COURSE TITLE: FOOD AND INDUSTRIAL MICROBIOLOGY
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Bioprocessing, Advantages of bioprocessing, Historical Developments of Bioprocessing, Criteria in the choice of organism, Media for industrial process, Upstream and downstream processing

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Bioprocessing is used in the production of pharmaceuticals, foods, flavours, fuels and chemicals with the help of a biocatalyst such as an enzyme, microorganisms, plant cell or animal cell in a bioreactor. It also involves genetic engineering for the manipulation of plants, animals and microorganisms such as yeasts, bacteria and fungi.

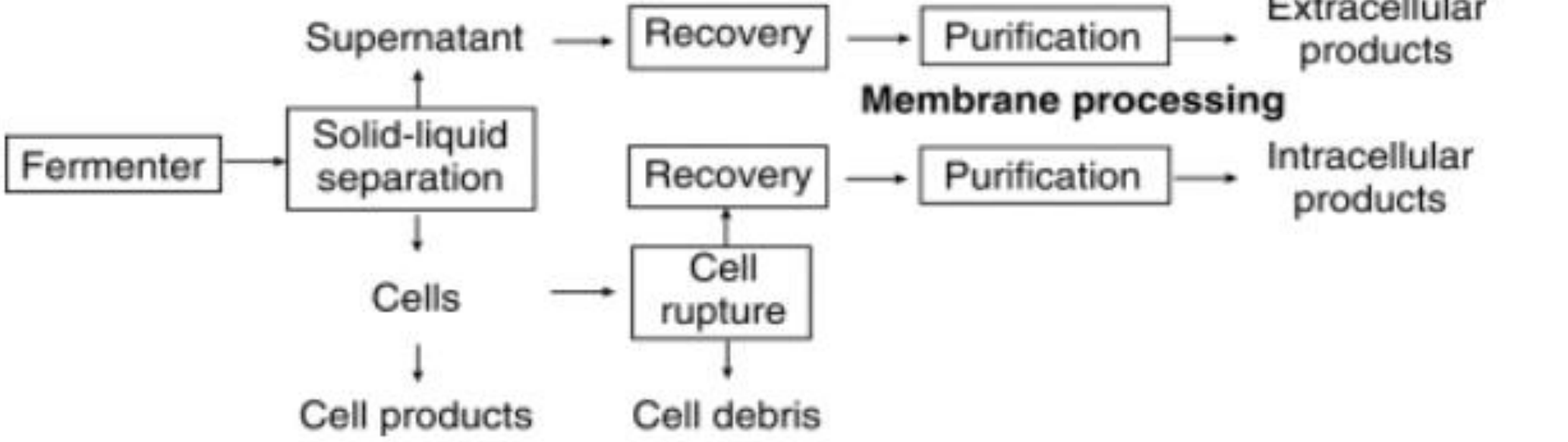
Bioprocessing is the use of biological materials (organisms, cells, organelles, enzymes) to carry out a process for commercial, medical or scientific reasons.
Bioprocess operations should ideally manufacture new products and destroy harmful wastes. Use of microorganisms to transfer biological material for production of fermented foods has been an essential part of many foods, chemical and pharmaceutical industries.

Bioprocesses have been developed for a number of commercial products, from relatively cheap materials such as industrial alcohol / organic solvents to expensive specific chemicals such as antibiotics, therapeutic proteins and vaccines.

Advantages of bioprocessing:

- ✓ work in mild conditions, energy saving
- ✓ Specific in their action
- ✓ Extremely efficient
- ✓ Biodegradable
- ✓ Safer

Modern biotechnology tools such as recombinant DNA, gene probes, cell fusion and tissue culture offer ways to produce new products or improve bioprocessing methods. Modern Biotechnology has allowed us to produce sophisticated medicines, cultured human tissues and organs, biochips for new age computers, environment friendly compatible pesticides and powerful pollution degrading microbes.



Historical Developments of Bioprocessing

10,000 - 7,000 BC --- Wine making develops in Eastern Mediterranean.
7,000 - 5,000 BC --- Beer develops in Egypt and Babylon
4,000 BC --- Yeast used for leavening bread.
500 BC–0 --- Vinegar referenced in old testament.
1600 --- Fermentation used (latin for “yeast” = fermentum), meaning a chemical change accompanied by effervescence.
1680 --- Van Leeuwenhoek observed yeast cells in fermented beer.
1781 --- Baker’s Yeast produced by Dutch Process.
1856 --- Pasteur demonstrated that living yeast cells ferment sugar into ethanol and carbon dioxide.
1877 --- Pasteur noted relationship between microorganisms and infectious disease.
1881 --- Koch developed techniques for the handling and maintenance of pure cultures of organisms.
1923 --- Commercial production of citric acid by surface cultures.
1929 --- Fleming demonstrated that a mold contaminant in a petri dish caused bacterial death.
1940 --- Florey and Chain isolated penicillin, elucidated its structure and demonstrated its bactericidal properties.

The development of genetic engineering and monoclonal antibody technology, which started in the 1970s, has led to the introduction of a large number of new products with application in many different areas. The most highly visible applications have been in the area of human health care, with product such as human insulin, interferon's, tissue plasminogen activator, erythropoietin, colony stimulating factors, and monoclonal antibody based products. New products for agriculture, food industry, fine chemicals and the environmental protection are also under intense development.

A **bioprocess** is a specific process that uses complete living cells or their components (e.g., bacteria, enzymes, chloroplasts) to obtain desired products.

Types of end products of fermentation include:

- Microbial cells (e.g. bacteria, yeast, fungal spores)
- Microbial enzymes (e.g. milk clotting enzymes or rennets, recombinant fungal and bacterial rennets for cheese manufacture)
- Microbial metabolites (e.g. alcohols - ethanol, butanol, 2, 3butanediol, isopropanol; chemicals– lactate, propionate, proteins, vitamins, antibiotics and fuels– methane)
- Recombinant products (e.g. hormones)

Examples of industrial products resulting from primary metabolism

Anabolic Products	*Catabolic Products*
1. Enzymes	1. Ethanol and ethanol-containing products, e.g. wines
2. Amino acids	2. Butanol
3. Vitamins	3. Acetone
4. Polysaccharides	4. Lactic acid
5. Yeast cells	5. Acetic acid (vinegar)
6. Single cell protein	
7. Nucleic acids	
8. Citric acid	

Source: Okafor, 2007

Examples of industrial products of microbial secondary metabolism

Product	*Organism*	*Use/Importance*
Antibiotics		
Penicillin	*Penicillium chrysogenum*	Clinical use
Streptomycin	*Streptomyces griseus*	Clinical use
Anti-tumor Agents		
Actinomyin	*Streptomyces antibioticus*	Clinical use
Bleomycin	*Streptomyces verticulus*	Clinical use
Toxins		
Aflatoxin	*Aspergiulus flavous*	Food toxin
Amanitine	*Amanita* sp	Food toxin
Alkaloids		
Ergot alkaloids	*Claviceps purpurea*	Pharmaceutical
Miscellaneous		
Gibberellic acid	*Gibberalla fujikuroi*	Plant growth hormone
Kojic acid	*Aspergillus flavus*	Food flavor
Muscarine	*Clitocybe rivalosa*	Pharmaceutical
Patulin	*Penicillium urticae*	Anti-microbial agent

Source: Okafor, 2007

Important criteria in the choice of organism

- Nutritional characteristics of the organism
- Optimum temperature of the organism
- The reaction of the organism with the equipment to be employed and the suitability of the organism to the type of process to be used
- Stability of the organism and its amenability to genetic manipulation.
- The productivity of the organism, measured in its ability to convert substrate into product and to give a high yield of product per unit time
- The ease of product recovery from the culture
- Irrespective of the origins of an industrial microorganism

Strain improvement

1. Regulating the activity of the enzymes secreted by the organisms
2. Increasing the permeability of the organism so that the microbial products can find their way more easily outside the cell.
3. Selecting suitable producing strains from a natural population
4. Manipulation of the existing genetic apparatus in a producing organism
5. Introducing new genetic properties into the organism by recombinant DNA
6. Technology or genetic engineering

Manipulation of the genome of industrial organisms in strain improvement can be done by mainly two ways

1. *Methods not involving foreign DNA*
   Conventional mutation

2. *Methods involving DNA foreign to the organism (i.e. recombination)*
   Transduction
   Conjugation
   Transformation
   Heterokaryosis
   Protoplast fusion
   Genetic engineering
   Metabolic engineering
   Site-directed mutation

Media for Industrial Inoculums Development

The use of a good, adequate and industrially usable medium is as important as the deployment of a suitable microorganism in industrial microbiology. Unless the medium is adequate, it will not be possible to harness the organism's full industrial potentials. Indeed not only may the production of the desired product be reduced but toxic materials may be produced.

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

1. Cost of the material

The cheaper the raw materials the more competitive the selling price of the final product will be. Due to these economic considerations the raw materials used in many industrial media are usually waste products from other processes. Corn steep liquor and molasses are, for example, waste products from the starch and sugar industries, respectively.

2. Ready availability of the raw material

The raw material must be readily available in order not to halt production. If it is seasonal or imported, then it must be possible to store it for a reasonable period.

3. Transportation costs

Proximity of the user-industry to the site of production of the raw materials is a factor of great importance, because the cost of the finished material and its competitiveness can all be affected by the transportation costs.

4. Ease of disposal of wastes resulting from the raw materials

The disposal of industrial waste is rigidly controlled in many countries. When choosing a raw material therefore the cost, if any, of treating its waste must be considered.

5. Uniformity in the quality of the raw material and ease of standardization

The quality of the raw material in terms of its composition must be reasonably constant in order to ensure uniformity of quality in the final product and the satisfaction of the customer and his/her expectations.

6. Adequate chemical composition of medium

The medium must have adequate amounts of carbon, nitrogen, minerals and vitamins in the appropriate quantities and proportions necessary for the optimum production of the commodity in question.

7. Presence of relevant precursors

The raw material must contain the precursors necessary for the synthesis of the finished product. Precursors often stimulate production of secondary metabolites either by increasing the amount of a limiting metabolite, by inducing a biosynthetic enzyme or both.

8. Satisfaction of growth and production requirements of the microorganisms

Many industrial organisms have two phases of growth in batch cultivation: the phase of growth, or the trophophase , and the phase of production, or the idiophase. Often these two phases require different nutrients or different proportions of the same nutrients.

Components of media for industrial inoculums development

The media should support the metabolic process of the microorganisms and allow biosynthesis of the desired products.

Carbon and Energy source+Nitrogen source+Nutrients Product+ Carbon Dioxide+Water+Heat+Biomass

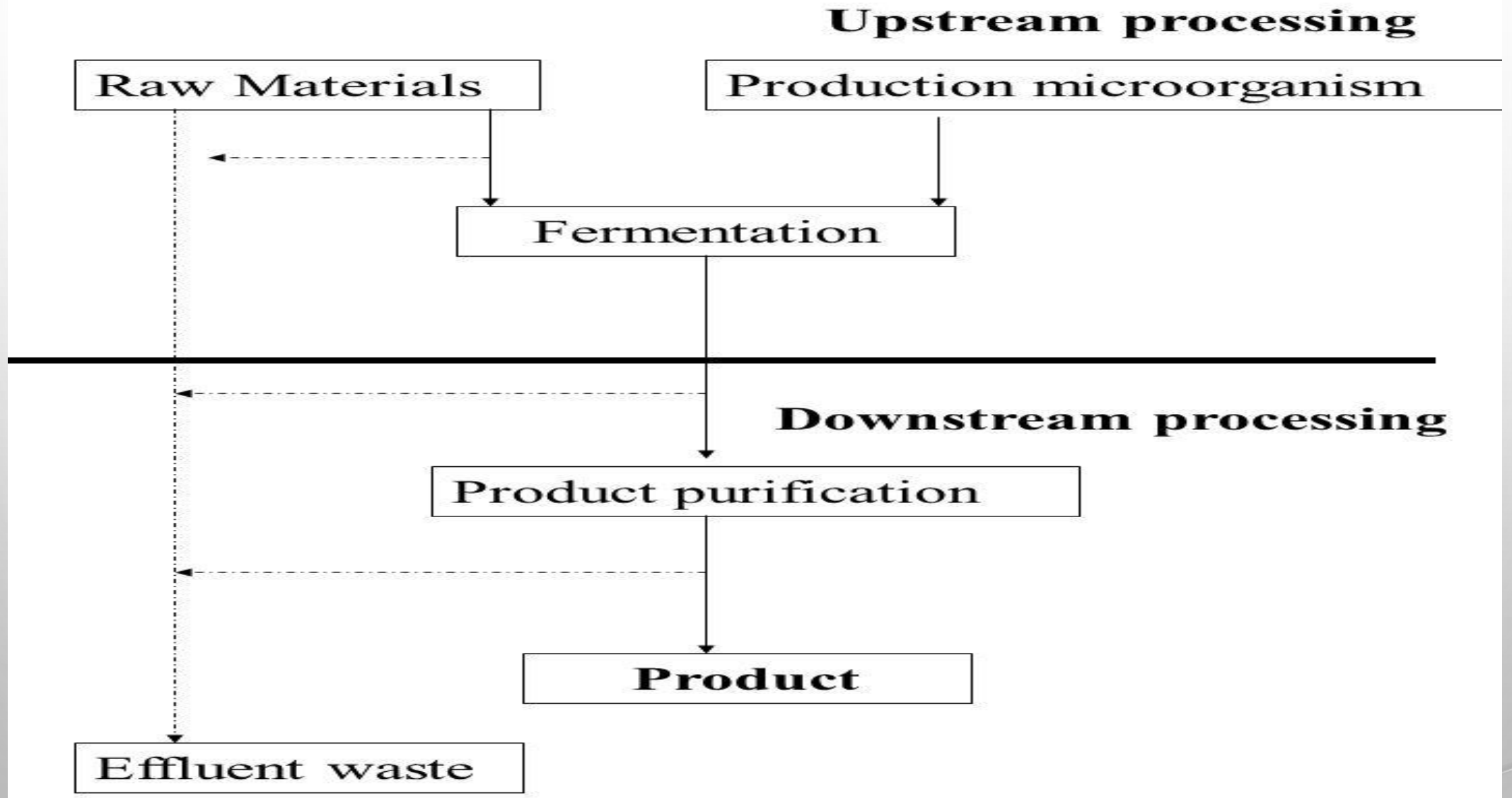
1. Carbon sources
    a. Molasses
    b. Malt Extract
    c. Starch and Dextrins
    d. Sulphite Waste Liquor
    e. Cellulose
    f. Whey
    g. Alkanes and Alcohols
2. Fats and Oils
3. Nitrogen sources
    a. Corn *Steep Liquor*
    b. *Yeast Extracts*
    c. Peptones
4. Water
5. Minerals
6. Vitamins and growth factors
7. Precursors
8. Inducers and elicitors
9. Inhibitors
10. Cell permeability modifiers
11. Oxygen
12. Antifoams

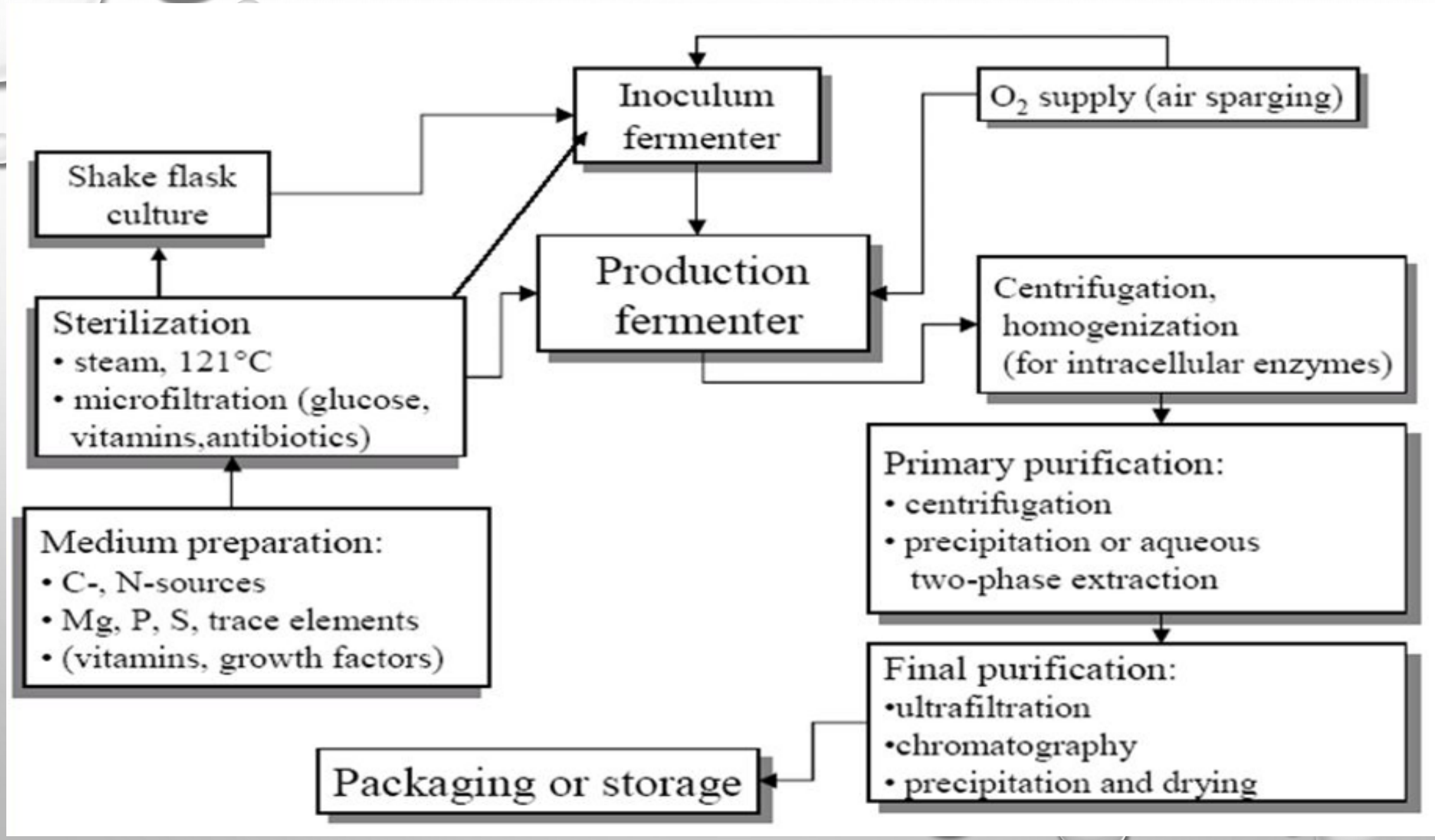
Industrial fermentation involves upstream and downstream processes

Upstream processes, include selection of a microbial strain characterized by the ability to synthesize a specific product having the desired commercial value. This selected strains are subjected to improvement protocols to maximize the ability of the strain to synthesize economical amounts of the product. Included in the upstream phase is the fermentation process itself which usually is carried out in large tanks known as fermenters or bioreactors. In addition to mechanical parts which provide proper conditions inside the tank such as aeration, cooling, agitation, etc., the tank is usually also equipped with complex sets of monitors and control devices in order to run the microbial growth and product synthesis under optimized conditions.

Downstream processing, the various stages that follow the fermentation process, involves suitable techniques and methods for recovery, purification, and characterization of the desired fermentation product. A vast array of methods for downstream processing, such as centrifugation, filtration, and chromatography, may be applied. These methods vary according to the chemical and physical nature, as well as the desired grade of the final product.

Outline of a fermentation process




Inoculum fermenter
O_2 supply (air sparging)
Shake flask culture
Production fermenter
Sterilization
• steam, 121°C
• microfiltration (glucose, vitamins,antibiotics)
Centrifugation, homogenization (for intracellular enzymes)
Primary purification:
• centrifugation
• precipitation or aqueous two-phase extraction
Medium preparation:
• C-, N-sources
• Mg, P, S, trace elements
• (vitamins, growth factors)
Final purification:
•ultrafiltration
•chromatography
• precipitation and drying
Packaging or storage

Downstream processing

The various stages of processing that occur after the completion of the fermentation or bioconversion stage, including separation, purification, and packaging of the product.

Stages in Downstream Processing

- ✓ Removal of insolubles
- ✓ Product Isolation
- ✓ Product Purification
- ✓ Product Polishing

A few product recovery methods may be considered to combine two or more stages. For example, expanded bed adsorption accomplishes removal of insolubles and product isolation in a single step. Affinity chromatography often isolates and purifies in a single step.

THANK YOU