Aspects of Biochemical Engineering
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Lecture - 29
Kinetics of enzyme catalyzed reactions using immobilized enzyemes – I

Welcome back to my course Aspects of Biochemical Engineering. In the last couple of lectures I try to discuss the immobilized enzyme system. Because initially I told that what are the, what do you mean by immobilized enzyme? And I told that immobilized enzyme is nothing but our confined or you know fixation of the shell on the solid matrix and then I try to discuss what is the advantage of using this immobilized enzyme system. Then I also try to tell you that that what are the different mobilization techniques we have.

And what are the methodology on the basis of that you can select the immobilized immobilization technique and one thing I want I highlighted that all immobilization technique is not suitable for all enzymes. Because whenever we select any kind of immobilization technique we shall have to first select the criteria.

There on the because whether we are looking for easy preparation or we are looking for strong bonding between the solid matrix and the enzymes and all this criteria is very important and during this lecture we emphasize on couple of things. One is the characteristics of the solid matrix and that place very important role.

And we also discuss about the porous and nonporous solid matrix how it affects in the immobilized enzyme system? Now today we already discuss the kinetics of enzymatic reaction and in these kinetics of that enzymatic reaction we assume the enzymes they are freely interact with the substrate. So, we considered that as a homogeneous system the whenever we consider that immobilize system I told you previously that I usually the enzyme surface on a solid matrix.

Now, solid matrix is insoluble in water. So, and your substrate actually present in the soluble form as soon as even your enzymes is soluble when you fix on the solid matrix. So, things will be insoluble and so there is a presence of two different phases one is solid and the liquid.

So, this is an example of the heterogeneous reaction system. Now in case of heterogeneous system that what we have we have your bulk, you have the substrate. The substrate has to defuse from the bulk to the surface of the solid matrix and where the enzymes are located and let the reaction take place and after the reaction is over that whatever product formation is there that product will diffuse from the surface to the to the bulk and then will and only then will get the product.

So, this kinetics that we are going to discuss that how these heterogeneous system kinetics can be can be discuss can be you know use for explaining the immobilized wholesale system.

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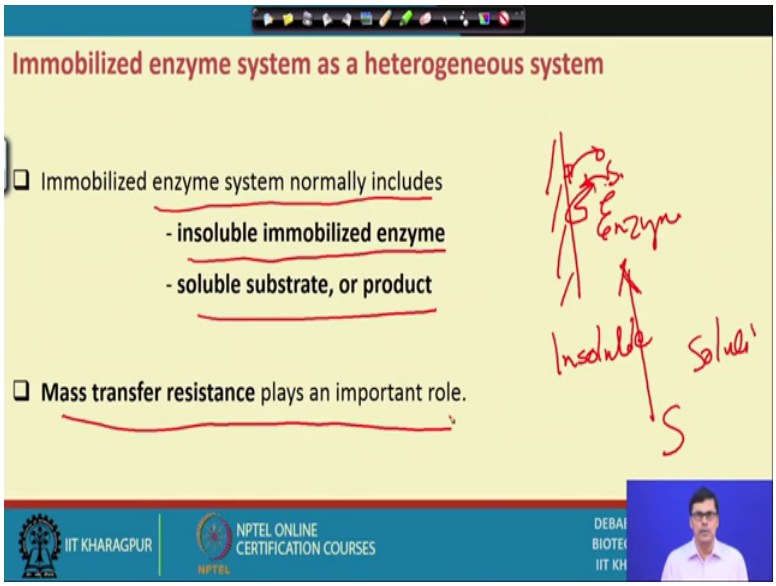

The first that let us let us discuss about that immobilized what do you mean by immobilized enzyme system normal include? I as I told you the insoluble immobilized enzyme and soluble products and substrate and product. So, what I what I told that suppose this is a solid matrix and this is insoluble.

And here the enzymes are this is the enzyme. So, when it fix on the solid matrix so this will be insoluble. Now when you pass your substrate like this here and this substrate is soluble and they where this substrate when it comes here and this has to defuse on the surface of the solid matrix then and only then the it will interact with the enzyme and product will form as soon as product is from product has to defuse out to the bulk.

So, you know that diffusion one of the important aspects of the heterogeneous reaction heterogeneous system and that is why we have written that mass transfer resistance plays very important role in case of heterogeneous reaction kinetics.

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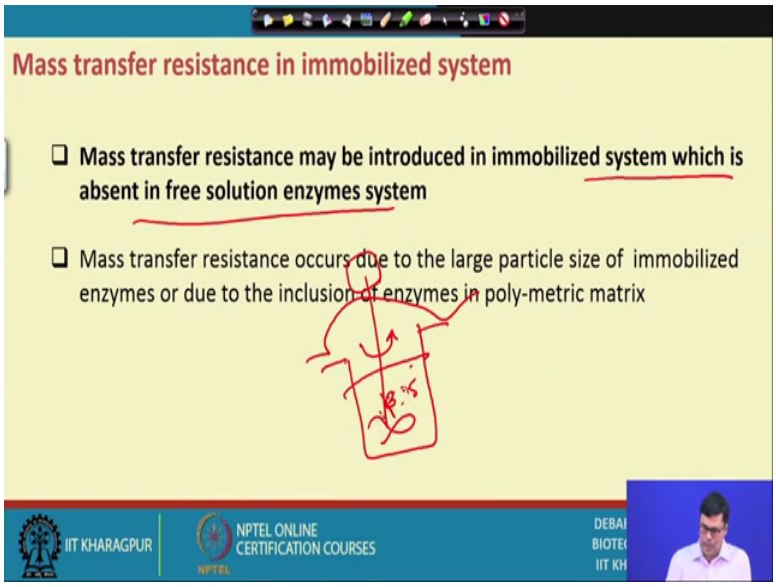

Now, mass transfer resistance may be introduced by the immobilized system which is absent in the free enzyme solution. This is now when you have suppose this is the reactor in this reactor when you have you have this enzyme and substrate. So, there when they are soluble they can freely interact with each other there is no diffusion problem.

But as soon as this is the insoluble then substrate has to defuse from the soluble that from the liquid to the surface of the solid matrix and then only the reaction as I mentioned before ok.

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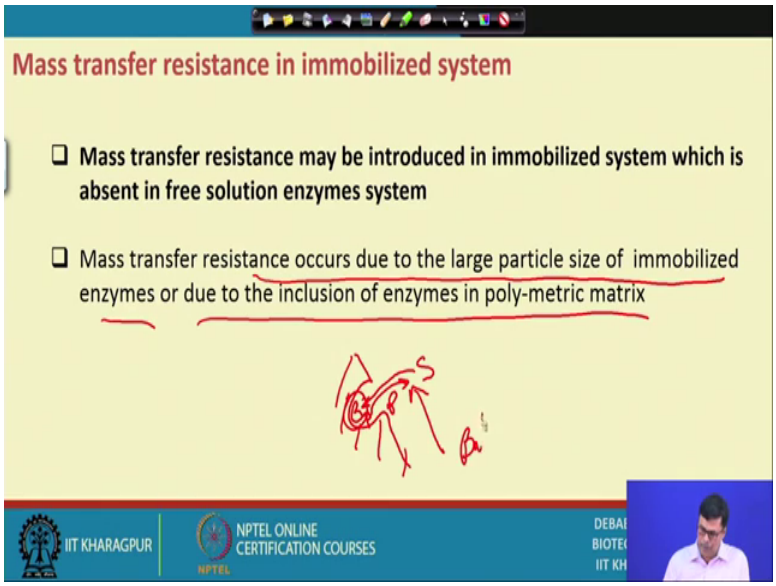

The mass transfer resistance occur due to large particle size of the immobilized enzyme and due to inclusion of the enzymes in the polymteric, polymetric matrix.

So, you know that that mass transfer limitation I can I can I can tell little bit in details suppose that this is the pore of the solid matrix in the inside the enzymes are there. So, naturally when substrate is there here substrate, substrate has to defuse inside this and then and only then it can interact with the enzyme. And when product form proper product has to defuse out from the surface of the solid matrix to the bulk this is we call bulk.

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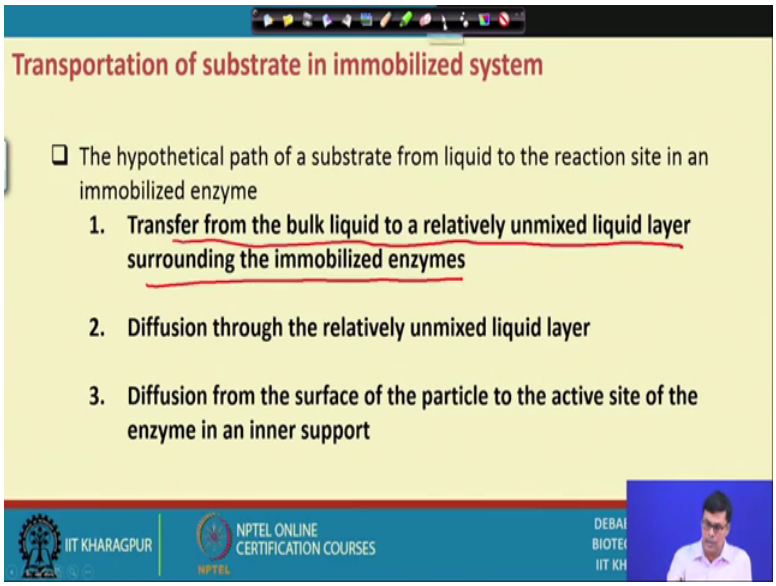

Now, transportation of substrate in immobilized system. How the transportation take place? Now you look at the say you see that the transfer from the bulk liquid to the relatively unmixed liquid layer surrounding the immobilized enzyme system.

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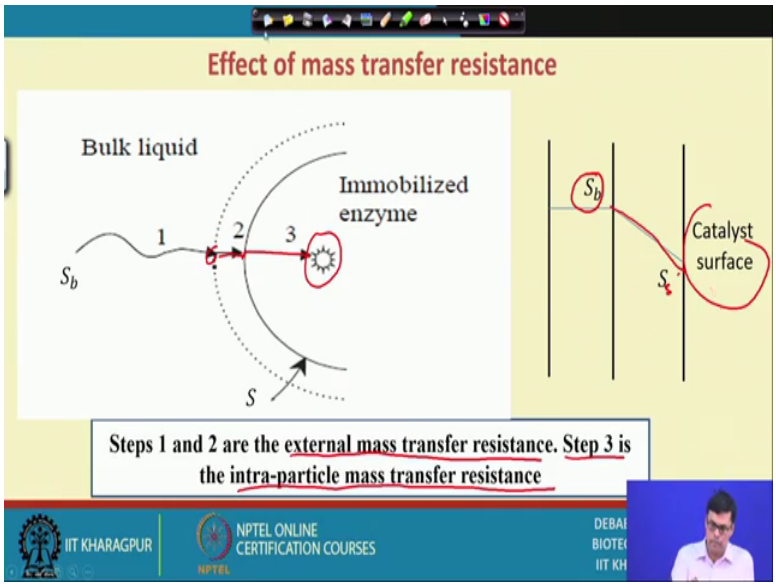

So, this is the this is I can explain on the on the basis of this. You see that here this is the substrate am I right this is a substrate and this should be defused this is the this is the solid surface and across the solid surface there is a fine layer and this we consider as unmixed layer you know that.

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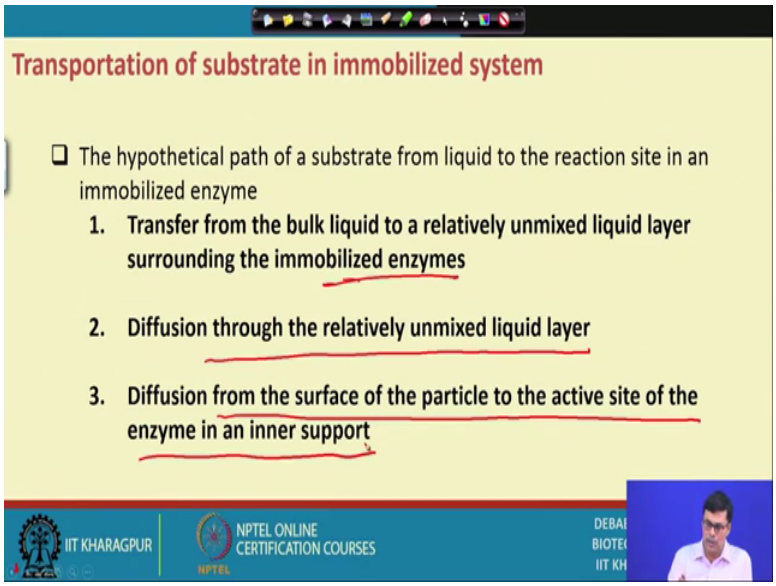

So, that is exactly what I we have written here that transfer of the bulk liquid to a relatively unmixed layer liquid layer surrounding the immobilized enzyme and diffusion through the relatively unmixed layer then diffuse take place and diffusion from the surface of the particle to the active sites of the enzyme which is the inner support material.

Now you can you can understand here let us assume that this is the enzyme enzyme we have inside that this is the enzyme. So, substrate is diffuse this is the unmixed layer then diffuse into the surface then it goes inside the solid matrix this is the nearby so 1 and 2 steps are the external mass transfer resistance. And third step is the intra particular mass transfer resistance.

So, this is the S b is the bulk substrate concentration and this is the catalytic surface when it goes the surface this is the S s. If you consider this is S s; S s means the substrate concentration at the bulk of the solid matrix. So, you know that this is the guidance that we have you this is a concentration most of the cases usually less as compare to all cases with less as compared to this bulk substrate concentration. And what is the gradient here? S b minus S s or S that we have here in this we have consider the substrate concentration at the bulk as S.

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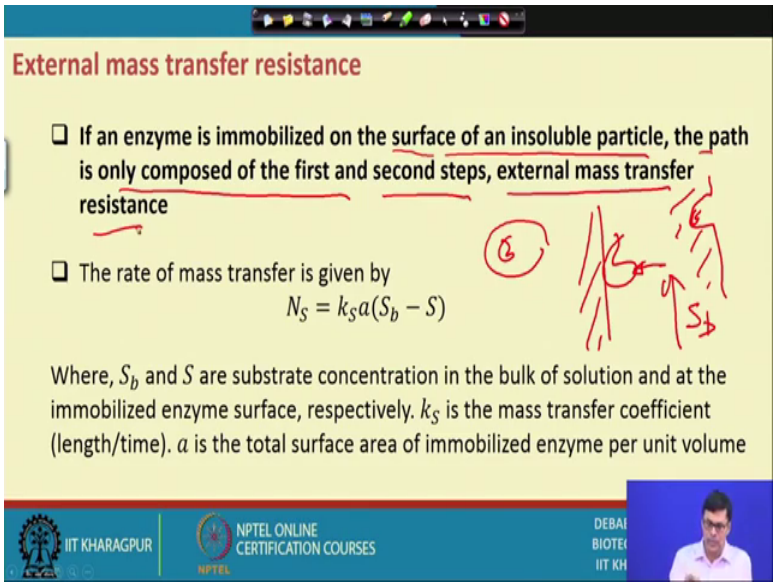

Now, question comes how you can calculate the external mass transfer resistance. Now here I want to point out that suppose this is a solid matrix and enzymes are immobilized on here.

So, when you have you have this is the bulk substrate concentration. So, it is at the surface it like this. So, the external surface this is called external surface and when the enzymes are in inside the solid matrix we call it internal surface. So, or you know that suppose it is inside the pore then it is inside the inside the solid internal surface.

So, if the enzyme is immobilized on the surface of the insoluble particle the path is only composed of first and second step what you call external mass transfer resistance. So, as for as for example if we consider the nylon solid matrix and which surface is we have little pore inside. So, in that case that you know that the enzymes will remain at the surface of the solid matrix, now in that case how you can express the mass transfer?

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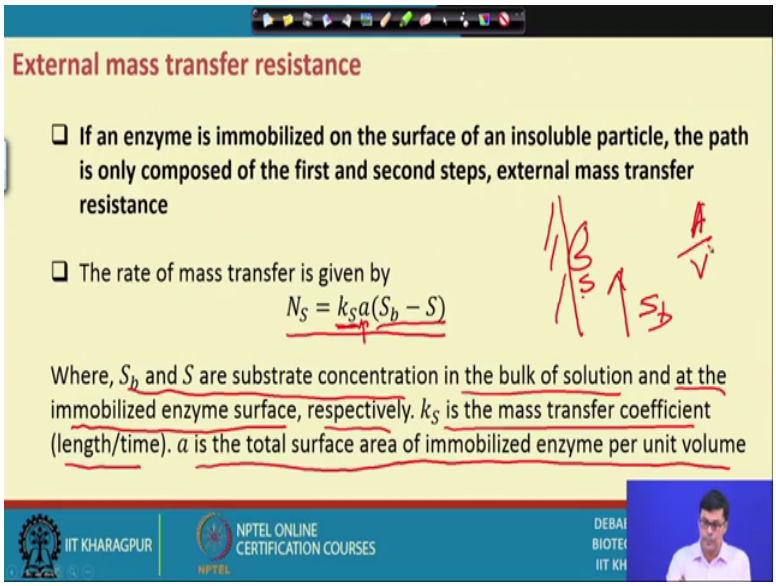

Mass transfer can be express as N s equal to k s into a S b minus S. Now I told you that here this is the enzyme and the here S is the substrate concentration in the bulk we have substrate concentration S b. So, what is the dragging force we have S b minus S b minus S this is the concentration gradient and this is the mass transfer coefficient this is k s is the mass transfer coefficient a is the interfacial area, the how much area this mass transfer take place?

So, we have what we have written here S b and S are the substrate concentration in the bulk of the solution at this at the at the immobilized enzyme surface respectively and k s is the mass transfer coefficient unit is the length per unit time, and a is the surface area of the immobilized system per unit volume per unit volume means this is the a if A is the surface area, and volume is V that is the per unit volume that is A is like this.

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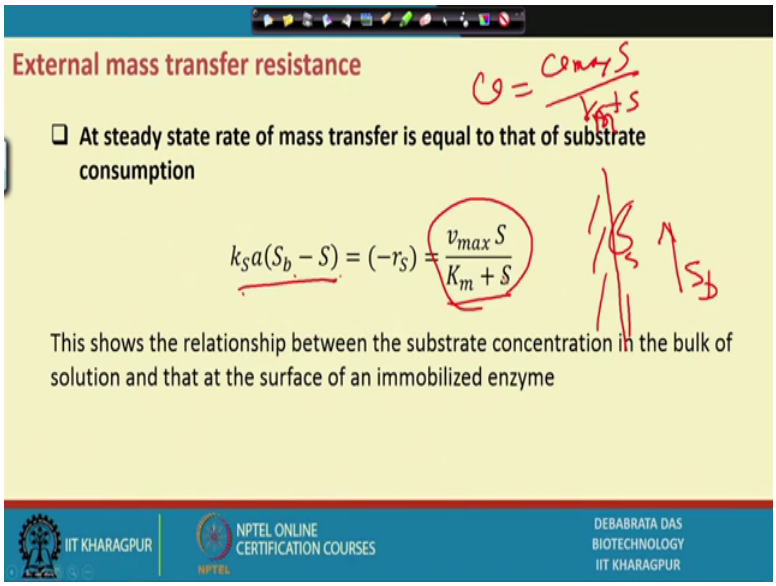

Now, that you know that very interesting thing is that that if you look at the reaction. Because here the reaction how it is taking place? Suppose this is the enzyme and this is the substrate that is diffusion and this is the S is the substrate concentration.

So, what will be the velocity of reaction because the velocity of reaction will be V equal to V max S k s plus S K m plus S am I right not k s K m plus S this is the what exactly we have written this is the velocity of reaction. Now this is the mass transfer am I right now under steady state condition.

Now, what do you mean by steady state condition? Steady state condition means rate of substrate diffuse from the bulk to the surface and rate of reaction. If the rate of reaction and rate of diffusion they are same then and only then substrate concentration in the in the surface of the enzyme surface of the solid matrix will be constant. Now if suppose rate of diffusion is more substrate diffusion is more than rate of reaction.

Then what will happen? The substrate concentration on the surface of the solid matrix will increase. Now, if rate of substrate diffusion is less as compared to rate of reaction then what will happen more substrate will be consume the substrate concentration at the surface of the solid matrix with that keep on decreasing with the with the time.

So, steady state condition will be what? When rate of mass transfer equal to rate of reaction and how we can write this is how what we can write k s into a S b minus S is

equal to v max is K m plus S this is how we can write. Now this shows the relationship between substrate concentration in the bulk of the solution and the surface of the immobilized enzyme. So, this way this we can we can visualize from that.

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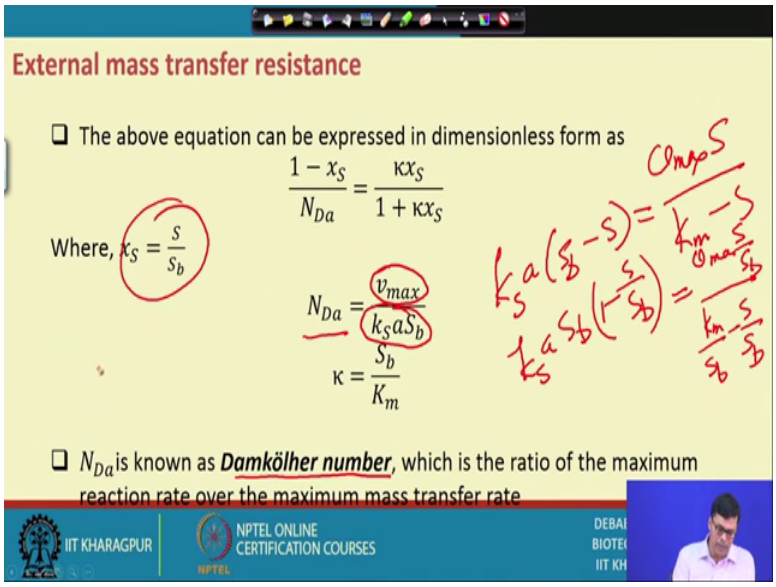

Now, this equation if you look at the previous equation that we have what we have this is we have the k s. What we have? We have k s into a what is S b minus S equal to v max S k m S am I right? So, now the interesting thing is that suppose k s a and S b you can take common then you this will be what S by S b am I right.

Now here if you divide this with S b then what we can write v max S by S b by K m by S b plus S by S b can I write like this? Now if we assume x S equal to S by S b then I can write what I can write this is equal to here this you can and and we can assume that N a D a.

What N D a? What N D a is the Damkolher number. What is the Damlolher number? Damkolher number you can see this is what v max am I right and this is what k S a into S b.

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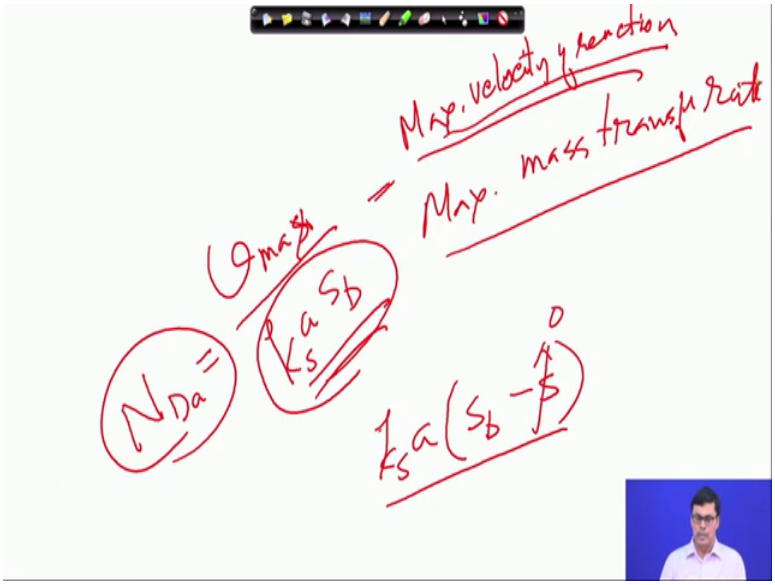

Now, what is v max? v max is the maximum. So, I was talking about the N D a am I right what is N D a is the v max into k S a into S b. Now the this is sorry this is v max what is v max is the v max is the maximum velocity of reaction velocity of reaction am I right. .

Now what is this may what is the rate of mass transfer mass transfer equal to the k S a rate of mass transfer S b minus S and when it will be maximum when this will be equal to 0. So, this is the, what we can did this is the maximum mass transfer rate am I right. So Damkolher number this is nothing but ratio between the maximum velocity of reaction and maximum mass transfer rate that is what how we can define this.

So, this is the exactly if we if we if we put all this value we will come across this equation. We will come across this equation that 1 minus x S, N D a equal to k x S one plus k x S now k is equal to S b by K m S b by K m that we can easily find it out.

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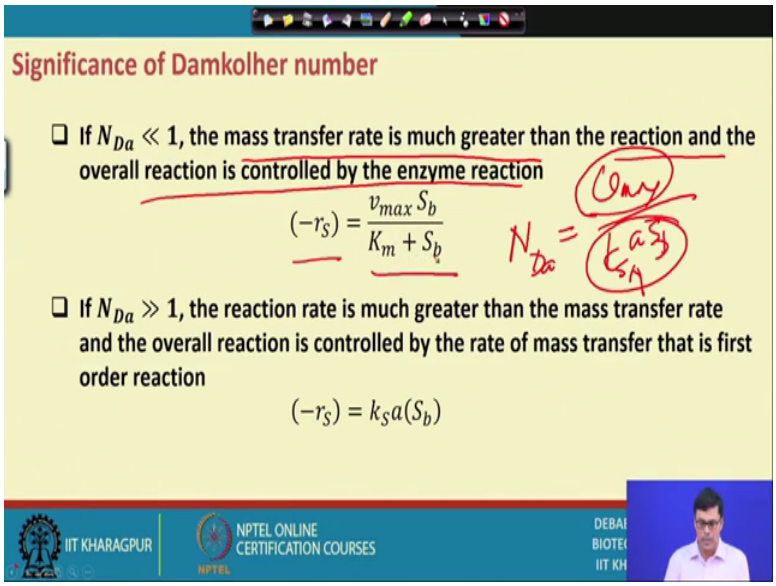

Now, from this equation what we can conclude? This equation that what we can conclude conclusion is that that if N D a is greater than 1, then the mass transfer rate is much higher than the reaction. So, the overall reaction is controlling by the enzymatic reaction.

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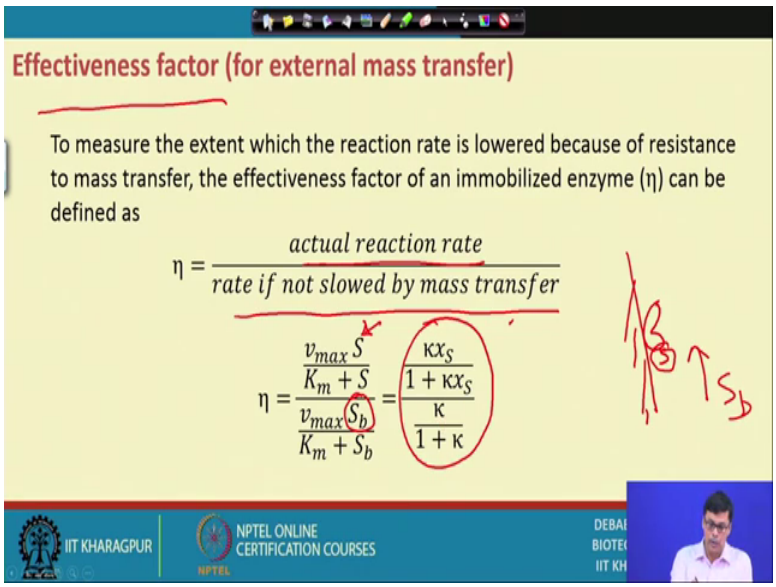

Let me let me tell you what is the N D a I told you? What is the N D a I told you N D a equal to N D a equal to what v max by k S into a into S b; that means, the maximum

velocity of reaction by maximum rate of mass transfer. Now if k S is less than one; that means, what that this should be very high rate of mass transfer will be very high.

So, you are say the substrate is going as the higher rate than as compared to the rate of reaction. Then what will happen? The mass transfer is much greater than the reaction and the overall reaction will control by the enzymatic reaction. So, the velocity of reaction what is the limiting factor here? The limiting factor is the enzymatic reaction because your are rate of mass transfer is more. So, then if you want to want to improve upon the rate of reaction then what will we shall have to increase the velocity of reaction and which is nothing but v max S b K m plus S b.

Now, what is the next case your next case what is what is there is the N D a is greater than 1, then velocity of reaction is more as compare to the mass transfer. So, in that case the velocity of reaction that is not a important factor that limiting factor limiting factor in the mass transfer.

What is the mass transfer? Mass transfer is equal to the k S a's into S b that is the maximum mass transfer. So, this is the first order with respect to bulk substrate concentration. This a this we can write this expression like this. Then there is a another important term that is associated with the immobilized enzyme system or heterogeneous reaction kinetics that is you have effectiveness factor.

What is the effectiveness factor? Effectiveness factor is the actual reaction rate the rate if not slowed by the mass transfer. What does it mean? It mean the, that you know suppose this is a solid matrix and your enzymes are immobilized on here. So, here substrate concentration is this and bulk is the substrate concentration S b.

Now what is actual rate of reaction depends on the this substrate concentration. Now when rate divided by rate if not slow by mass transfer; that means, if if if the all substrate is available at the surface then that is the if the if this is not slow by mass transfer that mean here S will be equal to S b and this will be S.

So, what is the effectiveness factor? Effectiveness factor is the actual rate of reaction divided rate of reaction when there is no mass transfer limitation because all substrate are available for the reaction. So, this is how we can write this is when S is the surface that we concentration is we can write this equation. When in the bulk you have the all

substrate available for the reaction is this and if you write in this form is the final equation we will get in this form because the I have already prepare and explain what is k and x S all this thing I explained if I solve it you can find this equation.

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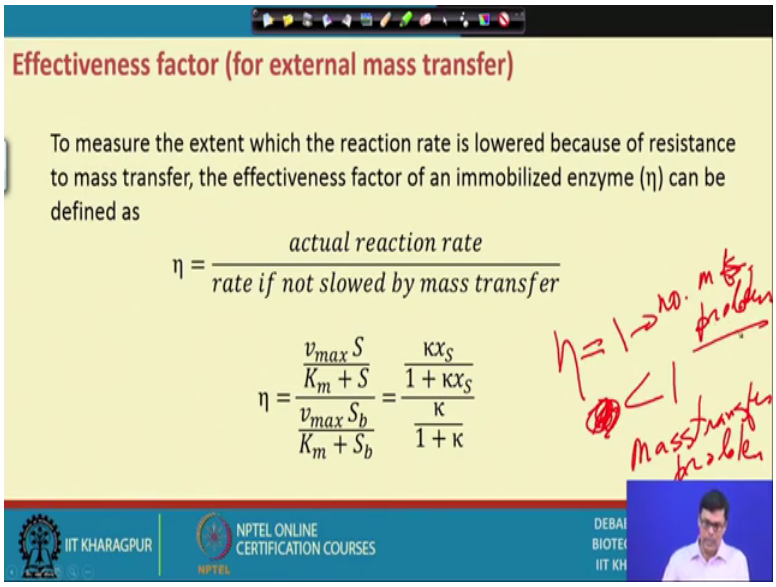

Now, here I want to point out one thing now if eta, eta cannot be more than 1 am I right? The maximum rate of reaction will be what that when all bulk substrate concentration available for the reaction. Now so eta value will be maximum it should be 1. Now if it is 1, I can assume that there is no mass transfer limitation problem. So, your heterogeneous reaction system will tends to the homogeneous system.

Now, when the eta value is less than 1, if it is less than 1 then what is happening? That means, your mass transfer diffusion problem is there; that means, actual rate of reaction will be less as compare to that of when the all substrate available for the reaction.

So, the eta value when it is less than 1, then we have mass transfer, mass transfer problem. So, here there is no mass transfer problem. I hope you understand that.

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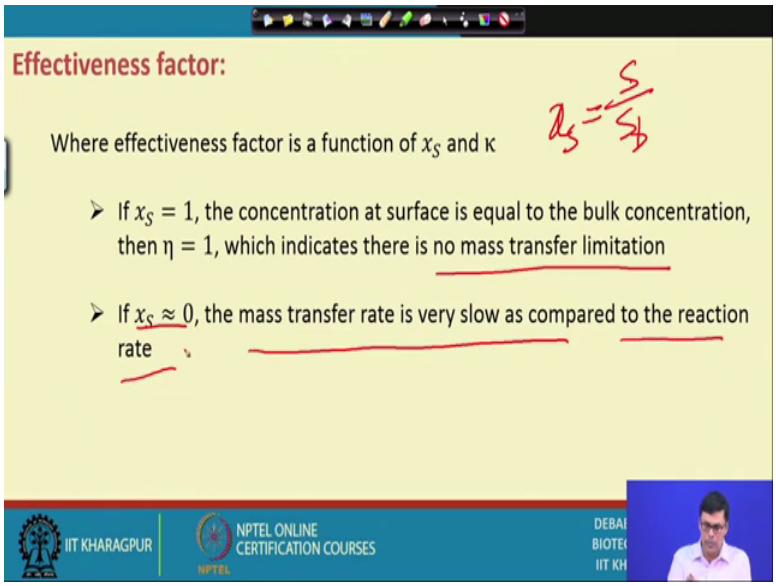

Now, how you explain that that if you look x S what is x S again, let me write it x S equal to what I told S by S b. What is l S is the substrate concentration at the surface of the solid matrix, S b is the bulk substrate concentration. Now if you say x S equal to 1; then what will happen that concentration at the surface is equal to the bulk substrate concentration then eta value will be 1 which indicate there is no mass transfer limitation what exactly I point out before.

But when x S is tends to 0; that means, that you know that this is very high as compared to this S S b is very high as compared to l then the mass transfer is very slow as compared to the reaction rate I hope you understand that.

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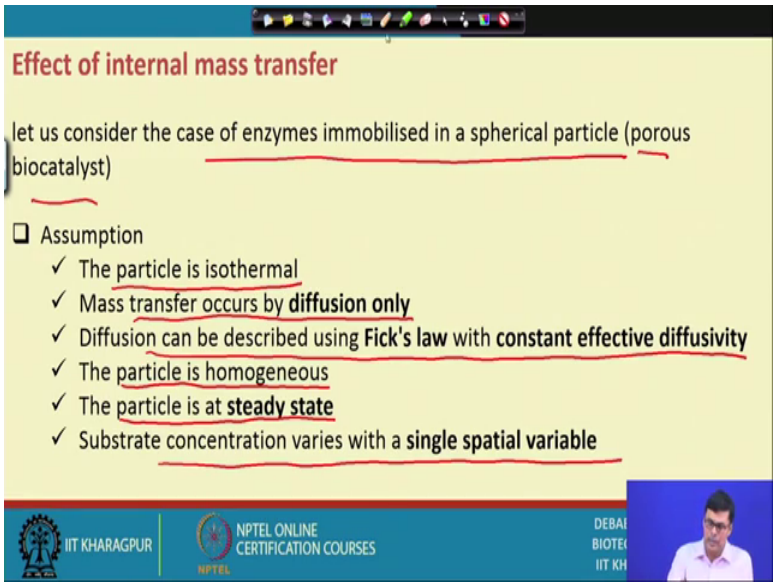

Now, this is the about the external mass transfer. Now let me switch over to the internal mass transfer when the particles that enzyme present inside the porous porous solid matrix. Now let us consider the case of enzymes immobilized system in a spherical particle porous and porous biocatalyst. My assumption is that the particle is isothermal; isothermal means you control the temperature is constant and mass transferred occurred by diffusion only and diffusion rate can be expressed by using the Fick's law with constant effective diffusivity.

And then the particle is homogeneous is distributed in the in the solution and particle is the stays at a steady state and substrate concentration varies with the single spatial variable.

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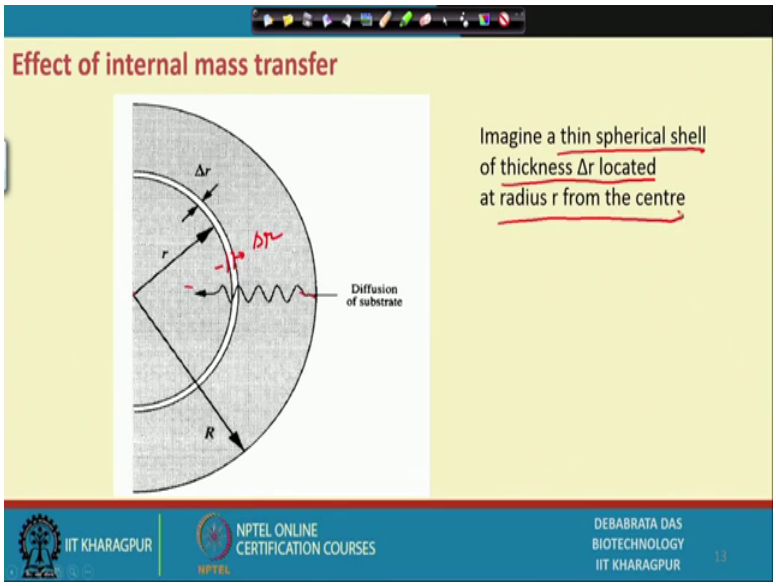

So, if we assume all this thing then we can we can write this here we can see that you know this is the core of the solid matrix and here the diffusion substrate is diffusing. How it diffused to the and let us assume this is a fraction that which is we consider as del r this is the fraction. Then we can integrate from 0 to r that we can integrate them. Imagine a thin spherical shell thickness of del r located at the radius r from the centre let us assume that.

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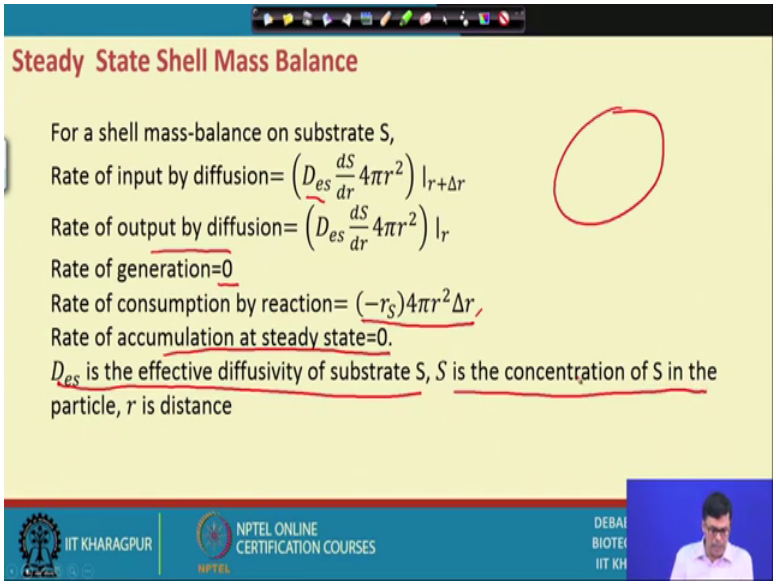

Now, here the here we can see that in the in the rate of input will be what will be equal to D e s D e s is the diffusivity and d s by d r into pi r 4 pi r square, because since we are considering the spherical sphere is shared spherical particles the surface area of the sphere is about 4 pi r square. So, this is r plus del r and then what is the output that we have this is the r you know that this is r we can D e s equal to r that is.

The rate of generation is equal to 0 because this since substrate in diffusion take place rate of consumption I can assume minus r S equal to this the r S into volume and this is this is 4 pi r square into del r is the nothing but volume. And rate of accumulation at the steady state condition is 0 and D e s is the effective diffusivity of the substrate S substrate is the concentration of S in the particle and r is the distance.

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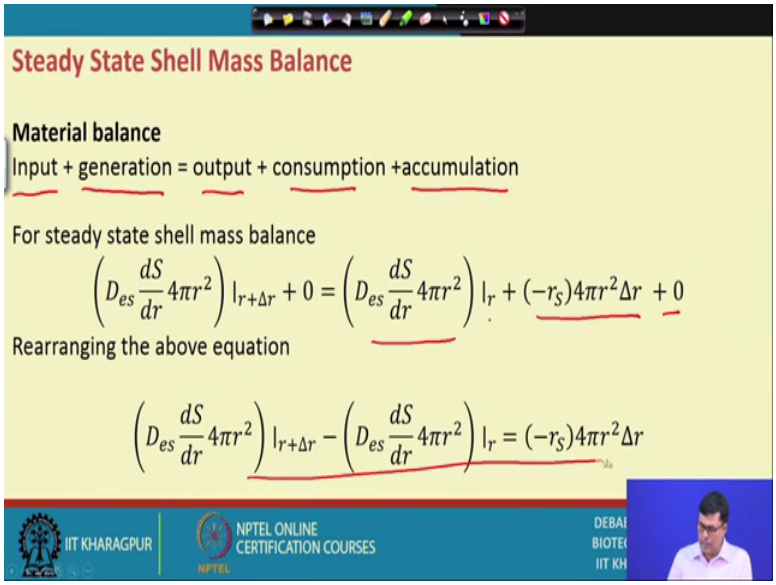

Then what is the our equation that we have that you know during the reactor analysis we use that equation for material balance equal to rate of input equal to plus generation equals the output plus consumption plus accumulation. Now here what is the rate of input this is D e s into d S by r 4 pi r square. What is r plus del r and what is the output of that generation no substrate generation take place that is equal to 0 and what is the output this is the output as the r and then this is the rate of reaction they del r by minus r square into 4 pi r square into del r and rate of accumulation that should be equal to 0 then we will finally, reach this equation.

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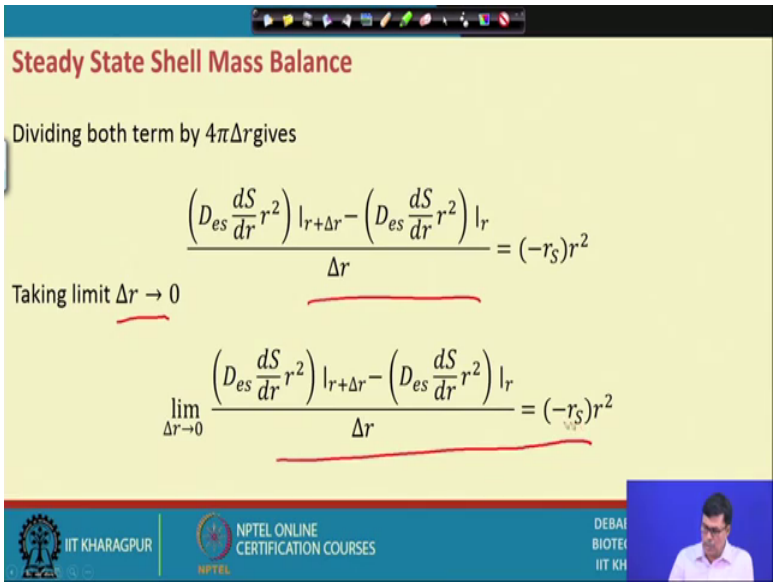

Now, if you divide this 4 pi del r give this equation. So, you will come across this equation and then from this we can and find out this equation. When del r tends to 0; then we can put a limit then we can we can find this equation there.

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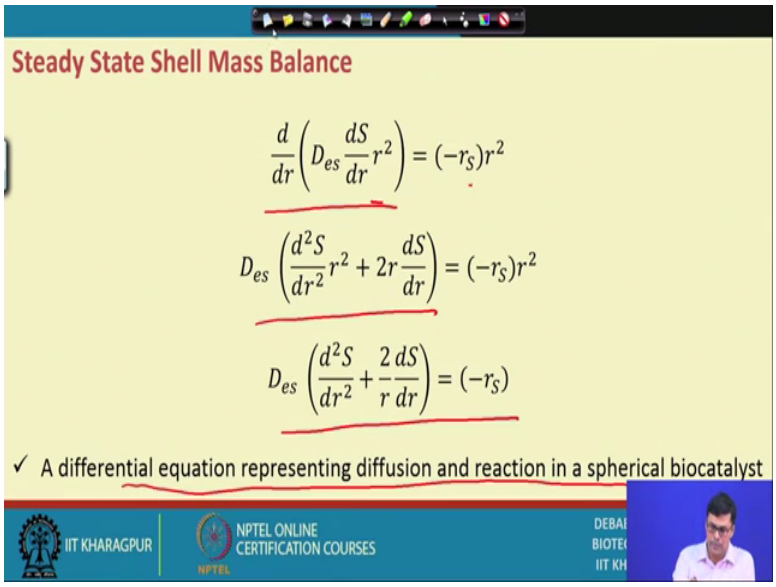

Now, finally we come across this steady state that shell mass balance is like this under steady state condition this d del this value this will be equal to minus r r square and this if you now we can differentiate this then we will get this kind of equation and finally, we

come across this equation the a differential equation representing the diffusion reaction of this spherical catalyst here.

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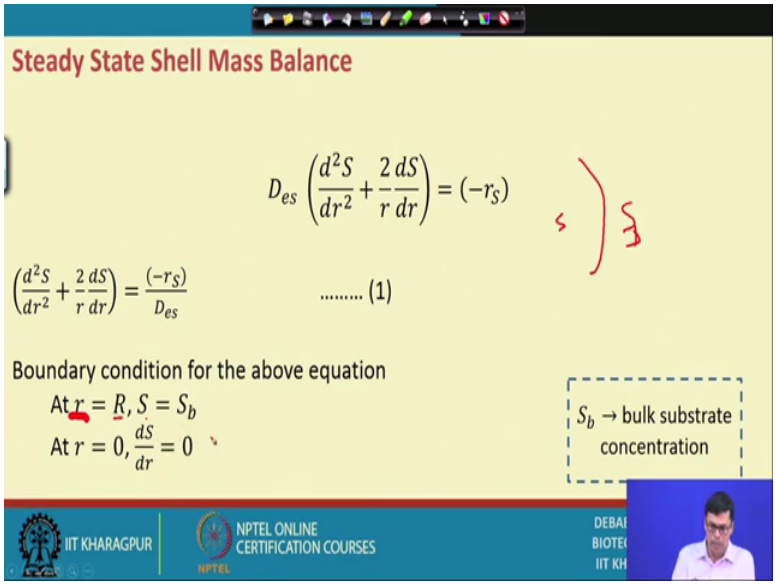

Now, finally, we come across this equation of the steady state this D e s d d d 2 S by d r 2 plus 2 by r d S by d r equal to minus r and this equation I can write in this form and then we can put the boundary condition at the r equal to how smaller when equal to the bigger S will be equal to S b that we have seen that that thing that is that here is the S b and here is the s. So, when this r equal to r then S will be equal to S here when r equal to zero this rate of mass transfer will be equal to 0.

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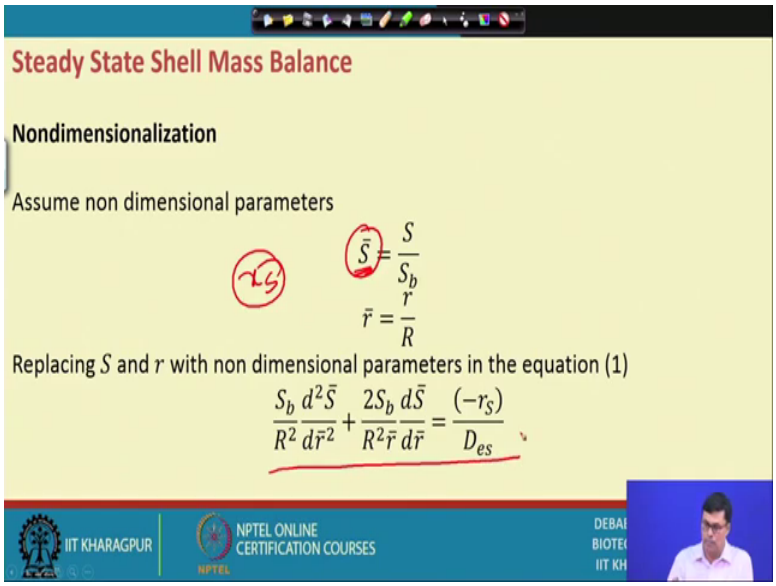

Now, if we assume now non dimensional parameters if we assume like this now previously this we assume at a x s, but you know in this case we assume this as the S bar to understand differently that S equal to for internal diffusion this is S by S b the same thing only we use the terminology r, r bar equal to smaller by capital r then this equation the previous equation can be rewrite it in this form.

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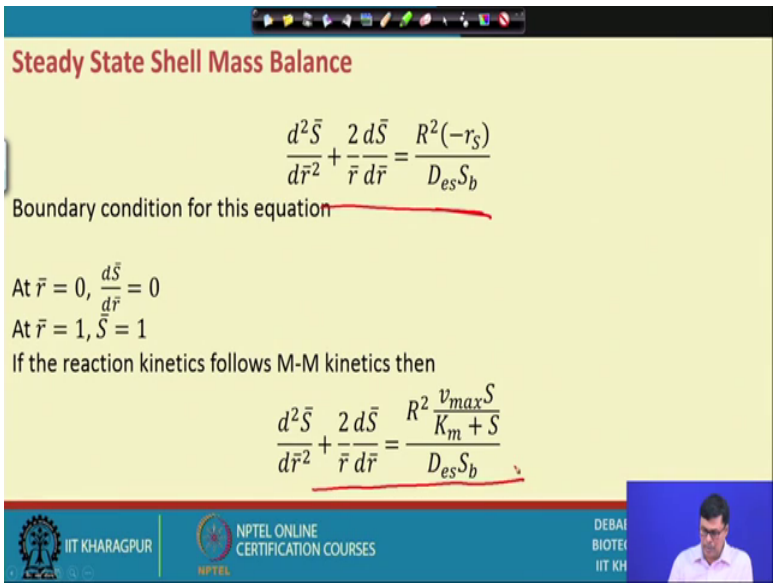

So, finally we come across this equation this is the equation that we have and again if we put the boundary condition this r bar equal to 0; then d S bar by d r bar equal to 0; r bar equal to 1; S bar equal to 1 then this equation will come across this particular equation.

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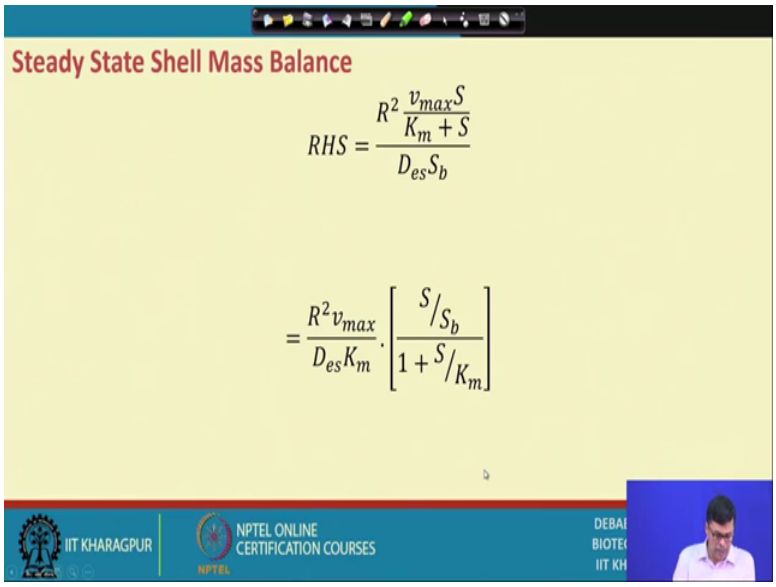

Now, finally, the right hand side if you look at the right hand side this is the this one I can write this equation in this form and this is equal to R square v and D e K m you can take it out this I can write S by S b 1 plus that just we divide by S b we can get this equation that you can write this equation like this.

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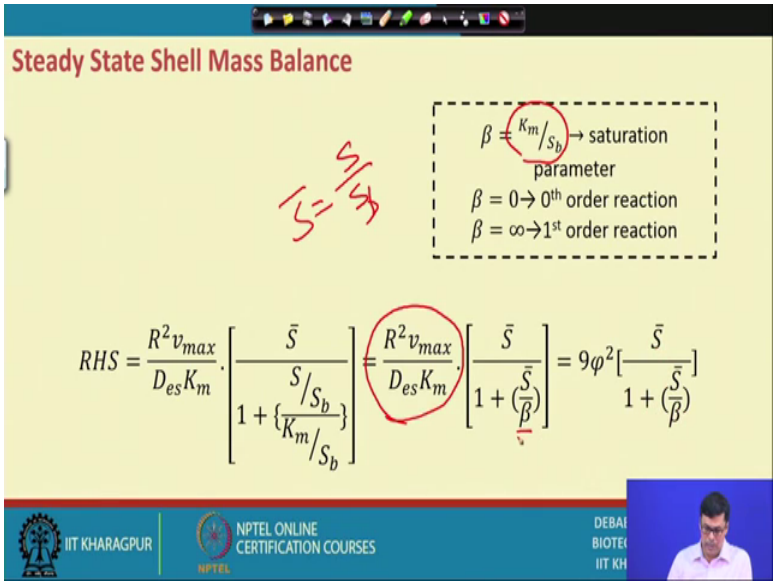

Then the right hand side again we can write in this form and what is the what is this form this is if you look at this is this is constant what we have written before.

Now, this is equal to S bar S by I told you told you S bar equal to what S by S b am I right? So, we can this is equal and beta equal to what K m by S b. So, we can write in this form and finally we come across this equation is the 9 9 phi square then S bar by this is why this is what is phi this is called Thiele modulus ah.

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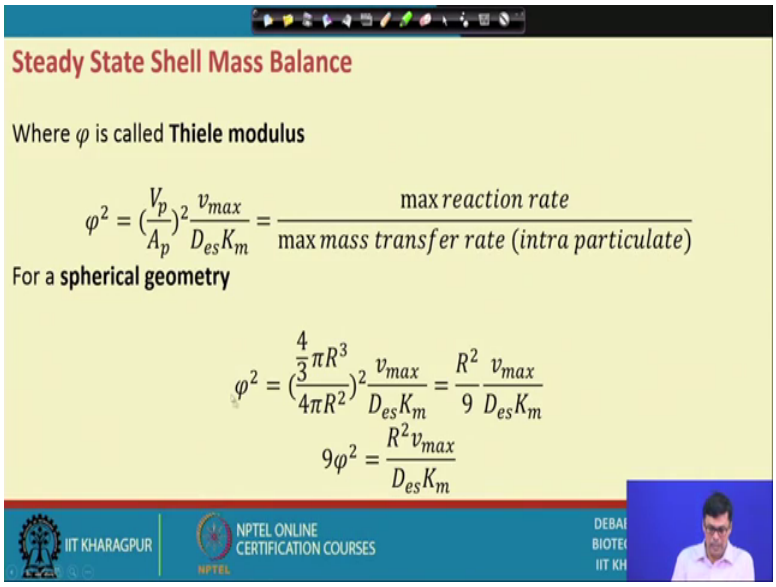

This is phi square equal to this and the this is equal to mass maximum reaction rate and divided by maximum mass transfer rate in case of intra particulate matters. Then this is then phi square equal to this is you can write in this form this is the final equation I can write.

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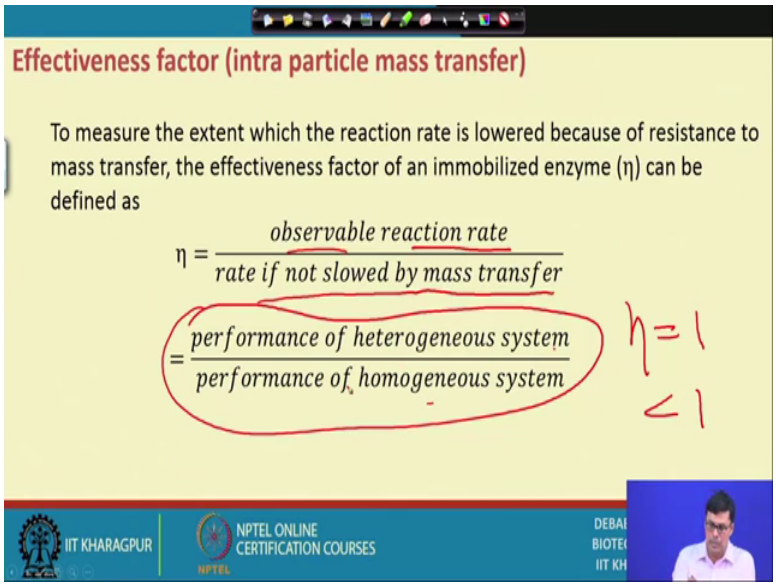

So, now if we go back to the effectiveness I we have explain before that is the what is the effectiveness factor that observe reaction rate divided by rate if not if not slowed by the mass transfer the so this can be explained like this when there is no mass transfer limitation.

The performance that means, this is actually the ratio of the performance of heterogeneous system divided by performance of the homogeneous system as I told you that when eta equal to 1; that means, your heterogeneous system is equal to homogeneous system. Now when it is less than 1; that means, your how then they we have the mass transfer limitation problem in this is how the eta can be explained.

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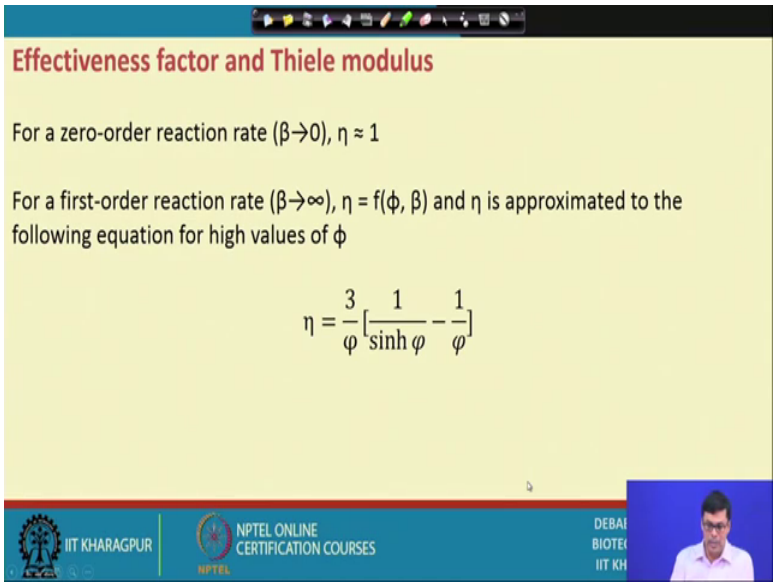

Now, this is this we can we can write that in case of zero-order reaction that beta tends to 0, then eta equal to 1 that I shall explained you and eta can be correlated with Thiele modulus in this particular equation.

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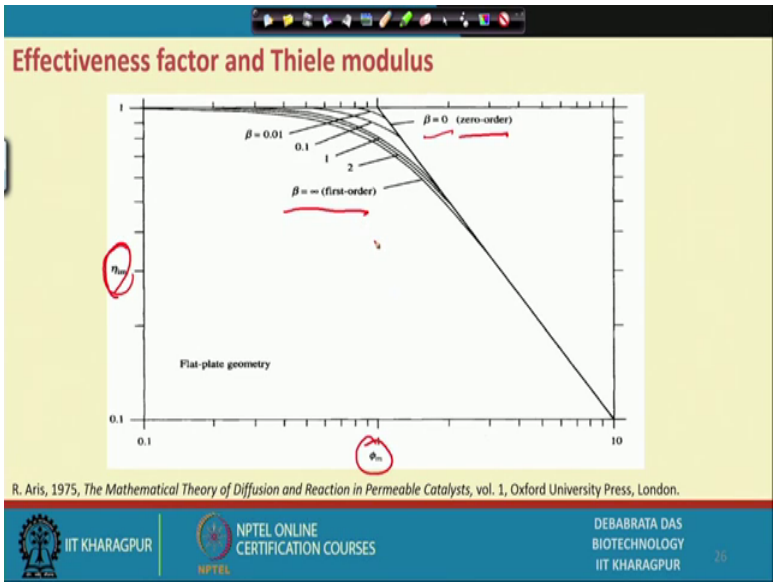

Now, this equation this graph tell us the correlation between eta and the phi value this is Thiele modulus and this is effectiveness factor. Now if you find out if a if a 0.3 that is you know that it is rate of eta is equal to 0 and part if it is more than 0.3 and then you know your eta value is less than less than 1 that is your mass transfer problem is more.

Now here if beta is equal to 0, it considered as zero-order and when beta is tends to infinity is considered as the first order reaction.

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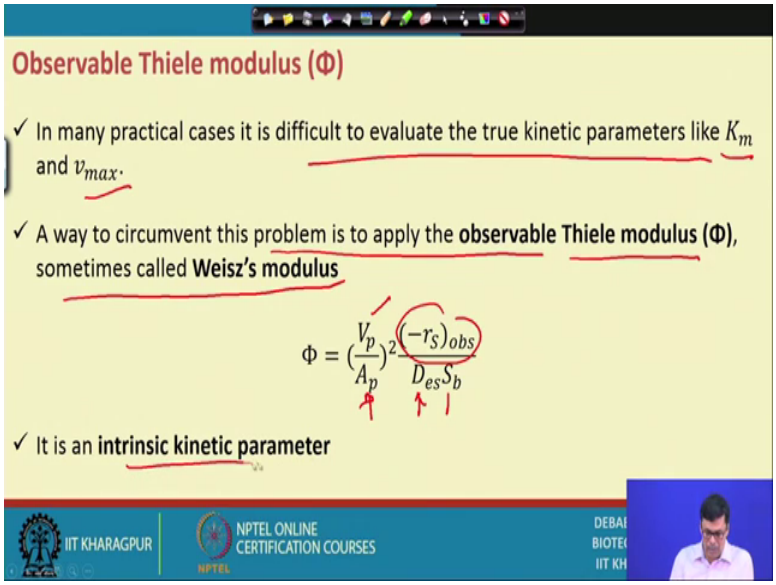

Now, finally we come across that you know in many practical cases that you know it is difficult to evaluate the kinetic parameters K m and v max which is Michaelis-Menten constant and maximum velocity of reaction. So, a way of cir[cum]- circumvent that problem is to applied the observable Thiele modulus as sometimes called the Weisz's modulus this is equal to phi equal to V V p by A p. What is V p? Volume of the particle A p is the area of the particle this is r S is the is the observed rate of reaction and D e s is the diffusivity, S b is the bulk substrate concentration and this is called intrinsic kinetic parameters.

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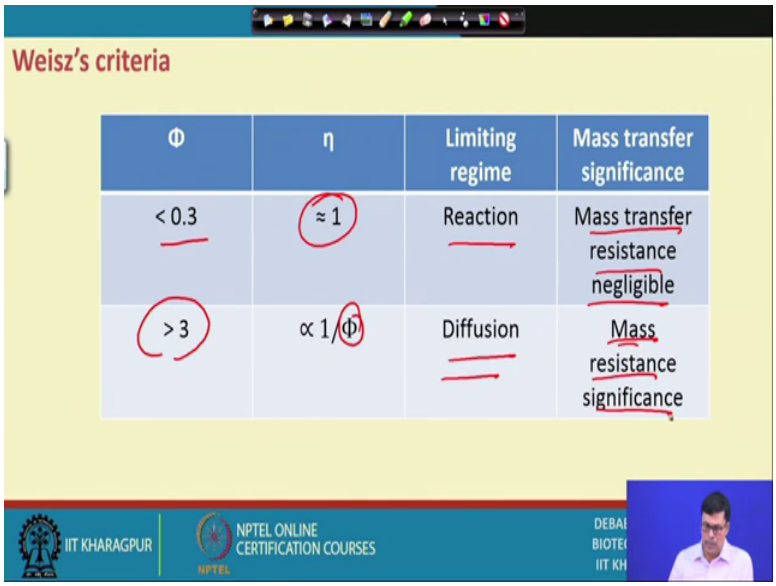

Now, this is the final thing that we have if phi value is less than from the figure we have seen that is the less then 0.3, then eta is close to 1, then the limiting reason is the reaction and mass transfer resistance is negligible. Now when eta value is greater than 3, then this eta is the inversely proportional to phi value then it will be diffusion resistance the mass res resistance will play the very important role.

So, in conclusion in this lecture what we try to learn that how the heterogeneous reaction kinetics can be explained? Now heterogeneous reaction kinetics, the substrate has to defuse from the bulk to the surface of the solid matrix then only then reaction take place. Two type of diffusion phenomena that works one is the external mass transfer and the internal mass transfer. Now how this the two parameters we have come across close one is Damkolher number, another is a effect membrane factor.

What is damkohler number? Damkolher number is the maximum velocity of reaction divided by the maximum mass transfer. What is effectiveness factor, rate of reaction actual rate of reaction divided by rate of reaction when there is no mass transfer limitation. So, when eta value is 1 your heterogeneous system tends to homogeneous system. When eta value is less than 1; that means, there is a mass transfer problem. How it is related with Thiele modulus that we try to explain in this particular lecture.

Thank you very much.