Module-27: Fermentation Product Recovery and Purification-III:

Cell Disruption

- Microbes are protected from the outside environment by rigid cell wall.
- The cell wall may beextremely hard and the recovery of the intracellular products requires the breakage of the cellwall.
- A number of cell disintegration methods are available but the choice of themethod depends on its suitability for the particular substance.
- Though in some cases by theapplication of a particular method, a specific product can be recovered from inside the cellswith greater yield and purity, most of the times these types of applications are not feasible.
- On the other hand, gross disruption of the cell releases a huge number of products and itsdownstream processing becomes difficult.
- Sometimes application of a particular process isonly feasible in the laboratory scale and therefore, choice of suitable methods for theindustrial application is a matter of investigation.
- In recent times, enzymes are the mostimportant intercellular products of interest.
- But the disruption methods applied for the releaseof enzymes must keep them active and properly folded, at the same time release yield mustbe high.
- Followings are the some cell disruption methods:

Chemical method

- Detergent treatment
- Alkali Treatment
- Osmotic shock

Physical method

- Liquid shear
- Enzyme treatment
- Solid-liquid shear
- Agitation wit abrasive
- Ultra sonication
- Freeze-thawing

Enzymatic method

Chemical Methods

1. Detergent treatment

- Detergents damage the cell wall by interacting with the lipoproteins of the microbial cellmembrane and release the intracellular enzyme.
- The detergents used may be anionic orcationic or nonionic.
- The widely used detergents are quaternary ammonium salts, sodiumdodecyl sulphate, Triton X-100 etc.
- While applying these substances for the release ofenzyme, it has to be kept in mind that the substances can cause protein denaturation and haveto be removed from the cell free extract during further purification as soon as possible.
- Theuse of Triton X-100 is widely known to release membrane bound enzymes.
- The applicationof Triton X-100 along with guanidine–HCl is very effective for the release of a number ofproteins.

2. Alkali Treatment

- This method is applied for the cell disruption in very limited cases only when the enzyme ishighly alkali stable and can tolerate pH up to at least 11.5.
- Only very few applications of thismethod are found for the release of the enzyme and one of the classical examples is for therelease of L-asparaginase.

Physical Methods

1. Liquid Shear

- Liquid shear force for cell disruption is used mostly for the large scale release of enzymes orintracellular products from the microbial cells.
- The method is based on the shear forcegenerated by cavitation in the cell slurry due to a large pressure drop.
- The machine consistsof a hollow cylinder made up of stainless steel and a piston with an appropriate system ofadjustable valves.
- Cell slurry is loaded inside the cylinder and the pressure inside the cylinderis increased to thousands of psi through a system of hydraulics.

- When the cell slurry isallowed to pass through a small orifice, the cells experience a sudden pressure drop.
- Thatpressure drop causes cavitation and the shock waves so produced disrupt the cells.
- Theamount of pressure drop experienced by the cells has a direct influence on the disruptiverelease of the enzymes from the cells.
- Therefore, it can be concluded that higher pressuredrop is more effective for cell disruption.
- Mechanical strength of the cell wall, shape and sizeof the microorganisms also play an important role in achieving effective disruption.
- Theprocess of cell disruption is exothermic and to prevent heat denaturation of the enzyme, aneffective cooling system is always needed.
- The whole process is operated within 0-4oC.
- Tomake the process less abrasive, it should be operated in multi-pass mode for a longer time.
- Inan industrial process, proper balance has to be made between the maximum release due toeffective breakage of the cells and the percentage of released enzyme to achieve costeffectiveness.

2. Solid-liquid shear

- This is a suitable method for laboratory and can also be operated in semi-continuous mode insmall scale in the industry.
- Frozen samples of microorganisms are passed at very lowtemperature (-25oC) through a small orifice at very high pressure.
- The breakage occurs due tothe liquid-shear and the presence of ice crystals.
- The process is especially applicable fortemperature labile enzymes.

3. Agitation with abrasives

- In this method cells are disrupted with the help of mechanically resistant beads made up ofglass, alumina or titanium compounds.
- Inside a hollow chamber, cells are agitated with beadsby a system of agitator shaft.
- The shear forces so generated cause cell disintegration.

- Theprocess is exothermic and an efficient cooling system must be associated to prevent thermaldenaturation of the enzyme.
- The process can be applied at a large scale after suitableinvestigation.

4. Ultrasonication

- Ultrasonic waves are used to break the cells at small scale.
- The process relies on thecavitation generated due to the sonic waves and the shear force generated thereby.
- A highelectrical power is converted in to mechanical energy in terms of sonic wave and propagatesthrough a horn in the liquid generating cavitation.
- The system suffers from several drawbacks: high power consumption, heat generation, small operable volume, etc.

5. Freeze-thawing

- This is a very mild process.
- Due to repeated freezing and thawing of the microbial cells, icecrystals generate, create pores in the cell wall and facilitate the release of enzyme.
- Theprocess is applicable in combination with other methods.

6. Osmotic Shock

- Osmotic shock is applied for the mild release for the enzymes from the cells.
- A suddenchange in the salt concentration changes the osmotic balance within the cells and the cell isdisrupted.
- But the method is not very efficient for the microbial cells having tough cell wall.
- To apply this method for the disruption of microbial cells, generally the cell wall is first madeweak by some other method.
- The method has proved to very efficient and unique for therelease of luciferase enzyme from *Photobacterium fischeri*.

Enzymatic method

- Various enzymes can break different bonds present in the cell wall and facilitate the releaseof enzymes.
- The process is the mildest one used for the release of intracellular enzymes.
- Theenzymes used are lysozyme, enzyme extracts from *Streptomyces* sp., *Penicillum* sp.,*Trichoderme* sp., snail etc.
- The method is very costly but highly effective for the release ofenzymes with lesser impurities.
- During the downstream processing, the used enzyme must beremoved.
- Sometimes this process is also used in combination with other cell disintegrationmethods.

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