Downstream Processing.
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Lecture - 07
Cell Breakage (Continued)

In the previous lecture, I introduced the various techniques that are used for breaking the cells, whether it is a bacterial, fungal or yeast, animal and so on. We will continue with the same topic this class as well. So, there are many different ways of breaking cells as I introduced in the last class. Mechanical methods are there, chemical methods are there, thermal methods are there.

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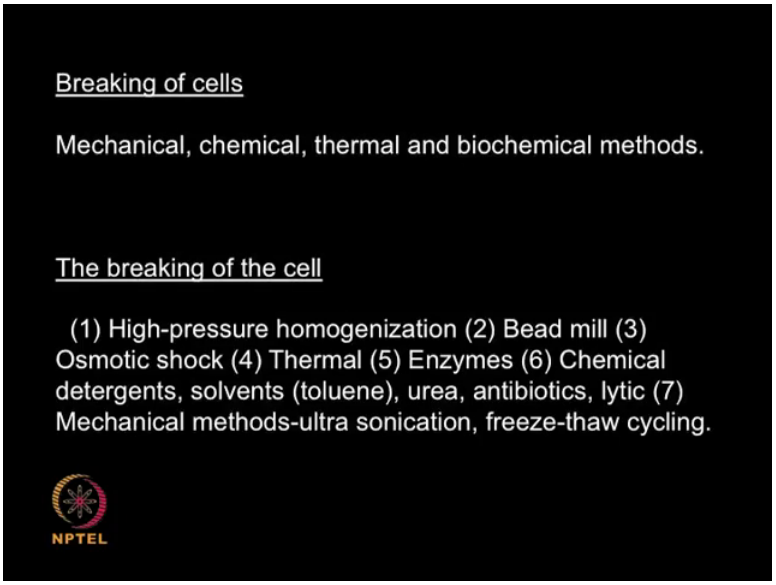

Each has advantages, disadvantages and each can be used for certain types of applications or purposes. So, to sum up, you can use high pressure homogenization, you can use bead mill; you can use osmotic shock, high temperatures. You can use enzymes to destroy the cell walls. You can use chemical detergents like solvents, toluene, urea, antibiotics. Mechanical methods like ultra sonication; even freeze thaw cycling type of methods you can use. Let us look at some of them, which we have missed out in the previous classes.

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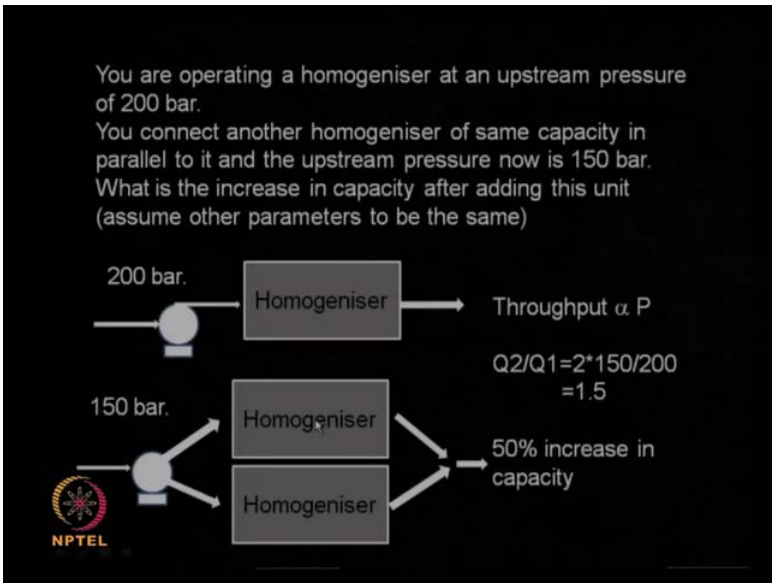

Let us look at a problem before we go further. Imagine you are operating a homogenizer. As you know, homogenizer operates at high pressure, so you are using 200 bars. This cell slurry is pumped inside the homogenizer and the outlet from the homogenizer comes out with broken cells and metabolites containing all the intracellular material. Now, imagine that you are putting another homogenizer of same capacity in parallel to the one that is already existing.

So, because you are putting that extra homogenizer, what happens is the pressure here goes down. So, it becomes 150 bar. So, you are connecting another homogenizer of same capacity in parallel. Now, the pressure goes down to 150 bar. Now, what will be the increased capacity after adding this homogenizer in parallel? So, we have not changed the pump. We have just added one homogenizer. So, because of that, the pressure has gone down. So, capacity is going to increase, but definitely it might not be double the capacity because the pressure has gone down.

So, what is the most important thing? Capacity is directly proportional to pressure. Capacity is directly proportional to pressure. So, here it is 200 bar, here it is 150 bar, but here you have two homogenizers of same capacity. So, what will be the increased capacity after I put in an extra homogenizer of the same type in parallel? So, Q is directly proportional to pressure. So, when I add two homogenizers, I would have multiplied by two, but the pressure has gone down from 200 to 150. So, if you consider that, what

happens?

Throughput is directly proportional to pressure. Agreed? So, Q2 is the capacity of this particular system divided by Q1 will be equal to directly proportional to pressure. So, I have put 150, 150 bar because that is 150. Here, I have put 200 bar because that is the pressure here. Then, I have put in a multiplication of 2 here because there are two homogenizers in parallel. Do you understand? This Q2 is the capacity of this particular system; Q1 is the capacity of this particular system. So, Q2 by Q1 will be equal to I have put a 2 here because there are two homogenizers.

Now, the pressure here is 150 bar, pressure is 200 bar. That is why; I have put 150 here and 200 here. Understand? So, when I do that, I will get 1.5. So, what is happening? The throughput increases by only 50 percent. So, it is not actually increasing twice. Although I have put in a same homogenizer in parallel, I am not getting two times the capacity increase because the pressure has gone down from 200 to 150 bar. This is because it has gone down from 200 to 150 bar, so I am getting only a 50 percent increase in capacity.

So, if I wanted two times increase in capacity what do I do? I need to increase the pressure of this pump. If I use the same pump, obviously the pressure will go down because I have to pump through two homogenizers. Once the pressure goes down, my throughput also goes down.

That is why; I am getting only a 50 percent increase in capacity. Understand? Another important assumption we have made here is the pressure here is 0 that means it is atmospheric pressure. That is why; we have to taken in only 150 and we have taken in 200 here so that means the pressure in the downstream is assumed as 0. So, the most important equation here is throughput is directly proportional to pressure. That is the most important equation in this whole problem.

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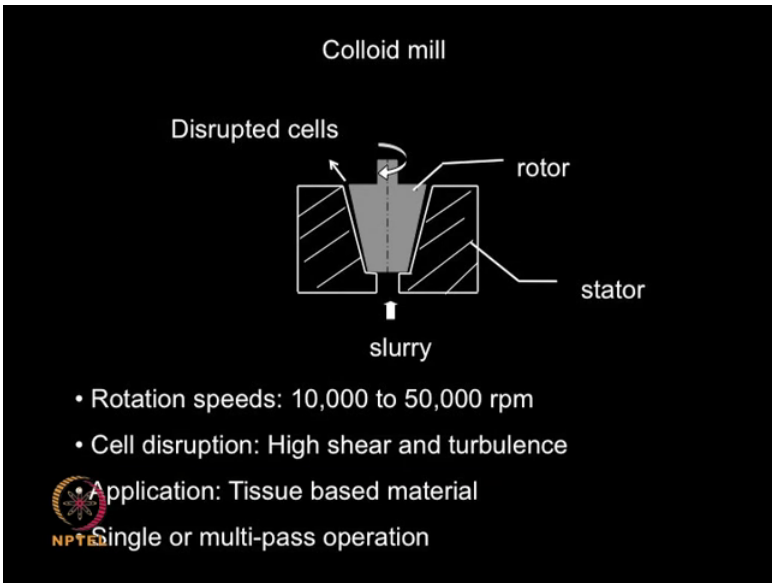

Now, there is another system, which can also help you to break the cells. It is a mechanical operation. This is called a colloid mill. So, how does it work? It has got a rotor here, which is rotating at very high speed. This is the rotor. It is rotating at very high speed and there is a stator. So, the whole unit is like a cone with a very small gap in between. So, your slurry with the cells is entering this gap and because the rotor rotation, which is at high rpm, there is a shear created on the cells. The cells break because of the high shear.

So, because there is a very small gap between the rotor and the stator, the shear created is also extremely high. So, as you can see, it rotates at almost 10,000 to 50,000 rpm. So, the shear created also is very high. It also creates a very high turbulence because the slurry is pumped through this small gap. So, a high turbulence is created. So, the cells break because of the high shear as well as because of the turbulent motion.

So, we can operate this at single pass or multiple pass. So, I can pass this slurry through this. Once it is coming out after the shear force, I can have a cooling system, I can cool the slurry. If necessary, I can remove the part of the liquid which may contain my metabolites. Then, I can put the slurry back.

So, I can have several passes, single or multiple passes. Generally, it is used for tissue based material so where we are interested in high shear and high turbulence for breaking the cells. So, this is another mechanical type of device, which can be used for cell

breakage.

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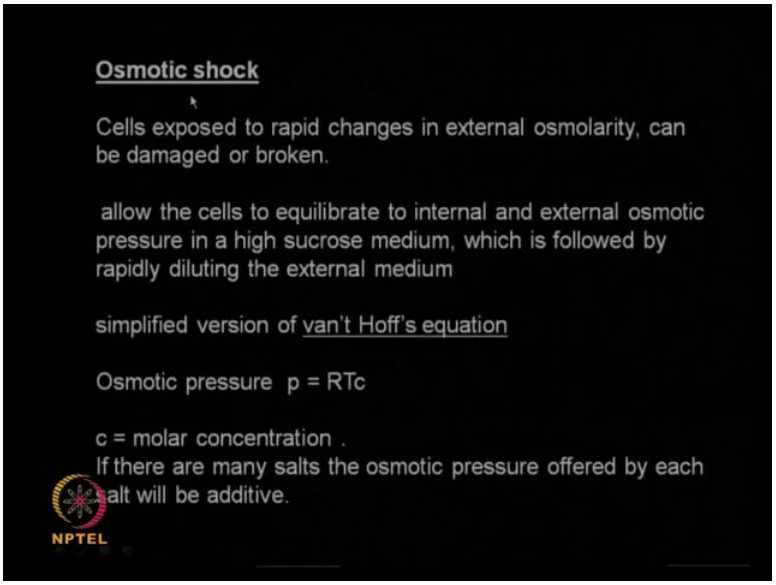

The next method, which is not mechanical, is called osmotic shock. It is based on the concept of osmotic pressure. So, when cells are exposed to rapid changes is in external osmolarity, the cells take much longer time to adjust itself between the internal and the external pressure. So, there is a breakage of the cell wall. So, what do you do is how do you create this high osmotic driving force? I can have a very concentrated salt solution, either inside the cell or outside the cell and I can have just water on the other side. So, there is a very large osmotic pressure created.

So, how do you do this type of osmolotic shock or osmotic shock? So, what you do is you first allow the cells to equilibrate in internal and external osmotic pressure like I can have a sucrose solution. So, inside the cells, the sucrose solution diffuses; outside the cells also, you have the sucrose solution. So, the whole system is at equilibrium. Now, you suddenly put water in the external medium. So, inside there will be sucrose; outside will be water. So, a very large osmotic pressure is created and because of that, the cells can break. So, this pressure depends upon the concentration of the salt that is present.

If you look at a equation called Van't Hoff's equation, you would have all studied Van't Hoff's equation in your thermodynamics, may be in a second year B. Tech or first year MSc, you might have studied this Van't Hoff's equation. So, the equation says that the osmotic pressure is equal to, it is simplified version, R into T into c. R is your universal

gas constant, T is your temperature and c is your molar concentration of the solute present. So, if there are many salts present, then you will have additive effect, salt one, salt two, and salt three and so on actually.

So, there will be osmotic pressure because of salt one, there will be osmotic pressure because of salt two, there will be osmotic pressure because of salt three and so all of them get added up. So, you can use this equation to calculate approximate osmotic pressure. So, osmotic pressure is equal to R into T into c. We will look at a problem later actually.

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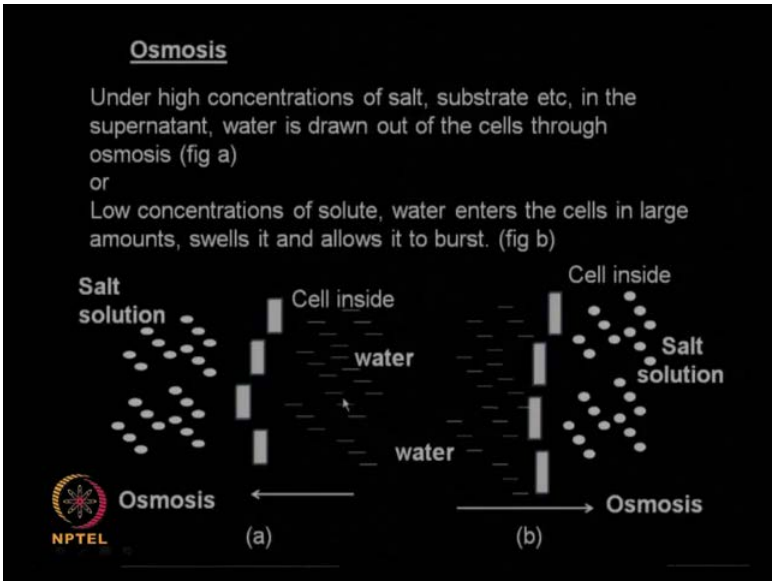

So, this sort of pictorially tells you how the concept of osmotic pressure works on it. So, outside you have a salt solution and inside you have water. So, the pressure here, pressure is much higher than this because you have added salt. The vapor pressure decreases. So, if you do this type of difference in pressure suddenly, the water will try to come out of this because of the pressure here. So, the cells can break. That means water is drawn out of the cells through osmosis. That is what this is.

Now, if you look at the other situation where you have a salt, concentrated salt solution inside the cells and you have water outside and vapor pressure of water is much higher than the salt solution, so the water tries to enter inside. So, the cells swell and then it bursts. So, both the situations, so you have salt outside, water inside. So, water is coming out. So, water is drawn out from the cells. So, it just collapses. The cells collapse.

In the other situation, you have salt solution inside the cells. You have water outside. So, the water tries to enter inside and it is, the cells get swollen and the cells break. So, in both types of situations, if this operation is done in a very dynamic condition, it can break the cell. This is how the concept of osmotic breakage works.

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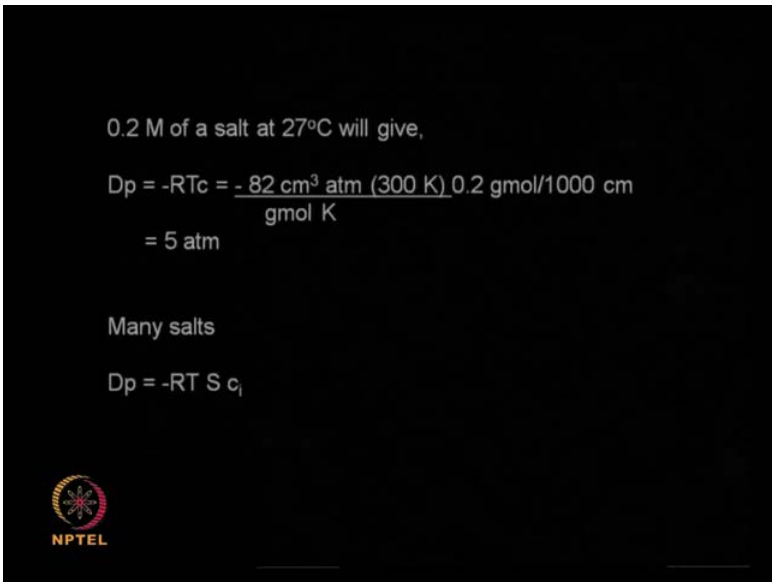

Let us look at a simple problem. Imagine I have a salt of 0.2 moles at 27 degree centigrade. So, the delta p, you calculate as RT c. So, R is the universal gas constant. You have to take the correct R depending upon the units. Do not forget. So, T is say 27 degrees. So, that comes to 300 K. Agreed? So, you have to take the correct R, which is 82 centimeter cube atmosphere divided by gram mol per K. Then, here you have 0.2. 0.2mol is point two gram mol per 1000 cc, 0.2 gram mol per liter. So, if you multiply all, you get 5 atmosphere.

So, you see a small concentration of salt 0.2 moles of salt can generate almost 5atmospheric osmotic pressure. So, if I have another salt of different molarity, then again I introduce that and that salt will also generate a similar osmotic pressure. So, we can keep on adding the pressure created by various salts to get the cumulative osmotic pressure.

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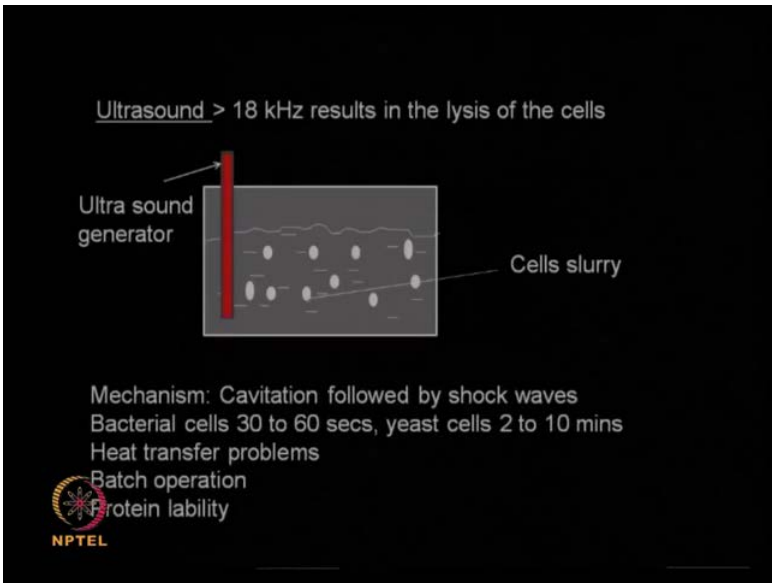

Another technique, which can be used for breaking your cells, is called the ultrasound. Ultra sound ultrasound above 18 kilo hertz can help in breaking the cells. So, what you have is you have a container and you have the slurry that means cells and the liquid or water is inside. Then, you have ultrasound generator is there. So, this generates ultrasounds, which gets transmitted inside the cell slurry. The cells feel the high energy and because of that, they break.

So, basically the mechanism is through cavitations or through shock waves. You need to use different time periods of ultrasound energy for different types of cells, say for bacterial cells it is enough if you do the ultrasound treatment for about 30 to 60 seconds. But, if you have yeast cells, which are very tough, you need time in minutes. You can see you need very large minutes also, whereas for bacterial cells, you do not have to go beyond 1 minute. By that time, the cell walls will break. Some of the problems in ultrasound are heat transfer problems. That means the heat generated inside the slurry are the bath is high.

So, if you use the ultrasound equipment for a very long time, you may be generating very high temperatures. If there are proteins or enzymes present, they can get deactivated. This is very good for batch operation. It is not very good for continuous operation. That means I take slurry in a vessel. Then, I apply the ultrasound and after so many minutes, I discharge the slurry. Then, again I charge another slurry system and then continue the

operation. So, it is ideal for batch operation. You cannot do it for continuous operation.

The third problem is protein liability. That means the protein can get denatured because of the shock waves or because of the cavitations, because of the high temperature or hot spots generated. So, you need to be very careful about this problem.

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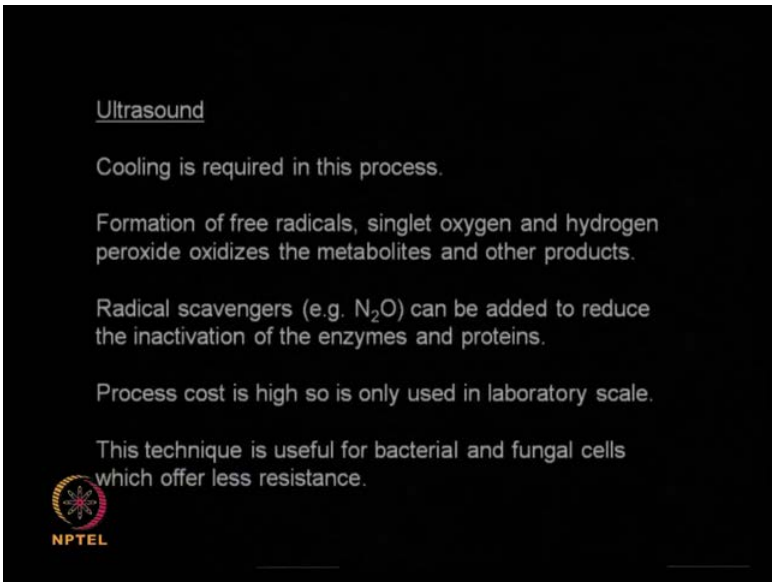

So, we need to have cooling in this type of situations. Another problem is it can even generate free radicals, singlet oxygen. So, if you get singlet oxygen, you may be getting hydrogen peroxide. Hydrogen peroxide, as you know, it is a very strong oxidizing agent. So, it can start oxidizing the metabolites. You may have some metabolites and you may end up having an oxidation product, which is not desired.

So, you need to be very careful about it because ultrasound produces radical. So, you need to add radical scavengers, example N2O. So, by adding radical scavengers, the radicals that are generated by the ultrasound treatment can be captured, but then the problem is how you remove the radical scavengers after you do your cell breakage. So, that is an issue, which you need to keep in mind. The process cost is high in ultra sound treatment.

So, generally it is used in laboratory scale application and not in a full scale industrial production. So, small scale applications, labs, if you are performing fermentation and if you are interested in recovering some intracellular products in lab scale application,

ultrasound is a very good treatment.

But, then in a large industrial scale, when I am talking in terms of thousands of liters, it is not a very good technique because the energy cost will be very high and generating such large amount of energy through ultrasound for large capacity tanks is always a big problem. It is also good for bacterial and fungal cells, which offers less resistance. So, it cannot be used for plant cell, which are very tough. So, it is ideal for these types of cells only.

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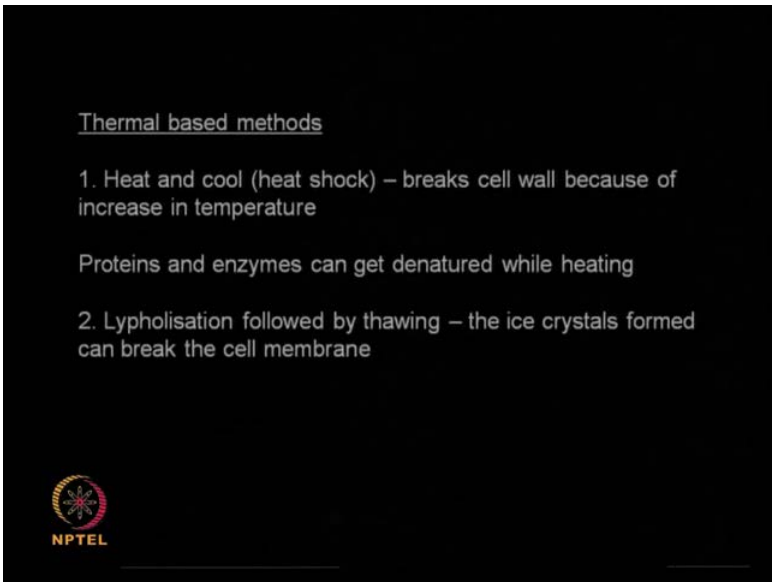

Thermal based methods, so we looked at some physical techniques, we looked at some mechanical techniques, and we will look at couple of thermal based methods. One is called the heat shock method. That means heat and cool. So, heat the cells to high temperature and then cool it like that you know. When the when the cells suddenly come across this type of shock, the cell wall breaks, but the problem here is of course, temperature. You may be heating it up too much. The protein or enzyme can get deactivated. We will look at a problem later on the effect of temperature on denaturalization of enzymes.

So, that is a big problem. Heating and cooling is quite a simple method. You just take the contents of the cell slurry and then heat it up and then suddenly cool it, again heat it up and so on. But, then you may be denaturing the protein or the bio molecule. Another method is lypholisation followed by thawing. That means you cool it. Then, again

convert bring it back using thawing. So, the ice crystals that are formed can break the cell membrane. So, this also is a good method. So, you completely cool it, solidify it and again bring it back to the normal condition. In that process, the ice that is formed can really break the cell walls.

So, this is really a good method because you are not using high temperature. So, your bio molecule will not get denatured, but then energy cost is also very high. But, then there are systems, lypholising systems and thawing systems are available, which can help you to break cells of small quantity.

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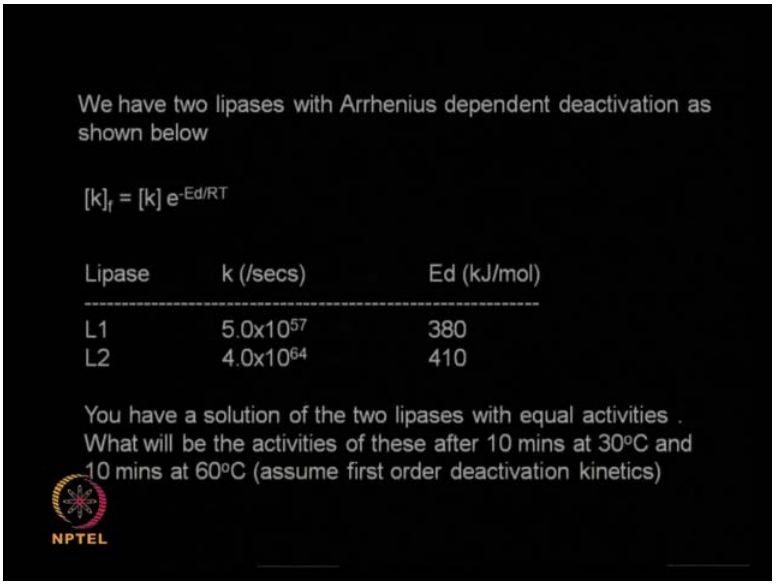

Let us look at a problem related to the deactivation of an enzyme when we go into higher temperature. Generally, we can assume an Arrhenius type of a deactivation parameter. See this is a typical Arrhenius type of relation. You might have all studied in your school itself. The k that is the rate constant and then you have another rate constant, the actual rate constant that will be dependent upon the temperature in this fashion e power minus Ed by RT, where E d is your deactivation activation energy.

So, if the temperature goes up, the deactivation is going to be much higher. If the temperature is low, the deactivation is low. That is a normal thing. Imagine I have two lipases. You know what lipases are used for. Lipases are used for esterification reactions, trans esterification and so on. So, lipase is good for converting oil and fat into esters.

So, we have two lipases. The deactivation activation energies are given like this, Ed in kilo joule per mol for lipase 1 it is 380, for lipase 2, it is 410. The rate constant is given like this in per second, 5 into 10 power 57 per second, 4 into 10 power 64 per second. Now, we have a solution with both these lipases with equal activities? What will be the activities of these after 10 minutes at 30 degree centigrade and 10 minutes at 60 degree centigrade? We will assume first order deactivation kinetics.

So, what does first order deactivation kinetics mean? It means the rate of decrease in the activity will be directly proportional to the concentration. That is what a first order deactivation kinetics means. That means dc by d t is equal to minus k into c, dc by dt equal to minus k into c. That is the first order rate constant and the k that is the rate constant depends on the temperature this way. Understand?

So, we are supposed to estimate the activity of these two lipases, which deactivate with these values of activation energies after 10 minutes when they are kept at 30 degrees centigrade and when they are kept at 60 degrees centigrade. Understand? So, the first step is to calculate their actual k at 30 degrees centigrade as well as at 60 degree centigrade using this data.

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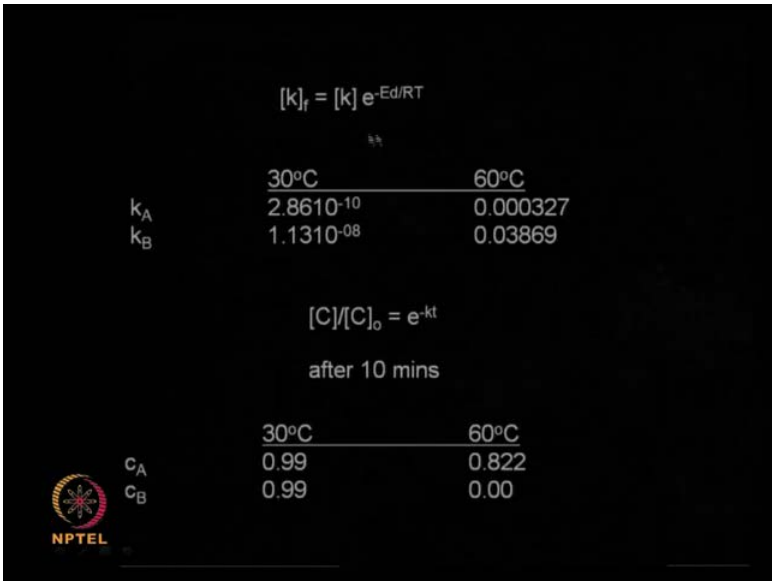

So, as you know, this is deactivation kinetics. So, if you take lipase one, the rate constant as kA, lipase two rate constant at Kb. So, at 30 degree centigrade, what do I do? Here, I put 30 that is in Kelvin and when it is 60 degrees, I will put 60, convert it to Kelvin. E

values for lipase one and lipase two are given. E value for lipase one and lipase two are given. Similarly, the k also is given. So, I can substitute here. I can calculate the actual k by substituting those values.

So, for the first lipase at 30 degrees, I get the k as this and for the second lipase at 60 degrees, I get the k as this. Similarly, for the second lipase at 30 degrees, I get the k as this and for the second lipase at 60 degrees; I get the k as this. So, you can see as the temperature increases, k value also increases. Do you see that? Now, I mentioned that the deactivation follows a first order deactivation kinetics. That means for a first order reaction, this is a typical equation. You must have all studied this long time back in your reaction kinetics.

So, C by C naught equal to e power minus k t. t is your time, k is your rate constant, first order rate constant, C naught is your initial concentration, C is your concentration at any time. So, all we have to do is we put t as 10 and k will be either this or this for lipase one at 30 or 60 degrees and either this value or this value for lipase two at 30 or 60 degrees. So, once I put it, I will be able to calculate the C value here. So, that is what I do. So, if I want to calculate for lipase one at 30 degrees, I will put t as 10 minutes and then k value, I will put it here.

So, I get 0.99 and for lipase two, if I want to calculate the concentration at 30 degrees, so I will put this k value here and t will be 10. Then, I calculate C by C naught. If I take C naught as 1, I will get C as 0.99. So, you see at 30 degrees, deactivation is very little. So, it is almost 1 and the concentration of lipase one and lipase two at 30 degrees is almost same. Understand? At 60 degrees, if I take this as the rate constant for lipase one and if I take this number as rate constant for lipase two and I calculate C assuming C naught as 1, I will get like this. So, what does this mean?

It means the lipase one at 60 degrees centigrade after 10 minutes will have an activity of 0.822, whereas the lipase two after 10 minutes activity has become almost 0. See a large change has happened at 30 degrees centigrade after 10 minutes. The activities of both the lipases are almost same. That means concentrations of both the lipases are almost same, but at 60 degrees centigrade, lipase one has lost some activity. It has come from 0.99 to 0.82. But, look at lipase two. It has come from 0.99 to almost 0. Lipase two has completely lost its activity. The concentration has become 0.

So, temperature makes a large difference. So, from moving from 30 to 60 degree, a dramatic difference has been seen on lipase two when compared to the lipase one. Lipase one has lost activity from 0.99 to 0.82, whereas lipase two has lost activity from 0.99 to become almost 0. So, it is all because of the Arrhenius type of behavior for the deactivation of the lipases. So, temperature that is how affects deactivation kinetics. Generally, it is reasonable to assume Arrhenius type of dependence for deactivation of proteins and enzymes.

We generally assume a first order type of relationship for the deactivation kinetics. So, these two equations are valid for most of our enzyme deactivation kinetics. So, all we need to know is the Ed value, the deactivation energy and the k value.

So, once you know, we can tell what will be the activity of any enzyme at different times, at any temperature. Understand? So, we can easily find out. Now, this method can also be used for removing one enzyme when you have a mixture of two enzymes. Suppose I had enzyme a and enzyme b. I am interested only in enzyme a. So, what did I do? I raised the temperature to 60 degree centigrade, enzyme b completely went away. I am left only with the enzyme a. So, you see this is a good way of separating these two enzymes. I am interested in enzyme a. I am not interested in enzyme b.

So, I just raise the temperature from 30. At 30, I have equal amounts of enzymes, 0.99, and 0.99. When I increase the temperature from 30 to 60, I lost some activity for enzyme a because it has come down from 0.99 to 0.82, but enzyme b has completely disappeared. Now, I have only pure a. So, I can use thermal based method for purification. If the difference between the deactivation energies is reasonably high, it is a very good method. It is a very cheap method.

All I have to do is just little bit heat it. Enzyme two totally gets deactivated. I will have only enzyme one, deactivate enzyme, which I am interested in. But, the main point is there has to be some sufficient difference between the deactivation energies, otherwise if the deactivation energies are almost same, if enzyme a loses activity when I raise the temperature and enzyme b also loses at the same value, then I will not be able to separate out. But, this is very dramatic. You can see 0.99 to 0.82 and here 0.99 to complete 0. That means enzyme two has completely got denatured. So, once it has got denatured, it will just precipitate out. So, only enzyme one will be inside my broth.

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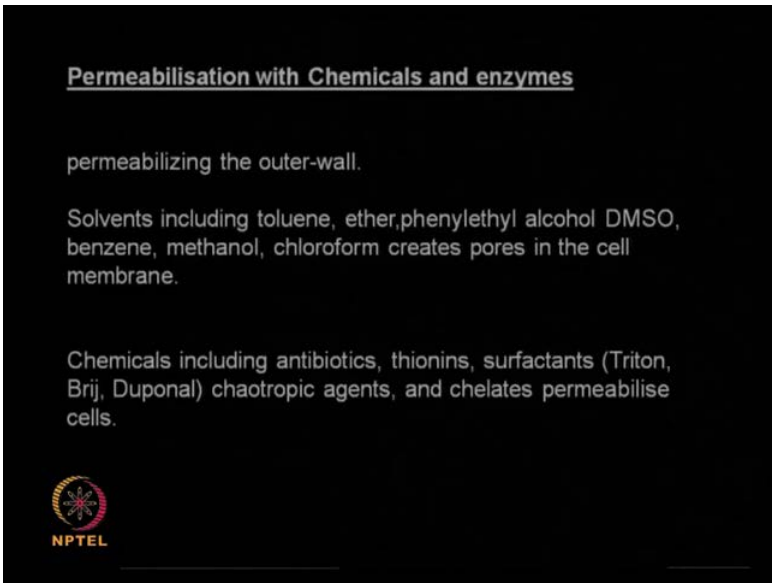

Now, let us go to other techniques in cell recovery and a breakage to extract an intracellular material. This method uses chemicals or enzymes. So, you use chemicals, which will permeabilize or make the cell wall porous. So, it can permeabilize the outer wall. So, sometimes people use solvents, toluene, ether, phenyl ethyl, alcohol DMSO, benzene, methanol, chloroform. So, all these solvents can permeabilize or create pores in the cell membrane. Once you create pores, slowly the intracellular material leaks out and then comes out to the external region. This is also a very mild method.

It is a good method because unlike your mechanical methods or thermal methods, where you are heating and you may be worried about deactivation of the bio molecule, here the method is so mild, you are not creating any high temperature or shock or sonication, but only problem is these chemicals should not deactivate or inhibit the protein or bio molecule, which you are looking at. That is the only point you need to keep in mind. So, for example, the ether should not deactivate the enzyme or protein, which you are trying to recover.

Just like solvents, we can also use chemicals to permeabilize the cell walls. We can use antibiotics. We can use thionins, sulphur based compounds, surfactants, anionic surfactants, cationic surfactants can be used. These surfactants can go and bind to the charged groups of available on the cell wall and disturb the cell wall integrity, thereby breaking it and allow the intracellular material to come out. We can also use chelates,

even chaotropic agents, all these can be used to permeabilize the cell wall.

So, here they either eat away the cell material or they disturb the charged anions and cations present on the cell, which keeps the cell integrity safe. So, that is what these chemicals do, whereas the solvents create pores in the cell membrane and allow the intracellular material to reach out.

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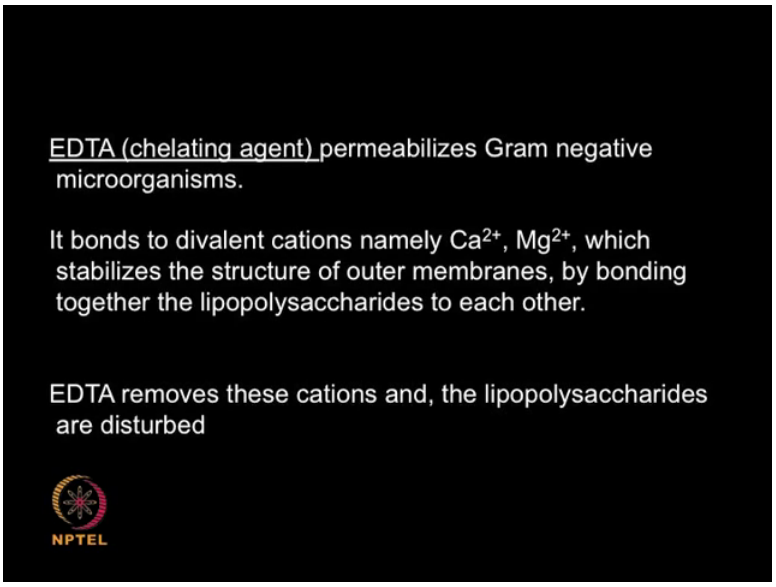

EDTA is a very common permeabilizer. It is chelating agent. It is very good for gram negative organisms. So, what it does is it binds to divalent cations like calcium, magnesium. These calcium and magnesium are present on the cell surface. It stabilizes the structure of the cell surface, especially the outer membrane.

They bond various lipopolysaccharides together. They keep the lipopolysaccharides together. So, when EDTA goes and removes these calcium and magnesium, so the bonding of lipopolysaccharides gets disturbed. So, they fall apart. So, you create pores in the cell membrane. So, any chemical or any chelating agent, which goes and binds to these calcium and magnesium, is a very good agent to create pores in the cell wall and EDTA is one among them actually.

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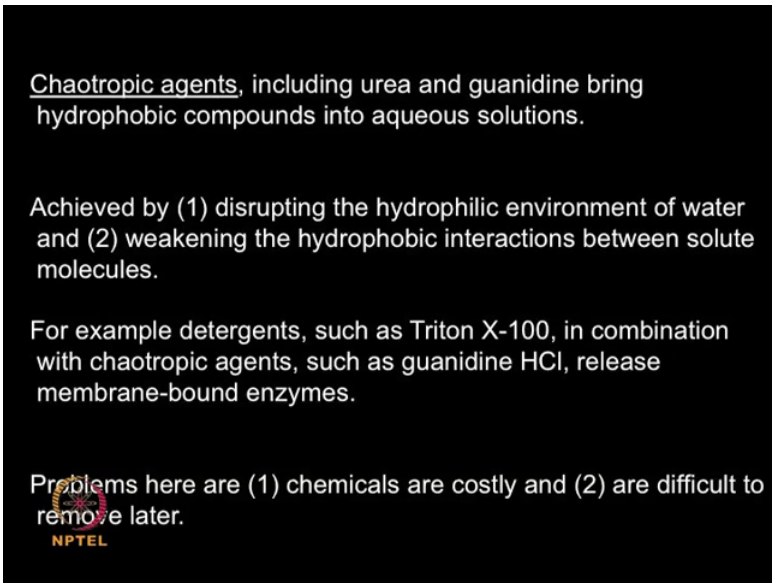

Then, you have chaotropic agents. We can use urea, guanidine. Uracil is another agent. So, here urea and guanidine, they bring the hydrophobic compounds into aqueous solutions. Basically that is what they do. They bring hydrophobic compounds into aqueous solutions. So, how do they do it? They disrupt the hydrophilic environment of water or they also weaken the hydrophobic interaction between the solute molecules. So, by doing that, all the hydrophobic compounds come into the aqueous medium actually.

So, suppose I have a triton X 100. Triton X hundred is a surfactant. Then, I use a chaotropic agent like guandine hydrochloride. They can be used for releasing membrane bound proteins or an enzyme, but then the main problem in these types of agents is that they are very expensive. Once you have done this particular cell breakage, you have these agents and chemicals present with your bio molecule.

So, how do you separate them all? If your metabolite of interest also is a small molecule, how do you separate? That becomes a big challenge afterwards. Although these methods are extremely mild and they do not affect the stability of the compounds, which you are extracting out, but how do you separate them later is always a big challenge. So, that is lot of research in that particular area.

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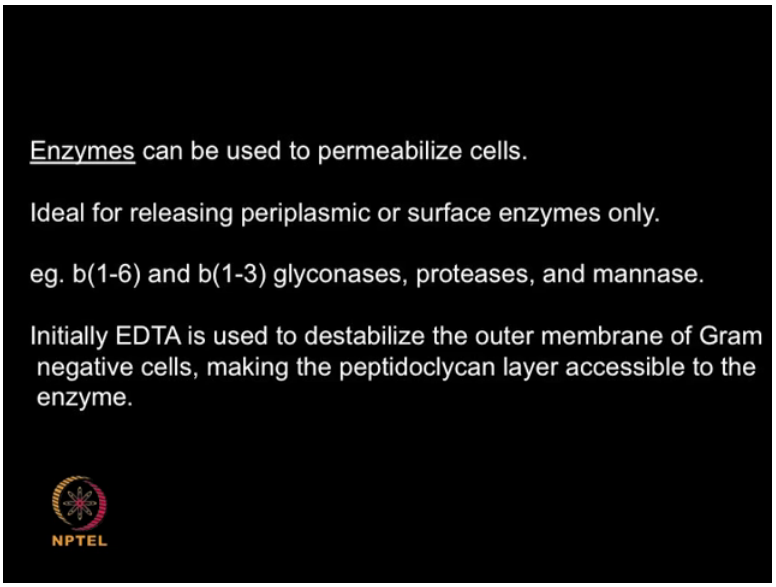

You can also use enzymes for permeabilizing cells. It is very good for periplasmic or surface enzymes actually like beta, beta 1, 6, beta 1, 3, glyconases, proteases, mannoses. All these enzymes, they are very good for that.

So, we can use EDTA. What does EDTA do? It goes and chelates with the calcium and magnesium, disturbs that. Then, we can use these enzymes, which go and eat away all the material on the cell walls. So, enzymes are very good, extremely mild and ideal, but then the main problem with the enzymes is they are extremely expensive. It adds to the total cost of your product recovery from intracellular region. It is very ideal if you are interested in very expensive material, which is produced intracellularly and you want to collect as much as possible without affecting its stability, but then otherwise for bulk chemicals or cheap chemicals, use of enzymes is not very good.

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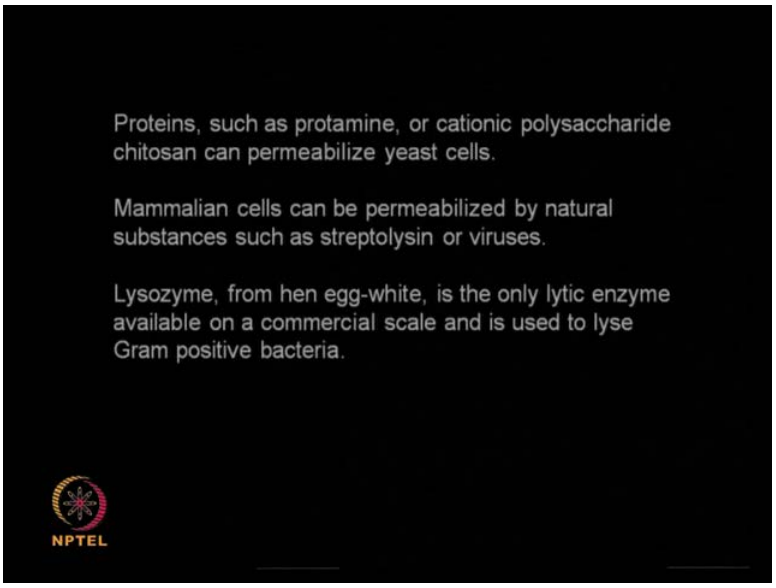

We can also use proteins like protamine, or cationic polysaccharide chitosan can permeabilize yeast cells. If you have mammalian cells, they can be permeabilized using even viruses, very interesting; we can use some virus, which can permeabilize mammalian cells. Lysozyme from hen egg white is also an enzyme, which is called lytic enzyme. That means they break the cell wall and they are also used in commercial scale as well to lyse gram positive bacteria. So, lysozyme is not very expensive. They can be used for breaking the cell walls.

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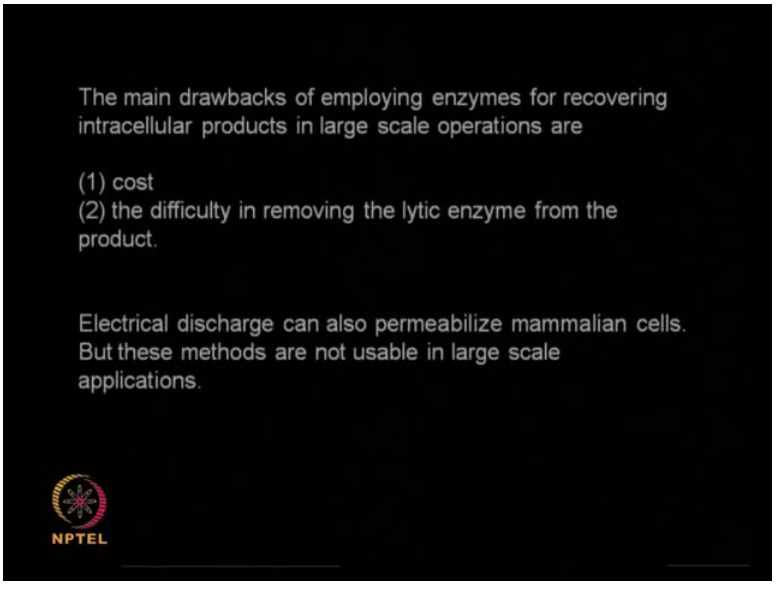

So, the main draw backs as I mentioned are the cost and then difficulty of removing these enzymes after you have done this job. This is because if intracellular product is an enzyme and this you are using an enzyme, then how do you separate both of them? Sometimes even electric discharge can be used for permeabilizing mammalian cells, but then you cannot use all these techniques for large scale applications.

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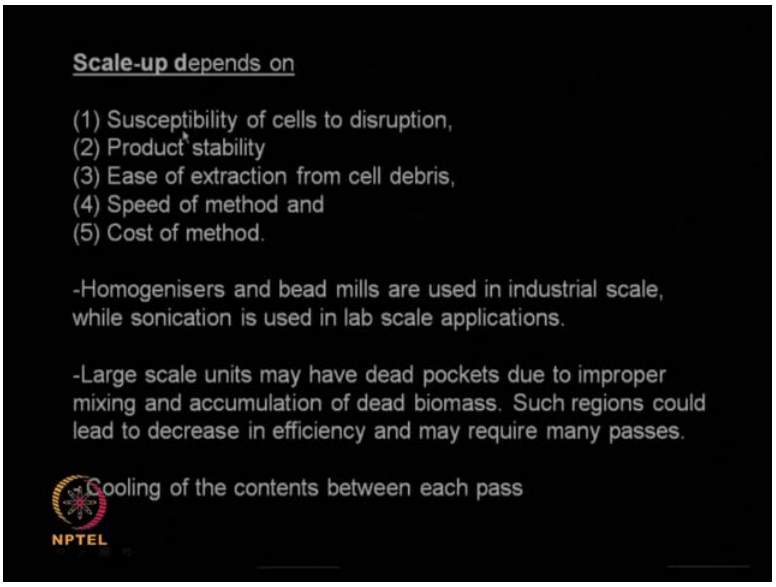

So, if I am looking at scale up from lab scale, I need to see susceptibility of cells to disruptions, product stability, how easy it is to extract from cell debris, speed of the method, how fast I can do this type of operation, what is the cost of the method. So, that is why; mechanical methods like homogenizers, bead mills, they are used in industrial scale, while sonication is used more in the lab scale. Large scale units will have dead pockets because of improper mixing, accumulation of dead biomass.

So, those reasons are very inefficient and you may have to again recycle the whole material again into it to do a better job. So, large scale units have this type of problems. Cooling is also an issue we need to consider in large scale applications. I need to cool after every pass because your temperature rises high and because of the temperature rise, you may have deactivation taking place.

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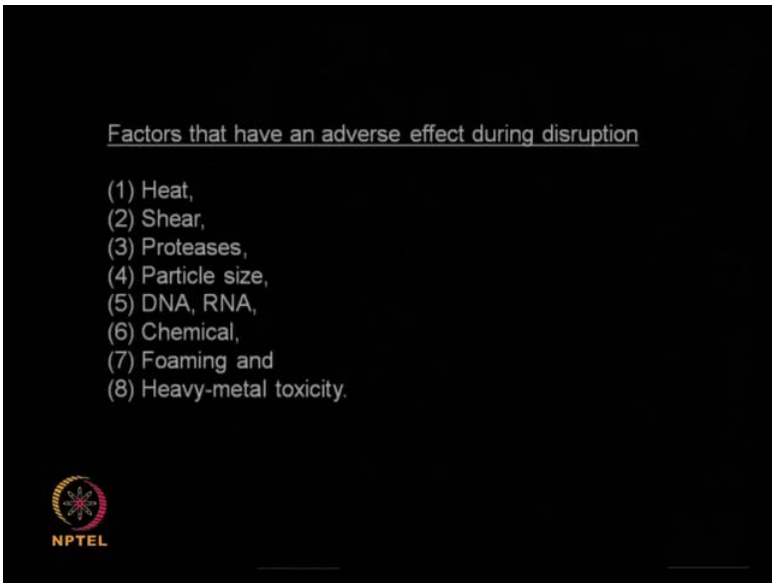

So, you need to consider these factors when you are looking at disruption of cells, heat, and shear. What type of shear forces I need to use? Am I generating too much heat? Am I generating proteases, thereby increasing the viscosity? What will be the particle size that means original biomass size? Am I liberating DNAs and RNAs? Chemicals, what type of chemicals I am going to use? Am I going to get foaming because of this? Is there going to be heavy metal and there is going to be toxicity because of the heavy metals? All these issues need to be considered.

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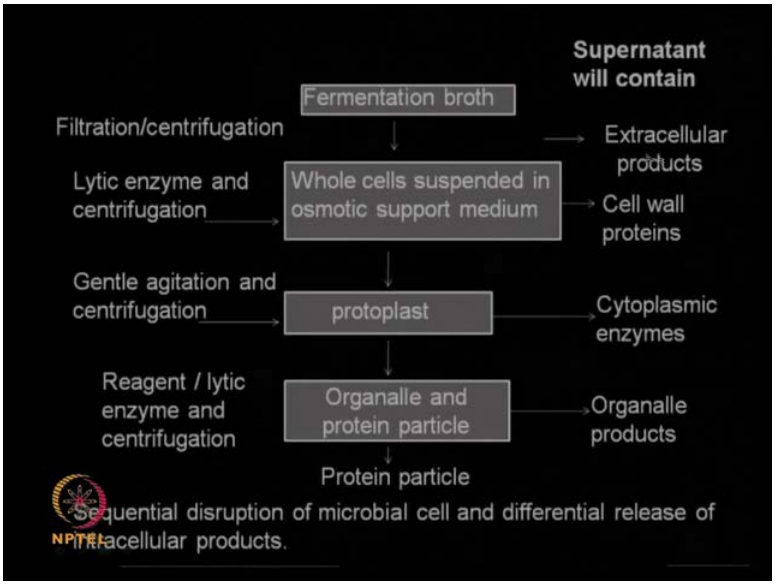

So, we have talked quite a lot. Let me sum up the whole thing. You may have extracellular products. You may have cell wall proteins. You may have cytoplasmic enzymes. You may have organelle products. So, for each one, comes at different situations. That means extracellular products are easily present, immediately collected. Cell wall proteins need little bit of cell breakage, but cytoplasmic enzymes, you need to break more. If it is present inside in organelles, then you need to resort to much higher disruption so that you get the products out.

So, the supernatant will first have extracellular products followed by cell wall proteins followed by cytoplasmic enzymes, and so finally followed by the organelle products actually. This is how a disruption, sequential disruption of microbial cells happens. So, when you have a fermentation broth, mostly it will be extracellular products. We can collect through filtration and centrifugation.

But, if you have an osmotic support medium, may be whatever is present near the cell wall proteins can be recovered. Agitation, centrifugation, cytoplasmic enzymes can be released. But, if you are interested in intracellular organelle products, we need to go for reagents, lytic enzymes, and centrifugations.

Finally, you will end up with the proteins. So, this slide tells you the order in which products due to fermentation come out into the extracellular medium and the difficulty with which you start collecting products as you have your intracellular products right inside the organelles. Each one of these types of products requires different types of a technique and different type of recovery and different type of purification. Each one add to extra cost, the breakage cost, the purification cost, the recovery cost and all these will add up of course, finally, to the overall product cost.