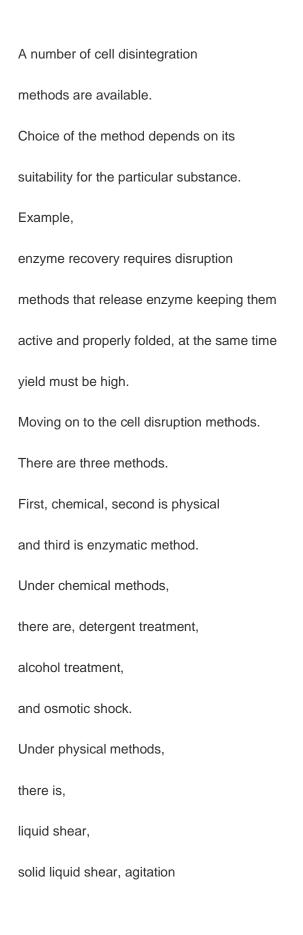
Hello students, today we're going to learn about downstream processing. That is cell disruption. I'm Maxwell Trindade, Assistant professor of Microbiology, Government College of Arts, Science and Commerce, Khandola So the outline of this lecture is cell disruption. An it's methods; that is physical, chemical and enzymatic methods. The learning outcomes of this lecture are the students will be able to describe various cell disruption methods, explained the principle of the methods. So the product of our interest is in the cell that is intracellular and we know microbes are protected from the outside environment by rigid cell wall.

To get this product we have to lyse the cell.



with abrasive, ultrasonication

and freeze-thawing.

Under enzymatic method,

that is enzyme treatment.

So the first one, physical

method that is liquid shear.

It uses high pressure homogenizer.

Most widely used in large

scale enzyme purification.

The principle; shear force is

generated by cavitation in the cell

slurry due to a large pressure drop.

So, the working, it consists of a

hollow cylinder of stainless steel

and a piston with an appropriate

system of adjustable valves.

The cell slurry is loaded inside the

cylinder and the pressure inside the

cylinder is increased to thousands of PSI.

When the cell slurry is allowed

to pass through a small orifice,

the cell experiences a sudden pressure drop, which causes cavitation and the shock waves produced disrupt the cell. See the diagram, So, the next one is solid shared. The principle of this is. A jacketed grinding chamber with a rotating shaft, running in its centre contains bead of glass or metal along with cell suspension. When the chamber starts rotating, it provides kinetic energy to the small beads. That makes the builds collide with each other, thus, leading to cell disruption. The next one is solid-liquid shear. Here pressure extrusion of frozen microorganisms at around minus 25 degrees celsius through a small orifice is done.

This disruption is due to a combination of

liquid shear through a narrow orifice and the presence of ice crystals. This is ideal for microbial products, which are very temperature labile. Moving on agitation with abrasives. OK, the principle here consists of a disintegrator containing a series of rotating disc and a charge of small beads. This beads are made of mechanical resistant material such as glass, aluminium, ceramics and some titanium compounds. Dissipation of heat generated can be done by provision of a cooling jacket. The next one is freeze thaw. Freezing of cell suspension in dry ice or ethanol bath or freezer and then thawing the material at room temperature.

The method of cell lysis

causes the cell to swell and

ultimately break as ice crystals formed

during freezing process contract

during thawing. Multiple cycles are

necessary for effective lyses.

It is a slow method with limited release

of cellular materials and are often

used in combination with other techniques.

So the next one is ultrasonication.

The principle. High frequency vibration

at the tip of the ultrasonication

probe causes cavitation

and shock waves which lead

to cell disruption.

A transducer converts the waves

into mechanical oscillations

through a titanium probe which is

immersed into the cell suspension.

It is used for both bacterial

and fungal cell disruption.

The bacterial cells can be

disrupted in 30 to 60 seconds, whereas the yeast cells take around 2 to 10 minutes. It is very effective on small scale. The drawbacks. On large scale, it requires high power. There is a large heating effect, so cooling is needed. The next one is chemical method. And in this we're going to look at the detergents. The principle. The detergents damage the lipoproteins of the microbial cell membrane and lead to release of intracellular components. That is, The detergents used are quaternary ammonium compounds, sodium lauryl sulphate, sodium dodecyl sulphate (SDS) and Triton X100.

So the drawbacks of these are; detergents may cause some protein denaturation and may need to be removed before further purification stages are taken. Next is osmotic shock. Osmotic shock caused by a sudden change in salt concentration will cause disruption of cells. The drawback of this is; The effect on microbial cell is normally minimal, so we cannot readily use this technique. It has, but it has proved to be successful technique for the extraction of luciferase enzyme from Photobacterium fischeri. The next one is alkali treatment. In this the cell lysis due to the increase in pH. The drawback. It can be only used if the desired enzyme

can tolerate the pH of 11.5 two 12.5 for 20-30 minutes. The last enzyme treatment. Enzymes hydrolyze specific bonds in cell wall of microorganisms. Example lysozyme an enzyme extract from leucocytes. Streptomyces species, Penicillium species. Trichoderma species and snails. Although it is one of the most gentle method available, it is expensive and the presence of enzymes may complicate further downstream purification processes. And then may also be used as pretreatment to partially hydrolyse cell walls prior to cell disruption by mechanical methods. These are your references, thank you.