Bioreactors
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Lecture – 05
Enzyme bioreactors, enzyme kinetics

Welcome to lecture number 5 of this NPTEL online certification course on Bioreactors.

In the last lecture, we saw that the two major products from a bioreactor could be cells themselves, or the products that are produced by the cell, we said that time; we will refine it a little better in this lecture. We also saw the various models for the variation of specific growth rate with substrate concentration, with product concentration, and essentially, the models for the growth kinetics. And, we also saw a model, the Luedeking Piret model for the product formation kinetics. Then, we saw a few concepts such as the yield coefficient, the maintenance, and that is where we finished up in the last lecture.

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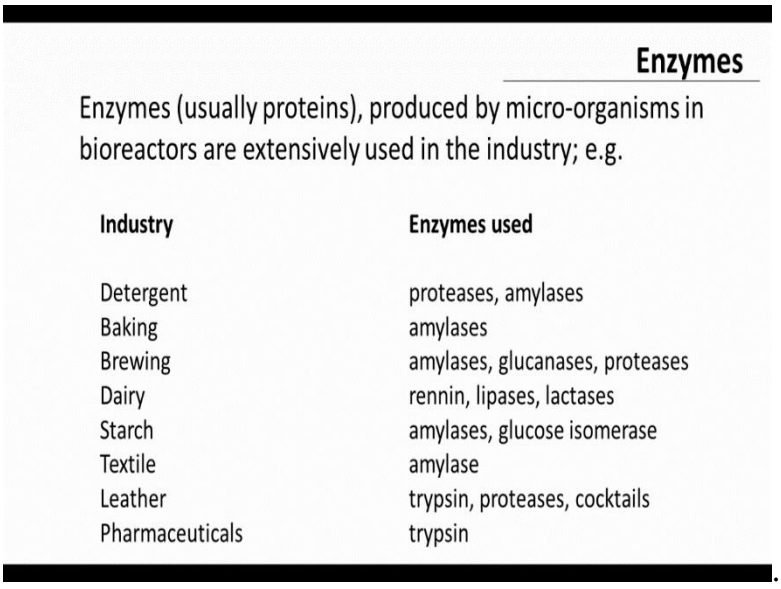

Let us begin this lecture with enzymes. We said we will slightly improve the outcomes from the bioreactor; cells themselves, and the products that are made. Earlier, we said that the products that were made by the cells, it could also be the products that result

from an enzyme mediated reaction. All of you would have heard of enzymes. Enzymes, usually proteins, which are produced by microorganisms in bioreactors, are used extensively in the industry. And, that is, the interest in the enzymes themselves. Then, the enzymes could mediate products, from, let us say, from A to B conversion and so on and so forth. The B could be the preferred product over A, and the transformation through enzymes, the bio-transformation through enzymes, could be carried out in a bioreactor. But before we look at that, let us look at enzymes themselves. These are produced by microorganisms and are extensively used in the industry. I have given a few examples here, and this use has been for a few decades now.

The listing is as, industry and the enzymes used. They are heavily used in the detergent industry, especially, enzymes such as proteases and amylases are used in the detergent industry to improve the cleaning ability of the detergents. In the baking industry amylases are used. In the brewing industry amylases, glucanases and proteases are used. In the dairy industry, rennin, lipases, lactases; in the starch industry, amylases, glucose isomerase. Remember, we said that, starch can be broken down to glucose for further fermentation; amylases are the ones that could carry that out. Textile industry amylase, leather industry- trypsin, proteases and cocktails of enzymes could be used; mixtures of enzymes could be used in the leather industry. Pharmaceuticals, trypsin, and that is just examples of the use of enzymes in the industry.

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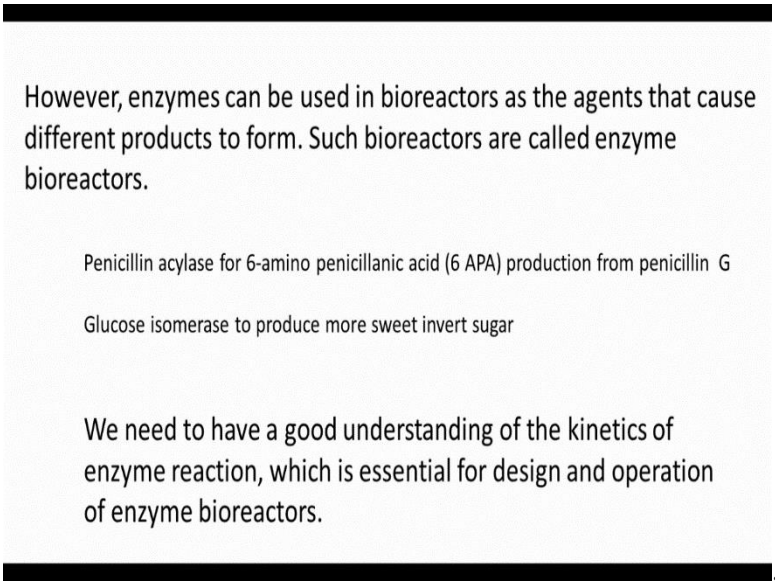

As was mentioned previously, enzymes can be used in the bioreactors as the agents that cause different products to form, and there is a special name for that bioreactor. Such bioreactors are called enzyme bioreactors. Examples here, Penicillin acylase for 6-amino penicillanic acid, or 6 APA, production from penicillin G. Penicillin G is converted to 6 APA through penicillin acylase, 6 APA is widely used. And, glucose isomerase is used to produce sweet invert sugar. This is also used heavily in the industry, in the food industry, beverage industry.

As before, we need to have a good understanding of the kinetics of the enzyme reaction. Earlier, we looked at the kinetics, the rates of growth, in the rates of products formation. Here, we need to have a good understanding of the kinetics, or the rates of enzymes reaction, and they become essential in the design and operation of enzyme bioreactors.

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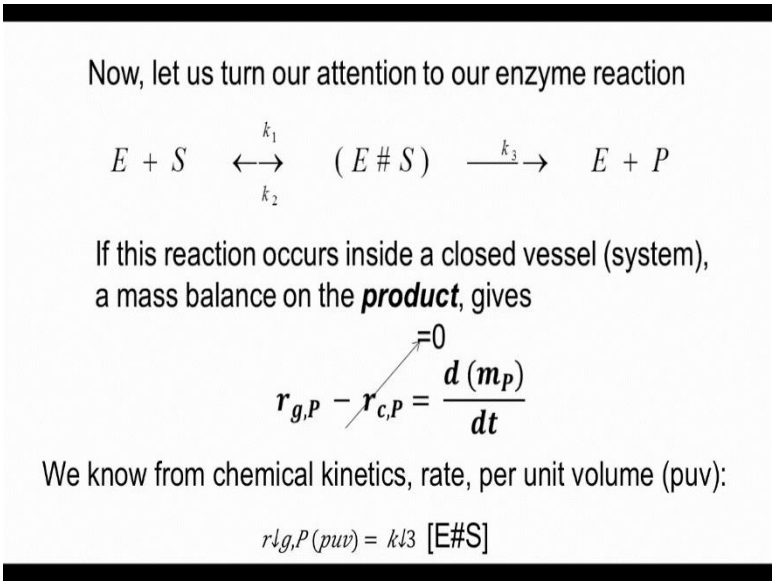

So, we will look at enzyme kinetics predominantly in this particular lecture. Let us make things as simple as possible. Let us see, how an enzyme reacts to form the products, or how the product is made in the presence of an enzyme? How the enzyme mediates the process? How does it get involved in the process? One of the simplest mechanisms that have wide acceptance for simple enzymatic reactions is as follows:

$$E + S \underset{k_2}{\overset{k_1}{\longleftrightarrow}} (E\#S) \xrightarrow{k_3} E + P$$

if E is the enzyme which converts substrate S to product P, the mechanism that is considered here is the enzyme reacts with the substrate, in a reversible fashion; the rate of the forward reaction is k 1; the backward reaction here is k 2. This is a reversible reaction, to form an enzyme substrate complex. And, this reaction is fast. This is also an equilibrium reaction. The k 1 is rather high, equilibrium reaction. And then, this enzyme substrate complex reacts further, converts further to enzyme and product. This is through, the rate constant here is k 3. This is the overall mechanism of simple enzyme mediated reaction.

If this is the case, and let us say, this is occurring inside a closed vessel, a batch reactor, which we take as our system, if we write a mass balance on the product, we are going to focus on the product, and write a mass balance for the product, that gives the rate of generation of the product, that is given here, minus the rate of consumption; remember, this is a batch reaction, and therefore, the input and output terms are zero; the input and output rates are zero. So, the other, those two terms disappear, and we have only the generation and consumption terms associated with the product. So, the rate of generation minus the rate of consumption of the product equals the rate of accumulation of the product; all this in mass terms.

$$\boldsymbol{r_{g,P} - r_{c,P} = \frac{d\,(m_P)}{dt}}$$

There is no, if we assume that there is no consumption of the product, then, the rate of generation of the product equals the rate of accumulation of the product here; rate of generation, rate of accumulation.

$$\boldsymbol{r_{g,P} = \frac{d\,(m_P)}{dt}}$$

And, we know from chemical kinetics that, the rate on a per unit volume basis, the volumetric rate as we call is, r g P per unit volume. This is the normal way in which we write our rates on a per unit volume basis. This rate is on a mass basis; mass per time; this is mass per volume per time. This is what you would have learnt in your high school, higher secondary school and so on. The rate on a per unit volume basis is k 3 into the concentration of the enzyme substrate complex, first order kind of a thing there.

$r_{g,P}(puv) = k_3$ [E#S]

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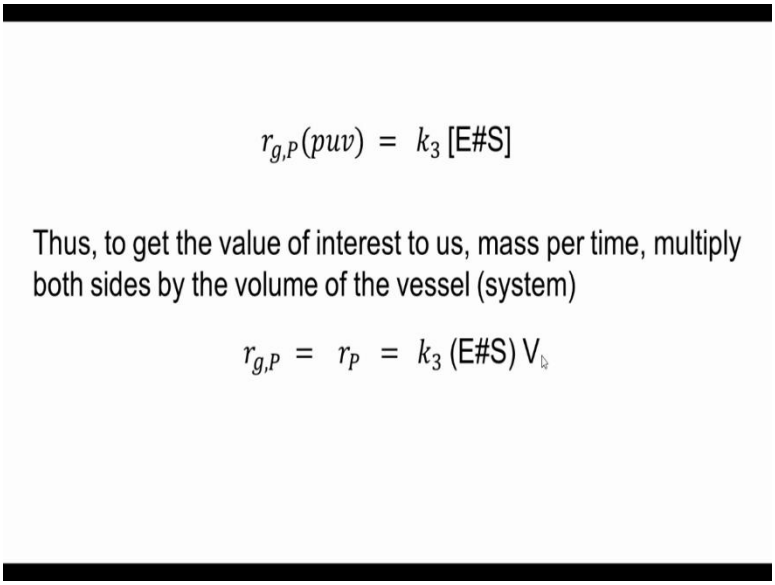

That is the earlier equation. So, to get to the value of interest to us, which is mass per time, we need to multiply both sides by the volume of the system; because this is on a volumetric basis, we are looking for a mass basis. Concentration is nothing, but mass per volume. If we multiply that, then we get rate on a mass basis, the rate of product generation on a mass basis; let us call it r P for simplicity, equals k 3 into the concentration of the enzyme substrate complex into the volume V.

$r_{g,P} = r_P = k_3$ (E#S) V

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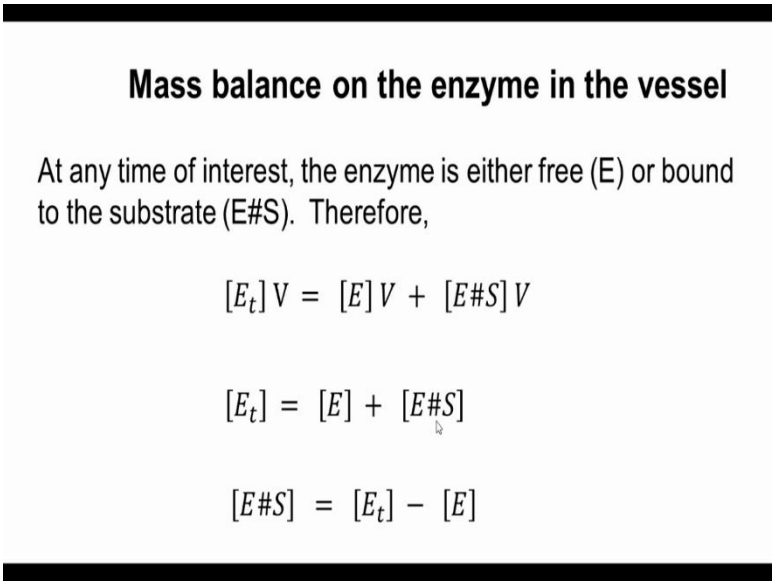

Now, let us write a mass balance on the enzyme in the vessel. Earlier, we wrote a mass balance for the product; now, let us write a mass balance on the enzyme. If we do that, at any time of interest, the enzyme is either free, or bound to the enzyme substrate complex, as we had viewed in our mechanism. Therefore, the mass of the total enzyme E t is the concentration of the total enzyme times the volume. The mass of the total enzyme is the mass of the free enzyme plus the mass of the enzyme that is bound to the enzyme substrate complex, right. Therefore, the mass of the free enzyme is concentration of the free enzyme into the volume, the same volume as this, plus the mass of the enzyme that is associated with the enzyme substrate complex, is the concentration of the enzyme substrate complex times the volume.

$$[E_t]\,V = [E]\,V + [E\#S]\,V$$

So, this is just the mass balance. This is what can be equated; the concentrations cannot.

Since the concentrations cannot be equated directly, but the masses can be equated. Now, since all the volumes are the same, we get equality in concentrations. The concentration of the total enzyme equals the concentration of the free enzyme, plus the concentration of the bound enzyme, or bound as enzyme substrate complex.

$$[E_t] = [E] + [E\#S]$$

Therefore, this is just transposition of this equation. The concentration of the enzyme substrate complex is total concentration of the enzyme minus the concentration of the free enzyme.

$$[E\#S] = [E_t] - [E]$$

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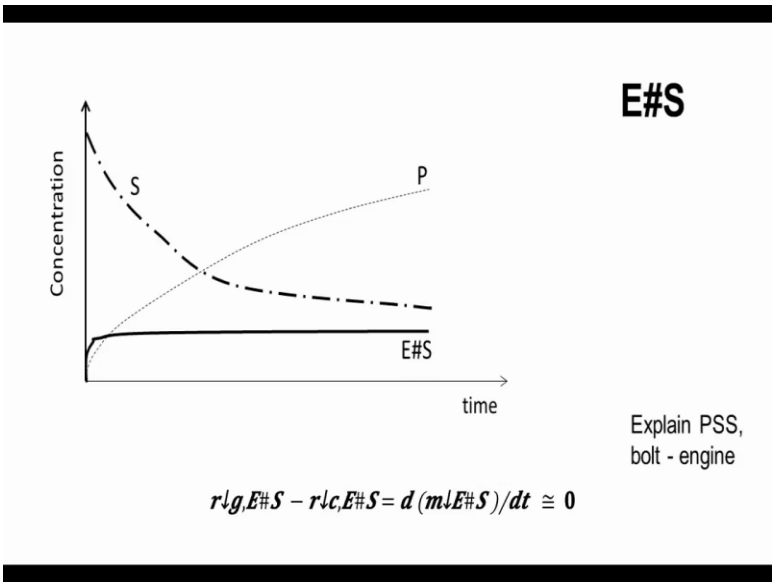

Now, if we plot the concentrations of, let us say, the substrate, the product and the enzyme substrate concentration, versus time, as the reaction proceeds, it will be something like this. The substrate is going to react to give the product. Therefore, that goes down. The product is going to get formed. Therefore, that keeps going up. And, the enzyme substrate complex behaves something like this. At very small times, there is an increase, and then, there is no change in the concentration of the enzyme substrate complex. So, apart from very short times here, early times, the concentration of the enzyme substrate complex does not change. Therefore, if you are interested in this region, this range of times, then, we could say that, the accumulation rate of the enzyme substrate complex is zero, because if it is accumulating, it should either go down, sorry, it

should either go up, or go down; that is not happening here, and therefore, this can be put as equal to zero.

This is the same equation as earlier generation rate of enzyme substrate complex minus consumption rate of enzyme substrate complex from the materiel balance equals the accumulation rate of the mass of enzyme substrate, in this system. And, since there is no change in concentration, that goes to be, that goes to zero.

$$r_{g,E\#S} - r_{c,E\#S} = \frac{d\,(m_{E\#S})}{dt} \cong 0$$

Now, this is what you might have seen, if you remember your enzyme kinetics from high school, or your pre-engineering preparations and so on and so forth. This is only a limited way of looking at this concept. In fact, this concept is very powerful. So, let us look at a generalized way of the consequences of this particular aspect. To do that, let us consider an example. Before I say that, I should also say that, the concentration here does not change; therefore, this rate is zero. Therefore, you can assume, take the enzyme substrate complex to be at pseudo steady state; that is what PSS means -pseudo steady state. This is, this concept is fine in the case of a batch system. Let us generalize that concept.

Let us, to generalize that concept, let us consider an example, a fictitious example of making an engine for a car. Let us say, that we are putting the engine together in-house, right from the bolt, to the engine. The time that is needed for the manufacture of a bolt is, on the average, about 5 seconds, let us say that. And let us say that, the time, on the average, for the manufacture of an engine is about an hour. This is an average of 5 seconds; it could be 6 seconds, 7 seconds, 3 seconds, 4 seconds, whatever it is, or even 2 seconds and so on; it could vary, right. And, that is the unsteady aspect in the manufacture of the bolt. Whether it takes 5 seconds to manufacture a bolt, or 7 seconds to manufacture a bolt, or 3 seconds to manufacture a bolt, has no impact on the time that it takes to build an engine; this is of the order of an hour.

This variation, in a matter of seconds, is not going to affect the rate at which the engine is being made. The unsteady nature, the time varying nature of bolt manufacture, does not affect the kinetics of engine manufacture. That is because, these two are widely separated, in terms of their characteristic times as they are called. This one is in a matter of seconds, we manufacture this. This one takes thousands of seconds, 60 into 60, about an hour, 3600 seconds. So, if the characteristic systems, characteristic times are widely different, the unsteady nature of the faster process does not affect the kinetics of the slower process, if our interest happens to be in the slower process. So, while looking at the slower process, we can always consider the faster process, or a much faster process, to be at pseudo steady state; in actuality, it could vary, but that variation does not affect the process, the slower process. So, the faster process, in comparison can be taken to be at pseudo steady state, and this is a concept that has wide applications. You can also look at it this way; the enzyme substrate complex formation variation is very fast compared to the times over which a product gets formed, and therefore, the enzyme substrate complex could be assumed to be at pseudo steady state, compared to the product.

So, whenever we use the pseudo steady state assumption, we need two processes which are widely separated in terms of the characteristic types. We will visit this later, but for now, whichever way you understand it, it is fine; it is not going to affect the understanding of the derivation of the kinetic equation.

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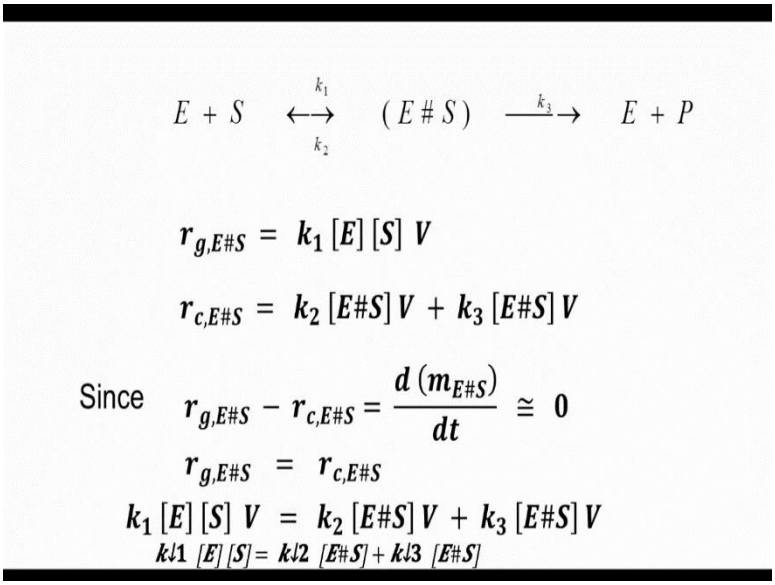

Let us go back to this. We had enzyme plus substrate giving you reversibly, an enzyme substrate complex, and irreversibly, an enzyme and the product.

$$E + S \underset{k_2}{\overset{k_1}{\leftrightarrow}} (E\#S) \xrightarrow{k_3} E + P$$

The rate of generation of the enzyme substrate complex, we are going to focus here, is nothing but k 1 into the concentrations of enzyme and substrate into the volume. This is on a volumetric basis; this is on a mass basis. Therefore, we need to multiply it by the volume.

$$r_{g,E\#S} = k_1 [E] [S] V$$

And, the rate of consumption of the enzyme substrate complex, as you see here, it is consumed through the reverse reaction with the rate constant k 2, and this reaction with the rate constant k 3, the consumption is k 2 into concentration of the enzyme substrate into the volume, plus, the k 3 into the concentration of the enzyme substrate complex into the volume, here.

$$r_{c,E\#S} = k_2 [E\#S] V + k_3 [E\#S] V$$

This is the rate of generation, rate of consumption. And, so, the mass balance turns out to be, the rate of generation minus the rate of consumption, rate of accumulation is zero; this is what we have already seen as the pseudo steady state assumption. Therefore, the rate of generation of the enzyme substrate complex equals the rate of consumption of the enzyme substrate complex, because this is equal to zero in the previous step.

$$r_{g,E\#S} - r_{c,E\#S} = \frac{d\,(m_{E\#S})}{dt} \cong 0$$

$$r_{g,E\#S} = r_{c,E\#S}$$

Replacing the rates by the concentrations k 1into E into S, both are concentrations, into volume, equals k 2 into enzyme substrate complex concentration into volume, plus k 3 into enzyme substrate complex concentration into volume.

$$k_1 [E] [S] V = k_2 [E\#S] V + k_3 [E\#S] V$$

The volumes are the same; therefore, they can be canceled, and therefore, you get k 1 into E into S equals k 2 into E S plus k 3 into E S.

$$k_1 [E] [S] = k_2 [E\#S] + k_3 [E\#S]$$

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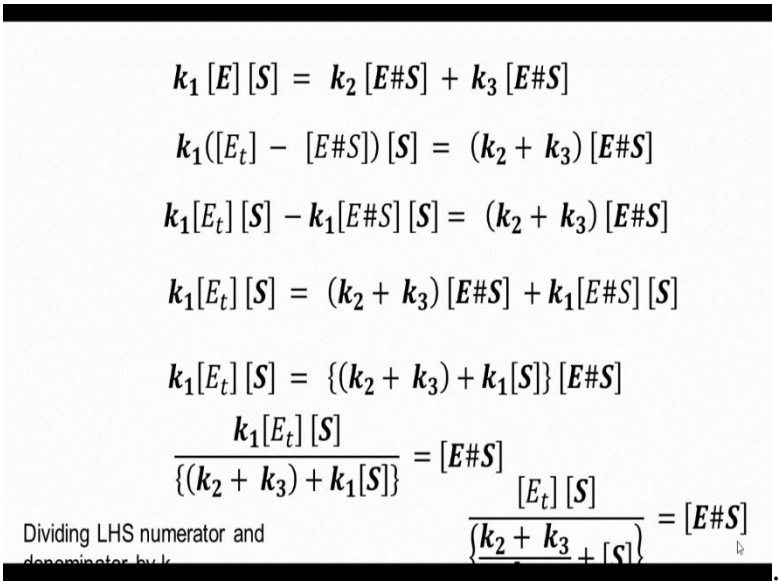

That is the same equation.

$$k_1[E][S] = k_2[E\#S] + k_3[E\#S]$$

And we are just transposing this; we are collecting all the k 1 into E;E is difficult to know, whereas E t, we can have a handle on. Therefore, let us write E in terms of E t and the enzyme substrate complex; we had derived this earlier through a mass balance on the enzyme. You can go back and check that. k 1 into E t minus enzyme substrate complex into this S; this is common here; therefore, k 2 plus k 3 into E S.

$$k_1([E_t] - [E\#S])[S] = (k_2 + k_3)[E\#S]$$

And, a little more of algebra; I am just taking this S inside the bracket, and then, I am combining the terms which have the enzyme substrate complex. So, k 2 into k 3 into enzyme substrate complex, plus k 1 into enzyme substrate complex into S. And therefore, you could write this as, the left-hand side remains the same; right hand side, if you take the enzyme substrate complex concentration common out, k 2 plus k 3, from here, plus k 1 into S; the enzyme substrate complex is out; therefore, k 1 into S here. And

therefore, the concentration of the enzyme substrate complex is the left-hand side, divided by the factor here, k 2 plus k 3 plus k 1 times S.

$$\boldsymbol{k_1}[E_t]\,[\boldsymbol{S}] \;=\; (\boldsymbol{k_2} + \boldsymbol{k_3})\,[\boldsymbol{E\#S}] \;+ \boldsymbol{k_1}[E\#S]\,[\boldsymbol{S}]$$

$$\boldsymbol{k_1}[E_t]\,[\boldsymbol{S}] \;=\; \{(\boldsymbol{k_2} + \boldsymbol{k_3}) + \boldsymbol{k_1}[\boldsymbol{S}]\}\,[\boldsymbol{E\#S}]$$

$$\frac{\boldsymbol{k_1}[E_t]\,[\boldsymbol{S}]}{\{(\boldsymbol{k_2} + \boldsymbol{k_3}) + \boldsymbol{k_1}[\boldsymbol{S}]\}} \;= [\boldsymbol{E\#S}]$$

If we divide the numerator and the denominator by k 1, right, we get, the k 1 disappears from here. So, E t into S and here, k 2 plus k 3 divided by k 1 plus S; there is a k 1 here which gets canceled, plus S, equals the enzyme substrate complex concentration.

$$\frac{[E_t]\,[\boldsymbol{S}]}{\left\{\frac{\boldsymbol{k_2} + \boldsymbol{k_3}}{\boldsymbol{k_1}} + [\boldsymbol{S}]\right\}} \;= [\boldsymbol{E\#S}]$$

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$$\frac{[E_t]\,[\boldsymbol{S}]}{\left\{\frac{\boldsymbol{k_2} + \boldsymbol{k_3}}{\boldsymbol{k_1}} + [\boldsymbol{S}]\right\}} = [\boldsymbol{E\#S}]$$

$$\frac{[E_t]\,[\boldsymbol{S}]}{\{\boldsymbol{K_m} + [\boldsymbol{S}]\}} = [\boldsymbol{E\#S}]$$

We had earlier seen $r_P = k_3\,(\mathrm{E\#S})\,\mathrm{V}$

Substituting from above $r_P = k_3 \frac{[E_t]\,[S]}{\{K_m + [S]\}}\,\mathrm{V}$

$$r_P = \frac{v_m\,[S]}{\{K_m + [S]\}}\,\mathrm{V} \qquad \text{where } v_m = k_3\,[E_t]$$

$r_P(puv) = v = \frac{v_m\,[S]}{\{K_m + [S]\}}$ **Michaelis – Menten**

Which is the same as this,

$$\frac{[E_t]\,[\boldsymbol{S}]}{\left\{\frac{\boldsymbol{k_2}+\boldsymbol{k_3}}{\boldsymbol{k_1}}+[\boldsymbol{S}]\right\}} = [\boldsymbol{E\#S}]$$

And if I call k 2 plus k 3 by k 1 as some capital K m, then the same equation can be written as this: E t S divided by K m plus S equals enzyme substrate complex concentration.

$$\frac{[E_t]\,[\boldsymbol{S}]}{\{\boldsymbol{K_m}+[\boldsymbol{S}]\}} = [\boldsymbol{E\#S}]$$

And, we had earlier seen that, the rate of product formation, in terms of a mass rate, is k 3 into E S into the volume; you can go back and check that equation.

$r_P = k_3$ (E#S) V

So, if you substitute the enzyme substrate complex concentration from above, we get the rate of product formation is k 3 into E t S by K m plus S in the place of enzyme substrate complex concentration, times V,

$$Substituting,\ r_P = k_3 \frac{[E_t]\,[\boldsymbol{S}]}{\{\boldsymbol{K_m}+[\boldsymbol{S}]\}}\ \text{V}$$

And if I say k 3 into E t, k 3 into the total enzyme concentration, if I call that as v m, r P becomes, v m S by K m plus S into V; note that r P is on a mass basis.

$$r_P = \frac{v_m\,[\boldsymbol{S}]}{\{\boldsymbol{K_m}+[\boldsymbol{S}]\}}\text{V},\quad \text{where}\quad v_m = k_3\,[E_t]$$

r P on a per unit volume basis, or a concentration basis, let us call that as v, to be consistent with the terminology that you would be familiar with. So, v equals v m S by K m plus S.

$$r_P(puv) = v = \frac{v_m [\boldsymbol{S}]}{\{\boldsymbol{K_m} + [\boldsymbol{S}]\}}$$

Does this appear familiar? Of course, it does. This is the well-known Michaelis - Menten equation. This is the simplest enzyme kinetics equation for a single link enzyme single substrate case, which goes as, enzyme and substrate reacting together in a reversible fashion, to form the enzyme substrate complex, which further goes to give you the product and the enzyme back.

So, this is v equals v m S by K s plus S. Does this form of the equation remind you of something that we saw in the previous lecture? Just keep that in mind; we will come back to that.

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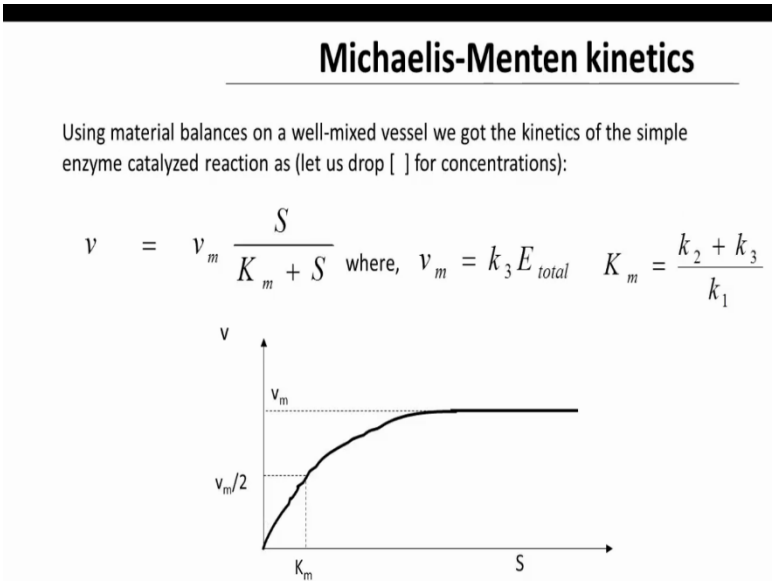

Right, so this is what we got, v equals v m S by K m plus S, where v m equals k 3 into E total, or E t, and K m is k 2 plus k 3 by k 1.

$$v = v_m \frac{S}{K_m + S}$$

Where

$$v_m = k_3 E_{total}$$

$$K_m = \frac{k_2 + k_3}{k_1}$$

Now, does it strike you where you saw this earlier? If you plot v verses S, the rate of the reaction, the volumetric rate of the reaction with the substrate concentration, you get a rectangular parabola. Yes, it is the same form that we got for the monod equation. The monod equation gave us the variation of growth rate; you know, the culture growth rate with substrate concentration. Here, it is very different. Here, it is the rate of the Michaelis - Menten enzyme reaction, the enzyme reaction which takes place in the Michaelis - Menten form, with again the substrate concentration.

The substrate concentration variation, that aspect is the same, but, this is for an enzyme reaction; that is for growth kinetics. You must make a distinction between these two. People tend to confuse one with the other. So, the same kind of, since this is the same form of the equation, the same kind of interpretations can be drawn. v m is the maximum rate, I mean, the rate increases, and then attains, asymptotically attains the maximum rate v m, and if we replace K m with S, then we get, v equals S by 2 S, therefore, v m by 2. Therefore, K m is nothing, but the substrate concentration at which we get the half maximal rate; same as the monod equation interpretations.

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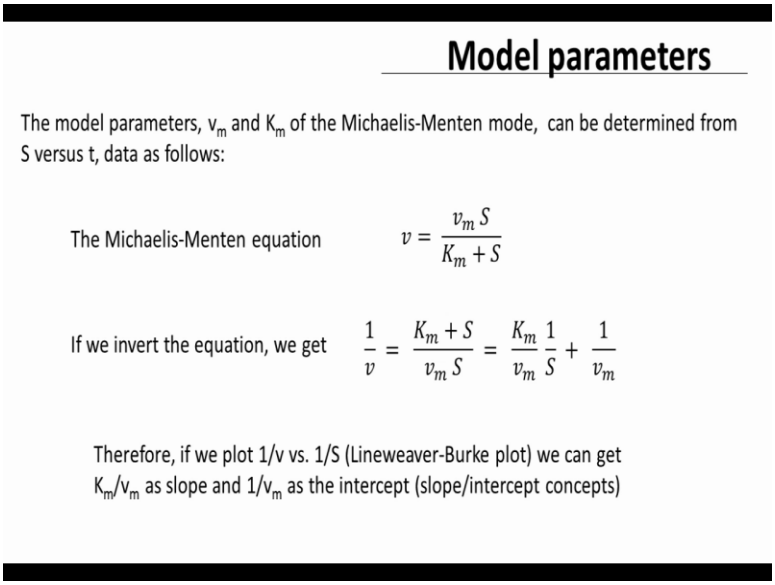

The model parameters v m and K m, these are the two model parameters that we need to completely describe a simple enzyme kinetics. They can be determined by doing an experiment where we follow the substrate concentration with respect to time in a batch reactor. This experiment is done in a batch situation, batch reactor. We collect S verses t data, and if we do that, the Michaelis-Menten equation is v equals v max S by K m plus S.

$$v = \frac{v_m S}{K_m + S}$$

If we invert this equation, take 1 by on both sides, we get 1 by v equals, inversion of this K m plus S by v m S; and, this can be written as, K, this is the plus here, therefore, K m by v m S plus S by v m S; K m by v m S becomes K m by v m into 1 by S; and here, the S cancels out. So, you have 1 by v.

$$\frac{1}{v} = \frac{K_m + S}{v_m S} = \frac{K_m}{v_m}\frac{1}{S} + \frac{1}{v_m}$$

So, if you look at this, as y equals m x plus c, if you take this as y, if you take this as x, you can call this as m, the slope, and c, the intercept. So, if you plot 1 by v verses 1 by S from the S verses t data, you should get K m by v m as a slope, and 1 by v m as the intercept. This is the way in which these model parameters are estimated. There are some issues associated with estimating it this way; let us not get into that for the time being. Let us assume that, the data is good, and we have a good spread in the data, and so on; that, the outliers are not going to affect the value itself; that is, the essential problem here, if you know what I am talking about; if you do not know, do not worry about it. We will have to be a little careful while using this; that you can learn later. A plot of 1 by v verses 1 by S which is actually called the Lineweaver-Burke plot, gives us K m by v m as the slope, and 1 by v m as the intercept.

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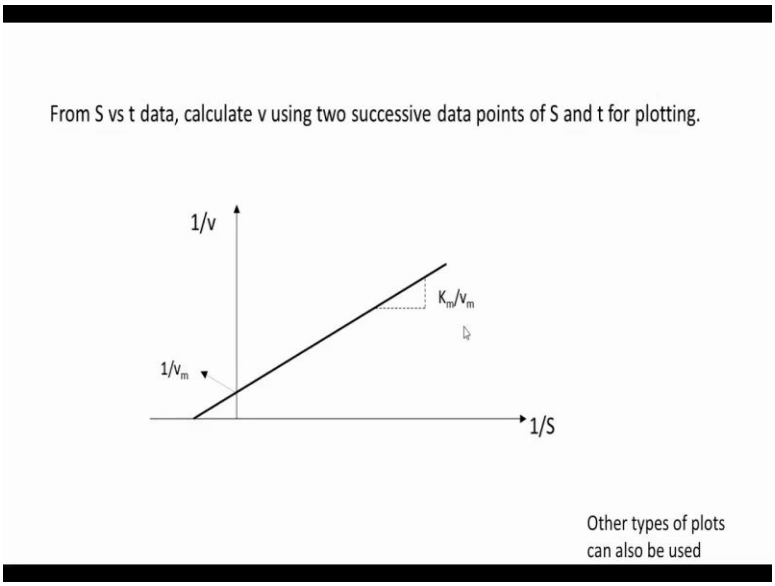

From S verses t data, calculate v using two successive data points of S and t for plotting; this is what you need to do, because, we have substrate concentration versus time. The rate is delta S by delta t, over that short interval, that is what we are approximating. And, we assign that delta S by delta t to the midpoint of the time; that is, that is how we need to do it. When we have the data, we need to use the complete set of data to get the required parameters; this is experimental data. So, 1 by v versus 1 by S, you will get a line like this. The y intercept is 1 by v m and the slope is K m by v m. Other types of

plots can also be used. Let us not get into that. You can look at any standard book on enzyme kinetics including your text book, and that will give you the other plots also.

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Practice problem 2.1

An industry scientist is studying a novel method of disposing a toxin that results from cell culture, during the course of cultivation. The aim is to enhance cell yields through toxin reduction and medium recirculation. The toxin does not affect cell growth if its concentration is below 7.5 mM. The particular toxin she is studying, X (secrecy requirements!) can be broken down enzymatically using the enzyme, E. The breakdown was studied at 30 ^{o}C at a pH of 7.2, and the following kinetic data was obtained under batch conditions:

Now, let a problem be assigned. You can try this problem out. This requires the concepts that we just picked up. Just to summarize, we started this lecture 5 with examples of enzymes being used in the industry, industrial enzymes. And then, we also said that, an enzyme can be used in a bioreactor to form a product of interest, and that is what we are looking at in detail. We said that, we need the kinetics of the enzyme reaction. We went through the derivation of Michaelis-Menten equation which has two parameters v m and K m. In form, it is very similar to the monod equation, which describes the variation of a growth rate with substrate concentration; here, it is a variation of enzyme volumetric rate with the substrate concentration. And then, we also saw that, we could find out v m and K m from the data of substrate concentration with time variation from a batch system by using the Lineweaver-Burke plot.

Now, we will use some of those concepts to address this closed ended problem, to solve this closed ended problem. Please try it out. And of course, in the next lecture, I will show you how to solve this. The question reads as follows: An industry scientist is studying a novel method of disposing a toxin that results from cell culture during the

course of cultivation. The aim is to enhance cell yields through toxin reduction and medium re-circulation. The toxin does not affect cell growth, if its concentration is below 7.5 millimolar. The particular toxin she is studying, X, she has a lot of secrecy requirements, can be broken down enzymatically using the enzyme E; again secrets. The breakdown was studied at 30-degree C at a pH of 7.2 and the following data was obtained under batch conditions.

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Time, min	0	10	20	50	100
X, mM	20.0	17.7	15.8	10.6	5.0

(a) Determine the Michaelis-Menten parameters for this enzymatic degradation

(b) If the total enzyme concentration is tripled, will the medium contain toxic levels of X after 30 min?

This is the variation of time with time of X. X is the substrate here, the breakdown of which we are studying. This gives 5 points; at time zero, it is 20 millimolar; time 10, it is 17.7 millimolar and so on. Part a, determine the Michaelis-Menten parameters for this enzymatic degradation; that is part a. And b, if the total enzyme concentration is tripled, what will the medium, will the medium contain toxic levels of X after 30 minutes. I think this is a nice place to stop lecture 5. We will meet again in lecture 6. See you there.