QUESTIONS

1. What are steps involved in downstream processing with diagram.
2. Explain in detail:
    a. High performance liquid chromatography.
    b. Ion-exchange chromatography.
3. Describe the methods of product recovery in bioprocess technology.
4. Give a short note on purification methods of bioprocess technology.
5. Comment on i) Drying ii) Rotary vacuum filter.

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SYLLABUS

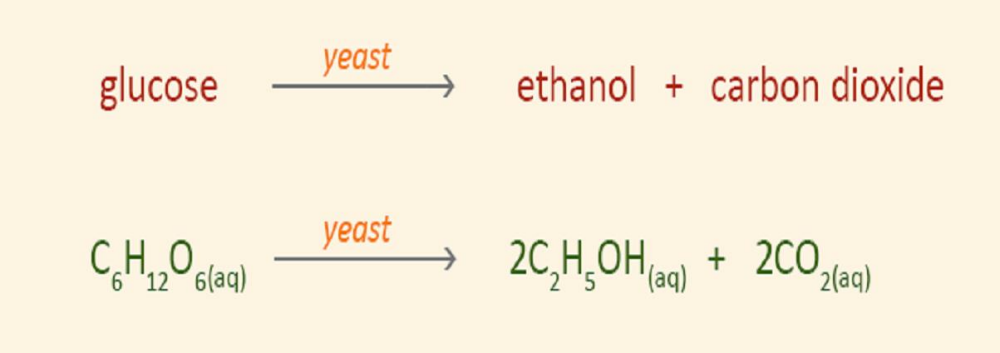
Application: Microbial production of ethanol, amylase, lactic acid, and Single Cell Proteins. Fermentation economics.

MICROBIAL PRODUCTION OF ETHANOL

Aqueous solutions of ethanol can be produced when sugar solutions are fermented using yeast. The fermentation method is used to make alcoholic drinks. Fruit juices, such as grape juice, contain a source of sugar glucose (C6H12O6). When yeast is added it feeds on the sugar in the absence of oxygen to form wine (a solution of ethanol) and carbon dioxide.

A chemical reaction called fermentation takes place in which the glucose is broken down to ethanol by the action of enzymes in the yeast.

The equation for the reaction is:



Yeast is a single cell organism and a type of fungi. It contains the enzyme zymase which acts as a catalyst for the reaction. The juices of other fruits i.e. apples, plums, pears etc. can be fermented to produce wines of various flavours.

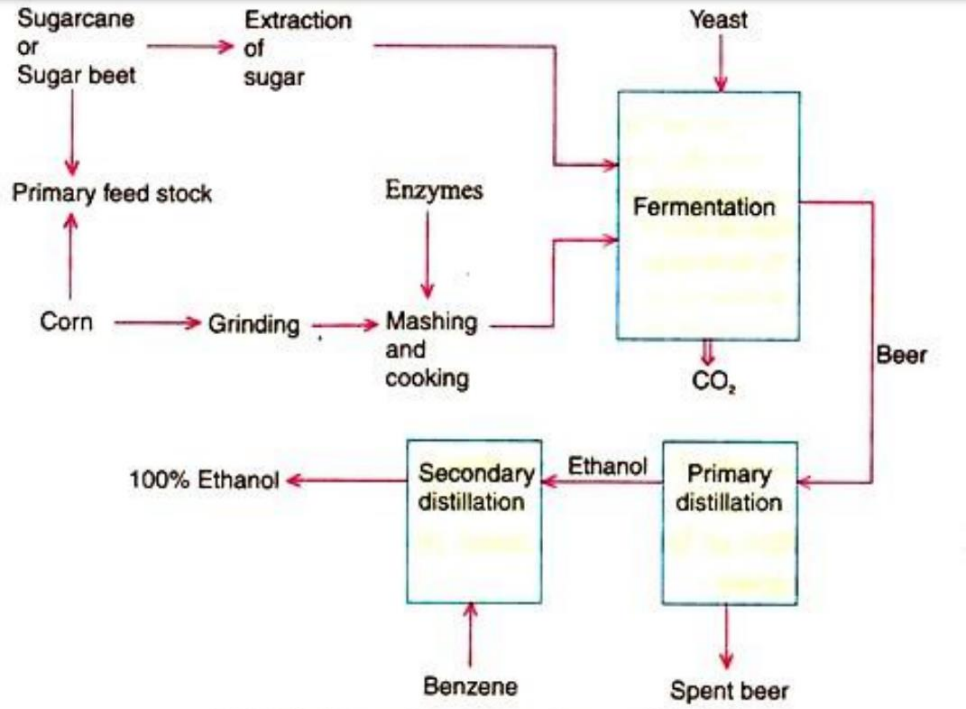


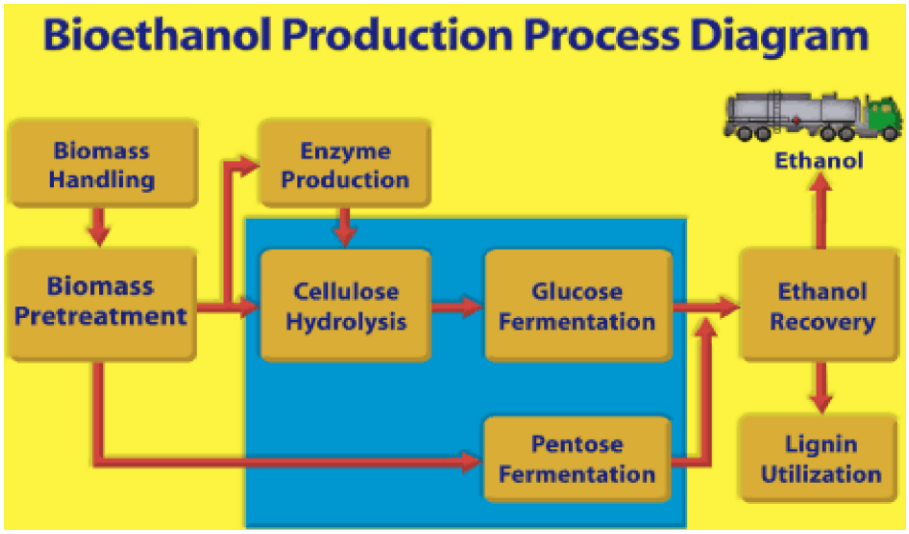
Fig. 20.6: Ethanol production from molasses.

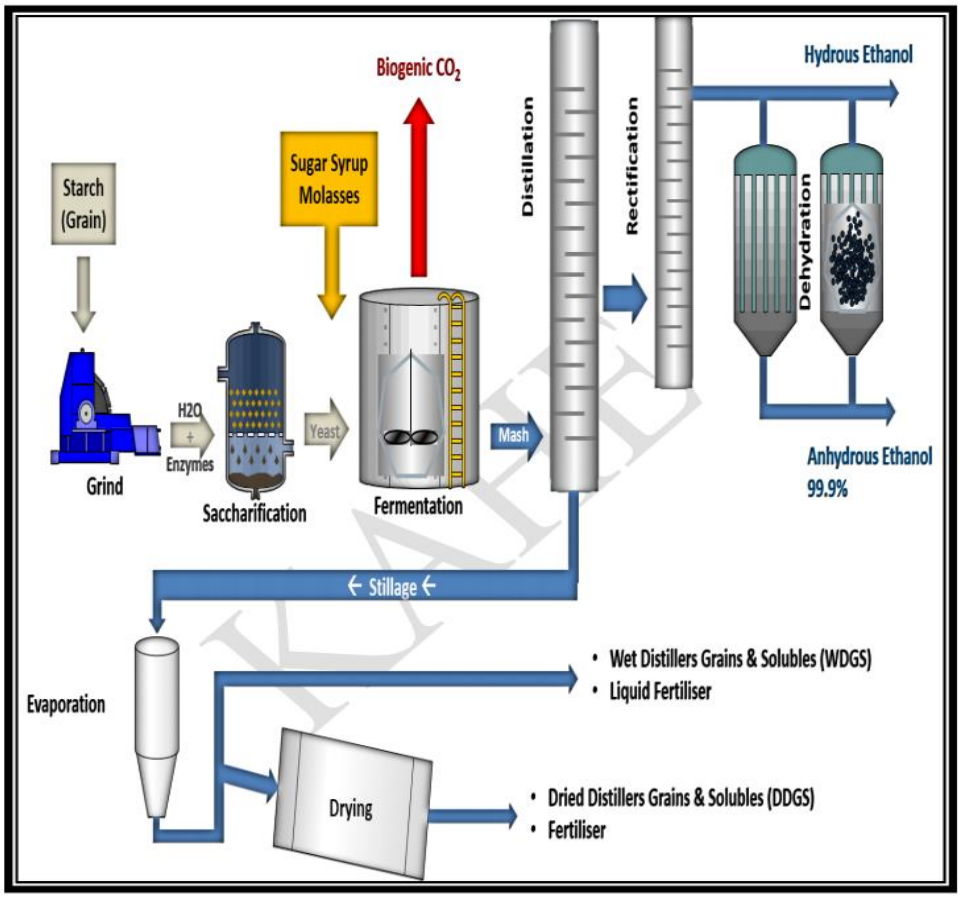
The fermentation reaction requires the following conditions:

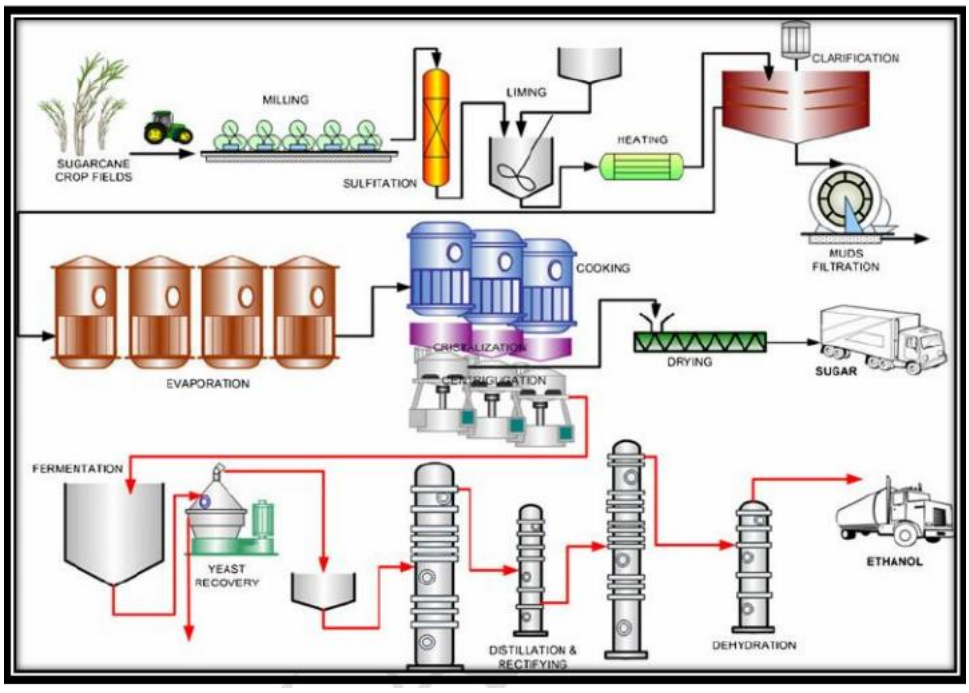
- **Temperature –** The temperature must be between the range of 25°C and 50°C. Enzymes are affected a great deal by temperature. If the temperature is too cold the enzymes move around too slowly to meet the substrate and for a reaction to occur. As the temperature increases though, so does the rate of reaction. This is because heat energy causes more collisions between the enzyme and the substrate. However, all enzymes are proteins and at too high temperatures the proteins break down. The active site of the enzyme becomes distorted and so the substrate no longer fits and hence the reaction does not occur, the enzyme is said to be denatured.
- **Substrate (the glucose solution) -** Enzymes work best when there is a high enough substrate concentration for the reaction they catalyse. If too little substrate is available the rate of the reaction is slowed and cannot increase any further.
- **Absence of Oxygen –** Air must be excluded from the vessel in which fermentation is being carried out. Air contains a large proportion of bacteria called

Acetobacter. *Acetobacter* bacteria use atmospheric oxygen from air to oxidise ethanol in the wine, producing a weak solution of ethanoic acid (vinegar).

- **Yeast –** the fermentation of the glucose solution to ethanol cannot take place without the presence of yeast. Yeast contains the enzyme zymase which acts as a catalyst for the reaction Wine contains ethanol of a concentration up to about 14 -15%. This is because above this level the ethanol kills the yeast and fermentation stops.




Biogenic CO_2
Hydrous Ethanol
Sugar Syrup
Molasses
Starch
(Grain)
Distillation
Rectification
Dehydration
H2O
+
Enzymes
Yeast
Mash
Grind
Saccharification
Fermentation
Anhydrous Ethanol
99.9%
← Stillage ←
Evaporation
• Wet Distillers Grains & Solubles (WDGS)
• Liquid Fertiliser
Drying
• Dried Distillers Grains & Solubles (DDGS)
• Fertiliser


SUGARCANE
CROP FIELDS
MILLING
SULFITATION
LIMING
HEATING
CLARIFICATION
MUDS
FILTRATION
EVAPORATION
COOKING
CRYSTALLIZATION
CENTRIFUGATION
DRYING
SUGAR
FERMENTATION
YEAST
RECOVERY
DISTILLATION &
RECTIFYING
DEHYDRATION
ETHANOL

MICROBIAL PRODUCTION OF AMYLASE

Amylase is an important and indispensable enzyme that plays a pivotal role in the field of biotechnology. It is produced mainly from microbial sources and is used in many industries. Industrial sectors with top-down and bottom-up approaches are currently focusing on improving microbial amylase production levels by implementing bioengineering technologies. The further support of energy consumption studies, such as those on thermodynamics, pinch technology, and environment-friendly technologies, has hastened the large-scale production of the enzyme. Herein, the importance of microbial (bacteria and fungi) amylase is discussed along with its production methods from the laboratory to industrial scales.

Bacterial Amylases

Among the wide range of microbial species that secrete amylase, its production from bacteria is cheaper and faster than from other microorganisms. A wide range of bacterial species has been isolated for amylase secretion. Most are *Bacillus species (B. subtilis,B. stearothermophilus, B. amyloliquefaciens, B. licheniformis, B. coagulans, B. polymyxa, B. mesentericus, B. vulgaris, B. megaterium, B. cereus, B. halodurans, and Bacillus sp. Ferdowsicous),* Halophilic strains that produce amylases include *Haloarcula hispanica, Halobacillus sp., Chromohalobacter sp., Bacillus dipsosauri, and Halomonas meridiana*. More studies involving the isolation and improvement of novel strains will pave the way to creating important strains.

Fungal Amylases

Fungal enzymes have the advantage of being secreted extracellularly. In addition, the ability of fungi to penetrate hard substrates facilitates the hydrolysis process. In addition, fungal species are highly suitable for solid-based fermentation. Efficient amylase-producing species include those of genus *Aspergillus (A. oryzae, A. niger, A. awamori, A. fumigatus, A. kawachii, and A. flavus)*, as well as *Penicillium, Streptomyces rimosus, Thermomyces*

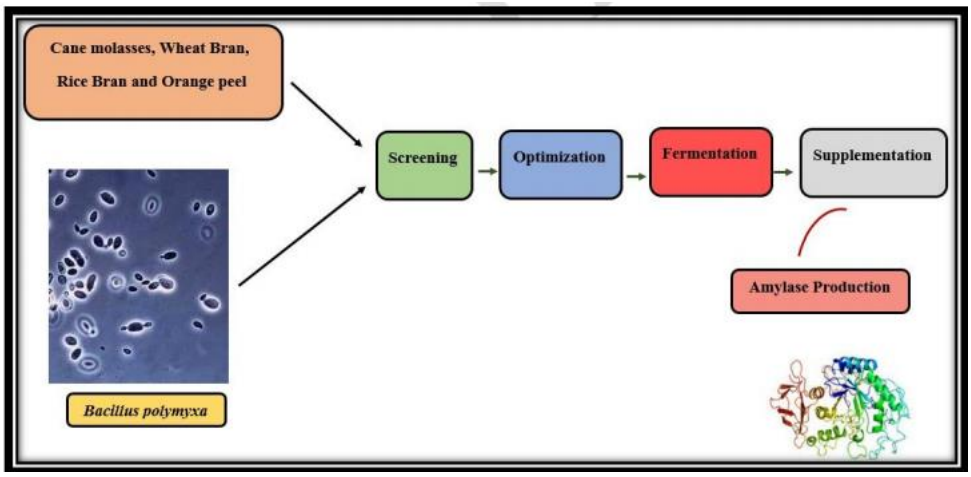
lanuginosus, Pycnoporus sanguineus, Cryptococcus flavus, Thermomonospora curvata, and Mucor sp.

Recombinant Amylase

Genetic engineering and recombinant DNA technology are the current molecular techniques used to promote efficient enzyme production. Recombinant DNA technology for amylase production involves the selection of an efficient amylase gene, gene insertion into an appropriate vector system, transformation in an efficient bacterial system to produce a high amount of recombinant protein (in the presence of an expression-vector promoterinducing agent), and purification of the protein for downstream applications.

Industrial Applications of Microbial Amylase

Amylase makes up approximately 25% of the world enzyme market. It is used in foods, detergents, pharmaceuticals, and the paper and textile industries. Its applications in the food industry include the production of corn syrups, maltose syrups, glucose syrups, and juices and alcohol fermentation and baking. It has been used as a food additive and for making detergents. Amylases also play an important role in beer and liquor brewing from sugars (based on starch). In this fermentation process, yeast is used to ingest sugars, and alcohol is produced. Fermentation is suitable for microbial amylase production under moisture and proper growth conditions. Two kinds of fermentation processes have been followed: submerged fermentation and solid-state fermentation. The former is the one traditionally used and the latter has been more recently developed. In traditional beer brewing, malted barley is mashed and its starch is hydrolyzed into sugars by amylase at an appropriate temperature.



MICROBIAL PRODUCTION OF LACTIC ACID

Lactic acid is an organic compound with the formula CH3CH(OH)CO2H. In its solid state, it is white and water-soluble. In its liquid state, it is colorless. It is produced both naturally and synthetically. With a hydroxyl group adjacent to the carboxyl group, lactic acid is classified as an alpha-hydroxy acid (AHA). In the form of its conjugate base called lactate, it plays a role in several biochemical processes.

Production

Lactic acid is produced industrially by bacterial fermentation of carbohydrates (sugar, starch) or by chemical synthesis from acetaldehyde, that is available from coal or crude oil. In 2009 lactic acid was produced predominantly (70–90%) by fermentation. Production of racemic lactic acid consisting of a 1:1 mixture of D and L stereoisomers, or of mixtures with up to 99.9% L-lactic acid, is possible by microbial fermentation. Industrial scale production of D-lactic acid by fermentation is possible, but much more challenging.

Fermentative production

Fermented milk products are obtained industrially by fermentation of milk or whey by *Lactobacillius* species: *Lactobacillus acidophilus*, *Lactobacillus casei*, *Lactobacillus delbrueckii* subsp. *bulgaricus* (*Lactobacillus bulgaricus*) and *Lactobacillus helveticus*, and

furthermore *Streptococcus salivarius* subsp. *thermophilus* (*Streptococcus thermophilus*) and *Lactococcus lactis*.

As a starting material for industrial production of lactic chemistry that is applied for chemical synthesis almost any carbohydrate source containing C5/C6 sugars could be used. Pure sucrose, glucose from starch, raw sugar beet juice are frequently applied. Lactic acid producing bacteria could be divided in two classes: homofermentative bacteria like *Lactobacillus casei* and *Lactococcus lactis*, producing two moles of lactate from one mole of glucose, hetero fermentative species producing one mole of lactate from one mole of glucose as well as carbon dioxide and acetic acid/ethanol.

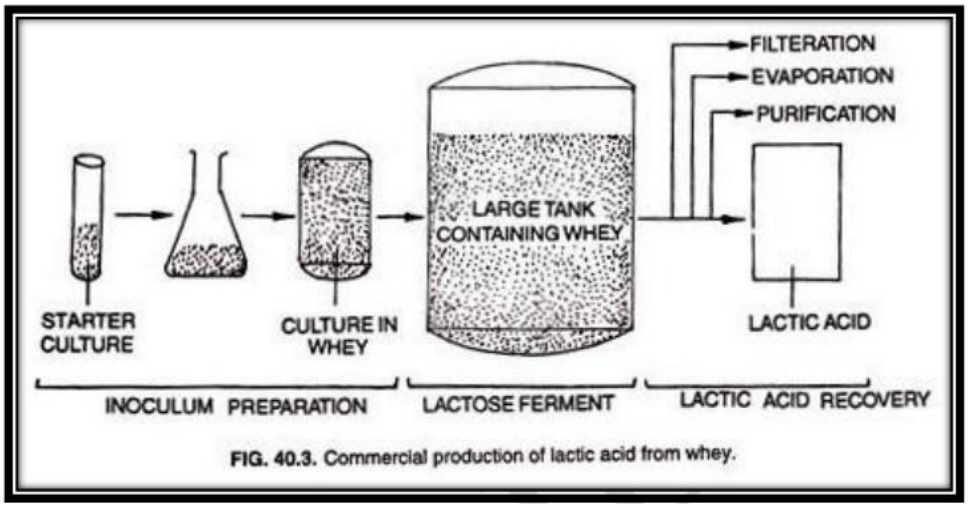


FIG. 40.3. Commercial production of lactic acid from whey.

Lactic acid has applications in the leather tanning industry, in descaling processes, in the textile industry as a mordant (fixative) for dyeing, and can replace ethylene glycol in antifreeze, which results in a higher efficiency and lower cost. In the chemical industry, lactic acid can be converted to ethanol, propylene glycol, and acrylic polymers. Lactic acid derivatives, salts, and esters are used as solvents, emulsifiers, and plasticizers. Lactic acid is also used in the production of propylene oxide, acetaldehyde, acrylic acid, propanoic acid, 2,3-pentanedione, ethyl lactate, lactide, and polylactic acid.

SEPARATION AND PURIFICATION PROCESSES

Separation processes are essential to the chemical industry and other related industries. Approximately 40% to 70% of operating and capital costs are associated with the separations steps. In lactic acid production processes, development of an effective method of lactic acid separation and purification from fermentation broth is extremely important for economic viability. Although the difference between the boiling point of lactic acid and water is relatively large, it is almost impossible to obtain pure crystalline lactic acid. This is because lactic acid has a high affinity for water and a dimer of lactate is formed when lactic acid concentrations are sufficiently high.

Lactic acid process in a classical way involves a series of downstream treatments such as precipitation, conventional filtration, acidification, carbon adsorption, evaporation, crystallization, and others. The number of downstream processing steps strongly influences the quality and the price of the product.

MICROBIAL PRODUCTION OF SINGLE CELL PROTEIN (SCP)

Single-cell protein (SCP) typically refers to sources of mixed protein extracted from pure or mixed cultures of algae, yeasts, fungi or bacteria (grown on agricultural wastes) used as a substitute for protein-rich foods, in human and animal feeds.

Production Process

Single-cell proteins develop when microbes ferment waste materials (including wood, straw, cannery, and food-processing wastes, residues from alcohol production, hydrocarbons, or human and animal excreta). The problem with extracting single-cell proteins from the wastes is the dilution and cost. They are found in very low concentrations, usually less than 5%. Engineers have developed ways to increase the concentrations including centrifugation, flotation, precipitation, coagulation, and filtration, or the use of semi-permeable membranes. The single-cell protein must be dehydrated to approximately 10% moisture content and/or acidified to aid in storage and prevent spoilage. The methods to increase the concentrations to adequate levels and the de-watering process require equipment that is expensive and not

always suitable for small-scale operations. It is economically prudent to feed the product locally and soon after it is produced.

Seed culture

Aspergillus niger used as seed culture to produce SCP.

The process of SCP production from any microorganism or substrate would have the following basic steps:

1. Provision of a carbon source; it may need physical and/or chemical pretreatments.
2. Addition, to the carbon source, of sources of nitrogen, phosphorus and other nutrients needed to support optimal growth of the selected microorganism.
3. Prevention of contamination by maintaining sterile or hygienic conditions. The medium components may be heated or sterilized by filtration and fermentation equipments may be sterilized.
4. The selected microorganism is inoculated in a pure state.
5. SCP processes are highly aerobic (except those using algae). Therefore, adequate aeration must be provided. In addition, cooling is necessary as considerable heat is generated.
6. The microbial biomass is recovered from the medium.
7. Processing of the biomass for enhancing its usefulness and/or storability.

The selection of certain microbial strain is very important, some of the criteria are:

1. Performance (growth rate, productivity, yield) on the specific. preferably low-cost substrates to be used
2. Temperature and pH tolerance
3. Oxygen requirements, heat generation during fermentation and foaming characteristics
4. Growth morphology and genetic stability in the fermentation
5. Ease of recovery, and requirements for further downstream processing
6. Structure and composition of the final product, in terms of protein

It has been calculated that 100 lbs of yeast will produce 250 tons of proteins in 24 hours, whereas a 1000 lbs steer will synthesize only 1 lb of protein 24 hours and this after consuming 12 to 20 lbs of plant proteins. Similar, algae grown in ponds can produce 20 tons (dry weight) of protein, per acre, per year.

Single cell protein can be produced by two types of fermentation processes, namely submerged fermentation and semisolid state fermentation. In the submerged process, the substrate to be fermented is always in a liquid which contains the nutrient needed for growth. The substrate is held in the fermentor which is operated continuously while the product biomass is continuously harvested.

The product is filtered or centrifuged and then dried. For semisolid fermentation, the preparation of the substrate is not as elaborate; it is also more conducive to a solid substrate such as cassava waste. Submerged culture fermentations are more capital intensive and have a higher operating cost when compared with semisolid fermentations which, however, have a lower protein yield.

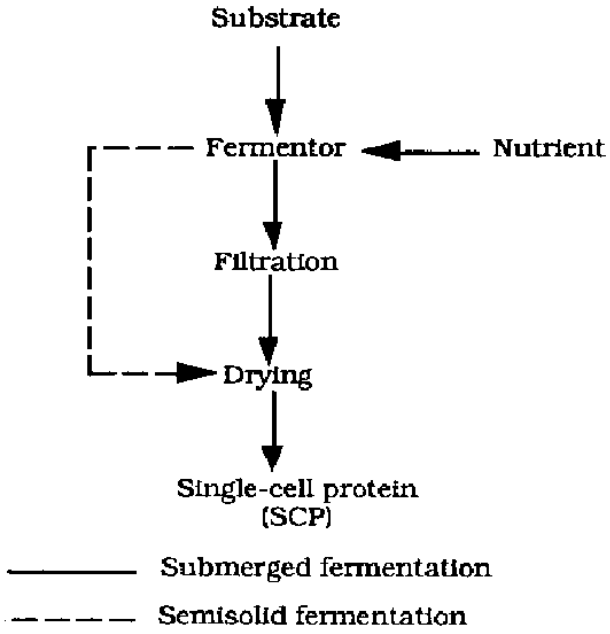
The major proportion of the production cost in most fermentation processes is the cost of the raw materials which can be up to 25-70 percent.

However, the main problem of SCP production is the relatively high cost in the downstream processing and marketing SCP as food. Using waste materials as substrate provides lower yield of biomass than using more defined substrates, many processes are required to increase the biomass concentrations including centrifugation, flotation, precipitation, coagulation and filtration, or the use of semi-permeable membranes. SCP also treated with other processes to kill the microbes, increase the digestibility, and to reduce the nucleic acid content.

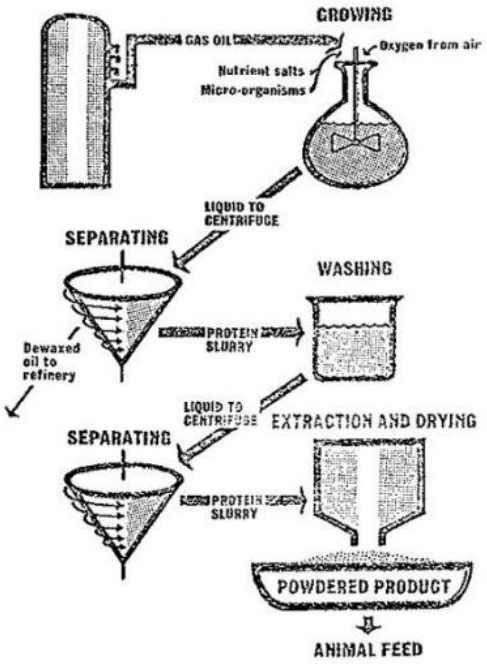
These processes require extra costs which may exceed the costs for conventional food production. The removal or reduction of nucleic acid content of various SCP's is achieved with one of the following treatments:

a. chemical treatment with NaOH;
b. treatment of cells with 10% NaCl;

c. thermal shock.



These methods aim to reduce the RNA content from about 7% to 1% which is considered within acceptable levels.



Single Cell Protein - Yeast

Yeast is another source of Single Cell Protein, and have been produced since a long time ago. In World War I, Torula yeast (*Candida utilis*) was produced in Germany and used in soups and sausages. Nowadays, the pet food industry is a major outlet of microbial biomass. The dog, cat. fish feed is supplemented with yeasts, it will make the product more palatable to the animals. Use of yeast as food seasoning is commonly found in vegetarian's diet, Torula yeast has been commercially used for this purpose, an example of this product is Hickory Smoked Dried Torula Yeast. Yeast has some advantages among other SCP sources, such as:

1. Easy to harvest because of their size (larger than bacteria)
2. High level of Malic acid content
3. High lysine content
4. Can grow at acidic pH
5. Long history of traditional use

This nutritious microbe unfortunately has few disadvantages that have to be taken as consideration, such as:

1. Lower growth rates compared to bacteria
2. Lower protein content than bacteria (45-65%)
3. Lower Methonine content than bacteria, solved by the addition of Methonine in the final product.

Single Cell Protein - Algae

Since a decade ago studies on Single Cell Protein (SCP) had drawn the attention of scientist to bridge the protein gap. The use of algae as food and feed is known since centuries as they form part of the diets is East Asian countries as well as the natives in Central Africa. Some of the algae like *Chloralla, Soenedesmus, Coelastrum* and *Spirulina* have been found to suitable for mass cultivation and utilization. The advantages in using algae include simple cultivation, effective utilization of solar energy, faster growth and high protein and nutrient content.

Cultivation of *Spirulina* and production

In tropical countries *Spirulina* cultured under authotrophic and heterotrphic conditions.

- Mass cultivation easier than other algal cultivation because aeration of CO2 is not necessary for this species since it can maximally utilizes the amounts of carbon that are supplied by using bicarbonate of the culture medium.
- Continues agitation is not very essential to boost up the yield.
- Because the studies proofing that continuous mixing with paddle wheels or pumps and sporadic manual stirring with brooms are not providing any considerable differences in yields.
- The optimum temperature for *Spirulina* is the temperatures in between 25 and 35°C.
    - The growth will be retarded when the temperatures below 20°C.
    - Although *Spirulina* seems to be a more thermophilic alga, it could not withstand longer periods at temperatures above 40°C.
- Light intensities of 30-40 klux were found to be optimal for *Spirulina.*
    - To maintain these intensities during summer months, scaffoldings were arranged around the ponds and roofed over sparsely with coconut fronds, which cut down the light by 40-50%, but left enough space for ventilation.

FERMENTATION ECONOMICS

The objective of any successful fermentation process is the ability to produce a fermentation product. Thus the product must be sold to recover all the costs along with desired profit. But manufacturing should be done in accordance with the market demand. So there could be 2 possibilities:

First possibility is: That the market for so called product already exists because the same or similar product has previously been sold by others.

Second possibility is: a newly manufactured or discovered product e.g. a new antibiotic will require a market to be established.

This might include the approval by FDA (food and drug administration).

There are certain obstacles regarding the marketing of a certain product like the semand of the product are low or it has relatively very few uses. so its quite obvious that for a product like this it could be challenging to get patent coverage because of lack of utility.

For the products which are already in the market, there could be a fierce competition. so to succeed in the competition the product must be cheap enough that it can be sold at or slightly less than the already existing selling price. So, in the nutshell, the whole fermentation process and its product must be able to compete on an economically sound basis with the similar products in the market.

The economic position of a fermentation product is closely tied to the costs associated with its production and distribution. These costs can be categorized into several classes as follows:

Media components

The competitive position and expected profits from a fermentation product are closely tied to the costs of the various components of the production medium. Usually inoculums medium is less expensive because it is required to provide rapid cell growth only and not for converting large amount of carbon substrate into a fermentation product. However any medium component may be subject to fluctuations in context to availability and costs. so it is always advantageous to have a alternative medium for use if any unusual situation happens.

Labor Costs

Labor costs involve technical ad non technical trained personnel at all levels of competence. This includes handling of cultures, inoculums, production, product recovery and purification, packaging, cleaning and administration and so forth. Labor costs vary from fermentation to fermentation.

Contamination and sterilization

Contamination always adds costs to any fermentation process. Most fermentations cannot survive serious contaminations so the medium must be discarded moderate fermentations does not require the medium to be discarded but it might affect the yields. Certain fermentations are more prone to contamination than others. This involves cases in which foaming is a problem. some are more sensitive to phage infections like bacterial fermentations. So there has to be an alternate method for the contaminant growth. Such methods include low pH of the medium, partial heat treatment of the medium and inclusion of certain chemicals so as to retard contaminant growth.

Yield and Product Recovery Product Yield and Recovery are the prime considerations of fermentation economics and in that context yield and recovery must be considered together, since high yield id of little value if the product cannot be recovered properly for sale.

Product Purity

At one end of the scale some products, like antibiotic preparations must be sterile and free from pyrogens. In contrast other antibiotic preparations are sold in crude form for mixture with animal feeds. Thus the purity level required for the marketing of a fermentation product has a major effect on the costs associated with the product. Specific fermentation products can also be marketed at more than one concentration as level of purity. For example, lactic acid is sold at strengths ranging from approx 20 - 85% and its purity levels range from crude technical grade to high purity edible and U.S.P. grades. Each of these grades of lactic acid has a place on the market.

Waste Disposal

Costs attributed to waste disposal vary from minimal to maximal factor in fermentation process. A critical consideration is the acceptance of waste by Municipal's STW (Sewage Treatment Works); as they might want pre treatment of wastes before the acceptance. In the alter case the fermentation company must have its own waste treatment plant. Disposal of wastes is no longer simple in contrast to historical disposal in the rivers, streams or other water bodies. Certain fermentations require the waste to be sterilized before disposal.

Research Costs

Fermentation process must include those expenses incurred in the research that actually discovered the process and developed it. These costs can be considerable for those fermentations where they provide new products .there are less tangible research costs that must also be considered in the overall cost of fermentation. This type of research is pursued in the hope that the resulting basic information obtained on the growth and synthetic activities of microbes will be of later value in defining areas of exploration and approaches for discovering new fermentation processed and bettering old processes.

An appraisal of the economic potential is required for the fermentation process which evaluates all the above categories under present and future market potential. But evaluation must be made as early as possible during process development. Also process must be evaluated at later stages during actual production. It is also important to consider present and future costs and a selling price for the product that market can bear.. All these points must be evaluated and then decide whether the fermentation product can be sold at an acceptable level of profit or not? If the decisions are negative then the alternatives are to abandon the whole process and carry out further research on the product recovery. After all a great deal of money is at stake in these decisions!!!

POSSIBLE QUESTIONS

TWO MARK QUESTIONS

1. What are the applications of ethanol?
2. Which bacteria produce amylase?
3. How is amylase used?
4. Draw the flow diagram for production of ethanol.
5. State the uses of lactic acid.
6. What are types of amylase?
7. Expand SCP.
8. Write any three advantages of Single Cell Protein.
9. Which fungi used for production of SCP?
10. Draw the flow diagram for production of SCP.
11. Mention about the industrial application of amylase.
12. Define the fermentation economics.

EIGHT MARK QUESTIONS

1. Explain the details about microbial production of ethanol with diagram.
2. What is amylase? Describe the production of amylase and its application.
3. Explain in detail about the fermentation economics.
4. Give a short note on Lactic acid production and their application.
5. Comment on i) Algal Production of SCP ii) Fungal Production of SCP

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