Bioreactors
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Lecture – 07
Inhibited enzyme kinetics

Welcome to lecture-7 in this NPTEL online certification course on Bioreactors. In the last lecture, we had looked at the solution to a practice problem - practice problem 2.1. So, let us continue with the material after that.

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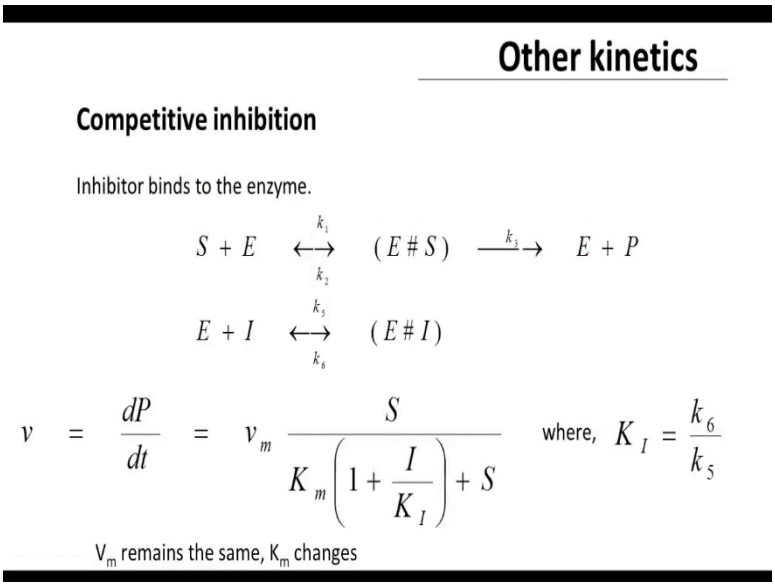

We had looked at the Michaelis-Menten kinetics for an enzyme reaction; there the mechanism was enzyme and substrate giving us an enzyme substrate complex. And the enzyme substrate complex breaks down to give you the enzyme and the product, product and the enzyme back, that was the mechanism. That is of course, a very simplistic mechanism; and as expected many other types of mechanisms exist for enzymatic reactions. And we would look at some of those as variations from this Michaelis-Menten kinetics. That way it will be easier for us to put it in a certain framework, get the appropriate parameters and so on.

Therefore, let us look at other kinetics with a single enzyme say single substrate kind of the situation with some inhibitors thrown in. The first inhibition that we are going to look at is called competitive inhibition. In this case, the inhibitor - inhibitor is the one that inhibits the reaction, that inhibits the reaction by binding to the enzyme and thereby providing competition, so called competitive inhibition thereby providing competition to the substrate in the process of binding to the enzyme. In other words, the scheme - reaction scheme is like this;

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

$$E + I \underset{k_6}{\overset{k_5}{\longleftrightarrow}} (E\#I)$$

The substrate plus enzyme they bind together to give you the enzyme substrate complex. This is the reversible reaction k 1 - forward rate, k 2 - the reverse reaction rate and enzyme substrate complex gives the enzyme and the product. This was the simple enzyme Michaelis-Menten enzyme kinetics. And here in addition, we have an inhibitor molecule that interacts with the enzyme, at the same time and reversibly gives an enzyme inhibitor complex. This kind of a reaction scheme is called competitive inhibition.

Now you can try this when you have time; it is going to take a lot of time, and therefore, I am not going to derive the whole thing. The derivation is on the same lines as that for the Michaelis-Menten kinetics. It will be a little more complex than that because there is one more reaction taking place; you need to look at how things are done. If you derive it, which will take you quite some time, a few pages and so on. We will get v in this, again this is a batch system, therefore the rate - the volumetric rate is, can be taken to be the accumulation rate of the product and can be given as:

$$v = \frac{dP}{dt} = v_m \frac{S}{K_m\left(1+\frac{I}{K_I}\right)+S}$$

Where I is the inhibitor concentration and

$$K_I = \frac{k_6}{k_5}$$

This has been deliberately arranged to be of this form.

Remember our Michaelis-Menten

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

Here, the K m has been modified Km(1+ I/Ki). Therefore, in the case of competitor inhibition, the K m gets modified. The mechanism of course, the inhibitor binds with the enzyme and that is how it is competing with the substrate for the enzyme and thereby hinders the reaction and in the case of the rate expression, the variation is only in K m, v m remains intact. The K I here is nothing but k 6 - the reverse reaction, by k 5 - the forward reaction rate constant; reverse reaction constant divided by the forward reaction rate constant. As mentioned earlier v m remains the same, so K m changes. So if we plot the Lineweaver -Burk plot, then we will get the appropriate things as this slope and appropriate things as intersect. I will let you figure that out. In fact, let's assign a problem so that you can figure that out.

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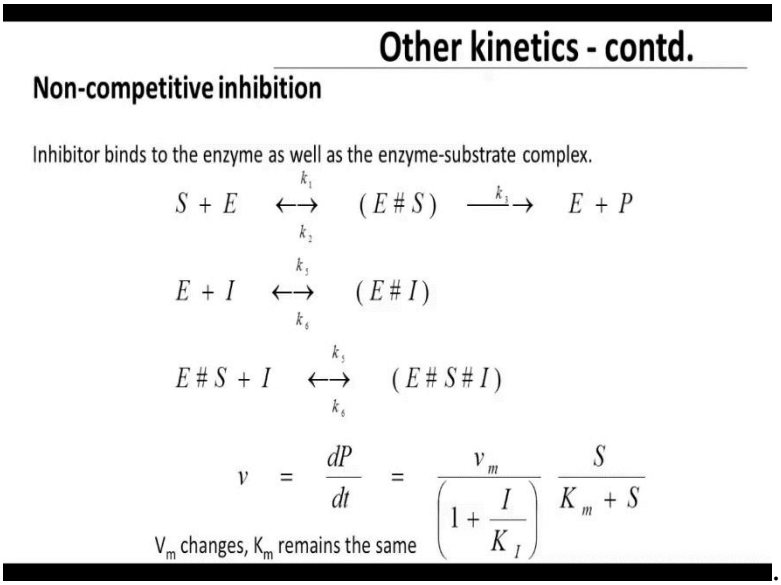

The next type of inhibition, the earlier one was competitive, this one is called, the second one is called the non-competitive inhibition. In the non-competitive inhibition case, the inhibitor binds to the enzyme as well as the enzyme-substrate complex. The scheme is like this;

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

$$E + I \underset{k_6}{\overset{k_5}{\leftrightarrow}} (E\#I)$$

$$E\#S + I \underset{k_6}{\overset{k_5}{\leftrightarrow}} (E\#S\#I)$$

this is the Michaelis-Menten scheme, the first reaction S plus E reversibly E S gives you irreversibly E plus P. The enzyme binds to the inhibitor to give you the enzyme inhibitor complex. The enzyme-substrate complex also binds to the inhibitor reversibly to give you enzyme-substrate inhibitor complex. So, this is the additional reaction here for non-competitive inhibition; in which case, the inhibitor binds to the enzyme as well as to the enzyme-substrate complex.

You can go through a longer process on the same lines as that for Michaelis-Menten, the material balance and so on. And if you do it right, you will get v equals, for a batch case,

$$v = \frac{dP}{dt} = \frac{v_m}{\left(1+\frac{I}{K_I}\right)} \frac{S}{K_m + S}$$

V m has been modified, K m has been not modified in the case of non-competitive inhibition; V m changes, K m remains the same. We will look at one other kind of inhibition, and then we will look at the problem, and I think that is good for this lecture which is other kinetics, a short lecture may be.

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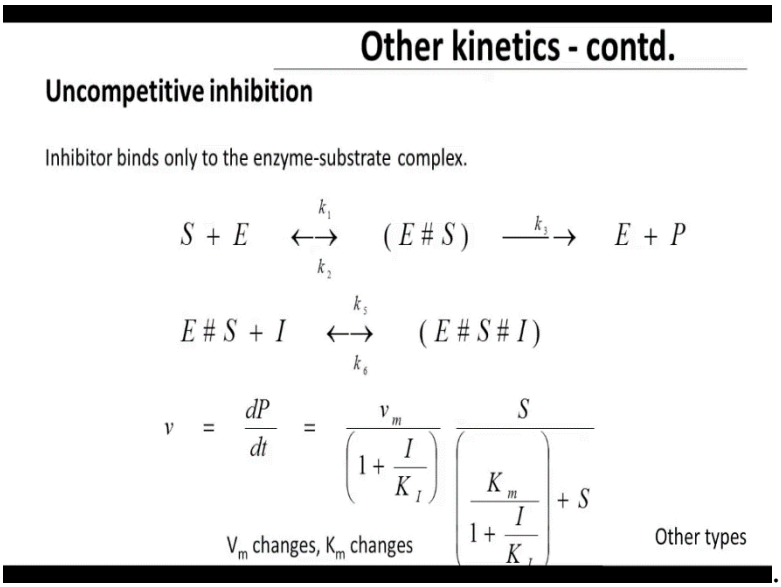

The third one is uncompetitive inhibition. The first one was competitive, then non-competitive, uncompetitive. The uncompetitive inhibition, in that, the inhibitor binds only to the enzyme-substrate complex. In the case of competitive, it bound to the enzyme-substrate complex; it bound to the enzyme, the inhibitor bound to the enzyme. In the case of a non-competitive inhibition, it bound to the enzyme as well as the enzyme-substrate complex. In the case of uncompetitive, it binds only to the enzyme-substrate

complex. In such a case the reaction mechanism is the Michaelis-Menten plus enzyme-substrate complex plus I, giving you reversibly enzyme-substrate inhibitor complex.

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

$$E\#S + I \underset{k_6}{\overset{k_5}{\longleftrightarrow}} (E\#S\#I)$$

The derivation of this would lead to

$$v = \frac{dP}{dt} = \frac{v_m}{\left(1+\frac{I}{K_I}\right)} \frac{S}{\left(\frac{K_m}{1+\frac{I}{K_I}}\right)+S}$$

As we can see here both v m and K m have been modified for uncompetitive inhibition. So, these are the three kinds of other kinetics that we will look at in this course. To better understand these types of kinetics, let us do a problem; and of course, there are other types of kinetics also, but we will not look at that in this course.

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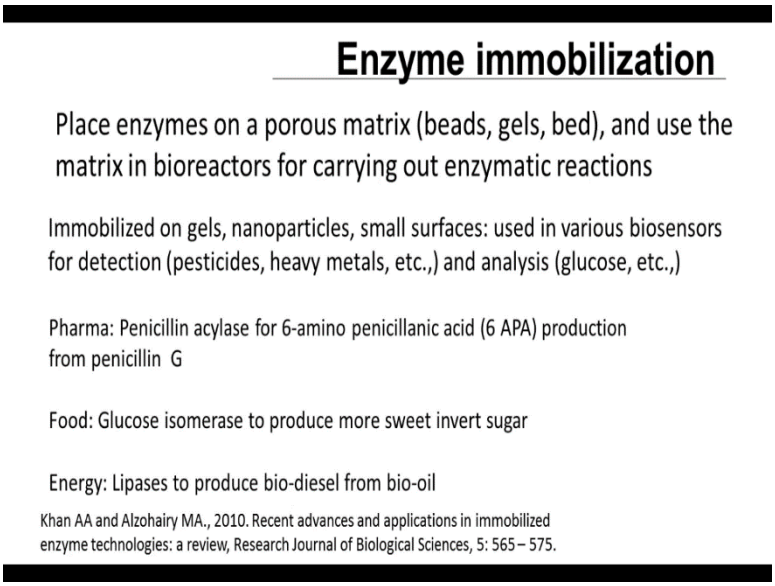

Before we do the problem, let me tell you one more thing. Enzymes are, lets say mostly proteins, let us not get into the other enzymes, mostly proteins; and their functionality depends on how they fold, how the protein folds, all of you know this from your basic biology course. So, if the folding is inappropriate or it gets disturbed by some means then the enzyme becomes inactive. If the active sites get changed, then the enzymes are going to become inactive. To avoid that as well as to reuse the enzyme, people have immobilized the enzyme. They have taken the parts that are not crucial for the activity of the enzyme, the non-active sites and then anchored them on some matrices, it could be a porous matrix such as a bead or a gel or even a bed and carried out the enzyme reaction on the immobilized or with the immobilized enzyme. They have placed enzymes on a porous matrix and we use the matrix in bioreactors for carrying out enzyme reactions.

The beads and gels, it is quite easy to see, you can just add them to the reactor, stirred environment may be stirred reactor. And then the substrate gets into these beads or gels, it interacts with the enzyme, the product gets made, the product comes out. Or it could happen in a bed, where we have a bed with this matrix, the substrate solution passes through this bed, and when it comes out at the end of the bed, the conversion is done. So, this is an example of enzyme immobilization. This has been heavily used in the industry for a few products, so it is an industrial process. They are also immobilized on gels, nano particles and small surfaces - these enzymes, and when done appropriately they can be used as bio sensors for the detection of let’s say pesticides or heavy metals, and also for analysis such as glucose. The enzyme immobilization, maybe glucose oxidase, immobilized glucose oxidase can be used as a glucose sensor in an appropriate format. That is what is typically done also. So, it can be used for various different purposes, not just for production purpose.

Some examples of industrial scale use of enzyme immobilization; in the pharma industry penicillin acylase has been immobilized and that immobilized enzyme has been used for 6 APA production from penicillin G. We saw that 6 APA is the preferred antibiotic, and the conversion of penicillin G to 6 APA is done in an enzyme immobilized reactor in the industry. The food industry, glucose isomerase to produce sweet invert sugar, a mixture of glucose and fructose, that is also done by immobilized glucose isomerase. In the

energy industry, lipases to produce bio-diesel from bio-oil is carried out using immobilized enzyme, immobilized lipases.

And this is a paper, this also there in your list of references, list of additional sources, you can go and take it from that. The complete reference is here; this is published in 2010 in the Research Journal of Biological Sciences; this by Khan and Alzohairy. The title is recent advances and applications in immobilization enzyme technologies: a review. So, if you are interested in knowing some details about immobilized enzymes, you may want to read that paper. Not just enzymes, the cells that we saw earlier, they can also be immobilized. That helps the cells overcome the harsh environment of a bioreactor with a lot of shear and so on, so that cells can be protected. There are again some down sides in terms of rates and so on, but in terms of a concept, you could use immobilized cells, immobilized enzymes or of course, in industrial processes, immobilized cells have been heavily studied, maybe they are used.

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Practice problem 2.2

An enzyme normally displays Michaelis-Menten (M-M) behaviour in converting a substrate, S, to a product, P. It gave the following data when its M-M parameters for the above conversion were estimated in the presence of another substance I at different concentrations. Determine the type of inhibition, and the inhibition constant.

M-M parameters/I, mM→	0	0.5	1	5	10
v_{max}, mM min^{-1}	0.33	0.33	0.33	0.33	0.33
K_m, mM	0.120	0.126	0.132	0.180	0.240

With this let us look at practice problem 2.2, and we will finish of this lecture here. In the next lecture, I will tell you how to solve this. Practice problem 2.2, before this, just a quick recap of the short lecture that we had. We had looked at the kinetic mechanisms for enzymes which are different from the Michaelis-Menten kinetics, especially we had

looked at inhibition kinetics. We looked at three types of inhibition kinetics - competitive, non-competitive and uncompetitive; what happens during competitive, what happens during non-competitive and what happen during uncompetitive in terms of the mechanism. What does an inhibitor bind to essentially that makes the difference. And the resulting kinetic expression also is different in the first case one is modified; in the second case, other one is modified; in third case, both are modified, so that is over all what we saw. And then we saw something about immobilized enzymes being used in the industry.

Having said that, let us look at the practice problem 2.2. An enzyme normally displays Michaelis-Menten behavior in converting a substrate, S, to a product, P. It gave the following data when its M-M parameters for the above conversion were estimated in the presence of another substance I at different concentrations. Determine the type of inhibition, and the inhibition constant. Here, given the Michaelis-Menten parameters here, v max and K m, v max as millimolar per minute and K m as millimolar and the inhibitor contraction in millimolar are given as 0, 0.5, 1, 5 and 10. So, these are the Michaelis-Menten parameters that were obtained, v m and K m at various inhibitor concentrations. You are asked to find the type of inhibition, and the inhibition constant K I. Go ahead and do it, when we meet in the next lecture, we will take things forward.