ENZYME SCIENCE AND ENGINEERING

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LECTURE–22

KINETIC PARAMETERS FOR IME SYSTEMS INVOLVING MASS TRANSFER & BIOCHEMICAL REACTION

So we have so far seen that the performance of idealized continuous flow immobilized enzyme reactors depends primarily on the reaction kinetics and the basic assumption for idealized reactors was that the immobilized enzyme preparation does not experience any mass transfer or partitioning effects. The immobilized enzymes experience in addition to the catalytic activity three major external effects during immobilization. One is the conformational changes as a result of which the basic turn over number of the enzyme might be altered; even the value of Michaelis Menten constant may also be altered. The other effect is partitioning of substrate and product which also results in alteration in the K_m value of the enzyme reaction. Then the third effect was mass transfer of substrate and product and this mass transfer as we noticed was at two levels.

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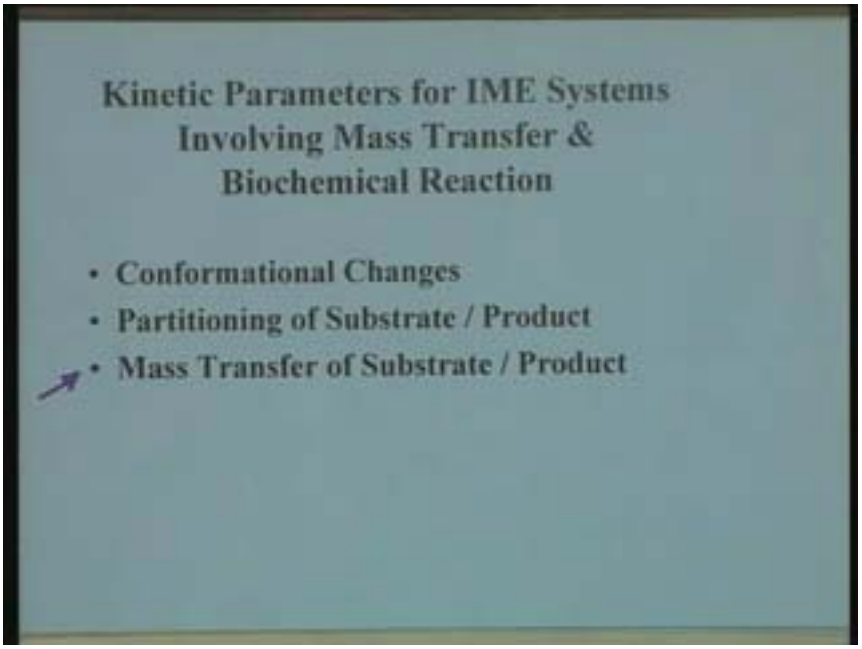

Or in a very broader sense if you consider the reaction in immobilized enzyme reactor it will imply that in addition to the biochemical reaction there will be four more resistances

involved. One will be transport of the substrate from the bulk to the surface; from surface the substrate has to transport to the site of the enzymatic reaction which means the enzyme molecules that are located throughout the immobilized enzyme particle and then the reverse process that means the product produced as a result of biochemical reaction has to transport back from the carrier interior to the surface. Therefore the two major matrices which provide resistance to the over all reaction is the thin film around the immobilized enzyme particle and the whole matrix itself and the pores in which the immobilized enzyme is distributed. I think you will also recall what we had seen earlier; the diagram in which you have a thin film, a stationary film around the particle. This film thickness will depend on various physical properties of the fluid. Then you have enzyme distributed all over the pores in the particle and the substrate has to transport from the surface to those materials and similarly reverse. Thus the product has to come back from the reaction site to the surface and then to the bulk.

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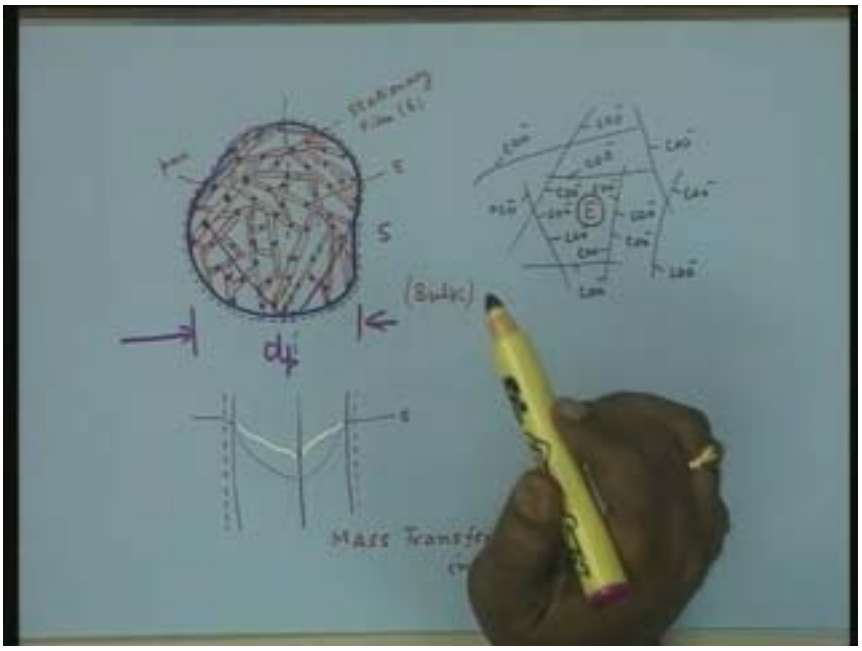

I like you to have a look at the substrate gradient. If you consider that this regime is the thin film then from the bulk there might be a linear substrate gradient across the thin film and the concentration might drop at the surface and from the surface to the interior of the particle that means to center of the particle there might be again a gradient as a result of biochemical reaction.

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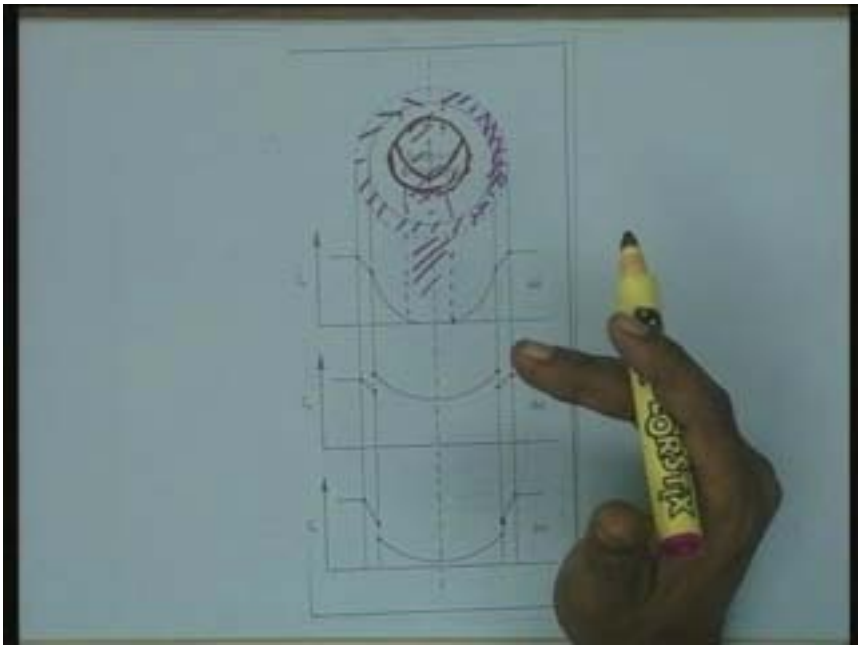

Because the substrate will be consumed and as the substrate progresses through the particle depth the concentration will be dropping down and there are two possibilities. One is that you have a rather more convenient situation that from here the substrate concentration goes like a profile like this which is more favorable in the sense that around the whole particle there is some substrate available for biochemical reaction to take place. On the other hand it can also happen that the substrate concentration drops to zero earlier than the substrate has reached to the middle point and therefore this portion which is highlighted here is deprived of the substrate and the overall reaction rate that is observed apparently is much smaller.

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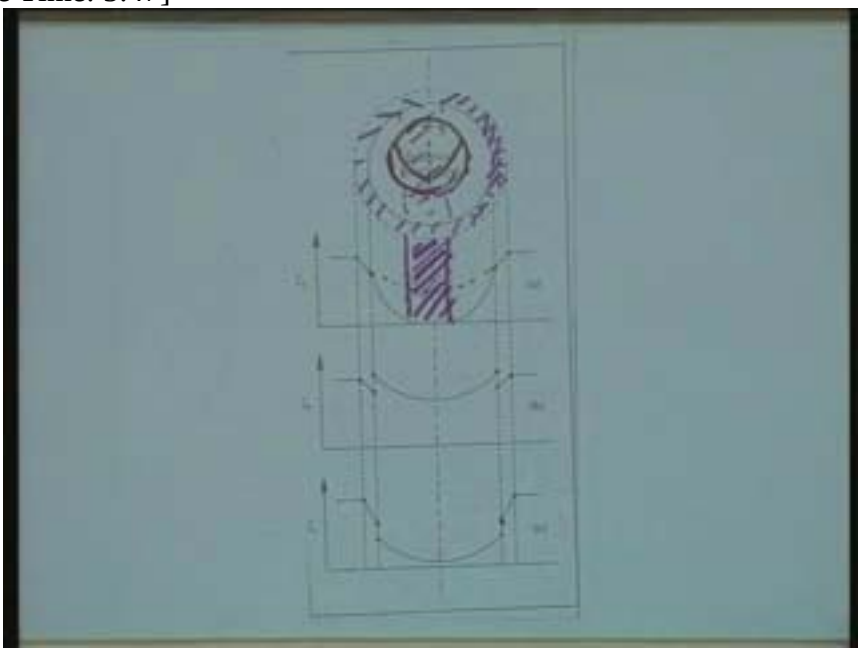

There might be coupled partitioning effects as I have shown in the lower figures in the gradient lower but basically these are the three major issues: biochemical reaction, external film diffusion and internal pore diffusion that are encountered in the case of an immobilized enzyme reactor which need to be considered while we consider the reactor performance. Although in the idealized reactor performance we have not taken into account but in the actual reactors they need to be taken care while analyzing the steady state reactor performance.

An important feature of the performance of immobilized enzyme reactor is that one needs to identify the limiting rate, the step which has a limiting rate the lowest reaction rate. That means a comparison between the biochemical reaction rate and the rate of diffusion by the …. film diffusion or the pore diffusion. One must be able to identify by some analytical method or experimental method that which is the rate limiting step and we must analyze or design our reactor keeping in view the rate limiting step. Consider that we have an immobilized enzyme preparation with a very high enzyme loading. That means the preparation is highly reactive, the rate of reaction can be very high and the rate of diffusion is slow either because of the ….. (7:34) of the substrate or the chemical nature of the matrix. If the rate of diffusion is slow in that case the diffusion will become rate limiting. If the difference between the rate of diffusion and the rate of biochemical reaction are too large then one of them will be the controlling factor and the whole reactor design must be based on that particular step. Alternatively if you take an immobilized enzyme preparation to be very lean loading that means the loading is very low and the catalyst is not very reactive or alternatively the enzyme …. that turn over number is very low and the rate of diffusion is very fast. In that case diffusion effectively will not play a key role in the reactor design and the reactor design can be easily taken care by idealized reactor performance considering the reaction kinetics in picture.

Based on these three parameters particularly as I mentioned earlier the conformational changes, mass transfer effects and the partitioning effects, the kinetic parameters for immobilized enzyme are classified into three major classes.

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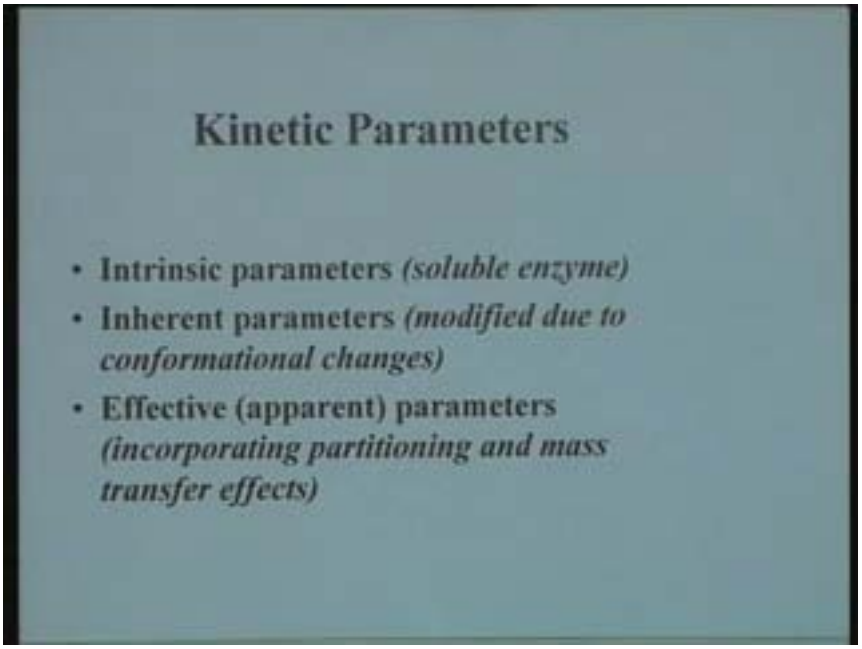

One is intrinsic parameters which are the same as that of soluble enzyme which means no partitioning in a soluble system, no mass transfer limitations; the sole parameter is the reaction rate and they are called the intrinsic parameters. If we consider the rate expression our classical rate expression

$$v = k_2E_0S/K_m + S$$

will apply to the situation.

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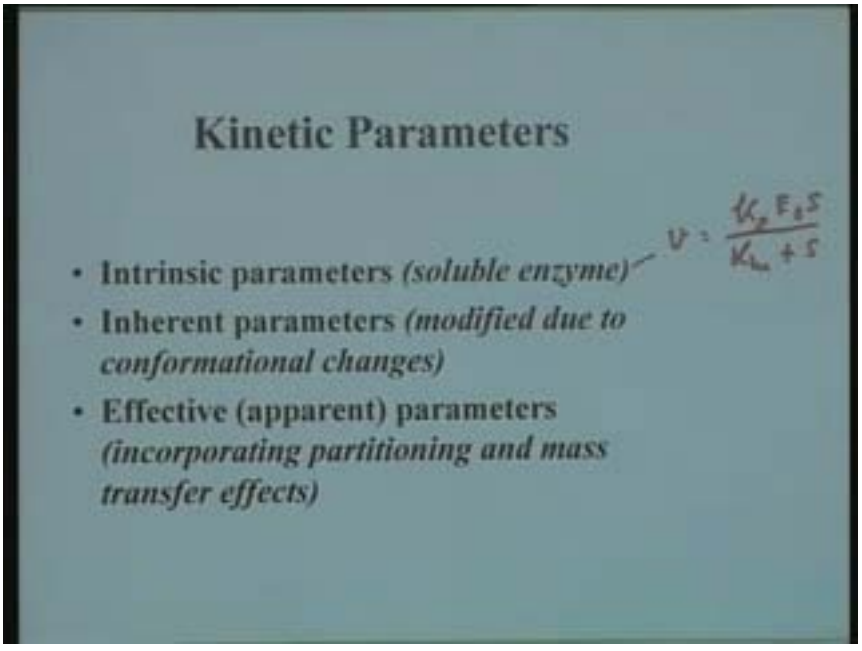

The second class of the kinetic parameters is defined as inherent parameter. Inherent kinetic parameters are those which are obtained as a result of conformational changes only. That means they are dependent on the method of immobilization. That means the kind of reactants we have used in immobilization, the kind of matrix we have used in immobilization and whatever the interaction between the matrix and the enzyme under the conditions of immobilization, whatever conformational changes have taken place, whatever kinetic parameters have been modified as a result of this interaction are considered in the inherent parameters and we define them as

$$v' = k'_2 E_0 S / K'_m + S$$

The parameters are just given prime and the inherent parameters have no role to play or not much can be done during the reactor design or reactor operation. They are the final property of the immobilized enzyme, kinetic property. I must again clearly stress that the inherent parameters do not consider the diffusional effects or the partitioning effects.

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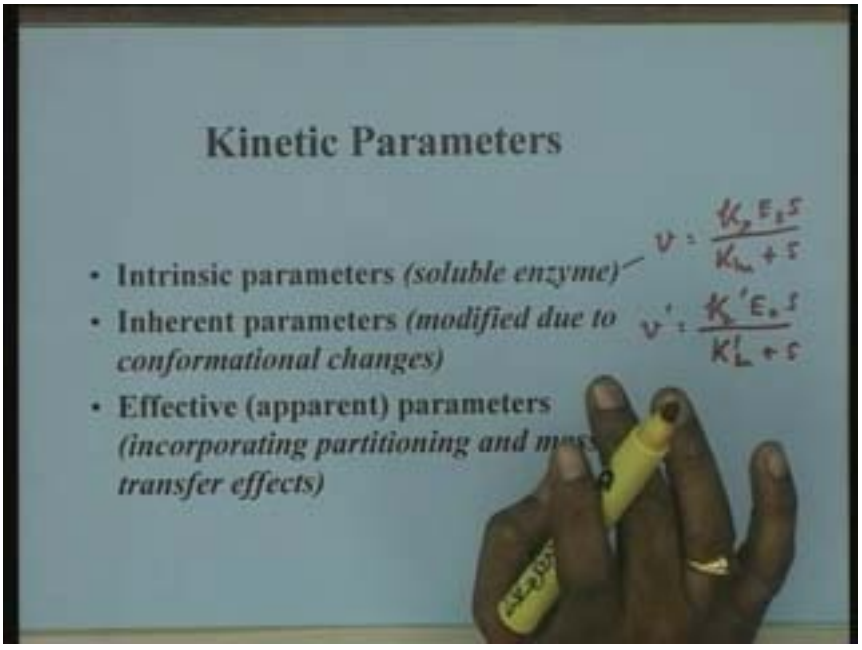

That means if the system has no partitioning behavior or no diffusional limitations; diffusion will be there because there is a phase difference. But if the diffusion is not the rate limiting step or rather it is quite fast compared to the reaction rate the inherent parameters will be useful. On the other hand in case the mass transfer effects are very severe they can be totally rate limiting steps. That means the whole rate of reaction is controlled by the rate of diffusion. Biochemical reaction is very fast and the rate of diffusion is slow. The rates may not be absolutely very far apart if the two are very comparable in that case we need to consider our apparent reaction velocity as equal to

$$v_{app} = k'_2 E_0 S / K^{app}_m + S$$

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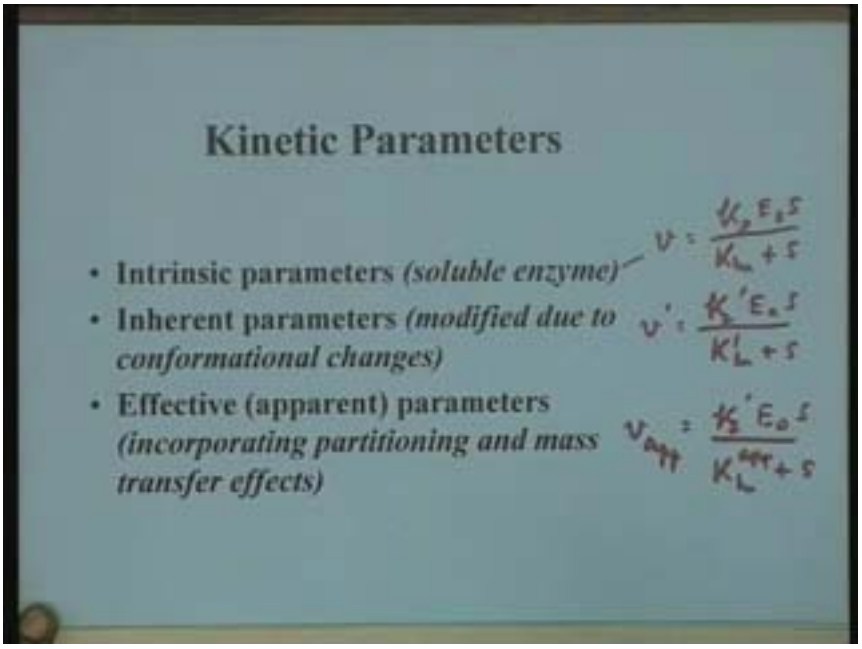

Here when we say that the apparent parameters are those which can be modified, which can be taken care by reactor operation. That means as I mentioned some time earlier that the thickness of the film can be controlled by either increased linear flow velocity in the case of packed bed reactor or by increasing the agitation speed in the case of CSTR. Similarly on the other hand the internal flow diffusion also can be regulated by controlling the particle size. That means the distance of transport of the substrate into the immobilized enzyme particle is minimized or reduced. Therefore the substrate will be available all across the particle and thus can be modified. Therefore they are called apparent parameters and in all possible analysis we must be interested in the apparent parameters, K^{app}_m. That will dictate if you want to consider diffusional limitations also as a part of our analysis.

The K^{app}_m has been a parameter which people have tried to define; quantitatively what are the parameters which can define the Km apparent and one of the most acceptable definitions of K^{app}_m can be

$$K^{app}_m = K'_m/PF$$

where P is the partitioning coefficient that is concentration of the substrate in solid phase divided by concentration of the substrate in the bulk phase that is the bulk liquid. On the other hand F is equal to tanh γl/ γl where γ is the ratio of the kinetic parameters to the diffusional parameters which is defined as

$$\gamma = \tfrac{1}{2}[k'_2 E_S/D.K'_m]^{1/2}$$

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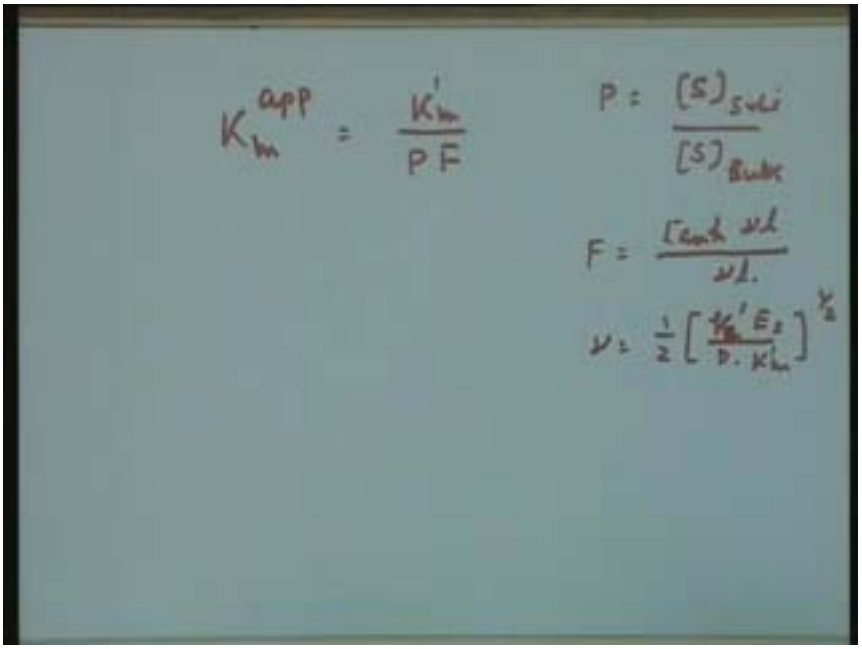

If you see here in this expression $k'_2 E_S$ instead of writing S_0 I am writing Es. That means enzyme concentration in the solid phase and not the E_0 because as a result of conformational changes the enzyme concentration interms of activity can get altered. D is the molecular diffusivity and k'_m is the Km value of the enzyme taking into account the conformational changes. The unique part is the parameter P is a fixed parameter and depends on the chemical nature of the substrate and the matrix. Once the method of immobilization is fixed, the immobilized preparation is fixed the value of P is fixed. It behaves almost like a constant parameter. Once the method of immobilization, the nature of the matrix and the substrate are defined the value of P will remain constant and the value of K^{app}_m is dictated by the parameter F and in the case of F what we call as a Thiele function, the form of F is very significant and this can be considered to have a very unique profile.

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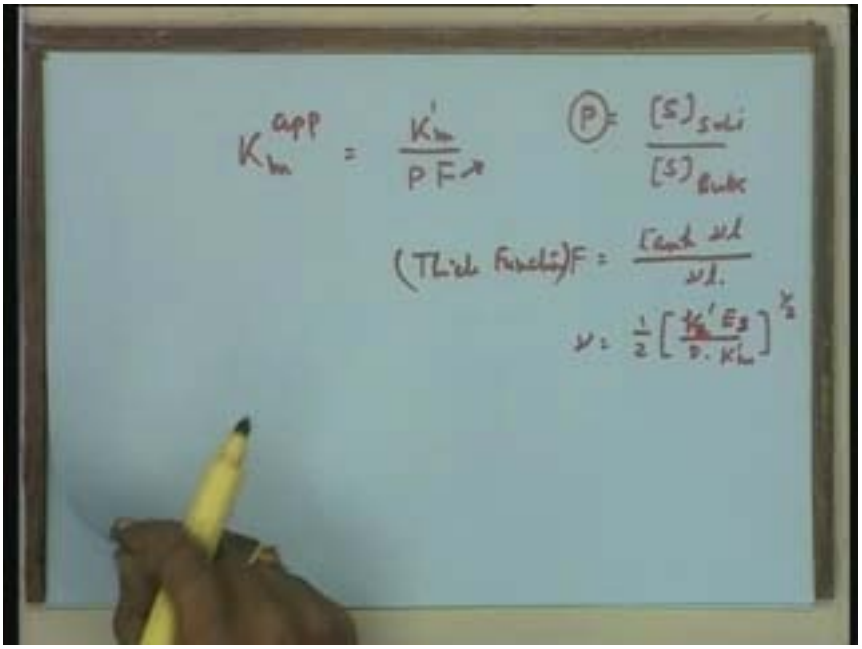

If you consider this function F that is tanh γl/ γ l, the characteristics of this function is that when γl is less than one that means in this region, the value of F is approximately equal to one. On the other hand when you go at γl value greater than two F is approximately 1/ γl.

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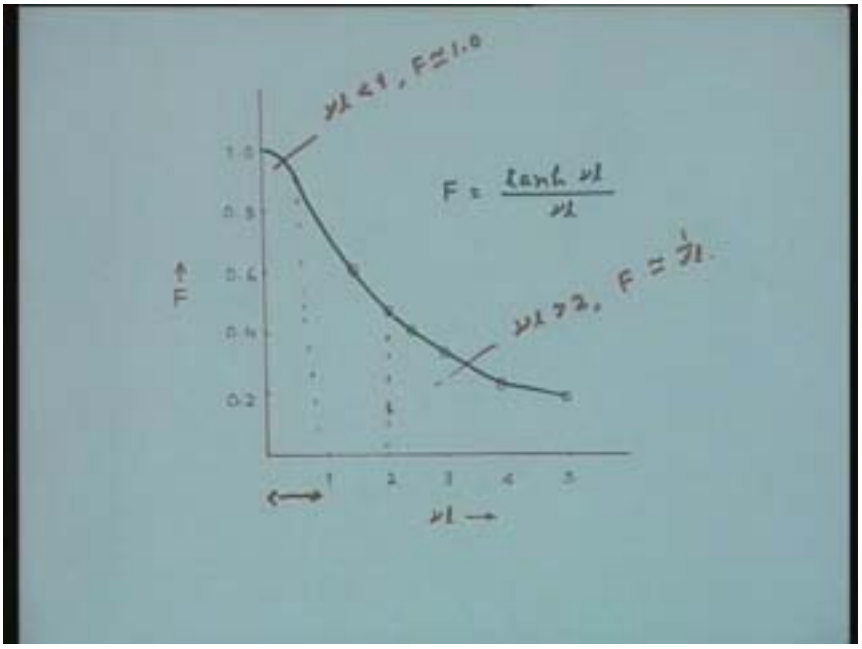

When you say F is equal to one, there is no effect on the kinetic parameter K_m or there is no diffusional limitation in other words. The K_m is not altered as a result of mass transfer, diffusion. F is equal to one and as we defined earlier K^{app}_m was equal to K'_m/PF and P we have considered as a constant parameter as far as the immobilized enzyme preparation is

concerned and the diffusion does not play a controlling role here if F is equal to one. On the other hand when the γl becomes higher than two the value of F is 1/γl or it implies that K^{app}_m will be equal to $K'_m.\gamma l/P$ and therefore the value of K^{app}_m gets altered by a factor of γl. "l" is the characteristic dimension of the immobilized particle. If it is a thin film it can be thickness of the film; if it's a spherical bead it can be diameter of the bead or any characteristics parameter l may be defined as. Yes only inherent kinetic constant and ….. (18:52). When we assume that γl is less than one F is approximately equal to one, no diffusion control.

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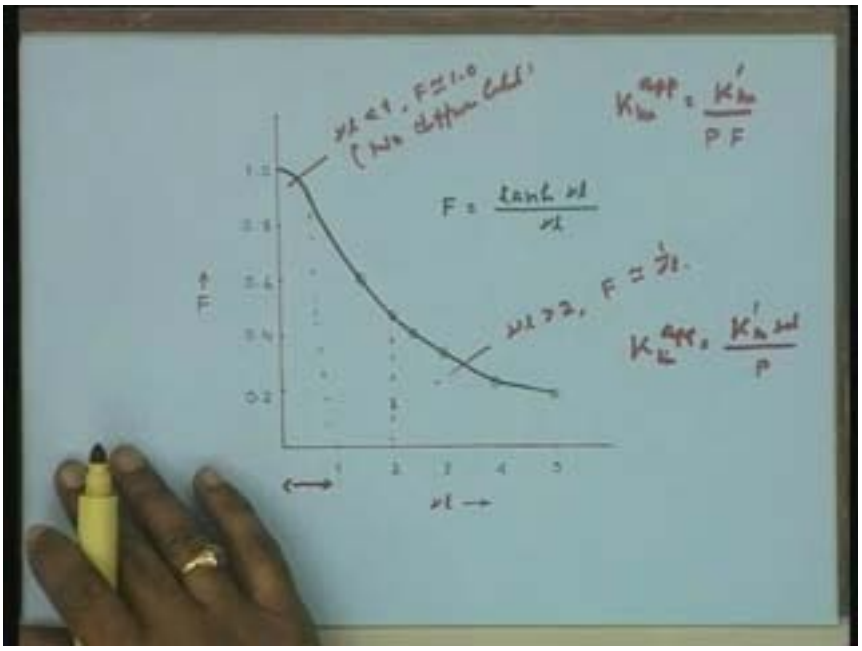

If you just consider the definition of γl

$$\gamma l = l/2[k'_2 E_S/D.K'_m]^{1/2}$$

This is γl. Under what conditions γl will be less than one or a smaller value? The very condition can be taken from these various parameters. When l is small that means it is a very thin film or very small diameter particles, the value of K^{app}_m will approach to that of K'_m and there will be no diffusion control and under ideal condition l is equal to zero for soluble enzyme. So under that condition there will be no diffusion control. Second is D is large. If the rate of diffusion or molecular diffusivity is very high also γl will be small and therefore also there will be no diffusion control. Third is E_s is small which means low enzyme loading in the matrix. If immobilized enzyme preparation has a small loading the diffusional resistances will not be very controlling. The fourth thing is that the ratio of K'_2/K'_m which you will recall from the basic definition of this parameter which is specificity constant or a pseudo first order rate constant for the enzyme catalyzed reaction K'_2/K'_m is small which refers to slow catalysis. That means catalyst is very slow; it's turn

over number is very low. k'_2 is the turn over number and if the catalyst is slow then also the diffusional limitations will be negligible.

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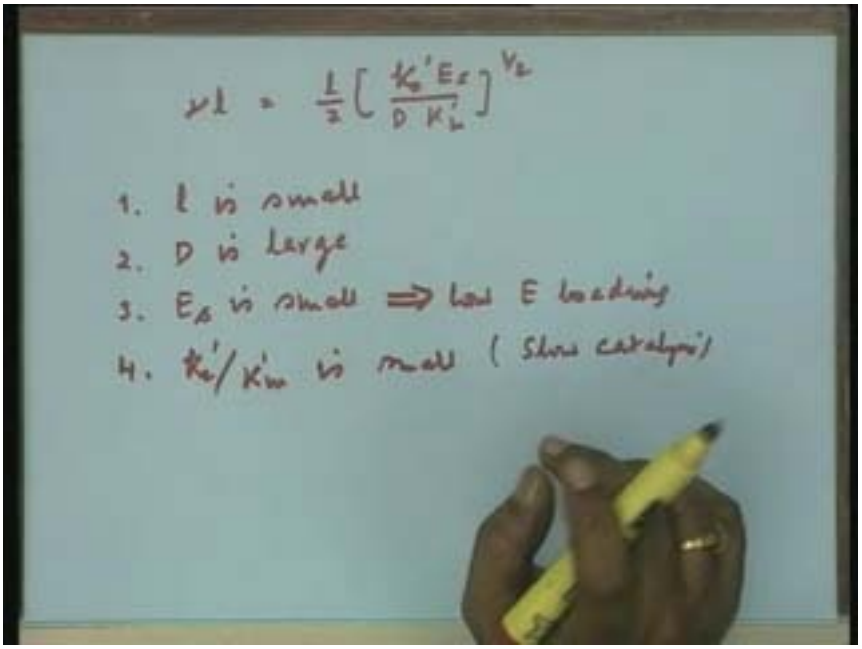

Considering these parameters one can design the immobilization enzyme preparation also in such a way that diffusion limitations are not there. It is always desirable but that is one of the reasons why we call this kinetic parameters apparent parameters. That means they are neither intrinsic nor inherent but they are just apparent and they can be modified if you design your system in a way that you can have a very low enzyme loading to the extent that the overall rate of the enzyme catalyzed reaction is smaller than the rate of diffusion. The diffusional limitations are wiped out straight away or if you take a smaller particle you can get rid of diffusion.

We are considering this parameter the γl. If the γl is less than one or smaller value then the magnitude of F is approximately one; no diffusional limitations. γl will be small when the E_S will be small and E_S is the enzyme concentration in the matrix in the solid phase, which is the enzyme loading. Physical interpretation of γl is the ratio between the biochemical parameters and the diffusional parameters. In the numerator you have k_2 and E_S. Both are related to biochemical reaction. On the denominator you have D. k_2/K_m is a combined parameter for the biocatalysis but D is a diffusional parameter so it is physically some parameter which defines the ratio between the two and by adjusting either numerator or increasing or decreasing the numerator or the denominator one can regulate the controlling parameter. If you look at higher value of γl, when γl is greater than two F is equal to 1/ γl by basic definitions and therefore F is usually less than 1. It is inverse of γl and therefore F is less than one which means the reaction is under diffusional control regime.

When γl magnitude is greater than two you can safely take that the reaction is diffusional limited and K^{app}_m equal to

$$K^{app}_m = K'_m \cdot \gamma l/P$$

This means K^{app}_m increases by a magnitude of γl. What are the experimental or analytical ways by which we can experimentally determine the rate limiting step; whether diffusion is rate limiting or not. Various approaches can be adopted. Consider zero order regime. That means at high substrate concentration when you are talking of γl less than one that means a thin flies or smaller particles reaction rate of the immobilized enzyme will be proportional to enzyme loading. If you make different preparation with different enzyme loadings the reaction rate will be proportional to the enzyme loading. That means as you increase the enzyme loading the reaction rate will also increase. But at low substrate concentration, for γl less than one and F is equal to one the reaction velocity of immobilized enzyme will become equal to

$$v_{IME} = k_{,2}\, P.F.S.E_S/K'_m$$

That means in the first order regime if you substitute in this parameter $K^{app}_m = K'_m/PF$ you get such an expression where F is equal to zero at γl less than one and v mobilized will be proportional to E_S.S. Because this is constant, this is constant. P we are considering as a constant. F is one. So the v immobilized becomes proportional to $S.E_S$, if you consider the low substrate concentration and at a small thickness of particles and γl is less than one.

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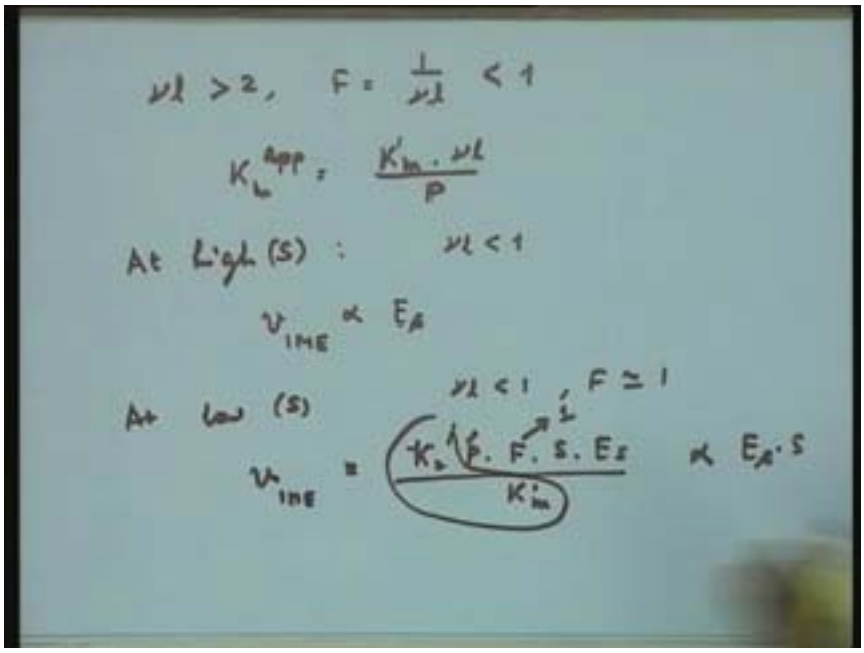

In the same case if you take γl greater than two K^{app}_m is

$$K^{app}_m = K'_m . \gamma l/P$$

If you substitute for v immobilized enzyme you get

$$v_{IME} = k'_2/K^{app}_m .S.E_S$$

That means a first order kinetics. We are talking of low substrate concentration. γl is greater than two that means the value of F is 1/ γl. Under that condition if you substitute the various terms v immobilized enzyme preparation will be proportional to $E_S^{1/2}$.

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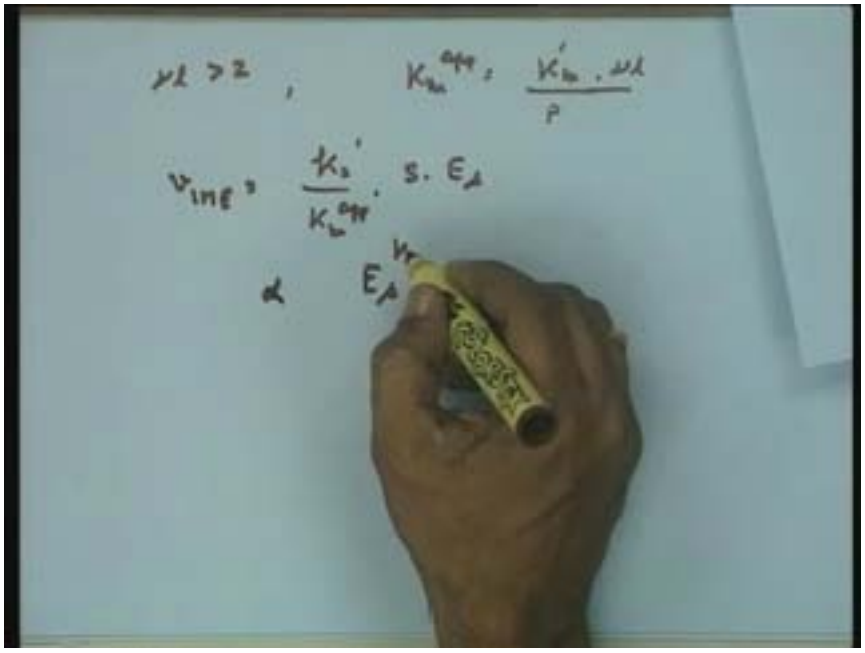

If you substitute here the value of gamma here you will get $E^{1/2}$. That means the enzyme reaction rate will not be proportional to the enzyme concentration in the particle but will be proportional to the square root of the enzyme concentration in the particle. In the case of immobilized enzyme systems which have no diffusional limitations the rate of reaction is proportional to enzyme loading and which is the usual understanding even for soluble enzyme. Up to a concentration range the rate of reaction is proportional to the enzyme concentration. But on the other hand when the immobilized enzyme preparation is controlled by diffusion, that relationship does not hold good. That means if you make a plot between ln v = 1/2ln E_S you will get a linear fit and that can be checked experimentally by making preparations of different enzyme loadings and merge in them enzyme reaction ….. . (29:27). That is one approach by which one can monitor or determine the concentration of the controlling parameter in the case of immobilized enzyme reaction. Or in other words as γl will increase from a small to large value either in thickness or in enzyme loading, behavior will change. Such an experiment can be even monitored. Either you increase the thickness of the particle or increase the enzyme loading. You can see the change in the behavior on the relationship between the enzyme

loading and the enzyme reaction rate. That can give you an indication of the controlling parameter.

Another way is to look at the Lineweaver-Burk plot. In the case of a classical enzyme catalyzed system we have 1/v Vs 1/S. When we are talking of S, we are talking of S_{bulk} because we are not monitoring the concentration of the substrate inside the reactor but we are concentrating on $1/S_b$. Normally we will get such a behavior where slope will be K'_m/v'_m or K'_2E_0. But in the case of immobilized enzyme preparation which are regulated by diffusional limitations the curve usually convex to the 1/S axis. That means it will be something like this, where the slope is K^{app}_m /v'_m.

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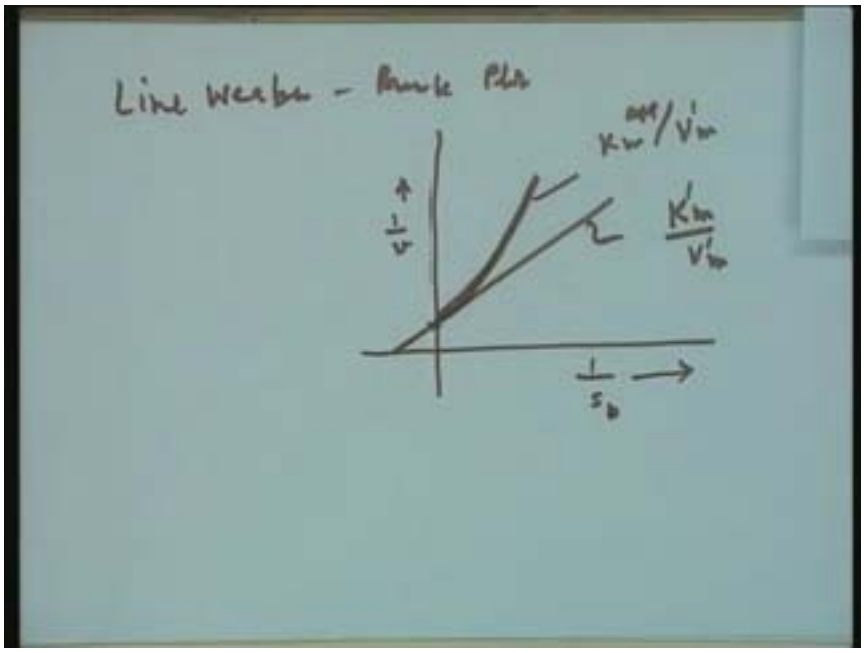

By plotting the Lineweaver Burk plot for immobilized enzyme preparation one can also identify the extent of diffusional limitations because then the K^{app}_m drastically changes. When you do the K^{app}_m, partitioning is fixed, the value of F can be noticed and F can give you a value of γl. So in that way also one can determine. One of the reference which has analyzed the diffusional limitations is given by Reagon and associates in Biotech Bioengineering, 16. 1081, 1974. In fact one of the earlier references in mass transfer analysis of an immobilized glucose oxidase particles was made and is given in this.

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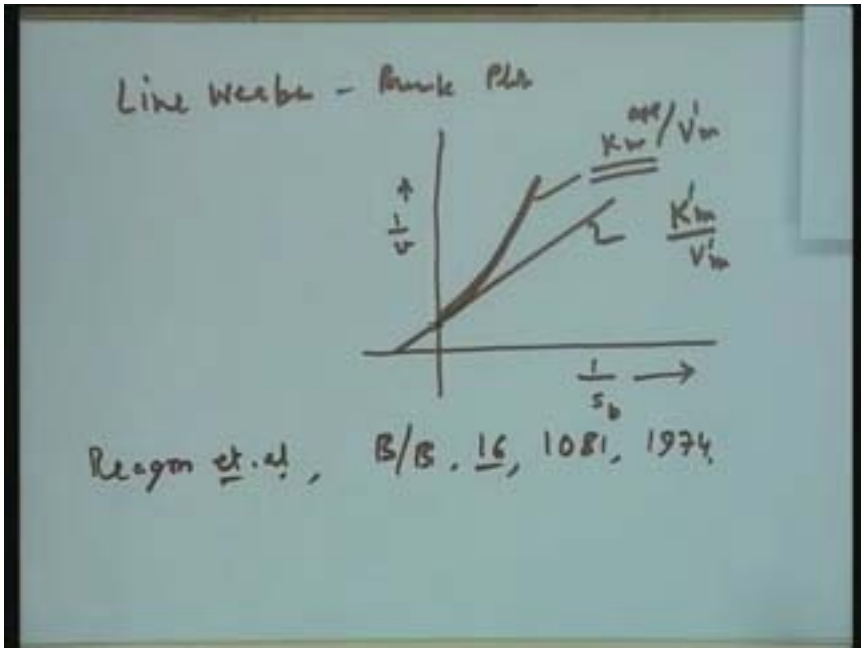

The third approach of analyzing the diffusional limitation is on the basis of activation energy. To illustrate my point I think I like to make you familiar with one of the experimental data which was reported. This is ln v verses 1/T for acetyl choline esterase immobilized in polyacrlyamide gel particles. When the estimation of activation energy was made under different substrate concentration regimes and with immobilized particles, one noticed a profile something like a biphasic profile. That means the activation energy got changed. Consider another situation in which at the low substrate concentration when γl is greater than two when diffusional control was established, the energy of activation noted was ΔE^* which was equal to 5.7kcal/mole. On the other hand at high substrate concentration, when γl was high one gets the same kind of energy of activation at high temperatures.

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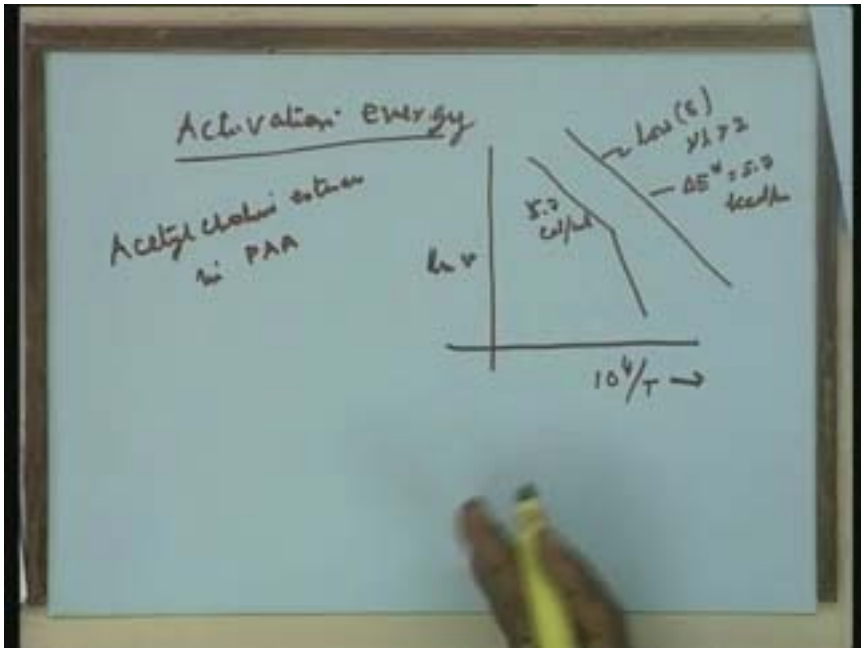

At high temperatures these similar activation energy was obtained even at high substrate concentration as in the case of low substrate concentration because at high temperatures the reaction rate was high. The reaction rate being high, the diffusion became limiting. The activation energy behaviour was almost similar as in the case of low substrate and at high γl which was established parameters for diffusional limitations.

The right hand side profile, a straight line profile refers to a thick particle where the thickness is high; γl is greater than two and at low substrate concentration. We know under these conditions the system will be diffusional limited and the activation energy computed in this case was 5.7 kcal/mole. When you go over to high substrate concentration over the range of substrate, over the range of temperature initially at high temperature range almost a similar activation energy was obtained 5.7. That means the system behaves almost like diffusional limited even at high substrate concentration which it should not. At high substrate concentration the system should not behave as a diffusion limited system because at higher temperatures the reaction rate is higher and the diffusional limitation was established. On the other hand when the temperature was reduced the system became diffusional unlimited and the activation energy was much higher, which was 9.4 kcal/mole.

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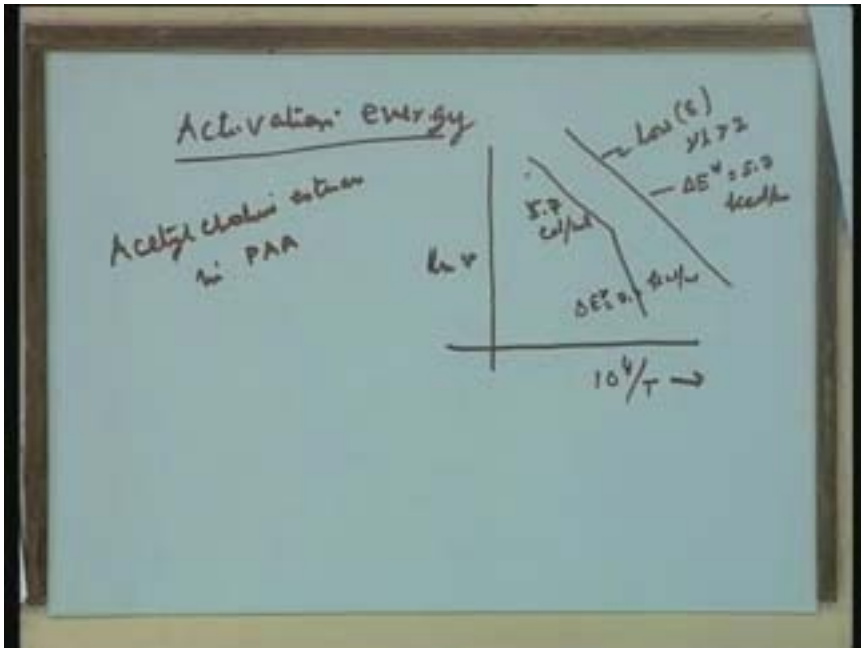

So a change in the activation energy over a range of temperature also indicates the diffusional limitation behavior. Therefore we have three distinct approaches to monitor by experimental methods the effect of diffusional limitation in the case of immobilized enzyme particles. One is by seeing the effect of enzyme loading on the reaction rate that means it's proportionality to the enzyme loading changes from linear to proportional to the power half. The second is the nature of the Lineweaver Burk plot which becomes convex to the 1/S in X-axis which means that the enzyme is more sensitive to diffusional limitations at lower substrate concentration. At higher substrate concentration the profile is more or less linear. But as it increases to higher substrate concentration the slope tends to increase and the K^{app}_m value increases. The third is the estimation of activation energy over a range of temperature.

By the experimental reactor operations in the laboratory many people have noticed that diffusional limitations play a key role either by varying the agitation speed in the case of a CSTR or by the varying the linear flow velocity in the case of PFR, the rate of reaction can be improved and so diffusional limitations can be overcome if any. The question is how to analyze a practical situation. That means if suppose you want to know how we can determine the actual value of the mass transfer coefficient. In that case before we go to a total mass transfer a closed box picture of diffusion we can break it into the external film diffusion and internal pore diffusion and look them separately and check if they can be analyzed along with the chemical reaction under steady state behavior because even the enzyme reactor that we have looked at are to be analyzed under steady state situation. If you look at immobilized enzyme reactor performance with external film diffusion that means the substrate may be very thick. There is a thick film of substrate around the immobilized enzyme particle which will cause a substrate gradient across the film and therefore the concentration of the substrate at the surface of the immobilized enzyme particle is not same as in the bulk. Ideal situation could be to consider a non-porous particle, where all the enzyme will be on the surface and the sole diffusional limitation is

as a result of transport through the thin film. One of the empirical methods to determine the K^{app}_m apparent was given as

$$K^{app}_m = K'_m + k'_2 E_0 \delta / D$$

Purely an empirical solution to describe the problem. That means the K^{app}_m will be dependent on the thickness of the film. Logical because of the thickness the mass transfer tends to be more. Then D is inversely proportional to the rate of the diffusion; that is also quite obvious. Now the problem is that experimental measurement of the film thickness is not an easy task. One cannot really, for any immobilized enzyme reactor, measure the film thickness and then make a design computation. So therefore an alternative analytical method has to be chosen and if you look at analytical methods the best way is to look under steady state reactor performance. Let us say you have an immobilized enzyme particle which is non-porous in nature. All the enzyme is immobilized on the surface, a typical particle on which enzyme is only on the surface. There is a layer of film through which the substrate has to diffuse to reach to the enzyme active site and as a result of this there is a concentration gradient. For simplicity one can assume a linear gradient and so therefore rate of mass transfer here will be equal to

$$r_m = k_l a_m (S_b - S_s)$$

This is S_b; this is S_s. That is the concentration gradient.

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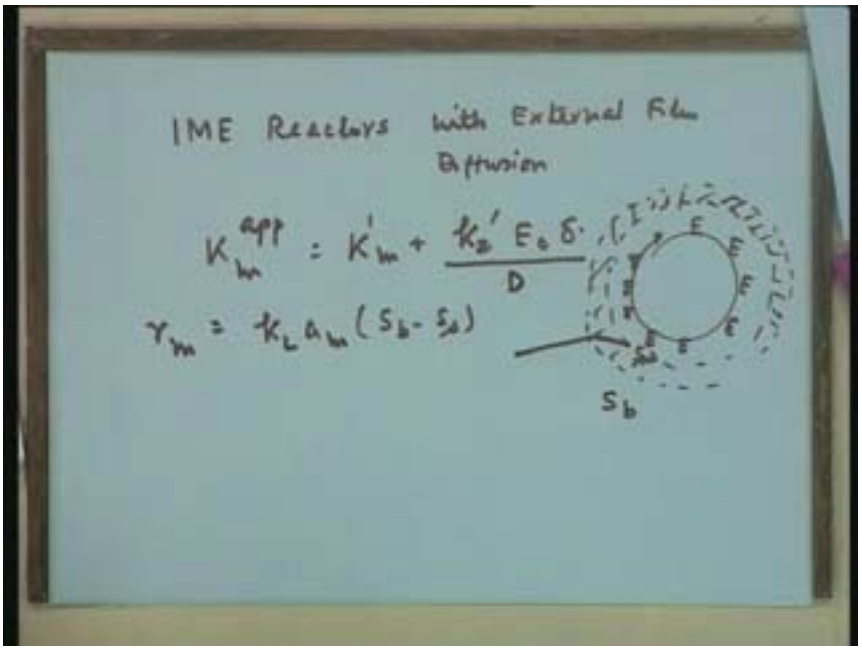

The rate of diffusion in such a situation let us assume the situation that this non-porous particle is packed in packed bed reactor and we operate it till a steady state is reached; steady state reached means the concentration of the substrate at the exit end becomes

constant with time. The fractional conversion becomes constant with time. The reactor performance is under steady state which would mean that the rate of reaction and rate of diffusion are same. Rate of mass transfer, rate of reaction are identical. So rate of mass transfer is $k_l a_m$. That's the mass transfer coefficient. a_m is the area of the mass transfer which is described as area cm^2/cm^3; that means the surface area per unit volume of the particle. k_l is the mass transfer coefficient cm/sec. So overall $k_l a_m$ will have unit of per second. A typical first order rate constant. S_b-S_s is the concentration gradient across the thin film. This under steady state conditions must be same as the rate of a reaction and that is

$$v = k'_2 E_0 S_S / K'_m + S_S$$

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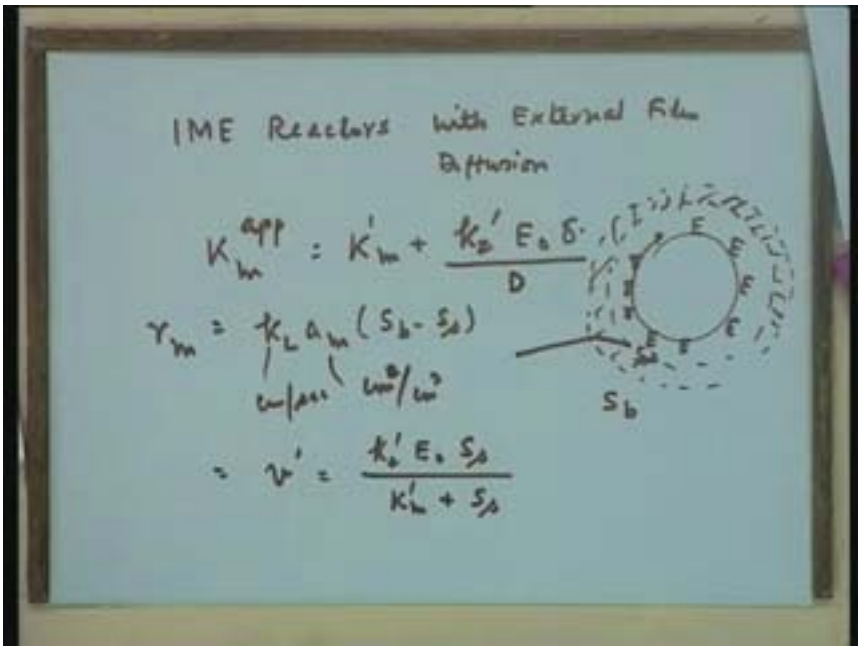

If you substitute the magnitude of the S_s from this expression and simplify you get

$$v' = \frac{k'_2 E_0 [S_b - v'/k_l a_m]}{K'_m + [S_b - v'/k_l a_m]}$$

The steady state rate of reaction will be given by this expression and the S_s will be equal to S_b- Rn or $v'/k_l a_m$ and therefore and $1/v'$ will be equal to

$$1/v' = K'_m / k'_2 E_0 \, (1/ S_b - v'/k_l a_m) + 1/k'_2 E_0$$

That gives you the 1/v'

If you consider the extreme conditions, that is when S_b is very high or we are considering zero order regime. From this expression you can get

$$1/v' = K'_m/k'_2E_0 \,.1/S_b + 1/k'_2E_0$$

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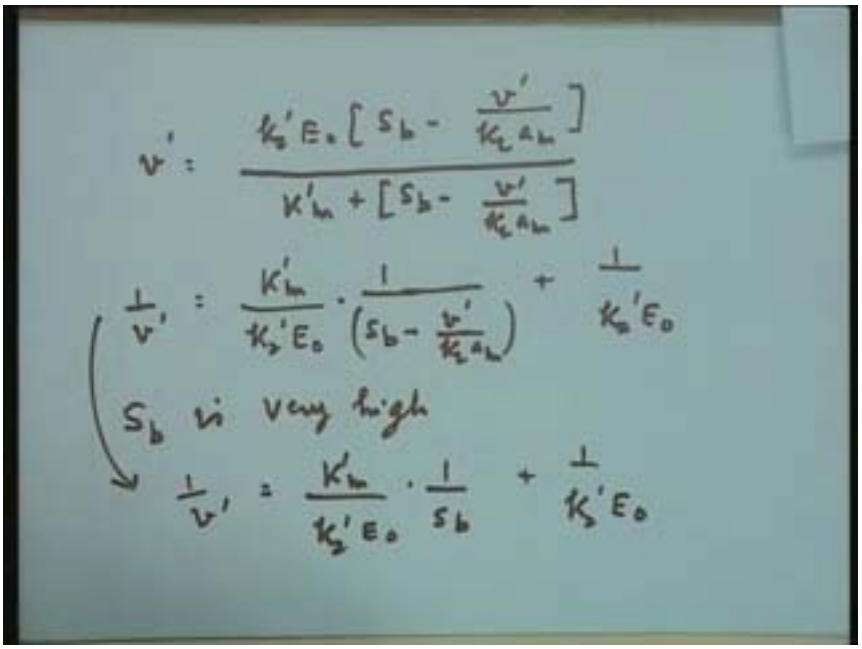

In the case of very high substrate concentration in a zero order regime. On the other hand when S_b is very small or a first order regime, if you look at the this equation from here you get

$$v' = k'_2E_0/K'_m\,(S_b - v'/k_la_m)$$

or if you simplify and take all the v' term onto the left hand side you will get

$$v' = \frac{k'_2E_0\, k_la_m.S_b}{K'_m\, k_la_m + k'_2E_0}$$

You can simplify or $1/v'$ for small Sb will be equal to

$$v' = \left(\frac{1}{k_la_m} + \frac{K'_m}{k'_2E_0}\right).\frac{1}{S_b}$$

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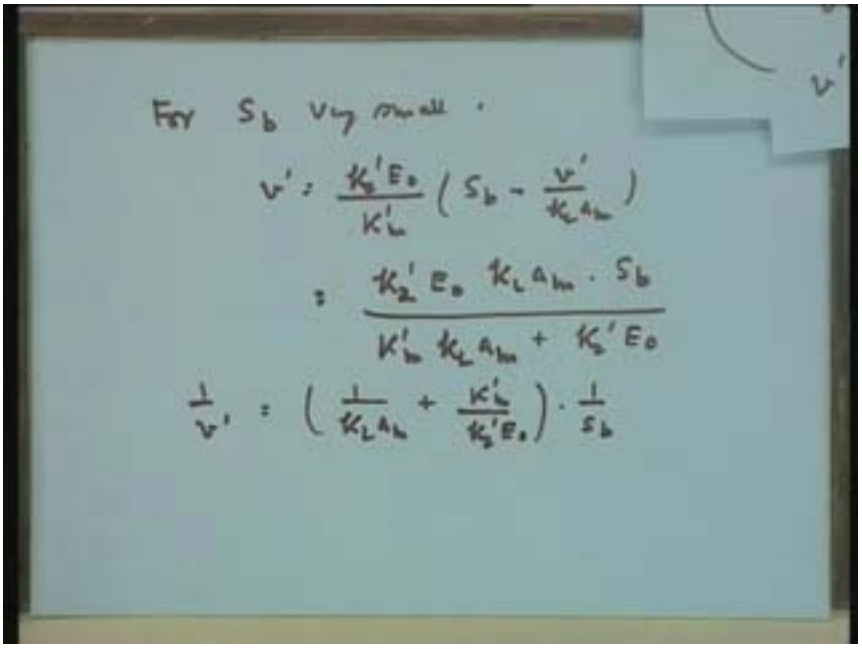

We have two different conditions of the Lineweaver Burk plot; one in the case where S_b is very high and the Lineweaver Burk plot will have the slope as K'_m/V'_m

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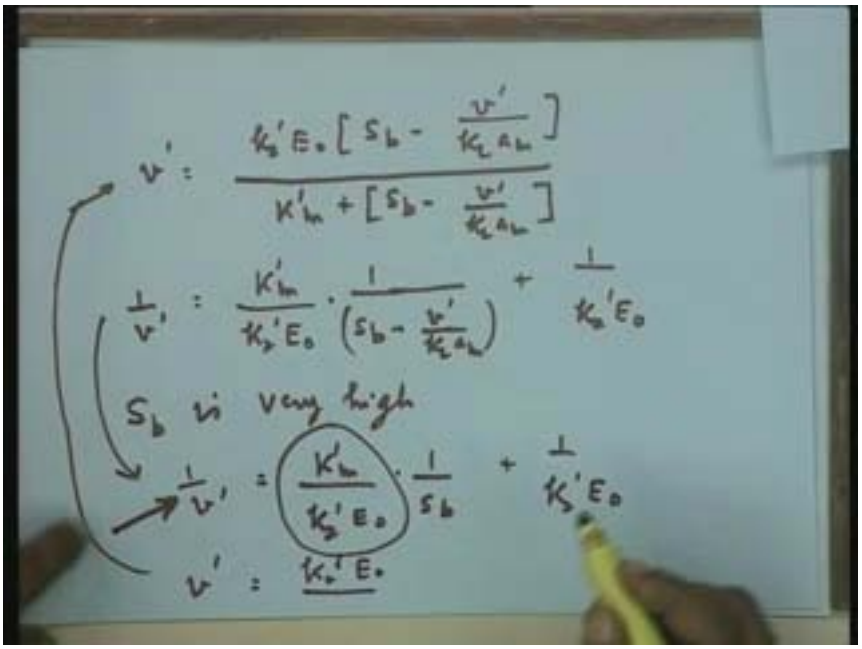

On the other hand when the substrate concentration is very low the slope will be $1/k_l a_m + K'_m/V'_m$.

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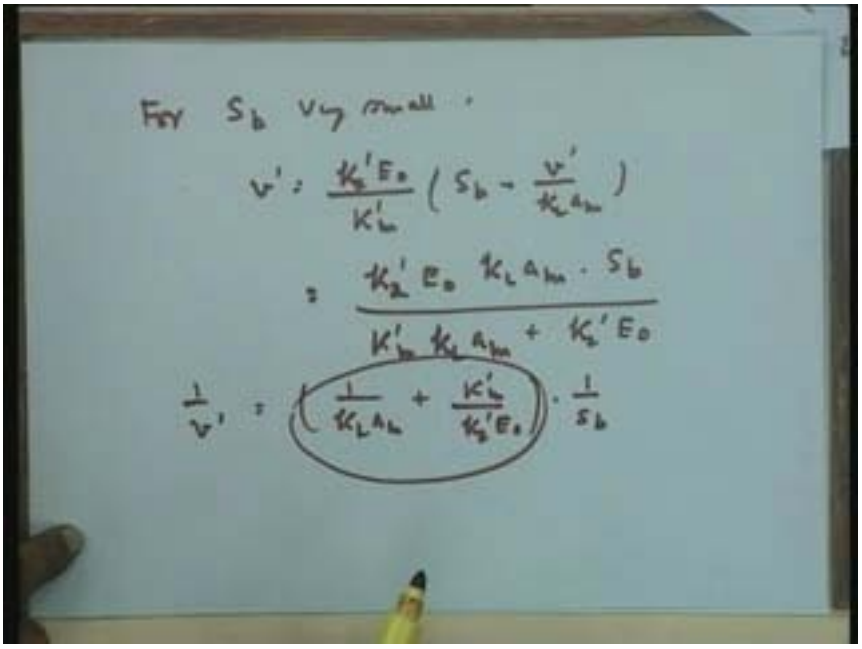

Or in other words you consider two in graphical form. You will get something like this; almost a typical profile as we discussed earlier that it becomes convex to $1/S_b$; $1/v'$. The slope of this straight line will be, at low substrate concentration, $1/k_l a_m + K'_m/k'_2 E_0$. On the other hand the slope here will be $K'_m/k'_2 E_0$.

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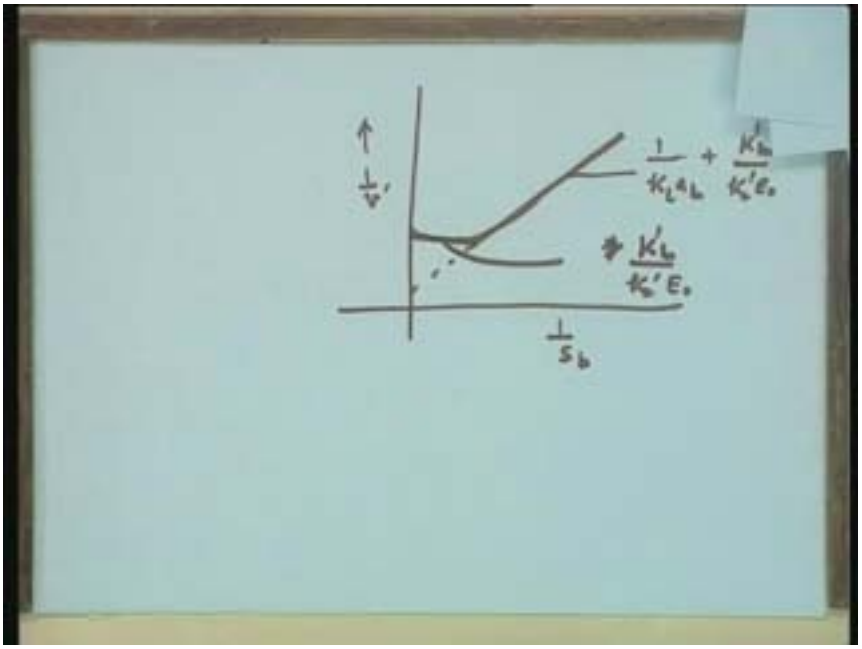

By knowing the two slopes one can calculate $k_l a_m$. The difference between the two slopes is the $1/k_l a_m$. So one can experimentally determine the magnitude of $k_l a_m$ by carrying out the immobilized enzyme reaction in a continuous reactor and getting the steady state performance of the $k_l a_m$ and from that K'_m and $k_2 E_0$ and once you know $k_l a_m$ you also know the substrate concentration gradient $S_b - S_s$ by the first equation where you have related the rate of mass transfer because that is $k_l a_m.(S_b - S_s)$. Rate of reaction and the mass transfer rate are identical under steady state situation and therefore they can be experimentally determined and one can then take the parameter which takes into account the immobilized enzyme reactor performance incorporating the external film diffusion. In this case we have only considered the external film diffusion; on the other hand actual reactor may also involve, if it is porous particle general pore diffusion also. Such an analysis is applicable only for non-porous particles or in case particle size is very small where the pore diffusional limitations are to be taken as negligible and we will continue with that in subsequent classes.