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Downstream Processing-An Overview

Introduction

Downstream processing refers to the isolation and purification of bioproducts for different end uses, in marketable quantity. The various stages of processing that occurs after the completion of fermentation or bioconversion stage, including separation, purification and packaging of product. The technique for bioseparations should be selected carefully as the cost of process steps generally goes more than 50%, concentration of the product may be very low compared to the impurities present and product may be lost at each unit process. Therefore, downstream process is selected carefully involving minimum steps (as possible) to recover fragile product of interest in purest form with highest yield in a cost-effective manner. Hence, downstream processing plays a major role in biotechnological process.

Need for Downstream processing

The products are manufactured using a variety of equipments. Besides fermenters or bioreactors other special reactors such as air-lift, membrane and immobilized cell reactors are also used. All the reactors operate under sterile conditions. A variety of microorganisms including genetically engineered species are used for the production of desired products.The products formed may be secreted into broth or may be retained within cell introducing complexity in recovery of product.

The products formed are usually in low concentration necessitating the handling of large volumes and in some cases, the broths are viscous creating additional problems of fouling of equipment.

In **classical biotechnology**products are produced naturally by living cells as in case of ethanol by yeast.The unit operations include filtration, centrifugation, sedimentation, adsorption and liquid-liquid extraction.

In **modern biotechnology** the microorganisms are induced under specific conditions to produce products. The recovery involves high resolution techniques such as affinity methods of adsorption, extraction, precipitation or chromatography. The choice of method depends to a large extent on nature of product, it's quantity and extent of purity required.

The choice for method of downstream processing mainly depends to a larger extent, on the:

i)The nature of the product

ii) Quantity of the product being manufactured

iii) Extent of purity required.

Steps in Downstream Processing

Downstream processing can be broadly classified into four steps.

1. Removal of Insoluble-In this step, separation of solid-liquid is achieved. The techniques used for this mainly involves-filtration, centrifugation, sedimentation and flocculation.

- **Filtration-** This process separates solid from a liquid by forcing the liquid through a solid support or filter medium. The filter medium allows the filtrate to pass through and retains the solids. Several filters like plate and frame filter, rotary vacuum filter, depth filter and membrane filters are in use.
- **Centrifugation-** Centrifugation is used to separate different densities material when gravitational force cannot be used for separation. In this the separation occurs with the help of centrifugal force. It is effectively used when solid particles are small and hard to

filter. Various centrifuges like tubular-bowl centrifuge, disc-stack centrifuge, multichamber bowl centrifuge is used.

- **Sedimentation-** It is a free settling process of heavier particles that depend on gravitational force. It is a very slow process.
- **Flocculation-** It is a process where a solute comes out of solution in the form of flocs. This is achieved by adding simple electrolytes or flocculants (like aluminum sulphate), which coagulate the colloidal particles to dense large sized particles. Flocculants have a disadvantage of fouling membrane.

2. Isolation/Recovery of Product- This step mainly involves volume reduction in which the highly diluted feed is reduced in volume. It is achieved by cell disruption (if the product is intracellular), extraction, adsorption, precipitation.

- **Cell disruption-**If the product is produced inside the cell, then cell disruption is carried out in order to release the intracellular product. Knowledge of the structure of cell wall of various organisms is required in selecting the cell disruption techniques. Physical, chemical or mechanical methods are employed for cell disruption.
- **Extraction-** This technique achieves isolation as well as purification of the product to some extent. This technique uses the relative solubilities of solute in immiscible solvents. Solvent dissolves in the solvent in which it has a higher solubility.
- **Adsorption-** It is a reversible surface phenomenon. It requires adsorbents to which solutes of interest bind reversibly. Common adsorbents used are carbon and synthetic resins.
- **Precipitation-** It is a technique of purifying biological solutes in a non-crystalline state. As the precipitated product is impure, this method is more appropriate as a concentration step than the purification. Neutral salts, organic solvents, high-molecular weight polymers are used for precipitation.

3. Product purification- In this step the product is purified using the high-resolution techniques of chromatography.

- **Chromatography-**It is a physical methodfor separation of compounds. In this the mixture is passed and allowed to interact with two immiscible phases- a stationary phase

and a mobile phase. The mobile phase moves the sample through the stationary phase and distributes the analyte to be measured from other molecules in the mixture and allows it to be isolated. This technique purifies the low molecular weight compound from the mixture of similar molecules. Various chromatographic procedures such as ion-exchange, gel filtration, affinity etc. are used for isolation and purification of products.

4. Product formulation-This stage aims at finishing or formulating the product according to the market requirement. The techniques used are of crystallization and drying.

- **Crystallization**-The formation of solid particles of defined shape and size from a homogeneous liquid phase is crystallization. The crystalline product obtained is of exceptional purity, facilitates subsequent finishing steps, like drying and also improves the product appearance.
- **Drying-** This technique removes the solvent from the desired product and in most cases stabilizes the product making it further amenable for packaging, storing and formulation. Spray drying, freeze drying or lyophilization is generally carried out in downstream processing of products.

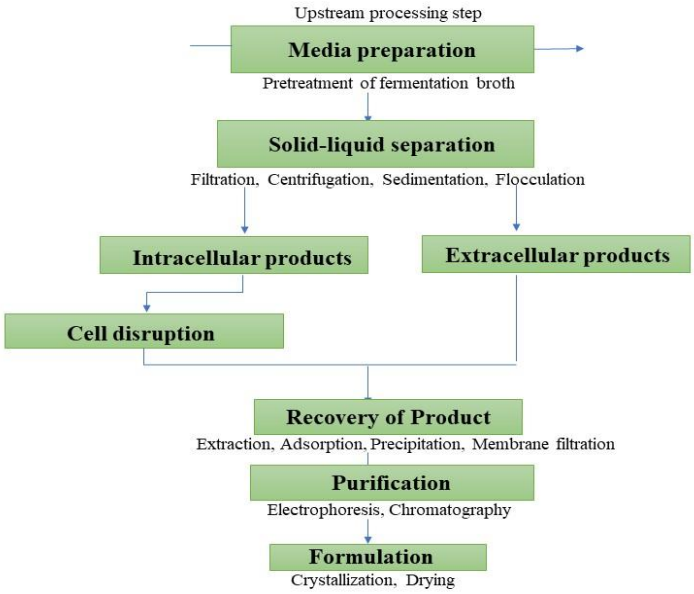

Figure: Steps in Downstream Processing

Challenges in Downstream Processing

- Low product formation.
- Heat-sensitive products.
- Wide range of impurities.
- Narrow operating physical and chemical properties (like pH).
- Low solubility and instability of biological products in organic solvents.

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