ENZYME SCIENCE AND ENGINEERING

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LECTURE–24

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

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

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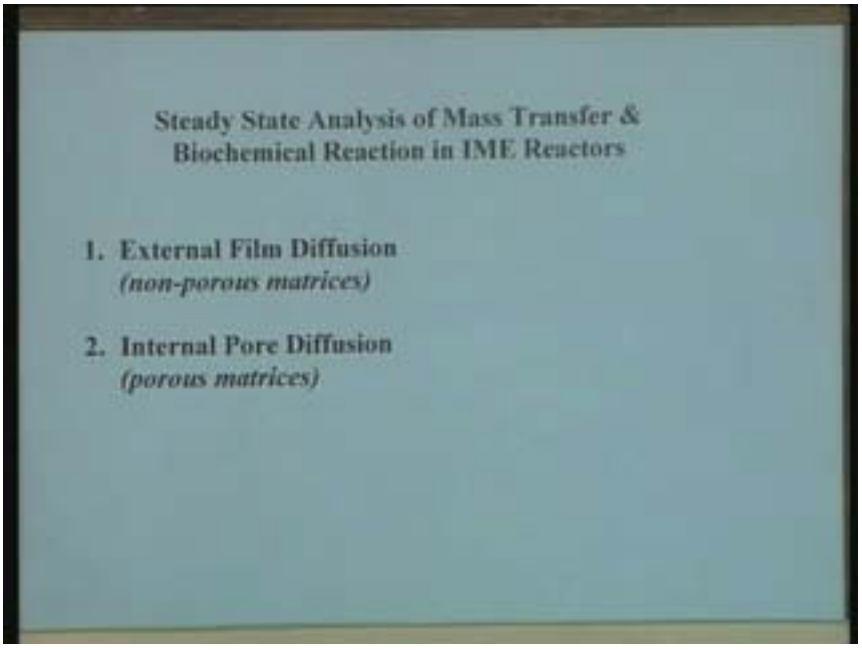

The effect of mass transfer is demonstrated in the performance of immobilized enzyme reactor by virtue of the effect of linear feed velocity in the case of plug flow reactors or the effect of agitation speed in the case of a stirred tank reactor or the effect of particle dimensions or particle size on the performance of enzyme reactor and these parameters influences the rate of enzyme reaction or the performance of the immobilized enzyme reactor demonstrate the role of mass transfer in the performance of the enzymatic catalysis.

We have earlier seen the role of external film diffusion as a result of a thin film that is present on the surface of the catalyst particle in the case of a enzyme reactor and we have

considered the interactions of the film diffusion along with the biochemical reaction in the case of a non-porous particle and a combined external film diffusion coefficient k_i along with the biochemical reaction constant was determined. The second case is about the pore diffusion which in fact is much more pronounced in the case of enzyme catalyzed reactions because in the case of porous matrices a very large surface area where the enzyme is present is through the pore surface in the catalyst particle and so the second case is if we consider a porous matrix in which the enzyme is immobilized throughout will provide you the case of a pore diffusion regimes.

In general for an isothermal reaction which usually most of the enzyme catalyzed reactions are the effect of internal pore diffusion can be clubbed or can be expressed in a parameter what we know as effectiveness factor. η is called effectiveness factor. η here is the ratio of actual reaction rate in the matrix in the presence of pore diffusion resistances. That means as a result of the substrate concentration gradient that develops in the matrix phase to that of maximum reaction rate obtainable in the absence of any pore diffusional resistance.

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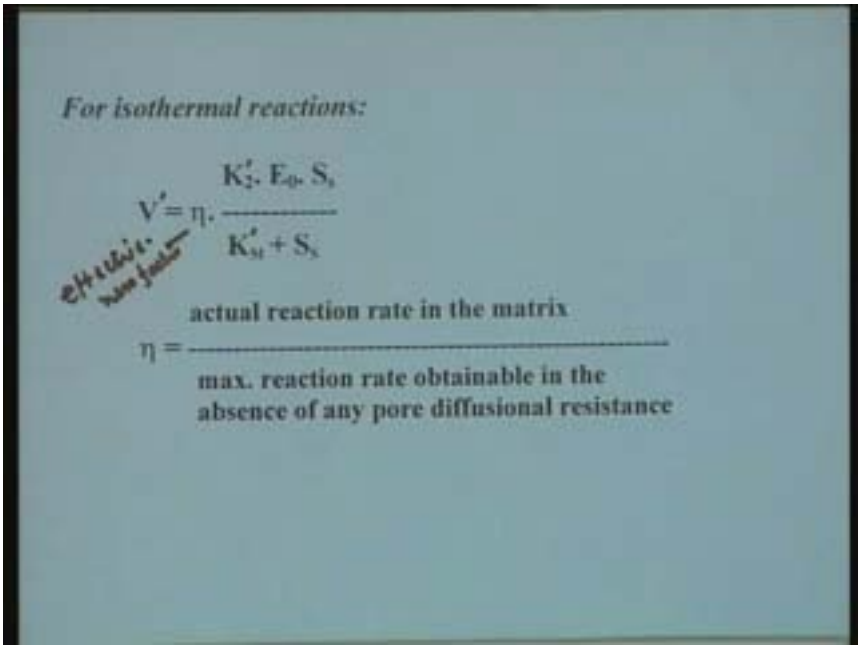

That means if you consider very fine particles in which the pore diffusional resistances may be negligible or there are no substrate concentration gradient across the particle physical boundaries and that means reaction is taking place in the particle at the same rate in the bulk solution and that ratio is called effectiveness factor. Physically one can conceive the ……. (4:52) diffusional resistances in the form of very small fine particles to that of the particle which are of larger diameter where the substrate concentration gradient becomes quite severe.
This effectiveness factor has a number of characteristics; one is that in most cases the effectiveness factor will be less than or equal to one because the present support

diffusional limitations will always reduce the substrate concentration in the particle surface. Therefore the substrate concentration at which the reaction will take place actually in the particle boundaries will be less than that in the bulk or at the surface and therefore the effectiveness factor will be less then or equal to one. As I mentioned earlier two specific cases demonstrate where the effectiveness factor can be more than one. One is in the case of enzyme reactions which are controlled by substrate inhibition kinetics. That means at higher substrate concentration the reaction velocity is inhibited. Therefore as the substrate concentration gradient developed in the particle the concentration drops and the reaction rate can be higher than at the surface and therefore the effectiveness factor can be more than one.

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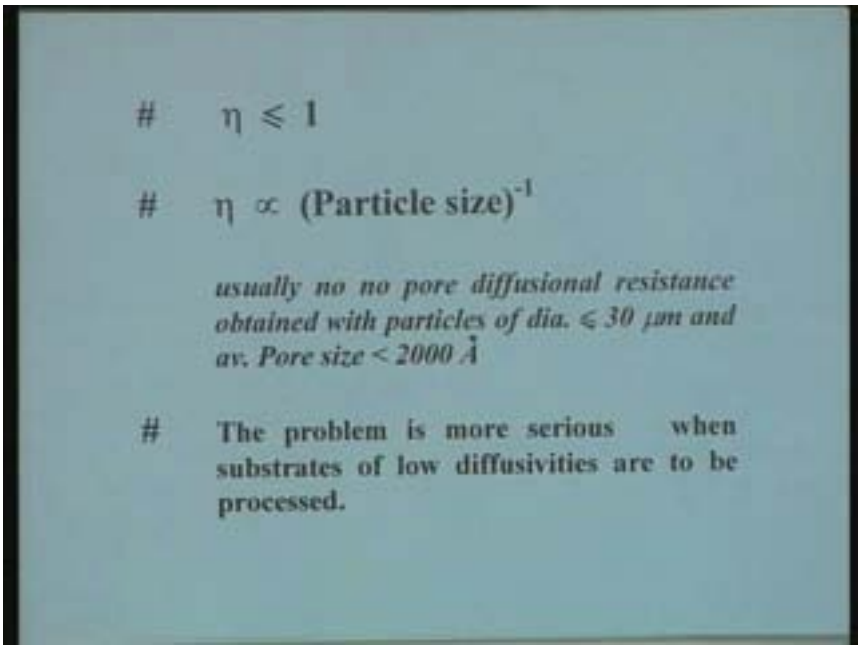

The second case is due to the partitioning effect. If suppose the matrix is charged and the substrate is also charged and if the two species have opposite charges then the concentration of the substrate inside the enzyme particle we will more than that in the bulk and therefore the enzyme reaction rate within the matrix may be more than what is obtained at the matrix surface. Therefore the effectiveness factor can be more than one in the two cases that we have just elaborated.

The second characteristics feature of the effectiveness factor is that it is inversely propositional to particle size. If you reduce the particle size the effectiveness factor can approach to unity which means that the whole of the matrix of the immobilized enzyme particle is able to get adequate substrate and carry out the biochemical reaction. In experimental conditions usually no pore diffusional resistance is obtained that means effectiveness factor is approaching one with particles of diameter less than thirty microns and average pore size greater than two thousand angstroms. That means either the pore diameter is large or the particle size is reduced. The effectiveness factor can be made to

go towards one but in these cases you face other operational problems like at the low particle size there is a risk of high pressure drop across the bed resulting in more power consumption for operating the enzyme reactor. Also the effectiveness problem can be more serious when substrates of low diffusivities are to be processed.

If you take a substrate which has low diffusivity in the matrix the problem is more serious because the rate of diffusion is slower than the biochemical reaction rate. Therefore the relative rate of diffusion and the relative rate of biochemical reaction are the other features which control the effectiveness factor. The diffusional coefficient, the ….. diffusivity of a particular substrate in an empty matrix and in the case of an immobilized enzyme particle where the path of diffusion is quite haphazard, it makes a quite a different diffusivity. Very often in the case of immobilized enzyme particle we call it effective diffusivity rather than diffusivity.

Normally we take an ordinary particle, a spherical particle where the pores are very uniform; say for example you have a sphere where the pores are identical, for example control pore glass then the path of diffusion is linear and of the same dimensions. Whereas in the case of actual particles what we have encountered in the case of immobilized enzyme systems the path of diffusion is really not linear and uniform through out the particle and it can happen that you have pores almost going in a random manner in the whole particle and therefore the diffusivity that we obtained from theoretical considerations of a spherical particle is in effect different than what is obtainable of a particle at that dimensions assuming a uniform cylindrical pore.

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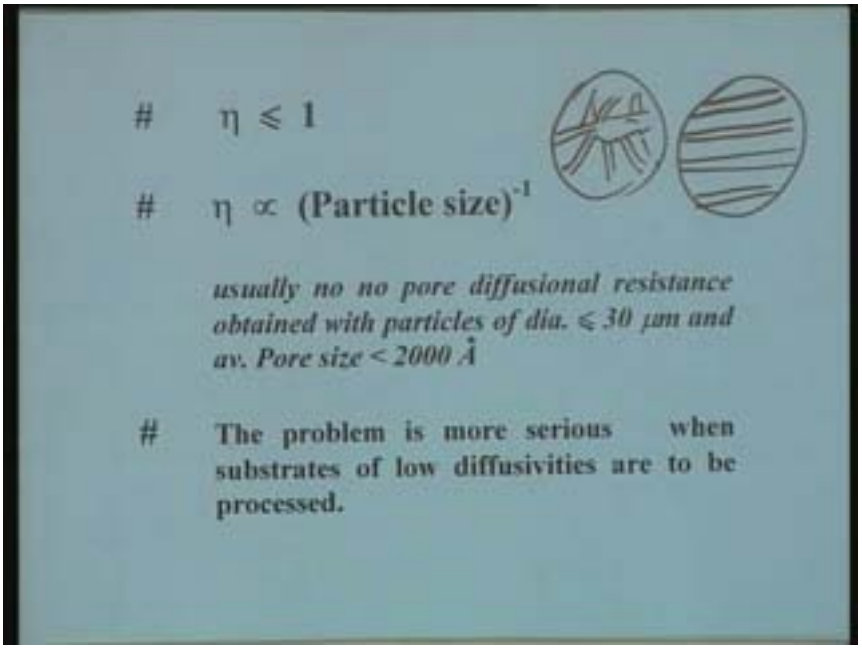

We have also mentioned last time that if you want to analyze the simultaneous mass transfer and biochemical reaction in immobilized enzyme particle it can be expressed

assuming a cylindrical pore model. That means you assume that the substrate diffuses into the particle in the form of a cylindrical pore. In practice it may not be actually like a cylindrical pore but just to simplify a geometric similarity and to analyze it we are just assuming as cylindrical pore and then instead of taking molecular diffusivity we consider effective diffusivity which takes care of the random nature of the pores.

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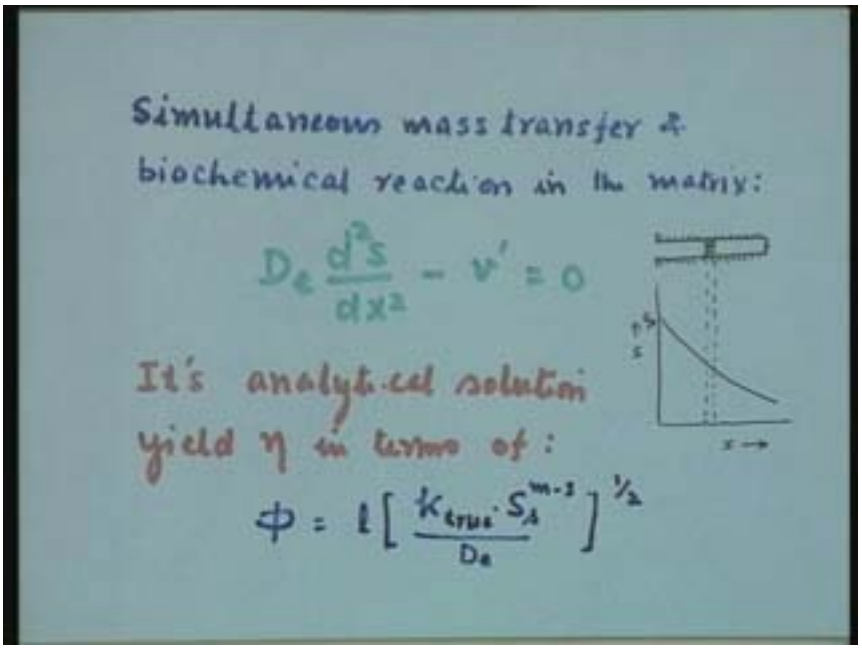

Simultaneous mass transfer and biochemical reaction in the matrix can be analyzed in the form of a second order differential equation.

$$D_e d^2S/dx^2 - v' = 0$$

v' is the apparent rate of reaction that means under apparent kinetic parameters in the case of immobilized enzyme and De is the effective diffusivity. In the substrate concentration profile here we consider this as single pore and this pore refers to half the particle depth. There will be another pore on this side of the same dimension so whatever dimension of the particle we are considering let us say if it is a pallet of thickness twelve we consider l as the thickness because the substrate will be accessible on both the sides. Similarly if it is a sphere the radius is the major dimension which is important here in terms of X and therefore one needs to consider only half the particle for getting an analytical solution for the substrate concentration profile. The substrate concentration profile for such a second order differential equation is usually obtained interms of a Thiele modulus. φ here is defined as Thiele modulus in general which is defined as a lump parameter like this which is

$$\varphi = l.[k_{true}.S_s^{m-1}/De]^{1/2}$$

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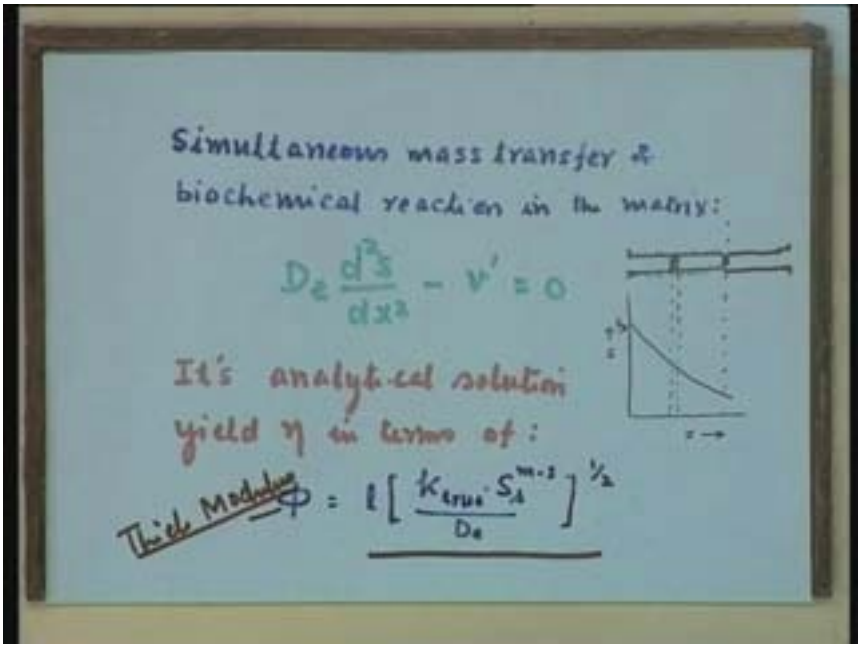

k_{true} is the reaction kinetic constant in the absence of any pore diffusional effects that means in the surface or in the bulk. S_s is the substrate concentration of the surface and m is the order of reaction whatever the order of reaction and De is the effective diffusivity. The solution of this differential equation is usually expressed in the form of Thiele modulus and a large number of workers have given the solution of this expression both for zero as well as the first order reactions for which the analytical solution can be easily obtained.

In the case of Michaelis Menten kinetics if we put the v' term which is a non-linear expression the analytical solutions are difficult and usually you approach numerical solutions which have been also reported in literature. But very often for practical purposes we try to look at our reaction conditions and consider if they are closer to zero order or first order depending upon the percentage error that is involved in calculating the space time under the given set of conditions which are the inlet substrate concentration and outlet substrate concentration in the reactor and with in that regime that means fractional conversion and initial substrate concentration in that regimes if the reaction is more close to zero order we very conveniently consider our system as zero order and make the design calculations or in case if it is more closer the percentage error is less in the case of first order kinetics we go for a first order design calculations. But alternatively …… solution using Michaelis Menten kinetics also can be obtained numerically.

The effectiveness factor for all those system is a function of the Thiele modulus in all cases whatever is the order of reaction whether it is first order, zero order or Michaelis Menten kinetics, the effectiveness factor is always a function of Thiele modulus and this function varies basically on the order of reaction as well as on the particle geometry.

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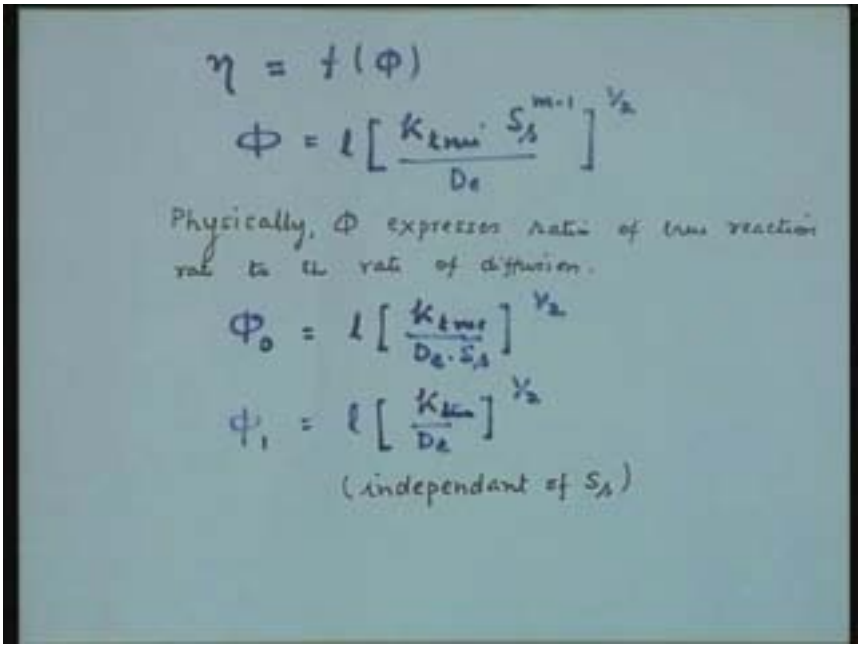

That means whether particle is a sphere or a chip or a cylindrical pallet or whatever be the shape, it varies; the relationship between the effectiveness factor and the Thiele modulus. As I mentioned earlier the Thiele modulus is a function of the true rate constant, the substrate concentration on the surface and the effective diffusivity and physically if you look at, the Thiele modulus gives you an indication although its not an exact ratio but it really gives you a ratio between the two reaction rate or reaction rate parameters to that of rate of diffusion. The numerator expresses the parameter resembling the rate of reaction and the denominator represents the rate of diffusion and the Thiele modulus is an expression of the ratio between the two things. So if the Thiele modulus is high, the reaction rate is faster. Smaller Thiele modulus means the rate of diffusion is smaller. If you simplify the Thiele modulus expressions either for a zero order or a first order reaction it becomes still simpler. For a zero order system it is

$$\varphi = l.[k_{true}./De.\ S_s]^{1/2}$$

That means the Thiele modulus is inversely proportional to the substrate concentration at the surface. On the other hand for the first order reactions the Thiele modulus is independent of substrate concentration because 1-1 is zero and so it becomes independent of substrate concentration.

There are ample literatures available from which one can write for pore diffusion control first order reactions, assuming spherical particles in the packed bed reactor the effectiveness factor is given as

$$1/\varphi = [1/\varphi\ (1/\tan 3\varphi - 1/3\varphi)]$$

That is the relationship between φ and effectiveness factor and using this effectiveness factor then you can write down the reaction rate expression for first order kinetics. One can write the reactor performance equation as

$$\varepsilon\tau = \frac{-K'_m \ln(1-X)}{k'_2 E_0} \left[\frac{1}{\varphi}\left(\frac{1}{\tanh 3\varphi} - \frac{1}{3\varphi}\right)\right]$$

The expression for effectiveness factor is given in the parenthesis. The expression in the parenthesis is same and you remember we have defined right at the beginning that the reactor performance can be simplified by taking a reaction rate expression which is multiplied by effectiveness factor. So therefore the tow the space velocity or the space time can be given by the same design expression which was used for idealized enzyme reactors excepting that the effectiveness factor for a case has been multiplied to the system. For the first order kinetics this is the effectiveness factor as we discussed earlier. This is in the case of spherical particles. The same expressions when you talk of packed bed of chips that means very thin films of very fine thickness let us say of the thickness l. In that case the tow is given as

$$\varepsilon\tau = \frac{-K'_m \ln(1-X)}{k'_2 E_0} \left[\frac{\tanh \varphi}{\varphi}\right]$$

The effectiveness factor here is different; tanh φ/ φ where the particle thickness is very, very small.

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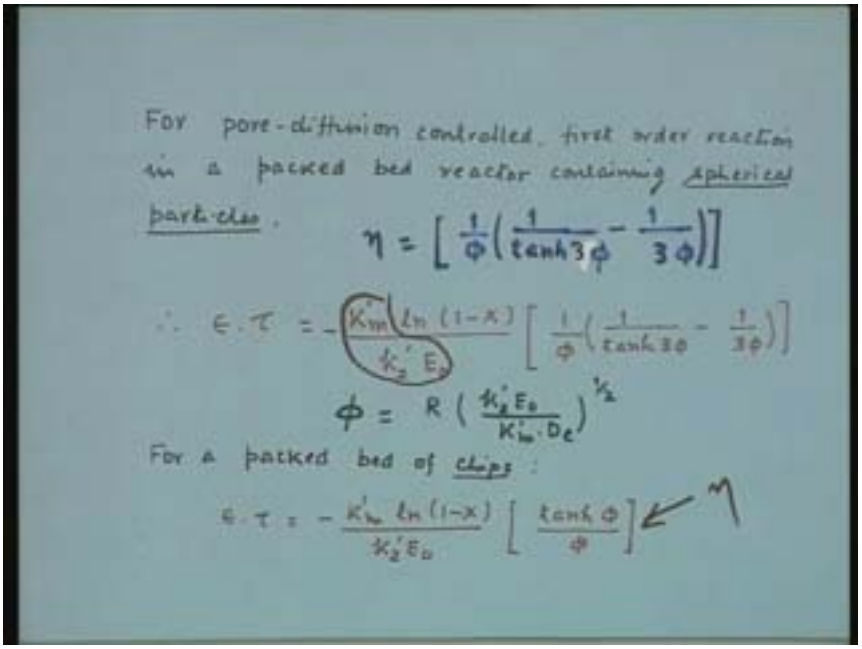

These expressions have been obtained analytically in the case of different particle size like in the case of thin films and for spheres. They are mentioned even for cylindrical pallets, discs. A number of such expressions are already available in the literature which can be used.

Based on this I think one of the major studies which were reported in seventies was particularly on the mass transfer in immobilized enzyme reactor by Rovito and Kitrell.

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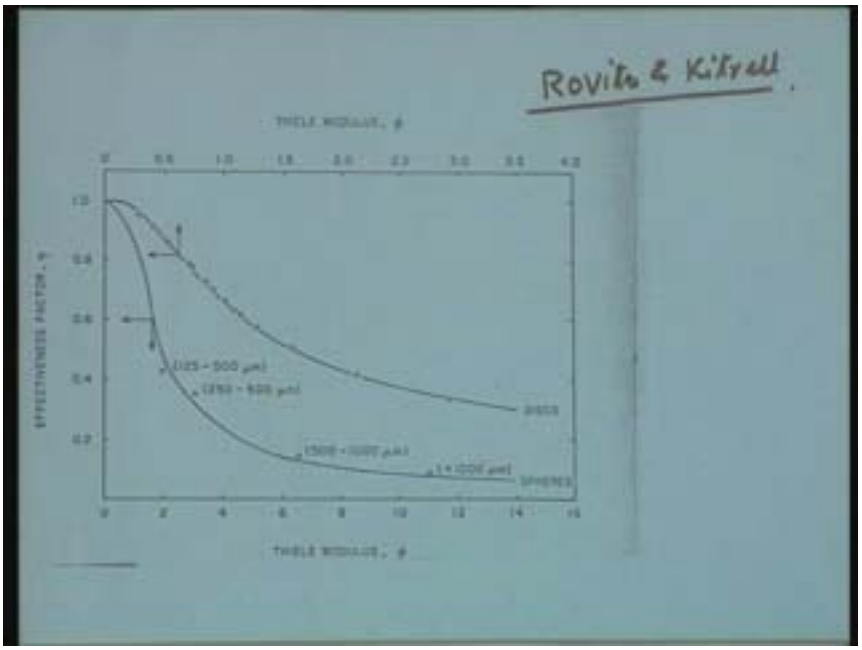

They made physical measurements experimentally of the Thiele modulus and also calculated the effectiveness factor based on these expressions and they gave for both sphere as well as discs of different thicknesses and confirmed the validity of the first order reaction rate expression applicable in many cases of packed bed reactors. The data points shown here are experimental data with different particle sizes say for example from hundred twenty microns to thousand microns. They used controlled pore glass and immobilized it with glucose oxidase and measured the reactor performance with different particles sizes, the value of effectiveness factor based on the experimental data that means by taking the ratio of the actual reaction rate for a particular particle diameter divided by the reaction rate at which particularly with a very small particle size say thirty microns. They also calculated the same reactor performance using the analytically developed reactor design equation and they found almost very good fit both in the case of sphere as well as in the case of disks. Y-axis is effectiveness factor and X–axis is Thiele modulus.

The relationship between pore diffusional limitations and the Thiele modulus is explained in terms of effectiveness factor because if you know effectiveness factor you can have reactor performance equation, where you multiply reactor rate expression which is $\eta.K_2E_0/K_m$, a pseudo first order rate constant for an enzyme catalyzed reaction or if zero order which is K_2E_0 and multiply by the effectiveness factor which you are aware of.

Another thing which also has been checked experimentally, another factor which influences the reactor performance as a result of pore diffusion is the effect of increasing the enzyme loading.

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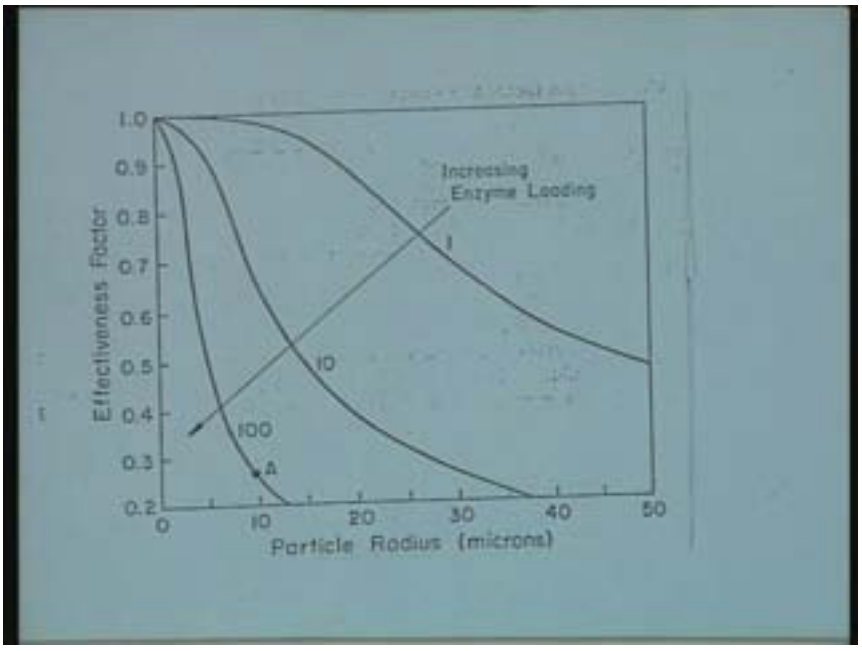

Basically what you are doing when you increase the enzyme loading is you are changing the Thiele modulus. The numerator in the Thiele modulus which gives you the reaction rate constant is increasing because the concentration is high. If it is first order rate constant, K_2E_0/K_m in the case of immobilized enzyme catalyzed reaction then the value of E_0 is increasing by enzyme loading and therefore as you increase the enzyme loading in this direction and some arbitrary values of let us say one, ten and hundred you notice that the effectiveness factor very sharply decreases. That means the enzyme reactor system becomes more and more controlled by diffusional limitations and when the enzyme loading is less the diffusional limitations are less. Up to quite a large particle size it can be considered almost free of diffusional limitations. Increasing the enzyme loading influences the increase in φ, the Thiele modulus. The Thiele modulus on the numerator is K_{true} that is the true rate constant which is K_2E_0/K_m in the case of enzyme reactor for first order or in the case of zero order it is K_2E_0. So increasing enzyme loading means the concentration of enzyme is being increased. E_0 is increasing so φ is increasing and increasing Thiele modulus gives you a lower effectiveness factor which means that the rate of reaction is must faster than the rate of diffusion and that is what the ratio is described by Thiele modulus and therefore the effectiveness factor decreases. If you keep on increasing the loading you will have a very significant effect upon the final reactor performance and ultimately it may happen that the loading may have no function; the whole reactor performance will be controlled by only rate of diffusion.

In many cases numerical solutions have been found in the case of Michaelis Menten kinetics which indicates the effect of Thiele modulus and effectiveness factor as a function of β something like a dimension less Michaelis Menten constant which is the ratio of K'_m/S_0. It shows the effect of shift from zero order to first order. The shift is when β is small the K'_m/S_0 is also small and whereby you see the effect of β on the effectiveness factor as a function of Thiele modulus. In this case also as the Thiele modulus or the beta value increases the reaction shifts from zero order to first order. β value is increasing means from zero order to first order and in that case the effectiveness factor decreases and the same effect from the Thiele modulus is obtained in either directions.

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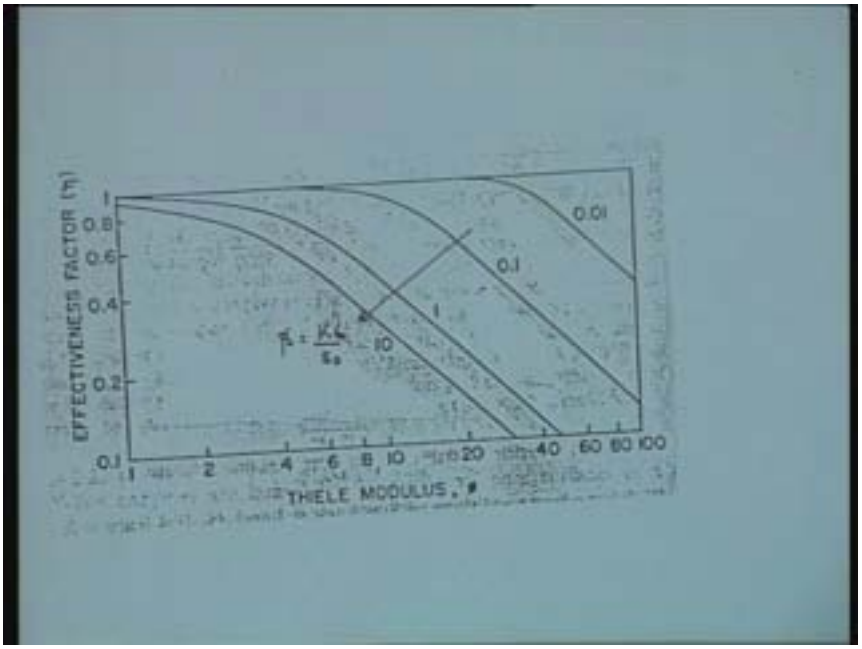

In the case of analysis of the internal pore diffusion in the case of an immobilized enzyme reactor, the analysis amounts to determination of effectiveness factor for a particular system and that is directly controlled by the value of effective diffusivity, the reaction rate constant and the size of particles what we are considering.

If you consider say for example a combined film and pore diffusional effects and for a simplistic case of a first order irreversible enzyme catalyzed reaction , we can club the two things for example the external film diffusion and internal pore diffusion along with the biochemical reaction and the flux of the substrate can be then written as

$$J = k_L(S_b - S_S)$$

You will recall that in the external film diffusion, flux of substrate along the particle surface is given by k_L which is the external film diffusion coefficient into the substrate concentration gradient that is developed in the film and that if we could equate to $\eta.K_f.S_S$

$$J = k_L(S_b - S_S) = \eta.K_f.S_S$$

This is the factor; this is the rate of reaction or the flux through the pore diffusional limitations. The pore diffusional resistance is expressed by η. K_f is the first order rate constant which can be also be written as k'_2E_0/K_m and S_s is the substrate concentration at the surface of the particle. If you simplify this you can write

$$(S_b - S_S)/J = 1/k_L$$

That is the inverse of the film diffusion coefficient and also

$$S_s/J = 1/\eta k_f \; ; \; S_b/J = 1/k_L + 1/\eta k_f$$

If this S_b/J is the main flux taking into account both external film diffusion as well as internal pore diffusion and the first factor $1/k_L$ is resistance provided by external film diffusion and the second expression is a resistance term due to pore diffusional limitations then the overall effect is a sum of these two resistances and S_b/J will be the flux which will really be expressed in the case of a reactor performance which experiences both film diffusion as well as internal pore diffusion.

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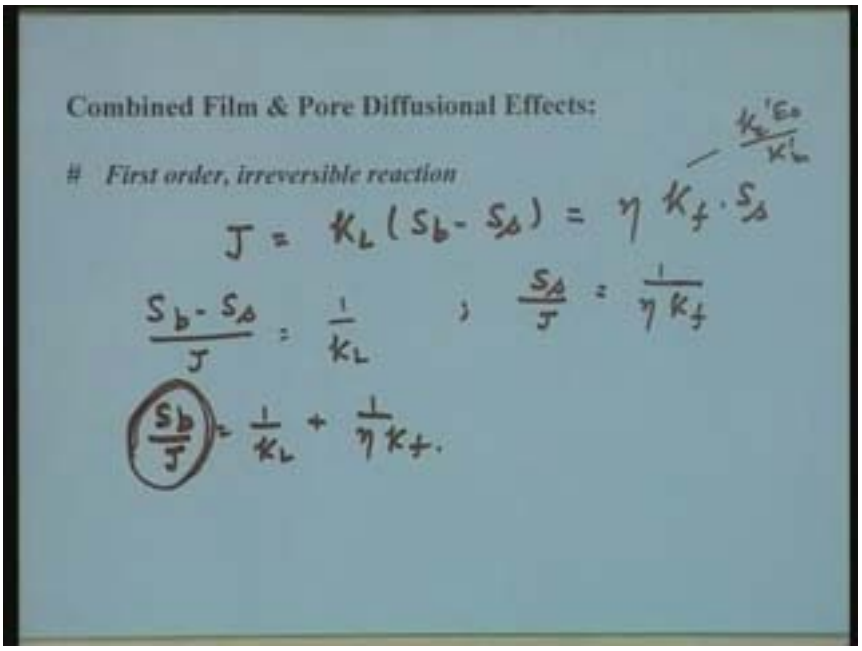

So therefore for example if you carry out continuous immobilized enzyme reactor and determine the reactor performance and if you assume that you carry out the steady state reactor performance at various feed velocities that is a linear flow velocities what you are doing is we are varying the k_L value. We know that increasing the linear flow velocity will influence the k_L value. $1/1/k_L$ will be increased by increasing the flow velocity the k_L will decrease and the $1/1/k_L$ will increase. If you plot flow rate inverse verses the overall rate constant, $1/k'$, which is defined as the apparent reaction rate constant which takes into account biochemical reaction and also the external film diffusion as well as internal pore diffusion, then you get almost linear velocity with the intercept at $1/ \eta.K_f$ where K_f is pseudo first order rate constant. To get such a performance if you carry out the reaction at a low substrate concentration or even at a high substrate concentration degree of conversion fractional conversion is very high though the reactor performance will be closer to the first order kinetics. If you consider the reactor performance in terms of zero order or first order kinetics and if you take any substrate concentration of the feed and carry out the substrate conversion to very high level then you will have the overall reactor performance which is much closer to a first order kinetics. If the conversion level is not very high then the reactor performance will be controlled by zero order kinetics. If the outlet substrate concentration is also very high compare to the inlet substrate concentration the reaction can be considered or atleast approximated to be equal to zero

order kinetics but if the conversion is very high so that the outlet substrate concentration is very low then you can always approximate to be a first order reaction. If you carry out the steady state reactor performance as a function of flow rate and determine the overall reaction rate constant based on the reactor performance, then you can determine by intercept $1/\eta k_f$. k_f is known to you depending on the kinetic parameters of the immobilized enzyme. So the value of the effectiveness factor can be theoretically calculated and also the $K^{'}$, the overall reaction rate constant which takes into account the biochemical reaction and combined film and pore diffusional effects. The $K^{'}$ can be used as a first order rate constant for design of the immobilized enzyme continuous reactor.

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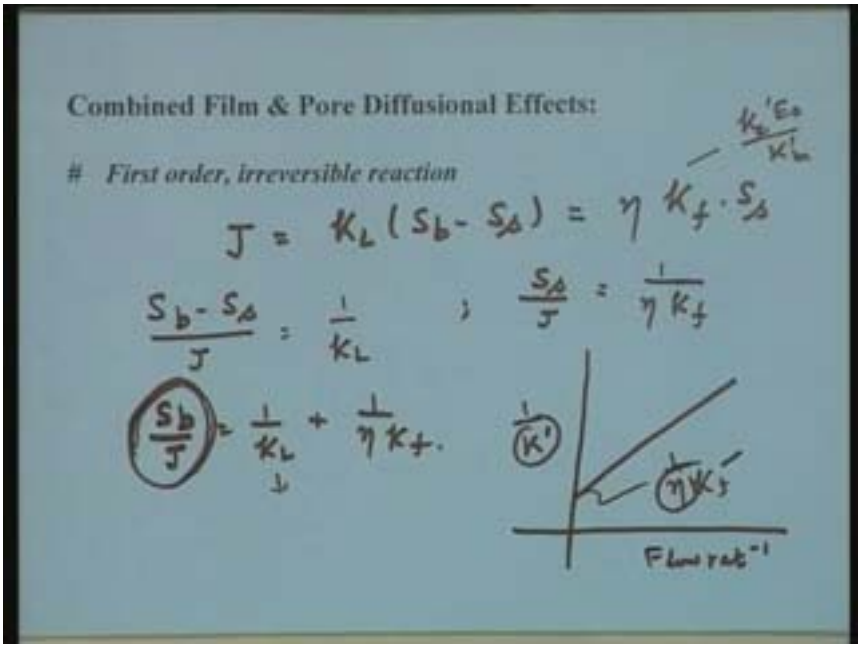

Finally I would like you to leave with a small exercise. Consider that the analytical solutions of a internal pore diffusion and biochemical reaction can be determined by a second order differential equation. Let us assume that we are given some solution for the first order reactions. If you assume that the reaction is towards zero order kinetics that means the initial substrate concentration used is high and fractional conversion will also be not very high. If you start the feed inlet concentration say at hundred times the Km value and the final substrate concentration from the effluent is let us say fifty Km. The whole reactor will operate almost closer towards zero order regimes and that expression can be given by

$$D_e d^2S/dX^2 - k^{'}_2 E_0 = 0$$

If you consider that we are talking of a cylindrical disk with a thickness of 2l, I would like you to develop this solved equation to get the substrate concentration profile as a function of distance from the centre. If this is the distance x on either sides that means the distance of the surface from the center is x on either sides. The substrate concentration

profile in such a thick membrane needs to be evaluated based on the second order differential equation. The boundary conditions in such a case will be x = l and S = S_s. You can consider the substrate concentration as Ss if you leave out the external film diffusion and on the other hand X = 0. That means at the center of the particle ds/dx is also equal to zero. Under these two boundary conditions you simplify and get an expression for S/S_s and rearrange that in the form of Thiele modulus. For zero order

$$\varphi_0 = l[k'_2 E_0/D_e.S_s]^{1/2}$$

This will be Thiele modulus and so rearrange the expression in the form of φ and get the reactor performance or you can also find it out that at what thickness from the center that is at what value of x let us say x is equal to some critical value of l when the substrate concentration approaches to zero. That means that fraction of enzyme will be ineffective and the ratio of the two that is l/l_c will give you effectiveness factor.

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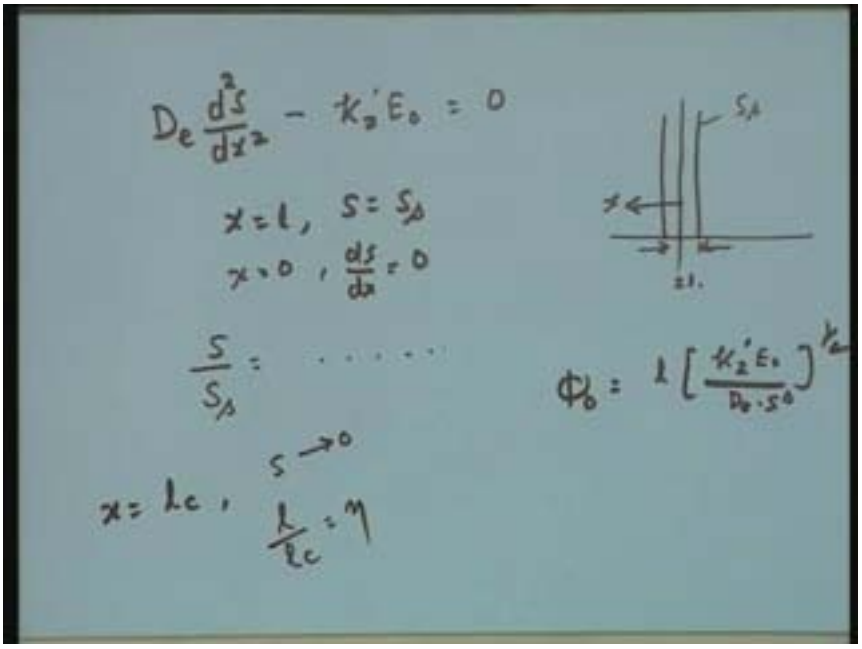

I like you to do this exercise and solve this differential equation under this boundary condition and arrive at two things; one is the substrate concentration profile within the immobilized enzyme particle and interms of surface substrate concentration and second thing is this length from the centre, the value of l_c at which the substrate concentration tends to be zero. If you assume that then the boundary conditions will change. Is that alright? So I think we will stop at this point.