Downstream Processing (DSP) of Products

The following points highlight the six main steps involved in the downstream processing of products. The steps are: 1. Separation of Particles 2. Disintegration of Cells 3. Extraction 4. Concentration 5. Purification 6. Drying.

Step # 1. Separation of Particles:

The first step in DSP is the separation of solids, usually cells, from the liquid medium.

This is generally achieved as follows:

1. Filtration:

It is used for the separation of filamentous fungi and filamentous bacteria, e.g., streptomycetes, and often for yeast flocks. Filtration uses pressure created by overpressure or vacuum, and its rate depends on filter area, fluid viscosity and the resistance generated by filter cake which increases with time.

The various techniques of filtration employed are: surface filtration, depth filtration, centrifugal filtration, cross-flow filtration, rotary drum vacuum filtration. Rotary drum vacuum filters are the most commonly used: the filter is in form of a rotating drum with a partial vacuum on the inside of the drum.

A portion of the drum rotates through the medium and the cells are sucked to form a coating on the drum; the cells are continuously scraped off to prevent blocking of the filter.

2. Centrifugation:

It may be used to separate bacteria and usually protein precipitates. But difficulties arise due to small differences in the densities of the particles and the medium. In addition, equipment cost, power consumption, temperature etc. are the other disadvantages.

3. Flocculation and Floatation:

Small bacterial cells, which are difficult to separate even by centrifugation, can be recovered as follows. Flocculation, i.e., sticking together of cells, can be induced by inorganic salts, mineral hydro-colloids and organic polyelectrolytes. Since sedimentation rate of a particle increases with size, flocculated cells can be recovered by centrifugation.

In cases, where flocculation is not effective, very fine gas bubbles can be created by sparging, release of overpressure or electrolysis. In any case the gas bubbles absorb to and surround the cells, raising them to the surface of medium in form of foam (floatation); long chain fatty acids or amines promote stable foam formation.

The cells collected in the foam are readily recovered. Flocculation and floatation are used for the most efficient recovery of microbial biomass in some single cell protein production systems.

Step # 2. Disintegration of Cells:

Disruption of microbial cells is usually difficult due to their small size, strong cell wall and high osmotic pressure inside cells. Generally, cell disruption is achieved by mechanical means, lysis or drying.

The method of cell disruption must not damage the product of interest; the suitability of the methods is usually assessed in terms of recovery of a cellular enzyme activity following cell disruption:

1. Mechanical Cell Disruption:

This approach uses shear, e.g., grinding in a ball mill, colloid mill etc., pressure and pressure release, e.g., homogenizer and ultrasound. A widely used method is as follows; the cell suspension is forced through a fine nozzle; the cells disintegrate due to hydrodyanic shear and cavitation.

2. Drying:

The cells may be dried, e.g., by adding the cells into a large excess of cold acetone and subsequently extracted using buffer or salt solutions. Drying induces changes in cell wall structure which facilitates extraction. This method is widely used.

3. Lysis:

Microbial cells may be lysed by chemical means, e.g., salts or surfactants, osmotic shock, freezing, or by lytic enzymes, e.g., lysozyme etc.

In general, recovery of enzyme activity is the best following cell disruption using enzymes or ultrasound, followed by thermal and osmotic methods, while mechanical methods are the least desirable. However, ultrasound method is confined to laboratory mainly due to difficulties in heat removal on a large scale.

The process of recovering a compound or a group of compounds from a mixture or from cells into a solvent phase is called extraction. Extraction usually achieves both separation as well as concentration of the product. It is especially useful for the recovery of lipophilic substances, and in antibiotic recovery; it is often an early step after cell separation.

1. Liquid-Liquid Extraction:

It employs two immiscible liquids into which the product is differentially soluble. Usually successively smaller volumes of the solvent are used for repeated extraction of a given sample; back-extraction also tends to increase the selectivity of extraction. The extraction may be performed in a single step, by multi-stage parallel-flow extraction, or by counter-current extraction (most complex but most effective).

2. Whole Broth (Medium + Cells) Extraction:

It should be used wherever possible since it reduces the number of steps as well as product loss. The effectiveness of extraction may, however, be reduced due to the presence of cells.

3. Aqueous Multiphase Extraction:

It is used for separation of enzymes from cells/cell debris. The enzymes are extracted in an aqueous polyethylene glycol-dextran mixture which form separate phases. Recovery of enzymes from these phases is rather easy and free from some of the difficulties encountered in centrifugation.

Step # 4. Concentration:

Some concentration of the product may occur during the extraction step.

Further concentration may be achieved by:

(i) Evaporation,

(ii) Membrane filtration,

(iii) Ion exchange methods and

(iv) Adsorption methods.

1. Evaporation: It is generally used in cases of solvent extraction using various devices, e.g., continuous flow evaporators, falling film evaporators, thin film evaporators, centrifugal thin film evaporators and spray- dryers. Efficient arrangements must be made for recovery of the evaporated solvent to reduce costs. For low grade products, often evaporation of the whole broth is undertaken using a spray-drier.

2. Membrane Filtration: It generally achieves both concentration and separation of the products usually based on the size of molecules. The different processes of membrane filtration are: microfiltration, ultrafiltration, reverse osmosis and electro-dialysis.

Micro- and ultrafiltration work as sieves and separate molecules of different sizes, but reverse osmosis can separate molecules of similar size. Microfiltration can be used for cell separation as well.

3. Ion Exchange Resins: These are polymers having firmly attached ionizable groups (anions or cations) which ionize under a suitable environment. These may be solid, e.g., dextran, cellulose, polyamine, acrylate etc., or liquid, e.g., a solvent carrying a functional group like phosphoric acid mono- or diester etc.

Solid ion exchangers may be used in two ways:

(i) They may be packed in columns or

(ii) They may be added to the extract and removed by decantation.

Liquid ion exchangers dissolve only in non-aqueous solvent carrier and the separation is similar to liquid-liquid extraction.

Some antibiotics are recovered directly from the whole broth using ion exchange resins. The product is recovered from the ion exchangers by ion displacement; this also regenerates the ion exchanger.

4. Absorption Resins: These are porous polymers without ionization. Most compounds are adsorbed to the resins in non-nonized state. The porosity of the resin determines the surface available for adsorption. These resins may be apolar (e.g., styrene-divinyl bcneze), polar (e.g., sulfoxide, amide etc.), or semipolar (e.g., acrylic ester). The products are recovered from such resins by solvent (organic) extraction, changed pH etc.

Step # 5. Purification:

The final step in the recovery of a product is purification which aims at obtaining the product in highly purified state. The earlier steps will have achieved variable degrees of purification which may determine the degree of resolution necessary during the purification step.

The degree of resolution will mainly depend on the similarities to the metabolite of other molecules present in the concentrate, and the degree of purity required in the final product.

Purification is achieved by:

(i) Crystallization and

(ii) Chromatographic procedures.

1. Crystallization: It is mainly used for purification of low molecular weight compounds like antibiotics, e.g., penicillin G is usually extracted from fermentation broth in butyl acetate and cystallized by the addition of potassium acetate in ethanolic solution. Crystallization is the final stage in purification of products like citric- acid, sodium glutamate etc.

2. Chromatographic Methods: These are used for purification of low molecular weight compounds from mixtures of similar molecules, e.g., homologous antibiotics, and of macromolecules, especially enzymes, which are similar in properties. The materials used for chromatography are generally coated on particulate carriers which are packed in columns through which the liquid containing the product is pumped either upward or downward.

The separated product is recovered in some sort of fraction collector. On a large, scale organic solvents are used for collection; therefore, the whole system has to be installed in a flame-proof and explosion- proof room.

The different chromatographic procedures are:

(i) Adsorption,

(ii) Ion exchange,

(iii) Gel filtration,

(iv) Hydrophobic,

(v) Affinity,

(vi) Covalent and

(vii) Partition chromatography.

Adsorption chromatography separates molecules due to their differential affinities for the surface of a solid matrix, e.g., silica gel, alumina, hydroxyapatite (all inorganic) or an organic polymer. In case of ion exchange chromatography, resins or polysaccharides, e.g., cellulose, sepharose, having attached ionized functional groups are used for a high resolution separation of macromolecules, e.g., proteins.

Gel filtration uses molecular sieves, composed of neutral cross-linked carriers (e.g., polymers like agarose, dextran's), of different pore sizes. Molecules smaller than the pore size enter the carrier and

are retained; they are later eluted (in order of molecule size) and collected. Gel filtration is used in aqueous systems. Hydrophobic carriers are used for purification of hydrophobic molecules, e.g., many enzymes and other proteins.

Affinity chromatography uses molecules, called effectors (Table 39.7), to which the product has high and specific affinity, e.g., using an antibody (effector) for the purification of the antigen to which it is specific. The effector is immobilized on a water insoluble carrier which is packed in a column through which the mixture is passed.

A list of some effectors used in affinity chromatography and the molecules purified by them

The effector binds only to the molecules for which it is specific and retains it in the column; it is later recovered by elution using a buffer solution of a specified pH (Fig. 39.4). For example, human leucocyte interferon is recovered in high yield and in high purity by affinity chromatography using monoclonal antibody immobilized on a sepharose column.

Affinity chromatography using an antibody for purification of the specific antigen from a mixture

Group specific affinity chromatography and covalent chromatography are based on chemical interactions between the carrier and the product molecules.

Step # 6. Drying:

Drying makes the products suitable for handling and storage. It should be accomplished with a minimum rise in temperature due to heat sensitivity of most products. Addition of sugars or other stabilizers improves the heat tolerance of some products like enzymes and pharmaceutical preparations.

The most common approaches to drying are as follows:

(i) Vacuum drying,

(ii) Spray drying and

(iii) Freeze drying.

In spray drying, the solution or slurry to be dried is atomized by a nozzle or a rotating disc. A current of hot (150-250°C) air is passed; the drying is so rapid that the temperature of particles remains very low.

Spray drying is used for enzymes, antibiotics, and food products. Vacuum drying uses both heat and vacuum for drying; it can be applied both in batch mode (e.g., chamber dryers) or in continuous mode (e.g., rotating drum vacuum dryers).

In freeze drying, the liquor to be dried is first frozen and the water is sublimed from the frozen mass. A very low pressure (partial vacuum) is maintained to promote sublimation of water. The energy needed for sublimation is provided by heated plates and radiation on to the surface.

The temperature of solid is regulated by regulating the pressure in the drying chamber. This is the most gentle method of drying, and is used for many pharmaceutical products, e.g., viruses, vaccines, plasma fractions, enzymes etc., and in food industries.