Semester – III

17MBU303 INDUSTRIAL MICROBIOLOGY (4H – 4C)

Instruction Hours / week: L: 4 T: 0 P: 0 **Marks:** Internal: **40** External: **60** Total: **100**

End Semester Exam: 3 Hours

COURSE OBJECTIVES

- To encompasses the use of microorganisms in the manufacture of food or industrial products.

Unit I

Brief history and developments in industrial microbiology. Sources of industrially important microbes and methods for their isolation, primary and secondary screening methods. Strain improvement method (protoplast fusion, mutation and recombinant DNA technology).

Unit II

Preservation and maintenance of industrial strains. Media formulation (molasses, corn- steep liquor, sulphite waste liquor, whey, yeast extract and protein hydrolysates).

Unit III

Types of fermentation processes – Solid-state and liquid-state (stationary and submerged) fermentations; batch, fed-batch and continuous fermentations. Components of a typical bio-reactor, Types of bioreactors – Laboratory, pilot- scale and production fermenters, constantly stirred tank and air-lift fermenters. Measurement and control of fermentation parameters - pH, temperature, dissolved oxygen, foaming and aeration.

Unit IV

Separation of cells – filtration and centrifugation. Cell disruption – physical, chemical and enzymatic methods. Product separation – solvent extraction and precipitation. Lyophilization and spray drying. Methods of immobilization, advantages and applications of immobilization.

Unit V

Microbial production of industrial products-Citric acid, Ethanol, Penicillin, Glutamic acid, Vitamin B_{12}, Enzymes (amylase, protease, lipase) Wine, Beer (micro-organisms involved, media, fermentation conditions, downstream processing and uses).

SUGGESTED READINGS

1. Sivakumar, P.K., Joe, M.M., Sukesh, K., (2010) An introduction to industrial microbiology. S. Chand Publishing.

2. Stanbury PF, Whitaker A and Hall SJ. (2006). Principles of Fermentation Technology. 2nd edition, Elsevier Science Ltd.

3. Crueger W and Crueger A. (2000). Biotechnology: A textbook of Industrial Microbiology. 2nd edition. Panima Publishing Co. New Delhi.

4. Okafor N. (2007). Modern Industrial Microbiology and Biotechnology. 1st edition. Bios Scientific Publishers Limited. USA.

5. Saxena., and Sanjai., (2015). *Applied Microbiology*. Springer, Germany.
6. Bebek R and Arun (2014) Fundamental Food Microbiology. 5th edition, Taylor and Francis, CRC Press, United States.
7. Glaze A.N. and Nikaido H. (1995). Microbial Biotechnology: Fundamentals of Applied Microbiology. 1st edition. W.H. Freeman and Company
8. Casida LE. (1991). Industrial Microbiology. 1st edition. Wiley Eastern Limited.
9. Patel A.H. (1996). Industrial Microbiology. 1st edition, Macmillan India Limited.
10. Waites M.J., Morgan N.L., Rockey J.S. and Higton G. (2001). Industrial Microbiology: An Introduction. 1st edition. Wiley – Blackwell.

WEBLINKS

1. https://www.generalmicroscience.com/industrial-microbiology/strain-improvement-micro-organisms-used-fermentation/
2. https://www.scribd.com/document/330142375/Preservation-and-Maintenence-of-Industrial-Microorganisms.

UNIT I

Duration	Topic	Reference
01	History and development in industrial microbiology.	R1:3-14
02	History and development in industrial microbiology	
03	Sources of industrially important microbes	R2:9-50
04	Isolation of industrially important microbes	
05	Primary screening of industrially important microbes	
06	Primary screening of industrially important microbes	
07	Secondary screening of industrially important microbes	
08	Strain improvement	R3:71-73
09	Unit revision and possible questions.	
	Total hours: 9 h	

R1: Patel A. H., 2003. Industrial Microbiology. Macmillan India ltd, NewDelhi.

R2: Wulf Cruegar & A. Cruegar, 2000. A Textbook of Industrial Microbiology, Panima Publishing Corp, Newdelhi.

R3: Kalaichelvan and Arulpandi, 2009. Bioprocess technology,MJP Publishers.

UNIT II

Duration	Topic	Reference
01	Preservation and maintenance of industrial strains.	R1:244-293
02	Preservation and maintenance of industrial strains.	
03	Crude and synthetic media	R2:315-317
04	Molasses	
05	Corn- steep liquor	
06	Sulphite waste liquor	
07	Yeast extract	R3:43- 53
08	Protein hydrolysates, Whey	
09	Unit revision and possible questions.	
	Total hours: 9 h	

R1: Peppler and Pearlman, 1979. Microbial Biotechnology. Academic Press.

R2: Kalaichelvan and Arulpandi, 2009. Bioprocess technology,MJP Publishers.

Umesh Kumar, 2014. Industrial Microbiology, MJP Publishers.

R3: Patel A. H., 2003. Industrial Microbiology. Macmillan India ltd, NewDelhi.

UNIT III

Duration	Topic	Reference
01	Types of fermentation processes	T1:43-57 T1:56-69 T1:97-104
02	Batch, fed-batch fermentations	
03	Continuous fermentations	
04	Solid-state and liquid-state fermentations	
05	Components of a typical bio-reactor,	T2:188-215 R1:765-773
06	Types of bioreactors – Laboratory, pilot- scale and production fermenters,	
07	Constantly stirred tank and air-lift fermenters.	
08	Measurement and control of fermentation parameters- pH, temperature	R2:223-225 375-395
09	Measurement and control of fermentation parameters-dissolved oxygen, foaming and aeration.	
10	Unit revision and possible questions	
	Total hours: 10 h	

T1: Kalaichelvan and Arulpandi, 2009. Bioprocess technology,MJP Publishers.

T2: Umesh kumar, 2014. Industrial Microbiology, SBW Publishers.

R1: Doran, 2013. Bioprocess Enggineering Principles. Academic press,

R2: Peppler and Pearlman, 1979. Microbial Biotechnology. Academic Press.

UNIT IV

Duration	Topic	Reference
01	Separation of cells – filtration and centrifugation.	R1: 111-123
02	Cell disruption – physical, chemical and enzymatic methods.	R2: 64-86
03	Product separation – solvent extraction and precipitation.	R3: 452-46
04	Lyophilization and spray drying.	438-563
05	Introduction to immobilization	R4:45-461
06	Methods of immobilization	
07	Advantages and applications of immobilization	
08	Large scale applications of immobilized enzymes-glucose isomerase	
09	Large scale applications of immobilized enzymes-penicillin acylase	
10	Unit revision and possible questions	
	Total hours: 10 h	

R1: Wulf Cruegar & A. Cruegar, 2000. A Textbook of Industrial Microbiology, Panima Publishing Corp, Newdelhi.

R2: Patel A. H., 2003. Industrial Microbiology. Macmillan India ltd, NewDelhi.

R3: Kalaichelvan and Arulpandi, 2009. Bioprocess technology,MJP Publishers.

R4: Biotechnology-B.D.Songh. Kalyani Publishers, 1998. NewDelhi.

UNIT V

Duration	Topic	Reference
01	Microbial production of Citric acid	
02	Microbial production of Ethanol	
03	Microbial production of Penicillin	R1: 162-169
04	Microbial production of Glutamic acid	R1: 112-119
05	Microbial production of Vitamin B_{12}	
06	Microbial production of Enzymes (amylase, protease, lipase)	
07	Microbial production of Wine & Beer	
08	Revision of all units and possible questions	
09	Last five year old question paper discussion	
	Total hours: 10 h	

R1: Patel A. H., 2003. Industrial Microbiology. Macmillan India ltd, NewDelhi.

Unit I

Syllabus

Brief history and developments in industrial microbiology. Sources of industrially important microbes and methods for their isolation, primary and secondary screening methods. Strain improvement method (protoplast fusion, mutation and recombinant DNA technology

History and development of Industrial Microbiology

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. 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, and components of microbial cells, intracellular or extracellular enzymes or chemicals produced by the microbes utilizing the medium constituents or the provided substrate.

Table 1 Dates and events in early biotechnology

Ancient handicraft	
6000 BC	Beer fermentation
3500 BC	Wine fermentation
3500 BC	Soja fermentation
Cheese and bread fermentation	
Fourteenth century	Industrial acetic acid fermentation
Early period up to 1850	Technical application
Scientific events	
1680 Leeuwenhoek observes microorganisms	
1783 Spallanzani observed protease action	
1793 Lavoisier and	
1810 Gay-Lussac: quantitative chemistry of alcoholic fermentation Gay-Lussac: hypothesis of spontaneous generation	Early eighteenth century: technical beer and wine fermentation; also industrial beer fermentation
1833 Payen and Persoz: diastase (enzyme) characterization	1823 Immobilized bacteria used for acetic acid production
1836 Berzelius: catalysis (including enzymes) [a]	
1837, 1838 Schwann, Cagniard-Latour: living cells as fermentation agents	
1834, 1838 Kützing, Quevenne: hypotheses of spontaneous generation, (see also before, Gay- Lussac); vital factor	
1839 Liebig: chemical decay hypothesis	1840s industrial enzymatic dextrin production (Payen)
1830s Major controversy on fermentation theories	

Microbes have been employed for product generation, e.g., wines, bread, etc., since thousands of years, but these activities were purely art. The science of industrial microbiology is only about 150 years old. The first observations of microorganisms by Leeuwenhoek were published in 1677.

1. Ancient industrial microbiology:

The origin of fermentation are lost in ancient history, perhaps even in prehistory. Fermented foods such as yoghurt, cheese, soya sauce and pickled cabbage have been intentionally produced by man for centuries and in some case millennia. Beer recovered from the pyramids of the ancient Egyptians reveal that the Egyptians were able to produced the fermented brew using almost pure cultures of yeast. In Europe, allowing soil bacteria to nitrify ammonia in horse urine produced nitrates required for gun powder manufacture. The Aztecs of Mexico cultured *Spirulina* for both waste treatment purposes and as a source of protein. In the 20th century, biotechnologists have tried again to produce single celled protein to solve the protein shortage of the world.

2. Industrial Microbiology: Modern era

The father of modern fermentation technology is however Louis Pasteur. This 19th century scientist discovered or perhaps rediscovered that the conservation of sugar to alcohol (in beer and wine production) and the conversion of sugar lactic acid (as occurs in cheese production) were both microbiological conversions and both needed a microbial seed to start them,. In this process, Pasteur disproved the theory of spontaneous generation, discovered that some microbes can grow in the absence of oxygen, and discovered a new way of sterilizing materials, which was subsequently called pasteurization. His discoveries allowed Fresh producers to dramatically improve the quality of two things, which the world still; Fresh wine and French cheese.

Following Pasteur, microbiology and fermentation technology grew hand in hand. The research of the 19th and early 20th century microbiologists such as Winogradsky and Beijerinck have lead the other scientists to isolate new and potentially useful microbes, the development of processes for growing them and new analytical methods.

Wildiers demonstrated in 1901 that yeast required growth factors (vitamins) for growth, especially at low inoculums level; vitamins are used in fermentation even today. In 1929, Alexander Fleming accidentally discovered penicillin produced by *Penicillium* growing as contaminant in a Petri plate of *Staphylococcus*. Fleming developed the technique for assay of antibacterial activity of penicillin using bacteria and showed its low toxicity to man and animals. This was followed by an intensive search for antibiotics during the Second World War leading to the discovery of streptomycin, chloramphenicol, tetracyclines, etc. Later developments have resulted in the use of metabolically blocked mutants of microorganisms, which accumulate large amounts of metabolic intermediates. This was followed by an intensive search for antibiotics during the Second World War leading to the discovery of streptomycin, chloramphenicol, tetracyclines, etc. Later developments have resulted in the use of metabolically blocked mutants of microorganisms, which accumulate large amounts of metabolic intermediates.

Table 2 The period from 1850 to 1890 (Scriban 1982, pp.13, 14; Buchholz and Collins 2010, chapters 3 and 4)

Time scientists	Scientific findings, events	Technical progress, industrial innovation
1837/1838 Schwann and Cagniard-Latour	Experimental demonstration of living yeast as agent in alcoholic fermentation	Growing importance of industrial fermentation of beer (production 23 million hL in 1840, Germany) [a]
1850 Rayer and Davaine	Detection of the origin of anthrax and the role of microorganisms in diseases	Technical-scale production of yeast, wine, soy sauce, sake. Industrial-scale beer fermentation in GB
1856–1877 Pasteur	Investigations on fermentation (from 1856 on): Investigations on alcohol fermentation (1858)	
	Studies on spontaneous generation (1859–1862)	1870s: Hansen breeding pure yeast for commercial application; 1874 Christian Hansen's Laboratory
	Detection of anaerobic fermentation (1861)	(Denmark): production of rennet (chymosin) for cheese manufacture
	Studies on wine fermentation, invention of	
	Pasteurisation (1864)	Beer production: 36 million hectolitres in 1873, Germany
	Studies on beer fermentation (1871)	
	Theory of fermentation (1876)	
	Detection of facultative anaerobic fermentation of yeast	New type of industrial beer fermenter (Pasteur; Fig. 1)
1866 Mendel	Heredity laws	
1876 Koch	Work the bacterium leading to anthrax; agar plate method	1895 Wehmer: Lactic acid production
1877-86 Pasteur	Begin of investigations on anthrax (1877)	
1880 Winogradsky	Soil microorganisms: the bacterial nature of nitrification	
1881 Pasteur	Vaccination against anthrax and rabies	

3. Fermentation technologies and the world war:

By 1914, Weizmann isolated *Clostridium acetobutylicum*, a bacterium that used inexpensive starch to produce high yield of butyl alcohol and acetone, which is in the production of synthetic rubber. However, World War I broke out in August 1914 and diverted attention away from synthetic rubber towards gunpowder (cordite). As acetone turns out into a solvent for making nitrocellulose and this cordite was acetone. Weizmann was instrumental in making available a source for the creation of acetone and was recruited by Winston Churchill and the British government to set up his microbial fermentation for the production of acetone from corn in London.

Table 3 The period from 1890 to 1940 (Buchholz and Collins 2010, chapter 4; Roehr 1996)

Time scientists[a]	Scientific findings, events	Technical progress, industrial innovation
1894 E. Fischer	Specificity of enzymes	Enzyme technology expanding (Takadiastase)
1897 Buchner	Fermentation due to enzyme action only	First waste disposal biogas reactor (Bombay)
1900s Buchner	Rersearch on fermentation intermediates	
1905 E. Fischer and others	Research in the nature of proteins	1907 Enzyme technology: Röhm and Haas company (Germany)
1910f Fernbach	Rersearch on fermentation intermediates	
1911f Fernbach and Strange; 1912f Perkin	Microbial formation of acetone and butanol [b]	Fermentation technology expanding: Production of butanol for rubber manufacture [b]
1915f Weizmann	Finding of Clostridium acetobutylicum	War requirements: acetone and butanol production
1915f Connstein and Lüdecke	Glycerol fermentation [b]	Glycerol production for explosives
1916 Thom and Currie	Citric acid fermentation [b]	
1920s		Pfizer: Industrial production of citric acid
1920s and 1930s Embden, Meyerhoff and others	Research on glycolysis	Large-scale industrial yeast production for bakeries
1925, 1930s Sumner, Northrup	Enzyme crystallization	
1928 Fleming	Finding of penicillin action	Large-scale waste water treatment (1928, Essen, Germany)
1933 Reichstein	Sorbitol transformation into L-sorbose	Reichstein process for vitamin C production
End of 1930s Florey and Chain	Resumed research on penicillin	Sterile enzyme fermentation for detergents etc.
1940	Protein structure solved	Peak alcohol production

4. Industrial Microbiology: Present scenario:

The world wars and their aftermath of the 20th Century continued to spur on developments in fermentation technology. Material shortages forced countries to look for biological sources of chemical feed stocks. Scientists found ways of producing or enhancing the production of ethanol and solvents such as acetone and butanol by fermentation. Antibiotic production was also scaled up to meet growing needs derived from diseases and injuries. The designs and technologies developed for improving antibiotic production found application in many other products such as amino acids (e.g. lysine and glutamic acid) and food chemicals e.g. gluconic acid and citric acid. The engineers and the scientist now had to work hand in hand to achieve these improvements. The discipline that we now call Biotechnology was being conceived.

In more recent times, threats of diseases, environmental disasters and food shortages has imposed tremendous demand in developed countries for new opportunities in fermentation technology. Today, the fermentation industry is a multi-million dollar industry. The fermentation skills and knowledge are now essential driving force for systematic research into drug/receptor interactions, function of membrane proteins in health and disease, and are powering an unparalleled expansion in our capability to combat serious diseases in the human population, including cancers, degenerative illnesses such as Alzheimer's, and increasingly common complaints of developed societies such as asthma. The new fermentation-derived medicines, including biopharmaceuticals, hold out the prospect of improved specificity of treatment, and decreased side effects. It is truly a revolutionary period in clinical medicine as these new agents manufactured by fermentation routes enter the market. The new fermentation products, therapeutic proteins, antibodies (simple and conjugated) are more cheaper and simple than previous products, and the need to focus upon the fermentation step is now clearer than ever. Basically, the 'quality' of these products (the potency, efficacy, stability and immunogenicity) is determined by the upstream or fermentation stage, so the need for a clear understanding of what happens in that stage, how it can best be monitored, controlled, and carried out in a reproducible fashion, is greater than ever.

Industrial microbiology is a very fast growing area of biotechnology, with ever increasing process and products. With a longer history than any area of biological sciences, industrial microbiology has a longer and brighter future, in the service of mankind, covering important areas of food, agriculture and medicine.

Table 4 The period from 1940 to 1975 (Buchholz and Poulson 2000; Bud 2007; Buchholz and Collins 2010, chapters 4 and 5)

Time scientists	Scientific findings, events	Technical progress, industrial innovation
End of 1930s Florey and Chain	Resume research on penicillin	
1940	Protein structure solved	
1940s Waksman	Extended research on antibiotics: actinomycin, streptomycin	
1941		USA: penicillin project, due to war requirements
1944		Large-scale industrial penicillin production; Pfizer: deep tank penicillin fermentation
1948 Brotzu and Oxford team	Cephalosporin, broad spectrum antibiotic	
1949	First biochemical engineering symposium	
1952/1953		Production of further antibiotics: Pfizer, Lederle: tetracycline; Eli Lilly: erythromycin
1953 Watson, Crick, Franklin	Structure of DNA	
1950s	Development of immobilized enzymes	Industrial steroid biotransformation (prednisolone)
1958 Gaden (Ed.)	First biotech journal [a]	Expanding waste water treatment due to government requirements
1959 Chain et al. with Beecham	Begin of research on 6-APA	
End of 1960s		Large-scale enzyme processes: detergents, starch processing;
1971		
1972		Industrial production of 6-APA (Bayer, Germany; Beecham GB))
1973 Cohen and Boyer	Gene cloning	Large-scale enzymatic glucose isomerisation
1974	Political level: Germany: DECHEMA-report, followed by other studies on biotechnology in UK, Japan, France	Expanding production of amino and organic acids, vitamins, enzymes in food manufacture
		Failures: SCP production; cellulosics utilization; biosensors[b,c]

Some important products of fermentation are discussed below:

Microbial biomass: Microbial biomass is produced commercially as single cell protein (SCP) using unicellular algae as species of *Chlorella* or *Spirulina* for human or animal consumption, or viable yeast cells needed for the baking industry.

Primary metabolites: During the log phase or exponential phase microorganisms produce a variety of substances essential for their growth, such as nucleotides, amino acids, lipids, carbohydrates etc or by-products of energy yielding metabolism such as ethanol, acetone, butanol, etc. These products are usually called as primary metabolites and the phase at which it is produced is termed as trophase.

Examples:

Product	Organism	Industrial importance
Citric acid	*Aspergillus niger*	Solvent
Lysine	*Corynebacterium glutamicum*	Nutritional additive
Glutamic acid		Flavour enhancer
Acetone and butanol	*Clostridium acetobutylicum*	Food industry
Riboflavin	*Ashbya gossypii*	Nutritional
Vit B_{12}	*Pseudomonas denitrificans*	

Secondary metabolites: The phase during which products are produced have ni obvious role in the metabolism of the organism and this phase is called as idiophase. The products produced in this phase are called secondary metabolites. Many secondary metabolites are produced as an end product of primary metabolism.

Examples:

Product	Organism	Industrial importance
Penicillin	*P. chrysogenum*	Antibiotics
Steptomycin	*Streptomyces greseus*	
Erythromycin	*Streptomyces erythreus*	
Gresofulvin	*P. griseofulvin*	
Gibberelin	*Gibberella fujikuroi*	Growth hormone

Traditional products

Bread, beer, wine and spirits - *Saccharomyces cerevisiae*
Cheeses, other dairy products - Lactic acid bacteria
Ripening of blue and Camembert-type cheeses - *Penicillium* species
Fermented meats and vegetables - Lactic acid bacteria
Mushrooms - *Agaricus bisporus, Lentinula edodes*
Soy sauce *Aspergillus oryzae, Zygosaccharomyces rouxii*
Sufu (soya bean curd) - *Mucor* species
Vinegar - *Acetobacter* species

Agricultural products

Gibberellins - *Fusarium moniliforme*
Fungicides - *Coniothyrium minitans*
Insecticides - *Bacillus thuringiensis*
Silage - Lactic acid bacteria

Amino acids

l-Tryptophan - *Klebsiella aerogenes*
l-Glutamine - *Corynebacterium glutamicum*
l-Lysine - *Brevibacterium lactofermentum*

Enzymes

α-amylase - *Bacillus subtilis*
β-amylase - *Aspergillus niger*
Amyloglucosidase - *Aspergillus niger*
Glucose isomerase - *Streptomyces olivaceus*
Invertase - *Kluyveromyces* species
Lactase (b-galactosidase) - *Kluyveromyces lactis*
Cellulases - *Trichoderma viride*
Lipases - *Candida cylindraceae*
Pectinases - *Aspergillus wentii*

Fuels and chemical feedstocks

Acetone - *Clostridium* species
Butanol - *Clostridium acetobutylicum*
Ethanol - *Zymomonas mobilis S.cerevisiae*
Glycerol - *Zygosaccharomyces rouxii*
Methane - Methanogenic archaeans

Organic acids

Acetic acid - *Acetobacter xylinum*
Citric acid – *A.niger, Yarrowia lipolytica*
Fumaric acid -*Rhizopus* species

Vitamins

Vit B_{12} (cyanocobalamin) - *Pseudomonas denitrificans*
β-Carotene (provitamin A) - *Blakeslea trispora*
Vit C - *Acetobacter suboxydans*

Gluconic acid -*Acetobacter suboxydans*
Itaconic acid -*Aspergillus itaconicus*
Kojic acid -*Aspergillus flavus*
Lactic acid -*Lactobacillus delbrueckii*
Riboflavin - Recombinant *B.subtilis, Ashbya gossypii*

Pharmaceuticals and related compounds &Hormones

Human growth hormone - Recombinant *Escherichia coli,* Recombinant *Saccharomyces cerevisiae*
Insulin - Recombinant *Escherichia coli,* Recombinant *Saccharomyces cerevisiae*
Cyclosporin - *Trichoderma polysporum*
Interferon - Recombinant *Escherichia coli,* Recombinant *Saccharomyces cerevisiae*
Steroids - *Arthrobacter* species *Rhizopus* species
Vaccines - *Bacillus anthracis, Clostridium tetani,* Recombinant *Escherichia coli, Salmonella typhi*

Polymers

Alginates - *Azotobacter vinelandii*
Cellulose - *Acetobacter xylinum*
Dextran - *Leuconostoc mesenteroides*
Gellan - *Sphingomonas paucimobilis*
Polyhydroxybutyrate - *Ralstonia eutropha*
Pullulan - *Aureobasidium pullulans*
Scleroglucan - *Sclerotium rolfsii*
Xanthan - *Xanthomonas campestris*

Single cell protein

Methylococcus capsulatus
Candida utilis
Paecilomyces variotii
Saccharomyces cerevisiae
Fusarium venenatum
Methylophilus methylotrophus
Kluyveromyces marxianus

Isolation and Screening of Microorganisms

The success of an industrial fermentation process chiefly depends on the microorganism strain used. An ideal producer or economically important strain should have the following characteristics.

1. It should be pure, and free from phage
2. It should be genetically stable, but amenable to genetic modification
3. It should produce both vegetative cells and spores; species producing only mycelium are rarely used
4. It should grow vigorously after inoculation in seed stage vessels
5. Should produce a single valuable product, and no toxic by-products
6. Product should be produced in a short time, e.g., 3 days
7. It should be amenable to long term conservation
8. The risk of contamination should be minimal under the optimum performance conditions

Isolation of Microorganisms

The first step in developing a producer strain is the isolation of concerned microorganisms from their natural habitats. Strategies that are adopted for the isolation of suitable industrial microorganisms from the environment can be divided into 2 types (i) shotgun and (ii) objective.

In shotgun approach, samples of free living microorganisms, biofilms and other microbial communities are collected from animal and plant material, soil, sewage, water and waste stream, particularly from unusual man-mad and natural habitats. These isolates are screened for desirable traits. The alternative

is to take a more objective approach by sampling from specific sites where organisms with the desired characteristics are considered to be likely components of the natural microflora.

Alternatively, microorganisms can be obtained as pure cultures from organization, which maintain culture collections.

Culture collection	Type of microorganisms held
American Type Culture Collection (ATCC)	All
European Collection of Animal Cell Collection (ECACC)	Animal cell cultures
National Collection of Food Bacteria (NCFB)	Food bacteria
National Collection of Industrial and Marine Bacteria (NCIMB)	Industrial and Marine bacteria
National Collection of Type Cultures (NCTC)	Medical microorganisms
National Collection of Yeast Cultures (NCYC)	Yeast
National Collection of Pathogenic Fungi (NCPF)	Pathogenic fungi
National Collection of Plant Pathogenic Bacteria (NCPF)	Plant pathogenic bacteria
Culture Collection of Algae and Protozoa (Marine) (CCAP)	Algae and Protozoa (Marine)
Culture Collection of Algae and Protozoa (Fresh water) (CCAP)	Algae and Protozoa (Fresh water)

The microorganisms of industrial importance are, generally, bacteria, actinomycetes, fungi and algae. These organisms occur virtually everywhere, e.g., in air, water, soil, surfaces of plants and animals, and plant and animals tissues. But most common sources of industrial microorganisms are soils, and lake and river mud. Often the ecological habitat from which a desired microorganism is more likely to be isolated will depend on the characteristics of the product desired from it, and of process development. For example, if the objective is to isolate a source of enzymes, which can withstand high temperatures, the obvious place to look will be hot water springs.

A variety of complex isolation procedures have been developed, but no single method can reveal all the microorganisms present in a sample. Many different microorganisms can be isolated by using specialized enrichment techniques, e.g., soil treatment (UV irradiation, air drying or heating at 70 – 120 °C, filtration or continuous percolation, washings from root systems, treatment with detergents or alcohols, pre-inoculation with toxic agents), selective inhibitors (antimetabolites, antibiotics, etc.), nutritional (specific C and N sources), variations in pH, temperature, aeration, etc.
The enrichment techniques are designed for selective multiplication of only some of the microorganisms present in a sample. These approaches however take a long time (20 - 40 days), and require considerable labour and money. The main isolation methods used routinely for isolation from soil samples are: sponging (soil directly), dilution, gradient plate, aerosol dilution, flotation, and differential centrifugation. Often these methods are used in conjunction with an enrichment technique. Microorganisms are used extensively to provide a vast range of products and services. They have proved to be particularly useful because of the ease of their mass cultivation, speed of growth, use of cheap substrates (which in many cases are wastes) and the diversity of potential products.

The most important factor for the success of any fermentation industry is of a production strain. It is highly desirable to use a production strain with the following four characteristics:

I. It should be a high-yielding strain
II. It should have all adoptable characteristics
III. It should not produce undesirable substances
IV. It should be easily cultivated on a large-scale

Screening techniques

Both detection and isolation of high-yielding species from the natural, such as soil, containing a huge microbial population is called screening. The screening process is generally carried out in two stages: (a) primary screening and (b) secondary screening.

(a) Primary Screening

A set of highly selective procedures, which allows the detection and isolation of microorganisms producing the desired metabolite, constitutes primary screening. The process is time consuming and labour intensive since a large number of isolates have to be screened to identify a few potential ones. However, it is most crucial steep since it eliminates the large number of unwanted isolates, which are either non-producers or producers f known compounds.

(i) Crowded plate technique

The crowded plate technique is the simplest screening technique employed in detecting and isolating antibiotic producing microorganisms.

The soil or other source of microorganisms is diluted only to a cell concentration such that agar plats prepared from these dilutions will be crowded with individual colonies. Colonies producing antibiotic activity a indicated by an area of agar sound the colony that is f of growth of the colonies. Such a colony is sub cultured to a similar medium and purified by staking. This technique is used when we are only interested in finding microorganisms capable of producing an antibiotic without regard to what type of microorganisms may b sensitive to antibiotic.

The crowded plate technique has a limited application, since it merely provides information regarding the inhibitory activity of colony against the unwanted microbes that may be resent by chance on the plate. Therefore the technique has been improved upon by introducing the use of a 'test-organism'. In this process, modified agar plates which give well-isolated colonies after incubation are flooded with a suspension of the test organism. Then the plates are subjected to further incubation to allow the growth of the test organism. The formation of inhibitory zones around certain colonies indicates their antibiotic activity.

(ii) Auxanography technique

This technique is rarely applied for detecting microorganisms able to produce growth factors (e.g. amino acids and vitamins) extracellularly. The two major steps of the technique are as under:

(a) Preparation of First Plate

1. A filter paper strip (15 X 12 cm) is put across the bottom of a petri dish in such a way that the ends pass over the edge of the dish
2. A filter, paper disc of petri dish size is placed over paper strip on the bottom of the dish
3. The nutrient agar (45 °C) is poured on the paper disc in the dish and allowed to solidify
4. Microbial source material such as soil, is subjected to dilution such that aliquots on plating will produce well isolated colonies
5. Plating of aliquots diluted soil sample is done

(b) Preparation of Second Plate

1. A minimal medium lacking the growth factor under consideration is seeded with the test organism

2. Tile seeded medium, is poured on the surface of a fresh petri dish
3. The plate is allowed to set

The agar in the first plate, as prepared in step (a), is carefully and aseptically lifted out with the help of tweezers and a spatula and, placed, without inverting, on the surface of the second plate as prepared in major steps (b). Growth factor produced by colonies present on the surface of the first layer of agar can diffuse into the layer of agar containing the test organism. The stimulated growth, of the test organism around the colonies is an indication that they produce growth factor(s) extracellularly.

(iii) Enriched culture technique

This technique was designed by a soil microbiologist, Martinus Beijerinck, to isolate the desired microorganisms from a heterogeneous microbial population present in soil. Either medium or incubation conditions are adjusted so as to favour the growth of the desired microorganisms. On the other hand, unwanted micro-organisms are eliminated, or develop poorly since they do not find suitable growth conditions in the newly created environment. Today, this technique has become a valuable tool in many screening programmes meant for isolating industrially important strains.

Generally, it consists of the following steps:

1. Nutrient broth containing an unusual substrate (e.g. Cellulose powder) is inoculated with the microbial source material (e.g. soil) and incubated.
2. A small portion of inoculums from step (1) is plated onto a solid medium having the same position. Well isolated colonies appear after incubation.
3. Suspected from plate of step (2) are subcultured on fresh media and they are also subjected to further testing.

An example of screening of enzyme, producing microorganisms may be cited.

Microorganisms excreting alkaline proteases may be detected from the soil as under:

- Selected to serial dilution.
- All soil dilutions are heated at 50 °C for 10 minutes. This treatment kills vegetative cells ,
- The plating of heat treated sample is done by spreading the samples (usually 0·1 ml.) from dilutions on the surface of nutrient agar containing casein at pH 10-12.
- The colonies surrounded by the clear zone are sub-cultured.

(iv) Differential plate technique

The pH indicating dyes may be employed in some screening methods for detecting microorganisms capable of producing organic acids or amines, since a pH indicating dye under colour changes according to its pH. Such dyes (e.g. neutral red, bromophenol blue) are added to the poorly buffered nutrient agar media. The change in the colour of a dye in the vicinity of the colony suggests the capability of colonial cells to produce anionic acids or amines, depending upon the nature of reaction. Such colonies are sub cultured to make stock cultures. Differential plate technique may also be employed in finding out whether microorganisms are capable of certain microbial transformations or not.

The presence of organic acids are also detected by the incorporation of calcium carbonate; indicated by a clear one of dissolved calcium carbonate around the colony.

(b) Secondary Screening

Secondary screening is strictly essential in any systematic screening process intended to isolate industrially useful microorganisms, since primary screening merely allows the detection and isolation of microorganisms that possess potentially interesting industrial application. Moreover, primary screening does not provide much information needed in setting up a new fermentation process. Secondary screening helps in detecting really useful microorganisms in fermentation processes:

The following points associated with the importance of secondary screening

- Provides information whether the product produced by a microorganism is a new one or not which accomplished thin layer, other chromatographic techniques.
- The compound produced by a microorganism under consideration is compared with previously known compounds.
- It gives an idea about the economic position of the fermentation process involving the use of a newly discovered culture. Thus, one may have a comparative study of this process with processes that are already known, so far as the economic status picture is concerned.
- It helps in providing information regarding the product yield potentials of different isolates Thus, this is useful in selecting efficient cultures for the fermentation processes.
- It determines the optimum conditions for growth or accumulation of a product associated with a particular culture.
- It provides information pertaining to the effect of different components of a medium. This is valuable in design in the medium that may be attractive so far as economic consideration is concerned.
- It detects the genetic instability in microbial cultures. This type of information is very important, since microorganisms tending to undergo mutation or alteration in some way may lose their capability for maximum accumulation of the fermentation products.
- It gives information about the number of products produced in a single fermentation additional major or minor products are of distinct value, since their recovery and sale as by-products can markedly improve the economic status of the prime fermentation.
- Chemical, physical and biological properties of a product are also determined during secondary screening. Moreover, it reveals whether a product produced in the culture broth occurs in more than one chemical form.
- It reveals whether the culture is homoferrnentative or hetero fermentative.
- Determination of the structure of the product is done.
- With certain types of products (e.g. antibiotics) determinations of the toxicity for animal, plant and human are made if they are to be therapeutic purposes.

Thus, secondary screening gives answers to many questions that arise during the final sorting out of industrially useful micro-organisms. This is accomplished by performing experiments on **agar plates**, in flasks or small bioreactors containing **liquid media**, or by a combination of these approaches.

A specific example of antibiotic producing *Streptomyces* species may be taken for an understanding of the sequence of events during a screening programme. Those streptomycetes able to produce antibiotics are detected and isolated in a primary screening programme. These streptomycetes exhibiting antimicrobial activity are subjected to an initial secondary screening where their inhibition spectra are determined. A simple, giant-colony', technique is used to do this. Each of the streptomycal isolates is streaked in a narrow band across the centres of the nutritious agar plates. Then, these plates are incubated until growth of a

streptomycete occurs. Now, the test organisms are streaked from the edges of the plates but not touching the streptomycete growth. Again, the plates are incubated. At the end of incubation, growth inhibitory zones for each test organism are measured in millimeters. Thus, the microbial inhibition spectrum helps in discarding poor cultures. Ultimately, streptomycete isolates that have exhibited interesting microbial inhibition spectra need further testing. With streptomycetes suspected to produce antibiotics with poor solubility in water, the initial secondary screening is done/in some different way which is out of the scope of this book.

Further screening is carried out employing liquid media in flasks since, such studies give more information than that which can be obtained on agar media. At the same time, it is advisable to use accurate assay techniques (e.g. paper disc-agar diffusion assay) to exactly determine the amounts of antibiotic present in samples of culture fluids. Thus each of the streptomycete isolates is studied by using several different liquid media in Erlenmeyer flasks provided with baffles. These streptomycete cultures are inoculated into sterilized liquid media. Then, such seeded flasks are incubated at a constant temperature. Usually, such cultures are incubated at near room temperature. Moreover, such flasks are aerated by keeping them on a mechanical shaker, since the growth of streptomycetes and production of antibiotics occur better in aerated flasks than in stationary ones. Samples are withdrawn at regular intervals under aseptic conditions and are tested in a quality control laborator. Important tests to be carried out include:

- Checking for contamination
- Checking of pH
- Estimation of critical nutrients
- Assaying of the antibiotic
- Other determinations, if necessary

Strain Improvement

After an organism producing a valuable product is identified, it becomes necessary to increase the product yield from fermentation to minimise production costs. Product yields can be increased by
(i) Developing a suitable medium for fermentation,
(ii) Refining the fermentation process and
(iii) Improving the productivity of the strain

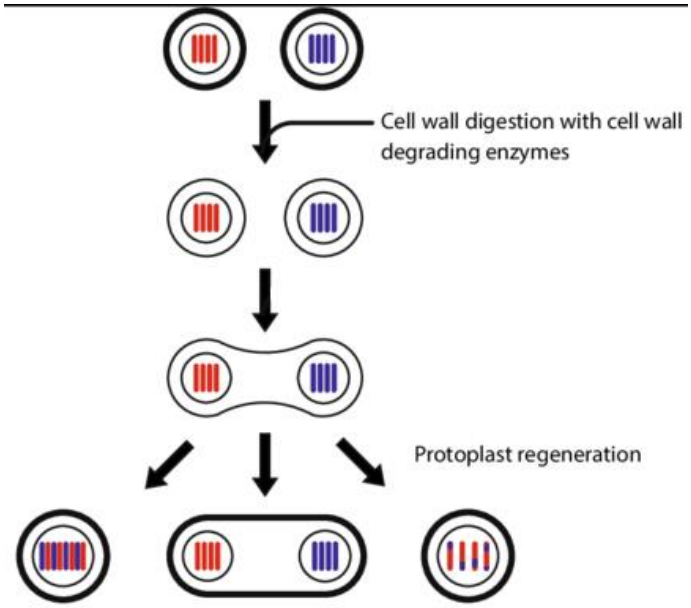
Generally, major improvements arise from the last approach; therefore, all fermentation enterprises place a considerable emphasis on this activity. The techniques and approaches used to genetically modify strains, to increase the production of the desired product are called strain improvement or strain development. Strain improvement is based on the following three approaches:
(i) Protoplast fusion,
(ii) Mutation,
(iii) Recombinant DNA technology

(i) Protoplast fusion

Protoplasts are the cells of which cell walls are removed and cytoplasmic membrane is the outermost layer in such cells. Protoplast can be obtained by specific lytic enzymes (pectinase, cellulase – plants; lysozyme - bacteria; Novozyme 234 - fungi) to remove cell wall. Protoplast fusion is a physical phenomenon, during fusion, two or more protoplasts come in contact and adhere with one another either spontaneously or in presence of fusion inducing agents. By protoplast fusion it is possible to transfer some

useful genes such as disease resistance, nitrogen fixation, rapid growth rate, more product formation rate, protein quality, frost hardiness, drought resistance, herbicide resistance, heat and cold resistance from one species to another.

Protoplast formation in Sterptomyces was first reported by Okanishi and his team in the year 1966. Protoplast fusion has been achieved using filamentous molds, yeasts, Streptomycetes and bacteria.



Methods of protoplast fusion:

Protoplast fusion can be broadly classified into two categories:

(i) Spontaneous fusion: Protoplast during isolation often fuses spontaneously and this phenomenon is called spontaneous fusion. During the enzyme treatment, protoplast from adjoining cells fuses through their plasmodesmata to form multinucleate protoplasts.

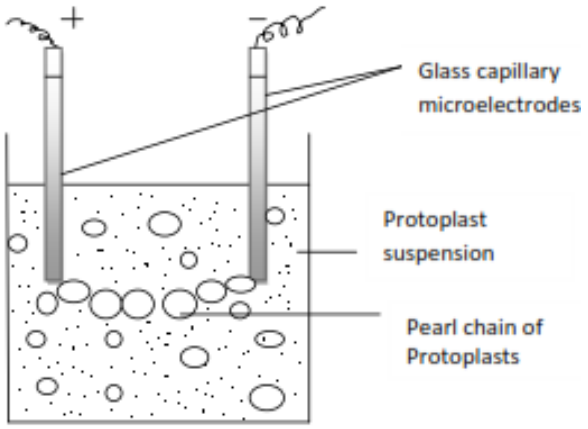
(ii) Induced fusion: Fusion of freely isolated protoplasts from different sources with the help of fusion inducing chemicals agents is known as induced fusion. Normally isolated protoplast do not fuse with each other because the surface of isolated protoplast carries negative charges (-10 mV to -30 mV) around the outside of the plasma membrane, and thus there is a strong tendency in the protoplast to repel each other due to their same charges. So this type of fusion needs a fusion inducing chemicals which actually reduce the electronegativity of the isolated protoplast and allow them to fuse with each others.

The isolated protoplast can be induced to fuse by three ways;

(i) Mechanical fusion: In this process the isolated protoplast are brought into intimate physical contact mechanically under microscope using micromanipulator or perfusion micropipette.

(ii) Chemofusion: Several chemicals have been used to induce protoplast fusion such as sodium nitrate, polyethylene glycol (PEG) and calcium ions (Ca^{2+}). Chemical fusogens cause the isolated protoplast to adhere each other and leads to tight agglutination followed by fusion of protoplast. Chemofusion is a non specific, inexpensive, can cause massive fusion product, can be cytotoxic and non selective and having less fusion frequency.

(iii) Electrofusion: Recently, mild electric stimulation is being used to fuse protoplast. In this two glass capillary microelectrode are placed in contact with the protoplast. An electric field of low strength (10 Kvm^{-1}) gives rise to dielectrophoretic dipole generation within the protoplast suspension. This leads to pearl chain arrangement of protoplasts. Subsequent application of high strength of electric fields (100 Kvm^{-1}) for some microseconds results in electric breakdown of membrane and subsequent fusion.



Protoplast fusion in fungi: Production and regeneration of protoplasts is a useful technique for fungal transformations. Commercial preparation of enzymes which contain mixture of products to digest fungal cell walls used. Novozyme 234 includes enzyme (glucanase and chitinase) mixture is added to rapidly growing fungal tissue suspended in an osmotic buffer (e.g.0.6 mol^{-1} KCl, 1.2 mol^{-1} Sorbitol or 1.2 mol^{-1} $MgSO_4$). The protoplasts and DNA are mixed in presence of 15 % (w/v) PEG 6000 and pH buffer (TRIS HCl). 10 mml^{-1} PEG causes clump formation in protoplasts. At 370 C, grow mycelium on cellophane placed on agar overnight. Incubate with enzyme at 300 C for 1.5 hours in empty Petri plate having KCl, than filter protoplasts, wash protoplast in KCl (Centrifuge and resuspended the pellets). Protoplast fusion frequency in fungi is 0.2 – 2 %.

Protoplast technology for *Streptomyces* species: *Streptomyces* spp also do not have natural means of mating. For obtaining protoplasts from *Streptomyces* lysozyme is used which can break glycan portion of peptidoglycan wall.

Protoplast fusion in bacteria: In bacteria protoplast can be obtained and fusion can be carried out with low frequency in some gram positive organisms. For gram negative bacteria it is possible to obtain protoplast but regeneration is difficult. The procedure is highly efficient and yields up to 80% transformants

(ii) Mutation

Each time a microbial cell divides there is a small probability of an inheritable change occurring. A strain exhibiting such a changed characteristic is termed a mutant and the process giving rise to it, a mutation.

A mutation without a known cause is called Spontaneous mutations. This occurs at low frequency leading to the chemical instability of purine and pyrimidine bases and also due to low level of metabolic errors, or mistakes during the DNA replication. Mutations that results from exposure of organisms to mutagenic agents such as ionizing irradiation, ultraviolet light or various chemicals that react with nucleic acids are called Induced Mutations. Generally, chemical mutagens induce point mutations, whereas ionizing radiations gives rise to large chromosomal abnormalities. Point mutations are simple changes in single base-pairs, the substitution of one base-pair for another, or duplication or deletion of single base-pairs. Point mutations occur at a single point on a chromosome Frame shift mutation, is a kind of mutation caused by the

addition or deletion of nucleotides which is not a multiple of three so that the codon is read incorrectly during translation.

In the early stages, selection of spontaneous mutants may be helpful, but induced mutations are the most common sources of improvements. The probability of a mutation occurring may be increased by exposing the culture to a mutagenic agent (physical mutagens are UV rays, X-rays and gamma rays; Chemical mutagens are nitro methyl guanidine, nitrous acid, N-nitroso guanidine, ethyl methyl sulphonate, ethidium bromide etc.). Such an exposure usually involves subjecting the population to a mutagen dose which results in the death of the vast majority of the cells. The survivors of the mutagen exposure may then contain some mutants, the vast majority of which will produce lower levels of the desired product. However, a very small proportion of the survivors may be improved producers. Thus, it is the task of the industrial geneticist to separate the desirable mutants (the superior producers) from the very many inferior types. This approach is easier for strains producing primary metabolites than it is for those producing secondary metabolites, as may be seen from the following examples.

The synthesis of a primary microbial metabolite (such as an amino acid) is controlled such that it is only produced at a level required by the organism. The control mechanisms involved are the inhibition of enzyme activity and the repression of enzyme synthesis by the end product when it is present in the cell at a sufficient concentration. Thus, these mechanisms are referred to as feedback control. It is obvious that a good 'commercial' mutant should lack the control systems so that 'overproduction' of the end product will result.

Removal of feedback control: The isolation of mutants of *Corynebacterium glutamicum* capable of producing lysine will be used to illustrate the approaches which have been adopted to remove the control systems. The control of lysine synthesis in C. *glutamicum* which it may be seen that the first enzyme in the pathway, aspartokinase, is inhibited only when both lysine and threonine are synthesized above a threshold level. This type of control is referred to as concerted feedback control. A mutant who could not catalyse the conversion of aspartic semialdehyde into homoserine would be capable of growth only in a homoserine-supplemented medium and the organism would be described as a homoserine auxotroph. If such an organism were grown in the presence of very low concentrations of homoserine the endogenous level of threonine would not reach the inhibitory level for aspartokinase control and, thus, aspartate would be converted into lysine which would accumulate in the medium. Thus, aknowledge of the control of the biosynthetic pathway allows a 'blueprint' of the desirable mutant to be constructed and makes easier the task of designing the procedure to isolate the desired type from the other survivors of a mutation treatment.

Approach	Chief feature	Example/Remark
Mutation		The main approach to strain improvement; produces new alleles of existing genes
Spontaneous Mutations	Occur without any treatment with a mutagen	Used in, the initial stages of strain improvement; also for maintenance of improved strains
Induced Mutations	Induced by chemical (mainly) or physical mutagens	Mutagenesis followed by selection; several cycles employed
Major Mutations	Affect the pattern of metabolite production	Production of 6-demethyl tetracycline in place of tetracycline by *S. aureofaciens*
Minor Mutations	Affect the rate metabolite production	Small gains in each cycle of selection; substantial improvement after several cycles

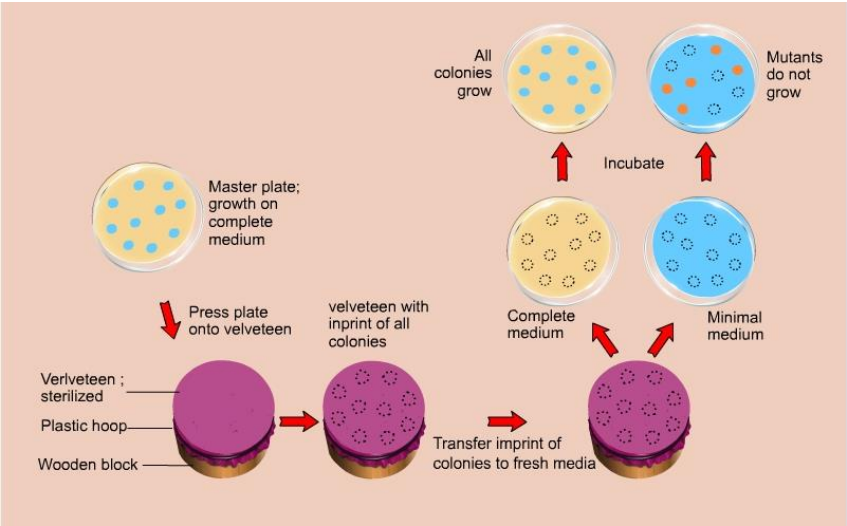
Many mutations bring about marked changes in a biochemical character of practical interest; these are called major mutations. Some major mutations can be useful in strain improvement.

For example, a mutant strain (S-604) of *Streptomyces aureofaciens* produces 6-demethyl tetracycline in place of tetracycline; this demethylated form of tetracycline is the major commercial form of tetracycline. In contrast, most improvements in biochemical production have been due to the stepwise accumulation of so called minor genes. These genes lead to small increases (or decreases) in the antibiotic or other biochemical production, and selection may be expected to result in a 10-15% increase in yield. The selected strains are usually subjected to successive cycles of mutagenesis and selection; after several cycles, a large increase is yield is likely to be obtained. Mutants of *Penicillium chrysogenum* were selected for increased penicillin production; each cycle of selection was preceded by mutagen (chemical) treatment and resulted in only small changes in penicillin yield.

A majority of desirable mutants, especially the 'minor gene' mutants, showing increased production are isolated by screening a large number of clones surviving the mutagen treatment; this is called secondary screening. But this approach requires a large amount of work. Therefore, efforts have increasingly focused on developing techniques for the isolation of particular classes of mutants, which are likely to be overproducers. Selective isolation of mutants is carried out using following techniques:

- Replica plating method: This technique is used to isolate auxotrophic mutants.

In this procedure, mutagenized culture (which is a mixture of both mutated and non-mutated bacteria) is spread on an agar plate which acts as a medium of growth for the bacteria. After incubation for some time, both the parent and mutant bacteria begin to grow. This is called the master plate. Using a sterile velvet pad, colonies are transferred from the master plate on to secondary plates (replica plating) that contain media that are capable of sustaining only mutants, and not the non-mutants. After further incubation, replica plates are compared with the master plates, which allows for the identification and isolation of mutant bacteria from the replica plate. In some cases, it may so happen that only non-mutants are able to grow on the replica plate, and not the mutants. This too helps in the identification of mutants.

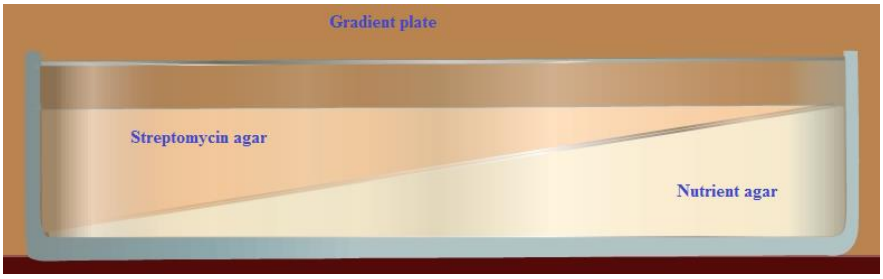


- Penicillin Enrichment Technique:

The isolation of bacterial auxotrophs may be achieved using the penicillin enrichment. Under normal culture conditions an auxotroph is at a disadvantage compared with the parental (wild-type) cells. However, penicillin only kills growing cells and, therefore, if the survivors of a mutation treatment were cultured in a medium containing penicillin and lacking the growth requirement of the desired mutant only those cells unable to grow would survive, *i.e.* the desired auxotrophs. If the cells were removed from the penicillin broth, washed, and resuspended in a medium containing the requirement of the desired auxotroph then the resulting culture should be rich in the required type.

- Resistance selection method or gradient plate method: This method is useful to isolate antibiotic resistant mutants.

An excellent way to determine the ability of organisms to produce mutants that are resistant to antibiotic is to grow them on a gradient plate of a particular antibiotic. The gradient plate consists of two wedges like layers of media: a bottom layer of plain nutrient agar and top layer of antibiotic with nutrient agar. The antibiotic in the top layer, diffuse into the bottom layer producing a gradient of antibiotic concentration from low to high. A gradient plate is made by using Streptomycin in the medium. *E. coli*, which is normally sensitive to Streptomycin, will be spread over the surface of the plate and incubated for 24 to 72 hours. After incubation colonies will appear on both the gradients. The colonies develop in the high concentration are resistant to the action of Streptomycin, and are considered as Streptomycin resistant mutants. For isolation of antibiotic resistant of gram negative enteric bacteria, the antibiotics commonly used are Rifampicin, Streptomycin, and Erythromycin etc.



- Substrate utilization method: In this method, mutants are isolated using chromogenic substrate.

A synthetic analogue of a natural substrate (food source) which has a dye linked to it is used in the growth medium. For example, the enzyme β-galactosidase, catalyzes the hydrolysis of lactose into its constituent sugars, glucose and galactose. A commonly used chromogenic substrate for β-galactosidase is a special kind of colorless galactose called X-gal, which is a synthetic analogue, containing a dye linked to galactose. The color of the dye is only observed when X-gal is hydrolyzed by β-galactosidase. Bacteria with a functional β-galactosidase gene turn blue on a growth medium containing X-gal, and bacteria that do not produce β-galactosidase stay white.

Recombination (recombinant DNA technology):

Recombination as any process which helps to generate new combinations of genes that were originally present in different individuals. Compared with the use of mutation techniques for the

improvement of industrial strains the use of recombination was fairly limited in the early years of improvement programmes. However, techniques are now widely available which allow the use of recombination as a system of strain improvement. *In vivo* recombination may be achieved in the asexual fungi *(e.g. Penicillium chrysogenum,* used for the commercial production of penicillin) using the parasexual cycle. The technique of protoplast fusion has increased greatly the prospects of combining together characteristics found in different production strains.

The application of *in vitro* recombinant DNA technology to the improvement of secondary metabolite formation is not as developed as it is in the primary metabolite field. However, considerable advances have been made in the genetic manipulation of the streptomycetes and the filamentous fungi and a number of different strategies have been devised for cloning secondary metabolite genes.

By employing restriction endonucleases and ligases, investigators can cut and splice DNA at specific sites. Some endonucleases have the ability to cut precisely and generate what are known as “sticky ends.” When different DNA molecules are cut by the same restriction enzyme, they possess similar sticky ends. Through a form of biological “cut and paste” processes, the lower parts of the one DNA is made to stick well onto the upper part of another DNA. These DNA molecules are later ligated to make hybrid molecules. The ability to cut and paste the DNA molecule is the basis of “genetic engineering.” A useful aspect of this cut and paste process involves the use of plasmid, phage, and other small fragments of DNA (vectors) that are capable of carrying genetic material and inserting it into a host microbe such that the foreign DNA is replicated and expressed in the host. A wide array of techniques can now be combined to isolate, sequence, synthesize, modify, and join fragments of DNA. It is therefore possible to obtain nearly any combination of DNA sequence. The challenges lie in designing sequences that will be functional and useful.

The protocol to modify and improve strains involves the following steps:

a. Isolate the desired gene (DNA fragment) from the donor cells.

b. Isolate the vector (a plasmid or a phage).

c. Cleave the vector, align the donor DNA with the vector, and insert the gene into the vector.

d. Introduce the new plasmid into the host cell by transformation or, if a viral vector is used, by infection.

e. Select the new recombinant strains that express the desired characteristics.

Unit-1; Possible questions

PART –A (1 Mark)

PART-B (2 Marks)

1. Name two important discoveries in field of industrial microbiology.
2. Name any four industrially important strains used in industries.
3. Who is the father of industrial microbiology?
4. Name the strategies adopted for the isolation of industrial microorganisms.
5. Mention any four characteristics of industrially important strains.
6. What are the methods of primary screening of industrial important strains?
7. Mention the importance of secondary screening of industrial important strains?
8. Name the agents used for protoplast fusion.
9. What is techniques for isolation of mutant strains
10. Mention any four advantages of using rDNA technology.

PART-C (8 Marks)

1. Discuss important historical developments in industrial microbiology
2. Explain the primary screening methods for strain isolation.
3. Discuss the importance of secondary screening for strain isolation.
4. How to isolate an antibiotic producing organism?
5. Write notes on strain improvement.

KARPAGAM ACADEMY OF HIGHER EDUCATION

CLASS: II B.Sc MB COURSE NAME: INDUSTRIAL MICROBIOLOGY

COURSE CODE: 17MBU303 UNIT: I BATCH-2017-2020

Sl. No	Question	Option A	Option B	Option C	Option D	Correct Ans
1	The first observations of microorganisms by Leeuwenhoek were published in _____.	1677	1673	1667	1679	1677
2	_____ made an important discovery that fermentation takes place in absence of oxygen.	Schwann	Pasteur	Koch	Bertholet	Pasteur
3	Which of the following are primary metabolites? (i)Lysine (ii)Gibberlin (iii)Vitamin B_{12} and (iv)Streptomycein	(i) and (iv)	(iii) and (ii)	(i) and (iii)	(ii) and (iv)	(i) and (iii)
4	In _____, Alexander Fleming accidentally discovered penicillin produced by *Penicillium* growing as contaminant in a Petri plate of *Staphylococcus.*	1925	1929	1927	1935	1929
5	Lysine and riboflavin a _____ metabolites.	Primary	Secondary	Tertiary	Quaternary	Primary
6	Gresofulvin is an _____.	Growth hormone	Antibiotic	Nutritional additive	Solvent	Antibiotic
7	Which of the following is not a type of protoplast fusion?	Biochemical fusion	Chemofusion	Mechanical fusion	Electrofusion	Biochemical fusion
8	A mutant strain (S-604) of *Streptomyces aureofaciens* produces _____ in place of tetracycline.	6-demethyl tetracycline	2-demethyl tetracycline	4-demethyl tetracycline	8-demethyl tetracycline	6-demethyl tetracycline
9	Industrial microbiology deals with areas of microbiology involving economic aspects, where valuable parts are prepared.	From costly substrates	From cheaper and disposable substrates	From unavailable substrates	From foreign countries	From cheaper and disposable substrates
10	Protoplast of fungi can be obtained by treating with _____.	Lysozyme	Cellulase	Glucanase and chitinase	Pectinase	Glucanase and chitinase
11	Strategies that are adopted for the isolation of suitable industrial microorganisms from the	Shotgun and objective	Shotgun and instinctive	Random and objective	Instinctive and objective	Shotgun and objective

	environment can be divided into _____.					
12	Citric acid is produced by _____	*Corynebacterium glutamicum*	*Aspergillus niger*	*Clostridium acetobutylicum*	*Pseudomonas denitrificans*	*Aspergillus niger*
13	Vit B_{12} is produced by _____	*Corynebacterium glutamicum*	*Aspergillus niger*	*Clostridium acetobutylicum*	*Pseudomonas denitrificans*	*Pseudomonas denitrificans*
14	Protoplast of plants can be obtained by treating with _____.	Novozyme 234	Lysozyme	Pectinase, cellulase	Glucanase	Pectinase, cellulase
15	Protoplast formation in Sterptomyces was first reported by _____ and his team in the year 1966.	Watsmann	Walksman	Websmann	Okanishi	Okanishi
16	Which of the following is used as single cell protein?	*Spirogyr*	*Acetabularia*	*Nostoc*	*Spirulina*	*Spirulina*
17	_____ is used for the strain improvement.	Diamines	Nitrogen oxide	Gamma rays	Organic acids	Gamma rays
18	_____agent is used for the inducing the protoplast fusion	Glycerol	Epichlorohydrin	Polyvinyl chloride	Poly ethylene glycol	Poly ethylene glycol
19	The chemical agent which causes mutation is _____.	Sodium sulfate	Ethyl sulfonate	Nitro methyl guanidine	Calcium Chloride	Nitro methyl guanidine
20	Protoplast fusion is used to improve _____.	Yeast cells	Fungi	Yeast cells	All the above	All the above
21	Which of the following is involved in the preservation and maintenance of industrially important bacteria?	NCIMB	NCPF	ECACC	NCTC	NCIMB
22	Indicator dyes are used in the medium to detect _____.	Moisture availability	Nutrient concentration	Temperature change	Ph change	pH change
23	Gibberelin is produced by _____.	*P. Griseofulvin*	*Ashbya gossypii*	*Aspergillus niger*	*Gibberella fujikuroi*	*Gibberella fujikuroi*
24	The phase at which primary metabolites are produced is termed as _____.	Idiosphase	Trophase	Idiophase	Tramphase	Trophase
25	The phase at which secondary metabolites are	Idiophase	Trophase	Idiosphase	Tramphase	Idiophase

	produced is termed as _____.					
26	In fermentation, yeast converts _____to _____.	Carbohydrate, alcohol,	Fatty acids, alcohol,	Starch, alcohol,	Starch, alcohol,	Sugar, alcohol,
27	which method is most satisfory method for long time preservation of microbes.	Mineral oil overlay	Lyophilisation	Cryopreservation	Periodic transfer	Lyophilisation
28	The major antibiotics such as Steptomycin and Erythromycin were isolated from _____.	*Bacillus sp*	*Staphylococcus sp*	*Streptococcus sp*	*Streptomyces sp*	*Streptomyces sp*
29	Lactic acid organism is a _____.	Fungi	Bacteria	Virus	Protozoa	Bacteria
30	who made an important discovery that fermentation takes place in absence of oxygen.	Schwann	Pasteur	Koch	Bertholet	Pasteur
31	The process of sterilization of wine introduced by Pasteur is called _____.	Pasteurization	Ultrafiltration	Low temperature,high holding time	High temperature,low holding time	Pasteurization
32	The acetone-butanol fermentation is also called as _____ process.	Watsmann	Walksman	Websmann	Weizmann	Weizmann
33	The important quality of production strain is _____.	Should be a high yielding strain	Unstable biochemical characteristics	Produce underisable substances	Not easily cultivate	Should be a high yielding strain
34	The screening techniques involve _____ and _____.	Primary and secondary	Secondary and tertiary	Primary and quartenary	Secondary and quartenary	Primary and secondary
35	Primary screening technology involves the isolation of new microbial species exhibiting the _____.	Desined color	Desired shape	Desired quality	Desired property	Desired property
36	Crowded plate technique is an example of _____screening.	Primary	Secondary	Tertiary	Quartenary	Primary
37	The primary screening technique which is employed for a detect and isolating antibiotic producing strain is _____.	Crowded plate technique	Auxanography	Enrichment culture technique	Use of indicator dye	Crowded plate technique
38	Enrichment culture technology was designed by soil	Pasteur	Koch	Ehrlich	Beijerinck	Beijerinck

	microbiologist_____.					
39	Which technique is largely employed to identify the growth factor producing strain extracellularly?	Crowded plate technique	Auxanography	Enrichment culture technique	Use of indicator dye	Auxanography
40	Neutral red, bromothymol blue dyes are added to partly buffered nutrient agar media to detect microorganisms capable of producing _____.	Vitamins	Growth factors	Organic acids	Amines	Organic acids
41	Which screening helps in segregation microbe that has real potential in fermentation industry?	Primary	Secondary	Tertiary	Quartenary	Secondary
42	Bread, beer, wine and spirits are produced by _____.	*Bacillus sp*	*Saccharomyces cerevisiae*	*Aspergillus niger*	*Clostridium acetobutylicum*	*Saccharomyces cerevisiae*
43	The fermentation product produced by the identified industrial strain should be _____.	Old	Novel	Gold	Critical	Novel
44	The selected industrial strain is _____ by secondary screening.	Optimized	Priotized	Compared	Deselected	Optimized
45	The important criteria in handling the industrially productive strain are to prevent _____.	Stability	Contamination	Oxidation	Reduction	Contamination
46	Mutation is done by_____ and _____methods.	Physical and chemical	Chemical and political	Physical and botanical	Chemical and zoological	Physical and chemical
47	Secondary screening reveals whether the culture is homofermentative and _____.	Zygofermentative	Heterofermentative	Perifermentative	Afermentative	Heterofermentative
48	An ideal producer or economically important strain should be _____.	Pure, and free from phage	Grow vigorously	Amenable to genetic modification	All the above	All the above
49	The lysine biosynthesis, the end products lysine and threonine inhibit _____ enzyme.	Aspartate kinase	Homoserine phosphatase	Serine kinase	Tryptophan synthase	Aspartate kinase
50	Alexander Fleming accidentally discovered penicillin produced by *Penicillium* growing as	*Streptococcus*	*Staphylococcus*	*Clostridium*	*Klebsiella*	*Staphylococcus*

	contaminant in a petri plate of _____.					
51	In---------- Weizmann isolated *Clostridium acetobutylicum*, a bacterium that used inexpensive starch to produce high yield of butyl alcohol and acetone.	1916	1913	1914	1918	1914
52	The wild strain of *Coryrebacterium glutamicus* secretes both _____ and _____.	Lysine and threonine	Lysine and methionine	Threonine and methionine	Threonine and pectin	Lysine and threonine
53	The father of modern fermentation technology is however _____.	Louis pasteur	Winogradsky	Beijerinck	Wildiers	Louis Pasteur
54	In Europe, allowing soil bacteria to nitrify ammonia in horse urine produced _____ required for gun powder manufacture.	Ammonia	Urea	Nitrates	Nitrites	Nitrates
55	Microbes are grown in especially designed vessels called _____, containing special media for its growth.	Fermentors	Batch cookers	Swap medium	Conical flasks	Fermentors
56	Secondary metabolites are produced during _____.	Lag phase	Log phase	Stationary phase	Death phase	Stationary phase
57	Wine is produced from _____.	Malt	Molasses	Grapes	Sugarcane	Grapes
58	The great breakthrough came with observation of-- _____ under simple microscope by Anton Van Leewanhoek.	Plant	Animals	Microbes	God	Microbes
59	The Aztecs of Mexico cultured _____ for both waste treatment purposes and as a source of protein.	*Cyanobacteria*	*Spirulina*	*Spirogyra*	*Nostoc*	*Spirulina*
60	Calcium carbonate is added to partly buffered nutrient agar media to detect microorganisms capable of producing _____.	Vitamins	Growth factors	Organic acids	Amines	Organic acids

Unit II

Syllabus

Preservation and maintenance of industrial strains. Media formulation (molasses, corn- steep liquor, sulphite waste liquor, whey, yeast extract and protein hydrolysates).

Preservation of Microorganisms

There are different methods for microbial preservation. Suitable methods are selected based on:

- Type of microorganism,
- Effect of the preservation method on the viability of the microorganism,
- Frequency at which the cultures are withdrawn,
- Size of the microbial population to be preserved,
- Availability of resources, and
- Cost of the preservation method.

Many methods of preservation for microorganisms have been developed. Here, it is to be noted that there exist different types of micro-organisms (bacteria, viruses, algae, protozoa, yeasts and molds).

The best known of these are the American Type Culture Collection (A.T.C.C.), founded in 1925 and the collection of the Common wealth Mycological Institute (C.M.I.), founded in 1947. Several other countries are developing their own national collections, and there are large collections belonging to industrial concerns as well as specialized government departments.

There are three basic aims in maintaining and preserving the micro-organisms. They are:

(i) To keep cultures alive

(ii) Uncontaminated, and

(iii) As healthy as possible, both physically and physiologically, preserving their original properties until they are deposited in any major collections (i.e. unchanged in their properties).

- **Serial Subculture:**

This is the simplest and most common method of maintaining microbial cultures. Microbes are grown on slants and are transferred to fresh media before they exhaust all the nutrients or dry out. The drying-up of the medium appeared to encourage good sporulation and the preserved specimen became simply a dried out strand of agar coated with spores which remained viable for a few years at room temperature. For some microbial cultures, no other methods have been found satisfactory, but for the majority of species other methods are available.

There are several factors to be borne in mind while choosing a suitable medium. Solid media should be chosen in preference to liquid media, as growth of a contaminant can be more readily observed.

The time period appropriate for subculture may range from a week to even a few years Under normal conditions cultures have to be re-grown at fairly frequent intervals (e.g. every four, six or eight weeks). With a large collection, this requires much labour. Moreover, there is a risk of occurring hazards, every time a culture is handled. To cut down the frequency of handling of the cultures, it is, therefore desirable to prolong the intervals between subculturing. There are various means to accomplish this (e.g. cold storage and mineral oil storage).

- **Preservation by overlaying cultures with oil:**

This method of preservation is a modification of serial subculture technique. It was first extensively used by Buell and Weston (1947).

This method is cheap and easy, since it does not require special skills or apparatus such as a centri fuge, dessiccator, or vacuum pump. The steps involved in this method are:

i. First of all, inoculation of the agar slant contained in a screw-cap tube with a given culture is practised.
ii. Inoculated agar slant is subjected to incubation until good growth appears.
iii. Using sterial technique, a healthy agar slant culture (from above step) is covered with sterile mineral oil to a depth of about 1 cm above the top of the agar slant. If a short slant of agar is used, less oil is required.
iv. Finally, oiled culture from step (iii) can be stored at room temperature. But better viability is obtained when stored at lower temperatures.

The oil used should be of good quality. British pharmacopoeia medicinal paraffin oil of specific gravity 0·830 to 0·890 is quite satisfactory. Sterilization of oil at the C.M.I is done in McCartney bottles for 15 minutes at 15Ib/in^2

The covering of the culture with oil prevents drying out. The oil allows slow diffusion of gases so growth continues at a reduced rate. This may induce change due to adaption to growth in oil. Some fungus isolates appear stable and survivals of over many years have been obtained at the C.M.I. Others change rapidly, producing a typical culture in a few months (e.g. *Fusarium* species). If the McCartney bottles are used the rubber liners should be removed from the metal caps as the oil tends to dissolve the rubber and this can be toxic to the cultures.

This method has the following advantages:

i. Practically all bacterial species or strains tested live longer under oil than in the control tubes without oil. Some bacterial species have been preserved satisfactorily for many years.
ii. Transplants may be prepared when desired without affecting the preservation of the stock cultures.
iii. The method is especially advantageous when working with unstable variants where occasional transfers to fresh media or growth in mass cultures results in changes in the developmental stages of the strains.
iv. This method also appears to be an ideal method of storage for a busy laboratory with limited funds and a relatively small collection.

- **Lyophilization or freeze-drying:**

Lyophilization is the most satisfactory method of long-term preservation of microorganisms. It is universally used for the preservation of bacteria, viruses, fungi, sera, toxins, enzymes and other biological materials. The process of lyophilization was first applied to microfungi on a large scale by Raper and Alexander in 1945. Lyophilization is perhaps the most popular form of suspended metabolism. It consists of drying cultures or a spore suspension from the frozen state under reduced pressure.

Major steps involved in this technique are:

(i) A cell or spore suspension is prepared in a suitable protective medium

(ii) Using a sterile technique, the suspension from (i) is distributed in small quantities into glass ampoules.

(iii) The ampoules are connected with a high vacuum system usually incorporating a desiccant (e.g. phosphorous pentoxide, silica gel or a freezing trap), and immersed into a freezing mixture of dry ice and alcohol (-70 to -78 °C).

(iv) The vacuum pump is turned on and the ampoules are evacuated till drying is complete, after which they may be sealed off.

Factors affecting the viability of freeze-dried cultures include:
(i) Chemical composition of the protective (suspending) medium;
(ii) Addition of certain compounds to the culture suspension before freeze-drying to give protection to the culture against the toxicity exerted by moisture and oxygen when stored in unsealed ampoules;
(iii) Sealing the ampoules after freeze-drying to stop access of oxygen and moisture;
(iv) Insufficient elimination of oxygen and moisture on the survival rate of freeze-dried culture;
(v) Storage temperature of freeze-dried and sealed cultures.

This method possesses the following advantages:

i. As the ampoules are sealed there is no risk of contamination or infection with mites.
ii. The prepared ampoules are easily stored, they are not readily broken and most species
iii. There is less opportunity for cultures to undergo changes in characteristics (i.e. they remain unchanged during storage period).
iv. Owing to the small size of glass ampoules, hundreds of lyophilized cultures can be stored in a small storage space. In addition to this, the ampoules' small size makes them ideal for postage. It remains viable for many years (more than 20 years in case of many bacterial species).

Checking of viability may be done at long intervals. This may be done when cultures are required or by routine sampling. Usually, many replicates are made so that the material from a constant source can be supplied over a considerable period. It makes the 'Seed Stock' system possible, which is used at the American Type Culture Collection for conserving living reference microbes over long periods.

- **Storage at very low temperatures or Nitrogen Storage**

This method is also called *cryogenic storage*. It is like lyophilization, a satisfactory method for the long-term preservation of microorganisms. The maintenance of microbes is done by suspended metabolism.

Major steps involved in the performance of this method are:
i) The culture is suspended as a cell or a spore suspension, as finely broken-up particles of mycelium, or as a piece of fungus mycelium in a suitable suspending medium (dimethyl sulfoxide / glycerol).
(ii) The suspension as prepared in step (i) is distributed into ampoules (These must be resistant to cold-shock). The manufacturers of ampoules warn us that they must not be overfilled (less than 0·5 ml, being recommended).
(iii) Ampoules filled with a culture suspension are frozen and are hermetically sealed. Freezing can be attained by plunging the ampoules straight into the liquid nitrogen by suspending them over the liquid nitrogen for a short period and then lowering them into the liquid nitrogen, or by controlled cooling. There are many interacting factors concerning the choice of the method of freezing and it will depend largely on the microorganisms to be frozen, the degree of revival required and the apparatus available.
(iv) The frozen ampoules prepared as in (iii) are usually clipped on metal (aluminium) canes, one above the other and six to each cane. The canes, in turn, are packed in metal boxes or canisters (aluminium), which hold about 20 canes. These are perforated to allow the free running of the liquid nitrogen. The cultures are revived by removing from the container, rapidly thawing and culturing them in the usual way.

The method has the following advantages:

I. It is an effective method of preservation
II. No subculturing is required
III. The cultural characteristics remain unchanged. This could be of particular importance while storing highly specialized strains employed in industrial processes
IV. The ampoules are not open to contamination or infection by mites, since they are sealed
V. The living material of a type which would not normally grow in a culture and would not be preserved in a culture collection can be retained in a viable state
VI. The method has also some disadvantages. They are:

- The method is expensive, since a costly apparatus is required
- A reliable supply of nitrogen is needed
- There is a possibility of a minor accident, since the method involves the use of explosive gas (the liquid nitrogen)

Other Methods or Storage for Fungi

Every laboratory cannot afford lyophilization or liquid nitrogen storage. Many technicians lack the time for the periodic transfer to fresh media and they would find oil storage messy. Indeed, in these days, when the safety of the personnel is the first consideration, the spatter made by oily needles when they are sterilized is undesirable. Therefore, various other methods of preservations nave been developed.

- **Soil cultures:** This method is particularly applied for the preservation of sporing microbes (e.g. *Bacillus, Penicillium, Aspergillus* and *Streptomyces).*

Steps involved in this method are listed:

(i) A spore suspension is first prepared which may involve the use of a special medium.
(ii) A mixture of soil (20 %), sand (78 %) and calcium carbonate (2 %) is prepared and distributed into tubes (a few grams per tube). They are sterilized for 8 to 15 hours at 130 °C and then cooled.
(iii) A spore suspension is added to the sterilized loam as prepared in step (ii) and allowed to grow for about 10 days.
(iv) The inoculated tubes as in step (iii) are kept in desiccators under vacuum. The reason behind this is to evaporate the excess water. Then the tubes are sealed.
(v) The culture tubes are stored in a refrigerator at about 5 to 8 °C.

- **Desiccation**

This involves removal of water from the culture. Desiccation is used to preserve actinomycetes **(**a form of fungi-like bacteria) for very long period of time. The microorganisms can be preserved by desiccating on sand, silica gel, or paper strips.

Culture Media - Inoculum preparation media are quite different from production media. These media are designed for rapid microbial growth, and little or no product accumulation will normally occur.

Bioreactor Media - The medium composition is as critical to product yields as high producing strains of microorganisms. The medium not only provides the nutrients needed for microbial growth but also for the metabolite production. The organisms vary in their nutrient requirements from autotrophs, which produce all the biochemicals

required from simple inorganic nutrients deriving their energy from oxidation of some inorganic component of the medium to the difficult organisms like lactic acid bacteria, which require many organic compounds for their growth.

The various media may be grouped into two broad categories:
(i) synthetic and
(ii) complex / crude

A synthetic or chemically defined medium is desirable for various studies, but product yields from such media are generally low with low foam. The complex media contain undefined constituents like soybean meal, molasses, corn steep liquor, etc., and give much higher yields of metabolites.

Carbon source can be simple, e.g., sugar, alcohol, etc., or complex carbohydrates, proteins, molasses, potatoes, sweet potatoes, etc. In many processes, precursors need to be provided, e.g., phenylacetic acid for penicillin G, inorganic cobalt for vit. B12. Buffers are also added to prevent drastic changes in pH, and anti foam would often be needed when complex media are used. For much fermentation, e.g., antibiotic production, medium suited for rapid cell growth is unsuitable for product formation. In such cases, specialized media for production have to be devised.

Media for Industrial fermentation process

Fermentation Medium: Most of the fermentation process requires liquid media, often referred to as broth, although some solid-substrate fermentations are operated.

Media formulation: All microbes require water, sources of carbon, mineral elements and probably vitamin and oxygen if aerobic on a large scale; manufacturers normally use sources of cheap nutrient to make a medium which will need the following criteria.
(i) It must produce the maximum yield of products or biomass per gram of substrate used.
(ii) It must permit the maximum rate of product formation.
(iii) The yield of undesirable products must be minimal.
(iv) It must be cheap and of consistent quality.
(v) It must cause minimum problems in the production process particularly other aspects as aeration, purification extraction and waste management.

Constituents of medium

1. Water
2. Carbon source / Nitrogen source / Sources of phosphorous and sulfur / Minor and trace elements / Vitamins such as biotin and riboflavin
3. Oxygen: even some anaerobic fermentations require initial aeration, e.g. beer fermentations
4. Buffers or controlled by acid and alkali additions
5. Antifoam agents
6. Precursor, inducer or inhibitor compounds

Considerations in media design:

Nutritional Requirements, Environmental Requirements and Techno-economic Factors.

<u>Environmental requirements</u>

1. Effect of growth temperature on cell yield / below optimal temperature for growth.

2. Environmental effect of substrate.

Techno-economic factors that affect the choice of individual raw materials

- Cost: transport and storage, e.g. temperature control
- Availability: consistent quality and year round availability
- Ease of handling: solid or liquid forms
- Sterilization: thermal damage and inhibitory byproduct
- Operational characteristics: formulation, mixing, complexing and viscosity characteristics that may influence agitation, aeration, foaming and recovery
- Supply: the concentration of target product attained, its rate of formation and yield per gram of substrate utilized
- Purification: levels and range of impurities, potential for generating undesired products

Major Carbon sources

➢ **Molasses**

1. Byproduct of cane or beet sugar production / residues remaining after most of the sucrose has been crystallized from the plant extract
2. Dark colored viscous syrup containing 50-60% (w/v) carbohydrate, primarily sucrose, with 2% (w/v) nitrogenous substances, along with some vitamins and minerals.
3. Overall composition varies depending upon the plant source, the location of the crop, the climatic conditions under which it was grown, and the factory where it was processed
4. Hydrol molasses, containing primarily glucose, is a byproduct of maize starch processing

➢ **Malt extract**

1. Concentrated aqueous extracts of malted barley to form syrups; particularly useful for the cultivation of filamentous fungi, yeasts and actinomycetes.
2. App. 90% carbohydrate (w/w) and some vitamins and app. 5% nitrogenous substances, proteins, peptides and amino acids / carbohydrate comprising 20% hexoses (glucose and small amounts of fructose), 55% disaccharides (maltose and traces of sucrose), 10% maltotriose, and additionally contain 15-20% branched and unbranched dextrins.

➢ **Starch and dextrin**

1. Can be directly metabolized by amylase-producing microorganisms, particularly filamentous fungi. Maize starch is most widely used.
2. To allow use in a wide range of fermentations, the starch is usually converted into sugar syrup, containing mostly glucose. It is first gelatinized and then hydrolyzed by dilute acids or amylolytic enzymes, often microbial gluco-amylases that operate at elevated temperatures.

➢ **Sulphite waste liquor**

1. Sugar containing wastes derived from the paper pulping industry are primarily used for the cultivation of yeasts
2. Waste liquors from coniferous trees contain 2-3% (w/v) sugar, 80% hexoses (glucose, mannose and galactose) and 20% pentoses (mostly xylose and arabinose) / Liquors derived from deciduous trees contain mainly pentoses

➢ **Cellulose**

1. Predominantly as lignocellulose (composed of cellulose, hemicellulose and lignin); Available from agricultural, forestry, industrial and domestic wastes
2. Relatively few microorganisms can utilize it directly / The cellulose component is in part crystalline, encrusted with lignin, and provides little surface area for enzyme attack
3. At present, mainly used in solid-substrate fermentations (e.g. mushrooms)

➢ **Whey**

1. An aqueous by-product of the dairy industry / Annual worldwide production is over 80 million tons, containing over 1 million tons of lactose and 0.2 million tons of milk proteins.
2. Expensive to store and transport / Lactose concentrates are often prepared for later fermentation by evaporation of the whey, following removal of milk proteins for use as food supplements.
3. Formerly used extensively in penicillin fermentation / still employed for producing ethanol, single cell protein, lactic acid, xanthan gum, vitamin B_{12} and gibberellic acid.

➢ **Fats and oils**

1. Hard animal fats (composed mainly of glycerides of palmitic and stearic acids) are rarely used in fermentation.
2. Plant oils (primarily from cotton seed, linseed, maize, olive, palm, rape seed and soy) and occasionally fish oil, may be used as the primary or supplementary carbon source, especially in antibiotic production. Plant oils are mostly composed of oleic and linoleic acids, but linseed and soy oil also have a substantial amount of linolenic acid.

Water

1. Used for media, cleaning, cooling purposes
2. A reliable source of large quantities of clean water, of consistent composition is essential
3. Before use, removal of suspended solids, iron, chlorine colloids and microorganisms is usually required
4. Water is becoming increasingly expensive / recycle / reuse wherever possible / minimizes water costs and reduces the volume requiring waste-water treatment

Special compounds

1. Precursors: Phenylacetic acid or phenylacetamide as side-chain precursors in penicillin production / D-threonine in L-isoleucine production by *Serratia marsescens* / anthranillic acid for L-tryptophan production by yeast *Hansenula anomala*
2. Inducers: Inducers are often necessary for genetically modified microorganisms (GMMs)
3. Inhibitors: Used to redirect metabolism towards the target product and reduce formation of other metabolic intermediates (example: sodium bisulphite in production of glycerol by *S. cerevisiae*)

Antifoams: Chemicals controlling foams have been classified into antifoams or defoamers. Most of the media used in culturing organisms contain protein which is susceptible to foam formation due to the fine bubble which easily induce foam.

One possibility is to ensure that there:

(1) Sufficient space available in the fermenter for the foam produced. However this reduces the effective volume of the fermenter as well as the additional changes of contamination. Foaming can also be hindered/counteracted using chemical & mechanical measures.

(a) Chemical antifoam agents such as animal vegetable oil, mineral oil, white oil, polydimthylsiloxane etc,. reduce the surface tension of the broth and at the same time they reduce the solubility of oxygen which in turn affects the aeration requirement. It may also make downstream processing more difficult.
(b) Mechanical defoamers can be employed instead of chemical agents. Mechanical elements mounted on the agitation shaft are that it will affect the speed of rotation for effective defoaming.

Properties of Antifoams:

1. Readily and rapidly dispersed with rapid action
2. High activity at low concentration
3. Prolonged action
4. Non-toxic to fermentation microorganisms, humans or animals
5. Low cost
6. Thermostable
7. Compatibility with other media components and the process.

Major Nitrogen sources

- **Corn steep liquor**

1. By-product of starch extraction from maize / first use in fermentations for penicillin production in the 1940s
2. Exact composition varies depending on the quality of maize and the processing conditions / Concentrated extracts generally contain about 4 % (w/v) nitrogen, including a wide range of amino acids, along with vitamins and minerals / Any residual sugars are usually converted to lactic acid (9-20 % w/v) by contaminating bacteria
3. Can sometimes be replaced by liquor derived from potato starch production

- **Yeast extract - 1**

1. **Yeast extract** is the common name for yeast products made by extracting the cell contents (removing the cell walls); Produced from waste baker's and brewer's yeast, or other strains of *S. cerevisiae* or *Kluyveromyces marxianus* (formerly *K. fragilis*) grown on whey and *Candida utilis* cultivated using ethanol, or wastes from wood and paper processing.
2. Extracts used in the formulation of fermentation media are normally salt-free concentrates of soluble components of hydrolyzed yeast cells / Extracts with sodium chloride concentrations greater than 0.05 % (w/v) cannot be used in fermentation processes due to potential corrosion problems
3. Yeast cell hydrolysis is often achieved by autolysis, which can be initiated by temperature or osmotic shock, causing cells to die but without inactivating their endogenous enzymes.
1. Extracts are available as liquids containing 50-65 % solids, viscous pastes or dry powders; They contain amino acids (35-40 %, w/v), peptides (30-45 %, w/v), water-soluble vitamins and some glucose derived from the yeast storage carbohydrates (trehalose and glycogen)

- **Peptones**

1. Peptones are usually too expensive for large-scale industrial fermentations
2. Prepared by acid or enzyme hydrolysis of high protein materials: meat, casein, gelatin, keratin, peanuts, soy meal, cotton seed, etc.

3. Amino acids compositions vary depending upon the original protein source / Gelatin-derived peptones are rich in proline and hydroxyproline, but almost devoid of sulfur-containing amino acids / Keratin peptone is rich in both proline and cystine, but lacks lysine
4. Plant peptones invariably contain relatively large quantities of carbohydrates

➢ **Soya bean meal**

1. Residuals after extraction of soy oil
2. Composed of 50% protein, 7% non-protein nitrogenous compounds, 30% carbohydrates and 1% oil
3. Often used in antibiotic fermentation because the components are only slowly metabolized, thereby eliminating the possibility of repression of product formation

Preparation of sterilized media

Liquid media which are sterilized in their final containers should be cooled down to room temperature as rapidly as possible. Screw caps should then be tightened. Containers of agar media which have been sterilized should be placed in a 50 °C water bath and the medium dispensed as soon as it reaches this temperature, or within a maximum of 3 hours in the bath. The medium should be mixed thoroughly, without bubble formation and aseptically dispensed into sterile containers. Do not expose dishes of agar media to sunlight; it causes excessive condensation on the lids and may cause the formation of inhibitory substances by photo-oxidation.

Heat-labile supplements should be added to the medium after it has cooled to 50 °C. Allow the sterile supplement to come to room temperature before adding it to the agar medium. Very cold liquids may cause agar to gel or form transparent flakes which can easily be seen e.g. in blood enriched agar. Mix all supplements into the medium gently and thoroughly, then distribute into the final containers as quickly as possible.

Blood used for the preparation of blood agar should be as fresh as possible and should have been stored at 2-8 °C (blood must not be frozen). Warm the blood in a 35 °C incubator before addition to sterile molten agar base, which has been cooled to 40-45 °C. Adequate mixing in a large head-space vessel is essential to ensure aeration of the blood. Poorly oxygenated blood plates are purplish in color whereas properly aerated blood agar is cherry-red. Defibrinated blood is recommended for use rather than blood containing an anticoagulant.

Unit-2; Possible questions

Part A (1 Mark)

Part B (2 Marks)

1. Difference between solid state fermentation and submerged fermentation?
2. How cultures are preserved?
3. Discuss the types of fermentation medium components?
4. Why media formulation is important?1. Write the types of fermentation.
5. What is lag phase?
6. What is batch fermentation?
7. What is continuous stirred tank bioreactor?
8. What is sparger?
9. Write the advantages of continuous stirred tank bioreactor?
10. Define tower bioreactor.
11. What is fluidized bed bioreactor?
12. Write the types of conventional bioreactor.

Part C (8 Marks)

1. What is media formulation and ideal characteristics of fermentation medium?
2. Elaborate on the various carbon sources for fermentation medium.
3. Give a note on lyophilization.
4. Comment on sulphite waste liquor, hydrocarbons and corn steep liquor

KARPAGAM ACADEMY OF HIGHER EDUCATION

CLASS: II B.Sc MB COURSE NAME: INDUSTRIAL MICROBIOLOGY

COURSE CODE: 17MBU303 UNIT: II BATCH-2017-2020

Sl. No	Question	Option A	Option B	Option C	Option D	Correct ans
1	_____first used in fermentations for penicillin production in the 1940s.	Malt extract	Molasses	Corn steep liquor	Sulphite waste liquor	Corn steep liquor
2	Common wealth Mycological Institute (C.M.I.) was founded in _____.	1949	1947	1943	1945	1947
3	The time period appropriate for subculture may range from a _____ to _____ months.	2-3	1-10	13-22	10-12	2-3
4	American Type Culture Collection was founded in _____.	1929	1928	1923	1925	1925
5	Which of the following is NOT an antifoaming agent?	Vegetable oil	Black oil	Mineral oil	White oil	Black oil
6	Corn steep liquor generally contain about ____ % (w/v) nitrogen.	4	6	8	10	4
7	*Candida utilis, S. cerevisiae* or *Kluyveromyces* sp. are used for the production of _____.	Yeast extract	Peptones	Both A and B	None of the above	Yeast extract
8	Sterile mineral oil to a depth of about ____ cm above the top of the agar slant.	1.0	2.0	3.0	2.5	1.0
9	Sterilization of oil at the C.M.I is done in McCartney bottles for _____.	20 minutes at 15 Ib/in^2	15 minutes at 20 Ib/in^2	15 minutes at 15 Ib/in^2	20 minutes at 20 Ib/in^2	15 minutes at 15Ib/in^2
10	Desiccation is used to preserve _____.	Mold	Actinomycetes	Yeast	Bactria	Actinomycetes
11	Chemicals controlling foams have been classified into _____.	Antifoams	Defoamers	All the above	None of the above	All the above
12	Hard animal fats are composed mainly of glycerid of _____ and _____.	Palmitic and linolenic acids	Oleic and stearic acids	Palmitic and stearic acids	Palmitic and oleic acids	Palmitic and stearic acids

13	Soya bean meal is residuals after extraction of	Soy oil	Sugarcane	Maize	Milk	Soy oil
14	Which of the following is NOT major source of nitrogen.	Corn steep liquor	Fats and oils	Peptones	Soybean meal	Fats and oils
15	_____ is composed of 50% protein, 7% no protein nitrogenous compounds, 30% carbohydrat and 1% oil.	Peptones	Yeast extract	Corn steep liquor	Soya bean meal	Soya bean meal
16	_____ is universally used for the preservation of bacteria, viruses, fungi, sera, toxins, enzymes and other biological materials.	Freeze-drying	*Cryogenic storage*	Desiccation	Serial subculture	Freeze-drying
17	_____ is prepared by acid or enzyme hydrolysis of high protein materials: meat, casein, gelatin, keratin, peanuts, soy meal, cotton seed, etc.	Soya bean meal	Peptones	Yeast extract	Corn steep liquor	Peptones
18	_____ is used as an inhibitor in production of glycerol by *S. Cerevisiae.*	Sodium bisulphite	Potassium bisulphite	Calcium bisulphite	Magnesium bisulphite	Sodium bisulphite
19	Which of the following are Techno-economic factors that affect the choice of individual raw materials.	Availability	Purification	Transport and storage	All the above	All the above
20	Sugar containing wastes derived from the paper pulping industry is called _____..	Sulphite waste liquor	Malt extract	Molasses	Whey	Sulphite waste liquor
21	_____ is the precursors for the production of L-isoleucine by *Serratia marsescens.*	D-threonine	Tyrosine	L-tryptophan	Lysine	D-threonine
22	Dark colored viscous syrup obtained as a byproduct of cane or beet sugar production.	Whey	Sulphite waste liquor	Malt extract	Molasses	Molasses
23	Molasses contains _____ % carbohydrate, ____ % nitrogenous substances, along with some vitamins and minerals.	50-60 , 2	40-90 , 3	20-50 , 2	50-80 , 4	50-60 , 2

24	Anthranillic acid is used as a precursor for the production of _____.	Tyrosine	L-tryptophan	Lysine	Threonine	L-tryptophan
25	_____ is an aqueous by-product of the dairy industry.	Sulphite waste liquor	Dextrin	Whey	Malt extract	Whey
26	Plant oils are mostly composed of _____.	Linolenic and oleic acid	Oleic and linoleic acids	Oleic and linolenic acids	None of the above	Oleic and linoleic acids
27	Sugar containing wastes derived from the paper pulping industry are primarily used for the cultivation of _____.	Molds	Bacteria	Yeasts	All the above	Yeasts
28	_____ is the simplest and common method of maintaining microbial cultures.	Serial subculture	Lyophilisation	Cryopreservation	Dessication	Serial subculture
29	Phenyl acetic acid is used as a precursor for the production of _____.	Amylase	Penicillin	Streptomycin	Erythromycin	Penicillin
30	_____ is the by-product of starch extraction from maize.	Sulfite waste liquor	Corn steep liquor	Wood molasses syrup	Distillers soluble	Corn steep liquor
31	Initially fermentation industries used corn steep liquor for _____ production.	Mushroom	Penicillin	Vitamin	Organic acid	Penicillin
32	_____ still employed for producing ethanol, single cell protein, lactic acid, xanthan gum, vitamin B_{12} and gibberellic acid.	Corn steep liquor	Molasses	Malt extract	Whey	Whey
33	The soil culture tubes are kept in refrigerator at above _____ temperature.	5-8 °c	4-6 °c	2-4 °c	6-8 °c	5-8 °c
34	The clean, yellow, fine powder prepared from embryo of cotton seed is called as _____.	Corn-steep liquor	Soya bean meal	Pharmamedia	Distiller's soluble	Pharmamedia
35	Foaming during fermentation process creates _____.	Oxidation	Reduction	Contamination	Production	Contamination
36	Hydrol molasses, containing primarily glucose, is a /product of _____ processing.	Sugar cane	Maize starch	Barley	Lignocellulose	Maize starch

37	The mineral oil overlay method was first used by Bwell and Weston in _____.	1945	1947	1949	1950	1947
38	Temperature of liquid nitrogen is _____.	-130˚c	-150˚c	-176˚c	-196˚c	-196˚c
39	____ was founded in 1925.	American type culture collection	African type culture collection	Auxenic type culture collection	Australian type culture collection	American type culture collection
40	The specific gravity of oil used in presence of cultures is _____.	0.830-0.860	0.865-0.890	0.752-0.812	0.718-0.835	0.830-0.890
41	Which is the common raw material source used in fermentation process.	Food waste	Agricultural waste	Industrial toxic waste	Biofuel waste	Agricultural waste
42	_____, _____and_____ are the main sources of starch.	Molasses and cereals	Cheese whey and tubers	Cereals, roots and tubers	Cereals and cornsteep liquor	Cereals, roots and tubers
43	_____ media is mainly used in fermentation process.	Synthetic	Semi-synthetic	Complex	Differential	Complex
44	Lyophilization is _____.	Short term preservation of microorganisms	Long term preservation of Microorganisms	Killing of Microorganisms	None of the above	Long term preservation of Microorganisms
45	Cellulose are carbohydrates made of repeating units of _____.	α-glucose	β-glucose	α-galactose	β-galactose	β-glucose
46	Sulphite waste liquor contains _____ sugars.	1-2 %	2-3 %	3-4 %	4-5 %	2-3 %
47	The process of lyophilization was first applied to microfungi on layers scale _____ and ____in 1942.	Raper and Alexander	Thomas and Alexander	Koch and Alexander	Koch and Thomas	Raper and Alexander
48	Initially fermentation industries used corn steep liquor for_____production.	Mushroom	Penicillin	Vitamin	Organic acid	Penicillin
49	Vegetable oils are used as _____.	Animal feed	Antifoams	Mushroom production media	pH adjustment	Antifoams

50	_____ is used in storage at very low temperature.	Glycerol	Mineral oil	Paraffin wax	All the above	Glycerol
51	Aim of ATCC is to _____.	Prevent contamination	To maintain antibiotics	Preserve cultures	None of the above	Preserve cultures
52	Oil should be autoclaved at _____.	15 lb/in2 for 2 hr	30 lb/in2 for 2 hr	45 lb/in2 for 2 hr	60 lb/in2 for 2 hr	15 lb/in2 for 2 hr
53	Dessicant used in lyophilizer are _____.	Phosphorus	Silica gel	Magnesium	Copper	Silica gel
54	The main success of fermentation industry depends mainly on _____.	Production temperature	Inoculums development	Production strain	Production medium	Production strain
55	The oil used in oil overlay method is _____.	British Pharmacopoeia Medicinal Paraffin oil.	American Pharmacopoeia Medicinal Paraffin oil.	Australian Pharmacopoeia Medicinal Paraffin oil.	African Pharmacopoeia Medicinal Paraffin oil.	British Pharmacopoeia Medicinal Paraffin oil.
56	In fermentation process, the _____ of product in various organic solvents has to be checked for purification process.	Solubility	Insolubility	Emulsion	Viscosity	Solubility
57	The important criteria in handling the industrially productive strain is to prevent _____.	Stability	Contamination	Oxidation	Reduction	Contamination
58	Phosphorous pentoxide silica gel/freezing trap are examples of _____.	Crypreservation	Desiccant	Preservants	Mineral oil overlay	Desiccant
59	Which of the following is NOT major source of carbohydrate?	Molasses	Whey	Peptones	Fats and oils	Peptones
60	The special medium used in soil culture method is soil, sand and calcium carbonate in ratio of ____, ____ and _____ respectively.	20%, 78% and 2%	18%, 78% and 4%	10%, 88% and 2%	30%, 68% and 2%	20%,78% and 2%

Unit III

Syllabus

Types of fermentation processes – Solid-state and liquid-state (stationary and submerged) fermentations; batch, fed-batch and continuous fermentations. Components of a typical bio-reactor, Types of bioreactors – Laboratory, pilot- scale and production fermenters, constantly stirred tank and air-lift fermenters. Measurement and control of fermentation parameters - pH, temperature, dissolved oxygen, foaming and aeration

Fermentation: The term "fermentation" derives from the Latin work *fevere* meaning "to ferment." Fermentation is the process by which alcoholic beverages or acidic dairy products (cheeses, yogurt) are manufactured. During the process, complex organic substances are broken down into simpler ones. The cell (microbial or animal) obtains energy through glycolysis– the splitting of a sugar molecule to extract its electrons. The by-product of this process is excreted from the cell in the form of substances such as alcohol, lactic acid, and acetone. With advances in the science of microbiology and technologies like biotechnology, micro-organisms are exploited to produce a wide variety of products using fermentation.

Procedure of Fermentation:

(a) Depending upon the type of product required, a particular bioreactor is selected.
(b) A suitable substrate is added at a specific temperature, pH and then diluted.
(c) The organism (microbe, animal/plant cell, sub-cellular organelle or enzyme) is added to it.
(d) Then it is incubated at a specific temperature for the specified time.
(e) The incubation may either be aerobic or anaerobic.
i. Aerobic conditions are created by bubbling oxygen through the medium.
ii. Anaerobic conditions are created by using closed vessels, wherein oxygen cannot diffuse into the media and the oxygen present just above is replaced by carbon dioxide released.
(f) After the specified time interval, the products are removed, as some of the products are toxic to the growing cell or at least inhibitory to their growth. The organisms are re-circulated.

Solid state fermentation (SSF): Solid state fermentation (SSF) deals with substrates that are solid and contain low moisture levels. The most regularly used solid substrates are starch based like cereal grains (rice, wheat, barley and corn), roots, tubers, legume seeds, wheat bran, lignocellulose materials such as straws, sawdust or wood shavings, and a wide range of plant and animal materials. Most of these compounds are polymeric molecules – insoluble or sparingly soluble in water – but most are cheap and easily obtainable and represent a concentrated source of nutrients for microbial growth. An example of solid state fermentation is koji fermentation.

SSF are normally multistep processes involving the following steps:
1. Pre-treatment of substrate raw materials either by mechanical, chemical or biochemical processing to enhance the availability of the bound nutrients and also to reduce the size of the components, e.g., pulverizing straw and shredding vegetable materials to optimize the physical aspects of the process. However, the cost of pre-treatment must be balanced with eventual product value.
2. Hydrolysis of primarily polymeric substrates, e.g., polysaccharides and proteins.
3. Utilization (fermentation) of hydrolysis products.
4. Separation and purification of end products.

ADVANTAGES OF SSF:

[1] Low moisture content of the substrates allow for minimal contamination.
[2] Can be carried out with small volumes of substrate, lowering the operational cost of the reactors.
[3] More energy economical.
[4] Product separations are easy and less cumbersome.

[5] Low cost media.
[6] No problems with foaming.
[7] Low waste water output.

EXAMPLE	SUBSTRATE	MICROORGANISM(S) INVOLVED
Mushroom production (European & Oriental)	Straw, manure	*Agaricus bisporus*, *Lentinus edodes*, *Volvariella volvacea*
Sauerkraut	Cabbage	Lactic acid bacteria
Soy sauce	Soya beans and wheat	*Aspergillus oryzae*
Tempeh	Soya beans	*Rhizopus oligosporus*
Ontjom	Peanut press cake	*Neurospora sitophila*
Cheeses	Milk curd	*Penicillium roquefortii*
Leaching of metals	Low-grade ores	*Thiobacillus* sp.
Organic acids	Cane sugar, molasses	*Aspergillus niger*
Enzymes	Wheat, bran etc.	*Aspergillus niger*
Composting	Mixed organic material	Fungi, bacteria, actinomycetes
Sewage treatment	Components of sewage	Bacteria, fungi, protozoa

DISADVANTAGES OF SSF:

[1] Media are heterogenous; hence the mash is not properly mixed.
[2] Substrate moisture level is difficult to control.
[3] Reactor parameters such as pH, temperature and dissolved oxygen need precise control.
[4] Continuous mixing or agitation of the medium is required to overcome control parameters.
[5] Continuous agitation of the medium often damages the mycelia, retarding their growth and resulting in poor growth of the organisms.

Liquid state fermentation (LSF): A controlled fermentation process in which microorganisms are grown in a liquid culture medium.

Submerged fermentation: Submerged fermentation is the cultivation of microorganisms in liquid nutrient broth. This liquid broth contains nutrients and it results in the production of industrial enzymes, antibiotics or other products. Since microbial enzymes are mostly low volume, medium cost products, the production methods using submerged liquid systems have generally relied on bioreactors similar in design and function to those used in antibiotic production processes. This involves growing carefully selected microorganisms (bacteria and fungi) in closed vessels containing a rich broth of nutrients (the fermentation medium) and a high concentration of oxygen. As the microorganisms break down the nutrients, desired product (enzymes or other bioactive compounds) are submerged in a liquid such as alcohol, oil, or nutrient broth. There are two common methods by which submerged fermentation takes place; they are batch-fed fermentation and continuous fermentation.

(i) Batch Fermentation: This type of fermentation is also called a closed culture system because nutrients and other components are added in specific amounts at the start of the process and are not replenished once the fermentation has started. At the end of the process the product is recovered; then, the fermenter is cleaned, sterilized, and used for another batch process. In the initial stages microorganisms grow at a rapid rate in the presence of excess nutrients but as they multiply in large numbers they use up the nutrients. They also produce toxic metabolites which retard further growth of microorganisms during the later stages of the fermentation process. The capacity of batch fermentor is 10 to 12 L.

(ii) Fed-batch Fermentation: It is a modified version of batch fermentation. In this process the nutrients and substrates are added at the start of the process and at regular intervals after the start. This is called controlled feeding. Inoculum is added to the fermentation vessel when microorganisms are in exponential growth phase and periodical addition of substrate keeps the prolonged log and stationary phase the microbes in the fermentor. Fed-batch culture is controlled by feed-back control and control without feed-back.

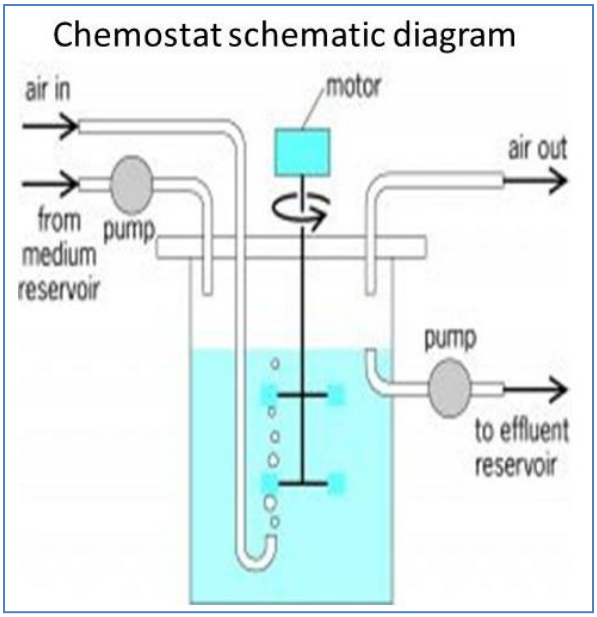
1. Feed-back control– The fermentation process is controlled by monitoring process parameters like dissolved oxygen content, carbon dioxide to oxygen ratio, pH, concentration of substrate, and concentration of the product.
2. Control without feed-back– The substrates and nutrients are added at regular intervals.

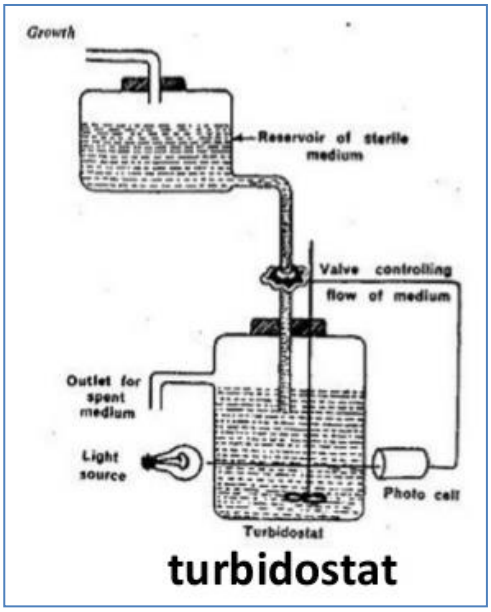
Fed-batch culture requires special equipment such as a reservoir which holds the nutrients, pH modifiers so that they can be added to the fermenter at regular intervals, and pumps to deliver culture medium aseptically to the fermenter

Continuous Fermentation: This method prolongs the exponential growth phase of microbial growth as nutrients are continually supplied and metabolites and other wastes are continually removed thus promoting continual growth of the microorganisms. Continuous culture fermentation is advantageous because of its high productivity. Two control methods are used in continuous culture fermentation, namely, chemostat and turbidostat.

Chemostat: is a bioreactor to which fresh medium is continuously added, while culture liquid containing left over nutrients, metabolic end products and microorganisms are continuously removed at the same rate to keep the culture volume constant. By changing the rate with which medium is added to the bioreactor the specific growth rate of the microorganism can be easily controlled within limits. Parameters, like temperature, pH, and concentration of dissolved gases in the growth medium, can be monitored and controlled.

Turbidostat: This medium contains excess of all nutrients so the microbial growth is at its maximum specific growth rate. The system consist of a photoelectric cell which is a turbidity sensor that detects changes in turbidity of the contents in the fermenter and then controls the amount of medium fed to the fermenter.





In other techniques, a fermenter variable, eg. turbidity or pH, will be monitored using an appropriate detector and the liquid flow rate will be automatically adjusted so as to maintain the variable at a constant level.

A turbidostat is a continuous culturing method where the turbidity of the culture is held constant by manipulating the rate at which medium is fed. If the culture is growing faster than the rate imposed by the current dilution rate, then the biomass concentration will rise as a result of the positive difference between the biomass

production rate and the rate of removal of cells from the culture vessel. In such circumstances, a control loop is activated that increases the dilution rate by increasing the speed at which a pump introduces fresh medium into the culture. This continues until the biomass concentration is decreased to some set point. Contrariwise, if the culture is growing slower than the current dilution rate, the biomass concentration will fall to below the set point, and the control loop acts to decrease the rate at which fresh medium is pumped into the growth vessel. Hence the turbidostat equilibrates at a dilution rate equal to the actual growth rate of the culture and the cell concentration stays constant.

FERMENTER DESIGN AND OPERATION

A fermenter is a vessel which does not permit contamination but provides conditions necessary for the maximum production of the desired product.

In designing and constructing a fermenter, a number of factors must be considered.

(1) The vessel must be capable of being operated aseptically for a number of days and should be reliable in long term operations.
(2) Adequate aeration and agitation should be provided to meet the metabolic requirements of microorganisms.
(3) Power consumption should be as low as possible.
(4) It must have a system of temperature control.
(5) It must have a system of pH control.
(6) Sampling facilities should be provided.
(7) Evaporation losses from the fermenter should not be excessive.
(8) The vessel should be designed to require the minimal use of labour in operation cleaning, harvesting and maintenance.
(9) The vessel should be suitable for a wide of range of processes.
(10) It should have smooth internal surface.
(11) The cheapest material which enables satisfactory result to be achieved should be used.
(12) The vessel should be of similar geometry to both smaller and larger vessels in the pilot plant to facilitate scale-up.
(13) There should be adequate service provision for industrial parts.
(14) It should have exit gas cooler (removes enough moisture from the gas leaving fermentor and prevents excess fluid loss)

In a fermenter with strict aseptic requirement, it is important to select materials that can withstand repeated steam sterilization. On a small scale, glass, stainless steel can be used. Glass gives smooth surfaces; it is not toxic and corrosion-free. The inoculum level introduced into a production tank is usually 5 – 25 %**.** It is usually easy to examine the process while it is going on. In order to achieve and maintain aseptic condition during fermentation, the following operations should be performed:

- Sterilization of the fermenter
- Sterilized air supply
- Addition of inoculums, nutrients and other supplements must be done aseptically.
- Presence of sampling point
- Foam control system
- Correct monitoring and control of various parameters such as pH, dissolved O_2, temperature etc.

PARTS OF THE FERMENTOR

Body construction: Construction material differs with small scale, pilot and large scale. The material used for designing a fermentor should have some important features:

- It should not be corrosive
- It should not add any toxic substance to the fermentation media
- It should have the capacity to tolerate steam sterilization process, high pressure and resist pH change

In small scale for vessel construction, glass or stainless steel may be used. For pilot and large scale process, stainless steel (> 4 % chromium), mild steel (coated with glass or epoxy material), wood, plastic or concrete are generally used.

Glass vessel (borosilicate glass):

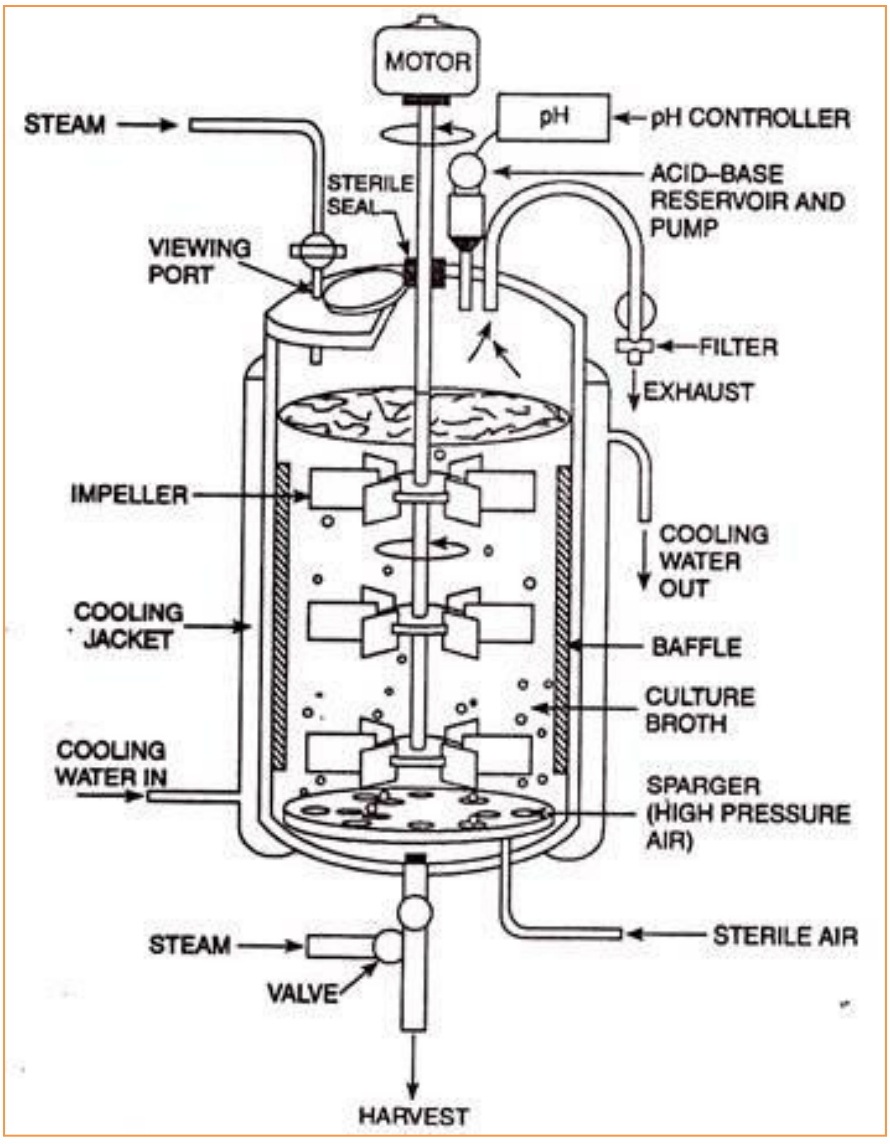
Type I – glass vessel round or flat bottom with top plate. It can be sterilized by autoclaving and the largest diameter is 60 cm.

Type II – glass vessel flat bottom with top and bottom stainless steel plate. This type is used in *in-situ* sterilization process and the largest diameter 30 cm.

Stainless steel: Large-scale industrial fermentors are almost always constructed of stainless steel of 304 and 316 type and these fermentors are mostly coated with epoxy or glass lining. Thickness of vessel should be increased with scale. Side plates have lower thickness than top and bottom plates. Top and bottom plate are hemispherical to withstand pressures.

Stainless steel is used as vessel construction material with the following modifications,

1. > 4 % chromium (at least 10-13 %) may be added
2. Film of thin hydrous oxide - non-porous, continuous, self healing, corrosion resistance
3. Inclusion of nickel - improves engineering
4. Presence of molybdenum - resistance to halogen salts, brine, sea water



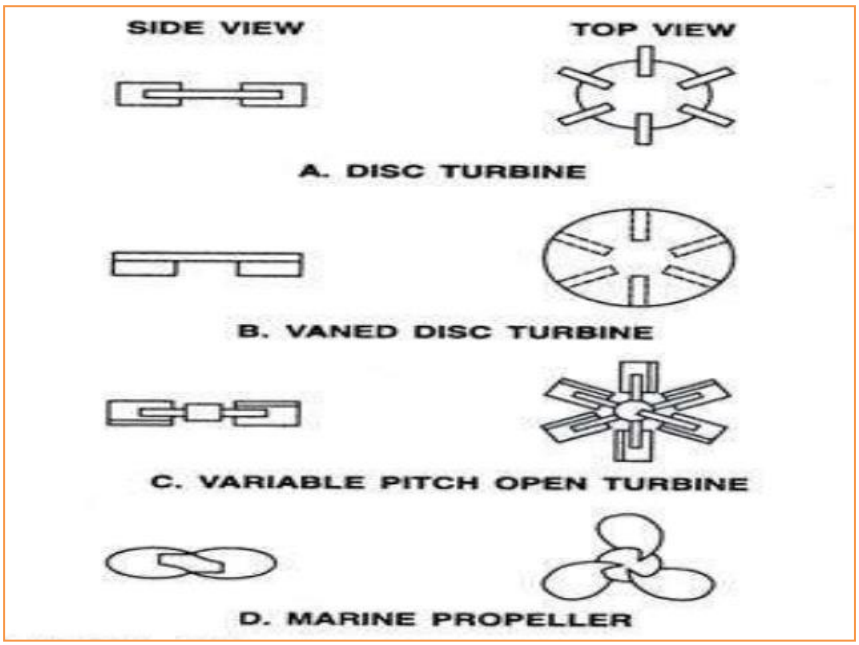
5. Tungsten, silicone - improve resistance

Aeration system (sparger): The sparger is typically just a series of holes in a metal ring or a nozzle through which filter-sterilized air (or oxygen-enriched air) passes into the fermentor under high pressure. The air enters the fermentor as a series of tiny bubbles from which the oxygen passes by diffusion into the liquid culture medium. Large bubbles will have less surface area than smaller bubbles which will facilitate oxygen transfer to a greater extent. For aeration to provide agitation the vessel height/diameter ratio (aspect ratio) should be 5:1. The size of the holes in the sparger ranges from 1/64-1/32 inches. Dissolved oxygen in fermentation process medium measured by using galvanic electrode. Gas flow rate is measured by rotameters

Air supply to sparger should be supplied through filter. There are diff types of sparger *viz.* porous sparger, orifice sparger, nozzle sparger and combined sparger agitator.

1. Porous sparger: made of sintered glass, ceramics or metal. It is used only in lab scale-non agitated vessel. The size of the bubble formed is 10-100 times larger than pore size. There is a pressure drop across the sparger and the holes tend to be blocked by growth which is the limitation of porous sparger.
2. Orifice sparger: used in small stirred fermenter. It is a perforated pipe kept below the impeller in the form of crosses or rings. The size should be ~ ¾ of impeller diameter. Air holes drilled on the under surfaces of the tubes and the holes should be at least 6mm diameter. This type of sparger is used mostly with agitation. It is also used without agitation in some cases like yeast manufacture, effluent treatment and production of SCP.
3. Nozzle sparger: is used widely large scale fermentation process. It is single open/partially closed pipe positioned centrally below the impeller. When air is passed through this pipe there is lower pressure loss and does not get blocked.
4. Combined sparger agitator: In this, the air supply is provided via hallow agitator shaft. The air is emitted through holes in the disc or blades of agitator.

Agitation (impeller): Agitation provides uniform suspension of cells in homogenous nutrient medium. The impeller (also called agitator) is an agitating device necessary for stirring of the fermenter. They are mounted on the shaft and introduced in the fermentor through the lid. Impellers consist of circular discs to which blades are fitted with bolts. This agitation provides bulk fluid and gas phase mixing, air dispersion, facilitates oxygen transfer and heat transfer and uniform environment throughout the vessel. torsion dynamometer is used for measuring the speed of agitator
The liquid flow rate is measured by using thermal mass flowmeter



The stirring accomplishes two things:
(i) It mixes the gas bubbles through the liquid culture medium and

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

The size and position of the impeller in the fermentor depends upon the size of the fermentor. In tall fermentors, more than one impeller is needed if adequate aeration and agitation is to be obtained. Ideally, the impeller should be 1/3 of the fermentors diameter and the number of impeller may vary from size to size to the fermentor. There are four classes, namely Disc turbine, Vaned disc, Open turbine of variable pitch and Marine impeller.

Temperature controlling system: Temperature control device usually contains a thermometer and the fermenter is fitted externally with a cooling jacket through which steam (for sterilization) or cooling water (for cooling) is circulated. Cooling jacket is necessary because sterilization of the nutrient medium and removal of the heat generated are obligatory for successful completion of the fermentation in the fermenter. For very large fermenters, insufficient heat transfer takes place through the jacket and therefore, internal coils are provided through which either steam or cooling water is circulated.

pH control: pH control sensors are used in fermenter for periodically checking of ph. Based on the requirement an appropriate acid or alkali is added into the fermenter. Peristaltic pump is mainly used for addition of acid and base

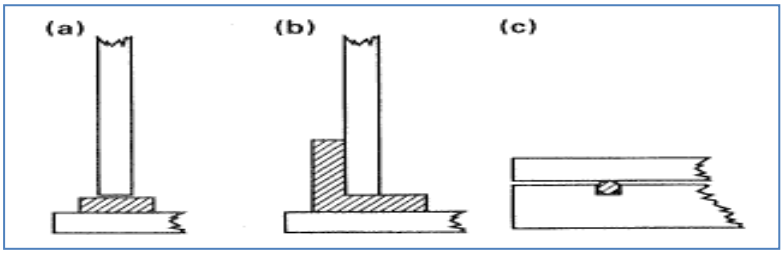
Inoculation port: Inoculation port (Feed pumps) is from which fermentation media, inoculums and nutrients added in the fermentation tank. Inoculums port should b easy to sterilize. Hence it is used to regulate the addition of culture, medium and nutrients.

Sampling point: Sampling point is used for time to time withdrawal of samples to monitor fermentation process and quality control. The sampling point should provide aseptic withdrawal of sample.

Foam controlling system: A foam sensing device is usually placed on the top of the fermenter. If the excessive foaming is not prevented, it results in the leakage of the medium from the lid of the fermentation vessel and hence leads to the contamination of the fermentation medium. When the foam rises and touches the probe tip, a signal is generated and passes through the circuit of the probe where antifoam agent (vegetable oil, silicon oil, mineral oil, etc.,) is released within seconds manually.

Baffles: Baffles are metal strips that prevent vortex formation around the walls of the vessel. These metal strips attached radially to the wall for every 1/10th of vessel diameter. Usually baffles are present but when the vessel diameter is over 3 dm^3 around 6-8 baffles are used. There should be enough gaps between wall and baffle so that scouring action around vessel is facilitated. This movement minimizes microbial growth on baffles and fermentation walls. If needed cooling coils may be attached to baffles.

Sealing: Sealing between top plate and vessel is an important criterion to maintain airtight condition, aseptic and containment. Sealing have to be done between three types of surfaces *viz.* between glass-glass, glass- metal and metal-metal. There are three types of sealing. They are gasket (a), lipseal (b) and 'O' ring (c). The materials used for sealing may be fabric-nitryl or butyl rubbers. The seals should be changed after finite time.



Stirrer glands and bearings: The entry point of stirrer into fermenter may be from top to bottom or sides. Mostly used from bottom so that that leaves more space for entry ports on top. There are four types of stirrer glands and bearings.
1) Stuffing box; 2) Mechanical seal; 3) Magnetic drives (some animal cell cultures); 4) Simple bush seals

Check valves: Valves used to prevent accidental reversal flow of liquid or gas due to break down. There are three types – swing check, lift check, combined stop and check.

Pressure control valves: These types of valves are used for two purposes.
a) Pressure reduction
b) Pressure retaining

Safety valve: There are types of safety valve by which the increase in pressure is released. They are,
a) A spindle lifted from its seating against the pressure – releases pressure
b) Bursting / rupturing of discs to release pressure. In case of releasing the gas, the escaping gas must be treated before release.

Types of fermentors: The main function of a fermenter is to provide a controlled environment for the growth of microorganisms or animal cells, to obtain a desired product. Few of the bioreactor types are discussed below:

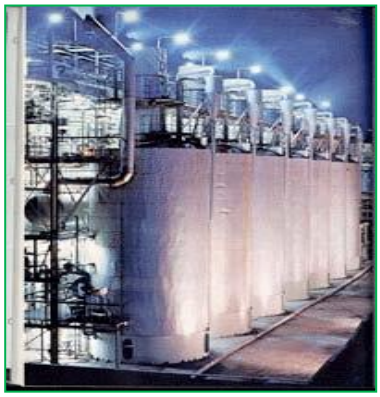
Laboratory fermenter: In fermentation with strict aseptic requirements it is important to select materials that can withstand repeated sterilization cycles. On a small scale, it is possible to use glass and/or stainless steel. Glass is useful because it gives smooth surfaces, is non-toxic, corrosion proof and it is usually easy to examine the interior of vessel. The glass should be 100 % borosilicate.
The following variants of the laboratory bioreactor can be made:

1. Glass bioreactor (without the jacket/ with the jacket) with an upper stainless steel lid.
2. Glass bioreactor (without the jacket) with the upper and lower stainless steel lids.
3. Two-part bioreactor - glass/stainless steel. The stainless steel part has a jacket and ports for electrodes installation.
5. Stainless steel bioreactor with peepholes. Vessels with two stainless steel plates cost approximately 50% more than those with just a top plate.

Pilot scale and Production fermenter: The first pilot fermenter was erected in India at Hindustan Antibiotic ltd, Pune in the 1950 year. When all bioreactors are sterilized in situ, any materials use will have to assess on their ability to withstand pressure sterilization and corrosion and their potential toxicity and cost. Pilot scale and large scale vessels are normally constructed of stainless steel or at least have a stainless steel cladding to limit corrosion. The American Iron and Steel Institute (AISI) states that steel containing less than 4% chromium are classified as steel alloys and those containing more than 4% are classified as stainless steel. Mild steel coated with glass or phenolic epoxy materials has occasionally been used. Wood, concrete and plastic have been used when contamination was not a problem in a process. Range of fermentation tank used in enzyme production 1500-30,000 Gallons (1 Gallons = 3.8 L). Large fermentors range from 2000 – 5,000 gallons .

Laboratory fermenter

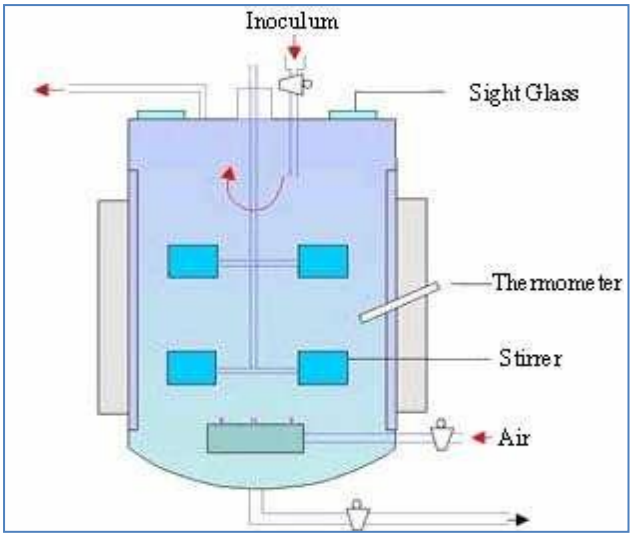
Pilot scale fermenter

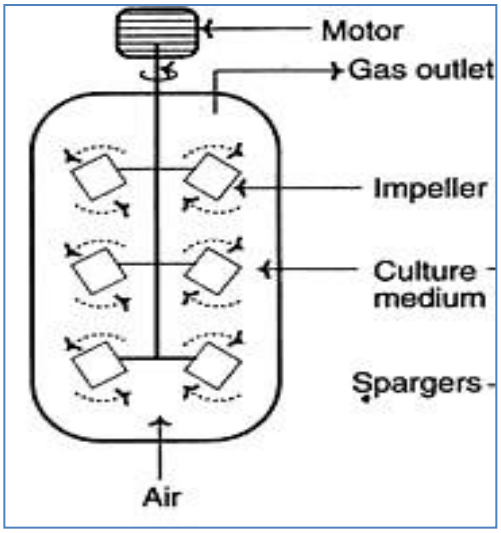


Production fermenter

(a) Stirred tank fermenter: A bioreactor system for suspension culture consists typically of a bioreactor vessel which is equipped with a drive for the stirrer in case of stirred tank bioreactors, a measurement and control unit, a heating and cooling circuit, gas supply and removal, feed tanks, sampling and harvest systems, sterile couplers, fittings, piping etc. Stirred tank reactor's have the following functions: homogenization, suspension of solids, dispersion of gas-liquid mixtures, aeration of liquid and heat exchange. The Stirred tank reactor is provided with a baffle and a rotating stirrer is attached either at the top or at the bottom of the bioreactor. The typical decision variables are: type, size, location and the number of impellers; sparger size and location. These determine the hydrodynamic pattern in the reactor, which in turn influence mixing times, mass and heat transfer coefficients, shear rates etc. The conventional fermentation is carried out in a batch mode. Since stirred tank reactors are commonly used for batch processes with slight modifications, these reactors are simple in design and easier to operate. Many of the industrial bioprocesses even today are being carried out in batch reactors though significant developments have taken place in the recent years in reactor design, the industry, still prefers stirred tanks because in case of contamination or any other substandard product formation the loss is minimal.

The batch stirred tanks generally suffer due to their low volumetric productivity. The downtimes are quite large and unsteady state fermentation imposes stress to the microbial cultures due to nutritional limitations. The fed batch mode adopted in the recent years eliminates this limitation. The Stirred tank reactor's offer excellent mixing and reasonably good mass transfer rates. The cost of operation is lower and the reactors can be used with a variety of microbial species. Since stirred tank reactor is commonly used in chemical industry the mixing concepts are well developed. Stirred tank reactor with immobilized cells is not favoured generally due to attrition problems; however by separating the zone of mixing from the zone of cell culturing one can successfully operate the system.





(b) Air-lift fermenter: Airlift fermenter (ALF) is generally classified as pneumatic reactors without any mechanical stirring arrangements for mixing. Multiple air lift fermenter was designed by Bakker.

1.The turbulence caused by the fluid flow ensures adequate mixing of the liquid.

2.The draft tube is provided in the central section of the reactor.

3.The introduction of the fluid (air/liquid) causes upward motion and results in circulatory flow in the entire reactor.

3.Even large fermentors doesn't require internal cooling coils as a jacket can normally provide sufficient heat transfer, due to the rapid movement of fluid within the vessel.

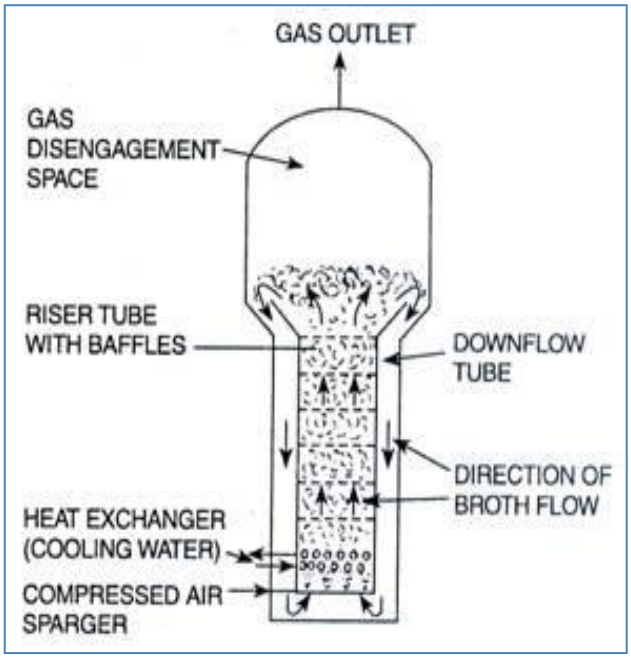
4.The air/liquid velocities will be low and hence the energy consumption is also low.

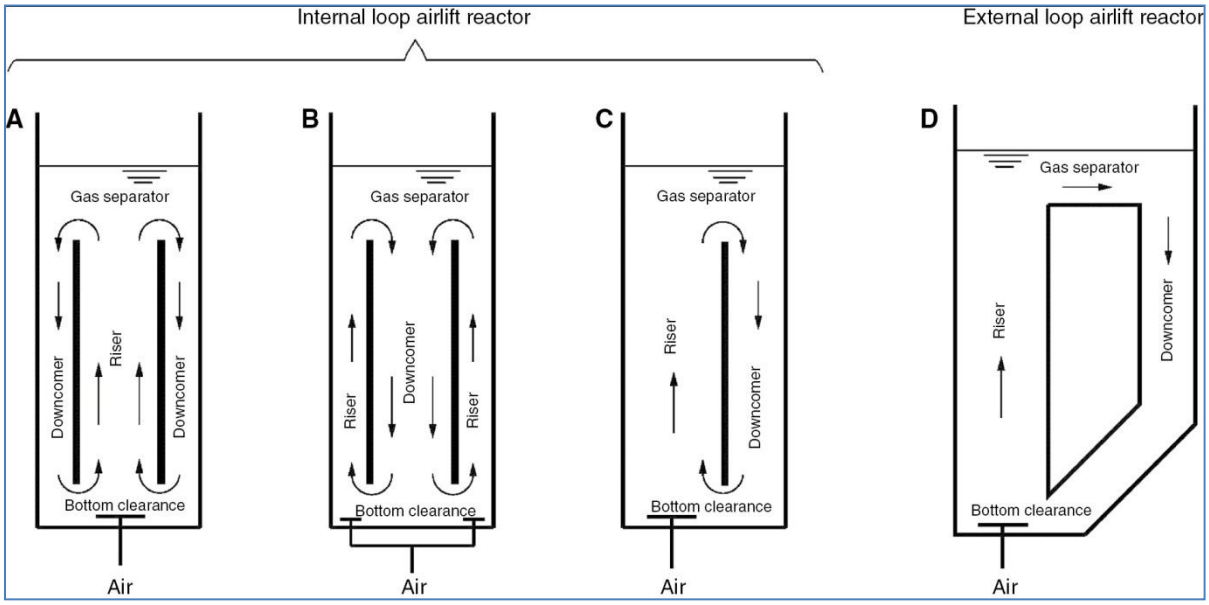
6.ALBs can be used for both free and immobilized cells.

7.There are very few reports on ALBs for metabolite production.

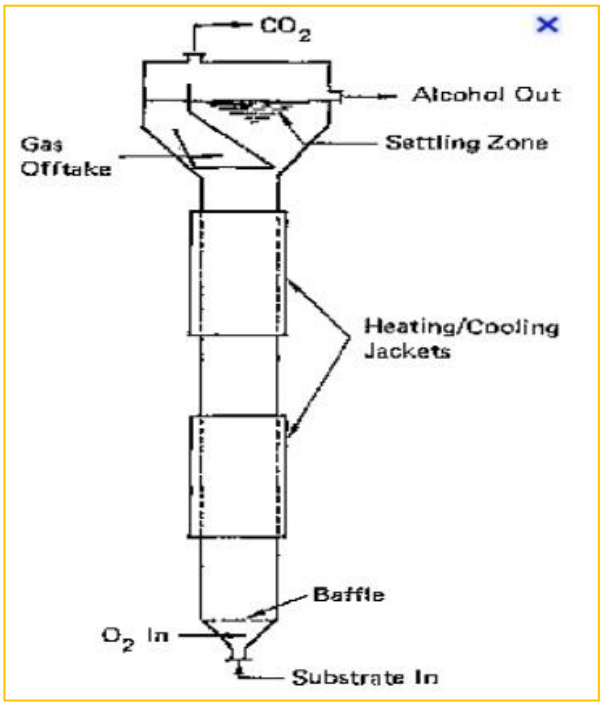
These fermentors do not have mechanical agitation systems (motor, shaft, impeller blades) but contents are agitated by injecting air from the bottom. Sterile atmospheric air is used if microorganisms are aerobic and "inert gas" is used if microorganisms are anaerobic. This is a gentle method of mixing the contents and is most suitable for fermentation of animal and plant cell cultures since the mechanical agitation produces high shearing stress that may damage the cells. Air-lift fermentors are most widely used for large-scale production of monoclonal antibodies.

There are two types of air-lift fermentors which are the internal loop and the external loop. The internal loop has a draft tube in its inner tube, in which the up-flowing gasses liquid and the down-flowing liquid is separated by the draft tube. Draft tubes are used in some processes to promote better mass transfer, mixing and inducing circulatory motion to reduce bubble coalescence. The external loop has two streams flow in two separate pipes connected at top and bottom. In this way, the air-lift fermentors improve the circulation and oxygen transfer and equalize shear forces in the reactor.





Tower fermentor: An elongated nonmechanically stirred fermentor having aspect ratio 6:1 for tubular sections and 10:1 for oval, though which there is a unidirectional flow of gases. The fermentor is two feet in diameter, about 20 ft tall in the tubular section with an expansion chamber of about four feet in diameter and six feet high. Simple tower fermentor are those with air sparger at the base; used for citric acid, beer, vinegar production.



Unit-3; Possible questions

Part- A (1 mark)

Part- B (2 marks)

1. What is sterilization?
2. What is aeration?
3. Define dissolved oxygen.
4. What is flotation?
5. Write the types of filters.
6. What is multi chamber centrifuge?
7. What is thermolysis?
8. Write the chemical methods of cell disruption.

Part- C (8 marks)

1. What are the salient features of fermentor?
2. Describe the sterilization process of fermentors.
3. Describe the sterilization process of medium.
4. How scale-up process is done in industrial fermentation?
5. How is air sterilized in fermentation process?
6. Explain types of impellers and baffles used in bioreactors.
7. Discuss about the batch and continuous fermentation.
8. With clear figure explain the principle and application of air lift reactors.
9. Explain the basic design of a fermentor with neat diagram
10. Explain about the computer control of fermentation process
11. Notes on tube and fluidized bed reactors.
12. Explain about the lab, pilot and production fermentors?

KARPAGAM ACADEMY OF HIGHER EDUCATION

CLASS: II B.Sc MB COURSE NAME: INDUSTRIAL MICROBIOLOGY

COURSE CODE: 17MBU303 UNIT: III BATCH-2017-2020

Sl. No	Question	Option A	Option B	Option C	Option D	Correct ans
1	Lignocellulose materials used in solid state fermentation _____.	Straws	Sawdust	Wood shavings	All the above	All the **a**bove
2	Pre-treatment of substrate raw materials is done to _____.	Enhance the availability of the bound nutrients	Reduce the size of the components	Both a and b	None of the above	Both a and b
3	"Inert gas" is used if microorganisms are anaerobic in Air-lift fermentors to provide _____.	Aeration	Agitation	pH control	Foam control	Agitation
4	_____ fermentor do not have mechanical agitation systems.	Air-lift	Tower	Stirred tank	All the above	Air-lift
5	_____ are common methods by which submerged fermentation .	Batch-fed fermentation	Continuous fermentation	Batch-fed fermentation	All the **a**bove	All the **a**bove
6	The amount of fresh media added to turbidostat is controlled by _____.	Control loop	Photoelectric cell	Turbidity sensor	Both b and c	Control loop
7	Which of the following is NOT used as a construction material of fermentor for large scale fermentation process?	Mild steel	Glass	Stainless steel	Wood	Glass
8	Air-lift fermentors are most widely used for large-scale production of _____.	Vitamins	Antibiotics	Monoclonal antibodies	Organic acids	Monoclonal antibodies
9	Simple ____ fermentor is those with air sparger at the base and is used for citric acid, beer, vinegar production.	Air-lift	Tower	Stirred tank	All the above	Tower
10	Stainless steel is used as vessel construction material has _____% of chromium.	> 4 % chromium	> 8 % chromium	> 6 % chromium	> 2 % chromium	> 4 %
11	Which of the following sealing is NOT used for large scale fermentation process?	'O' ring	Lipseal	Gasket	Magnetic drives	Magnetic drives
12	Baffles metal strips attached radially to the wall for every	1/5 th	1/8 th	1/10 th	¼ th	1/10th

	1/10th of vessel diameter.					
13	Sampling point is used for time to time withdrawal of samples to monitor _____.	Quality control	Contamination	Aeration	Agitation	Quality control
14	Inoculation port is also called as _____.	Syringe pumps	Peristaltic pumps	Feed pumps	Pressure pumps	Feed pumps
15	Which of the following sparger is used for large scale fermentation process?	Porous sparger	Orifice sparger	Nozzle sparger	Combined sparger agitator	Nozzle sparger
16	_____ is also used without agitation in some cases like yeast manufacture, effluent treatment and production of SCP.	Porous sparger	Orifice sparger	Nozzle sparger	Combined sparger agitator	Orifice sparger
17	Which of the following is NOT an agitator?	Disc turbine	Vaned open disc turbine	Open turbine of variable pitch	Marine impeller	Vaned open disc turbine
18	Which of the following sparger is used for laboratory scale?	Porous sparger	Orifice sparger	Nozzle sparger	Combined sparger agitator	Porous sparger
19	Usually baffles are present but when the vessel diameter is over 3 dm^3 around _____ baffles are used.	6-12	6-10	3-8	6-8	6-8
20	_____ bioreactor are so designed that adequate supply of oxygen is obtained without agitation.	Air lift	Cstr	Packed bed	Fluidised bed	Air lift
21	Stainless steel of _____ and _____ type are used for the construction of fermentor.	304, 318	304, 314	304, 316	306, 314	304, 316
22	Presence of tungsten and silicone in the construction of the body of fermentor _____.	Improves engineering	Resistance to brine, sea water	Resistance to halogen salts	Improve resistance	Improve resistance
23	Microbes are grown in especially designed vessels called _____, containing special media for its growth.	Fermentors	Batch cookers	Swap medium	Conical flasks	Fermentors
24	pH denotes the presence of _____ in aqueous solution.	Hydrogen ion	Hydroxyl ion	Carboxyl ions	Carbonyl ion	Hydrogen ion

25	_____ are used in side of fermentors to avoid vortex formation.	Spargers	Bearing glands	Rotameter	Baffles	Baffles
26	_____ tanks are used in production of all and lactic acid fermentation.	Stainless steel	Glass	Copper	Wooden	Wooden
27	The _____ in stainless steel fermentor gives resistance to halogen salts, lodine and sea water.	Chromium	Molybdenum	Nickel	Tungsten	Molybdenum
28	_____ substrate is used for the production of Tempeh.	Soybeans	Sawdust	Straws	Wheat bran	Soybeans
29	Fed-batch culture is controlled by _____.	Control without feed-back	Feed-back control	Feed-back control and control without feed-back	None of the above	Feed-back control and control without feed-back
30	Dissolved oxygen in fermentation process medium measured by using _____.	Galvanic electrode	Ph electrode	Thermometers	Thermistors	Galvanic electrode
31	_____ spargers are used widely large scale fermentation process.	Porous	Orifice	Nozzle	Combined sparger agitator	Nozzle
32	_____ removes enough moisture from the gas leaving fermentor and prevent excess fluid loss.	Baffles	Heat exchange	Cooler	Exit gas cooler	Exit gas cooler
33	_____device is used for giving air into fermentor.	Sparger	Baffles	Shaft	Bearings box	Sparger
34	_____type of bubbles facilitate high oxygen transfer than _____ bubbles.	Larger, smaller	Smaller, larger	Medium, large	Very small, very medium	Smaller, larger
35	The ideal aspect ratio for a fermentor is _____.	3:01	4:01	5:01	6:01	5:01
36	_____ ensures uniform suspension on microbial cells.	Aeration	Agitation	Sparger	Baffler	Agitation
37	The fermentor vessel _____ should be increased with scale.	Diameter	Thickness	Height	Design	Thickness
38	_____ between top plate and vessel is very important to maintain airtight / aseptic condition.	Baffles	Sealing	Sparger	Clamp	Sealing

39	_____ substrate is used for the production of mushroom.	Sawdust	Straws	Wheat bran	Legume seeds	Straws
40	Solid state fermentation (SSF) deals with substrates that are _____ and contain _____ moisture levels.	Semi solid, high	Solid, low	Solid, high	Semi solid, low	Solid, low
41	Gas flow rate is measured by _____.	Thermometers	Rotameters	Pistonmeters	Torsion dynamometer	Rotameters
42	The liquid flow rate is measured by using _____.	Thermometers	Thermal mass flowmeter	Pistonmeters	Torsion dynamometer	Thermal mass flowmeter
43	_____device is used to introduce air in fermenter.	Spargers	Impellers	Baffles	Turbines	Spargers
44	At _____ cultures may be aerated by means of the *shake-flask technique* where the culture is grown in a conical flask shaken on a platform contained in a controlled environment of chamber.	Pilot scale	Laboratory-scale	Industrial scale	Semi-industrial scale	Laboratory-scale
45	In ____ fermentations broth or culture is aerated by stirrers or agitators.	Pilot- and industrial-scale	Pilot scale	Industrial scale	Laboratory-scale	Pilot- and industrial-scale
46	In fermentation, it is very important to find the _____or _____ of product from the given carbon source.	Gases or distribution	Transport or energy	Productivity or conversion yield	Concentration or consumable	Productivity or conversion yield
47	_____is used for measuring the speed of agitator	Voltmeter	Torsion dynamometer	Ammeter	Taometer	Torsion dynamometer
48	_____ is generated due to mixing by agitator and microbes' action on substrates during fermentation process.	Energy	Heat	Resistance	Current	Heat
49	Peristaltic pump is mainly used for addition of _____ and _____.	Medium and inoculums	Acid and base	Buffers and antifoamers	Salts and growth factors	Acid and base

50	Aeration and agitation of a liquid medium may lead to the formation of_____.	Acid	Alkali	Foam	Air	Foam
51	Sparger size ranges from _____.	1/64 – 1/32 inch	1/32 – 1/18 inch	1/48 – 1/32 inch	1/24 – 1/12 inch	1/64 – 1/32 inch
52	The first pilot fermenter was erected in India at Hindustan Antibiotic ltd, Pune in the _____ year.	1920	1930	1940	1950	1950
53	_____ are provided to maintain constant temperature inside the bioreactor	Baffles	Cooling coils	Stirrer gland	Sparger	Cooling coils
54	The impeller should be _____ of the vessel diameter.	1\1	1\3	1\4	None of the above	1\3
55	Range of fermentation tank used in enzyme production.	1500 30,000	1000 – 30,000 c	Gallons	None of the above	1500-30,000
56	The capacity of the batch fermentors _____.	10 – 12 litre	12 – 15 litre	20 -40 litre	6 – 8 litre	10 – 12 litre
57	Large fermentors range from _____.	2000 – 5,000 gallons	5000 – 10,000 gallons	10,000 gallons	None of the above.	2000 – 5,000 gallons
58	Multiple air lift fermenter is designed by _____.	Bakker etal	Okabe etal	Bacon etal	Dawsa	Bakker etal
59	The inoculums level introduced into a production tank is usually _____.	0.5-5%	5 – 25%	20 – 40%	50%	5 – 25%
60	_____ fermenter is called as elongated non-mechanically stirred fermenter.	Tower	Airlift	Cylindraconical	Deep jet	Tower

Unit-4

Syllabus

Separation of cells – filtration and centrifugation. Cell disruption – physical, chemical and enzymatic methods. Product separation – solvent extraction and precipitation. Lyophilization and spray drying. Methods of immobilization, advantages and applications of immobilization

Downstream process (DSP): Downstream processing is an essential part of bioprocess technology in that the desired product needs to be isolated, purified and for different end uses. The products formed may be secreted into the broth or may be retained within the cell introducing complexity in the recovery of the product. In view of the complexity, downstream processing involves various techniques and methodologies. Bioproducts differ greatly in their nature hence different separation principles and mechanisms depending on molecular mass, charge distribution, hydrophobicity, distribution coefficient, structure and immunogenic structure and specific affinity towards other biomolecules becomes necessary for their isolation and purification. The choice of the separation methodology depends to a large extent on the nature of the product, its quantity and the extent of purity required.

The various processes used for the actual recovery of useful products from fermentation or any other industrial process are called downstream processing. The cost of downstream processing (DSP) is often more than 50 % of the manufacturing cost, and there is product loss at each step of DSP. In addition, the product is either present in the cells, in the medium or both. In either case, the concentration of product is usually rather low, and it is generally mixed with other molecules from which it has to be separated. Therefore, the DSP should be efficient, involve as few steps as possible (to avoid product loss), and be cost effective.

Downstream processing involves the following primary steps:

- Fermentation process
- Foam separation
- Primary separation – removal of insoluble products/cell (centrifugation, filtration and sedimentation)
- Cell disruption (physical, chemical and enzymatic)
- Product isolation and separation – solvent extraction, adsorption, aqueous two-phase system and precipitation
- Purification techniques (Chromatography - ion exchange, gel permeation and affinity)
- Membrane separation (micro-filtration, ultra-filtration and reverse phase electrophoresis)
- Product polishing (crystallization, drying and diafiltration)

Foam separation: Initial step in the downstream processing is the removal of foam without loss of cells and products. Whole cells or proteins gets attached to the surface of the air bubbles rising through liquid forming foam and when separated will be lost. To minimize the loss, two ways of foam separation has been adapted.

1. Excess foam collected in a separate outlet and the foam is mechanically broken. The cells and protein from the foam is collected for further usage.
2. Materials are made surface active and collected termed as colligends and the surfactants used for that purpose are termed collectors. Addition of surfactants is found to improve the percentage of removal of cells.

Separation of cells and insoluble products

Filtration: Filtration is defined as the separation of solid in a slurry consisting of the solid and fluid by passing the slurry through a septum called filter medium. For filtration in some cases, filter aids (diatomaceous earth) are used to improve porosity and faster flow rate.

1. Batch Filters:

(a) Plate and frame filters: In this, plate and frame are arranged alternately assembled on a horizontal framework. Plates are covered with filter clothes and held together by hand screw to prevent leakage between frames. The slurry is fed through the continuous channel by the holes in the corners of the plates and frames. The filtrate passes through the filter cloth or pad, runs down grooves in the filter plates and is then discharged through outlet taps to a channel.

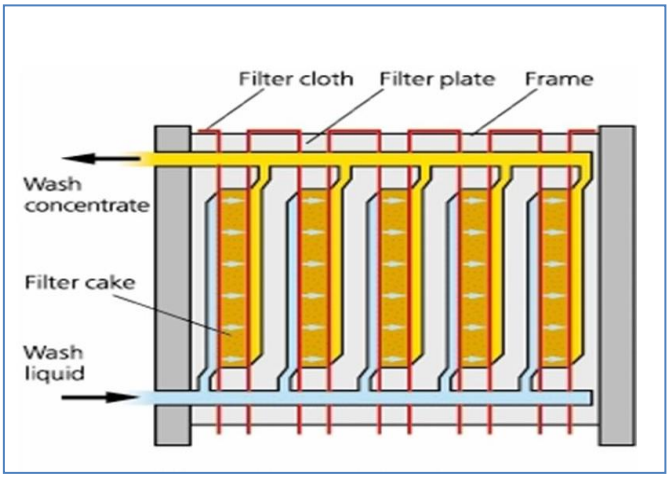
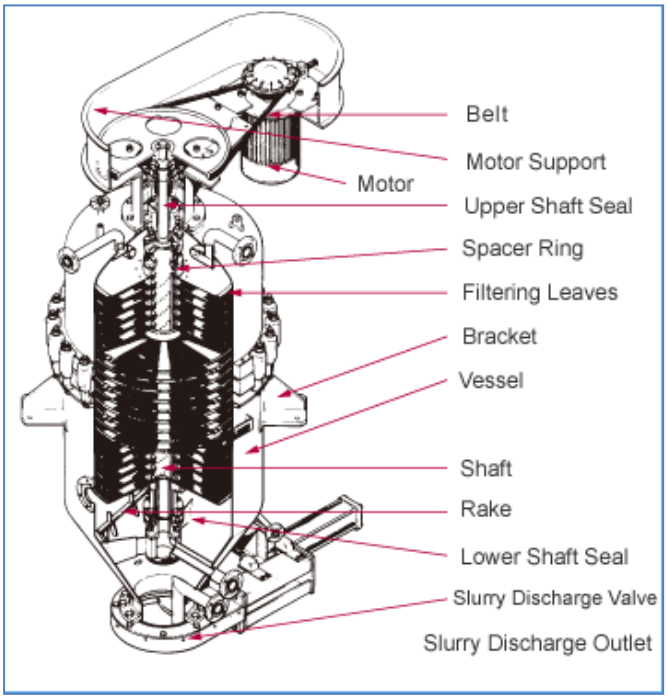


Plate and frame filters



Pressure leaf filters

(b) Pressure leaf filters: These filters incorporates 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 pre-coated with a layer of cellulose fibres. The slurry is fed into the filter which operated under pressure or by suction with a vacuum pump. There are three types of pressure leaf filters based on the arrangement of filters.

(i) Vertical metal-leaf filter – consists of number of vertical porous metal leaves mounted on a hollow shaft in a cylindrical pressure level. The solids from the slurry gradually build up on the surfaces of the leaves and the filtrate is removed from the plates via the horizontal hollow shaft

(ii) Horizontal metal-leaf filter – consists of metal leaves mounted on a vertical hollow shaft within a pressure vessel. Filtration is continued until the cake fills the space between the disc shaped leaves or when the operational pressure has become excessive

(iii) Stacked disc filter – this metal filter consists of a number of precision made rings which are stacked on a fluted rod. The filtrate passes between the discs and is removed through the grooves of the fluted rods, while solids are deposited on the filter coating

2. Continuous filters

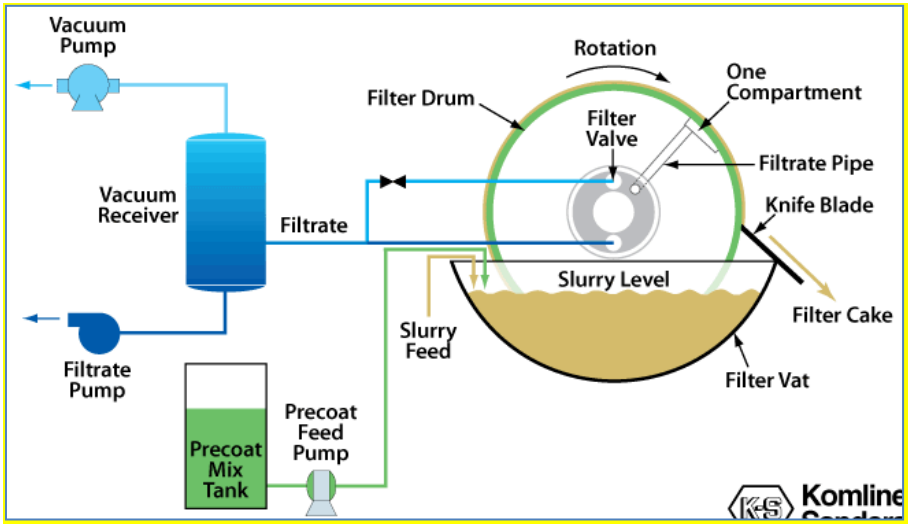
Rotary vacuum filters: Drum covered with diatomaceous earth and allowed to rotate under vacuum with half immersed in the slurry tank. Small amount of coagulation agent added to broth and pumped into the slurry tank. As drum rotates in the slurry tank under vacuum thin layer of coagulated particles adhere to drum. The layer thickens to from cake. As the cake portion in the drum comes to the upper region which is not immersed in the liquid it is washed with water and dewatered immediately by blowing air over it. Then

before the dried portion is again immersed into the liquid it is cut off from drum by knife. The mechanism of cake discharge is achieved by three ways.

(i) String discharge – Long lengths of string 1.5 cm apart are threaded over the drum and round two rollers. The cake is lifted free from the upper part of the drum when the vacuum pressure is released and carried to the small rollers where it falls free

(ii) Scraper discharge – by using a knife or scraper positioned accurately to slice off the cake

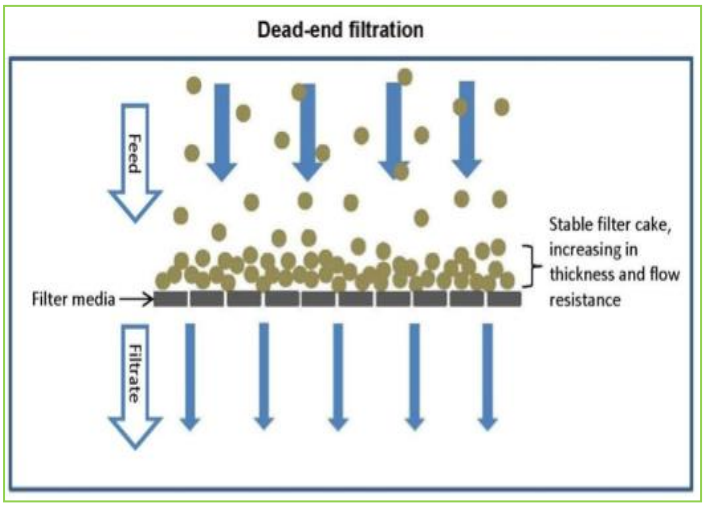
(iii) Scraper discharge with precoating – to avoid blockage of filter cloth in the drum by cells a scraper which is coated with a layer of filter-aid 2 to 10 cm thick

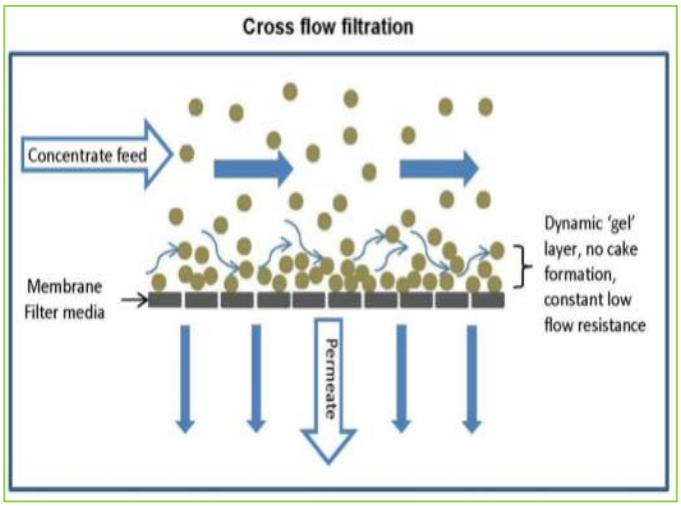


3. Micro or Ultra Filtration: Filtration of suspended particles can be achieved by either dead end filtration or cross flow filtration.

(i) Dead end filtration – Solution poured over the membrane and the filtrate is collected at the bottom. On prolonged filtration pores become blocked which reduce the filtering capacity

(ii) Cross flow filtration – To prevent the blockage the solution is passed over the membrane. Cell suspension enters laterally and flows over the membrane. The filtrate gets collected at the bottom whereas the cells are pushed to the opposite end by the continuous flow of suspension which is sent out via an outlet at the opposite end. The liquid is again passed through a tube which recycles back to the flow. Since the cells do not block the pores the filtration process can be performed continuously

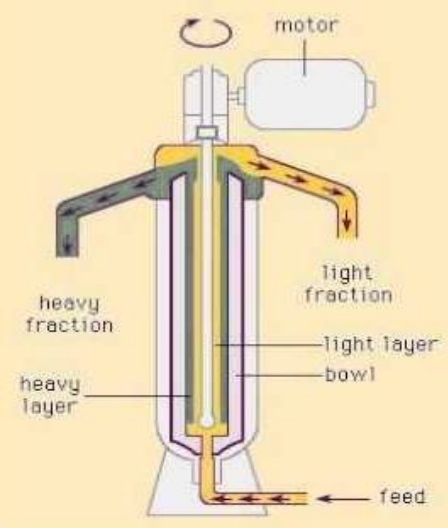




4. Adsorption on filter aids: Filter aids, are inert incompressible discrete particles of high permeability. Solids such as wood pulp, starch powder, cellulose, inactive carbon, when added as filter aid enhances their filterability. Filter aids absorb small particles, which otherwise clog the filter pores. Filter aids also reduce the compressibility of the accumulated biomass by adsorbing the colloidal particles.

Centrifugation: Centrifugation is a common method used to separate cells from cultured broth. It employs centrifugal force to promote accelerated settling of particles in a solid-liquid mixture. Separation is achieved by means of accelerated gravitational force by rapid rotation. Microorganisms and other cells from the fermented slurry can be removed by using centrifugation when filtration is not a satisfactory separation method. The particle size that can be separated range from 0.1 μm to 100 μm.

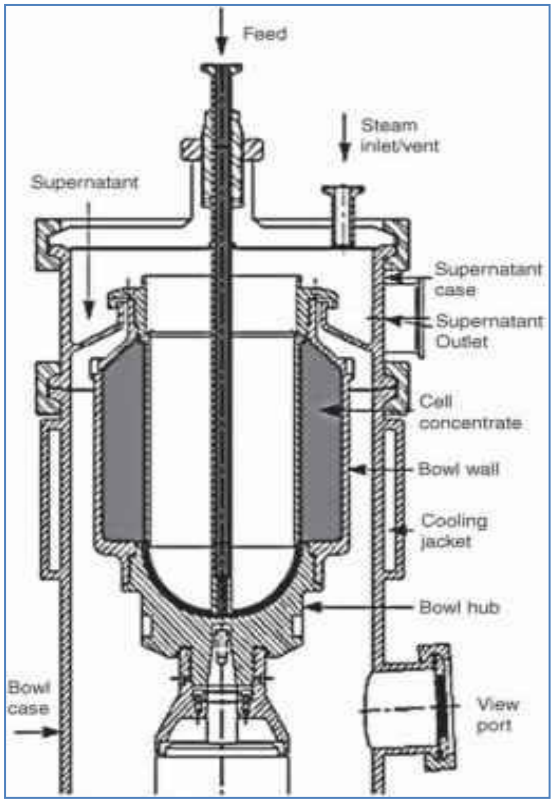
(a) Tubular centrifuges: This is used of separate particle size of 0.1 - 200 μm. This is simple machine made of a tube rotating between bearings at each end. The suspension enters at the bottom of the centrifuge and high centrifugal forces act to separate the solids and liquids. The bulk of solids will adhere to the walls of the bowl, while the liquids exit at the top of the centrifuge. It is employed for light phase/heavy phase liquid separation and solid-liquid phase separation.
Application: Pharmaceutical industries, edible oil industries, chemical and food industries



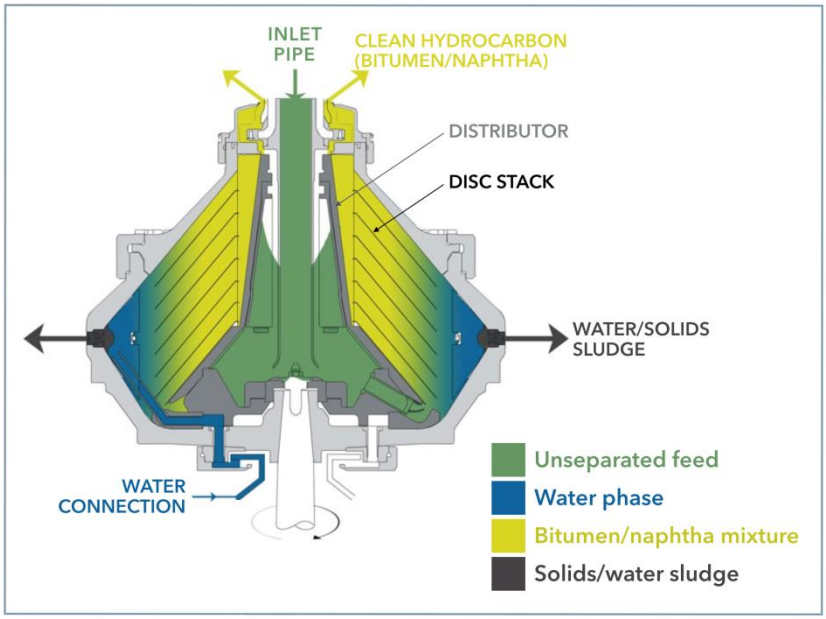
(b) Chamber bowl centrifuge: In chamber bowl centrifuge, a number of tubular bowl arranged co-axially. It has a main bowl containing cylindrical inserts that divides the volume of the bowl into a series of annular chambers, which operate in series. Feed enters the centre of the bowl and the suspension passes through each chamber in turn, at increasing distances from the axis. The solids settle onto the outer wall of each chamber and the clarified liquid emerges as an overflow from the largest diameter chamber. This device provides also a classification of the suspended solids: the coarse particles deposit in the inner chamber and the increasingly fine particle deposit on the subsequent chambers. The removal of sedimented solids requires the stopping of rotation for manual cleaning.

(c) Disc centrifuge: The simplest design is a closed bowl, containing the disk stack, with any solids present collecting at the outer part of the bowl, from which they have to be removed manually after stopping rotation. The solids are discharged from the bowl by a number of methods, including the basic use of nozzles, which are open continuously, allowing thick slurry to discharge. In the more complicated design valves, nozzles open automatically when the solid depth in the bowl reaches a certain value, and then close

again when most of the solids have been discharged. In the most complicated design the bowl is opened: its shell splits circumferentially for a short period, with the opening also controlled by solids depth in the bowl.

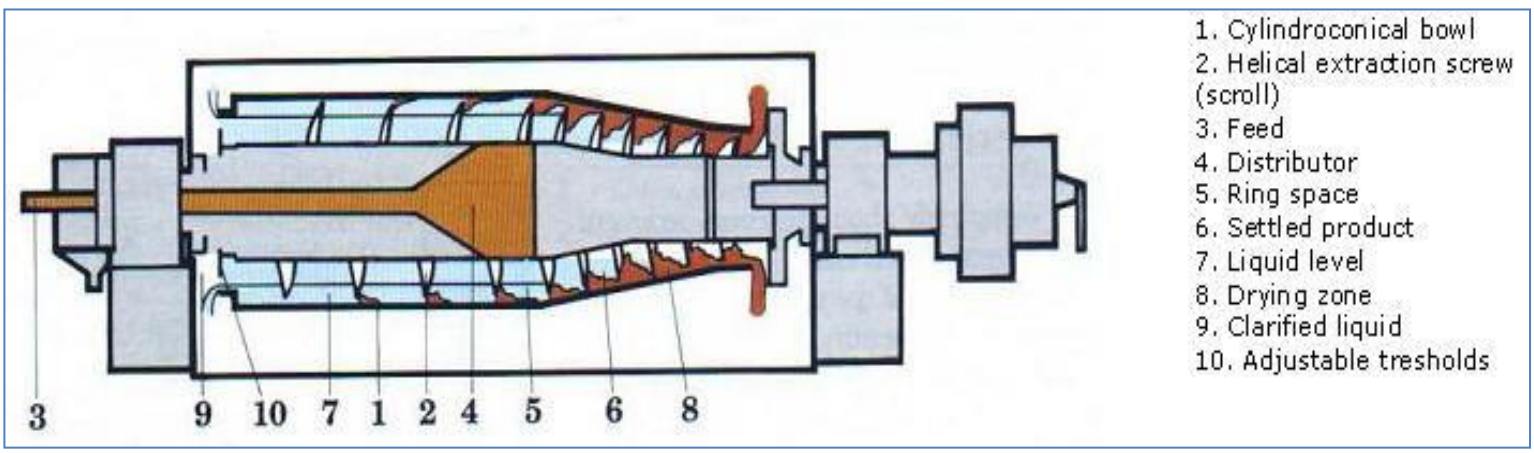


Chamber bowl centrifuge



Disc centrifuge

(d) Decanter centrifuge: This used for continuous handling of slurry. This sedimentation centrifuge is designed to handle significant solid concentration in feed suspension. It consists of horizontal cylindrical bowl rotating at a high speed, with a helical extraction screw placed co-axially. The screw perfectly fits the internal contour of the bowl, only allowing clearance between bowl and scroll. The differential speed between the screw and scroll provides the conveying motion to collect and remove solids that accumulate at the bowl wall.

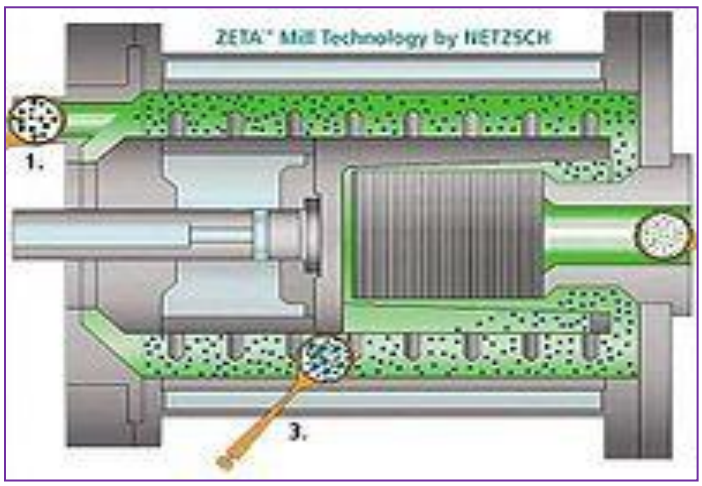


Cell disruption: Cell disruption is the process of obtaining intracellular fluid via methods that open the cell wall. The overall goal in cell disruption is to obtain the intracellular fluid without disrupting any of its components. Though many cell disruption methods exist, certain factors must be considered in order to obtain viable cellular products such as

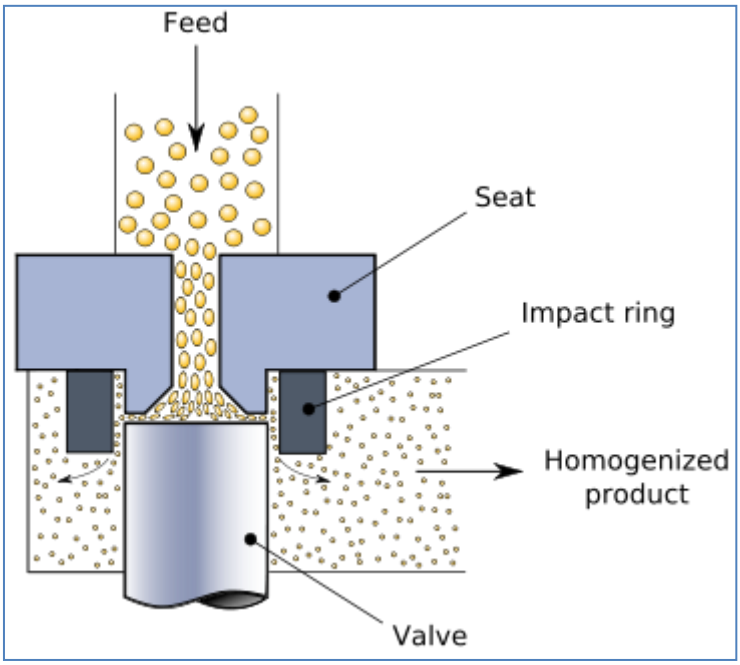
1. Sample Size
2. Ability to disrupt the cell and the necessary conditions
3. Efficiency of disruption
4. Stability of the component needed to be isolated
5. Problems with Cell Disruption methods
   - i. Heat generation
   - ii. Release of proteases
   - iii. Contamination (Nucleic acids, heavy metal, etc.)
   - iv. Foaming

(a) Physical methods / Mechanical methods: In Ultrasonic vibrators (sonicators) the ultrasound waves of frequencies greater than 20 KHz/s ruptures the cell wall by a phenomenon known as cavitation. The passage of ultrasound waves creates alternating areas of compression and rarefaction which change rapidly. The cavities formed in the areas of rarefaction rapidly collapses as the area changes to one of compression. The bubbles produced in the cavities collapse creating shock waves which disrupt cell walls.

In laboratory, grinding with a bead mill or a waring blender may be used. Waring blender is particularly effective with animal cells and tissues as well as with mycelial organisms. In industrial scale, cell disruption is carried out using a bead mill or high pressure homogenizer. Vertical or horizontal bead mill consists of a grinding cylinder with a central shaft fitted with a number of impellers and driven by motor. The cell suspension is pumped into the cylinder and cell disruption occurs due to shear forces produced between velocity gradients because of the rotary motion of cells and beads. In addition, collision between beads and cells and grinding of cells between rolling beads also contribute to the disruptive forces. High pressure homogenization consists of a high pressure positive displacement pump couple to an adjustable discharge valve with a restricted orifice. The cell suspension is pumped through the homogenizing valve at 200 – 1000 atmospheric pressure depending on microbes and cell concentration. Cell disruption occurs due to stress due to impingement, normal stress during passage through narrow channel and shear stress due to pressure drop.



In High pressure homogenization, the cell suspension is drawn through a valve into a pump cylinder. Then it is forced under pressure of up to 1500 bar, through a narrow annular gap and discharge valve, where the pressure drops to atmospheric. Cell disruption is achieved due to the sudden drop in pressure upon the discharge, causing the cells to explode. This method is one of the most widely known and used methods. It is mostly used for yeast cells. It is a vital unit in the dairy production industry, for milk homogenization. By operating the press at higher pressures, the number of passes of the slurry through it can be decreased in order to obtain the desired degree of disruption. However, the operating pressure may be limited due to the deactivation of certain heat-sensitive proteins, which may increase the number of passages required.



Non mechanical physical methods: Osmotic shock is physiological dysfunction caused by a sudden change in the solute concentration around a cell, which causes a rapid movement of water across the cell membrane which ultimately results in bursting of cell.

Freezing thawing cycles also cause loss of membrane integrity and cell wall is ruptured. In Thermolyis the heat inactivates the organism by disrupting the cell walls without affecting the products. The effect of heat shock depends on pH, ionic strength, and presence of chelating or sequestering agents such as EDTA.

(b) Chemical methods: Chemical treatment for disrupting cell includes the use of alkali, organic solvent, detergents and chaotropic agents. Alkali acts on the cell wall and results in saponification of membrane lipids. Alkali such as sodium hydroxide (NaOH) addition alters the pH and affects the integrity of the cell membrane (It is carried out at pH range of 11 to 12 for about 20 to 30 min).

The use of organic solvents (alcohols, dimethyl sulfoxide, methyl ethyl ketone or toluene) leads to disruption of cell wall where the cell wall absorbs the solvent resulting in swelling and rupture of cell wall. At low concentration of solvents, the cell wall is not ruptured but the permeability is increased. Product stability should be considered when choosing the solvent.

Eg. Toluene is used for *Agrobacterium radiobacter*, Ethyl acetate used for yeast and Dimethyl sulfoxide (DMSO) for plant cell wall. Other organic solvents used are benzene, chlorobenzene, xylene, cumeme, octanol, etc.

Detergents permeabilize cells by solubilising cell membranes. They are amphipathic in nature capable of interacting with both water and lipids.

Eg. Anionic detergents such as sodium dodecyl sulphate (SDS), sodium sulphonate; cationic detergents such as cetyltrimethyl ammonium bromide (CTAB); Non-ionic detergents such triton X100.

Chaotropic agents disrupt the structure of water making it less hydrophilic and weaken the hydrophobic interactions and increase permeability.

(c) Biological methods: Enzymatic digestion is involved in two stages (i) cell wall disruption resulting in the release of cell wall proteins leaving the protoplast intact and (ii) digestion of organelle membrane to release the organelle proteins.

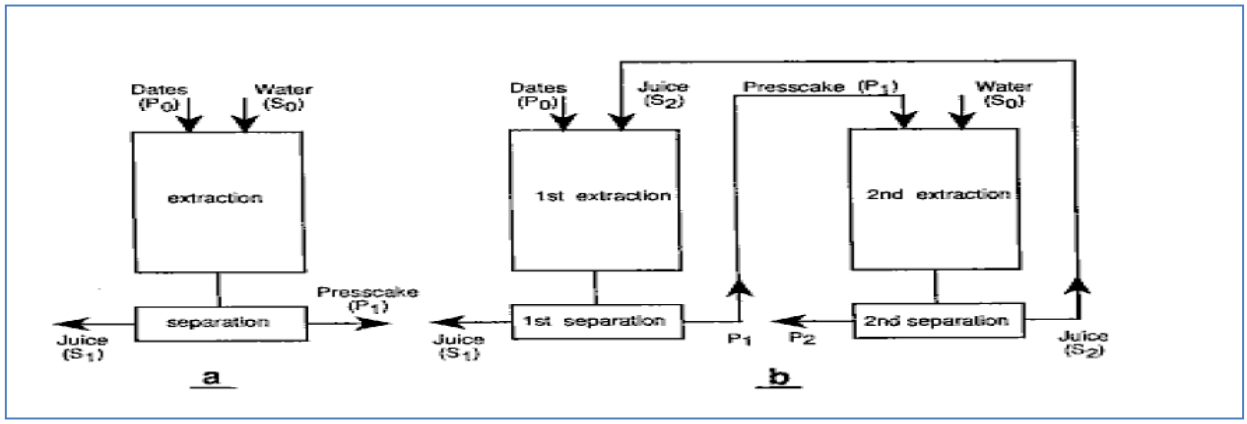
Digestion may be achieved by hydrolyzing cell walls by specific enzymes such as Lysozyme. Hydrolyzing cell wall by combination of enzymes (1,3-glucanase, 1,6-glucanase, mannanase, chitinase etc) may be also used for digestion in cases like plants cell wall. The cell wall of yeast and mold differs significantly from the cell wall bacteria. One commonly used enzyme mixture for degradation of cell wall of yeast and mold are Zymolyase (β-1,3-glucanase and β-1,3-glucan laminaripentao-hydrolase activities). In addition, the enzymes that are commonly used for degradation of cell wall of yeast and fungi include different cellulases, pectinases, xylanases and chitinases.

Product Separation

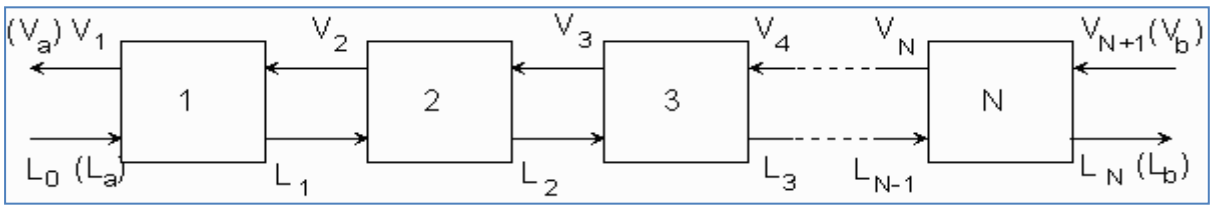
(a) Solvent extraction or Liquid-liquid extraction: It is a classical method for recovery as well as concentration of various products. Solvent extraction has several advantages such as selectivity of extraction directly from broth or reaction medium, reduction in product loss as the product is just transferred to a second phase and easy scale up. Solvent extraction involves extraction of compound in a liquid phase to another liquid. The solute originally present in aqueous phase gets partitioned in both the phases. The distribution between the two immiscible liquids and solubility in two liquids decide the efficacy of extraction. The choice of solvent selection was based on dielectric point. The dielectric constant is a measure of the degree of molar polarization of a compound. An increase dielectric pole increases the polarity of the solute. In single stage batch extraction, the aqueous feed is mixed with the organic solvent and after equilibration; the extract phase containing the desired solute is separated out for further processing. In some cases, a single stage extraction may not be enough and multi stage process is required wherein fresh volume of solvent is contacted with the raffinate.

Continuous extraction can be carried out by co-current or counter current methods. In Cocurrent extraction there are n mixer vessels in line and the raffinate goes from vessel 1 to vessel n. Fresh solvent is added to each stage and the extracting solvent pass through the cascade in the same direction. At every stage the extract is recovered. In Counter current extraction the extracted raffinate passes from vessel 1 to vessel n while the product-enriched solvent is flowing from vessel n to vessel 1. This is the most efficient method of extraction.

Extraction is achieved by three mechanisms *viz.*, physical extraction, dissociative extraction and selective extraction. Physical extraction involves preferential dissolution of the desired solute in a chosen organic solvent. Dissociative extraction involves the modification of the physical property of the solute to increase the solubility in organic phase. For example for extraction of organic acids pH is adjusted below *pK* value enhancing dissociation and thus extraction. Selective extraction involves modifying the solute solubility through ion pair or complex or adducts formation. For example long chain aliphatic amines are used for citric acid extraction. Solvent recovery after extraction process is an essential one which is usually done by distillation. The distillation is performed in three stages (i) evaporation of solvent into vapour phase, (ii) vapour-liquid separation and (iii) condensation to collect solvent.



(a) Single stage and (b) double stage extraction process



Counter current extraction process

Aqueous two phase extraction: The basic principle involves differential partitioning of solute in two immiscible phases. Phase separation occurs when hydrophilic polymers are added to an aqueous solution. At low concentration of polymers, homogenous solution is formed but at discrete concentration rise, two immiscible phases are formed using two aqueous phases with incompatible polymers such as PEG and dextran. Eg. PEG water / dextran water and PEG water/K-phosphate water, PEG phosphates. Homogenates are prepared with the two incompatible polymers after which mixer – phase separation is done by keeping idle. The bottom phase and top phase separated. Then the soluble and nonsoluble substances are separated by ultra filtration and product recovered. The retentate may be recycled for further recovery.

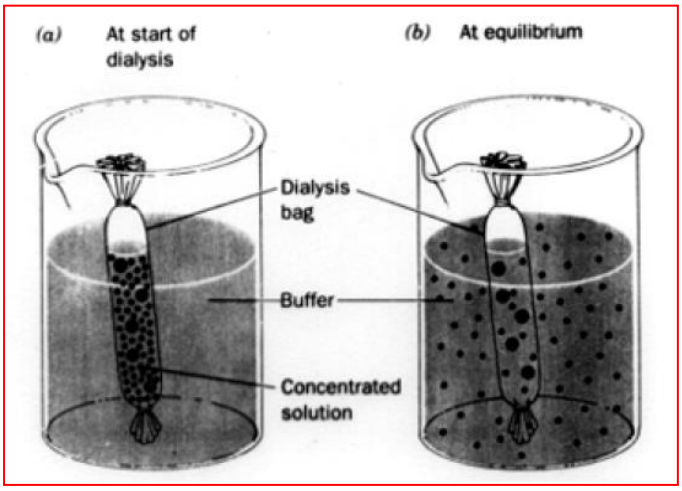
Precipitation - by decreasing the solubility of the solutes, the solute can be separated by precipitation Solubility of the particle can be changed by,

1) Salting out – by increasing ionic strength by adding salts as ammonium sulphate, disodium sulphate
2) Solubility reduction at low temperature – by adding organic solvents at low temperature
3) Solvent precipitation – adding solvent (chilled ethanol and acetone), pH adjustment and low temperature
4) Isoelectric precipitation – by the changing the pH to isoelectric pH (no charge in proteins)
5) Use of electrolytes – ionic polymers (ionic polysaccharides), non ionic polymer (dextrans)

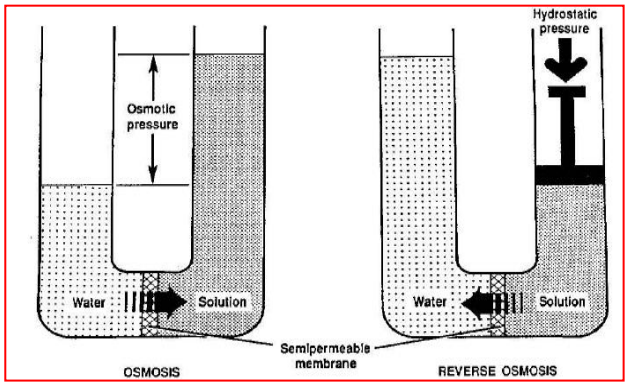
Adsorption - This refers to the binding of molecules or particles to a surface or adsorption of solutes from liquid media onto solids. Adsorption occurs due to van der Waals' force, strong ionic bonds. There are different types of solid – liquid contactors to facilitate adsorption *viz.*, packed bed, moving bed, fluidized bed, and agitated vessel contactors. Packed beds are adsorbent bed packed and liquid sent through it. In moving bed, adsorbent solid is continuously supplied, after adsorbing get removed from vessel. Fluidized bed is in which adsorbent solid is suspended in liquid. Packed and moving beds are widely used.

Dialysis is a membrane separation used to remove low molecular weight solutes (organic acids, inorganic ions). Dialysis is the movement of molecules by diffusion from high concentration to low concentration through a semi-permeable membrane. Only those molecules that are small enough pass through the membrane pores and reach equilibrium with the entire volume of solution in the system. Once equilibrium is reached, there is no further net movement of the substance because molecules keep moving through the pores in and out of the dialysis unit at the same rate.

Factors affecting dialysis: dialysis buffer volume, buffer composition, number of buffer changes, time, temperature and particle size. Membranes containing convoluted pores, not the tube-like pores often found in traditional dialysis tubing.



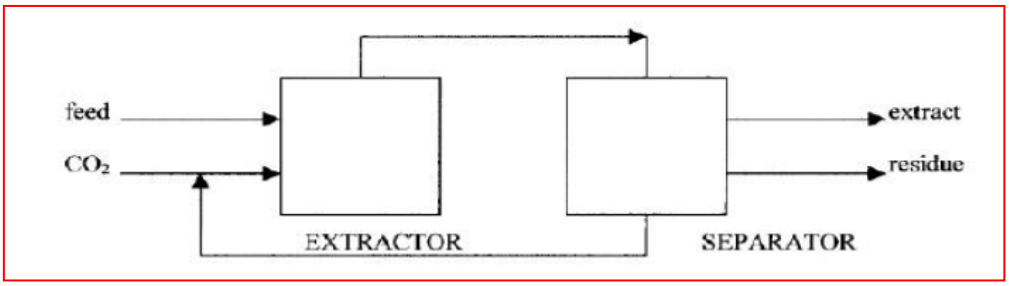
Dialysis



Reverse osmosis

Reverse osmosis is a process where the solvent molecules are forced by an applied pressure to flow through a semi permeable membrane in the opposite direction. Reverse osmosis from low to high by applying pressure uses membrane (pore size 0.0001 – 0.001 µm) permeable to water but not dissolved salts of low molecular weight. This method is applicable to concentrate smaller molecules

Supercritical fluid extraction: Supercritical fluid extraction involves the dissolution power of super critical fluids *ie.* fluids above their critical temperature and pressure. Critical temperature is defined as the temperature above which a distinct liquid phase cannot exist regardless of pressure. The vapour pressure of the substance at its critical temperature is called the critical pressure. Alternately, pressure and temperature required to liquefy a gas are critical temperature and pressure. At temperature and pressure above but close to the critical point a substance exists as a supercritical fluid. For example Carbon dioxide, NO, SO_2 are used in extraction of β-carotene, vanilla, vegetable oil, etc.



Concentration: Some concentration of the product may occur during the extraction step. Further concentration may be achieved by: (i) evaporation, (ii) membrane filtration, (iii) ion exchange methods and (iv) adsorption methods.

(a) Evaporation. It is generally used in cases of solvent extraction using various devices, *e.g.*, continuous flow evaporators, falling film evaporators, thin film evaporators, centrifugal thin film evaporators and spray-dryers. Efficient arrangements must be made for recovery of the evaporated solvent to reduce costs. For low grade products, often evaporation of the whole broth is undertaken using a spray-drier.

(b) Membrane Filtration. It generally achieves both concentration and separation of the products usually based on the size of molecules. The different processes of membrane filtration are: microfiltration, ultrafiltration, reverse osmosis and electrodialysis. Micro and ultra-filtration work as sieves and separate molecules of different sizes, but reverse osmosis can separate molecules of similar size. Microfiltration can be used for cell separation as well.

(c) Ion Exchange Resins. These are polymers having firmly attached ionizable groups (anions or cations) which ionize under a suitable environment. These may be solid, *e.g.*, dextran, cellulose, polyamine, acrylate etc., or liquid, e.g., a solvent carrying a functional group like phosphoric acid mono or diester etc. Solid ion exchangers may be used in two ways: (i) they may be packed in columnsor (ii) they may be added to the extract and removed by decantation. Liquid ion exchangers dissolve only in nonaqueous solvent carrier and the separation is similar to liquid-liquid extraction. Some antibiotics are recovered directly from the whole broth using ion exchange resins. The product is recovered from the ion exchangers by ion displacement; this also regenerates the ion exchanger.

(d) Adsorption Resins. These are porous polymers without ionization. Most compounds are adsorbed to the resins in non-ionized state. The porosity of the resin determines the surface available for adsorption. These resins may be apolar (e.g.,styrene-divinyl beneze), polar (e.g., sulfoxide, amide etc.), or semipolar (e.g., acrylic ester). The products are recovered from such resins by solvent (organic) extraction, changed pH etc.

Purification: The final step in the recovery of a product is purification which aims at obtaining the product in highly purified state. The earlier steps will have achieved variable degrees of purification which may determine the degree of resolution necessary during the purification step. The degree of resolution will mainly depend on the similarities to the metabolite of other molecules present in the concentrate, and the degree of purity required in the final product.
Purification is achieved by: (a) crystallization and (b) chromatographic procedures.

(a) Crystallization*:* It is mainly used for purification of low molecular weight compounds like antibiotics, *e.g.*, penicillin G is usually extracted from fermentation broth in butyl acetate and cystallized by the addition of potassium acetate in ethanolic solution. Crystallization is the final stage in purification of products like citric acid, sodium glutamate etc.

(b) Chromatographic Methods: These are used for purification of high molecular weight compounds from mixtures of similar molecules, *e.g.*, homologous antibiotics, and of macromolecules, especially enzymes, which are similar in properties. The materials used for chromatography are generally coated on particulate carriers (stationary phase) which are packed in columns through which the liquid containing the product is pumped either upward or downward. The separated product is recovered in some sort of fraction collector.

On a large scale, organic solvents are used for collection. Therefore, the whole system has to be installed in a flame-proof and explosion-proof room.

The different chromatographic procedures are: (a) adsorption, (b) gel filtration, (c) hydrophobic, (d) affinity, (e) covalent and (f) partition chromatography.

Adsorption chromatography separates molecules due to their differential affinities for the surface of a solid matrix, *e.g.*, silica gel, alumina, hydroxyapatite (all inorganic) or an organic polymer. In case of ion exchange chromatography, resins or polysaccharides, *e.g.*, cellulose, sepharose, having attached ionized functional groups are used for a high resolution separation of macromolecules, *e.g.*, proteins. Gel filtration uses molecular sieves, composed of neutral cross-linked carriers *(e.g.*, polymers like agarose, dextrans), of different pore sizes. Molecules smaller than the pore size enter the carrier and are retained; they are later eluted (in order of molecule size) and collected.

Gel filtration is used in aqueous systems. Hydrophobic carriers are used for purification of hydrophobia molecules.

Affinity chromatography uses molecules, called effectors, to which the product has high and specific affinity, *e.g.*, using an antibody (effector) for the purification of the antigen to which it is specific. The effector is immobilized on a water insoluble carrier which is packed in a column through which the mixture is passed. The effector binds only to the molecules for which it is specific and retains it in the column; it is later recovered by elution using a buffer solution of a specified pH.

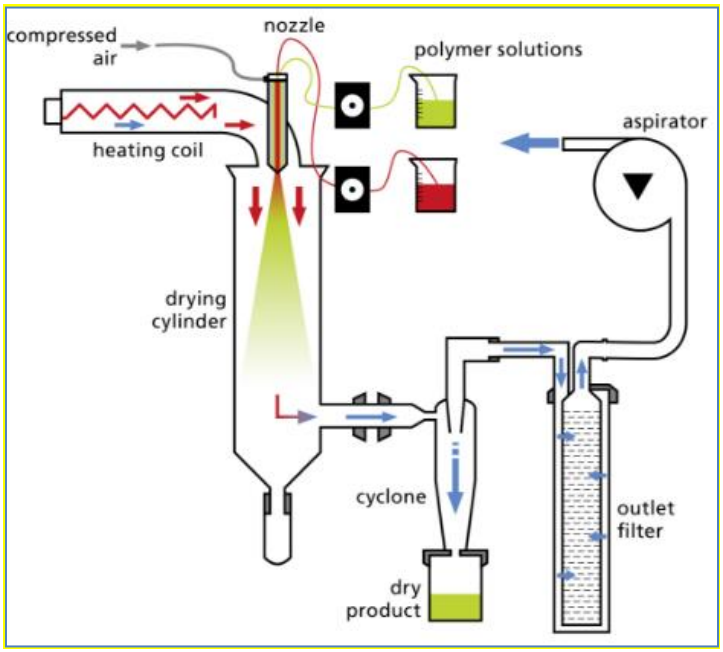
Drying: Drying makes the product suitable for handling and storage. It should be accomplished with a minimum rise in temperature due to heat sensitivity of most products. Addition of sugars or other stabilizers improves the heat tolerance of some products like enzymes and pharmaceutical preparations.

The most common approaches to drying are as follows: (/) vacuum drying, *(ii)* spray drying, and *(iii)* freeze drying.

In spray drying, the solution or slurry to be dried is atomized by a *nozzle* or a rotating disc. A current of hot (150-250 °C) air is passed; the drying is so rapid that the temperature of particles remains very low. Spray drying is used for enzymes, antibiotics and food products.

Vacuum drying uses both heat and vacuum for drying; it can be applied both in batch mode *(e.g.*, chamber dryers) and in continuous mode *(e.g.*, rotating drum vacuum dryers).

In freeze drying, the liquid to be dried is first frozen and the water is sublimed from the frozen mass. A very low pressure (partial vacuum) is maintained to promote sublimation of water. The energy needed for sublimation is provided by heated plates and radiation on to the surface. The temperature of solid is regulated by regulating the pressure in the drying chamber. This is the most gentle method of drying, and is used for many pharmaceutical products, e.g., viruses, vaccines, plasma fractions, enzymes etc., and in food industries.



Spray dryer

Immobilization: Immobilization is defined as the imprisonment of cell or enzymes in a distinct support or matrix. The support or matrix which the enzymes are immobilized allows the exchange of medium containing substrate or effector or inhibitor molecules. The practice of immobilization of cells is very old and first immobilized enzyme was amino acylase of *A. oryzae* for the production of L-amino acids in Japan.

The matrix immobilizes the enzyme by holding it permanently or temporarily for a brief period of time. There are a wide variety of supports available for immobilization and are grouped into following categories: (a) Natural polymers, (b) Synthetic polymers and (c) Inorganic materials

(a) Natural polymers:

i. Alginates: It is a polysaccharide distributed widely in the cell walls of brown algae. Calcium or magnesium alginate is most commonly used matrix. They are inert and show high water holding capacity
ii. Chitosan and chitin: They are structural polysaccharides occurring naturally in the cell wall of fungi and the exoskeleton of arthropods. The various functional groups in enzymes can bind to hydroxyl and amino groups of chitin and can form a covalent bond
iii. Collagen: It is the protenaceous support with good porosity and water holding capacity. The side chains of the amino acids in the collagen and that of enzyme can form covalent bonds to permanently hold the enzyme to the support
iv. Carrageenan: They are linear sulphated polysaccharides that are extracted from red edible seaweeds. Their high protein holding capacity makes them good support for immobilizing enzymes
v. Gelatin: it is partially hydrolysed collagen with good water holding capacity
vi. Cellulose: Most abundant polymer of nature and it is cheapest support available as carrier of enzymes. The hydroxyl group of the monomer units can form covalent bonds with that of the amino acids of enzymes
vii. Starch: A natural polymer of amylose and amylopectin which has a good water holding capacity
viii. Pectin: it is a structural polysaccharide of plants found in their primary cell wall and they also act as the inter-cellular cementing material in plant tissues. It is gelling agent with good water holding capacity

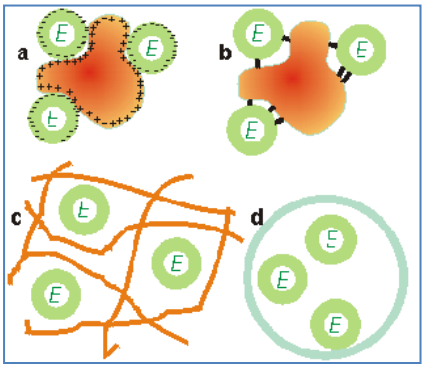
(b) Synthetic polymers: They are ion exchange resins or polymers and are insoluble supports with porous surface. Their porous surface can trap and hold the enzymes or whole cells. Example: UV activated poly ethylene glycol (PEG), Polyvinyl chloride (PVC), Diethylaminoethyl cellulose (DEAE)

(c) Inorganic polymers:

i. Zeolites: They are microporous, aluminosilicates of sodium, potassium, calcium, and barium with good adsorbing properties
ii. Ceramics: A ceramic is a non-metallic solid material comprising an inorganic compound of metal, non-metal or metalloid atoms primarily held in ionic and covalent bonds
iii. Diatomaceous earth: is a naturally occurring, soft, siliceous sedimentary rock that is easily crumbled into a fine white to off-white powder. It has a particle size ranging from less than 3 μm to more than 1 mm, but typically 10 to 200 μm. The typical chemical composition of oven-dried diatomaceous earth is 80–90 % silica, with 2–4 % alumina (attributed mostly to clay minerals) and 0.5–2 % iron oxide.
iv. Silica: Silicon dioxide, also known as silica, silicic acid or silicic acid anydride is an oxide of silicon with the chemical formula SiO_2, most commonly found in nature as quartz and in various living organisms.
v. Glass
vi. Activated carbon: Activated carbon is usually derived from charcoal . Activated carbon, also called activated charcoal, is a form of carbon processed to have small, low-volume pores that increase the surface area available for adsorption or chemical reactions.
vii. Charcoal: Charcoal is the lightweight black carbon and ash residue hydrocarbon produced by removing water and other volatile constituents from animal and vegetation substances. Charcoal is usually produced by slow pyrolysis (the heating of wood or other substances in the absence of oxygen).

Immobilized enzymes are very important for commercial uses as they possess many benefits which include:
(a) Economical: The immobilized enzyme can be easily removed from the reaction thus making it easy to recycle, multiple or repetitive use
(b) Stability: Immobilized enzymes typically have greater thermal and operational stability than the soluble form of the enzyme and product is not contaminated with the enzyme
(c) Convenience: The ability to stop/start the reaction rapidly by removing/adding the enzyme from the reaction solution, long half life, predictable decay rates, elimination of reagent preparation, etc.

Methods of immobilization: It is essential to choose a method for immobilizing an enzyme to a surface which will prevent loss of enzyme activity. There are mainly three methods available for immobilization of enzymes:
(a) Adsorption: the binding of enzymes by physical / ionic interaction to water insoluble carriers.
(b) Covalent Binding: the binding of enzymes by covalent coupling to water-insoluble carriers.
(c) Entrapping: incorporating enzymes into the lattices of a semi-permeable gel or enclosing the enzymes in a semi-permeable polymer membrane.



(a) Adsorption; (b) Co-valent Bonding; (c) and (d) Entrapment

(a) Adsorption: Adsorption is a very simple method of an enzyme immobilization which has wide applicability. The immobilized enzyme in a directly usable form can be obtained by simply mixing an enzyme with a suitable adsorbent under appropriate conditions of pH, ionic strength, incubation period and washing off unbound enzyme. It is possible to achieve high enzyme loading using adsorption. The binding between adsorbent and the enzyme is mostly due to a combination of hydrophobic effects and the formation of several salt links per enzyme molecule. The physical links between the enzyme molecules and the support generally weakened during use by inappropriate changes in pH or ionic strength.
Examples: ion-exchange matrices, porous carbon, clays, hydrous metal oxides, glasses and polymeric aromatic resins etc.

(b) Covalent Binding: In comparison to other methods, the covalent binding method is most effective and widely studied. The ultimate aim of the covalent binding is to avoid leaching of an enzyme into the surrounding solution. In general, the functional groups of proteins suitable for covalent binding and which do not cause loss of the catalytic activity of the enzyme include (a) the alpha amino groups of chain and epsilon amino groups of lysine and arginine, (b) the alpha carboxyl group of chain end and beta and gamma carboxyl groups of aspartic and glutamic acids, (c) the phenol ring of tyrosine, (d) the thiol group of cysteine, (e) the hydroxyl groups of serine and threonine, (f) the imidazile group of histidine, and (g) the indole group of tryptophan. Commonly used methods for the covalent immobilization of enzymes:
(i) Activation of sepharose by cyanogen bromide.
(ii) Chloroformates may be used to produce similar intermediates to those produced by cyanogen bromide but without its inherent toxicity.
(iii) Carbodiimides may be used to attach amino groups on the enzyme to carboxylate groups on the support or carboxylate groups on the enzyme to amino groups on the support.
(iv) Glutaraldehyde is used to link enzymes to supports.
(v) The use of trialkoxysilane to derivatise glass.

(c) Entrapping: In general, entrapment is a useful method of enzyme immobilization using gels or fibers where substrates and products are low molecular weight. A purely physical caging or covalent binding of an enzyme can be achieved using entrapment method. Generally, calcium alginate has been widely used for the entrapment of microbial, animal and plant cells. Another method of entrapment is the confinement of enzymes using membranes however, semipermeable nature of the membrane plays a critical role. The entrapment using membranes should ensure confinement of the enzyme and free passage for the reaction products. The entrapment of an enzymes using membrane can be achieved by simply placing the enzyme on one side of the semipermeable membrane whereas the reactant and product on the other side. Hollow fibers are the best example of entrapment of an enzyme using membranes. They are available commercially with large surface areas relative to their contained volumes (> 20 m2 l-1) and permeable only to substances of

molecular weight substantially less than the enzymes. Hollow fibers are very easy to use for a wide variety of enzymes (including regenerating coenzyme systems) and the cost effective. Another method is the encapsulation of an enzyme within small membrane bound droplets or liposomes.

Advantages of immobilized enzymes:

- Increased functional efficiency of enzyme
- Enhanced reproducibility of the process they are undertaking
- Reuse of enzyme
- Minimum reaction time
- More stable products
- Less chance of contamination
- Less labour
- Saving investment
- Improved process control

Applications of immobilized enzymes

- Industrial Production
- Biomedical applications
- Food industry
- Research
- Production of bio-diesel
- Waste water management
- Textile industry
- Detergent industry

Unit-4; Possible questions

Part-A (1 Mark)

Part- B (2 Marks)

1. What is cell disruption and name the methods used for cell disruption.
2. Name the types of batch filters.
3. Name the types of centrifuges.
4. Principles of affinity chromatography.
5. Mention the advantages of immobilization
6. Name the type of supports used in the immobilization process

Part-C (8 Marks)

1. Write about aeration and agitation in a bioreactor.
2. Comment on packed bed and trickle flow reactors.
3. Comment on configuration of bioreactor.
4. Discuss on the measurement and control of fermentation process.
5. Write notes on cell disruption methods.
6. What are the methods of immobilization? Explain with figures.
7. Discuss on solvent extraction techniques used in down streaming process.
8. Describe the different filters used in downstream processing.

KARPAGAM ACADEMY OF HIGHER EDUCATION

CLASS: II B.Sc MB COURSE NAME: INDUSTRIAL MICROBIOLOGY

COURSE CODE: 17MBU303 UNIT: IV BATCH-2017-2020

Sl. No	Question	Option A	Option B	Option C	Option D	Correct ans
1	Chromatography is based on the_____.	Different rate of movement of the solute in the column	Separation of one solute from other constituents by being captured on the adsorbent	Different rate of movement of the solvent in the column	Solvent differnces	Different rate of movement of the solute in the column
2	The purification and recovery of the production after fermentation is called_____.	Upstream process	Downstream process	Surface fermentation	None of these	Downstream process
3	Cell lysis becomes an important operation if the product is _____.	Extra cellular	Heat labile	Toxic	Intracellular	Intracellular
4	A centrifuge is used to separate molecules on the basis of their _____.	Size	Shape	Density	Size, shape and density	Size, shape and density
5	Which process uses a porous medium for the separation of the solid material from gas or liquids_____.	Precipitation	Filtration	Centrifugation	Foam separation	Filtration
6	The sterilization temperature of the fermentation equipment is _____.	121°C – 15 min	120°C – 20 min	115°C – 15min	115°C – 20min	121°C – 20 min
7	Example for non ionic detergents.	Tween 80	Tween X100	Tween 40	None of the above	Tween X100
8	Ultrasonication has frequency of _____khz.	200	2	20	2000	20
9	First immobilized enzyme was _____ of *A. oryzae* for the production of L-amino acids.	Amino reductase	Amino acylase	Amino kinase	Amino acylase	Amino acylase
10	Example of an inorganic polymer used as support in immobilization.	Alginates	Carrageenan	Ceramics	Polyvinyl chloride	Ceramics
11	Example of synthetic polymer used as support in immobilization.	Alumina	Poly ethylene glycol	Glass	Diethylaminoethyl cellulose	Diethylaminoethyl cellulose

12	Example of natural polymer used as support in immobilization.	Gelatin	Activated carbon	Silica	Diatomaceous earth	Gelatin
13	_____ is most gentle method of drying, for many pharmaceutical products (viruses, vaccines, plasma fractions, enzymes) and in food industries.	Spray drying	Freeze drying	Vacuum drying	All the above	Freeze drying
14	In antibiotic manufacturing processes, the fermentation time ranges from_____.	2-3 weeks	1-2 weeks	4-5 weeks	2-4 weeks	4-5 weeks
15	There should be no permanent direct connection below _____ and _____parts of the fermentor system.	Medium and air	Sterile and non-sterile	Mixing and air	Probes and medium	Sterile and non-sterile
16	After sterilization, all parts of fermentor are kept sterile by maintaining at _____ pressure.	Positive	Negative	No	Zero	Positive
17	The cell suspension is pumped through the homogenizing valve at _____ atmospheric pressure depending on microbes and cell concentration.	200 – 1000	100 – 1500	200 – 100	20 – 100	200 – 1000
18	Example for apolar adsorption resins	Styrene-divinyl beneze	Silica	Charcoal	Pectin	Styrene-divinyl beneze
19	Example for polar adsorption resins.	Pectin	Diethylaminoethyl cellulose	Sulfoxide	Diatomaceous earth	Sulfoxide
20	Filtration of suspended particles can be achieved by either _____ or _____.	Dead end filtration	Cross flow filtration	Both A and B	None of the above	Both A and B
21	Example for semipolar adsorption resins	Styrene-divinyl beneze	Sulfoxide	Zeolites	Acrylic ester	Acrylic ester
22	Example for ion exchange resins _____.	Diethylaminoethyl cellulose	Polyvinyl chloride	Peg	Dextran	Dextran
23	The different processes of membrane filtration are	Microfiltration	Electrodialysis	Reverse osmosis	All the above	All the above

24	Factors affecting dialysis are _____.	Dialysis buffer volume	Buffer composition	Number of buffer changes	All the above	All the above
25	Example for adsorption resins _____.	Alginates	Styrene-divinyl beneze	Diatomaceous earth	Silica	Styrene-divinyl beneze
26	Concentration of the product can be achieved by _____.	Membrane filtration	Ion exchange methods	Evaporation	All the above	All the above
27	Membrane of pore size _____ a used for reverse osmosis.	0.001 – 0.01 mm	0.0001 – 0.001 µm	0.01 – 0.1 µm	0.0001 – 0.001 mm	0.0001 – 0.001 µm
28	Dialysis separation is based on the _____.	Movement of low molecular weight solutes	Membrane pores	Movement of molecules from high concentration to low concentration	All the above	All the above
29	Adsorption occurs due to _____	Van der Waals' force	Strong ionic bonds	Both A and B	None of the above	Both A and B
30	_____ of any product is often the last stage of a manufacturing process.	Filtration	Centrifugation	Drying	Packing	Drying
31	Dextrans can be precipitated out of a broth by the addition of _____.	Methanol	Ethanol	Butanol	Alcohol	Methanol
32	Zymolyase is mainly composed of _____	β-1,3-glucanase	β-1,3-glucan laminaripentao-hydrolase	Both A and B	None of the above	Both A and B
33	Which of the following organic solvents used for the cell disruption?	Benzene	Chlorobenzene	Xylene	All the above	All the above
34	Which of the following organic solvent used for the cell disruption of plant cell wall?	Triton X100	Sodium dodecyl sulphate	Dimethyl sulfoxide	Sodium sulphonate	Dimethyl sulfoxide
35	Example for anionic detergents	Cetyltrimethyl	Sodium dodecyl	Sodium	Both B and C	Both B and C

		ammonium bromide	sulphate (SDS),	sulphonate		
36	Which of the following organic solvent used for the cell disruption of yeast?	Ethyl acetate	Sodium dodecyl sulphate	Cetyltrimethyl ammonium bromide	Sodium sulphonate	Ethyl acetate
37	Which of the following organic solvent used for the cell disruption of *Agrobacterium radiobacter?*	Sodium sulphonate	Toluene	Cetyltrimethyl ammonium bromide	Sodium dodecyl sulphate	Toluene
38	Example for cationic detergents	Cetyltrimethyl ammonium bromide	Sodium sulphonate	Both A and B	None of the above	Cetyltrimethyl ammonium bromide
39	Micro filtration refers to the separation of suspended material such as bacteria by using a membrane with spore sizes of_____	0.02 to 10 µm	1-10 A°	20-30µm	10-200 A°	0.02 to 10 µm
40	_____ is added along with stainless steel fermentor during construction improves engineering.	Chromium	Nickel	Tungsten	Silicone	Nickel
41	_____ damage the cell membrane and lead to the release of intracellular components.	Osmotic shock	Alkali treatment	Detergent	Enzyme	Osmotic shock
42	_____ caused by a sudden change in salt concentration will cause disruption of a number of cell types.	Osmotic shock	Alkali	Protease	SDS	Osmotic shock
43	_____is the separation process where the solvent molecules are passed to flow through semipermiable membrane in the opposite direction.	Ultra filtration	Reverse osmosis	Liquid membranes	Pumping	Reverse osmosis
44	_____ is added to adjust pH if too acidic.	Ammonia	Sodium hydroxide	Both a or b	Sulphuric acid	Sodium hydroxide

45	_____ is an established and final purification step of a diverse range of compounds.	Drying	Crystallization	Filtration	HPLC	Crystallization
46	_____ are metal strips roughly one- tenth of the vessel diameter and attached radically to the wall.	Sparger	Baffler	Magnetic devices	Impellers	Baffler
47	_____ chromatography separates according to the affinity of the protein, for the surface of the solid matrix.	Adsorption	Affinity	Ion exchange	Column	Adsorption
48	_____ chromatography is a powerful and highly selective purification technique.	Adsorption	Affinity	Ion exchange	Column	Affinity
49	Microbial cells and other insoluble materials are normally separated from the harvested broth by_____ or _____.	Filtration	Centrifugation	Filtration or centrifugation	Sedimentation	Filtration or centrifugation
50	Which of the following is NOT a batch filter?	Frame filters	Pressure leaf filters	Plate filters	Rotary vacuum filters	Rotary vacuum filters
51	Chilled_____ and_____ can be used in the precipitation of proteins mainly due to changes in the dielectric property of solution.	Ethanol, ethane	Acetone, ketone	Glycol, glycerol	Ethanol, acetone	Chilled Ethanol, acetone
52	Which separation technique is based on differential partitioning between two phases that is mobile and stationary?	Filtration	Precipitation	Centrifugation	Chromatography	Chromatography
53	Vacuum drying uses _____.	Heat	Vacuum	Solvent	Heat and vacuum	Heat and vacuum
54	In spray drying, a current of hot air of ____ is passed.	100-200 °C	10-150 °C	50-150 °C	150-250 °C	150-250 °C
55	The different chromatographic procedures are _____.	Adsorption chromatography	Affinity chromatography	Partition chromatography	All the above	All the above

56	Example of polysaccharides used for an ion exchange chromatography.	Cellulose,	Sepharose	Both A and B	None of the above	Both A and B
57	Penicillin G is usually crystallized by the addition of _____ in ethanol solution.	Potassium acetate	Potassium nitrate	Calcium nitrate	Calcium acetate	Potassium acetate
58	Chromatographic methods are used for purification of _____ compounds from mixtures of similar molecules.	Hydrophillic	Low molecular weight	Hydrophobic	High molecular weight	High molecular weight
59	_____ generally achieves both concentration and separation of the products usually based on the size of molecules	Drying	Immobilization	Membrane filtration	Crystallization	Membrane filtration
60	In gel filtration _____ carriers are used for purification of hydrophobia molecules.	Hydrophillic	Hydrophobic	Both A and B	None of the above	Hydrophobic
61	CSTF is expanded as _____.	Continuous solid tank fermentor	Cell suspended tank fermentor	Continuous solid type fermentor	Continuous stirred tank fermentor	Continuous stirred tank fermentor
62	The enzymes are purified using solvents such as _____.	Acetone	Inorganic-salts(ammonium sulfate)	Alcohols	Acetone, salts and solvents	Acetone, salts and solvents

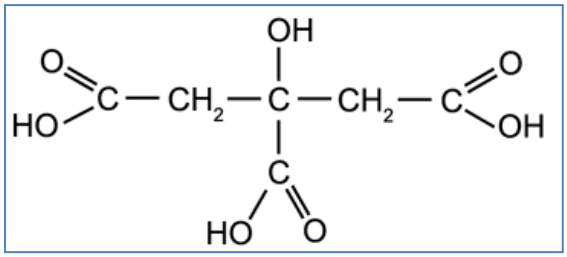
Unit V

Syllabus

Microbial production of industrial products-Citric acid, Ethanol, Penicillin, Glutamic acid, Vitamin B_{12}, Enzymes (amylase, protease, lipase) Wine, Beer (micro-organisms involved, media, fermentation conditions, downstream processing and uses).

1. MICROBIAL PRODUCTION OF CITRIC ACID

Introduction and applications: Citric acid ($C_6H_8O_7$, 2 - hydroxy - 1,2,3 - propane tricarboxylic acid), a natural constituent and common metabolite of plants and animals, is the most versatile and widely used organic acid.



Industrial-scale citric acid production first began in 1890 based on the Italian citrus fruit industry, where the juice was treated with hydrated lime (calcium hydroxide) to precipitate calcium citrate, which was isolated and converted back to the acid using diluted sulfuric acid. In 1893, C. Wehmer discovered *Penicillium* mold could produce citric acid from sugar. However, microbial production of citric acid did not become industrially important until World War I disrupted Italian citrus exports. Currently, the global production of citric acid is estimated to be around 736000 tones/year, and the entire production is carried out by fermentation. In Brazil, almost the entire demand of citric acid is met through imports. More than 50 % of this volume was produced in China. More than 50 % was used as acidity regulator in beverages, some 20 % in other food applications, 20 % for detergent applications and 10 % for related applications other than food, such as cosmetics, pharmaceutics and in the chemical industry. There is constant increase (3.5-4 %) each year in its consumption, showing the need of finding new alternatives for its manufacture.

Citric acid is mainly used in food industry because of its pleasant acid taste, an high solubility in water. It is worldwide accepted as "GRAS" (generally recognized as safe), approved by the Joint FAO/WHO Expert Committee on Food Additives. The pharmaceutical and cosmetic industries retain 10 % of its utilization and the remainder is used for various other purposes.

Micro-organisms used: A large number of micro-organisms including bacteria, fungi and yeasts have been employed to produce citric acid. The fungus *Aspergillus niger* is most commonly used for industrial production of citric acid. The main advantages of using this micro-organism are: (a) its ease of handling, (b) its ability to ferment a variety of cheap raw materials, and (c) high yields. For improved industrial production of citric acid, mutant strains of *A. niger* have been developed. The strains that can tolerate high sugar concentration and low pH with reduced synthesis of undesirable by-products (oxalic acid, isocitric acid and

gluconic acid) are industrially important. Other microorganisms used for the production of citric acid are tabulated.

Table 1. Applications of citric acid

Industry	Applications
Beverages	Provides tartness and complements fruits and berries flavors. Increases the effectiveness of antimicrobial preservatives. Used in pH adjustment to provide uniform acidity.
Jellies, Jams and Preserves	Provides tartness. pH adjustment.
Candy	Provides tartness. Minimizes sucrose inversion. Produces dark color in hard candies. Acts as acidulant.
Frozen fruit	Lowers pH to inactivate oxidative enzymes. Protects ascorbic acid by inactivating trace metals
Dairy products	As emulsifier in ice creams and processed cheese; acidifying agent in many cheese products and as an antioxidant.
Fats and oils	Synergist for other antioxidants, as sequestrant.
Pharmaceuticals	As effervescent in powders and tablets in combination with bicarbonates. Provides rapid dissolution of active ingredients. Acidulant in mild astringent formulation. Anticoagulant.
Cosmetics and toiletries	pH adjustment, antioxidant as a metallic–ion chelator, buffering agent.
Industrial applications	Sequestrant of metal ions, neutralizant, buffer agent
Metal cleaning	Removes metal oxides from surface of ferrous and nonferrous metals, for preperational and operational cleaning of iron and copper oxides
Others	In electroplating, copper plating, metal cleaning, leather tanning, printing inks, bottle washing compounds, floor cement, textiles, photographic reagents, concrete, plaster, refractories and moulds, adhesives, paper, polymers, tobacco, waste treatment, etc.

Micro-organisms		
Mold	**Yeast**	**Bacteria**
A. aculeatus	*Candida tropicalis*	*Bacilluslicheniformis*
A. awamori	*C. oleophila*	*Arthrobacter paraffinens*
A. carbonarius	*C. guilliermondii*	*Corynebacterium sp.*
A. wentii	*C. parapsilosis*	
A. foetidus	*C. citroformans*	
A. clavatus	*Hansenula anamola*	
Penicillium luteum	*C. catenula*	
P. citrinium	*C. lipolytica*	
P. janthinelum	*C. intermedia*	
Mucor piriformis	*C. zeylanoides*	
Sacharomycopsis lipolitica	*Yarrowia lipolytica*	
Trichoderma viride	*C. fibriae*	

Paecilomyces divaricatum	*C. subtropicalix*	
P. purpurogenum		
A. fumaricus		
A. luchensis		
A. fonsecaeus		
Mutants- *Aspergillus niger* C192, *A. niger* GCB-75, *A. niger* YW-112, *A. niger* CBX-209		

Media formulation: Although citric acid is mostly produced from starch or sucrose based media using liquid fermentation, a variety of raw materials such as molasses, several starchy materials and hydrocarbons have also been employed. Several attempts have been made to produce citric acid using molasses, which is preferred due its low cost and high sugar content (40-55 %). The composition of molasses depends on various factors, e.g. the kind of beet and cane, methods of cultivation of crops and fertilizers and pesticides applied during cultivation, conditions of storage and handling (e.g. transport, temperature variations), production procedures, etc. Both, cane and beet molasses are suitable for citric acid production. However, beet molasses is preferred due to its lower content of trace metals. Generally, cane molasses contains calcium, magnesium, manganese, iron and zinc, which have a retarding effect on the synthesis of citric acid. Consequently, some pre-treatment is required for the removal/reduction of trace metals. Despite that, cane molasses posses difficulties in achieving good fermentation yields. The Aspergillus niger spores are developed by growing stock culture onto a solid sporulation medium (glycerol, cane molasses, corn-steep liquor) at 25 °C for 4 to 14 days.

Fermentation and fermentation conditions: The industrial citric acid fermentation can be carried in different ways: submerged fermentation and solid-state fermentation. The production vessel for citric acid must be made of stainless steel and aluminium.

The submerged fermentation is the commonly employed technique for citric acid production. It is estimated that about 80 % of world production is obtained by submerged fermentation. Two types of fermentors, conventional stirred fermentors and tower fermentors are employed, although the latter is preferred due to the advantages it offers on price, size and operation. Inoculation is performed either by adding a suspension of spores, or of pre-cultivated mycelia. When spores are used, a surfactant is added in order to disperse them in the medium. For pre-cultivated mycelia, an inoculum size of 10 % of fresh medium is generally required. pH of 2.5 and below (2.20-1.60; oxalic acid formation is suppressed) is maintained for the maximum production of citric acid. The temperature of incubation can vary from 28 to 30 °C. Normally, submerged fermentation is concluded in 7 to 10 days depending on the process conditions. It is carried out in batch mode.

Solid-state fermentation (SSF) has been termed as an alternative method to produce citric acid from agro-industrial residues. Citric acid production by SSF (Koji process) was first developed in Japan and is as the simplest method for its production. SSF can be carried out using several raw materials. Generally, the substrate is moistened to about 70 % moisture depending on the substrate absorption capacity. The initial pH is normally adjusted to 4.5-6.0 and the temperature of incubation can vary from 28 to 30 °C. The

fermentation period varies depending on the type of the raw material used. It is important to maintain the oxygen concentration above 25 % saturation.

Downstream processing: The recovery of citric acid from liquid fermentation is generally accomplished by three basic procedures: filtration, precipitation, extraction, and adsorption (mainly using ion exchange resins). Usually the recovery starts with the filtration with rotoary vacuum filter of the culture broth and washing of mycelium (which may contain about 10 % of citric acid produced). Oxalic acid is an unwanted by-product and it can be removed by precipitation by adding lime at pH < 3. The culture broth is then subjected to pH 7.2 and temperature 70-90 °C for precipitating citric acid. For further purification, citric acid is dissolved in sulfuric acid (calcium sulfate precipitate separates). The final steps for citric acid recovery are treatment with activated charcoal, cation and anion-exchangers and vacuum crystallization at 20-25 °C, forming citric acid monohydrate which is the main commercial product. Above 40 °C, citric acid crystallizes in an anhydrous form.

2. MICROBIAL PRODUCTION OF ETHANOL

Introduction and applications: Ethanol is a volatile, flammable, colourless liquid with a slight characteristic odour. *Sacchcromyces cerevasiae* is usually employed for commercial production of ethyl alcohol One gram of glucose yields 0.5111 gms of ethanol in microbial fermentation process.
It has many applications such as

- Ethanol is used in medical wipes and most common antibacterial hand sanitizer gels as an antiseptic
- Ethanol may be administered as an antidote to methanol and ethylene glycol poisoning
- Ethanol is one of the most commonly consumed psychoactive drugs
- The largest single use of ethanol is as an engine fuel and fuel additive
- It is found in paints, tinctures, markers, and personal care products such as mouthwashes, perfumes and deodorants
- Ethanol is present in alcoholic beverages such as beer, wine, and whisky
- Ethanol is used as hypnotic

Micro-organisms used: Many of the yeast species are known to ferment hexoses (mainly D-glucose) to ethanol with significantly high yields. With respect to xylose fermentation, yeasts which have been studied extensively are *Pachysolen tannophilus, Candida shehatae, Pichia stipitis* and *Kluyveromyces marxianus*. Various other yeast species which have been investigated for their xylose fermenting ability include *Brettanomyces, Clavispora, Schizosaccharomyces,* several species of *Candida viz. C. tenuis, C. tropicalis, C. utilis, C. blankii, C. friedrichii, C. solani, C. parapsilosis, C. sake*, and species of *Debaromyces* viz. *D. nepalensis, D. polymorpha*. Other microorganisms used for the production of ethanol are tabulated.

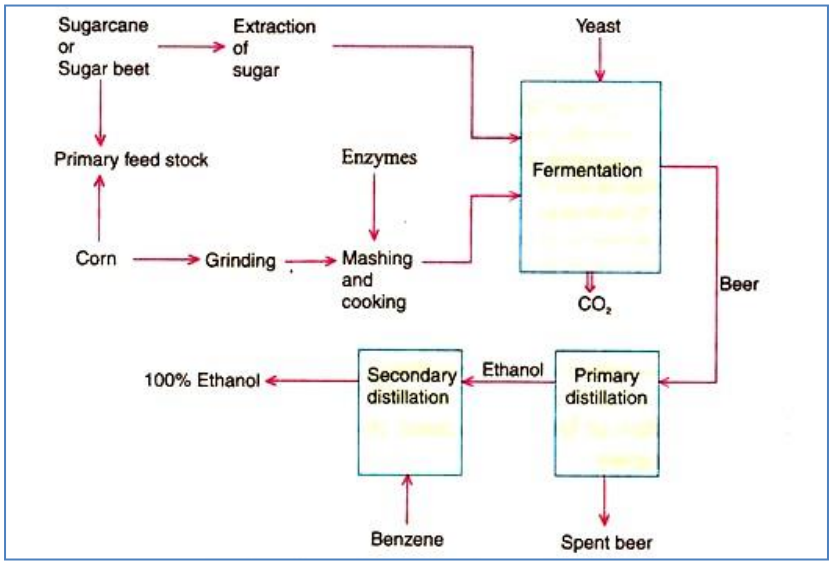
Micro-organisms	
Mold	**Bacteria**
Fusarium oxysporum	*Bacillus macerans*
Neurospora crassa	*Clostridium thermocellum*
Paecilomyces sp.	*C. saccharolyticum*
Monilia sp.	*Thermoanaerobacter ethanolicus*

Media formulation: Three types of substrates are used for ethanol production:
(a) Starch containing substrate,
(b) Juice from sugarcane or molasses or sugar beet,
(c) Waste products from wood or processed wood. Production of ethanol from whey is not viable.

If yeast strains are to be used, the starch must be hydrolysed as yeast does not contain amylases. After hydrolysis, it is supplemented with celluloses of microbial origin so as to obtain reducing sugars. About 1 ton of starch required 1L of amylases and 3.5 L of glucoamylases.

On the other hand, if molasses are used for ethanol production, the bagasse can also give ethanol after fermentation. Several other non-conventional sources of energy such as aquatic plant biomass, wood after hydrolysis with celluloses gives ethanol. Sulphite waste-liquor also contains hexose as well as pentose sugar.

Fermentation and fermentation conditions: Ethanol is produced by continuous fermentation and batch fermentation und anaerobic conditions. Other fermentation conditions are almost similar (pH 5.0, temperature 35 °C) but the cultures and culture conditions are different. The fermentation is normally carried out for several days but within 12h starts production. After the fermentation is over, the cells are separated to get biomass of yeast cells which are used as single cell protein (SCP) for animal's feed. The culture medium or supernatant is processed for recovery of ethanol.

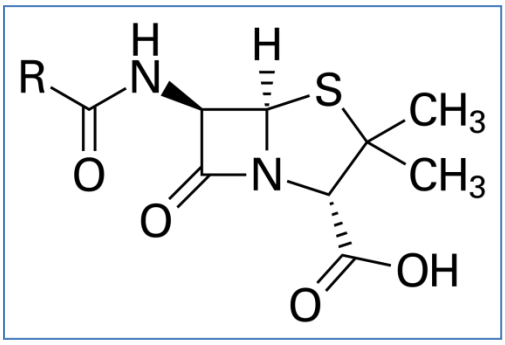


Downstream processing: Fermentation by-products are mostly removed by distillation. However, volatile by-products tend to lodge more in ethanol. Also, especially for drinking or pharmaceutical purpose, high concentration of ethanol is not required. In this case, further distillation is just waste of energy and money. In ethanol production, a distillation tower is designed to separate water and ethanol effectively. Water is obtained from the bottom of the tower and ethanol is obtained from the top of the tower. It is expected that impurities with similar boiling points to ethanol lodges in ethanol even after distillation. Ethanol can be recovered up to 95 percent by successive distillations. To obtain 100 percent, it requires forming an azeotropic mixture containing 5 percent water. Thus 5 percent water is removed from azeotropic mixture of

ethanol, water and benzene after distillation. In this procedure, benzene water ethanol and then ethanol-benzene azeotropic mixture are removed so that absolute alcohol is obtained.

3. MICROBIAL PRODUCTION OF PENICILLIN

Introduction and applications: Penicillin was the first naturally occurring antibiotic discovered. It is obtained in a number of forms from *Penicillium* moulds. Penicillin is not a single compound but a group of closely related compounds, all with the same basic ring-like structure (a β-lactam) derived from two amino acids (valine and cysteine) via a tripeptide intermediate. The third amino acid of this tripeptide is replaced by an acyl group (R) and the nature of this acyl group produces specific properties on different types of penicillin.



There are two different types of penicillin.

Biosynthetic penicillin is natural penicillin that is harvested from the mould itself through fermentation. Other naturally occurring penicillins are Penicillin G (Benzyl penicillin), Procaine Penicillin G, Penicillin V (phenoxymethyl penicillin), and Benzathine. Penicillin resistant *Staphylococcus aureus* produces an enzyme called penicillinase o β-lactamase which cleaves the β-lactum ring of penicillin.

Semi-synthetic penicillin includes semi synthetic derivatives of penicillin - like Ampicillin, Penicillin V, Carbenicillin, Oxacillin, Methicillin, etc. These compounds consist of the basic Penicillin structure, but have been purposefully modified chemically by removing the acyl group to leave 6-aminopenicillanic acid and then adding acyl groups that produce new properties. These modern semi-synthetic penicillins have various specific properties such as resistance to stomach acids so that they can be taken orally, a degree of resistance to penicillinase (or β-lactamase) (a penicillin-destroying enzyme produced by some bacteria) and an extended range of activity against some Gram-negative bacteria. Penicillin G is the most widely used form and the same one we get in a hypodermic form.

Micro-organisms used: Various species of *Penicillium* (*P. Notatum, P. allii-sativi, P. dipodomyis, P. flavigenum, P. nalgiovense, P. rubens, P. tardochrysogenum* and *P. vanluykii*) are most commonly used for industrial production of Penicillin. For improved industrial production of Penicillin, mutant strains of *Penicillium* have been developed.

Media formulation: The production medium contains the following compounds:

- Lactose: 3-4 %
- Calcium carbonate: 1%
- Glucose or molasses: 10 %

- Corn steep liquor: 4 %
- Phenyl acetic acid: 0.5-0.8 %
- Potassium dihydrogen phosphate: 0.4 %
- Antifoaming agent (Vegetable oil): 0.25-0.5 %

Fermentation and fermentation conditions: Production fermentors are agitated tank 200-250 m^3 in volume made of stainless steel. Mechanical agitation is provided at the rate of 100-300 rpm in batch or fed batch mode. Temperature is controlled around 25-28 ºC by using cooling coils. Most filamentous fungi are difficult mix in the both due to the high (and not constant) viscosity of the medium. Dissolved oxygen is controlled at >2 mg/L and pH at 6.5. Biomass doubling is about 6 h. Three phases of growth can be differentiated during cultivation of *Penicillium chrysogenum*.

(i) First phase- In this phase, growth of the mycelium occurs, yield of antibiotic is quite low. Lactic acid present in corn steep liquor is utilized at the maximum rate by the microorganisms. Lactose is used slowly. Ammonia is liberated into the medium resulting into the rise in pH

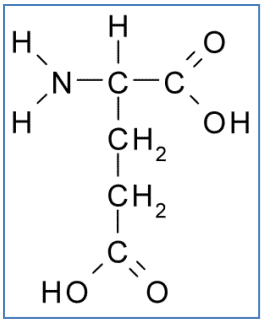
(ii) Second phase- There was intense synthesis of penicillin in this phase, due to rapid consumption of lactose and ammonia nitrogen. The mycelia mass increases, the pH remain unchanged

(iii) Third phase- The concentration of antibiotic decreases in the medium. The autolysis of mycelium starts, liberation of ammonia and slight rise in pH

Downstream processing: Once the formation is completed the broth is separated from fungal mycelium by filtration with rotoary vacuum filter and processed by adsorption, precipitation and crystallization to yield the final product. Penicillin is recovered by solvent extraction at an acidic pH at temperature below 10 ºC. The solid can be removed by ultrafilteration. Mycelium can be treated, dried and used as soil conditioner. The penicillin rich solvent can be treated with activated carbon, to remove pigments and other impurities and the penicillin recovered as the potassium or the sodium salt by adding potassium or sodium acetate to the solvent. Further impurities can be removed by washing the recovered salt with a dry solvent such as isopropanol or n-butanol.

4. MICROBIAL PRODUCTION OF GLUTAMIC ACID

Introduction and applications: Glutamic acid is an α-amino acid containing a α-carboxylic acid group and a side chain of carboxylic acid. It is nonessential amino acid in humans. L-glutamic acid was the first amino acid produced commercially. Glutamic acid commercial production by microbial fermentation provides 90% of world's total demand, and remaining 10% is met through chemical methods. The world production of glutamic acid is to the tune of 800,000 tonnes/year.



Applications of Glutamic Acid as follows:

- L-Glutamic acid is used as nutritional supplement in food such as in processed cheeses, processed fruits, processed meats, soups, wines, seasonings, and yogurts, canned shrimps, crabs etc.
- L-Glutamic acid can be used as flavor enhancer in beverage such as soft drink and wine.
- L-Glutamic acid can be used as nutritional supplement in Agriculture/Animal Feed/Poultry feed.
- L-Glutamic acid is the raw materials manufacturing of monosodium glutamate (MSG).
- L-Glutamic acid can be used in cosmetics and personal care products. L-Glutamic Acid can be used in baby products, bath products, cleansing products, eye makeup, shaving preparations and hair and skin care products. In cosmetics and personal care products, L-Glutamic Acid function primarily as hair conditioning agents and skin conditioning agents – miscellaneous

Micro-organisms used: *Corynebacterium* glutamicum is used for economic production of glutamic acid by submerged fermentation. Other organisms found to accumulate significant quantities of glutamic acid were; *Corynebacterium lilium, C. callunae, Brevibacterium devaricatum, Br. lactofermentum, Br. saccharolyticum, Br. flayum, Br. immariophilium, Br. roseum* and *Bacillus megaterium*

Media formulation: α-ketoglutaric acid serves as the precursor of glutamic acid and the conversion of the α-ketoglutaric acid to glutamic acid occurs in presence of enzyme glutamic acid dehydrogenase.
Biotin concentration in the fermentation medium has a significant influence on the yield of glutamic acid.

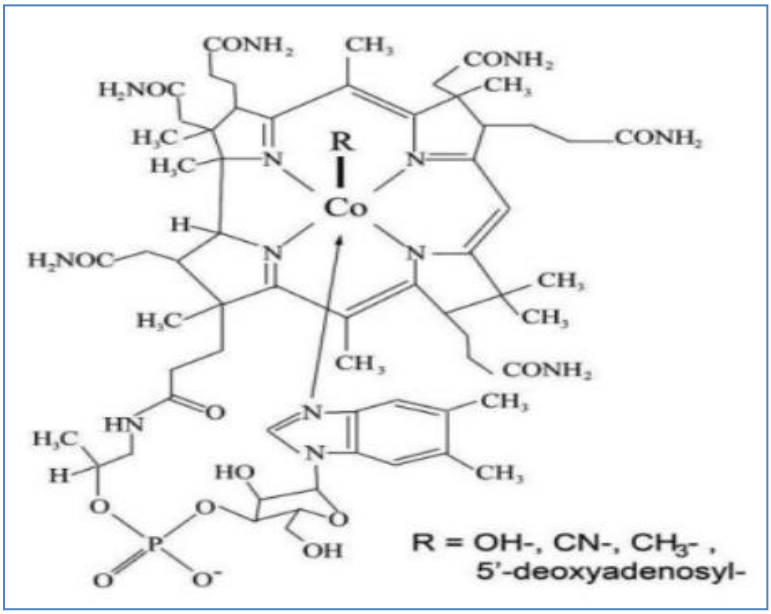
- Molasses- 50 %
- Corn steep liquor: 8.0 %
- Biotin- 1.0 %
- α-ketoglutaric acid- 5.0 %
- K_2HPO_4- 1.0 %
- $MgSO_4$- 2.5 %
- $CaCO_3$- 1.6 %
- Urea- 4.0 %

Fermentation and fermentation conditions: Stirred tank fermentor is used for economic production of glutamic acid by submerged fermentation. The volume of bacterial inoculum used is 0.5-5 % of total fermentation medium. Fermentation conditions such as aeration, agitation, temperature- 30 °C, pH- 6 were maintained for the maximum production of glutamic acid. Fermentation completes within 2-4 days and at the end of the fermentation, the broth contains glutamic acid in the form of its ammonium salt.

Downstream processing: In a typical downstream process, the bacterial cells are separated though filtration with rotoary vacuum filter and the culture free broth is passed through a basic anion exchange resin. Glutamic acid anions get bound to the resin and ammonia is released. This ammonia can be recovered via distillation and reused in the fermentation. Elution is performed with NaOH to form monosodium glutamate (MSG) in the solution and to regenerate the basic anion exchanger. From elute, MSG may be crystallized directly followed by further conditioning steps like decolourization and serving to yield a food-grade quality of MSG.

5. MICROBIAL PRODUCTION OF VITAMIN B_{12}

Introduction and applications: Vitamin B12, like so many other members of the B complex, is not a single substance; rather, it consists of several closely related compounds with similar activity.



VitaminB-12, which is the most active member, is cyanocobalamin, name after the cyanide ion in the~molecule. Other chemically related compounds known to have vitamin B12 activity include hydroxocobalamin, nitritocobalamin, and thiocyanate cobalamin. Vitamin B_{12} is present in animal tissue at a very low concentration (e.g. 1 ppm in the liver). It occurs mostly in the coenzyme forms- methylcobalamin and deoxyadenosylcobalamin. Isolation of vitamin B_{12} from animal tissues is very expensive and tedious. It is estimated that the world's annual production of vitamin B_{12} is around 15,000 kg.

Applications of Vitamin B12 as follows:

- Vitamin B12 is used as nutrition supplements in food and beverage
- Vitamin B12 can be used as Nutritional therapy in Pharmaceutical in treatment of vitamin B12 deficiency and pernicious anemia, liver disease, neuritis, neuralgia
- Vitamin B12 used as Skin-Conditioning Agent.
- Vitamin B12 can be used as supplement in Agriculture/Animal Feed/Poultry products such as in cattle, sheep.

Micro-organisms used: For the commercial production of vitamin BI2, chemical or semi synthetic procedures are feasible. Various types of microorganisms are used for commercial production of vitamin BI2. Because of their already naturally high vitamin B12 productivity and their rapid growth, mainly *Propionibacterium shermanii* (GRAS) and *Pseudomonas denitrificans* strains are employed for industrial production.

Bacteria	Yield (mg/L)
Propionibacterium freudenreichii	206.0
Rhodopseudomonas protamicus	135.0
Propionibacterium shermanii	60.0
Pseudomonas denitrificans	60.0
Nocardia rugosa	18.0
Rhizobium cobalaminogenum	16.5
Micromonospora sp.	11.5
Streptomyces olivaceus	6.00
Nocardia gardneri	4.50
Butyribacterium rettgeri	3.60
Arthrobacter hyalinus	1.10
Bacillus megaterium	0.51

Media formulation: The production medium contains the following compounds:

- Corn steep liquor
- Beet molasses
- Glucose/soybean meal
- Ammonium phosphate
- Ammonium hydroxide
- Cobalt chloride

Fermentation and fermentation conditions: The bulk production of vitamin B12 is mostly done by submerged fermentation in a stirred tank fermentor. The process is carried out by adding cobalt in two phases. Anaerobic phase: This is a preliminary phase that may take 2-4 days. In an anaerobic phase 5′-deoxyadenosylcobinamide is predominantly produced.
Aerobic phase: In this phase, 5, 6-dimethyl- Benz imidazole is produced from riboflavin which gets incorporated to finally form coenzyme of vitamin B-p namely 5′-deoxyadenosylcobalamin. This is a phase that may take 3-5 days. In recent years, some fermentation technologists have successfully clubbed both an anaerobic and aerobic phases to carry out the operation continuously in two reaction tanks. The temperature 25 C-37 °C and a pH in the range of 6-7 are maintained during the fermentation. Vitamin B12 is formed by linking of a cobinamide linked to a nucleotide.

Downstream processing: The cobalamins produced by fermentation are mostly bound to the cells. The extraction process depends on the release of unstable cobalamin from the cells lysed and subsequent treatment of this with cyanide to convert into more stable cyanocobalamin. The solids and mycelium are filtered and the fermentation broth collected. Cells are lysed by heat treatment at 80-120 °C for about 30 min at pH 6.5-8.5. These are then solubilized with potassium cyanide in the presence of sodium nitrite. The obtained cobalamin gets converted into cyanocobalamin. The purification of the product is done using adsorption method for substances like amberlite IRC SO, Dowex Ix2, alumina, silanized silica gel, and Amberlite XAD2. It is then followed by elution with water-alcohol or water-phenol mixtures. This vitamin

B_{12} is around 80 % purity and can be directly used as a feed additive. However, for medical use (particularly for treatment of pernicious anemia), vitamin B_{12} should be further purified (95-98 % purity).

6. MICROBIAL PRODUCTION OF AMYLASE

Introduction and applications: Enzymes are biological catalysts which are an indispensible component of biological reactions. The use of chemical catalysts has been followed for a very long time. Amylases are among the most important enzymes and are of great significance for biotechnology, constituting a class of industrial enzymes having approximately 25 % of the world enzyme market. Among the many enzymes that are widely used, α-Amylase has been in increasing demand due to its crucial role of starch hydrolysis and the applications of this hydrolytic action. α-Amylases have potential application in a wide number of industrial processes such as food, fermentation, textile, paper, detergent, and pharmaceutical industries.

Micro-organisms used: α-Amylase can be isolated from plants, animals or microorganisms. The enzyme has been isolated from barley and rice plants. α-Amylase is produced by several bacteria, fungi and genetically modified species of microbes. The fungal source used predominantly for commercial production of α-Amylase is the strains of *Aspergillus spp*. (*Aspergillus oryzae, A. niger and A. awamori* and *A. fumigates*). Other microorganisms which have been explored for production of the enzyme include *B.cereus, B. subtilis, B. licheniformis, B. stearothermophilus, B. amyloliquefaciens, B. licheniformis, Chromohalobactersp., Halobacillus sp., Haloarcula hispanica, Halo monas meridiana, Bacillus dipsosauri, Penicillium, P. Brunneum, Penicillium fellutanum, Penicillium expa nsum* MT-1, *Penicillium chrysogenum , Bacillus amyloliquefaciens* UNG-16, *B.subtilis* YN9

Media formulation: Starch, wheat bran, rice bran, maize bran, sugar cane bagasse, wheat straw, rice straw, rice husk, banana waste, tea waste, cassava waste, Corn steep liquor, cassava flour, corn flour, wheat flour, steamed rice, molasses. In addition to the cud media certain minerals (KCl, Na_2HPO_4, $CaCL_2$, $MgCL_2$) are also added for the maximum production of enzyme.

Fermentation and fermentation conditions: There are two methods used for production of α-Amylase on a commercial scale. These are: 1) Submerged fermentation (batch-fed and continuous fermentation); 2) Solid State fermentation.

Submerged fermentation employs the use of batch-fed and continuous fermentation methods to produce industrial enzymes by cultivation of microorganisms in closed containers (1,000 cubic meters volumes) having a high concentration of oxygen. SSF systems appear promising due to the natural potential and advantages they offer. SSF resembles the natural habitat of microorganism and is, therefore, the preferred choice for microorganisms to grow and produce useful value added products. An optimum temperature (molds- 30 to 50 °C; bacteria- 35 °C) and pH (molds-5.0–6.0; bacteria-7.0) are maintained during fermentation period (3-4 days).

Downstream processing: Purification methods commonly employed are filtration, precipitation, chromatography and liquid-liquid extraction depending on the properties of the enzyme desired. The crude extracellular enzyme sample can be obtained from the fermented mass by filtration and centrifugation. In the case of intracellular enzymes, raw corn starch may be added followed by filtration and subsequent steps. The

crude amylase enzyme can be precipitated and concentrated using ammonium sulphate precipitation or organic solvents (acetone and salts). The precipitated sample can be subjected to dialysis against water or a buffer for further concentration. This can be followed by any of the chromatographic techniques like ion exchange, gel filtration and affinity chromatography for further separation and purification of the enzyme.

7. MICROBIAL PRODUCTION OF PROTEASE

Introduction and applications: Protease constitutes a large and complex group of enzymes that plays an important nutritional and regulatory role in nature. Proteases are (physiologically) necessary for living organisms; they are ubiquitous and found in a wide diversity of sources. Protease is the most important industrial enzyme of interest accounting for about 60 % of the total enzyme market in the world and account for approximately 40 % of the total worldwide enzyme sale. These enzymes are found in a wide diversity of sources such as plants, animals and microorganisms but they are mainly produced by bacteria and fungi. Microbial proteases are predominantly extracellular and can be secreted in the fermentation medium.

They are generally used in detergents, food industries, leather, meat processing, cheese making, silver recovery from photographic film, production of digestive and certain medical treatments of inflammation and virulent wounds. They also have medical and pharmaceutical applications. Microbial proteases are degradative enzymes, which catalyze the total hydrolysis of proteins.

Micro-organisms used: Several species including *Aspergillus flavus, Aspergillus melleu, Aspergillus niger, Chrysosporium keratinophilum, Fusarium graminarum, Penicillium griseofulvin, Scedosporium apiosermum, Bacillus licheniformis, Bacillus firmus, Bacillus alcalophilus, Bacillus amyloliquefaciens, Bacillus proteolyticus, Bacillus subtilis, Bacillus thuringiensis* are reported to produce proteases.

Media formulation: wheat or rice bran , beef extract, tryptone, peptone, glycine, casein, con steep liquor, soya bean meal, KNO_3, $MgSO_4$, K_2HPO_4, $ZnSO_4$, $FeSO_4$, $MnSO_4$.

Fermentation and fermentation conditions: There are two methods used for production of protease on a commercial scale. These are: 1) Submerged fermentation, 2) Solid State fermentation.

The moist bran, inoculated with spores of the appropriate fungi, is distributed either in flat trays or placed in a revolving drum. Moisture (about 8 %) is maintained by occasionally by providing the moist air and by circulating moist air over the preparation. The pH and temperature of the bran is kept at 5.0 and 30 °C respectively. The production period is usually 4 days, but could be as long as 7 days.

Downstream processing: In order to limit contamination and degradation of the enzyme the broth is cooled to about 20 °C as soon as the fermentation is over. Stabilizers such as calcium salts, proteins, sugar, and starch hydrolysates may be added and destabilizing metals may be removed with EDTA. Antimicrobials if used at all are those that are normally allowed in food such as benzoates and sorbate. Enzymes precipitated using a variety of chemicals such as methanol, acetone, ethyl alcohol or ammonium sulphate. The precipitate is further purified by dialysis, chromatography, etc., before being dried in a drum drier or a low temperature vacuum drier depending on the stability of the enzymes to high temperature.

8. MICROBIAL PRODUCTION OF LIPASE

Introduction and applications: Lipases catalyze the hydrolysis and the synthesis of esters formed from glycerol and long-chain fatty acids. Lipases occur widely in nature, but only microbial lipases are commercially significant. The many applications of lipases include specialty organic syntheses, hydrolysis of fats and oils, resolution of racemic mixtures, chemical analyses, dairy products, Beverages, Laundry/surfactant, Pharmaceutical, Textile, Cosmetics, Agrochemicals and Fuel industries.

Micro-organisms used: *Acinetobacter radioresistens, Pseudomonas aeruginosa, Staphylococcus caseolyticus, Bacillus spp. (B. stearothermophilus, B. coagulans, B. subtilis), Burkholderia cepacia, Serratia rubidaea, Rhizopus arrhizus, R. oryzae, R. chinensis, R. homothallicus, Aspergillus carneus, A. niger, Penicillium citrinum, P. restrictum, P. simplicissimum, P. verrucosum, Geotrichum candidum, Colletotrichum gloesporioides, Candida utilis, C. rugosa, C. cylindracea, Yarrowia lipolytica, Rhodotorula mucilaginosa, Aureobasidium pullulans, Saccharomyces cerevisiae, Williopsis californica*

Media formulation: Palm kernal cake, wheat bran, rice bran, sunflower oil, olive oil, castor oil cake, coconut oil cake, sugar cane bagasse, gingelly oil cake, babassu oil cake, mustard oil cake, shea butter cake, soya bean, rice husk, deoiled rice bran, rice bran oil and mineral salts. Inducers such as cetyl trimethylammonium bromide, tween 20, triton x-100, olive oil, sesame oil and sunflower oil, canola oil are also used.

Fermentation and fermentation conditions: The bulk production of lipase is mostly done by submerged fermentation in a fermentor. The volume of bacterial inoculum used is 0.5-5 % of total fermentation medium. Fermentation conditions such as aeration, agitation, temperature- 25 to 35 °C, pH- 3 to 8 were maintained for the maximum production. Fermentation completes within 4-7 days.

Downstream processing: The usual method used to extract this enzyme is homogenization of the substrate or mixing it in a rotary shaker with one of the various solvents including water, NaCl, $(NH_4)_2SO_4$ and NaCl with tween 80, triton X-100. The temperature at which the mixing is done is kept high enough for maximum extraction of the enzyme. However, there is no marked difference in the recovery of enzyme using various solvents just by themselves. The mixture is then filtered using a double layer muslin cloth and the filtrate is centrifuged at around 5000g for 20 min at low temperature of around 4 °C to prevent enzyme denaturation. The clear supernatant is used as the extracellular enzyme. Supplementation of the solvent with surfactants like Triton X-100 increases recovery by increasing the membrane permeability**.** Phosphate buffer is also used for extraction of these enzymes with suitable pH values.

9. MICROBIAL PRODUCTION OF WINE

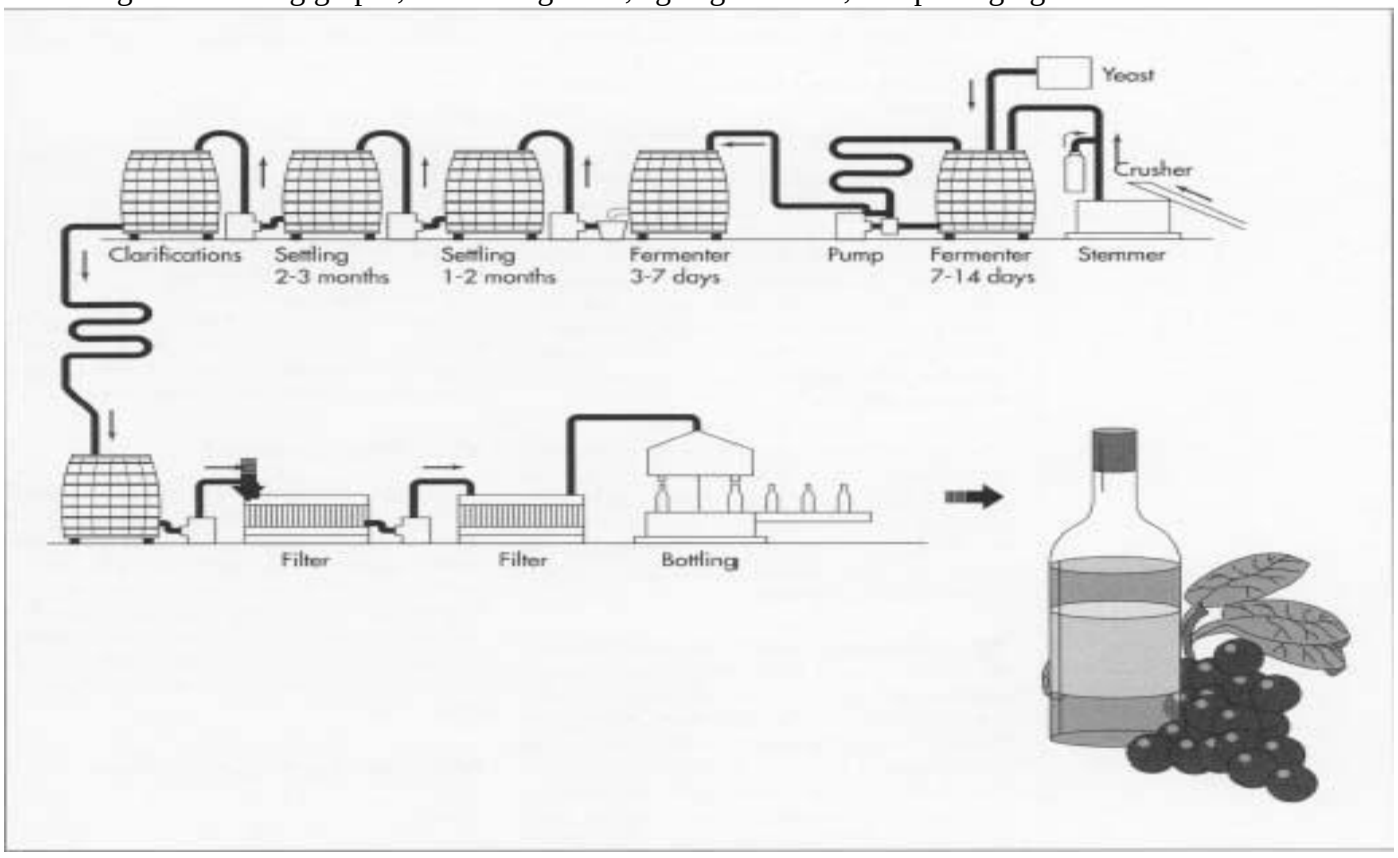
Introduction and applications: Wine is an alcoholic beverage made from grapes fermented without the addition of sugars, acids, enzymes, water, or other nutrients. Wine is a popular and important beverage that accompanies and enhances a wide range of cuisines, from the simple and traditional stews to the most sophisticated and complex haute cuisines. Wine is often served with dinner. Sweet dessert wines may be served with the dessert course. Wine is important in cuisine not just for its value as a beverage, but as a flavour agent, primarily in stocks and braising, since its acidity lends balance to rich savoury or sweet dishes. Wine sauce is an example of a culinary sauce that uses wine as a primary

ingredient. Natural wines may exhibit a broad range of alcohol content, from below 9 % to above 16 % ABV, with most wines being in the 12.5–14.5 % range. Fortified wines (usually with brandy) may contain 20 % alcohol or more.

Micro-organisms used: Various species of *Saccharomyces, Candida, Pichia, Brettanomyces, Hanseniaspora, Kluyveromyces, Issatchenkia, Torulaspora, Debaryomyces, Zygosaccharomyces* and *Schizosaccharomyces*

Media formulation: The wine grape itself contains all the necessary ingredients for wine: pulp, juice, sugars, acids, tannins, and minerals. However, some manufacturers cane or beet sugar to increase alcoholic content. During fermentation, winemakers also add sulphur dioxide to control the growth of wild yeasts.

Fermentation and fermentation conditions: In winemaking, the temperature and speed of fermentation are important considerations as well as the levels of oxygen present in the must at the start of the fermentation. The risk of stuck fermentation and the development of several wine faults can also occur during this stage, which can last anywhere from 5 to 14 days for *primary fermentation* and potentially another 5 to 10 days for a *secondary fermentation*. Fermentation may be done in stainless steel tanks, which is common with many white wines like Riesling, in an open wooden vat, inside a wine barrel and inside the wine bottle itself as in the production of many sparkling wines. The wine-making process can be divided into four distinct steps: harvesting and crushing grapes; fermenting must; ageing the wine; and packaging.



The grapes are crushed and the stems are removed, leaving liquid must that flows either into a stainless steel fermentation tank or a wooden vat (for fine wines). For white wine, all the grape skins are separated from the "must" by filters or centrifuges before the must undergoes fermentation. For red wine, the whole crushed grape, including the skin, goes into the fermentation tank or vat. (The pigment in the grape skins gives red wine its colour. The amount of time the skins are left in the tank or vat determines how dark or light the color will be. For rose, the skins only stay in the tank or vat for a short time before they are filtered out.)

During the fermentation process, wild yeast is fed into the tank or vat to turn the sugar in the must into alcohol. To add strength, varying degrees of yeast may be added. In addition, cane or beet sugar may be added to increase the alcoholic content. Adding sugar is call *chaptalization*. Usually chaptalization is done because the grapes have not received enough sun prior to harvesting. The winemaker will use a handheld hydrometer to measure the sugar content in the tank or vat. The wine must ferments in the tank or vat for approximately seven to fourteen days, depending on the type of wine being produced.

Downstream processing: After crushing and fermentation, wine needs to be stored, filtered, and properly aged. In some instances, the wine must also be blended with other alcohol. Many wineries still store wine in damp, subterranean wine cellars to keep the wine cool, but larger wineries now store wine above ground in epoxy lined and stainless steel tanks. The tanks are temperature-controlled by water that circulates inside the lining of the tank shell. Other similar tanks are used instead of the old redwood and concrete vats when wine are temporarily stored during the settling process.

After fermentation, certain wines (mainly red wine) will be crushed again and pumped into another fermentation tank where the wine will ferment again for approximately three to seven days. This is done not only to extend the wine's shelf life but also to ensure clarity and colour stability.

The wine is then pumped into settling ("racking") tanks or vats. The wine will remain in the tank for one to two months. Typically, racking is done at 10 to 16 °C for red wine, and 0 °C for white wine.

After the initial settling (racking) process, certain wines are pumped into another settling tank or vat where the wine remains for another two to three months. During settling the weighty unwanted debris (remaining stem pieces, etc.) settle to the bottom of the tank and are eliminated when the wine is pumped into another tank. The settling process creates smoother wine. Additional settling may be necessary for certain wines.

After the settling process, the wine passes through a number of filters or centrifuges where the wine is stored at low temperatures or where clarifying substances trickle through the wine.

After various filtering processes, the wine is aged in stainless steel tanks or wooden vats. White and rose wines may age for a year to four years, or far less than a year. Red wines may age for seven to ten years. Most large wineries age their wine in large temperature-controlled stainless steel tanks that are above ground, while smaller wineries may still store their wine in wooden barrels in damp wine cellars.

The wine is then filtered one last time to remove unwanted sediment.

10. MICROBIAL PRODUCTION OF BEER

Introduction: Beer is one of the oldest and most widely consumed alcoholic drinks in the world, and the third most popular drink overall after water and tea. The strength of modern beer is usually around 4 % to 6 % alcohol by volume (ABV), although it may vary between 0.5 % and 20 %, with some breweries creating examples of 40 % ABV and above.

Micro-organisms used: The dominant types of yeast used to make beer are *Saccharomyces cerevisiae*, known as ale yeast, and *Saccharomyces pastorianus*, known as lager yeast; *Brettanomyces* ferments lambics, and *Torulaspora delbrueckii* ferments Bavarian weissbier and flavoured with hops (Hops are the female flower clusters or seed cones of the hop vine *Humulus lupulus*, which are used as flavouring and preservative agent in nearly all beer made today. Hops contain several characteristics that brewers desire in beer: they contribute a bitterness that balances the sweetness of the malt; they provide floral, citrus, and herbal aromas and flavours; they have an antibiotic effect that favours the activity of brewer's yeast over less desirable microorganisms).

Hops flower clusters

Media formulation: The basic ingredients of beer are water and a fermentable starch source such as malted barley. Less widely used starch sources include millet, sorghum and cassava. Secondary sources (adjuncts), such as maize (corn), rice, or sugar, may also be used, sometimes to reduce cost, or to add a feature, such as adding wheat to aid in retaining the foamy head of the beer. The proportion of each starch source in a beer recipe is collectively called the grain bill.

Fermentation and fermentation conditions: Fermentation may take place in an open or closed fermenting vessel; a secondary fermentation may also occur in the cask or bottle. Most breweries today use cylindroconical vessels, or CCVs, which have a conical bottom and a cylindrical top. The cone's aperture is typically around 60°, an angle that will allow the yeast to flow towards the cone's apex, but is not so steep as to take up too much vertical space. Steps in the brewing process include malting, milling, mashing, lautering, boiling, fermenting, conditioning, filteringand packaging. There are three main fermentation methods, warm, cool and spontaneous.

Fully ripened barley grains are "steeped," or soaked in cold water until they are fully saturated. The water is changed once a day, and after 45-72 hours the grains are placed in shallow tanks. The grain is aerated and stirred, which causes it to germinate, releasing enzymes such as malt diastase. Malt diastase converts the starches contained in the grain to sugar for fermentation. As soon as the germination is adequately complete, usually six days, the grain is roasted to stop the germination process. The exact point at which the roasting starts and ends affects the flavor and color of the beer. The product at this point is referred to as malt.

The malt is crushed using iron rollers and transferred to the mash tank (or "tun"). This tank is a large copper or stainless steel vessel that mixes the malt with warm water until it is of porridge-like consistency. This mixture is called mash. After mixing with similarly prepared cereal grains, the temperature of the mash

is raised incrementally from 38-77 °C so that the enzymes react. The enzymes break down the starch in the grain and convert it to simple sugars. Later, the yeast will convert the sugars into alcohol. Once complete, the mash is allowed to sit undisturbed so the solids can descend to the bottom of the tank

The liquid contained in the mash is transferred into another tank called a lauter tun. This is accomplished by drawing the liquid out through the bottom layer of mash solids, which acts as a filter. Hot water is added to the top of the mash tank to rinse the remaining liquid, now called wort, from the mash. The solid remains of the grain are dried and sold by the brewery as animal feed. The wort travels on to the brew kettles, where it is boiled to sterilize it, and where the carefully prepared hops are added. The addition of the hops is important because they contribute to the bitterness of the beer. The brew kettles are the most impressive equipment in the process. Gleaming copper, they can be 7-12 feet (2-3.6 m) in diameter and two stories high. Steam usually provides the heating energy to the brew kettles. After brewing is complete, the finished wort is filtered again and pumped to the fermentation tanks

In the fermentation tanks, the atmosphere is carefully controlled to prevent any "rouge" bacteria from interfering with the yeast. Carefully maintained yeast (approximately one pound per barrel of wort) is added to the wort in presence of hops and the temperature of the mixture is slowly reduced over a period of days to between 10-15 °C. In this temperature range, the yeast grows, consuming the sugar in the wort, and bubbles of carbon dioxide form. The wort has now become beer. The new beer is filtered and transferred once more into the aging casks, where the temperature is controlled at 0.5 °C for 2-24 weeks (lagering). The shorter storage time produces a pale lager beer while the European lagers (called Pilsner) are aged longer to increase the alcohol content

Downstream processing: After aging, the beer can be pasteurized to kill the remaining yeast and prevent further alcohol production. This is accomplished by heating the beer above 57 °C. These beers are also known as "ice" beers, since they must be kept refrigerated to preserve their flavour and slow the remaining yeast activity. Many consider the draft beers best in aroma as well as taste.

Some brewers add one or more clarifying agents to beer, which typically precipitate (collect as a solid) out of the beer along with protein solids and are found only in trace amounts in the finished product. This process makes the beer appear bright and clean, rather than the cloudy appearance of ethnic and older styles of beer such as wheat beers. Examples of clarifying agents include: isinglass, obtained from swim bladders of fish; Irish moss, a sea weed; kappa carrageenan, from the seaweed *Kappaphycus cottonii*; Polyclar (artificial); and gelatin.

Unit-5; Possible questions

Part-A (1 Mark)

Part- B (2 Marks)

1. Mention the microorganisms used for the production of citric acid.
2. Mention the fermentation conditions of penicillin production.
3. Mention the cud media used for the production of lipase
4. Mention the cud media used for the production of amylase and lipase
5. What are the microorganisms used for the production of wine?
6. Mention the steps involved in the beer production.
7. What are the microorganisms used for the production of Vitamin B12?
8. Mention the media components used for the production of Penicillin
9. What are the microorganisms used for the production of citric acid?

Part-C (8 Marks)

1. Detail on the microbial production of ethanol.
2. Brief on production of amylase enzyme.
3. Detail on the microbial production of citric acid.
4. Detail on microbial production of beer.
5. Brief on microbial production of Vitamin B12.
6. Describe the methodology for production of glutamic acid
7. Brief on production of amylase enzyme Wine.
8. Describe the methodology for production of Penicillin.
9. Brief on production of protease enzyme.
10. Detail on microbial production of lipase.

KARPAGAM ACADEMY OF HIGHER EDUCATION

CLASS: II B.Sc MB COURSE NAME: INDUSTRIAL MICROBIOLOGY

COURSE CODE: 17MBU303 UNIT: V BATCH-2017-2020

Sl. No	Question	Option A	Option B	Option C	Option D	Correct Ans
1	The *Aspergillus niger* spores are developed by growing stock culture onto a solid _____ at 25 ℃ for 4 to 14 days.	Production medium	Sporulation medium	Growth medium	Optimization medium	Sporulation medium
2	The most efficient industrial strain used in production of citric acid is ----------.	*Aspergillus niger*	*Aspergillus terreus*	*Aspergillus clavatus*	*Aspergillus oryzae*	*Aspergillus niger*
3	_____ is often the best carbon substrate for production of citric acid.	Sugarcane molasses	Beet molasses	Wood molasses	Corn steep liquor	Beet molasses
4	The optimum pH for citric acid production in submerged fermentation is _____.	< 2.50	3.6 to 4.20	4.5-6.0	1.2 to 2.6	< 2.50
5	The optimum temperature for citric acid production is _____.	25 ℃ to 27 ℃	26 ℃ to 28 ℃	28 ℃ to 30 ℃	27 ℃ to 29 ℃	28 ℃ to 30 ℃
6	The citric acid fermentation using *Aspergillus niger* at low pH is highly favourable as _____.	Sterilization of medium is not effective	Citric acid is produced at very low yields	Oxalic acid formation is suppress	There is high rich of contamination	Oxalic acid formation is suppress
7	The production vessel for citric acid must be made of _____ as they are less prone to conversion with iron.	Stainless steel	Iron	Aluminium	Stainless steel and aluminium	Stainless steel and aluminium
8	The citric acid fermentation takes up to _____ days.	5 to 8 days	6 to 9 days	7 to 10 days	8 to 11 days	7 to 10 days
9	_____ have various structures, composition and are of low molecular weight compounds.	Antibiotics	Hydrocarbons	Proteins	Lipids	Antibiotics
10	For the commercial production of penicillin high yielding strains have been selected from the ancestral fungus_____.	*Aspergillus* spp.	*Mucor* spp.	*Candida* spp.	*Penicillium* spp.	*Penicillium* spp.

11	In _____ group of antibiotics, β-lactum ring is found.	Peptide	Antifungal	Macrolide	Pencillin	Pencillin
12	_____ employed in production media yields high amounts of penicillin.	Pharmamedia	Corn-steep liquor	Sulfite-waste liquor	Hydrocarbons	Corn-steep liquor
13	The examples of two important naturally occurring pencillin are _____.	Pencillin G and V	Penicillin B and V	Penicillin M and V	Penicillin O and V	Pencillin G and V
14	Staphylococcus aureus produces an enzyme called _____ which cleaves the β-lactum ring of penicillin.	Amylase	Pectinase	Protease	β-lactamase	β-lactamase
15	The precursor for penicillin G is _____.	Phenyl sulphate	Phenylacetic acid	Phenyl phosphate	Phenychloride	Phenylacetic acid
16	The _____ medium contains glycerol, cane molasses, corn-steep liquor for production of large number of spores.	Production	Sporulation	Preservation	Mother culture	Sporulation
17	For removal of fungal mycelium _____ with rotoary vacuum filter has been employed.	Centrifugation	Filtration	Heating	Precipitation	Filtration
18	During penicillin production, further impurities can be removed by washing the recovered salt with a dry solvent such as _____.	Isopropanol	n-butanol	Both A and B	Non of above	Both A and B
19	_____ is a penicillin without an acyl group, prepared from 6-amino penicillanic acid.	Natural penicillin	Synthetic penicillin	Semi-synthetic penicillin	Gn penicillin	Semi-synthetic penicillin
20	_____ of 6-amino penicillanic acid is carried out by using derivative of carboxylic acid.	Acylation	Chlorination	Carboxylation	Methylation	Acylation
21	Citric acid is used as _____ in pharmaceutical industries.	Acidulant	Basifier	Precursor	Anti-foaming agent	Acidulant
22	Several species such as *Aspergillus clavatus, Penicillium luteum, Penicillium citrinium, Mucor piriformis* are used for production of	Lactic acid	Succinic acid	Tartaric acid	Citric acid	Citric acid

	_____ in large scale.					
23	The problems faced during recovery of citric acid are _____.	Presence of unconverted sugars	Presence of oxalic acid	Presence of trace salts	All the above	All the above
24	It is estimated that about ___ % of world citric acid production is obtained by submerged fermentation.	45	50	80	20	80
25	Vacuum crystallization during citric acid production at ____ °C yields citric acid monohydrate	20-55	20-25	10-25	30-45	20-25
26	It is important to maintain the oxygen concentration above ____ % saturation for citric acid production.	25	15	75	95	25
27	For pre-cultivated mycelia, an inoculum size of ___ % of fresh medium is generally required for citric acid production.	20	30	10	5	10
28	Microorganisms namely *Streptomyces griseus*, *Streptomyces olivaceus*, *Bacillus megaterium*, *Pseudomonas denitrificans* have been employed for production of _____.	Vitamin A	Vitamin B12	Vitamin C	Vitamin D	vitamin B12
29	_____ is generally added in alcoholic production medium.	sulfur dioxide	sulfur oxide	ethylene gas	nitrous oxide	sulfur dioxide
30	_____ inhibits the growth and activity of unwanted bacteria and yeasts.	sulfur dioxide	sulfur oxide	ethylene gas	nitrous oxide	sulfur dioxide
31	One gram of glucose yields ---------- gms of ethanol in fermentation process.	0.4111	0.5111	0.6111	0.7111	0.5111
32	Alpha amylase are produced by _____	*Aspergillus*	*Mucor*	*Pencillium*	*fusarium*	*Aspergillus*
33	The enzymes are purified using solvents such as--	acetone	alcohols	inorganic-	acetone, salts	acetone, salts

	----------			salts(ammonium sulfate)	and solvents	and solvents
34	Vitamin B12 is formed by linking of a cobinamide linked to a _____.	Nucleotide	Nucleoside	DNA/RNA	Nucleode	Nucleotide
35	The presence of ----------inhibits the formation of α-amylase enzyme.	glucose	lactose	sucrose	galactose	glucose
36	The french word “vinaigre” means ----------	fresh grape juice	fresh wine	sour wine	ethanol	sour wine
37	The optimum temperature & pH for protease enzyme fermentation is----------	20-30°C, 7	30-40°C, 7	30-40°C, 8	40-50°C, 7	30-40°C, 7
38	Lipase is produced industrially using ---------- -----.	*Aspergillus niger*	*Rhizopus sp*	*Candida sp*	*Aspergillus niger, Rhizopus sp and Candida*	*Aspergillus niger, Rhizopus sp and Candida*
39	The bioconversion of sugars to alcohols are mediated by ---------- of years.	proteins	lipids	saccharides	enzymes	enzymes
40	Acid proteases are produced by the fungi	*Aspergillus oryzae*	*Rhizopus stoleniferous*	*fusarium sp*	*Altemaria sp.*	*Aspergillus oryzae*
41	_____ are usually employed for commercial prodution of ethyl alcohol	*Sacchcromyces cerevasiae*	*Candida albucans*	*Cryptococcus*	*none of the above*	*Sacchcromyces cerevasiae*
42	There are ---------- stages in alcoholic fermentation.	two	three	four	five	two
43	The primary stage of alcoholic fermentation is carried out in open fermentation vats for ---------- days.	2 to 5	3 to 7	4 to 8	5 to 10	3 to 7
44	The secondary stage of alcoholic fermentation proceeds for several weeks in ---------- containers.	closed	open	plastic	iron	closed
45	The final industrial product is treated with ---------- -- to eliminate pyrogens.	alkali	charcoal	glycerol	acid	charcoal

46						
47	The microorganismis namely Aspergillus niger, Rhizopus niveus, Rhizopus delemar are sources of _____enzyme.	α-amylase	Glucoamyase	Protease	Cellulase	Protease
48	Proteases are produced by _____.	*B .subtilis*	*B.sterothermophiles*	*B.tumefashions*	*none.of the above*	*B .subtilis*
49	During completion of enzymatic fermentation, the spent liquid is subjected to _____to reduce deterioration.	rapid heating	precipitation	condensation	rapid cooling	rapid cooling
50	The enzyme protease is produced from bacteria such as _____.	*Bacillus subtilis*	*Bacillus lichenifermis*	*Bacillus subtilis & Bacillus lichenifermis*	*Bacillus anthrax*	*Bacillus subtilis & Bacillus lichenifermis*
51	The recovered fermentation liquor is treated with _____ forming precipitate of calcium citrate.	NaOH	Sulphuric acid	Nacl	Milk of lime	Milk of lime
52	Which enzyme is very important in bread making, beer making for flood industry.	α-amylases	pectinase	cellulase	proteases	α-amylases
53	The preservation of bacterial α-amylase enzyme in liquid formulation is _____% NaCl	5	10	15	20	20
54	The optimum pH for amylase industrial fermentation process is_____.	5	6	7	8	7
55	Proteases enzymes are a complex mixture of _____.	amylases & amylopectinases	cellulase & cellulobiase	proteinases & peptidases	invertase& phosphatase	proteinases & peptidases
56	Which enzyme is unstable & loss of activity can occur during fermentation process.	α-amylase	Glucoamyase	Protease	Cellulase	Protease
57	The vitamin B12 fermentation (Aerobic phase) takes about_____ days.	2 to 4	4 to 6	3 to 5	5 to 7	3 to 5
58	Isinglass is obtained from _____.	Seaweed	*Kappaphycus cottonii*	swimbladders of fish	None of the above	swimbladders of fish

59	_____ is used as a flavoring and preservative agent in beer made today.	*Humulus lupulus*	*Humia lupulus*	*Humulus conopus*	None of the above	*Humulus lupulus*
60	The purification and recovery of the production after fermentation is called _____.	Downstream process	Submerged fermentation	Surface fermentation	Upstream process	Downstream process

Reg. No. : ----------

[17MBU303]

KARPAGAM ACADEMY OF HIGHER EDUCATION

(Under Section 3 of UGC Act 1956)

COIMBATORE – 641 021

B.Sc. DEGREE EXAMINATION, AUGUST 2018

DEPARTMENT OF MICROBIOLOGY

I INTERNAL TEST – THIRD SEMESTER

INDUSTRIAL MICROBIOLOGY

Time: 2 hours **Maximum: 50 marks**

Date / Session:

Part A

Multiple Choice Questions: No 1 to 20 **20x1 = 20 marks**

1. _____ is the father of industrial microbiology
   A.Sergei Winogradsky B. Alexander Flaming
   C. Louis Pasteur D. Chaim Weizmann
2. In 1914, Chaim Weizmann isolated *C. acetobutylicum* which uses _____ to produce ____ and ____
   A. Cellulose, butyric acid and acetone B. Starch, butanol and acetic acid
   C. Starch, butanol and acetone D. Cellulose, butyric acid and acetic acid
3. Which of the following are primary metabolites?
   (i) Lysine (ii) Gibberlin (iii) Vitamin B_{12} and (iv) Streptomycein
   A. (i) and (iv) B. (iii) and (ii)
   C. (ii) and (iv) D. (i) and (iii)
4. Secondary metabolites are produced during _____.
   A. Lag phase B. Log phase
   C. Stationary phase D. Death phase
5. Antibiotic producing organisms are identified by using _____
   A. Crowd plate technique B. Auxanographic technique
   C. Enriched culture technique D. Differential culture technique
6. Enrichment culture technique was designed by _____.
   A. Martinus Beijernick B. Louis Pasteur
   C. Bacon D. Robert Hooke
7. Indicator dyes are used in the medium to detect _____.
   A. Moisture availability B. Temperature change
   C. Nutrient concentration D. pH change
8. Chemical stability of the product is identified during _____.
   A. Primary screening B. Secondary screening
   C. Enrichment method D. Tertiary method
9. Which of the following is used as single cell protein?
   A. *Spirogyra* B. *Nostoc*
   C. *Spirulina* D. *Acetabularia*
10. _____ is used for the strain improvement.
    A. Nitrogen oxide B. Gamma rays
    C. Diamines D. Organic acids
11. _____ agent is used for the inducing the protoplast fusion.
    A. Glycerol B. Epichlorohydrin
    C. Poly ethylene glycol D. Polyvinyl chloride
12. The chemical agent which causes mutation is _____.
    A. Sodium sulfate B. Nitro methyl guanidine
    C. Calcium Chloride D. Ethyl sulfonate

13. The clean, yellow, fine powder prepared from embryo of cotton seed is called as _____.

A. Corn-steep liquor B. Soya bean meal

C. Pharmamedia D. Distiller's soluble

14. Buell and Weston introduced the preservation of microorganisms using mineral oil in the year ____.

A. 1937 B. 1941

C. 1957 D. 1947

15. _____ drying is most commonly used for drying of biological materials

A. Freeze B. Spray

C. Drum D. Tray

16. The specific gravity of paraffin oil used for the preservation of cultures is _____.

A. 0.821-0.860 B. 0.830-0.890

C. 0.856 -0.898 D. 0.718-0.835

17. The stabilizing agent used in cryopreservation of cultures is _____.

A. Carbon disulfide B. Glycol ether

C. Tetrahydrofuran D. Dimethyl Sulfoxide

18. The lyophilized pure cultures are stored at _____.

A. 0 °C B. -196 °C

C. -70 °C D. 4 °C

19. Which of the following is involved in the preservation and maintenance of industrially important bacteria?

A. NCTC B. NCIMB

C. ECACC D. NCPF

20. Protoplast fusion is used to improve _____ industrial microbes.

A. Bacteria B. Fungi

C. Yeast cells D. All the above

Part B

Answer all the questions **3x2 = 6 marks**

21. Mention any four characteristics of industrially important strains?

22. What are primary and secondary metabolites? Give example.

23. Write any two differences between crude and synthetic media.

Part C

Answer all the questions **3x8 = 24 marks**

24. A. Discuss important historical developments in industrial microbiology

Or

B. Outline the primary screening methods for strain isolation

25. A. Discuss the importance of secondary screening for strain isolation

Or

B. Define stain improvement and give a brief note on it.

26. A. Describe the methods of preservation of microorganisms with its advantages and disadvantages

Or

B. Write a detail note on media formulation.

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[17MBU303]

KARPAGAM ACADEMY OF HIGHER EDUCATION

(Under Section 3 of UGC Act 1956)

COIMBATORE – 641 021

B.Sc. DEGREE EXAMINATION, AUGUST 2018

DEPARTMENT OF MICROBIOLOGY

II INTERNAL TEST – THIRD SEMESTER

INDUSTRIAL MICROBIOLOGY

Time: 2 hours **Maximum: 50 marks**

Date / Session: **Class: II B.Sc**

PART – A

Multiple Choice Questions: No 1 to 20 **20x1 = 20 marks**

1. Sugar containing wastes derived from the paper pulping industry are primarily used for the cultivation of _____.
   a) Bacteria b) Molds c) Yeasts d) All the above
2. Hydrol molasses, containing primarily glucose, is a byproduct of _____ processing.
   a) Sugar cane b) Maize starch c) Barley d) Lignocellulose
3. _____ is used as a precursor for the production of L-tryptophan.
   a) Citric acid b) Anthranillic acid c) Sodium bisulphite d) None of the above
4. _____ still employed for producing ethanol, single cell protein, lactic acid, xanthan gum, vitamin B_{12} and gibberellic acid.
   a) Corn steep liquor b) Molasses c) Malt extract d) Whey
5. Range of fermentation tank used in enzyme production _____.
   a) 1,000-30,000 Gallons b) 500-1,000 Gallons
   c) 1,500-30,000Gallons d) None of the above
6. Phenyl acetic acid is used as a precursor for the production of _____.
   a) Penicillin b) Amylase c) Streptomycin d) Erythromycin
7. Size of the holes in the sparger ranges from_____.
   a) 1/64 -1/64 b) 1/32- 1/32 c) 1/32- 1/64 d) 1/64-1/32
8. Dissolved oxygen in fermentation process medium measured by using _____.
   a) Cooler b) Baffles c) Exit gas cooler d) Heat exchange
9. Initially fermentation industries used corn steep liquor for _____ production.
   a) Mushroom b) Penicillin c) Vitamin d) Organic acid
10. _____ spargers are used widely large scale fermentation process.
    a) Porous b) Orifice c) Combined sparger agitator d) Nozzle
11. The capacity of the batch fermentors _____.
    a) 10-12 litre b) 12-15 litre c) 20-40 litre d) 6-8 litre
12. Foaming during fermentation process creates _____.
    a) Oxidation b) Reduction c) Contamination d) Production
13. _____ are used in fermentors to avoid vortex formation.
    a) Spargers b) Bearing glands c) Rotameter d) Baffles
14. The _____ in stainless steel fermentor gives resistance to halogen salts, lodine and sea water
    a) Chromium b) Molybdenum c) Nickel d) Tungsten
15. The first pilot fermenter was erected in India at Hindustan Antibiotic ltd, Pune in the --------- year
    a) 1920 b) 1950 c) 1930 d) 1980
16. _____ consists of circular discs to which blades are fitted with bolts.
    a) Impellers b) Sparger c) Baffler d) Aerator
17. Aeration and agitation of a liquid medium may lead to the formation of _____.
    a) Acid b) Air c) Foam d) Alkali
18. _____ fermenter is called as elongated non-mechanically stirred fermenter.
    a) Tower b) Airlift c) Cylinder conical d) None of the above
19. Multiple air lift fermenter is designed by _____.

a) Bacon b) Dawsa c) Bakker d) Okabe

20. _____ removes enough moisture from the gas leaving fermentor and prevents excess fluid loss.

a) Cooler b) Baffles c) Exit gas cooler d) Heat exchange

PART - B

Answer all the questions **3x2 = 6 marks**

21. Mention any four carbon and nitrogen sources used in the complex/crude media?
22. What are sparger and buffles?
23. What is continuous fermentation?

PART - C

Answer all the questions **3x8 = 24 marks**

24. A. What is media formulation and ideal characteristics of fermentation medium?

Or

B. Comment on molasses, sulphite waste liquor, malt extract, fats and oils.

25. A. Write a note on types of fermentors.

Or

B. Explain about the aeration and agitation system in fermentor.

26. A. Explain basic design of a fermentor with neat diagram.

Or

B. Write a detail note on antifoams.

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Reg. No. : ----------

[17MBU303]

KARPAGAM ACADEMY OF HIGHER EDUCATION

(Under Section 3 of UGC Act 1956)

COIMBATORE – 641 021

B.Sc. DEGREE EXAMINATION, OCTOBER 2018

DEPARTMENT OF MICROBIOLOGY

III INTERNAL TEST – THIRD SEMESTER

INDUSTRIAL MICROBIOLOGY

Time: 2 hours **Maximum: 50 marks**

Date / Session: 09.10.2018/FN **Class: II B.Sc**

PART – A

Multiple Choice Questions: No 1 to 20 **20x1 = 20 marks**

1. The purification and recovery of the production after fermentation is called_____.
   a) Upstream process b) Downstream process
   c) Surface fermentation d) None of the above
2. The citric acid fermentation takes up to _____ days.
   a) 5 to 8 days b) 6 to 9 days c) 7 to 10 days d) 8 to 11 days
3. Micro filtration refers to the separation of suspended material such as bacteria by using a membrane with spore sizes of_____.
   a) 0.02 to 10 μm b) 1-10 A° c) 20-30 μm d) 10-200 A°
4. Example of an inorganic polymer used as support in immobilization.
   a) Alginates b) Carrageenan c) Ceramics d) Polyvinyl chloride
5. The glutamic acid fermentation takes up to _____ days.
   a) 2 to 4 days b) 2 to 6 days c) 4 to 6 days d) 4 to 8 days
6. _____ is most gentle method of drying, for many pharmaceutical products (viruses, vaccines, plasma fractions, enzymes) and in food industries.
   a) Spray drying b) Freeze drying c) Vacuum drying d) All the above
7. Cell lysis becomes an important operation if the product is _____.
   a) Extra cellular b) Heat labile c) Toxic d) Intracellular
8. Example for non ionic detergents.
   a) Tween 80 b) Tween X100 c) Tween 40 d) None of the above
9. Ultrasonication has frequency of _____khz.
   a) 200 b) 20 c) 2 d) 2000
10. Which of the following is NOT a batch filter?
    a) Frame filters b) Pressure leaf filters c) Plate filters d) Rotary vacuum filters
11. Membrane of pore size _____ a used for reverse osmosis.
    a) 0.001 – 0.01 mm b) 0.0001 – 0.001 μm c) 0.01 – 0.1 μm d) 0.0001 – 0.001 mm
12. In spray drying, a current of hot air of ____ is passed.
    a) 100-200 ℃ b) 10-150 ℃ c) 50-150 ℃ d) 150-250 ℃
13. The cell suspension is pumped through the homogenizing valve at _____ atmospheric pressure depending on microbes and cell concentration.
    a) 200 – 1000 b) 100 – 1500 c) 200 – 100 d) 20 – 100
14. First immobilized enzyme was _____ of A. oryzae for the production of L-amino acids.
    a) Amino reductase b) Amino acylase c) Amino kinase d) None of the above
15. *Staphylococcus aureus* produces an enzyme called _____ which cleaves the β-lactum ring of penicillin
    a) Amylase b) Pectinase c) Protease d) β-lactamase
16. The presence of _____ inhibits the production of alpha-amylase enzyme.

a) Glucose b) Lactose c) Sucrose d) Galactose

17. The optimum temperature & pH for protease enzyme fermentation is _____

a) 20-30 ℃, 7 b) 30-40 ℃, 7 c) 30-40 ℃, 8 d) 40-50 ℃, 7.

18. _____ are usually employed for commercial production of ethyl alcohol.

a) *Sacchcromyces cerevasiae* b) *Cryptococcus*

c) *Candida albucans* d) None of the above

19. _____ is used as a flavoring and preservative agent in beer made today.

a) *Humulus lupulus* b) *Humulus conopus*

c) *Humia lupulus* d) None of the above

20. Lipase is produced industrially using _____

a) *Aspergillus niger* b) *Rhizobium* c) *Streptococcus* sp. d) *Mucor* sp.

PART - B

Answer all the questions **3x2 = 6 marks**

21. Mention the types of filters used in the downstream processing.
22. Name the types of centrifuges.
23. Mention the media components used for the production of Penicillin.

PART - C

Answer all the questions **3x8 = 24 marks**

24. A. Write a detail note on immobilization.

Or

B. Comment on methods used for drying of product.

25. A. Write a note on chemical and biological methods used for cell disruption.

Or

B. Detail on the microbial production of citric acid.

26. A. Describe the methodology for production of vitamin B12

Or

B. Detail on the microbial production of beer.

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