Isolation methods of fermentative products

The extraction and purification of fermentation products may be difficult and costly. The choice of recovery process is based on the following criteria:

1. The intracellular or extracellular location of the product
2. The concentration of the product in the fermentation broth
3. The physical and chemical properties of the desired product
4. The intended use of the product
5. The minimal acceptable standard of purity
6. The impurities in the fermented broth
7. The marketable price for the product

The main objective of the first stage for the recovery of an extracellular product is the removal of large solid particles and microbial cells usually by centrifugation or filtration. In the next stage, the broth is fractionated or extracted into major fractions using ultrafiltration, reverse osmosis, adsorption/ion-exchange/gel filtration or affinity chromatography, liquid–liquid extraction, two phase aqueous extraction, supercritical fluid extraction, or precipitation. The methods including:

1- Foam separation (floatation)

Foam separation depends on using methods, which exploit differences in surface activity of materials. The material may be whole cells or molecular such as a protein or colloid, which is selectively adsorbed or attached to the surface of gas bubbles rising through a liquid, to be concentrated or separated and finally removed by skimming. It may be possible to make some materials surface active by the application of surfactants such as long-chain fatty acids, amines, and quaternary ammonium compounds.

2- Precipitation

Precipitation may be conducted at various stages of the product recovery process. It is a particularly useful process as it allows enrichment and concentration in one step, thereby reducing the volume of material for further processing. It is possible to obtain some products (or to remove certain impurities) directly from the broth by precipitation, or to use the technique after a crude cell lysate has been obtained.

3- Filtration

Filtration is used at all scales of operation to separate suspended particles from a liquid or gas, using a porous medium which retains the particles but allows the liquid or gas to pass through. A number of factors may be influenced the choice of the most suitable type of filters including:

1. The properties of the filtrate, particularly its viscosity and density.
2. The nature of the solid particles, particularly their size and shape, the size distribution and packing characteristics.
3. The solids:liquid ratio.
4. The need for recovery of the solid or liquid fraction or both.
5. The scale of operation.
6. The need for batch or continuous operation.
7. The need for aseptic conditions.
8. The need for pressure or vacuum suction to ensure an adequate flow rate of the liquid.

4- Centrifugation

Microorganisms and other similar sized particles can be removed from a broth by using a centrifuge when filtration is not a satisfactory separation method. Although a centrifuge may be expensive when compared with a filter it may be essential when:

1. Filtration is slow and difficult.

2. The cells or other suspended matter must be obtained free of filter aids.

3. Continuous separation to a high standard of hygiene is required.

5- Cell Aggregation and Flocculation

Following an industrial fermentation, it is quite common to add flocculating agents to the broth to aid dewatering for the removal of microbial cells and suspended colloidal matter. It is well known that aggregates of microbial cells, although they have the same density as the individual cells, will sediment faster because of the increased diameter of the particles.

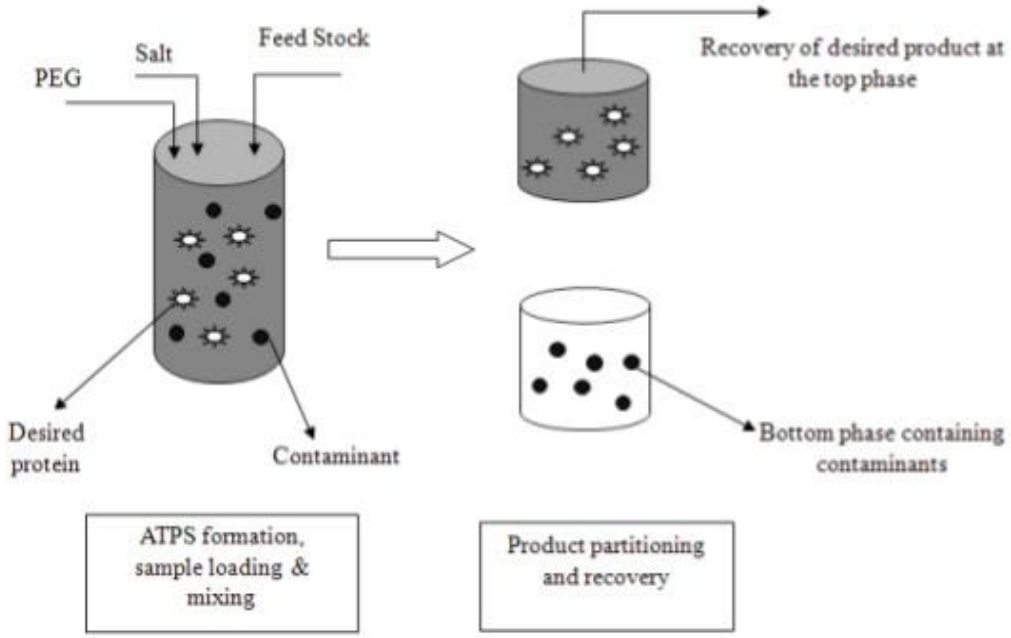
The majority of flocculating agents are polyelectrolytes such as tannic acid, titanium quaternary ammonium compounds, alkyl amines, and alkyl pyridinium salts which act by charge neutralization and hydrophobic interactions to link cells to each other.

6- Solvent extraction

Also known as Liquid–liquid extraction and partitioning, is a method to separate compounds based on their relative solubilities in two different immiscible liquids, usually water and an organic solvent. It is an extraction of a substance from one liquid into another liquid phase. It consists of transferring one (or more) solute(s) contained in a feed solution to another immiscible liquid (solvent) and concentrated in a smaller volume of solvent.

7- Aqueous biphasic systems (**ABS**) or **aqueous two-phase systems** (**ATPS**)

Aqueous two-phase extraction (ATPE), unique liquid-liquid extraction, involves a transfer of solute from one aqueous phase to another. ATPE includes polymer–polymer type and polymer–salt type systems for the recovery of proteins.



8- Reversed Micelle Extraction

Reversed micelles are aggregates of surfactant molecules in the organic solvents. These surfactant aggregates consist of a polar inner core and an inner layer made of the surfactant hydrophilic head.

A reversed micellar extraction cycle is basically composed of two steps: forward and back extraction. In the forward extraction process, biomolecules are transferred from the initial aqueous phase to the reversed micelles. In the back extraction process, biomolecules are transferred from the reversed micelles back to the aqueous stripping solution.

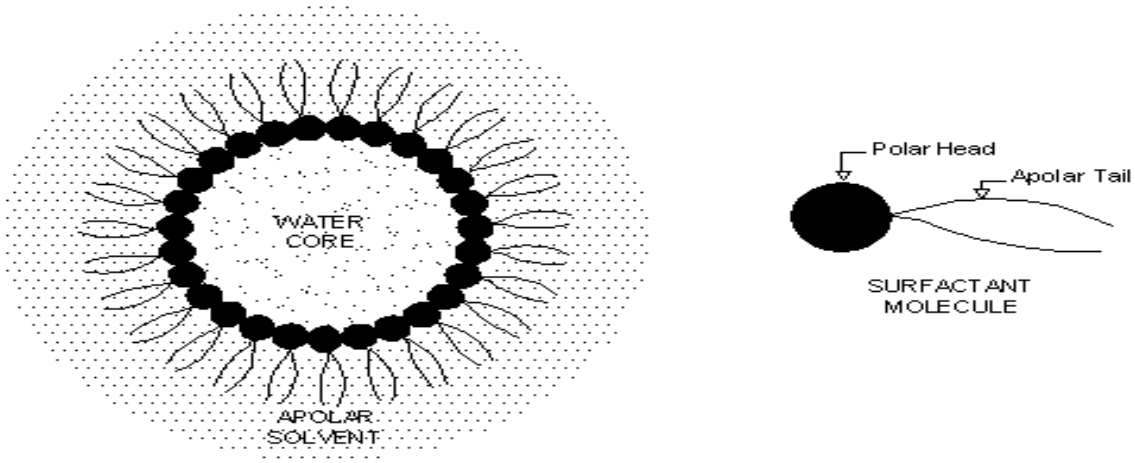


Figure 1: Reversed micelle structure.

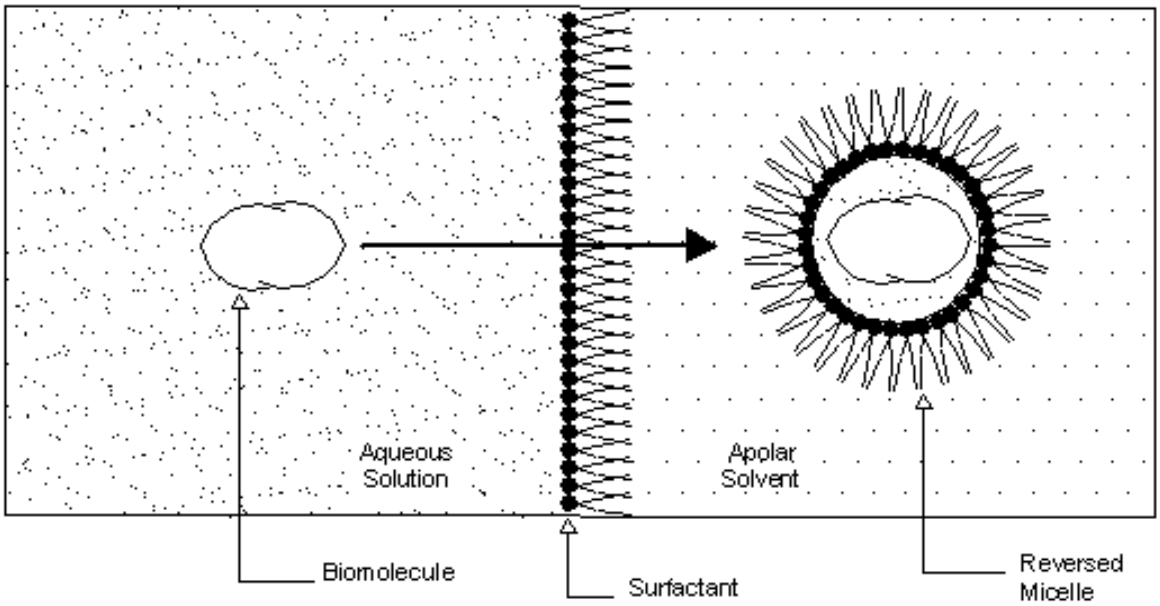


Figure 2: Schematic representation of the liquid-liquid extraction of a biomolecule between an aqueous phase and an apolar phase containing reversed micelles.

9- Chromatography

In many fermentation processes, chromatographic techniques are used to isolate and purify relatively low concentrations of metabolic products. Chromatographic methods separate solutes based on charge, polarity, size, and affinity.

9- Adsorption

Adsorption can be a useful technique for the separation of a product from a dilute aqueous phase and the use of polymer absorbers for the recovery of small molecules. A range of polymers (eg, ion-exchangers) are available on a large scale. After extraction of the product onto the absorber, the product can then be recovered by solvent elution/extraction and the absorber can then be recycled.

11- Distillation (evaporation)

It is used for the separation of volatile products from less volatile materials such as ethanol (both alcoholic beverages and biofuel), flavors, and fragrances.

12- Ultrafiltration

Ultrafiltration is a process in which solutes of high molecular weight are retained when the solvent and low molecular weight solutes are forced under hydraulic pressure (between 2 and 10 atmospheres) through a membrane of a very fine pore size, typically between 0.001 and 0.1 µm. It is therefore used for product concentration and purification. A range of membranes made from a variety of polymeric materials, with different molecular weight cut-offs (500–500,000), for separation of macromolecules such as proteins, enzymes, hormones, and viruses.