Enzyme Science and Technology
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Module - V
Enzyme Production (Part 3: Purification)
Lecture - 23
Host Cell Disruption Methods

Hello everyone, this is Dr. Vishal Trivedi from Department of Biosciences and Bioengineering IIT, Guwahati. And what we were discussing? We were discussing about the different properties of the enzyme in the course, Enzyme Science and Technology. And so far, what we have discussed? We have discussed about the history of the development of the enzymology, we have also discussed about the naming and as well as the classification of the different enzymes.

And then previous two modules, we are discussing about how you can be able to produce the protein or the enzyme in the bulk quantities. In this context, we have discussed about the cloning of the enzyme from the genome, either you use the genomic library or the CGNA library to select the gene of your interest. Or you can use the polymerase chain reaction to amplify the gene of your interest using the side specific primers.

Once you have the gene of your interest, then you can you know digest that with the restriction enzyme and you can clone that into a suitable vector. Once you got the clone into a suitable vector, then you can transform that into the, you know, the into the host and that is how you can devise the different strategies to over express the protein into the host.

So, all these processes, what we have discussed so far are called as upstream events or upstream processes. Where you are going to clone the protein or your, and then you are going to transform the protein and then you are going to over express the protein come to the host us. Now, what we are going to discuss today is, once you have the protein in the host cells, how you can be able to recover that protein and how you can be able to verify that protein using the different chromatography techniques.

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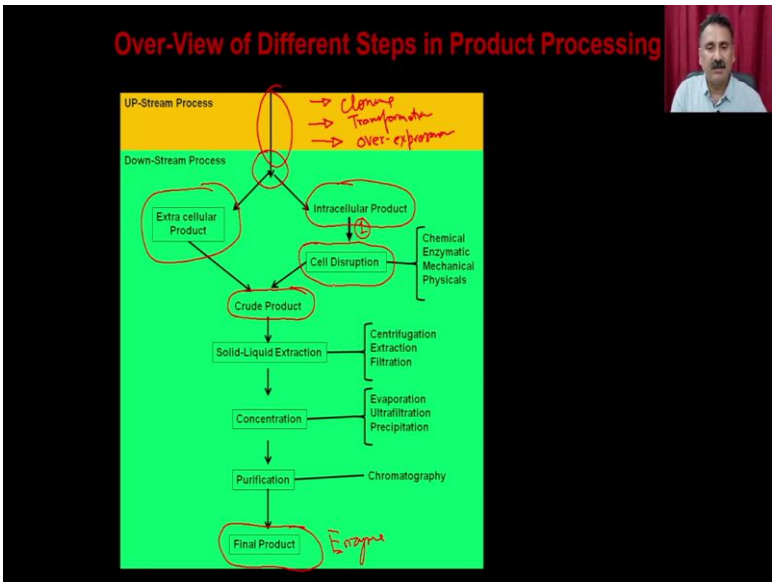

So, this is what we said, right? We are, we have discussed so far about the different types of upstream events. So, in the upstream processes, we are going to do the cloning of the gene, you are going to do the transformations or the entry of the gene into the host and then you are going to do the over expression. Once you are going to do the over expression, you are going to get the over expressed cells, ok.

This over expressed cells actually can provide you the protein either into the extracellular media or it can be intracellular proteins. If it is a intracellular protein, then it has to be, you know, the cell has to be break open; so, that you can be able to get the, you know, the crude product or the crude protein, right.

Whether you are going to have the protein of your interest and along with that, you are going to have the other proteins. From this, you can actually be able to perform the different types of procedures to purify these proteins; so, that you are going to get the final enzyme at the end. So, in this process, what you see here is that the first event or the first process is that you are actually going to do the cell disruption.

You are going to do a cell disruption; so, that the material, what is present inside the cell is going to be released and cell disruption can be done by many methods. So, in today's lecture, we are going to discuss about the cell disruption method and then we are also going to discuss about the basics of chromatography; so, let us start that.

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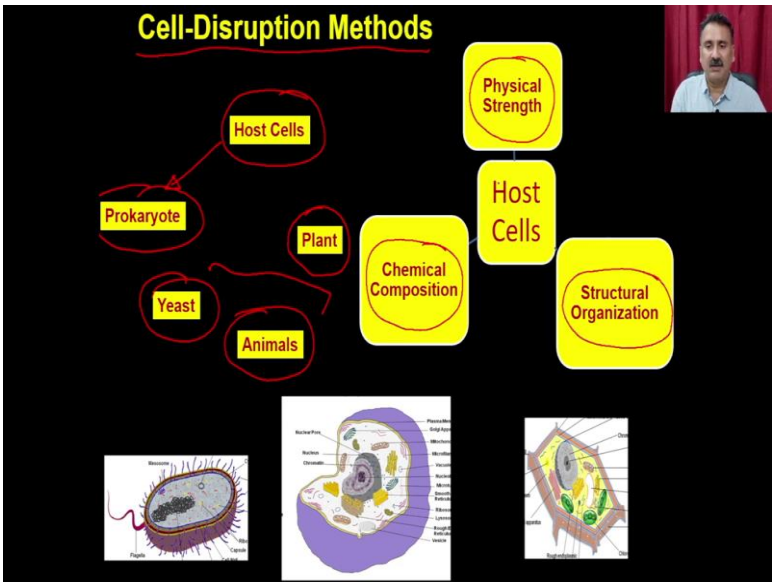

So, when we talk about the cell disruption methods, cell disruption methods are dependent on to the host cells. So, you can remember that we were talking about the different types of host cells, we are talking about the prokaryotic cells, we are talking about the plant cells, we are talking about the yeast cells and we are talking about the animal cells.

So, the prokaryotic cells are the bacteria where we can actually over express the protein or you can have the eukaryotic choices like, we plant yeast or animals. So, depending on the composition of these host cells, you can be able to devise the different types of disruption methods. So, you can have the disruption method which are actually going to withstand, which are going to challenge the physical strength of these cells.

Or you can actually be able to use the you know disruption methods which are actually going to destroy the chemical composition of these cells or you can also try to describe, you know, try the disruption method which are actually going to destroy the structure of the cells; so, that the cells are going to be break open.

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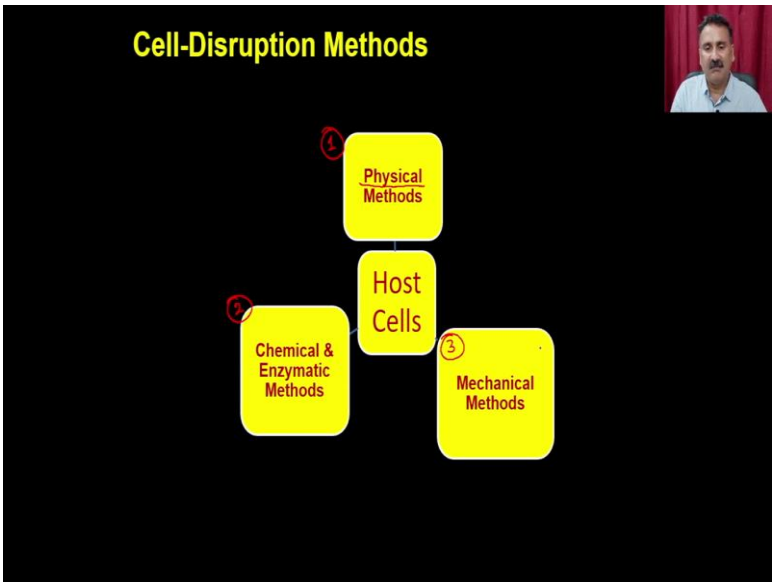

So, depending on this, you can actually have the three different types of method, you can have the physical method where you are going to use the physical parameters. You are going to vary the physical parameter and that is how it is actually eventually going to lead to the, you know, the break lysis of the cells.

You can actually be able to use the chemical or the enzymatic method which are where you are going to use the different types of chemicals. And they will actually going to react with the plasma membrane of the cell and that is how they are actually going to digest, you know the release the content. Or, you can use the mechanical method; so, you can use the different types of mechanical method and that mechanical method is also going to disrupt the cells.

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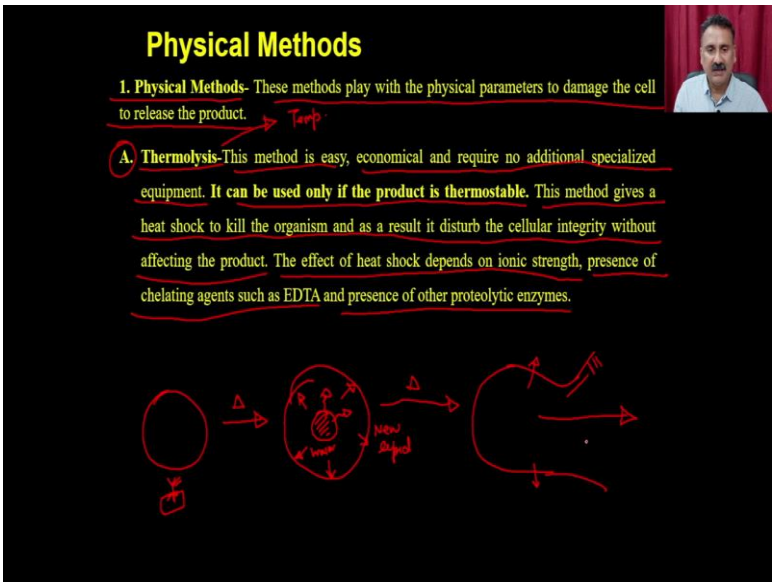

So, let us start first with the physical method; so, physical method, these methods play with the physical parameter to damage the cells to release the product. The first is the thermolysis; so, thermolysis is the, is a step where you are actually going to use the temperature. So, this method is easy, economical and require no additional specialized equipment, it can be used only if the product is thermostable.

Which means, thermostat lysis you can do only when the product is thermostable; which means, it is not sensitive for the temperature changes. So, this method gives a heat shock to kill the organism and as a result, it disturbs the cellular integrity without affecting the product. The effect of heat shock depends on the ionic strength presence of chelating agents such as EDTA and the presence of other proteolytic enzymes.

So, what happens is that when you are actually going to have a cell and if suppose you are actually going to increase the temperature, right. For example, if we heat the cell, right; so, what will happen is that it is actually going to expand, right. So, it is going to expand to some degree, right, because you open you know that when you are heating that any material, it actually going to expand, right. When it expands, it actually is required to synthesize the new lipid molecules, right.

So, it has to be synthesized the new lipid molecule, because the now the surface area or the volume of the cell is gone up, right. And that is actually going to be linked to the

production of the new lipid molecules, right, and as well as the protein molecules. This means you are eventually going to put a stress on to the cellular production machinery.

Now, if you increase the temperature further; so, what will happen is that this cell has a capacity until it can be able to provide the new lipid as well as the new protein molecules for the synthesis of the increased surface area of the plasma membrane. And as a result, eventually what will happen is that it is actually going to break, right, it is going to disrupt because it cannot synthesize that much amount of lipids and as a result, it is going to break open.

So, apart from that, the another way to explain this is that when you increase the temperature, actually the cellular molecules what is present inside the cell is actually going to push the cell from all the ends, right. Especially that water molecule, right and they will actually going to create the pressure.

So, if it is under the high pressure, it is actually going to stand that pressure, if it is; if it is actually going to be increase the volume, right. But if it cannot withstand that pressure is going to break the plasma membrane and that is how the content is going to be released.

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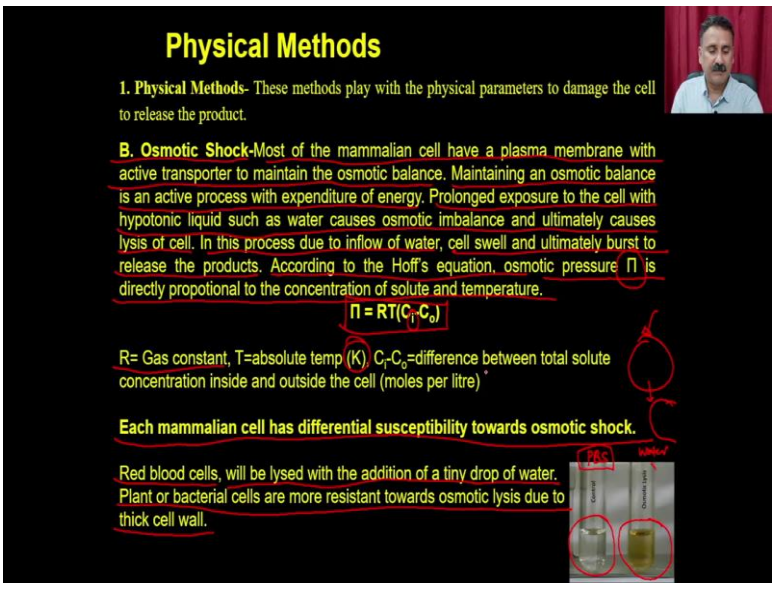

Then we have the second method and second method is the osmotic shock. So, most of the mammalian cells have a plasma membrane with the active transporters to maintain

the osmotic balance. Maintaining an osmotic balance in as active process with the expenditure of the energy; prolonged exposure to the cell with hypotonic liquids such as water causes osmotic imbalance and ultimately causes the lysis of the cell, right.

In this process due to the inflow of water, cell swells and ultimately burst to release the product. According to the Hoffman's equation, the osmotic pressure is directly proportional to the concentration of the solute and the temperature and it can be expressed with this particular formula that is the osmotic pressure is equivalent to RT C i minus C o, whereas, the C i is the, you know C i minus C o is the difference between the total solute concentration inside and outside the cell.

Whereas, T is the absolute temperature which is going to be given in the Kelvin and R is the gas constant. So, if you put all these values, it is actually going to tell you what is the osmotic pressure it is going to you know cause and accordingly you can be able to predict when the cell is going to be lysed. So, each mammalian cells has a different susceptibility towards the osmotic shock.

For example, I have shown you here the red blood cells; so, these are the control cells where I have incubated the cell in a isotonic solution such as PBS. Whereas, when I am adding the water to this, the cells are actually taking up the water and that is how you see the cells are actually going to be get lysed; so, this is the osmotic lysis. So, red blood cell is a very simple example; so, that to monitor or to actually demonstrate the osmotic lysis.

So, what you can do is just take the small amount of red blood cells and add one drop of water. So, when you add one drop of water, what is going to do is, it is the water is going to try to enter into the cell and as a result the cell is actually going to swell right. So, it is going to swell in size right and when it is swell in size, it cannot be able to synthesize or provide the additional amount of lipids and protein and that is how the cell is going to be burst.

RBC cell lysis will lysis with the addition of a tiny drop of blood. Plant or Bacteria cells are more resistant towards the osmotic lysis with because of the thick cell wall. So, bacteria or the plant cells are not susceptible for the water lysis, that is why you have seen that we actually wash the plants right.

We actually wash the plants and we provide them before you know cook any kind of vegetables, you actually wash it very thoroughly and that does not mean that it is actually going to cause any kind of damage to the plant cells. Same is true that plants are also you know you are putting the watering the plants also and that does not cause any kind of lysis of the stem cells or the root cells.

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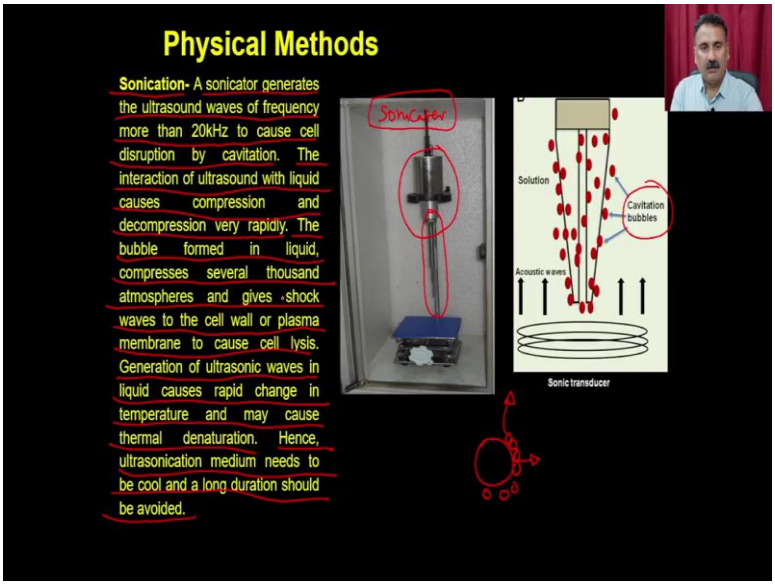

Then the third method is called as the Sonications; a sonicator generates the ultrasonic base of the frequency more than 20 kilohertz to cause a cell disruption by the cavitation method. That interaction of the internal sound with the liquid causes the compression and the decompression very rapidly. The bubble formed in this in liquid compresses several thousand atmosphere and gives atmospheric wave shock wave to the cell wall or the plasma membrane to cause the cell lysis.

This is what you see here is actually a sonicator where you have the; so, this is actually you see there is a glass actually; so, within that the sonicator is being placed. And what you see here is this is the machine through which you are actually going to generate the sonic waves and this is the probe. And so, this probe you have to insert into the vessel where you want to do the sonication.

And when you do the sonication, it is actually going to generate bubbles and these bubbles are actually going to roll over the cell. So, this you can imagine that if I have generated a bubble here, what will happen is the bubble is actually going to roll over the

cell and that is how it is actually will go right. When it roll over the blood the cell, it is actually going to expand the cell because it is actually going to cause a pressure difference.

And because of that pressure difference the cell is going to expand and in this process when it happens very rapidly, the cell cannot be able to adjust its volume and that is how it going to be lysed. Generation of the ultrasonic waves in the liquid causes rapid change in the temperature and may cause the thermal denaturation.

Hence the ultrasonication medium need to be cool and a long duration should be avoided. So, all this sonication, because the sonic waves are very high energy waves it is actually going to you know cause the temperature change in the media where you are doing the sonication. So, sonication has to be done under the cold conditions and you are going to put the ice and other kinds of things.

Hi everyone, myself Sooram Banesh, research scholar Department at Biosciences and Bio-engineering, IIT Guwahati. In this video we will demonstrate how to sonicate the bacterial pellet, how to lyse the cells bacterial cells. So, we can lyse bacterial cells in multiple ways like, sonication one of the method apart from that homogenizer we can use. In this video we will show you how to use a sonicator and lyse this cells.

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So, after centrifugation we will get the pellet; so, for this pellet we have to add lysis buffer of your choice. If you are lysing GST containing protein; so, for that you need special buffers or if you are using minus binding protein containing protein for that you need special buffer. So, it depends on which insert you have and conjugated to what protein.

So, here this is a (Refer Time: 15:40) protein, in further videos we will show how to purify this protein using nickel into a carbon. So, for now after pelleting down, we have to add lysis buffer to cells.

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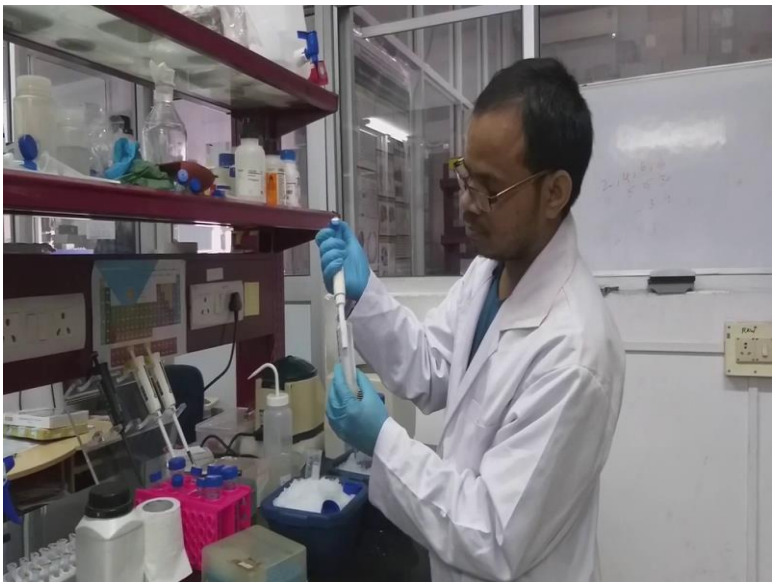

And suspend and we have to resuspend the pellet in lysis buffer; so, while doing this make sure that there is no clumps in cell pellet and always keep on ice. So, while we are keeping on ice? Here we have to remember most of the proteins heat sensitive. So, to prevent degradation or dis-functioning of the proteins, we will use ice as a medium.

Like, if you keep on ice at least they will be stable and also during sonication we should note that high amount of energy generated. So, to dissipate that heat we will use ice; so, I will show you how to operate the sonicator instrument.

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This is the sonicator; so, this is (Refer Time: 17:29)

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Where we can adjust the height of the sonicator cell; so, it is constitutively the sample you want to (Refer Time: 17:45), we can adjust the height by changing the this nobe.

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So, actually this is the probe we are using probe sonicator, normally for larger amount of samples we use this one. We have small one also, if you are using a pen wrap for that you can use small one; so, while sonication is going on we have to properly close the door.

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If you are standing there you have to use headphones; so, whatever the sonic waves are coming it will not affect the ears. Sonic waves are very powerful and the most affected

organism in our body is ears. So, it may affect internal organs; so, it is better to keep headphones while sonification is (Refer Time: 19:04)

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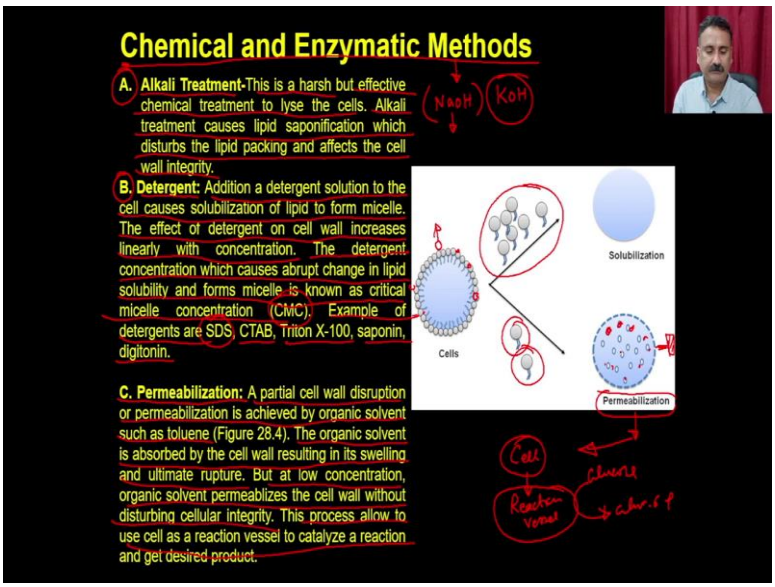

Then we are going to talk about the chemical and as well as the enzymatic method. So, in the chemical and enzymatic method you are going to use the different types of chemicals and these chemicals are actually going to react with the cellular machinery and that is how they are going to cause the cell lysis. So, the first is you are going to use the alkali

treatments; so, alkali treatments, this is harsh, but effective chemical treatment to the to lyse the cells.

Alkyl treatment causes lipid saponification which disturb the lipid packing and at effect the cell wall integrity. So, lipid means the alkali treatment is you are going to treat it with the sodium hydroxide or other kinds of alkali like KOH and other alkali. So, what will happen is that it is actually going to you know it is actually going to dissolve the lipid molecules and that is how it is actually going to disturb the lipid packaging and it is going to affect the cell wall integrity.

Then the second what you are going to use is you are going to use the detergents. Adding a detergent solution to the cell causes solubilization of lipid to form the micelles. The effect of detergent on the cell wall increases linearly with the concentration. The detergent concentration which causes abrupt change in the lipid solubility and form micelle is known as a Critical Micelle Concentration or CMC.

For example, the detergents, the detergents are the SDS, Sodium Doticide Sulfate, CTAB, triton X-100, saponin and digitonin. So, what will happen is that if you are taking a cell and if you are adding the detergent, right; if you are adding the detergent. So, detergent is what? Detergent is an amphipathic molecule; so, it is actually going to have the hydrophobic surfaces. So, what will happen is that this lipid molecules are actually going to get dissolved into that.

And as a result, the cell is going to be devoid of the lipid and when you do not have the lipid it is actually going to disrupt the plasma membrane. It is some slightly different process where also you can use the detergent is called as the permeabilization. So, if you use a very high concentration of detergent, it is actually going to remove the large quantity of lipids from the plasma membrane and that is how it is actually going to solidify the cell.

So, there will be no plasma membrane; so, all the cytosolic content is going to be released. Whereas, if you remove the lipids on a partial matter, right; if you remove the lipids like from the, this lipid, this lipid, this lipid, this lipid, you are not going to remove all the lipid molecule then this process is called as the permeabilization. So, permeabilization is actually not going to remove the all the lipid molecule, but it is

actually going to make the tiny holes and these holes can be used for delivering the content into the cell.

A partial cell wall disruption or the permeabilization is achieved by the organic solvent such as toluene. The organic solvent is absorbed by the cell wall resulting in its swelling and ultimate rupture, but at low concentration the organic solvent, permeabilizes the cell wall without disturbing the cellular integrity ok. This process allows to use cell as a reaction vessel to catalyze the reaction and get desired product.

So, permeabilization has very very good advantage because it actually going to give you, it actually going to make the cell as the vessel right; so, you can convert the cell which is into reaction vessel right. So, you can imagine that if I am making it as a reaction vessel, I can actually be used that for catalyzing the reaction. Because all these tiny pores will not allow the release of the content; so, all the content is there, but this cell is dead actually.

And I can put like, for example, if I want to convert the glucose into glucose 6 phosphate, right, I can do that because it actually has the hexokinase. So, it actually can convert the glucose into glucose 6-phosphate; so, what I can do is I can just formalize the cell and immobilize to some surface right. And in that case, this will work as an as a bag of enzymes actually; so, I can do all the chemical reactions, all the cards of modifications and so on.

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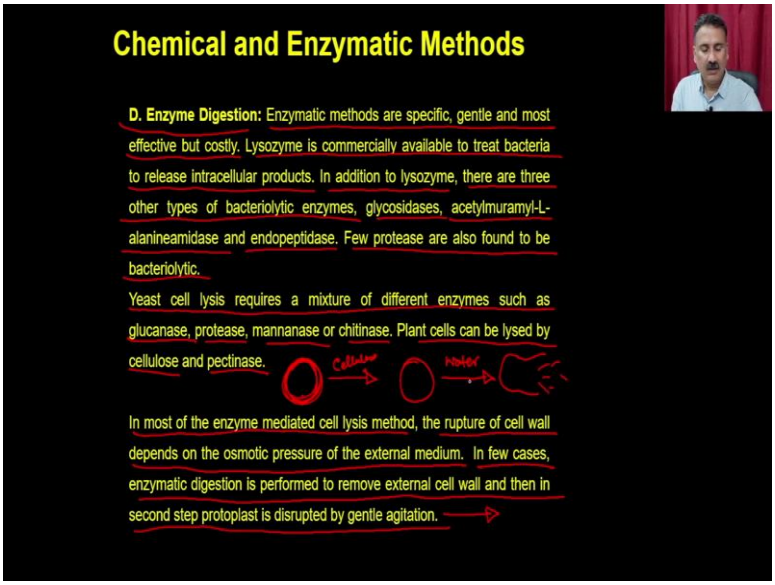

Then you have the enzymatic method; so, enzymatic methods are specific, gentle and most effective, but costly. Lysozyme is commercially available to treat the bacteria to release the intracellular product. In addition to lysozyme, there are three other types of bacteriolytic enzymes like the glycosidases, acetylmuramyl-L-alanineamidase and the endopeptidase.

Full proteins are also found to be bacteriolytic. Each cell lysis require a mixture of different enzymes such as glucanases, proteases, mannanases or chitinases. Plant cell can be lysed by the cellulases and the pectinases. In most of the enzyme mediated cell lysis method, the rupture of the cell wall depends on the osmotic pressure of the external media. In few cases, the enzymatic digestion is performed to remove the external cell wall and then in the second step, the protoplast is disrupted by the gentle addition.

So, the enzymatic digestion is being used sometime to even remove the outer covering. For example, you have you take the plant cell; so, what will happen is in the plant cell, you have a cell wall and within this, you are actually going to have the plasma membrane; so, what you are going to do is you just treat it with the cell cellulase.

So, what will happen is cellulose is, cellulase is actually going to chew up all the cellulose molecules that is present in the cell wall and that is how it is actually going to generate a plant cell without the cell wall. Now, this cell is actually sensitive for the osmotic damage; so, what you can do is then you can just add the water and it is actually going to break open the cell.

Because, as I told you, right the plants are not sensitive for the osmotic damage. But that is only because there is a cell wall present, right, but if you treat it with the cellulase enzyme or pectinase enzyme, it is actually going to remove the cell wall and that is how you are going to have the plant cell without cell wall. And then if you add the water, it is going to be susceptible for the osmotic lysis and that is how you can be able to release the content of the cell.

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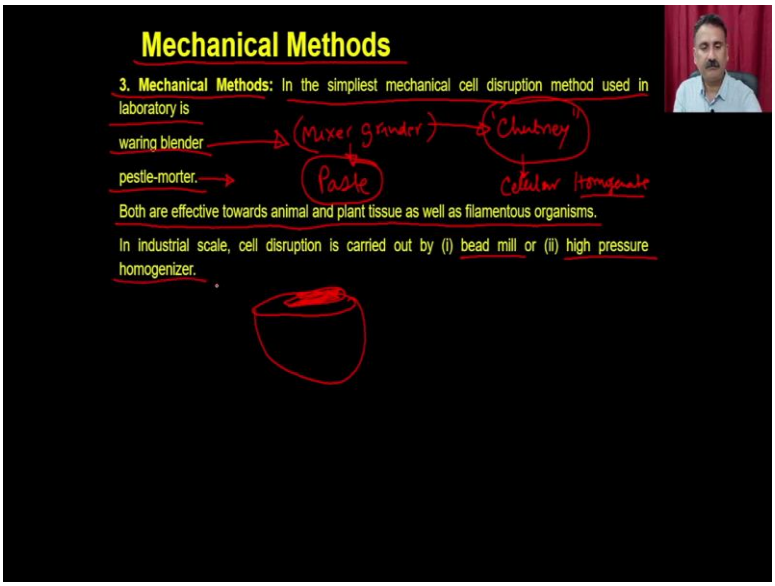

Now, let us talk about the mechanical method; so, mechanical method are going to be the method in which you are actually going to use the you know the some kind of machines. So, it in the simplest mechanics used in the laboratory is the waring blenders or the pestle morter. So, waring blender is nothing but the mixer grinder actually ok.

I am sure you might have used the mixer grinder for making the chutney right in your home right. So, when you prepare the chutney, chutney is nothing but the cellular homogenate right, it is a homogenate actually. Because, where you are actually taking the different types of constituents like you are taking the corianders, you are taking the onions, garlic and all that.

And then you are running the mixer grinder and that is how it is actually going to crush all the cells and it is giving you a paste, that paste is nothing but the cellular homogenate. So, it actually contains all the different types of enzyme and so on. Pestle morter is also the same, I am sure you might have seen the pestle morter is where you have a bowl and you also have a rod, right.

So, that rod is actually going to be used for grinding the I mean grinding the substance. But these are mechanical methods; these are the manually mechanical methods. Whereas, you can actually be able to use some more effective method where you can be able to run the motors and that is how the it is going to be more and more effective.

Both are effective towards the animal and plant tissue as well as the filamentous organism. So, you can use the waring blender or pestle morter which are effective for the animal and plant cells, but they are not good for the hard tissues right. And they are also not good if you are trying to do this at an industrial scale, because you cannot do the industrial scale mixer grinders right. So, in those cases you have to use the bead-mill homogenizer or the high pressure homogenization.

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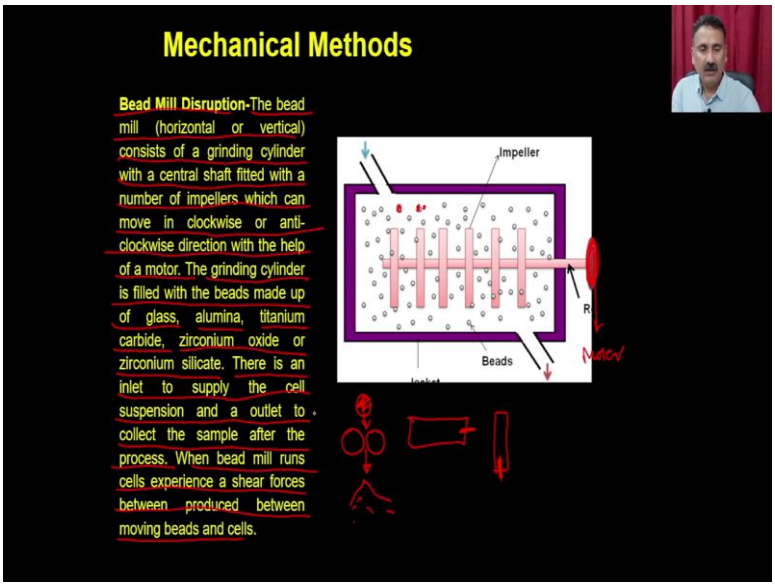

So, in a bead-mill homogenizer disruptions what you are going to have is, you are going to have the bead-mill homogenizer right. So, the bead-mill homogenizer either it can be or a horizontal model or it can be a vertical model which means it is either going to be in this direction where you are going to have the shaft on this side or it can be actually this way; so, that you can have a shaft on this side.

Either of these consist of a grinding cylinder with a central shaft fitted with a number of impellers. So, this is the central shaft what you have and then these are the impellers; so, they run in a clockwise direction or the anti-clock directions which can move in a clockwise or the anti-clockwise direction with the help of a motor. So, this is actually being connected to a motor right.

So, this is being connected to a motor and that motor can run this impellers into the positive, you know, the clockwise direction or the anti-wise directions. The grinder cylinder is filled with the bead made up of the glass, aluminium, titanium, carbide, zinc-

onium oxide or the zinc-onium silicate. There is an inlet to supply the cell suspension and outlet to collect the sample after the process.

When the bead mill runs, cell experiences the shear strength between the produced, between the moving beads. So, what will happen is you are actually going to add the beads and so, this is the, you know, the bead mill homogenizers. And in this you have a central shaft, this shaft is connected to the motor and on this shaft, you have the impeller.

So, when this runs, it and you also going to add the beads, right; so, when these beads actually moves. So, what will happen is that you can imagine that if these two beads are moving, the cell is actually passing through to these beads; so, cell is passing through these tiny pores which are being formed between these beads.

So, as a result, the cell is actually going to experience a shear stress, which means these two beads are actually moving and the cell is hitting with these beads and it is actually passed through. So, it actually has to squeeze and then only it can actually be passed through. But in that process, it is actually going to experience a very significant change in pressure, because there is a very high shear stress and as a result content is going to be released.

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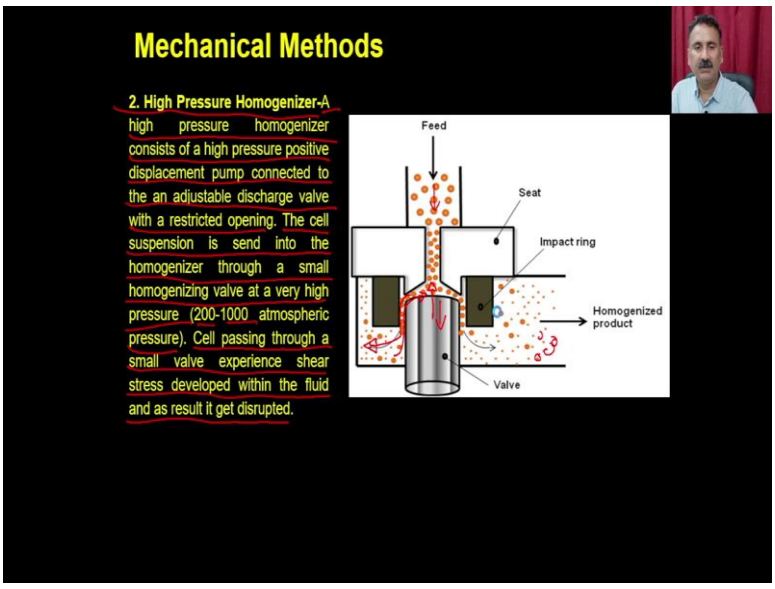

Then we have the high pressure homogenizers, high pressure homogenizers consists of a high pressure positive displacement pump connected to the adjustable discharge wall

with a restricted opening. The cell suspension is sent into the homogenizer through a small homogenizing wall at a very high pressure like 200 to 1000 atmospheric pressure.

The cell passing through a small wall experiencing the shear stress that developed within the liquid and as well it gets disrupted. So, what you are going to do is you are going to add the cell and then you have actually the, this cells the high pressure homogenizer where this valve is actually can move in the up and down direction.

So, when it goes up, it at all the cells are actually passing through this tiny pore and it is actually coming out, right, and when it is coming down, then all these cells are going again back. So, when the cells, these cells are passing through these small pores; they are experiencing a shear stress. And the pressure difference what you see is from 200 to 1000 atmospheric pressure, this means the pressure is going to be very high.

So, when this, it happens with a very high pressure, all the cells are actually going to be released going to be the lysed and they are actually going to release their product. And then this product can be recovered from the, another opening. So, this is all about the cell disruption method, right; so, now once you have done the cell disruption, you are going to have the crude product. What is there in this crude product?

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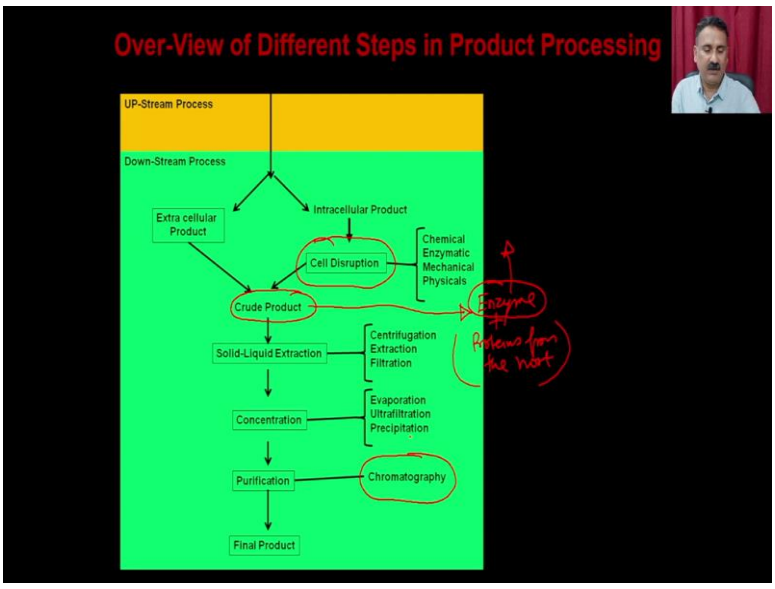

The crude product is actually going to have the enzyme, what you are going to be purified and then it is actually going to have the protein from the host, right. So, it is

going to have a mixture of the two species, one, the proteins from the host and the enzyme, what you are over expressing in the large quantities.

Now, when our objective is that we want to purify this particular enzyme. So, we want to isolate this enzyme and that is how we are actually going to do a technique which is called as the chromatography. So, you are going to purify this with the help of a technique which is called as chromatography; so, with this, I would like to conclude my lecture here.

Thank you.