**An Introduction to Bio separations**

Bio separation is the name given to the practice of purifying biological products on large-scale, using fundamental aspects of engineering and scientific principles. The end goal of bio separation is to refine molecules, cells and parts of cells into purified fractions.

Bio separation study the processes used to recover, isolate, predicate and polish products made by/of biological materials

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

 Bio separation or downstream processing: - refers to the recovery, isolation, purification and polishing of products synthesized by biotechnological processes (e.g. r-DNA technology, conventional microbial fermentation, enzyme technology, and hybridism technology).

**Differences between Bio separation and Chemical Separation**

Although bio separation is based on traditional chemical separation processes, they do differ in significant ways. This is because the materials being purified and separated in bio separation are biological substances rather than the synthetic chemicals used in traditional techniques. As such, substances such as proteins, carbohydrates and nucleic acids are not suitable for the rigors of traditional techniques like packed-bed adsorption and evaporation.

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

Because biological products are more apt to degradation than chemical ones, this rules out the use of many common organic solvents in bio separation, 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.

**Bio separation Techniques**

Many different techniques by which bio separation can be achieved – however, there is none currently works effectively on their own. This is because bio separation requires a combination of high resolution (also known as selectivity) with high throughput (also called productivity). As you will, notice in the table of techniques below, not even one combines those two traits.

One of the more commonly used methods of achieving bio separation is through the deployment of a RIPP scheme (Recovery, Isolation, Purification, and 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.

**Common stages of bio separations**

**1-R**emoval of solids

**2-I**solation (volume reduction)

**3-P**urification

**4- Polishing**

**Unit operations:-**

**A-Removal** of solids: Filtration, Centrifugation, Sedimentation

**B-Isolation** (volume reduction): Adsorption, Extraction

**C-Purification:** Chromatography. Electrophoresis, precipitation

**D- Polishing:** Crystallization, Drying

**Methods of bios products extraction:**

The importance of the selection the ideal extraction:

Enzymes and active proteins are the most important biological products and are most sensitive to the extraction circumstances, so the goal of these methods are:

1- Getting the enzyme with highest activity

2- Keeping the enzyme with highest stability grades

3-High extraction selectivity increase the purification efficiency

**M.Os enzymes extraction:**

This point should be priority for the extraction of the enzymes from the M.O:

(**1)Type of enzymes:** the micro enzymes are classified according to their production nature in to two types: (a) constitutive enzymes, is the enzymes which produce by the cells always with good quantity during their growth stages like Glycolysis enzymes and the respiratory chain enzymes. While the other type is the inducible enzymes, the enzymes which produce only when the basic reaction substance is present in the growth media.

**Principles methods of the M.Os cell lysis:**

**1-Mechanical lysis method**: it’s characterized by the flowing **which make it the beast.**

-No need for chemical substances

-Decrease the differences among the strains

-Economically good

**While the disadvantages are**:

a-The high temperature produced by the mechanical action which need control

b-Surface denaturation and foaming which should be controlled to prevent the surface distortion and allow oxidation

c-High mechanical action lysis cause to form fine insoluble particles which affect the purification process later.

d-DNA releasing which cause high viscosity.

The most important mechanical lysis are three:

1-Agitation with abrasive 2-Liquid shear lysis 3-Sonication

**2-The enzymes lysis**: the enzymes lysis characterized by

1-Determenation of denaturation

2-Can be used without the determination of the cells

3-There is some selectivity in the production of cellular components

**The disadvantages**: the lysis efficiency depending on many substances interfere with purification steps, the success of the lysis determined by the following:

1-The differences among the types and strains

2-The growth media

3-The cells situation during the lysis(frozen or fresh)

4-protease suppressers presence

5-The buffer solution presence

6-Cells concentration in the suspension

7-Osmotic pressure of the buffer solution

8-The growth phase when the cells collected

The enzymes, which used in the cell lysis are:

-Lysozymes enzyme for G+ve bacteria, lysostaphin for *S.aureus*  - Lysozymes enzyme for G-ve bacteria

-Glucuronidase, Zymolyase, Glusulase for yeast lysis.

**Plant enzyme extraction: we should follow the following for the extraction design**

1-Plant age

2-The plant parts (leaves, stems, roots, flours, seeds)

3-The seasonal changes for the desired enzymes activity in the selected part or the tissue as a source of enzyme

4-Position of the enzyme in the cell (membrane, cytoplasm, mitochondria or nucleus)

5-The capacity for the probably effects of some cellular components and the determination of the necessary principles to decrease them, like the vacuoles which contain the in organic components and the middle compounds for the Tri carbonate, water lysis enzymes especially the protease and the phenol compounds.

**We should follow the following in the extraction process:**

1-Using buffer solutions with pH and buffering capacity can withstand the organic acids, which collect in the holes

2-Reduction agent should be used like ascorbic acid for deoxidizing the oxidizing enzymes produced

3-Using the chelating agents like EDTA for pulling the multi equivalent ions and suppressing the oxidizing enzymes

4-Using of some insoluble polymers which adsorb the phenol substances from the extraction media

5-Protease suppressors using in order to control the protease which presence in the plants tissue and the mostly used suppressors are EDTA for Metalloprotease and PMSF for serine protease

**Animal enzymes extraction:**

The cellular enzymes do not need the extraction processes like the enzymes with in the blood, milk and urine while, the intracellular enzymes need to extract but the extraction is simpler than in the plant and the microbial enzymes because the structural differences and the absences of cell wall in the animal cells in compare to the plant and microbial cells.

**We must follow the following for the extraction the animal enzymes**:

1-age of the animal (the enzymatic component of the tissue depend on the age of the animal (rennin enzyme)

2-determenation of the target tissue or organ (liver, pancreas, salivary glands,)

3-identification of enzyme location within the cell ( cytoplasm, cell membrane, mitochondria, …..) that will help for increasing selectivity to the extraction method by surrounding the target part first then extraction processes. Generally for the extraction circumstances the pH should be perfect for the enzyme activity and the temperature should be low at 4c◦

4-carfely keeping the selected tissue or the organ for the extraction from the protease activity and its effect on the target enzyme for example temp. Should be less than 50c◦ to avoid the formation of ice crystals, which cause the lissome slippage, and liberation of its protein destructive enzymes.

There are two methods for the animal proteins extraction:

1-Grinding to the target tissue with buffer solution for extraction and the removal of the remnants of the tissue by cold centrifuge then collection of the clear extract which contain the enzyme or by using the filtration

2-Homogenization of the tissue and the buffer solution in a mixer and following same the previous steps

The term **Crud extract :** is called on the solution which produced by extraction process and it a general term for all the extracted enzymes from all the sources .

**Purification**: there are many ways to purify the animal products like the enzymes and the protein. **The purification** is the chain of steps, which through them the enzyme, the protein and biological products purified efficiently, economically and fast.

**The parameters should be respected for the selection of the purification method:**

1-Concentrating the products with the proper way which often start the purification with 1 or more steps to remove the water like the precipitation method

2-Obtaining high purification with concentration increase is done by ionic exchange

3-Excluding the purification methods which cannot include high volume is not to use

4- Excluding the highest number of inactive protein by the ionic exchange or the hydrophobic tendency or the polarized attraction or the tendency chromatographic

5-Getting the enzyme alone by gel filtration by one or more type of columns

**(2)Enzymes Purification:**

**Why we do purification?**

1-Recognition and diagnosis of the enzyme, determination the structure, dynamic feature and the reaction mechanisms.

2-Eliminating the enzymes which interfere with the desired enzyme activity like the protease which preset with alkaloid protease

3-Eliminating the color, dies and the odors in the bio products like the microbial enzyme which used for cheese production and the amylase which used for bread

4-Production of the pure enzyme, which used for analysis like clinical analysis and food analysis

5-comparison among different living organisms extracted enzymes features especially for the molecular development study

**The differences in the proteins features which can be used for purification:**

1-The wide differences in the molecules size and in the molecular weight of the protein for example the MW of insulin=6000 Dalton while the glutamate dehydrogenase =

106 ×2Dalton

2-The charge density for the proteins which equal to the MW

3-The difference in the charge distribution on the molecules surface and that lead to the differences in those molecules behavior in the solution

4-Difference in the PH of the solution and the Temp. For example the trypsin and chymotrypsin (protease enzyme) extracted by 0.25M of H2SO4 while most of other enzymes are destroyed in this solution

5-The difference in the molecule density

6-The differences in the bioactivity of the proteins and the specialization for the enzymes

**Purification level:**

As we mentioned before the purification: is doing a group of studied consequence steps which them goal is the separation of the protein and the enzymes from the extract.

Purification level: the enzymatic products are classified according to them purity in to three types:

1-Crud enzymes: usually are crude extracts in liquid media in the case of external microbial enzymes

2-Partially purified enzymes: which purified by one or more steps by purification while the solution still contain the enzyme, protein and other cellular contents

3-Highe purity enzymes: enzymatic product purified until it reach the homogeneity which solution do not contain another enzymes or protein

Methods of enzymes concentration: The importance of the enzymatic concentrating is to eliminate the more volume of the water to increase the concentration of the enzyme and often with this process the purity will increased too by different ratio because of the different protein features and the most important methods are:

1-Salting out: the salt are used for precipitation the protein and the enzymes like Magnesium Chloride, ammonium sulfate, sodium sulfate, NaCl

2-Organic solvent precipitation: depend on using water mixable organic solvents with electrical separation constant much less than the water, which lead to decrease the value of this constant and increase in the force value, which attract the molecules to each other and from masses precipitate in the solution.

3-The ultrafiltration: it is one of the methods which highly used on laboratories, especially for microbes enzyme because it provide the following advantages:

1-No need for large amounts of chemical substances

2- it is done at low Temp.

3- There is no change happened to the protein (no change from liquid to solid)

4- Can be used as continuous steps

5-Easy to work on high levels

6-Can be done in sterile condition