

### **An Introduction to Bioseparation**

**Bioseparation** is the name given to the practice of purifying biological products on a large-scale, using fundamental aspects of engineering and scientific principles. The end goal of bioseparation is to refine molecules, cells and parts of cells into purified fractions, therefore decreasing the overall cost of the product.

Biological products can be separated and purified depending upon the following characteristics: density, diffusivity, electrostatic charge, polarity, shape, size, solubility and volatility.

### **Differences between Bioseparation and Chemical Separation**

Although bioseparation is based on traditional chemical separation processes, they do differ in significant ways.

- 1- The materials being purified and separated in bioseparation are biological substances rather than the synthetic chemicals used in traditional techniques.
- 2-The substances such as proteins, carbohydrates and nucleic acids are not suitable for the rigorous of traditional techniques like packed-bed adsorption and evaporation.
- 3-The desired final product is only found in very minute quantities in the starting substance from which they are refined. Because of this, vast quantities of dilute product streams must undergo processing in order to obtain a small amount of pure product.

4- there are often unwanted impurities in the starting substance which have similar genetic makeup to the desired product, thus making separation very difficult.

5- The biological products are more sensitive to degradation than chemical ones, this rules out the use of many common organic solvents in bioseparation, since they have a tendency to act as a catalyst for degradation. Furthermore, many biological substances are unstable when heated and as such have to be handled in sub-ambient temperatures.

### **Bioseparation Techniques**

There are many different techniques by which bioseparation can be achieved, however, there are none which currently work effectively on their own. This is because bioseparation requires a combination of high resolution (also known as selectivity) with high throughput (also called productivity).

As a result, bioseparation must incorporate two or more techniques to achieve dual proficiency in the two categories.

<u>High Throughput &amp; Low Resolution</u>	<u>High Resolution &amp; Low Throughput</u>
Adsorption	Affinity Separation
Centrifugation	Chromatography
Filtration / Microfiltration / Ultrafiltration	Counter-current extraction
Precipitation	Electrophoresis
Solvent extraction	Ultracentrifugation
Supercritical fluid extraction	

One of the more commonly-used methods of achieving bioseparation is through the deployment of a RIPP scheme (Recovery, Isolation, Purification,

Polishing). This technique will first utilize one of the low resolution methods from the left column above to achieved recovery and isolation of the desired product. Then, one of the high resolution methods from the right column will purify the product and “polish” it. Polishing can refer to sterilization, removal of contaminants and any other final processing steps before it is packaged into a marketable form.

### **Physical forms separated in bioseparation**

**1-Particle-liquid separation:** the separation of cells from cell culture medium

**2-Particle-particle separation in liquid medium:** the separation of plasmid DNA from chromosomal DNA

**3-Particle-solute separation in liquid medium:** the separation of dissolved antibiotics from cells

**4-Solute-solvent separation:** the removal of dissolved impurities from a liquid product

**5-Solute-solute separation in liquid medium:** the separation of serum albumin from other serum proteins.

**6-Liquid-liquid separation:** the separation of solvents such as acetone and ethanol from an aqueous medium.

### **Bioprocessing categories:**

**1- Reactive bioprocessing:** the reactive bioprocessing involves steps such as biocatalyst screening, enrichment, isolation and propagation, cell

manipulation by recombinant DNA technology or hybridization technology, media optimization and formulation.

**2- Extractive bioprocessing:** the extractive bioprocessing almost entirely involves bioseparation.

The materials that separated in bioseparation involved:

Categories	Examples
Solvents	Ethanol, acetone, butanol
Organics acids	Citric acid, lactic acid, butyric acid
Vitamins	Ascorbic acid, vitamin B12
Amino acids	Lysine, phenylalanine, glycine
Antibiotics	Penicillins, rifampicin, streptomycin, framycetin, gentamycin
Sugars and carbohydrates	Glucose, fructose, starch, dextran, xanthan, gellan
Lipids	Glycerol, fatty acids, corticosteroids, prostaglandins
Nucleic acids	Plasmids, therapeutic DNA, retroviral vectors, anti-sense oligonucleotides, ribozymes
Semi-purified proteins	Industrial enzymes, egg proteins, milk proteins, whey protein, soy proteins
Purified proteins	Therapeutic enzymes, monoclonal antibodies, plasma proteins, cytokines, interleukins, hormones, growth factors, diagnostic enzymes, vaccines
Cells	Bakers yeast, brewers yeast, freeze dried lactobacillus
Crude cellular extracts	Yeast extract, soy extracts, animal tissue extract
Hydrolysates	Soy hydrolysates, whey hydrolysates, animal tissue hydrolysates

Industrial chemicals	Solvents, organic acids, industrial enzymes
Agrochemicals	Biofertilizers, biopesticides
Biopharmaceuticals	Antibiotics, hormones, monoclonal antibodies, plasma proteins, vaccines, hormones, cytokines, therapeutic nucleic acids
Food and food additives	Whey proteins, milk proteins, egg proteins, soy proteins, protein hydrolysates
Nutraceuticals	Vitamins, enzymes, coenzymes, cofactors, amino acids, purified whey proteins
Diagnostic products	Glucose oxidase, peroxidase, HCG
Commodity chemicals	Detergent enzymes, insecticides
Laboratory reagents	Bovine serum albumin, ovalbumin, lysozyme
Cosmetic products	plant extracts, animal tissue extracts