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INDUSTRIAL MICROBIOLOGY

Subject code – 16SCCMB9

UNIT I

Historical development of Industrial Microbiology, Industrially important microorganisms, sources and characters; Primary and secondary screening and preservation of industrially important strains, Major classes of products and processes. Strain improvement.

UNIT II

Fermenter - Design, types and basic functions. Characteristics of production media, Fermentation media - formulation strategies, economical means of providing energy, carbon, nitrogen, vitamin and mineral sources, role of buffers, precursors, inhibitors, inducers and antifoams. Sterilization of fermentation equipment, air and media. Types of fermentation.

UNIT III

Downstream processing - recovery and purification of fermentations products (intracellular and extracellular), cell disruption, precipitation, filtration, centrifugation, solvent recovery, chromatography, Ultrafiltration and drying, Quality assurance (QC) of finished product. Immobilization of cell and enzymes.

UNIT IV

Microbial products of pharmaceutical value – raw materials, organism and industrial processes involved in the production of Penicillin, Streptomycin, Vitamin B12, Riboflavin and rabies vaccine.

UNIT V

Microbial products of industrial value – raw materials, organism and industrial processes involved in the production of ethanol, vinegar, amylase, protease, glutamic acid. Recycling and disposal of industrial wastes through microbes.

Industrial microbiology

Industrial microbiology is a branch of Microbiology that applies microbial sciences to create industrial products in mass quantities. There are multiple ways to manipulate a microorganism in order to increase maximum product yields. Introduction of mutations into an organism may be accomplished by introducing them to mutagens. Another way to increase production is by gene amplification, this is done by the use of plasmids, and vectors.

- The manipulation of organisms in order to yield a specific product has many applications to the real world like the production of some antibiotics, vitamins, enzymes, amino acids, solvents, alcohol and daily products.
- They can also be used in an agricultural application and use them as a bio pesticide instead of using dangerous chemicals or as inoculants and help plant proliferation.
- Industrial microbiology came into existence, primarily, based on a naturally occurring microbiological process called fermentation.

Despite the necessity for team work emphasized above, the microbiologist has a central and key role in his organization. Some of his functions include:

a) the selection of the organism to be used in the processes;
b) the choice of the medium of growth of the organism;
c) the determination of the environmental conditions for the organism's optimum productivity i.e., pH, temperature, aeration, etc.
d) during the actual production the microbiologist must monitor the process for the absence of contaminants, and participate in quality control to ensure uniformity of quality in the products;
e) the proper custody of the organisms usually in a culture collection, so that their desirable properties are retained;
f) the improvement of the performance of the microorganisms by genetic manipulation or by medium reconstitution.

History of Industrial Microbiology

The history of industrial microbiology can be divided into five phases, which are précised in follow,

- **Phase I up to 1900 Alcohol fermentation period,**
  - *Alcohol, Vinegar, Bakery Yeast, Glycerol, Citric acid, Lactic acid, Acetone/Butanol*
- **Phase II 1900-1940 Antibiotic periods,**
  - *Penicillin, Streptomycin, Other Antibiotics*
- **Phase III 1940-1964 Single cell protein periods,**
  - *Gibberellins, Amino acids, Nucleotides, Enzyme, Transformation*
- **Phase IV 1964-1979 Metabolite production period and**
  - *SCP using hydrocarbons & other feed stocks*
- **Phase V 1979 onward Biotechnology period.**
  - *Heterogeneous protein by Microbial cell, Monoclonal antibodies by animal cell*

Important contribution

1. Antonie van Leeuwenhoek: Visualization of bacteria, yeasts, and protozoa using microscopes (1674 ~ 1723).
2. Louis Pasteur & Robert Koch –
    a. 1857: Demonstrating yeasts were responsible for the production of alcohol and the rod-shaped bacteria produced the lactic acid that caused the wine to sour; demonstrating the souring of milk was caused by the action of microorganisms (Pasteur)
    b. 1860: Invented "pasteurization" (Pasteur)
    c. 1873 ~ 1876: Investigated anthrax; developed techniques to view, grow, and stain microorganisms (Koch)
    d. 1879: Grew weakened strains of microorganisms that could not cause disease but protected against severe forms of the same disease (Pasteur).
3. 1860 to 1900: Production of lactic acid; Anaerobic fermentor used to grow baker's yeast was changed to aerated fermentor
4. 1915 to 1916: Fermentative production of glycerol, butanol and acetone in Germany ca. 1920: Manufacture of citric acid in a surface process ca.
5. 1920: Invented activated sludge process and aerobic production of yeast with continuous sugar addition
6. 1920~1940: sorbose production (D-sorbitol → L- sorbose)
7. 1928~1929: Noticed the inhibition of growth of bacteria (Staphylococcus) by fungi (Penicillium) (Alexander Fleming)
8. 1938~1940: Isolated the antimicrobial agent penicillin (Howard Florey and Ernst Chain)
9. 1930~1940: the invention of agitated and aerated fermentor, the shake flask, and the sterilization of air with fibrous filters
10. 1940 ~ 1945: Establishment of large-scale production of penicillin in USA (submerged liquid culture, aerobic, filamentous fungus, secondary metabolite
11. 1944: Streptomycin from Streptomyces
12. 1949: Vitamin B 12
13. 1950s: Manufacture of cortisone (11-hydroxylation)
14. 1956: Manufacture of glutamic acid in Japan
15. 1959: Manufacture of 5'-IMP and 5'-GMP (enzymatic hydrolysis of yeast RNA)
16. 1965: Microbial rennin (for cheese)
17. 1967: Production of fructose by glucose isomerase
18. 1969: L-amino acid by immobilized enzyme
19. 1970: Single cell protein (yeasts and bacteria) from gas oil and n-paraffin by continuous fermentation
20. 1970s: Gibberellins, enzyme inhibitors, Bt-toxin (*Bacillus thuringiensis*)
21. 1972: Created the first recombinant DNA technology
22. 1977: Reported the production of the first human protein (somatostatin) manufactured in a bacteria
23. 1982: Received approval from the Food and Drug Administration to market genetically engineered human insulin

24. 1986: Beginning the production of amino acid by genetically engineered bacteria in Japan
25. 1994: Completed the world's first large-scale trials of cheeses made with rennin produced by genetically engineered bacteria.

<u>Isolation of suitable microorganisms from the environment</u>

Strategies that are adopted for the isolation of a suitable industrial microorganism from the environment can be divided into two types,

1. shotgun and
2. Objective approaches.

In the **shotgun** approach, samples of free living microorganisms, biofilms or other microbial communities are collected from animal and plant material, soil, sewage, water and waste streams, and particularly from unusual man-made and natural habitats. These isolates are then 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. For example, when attempting to isolate an organism that can degrade or detoxify a specific target compound, sites may be sampled that are known to be contaminated by this material. These environmental conditions may select for microorganisms able to metabolize this compound.

<u>Examples of industrial fermentation products and their producer microorganisms</u>

Products	Bacteria	Fungi
Traditional products		
Bread, beer, wine and spirits		Mainly *Saccharomyces cerevisiae*
Cheeses, other dairy products	Lactic acid bacteria	
Ripening of blue and Camembert-type cheeses		*Penicillium species*
Fermented meats and vegetables	Mostly 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-Glutamine	*Corynebacterium glutamicum*	
l-Lysine	*Brevibacterium*	

	lactofermentum	
l-Tryptophan	*Klebsiella aerogenes*	
Enzymes **Carbohydrases**		
1. a-amylase	*Bacillus subtilis*	
2. b-amylase		*Aspergillus niger*
3. amyloglucosidase		*Aspergillus niger*
4. glucose isomerase	*Streptomyces olivaceus*	
5. invertase		*Kluyveromycesspecies*
6. lactase (b-galactosidase)		*Kluyveromyces lactis*
Cellulases		*Trichoderma viride*
Lipases		*Candida cylindraceae*
Pectinases		*Aspergillus wentii*
Proteases subtilisin (alkaline) neutral microbial rennet (acid)	 *Bacillus licheniformis*	 *Aspergillus oryzae* *Rhizomucor miehei*
Fuels and chemical feedstocks		
Acetone	*Clostridium species*	
Butanol	*Clostridium acetobutylicum*	
Ethanol	*Zymomonas mobilis*	*Saccharomyces cerevisiae*
Glycerol		*Zygosaccharomyces rouxii*
Methane	*Methanogenic archaeans*	
Nucelotides		
5'-Inosine monophosphate	*Bacillus subtilis*	
5'-Guanosine monophosphate	*Brevibacterium ammoniagenes*	
Organic acids		
Acetic	*Acetobacter xylinum*	
Citric		*Aspergillus niger, Yarrowia lipolytica*
Fumaric		*Rhizopus species*
Gluconic	*Acetobacter suboxydans*	
Itaconic		*Aspergillus itaconicus*
Kojic		*Aspergillus flavus*
Lactic	*Lactobacillus delbrueckii*	
Pharmaceuticals and related compounds		
Alkaloids		*Claviceps purpurea*
Antibiotics		
Aminoglycosides streptomycin	 *Streptomyces griseus*	
b-Lactams penicillins cephalosporins clavulanic acid	 *Streptomyces clavuligerus*	 *Penicillium chrysogenum* *Acremonium chrysogenum*
Lantibiotics nisin	 *Lactococcus lactis*	
Macrolides		

erythromycin	*Saccharapolyopora erythraea*	
Peptides Bacitracin Gramicidin	 *Bacillus licheniformis* *Bacillus brevis*	
Tetracyclines chlortetracycline	 *Streptomyces aureofasciens*	
Hormones Human growth hormone Insulin	 Recombinant *Escherichia coli*	 Recombinant *Saccharomyces cerevisiae*
Interferon	Recombinant *Escherichia coli*	Recombinant *Saccharomyces cerevisiae*
Steroids	*Arthrobacter species*	*Rhizopus species*
Vaccines	*Bacillus anthracis* , *Clostridium tetani*, Recombinant *Escherichia coli*, *Salmonella typhi*	
Vitamins		
B12 (cyanocobalamin)	*Pseudomonas denitrificans*	
b-Carotene (provitamin A)		*Blakeslea trispora*
Ascorbic acid (vitamin C)	*Acetobacter suboxydans*	
Riboflavin	*Recombinant Bacillus subtilis*	*Ashbya gossypii*
Polymers		
Alginate	*Azotobacter vinelandii*	
Cellulose	*Acetobacter xylinum*	
Dextran	*Leuconostoc mesenteroides*	
Gellan	*Sphingomonas paucimobilis*	
Polyhydroxbutyrate	*Ralstonia eutropha*	
Pullulan		*Aureobasidium pullulans*
Scleroglucan		*Sclerotium rolfsii*
Xanthan	*Xanthomonas campestris*	
SCP	*Methylococcus capsulatus, Methylophilus methylotrophus*	*Candida utilis, Fusarium venenatum, Kluyveromyces marxianus, Paecilomyces variotii, Saccharomyces cerevisiae*

Screening of Microorganisms: Primary and Secondary Techniques

The economics of a fermentation process largely depends upon the type of microorganism used.

The microorganism with a desired character is generally isolated from natural substrates like soil etc. Such an organism is generally called as a producer strain.
Although there are many screening techniques, all of them are generally grouped into two broad categories.

They are:

1. Primary screening, and
2. Secondary screening.

1. Primary Screening of Microorganisms:

Primary screening may be defined as detection and isolation of the desired microorganism based on its qualitative ability to produce the desired product from a natural environment like soil, which contains several different species.

The following are some of the important primary screening techniques:

(i) The crowded plate technique
(ii) Indicator dye technique
(iii) Enrichment culture technique
(iv) Auxanographic technique
(v) Technique of supplementing volatile and organic substrates.

(i) The Crowded Plate Technique:

This technique is primarily employed for detecting those microorganisms, which are capable of producing antibiotics. This technique starts with the selection of a natural substratum like soil or other source consisting of microorganisms.

- Progressive serial dilution of the source is made.
- Suitable aliquot of the serial dilution is chosen which is able to produce 300 to 400 individual colonies when plated on an agar plate, after incubation.
- Such a plate is called as crowded plate.

The antibiotic producing activity of a colony is indicated by no growth, is indicated by the formation of a clear and colorless area around the antibiotic producing microorganism's colony on the agar plate. This region is called as growth inhibitory zone.

Such a colony is isolated from the plate and purified either by making repeated sub-culturing or by streaking on a plate containing a suitable medium, before stock culture is made. The purified culture is then tested for its antibiotic spectrum.

(ii) Indicator Dye Technique:

- Microorganisms capable of producing acids or amines from natural sources can be detected using this method by incorporating certain pH indicator dyes such as neutral red or bromothymol blue into nutrient agar medium with calcium carbonate.
- The change in the color of a particular dye in the vicinity of a colony will indicate the ability of that colony to produce an organic acid or base.
- The production of organic acid is indicated by the formation of a clear zone around those colonies which release organic acid into the medium.
- The identified colonies are isolated and purified either by repeated sub-culturing or by streaking methods and a stock culture is made which may be used for further qualitative or quantitative screening tests.

(iii) Enrichment Culture Technique:

- This technique is generally employed to isolate those microorganisms that are very less in number in a soil sample and possess specific nutrient requirement and are important industrially.
- They can be isolated if the nutrients required by them is incorporated into the medium or by adjusting the incubation conditions.

(iv) Auxanotrophic Technique:

- This technique is employed for the detection and isolation of microorganisms capable of producing certain extracellular substances such as growth stimulating factors like amino acids, vitamins etc.
- A test organism with a definite growth requirement for the particular metabolite is used in this method.

(v) Technique of Supplementing Volatile Organic Substances:

- This technique is employed for the detection and isolation of microorganisms capable of utilizing carbon source from volatile substrates like hydrocarbons, low molecular weight alcohols and similar carbon sources.

2. Secondary Screening of Microorganisms:

Primary screening helps in the detection and isolation of microorganisms from the natural substrates that can be used for industrial fermentations for the production of compounds of human utility, but it cannot give the details of production potential or yield of the organism. Such details can be ascertained by further experimentation.

This is known as secondary screening, which can provide broad range of information pertaining to the:

i. Ability or potentiality of the organism to produce metabolite that can be used as an industrial organism.

ii. The quality of the yield product.

iii. The type of fermentation process that is able to perform.

iv. Elimination of the organisms, which are not industrially important.

Methods of Secondary Screening:

(i) Giant Colony Technique:

- This technique is used for isolation and detection of Streptomyces in solid medium, is capable of producing antibiotics during primary screening.
- The isolated Streptomyces culture is inoculated into the central area of a sterilized nutrient agar medium and incubated until sufficient microbial growth takes place.
- The relative inhibition of growth of different test organisms by the antibiotic is called inhibition spectrum.
- Such species of Streptomyces, which have potentiality of inhibiting microorganisms, is preserved for further testing.

(ii) Filtration Method:

- This method is employed for testing those antibiotics which are poorly soluble in water.
- The Streptomyces is grown in a broth and its mycelium is separated by filtration to get culture filtrate. Various dilutions of antibiotic filtrates are prepared and added to molten agar plating medium and allowed to solidify.
- Later on cultures of various test organisms are streaked on parallel lines on the solidified medium and such plates are incubated. The inhibitory effect of

antibiotic against the test organisms is measured by their degree of growth in different antibiotic dilutions.

(iii) Liquid Medium Method:

- This method is generally employed for further screening to determine the exact amount of antibiotic produced by a microorganism like Streptomyces.
- Nutritive medium are inoculated with Streptomyces and incubated at room temperature. They are also aerated by shaking continuously and vigorously during incubation period to allow Streptomyces to produce the antibiotic in an optimum quantity.

Industrial strains and strain improvement

Irrespective of the origins of an industrial microorganism, it should ideally exhibit:

1. genetic stability;
2. efficient production of the target product, whose route of biosynthesis should preferably be well characterized;
3. limited or no need for vitamins and additional growth factors;
4. utilization of a wide range of low-cost and readily available carbon sources;
5. amenability to genetic manipulation;
6. safety, non-pathogenicity and should not produce toxic agents, unless this is the target product;
7. ready harvesting from the fermentation;
8. ready breakage, if the target product is intracellular; And
9. production of limited byproducts to ease subsequent purification problems.

Other features that may be exploited are thermophilic or halophilic properties, which may be useful in a fermentation environment. Also, particularly for cells grown in suspension, they should grow well in conventional bioreactors to avoid the necessity to develop alternative systems. Consequently, they should not be shear sensitive, or generate excessive foam, nor be prone to attachment to surfaces.

Methods

In many cases strain improvement has been accomplished using natural methods of

Genetic recombination: which bring together genetic elements from two different genomes into one unit to form new genotypes.

An alternative strategy is via **Mutagenesis**.

Those recombinants and mutants are then subjected to screening and selection to obtain strains whose characteristics are more specifically suited to the industrial fermentation process.

Optimization of microbial activity

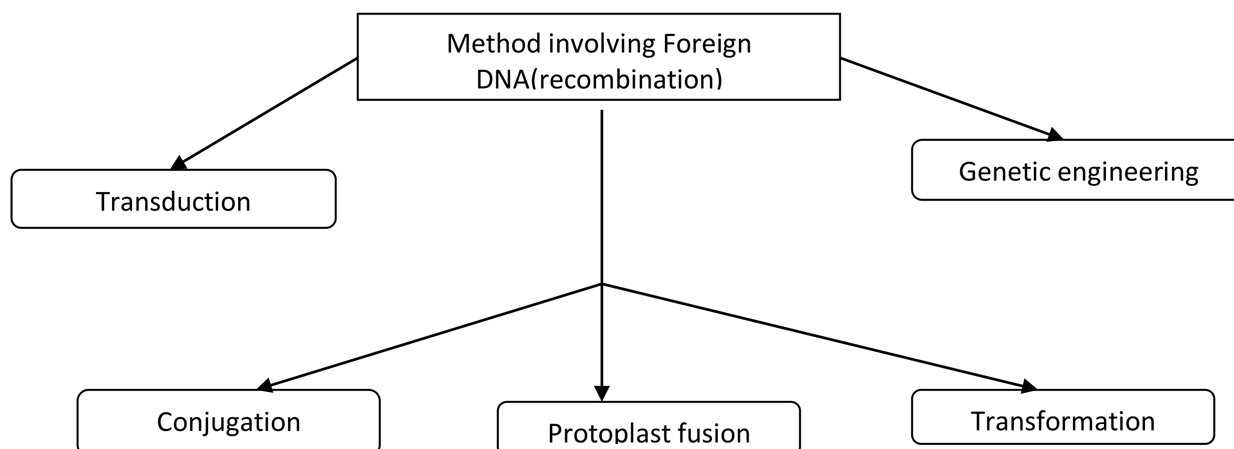
It can be done by

Optimizing environmental conditions

Optimizing nutrition of microorganisms

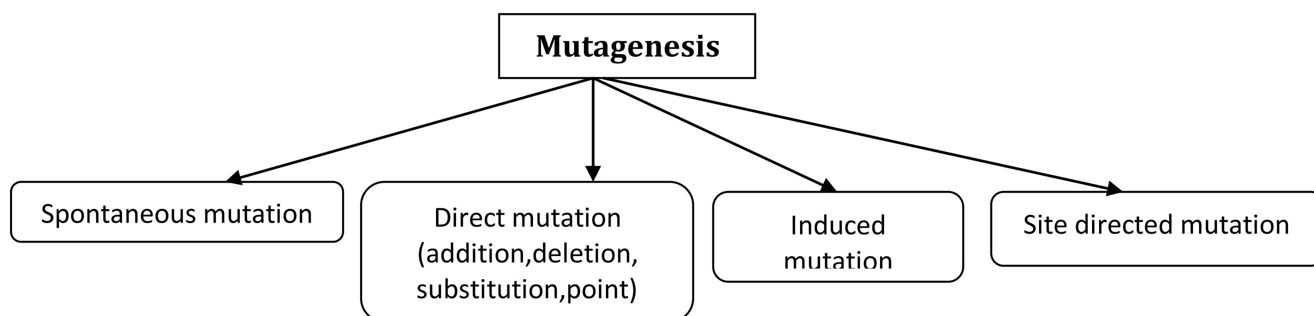
Other includes

1. Method not involving foregin DNA—Mutagenesis
2. Method involving foregin DNA—Genetic Recombination



1. MUTAGENESIS

Mutagenesis is a process of treatment given to microorganism which will cause an improvement in their genotypic and phenotypic performances



Types of mutation

1. **Spontaneous mutation**: Occur spontaneously at the rate of 10^{-10} and 10^{-15}per generation.
2. **Induced mutation**: The rate of mutation can be increased by various factors and agents called mutagens. ionizing radiations (e.g. X-rays, gamma rays) non-ionizing radiations (e.g. ultraviolet radiations) various chemicals (e.g. mustard gas, benzene, ethidium bromide, Nitrosoguanidine-NTG).

Table-1

S.No	MUTAGEN	MUTATION INDUCED	IMPACT ON DNA	RELATIVE EFFECT
1	Ionizing Radiations-X Rays,gamma rays	Single or double strand bearkage of DNA	Deletion/structural changes	high
2	UV rays,chemicals	Pyrimidine dymerisation	Trnsversion,deletion,frameshift transitions from GC---AT	Medium
3	Hydroxylamine(NH2 OH	Deamination of cytosine	GC ---A T transitions	low

4	N-Methyl –N’- Nitro NNitrosoguanidine	Methylation of bases and high pH	GC ---A T transitions	high
5	Nitrous acid(HNO2)	Deamination of A,C & G	Bidirectional transitions,deletion, AT ---GC/GC ----AT	Medium
6	Phage,plasmid,D NA transposing	Base substitution,break age.	Deletion,duplicati on,insertion.	high

Reports on strain improvement by mutation

- Lipase from *Aspergillus japonicus* MTCC 1975- mutation using UV, HNO2, NTG showed 127%, 177%, 276% higher lipase yield than parent strain respectively.
    - Lipase from *A. niger* -Nitrous acid induced mutation – showed 2.53 times higher activity.
- Medically useful products Demethyltetracycline and doxorubicin were discovered by mutations from tetracycline and daunorubicin. Hybramycines were also made by this way.
- First superior penicillin producing mutant, *Penicillium chrysogenum* X-1612, was isolated after X ray treatment.

2.Transduction

- Transduction is the transfer of bacterial DNA from one bacterial cell to another by means of a bacteriophage.

Two types:

- *general transduction* and
- *specialized transduction*.

3.Transformation

- When foreign DNA is absorbed by, and integrates with the genome of, the donor cell.
- Cells in which transformation can occur are ‘competent’ cells.
- The method has also been used to increase the level of protease and amylase production in *Bacillus* spp.

4.Conjugation

- Conjugation involves cell to cell contact or through sex pili (*singular*, pilus) and the transfer of plasmids.

5.Protoplast Fusion

- Protoplasts are formed from bacteria, fungi, yeasts and actinomycetes when dividing cells are caused to lose their cell walls.
- Fusion from mixed populations of protoplasts is greatly enhanced by the use of polyethylene glycol (PEG).
- Protoplast fusion has been successfully done with *Bacillus subtilis* and *B. megaterium* among several species of *Streptomyces spp. Like S. coeli-color,*

S.acrimycini, S. olividans, S. pravulies between the fungi *Geotrichum* and *Aspergillus* and Yeasts.

- Strain improvement of ***Aspergillus oryzae*** for protease production by both mutation and protoplast fusion.
    - UV radiation – 14 times higher yield.
    - Ethyl methane sulphonate – 39 times higher yield.
    - Protoplast fusion – using PEG and $CaCl_2$ – 82 times higher yield.
- An intergeneric hybrid was obtained from ***Aspergillus niger*** and ***Penicillium digitatum*** for enhancing the production of verbenol, a highly valued food flavorant.

6.Genetic Engineering

- Genetic engineering, also known as recombinant DNA technology, molecular cloning or gene cloning.
- Recombinant DNA Technology enables isolation of genes from an organism; this gene can be amplified, studied, altered & put into another organism.

Conclusion

The tremendous increase in fermentation productivity and resulting decreases in costs have come about mainly by using mutagenesis. In recent years, recombinant DNA technology has also been applied.
The promise of the future is via extensive of new genetic techniques-

- Metabolic engineering
- Genomic shuffling

The choice of approaches which should be taken will be driven by the economics of the biotechnological process and the genetic tools available for the strain of interest.

Unit - II
Fermentation systems

Microbiologists use the term fermentation in two different contexts.

- First, in metabolism, fermentation refers to energy-generating processes where organic compounds act as both electron donor and acceptor.
- Second, in the context of industrial microbiology, the term also refers to the growth of large quantities of cells under aerobic or anaerobic conditions, within a vessel referred to as a fermenter or bioreactor.

Fermentor:

A fermentor (bioreactor) is a closed vessel with adequate arrangement for aeration, agitation, temperature and pH control, and drain or overflow vent to remove the waste biomass of cultured microorganisms along-with their products.

- Fermentors are extensively used for food processing, fermentation, waste treatment, etc.
- De Beeze and Liebmann (1944) used the first large scale fermentor for the production of yeast.

Design of Fermentors:

- All bioreactors deal with heterogeneous systems dealing with two or more phases, e.g., liquid, gas, solid.

A bioreactor should provide for the following:

(i) Agitation (for mixing of cells and medium),
(ii) Aeration (aerobic fermentors); for O_2 supply,
(iii) Regulation of factors like temperature, pH, pressure, aeration, nutrient feeding, liquid level etc.,
(iv) Sterilization and maintenance of sterility, and
(v) Withdrawal of cells/medium (for continuous fermentors).

Generally, 20-25% of fermentor volume is left unfilled with medium as "head space" to allow for splashing, foaming and aeration.

Size of Fermentors:

The size of fermentors ranges from 1-2 litre laboratory fementors to 5,00,000 litre

Construction of Fermentors:

Industrial fermentors can be divided into two major classes, anaerobic and aerobic.

- Anaerobic fermentors require little special equipment except for removal of heat generated during the fermentation process, whereas
- aerobic fermentors require much more elaborate equipment to ensure that mixing and adequate aeration are achieved.

1. Cooling Jacket:

A fermentor is a large cylinder closed at the top and the bottom and various pipes and valves are fitted into it. It is fitted externally with a cooling jacket through which steam (for sterilization) or cooling water (for cooling) is run.

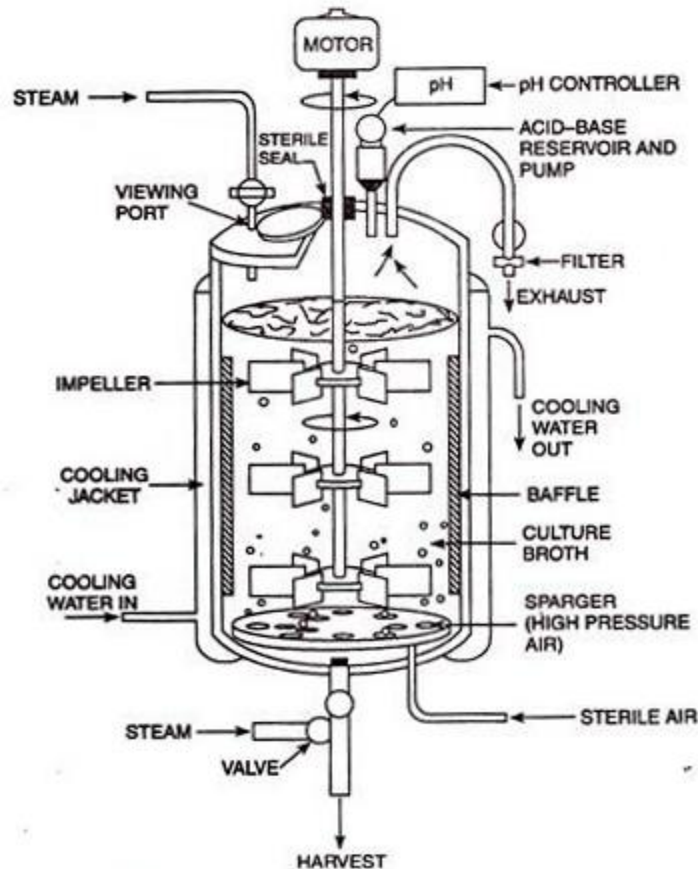
Cooling jacket is necessary because sterilization of the nutrient medium and removal of the heat.

Internal coils are provided through which either steam or cooling water is run.

2. Aeration System:

- In a fermentor with a high microbial population density, there is a tremendous oxygen demand by the culture, but oxygen being poorly soluble in water hardly transfers rapidly throughout the growth medium.

- These aeration devices are 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.



3. Agitation:

- The impeller is an agitating device necessary for stirring of the fermentor.
- It mixes the gas bubbles and microbial cells through the liquid culture medium.
- The impeller should be 1/3 of the fermentors diameter fitted above the base of the fermentor.

4. Baffles:

- The baffles are prevent a vortex and to improve aeration efficiency. They are metal strips roughly one-tenth of the fermentors diameter and attached radially to the walls.

5. Controlling Devices for Environmental Factors:

- In any microbial fermentation, to control the process by altering environmental parameters as the process proceeds includes temperature, oxygen concentration, pH, cells mass, levels of key nutrients, and product concentration.

Types of Fermentor

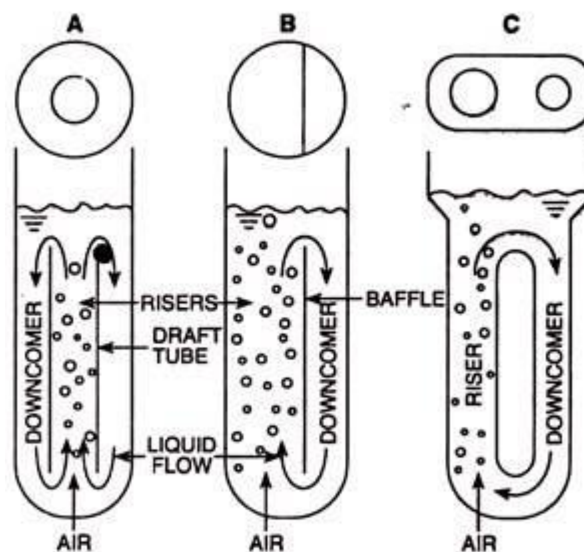
The fermentor (bioreactor) types used extensively in industries are the stirred tank fermentor, airlift fermentor, and bubble column fermentor.

(i) Stirred Tank Fermentor:

- Stirred tank fermentors consists of a cylindrical vessel with a motor driven central saft that supports one or more impellers.

(ii) Airlift Fermentor:

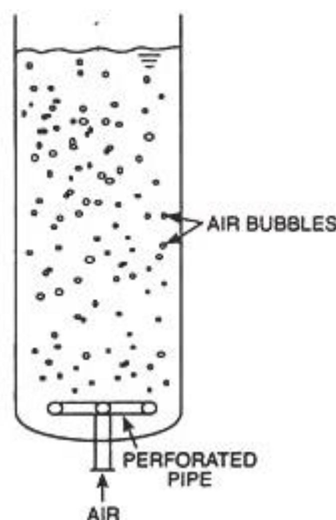
- In airlift fermentor the liquid culture volume of the vessel is divided into two interconnected zones by means of a baffle or draft tube.
- Only one of the two zones is sparged with air or other gas and this sparged zone is known as the riser.
- The other zone that receives no gas is called down-comer.
- The bulk density of the gas-liquid dispersion in the gas-sparged riser tends to be lower than the bulk density in the down-comer, consequently the dispersion flows up in the riser zone and down-flow occurs in the down-comer.
- Airlift fermentors are highly energy-efficient and are often used in large-scale manufacture of biopharmaceutical proteins obtained from fragile animal cells.
- Heat and mass transfer capabilities of airlift reactors are at least as good as those of other systems, and airlift reactors are more effective in suspending solids than are bubble column fermentors.
- All performance characteristics of airlift -fermentor are related ultimately to the gas injection rate and the resulting rate of liquid circulation.



- Usually, the rate of liquid circulation increases with the square root of the height of the airlift device.
- Because the liquid circulation is driven by the gas hold-up difference between the riser and the down-comer, circulation is enhanced if there is little or no gas in the down-comer.
- All the gas in the down-comer comes from being entrained in with the liquid as it flows into the down-comer from the riser near the top of the reactor.

(iii) Bubble Column Fermentor:

- A bubble column fermentor (Fig. 39.3) is usually cylindrical with an aspect (height-to-diameter) ratio of 4-6.
- Gas is sparged at the base of the column through perforated pipes, perforated plates, or sintered glass or metal micro-porous spargers.
- O_2transfer, mixing and other performance factors are influenced mainly by the gas flow rate and the rheological properties of the fluid.
- Internal devices such as horizontal perforated plates, vertical baffles and corrugated sheet packing's may be placed in the vessel to improve mass transfer and modify the basic design.



- The column diameter does not affect its behaviour so long as the diameter exceeds 0.1 m. One exception is the axial mixing performance.
- For a given gas flow rate, the mixing improves with increasing vessel diameter.
- Mass and heat transfer and the prevailing shear rate increase as gas flow rate is increased.

Fermentation media

Fermentation media must satisfy all the nutritional requirements of the microorganism and fulfil the process of industrial product. The nutrients should be formulated to promote the synthesis of the target product, either cell biomass or a specific metabolite.

Typically, the main elemental formula of microbial cells is approximately **$C_4H_7O_2N$**, which on a basis of dry weight is

48% C, 7% H, 32% O and 14% N

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

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

I. Carbon sources

- A carbon source is required for all biosynthesis leading to reproduction, product formation and cell maintenance. Carbohydrates are traditional carbon and energy sources for microbial fermentations, although other sources may be used, such as alcohols, alkanes and organic acids. Animal fats and plant oils may also be incorporated into some media, often as supplements to the main carbon source.

1. MOLASSES

- A dark coloured viscous syrup containing 50–60% (w/v) carbohydrates, primarily sucrose, with 2% (w/v) nitrogenous substances, along with some vitamins and minerals.

2. MALT EXTRACT

- Aqueous extracts of malted barley can be concentrated to form syrups that are particularly useful carbon sources for the cultivation of filamentous fungi, yeasts and actinomycetes.

3. STARCH AND DEXTRINS

- These polysaccharides are not as readily utilized as monosaccharides and disaccharides, but can be directly metabolized by amylase-producing microorganisms, particularly filamentous fungi.

4. SULPHITE WASTE LIQUOR

- Sugar containing wastes derived from the paper pulping industry are primarily used for the cultivation of yeasts.

5. CELLULOSE

- Cellulose is predominantly found as lignocellulose in plant cell walls, which is composed of three polymers: cellulose, hemicellulose and lignin. Relatively few microorganisms can utilize it directly, as it is difficult to hydrolyse.

6. WHEY

- Whey is an aqueous byproduct of the dairy industry. This disaccharide was formerly used extensively in penicillin fermentations and it is still employed for producing ethanol, single cell protein, lactic acid, xanthan gum, vitamin B12 and gibberellic acid.

7. FATS AND OILS

- Hard animal fats that are mostly composed of glycerides of palmitic and stearic acids are rarely used in fermentations. However, plant oils (primarily from cotton seed, linseed, maize, olive, palm, rape seed and soya) and occasionally fish oil, may be used as the primary or supplementary carbon source, especially in antibiotic production.

8. ALKANES AND ALCOHOLS

- *n*-Alkanes of chain length C10–C20 are readily metabolized by certain microorganisms. Methanol has high percent carbon content and is relatively cheap, although only a limited number of organisms will metabolize it.

II. NITROGEN SOURCES

Most industrial microbes can utilize both inorganic and organic nitrogen sources. Inorganic nitrogen may be supplied as ammonium salts, often ammonium sulphate and di-ammonium hydrogen phosphate, or ammonia.

1. CORN STEEP LIQUOR

- Corn steep liquor is a byproduct of starch extraction from maize and its first use in fermentations was for penicillin production.

2. YEAST EXTRACTS

- Yeast extracts may be produced from waste baker's and brewer's yeast from wood and paper processing.

3. PEPTONES

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

4. SOYA BEAN MEAL

- Residues remaining after soya beans have been processed to extract the bulk of their oil are composed of 50% protein, 8% non-protein nitrogenous compounds, 30% carbohydrates and 1% oil.

III. Water

- All fermentation processes, except solid-substrate fermentations, require vast quantities of water. In many cases it also provides trace mineral elements and it is important for ancillary equipment and cleaning.

IV. Minerals

- Normally, sufficient quantities of cobalt, copper, iron, manganese, molybdenum, and zinc are present in the water supplies, and as impurities in other media ingredients.

V. Vitamins and growth factors

- Many bacteria can synthesize all necessary vitamins from basic elements. For other bacteria, filamentous fungi and yeasts, they must be added as supplements to the fermentation medium.

VI. Precursors

- Some fermentation must be supplemented with specific precursors, notably for secondary metabolite production.
  Eg: Phenylacetic acid, Phenylacetamide, D-threonine, Anthranillic acid

VII. Inducer

- Inducers are often necessary in fermentations of genetically modified microorganisms (GMMs).

VIII. Inhibitors

- Inhibitors are used to redirect metabolism towards the target product and reduce formation of other metabolic intermediates.

IX. Antifoams

- Antifoams are necessary to reduce foam formation during fermentation.
  Eg: Natural antifoams include
    i. plant oils,
    ii. deodorized fish oil,
    iii. mineral oils and
    iv. tallow.

  The synthetic antifoams are mostly
    i. silicon oils,

ii. poly alcohols and
iii. alkylated glycols.

> If strains are naturally unstable, they should be maintained on media selective for the specific characteristic must be retained.

Fermentation

Fermentation is a metabolic process that produces chemical changes in organic substrates through the action of enzymes.

Industrial fermentation is the intentional use of fermentation by microorganisms such as bacteria and fungi as well as eukaryotic cells like CHO cells and insect cells, to make products useful to humans.

In general, fermentations can be divided into four types:

- Production of biomass (viable cellular material)
- Production of extracellular metabolites (chemical compounds)
- Production of intracellular components (enzymes and other proteins)
- Transformation of substrate (in which the transformed substrate is itself the product)

The following points highlight the eight main types of fermentations. The types are:-

1. Batch Fermentation
2. Continuous Fermentation
3. Fed Batch Fermentation
4. Anaerobic Fermentation
5. Aerobic Fermentation
6. Surface Fermentations
7. Submerged Fermentations
8. State Fermentation.

1. Batch Fermentation:

- Batch fermentation is a closed culture system, because initial and limited amount of sterilized nutrient medium is introduced into the fermentor.
- The medium is inoculated with a suitable microorganism and incubated for a definite period for fermentation to proceed under optimal physiological conditions.
- Oxygen in the form of air, an antifoam agent and acid or base, to control the pH, are being added during the course of fermentation process.
- During the course of incubation, the cells of the microorganism undergo multiplication and pass through different phases of growth and metabolism.
- Batch fermentation may be used to produce biomass, primary metabolites and secondary metabolites under cultural conditions.

2. Continuous Fermentation:

It is a closed system of fermentation, run for indefinite period. In this method, fresh nutrient medium is added continuously or intermittently to the fermenter and equivalent amount of used medium with microorganisms is withdrawn continuously or intermittently for the recovery of cells or fermentation products.

- In continuous mode with cell cycle, the cell mass is returned to the fermenter using micro filtrations with bacteria or screens with fungal mycelium.

A continuous fermentation is generally carried out in the following ways:

(a) Single stage fermentation

(b) Recycle fermentation
(c) Multiple stage fermentation

<u>3. Fed Batch Fermentation:</u>

It is a modification to the batch fermentation. In this process substrate is added periodically in instalments as the fermentation progresses at an optimal concentration.

- This is essential as some secondary metabolites are subjected to catabolite repression by high concentration.

The fed-batch fermentation may be of three types:

(i) Variable Volume Fed Batch Culture
(ii) Fixed Volume Fed Batch Culture
(iii) Cyclic Fed Batch Culture

<u>4. Anaerobic Fermentation:</u>

A fermentation process carried out in the absence of oxygen is called as anaerobic fermentation. There are two types of anaerobic microorganisms viz, obligate anaerobic microorganisms and facultative anaerobic microorganisms.

- Anaerobic conditions in the fermenter are created either by withdrawing the oxygen present in the head space by an exhaust pump and pumping some inert gases like nitrogen, argon etc. or by flushing it out, by the emergence of certain gases like carbon dioxide or hydrogen.

<u>5. Aerobic Fermentation:</u>

- A fermentation process carried out in the presence of oxygen is called as aerobic fermentation. In most of the commercial processes and majority of the products of human utility are produced by this type of fermentation.

<u>6. Surface Fermentations:</u>

- Surface fermentations are those where the substratum may be solid or liquid. The organism grows on the substratum and draws the nutrients from the substratum. These types of fermentations are desirable where the products are based on sporulation.

<u>7. Submerged Fermentations:</u>

- Submerged Fermentations are those in which the nutrient substratum is liquid and the organism grows inside the substratum. The culture conditions are made uniform with the help of spargers and impeller blades.

<u>8. Solid Substrate/State Fermentation:</u>

Solid state (substratum) fermentation (SSF) is generally defined as the growth of the microorganism on moist solid materials in the absence or near the absence of free water.

It distinguish between two processes,

- Solid substrate fermentation - substrate itself acts as carbon source occurring in absence
- Solid state fermentation- natural substrate or an inert substrate used as solid support.

For large-scale SSF bioprocess, three types of fermenters are in operation:

(a) Drum Fermenter
(b) Tray Fermenter
(c) Column Fermenter

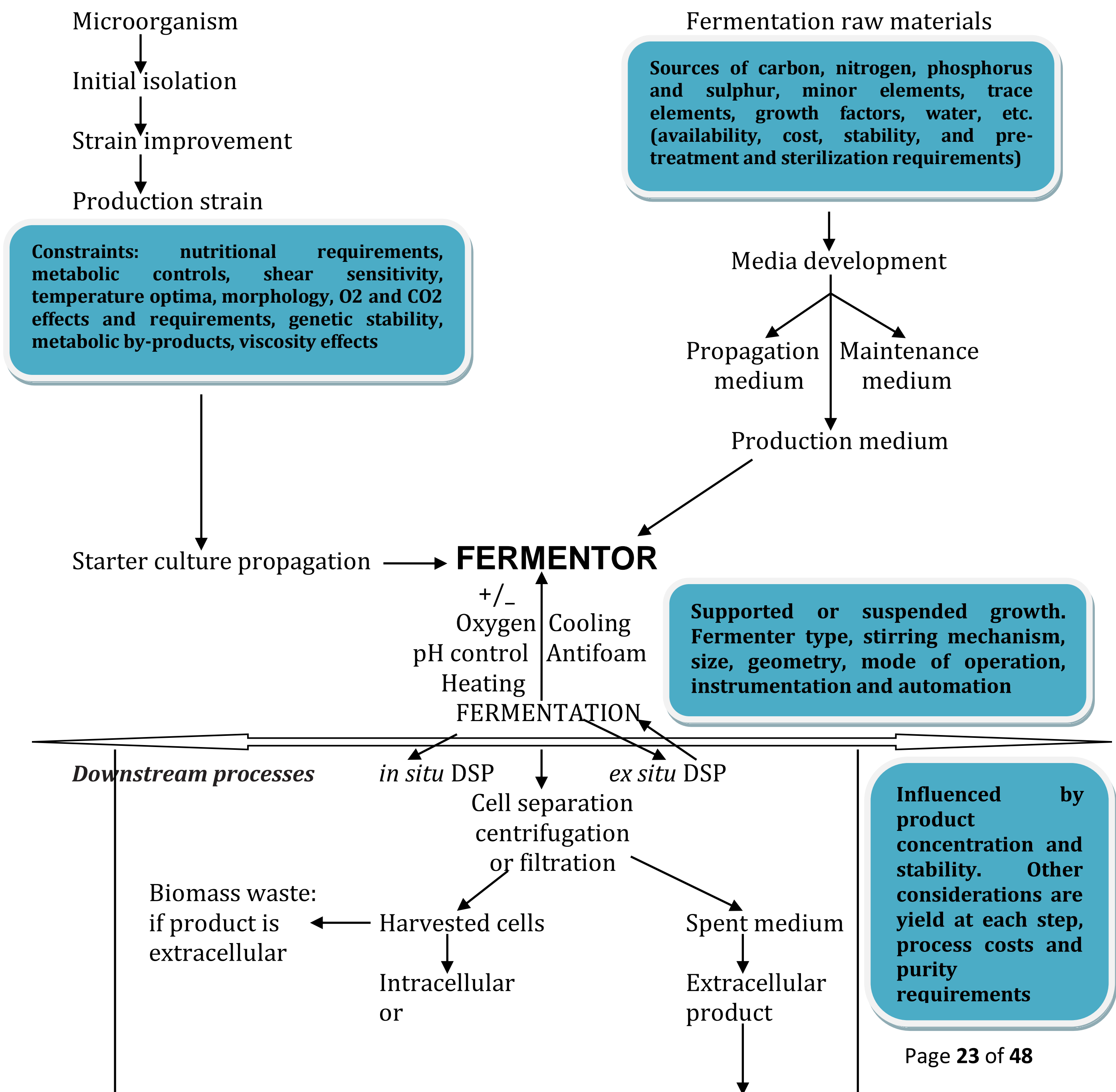
Unit III
Downstream processing

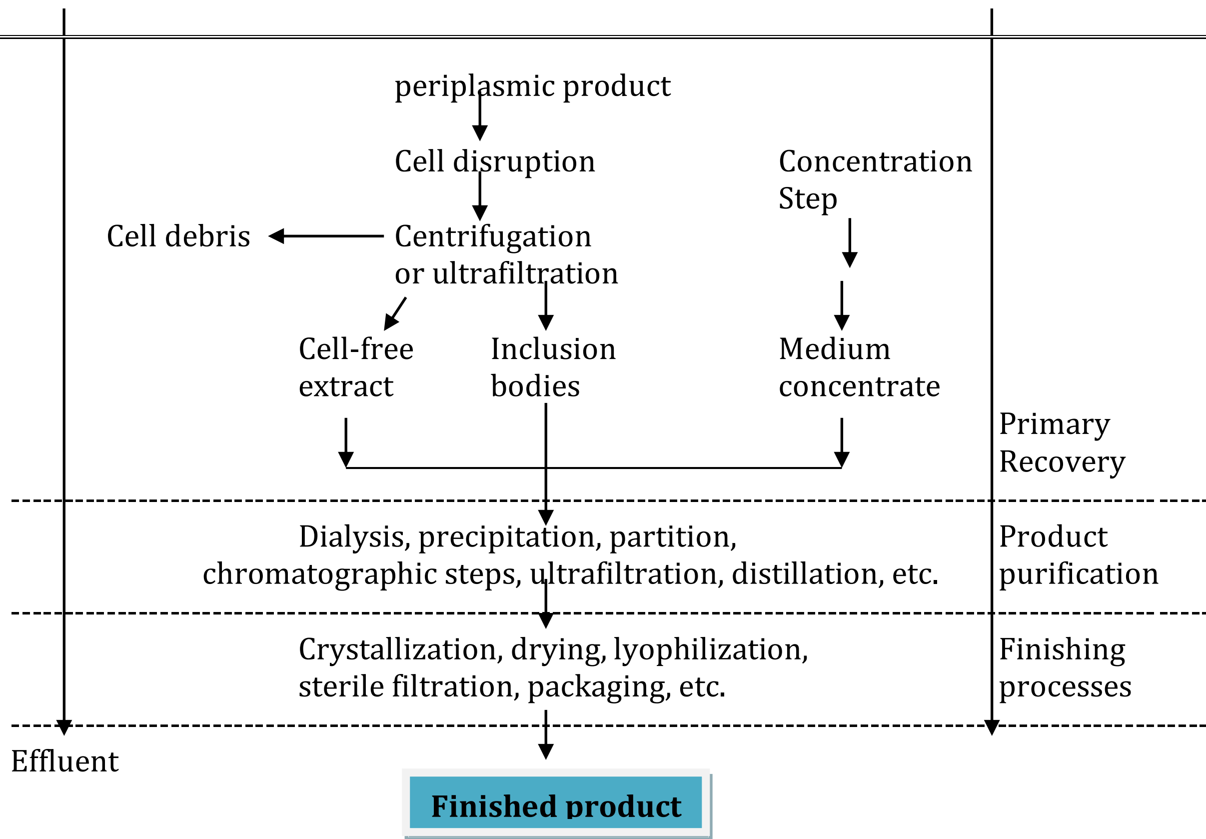
Industrial fermentations comprise both **upstream processing** (USP) and **downstream processing** (DSP) stages.

USP involves all factors and processes leading to the fermentation and consists of three main areas.

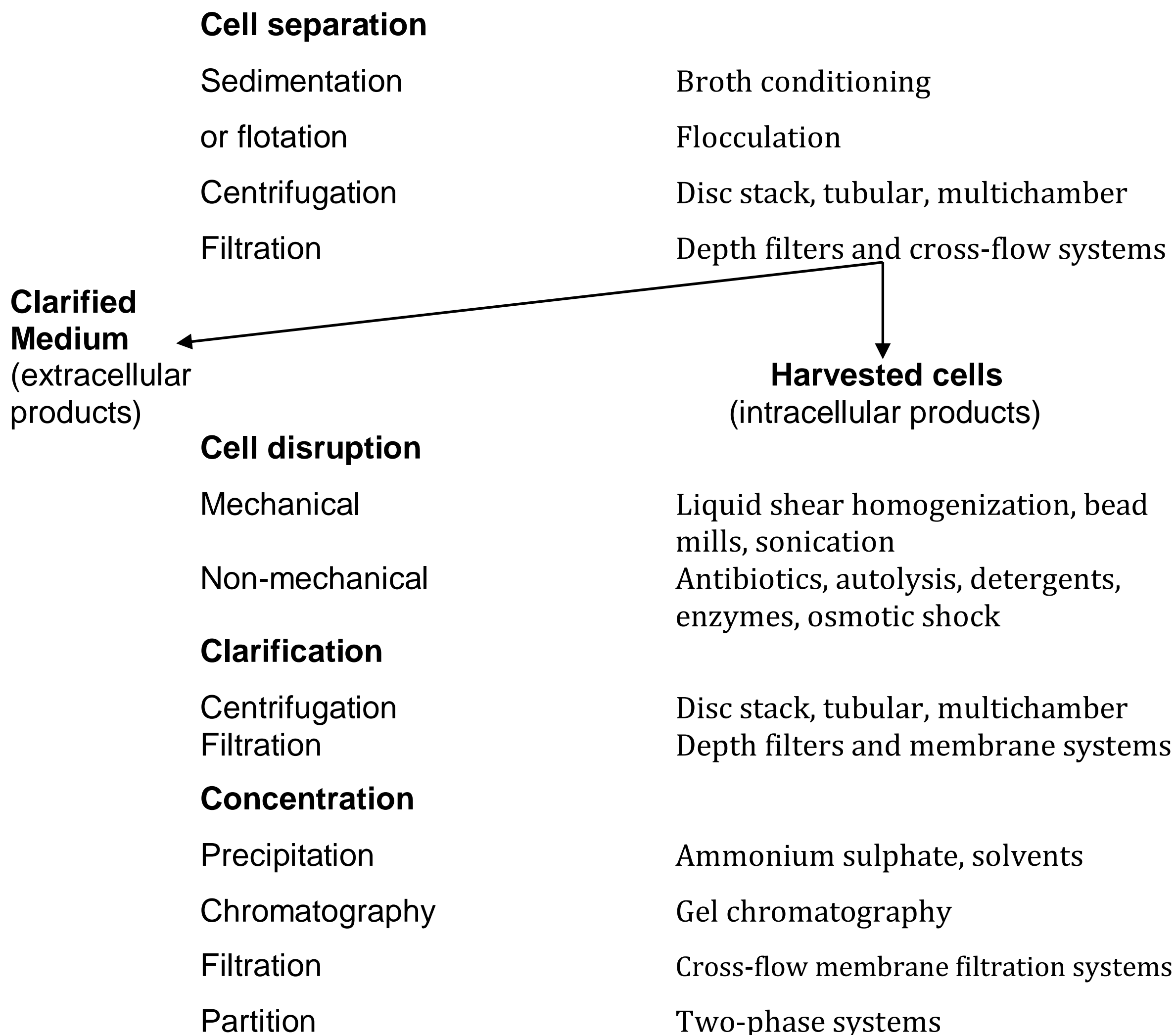
1. Producer microorganism- suitable microorganism, strain improvement.
2. Fermentation media- cost-effective carbon and energy sources, essential nutrients and optimization.
3. Fermentation- growth of the organism or the production of a target microbial product under rigorously controlled conditions.

DSP encompasses all processes following the fermentation. It has the primary aim of efficiently, reproducibly and safely recovering the target product to the required specifications (biological activity, purity, etc.), while maximizing recovery yield and minimizing costs.





DSP can be divided into a series of distinct **unit processes** linked together to achieve product purification.



Distillation	Pot still, continuous still
High-resolution techniques	
Chromatography	Adsorption, affinity, gel filtration, HPLC, hydrophobic, ion exchange, Metal chelate, etc.
Electrophoresis	Isoelectric focusing
Dialysis	Diafiltration, electrodialysis
Finishing/Packaging	
Crystallization	Added salts, solvents
Filtration	Membrane systems
Gel chromatography	Finishing, not concentration
Drying	Freeze-drying (lyophilization), spray drying, tray drying

Cell harvesting

The first step in the DSP of suspended cultures is a solid–liquid separation to remove the cells from the spent medium.

Choice of solid–liquid separation method is influenced by

- the size
- morphology of the microorganism (single cells, aggregates or mycelia),
- the specific gravity,
- viscosity and
- rheology of the spent fermentation medium.

Broth conditioning

It used in sedimentation for separation of cells from liquid media. They alter some property of a microorganism, such that it flocculates and usually precipitates.

Sedimentation

The rate of particle sedimentation is a function of both size and density. The relatively slow and is suitable only for large flocs (greater than 100 μm diameter).

Eg: primary yeast separation in the production of alcoholic beverages

Centrifugation

- Using gravitational force to separate suspended particles, a centrifugal field is applied, the rate of solid–liquid separation is significantly increased and much smaller particles can be separated.
- Centrifugation may be used to separate particles as small as 0.1 μm diameter and is also suitable for some liquid–liquid separations depends on particle size, density difference between the cells and the medium and medium viscosity.

Four main types of industrial centrifuge

1. **Tubular centrifuges**-highest centrifugal force of 13000–17000*g*
2. **Multi-chamber bowl centrifuges**-operating at 5000–10000*g*
3. **Disc stack centrifuges**-operate at 5000–13000*g*
4. **Screw-decanter centrifuges**-operate continuously at 1500–5000*g*

Filtration

Conventional filtration of liquids containing suspended solids involves depth filters composed of porous media (cloth, glass wool or cellulose) that retain the solids and allow the clarified liquid filtrate to pass through.

As filtration proceeds collected solids accumulate above the filter medium, resistance to filtration increases and flow through the filter decreases.

Eg: Harvesting the filamentous fungi.

The two main types

1. **Plate and frame filters** or **filter presses-**Preparation of blocks of baker's yeast, the recovery of protein precipitates and the dewatering of sewage sludge.
2. **Rotary vacuum filters-**antibiotic manufacture, baker's yeast production and in dewatering sludge during waste-water treatment.

MEMBRANE FILTRATION

Modern methods of filtration involve absolute filters rather than depth filters. These consist of supported membranes with specified pore sizes that can be divided into three main categories. They are, in decreasing order of pore size, **microfiltration**, **ultrafiltration** and **reverse osmosis** membranes.

Cell disruption

Some target products are intracellular, several of which form inclusion bodies. Therefore, methods are required to disrupt the microorganisms and release these products.

Eg: Many enzymes and recombinant proteins,

Mechanical cell disruption methods

- Several mechanical methods are available for the disruption of cells. Those based on **solid shear** involve extrusion of frozen cell preparations through a narrow orifice at high pressure.
- Methods utilizing **liquid shear** are generally more effective.
- The French press (pressure cell) is often used in the laboratory and the high-pressure homogenizers.
    - Eg: Bacterial and yeast cells, and fungal mycelium.

In these devices the cell suspension is drawn through a check valve into a pump cylinder. At this point, it is forced under pressure (up to 1500 bar) through a very narrow annulus or discharge valve, over which the pressure drops to atmospheric.

- In industry, **high-speed bead mills**, equipped with cooling jackets, are often used to agitate a cell suspension with small beads (0.5–0.9μm diameter) of glass, zirconium oxide or titanium carbide. Cell breakage results from shear forces, grinding between beads and collisions with beads.
- **Ultrasonic** disruption of cells involves **cavitation**, microscopic bubbles or cavities generated by pressure waves.

Non-mechanical cell disruption methods

- An alternative to mechanical methods of cell disruption is to cause their permeabilization. This can be accomplished by autolysis, osmotic shock, rupture with ice crystals (freezing/thawing) or heat shock.
- Autolysis, for example, has been used for the production of yeast extract and other yeast products. Osmotic shock is often useful for releasing products from the periplasmic space.

- A wide range of other techniques have been developed for small-scale microbial disruption using various chemicals and enzymes.
    - Eg: acetone, butanol, chloroform or methanol, alkali or detergents, such as sodium lauryl sulphate or Triton X-100, can also be effective.

Product recovery

- Recovery of extracellular proteins is from the clarified medium, whereas disrupted cell preparations are used for both intracellular proteins and those held within the periplasmic space.
- Following cell disruption, soluble proteins are usually separated from cell debris by centrifugation, followed by the recovery of precipitated proteins by centrifugation. Precipitation is achieved by the addition of inorganic salts at high ionic strength, usually in the form of solid or saturated solutions of ammonium sulphate.
- Aqueous two-phase separation involves partitioning the protein between the two phases, depending upon its molecular weight and charge.
- Commonly used systems include dextran and polyethylene glycol (PEG) and potassium phosphate.

Chromatography

Chromatographic techniques are usually employed for higher-value products. These methods, normally involving columns of stationary phase, are used for desalting, concentration and purification of protein preparations.

For protein products these factors include molecular weight, isoelectric point, hydrophobicity and biological affinity.

- **Adsorption chromatography** separates according to the affinity of the material, for the surfaces of the solid matrix.
    - Eg: Alumina, hydroxyapatite or silica are used for purifying non-polar molecules.
- **Gel filtration chromatography** essentially involves separation on the basis of molecular size can also influence separation performance.
- **Ion-exchange chromatography** involves the selective adsorption of ions or electrically charged compounds onto ion-exchange resin particles by electrostatic forces.
- **High-performance liquid chromatography** (HPLC) was originally developed for the separation of organic molecules in non-aqueous solvents. This method uses densely packed columns containing very small rigid particles, 5–50μm diameter of silica or a cross-linked polymer.
- **Hydrophobic chromatography** relies on hydrophobic interaction between hydrophobic regions or domains of a solute protein and hydrophobic functional groups of the support particles.
- **Metal chelate chromatography** utilizes a matrix with attached metal ions,
    - e.g. agarose containing calcium, copper or magnesium ions.

Dialysis and electrodialysis

These membrane separation techniques are primarily used for the removal of low molecular weight solutes and inorganic ions from a solution. The membranes involved are size selective with specific molecular weight cut-offs. Low molecular weight solutes move across the membrane by osmosis from a region of high concentration to one of low concentration.

Electrodialysis methods separate charged molecules from a solution by the application of a direct electrical current carried by mobile counter-ions.

Distillation

Distillation is used to recover fuel alcohol, acetone and other solvents from fermentation media.

- Incoming fermentation broth is heated, as it passes down a coiled pipe within the rectifier column, by the ascending hot vapour produced by the analyser column.
- The now hot broth is released into a trough at the top of the analyser column and as it falls down the column it is heated by steam.
- Hot vapours generated are then conveyed from the top of the analyser column to the bottom of the rectifier column.
- As it passes upwards it is condensed on the coils carrying incoming broth.
- There is a temperature gradient in the rectifier column and each volatile compound condenses at its appropriate level, from where the fraction is collected.

Finishing steps

Crystallization

Product crystallization may be achieved by evaporation, low-temperature treatment or the addition of a chemical reactive with the solute. The product's solubility can be reduced by adding solvents, salts, polymers (e.g. non-ionic PEG) and polyelectrolytes.

Drying

Drying involves the transfer of heat to the wet material and removal of the moisture as water vapour. Usually, this must be performed in such a way as to retain the biological activity of the product. Parameters affecting drying are the physical properties of the solid–liquid system, intrinsic properties of the solute, conditions of the drying environment and heat transfer parameters.

- **Rotary drum driers** remove water by heat conduction.
- **Freeze-drying** (lyophilization) is often used where the final products are live cells, as in starter culture preparations, or for thermolabile products. This is especially useful for some enzymes, vaccines and other pharmaceuticals.

Immobilization of Enzymes and Cells

Immobilization of enzymes (or cells) refers to the technique of confining/anchoring the enzymes (or cells) in or on an inert support for their stability and functional reuse. By employing this technique, enzymes are made more efficient and cost-effective for their industrial use. Some workers regard immobilization as a goose with a golden egg in enzyme technology. Immobilized enzymes retain their structural conformation necessary for catalysis.

There are several advantages of immobilized enzymes:

a. Stable and more efficient in function.
b. Can be reused again and again.
c. Products are enzyme-free.
d. Ideal for multi-enzyme reaction systems.
e. Control of enzyme function is easy.
f. Suitable for industrial and medical use.
g. Minimize effluent disposal problems.

Methods of Immobilization:

The commonly employed techniques for immobilization of enzymes are—adsorption, entrapment, covalent binding and cross-linking.

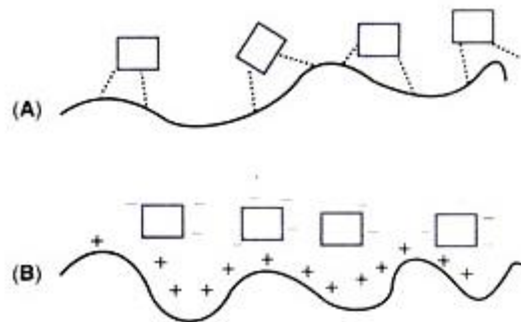
Adsorption:

Adsorption involves the physical binding of enzymes (or cells) on the surface of an inert support.

The support materials may be inorganic (e.g. alumina, silica gel, calcium phosphate gel, glass) or organic (starch, carboxymethyl cellulose, DEAE-cellulose, DEAE-sephadex).

Adsorption of enzyme molecules (on the inert support) involves weak forces such as van der Waals forces and hydrogen bonds.

Therefore, the adsorbed enzymes can be easily removed by minor changes in pH, ionic strength or temperature. This is a disadvantage for industrial use of enzymes.

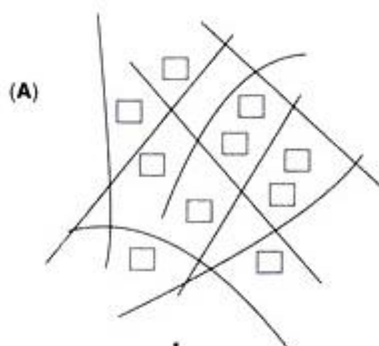


Entrapment:

Enzymes can be immobilized by physical entrapment inside a polymer or a gel matrix.

The size of the matrix pores is such that the enzyme is retained while the substrate and product molecules pass through.

The matrices used for entrapping of enzymes include polyacrylamide gel, collagen, gelatin, starch, cellulose, silicone and rubber.



Microencapsulation:

Microencapsulation is a type of entrapment. It refers to the process of spherical particle formation wherein a liquid or suspension is enclosed in a semipermeable membrane.

The membrane may be polymeric, lipoidal, lipoprotein-based or non-ionic in nature.

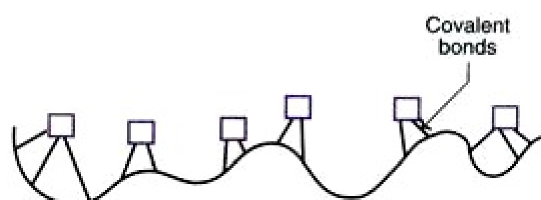
There are three distinct ways of microencapsulation.

1. Building of special membrane reactors.
2. Formation of emulsions.
3. Stabilization of emulsions to form microcapsules.

Covalent Binding:

Immobilization of the enzymes can be achieved by creation of covalent bonds between the chemical groups of enzymes and the chemical groups of the support.

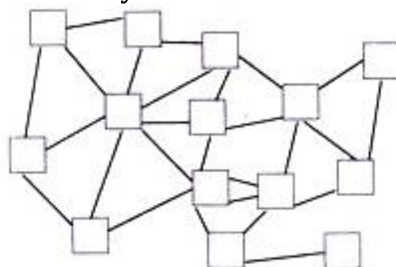
The inert support usually requires pretreatment (to form pre-activated support) before it binds to enzyme.



Cross-Linking:

The absence of a solid support is a characteristic feature of immobilization of enzymes by cross- linking, through the involvement of poly-functional reagents which form the backbone to hold enzyme molecules.

There are several reagents in use for cross-linking. These include glutaraldehyde, diazobenzidine, hexamethylene diisocyanate and toluene di- isothiocyanate.



Glutaraldehyde is the most extensively used cross-linking reagent. The technique of cross-linking is quite simple and cost-effective.

Unit IV
Penicillin production

Antibiotics are probably the most important group of compounds synthesized by industrial microorganisms.

- ❖ Most antibiotics are secondary metabolites produced by filamentous fungi and bacteria, particularly the actinomycetes.
- ❖ Penicillin was discovered by Fleming in 1928 following his famous observation of an inhibitory zone surrounding a fungal contaminant, *Penicillium notatum*, on a plate of *Staphylococcus aureus*.
- ❖ The basic structure of the penicillins is **6-aminopenicillanic acid (6-APA)**, composed of a **thiazolidine ring** fused with a b**-lactam ring** whose 6-amino position carries a variety of **acyl** substituents

The major steps in the commercial production of Penicillin are:

(1) Preparation of inoculum.
(2) Preparation and sterilisation of the medium.
(3) Inoculation of the medium in the fermentor.
(4) Forced aeration with sterile air during incubation.
(5) Removal of mould mycelium after fermentation.
(6) Extraction and purification of the penicillin.

Strain:

Penicllium chrysogenum* and *Penicillium notatum

Inoculum development is usually initiated by adding lyophilized spores to a small fermenter at a concentration of 5×10^3 spores/ml.

They were produced through natural selection and mutation using ultra violet irradiation, x-irradiation or nitrogen mustard treatment.

Production medium

Various carbon sources have been adopted for penicillin production, including glucose, lactose, sucrose, ethanol and vegetable oils. About

- **65% of the carbon source is metabolized for cellular maintenance,**
- **25% for growth and**
- **10% for penicillin production.**

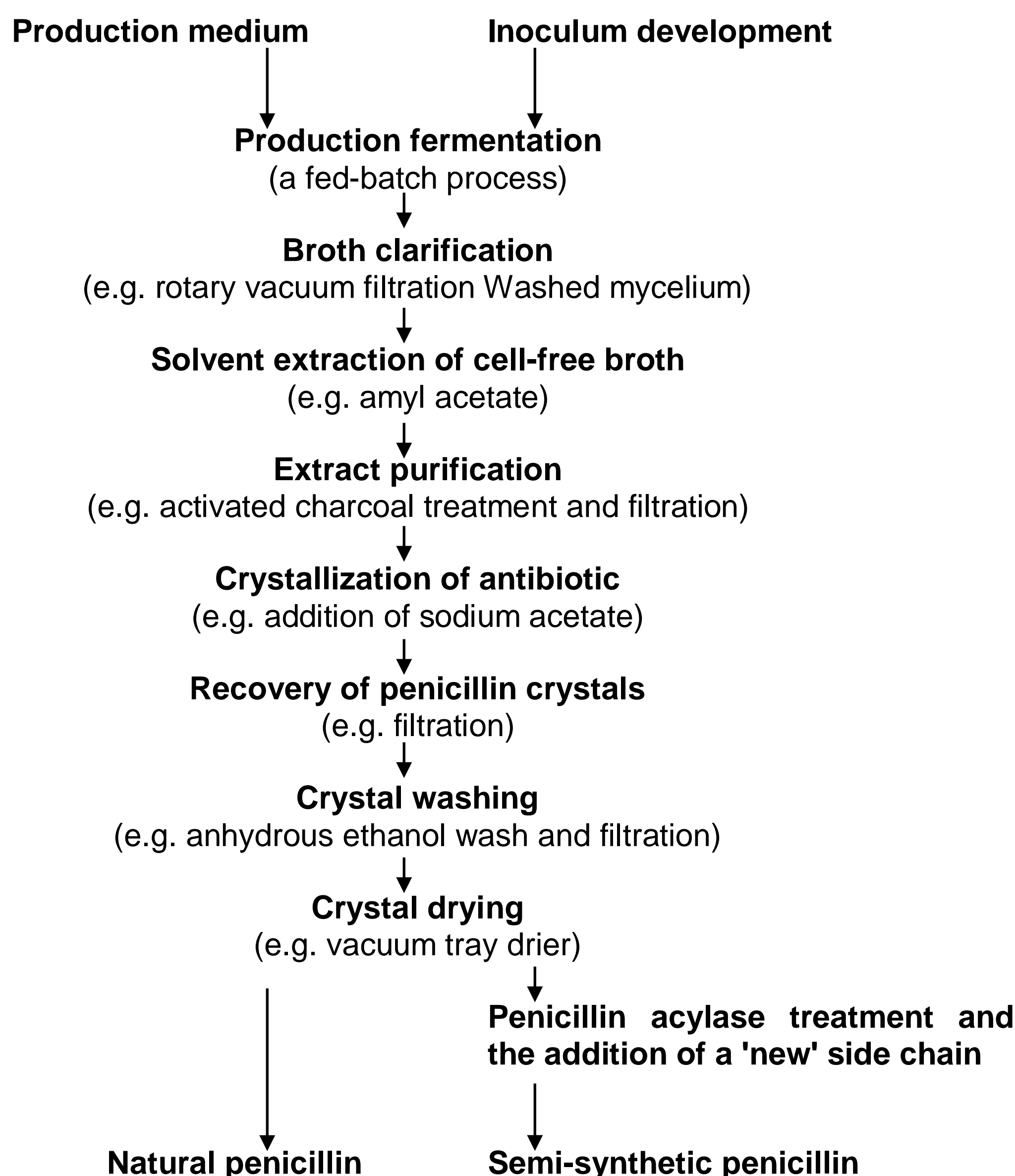
- **Corn steep liquor** is still used as a source of nitrogen, additional nutrients and side-chain precursors.
- Its acidic nature creates a requirement for **calcium carbonate** (1%, w/v) and a phosphate buffer to neutralize the medium, thereby optimizing its pH for penicillin production.
- **Ammonia, mineral salts** and specific side-chain precursors, e.g. **phenyl acetic acid** or phenoxyacetic acid, may also be added.

Bio-parameters

- ❖ Fed-batch fermentation process carried out aseptically
- ❖ Stirred tank fermentors of 40000–200000L capacity, although airlift systems are sometimes used.
- ❖ The fermentation involves an initial vegetative growth phase followed by the antibiotic production phase.
- ❖ Throughout the process, the oxygen level is 25–60mmol/L/h.
- ❖ These processes are maintained at 25–27°C and pH 6.5–7.7, the specific conditions depending upon the *P.chrysogenum* strain used.
- ❖ This continues for a further 6–8 days.

Recovery

1. Usually, penicillin recovery follows removal of mycelium using rotary vacuum filters.
2. Recovered mycelium is then washed to remove residual penicillin
3. Antibiotic recovery is often by solvent extraction of the cell-free medium
4. Any pigments and trace impurities are removed by treating with activated charcoal.
5. The penicillin is then retrieved from the solvent by addition of sodium or potassium acetate.
6. This reduces the solubility of the penicillin and it precipitates as a sodium or potassium salt.
7. Resultant penicillin crystals are separated by rotary vacuum filtration.



8. Penicillin crystals are mixed with a volatile solvent, usually anhydrous ethanol, butanol or isopropanol, to remove further impurities.
9. The crystals are collected by filtration and air dried. At this stage the penicillin is 99.5% pure.

Production of Streptomycin

Streptomycin is useful in the control of plant diseases caused by bacteria as it acts systemically in plants.

It produced by *Streptomyces griseus* is active against Gram (-) ve bacteria and against tuberculosis bacterium.

- Streptomycin is an aminoglycoside antibiotics.
- Streptomycin is directly derived from glucose into streptomycin in the synthesis of N-methyl glucosamine.
- Prolonged treatment by streptomycin at high dosage can produce neurotoxic reactions such as hearing impairment, loss of balance maintenance in man.

The Inoculum Production:

Spores of *Streptomyces griseus* maintained as lyophilized in a sterile skimmed milk, is employed as stock culture. After sufficient mycelial growth, it is fed to production fermentor.

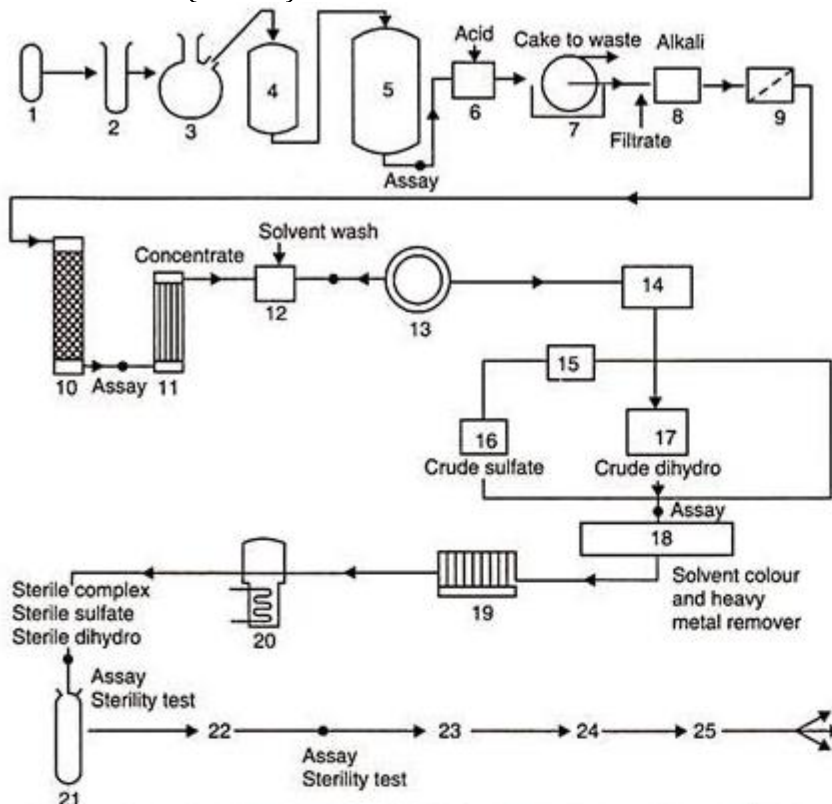
Preparation of the Medium:

A production medium contains carbon source and nitrogen source. Glucose is one of the best carbon sources which helps in the greater yield of streptomycin.

1. Soyabean meal (1%),
2. Glucose 1% and
3. Sodium chloride (0.5%),

Hocken hull (1963) recommended the medium consisting of

1. Glucose (2.5%),
2. Soyabean meal (4.0%),
3. Distillers dry soluble (0.5%) and
4. Sodium chloride (0.25%).



1. Master culture 2. Agar slopes 3. Shaker flask 4. Seed vessel 5. Fermentor 6. Acidification 7. Filtration 8. Neutralization 9. Filter clarification 10. Ion-exchange reagent 11. Evaporator 12. Crystallization 13. Vacuum oven 14. Calcium chloride crude complex 15. Calcium chloride removal 16. Crystallization 17. Catalytic hydrogenation 18. Finishing 19. Seitz filter 20. Freeze drying 21. Vial filling machine 22. Capping 23. Labelling 24. Packing 25. Despatch

Fermentation:

- Submerged culture method
- Appropriate volume of inoculum (4-5%)
- Temperature is in the range of 25 to 30°C and
- The optimum pH range is between 7.0 and 8.0. High rate of streptomycin production, however, occurs in the pH range of 7.6 to 8.0.

Harvest and Recovery:

1. After completion of fermentation the mycelium is separated from the broth by filtration. Streptomycin is recovered by several methods.
2. The fermentation broth is acidified, filtered and neutralized.
3. It is then passed through a column containing a cation exchange resin to adsorb the streptomycin from the broth.
4. The column is then washed with water and the antibiotic is eluted with hydrochloric acid or cyclohexanol or phosphoric acid.
5. It is then concentrated at about 60°C under vacuum.
6. The streptomycin is then dissolved in methanol and filtered and acetone is added to the filtrate to precipitate the antibiotic.
7. The precipitate is again washed with acetone and vacuum dried.
8. It is purified further by dissolving in methanol.
9. The streptomycin in pure form is extracted as calcium chloride complex.

Production of Vitamin – B12

Vitamin B12 also called cobalamin, is a water-soluble vitamin with a key role in the normal functioning of the brain and nervous system and for the formation of blood.

It is normally involved in the metabolism of every cell of the human body, especially affecting DNA synthesis and regulation, but also fatty acid synthesis and enegy production.

Vitamin B12 is important for the way the body works.

STARTER CULTURE

The following microbes were suitable for industrial fermentation of cyanocobalamine.

1. Bacillus megaterium
2. Streptomyces olivaceous
3. Propiombacteriumshermanii
4. Pseudomonas denitrificans
5. Rhodopseudomonaspalustris

Improved strains produce 50,000 times more vitamin B12 than wild strain by biotechnological treatment to produce vit B12.

MEDIA PREPARATION

Carbon source as

1. Corn steep glucose
2. Beet molasses
3. Soyabean meal / Glucose

Nitrogen source as

1. Ammonium phosphate
2. Ammonium hydroxide and Cobalt salt for minerals.

Prepared medium is sterilized by autoclaving. The sterilized medium is then used for fermentation.

Composition of seed medium (g/l)

Yeast extract - 1
Beef extract - 1
N-Z amine - 2
Glucose - 10
Agar - 15

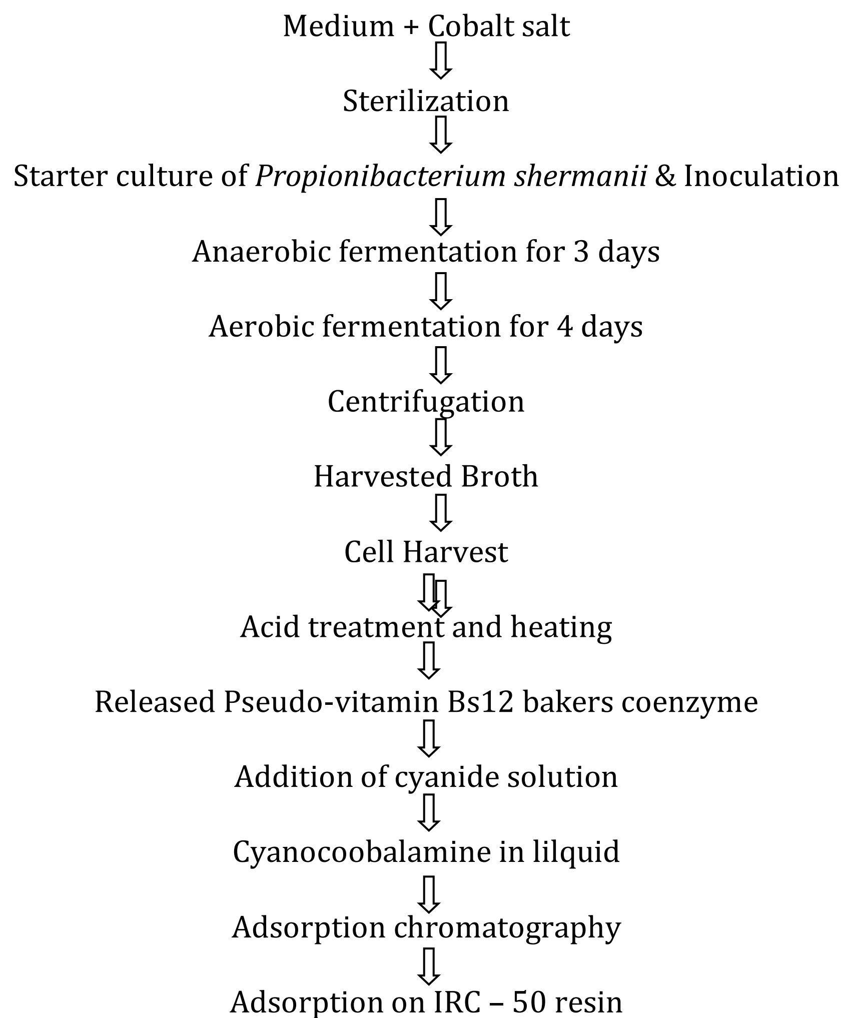
Composition of Production medium

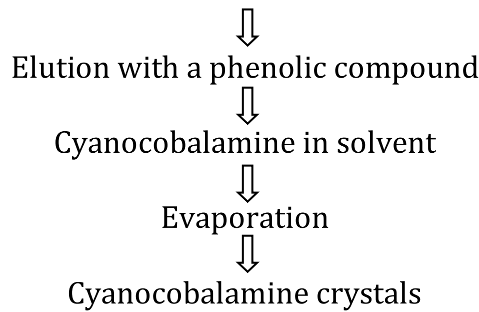
Distlled soluble - 4%
Dextrose - 0.5%
Calcium carbonate - 0.5%
Cacl2 - 1-5ppm
pH - 7

Fermentation

- Batch fermentation in aerobic status.
- Stirred tank fermentor.
- Tempeture-25-27c;
- PH- 5.5 – 7.5
- Cobalt is needed to attain maximum yield
- Aeration – 0.5 volume using activated charcoal
- Antifoam agent used are soyabean oil, corn oil and Lard oil.
- Yield of Vitamin b12 fermentation is 1-2 mg.litre

RECOVERY





Production of Riboflavin

Riboflavin (vitamin B_2) is a water soluble vitamin, essential for growth and reproduction in man and animals. Deficiency of riboflavin in rats causes growth retardation, dermatitis and eye lesions.

Riboflavin occurs in milk and milk products, meat, eggs, liver and kidney.

The overproduction of riboflavin in these organisms takes place mainly due to the constitutive nature of the riboflavin synthesizing enzymes.

Microorganisms and yields of riboflavin:

- Several microorganisms (bacteria, yeasts and fungi) can be employed for the production of riboflavin. In the acetone-butanol fermentation, employing the organisms *Clostridium acetobutylicum* and *Clostridium butylicum*, riboflavin is formed as a byproduct.
- Commercial production of riboflavin is predominantly carried out by direct fermentation using the ascomycetes.
- *Ashbya gossypii* and *Eremothecium ashbyii* are most commonly employed due to high yield.
- High yielding strains of *Ashbya gossypii* have been developed by genetic manipulations. Such strains can yield as high as 15 g/1 riboflavin.
- *Candida* sp can also produce riboflavin.

Production media

- Simple sugars such as glucose and corn steep liquor. Glucose can be replaced by sucrose or maltose for the supply of carbon source.
- In recent years, lipids such as corn oil, when added to the medium for energy purpose.
- Further, supplementation of the medium with yeast extract, peptones, glycine, inositol, purines also increase the yield of riboflavin.

There are three processes employed for the large scale production of riboflavin.

1. **Biotransformation**
2. **Chemical synthesis**
3. **Fermentation**

Fermentation

The initial pH of the culture medium is adjusted to around 6-7.5. The fermentation is conducted at temperature 26-28°C with an aeration rate 0.3 vvm.

The process is carried out for about 5-7 days by submerged aerated fermentation.

It is now accepted that the fermentation occurs through three phases.

Phase I:

- This phase is characterized by rapid growth of the organism utilizing glucose.

Phase II:

- Sporulation occurs in this phase

Phase III:

- In this last phase, cells get disrupted by a process of autolysis.

<u>Recovery:</u>

1. Riboflavin is found in fermentation broth and in a bound form to the cells.
2. The latter can be released by heat treatment i.e. 120°C for about 1 hour.
3. The cells can be discarded after filtration or centrifugation.
4. The filtrate can be further purified and dried, as per the requirements.

Production of Rabies Vaccines

Viruses multiply only in living cells. Viruses to be used for vaccine production must therefore be grown in such cells. In practice they are grown for vaccine purposes in tissue cultures.

<u>Tissue cultures and their cultivation</u>

The growth of animal cells *in vitro* in monolayers is known as tissue or cell cultures.

<u>Medium for tissue culture</u>

Various media are available for the cultivation of cells.

They consist essentially of

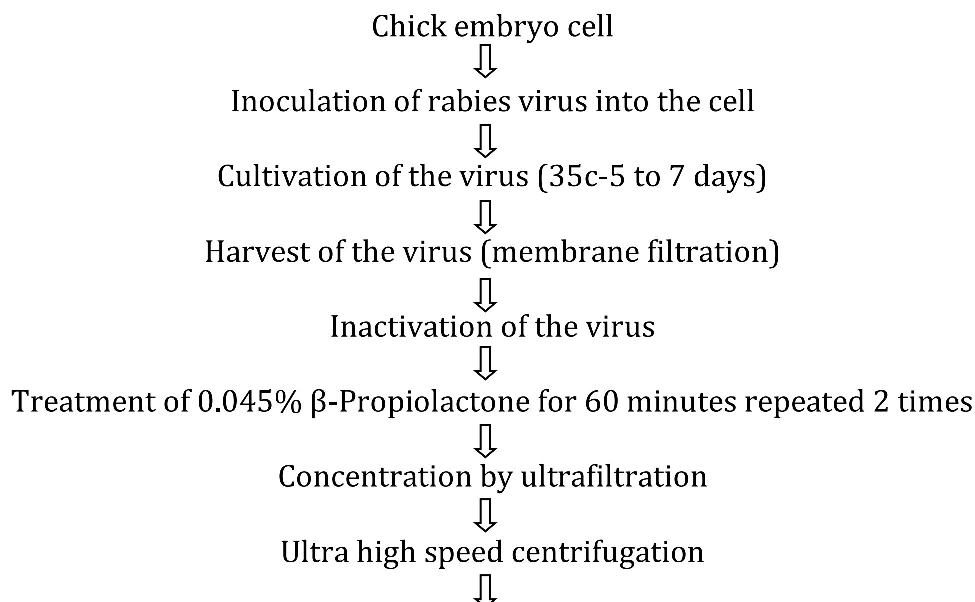
- inorganic salts,
- amino acids,
- vitamins,
- nucleotides, and
- low molecular growth factors such as hormones, steroids, and fatty acids.

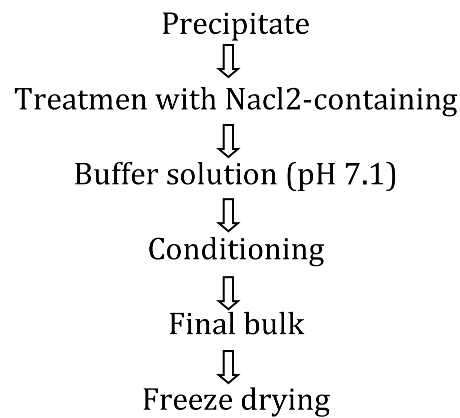
Another important component is serum and this is often used in conjunction with peptones, tryptic digests and albumin hydrolysates.

A mixture of antibiotics is also often added to remove contaminants.

<u>Other preparation methods</u>

1. When embryo of chicks or ducks are used, the avian embryos are harvested from eggs, obtained from special isolated flocks, and then minced and treated with disaggregating enzymes such as trypsin, collagenase, hyaluronidase and pronase.





2. The fibroblast cells released by this process are attachment dependent, requiring solid surfaces for growth.
3. The successful commercial manufactures of viral vaccines in attachment-dependent cell systems rely on the establishment and maintenance of healthy cell monolayers.
4. The appropriate growth and maintenance media must be carefully selected and careful attention must be paid to nutrient depletion, waste accumulation and changes in pH over time.

Cell harvest

Cells may be harvested by the use of trypsin (or other proteolytic enzymes such as papain or pronase), by the use of chelating agents e.g. EDTA, by physical scraping off or a combination of one or more of these methods.

Unit V
Ethanol production

Ethanol is an attractive fuel because it may be used alone or mixed with other liquid fuels, e.g. 'gasohol'.

Ethanol is produced from biomass mostly via a fermentation process using glucose derived from sugars (sugar cane, sugar beet and molasses), starch (corn, wheat, grains) or cellulose (forest products) as raw materials.

Strains:

- The ethanol was produced by fermentation of sucrose, derived from sugar cane, using *Saccharomyces cerevisiae.*
- Apart from sucrose, other conventional fermentation substrates include simple sugars derived from plants and dairy wastes.
- However, use of root and tuber starch (cassava, potato, etc.), grain starch (maize, wheat, rice, etc.), corn oil and lignocellulosic plant materials demands energy-consuming processing operations to achieve hydrolysis.
- Fermentation of the resulting sugars, mostly glucose, is carried out by selected strains of *S. cerevisiae* at 32–38°C and pH 4.5–5.0. Fermentations may be batch or continuous Processes.
- These alcoholic fermentations generate a 'beer' containing approximately 10% (v/v) ethanol from which the yeast is usually separated prior to distillation.
- The ethanol may be recovered by flash vaporization and the non-volatile solvent is reused. Genetic engineering of *S. cerevisiae* for strain improvements.

Biochemistry

Enzymes in the yeast first convert carbohydrates like maltose or sucrose into glucose and fructose and then convert these in turn into ethanol and CO2.

Sucrose----------- glucose-------- Pyruvic acid--------- Acetaldehyde-------- Ethanol+CO2

Raw materials

3 types

a) Saccharine materials : Sugar cane/beet juice, molasses, black strap molasses, fruit pulp, Juice waste, Cane sorghum and Whey.
b) Starch materials : Cereal grains (Corn, Wheat, Rice, Barely & Sorghum), Starch root plants.
c) Cellulosic materials : Straw components of corn, wheat, rice and Sulphide waste liquor.

Bio parameter

- 0.5-5% to 10% maximum of inoculums is used for fermentation.
- Continuous fermentation method using submerged process.
- Alcohol production starts within 12 hours of inoculums of seed culture.
- Temperature-28-34c
- pH- 4.5-4.9
- Duration of fermentation 24 hours.
- Agitation is required for maintaining uniform temperature.
- The supernatant is separated of ethanol by distilled analysis and rectified column to obtain rectified sprit.

Fermentation

Step-by-step explanation of the seven stages:

Stage 1: Extraction

Stage 2: Juice Treatment (leading to sugar)

Stage 3: Juice Treatment (leading to ethanol)
Stage 4: Multi Effect Evaporator
Stage 5: Crystallization, Drying
Stage 6: Fermentor
Stage 7: Ethanol Distillation

Whey and other dairy wastes

Starch crops cereals, roots and tubers

Sugar crops beet or cane

Lignocellulosic materials wood chips, sawdust, straw, waste paper, etc.

Pretreatments Mechanical size reduction & delignification

Deproteination

Gelatinization & saccharification to glucose, maltose and maltodextrins

Juice extraction (sucrose)

Lignin

Cellulose & hemicellulose

Acid or enzymic Hydrolysis to glucose, cellobiose, xylose, arabinose, etc.

Fermentation medium supplements (nitrogen, phosphate, vitamins, etc.)

Fermentation: batch, semi-continuous, continuous +/− continuous ethanol removal

Fermentation by-products (animal feed, etc.)

Fermentation by-products (animal feed, etc.)

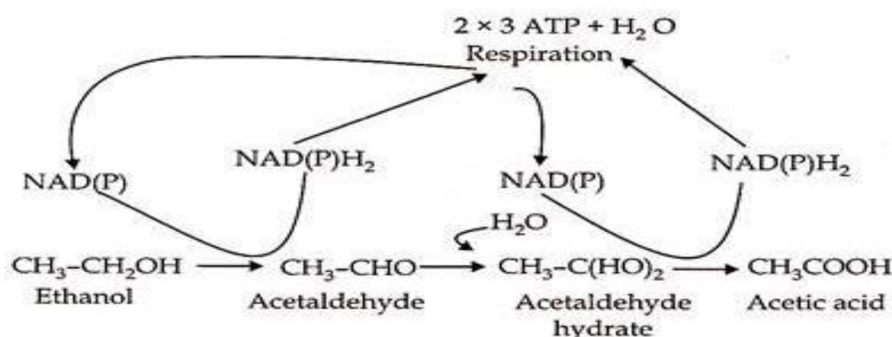
Azeotropic Distillation 99.8% (v/v) ethanol

Vinegar production

Vinegar is an alcoholic liquid. It is a sour taste. We use vinegar primarily to flavor and preserve foods and as an ingredient in salad dressings and marinades. It also acts as a cleaning agent.

Fermentable sugars is exposed to indigenous microbial activities is an alcoholic fermentation by yeasts, followed by bacterial oxidation of the ethanol to acetic acid (acetification).

The acetic acid fermentation is a highly aerobic process, essentially a biotransformation by acetic acid bacteria, involving incomplete oxidation of ethanol to acetic acid.



Strains

- Industrial acetic acid bacteria are members of the genera *Acetobacter* and *Gluconobacter*, mostly *A. aceti*, *A. europaeus*, *A.hansenii*, *A. rancens*, *A. xylinum* and *G. oxydans*.
- An acetic acid bacterium is an *Acetobacter aceti*, Obligatory aerobic bacteria.
- These bacteria convert alcohol to acetic acid.

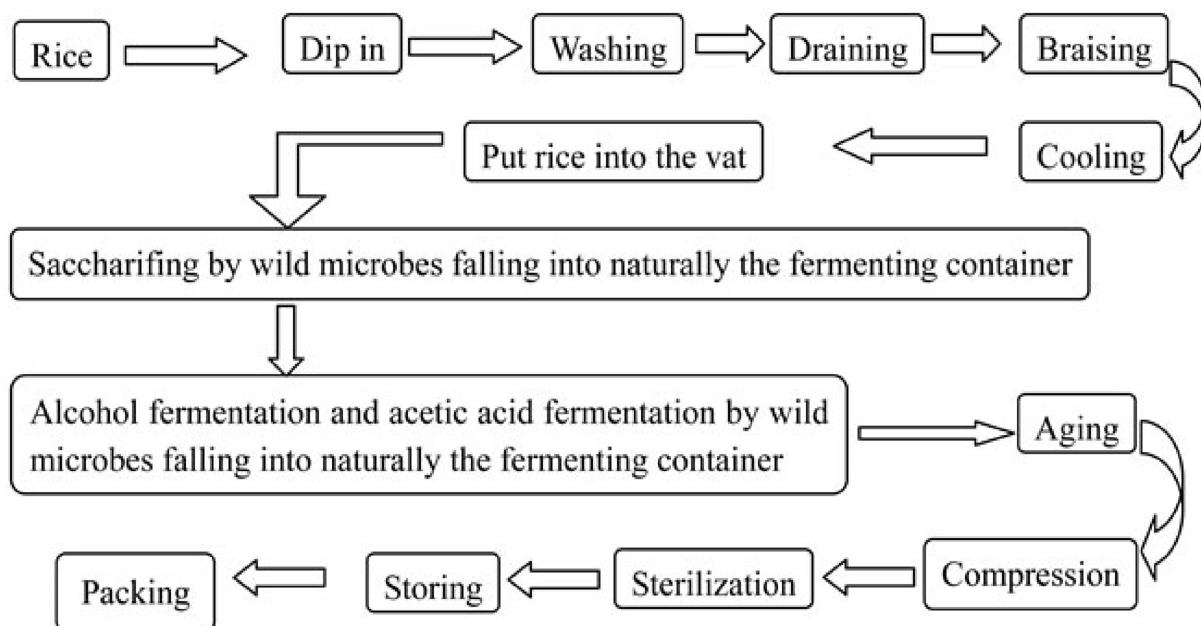
Fermentation

- Submerged methods
- Acetic acid fermentation
- Highly aerated stainless steel stirred tank reactor
- Normally maintained at 24–30°C & pH is 2.5.
- Initial inoculums concentrations of around 1.5×10^8 cells/ml.
- Material with low alcohol concentration such as fruits, wines and special mashes were first used

Vinegar Production Process:

There are three well-known processes of producing vinegar.

1. The Orleans Method
2. The Trickling, Quick Process
3. The Submerged Fermentation Method



Submerged Fermentation Method

1. In this method, a high-speed motor breaks down air that is brought down from a stainless steel tank into tiny bubbles and is forced into the solution of alcoholic liquid and the bacteria for even faster oxidisation.
2. The final steps are filtration and pasteurisation of the vinegar to stop any more bacteria growth and enzyme actions.
3. As this process usually takes one to two days to process so this method is mainly used by big industries.
4. Finally, the liquor is pasteurized at 75–80°C for 30–40 s prior to bottling, during which SO2 may be added at levels of 50–100mg/L.

Amylase Production

Amylase is one of the most important of these industrial enzymes.

α-Amylase is endo-enzyme are ultimately generates glucose, maltose and maltotriose units.

β-Amylase is an exo-hydrolase enzyme that acts from the non-reducing end of a polysaccharide chains.

Fungal amylases have been used to manufacture sugar syrups.

In addition, a-amylases are employed in brewing and baking, where starch liquefaction and dextrin hydrolysis are required.

Strains:

- They are also secreted by many other microorganisms, including bacteria and some yeasts.
- The thermostable a-amylases from *Bacillus* species, e.g. *B. licheniformis.*
- A further success has been **amyloglucosidase (glucoamylase)** from *Aspergillus niger* and *Rhizopus* species.
- This enzyme can completely break down starch and dextrins into glucose.
- The process was further improved by using highly thermostable, Mutation and genetically modified.

Production Medium:

Carbon sources:

- Maltose, Sucrose, Glucose and oil cakes

Nitrogen Sources:

- Ammonium sulphate, Ammonium chloride, Ammonium hydrogen phosphate

Organic sources:

- Peptone, Yeast extract, Soyabean meal

Fungal Amylase (g/l)

Surface culture system using corn starch containing media.

Corn starch - 24
Corn steep liquor - 36
KCL
Na2HPo4 - 4.7
Cacl2 - 1
Mgcl2 - 0.2

Bacterial Amylase

Submerged culture using the soybean meal media

Ground soya bean - 1.35 %
Autilysed brewers yeast - 1.5%
Distillers dried soluble - 0.76%
N-2 amines - 0.65%
Lactose - 4.75%
Mgso4 - 0.64%
Antifoam - 0.05%

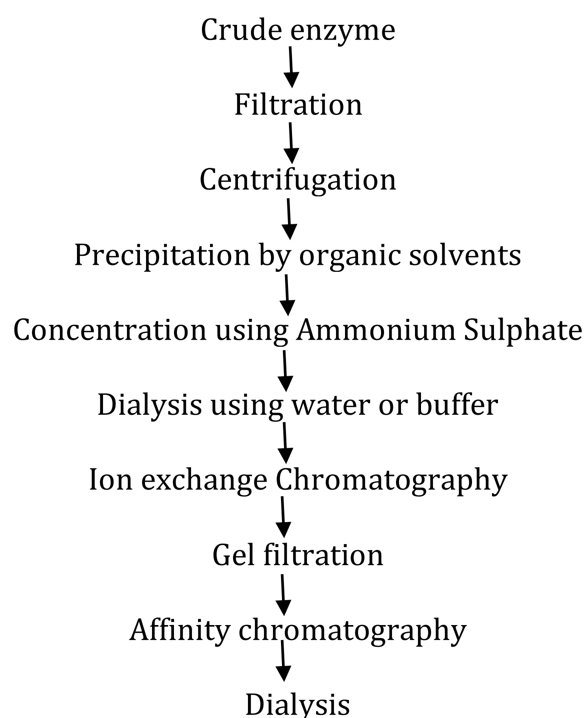
Fermetation:

Temperature : 30-55°c
pH : 6-7.0
Incubation : 7Hours

There are mainly two methods.
These are: 1) Submerged fermentation and 2) Solid State fermentation.
1. Submerged fermentation (SmF) employs free flowing liquid substrates, such as molasses and broths.
This fermentation technique is suitable for microorganisms such as bacteria that require high moisture content for their growth.
The sterilization of the medium and purification process of the end products can be done easily. Also the control of process parameters like temperature, pH, aeration, oxygen transfer and moisture can be done conveniently.

2. Solid state fermentation is a method used for microbes which require less moisture content for their growth.
The solid substrates commonly used in this method are, bran, bagasse, and paper pulp.
The main advantage is that nutrient-rich waste materials can be easily recycled and used as substrates in this method.

Recovery:
Purification methods commonly employed are precipitation, chromatography and liquid-liquid extraction depending on the properties.



Protease Production

Protease enzyme conducts proteolysis by hydrolysis of the peptide bonds that link amino acids together in the polypeptide chain.

They find increased application in food, pharmaceutical, detergent, leather, tanning industry and silver recovery and peptide synthesis.

Though plants and animals also produce extra cellular proteases, microorganisms are the preferred source because of their rapid growth.

Extra cellular proteases help in hydrolysis of protein in the cell free environment and their cellular uptake.

Strain:
Rhizopus microsporus was maintained at 4°C on potato dextrose agar (PDA).

They are 2 types

i) Alkaline serine protease – *Bacillus licheniformis*
The enzymes were produced using basal medium.

The basal medium contained the following ingredients (g/l):

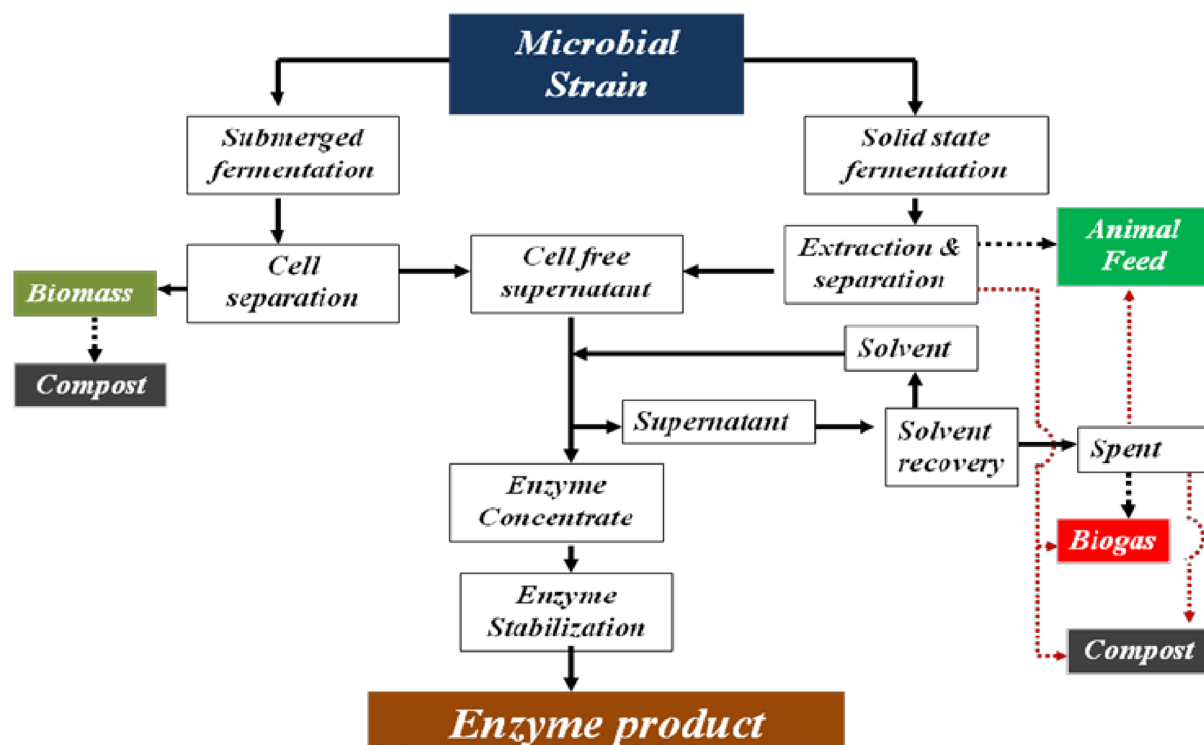
1. Starch hydrolysate - 50
2. Soya bean meal - 20
3. Casein - 20
4. Na2HPo4 - 3.3

ii) Acid protease - *Mucor pusillus, Aspergillus niger*

The basal medium contained the following ingredients:

1. Starch hydrolysate - 4%
2. Soya bean meal - 3%
3. Ground barley - 10%
4. Calcium carbonate - 0.5%

Fermentation:
Medium – Basal Medium (Manomani *et al.,* 1983)
Incubation time- 96 h; Submerged fermentation
Temperature – 30± 2°C; pH - 5.5-7.0



Recovery

1. Protease was isolated and partially purified with isopropanol.
2. The mold filtrate (20 ml) was chilled at 4°C in a refrigerator and 25 ml of chilled isopropanol was added to it.

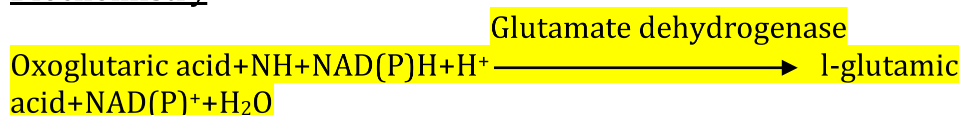
3. The precipitates were separated by centrifugation.
4. The precipitates were dissolved in 5 ml of 0.05 M citrate phosphate buffer having pH 5.2 to obtain partially purified enzymes.

Production of Glutamic acid

L-glutamic acid is one of the major amino acids that is present in a wide variety of foods. It is mainly used as a food additive and flavor enhancer in the form of sodium salt.

Monosodium l-glutamate (MSG) is naturally present in certain foods.

Biochemistry



Glutamate dehydrogenase

Oxoglutaric acid+NH+NAD(P)H+H^+ ⟶ l-glutamic acid+$NAD(P)^+$+H_2O

Strains:

Glutamic acid-producing microorganisms include species of the closely related genera *Arthrobacter*, *Brevibacterium*, *Corynebacterium*, *Microbacterium* and *Micrococcus*.

Corynebacterium species are used for economic production of glutamic acid by submerged fermentation.

Genetic Algorithm (GA) was adopted for the optimization of glutamic acid. The wild-type *Corynebacterium glutamicum*, for example, exhibits feedback inhibition when cellular glutamic acid oncentrations rise to 5% on a dry weight basis.

Production medium

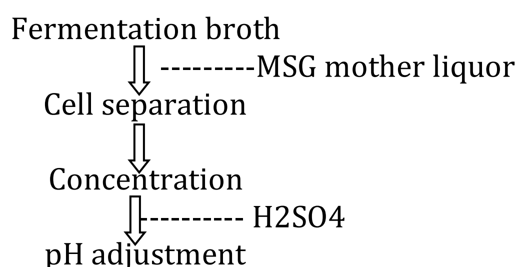
The medium composition as the following: (*g/l*)

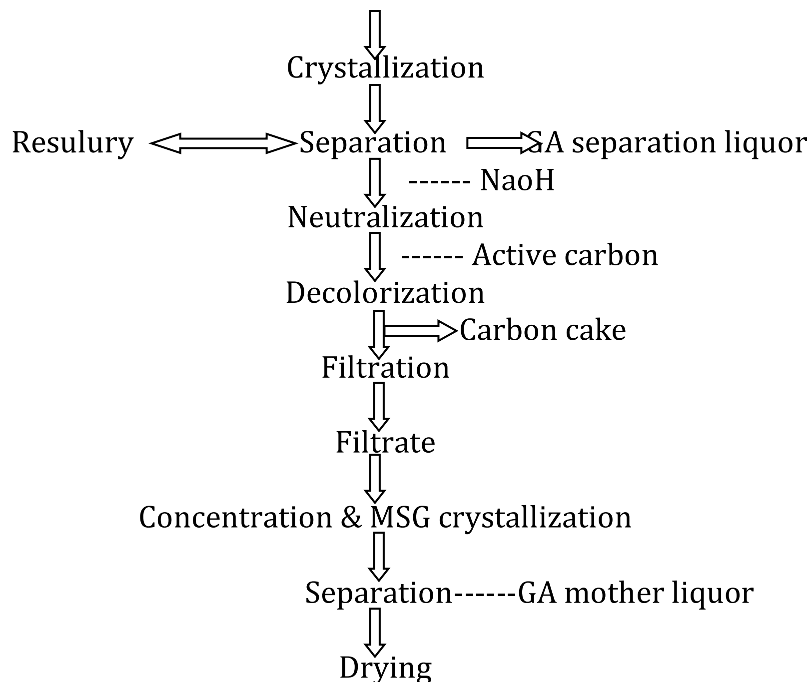
1. Glucose-50.0,
2. Urea-8.0,
3. Biotin-0.002,
4. K_2HPO_4-1.0,
5. $MgSO_4.7H_2O$-2.5,
6. $MnSO_4$. $7H_2O$-0.1,
7. $CaCO_3$-1.6.

The medium pH was adjusted to 7.0 with 1N sodium hydroxide or 1N hydrochloric acid.

Fermentation

- Industrial-scale fermenters are normally stainless steel stirred tank reactors of up to 450m^3
- Submerged fermentation.
- Operated aerobically at 30–37°C, the specific temperature depending on the microorganisms used.
- Medium pH is maintained at 7–8 by the addition of alkali.
- Normally lasts for 35–40 h and achieves l-glutamic acid levels in the broth of 80 g/L.





Recovery

1. Product recovery involves separation of the cells from the culture medium.
2. The l-glutamic acid is then crystallized from the spent medium by lowering the pH to its isoelectric point of pH 3.2 using hydrochloric acid.
3. Crystals of l-glutamic acid are then filtered off and washed.
4. MSG is prepared by adding a solution of sodium hydroxide to the crystalline l-glutamic acid followed by recrystallization.

Recycle and disposal the industrial waste

The compositions of industrial wastes depend on the industry. Wastes from three key industries for illustration: the oil, the pulp and paper and the food industries.

SYSTEMS FOR THE TREATMENT OF WASTES

The basic microbiological phenomenon in the treatment of wastes in aqueous environments is as follows:

(i) The degradable organic compounds in the waste water (carbohydrates, proteins, fats, etc.) are broken down by aerobic micro-organisms mainly bacteria and to some extent, fungi. The result is an effluent with a drastically reduced organic matter content.

(ii) The materials difficult to digest form a sludge which must be removed from time to time and which is also treated separately.

The discussion will therefore be under two headings: aerobic breakdown of raw waste-water and anaerobic breakdown of sludge.

Aerobic Breakdown of Raw Waste Waters

The two methods which are usually employed include the activated sludge and the trickling filter.

1. Activated sludge treatments

The efficiency of any system is usually determined by a reduction in the BOD of the waste water before and after treatment.

Efficiency depends on the amount of aeration, and the contact time between the sludge and the raw waste water.

Thus in conventional activated sludge plants the contact time is about 10 hours, after which 90-95% of the BOD is removed.

When the contact time is less (in the high-rate treatment) BOD removal is 60-70% and the sludge produced is more. With longer contact time, say several days, BOD reduction is over 95% and sludge extremely low.

With systems where oxygen is introduced as in the closed tank system or where there is great oxygen solubility as in the deep shaft system, contact time could be as short as 1 hour but with up to 90% BOD reduction along with substantially reduced sludge.

Organisms involved in the activated sludge process

The organisms involved are bacteria and ciliates (protozoa). It was once thought that the formation of flocs which are essential for sludge formation was brought about by the slime-forming organism, *Zooglea ramigera.* It is now known that a wide range of bacteria are involved, including *Pseudomonas, Achromobacter, Flavobacterium.*

2. The trickling filter

In the trickling filter no sludge is returned to the incoming waste water. Rather the waste water is sprayed uniformly by a rotating distributor on a bed of rocks 6-10 ft deep. The rotation may be powered by an electric motor or a hydraulic impulse.

The water percolates over the rocks within the bed which are 1-4 in diameter and is collected in an under-drain. The liquid is then collected from the under drain and allowed in a sedimentation tank which is an integral component of the trickling filter.

The sludge from the sedimentation tank is removed from time to time. Two filters may be placed in series and the effluent may be recycled.

Microbiology of the trickling filter:

A coating of microorganisms on the stones as the waste water trickles down the filter and these organisms break-down the waste. Fungi, algae, protozoa and bacteria form on the rocks. Aerobic bacteria are responsible for the breakdown of the organic matter.

ANAEROBIC BREAKDOWN OF SLUDGE

The goals of sludge treatment are to stabilize the sludge and reduce odors, remove some of the water and reduce volume, decompose some of the organic matter and reduce volume, kill disease causing organisms and disinfect the sludge.

1. Untreated sludges are about 97% water. Settling the sludge and decanting off the separated liquid removes some of the water and reduces the sludge volume. Settling can result in sludge with about 96 to 92% water.
2. More water can be removed from sludge by using sand drying beds, vacuum filters, filter presses, and centrifuges resulting in sludges with between 80 to 50% water. This dried sludge is called a sludge cake.
3. Anaerobic digestion is used to decompose organic matter to reduce its volume.
4. Digestion also stabilizes the sludge to reduce odors.
5. Caustic chemicals can be added to sludge or it may be heat treated to kill disease-causing organisms.

Following treatment, liquid and cake sludges are usually spread on fields, returning organic matter and nutrients to the soil.

Anaerobic digestion method

Anaerobic digestion consists of allowing the sludge to decompose in digesters under controlled conditions for several weeks. Digesters themselves are closed tanks with provision for mild agitation, and the introduction of sludge and release of gases. About 50% of the organic matter is broken down to gas, mostly methane. Amino acids, sugars alcohols are also produced. The broken-down sludge may then be de-watered and disposed. Organisms responsible for sludge breakdown are sensitive to pH values 7-8.

Recycling

Resource recovery is the process of taking useful discarded items for a specific next use. These discarded items are then processed to extract or recover materials and resources or convert them to energy in the form of useable heat, electricity or fuel.

Recycling is the process of converting waste products into new products to prevent energy usage and consumption of fresh raw materials. Recycling is the third component of Reduce, Reuse and Recycle waste hierarchy.

The idea behind recycling is to reduce energy usage, reduce volume of landfills, reduce air and water pollution, reduce greenhouse gas emissions and preserve natural resources for future use.