Plant Cell Bioprocessing
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Lecture – 21
Immobilization of plant cells

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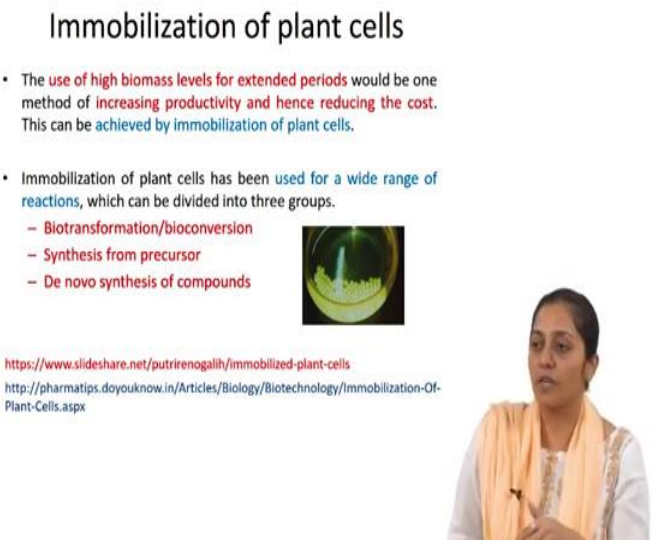

So, one other strategy which can lead to productivity enhancements in plant cell technology is immobilization of plant cells. So, the use of high biomass levels for an extended period, involves a number of cycles. So, it would be one method to increase productivity and hence reduce the cost because, you can use the same biomass for a number of processing cycles. So, this would in turn reduce the cost, this can be achieved by immobilizing the cells. There are different ways in which the cells can be immobilized.

Generally, the kind of reactions which are carried out using immobilized cells involve your biotransformations or your bioconversions. The precursor will directly get converted to your product of interest or synthesis from precursor with a greater number of steps, where the precursor will reach the cells and being a repository of enzymes there will be defined set of reactions leading to the product formation or de novo synthesis.

Which means that as a result of the metabolism of the cell and the development of the cell, the substrate which is added, finally gets metabolized as we have been seeing in free cells into your desired product. So, this is how the immobilized beads look like.

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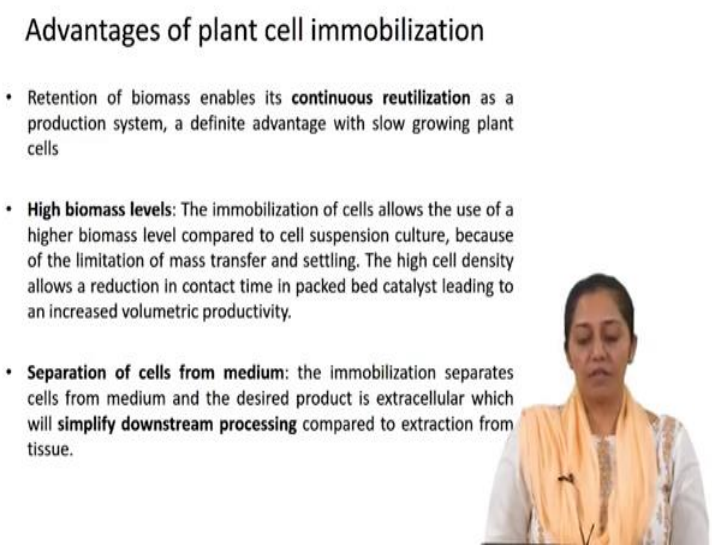

Now, what are the advantages of plant cell immobilization? Continuous reutilization can be done for plant cells. Now here the key or the condition which has to be taken care is the viability of the cells. How many cycles can they remain viable? So, that you can use them or the cells do not leach out from the immobilized beads.

Now, immobilization of cells will allow the use of higher biomass levels for the bioconversion. Generally, for free cells we know there are certain limitations in plant cell fermentations like the mass transfer limitation as they tend to aggregate. So, to get high cell density for maximum productivity and then converting it for product productivity is sometimes limited depending on the species on which we are working.

Now, but in case of immobilized cells, product production phase can be separated from the biomass production phase. Then if you have high cell density than you can use those cells, immobilize them in the form of beads and then make it into a packed bed reactor and use the substrate of the production medium and continuously utilize that high biomass density thereby leading to higher productivity levels.

So, separation of cells from the medium, is one of the advantages of plant cell immobilization, which will reduce your downstream processing cost or which can ease out the downstream processing.

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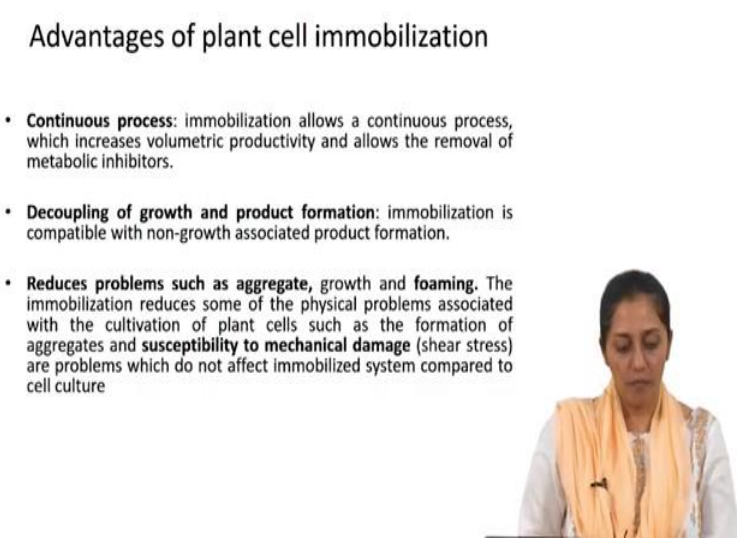

Now other advantages, it thereby makes your process continuous and therefore, increasing the productivity. Continuously you can have the product leached out from the cells in the effluent which is leaving the packed bed.

Decoupling of growth and product formation:

So, actually it works very well for those fermentations where the product formation phase and the biomass formation phase are decoupled. Therefore, in comparison to free plant cell suspensions, it can reduce the problem of aggregate formations, foaming which can happen and the scale up to reach high cell density and the shear effect on the cells. Susceptibility to mechanical damage can be also be avoided.

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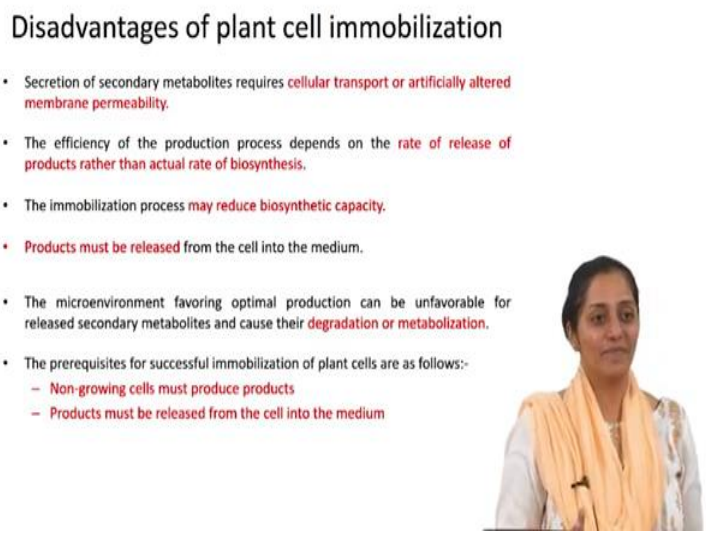

So, there are certain disadvantages also associated. Now what are those? There can be cellular transport or artificially altered membrane permeability. Now what happens is that you need to ensure that the process will be successful only if the product comes out, so which needs the membrane to be permeable for the product. So you need to alter the permeability of the membrane which you may need to work around with the kind of immobilization which you would use.

So, that can incorporate cost or even if you are altering the membrane permeability then the cells may also leach out or the viability of the cells might get affected. Then the efficiency of the production process, now rather than depending on the conversion rate of the substrate to product is found to be rate limiting step and is found to be the rate at which the product is leached out from the capsule or the capsule to the outside medium. So, the rate of release of the product rather than the actual rate of biosynthesis, now becomes the rate limiting step. The immobilization process may lead to reduction in the biosynthetic capacity. What do you think may lead to reduced biosynthetic capacity?

It depends on the micro environment which is around the biomass which is inside that encapsulation. This may lead to the material which has been used or there can be nutrient gradients, gas transfer which may get affected, which may in turn affect the biosynthetic capability of the immobilized cells. Now this is same whether it is plant cell or microbial cell. Products must be released from the cell into the medium that is the prerequisite, the

product has to come out then only the process is continuous and higher productivity holds true.

The micro environment favoring optimal production can be unfavorable for the release of secondary metabolites, which may then cause what? If the rate of release is not able to balance out the rate of biosynthesis, it may lead to product inhibition, which may in turn cause reduction in the biosynthesis or it will not be able to drive the rate of biosynthesis forward.

So, in this way that is the bottleneck, the product has to come out of the immobilized bead. The prerequisites therefore, for successful immobilizations include non growing cells must produce product, which means the growth phase and the production phase have to be decoupled and it has to be a non growth associated product formation. Products must be released from the cell into the medium.

Now when we say that it has to be non growth associated product formation, why it cannot be growth associated product formation in the immobilized bead? Why am I saying decoupling is needed? They will have a limitation, if they start growing then the oxygen demand will keep on increasing. So, then the steps which involve mass transfer and oxygen transfer of the nutrients or the gas will start becoming your rate limiting steps.

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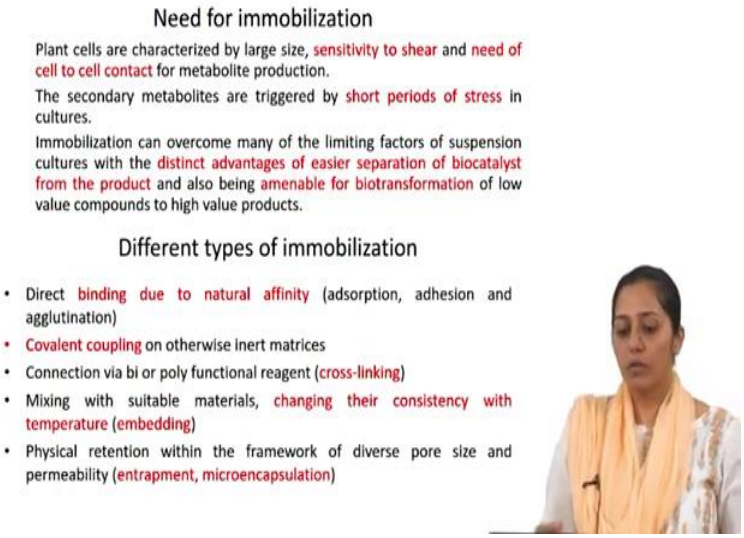

So, as I said the need for immobilization in plant cell fermentation is because of their sensitivity to shear, because of the large size. The biochemical capability is also dependent on cell to cell contact. So, sometimes immobilization gives them biochemical signals which improve the secondary metabolism in plant cells.

Because, you attach to supports may lead to differentiation signals, such that synthesis of the secondary metabolite is induced or accelerated. Immobilization can overcome many of the limiting factors like easier separation of the biocatalyst from the product. So, that is one of the advantages which the immobilization can provide. Plant cells are amenable for biotransformations of low value compounds to high value products.

So, it is found to be very useful in case of immobilized plant cells, where biotransformation can be easily done till the plant cells remain viable in the immobilized beads. So, what are the different ways in which immobilization is done, either directly binding to a natural substance or using natural affinity. For example, adsorption adhesion agglutination, I hope you people know what is adsorption adhesion and agglutination. Agglutination? This is surface phenomena isn't it. So, it involves surface energies. In simple words, agglutination means coming together and thereby,

Student: They form bonds or

There are no chemical bonds formed in this.

What is adhesion?

Attraction of one substance to a similar kind of substance to or to another?.

Student: To different different material.

Fluids, a solid?.

Where do you see adhesion every day? Common example.

Student: Water beads on a surface

Very good.

Cohesion is the attraction : intermolecular forces or intra molecular forces? So, inter molecular forces is the interaction between same atoms and intra would be between

different atoms. So, when it is attracted, is cohesion similar atoms or adhesion similar atoms?

Student: Cohesion similar

So agglutination is also a similar interaction which may lead to same kinds of molecules coming together. Now covalent coupling, this is where chemical bonds will form. Covalent coupling with the inert matrices. So, using functional groups, for example this functional groups may be responsible on the inert surface or the covalent coupling of the cells to the inert surface. Then connection via bi or polyfunctional reagent which is called as cross linking, which means you can have a polymer matrix and your cells.

Now these polymer matrices it is these chains which will join to each other to form a matrix using a cross linker. Now this is like poly functional agents they will have different functional groups which will facilitate joining of these polymer chains. Once these polymer chains interact then they would form a matrix and if the cells are captured within that matrix then they will get encapsulated. Mixing with suitable materials which can change it’s consistency with pH or temperature. example?

Student: Agar

Agar, gelatin. Physical retention within the framework of diverse pore size and permeability which is called as entrapment or microencapsulation, which you people must have done earlier in your lab classes which is like?

Student: sodium alginate.

Sodium alginate.

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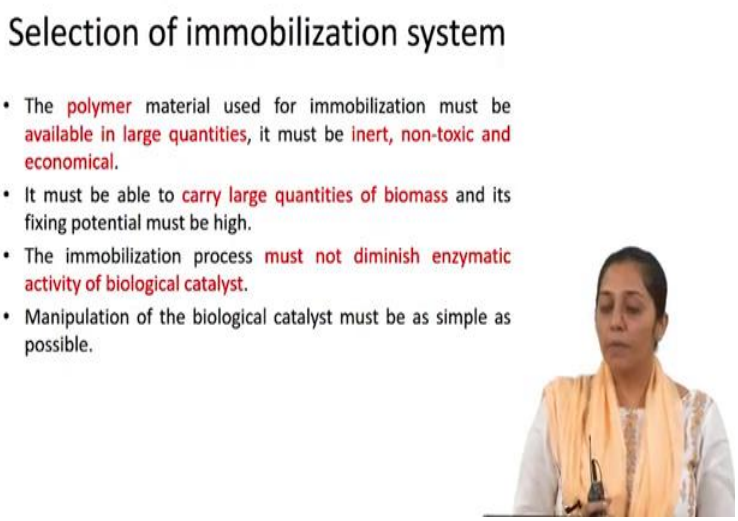

Now the polymer material, which is used for immobilization first must be available in large quantities, easily available. It should be inert such that it does not affect the viability of the cells- the biological material, should be non-toxic to the plant cells and should be economical. It must be able to carry large quantities of biomass and its fixing potential should be high; the retention potential of the cells should be high.

The immobilization process must not diminish the enzymatic activity of the biological catalyst. Let us take an example of enzyme immobilization; you must have already read that the kind of immobilization or the immobilizing matrix may sometimes impact the activity site of the enzyme, thereby disrupting the function of the enzyme.

So, similarly the plant cells are repository of enzymes. So, it should be taken care that the biological activity of the cells is not impacted by its interaction with the cross linker or the inert matrix if it is a chemical bonding. The cells should be easily available for biotransformation reaction. Manipulation of the biological catalyst must be therefore as simple as possible.

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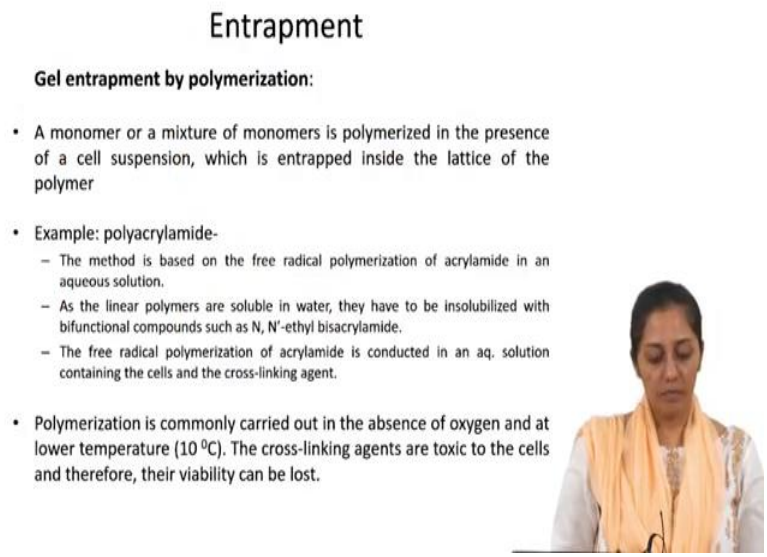

So, what are the different ways in which entrapment can be done? Gel entrapment by polymerization. A monomer or a mixture of monomers is polymerized in the presence of cell suspension, which is entrapped inside the lattice of the polymer, example polyacrylamide gels. Now everybody knows what is polyacrylamide; it is a polymer. So, what kind of polymerization happens to make it into a gel?

It is called as free radical polymerization. Now in this, care is taken that the polymerization process in the presence of cell suspension is carried out in the absence of oxygen and at lower temperatures. So, because the free radical monomer which is responsible to form the free radical polymer may interact with oxygen preferentially and thereby causing an oxygen radical and then the polymer formation rate reduces drastically.

Therefore, it is recommended to be carried out in the absence of oxygen. So, the free radical polymerization of acrylamide is conducted in an aqueous solution containing the cells and the cross-linking agent. Cross linking agent generally used is N, N' ethyl bisacrylamide. So, different polymer chains can be connected with the cross linkers so as to form a lattice or a matrix.

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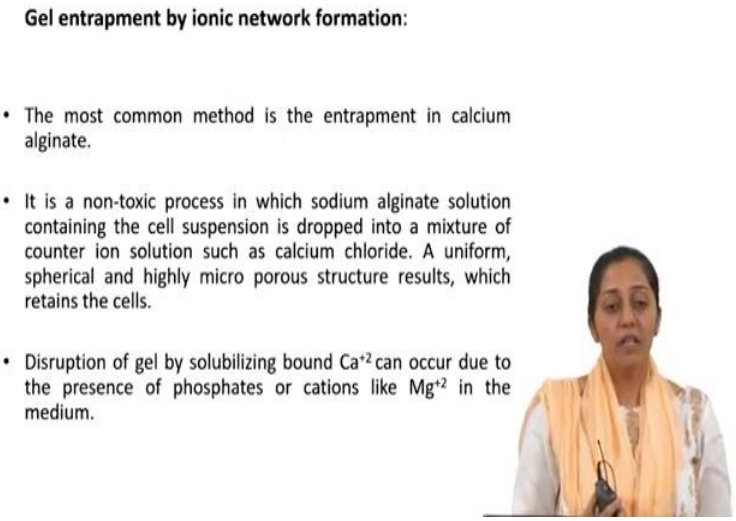

Gel entrapment by ionic network formation, the most common method is entrapment in calcium alginate. So, what happens it is a non-toxic process in which sodium alginate solution containing the cell suspension is dropped in a mixture of counter ions such as calcium chloride. A uniform spherical and high microporous structure will result so disruption of gel can happen if something else can disrupt calcium.

So, that is going to happen if you are using any other divalent ions like magnesium or even people say one should avoid sulphates. So, it may happen which gives us indication that if you are using an immobilized calcium alginate bead for plant cell fermentation, the media composition in which the beads are going to be put in can disrupt the beads depending on the concentration of your magnesium which is one of the major salts used in.

Sulphates, magnesium these are used in your media for plant cell fermentations. So, which means what it may impact the bead structure, so care should be taken.

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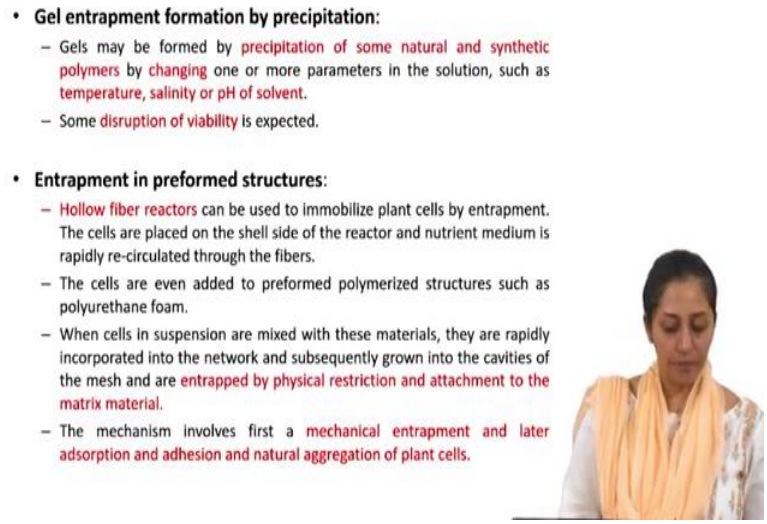

Gel entrapment formation by precipitation, gels may be formed by precipitation of some natural and synthetic polymers by changing one or more parameters in the solution. Like either you change the temperature or salinity or pH of the solution then the precipitation might happen. Some disruption in viability is expected in this process. So, after doing this a check on the viability should be done. So, we have already studied about different methods to check the plant cell viability free plant cell viability. What were those methods?

So, entrapment in preformed structures can also be done in which hollow fiber, an example is hollow fiber reactors, they can be used to immobilize plant cells by entrapment. So, generally they work on the principle of shell and tube bioreactors, there will be a number of very thin fibers and through the fibers the media is sent in and around the fibers, on the shell side plant cells are immobilized.

Now to facilitate immobilization around these fibers even agents like polyurethane foam are used. Now once the cells begin to divide and grow, they will multiply and they will start growing around these fibers. Now these fibers through which the media is flown in they are semi permeable membranes, through which the nutrients can flow from the inner tube to the outer lumen.

And similarly, the product which forms after the bioconversion can diffuse in these fibers and come out of the reactor in the effluent. So, they work on the principle of shell and tube reactors generally shell and tube is this term is used in which cases.

Student: Heat exchangers

Heat exchanger; the cells are even added to performed polymerized structures such as polyurethane foam. So, why do you think the cells are able to stick to polyurethane foam? Have you seen polyurethane foam?

There are different forms of polyurethane form the simplest everyday life those foams which are used for cleaning cars and all spongy material.

So, there are lot of gaps you can see and pores of different size and shapes. So, polyurethane foam because of these pores which are there, the cells first enter these pores and then as they divide the retention happens. So, we use the polyurethane foam for as a support for hairy root cultivations because, being an inert matrix once you inoculate at the time of inoculation they will not stick to it.

But in due course of cultivation time as they begin to multiply you will see that it will stick to the foam. It will enter the pores which are there the porous membrane and they will then stick that how they are used as support materials for plant cells or even for immobilization.

When cells in suspension are mixed with these materials, they are rapidly incorporated into the network and subsequently grow into the cavities of the mesh and are entrapped by physical restriction and attachment to the matrix material. There is no chemical interaction which is happening, no bonding, but it is the physically retention which happens as they grow. The mechanism involves first a mechanical entrapment and later adsorption and adhesion and natural aggregation of plant cells.

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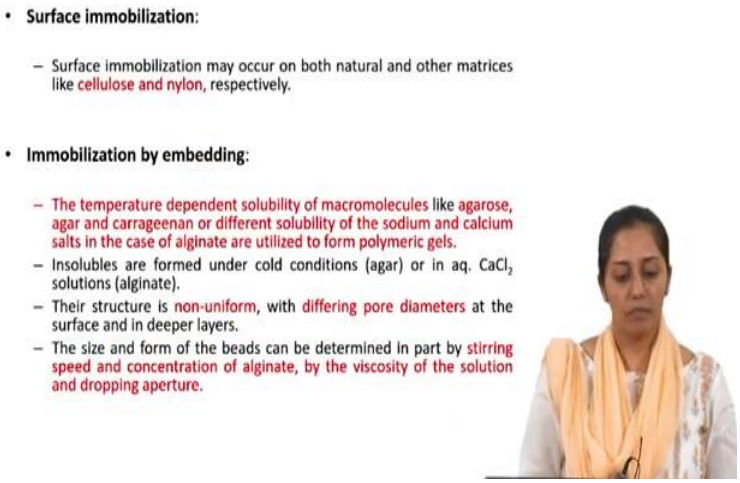

Surface immobilization; surface immobilization may occur on both natural and synthetic matrices like natural would be cellulose synthetic like nylon. Immobilization by embedding this is another way, the temperature dependent solubility of macromolecules like agarose, agar, carrageenan or different solubilities of the sodium and calcium salts.

Now, you will come across in literature different terms for similar methods. But you need to understand what does it mean, the concept behind the method. So, then you may understand whatever the terms may be given like for example embedding;

Embedding can be the cells have been embedded in a matrix. Now that can happen even by precipitation, changing the nature of the matrix from liquid to solid by changing the temperature, like for example in case of agar, agarose or carrageenan or by changing the pH or by using ionic interactions like calcium or sodium alginate interaction.

So, insolubles are formed under cold conditions in aqueous calcium chloride solutions, their structure is non uniform with different pore diameters. So now, let us take an example of calcium alginate beads. So, how can you improve the efficiency of immobilization if given a chance of calcium alginate immobilization? How will you improve the efficiency in this process? What factors do you think you can play around which will affect?

Student: Polarity of the solution.

Student: Polarity of the solution calcium chloride solution; strength.

How is that going to affect?

Student: The ionic strength because the beads form because of ionic interactions. So, if we change it through it may interact it may form better beads.

Better in what sense?

Student: The width. The porosity can be better

Right what else can be done?

Student: Size of the beads.

The size of the beads can be changed very good.

Student: Maybe if the strength is low it is not that solid.

That is what; which will impact what else?

Student: Time of incubation, sodium alginate to calcium chloride. So, how long we are keeping the beads in the calcium chloride solution. So, that will also porosity of the beads. The time of incubation of sodium alginate in the calcium chloride. How long it will take

So, all this is impacting the quality of?

Student: Beads porosity

Like for example, the porosity will get affected, maybe the cells will get leached out and the permeability of the membrane will get affected isn't it.

So, the viscosity of the solution, concentration of alginate, stirring speed and dropping aperture size. Stirring speed how is that going to impact?

Student: It will form smaller beads if the stirring is more.

Why?

Student: Surface area will increase because it will break down.

Beads will break down.

Student: Cells are adhered by forces so it can get affected

Speed will affect what?

Student: Mixing

Speed will affect what? What all things do you have in solution?

Student: sodium alginate and calcium alginate

Only calcium alginate and sodium alginate we keep talking about only these two things what are you why are you doing all this?

Uniform distribution of cells can also get impacted. So, you have forgotten about why we were doing all this.

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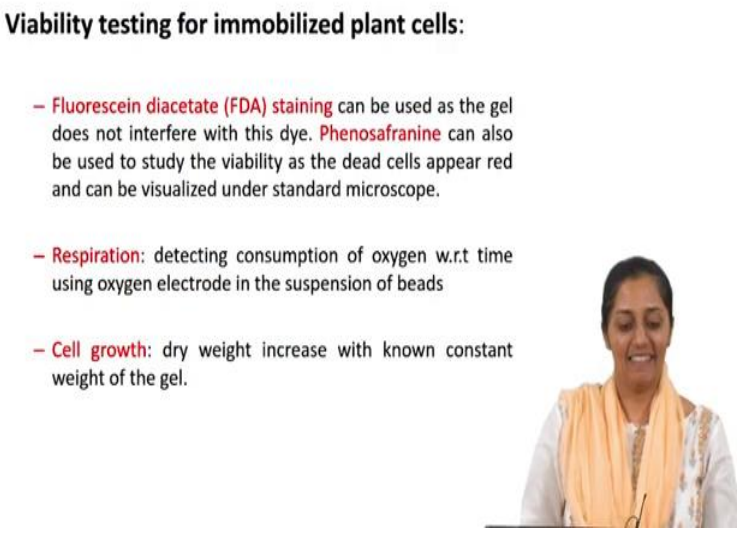

So, I was talking about viability testing, once the beads are in place, we would like to use them and all the cells must remain viable. How to find out how the cells or whether the cells are viable or not. So, if you remember we had spoken about fluorescein diacetate staining which in immobilized systems are generally used and the gel does not interfere with this dye.

So, that it can be taken in and it can measure the viability of the cells. Now similarly phenosafranine, it is another dye which stains dead cells. So, you can find out whether the cells present inside the beads are viable or nonviable. Then another way is looking at the respiration capability or their metabolic activity, metabolic activity in turn can be looked as the respiration ability.

So, if you drop the beads in a solution and there is a change in the specific oxygen demand or the dissolved oxygen levels, then you can assume that their viability is getting affected. Cell dry weight increases with known constant weight of the gel, generally because it is decoupled and you assume that significant multiplication of cells is not happening. They are already now well grown and these well grown cells are immobilized.

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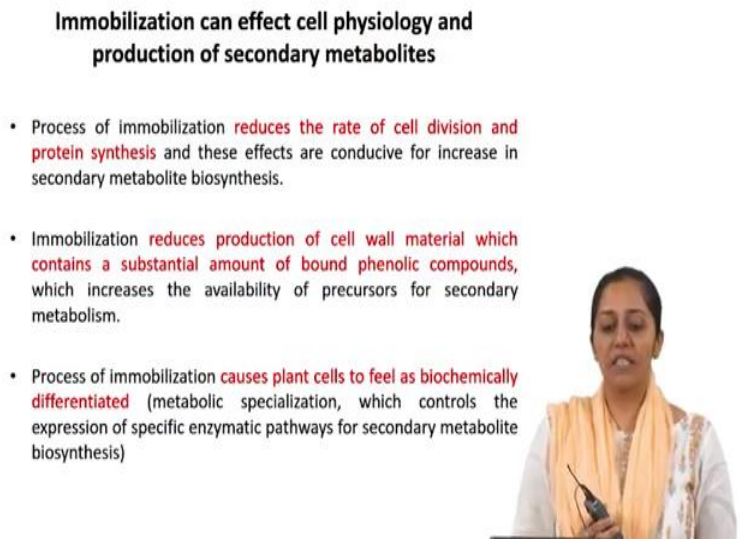

Immobilization can affect cell physiology and therefore, the production of secondary metabolites. Process of immobilization reduces the rate of cell division and protein synthesis and these effects are conducive for increase in secondary metabolite synthesis.

Immobilization reduces production of cell wall material which contains a substantial amount of bound phenolic compounds, which increases the availability of precursors for secondary metabolism. So, which means what that if the cells are in division phase then a lot of phenolics or the carbon flux has to go into the growth. But once you are

immobilizing and driving the secondary metabolism, the same carbon flux can now be utilized for secondary metabolite biosynthesis, thereby increasing the productivity.

Process of immobilization causes plant cells to feel as biochemically differentiated, metabolic specialization which controls the expression of specific enzymatic pathways for secondary metabolite biosynthesis can be induced.

Student: Mam why would immobilization reduce production of cell wall material because if cell has genetic material for cell wall production.

Right, so can somebody answer? She is asking why would cell immobilization inhibit cell wall production. Cell wall synthesis or rejuvenation continuously keeps on happening and also dependent on the protection which is required for the cell to survive and also dependent on the cell division.

So, when the cell division has been restricted, obviously the carbon flux flowing towards the cell wall material is also reduced. And generally, because these are made of phenolics, so many a times phenolics hamper the production of other secondary metabolites, being toxic to the cells and reduce viability. So, there it may improve the production on the flow of carbon flux towards the other desired secondary metabolites.

So, when the cells are in close contact, they get such biochemical signals such that they feel differentiated, thereby leading to improvement in the secondary metabolite yield. There is a possibility that it can lead to improvement in secondary metabolite production.

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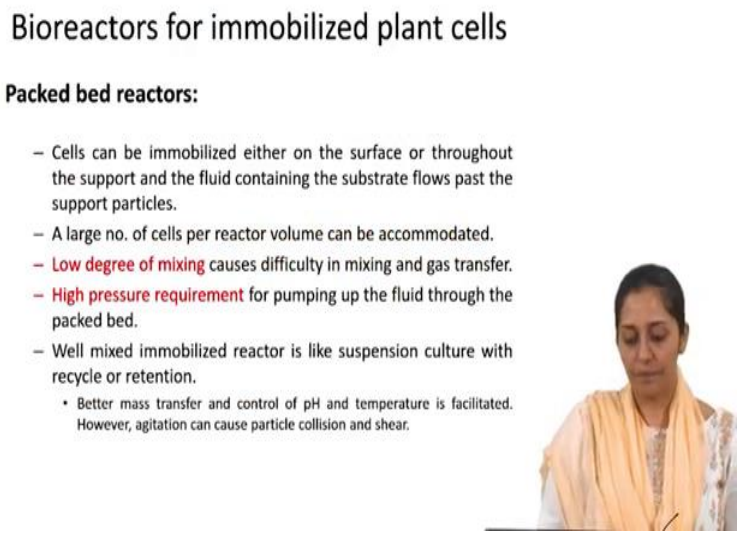

What are the different bioreactors which are used for plant cell immobilization? Packed bed reactors. Now cells are immobilized either on the surface or throughout the support. So, even if you remember your immobilized enzymes the enzymes either can be embedded inside the bead itself or they can be embedded on the surface. So, if they are embedded on, similarly the cells either can be attached to the surface or they can be embedded inside the bead.

Now, if they are inside the bead then if you remember your enzyme bioreactors or enzyme kinetics then external mass transfer limitation and internal mass transfer limitation both become crucial. But if they are placed only on the external surface then only external mass transfer limitations are limiting. So, the cells can be immobilized either on the surface or throughout the support and the fluid containing the substrate flows past the support material.

Now one thing is that mass transfer limitations, the substrate first has to diffuse to the surface, from the surface till the inner core if they are embedded inside and similarly the gas. A large number of cells per reactor volume can be accommodated. Low degree of mixing causes difficulty in mixing and gas transfer. These are the limitations, high pressure requirements being a packed bed there will be higher pressure drops.

So, higher pumping or suction pressure is needed; Well mixed immobilized reactors is like suspension culture with recycle or retention why?

Because spent medium is going out and the substrate is flowing in and it is a high cell density culture. So, better mass transfer and control of pH and temperature is facilitated; however, agitation can cause particle collision and shear.

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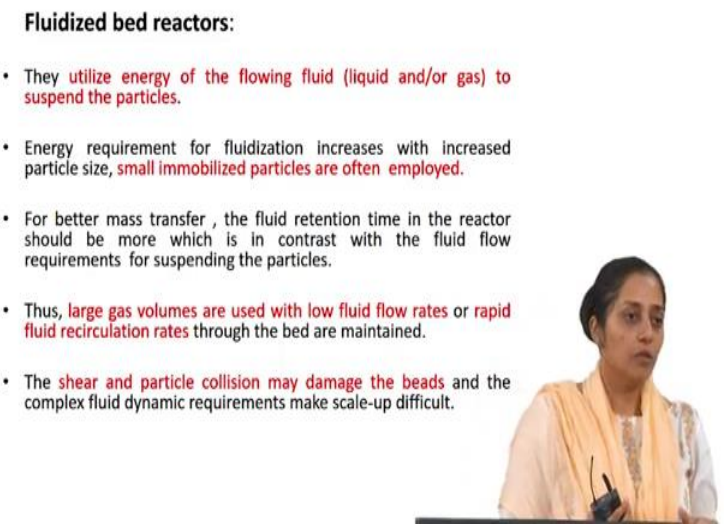

Fluidized bed reactors: They utilize energy of the flowing fluid, liquid or gas to suspend the particles. Now, in comparison to packed bed reactors, fluidized bed reactors are better in what sense?.

In terms of mixing. Energy requirement for fluidization increases with increase in particle size because, more power is required to suspend the particles. Small immobilized particles are therefore often employed. For better mass transfer, the fluid retention time in the reactor should be more. So, if the recirculation can be done you are increasing the retention time of the gas phase or the liquid to interact with the cells for the bioconversion.

Large gas volumes are used with low flow rates, now if in order to see it is balancing between the merit and demerit. In order to keep the cell suspended you would like to have high flow rates. Now with high flow rates the retention time is less the time to interact with the cells reduces. So, your productivity and your cost of production increases, because now all the substrate which are feeding is not getting converted. So, you need to increase the retention time at the same time you need to keep it suspended.

So, high gas flow rates are used, so as to create recirculation currents and thereby improving the retention time before the fluid moves out. The shear and the particle collision may damage the beads and the complex fluid dynamic requirements make scale up difficult.

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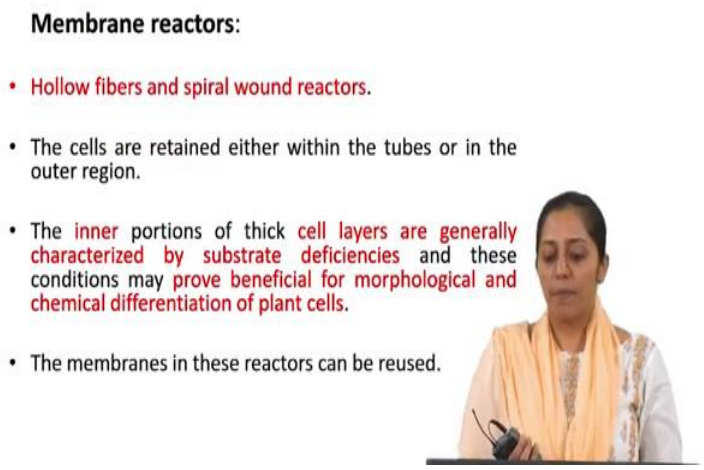

Membrane bioreactors we were talking about hollow fibers, similarly spiral wound reactors. The cells are retained either within the tubes or in the outer region, it can be shell side or the tube side immobilization. The cells are retained ether within the tubes or in the outer region and the inner portion of the thick cell layers are generally characterized by substrate deficiencies, because they keep accumulating one on top of the other. So, this may lead to in turn nutrient mass transfer and oxygen limitations.

What else? Increase in the cell number may eventually clog the membrane pores, thereby not allowing the media exchange - the nutrient and the product exchange to happen.

The membranes in these reactors can be reused.

So, there are merits and demerits both are associated to every kind of reactor configuration.