Bioseperation

Upstream process : refers to the production of bio - product in the raw form. In biochemical engineering , this part is divided into somesteps which include growing microbes for culturing process (inoculums development), media development, improvement of inculum (by genetic engineering process)., and optimization of growth kenetics so that product development: can be improved significantly.

Downstream process : refers to cell purification. A purification section , recovery of product and a polishing section. If it is in bio- separation process, the upstream refers to the first steps of separation which are removal of insolubles and product isolation. Removal of insolubles can be done by filtration (sometimes microfiltration is used), centrifugation , and cell disruption. Meanwhile product isolation can be done by extraction or adsorption.

Is a process of new bio-products separation techniques by increasing the seperation selectivity (extraction, purification and formulation).

Basis of separation in bioseparation processes:

Biological products are separated based on one or more of the following factors:

1. **Size:** e.g. filtration, membrane separation, centrifugation
2. **Density:** e.g. centrifugation, sedimentation, floatation
3. **Diffusivity:** e.g. membrane separation
4. **Shape:** e.g. centrifugation, filtration, sedimentation
5. **Polarity:** e.g. extraction, chromatography, adsorption
6. **Solubility:** e.g. extraction, precipitation, crystallization
7. **Electrostatic charge:** e.g. adsorption, membrane separation, electrophoresis
8. **Volatility:** e.g. distillation, membrane distillation, pervaporation

The steps of downstream processing are:

1. Cell harvesting

2. Lyses/breakage of cells

3. Concentration

4. Purification

5. Formulation

Cell harvesting: If the desired product is intra cellular the cell biomass can be disrupted so that the product should be released. The solid-liquid is separated by centrifugation or filtration and cell debris is discarded.

The solid phase (cell bio-mass) is separated from the liquid phase by any of the following methods: Centrifugation, Filtration, Settling, Flotation and Flocculation.

Lab 2

Centrifugation

Centrifugation: is a separation technique where different components of mixture are separated based on their density or particle size. The separation of different substances is based on centrifugal force that is produced by high speed rotation.

Centrifugation is one of the most important and widely applied research techniques in biochemistry, cellular and molecular biology, and in medicine. Current research applications rely on isolation of cells, subcellular organelles, and macromolecules, often in high yields.

Centrifuge used to separate bacteria, and can be used efficiently in the case of significant differences in density between the solid particles and liquid portion.

There are many applications of centrifugation include:

1) Sedimentation of cells, bacteria and viruses.

2) Separation of sub cellular organeles

3) Isolation of macromolecules such us RNA, DNA and proteins.

The purpose of using centrifuge is to increase the effect of gravity, when a suspension is rotated at a certain speed or revolutions per minute(RPM), centrifugal force causes the particles to move radially away from the axis of rotation.

Relative centrifugal force (RCF): The force on the particles (compared to gravity) For example, an RCF of (500 x g) indicates that the centrifugal force applied is 500 times greater than earth gravitational force.

Types of centrifuge depends on:

1- Maximum speed of sedimentation

2- Presence or absence of vacuum

3- Temperature control refrigeration

4- Volume of sample

5- Capacity of centrifugation tubes

Types of centrifuge:

1. Small Benchtop

– Slow speed (up to 4000 RPM)

– Common in clinical labs (blood/plasma/serum separation

– Can take approx (up to) 100 tubes, depending on diameter

- With or without refrigeration

2. **Microcentrifuges (microfuge, Eppendorf)**

– Generate forces up to 15000RPM

– Common in biochemistry/molecular biology/ biological labs

– Take tubes of small vols (up to 2 mL)

– With or without refrigeration

3. High Speed centrifuges

– Generate forces 15,000 – 20,000 RPM

– Large sample capacity depending on rotor

– Normally refrigerated

– Research applications

4. Ultracentrifuges

– Generate forces 65,000 RPM (100,000 x g)

– Limited lifetime

– Expensive

– Require special rotors

– Care in use balance critical

– Research applications

Sample Containers:

- Centrifuge tubes and bottles are available in different range of sizes, thickness and rigidity from different variety of materials including glass, cellulose, esters, polyallomer, polycarbonate, polyethylene, nylon and stainless steel.
- The type of container used will depend upon nature and volume of sample to be centrifuged along with centrifugal forces to be withstood.
- Glass centrifuge tubes are suitable only for centrifugation at low speeds as they disintegrate at higher centrifugal fields.
- The centrifuge tubes should be filled to accurate level and need to cap the tube or bottle depends upon the speed and type of the container used.

Classes of centrifuges and their applications

Maximum speed (RPM x10^3	Centrifuge classes		
	Low speed (10)	High speed (28)	Ultra/microultra (100/150)
Maximum RCF)(x10^3)	7	100	800/900
Pelleting applications			
Bacteria	yes	yes	Yes
Animal and plant cells	yes	yes	Yes
nuclei	yes	yes	Yes
precipitates	some	most	Yes
Membrane fractions	some	some	Yes
Ribosomes/polysoms	-	-	Yes
macromolecules	-	-	Yes
viruses	-	some	Yes