Course on Industrial Biotechnology
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Lecture 15 Immobilization Techniques (Contd.)

Now next present I will continue my previous lecture on immobilization technique now in the last lecture I try to point out that What is the different techniques of immobilization how how the enzymes can be immobilized on the solid matrix what is the classification that we have and and to the properties of the solid matrix that what is the required and what is the different applications we have immobilization immobilized enzyme system.

Now in this presentation I try to tell you something on the enzymatic reaction which is very important very much important for this process and then I want to do certain I want to do some problem because I know when you I told you that the enzymes are usually fix on the solid Matrix then we pass the substrate and we take the product in other end their question comes that what should be the height of the columns.

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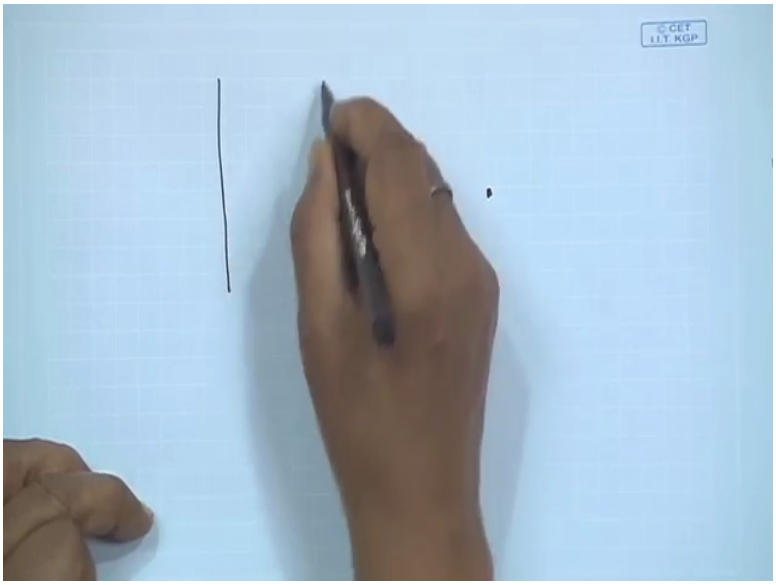

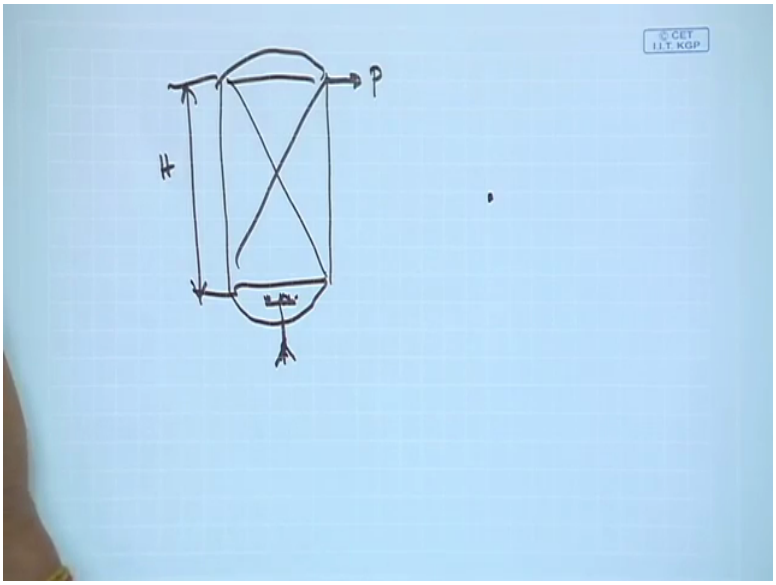

So I will try to find out this suppose this is a column and this is this is how you this is this going this substrate is going and and this is product is formed so this is the height that how the height of the column we can find out this I should show you at the end of the lecture now let me start at the beginning that this is.

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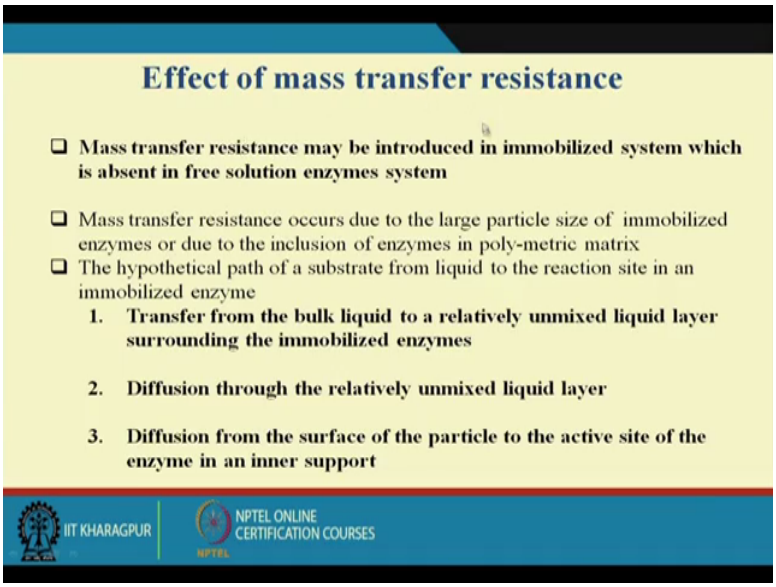

The effect of mass transfer the whenever we immobilized the enzyme on the solid Matrix that solid matrix I told you it is insoluble mass so when you fix with the solid Matrix the ad and your substrate usually remain in the soluble form so your in the in the reaction mixture both we have solid and liquid and if the reaction mixture comprises of the solid.

And liquid we call it heterogeneous mixture the in case of heterogeneous system that we have some problem I told you at the when I discuss the reaction kinetics that heterogeneous problem that a substrate has to diffuse on the on to the surface of the solid metric and then and only then the reaction take place and after the reaction is over again product has to defuse out to the bulk of the liquid.

The diffusion problem is a very important problem of the heterogeneous reaction kinetic so and two things simultaneously take place one is the diffusion as well as the reaction so we will do some exercise here and try to find out that which one is the controlling for your reaction whether it is diffusion controlling or if the reaction controlling suppose it is diffusing controlling means your rate of reaction is higher as compared to diffusion.

So if you want to improve upon the product formation you have to improve the diffusion if is the reaction controlling means your rate of reaction is smaller than as compared to rate of diffusion so we have to improve the rate of reaction so that your product formation will increase now effect of mass transfer resistance.

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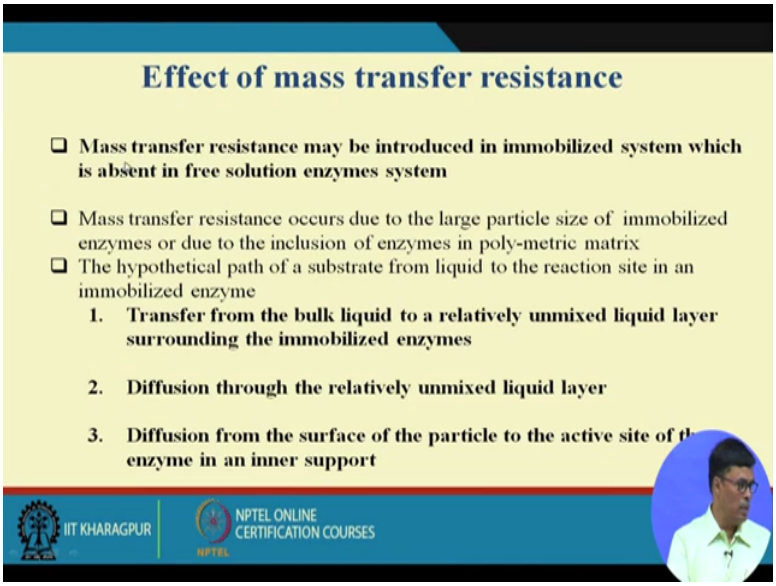

The mass transfer resistance may be introduced in enzyme immobilized system which is absent in free solution of the free solution enzyme system then in free enzyme solution your substrate and enzyme they are freely interact with each other there you don't have any kind of diffusion problem.

But as soon as I told you as soon as they immobilized then it is a heterogeneous mixture is a solid and liquid or solid has to diffuse on the surface of the liquid has to diffuse on the surface of the solid then the reaction take place after that it will go out.

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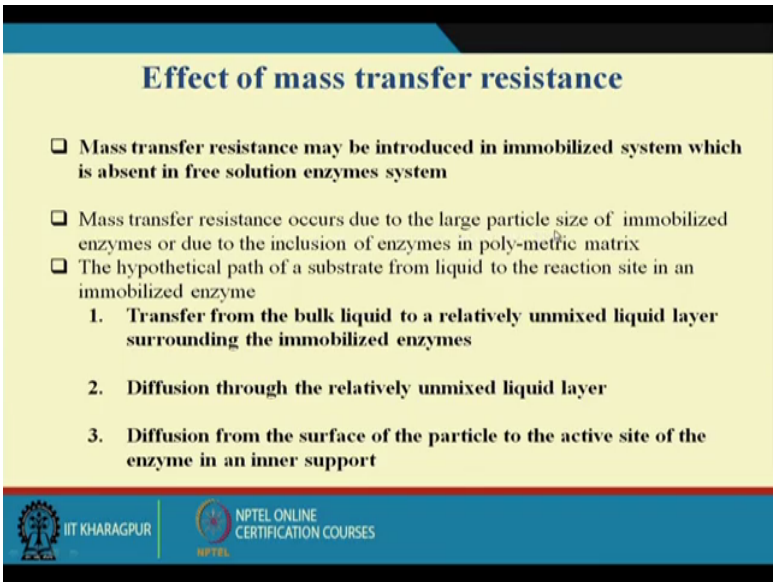

The mass transfer resistance occurred due to the large particle size of immobilized enzyme and or due to inclusion of the enzyme in poly Poly-metric matrix the hypothetical path of substrate from liquid to the reaction side in immobilized enzyme the transfer from the bulk liquid to relatively unmixed that liquid layer surrounding the immobilized enzyme then diffuse through the relatively unmixed layer liquid layer and diffusion from the surface of the particle to the active site of the enzyme to the into an inner support.

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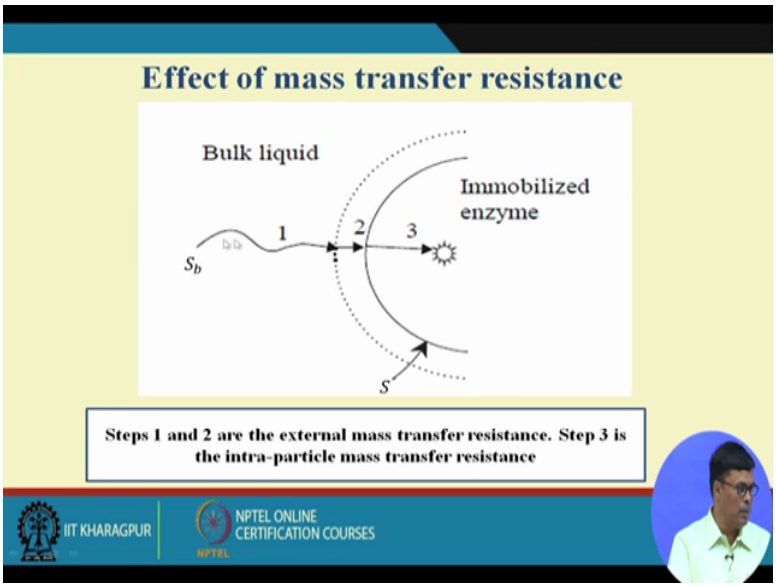

Now I can give the example here like this the substrate define diffuse here that is the unmixed layer then here to here surface then it goes to the inside of the code of the solid Matrix that we had that this is the three states that we required in in case of Mass transfer resistance.

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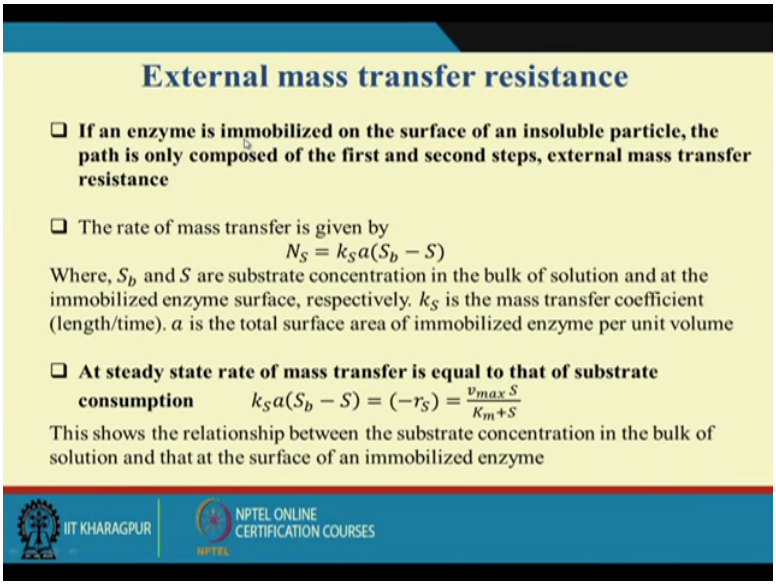

Now now there so we have 2 type of mass transfer we have most easy simplier technique is the if you have the external mass transfer resistance that means suppose.

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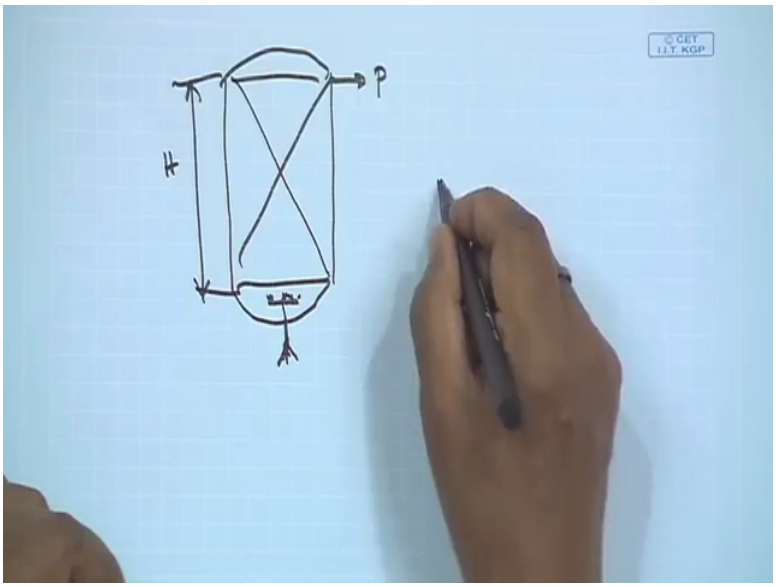

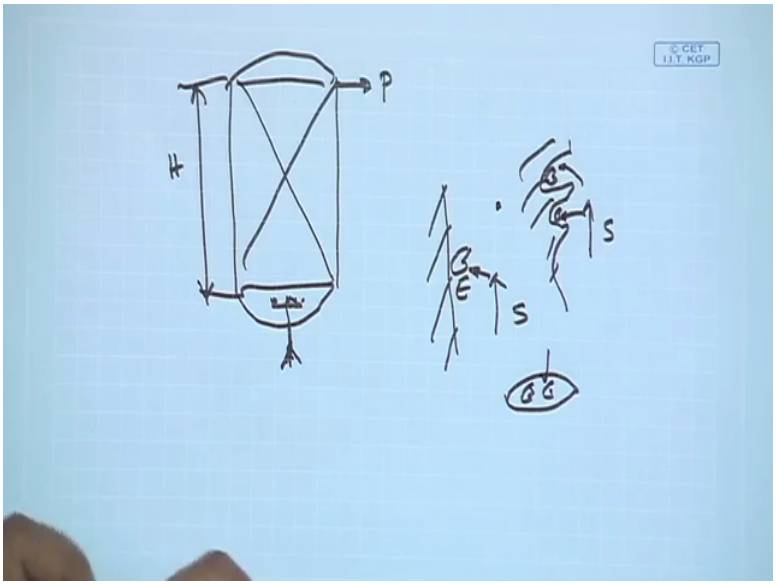

This is a solid Matrix and in the solid Matrix the enzymes is there and this is your this is your substrate this is your enzyme so it is at the surface so it is the external surface to your substrate can diffuse here very easily. What you call external mass transfer resistance now suppose your enzymes in a (())() suppose this is like this this the solid matrix.

And we have pour inside and inside this pour you have been enzymes located now when you pass here substrate the substrate have to diffuse inside this pour then we have inter particular diffusion that we have . We have been if you have the membrane inside the membrane we have then also you has to diffuse in inside the membrane that is that is the in kind of this is not the external solvent resistance.

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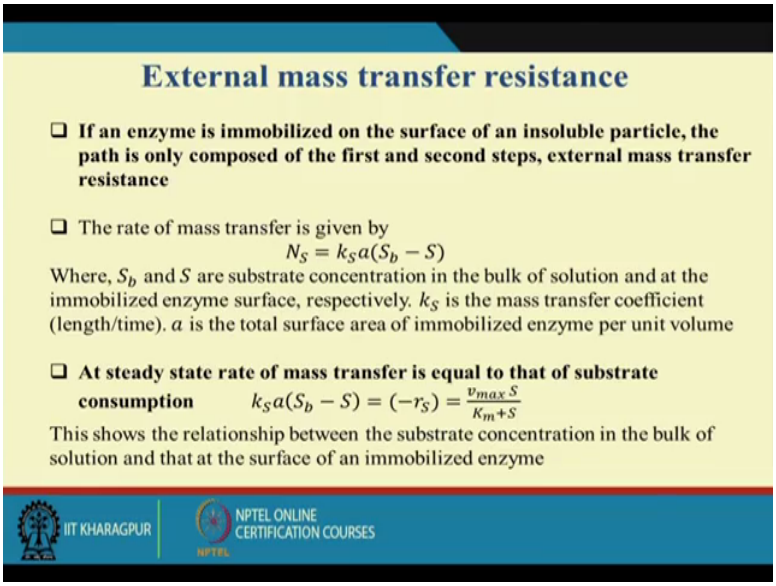

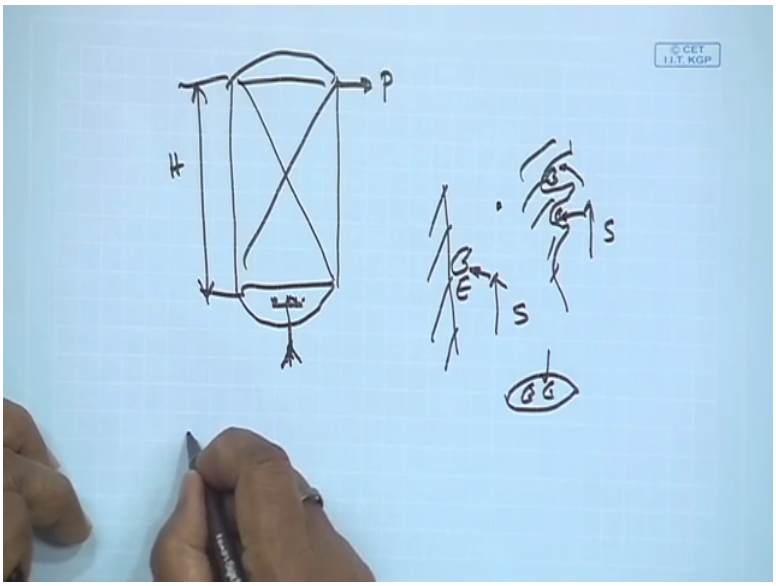

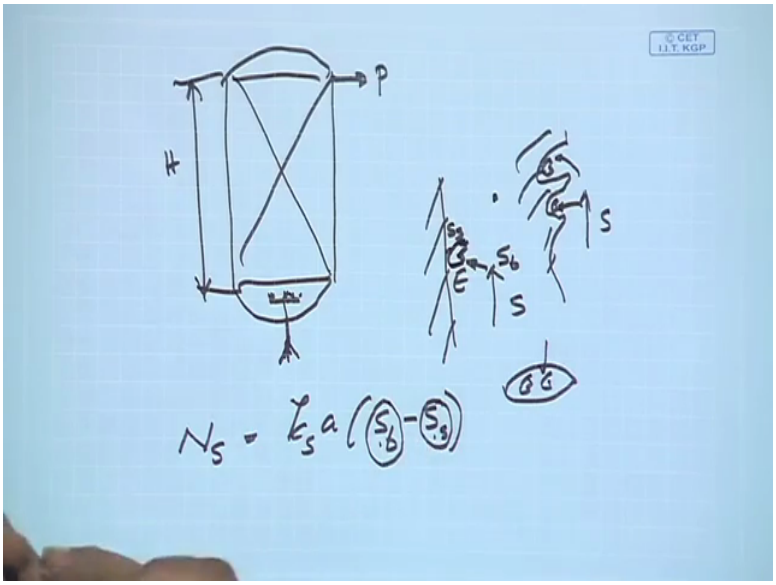

This is a inter particular resistance that will take place to for the simplest things we considered here that mostly the external mass transfer resistance if the enzymes are immobilized on the surface of insoluble particles the path is only composed of fisrt and second step and external mass transfer resistance now rate of mass transfer we usually expressed as NS is equal to KS A SB minus S this S actually SS.

SS actually SS is the here at the surface of the solid matrix whatever substrate concentration is very important because your enzymes is immobilized on the surface of the solid matrix now this is the bulk this is the liquid is flowing then here at the surface is SS so this is the substrate concentration at the surface of the solid matrix or at the surface of the enzyme or this is the bulk substrate concentration so what is the dragging force then you have SB minus SS so that is why I have written here.

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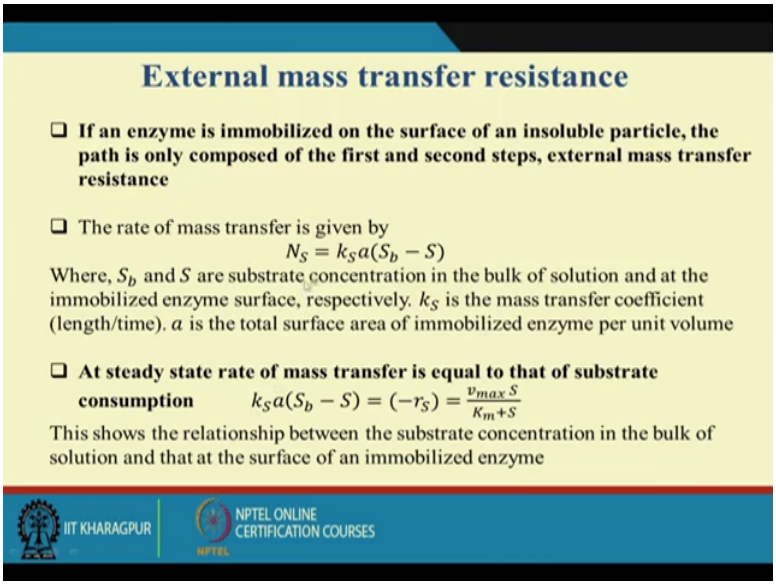

The rate of mass transfer equal to KS into A SB minus S under now under steady state condition what is steady state condition under steady state condition.

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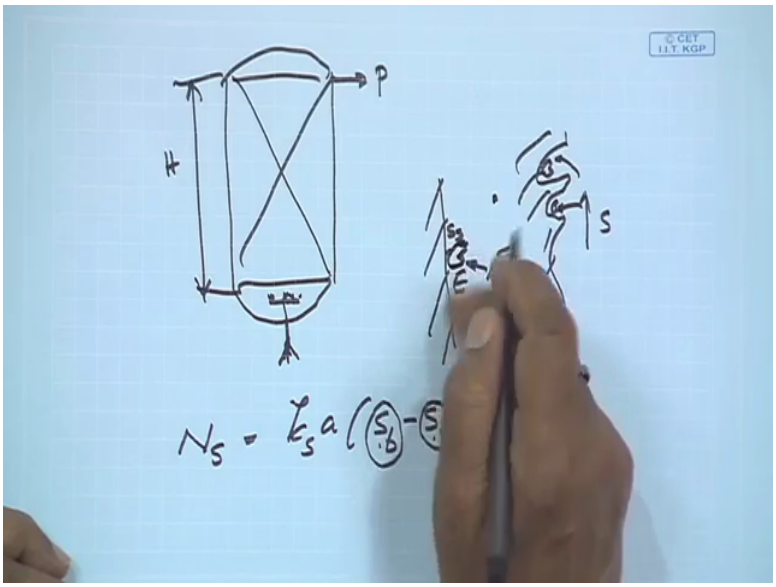

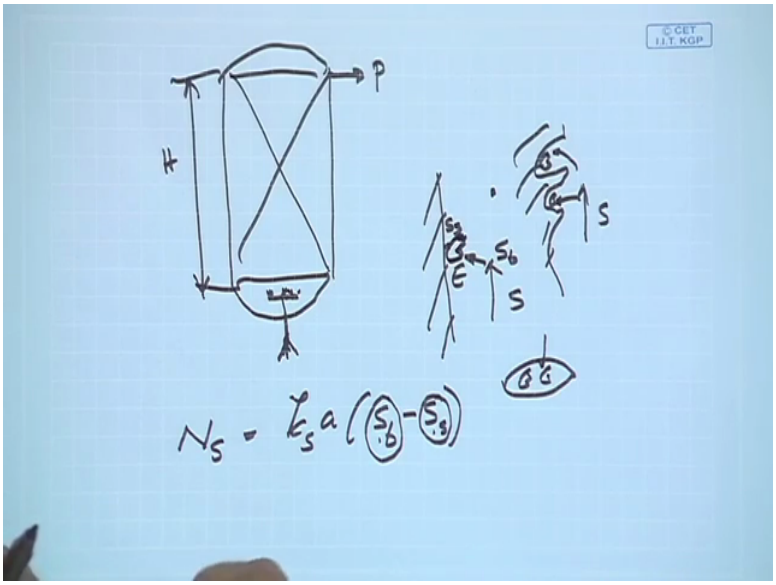

The SS the substrate concentration at the surface of the solid Matrix that should remain constant now suppose in in the enzyme substrate rate of reaction is more then what will happen if the rate of reaction is more then more substrate will consume the substrate concentration keep on decreasing with respect to time . If the rate of substrate consumption is less then rate of substrate keep accumulating on the surface of the solid matrix.

So that is the un steady state condition under steady state condition what will happen if your rate of diffusion is equal to rate of reaction then if substrate the rate at which the substrate is coming in the surface of the solid Matrix and at the same rate if the reaction take place then

and only then your substrate concentration on the surface of the solid Matrix will remain same so under the steady state condition rate of mass transfer is equal to rate of reaction.

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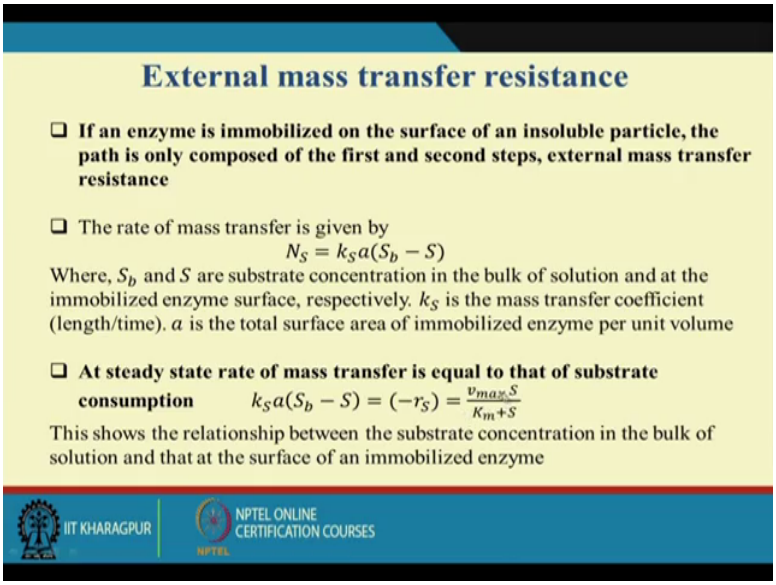

So we can write this this is the rate of mass transfer and rate of reaction we know this is a Michaels menten equation Vmax now this is obviously the substrate concentration at the surface of the solid Matrix.

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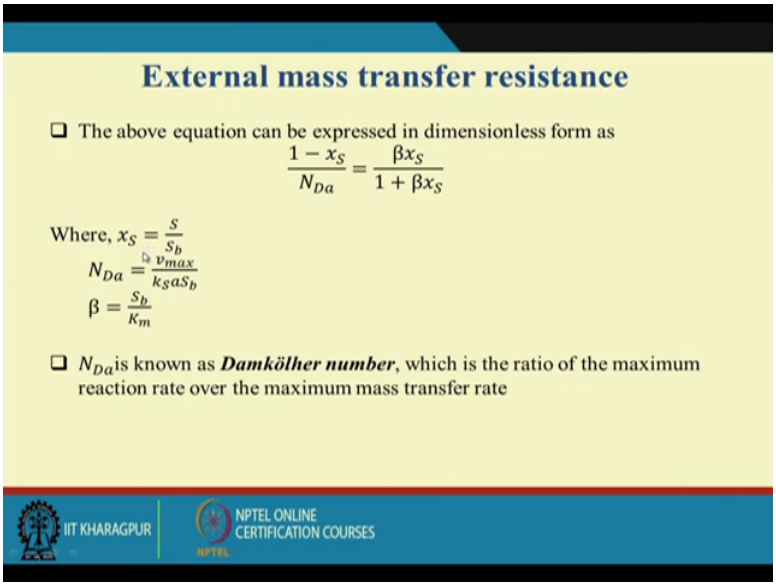

Now if you if you divide by SB in the both side and we can we can write this equation in this form 1 minus XS my Beta XS XS XS is equal to a SB S by SB S is the substrate

concentration at the surface of the solid matrix this is the bulk substrate concentration NDA is the VMax so here I want to elaborate little bit NDA is.

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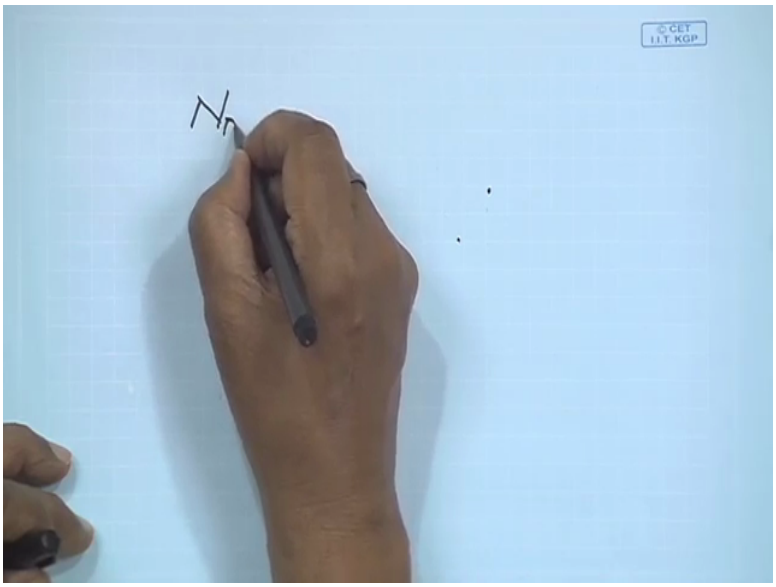

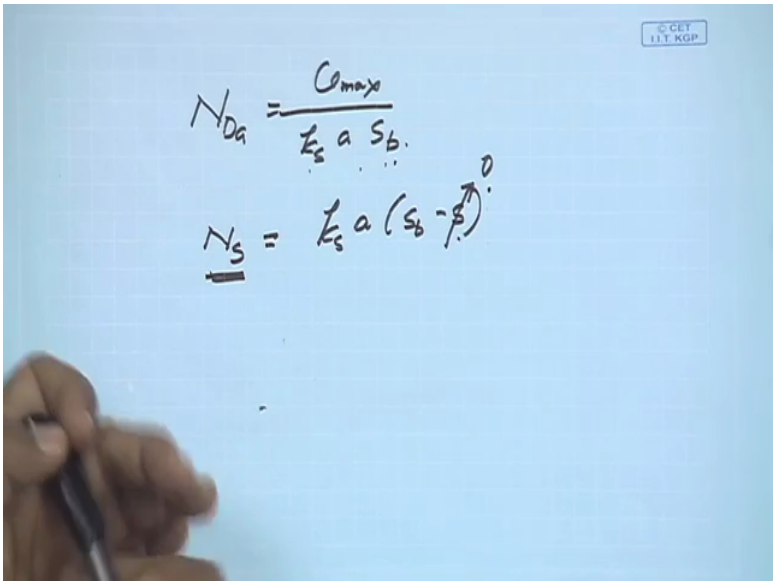

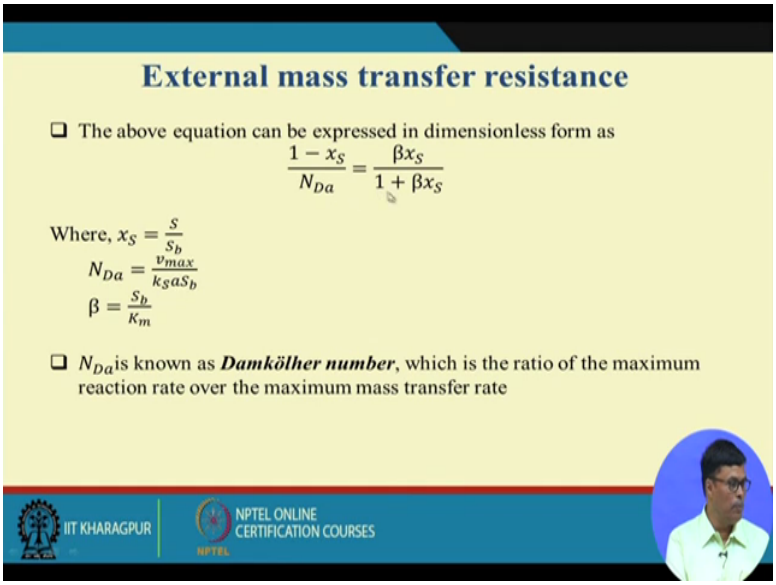

We can write NDA is Vmax KS a into SB now what what you can Remember what is the rate of mass transfer what we have written this is case A SB minus S am I right now S is the substrate concentration at the surface of the solid Matrix now when this rate of mass transfer will be maximum when this gradient will be maximum this gradient will be maximum when this is equal to zero if it is equal to zero then it will be KS A into S.

So the dam cooler number basically Vmax is the maximum rate of reaction and this is maximum rate of mass transfer so dam cool number this is NDA is called as this dam cooler number which is the ratio of maximum rate of reaction over the maximum mass transfer rate so beta s the SB by Km so these are the if you put this value then this equation.

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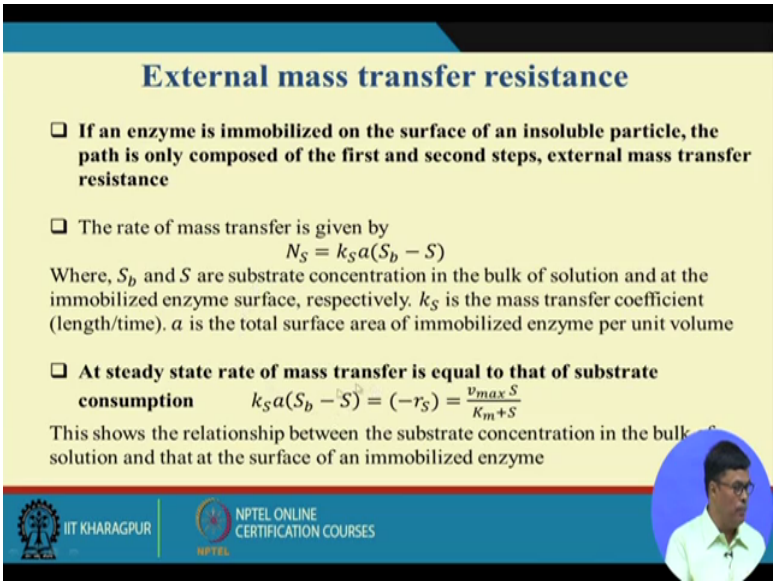

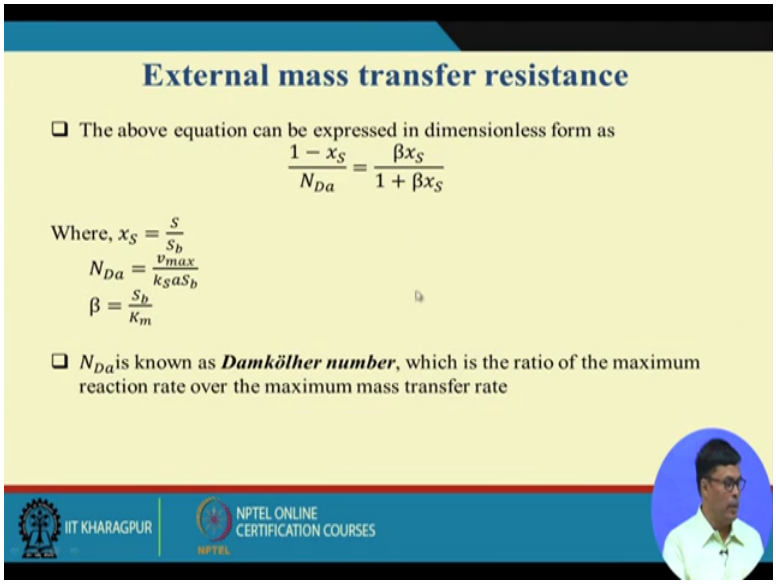

This equation we can write in this form we can write in this form very easily.

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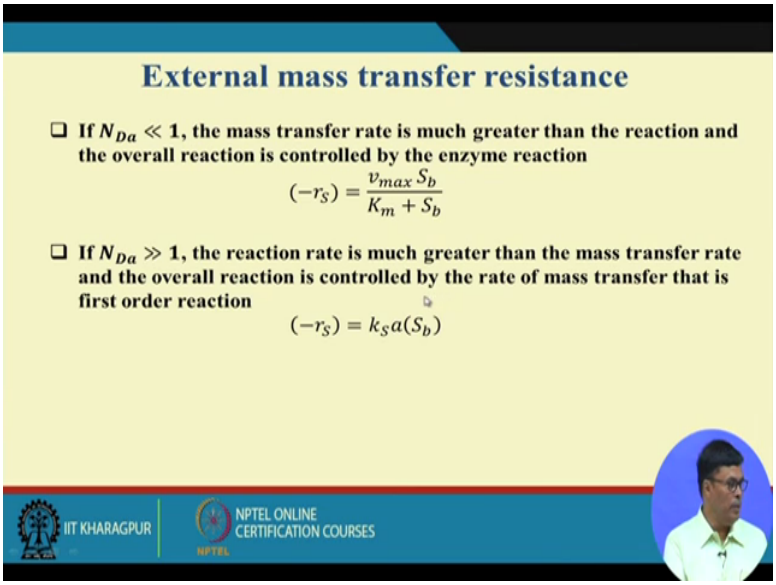

And then now and now what is the 3 of this dam coolant number now we have two different situation deep.

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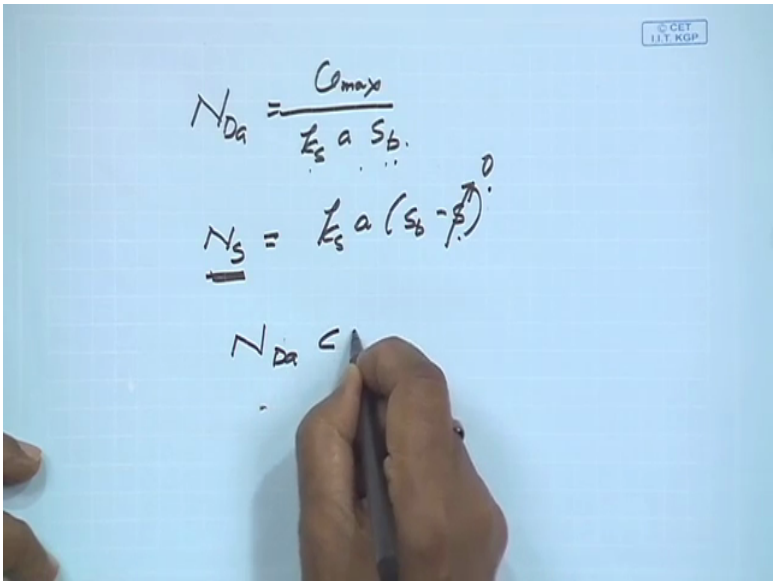

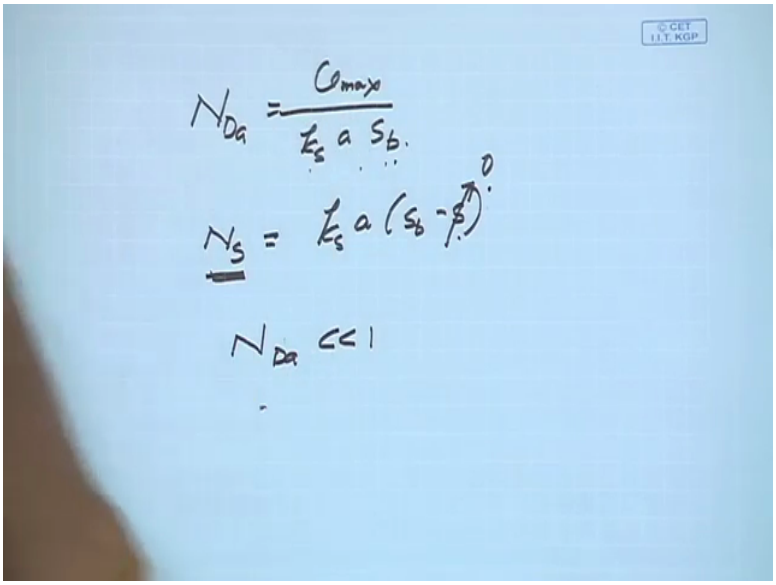

NDA is less than one is much much less than 1 then what will happen if it less than 1 then your this will be very high as compared to this will be very low as compared to the rate of mass transfer will be very high as compared to rate of reaction.

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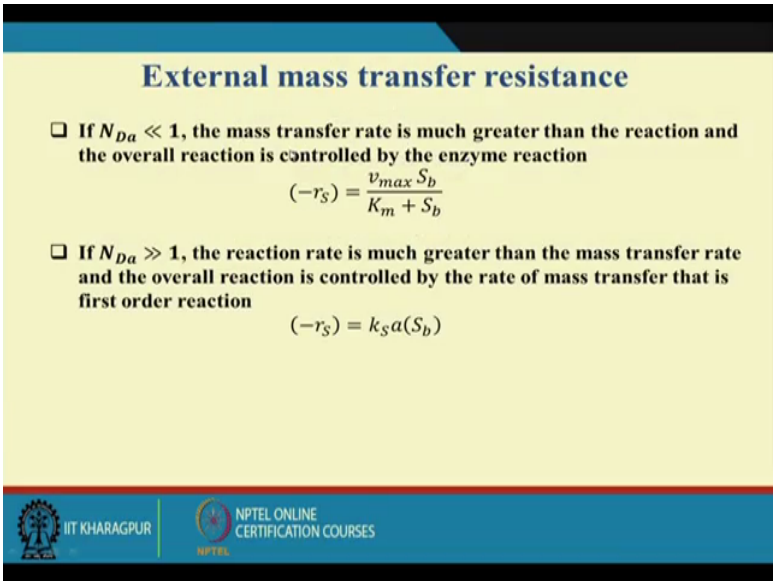

So if rate of mass transfer is very high as soon what will you have mass transfer is greater than overall reaction will be mass transfer control mass transfer control that the rate of mass transfer this will be very high.

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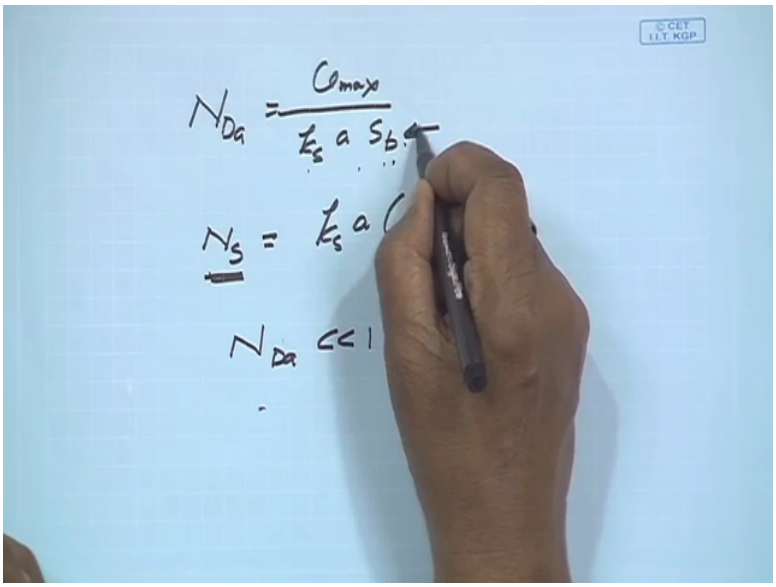

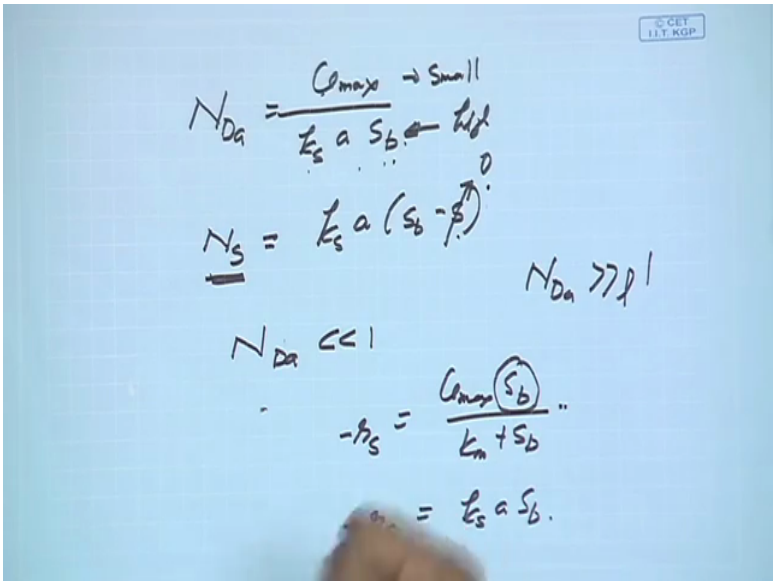

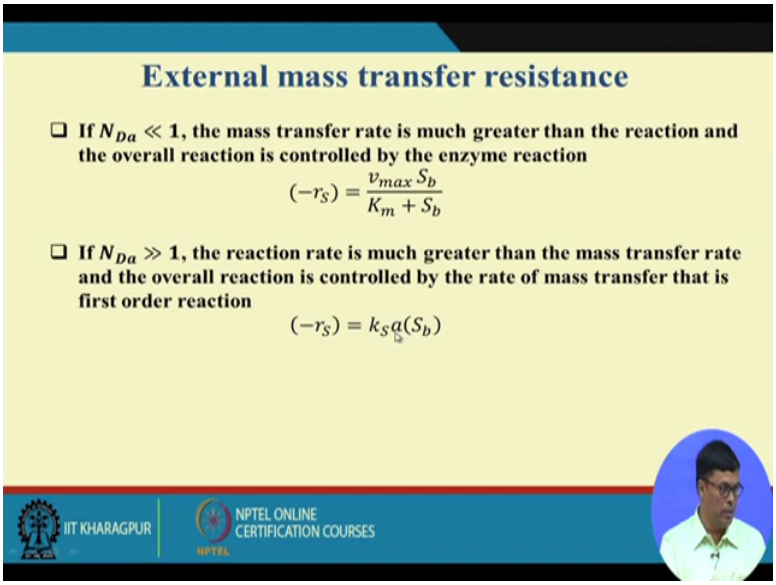

In case of this this will be very high and this will be very small ok now if it is like this then what will happen that you have to the rate of reaction that rate of product formation it control by the rate of reaction of reaction.
So your velocity of reaction minus R is we can write that V Max SB Km plus SB because you know that because it is controlled by this rate of reactions but in case of reverse suppose NDA is much greater than 1 then in that case what will happen the VMax is very high as compared to rate of mass transfer so it is a mass transfer control the since it is a mass transfer control then your reaction will be equal to KS A into SB.

Then it will not be the reaction control this is a mass transfer control if you want to improve upon the reaction then you have to mass transfer have to do if in NDA the reaction rate is much higher than mass transfer over reaction is controlled by the rate of mass transfer that is the the first order reaction this is constant and this is follow the first order reaction.

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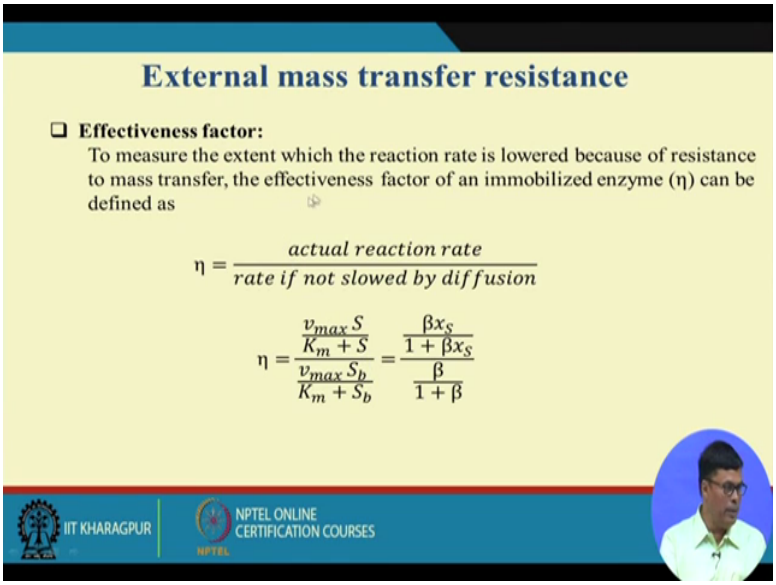

Now another another things we came across in case of immobilized system that is heterogeneous reaction we call this effectiveness factor what is effectiveness factor to measured the extent which the reaction rate is lower because the of resistance of mass transfer the effectiveness factor of immobilized enzyme can be defined as actual rate of reaction and rate if not slowed by diffusion let me let me elaborate little bit.

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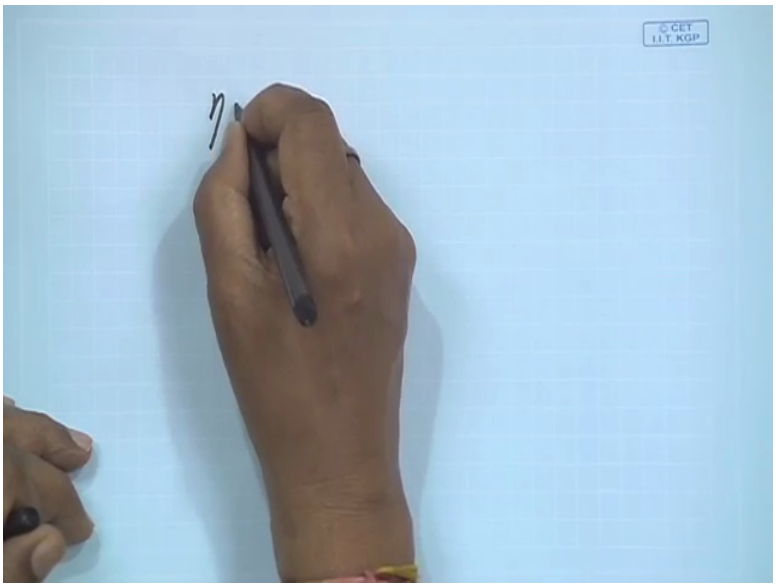

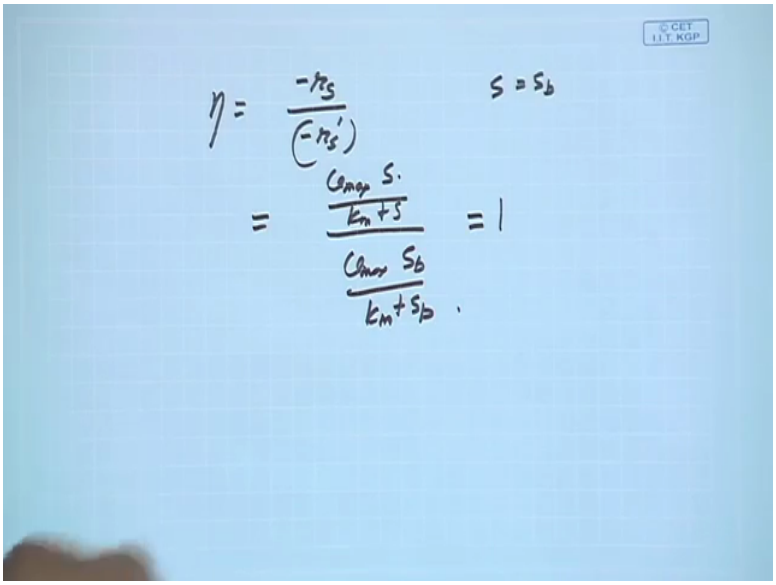

Suppose eeta eeta is effectiveness factor now rate of reaction actual rate of reaction how we can write minus RS am I right we can write RS that now this rate of reaction that this normal rate of reaction now if we assume all substrate there is no mass transfer limitation then all substrate will be available then S will be equal to SV if S will be equal to SB then then there is no mass transfer diffusion problem.

Then then the reaction that we have that is the SB the special case as per here I can write in case of normal reaction what will be this VmaxS this is S Km plus S but when all substrate available then then this S will be replaced by SB Sb means in the bulk whatever the substrate

the whole substrate concentration available on the surface of the enzyme then this is like this so this is the effectiveness factor.

Now the interesting thing is that if this is equal to 1 what will happen then this equal to that means all substrate is available for the enzymatic reaction then there is no mass transfer limitation problem so you immobilization system will approach to the heterogeneous reaction system approach to the homogeneous system.

Because I told you 2 type of reaction is there one is homogeneous and heterogeneous so when eeta value is one that will all substrate available on the surface of the enzyme for enzymatic reaction so there is no any kind of mass transfer limitation problem.

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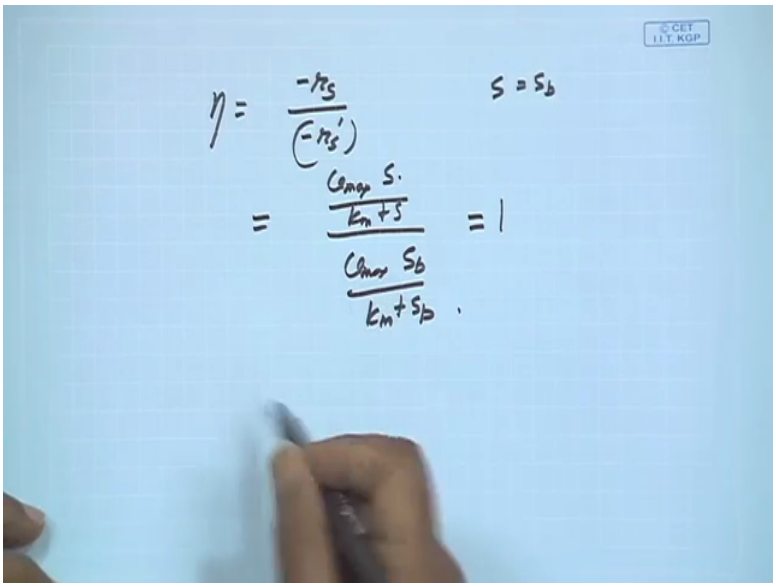

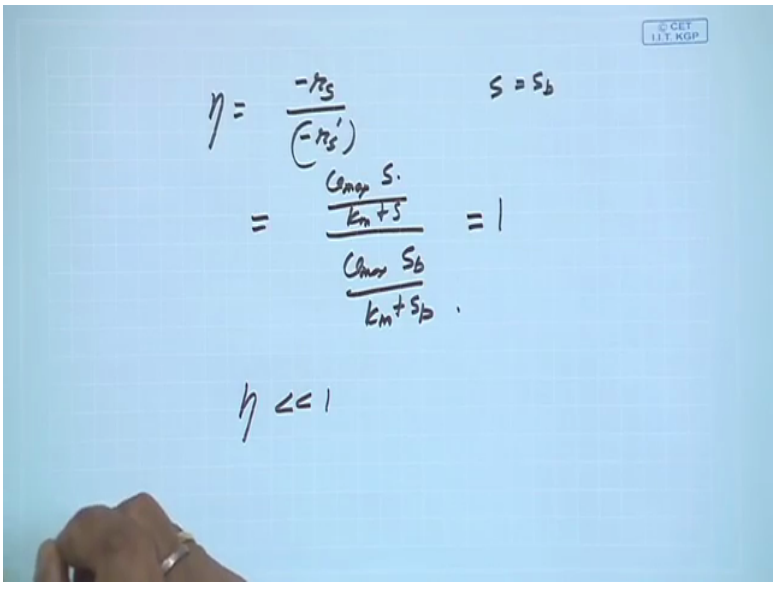

So in that case this eeta is equal to one but eeta in case it is very less than 1 then what will happen if it is less or tends to zero what will happen then your this will be very small as compared to this that means less amount of substrate available on the surface of the solid matrix then really we have the diffusion problem. We will be having the diffusion problem so from this effectiveness factor we can easily find out that whether you have diffusion problem or not we can easily determined that what is the titles of system that we have.

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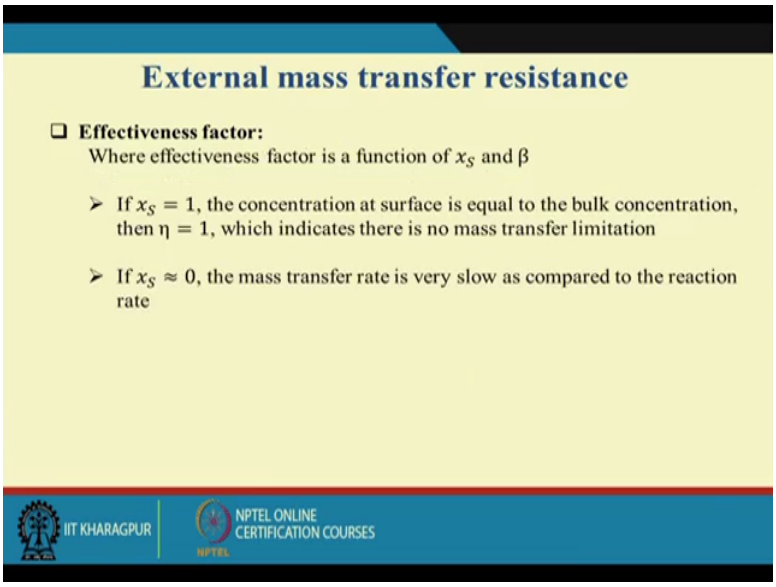

Now here we can have written that XS when the effectiveness factor is a function of XS and be the XS is the one the concentration at surface is equal to the bulk of the solution then eeta equals to one that is exactly what I am saying that if if the surface is the concentration equal to the bulks are there is no mass transfer limitation but when X is equal to zero X is this is the substrate concentration at the surface of the solid matrix when it is zero then mass transfer is slow as compared to the rate of reaction.

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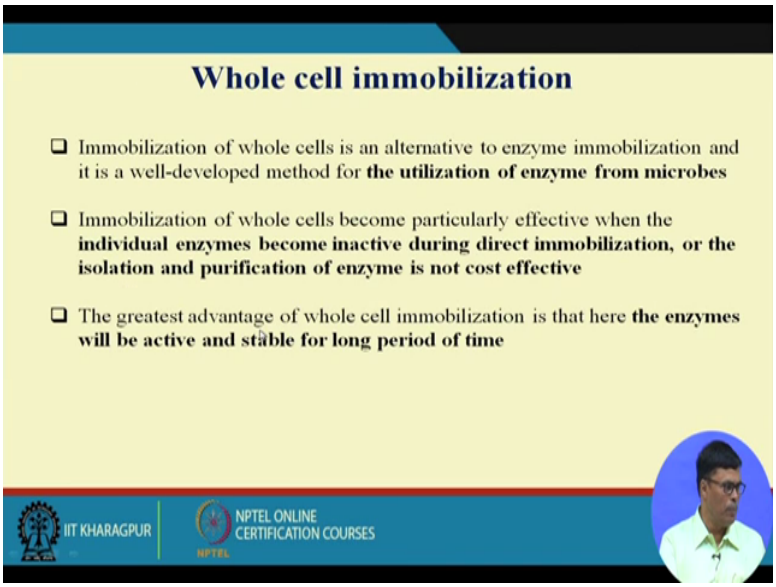

So this is you have to improve upon this process now in the last lecture I try to concentrate on the enzyme now let me talk little bit of whole cell immobilization technique immobilization whole cell is a is a alternative to the enzyme immobilization and well developed method for utilisation of enzyme from microbe.

The immobilization of the whole cell become particularly effective when the individual enzymes becomes inactive during the direct immobilization and the isolation and purification enzyme is not cause is not cost effective so what I want to point out.

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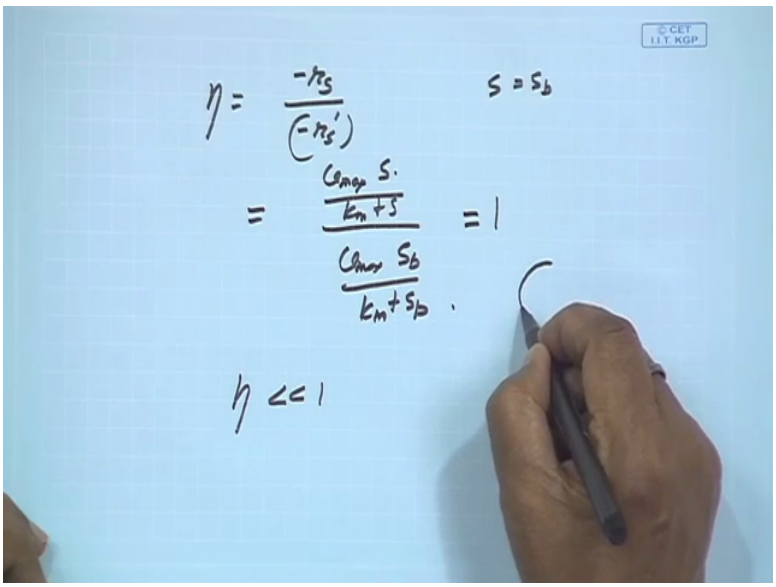

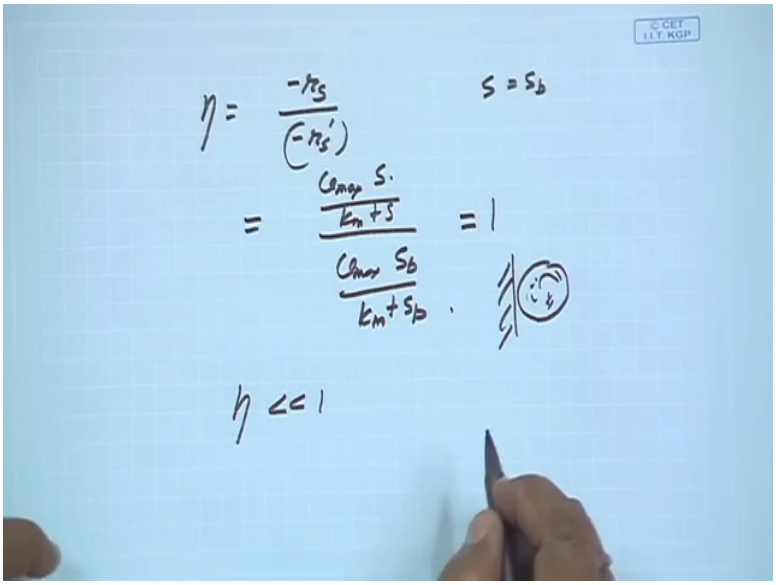

When you when you do the cells that cells inside the cells you have multi enzyme system so when you fix this solid Matrix on the itself you immobilized under surface then enzyme is in inside this cell that will not be deactivated or you don't have to purify the enzyme that purification of enzyme require additional cost so your system will be very very cost effective so I can tell you I walk with different type of immobilization system.

And particularly for the (())(18:48) digestion process I walks with methanosions and we use the poly propylene solid matrix and we immobilized the methanogenic organism on the solid matrix and pass there waste water from the bottom and it converted to methane and carbon dioxide and you know that why it is advantageous because methanosions they are very sensitive to dissolve oxygen because they are (())(19:16) .

And if the concentration little oxygen is there then the most of the organism will be killed so if you have immobilization system even when there is little contamination in the oxygen that takes place in the system will not directly affect the organism to a great extend so it is little bit stable system as compare to free cell system.

And another advantage of that I told you that in case of when you operate any CSTR what is the major disadvantage of this CSTR when you use the cell that you have continuous inflow and continuous outflow and when you are taking out the liquid you are taking out the liq with the liquid you take out some cell also now if the amount of cell mass that is going out of the of the reactor is more as compare to cell mass that is growing inside the reactor.

Then what will happen the cell mass concentration of the in the reactor will go down and and rate of reaction depends on the cell mass concentration now if the cell mass concentration is reduced then rate of reaction will there is a whole system will be affected so the immobilization system appears with data system because you can fix this solid matrix you are not allowing the cell to go out of the reactor and your rate of reaction will more less uniform in the react.

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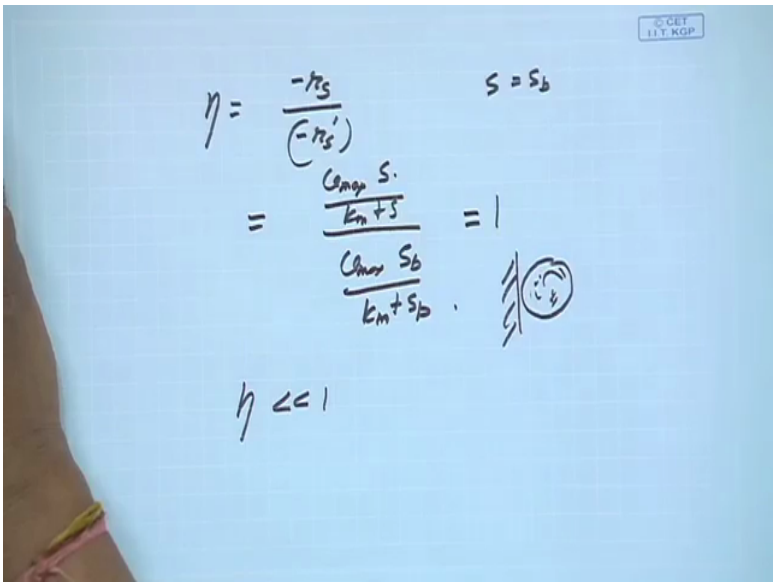

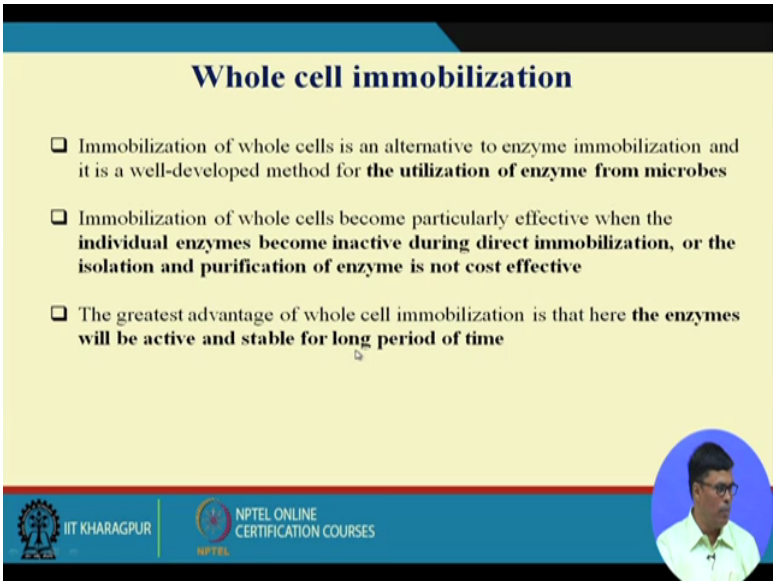

So that is the advantage of the whole cell immobilization is that the enzymes is that they here the enzymes will be active and stable for here the organism that all the active and stable for longer period of time.

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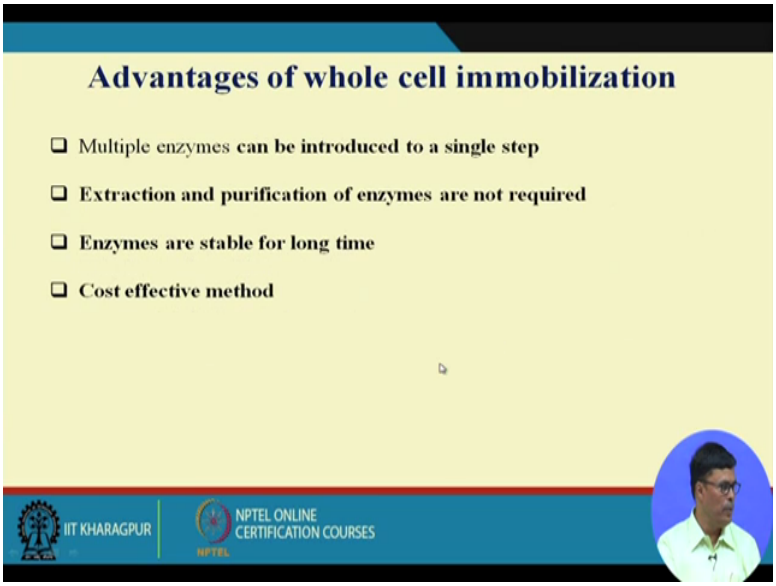

Now advantages of the whole cell immobilization is the multi enzymes can be introduced in a single step because you can change of reaction we can have in the as for examples that I can give the example of bacillus coagulant bacillus coagulant actually it produces this glucose isomerism enzyme so if we immobilize the bacillus coagulant pass glucose through this membrane.

Then it converted the glucose to fructose so you don't have to purify that glucose isomerism enzyme the enzyme that is produced from the glucose bacillus coagulant that came itself use to convert the glucose to fructose this will be very the single step reaction.

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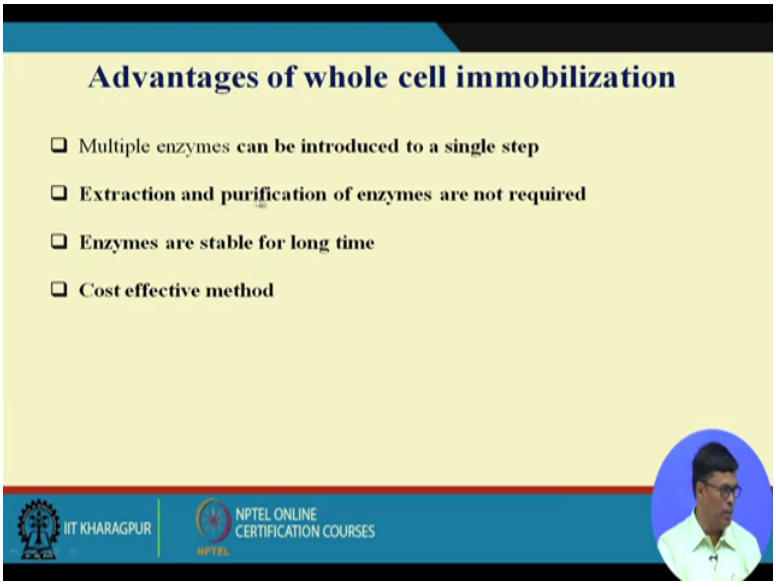

Extraction and purification enzyme are not required enzymes are stable for longer period of for longer period it is cost effective method.

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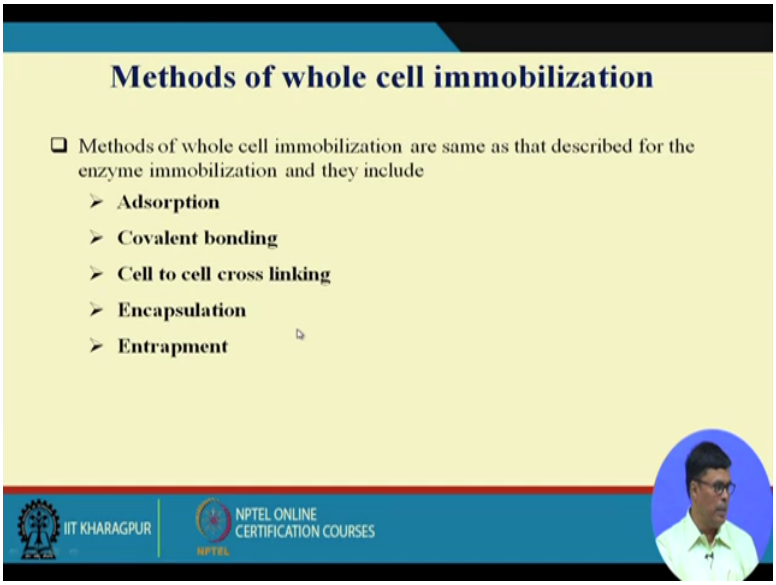

So this is the thing that we have and here also we use we can use the different techniques like absorption, covalent binding, cell to cell cross linking encapsulation entrapment like I discuss in my last lecture about this so I don't like to elaborate further.

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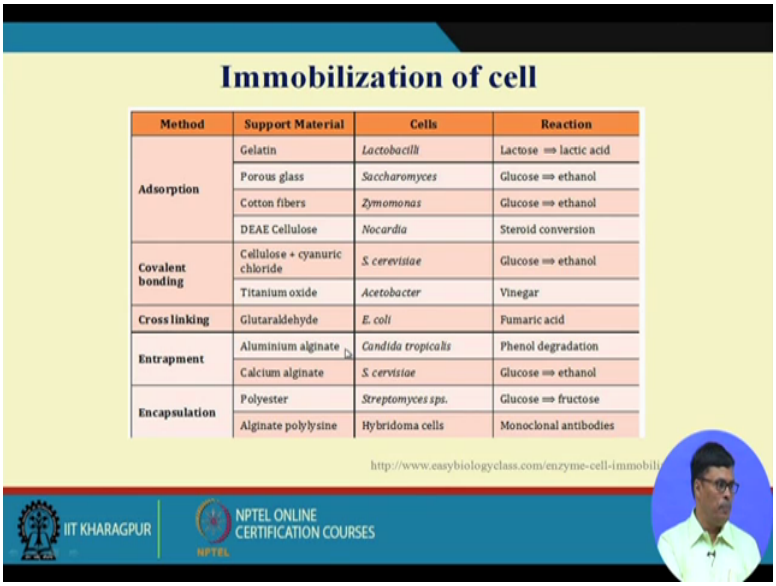

Now the examples we have Sable we have different methods absorption, covalent bonding then this cross linking entrapment and encapsulation. Now absorption we have gelatin example is this lactobacilli is used to convert lactose to lactic acid now now you might be

award the lactic acid as tremendous potentiality in the market particularly poly lactic acid is the largely used in the pharmaceutical industry for operational purpose.

So this is tremendous potentiality in the market and also used for the preparation of (()) (23:02) that in the household requirement then porous glass we use as a solid matrix for sacchromyces to convert the glucose to ethanol this is for immobilization of the sacchromyces (())(23:17) then cotton fibre zymomonas we use we use to convert glucose to ethanol DEAE cellulose.

We use this is use as the solid matrix or nocordia this is steroid conversion this is the covalent binding we have several cellulose and cyanuric this is chloride this is sacchromyces cerevisiae glucose to ethanol then cross linking we have E. Colli this is used for the production of fumatic acid now covalent bonding we have another example titanium oxide Acetobactor acetic that convert ethanol to acetic acid for the vinegar production.

Then we have entrapment aluminium alginate candida tropicallis this is for phenol degradation then we have calcium alginate S.cervisiae this is again for alcohol production. Encapsulation we have polyester streptomyces species glucose to fructose then algenate polylysine this is the hybridoma cells for monoclonal antibodies producton this is the different way we can use the immobilized cells.

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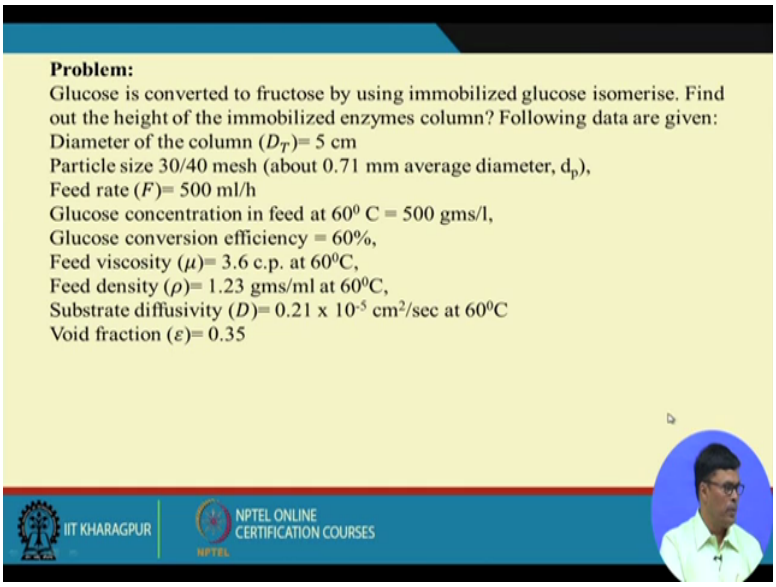

As I told you in the beginning that I want to discuss a problem just to find out that how you find out the height of immobilization column to get a desired reaction so this problem I have

chosen there is glucose converted to fructose by using immobilized glucose isomerism enzyme the find out the height of immobilized enzymes this spelling is mistake you can correct it isomeri the following data are given diameter is 5 Cm the particle size 30 to 40 mesh this is very interesting.

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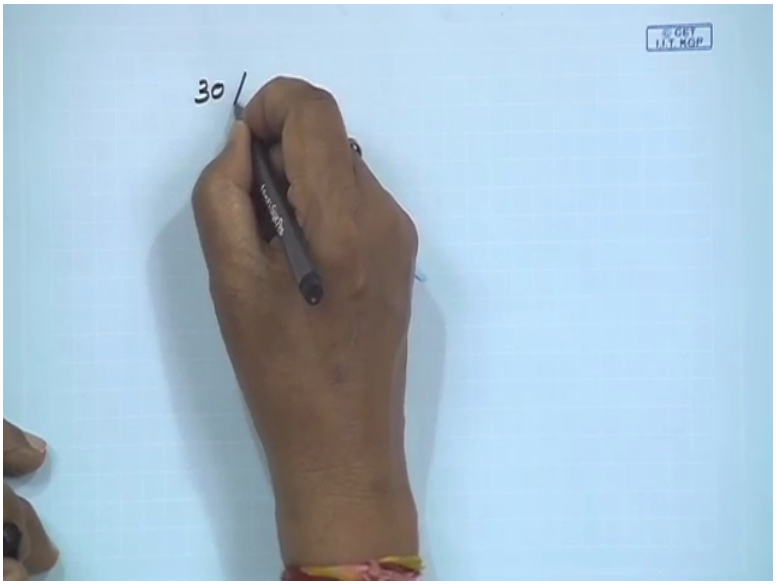

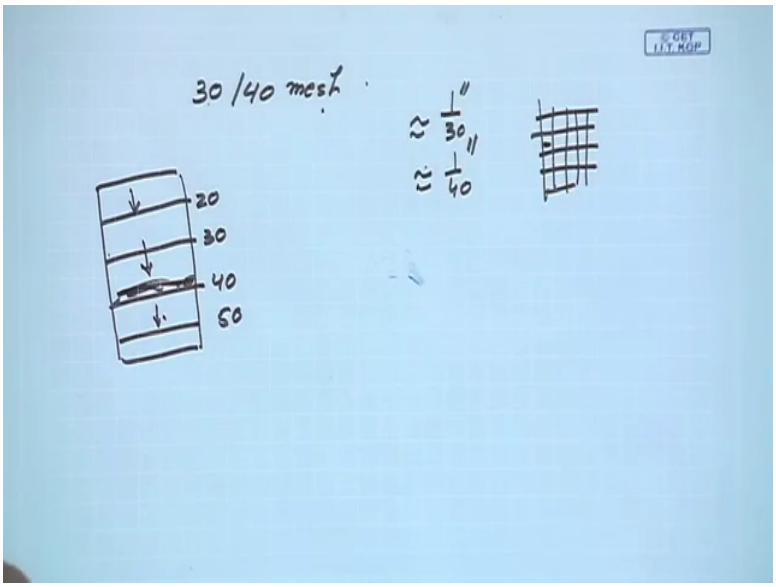

What do you understand by 30 to 40 mesh so you know that they usually that thumb of the rule is that 30 mesh is close to that you know that this is close to 1 by 30 inch it is approximately equal not exactly this is approximately equal to 1 by 30 inch 40 mesh means it is approximately equal to 140 that is the pore size suppose in the in the solid Matrix we have we have pore size.

And this pore size is this 1 by so if you have higher pore size lower number that means pore size will be bigger and higher number pore size will be smaller so we have we have sewing plate suppose we have sewing plate and we have different pore size this is like this so this is 20 mesh this is 30 mesh this 40 mesh this 50 mesh.

Let us assume that if the here you can have difference size of screens now if you put your solid material here and you do the vibration then what will happen that some particle will cross this and retain here so and some particle will cross this so whatever particle retain here we call it 30 by mesh that means particle size lies in between 1 by 30 inch to 1 by 40 inch.

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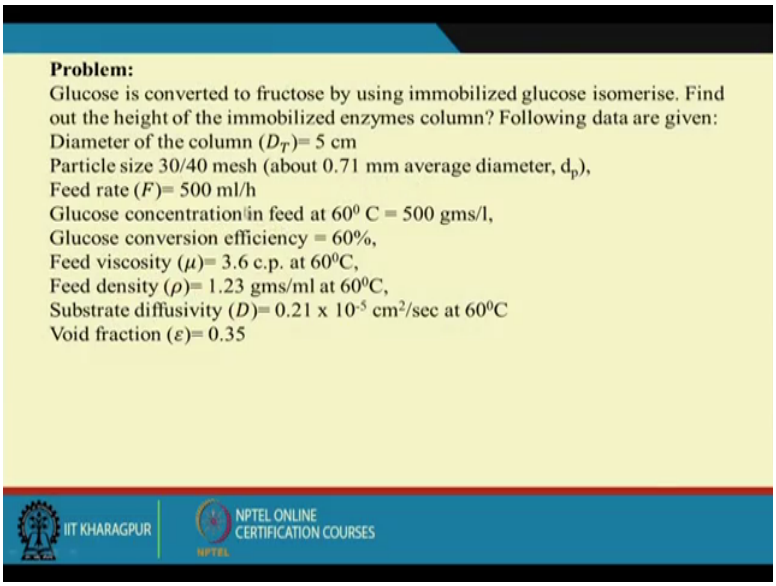

So here the approximately that that this is equal to about point seven one millimetre average diameter now feed rate is 500 millilitre per hour the glucose concentration in feet is 60 degree centigrade that is 500 grams per litre and glucose conversion rate is 60% feed viscosity is 3.7 centipoise at 600 degree centigrade feed density is 1.23 grams per litre at 60 60 degree centigrade substrate diffusivity is point 21 10 to the power minus 5 centimetre square per second at 40 60 degree centigrade and void fraction point 35 now I want to mention little bit of void fraction.

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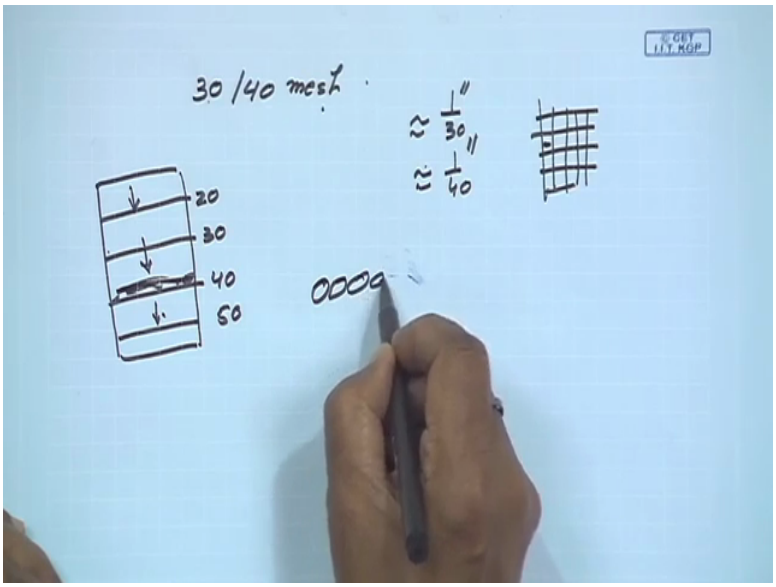

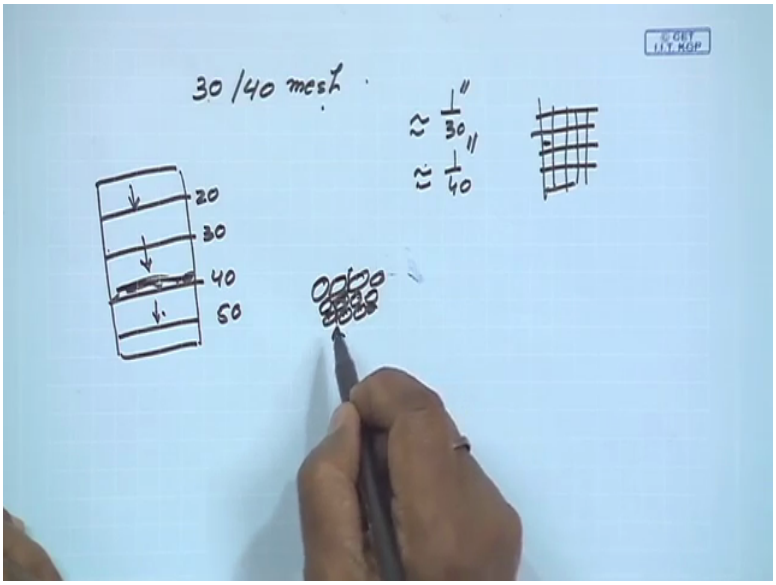

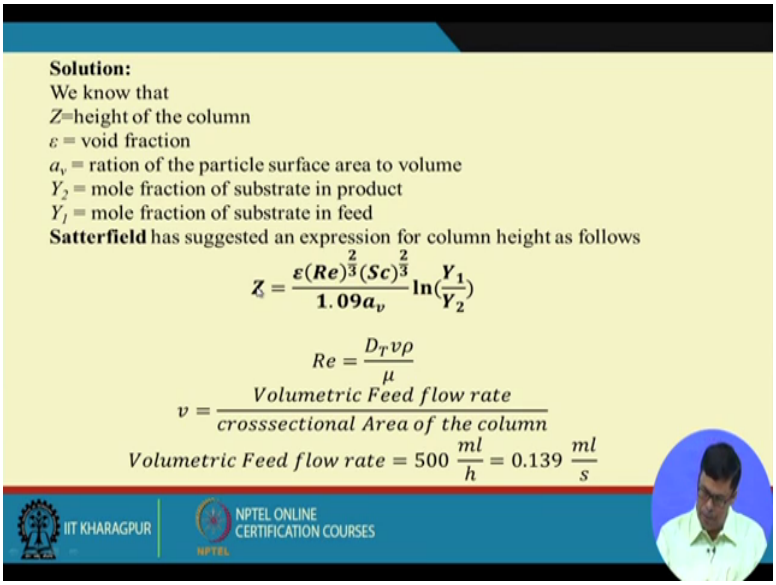

Suppose this is a solid Matrix and when you have solid Matrix and you are passing the liquid through this liquid can only travel in the void part you know that they cannot travel wherever there is no void space is there so the void the the the void void portion of the reactor plays very important role because void that liquid goes through that and that walking volume is equal to the void fraction of the particular immobilized system.

Now for solving this equation that we have the satterfield he suggested equation equation is this is the Z is the height of the column because I was talking about.

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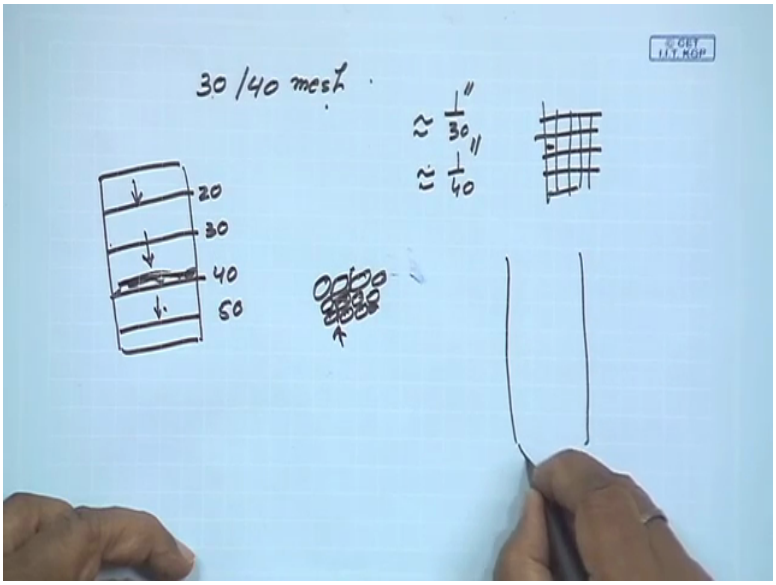

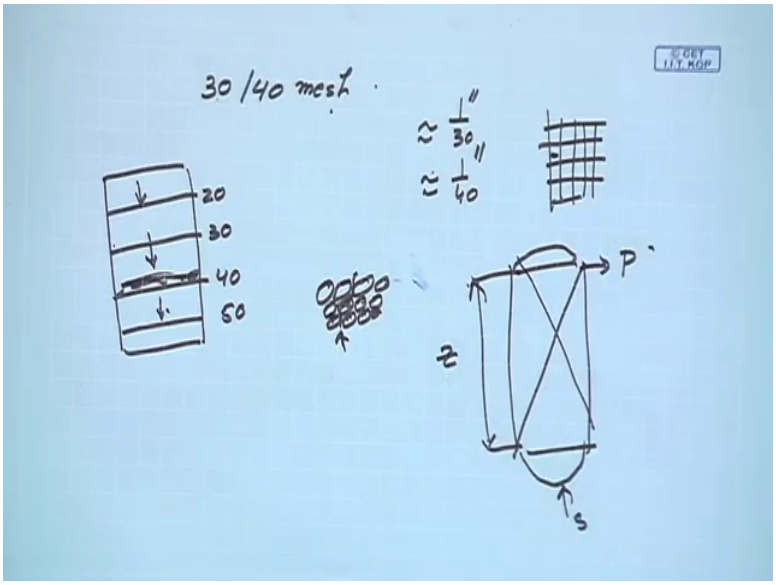

Suppose this is this is the this is the immobilize system that we have and we want to find out this and this height we considered as Z and this is the substrate is coming and product is going out like this.

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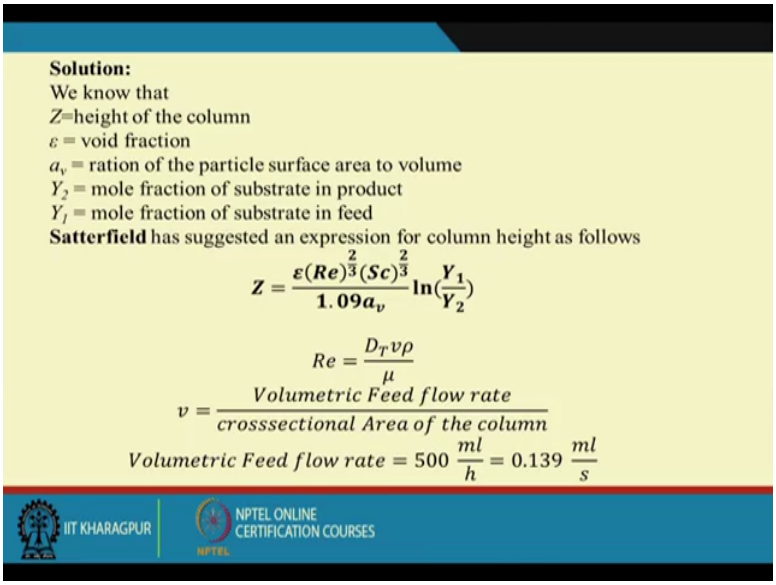

Now this the height of the column is equal to a epsilon this is the void fraction this is Reynolds number to the power 2 by 3 scmit number this is 2 by 3. One one point zero eight AV is the ratio of this surface per unit volume surface area per unit volume and this is the mole fraction of the surface in the entrance here what is the this is Y1.

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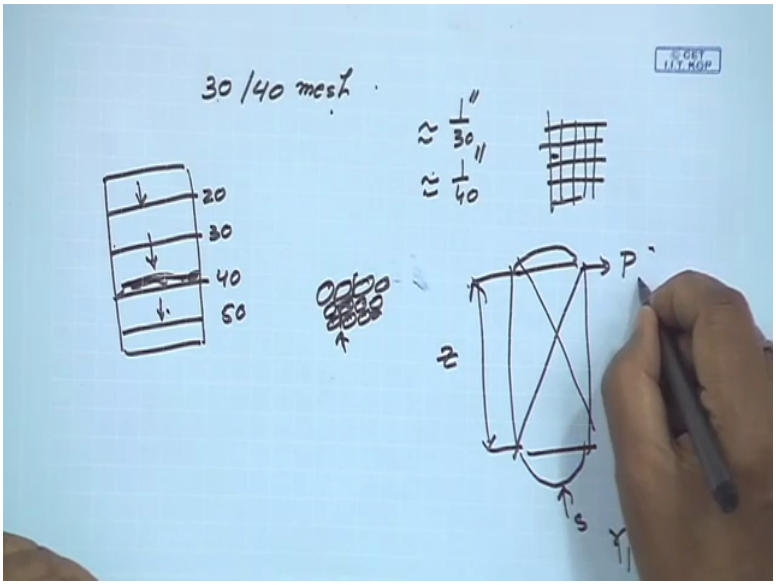

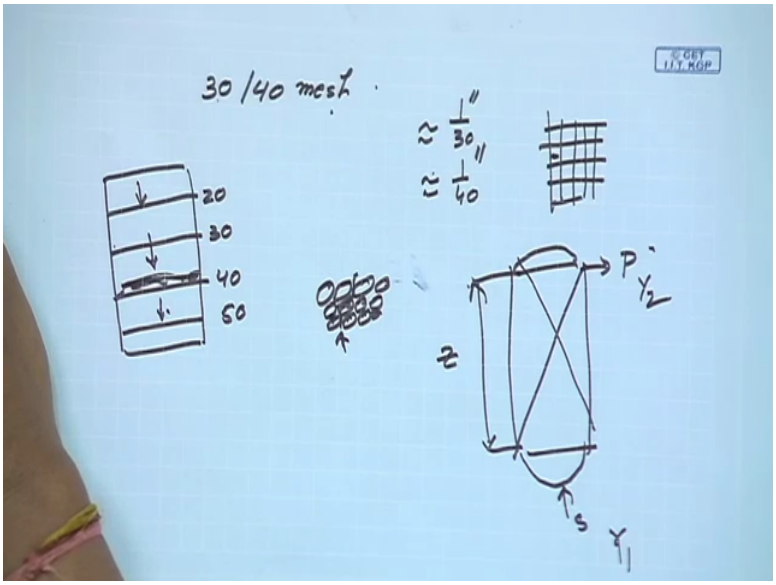

And this is Y2 this mole fraction of substrate in the incoming stream and outgoing stream now so we can first find out the product to the volumetric flow rate is given as 500 millilitre so we have the expression we can see the in the substrate diffusivity the unit is in second so we still have to convert in the second that is we divided by 16 to 60 then we get this is point 39 millilitre per second and then we find try to find.

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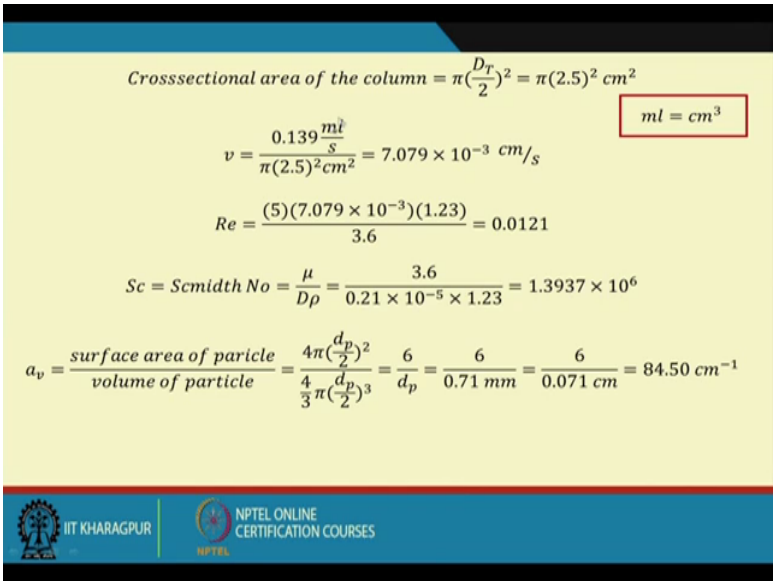

The A by V ratio A by B ratio is the point that you know surface cause the surface area this is A by V ratio is the surface area by volume of the surface 4 Pie S square is the we consider this spherical particles this is 4 Pie S square and this is volume of the spherical particle is 4 by 3 Pie R Cube so we can do that we can find out this now cross sectional area we can find out this why is required cross-sectional area we know the flow rate if you divide by cross sectional area.

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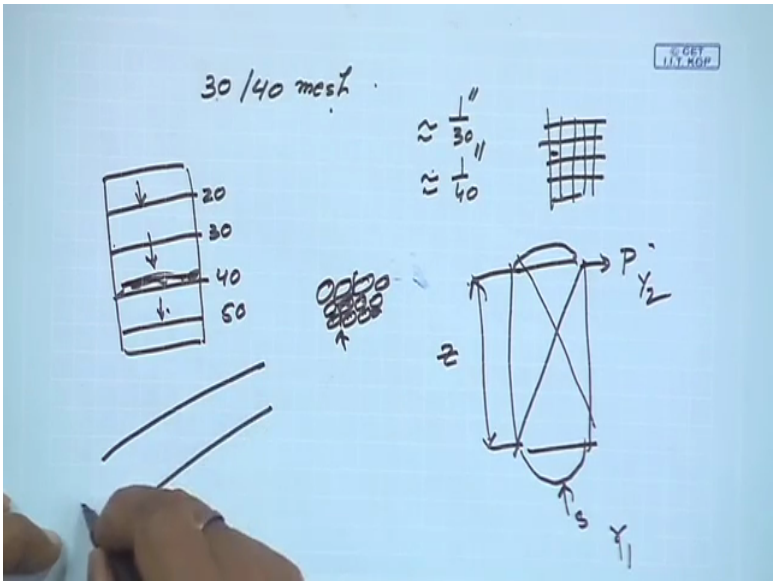

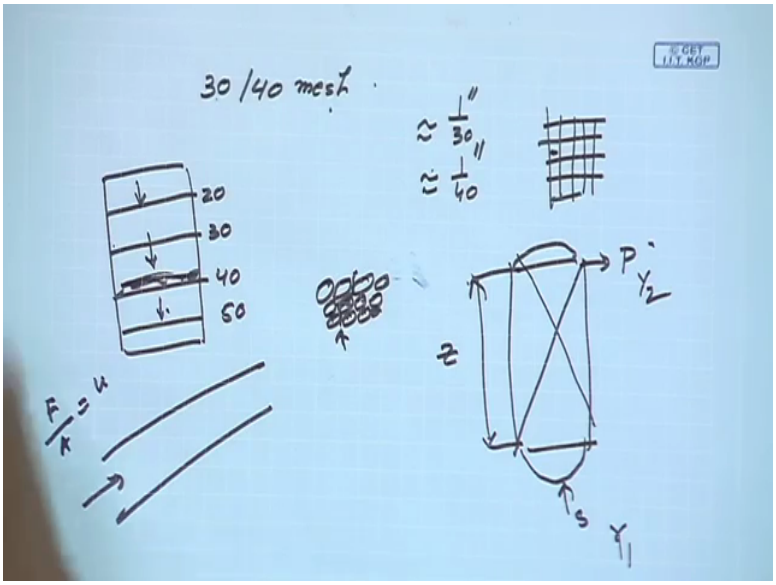

Because this is this is the liquid when going through this if you know the flow rate and if you divide by area you will get the velocity this is the velocity this is the velocity this is the how we have calculate the velocity centimetre per second.

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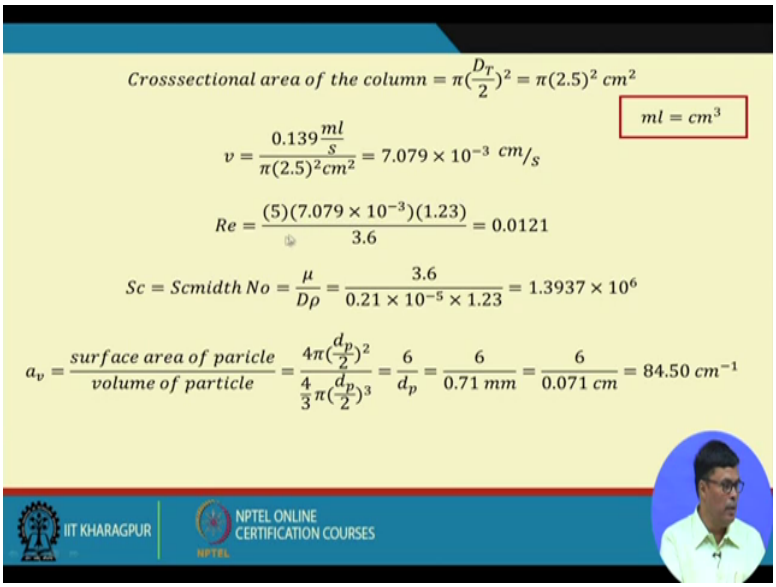

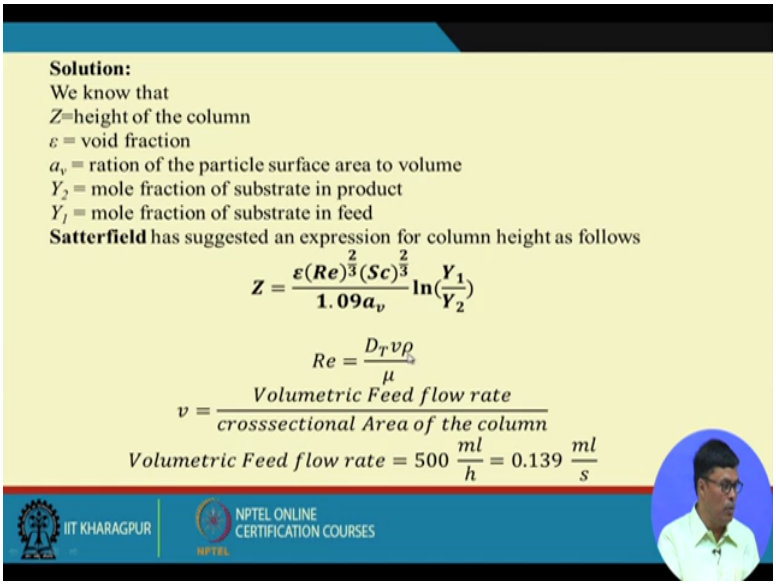

Reynolds number where we can easily calculate because I have already shown your here that you know somewhere DTV row by mu row is the density of the liquid mu is the vicosity V is the velocity and DT is the diameter of the tubes.

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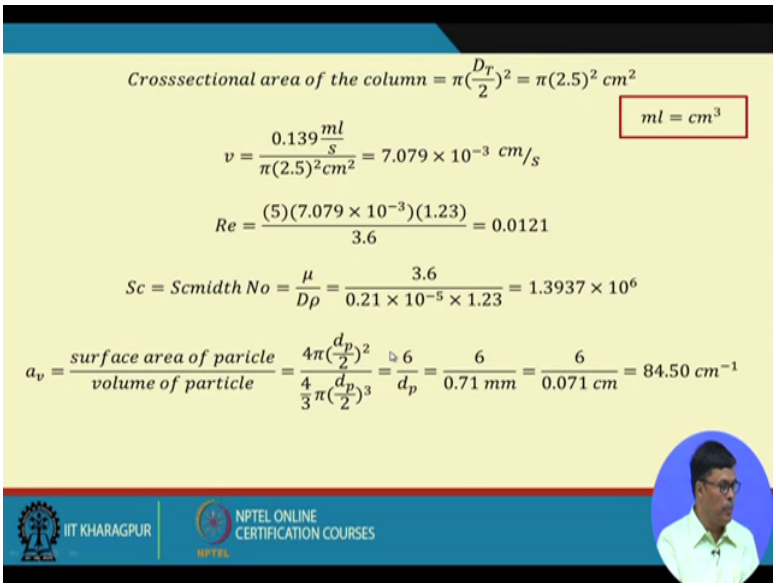

Then our reactors that we have so we can calculate scmit number we can calculate at Mu by D into P mu is the viscosity D is the diffusivity row is the density so we can easily calculate.

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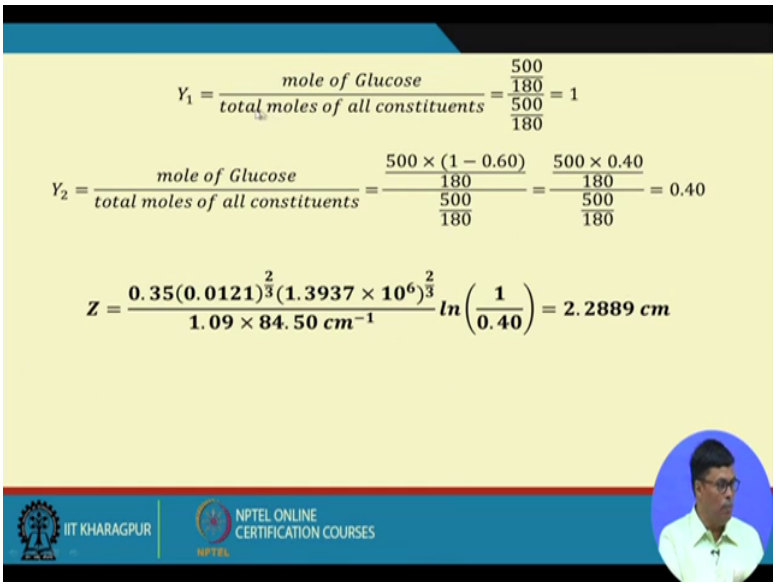

And if you now we mole fraction of the substrate the incoming stream is a will be equal to one because S content whatever substrate is content the initially that we put it but it is now 60 percent of the substrate is converted into the product that's means the remaining is 1 minus point 6 that is point 4 and it will coming Y2 is coming is point 4 now in food all this value is in this equation we get Z equal to 2.2889 centimetre.

So we can easily calculate the height of the that immobilized column very easily now in this so let me conclude this lecture that we in the immobilization technique we lecture we try to highlight that what is the purpose of immobilization what are the advantages of immobilization? What is the use of immobilization? What are the different techniques of immobilization?

What are the what is the methodology through which we can select the immobilization technique all all immobilization techniques under suitable for all enzymes that also we should remember we should select the immobilization technique on the basis of our requirement and then we consider the this is a reaction heterogeneous reactions systems both diffusion as well as the reaction it will take place now now question we discuss the that external.

And mass transfer diffusion problem and try to find out how the damkohler number and the effectiveness factor plays important role to find out whether the reaction is diffusion controlled or it is just whatever that is the rate of reaction control so and finally we try to solve 1 problem and try to find out the height of the column of the reactor thank you very much!