ENZYME SCIENCE AND ENGINEERING

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LECTURE–23

STEADY STATE ANALYSIS OF MASS TRANSFER
&
BIOCHEMICAL REACTION IN IME REACTORS

So we have been discussing so far the steady state analysis of mass transfer and biochemical reaction in immobilized enzyme reactors.

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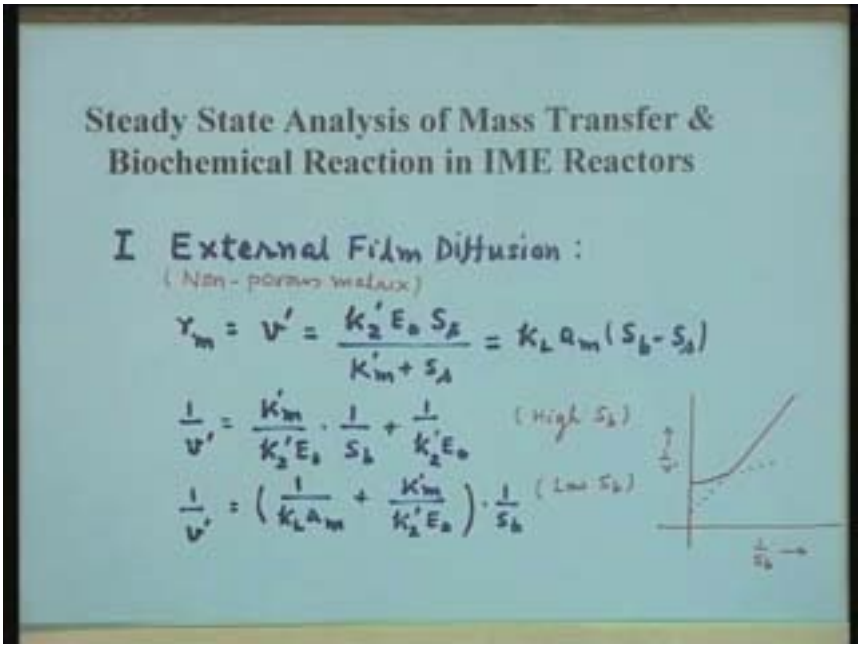

We have discussed yesterday about the external film diffusion that is in cases where we consider nonporous matrices. Under steady state condition, the rate of mass transfer can be equated to the rate of biochemical reaction and then by analysis one can come under two extreme conditions; one under high substrate concentrations we get a Lineweaver Burk profile which is analogous to ordinary soluble enzyme kinetics and in the case of low substrate concentration that is in this case; low substrate concentration and high substrate concentration you get differences in slopes and the measurement of slope at two distinct stages of Lineweaver Burk plot gives you an estimate of the value of mass transfer coefficient. You do film diffusion $k_l a_m$ can be computed.

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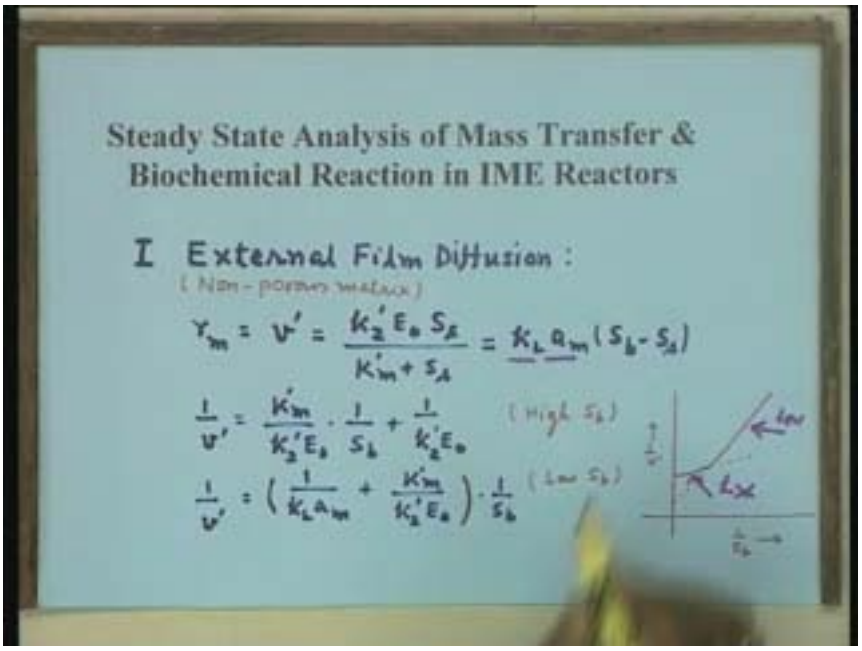

Therefore based on the observed reaction velocity in the case of an immobilized enzyme reactor one can determine the value of S_b-S_s that is the substrate concentration gradient and the magnitude of this determines whether the reaction is diffusional limited or otherwise. If the S_b-S_s is zero or very small quantity then there is no diffusional limitation. If the magnitude is large or S_b is much, much larger than S_s in that case film diffusion is the dominant player and so the nature of Lineweaver Burk plot under the range of condition of substrate concentration can give you an idea of the mass transfer coefficient.

We are now looking at a general approach to understand the external film diffusion. We determine by some experimental method or an analytical method or an empirical equation the magnitude of the mass transfer coefficient and the magnitude of mass transfer coefficient will give you the magnitude of the concentration gradient and so another alternative route to estimation of mass transfer coefficient has been from empirical relationship what we call as JD factor. A JD factor has been defined in the case of heterogeneous catalysis in general and which applies very well to even enzyme catalyzed reaction. JD factor is defined as a lumped parameter which takes into an account the mass transfer coefficient particularly field diffusion coefficient as well as the reactor operational parameters. The JD factor is

$$\text{JD factor} = (k_L\rho /G)\,(\mu /\rho D)^{2/3}$$

This is the …. (4:17) number, N_{sc}. The first term in the parenthesis represents the mass transfer function; the other is dimensional number which gives you the operational parameters and here k_L is a mass transfer coefficient; ρ is density, G is the superficial mass velocity and μ is the viscosity; row is again density and D is the diffusivity. It has been shown at least empirically in a large number of experimental data that JD is equal to

$$JD = C(Re)^{-n}$$

A very general relationship for JD factor has been given that it's a function of Renault number to the power minus n and under different conditions of particle shape and the range of Renault number the magnitude of C and n varies and that can be estimated for a particular system. A large number of correlations giving the values of C and n have been proposed under different conditions and one of them which needs the actual reaction conditions can be chosen from the literature and used for estimation of JD.

If you look at this one, you will notice that from the estimation of JD factor as a function of C, Renault number to the power minus n one can determine the value of k_L. Because k_L will be equal to

$$kL = JD.G/\rho \, .N_{sc}^{\ 2/3}$$

If you convert this magnitude of k_L is related to JD factor by JD value and also a combination of the reactor operational parameters; superficial mass velocity, density and ….. number. The magnitude of k_L then gives you at steady state, the reaction rate. That is

$$v' = k_L a_m (S_b - S_S) = r_m$$

Under steady state both the biochemical reaction and the mass transfer rate are equal. This v' can be considered as the observed reaction rate which has been monitored experimentally and therefore one can determine the magnitude of S_b-S_s and that determines whether the diffusional control is there or not and accordingly one can design the reactor.

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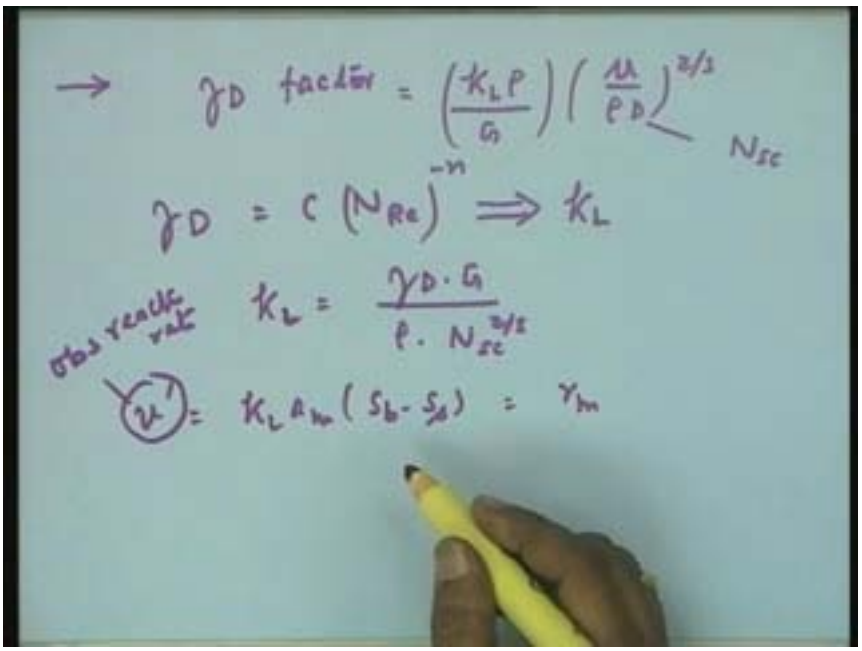

JD can be computed from a variety of correlations as I mentioned. One of them which have been applied is developed by McCune and Wilhelm. This is only one of probably a large number of relationships that are reported in the literature of heterogeneous catalysis in chemical engineering. For spherical particle JD is equal to

$$JD = 1.625 (N_{Re})^{-0.507}$$

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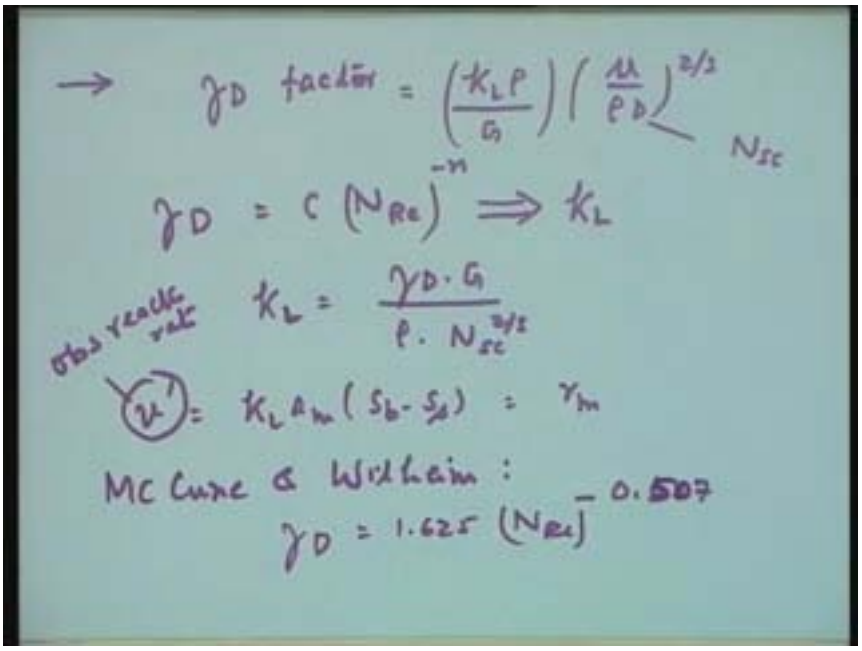

This is one of the relationships which have been proposed but a large number of such empirical correlations are known. Once the magnitude of k_L is known then one can really incorporate the value of k_L into the rate constant of a reactor performance equation. In most of the practical cases whether it is a plug flow reactor or a CSTR, the error involved in assuming the whole reaction to be under a first order regime or a zero order regime is not very large depending on initial substrate concentration. For example in the case of CSTR, life is much simpler; if the fractional conversion desired is very high even at a high initial substrate concentration, the substrate concentration in the reactor will be quite low and that is the substrate concentration which is going in the outlet stream. So a first order approximation is not something which is invalid and as I mentioned earlier if you take certain data roughly an error of about ten percent in calculation of the space velocity is encountered.

Similarly in the case of PFR, because the enzyme loading is very high usually in PFR, a large fraction of the reactor also experiences a low substrate concentration. It also experiences high product concentration. But substrate concentration keeps reducing and towards the end of the reactor, the substrate concentration will be very low and one can assume a first order kinetics.

If you consider a first order case, then the reaction velocity will be

$$v' = k_f a_m S_S$$

k_f I have defined here as a pseudo first order rate constant which is based on surface area; not volumetric rate constant; but the surface area based rate constant just to have a dimensional homogeneity with the mass transfer correlation. k_f is equal to a pseudo first order rate constant for enzyme catalyzed reaction which is $k'_2 E_0 / K'_m$. Therefore rate of mass transfer is

$$r_m = k_L a_m . (S_b - S_S)$$

In the case of a non-porous particle the issue is to develop an expression which gives you the substrate concentration at the surface because the reaction will take place at the surface of substrate concentration. If you equate the two that is $v' = r_m$, that will imply that

$$k_f a_m S_S = k_L a_m . (S_b - S_S)$$

Therefore from this substrate concentration on the surface can be calculated as

$$S_S = k_b . S_b / k_f + k_L$$

or the observed rate is

$$v' = \frac{k_L . k_f . a_m . S_b}{k_f + k_L}$$

Then the actual rate constant which incorporates the film diffusion coefficient is this parameter. k_f apparent will be

$$k^{app}_f = \frac{k_L . k_f . a_m}{k_f + k_L}$$

In your reactor design equation you can incorporate, instead of first order rate constant as this one which is based only on biochemical reaction, this rate expression. k_L can be determined either from experimental data from the Lineweaver Burk plot or from empirical equation of the JD factor. a_m is determined experimentally based on the geometry of the particle and k^{app}_f can be determined.

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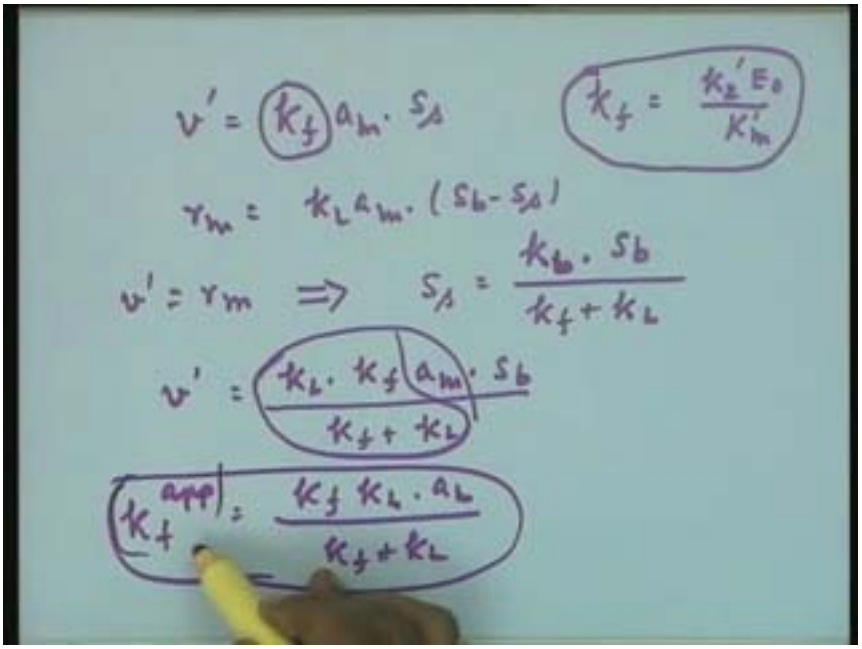

k^{app}_f can be used to designate the reactor performance of the first order immobilized enzyme reactor with film diffusion limitations or film diffusion control.

Another way again an empirical way to look at the film diffusion has been proposed by Satterfield. I must here again point out that the whole analysis of the immobilized enzyme reactor has been largely dominated by chemical engineering literature particularly with reference to heterogeneous catalysis because the systems are very identical excepting the thermal, specificity aspects which are attributed to enzymatic reactions but the catalysis part whether it is a heterogeneous catalyst in the case of chemical engineering that means a catalyst supported on a solid matrix. Even in chemical engineering that is very conventional thing; only difference is that those catalysts are very, very stable interms of their thermal, operational as well as storage stability where as immobilized enzymes are not as stable. But all the analysis is identical and Satterfield also provided a single point reactor performance equation based on film diffusion control for heterogeneous catalyst. He defined a parameter Z as equal to

$$Z = \frac{\varepsilon \, N_{Re}^{2/3} . N_{Se}^{2/3}}{1.09 \, a_v} \ln \frac{Y_1}{Y_2}$$

Here sigma is voidage as we are familiar; Renault number and the semite number and a_v is the ratio of particle surface area to reactor volume and Y_1 is the mole fraction of substrate in feed; Y_2 is equal to mole fraction of substrate in the product stream and Z is the length or the height of packed bed required for mass transfer. That means Z is the height of the length of the packed bed required to transfer the substrate concentration across the film as a function of Renault number and Semite number purely on the basis of mass transfer control; no biochemical reaction is assumed here. If the mass transfer has to be incorporated this much of length of the reactor is required under these operational

conditions to have the substrate concentration equivalent to biochemical reaction. That means to make S_b-S_s equal to zero, without any biochemical reaction this much of height of the reactor is required under these operational conditions. Based on the operational data that means the concentration of substrate in the feed and the product stream as well as the parameters of the reactor operation and the particle size, one can determine the length of the reactor required and if this length of the reactor required is relative to the length of the reactor required for biochemical reaction, if you compare this height required, let us say this is Z_1 to that of the height required Z_2 based on biochemical reaction if you calculate idealized reactor performance equation and calculate the height of the reactor required and compare the two values that gives you an identification whether film diffusion is controlling or not. The one which is larger has to be used. So for example let us say if this Z is much, much smaller than the height required for biochemical reaction then you don't have to worry about diffusion.

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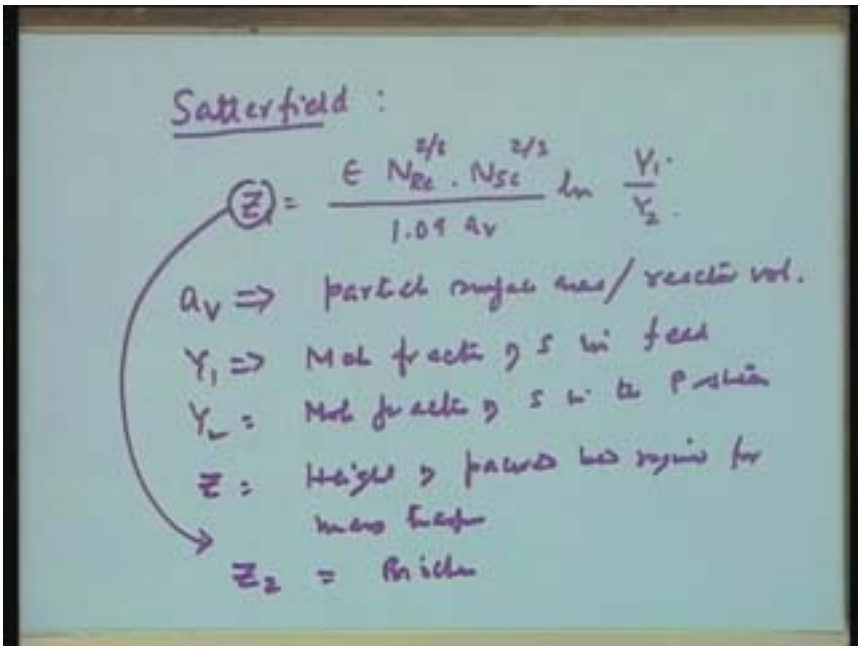

You simply design your reactor on the basis of biochemical reaction and be happy. On the other hand if this height required is reasonably high then you forget about biochemical reactions and design the reactor based on diffusional requirements based on the mass transfer coefficients.

This is the height of the reactor. This is basically empirical equation depending upon the reaction operational data the height required for effecting the mass transfer across that film. Right; only Z_1; you don't bother about Z_2. You then don't have to bother about the biochemical reaction because in that height reaction would have already taken place. Yes. Exactly what we are assuming here is that there is no reaction within the pores of the particle. All the enzymes are present on the surface and in the thin film there is no reaction taking place. You must appreciate the difference between the internal pore

diffusion and external film diffusion while in the case of internal pore diffusion during the transport of the substrate in the particle reaction is also taking place whereas here there is no reaction taking place along with the mass transfer; its only mass transfer. Only difference is that between the bulk and the surface where the reaction is taking place there is concentration gradient and ideally if the diffusion control has to be out of picture in that case the concentration gradient should not be large and this concentration can be reached if you assume that there is no substrate consumption at the surface. Normally what is happening is that this substrate is getting transported from bulk to the surface and at the surface reaction is taking place. If suppose there is no reaction taking place then along the length of the reactor at Z the substrate concentration will reach to S_b same as in bulk. It will take some time whereas if the biochemical reaction is also taking place at the substrate then that length required will be given by Z_2 at substrate concentration S_s.

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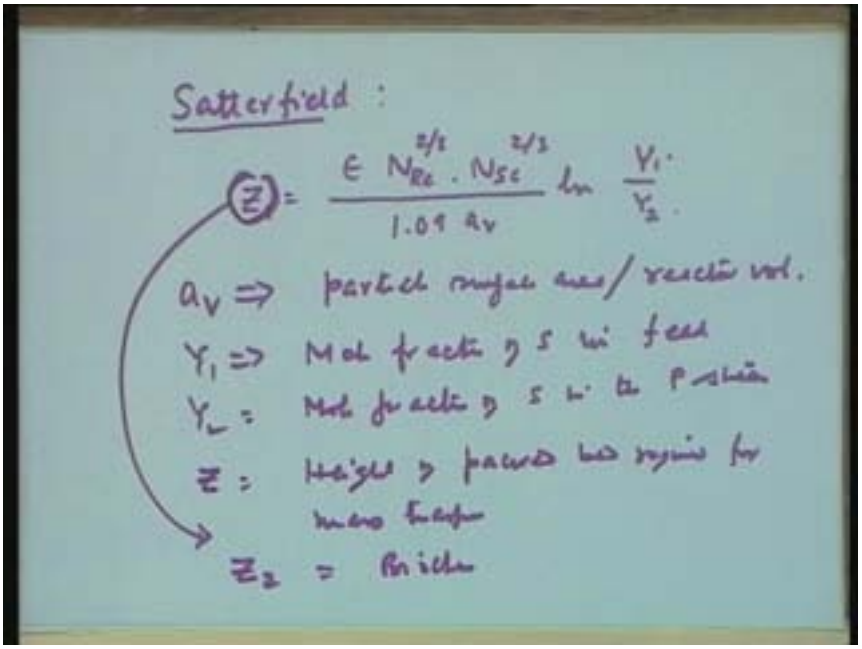

One of the two, which is larger, is required to be considered.

So this is what we were talking about the external film diffusion and the other part of the whole story of the steady state analysis is related to internal pore diffusion. In the case of porous matrices a very large quantity of fraction of total enzyme which is immobilized is within the pores of the particle. The quantity of enzyme immobilized on the surface is in fact very, very small compared to total surface area in which the whole enzyme is distributed inside the pores of the reactor. Therefore it will become very important that we look at the concentration of substrate available for the enzymatic reaction within the pores of the reaction. Very broadly the effect of internal pore diffusion in a very simplistic way is described for isothermal reaction incorporated in the pore diffusional effects by

$$v' = \eta \frac{k'_2 E_0 S_s}{K'_m + S_s}$$

I mentioned S_s that means I am excluding the film diffusion. Whatever concentration of substrate that has reached at the surface from there to the pores of the particle we are considering. We will consider the case where the two parameters or rather all the three biochemical reaction, external film diffusion and internal pore diffusion are taking place. But at the moment we are talking only of biochemical reaction and internal pore diffusion assuming that the transport through a film is fast enough and it does not create any problem. That means there is no concentration gradient across the film. In this case this is defined by a factor η which is called as effectiveness factor. This effectiveness factor is the ratio of the rate of actual reaction in the matrix divided by the maximum reaction rate obtainable if there was no pore diffusional resistance.

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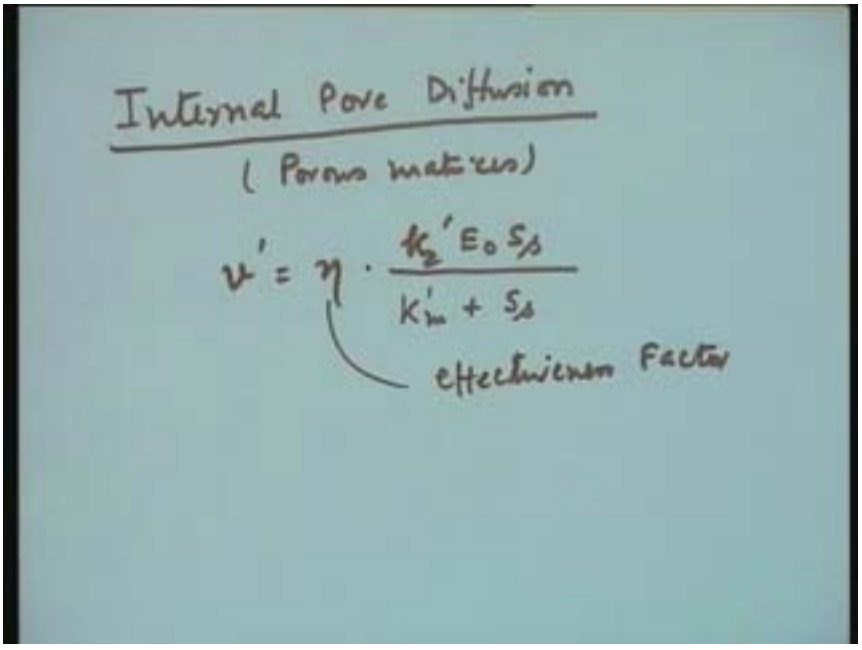

That means a very physical conceptual picture of effectiveness factor can be considered. That is if for example you take a particle let us say of one millimeter diameter; determine the reaction rate and then also take a particle let us say of ten microns or twenty microns diameter in which we assume that the pore length is very, very small. Even if the diffusional rate is very slow the substrate can reach to all parts of the enzyme go down to as low as possible and the ratio of the two that means the maximum reaction rate which you can get when you go down in the particle size to that of the reaction rate which is obtained by a particular particle size is defined as the effectiveness factor.

If you consider a very hypothetical condition a single pore as a part of the matrix. This is surface; this is thin film. You have Sb here; you reached S_s; there is no substrate concentration gradient but then here onwards there is a gradient. There are two things you must appreciate in contrast to the film diffusional effects. One is that there is a rate of diffusion; the substrate will take some time to reach to the different sites of the enzyme present in the pores. The other is the substrate is also getting consumed during the reaction. So therefore if suppose the bulk substrate concentration is itself very low there is likely hood that by the time the substrate reaches to the inside pores, center of the particle there is no substrate present and reaction cannot take place even if enzyme is there. Ultimately when you will measure the enzyme activity you will see that the enzyme activity is low because per unit enzyme loading the activity which will be expressed by the immobilized enzyme particle will be lower. So therefore if particle size is reduced you get an advantage that the fraction of the total particle which is receiving the substrate increases coming to one when the particle size is too small and usually in most cases as per the experimental experiences that a particle size about thirty microns in most cases of the biochemical reactions has been considered to give you an effectiveness factor of one. You will see from this relationship that maximum effectiveness factor can be one because that means the you have a enzyme reaction rate under the condition when the whole enzyme particle is flooded with the substrate, the same substrate concentration what is present at surface is present across the particle, at all surface at all the pores and under that condition the effectiveness factor will be one.

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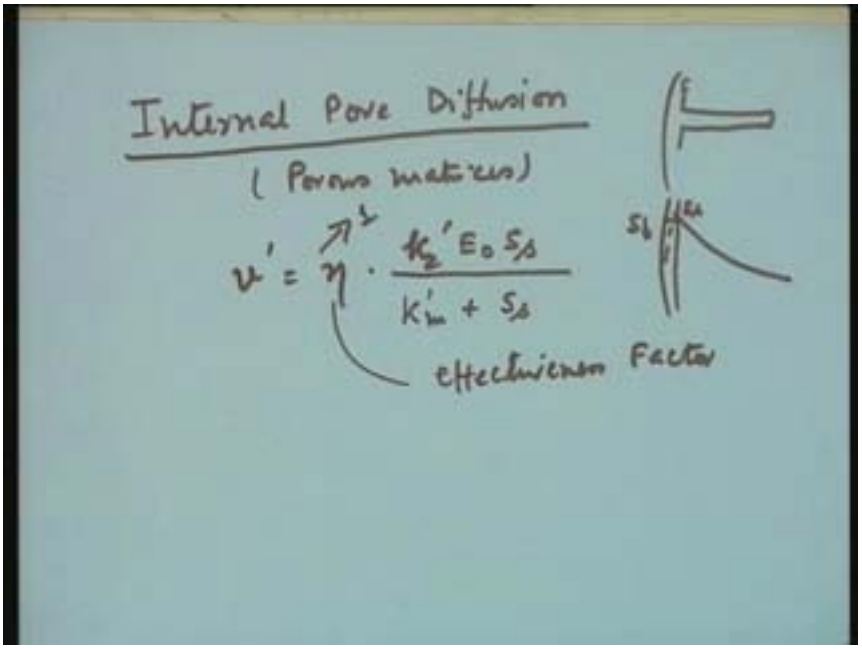

That is what is the ultimate limit and the internal pore diffusion effects are described by effectiveness factor. There is very tricky situation; in many cases people have also reported effectiveness factor exceeding unity; experimental observations. You measure the enzyme activity lets say for example for different particle size in a reactor and the

effectiveness factor works out to be more than one. Under what conditions the effectiveness factor should be more than one? The first condition will be the enzyme kinetics where substrate inhibition is very strong. The enzymes that are very strongly substrate inhibited may show an effectiveness factor greater than one because the reaction rate when the substrate concentration drops becomes higher than at the surface. If the substrate concentration is high at the surface the reaction rate is low. When the concentration goes down, reaction rate increases and therefore the effectiveness factor is one.

The other alternative case of the effectiveness factor exceeding unity is that of a case where we experience partitioning effects particularly …. pH. If suppose the product of the reaction provides you a change in the micro environment; let us say hydrogen ions are produced. Take a typical example of alcohol dehydrogenase. The reaction product ends up in hydrogen ions; there will be a pH change and that pH change enhances the enzyme activity. Then also we will notice the effectiveness factor to be greater than one. So there are two extreme cases where effectiveness factor show discrepancy from the theoretical understanding. In the case of strongly substrate inhibited enzymes, if the concentration of substrate at the surface is high then for substrate inhibited reaction, the reaction rate will be low and because of the pore diffusional parameter the substrate concentration across the pores is reducing. As we saw here the substrate concentration goes down along the pores and substrate concentration going down means the react rate of reaction increases. When you take the whole particle in account then the bulk of the particle experiencing lower substrate concentration means higher reaction velocity and so therefore this ratio will become more than one or in other words if you take two particles one of larger diameter other is smaller diameter the reaction rate in the larger particle may be more than in the smaller particle. That is what it means by effectiveness factor of one and in many cases of substrate inhibitor systems this is a usual pattern which is noticed. No. When there is a partitioning of hydrogen ions and the conditions are such that the rate of reaction as a result of partitioning increases that is the condition. If the partitioning creates unfavorable conditions for the reaction then it will not be more than one. Only when the hydrogen ions are produced and the micro environment created by the reaction in the form of product. No. The comparison will be only when we take the same reaction and in reactor performance equation in addition to internal pore diffusion you also consider partitioning effects. Then you cannot really compare the two only by a steady state analysis or mass transfer and biochemical reaction. You must incorporate partitioning effects also in the steady state analysis. Then only you can compare. At the moment we are talking only of mass transfer and biochemical reaction. Partitioning also can be incorporated and which can give you a real performance.

In the case of internal pore diffusion a steady state analysis can be made. In a simplistic way we consider a pore to be a single cylindrical catalyzed pore. We assume in a very simplistic way that the enzyme is taking place in a cylindrical pore. Consider a small differential element in this pore and along which the mass balance can be made. This is S_s, the substrate concentration on the surface and this goes down across the pore. This is dx the length of differential element. If you make a mass balance across this differential element at steady state you see the output of the substrate will be $-\pi R^2.D\ (ds/dx)_{out}$

R is the radius of the pore. dS/dx is negative and this is output of the substrate from the pore. Input is $\pi R^2.D\ (ds/dx)_{in}$. The conversion as a result of biochemical reaction is $v'(2\pi R)dx$.

At steady state:

$$-\pi R^2.D\ (ds/dx)_{out} + \pi R^2.D\ (ds/dx)_{in} + v'(2\pi R)dx$$

This should be equal to zero as at steady state we make a mass balance of substrate across the differential element we get this. I must again here remind one thing; this reaction velocity is based on surface area and not volumetric reaction velocity as we are usually used to in the case of soluble enzymes. v' is based on surface area unlike in the case of soluble enzyme where we usually represent based on the volumetric reaction velocity and that's why we have multiplied by $2\pi R$.

If you rearrange you get

$$\frac{(ds/dx)_{out} - (ds/dx)_{in}}{dx} - \frac{2}{RD}.v' = 0$$

If you take limit dx tends to zero you can write simply as the second order differential equation

$$d^2s/dx^2 - 2/DR\ .\ v' = 0$$

This gives you a generalized second order differential equation which can be set for internal pore diffusion cum biochemical reaction. Here you can make modification; if you transfer the reaction velocity in the volumetric form like for example

$$kV = k_S.S$$

These are the two rate constants; zero order or first order based on volumetric and surface area and in that case for a cylindrical pore the k will be equal to

$$k = k_S.S/v = 2k_S/R$$

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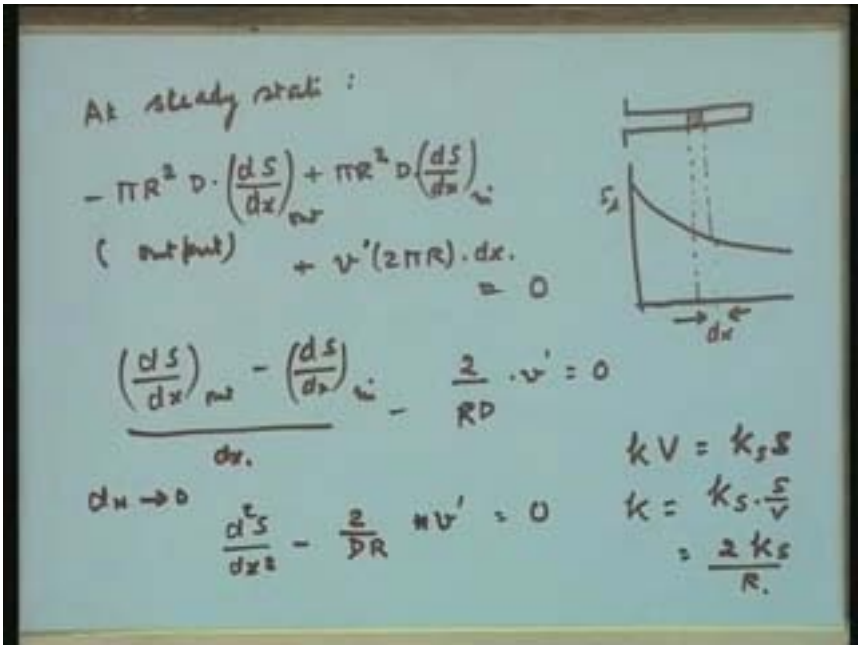

Therefore one can write here as

$$d^2s/dx^2 - v' = 0$$

as the basic second order differential equation a solution of which can give you if you substitute the reaction rate term here depending on the nature of the reaction either by assuming a first order kinetics or zero order kinetics or even Michaelis Menten kinetics and Michaelis Menten kinetics will require a numerical (35:44) solution and analytical solution may be difficult and you can get the solution of the substrate concentration as a function of the distance x from the surface.

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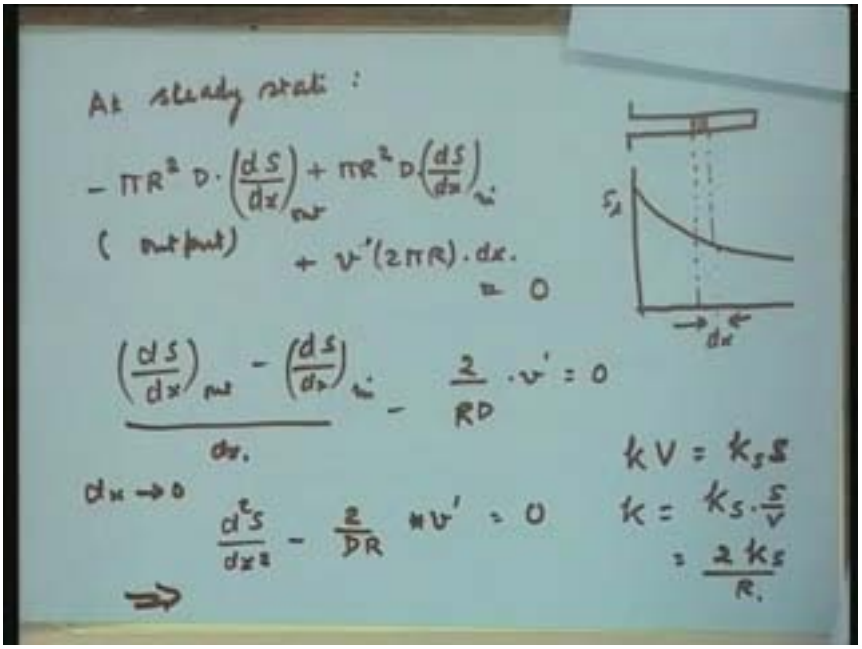

A solution of this will give you the substrate concentration profile in the pore or inside the particle. No. Sorry diffusional coefficient will come here. It is not going. We have multiplied here too or v/ D either way. This can be written for variety of cases and solved within boundary conditions so as to give you the reactor performance.

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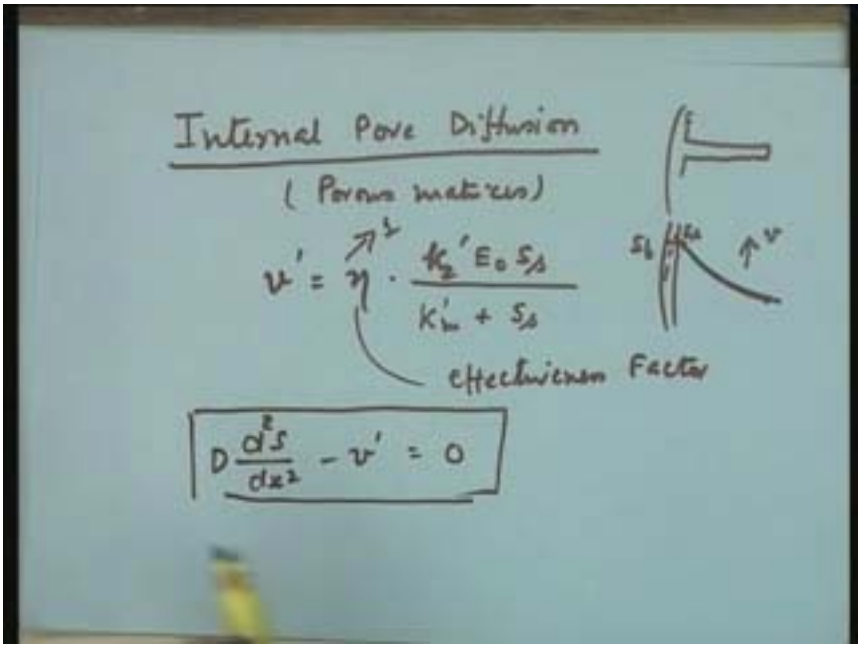

I think we will now stop at this point.