Quadrant II – Transcript and Related Materials

Programme: Bachelor of Science (Third Year)

Subject: Microbiology (HONS.)

Course Code: MIC106

Course Title: Industrial Microbiology

Unit 3: Down-stream processing

Module Name: CELL DISRUPTION- Mechanical methods

Name of the Presenter: Ms. Arina Frank

Notes:

Down-stream processing (DSP) encompasses all processes following the fermentation.

It has the primary aim of efficiently, reproducibly and safely recovering the target product to the required specifications (biological activity, purity, etc.), while maximizing recovery yield and minimizing costs.

Fermentation factors affecting DSP include the properties of microorganisms, particularly morphology, flocculation characteristics, size and cell wall rigidity. These factors have major influences on the filterability, sedimentation and homogenization efficiency.

DSP can be divided into a series of distinct unit processes linked together to achieve product purification.

The number of steps is kept to a minimum because:

The extraction and purification of fermentation products may be difficult and costly.

Even though individual steps may obtain high yields, the overall losses of multistage purification processes may be prohibitive.

The choice of recovery process is based on the following criteria:

- ✓ The intracellular or extracellular location of the product.
- ✓ The concentration of the product in the fermentation broth.
- ✓ The physical and chemical properties of the desired product (as an aid to select separation procedures).
- ✓ The intended use of the product.
- ✓ The minimal acceptable standard of purity.

- ✓ The magnitude of biohazard of the product or broth.
- ✓ The impurities in the fermenter broth.
- ✓ The marketable price for the product.

The main objective of the first stage for the recovery of an extracellular product is the removal of large solid particles and microbial cells usually by centrifugation or filtration.

The level of purity that must be achieved is usually determined by the specific use of the product.

Cell Disruption

Microorganisms are protected by extremely tough cell walls.

Some target products are intracellular, including many enzymes and recombinant proteins, several of which form inclusion bodies.

These target products/ cellular contents must be released from it. This is achieved by cell disruption (lysis).

Any potential method of disruption must ensure that labile materials are not denatured by the process or hydrolyzed by enzymes present in the cell.

Physical Methods

Mechanical Methods

Agitation with abrasives / Bead mills

In a bead mill, cells are agitated in suspension with small abrasive particles such as glass beads (ballotini), sand, silica, alumina, etc.

Cells break because of shear forces, grinding between beads, and collisions with beads.

The beads disrupt the cells to release biomolecules.

Factors that affect cell disruption: agitation speed, concentration of beads, bead density and diameter, broth density, cell concentration, age of cells, cell size, flow rate and temperature (Higher temperature results in faster breaking).

High Pressure Homogenisation / Liquid Sheer

The French press (pressure cell) is often used in the laboratory and the high-pressure homogenizers are employed for pilot- and production-scale cell disruption.

They may be used for bacterial and yeast cells, and fungal mycelium.

In these devices the cell suspension is pumped through restricted orifice or valves at a very high pressure (up to 1500 bar).

When the organism is under such pressure, the cell gets condensed and cytoplasm density increases due to sheer force.

This is followed by an instant expansion through a special exiting nozzle and the pressure drops to atmospheric pressure.

This high liquid shear in the orifice and the sudden pressure drop upon discharge results in the rapid decrease in cytoplasm density thus causing

explosion of the cells.
The method is applied mainly for the release of intracellular molecules.