Module-25: Removal of microbial cells and other solid matters

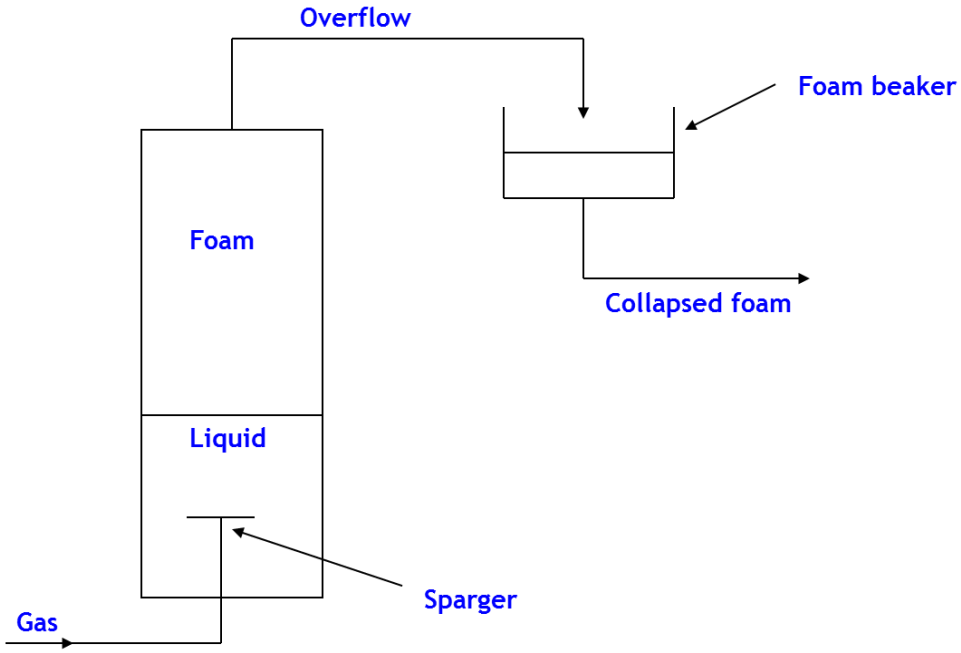
- There are various stages of recovery & purification of an extracellular product.
- The first stage for the downstream processing is the removal of large solid particles and microbial cells.
- It can be done by a number of processes like by centrifugation, filtration, sedimentation, precipitation, flocculation, electro-precipitation etc.
- Centrifugation is widely adopted technique for this purpose but requires large energy input per unit mass of the cells separated.
- So many efforts have been made to develop energy-saving separation methods.
- During filtration, the use of filter aids is necessary to improve filtration rates because many microbial cells are very minute.
- Sedimentation employs surface-active agents to obtain separation.
- To improve sedimentation rates in centrifugation heat and flocculation treatments are employed.
- However, the surfactants reduce the metabolic activities of the cells, so separated cells cannot be reused.
- Therefore, such methods can be used in operations where the reuse of the cells is not required such as sewage treatment.
- The methods of microbial cells separation have been accomplished for many years.
- Bowden *et al.* (1987) examined the use of electrophoresis and dielectrophoresis to exploit the charged properties of microbial cells as well as ultrasonic treatment to improve flocculation characteristics and magnetic separations.
- The problems associated with all these techniques include high cost and scale-up difficulties.
- Solution of this problem is the use of two-phase liquid extraction.

FOAM SEPARATION

- Foam separation is well known method of separation of components of a solution which is based on the differences in their surface activities.

- It is particularly suitable as foams are having large interfacial area per unit volume of the liquid.
- It allows separation of whole cells or molecular such as a protein or colloidal.
- Materials first selectively adsorbed to the surface of gas bubbles rising through a liquid & then be concentrated and finally removed by skimming.

Schematic flow diagram for foam fractionation



- By the use of surface active agents such as long-chain fatty acids, amines and quaternary ammonium compounds, surface activity of some materials can be improved.
- Materials made surface active and concentrated are termed colligends whereas the surface active agents used are termed collectors.
- During foam separation, some parameters should be checked such as pH, air-flow rates, surfactants and colligend-collector ratios.
- Rubin *et al.* (1966) separated 90% of the *E. coli* cells in 1 minute and 99% 10 minutes by foam separation using lauric acid, stearyl amine or *t-octyl* amine as surfactants.

- This method also proved effective with other organisms like *Chlorella* sp. and *Chlamydomonas* sp.
- Grieves and Wang (1966) have used ethyl-hexadecyl-dimethyl ammonium bromide for *E. coli* enrichment.

PRECIPITATION

- Precipitation is the chemical process in which solid gets formed in a solution or inside another solid.
- The solid formed in a solution is called the Precipitate & the liquid remaining above the solid is called the supernatant.
- Precipitation may be carried out at various stages of the product recovery process.
- This is the simplest method for isolation of fermentation products.
- To allow enrichment and concentration of desired product in one step precipitation is used.
- Therefore it reduces the volume of material for further processing.
- Following agents are used in precipitation of the compound of interest:
    1. **Acids and bases:** They are used to change pH of a solution until isoelectric point of the compound is achieved. At that pH molecules precipitate due to decrease in the solubility.
    2. **Salts such as ammonium and sodium sulphates:** They are used for the precipitation of proteins. The salt removes water from the surface of the protein & allows the exposure of the non-polar sites by that facilitate the aggregation & precipitation.
    3. **Organic solvents:** For example proteins can be precipitated out of a broth by the addition of Chilled ethanol and acetone. Methanol can be used in the precipitation of dextran. Some Nonionic polymers such as polyethylene glycol can also be used to precipitate proteins.
    4. **Polyelectrolytes:** They can be used in the precipitation of a range of compounds.
    5. **Protein binding dyes:** Proteins can precipitate by some protein binding dyes like triazine dyes which precipitate various classes of protein.
    6. **Affinity precipitation:** It is new method which allows the selective precipitation of Compounds.

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