Aspects of Biochemical Engineering
Prof. Debabrata Das
Department of Biotechnology
Indian Institute of Technology, Kharagpur

Lecture - 35
Kinetics of Substrate Utilization, Product Formation and Biomass Production of Microbial Cells – V

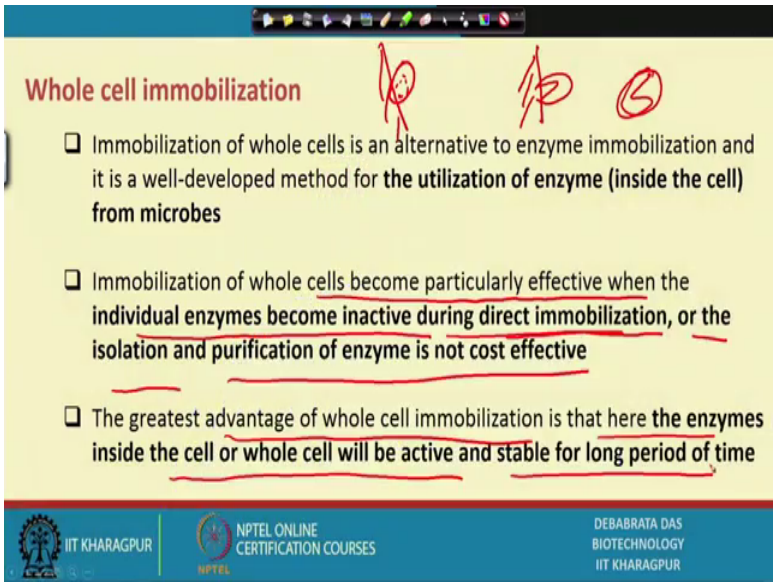
Welcome back to my course that aspects of biochemical engineering and we are now discussing about the kinetics of substrate utilization, product for formation and biomass production of microbial cells. Now last couple of lectures I try to discuss that regarding the cell growth kinetics by using monod and the other equation, which is followed by analysis of the batch process then CSTR or chemostat and the plug flow reactor.

Now, what we observed that chemostat may be the better process as compared to other process, the reason is that it is easy to operate it and your productivity also it is quite high as compared to other processes. Now been and chemostat is such a process we can maintain a particular phase of growth for infinite period of time.

N when we discuss about the chemostat, the major drawback of the chemostat is the cell mass that wasting from the reactor. So, cell mass that is going out from the reactor if it is more as compared to cell mass that is growing in the reactor, then a time will come there will be no cell present in the reactor what you call d washout or in other way if the hydraulic detention time is less than the generation time, the before the cell multiply you are taking up the cell from the reactor and we mating the situation of cell washout. And when there is no cell present in the reactor. So, there is no reaction take place inside the reactor.

Now, to so, to overcome this problem, one approach that we have seen that is the cell mass recycling. Now besides the cell mass recycling, there is other techniques that also we can use that is called immobilization of the whole cell. What we can do, we can hold the cell on the solid matrix and then we pass our substrate, then we will find that you know that no cell most of the cell that retain inside the reactor. So, we do not have we can easily overcome the cell washout problem.

(Refer Slide Time: 02:44)



Now, if you look at this, what we mean by whole cell immobilization. Now whole cell immobilization is an alternative to the enzyme immobilization and is well developed method for the utilization of enzyme inside the cell from the microbes. So, you know that in case of this microbial cells what is they have the metabolic process; when we immobilize the cell, then the substrate that go inside the cell and undergoes a metabolic process, and give the respective products and take it out.

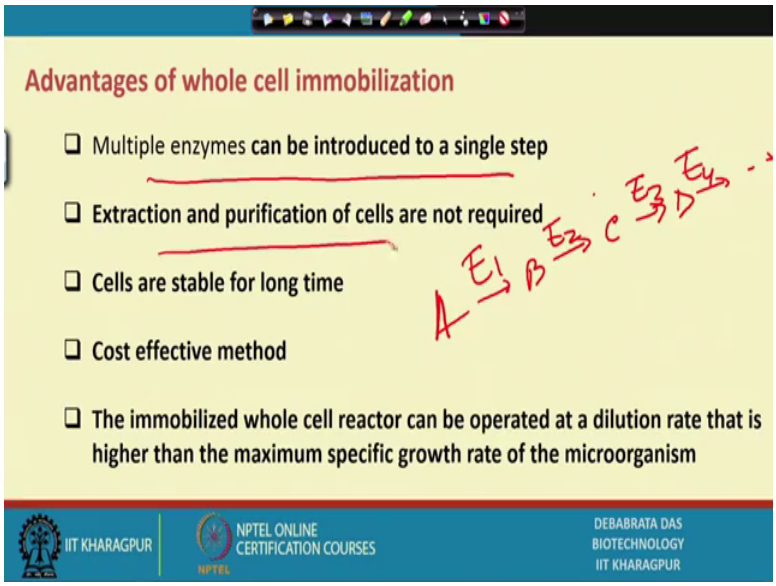
The immobilization of the whole cell become particularly effective, when the individual cells become inactive during the direct immobilization technique. So, what I want to mean, suppose this is the solid matrix and we know the active side is this one, now by chance this active side of the enzyme is fixed on the solid matrix then the enzyme will be inactive.

Now, here in case of the organism and this is not happened, because this is the cell and inside the cell we have the biomolecules. So, there is no such inactivation is possible. So, when the inactive individual enzyme become inactive during the direct immobilization or the isolation and purification of enzyme is the is not cost effective. Because we always find that enzyme proteins are costly that all enzyme all proteins are not enzyme the proteins with the active side their enzyme.

So, enzymes are more costlier. So, if you consider any kind of pure enzyme; obviously, it will very costly, but when you consider any kind of cell will find that it is less costly; and

the greatest advantage of the whole cell immobilization is the enzymes inside the whole cell will be active and stable for longer period of time. That is what is not possible in case of this enzymes, though you know that you can keep the organism active for a longer period of time. So, this is the advantage that we have in this particular process.

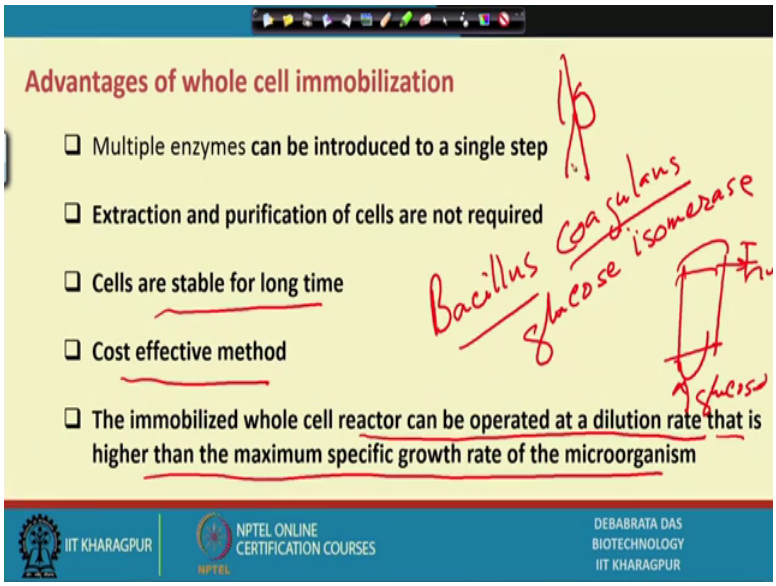
(Refer Slide Time: 05:45)



Now, what are the other advantages of this of the whole cell immobilization? If you look into the multiple enzymes can be introduced in a single step. So, you know that I told you inside the cell we have metabolic pathway. In the metabolic path is that the number of steps are involved. So, A to B, B to C, C to C. So, you know that like this A to B, B to C, C to D like this is n number of process we there and every step you have enzymes you know you required enzyme.

So, what we considered this is the multi enzyme system. So, multi enzyme system we can introduce as a single step, that is the major advantage of this particular whole cell immobilization system, and if you look at this extraction and purification of the cells are not required. Because you can use the cell directly you do not have to purify it. Only the organism that you are you are supposed to use that you have to immobilized on the system. Cells are stable for a longer period of time this is the one of the advantages I mentioned before and it is a cost effective method.

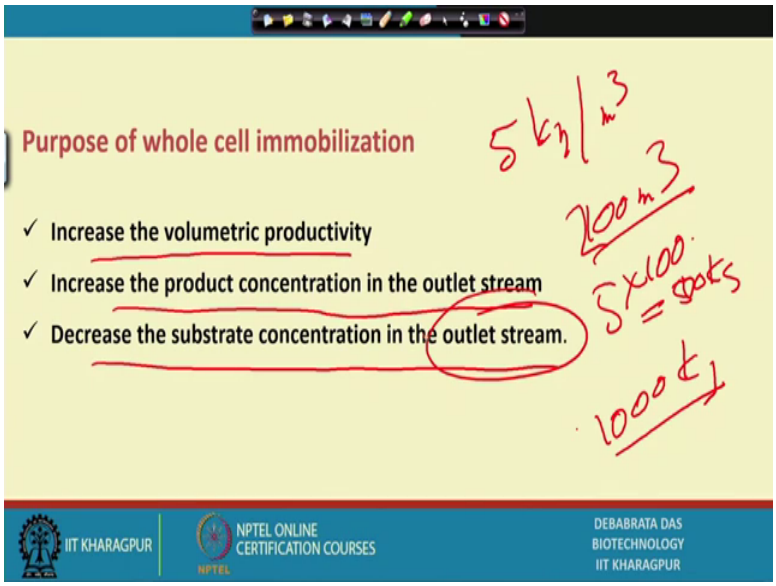
(Refer Slide Time: 07:03)



I can give a typical example that Bacillus Coagulans; Bacillus the this Bacillus Coagulan is it has the glucose isomerase enzyme.

So, if you, if suppose we have a column, in this column we immobilize this Bacillus Coagulan and pass the glucose this is one end and other end we can we can we can produce the fructose. So, obviously, it is the cost effective method we will do not have to purify the enzymes. The immobilized whole cell reactor can be operated at a dilution rate that is higher than the maximum specific growth rate of the organism. I told you since you are holding the cells, you are not allowing the cell to go out of the system. So, the dilution rate has little role to play because the dilution even you cross the d washout that in the normal the chemostat process that, that problem can be overcome here. So, this is the major advantage of this whole cell immobilization process.

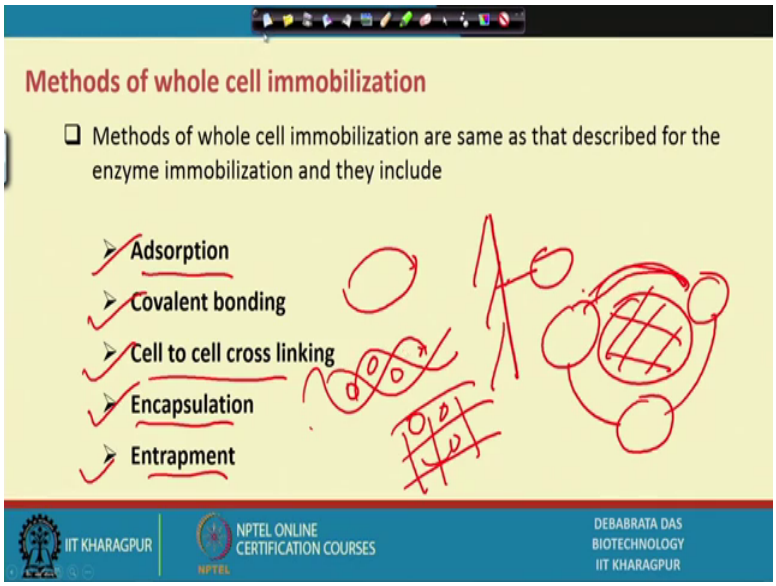
(Refer Slide Time: 08:16)



Now, purpose of whole cell immobilization is increase the volumetric productivity, this is one then increase the product concentration in the outlet stream and decrease the substrate concentration in outlets stream this is very important. So, increase the volumetric productivity what do you mean by volumetric productivity? Volumetric productivity is the amount of product form per unit volume. Now if we have more volumetric productivity our recovery cost will be very less which is more desirable, and product concentration that you know that also very important that if that also if a product concentration is high, then also cost of recovery also very high then with the. So, first is the volume. So, if we assume suppose 5 kg per cubic meter of product and if you increase the volume 100 cubic meters. So, how much volume of product amount of product you can have? 5 into 100 that is 50 kg am I right now 500 kg.

Now, if he has 200 then this will 5 into 200 will be 1000 kg. So, as we increase the volume of the liquid that your productivity increases. And decrease substrate concentration in the upstream that the outlet stream is very important. If the concentration in the outlet stream is less; that means, whatever your load for the waste of the water treatment process will be very less, which is that would be that will help to reduce the product cost to a great extent.

(Refer Slide Time: 10:02)



Now, question comes what are the methods of whole cell immobilization, the we have adsorption, then covalent bonding, then cell to cell cross linking encapsulation and entrapment. Now what do you mean by adsorption we told you, the adsorption is the physical phenomena physical phenomena means this cell is simple added on the surface of the solid matrix. So, the bonding between the cell and the solid matrix is due to the banderol type of folds.

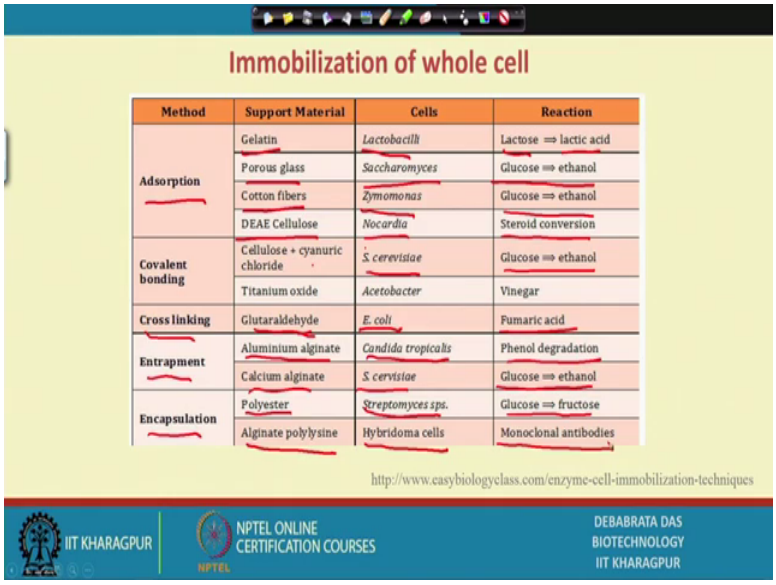
So, if you pass your liquid at the very high flow rate, then there will be and their axial cr force. Due to presence of the axial cr force there is the every possibility cell may dislodge from the surface of the solid matrix. So, that is the major disadvantage of the adsorption process, but it is considered as the cheapest process because you just pack the material on the solid matrix and pass the cell through the solid matrix. So, that it can absorb.

But when you consider the covalent binding, it is appear to be the very strong binding this is like this. So, I told you covalent binding is the kind of electron sharing, where the electrons we shared that bond is very strong. Cell to cell cross linking; that means, I told you this cell with the help of cross linking is not that like glucoside glucose aldehyde we can have the cross linking and inside maybe you have solid matrix, who it may be that embedded on the surface of the solid matrix.

This bonding also covalent bonding, this also very strong bonding; the encapsulation means I told you that day to day life we take lot of capsule, and in the capsule you know that we have we put the medicine and it is in clip inside the capsule like you know we know the envelope inside the envelope we put the later. Similarly inside the that you know that coating we put the whole cell and entrapment. Entrapment means I told you the fiber entrapment gel entrapment we have gel that inside the gel, the organism might be entrapped. Now inside the fiber the this organism might be entrapped.

So, these are the different immobilization techniques that we have.

(Refer Slide Time: 12:27)



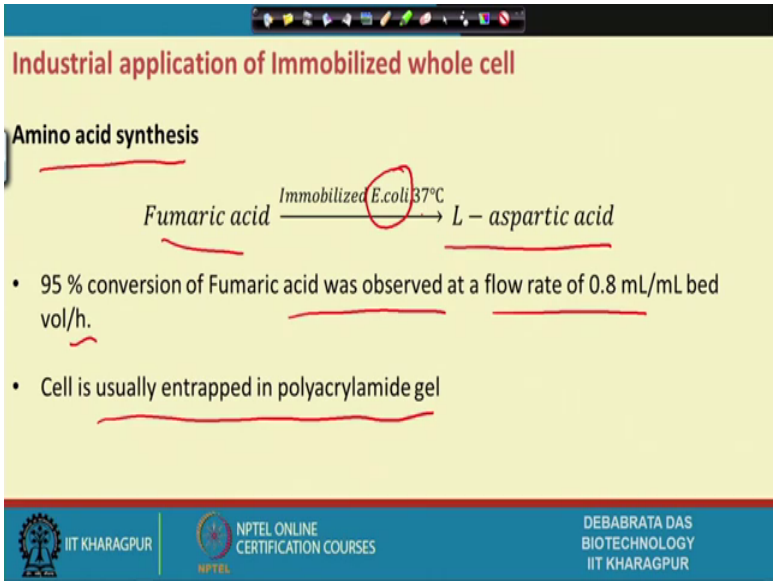
Now, let us say give you some application of this, now when you talk about the adsorption process and supporting solid metric is gelatin, porous glass, the cotton fiber and DEAE cellulose. So, these are the different solid matrix we can use and the cells. So, the one is lactobacillus where which convert the lactose to lactic acid. I told you that you know that lactic acid is very good they have raw material, that chemicals or the preservation of different food products I have given the example of the cheese is a food product and which can preserve the milk protein and fat for a longer period of time, due the presence of this lactic acid.

Now, in case of porous glass if we use the saccharomyces cerevisiae, then it convert the glucose to alcohol. Zymomonas Nobel is also can convert glucose to ethanol. And Nocardia it can in the undergoes some kind of standard steroid transformation process.

The covalent binding we have cellulose cyanuric this chloride, we have saccharomyces cerevisiae glucose to ethanol can be used. The cross linking we have we use the glossary glutaraldehyde is use as a cross linking essence and e coli when you immobilized then it produce the fumaric acid.

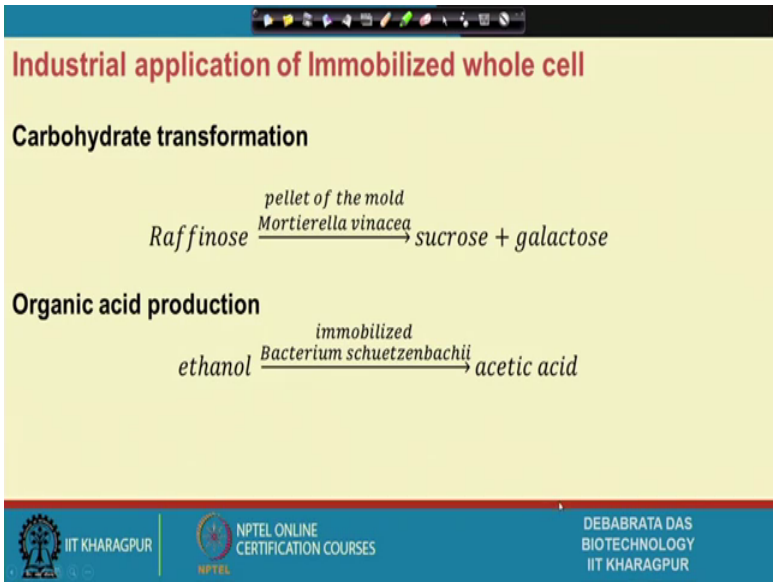
Now, then the entrapment we have aluminum alginate calcium alginate, we have candida tropicalis, we have saccharomyces cerevisiae, the one is candida tropicalis used for phenol degradation and saccharomyces cerevisiae convert glucose to ethanol. Then encapsulation will be polyester alginate poly lysine this is a hydro hybridoma cells and streptomyces spaces one is use for glucose to fructose than another use for monoclonal antibody production.

(Refer Slide Time: 14:32)



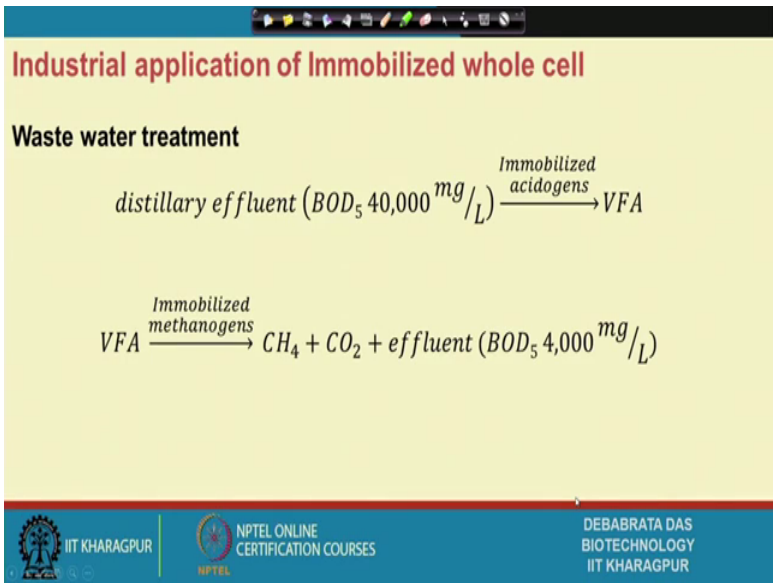
Now, there are other some examples that I have given here that is the amino acid synthesis Fumaric acid, when immobilized on e coli at thirty seven degree centigrade it converted to a [laufter]-aspartic acid. 95 percent conversion of Fumaric acid was observed at the flow rate of 0.8 milliliter per milliliter of bed volume per hour cell is usually entrapping the polyacrylamide gel.

(Refer Slide Time: 15:05)



Now, another industrial application is that Raf f inose that is carbohydrate transformation that when Raffinose, it is Raffinose is in presence of Mortierella that is spaces vinacea this converted to Raffinose to sucrose and galactose. Now organic acid ethanol in presence of bacterium spaces that is converted to acetic acid ethanol is converted to acetic acid.

(Refer Slide Time: 15:40)



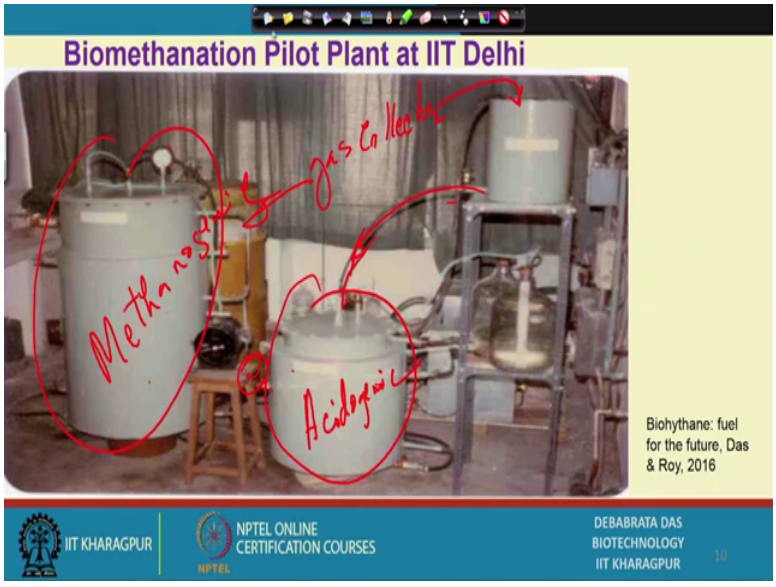
Now, another very intera interesting application that we have that is waste water treatment process. Particularly I personally involved that in IIT Delhi that in a conversion

of distillary effluent to methane and carbon dioxide. So, what we have done we have it is a basically two stage process one is acidogens another is methanogens we immobilize the cell in the poly propylene chloride, that solid matrix and I also show you how is the solid matrix then I said also show you after the immobilization that how the solid matrix look.

So, you know that we just pick the solid matrix and pour pass the, that distillary effluent and we will continuously produce higher the methane and carbon dioxide, and this process we can operate continuously for four month. After that the performance of the reactor decreases. So, we can stop the reactor and degenerate the system again.

So, this is like this the distillary effluent initially if you look at the bod is very high, initially bod was 40,000, and this is converted to volatile fatty acid like acetic acid propionic acid butyric acid and this acid converted to methane and carbon dioxide and so, 40,000 bod 5 that will be converted the 4000 greater than 90 percent removal of the bod that has been taking place.

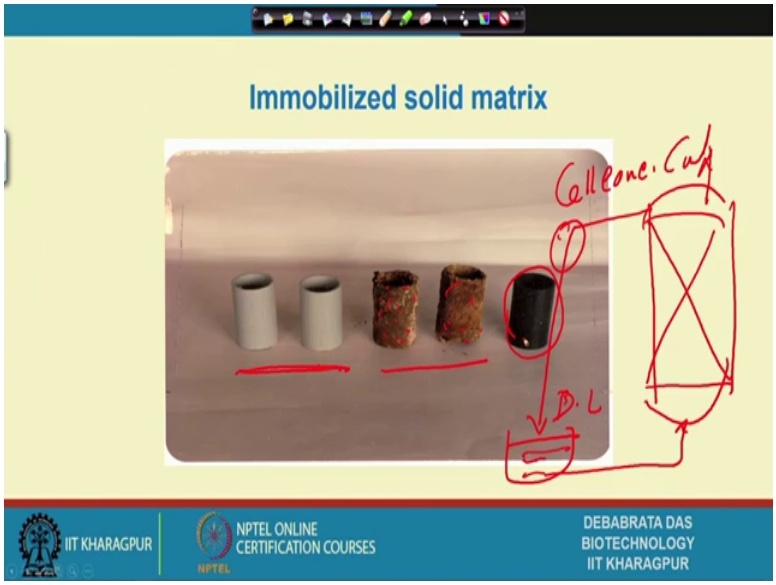
(Refer Slide Time: 17:24)



Now, if you show the see the experimental setup, it is like this is what this is I call this is a acidogenic reactor and this is the methanogenic reactor. So, this is Acidogenic and this is Methanogenic. So, what is happening that here with the help of palm, that you know this is the this we put the we take the distillary effluent here, and we pause the this is the solid matrix inside we trickle down and in process this convert to organic acid. Then with the help of pump we one pump is there we pump this here and we when we pause here

then this acid will convert it to methane and carbon dioxide, and this is the gas collector where we collect the gas.

(Refer Slide Time: 18:25)

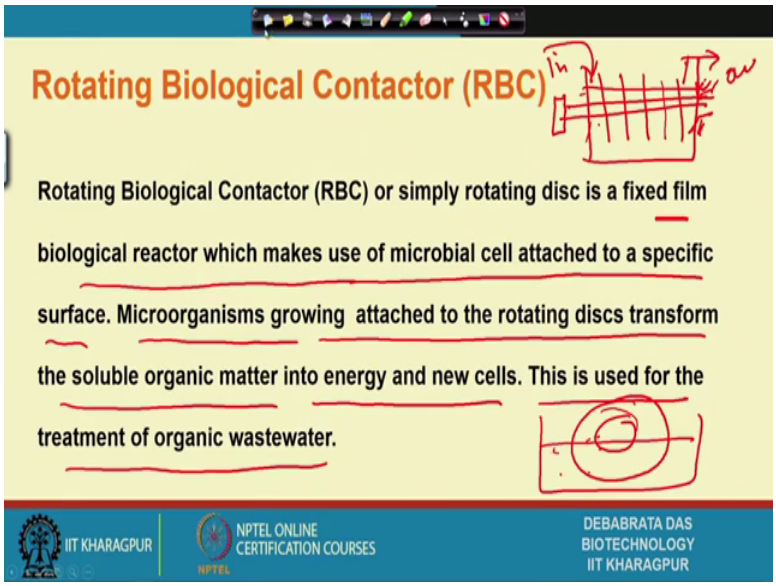


Now, as I told you that if we look at the solid metric, the polypropylene chloride looks like this we got even that this is one inch diameter, we cuts it one inch by one is that we cut it into different size this size, then we pack it in the solid matrix. So, it is it looks like this that in the solid matrix, we pack this material and then we prepared the cell suspension acidogenic culture or methanogenic culture we pass it like this and you know continuously we do until unless we find the cell concentration here constant cell concentration is constant.

Then we hold it for some time that with the media and then let the reaction take place when your gas production is maximum then is the replaced by the distillary effluent. So, we continuously pause and produce the produce the acid, and this acid we similarly used for the second reactor what converting acid to methane.

Now, this is before immobilization the solid matrix looks like this, after the immobilization you can see, this is how the cell that you know that you know attached with the surface of the solid matrix. Now when you take out the, this is the this cell out, it is becoming black this back to the color might be due to the astrious that you know prove form during the anaerobic digestion process. This reacts with the polypropylene and it change the color of the solid matrix.

(Refer Slide Time: 20:06)



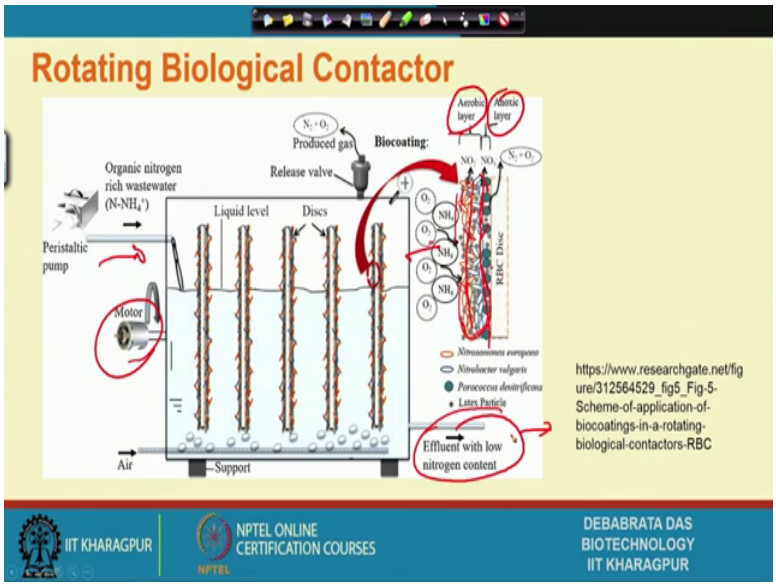
So, another very interesting thing I want to share with you that is the rotating disc, biological contactor rotating this biological contactor is a simple rotating disc with a fixed film and biological reactor, which makes use of the microbial cell attached to a specific surface, and microorganism growing attached to the rotating disc transformed the soluble organic matter into energy and new cell, this is used for the treatment of organic waste water.

Now, let me explain that because you know that suppose I have some picture because before I show you the picture, let me give you a simple diagram; suppose this is a rotating shaft this is the rotating shaft now here we can have the bearing. So, we can have the bearing and here we can have a motor. So, here the discs are embedded on the surface of the solid matrix, these are embedded and here you put the liquid waste water in and this is waste water out, this is the in and this is the out.

So, it is rotating at the very low rpm, when it rotates the disc that you know this is circular disc when immersed in the water. So, it will touch the surface of the liquid. So, it has the weighted surface. In the weighted surface the sail will be mobilized on the surface, and the organic material that present in the liquid that will have that will be utilized by this cell and produce the cell mass and other compound when carbon dioxide and other compounds. So, some kind of degradation of the organic matter will take place.

Now, if you see the picture how it occurs.

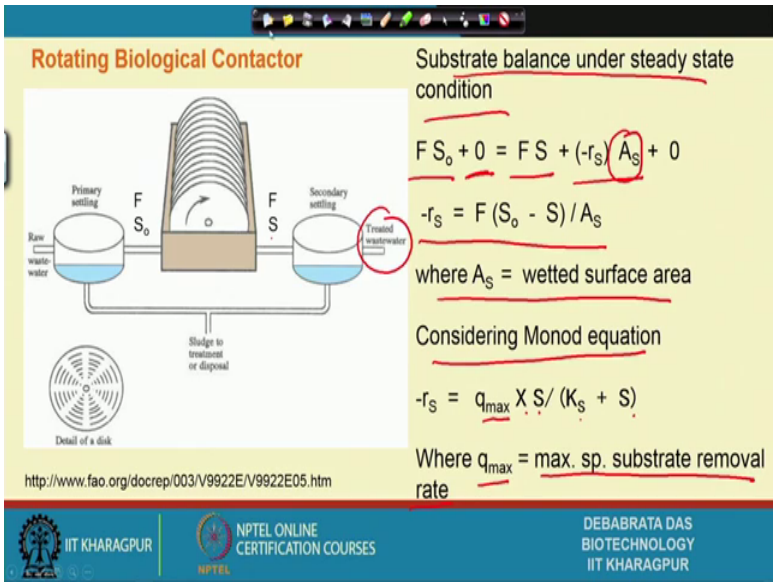
(Refer Slide Time: 21:59)



It is like this. So, you know that it is with the help of peristaltic pump, you can you can put the liquid like this. And this is the disc and this is rotating with the motor you know this is a disc and it is rotate like this. So, you know the cells are that grow on the surface of the solid matrix this is the disc, and this is how cells are growing on the solid matrix. So, in the outside layer they will come contact with the liquid and there we call the aerobic layer, and inside we call the anaerobic layer.

So, the inside organisms, it can carry out some kind of anaerobic reaction and outside we carried out some kind of aerobic reaction. So, this is we call it bio coating, and then we take the liquid how it in this way we take in and we are taking the effluent out this way. So, in that way we reduce the organic content of the waste water to a great extent.

(Refer Slide Time: 23:05)

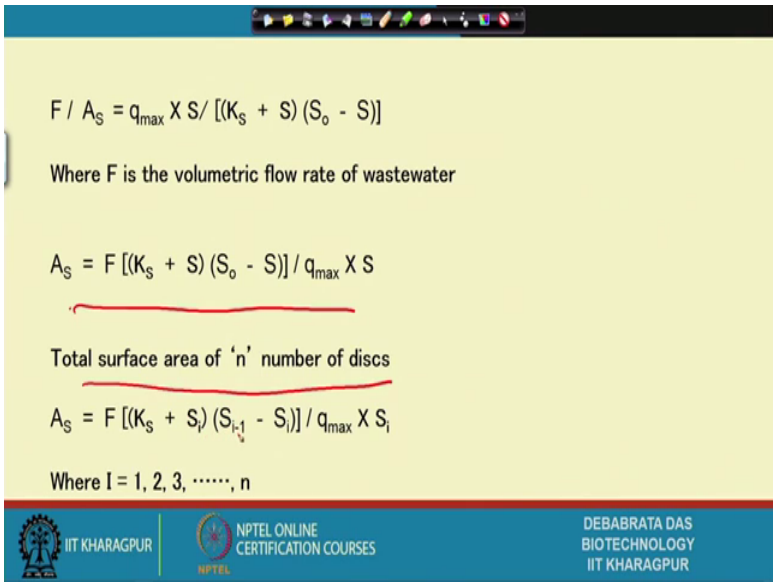


Now, if you look at this process can be represented in another very simple way, this is the disc of they mounted on a particular shaft and inverse on a trough. And this is the primary settling and that tank and operating from the primary settling tank the supernatant, you passes through this rotating with these biological contactor and in the reaction take place and the S 0 will be converted to S, and this is the secondary settling tank then this is the treated was waste water that we have.

Now, this we here we can do the substrate balance under steady state condition, what is the rate of input that is F into S 0 am I right? And what is the what is generation will be 0 and what is the output F into S and what is the reaction that we have minus rs that is the rate of substrate utilization per unit surface area it depends on the because cells we assume it presence in the disk and not in the suspension. So, it depends on the area. So, the As is the area of the weighted surface.

So, your rs can be represented like this where as is considered As the weighted surface area. Now considering the Monod equation or Michaelis Menten equation, we can write in the similar way that monod equation minus rs equal to q max into X into S, Ks plus s where q max is the maximum specific substrate removal rate. So, this is multiplied by X then it will remain maximum substrate removal rate and into S Ks plus S this how we can write this equation.

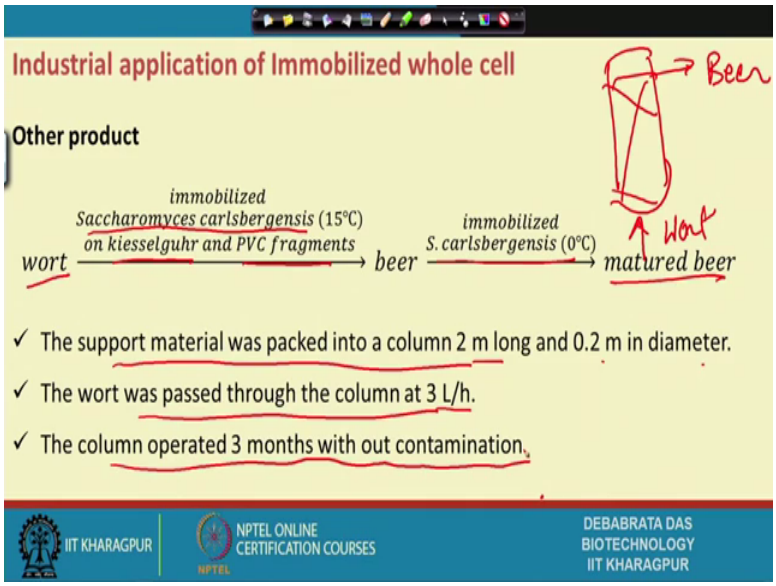
(Refer Slide Time: 24:58)



Now, we can write that BPS equation that this equation now we can write in this form, that F by A. Now here we have F and we had here we have F and A, now we can this is equal to this am I right this is equal to this we can write there, now we and then we can write this equation F by S equal to q max into X into S Ks plus S, S minus S 0 F is the volumetric flow rate.

So, then we can write the equation in this form that is as equal to like this, and this and total surface area this n number of discs there. So, we can write this a generalized equation F into Ks S plus Si, Si minus 1 minus S i q max by Si the. So, this one i is stands for 1 2 3 4 to n this is how we can find out total weighted area, because this weighted area responsible for carrying out the reaction.

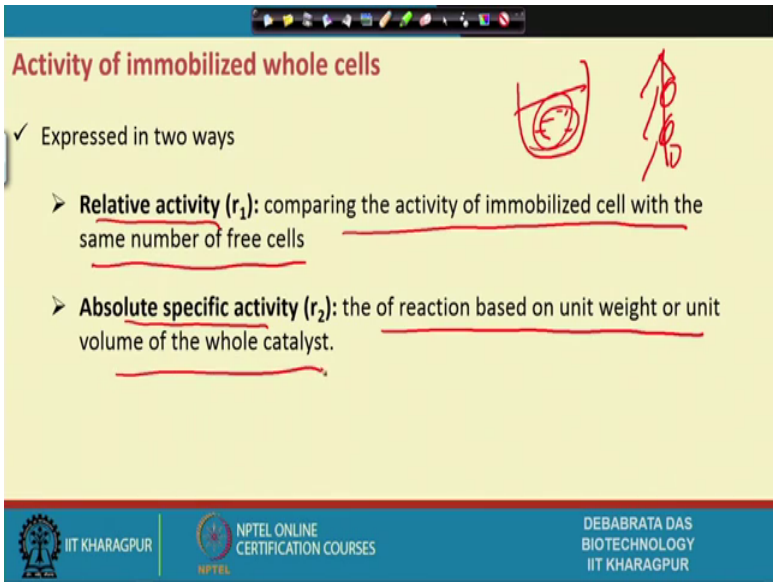
(Refer Slide Time: 26:17)



Now, another industrial application is that is a kind of beer industry that we have. Now in the beer industry what we have we have this is the wort now what is the wort? Wort is the fermentation media and this is when this is saccharomyces carlsbergensis, we this is used for the mostly four legged beer formation and this is the when we need immobilized on kiesselguhr or PVC fragments and then it will continuously pass through this column and we can produce the beer this is we can parts of wort. And here we will get the beer that is what is then again we are passing is saccharomyces carlsbergensis with another column, to get the matured beer whatever unconverted substrate is their glucose is there that will be further converted to alcohol.

So, the support material was packed with a column, two meter long and point two meter in diameter. The wort pass through a column at the rate of three liter per hour the column operated three month with outlet without contamination. So, this is one of the application that we have of this whole cell immobilized system.

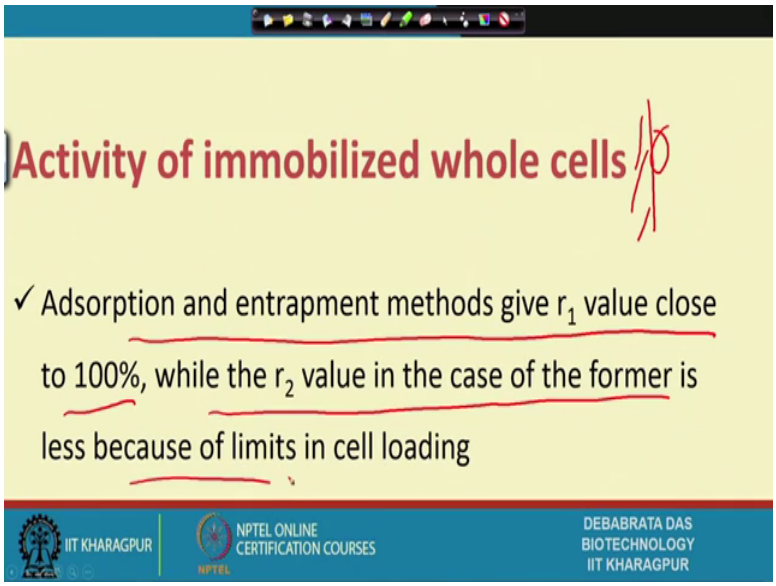
(Refer Slide Time: 27:36)



Now, activity of the whole cell immobilization system can be expressed as the relative activity, the comparing the activity of the immobilized cell with the same number of free cells. Suppose you we have in the suspension we have cell, and in the immobilized system we have immobilized cells now if you have number of cells is same and we if we compare that what is the performance of this suspended cell with respect to immobilized cells, that we call relative activity.

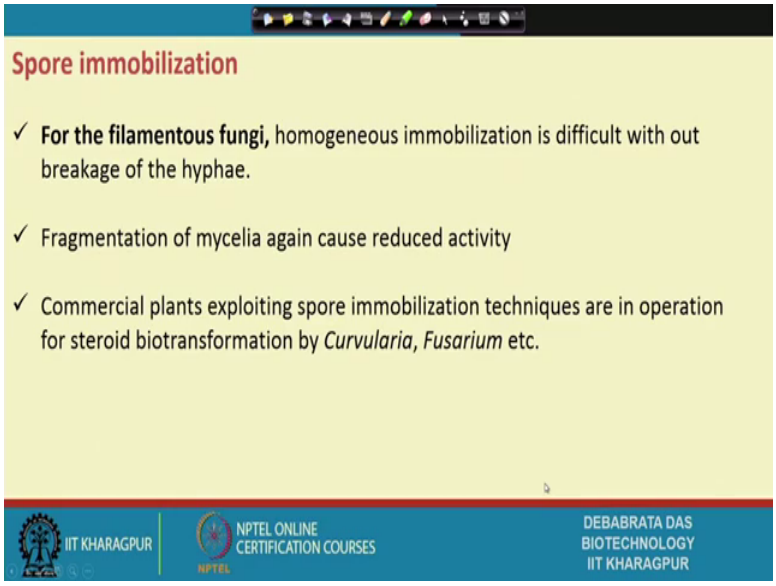
Absolute specific activities the reaction based on the unit weight or the unit volume of the whole catalyst; that means, per unit cell must the immobilize cell must that what is the activity what is the rate of substrate removal, that is actually that call absolutely specific activity of the. So, we I in this connection I want to tell you that, in case of immobilized enzyme with specific activity ie we express as the micro moles of substrate converted per minute per gram of solid matrix, though this is something similar to that.

(Refer Slide Time: 28:47)



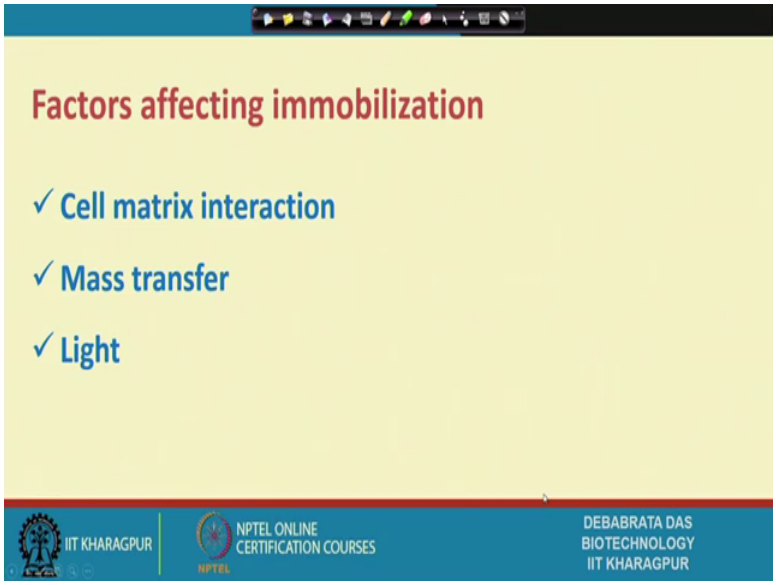
Now, activity of immobilized whole cells the adsorption and entrapment methods gives r 1 value close to 100 percent, because we assume since the cells are loosely connected on the surface and your diffusion problem is very less we can expect at the 100 percent of reaction, while the r 2 in case of the former where cell are loading inside the cell, then we have some problem because we have the rate of reaction will be little bit less as compared to them were the adsorption technique or you know in the suspended cells.

(Refer Slide Time: 29:26)



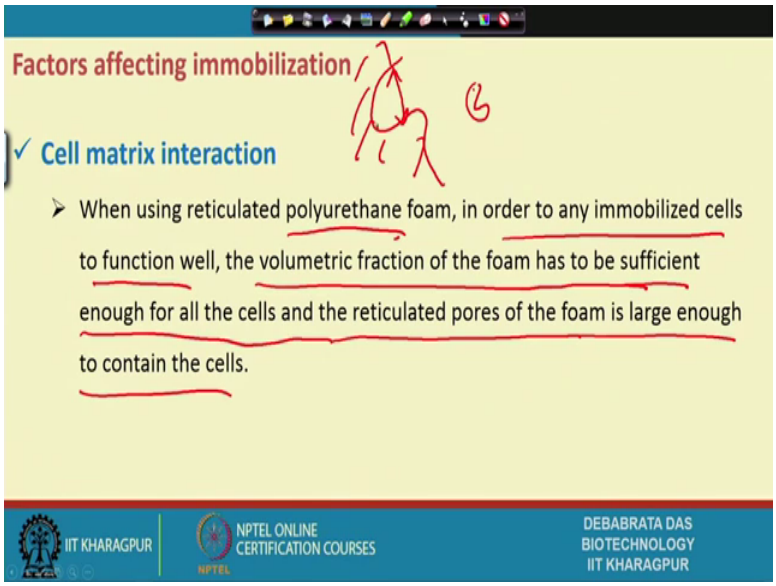
Now,. So, in case of spore immobilization let me tell you, in case of filamentous organism and that we have seen that it is difficult, that with the breakage due to the breakage of this hyphae and fragmentation of mycelia, again caused the reducing the activity, but commercial plant exploited the spore immobilization technique are in operation for the steroid transformation as usually at the Fusarium is they use, and this is largely use for steroid transformation process. So, this is another problem that we have in case of fungal cell.

(Refer Slide Time: 30:07)



Now, factors that affect the immobilization technique there is a goal one is cell metric interaction mass transfer and light, these are the different factors that influence this process.

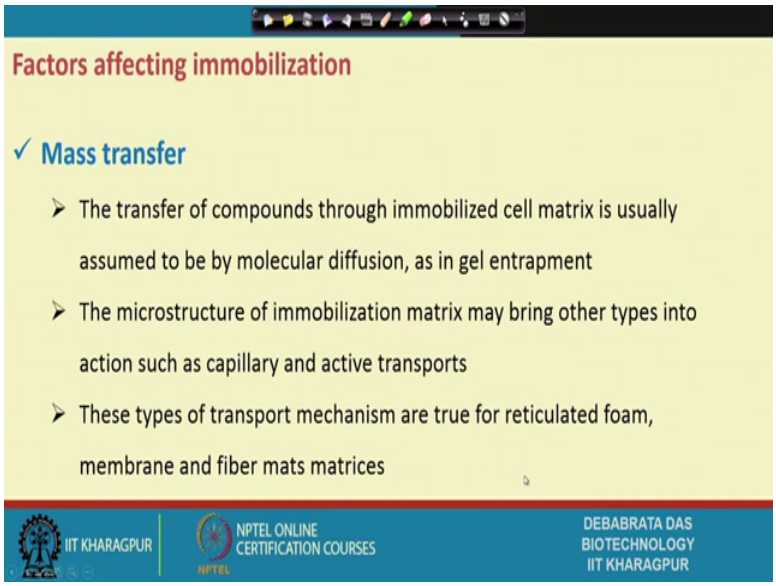
(Refer Slide Time: 30:23)



Now, if you look at cell metric interaction, when using the riticulous polyurethane foam this polyurethane foam we know this is largely used for insulation purpose, in order to immobilized any cell to function well the polymeric fraction of the foam has to be sufficient enough. So, that all the cells and the riticulous pores of the foam is large enough to contain the cells. So, I told you during the immobilization enzyme that you know when you have pores of the solid matrix, the size of this pore it should be double the size of the enzyme, otherwise the enzyme can you in say enter into the inside the pore.

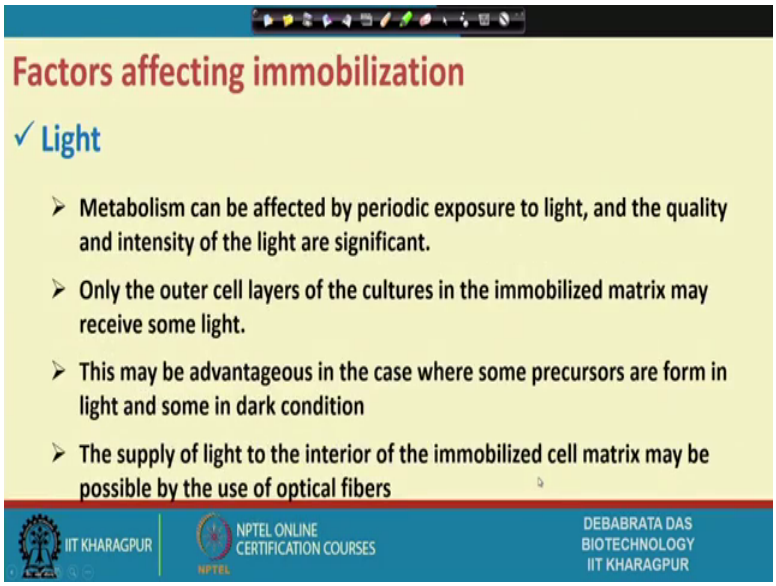
Now, if the pore size is more then there is every possibility of leaking of the pore. So, pore size plays very important role as for the immobilization of the whole cell.

(Refer Slide Time: 31:15)



Now, mass transfer as you know that mass transfer I told you previously also that as soon as the immobilized the cell, the mass transfer limitation problem that we take place the, that the substrate has diffused. Since it is co containing two phases liquid and solid then the substrate to diffuse from the liquid surface to solid surface and then when the product formation take the product has to diffuse from the surface to the. So, that kind of problem that we have that has been, I am not going in details you can just go through that and the mass transfer resistance and that has been decreases the nutrient transport that if you mass transfer resistance is more the nutrient transfer, will be reduced your growth of the organism that will be reduced to a great extent.

(Refer Slide Time: 32:05)



Now, light also plays very typical role in case of immobilized whole cell system, particularly photosynthetic organisms they require light. So, it gives some kind of barrier to the light penetration. So, we shall have to put the led light in the inside the reactor. So, that light transfer light transfer that transmission may be proper in that way, we can reduce the light penetration problem to some extent.

So, what I wanted to tell in this particular lecture that, how the immobilized whole cell system can be used for in the biochemical industries. It can be used for the production of certain product, it can use for carrying out the series of reaction in a particular one cell we can use for carrying out a single stage reaction in the by using a particular organism. I have given the example of bacillus coagulant how it is converted glucose to fructose.

I also told you the how in the biomethanation process, how distillary effluent can be converted to methane and carbon dioxide that this is the multistep process, both the acidogens and methanogens they how they involve and we can get the methane and carbon dioxide. Then I have given you the example of pure formation process, then what are the different physical parameters with what are the different parameters that influence the and mobilized whole cell system, that also you discussed and the activity of the whole cell how is the effect by this mass transfer light penetration that I discussed.

Thank you very much.