**Genetic Engineering: Theory & Applications**
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**Module 7: Isolation and Purification of Product (Part-1)**
**Lecture 19: Product Recovery from Host Cells**

Hello everybody, this is Dr. Vishal Trivedi from Department of Biosciences and Bioengineering, IIT Guwahati and what we were discussing, we were discussing about the Generation of Recombinant DNA or Generation of Genetically Modified Organisms and in this particular kind of discussion what we have discussed so far, we have discussed about the Host as well as the transforming agents.

So in detail we have discuss how to produce a Genetically Modified Organisms and subsequently we have also discussed the strategies how to produce the protein Intracellular as well as Extracellular in different Host strains whether it is Bacteria, Yeast, Insect Cell Lines or the Mammalian Cell Lines.

Now today, what we are going to discuss, we are going to discuss about the extraction of this particular product from the over expressed cells. So, as you know that the complete process of the product development within the biotechnology is considered to be in two phases and what are these phases?

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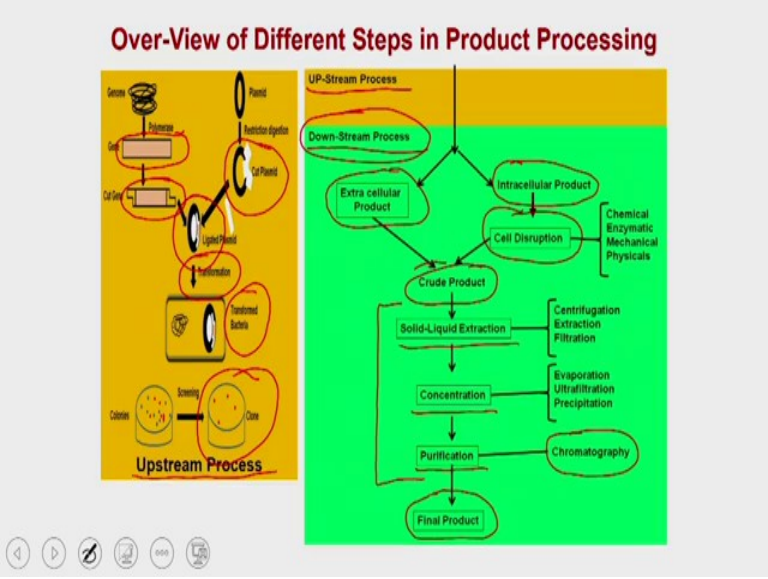


So, we have two different types of processes to get a final product, these are called Up-Stream Process or the Down-Stream Process. Whatever we have discussed so far which is like getting the gene from the host strain, then cutting the gene and generating the sticky ends, similarly you are processing the transforming agents and getting the Cut Plasmid and then you are putting the

gene as well as the Cut Plasmid to generate the recombinant clone then we are putting this recombinant clone into the bacteria and generating a genetically modified organisms.

Then we were screening these organisms to know (the screen) the desired organisms with the desired features, and all these processes what we have discussed so far are considered to be up-stream processes which are been involved. Now, once you have generated the product then it comes into the down-stream processing. Within the down-stream processing, you can have two possibilities, either the product what you are generating is a extra cellular Product or it is a Intracellular Product. For Extra cellular product, that product can directly been taken into the down-stream processing and whereas for Intra cellular product, the first step would be to disrupt the cells and extract the product into the supernatant.

Once you got the crude product, which is either from the extra cellular product or the intracellular product, then it will be go through with the different types of purification stages where either you do the solid liquid extractions, then you will do the concentration and then you will do the purifications using the Chromatography techniques and then finally you are going to get purified product. So in this whole step of down-stream processing, if the product is intracellular the first step is that you have to do the cell disruption and that is what we are going to discuss today. What we are going discuss is, what are the methods you can use for this cell disruption so that you can get your product into the liquid phase, so that you can use them for the down-stream processing events or the down-stream processing and then finally you can get the final product.

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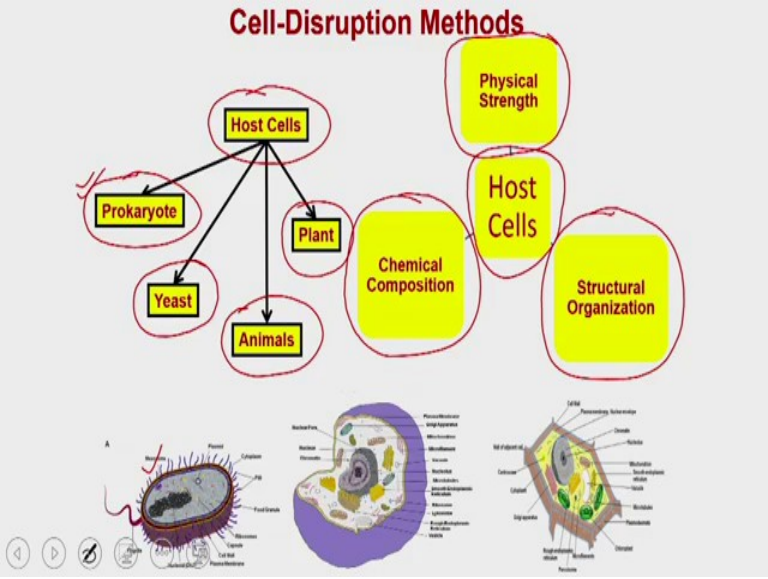


If you remember, when we were discussing about the different types of host cells, we have said that you can use either the Prokaryotic host cells such as the bacteria or you can use the

Eukaryotic host cells such as the animal or the plant origin and you can use the single cell Eukaryotic host which is the yeast. So you have the different choices of host cells and accordingly you can use the transforming agent as well.

So, all these host strains if they are containing your product which is inside the cell has to be taken out and what you see is that when you see the host cells, the host cells are varying in terms of their Physical Strength, which means the cell is going to have the different variable level of physical strength to be broken down.

Then you have the varying Chemical Composition of these host cells so that can also be taken care into the disruption processes and then at the end, the structural organization is also different between the all these four different streams. For example, in the case of Prokaryotes there is no organelle present so once you broken the cell wall and once you broken the bacterial cell, the product will come out whereas in the case of yeast, animal or the plant they all are going to contain the organelles.

So, even if you have broken the outer plasma membrane that will not ensure that the product will be released into the supernatant. You may have to broken down to the particular type of organelles and then you can get product into the very, very high consideration into the supernatant. So depending on the type of host strain, you also have to devise the strategies to disrupt the cell wall or disrupt the cells.

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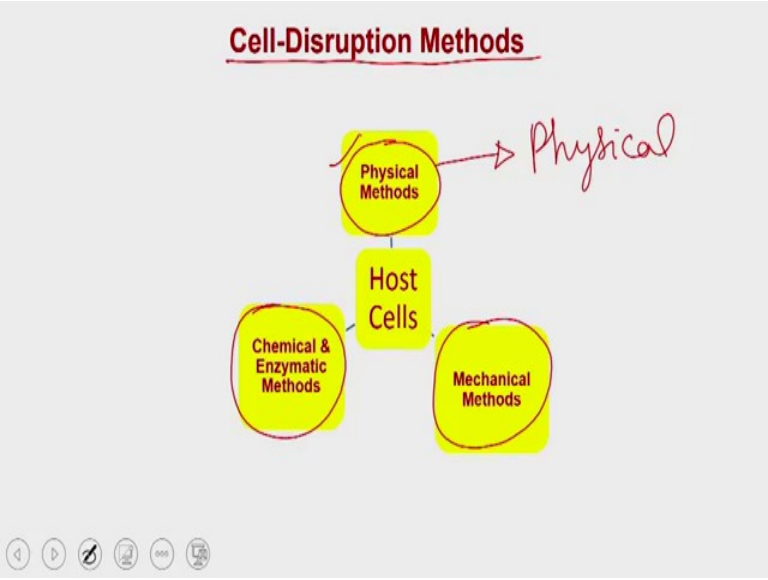


Depending on the different types of properties in which the host cells are varying from one to each other, the disruption methods are also being developed accordingly. So you have the physical method, physical methods are the method where you are using the physical properties or physical parameters such as pressure, temperature and all other kind of parameters.

So that will actually going to affect the cellular integrity of the host and (then) that will eventually going to lyse the cell. Similarly you can use the Chemical or the Enzymatic Method. So since the host strains are varying from one to another simply by having the different types of chemical compositions you can use the chemical as well as the enzymatic method to disrupt the different types of host cells. And then lastly you have the Mechanical Methods.

So Mechanical Methods are mostly being developed to break the very, very difficult cells to broken down. Mostly the mechanical methods are developing the sheer stress within the cell or within the liquid and that sheer stress is disrupting the cells. So let us discuss and start our discussion about the different types of cell disruption method with the physical method and as I said the physical method are going to exploit the different physical properties to disrupt the cells.

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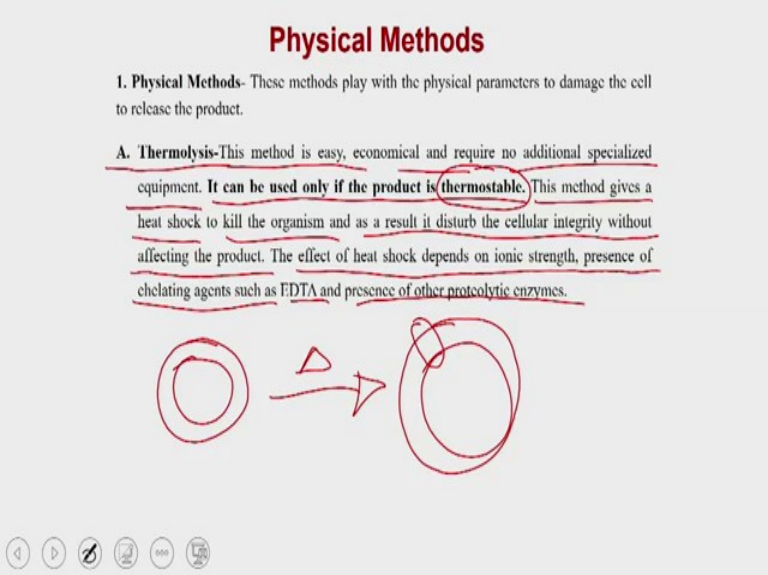


The idea of physical method is that when you apply a physical force that actually is going to give the stress to the cell and in that response the cell will either going to shrink or either going to expand. In both of these process, in both of these events the cells is eventually going to burst and that is how it is going to release the cellular content into the supernatant.

The first method what we are going to discuss is Thermolysis, as the name suggest, the thermolysis means that you are going to give the thermal stress which means you are going to vary the temperature of that particular cell in a very, very short span of time and that process is known as the Heat Shock.

So, this method is very easy, economical and requires no additional specialized equipments which means you can simply do a thermolysis by having a incubators or by having a water bath which can actually give you the particular type of precise temperature. The only way when you

can use the thermolysis is when your product is thermostable which means if you vary the temperature it should not destroy or it should not degrade your products.

This method gives a heat shock to kill the organism and as a result it disturb 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 presence of other protcolytic enzymes. So you can imagine that if you give a heat shock, so might have seen that when we were discussing about the bacterial cell, if you give the very, very brief period of heat shock, what will happen is the cell is going to expand, okay. And in that process when the cell will going to expand, since this process is going to happen so fast that the cell is going to utilize the whatever the lipids it has and for a smaller circle.

So, when it expanding from that particular volume to a larger volume in a very, very short span of time it does not get the time to synthesize the additional lipids which is required as per the surface area of the larger surface, larger volume or larger wall. In that case what happen is it actually expands and actually get burst instead. Because the cell has a capacity until it can expand and keep using the similar kind of, keep using the same lipids what it was using for the smaller volumes. But, beyond that it may get just burst and that actually will allow the cellular content to come out. And that is exactly what happens if you do a thermolysis, the cell will expand and eventually it will cannot sustain that particular volume and that will burst.

Let me give a simple example or daily life example. If you take a balloon okay, and suppose you imagine that the balloon is a cell okay. Now what you do is, take this balloon and put it into a warm water or if you just dip this balloon into a hot water bath. What will happen, once you put the balloon into the water bath, the balloon will have a elastic rubber okay. So balloon will expand to a certain extent, but beyond the elasticity of the rubber, the balloon because the air which is present inside the balloon is giving the pressure on to the outer layer and as a result the outer layer is expanding but it cannot expand beyond a limit and ultimately what will happen is the balloon will burst.

Exactly the same thing happens when you put the cell into the thermolysis. You change the temperature, as you change the temperature the cellular content which is present inside mostly the water is going to expand or going to acquire larger volume and as a result what happen is it actually asks the cell to expand as well, but the cell cannot expand beyond a limit and ultimately the cell is going to burst and it will going to release the cellular content. But, as I said the thermolysis method can be used only if you are sure that the product what you are generating or whatever you are producing inside these cells are thermostable.

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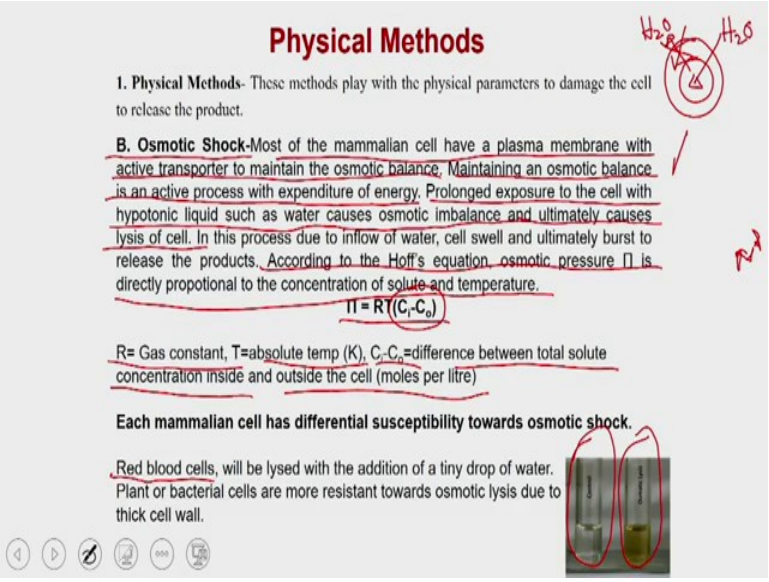


Now, the next method is called Osmotic Shock. Most of the mammalian cells have a plasma membrane with active transporter to maintain the osmotic balance. Maintaining an osmotic balance is an active process with expenditure of energy okay. So prolonged exposure to the cell with a hypotonic liquid such as water causes osmotic imbalance and ultimately causes lysis of the cell. So what happen is, if you a cell and (this is) this osmotic shock is only true for the cell which contains the plasma membrane. For example, if you take a bacteria and put it into the water or hypotonic solutions it is not going to be experiencing the osmotic shock. Because the bacteria or the plant cell is going to have the very thick cell wall and thick cell wall is going to make the things impermeable and as a result it is not going to experience any change in concentration of the solutes from inside to outside and that's why it will not experience any kind of osmotic imbalance.

But in the case of mammalian cells which do not contain the cell wall except the fungi or the yeast, they will experience a osmotic imbalance and as a result if you are putting a cell into the hypotonic solutions the concentration variant would be in such a way that it is going to take up the water from inside to outside. But, if you put the cell in a hypotonic solution and water will be coming inside, the water pumps which are going to be operating on to the cell will be keep exporting this water outside until the cell is going to be exhausted with the energy. Once the cell is getting exhausted with the energy, there will be no export of water from the cell and as a result the cell will start acquiring the water from the outside, because the outside environment is hypotonic which means the concentration of solute is less. So, the water will come inside and will be keep coming until the cell can sustain that particular type of expansion but beyond that it is going to do the exactly the same what we have discussed just now about the thermolysis.

It will going to burst the cells because the cell cannot sustain that much expansion and as a result the cell will not be able to hold the cellular content and the cellular content will be released into the external media or it will going to be burst. According to the Hoff's equation, osmotic pressure is directly proportional to the concentration of solute and the temperature and the equation what is given is RT C1 minus CO, which actually a C1 minus CO is the consideration differences the solute which is inside and outside, where R is the Gas constant, T is the Temperature and C1 minus CO is the difference between the total solute concentration inside and outside of the cells which is in the unit of moles per litre.

Now, every mammalian cell is different so every mammalian cell susceptible for the osmotic shock differentially. For example the cell which are containing the organelles or which are containing the high energy, they mat sustain for a longer period of time to the osmotic shock whereas the cells which are of the lower energy and does not contain the organelles they will be going to be more susceptible for the osmotic shock. One of the example I have shown here is, that suppose you take the red blood cells, so red blood cells are the cells which carry the oxygen inside the body and these are the very essential cells which carry the oxygen from one part of the body to another part and the in return it carry the carbon dioxide from the tissue and releases outside.

So if you take the red blood cells and just add a very small amount of may be one drop of water and that one drop of water is good enough to give such a large osmotic shock that it will going to lysed all the cells. What you can see here that we have done a simple experiment in our lab that if you take the cells and put it into the PBS, the cells will remain intact. PBS means Phosphate Buffer Saline, so that will be the isotonic solution and the cell will remain maintained. But if you put the same cells under the hypotonic solution such as the water, the cells are going to lysed and it will give you the bright red color or the slightly brownish color.

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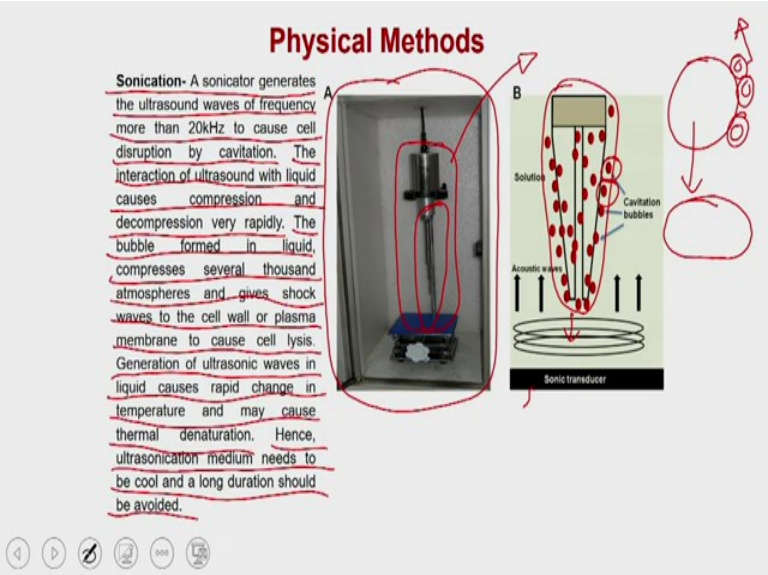


Now, let us go to the next method. The next method is known as the Sonication. The Sonication is been done with a specialized instrument called as the sonicator. So what you see is in a sonicator, the two component, one sonic waves generator which is actually outside of this box and inside this box what you see is the probe. So you can have the probe which can actually go into the eppendorf or you can have the probe which can go into the falcon tubes. You can have the sonicators which are of the bass sonicators where the sonic waves are been generated inside the water and you can have the probe like sonicator which actually, in which the sonic waves will be generated through this probe and that actually going to allow this sonic waves to transmit into the liquid which you will put inside this chamber.

This chamber has a lid from outside so that you can close, because the sonic waves are not good for the human being that's why it is always advisable that you should were the  or you should put the cotton plugs before you do the sonications. So, the sonicator is generating the ultrasound waves of frequency more than 20 kilo hertz to cause the cell disruption by a process known as the Cavitation. In the interaction of the sonic waves, interaction of this ultrasound with the liquid causes compression and decompression very rapidly which means when these ultrasound waves are interacting with the liquids, it is actually transmitting the energy from the different layer of the liquid differentially.

As a result what will happen is, it is actually compressing because if you put the pressure, if you put the energy into the liquid, the liquid is going to compress, okay. But, once the liquid is going to compress the other part of the liquid is going to decompress because it has to release that amount of the pressure okay. And as the result of this compression and decompression these waves actually generates the large quantity of bubbles into the liquid and because of that the

bubbles are formed in liquid and compresses several thousand atmosphere and gives the shock waves to the cell wall or the plasma membrane to cause the cell lysis.

So because there is a huge pressure difference, huge compression and decompression it actually produces a bubble and as well as because the change in pressure is very high it actually generates or it actually allow it, it gives a shock wave to the cell wall or to the plasma membrane and as a result the cell is going to be burst and releases the content. So, the sonic waves are also producing very high quantity because there is a rapid change in temperature because once you are doing the compression and decompression you are also changing the pressure inside the liquid and as a result you are actually causing the liquid layers causing the friction to each other and as a result of this friction, the temperature of the liquid is also changing.

As a result, the temperature of this particular whole vessel which you are going to put for sonication has to be maintained and that's why all these vessels are been kept in a ice bucket or some kind of ice bath so that while you are doing the sonication the temperature of the liquid should not change because that eventually is going to hamper or going to affect the quality of the product which you are going to get. For example, if you do not, if you allow the temperature to change, the temperature will go beyond a permissible limit and that actually is going to eventually damage the product what you are going to produce especially if the product is thermolabile. So, the generation of the waves in the liquid causes rapid change in temperature and may cause the thermal denaturation. Hence, the ultrasonication medium need to be cool and a long duration should be avoided.

So, that's why the doing a sonication of the bacterial cell or if you do it a Eppendorf or to the Falcon has to be done in a very, very sophisticated manner and that is why it requires the some kind of training, because what you are supposed to do is you also have to protect yourself from the sonic waves which you are going to produce inside this chamber and at the same time you also have to ensure that you are getting the sonication done without affecting the product. So we have prepared a small clip of movie to show you how to a sonication and what are the precaution you should take. So this movie is been prepared in our laboratory and I hope this movie will give you a better chance of learning how to do the sonication to disrupt the cells and recover your product.

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In this video, we will demonstrate how to sonicate the bacterial pellet, how to lyse 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 the cells. So, after centrifugation we will get the pellet okay. So, for this pellet we have to add lyses buffer of your choice.

If you are lysing a GST containing protein so for that you need special buffers or if you are using mannose 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 ((histyle))(24:16) protein, in further videos we will show how to purify this protein using Nickel-NTA column.

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So, for now, after pelleting down we have to add lysis buffer to cells and suspend and we have to re-suspend 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 why we are keeping on ice, here we have to remember, most of the proteins heat sensitive.

So, to prevent the degradation or dysfunctioning of the proteins we use ice as medium, like if you keep on ice at least they will be stable and also during sonication we should know 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 the (( ))(26:03) where we can adjust height of the sonicator inside. So this is consisting the sample you want to sonicate, you can adjust the height by changing this knob.

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So, actually this is the probe, we are using the probe sonicator. Normally for larger amount of samples we use this one. We have small one also, if you are using the Eppendorf, for that you can use the small one. So, while sonication is going done we have to properly close the door. 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 organ in our body is ears. So, it may affect internal organs also, so it is better to keep headphones while sonication is going on.

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So, after adjusting you make sure that the probe is not touching the bottom of this. But, it is just above the bottom, so we can check like this. After this to close it properly. These are high energy sound waves are produced during this process, so it is better to close this door.

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So, here this is where we can adjust the time, so I am going to adjust 1 hour and pulse it give 5 seconds on and 25 seconds off cycle. So, if you give the sonicator probe, the probe sonicator is very big, if you give more time on pulse then it will give high, it will release a high amount of energy which will ultimately destroy the protein. So, it is better to give low on pulse cycle and high off pulse cycle, so that will take care of the proper lysis. So once this process is over we can check the whether the lysis is taken place or not.

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So we just press start here, you can hear the sound whether the sonication is happening or not. So, after 1 hour we will come back and check the lysis.

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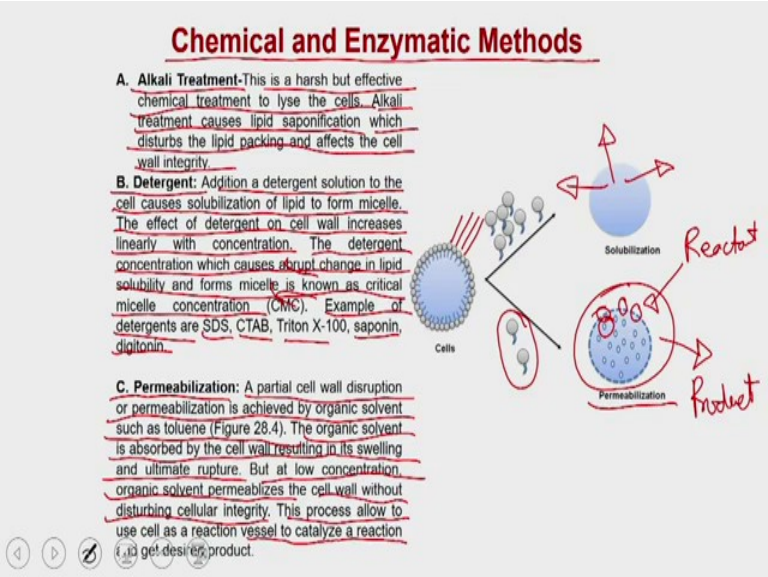


Now, let us go to the next method, so next method is called Chemical and Enzymatic Methods. So Chemical and Enzymatic Method is, in those methods you use the chemicals or the enzymes for the cell disruptions. So let us start with the chemical method, in a chemical method the first method is called as the Alkali Treatment.So Alkali Treatment this is a harsh but effective chemical treatment to lyse the cells. Alkali treatment causes lipid saponification which disturbs the lipid packing and affects the cell wall integrity.

Now the next method is the Detergent. So Detergent is a very, very popular method of the cell disruption. In detergent method you have the two possibilities, one is called as the lysis, the other method is called as a Permeabilization. Addition of a detergent solution to the cell causes solubilization of lipids to form the micelle. The effect of detergent on cell wall increases linearly with the concentration. The detergent concentration which causes abrupt change in lipid solubility and may form micelle is known as the critical micelle concentration or CMC.

Examples of different lipids or detergent which people are using very often for cell disruption methods are SDS sodium dodecyl sulfate, CTAB, Triton X 100, saponin and digitonin. So what happen is that when you do a detergent treatment, detergent is nothing but the hydrophobic liquid. So, it could be positively charged, it could be negatively charged or it could be neutral in nature. But, whatever it has a hydrophobic core and it has a polar chain. So, in that case what happen is, the hydrophobic core goes and bind the hydrophobic lipids and as a result it dissolves the lipids into the detergent.

So once the lipid is dissolved into the detergent, that particular part of the plasma membrane is going to be not present or it is going to be washed away okay. And as a result, that particular cell not going to be intact and as the result it is going to lyse the cells. But in that case you can have the option of either to cause the lyses or to cause the permeabilization. What is mean by

Permeabilization, a partial cell wall disruption or the permeabilization is achieved by the organic solvent such as the toluene. The organic solvents is absorbed by the cell wall resulting in its swelling and ultimate rupture.

But at low concentration, organic solvent permeabilizes the cell wall without disturbing the cellular integrity. This process allows to use the cell as a reaction vessel to catalyze the reaction and to get the desired product. Which means, if you have the cell which actually contains the lipids by layer. If you add the detergent you are actually going to remove these lipid molecule, because they are going to be dissolved into the detergent and as a result the cell is not going to contain as any plasma membrane and as a result this cell is going to release the content into the liquid.

Whereas, in the other method either you use the organic solvents or even if you use the detergent, what will happen is, if you use the detergent in a very, very controlled concentration. It is not going to remove all the lipids which are present on the plasma membrane, instead it is going to release the lipids in a discrete manner and as a result it is going to make the holes on to the cell. So that hole does not allow the content to be released. Because if you change the concentration or if you change the exposure period you could be able to very completely, you could be able to control the pore size in such a way that the content may not be released from the cell.

And as a result you are going to making the holes and this process is known as the permeabilization. And that permeabilization event actually gives you a cell which may loses its content, which may loses its water and all other kind of liquid content but it may not loses its organelles and other kind of content and so it becomes a empty vessel. And in that empty vessel what you can do is you can add the reactants and you can get the product.

So you can actually use these cells as a cellular factory, which means if you immobilize these cells to a bio reactor or some kind of surfaces and then you use very small quantity of detergent or the organic solvent and you achieve a permeabilization event in such a way that it loses its cellular content but it does not loses its organelles and other kind of enzymes.

Then in those cases, you can use these cells to catalyze a bio-transformations or other kind of chemical reactions. Like if you change one product to another product or a toxic product to a non toxic product those kind of events can be done and these permeabilization can be exploited in that way for the study purpose as well as for utilizing these cells in a industrial application as well.

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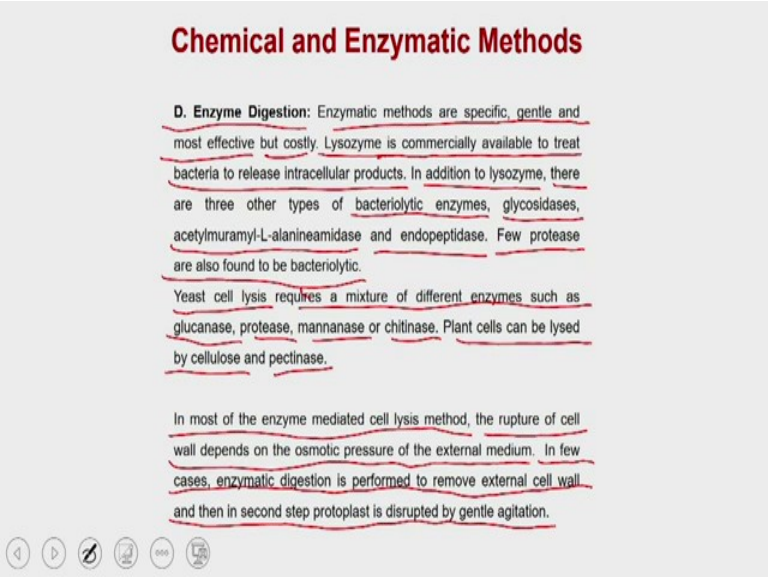


Apart from that we have the Enzymatic Digestion. So, one of the classical enzymes which we have already been discussing is Lysozyme. So, enzymatic methods are very specific, they are gentle, most effective because enzymes are very specific for a particular type of bio molecules so they will be very, very specific, gentle and effective. But, the enzymatic methods are very costly, because all these enzymes you have to procure from the in a very, very purified form and all these enzymes are commercially available so they will be very costly.

So, one of the classical enzyme which we people use is the Lysozyme. So Lysozyme is commercially available to treat bacteria to release the intercellular products.In addition to lysozyme, there are other kinds of bacteriolytic enzymes which you can use, we can use the glycosidases, you can use the other enzymes and as well as the endopeptidase. Some of the proteases are also found to be a bacteriolytic enzymes.

For example, for the yeast cell lysis require a mixture of different enzymes such as the glucanase, protease, mannanase or chitinase. Plant cells can also be lysed by the cellulose and pectinase. because the lysis requires you to degrade different barriers. For example, in the case of the plant cells, the plant cell has a cell wall, the cell wall is made up of cellulose. So, within the plant cells you have the lignin, which is actually the material which is present within the matrix of the two plant cells and which is actually making the two plant cells, joining the two plant cells.

So this lignin also has to be degraded, that is why if you want to disrupt the plant cells you have to first disrupt the cell wall. So you have to use the cellulose enzyme and then since you want to degrade these matrix protein also then you have to use the pectinase, to degrade the pectin or you have to use the ligninases to degrade the lignin and then only the plant cells will be free to present and then you can give the osmotic shock or eventually the plant cell will experience

osmotic shock if your outer environment is the hypotonic in nature and then eventually it is going to lyse the cells.

In most of the enzymatic mediated cell lysis method, the rupture of the cell wall depends on the osmotic pressure of the external medium. As I said you know, once you remove the cellular barriers then just need to give the osmotic shock and that will depend on the what will have in the outer medium. In few cases the enzymatic digestion is performed to remove the external cell wall and then in second step the protoplast which is been generated once you remove the cell wall is been disrupted by the gentle agitations.

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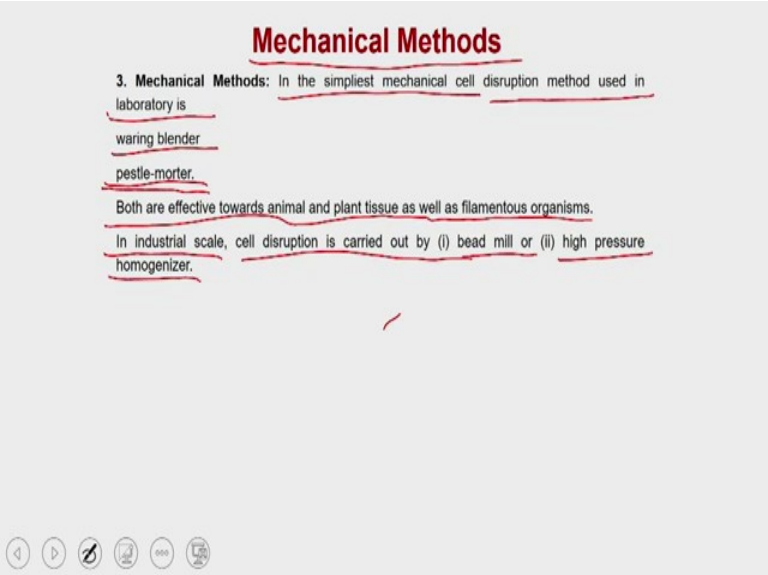


Now let us move on to the third method, the third method is called as the Mechanical Method. The Mechanical Method, in the simplest mechanical method the cell disruption method used in the laboratory is called as the waring blender as well as the pestle-morter. You might have seen waring blender, the waring blender is nothing but a simple mixy what people use in our home. You might have seen that these this mixy people in your mother or your grandmother is always using to prepare the chutney.

So, what you do is, you just take the small pieces of coriander and you put the onion, garlic and all other thing and then you run the grinders then you run the mixy. So mixy is nothing but containing a bowl and then it contains the blades. These blades are actually cutting the cells and actually grinding them and grinding them in such a high speed that it eventually cutting all the cells and giving you a soup actually and that soup is nothing but the cellular content. So the waring blender is nothing but the mixy, where as the pestle morter is the simplest form of the homogenizer.

You might see a pestle morter, a typical pestle morter is look like a bowl which is could be made up of glass or could be made up of ceramics and then you have the (pestle). What you have to do is, you have to put your content which you want to homogenize into this bowl and then you use the pestle and grind them. So in this grinding process what happen is the material is been crushed between the two layer of pestle and morter and as a result when you grind them it actually crush the cells and as a result the content comes out and it becomes a simple paste. Both are effective towards animal and plant cells as well as the filamentous bacteria, whereas in the industrial setup the cell disruption is carried out by bead mill or the high pressure homogenizers.

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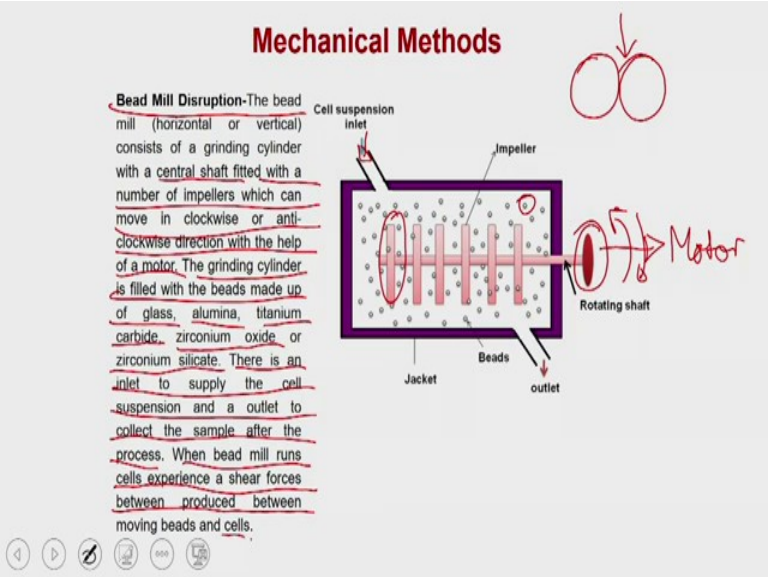


So the bead mill disrupter, so a typical bead mill disrupter is shown here. It could be a horizontal bead mill disrupter or the vertical bead mill disrupter. So what you have in this disrupter, you have a central shaft which is a central shaft fitted with a number of impellers. So these are the impellers. So, which can move either clockwise or the anti-clockwise with the help of a motor. So these you have a central shaft and then you have the impellers which are attached to this and then you can connect to this shaft to a motor.

This motor will allow this to go into the clockwise or to the anti-clockwise. Then what you do is, you take this, you fill this chamber with the grinding cylinder is filled with the beads which are made up of glass, alumina, titanium carbide, zirconium oxide or zirconium silicate. So, then you fill this whole chamber with the beads which could be either glass, alumina, titanium carbide, zirconium oxide or zirconium silicate. So depending on the type of material what you would like to homogenize, you can use or you can choose the beads of that particular type.

Suppose you want to use the gentle cells, then you can use the glass beads but if you are having the plant or some very hard material, then you can use the titanium beads or the zirconium beads. Then what you do is, from the inlet you put your cell suspension and then you let these impellers to move. When the impellers will move, they will also going to move the beads along with that. So, what will happen is, the beads are actually going to move and they will going to run over the cells. So when they will run over the cells or the cell will pass through these beads because, the area is very, very narrow, the beads are going to crush these cells and as a result the cell is going to experience very high level of sheer stress.

Because when the cell is passing through this narrow pass, it is going to experience the sheer stress and as a result of sheer stress the cell will try to expand and the expansion would be uneven or sometimes the expansion would be so much that it is going to lyse the cells. So there is a inlet to supply the cell suspension and a outlet to collect the sample after the process. When the bead mill runs cells experience a shear forces between the produce and moving beads and the cells.

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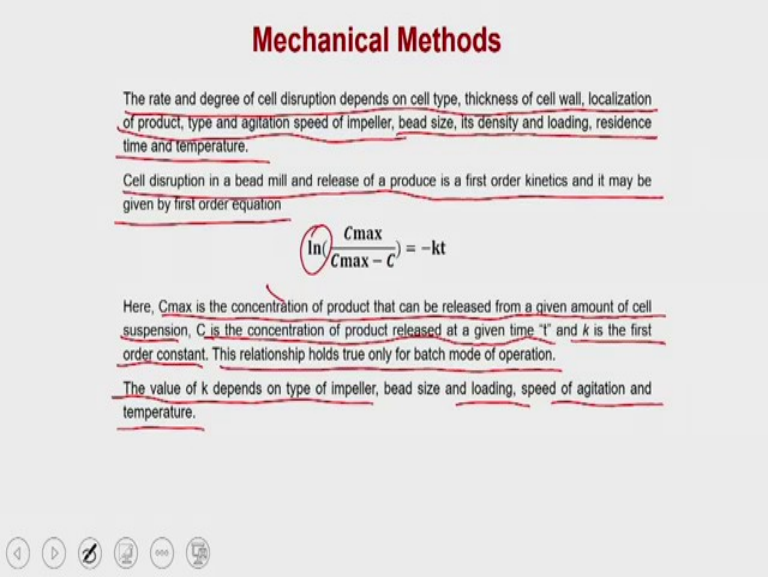


So what will happen is, the rate and the degree of disruption depends on the cell type, the thickness of cell wall, localization of the product, types and agitation speed of impeller, bead size, its density, loading, residence time and temperature. Cell disruption in a bead mill and release of product is a first order kinetics and it may be given by the equation lin Cmax by Cmax minus C is equal to minus KT where Cmax is the concentration of the product that can be released from a given amount of cell suspension.

C is the concentration of the product released at given time T and K is the first order (kinec) constant. This relationship holds only for the batch mode of operation which means it is not the continuous mode of operation. Once you fill that chamber, then you keep running and that actually will maintain this particular type of relationship. The value of k will depends on the type of impeller, the size of the impeller and the loading, the speed of the agitator and the temperature. And depending on the K, you will get the better grinding, better homogenizations.

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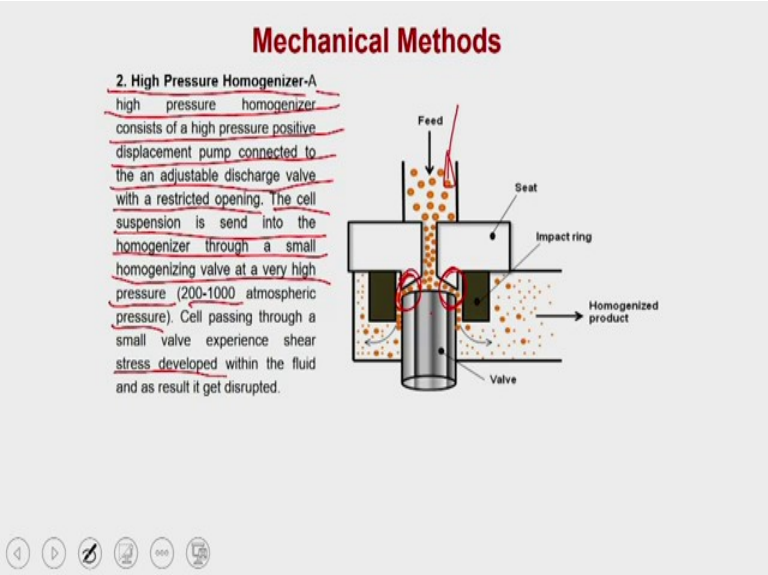


Now, let us go to the high pressure homogenizers. The high homogenizers are consist of a high pressure positive displacement pump connected to the adjacent discharge wall with a restricted opening. So, this is the restricted opening which is present between the wall and the pump. The cell suspension is sending into the homogenizer through a small homogenizing wall at a very high pressure which is approximately 200 to 1000 atmospheric pressure.

So what happen is when you are feeding the cell into this high pressure homogenizer, the cell are passing through this narrow space and they will do the exactly the same when they were passing through this narrow space they are actually experiencing the sheer stress and because they are passing through a very, very high pressure which is up to 200 to 1000 atmospheric pressure the cells are experiencing very high level of sheer stress and as a result the cell are going to be broken down exactly with the same mechanism that, when you put a sheer stress, the cell will try to expand to reduce stress level and in that process the cell is going to be disrupted.

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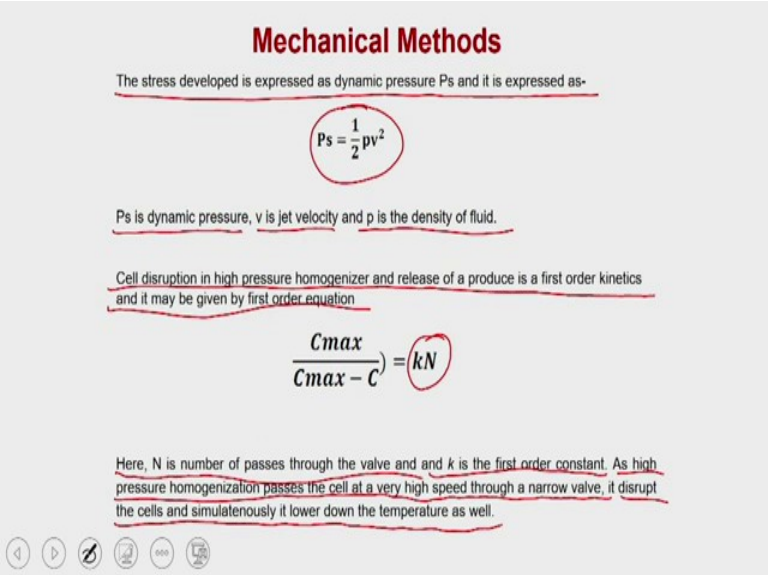


The sheer stress is developed as a dynamic pressure PS and it is expressed as PS is equal to half PV square, where PS is the dynamic pressure, V is the jet velocity and the rho is the density of the fluid which you are using for homogenization. The cell disruption is in a high pressure homogenization and a release of product is also following the first order kinetics with a question known as Cmax divided by Cmax minus C is equal to kN where N is the number of passes through the wall, which means how many times you will pass through this cell suspension to this narrow pass and the k is the first order constant.

As high pressure homogenization passes the cell at a very high speed through a narrow wall it disrupt the cells and the simultaneously it lower down the pressure as well. So that is the advantage. In most of the cases we have seen the cell disruption, we have seen that while the process is going on it actually not giving the sheer stress but at the same time it is increasing the temperature of the media because there is a friction forces also. Where as in this cases, while it is giving the high pressure or passing the liquids through a high pressure it is also making the system cool because it is lowering down the temperature as well.

So, with this we have we have discussed about different cell disruption method which are available and which can be used as per the host as well as the physical as well as the chemical properties of the host and there are different advantages as well as the disadvantages of all these methods. So depending on the requirement as well as the infrastructure, you can choose either of these methods and you can use them into your laboratory to extract or release the content from the cells which are having the over expressed proteins or over expressed material. So with this we would like to conclude our lecture here. In our next lecture, we are going to discuss about how to purify this product which you have taken out from the cells. Thank you.