Hello students, today we're going to

learn about downstream processing.

That is cell disruption.

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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.

A number of cell disintegration

methods are available.

Choice of the method depends on its

suitability for the particular substance.

Example,

enzyme recovery requires disruption

methods that release enzyme keeping them

active and properly folded, at the same time

yield must be high.

Moving on to the cell disruption methods.

There are three methods.

First, chemical, second is physical

and third is enzymatic method.

Under chemical methods,

there are, detergent treatment,

alcohol treatment,

and osmotic shock.

Under physical methods,

there is,

liquid shear,

solid liquid shear, agitation

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.