COVENANT UNIVERSITY
NIGERIA

TUTORIAL KIT
OMEGA SEMESTER

PROGRAMME: MICROBIOLOGY

COURSE: MCB 422

DISCLAIMER

The contents of this document are intended for practice and leaning purposes at the undergraduate level. The materials are from different sources including the internet and the contributors do not in any way claim authorship or ownership of them. The materials are also not to be used for any commercial purpose.

MCB 422: Fermentation and Substrate-Enzyme Biotechnology

Contributors: Dr Oranusi S.U., Dr Ajayi A.A.

1. Discuss the next stage after the completion of Fermentation in industrial processes
2. What are the end products of the down streaming Process?
3. Discuss the first step in product Recovery
4. What is the difference between intracellular and extracellular metabolites?
5. Write short notes on the types of chromatography techniques used for separation and their principles
6. Discuss the principle of Lyophilization
7. What are single celled proteins?

SOLUTION

1. When fermentation is complete, it is necessary to recover the end products.The process of recovering the end products is known as the downstreaming process. Downstream processing also known as Product recovery is the extraction and purification of a biotechnological product from fermentation. It is a complex procedure. It is as important as the fermentation process itself. It requires expertise and technical skills of chemists, process engineers and bioscientists
2. The end products of the down streaming process are the following:

   Antibiotics
   Amino Acids
   Vitamins
   Organic Acids
   Industrial enzymes
   Vaccines

3. The downstream processing of metabolites is a multistage operation divided into the following:

- Solid-liquid separation
- Release of intracellular products
- Concentration
- Purification
- Formulation

Solid-liquid Separation is the first step in product recovery. It is the separation of whole cells (Cell biomass) and other insoluble ingredients from the culture broth.The term harvesting of microbial cells can also be used for the separation of cells from the culture medium

4. Intracellular metabolites: These are products located within the cells e.g. vitamins and enzymes

Extracellular metabolites: These are present outside the cells (Culture fluids) e.g. most antibiotics (Penicillin ,Streptomycin), amino acids, alcohol, citric acid, some enzymes (amylases, proteases)

5. The biological products of fermentation (Proteins, pharmaceuticals, diagnostic compounds and research materials) are effectively purified by chromatography.Chromatography is an analytical technique dealing with the separation of closely related compounds from a mixture

   The following are the types of chromatography techniques used for separation and their principles

Gel Filtration or size exclusion	Size and Shape
Ion-exchange	Net Charge
Chromatofocussing	Net Charge
Affinity	Biological affinity and molecular recognition
Hydrophobic interaction	Polarity (hydrophobicity of molecules)
Immobilized metal-ion affinity	Metal ion binding

6. Lyophilization is the most preferred method of drying and formulation of a wide range of products – pharmaceuticals, foodstuffs, diagnostics, bacteria, viruses. It is also known as freeze drying. This is mainly because freeze drying usually does not cause loss of biological activity of the desired product. Lyophilization is based on the principle of sublimation of a liquid from a frozen state. Lyophilization is a process more commonly known as freeze-drying. The word is derived from Greek, and means "made solvent-loving". This process is a way of drying something that minimizes damage to its internal structure. Because it is a relatively complex and expensive form of drying, it is limited to those materials which are sensitive to heat and have delicate structures and substantial value. One of the only substances which cannot be preserved effectively by freeze-drying is mammalian cells, which are too fragile.The preferred method of preservation in the biotechnology industry, lyophilization is regularly used to preserve vaccines, pharmaceuticals, and other proteins. Freeze-drying is also used to preserve special food products, eliminating the need for refrigeration. Freeze-dried food is eaten by mountain climbers and astronauts. Lyophilization is used by botanists to preserve flower samples indefinitely. Because the process of freeze-drying removes most of the water from the sample, freeze-dried materials become highly absorbent, and merely adding water can restore the sample to something close to its original state.

7. **Single-cell protein** (**SCP**) typically refers to sources of mixed protein extracted from pure or mixed cultures of algae, yeasts, fungi or bacteria (grown on agricultural wastes) used as a substitute for protein-rich foods, in human and animal feeds. Single-cell proteins develop when microbes ferment waste materials (including wood, straw, cannery, and food-processing wastes, residues from alcohol production, hydrocarbons, or human and animal excreta). The problem with extracting single-cell proteins from the wastes is the dilution and cost. They are found in very low concentrations, usually less than 5%. Engineers have developed ways to increase the concentrations including centrifugation, flotation, precipitation, coagulation, and filtration, or the use of semi-permeable membranes. The single-cell protein must be dehydrated to approximately 10% moisture content and/or acidified to aid in storage and prevent spoilage. The methods to increase the concentrations to adequate levels and the de-watering process require equipment that is expensive and not always suitable for small-scale operations. It is economically prudent to feed the product locally and soon after it is produced.

MCB 422: FERMENTATION AND SUBSTRATE-ENZYME BIOTECHNOLOGY

1a. Identify the environmental factors that must be controlled in a fermentor operation

b. Compare and contrast anaerobic and aerated fermentor operations

c. Discuss the control of each of the factors identified in 2a above

2a. Discuss brewing processes

b. In a tabular form, identify the functions of the raw materials for brewing

c. Compare and contrast continuous and batch fermentor operations

3a. Describe the major parts of a fermentor, stating the functions of each of the parts and the overall role of fermentors in enzyme technology

b. Discuss the important differences between intracellular and extracellular enzymes in relation to their relevance in enzyme technology

4a. Discuss the roles of five important Microbial enzymes used for industrial processes

b. Discuss the major steps involved in downstream processing after fermentation

5a. State the various microorganisms involved in the production of the following fermented Alcoholic Beverages: (1.) Pito. (2.) Keffir Beer. (3.) Burukutu.

b.(i). What are the problems associated with the spoilage of palm – wine?

(ii). What are the research effort made by the Federal Institute of Industrial Research, Oshodi

(FIIRO) in ensuring an enhanced shelf life of the drink.

6a. Define fermentation with reference to food, stating precisely its unique function.

(b). Explain the following fermentation processes:

(i). Natural fermentation

(ii). Back slopping

(iii). Controlled fermentation

(c). Describe the processes involved in the production of the following:

(i). Ogi

(ii). Yoghurt

7a. Draw and label a typical aerated stirred tank batch fermentor

b. Explain the function of each labeled parts in 1a above.

c. Write short notes on materials for constructing fermentor

d. List ten areas/products for which continuous fermentation has found use

8a. Discuss the undesirable consequences of foaming (foam formation) in industrial fermentation

b. Write short notes on any seven parameters that must be controlled in a typical fermentor operation

c. With the aid of diagram only represent the following continuous fermentation processes:

i. Single state continuous fermentation

ii. Re-cycled single state continuous fermentation

iii. Re-cycled multiple stage simple chain continuous fermentation

iv. Multiple substrate addition simple chain continuous fermentation

v. Re-cycled pipe flow continuous fermentation.