**Lec4: Cell disruption (Non Mechanical Methods)**

Non mechanical methods are further divided into three class which are following:

**a- Physical methods**

**1- Freeze thaw**

* This method used when working with soft plant material and algae.
* freezing is used to achieve cell disruption via a series of freezing and thawing cycles.
* Freezing forms ice crystals, which expand upon thawing, and this ultimately causes the cell wall to rupture.

**2- Microwave/ Thermolysis**

Microwave (along with autoclave and other high temperature methods) are used to disrupt the bonds within cell walls, and also to denature proteins. This is a somewhat risky method, as the excess heat can quickly damage the rest of the cell.

**3- Osmatic shock**

* Through the process of osmosis, water can be moved into the cell causing its volume to increase to the point that it bursts.
* Note that this method can only work with animal cells and protozoa, since they do not have cell walls.

**4- Electric discharges**

* It is also possible to achieve cell disruption via electrical discharges in mammalian cells.
* Cells that are bounded by plasma membranes, unlike plant cells, have no cell membrane.
* This method allows researchers to examine secretion by exocytosis, which is a process during which the membrane-bounded sphere (intracellular vesicle) shifts to and fuses with the plasma membrane

**b- Chemical Method**

* Often used with plant cells (and sometimes in combination with shearing), organic solvents such as toluene, ether, benzene, methanol, surfactants, and phenyl ethyl alcohol DMSO can be used to permeate cell walls.
* EDTA (ethylene diamine tetraacetic acid) is a **chelating agent**  can be used specifically to disrupt the cell walls of gram negative bacteria, whose cell walls contain lipopolysaccharides that are stabilized by cations like Mg2+ and Ca2+. EDTA will chelate the cations leaving holes in the cell walls.
* **Surfactants (commonly called detergents)**disrupt the distinct interface between hydrophobic and hydrophilic systems.  Detergents are used in cell lyses buffers and they help to solubilize membrane proteins and lipids thereby causing the cell to lyse and release its contents. Widely used detergents include Triton X – 100, sodium dodecyl sulfate (SDS), Tween 20, and 80 and CHAPS
* **Chaotropic agents**, such as urea and guanidine, are also used for cell lysis. They are capable of bringing some hydrophobic compounds into aqueous solutions. They do this by disrupting the structure of water and making it a less hydrophilic environment, and weakening the hydrophobic interactions among solute molecules.

 **c- Enzymatic methods**

* Lysozyme, Chitinase, Pectinase as enzymatic methods are easy and fast but cost for these methods are also high. Lysozymes are used for Bacterial cell lysis and Chitinase for Yeast cell lysis. Pectinases are used for the lysis of plant cell walls.
* Enzymes such as beta(1-6) and beta(1-3) glycanases, proteases and mannase can be used to disrupt the cell wall.
* This method is particularly useful for isolating the cell without the wall (protoplast).

**Applications of Cell-disruption**

1. Isolating intracellular Proteins.
2. Downstream Processing.
3. Isolating intracellular organelles.
4. Nucleic acid isolation.