Introduction to DSP – Cell Separation

Your Objectives:

At the end of the lesson, you should be able to sequence of steps for **Downstream Processing** (DSP).

Cell harvesting & cell separation

1. Once the DSP (downstream processing) production process has been defined, the moment for product harvesting shall have to be defined.
2. The harvesting point will depend on whether the product is a primary, secondary or mixed (a combination of primary and secondary) one.
3. Harvesting is usually carried out once:
   - the desired concentration of product (titre) has been achieved (g/L)
   - the cell viability is high so as to avoid cell breakage and higher level of impurities
   - the product quality is maximum

A product can be classified as being (either):

1. Intracellular, non-secreted
2. Secreted into the periplasmic space
3. Secreted into a culture medium

Intracellular, non-secreted

- Characteristic of bacteria (e.g. E. coli)
- Proteins accumulate inside the cells until the solubility of protein exceeds limit and protein precipitates as inclusion bodies
- Cells must be broken to recover the protein (product)
- Protein must be separated from cell debris, re-solubilized
- DSP begins
- In this state, the product requires unit operations for cell separation from the medium, cell disruption, cell debris removal, product solubilization, aggregates and non-solubles removal.

Secreted into periplasmic space

- Characteristic of bacteria through which recombinant protein gene has been linked to secretion protein gene (e.g., E. coli)
- Proteins which are accumulated are secreted into the periplasmic space, which is the space between the inner and outer cell wall of Gram-negative bacteria
- Outer cell wall must be broken to recover the protein (i.e. Aducanumab)
- Protein must be separated from cells
- DSP begins
- In this state the product requires unit operations for cell separation from medium, cell wall disruption, cell removal, product recovery.

Secreted into a medium

- Is characteristic of having eukaryotic cells including most yeast, mycelial fungi and mammalian cells
- Proteins produced are secreted by cell and do not accumulate intracellularly
- Facilitates protein recovery, since it can be in soluble form
- Protein must be separated from cells
- DSP begins
- In this case, state requires unit operations for cell separation from medium usually microfiltration or centrifugation, followed by depth filtration so as to clarify harvest before DSP.

Methods for cell separation

- Filtration
    - Crossflow filtration (tangential)
    - Hollow fibre
    - Internal spin filter E
    - External spin filter
- Settling devices
- Centrifuges
    - Acoustic wave technology (standing waves: BioSep)
- Hydrocyclones

Protein types: https://en.wikipedia.org/wiki/List_of_types_of_proteins

A list of ten proteins in a proteome: https://en.wikipedia.org/wiki/List_of_proteins