# ENZYME SCIENCE AND ENGINEERING

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LECTURE–21
**IDEALIZED ENZYME REACTOR PERFORMANCE (CONTD…)**

So we had earlier discussed the reactor performance equations for three different types of reactors that I used for carrying out the enzyme catalyzed reactions; that is batch, plug flow reactor and continuous stirred tank reactor.

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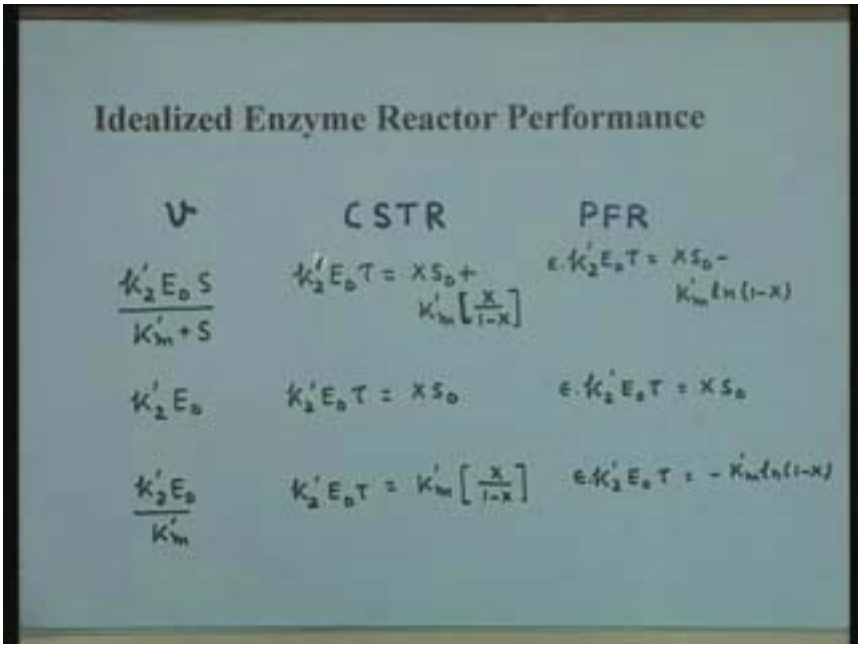


While the choice of the batch reactor is primarily based on many factors other than the reactor performance mainly on the basis of the additional requirements of the reaction. For example if you require a very stringent pH control or a temperature control or addition of a secondary substrate at a different period other than the beginning of the reaction then batch reactor provides you operational convenience; a better term would be the use of batch reactor is based on certain operational conveniences. But based on the reactor performance the choice remains between use of a CSTR and a PFR and here I have given a summary of the reactor performance equations for CSTR and PFR and three different kinetic designs.

The first line indicates the reactor performance with respect to Michaelis Menten kinetics and the second line refers to when the reactor performance is in the zero order of the design that means when substrate concentration is much higher than the $K_m$ value and the third line is in the first order design when the substrate concentration is less than the $K_m$ value. When we say higher or less than the $K_m$ value, when we compare substrate concentration with the Km value, what we are looking at in the case of zero order regime, we often look at the $K_m$ value; $K'_m$ is usually less than 0.01 $S_0$. That means substrate concentration is almost hundred fold or more than the $K_m$ value. Similarly in the case of first order regime it is again the same thing that the $K'_m$ is usually greater than $10S_0$ ten fold increase usually gives you a reasonable performance towards a first order regime

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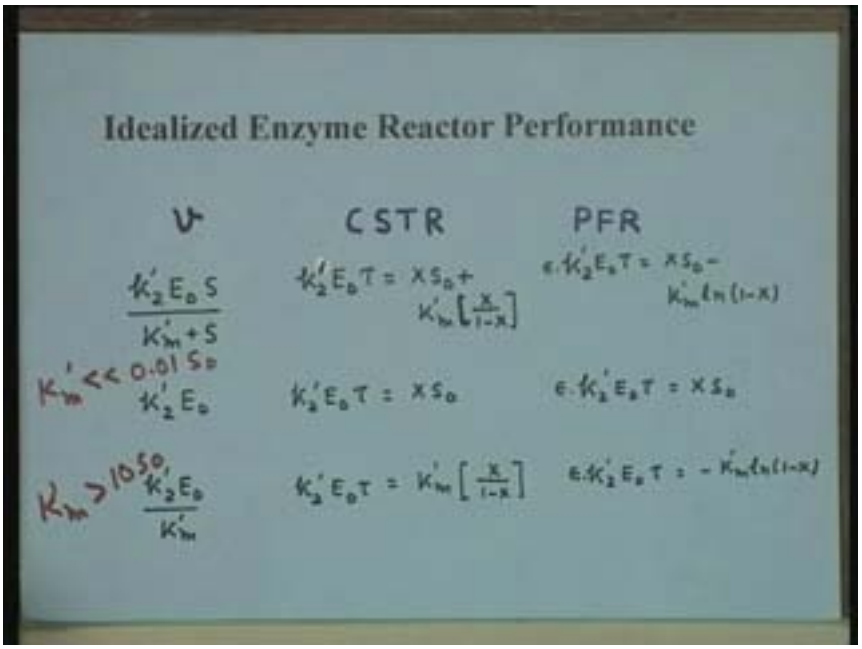


As you have noticed that in the case of zero order regime the reactor performance is identical in both the cases in CSTR and plug flow reactor excepting that the magnitude of the tau is altered by epsilon that is voidage. Otherwise the reactor performance in the zero order regime is identical. On the other hand in the case of a first order regime the performance of PFR, if you look from the point of view of fractional conversion the fact of fractional conversion on the quantity of enzyme required or the value of the space time, to be able to compare the reactor performance, what we need is to look at two different things: the fraction of conversion and the quantity of enzyme required.

These two parameters can be looked at from these expressions. We also looked at the relative quantity of enzyme required in CSTR to PFR which is given by this ratio and here you will notice that as we increase the fractional conversion the relative quantity of enzyme required for a first order regime significantly increases in the case of CSTR. In the zero order regime there is no difference. So therefore when we shift from zero order to first order regime the efficiency of plug flow reactor improves interms of its enzyme

requirement or even in terms of space time. For example there are two parameters: either you can keep the space time constant and increase the enzyme loading; or keep the enzyme loading constant and the space time can be varied. In the case of PFR the space time required will be much less compared to CSTR in the first order regime. In the zero order regime the space time will be identical in the two cases.

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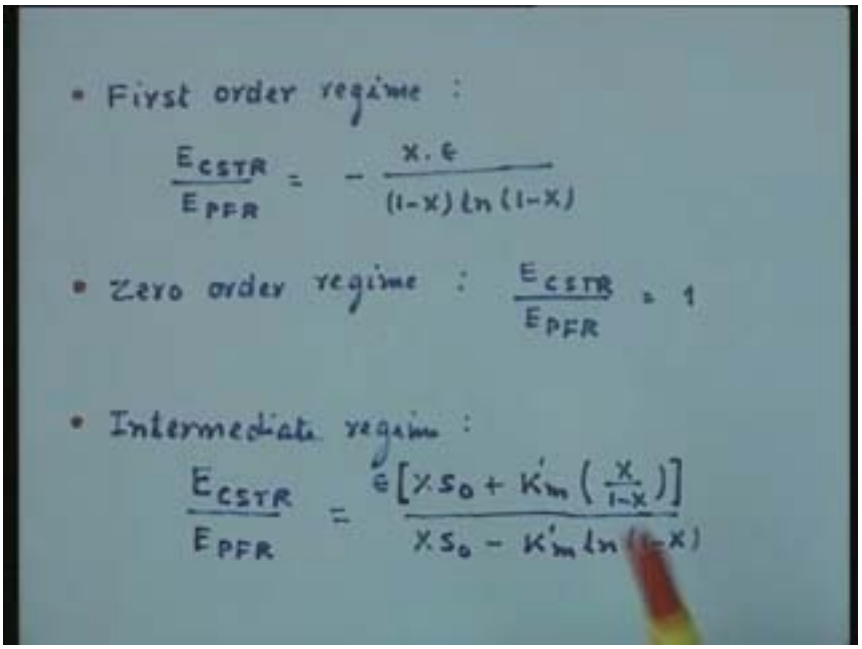


The intermediate regime is that where $K_m$ and $S_0$ are of comparable value. That means let us say between one and ten. That means $S_0$ is something like about five $K_m$ values. In that case the performance will be dependent not only on the magnitude of the fractional conversion but also on the magnitude of $K_m$ value and the relation between $S_0$ and $K_m$. the performance will be dictated.

To give you a quantitative picture under three distinct conditions here I have taken three different fractional conversions 0.8, 0.9 and 0.99. I must also state here that ideally for most of the enzyme catalyzed reactions we will try to aim at the fractional conversion as high as possible. Hundred percent is perfect but very often you may not be able to test that and one will like to have fractional conversion anywhere between 0.9 and 0.99. That is usually the pattern if the reaction is irreversible practically irreversible. So under those conditions and assuming $S_0$ equal to1M and epsilon of 0.5, the $E_{rel}$ in the case of first order regime at 0.8 is 1.24. They are any units; don't bother about units. But these are relative ratio; so the 1.24. As soon as you go to 0.9 conversion, the relative quantity of enzyme required is 1.95; almost about one and half times. But when you go from 0.9 to 0.99, the relative quantity of enzyme becomes more than five times and it very significantly increases the relative quantity in the first order regime. If you consider from 0.8 to 0.99, the ratio is still higher and the same relationship will also hold good for space time.

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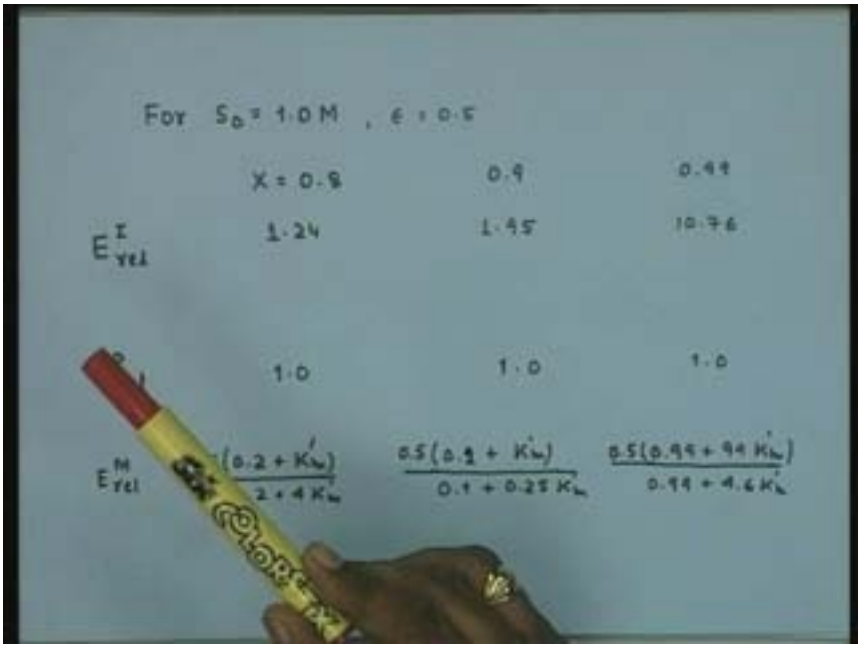


If the enzyme loading is constant, then the tau will also have the similar ratio because in our reactor performance equation the left hand side is $K_2E_0\tau$ equal to so and so. Therefore both have the same relationship as far as the fractional conversion is concerned. In the case of zero order regime there is no complication whatever is the fractional conversion the relative quantity required are identical; the same space time. On the other hand in the case of Michaelis Menten kinetics when the $E^m_{rel}$ and $S_0$ are comparable, it will vary and it will be a function of $K_m$; what the magnitude of $K_m$ is in comparison to substrate concentration. We have chosen one molar here. There is another point besides the fractional conversion; the enzyme quantity required and the space time required.

Another important parameter of the reactor design when we defined the parameters is reactor productivity that is Pr and is defined as $XS_0/\tau$. You will notice that the reactor productivity also is linked with the fractional conversion. In the case of the two types of reactor, the reactor productivity is directly linked with the fractional conversion and as it is also linked with the tau, inversely propositional to tau if the tau increases the productivity goes down. Therefore the fractional conversion, whatever we have discussed about relative quantity of enzyme or the space time as a function of fractional conversion a reverse phenomena or the reverse behavior is true for productivity. When you increase fractional conversion the productivity will go down and for the first order regime the productivity of PFR will be much, much higher than the CSTR.

I was trying to relate productivity and the quantity of enzyme or the space time. The quantity of enzyme required and the space time are almost analogous parameters. Their behavior on the basis of the fractional conversion is identical. But the effect of the fractional conversion on reactor productivity is just reverse because tau appears on the denominator, $XS_0/\tau$. So if the tau decreases more significantly with fractional conversion

the productivity will increase more significantly and therefore in the case of first order regime, the productivity as a function of fractional conversion increases much faster in the case of PFR than CSTR.

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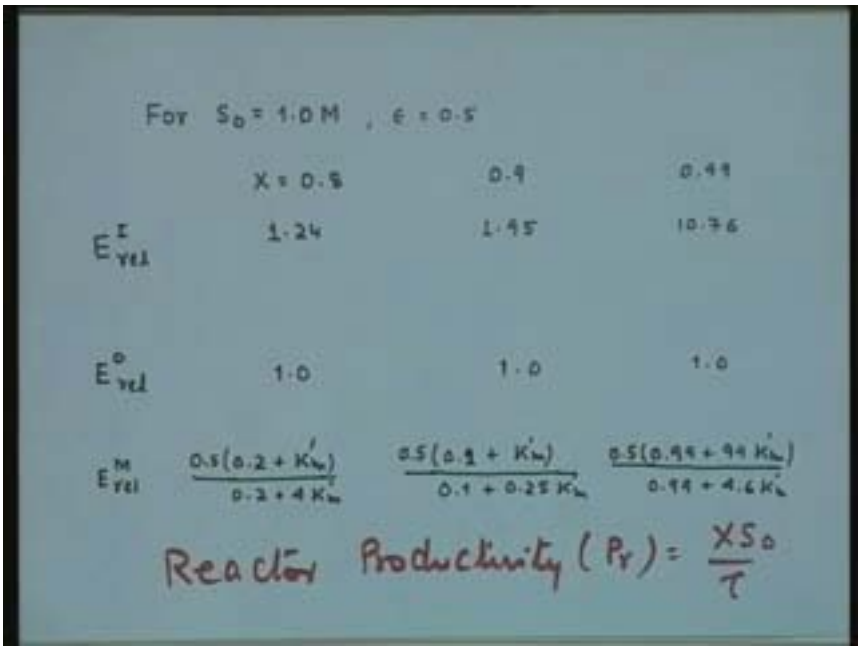


The quantity of enzyme required or the value of tau increases more in the case of CSTR with increasing fractional conversion but the productivity increases more drastically in the case of PFR when fractional conversion is increased. But in general with increase in fractional conversion the productivity will go down in both the cases because in the reactor performance equation the decrease in tau will be much faster compared to increase in fractional conversion.

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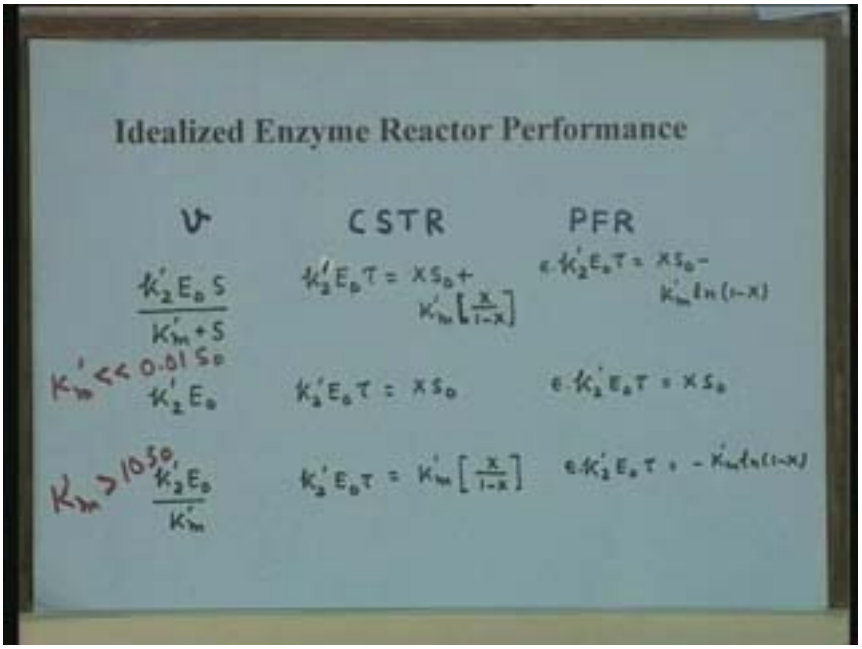


So in general there will be a decline in productivity as fractional conversion increases but the relative decrease will be more pronounced in the case of CSTR than in PFR.

No tau. Relative decrease of productivity; the tau and productivity are related inversely. Therefore the tau increases with increase in fractional conversion more pronouncedly in the case of CSTR, the reverse may happen in the case of productivity. Therefore I think one should very clearly understand the relationship between fractional conversion, enzyme quantity required or the space time and productivity.

Very often the choice of the right type of reactor whether to use a CSTR or a PFR is dependent upon the type of the reaction system we are looking at. For example in case we are talking of a very high value product say for example …..(12:52) transformations, a pharmaceutical product where the cost of product or the cost of substrate is very high. You don't want to waste the substrate. Then in such case we would like very high fractional conversion. We will not like any substrate to remain unconverted because the cost of substrate is very high and even at the cost of productivity you go for higher fractional conversion. The productivity will decrease; but even that is desirable to have a higher fractional conversion and a lower productivity. On the other hand if you are talking of a lower or reasonable value product or in those cases where even the unconverted substrate can be used along with the product you don't have to separate the product for application as in the case of glucose fructose syrup when you isomerise glucose to fructose the final product is a mixture of glucose and fructose. We never attempt to get the reaction converted to completion because then the residence time, space time required in the reactor will be very, very high because it's a reversible reaction. Therefore the final reaction product usually is an equilibrium mixture of glucose and fructose where the fractional conversion is only 0.4 or 0.45 not even 0.8. But the advantage is that the ultimate mixture of glucose and fructose is used as the product. No separation is attempted. So in those cases lower fractional conversion but we keep very

high stake on productivity; productivity is tried to be maximized. The cost of the product is not very high so productivity becomes very important parameter. But if the cost of the product is very high which also requires to be separated from unconverted substrate then it will be desirable to have a high fractional conversion even at the cost of productivity. So we must clearly understand the inter relationship between the kinetic order regimes, the fractional conversion and the productivity and the parameter which is very important in the case of reactor performance is the effect of substrate and product inhibition.

Most of the enzyme catalyzed reactions will experience some kind of inhibition. One inhibition as we talked that you can add a third component in addition to substrate and product and add another inhibitor either for any purpose of study or anything usually but that doesn't come into picture when we talk of industrial reactions using enzymes because we will not like to add inhibitor to the system for carrying out the enzyme catalyzed reaction for commercial purposes.

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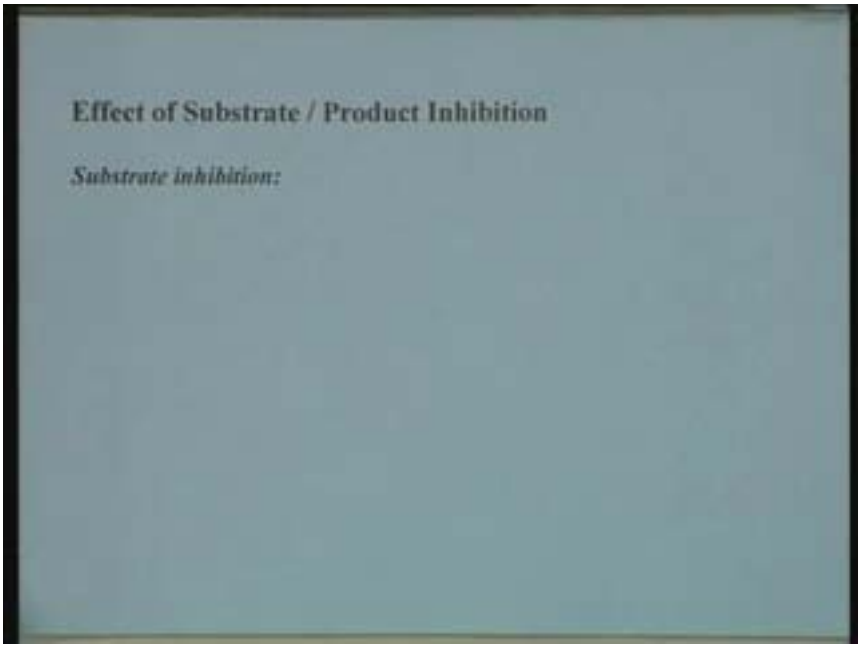


But still most of the enzyme catalyzed reactions are inhibited naturally by either substrate or the product; the product is a very common case. Inhibition by product is a very general feature for all enzyme catalyzed reactions but some enzyme reactions are also inhibited by substrate and this effect because the concentration profile of substrate and product in the two cases of PFR and CSTR are different. For example in the case of CSTR the concentration of substrate in the reaction mixture is very, very low, same as in the outlet or in other way the concentration of product is very high in the reaction mixture. So if a system experiences product inhibition and you use a CSTR you are making the reaction going at very high product concentration. That means in extremely inhibited conditions the reaction rate can be very, very slow.

On the other hand the reverse might be true in the case of plug flow reactor and so the incorporation of effect of substrate and product inhibition on the reactor performance equation becomes very important. While we are considering all other conditions of the isothermal operation or partitioning effect, diffusional limitations are still valid. We are still talking about idealized the vectors we are only changing the kinetic part. That means if we consider and if we let us take a general case of substrate inhibition where the reaction rate is given by

$$v = \frac{k_2 E_0}{(1 + K'_m/S + S/K_s)}$$

This is a general kind of expression. $K_s$ is the substrate inhibition constant, dissociation constant and $K_m$ is the natural $K_m$ value for the reaction. If you recall we discussed substrate inhibition kinetics in which the excess of substrate causes binding of two substrate molecules to the enzyme molecule and which becomes a dead end complex. Under that condition at in the case of CSTR you can develop the reactor performance equation for such system also on the same lines as we did for Michaelis Menten kinetics. That means you make a mass balance across the reactor, simplify it and the final reactor performance equation for this case for CSTR is

$$XS_0 + K'_m (X/1-X) + S^2_0/K_S (X-X^2) = k_2 E_0 \tau$$

In the case of PFR the same reaction expression will result in

$$XS_0 - K'_m \ln(1-X) + S^2_0/2K_S (2X-X^2) = k_2 E_0 \varepsilon\tau$$

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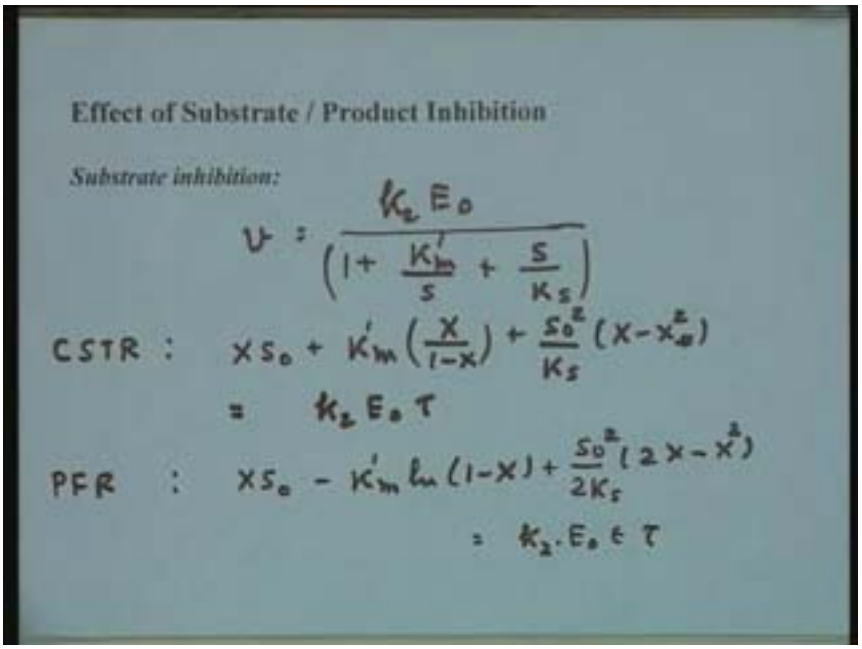

So the reaction performance equation gets modified and as you will notice that in the case of substrate inhibition, the performance of CSTR will be far superior. If you can use these two equations and substitute arbitrary values for fractional conversion at a given substrate concentration the performance of CSTR works out to be far superior compared to a plug flow reactor because the concentration of substrate at any given time physically is much lower than in the case of PFR which for bulk part, more than half of the part, substrate concentration is very high only in the upper half. The substrate concentration goes down and the reaction rate varies in the PFR along the length of the reactor.

If you look at product inhibition, product inhibition also has influence in two modes. Either the product can inhibit competitively or non-competitively. Uncompetitive cases are rare and as I mentioned earlier also in most cases the inhibition patterns are usually either competitive or non competitive. In the case of product inhibition the reaction rate is given by

$$v = \frac{k_2 E_0}{1 + K'_m/S\,(1+P/K'_p)}$$

$K'_p$ is the inhibition constant which is the competitive inhibitor of the reaction. In this case also one can write down the reactor performance equation for CSTR as

$$S_0X + K'_m\,(X/1-X) + K'_m/K_P(S_0X^2/1-X) = k_2E_0\tau$$

In the case of plug flow reactor the reactor expression will be

$$S_0X(1-K'm/K_P) - K'_m \ln(1-X)\,[1+ S_0/K_P] = k_2E_0\,\tau\varepsilon$$

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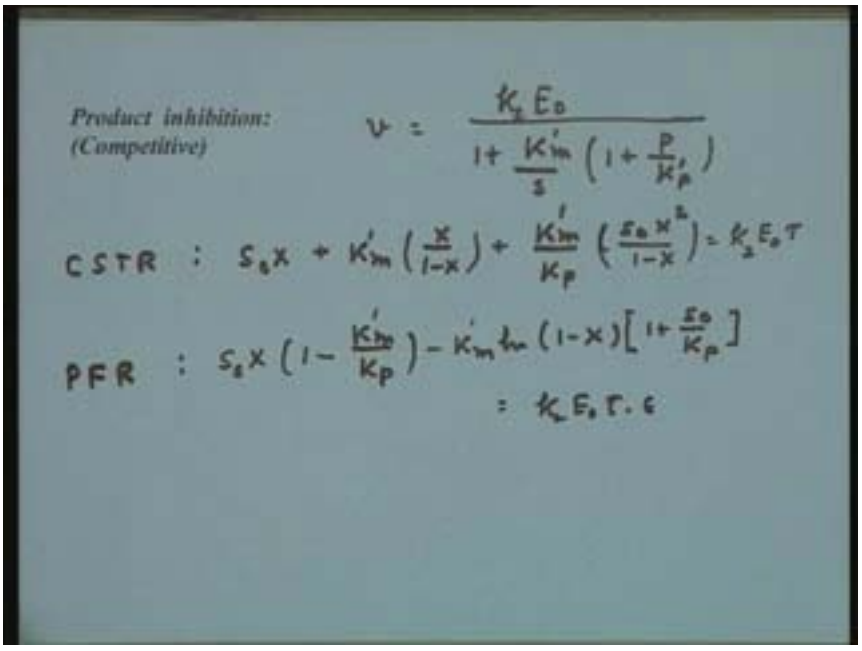

Even in the case of an uninhibited reaction the relative performance of PFR as the fractional conversion increases is superior. But in the case of a reaction that undergoes product inhibition the performance still becomes more superior. Relative quantity of enzyme required or the relative value of the space time required in PFR for increased fractional conversion is much, much higher in the case of CSTR compared to PFR. On the other hand the analogous relations in the case of non-competitive product inhibition where the reaction rate can be given by

$$v = \frac{k_2 E_0}{[1 + K'_m/S]\,[1+P/K'_p]}$$

The CSTR reactor performance comes out at

$$XS_0 + K'_m\,(X/1-X) + K'_m/K_P.1\text{-}X/\ S_0X^2 + X^2S^2_0/K_p = k_2E_0\tau$$

In the case of PFR the expression is arrived

$$XS_0(1\text{-}K'm/K'_P) - K'_m \ln(1\text{-}X)\,[1+ S_0/K'_P] + S^2_0X^2/2K'_P = k_2E_0\,\tau\varepsilon$$

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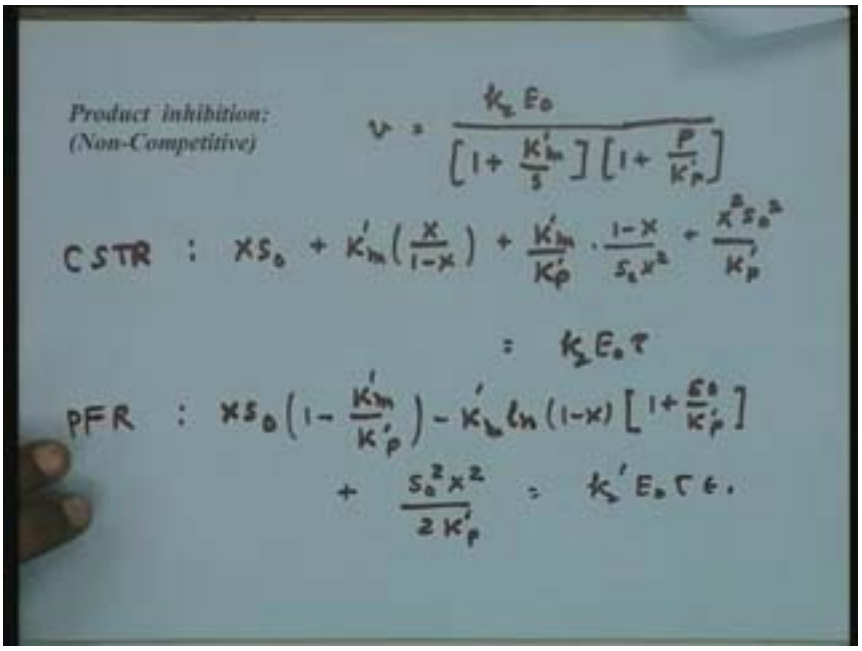


When the enzyme is subjected to inhibition by excess substrate, it is more serious in the case of a plug flow reactor; effect of inhibition on the reactor performance is more detrimental in the case of PFR. The situation can be slightly improved if you make intermittent feeding of the line; for example you use a plug flow reactor in which the feeding is done at more than one point. That means you don't use a high substrate concentration right at the beginning. Such modifications can always be looked at.

On the other hand the effect of product inhibition is more serious particularly when high conversions are required. So it is not only the effect of product inhibition which is more serious in the case of a CSTR compared to PFR but when high fractional conversions are required the product inhibition becomes even more serious. The product inhibition is more detrimental in CSTR compared to PFR because the concentration of product is much higher in the case of CSTR. But when higher fractional conversions are required the problem becomes more and more serious because the product concentration is high when the fractional conversion required is high and therefore in those cases the plug flow reactor becomes almost a necessity. That is one of the reasons that in the literature a large number of immobilized enzyme systems have been reported to be used in a plug flow reactor mode rather than a CSTR mode unless there is the requirement of pH control or in some cases when you want to add another constituent at a later stage.

These are the standard kind of inhibition patterns which we are familiar with; substrate inhibition or a product inhibition which is non competitive or competitive. I don't consider it necessary that a third inhibitor to be added it can be either substrate or product which can cause inhibition which has to be taken into account while making a reacted regime while analyzing the reactor performance. These are theoretical situations which are comparatively simpler. In many practical situations the inhibition patterns are usually complex. They are not as simple. For example I have illustrated here the analysis of penicillin to six APA and phenylacetic acid by penicillin acylase.

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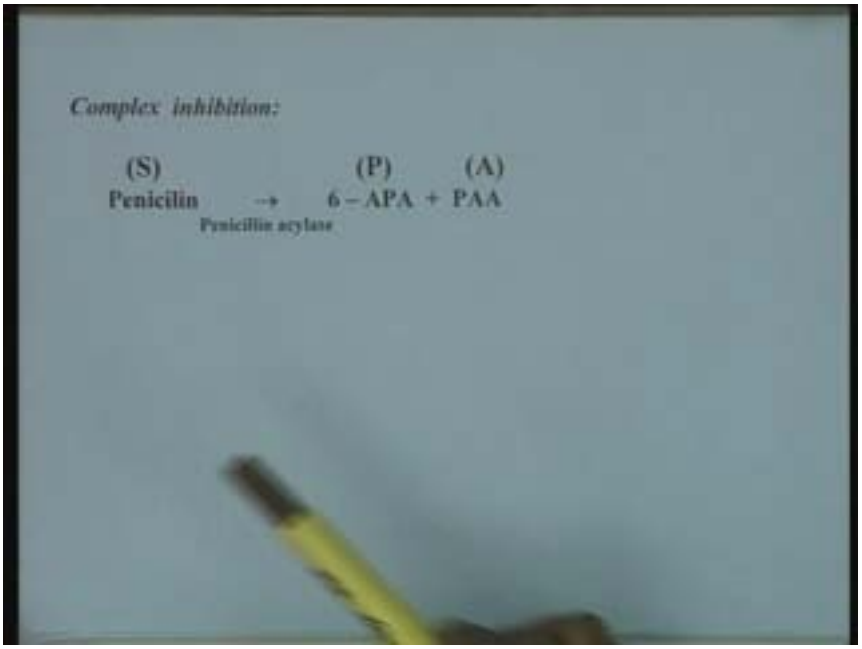


This is an enzyme catalyzed reaction which is used commercially all over the world and particularly 6- amino pencillanic acid is used as the intermediate for synthesis of ampicillin and today because the use of penicillin as an oral drug has reduced significantly excepting for application …. (28:55) ointment. But whenever it has to be

administrated orally you always look for derivatised penicillin and ampicillin is a major product where six APA is …. (29:08)here.

In such a case you see the reaction is inhibited both by P as well as A. Both the products inhibit the reaction. For the sake of writing the rate expression I named as P and A and in the case of A it is inhibited competitively. A is a competitive inhibitor and P is a non-competitive inhibitor. You can also arrive at cases we there is substrate inhibition also and life can be very, very complex in such cases. For example in this practical case of penicillin acylase catalyzed hydrolysis of penicillin the kinetic rate expression comes as

$$v = \frac{k_2.E_0}{1+K'_m/S\ (1+A/K_{ia}) + P/\ K_{ip}\ (1+K'_m/S)}$$

They have developed the reaction performance equations but the quantitative comparison of CSTR and PFR in such cases will become more and more complex. Before taking of the final design or analysis of the reactor system one need to look at the reactor performance equation based on actual reaction kinetics which is applicable and in this case I can site you the reference. The case of penicillin acylase in detail manner has been analyzed and the reactor performance compared by Warburton and associates in Biotech. Bioengineering volume fifteen, page thirteen, nineteen seventy three.

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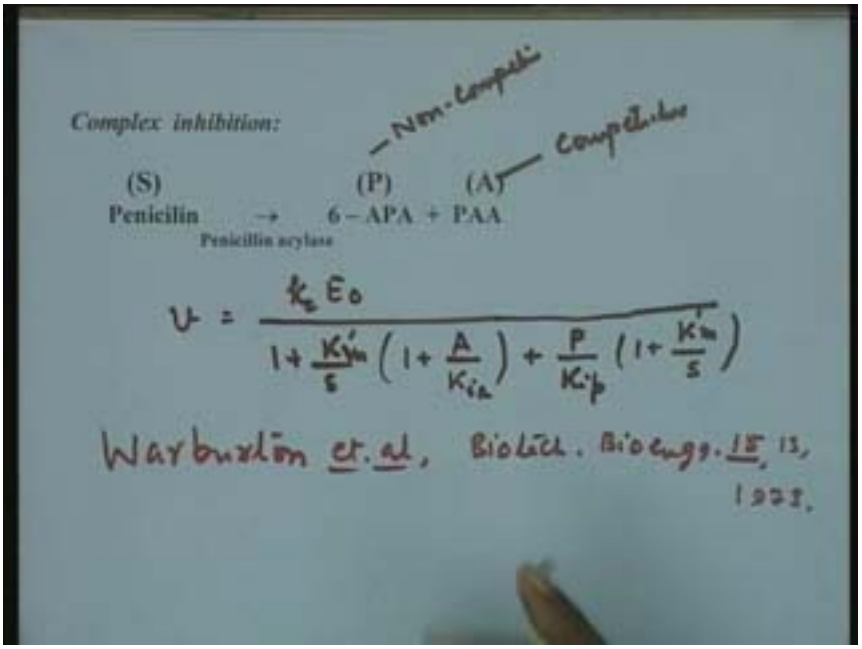


That is one case which illustrates the use of reaction kinetics for the design of or for the analysis of the reactor performance for any given practical enzyme catalyzed reaction which is not a theoretical one but which has been used practically and the process of the system is commercially used all over the world.

Besides the inhibition pattern the next step if you recall is the relative concentration of feed substrate and the magnitude of $S_0/K_m$ and the kinetic regime of the reaction. We have also seen the fractional conversion and productivity. The third item we have seen is the inhibition pattern which the kinetic reaction follows and probably another pattern which is very, very important in analyzing the immobilized enzyme reactors is the reactor type and operational stability. One of the most important motivating factors for looking at immobilized enzyme was the operational stability. That means you can use them continuously over a very long period of time and we must also look at how the reactor type influences the operational stability. The system which is more operationally stable has also some desirable features even at the cost of productivity or fractional conversion all those parameters taken separately.

We can divide the operational stability in two major patterns. One of the major patterns is where the thermal deactivation or the loss of enzyme activity during continuous use is independent of substrate concentration. That means irrespective of substrate concentration in the reactor the inactivation rate is constant and which can be given by

$$-dE/dt = k_d.E$$

A first order decay rate constant that is what we are familiar with. In such cases the life is simple because the substrate concentration is not involved. The reactor performance can be simply considered on the basis of thermal deactivation and if you replace in your idealized reactor performance equation instead of $E_0$, if you just transfer it to $E_0.e^{-kdt}$ where t is the operational time, you can get the modified reactor performance of the effect of operational stability on the reactor performance. In such cases you see the performance for CSTR. We are comparing the reactor performance at two different times say from zero time to time t how does the reactor performs. In the case of CSTR

$$k_d.t = \ln \left[ \frac{S_0X_0 + K'_m (X_0/1-X_0)}{S_0X_t + K'_m (X_t/1-X_t)} \right]$$

Here the $X_0$ and $X_t$ are the fractional conversion in the case of CSTR with a time zero and t. We are considering all this reactor performance under steady state conditions. Once the steady state has been achieved at time t equal to zero, the fractional conversion is $X_0$ and after the elapse of time t fractional conversion declines as a result of enzyme deactivation and that is the fractional conversion $X_t$. In the relationship $K_d.t$, t is the time of reactor operation. Mind it; this t should not be confused with the space time. Space time is kept constant over the reactor operation, over a period of time t and only the fractional conversion is declining from $X_0$ to $X_t$ and this gives you the reactor performance or the effect of operational stability on the reactor performance in CSTR.

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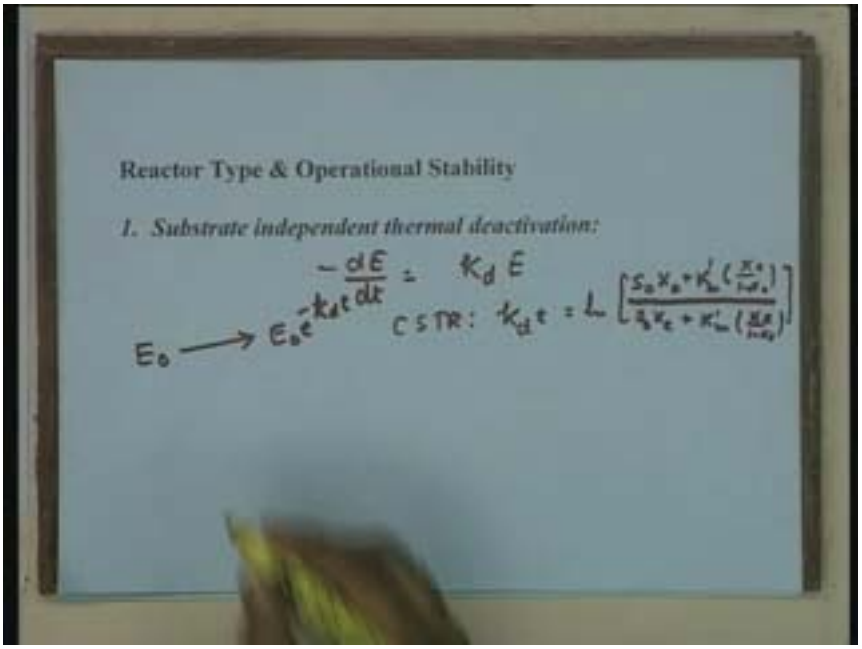


On the other hand in the case of PFR the corresponding relationship is

$$k_d.t = \ln\left[\frac{S_0X_0 - K'_m \ln(1-X_0)}{S_0X_0 - K'_m \ln(1-X_t)}\right]$$

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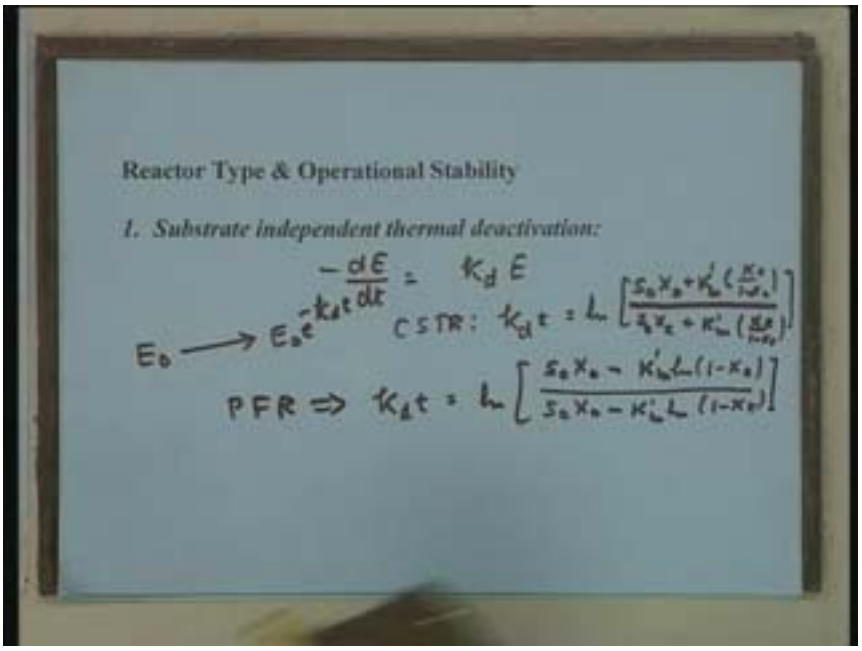

Here also you can consider the effect of the operational stability on the reactor performance. If the kinetic regime of the reaction is in the zero order that means the $K'_m$ in both the expressions will be deleted. It will be only first term and the effect of operation stability is independent of the type of reactor. The factor operational stability will be identical in the case of two types CSTR and PFR if we are operating on a system in the zero order regime whereas if you are operating in the case of first order regime, the first term will get out will be neglected and then the difference in conversion …… (38:03) between PFR and CSTR will be magnified. The $X_t$ value in the two cases after the elapse of time t will be different in the two cases and the output of the PFR, plug flow reactor will decrease much faster with time than in the case of CSTR. When you put some arbitrary values you can see that in the case of PFR or a substrate independent thermal deactivation the output that means the fractional conversion after time t will decrease much faster than CSTR. The deactivation is not linked to the substrate concentration and therefore in such cases the CSTR will be a desirable parameter.

On the other hand if you look at the kinetic pattern of thermal deactivation, the thermal deactivation is also very common situation, because in most cases it can be understood that the enzyme is more stable in the presence of a substrate. So higher the substrate concentration the thermal stability is more; its $K_d$ value is much, much less in the presence of substrate and the deactivation expression can be written as

$$-dE/dt = (k'_d/s).E$$

Here the deactivation rate is inversely propositional to substrate concentration. Higher the substrate concentration the deactivation rate is slow. In such a situation for a CSTR you can very easily write the reactor performance.

$$k_d.t = S_0(X_t - X_0) + S_0 \ln(X_0/X_t) + K'_m \ln\left[\frac{K'_m + S_0(1-X_0)}{K'_m + S_0(1-X_t)}\right]$$

This is for CSTR.

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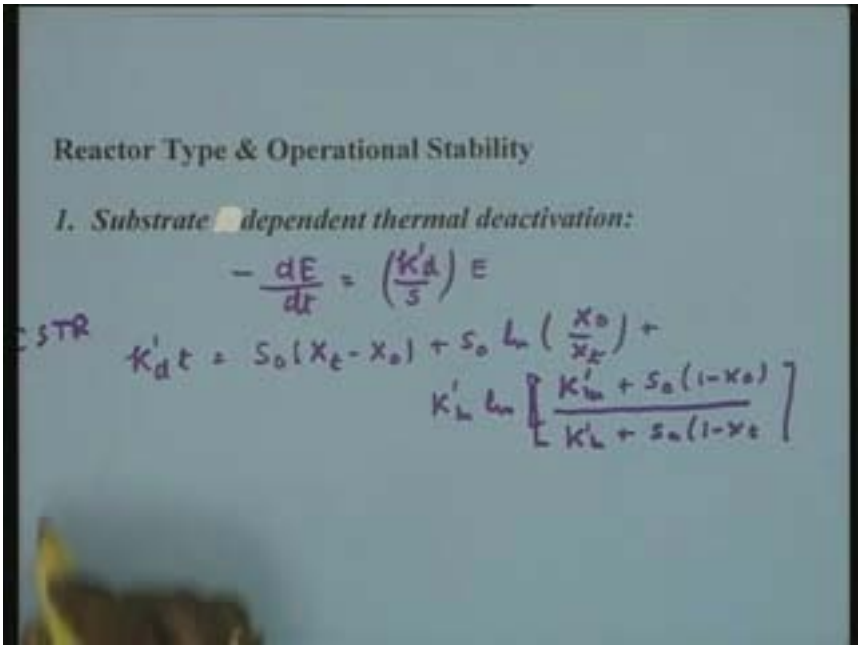


In the case of PFR the situation will be much more complex because the concentration of substrate across the reactor is not constant. So the rate of deactivation also along the length of the reactor is different and therefore a simple analytical solution is difficult. One has to probably get the reactor performance based on operational stability by a numerical solution and an analytical solution will be difficult. But in general you will notice that in the case of substrate dependent thermal deactivation the performance of PFR will be superior compared to a CSTR. The PFR is a desirable type of reactor where the effect of drop in the reactor performance as the result of enzyme deactivation will be much slower for substrate dependent thermal deactivation. Or in other words in the case of substrate independent thermal deactivation the reverse is true. The decay in the reactor performance will be more pronounced in the case of substrate independent thermal deactivation compared to CSTR. CSTR is much more desirable.

To summarize what we have done as far as the idealized reactor systems are concerned we have originally defined our idealized reactor systems which means that we are considering the extreme fluid dynamic regimes; back mix and plug flow. There is no significant heat of reaction, isothermal operation. We have not considered any of the mass transfer limitations or partitioning effect in the system and in the worst situation if you compare with the two major types of reactor systems, continuous flow reactor system, CSTR and PFR we can analyze the reactor performance for a given reaction on the basis of the relative concentration of substrate to the $K_m$ value of the reaction. Basically it amounts to looking at the reaction kinetics.

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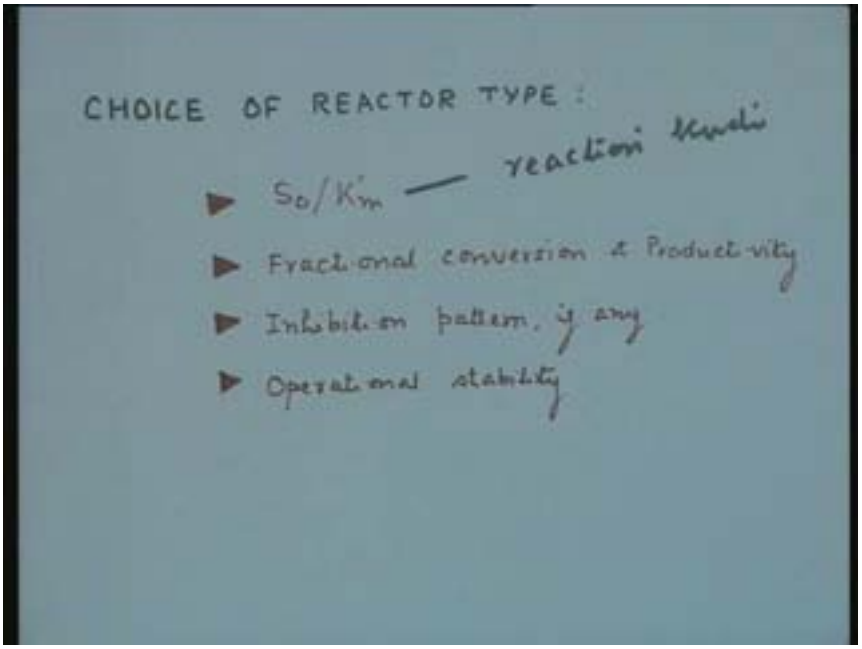


Then we need to look at the effect of fractional conversion and productivity; what is the desirable feature? Whether we are looking for high fractional conversion and that is the demand of the system or high productivity depending on the nature of the product stream. Then we have to look also the inhibition pattern if any. In some cases if the kinetics is much simpler one can very easily approximate it to first order or zero order or even Michaelis Menten; the life if simpler. But in case if there is inhibition pattern that must be taken into account for analyzing the reactor performance. Finally the operational stability of the system as a result of or the effect of the enzyme deactivation during the continuous operation of the two different types of reactor will also be influenced by the deactivation pattern and one cannot decide absolutely whether a CSTR is desirable or PFR is desirable. Under different sets of conditions, under the requirement of the reaction systems, the process requirement one has to make a choice and compare interms of the overall productivity or the desired parameter.

That is the sort of system that we have to look for our ideal reactor performance. Besides ideal reactor performance we have so far not considered anything about mass transfer, non-ideal flow. This is the practical reactor which we are talking like we talked many of the configurations like which were a combination of CSTR and ultra filtration reactor or a hollow fiber module. They may not to be an ideal flow dynamics. They may vary somewhere in between the two and that flow behaviour also might influence the reactor performance. So we will take those cases individually in subsequent classes.