Mathematical Modelling and Simulation of Chemical Engineering Process

Professor Dr. Sourav Mondal
Department of Chemical Engineering
Indian Institute of Technology, Kharagpur

Lecture 29
Modelling enzymatic reactions

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Hello everyone, in this lecture we are going to study about enzymatic reactions and how enzymatic reactions play a big role in our system or in any biological reactions, cellular reactions, then you have physiological reactions. Everywhere you see that there is a presence of enzymes and enzymatic reactions are often influenced by several factors, which is something we are going to talk about in this lecture in detail, but first let us try to understand the role of enzymes and how enzymatic reactions take place.

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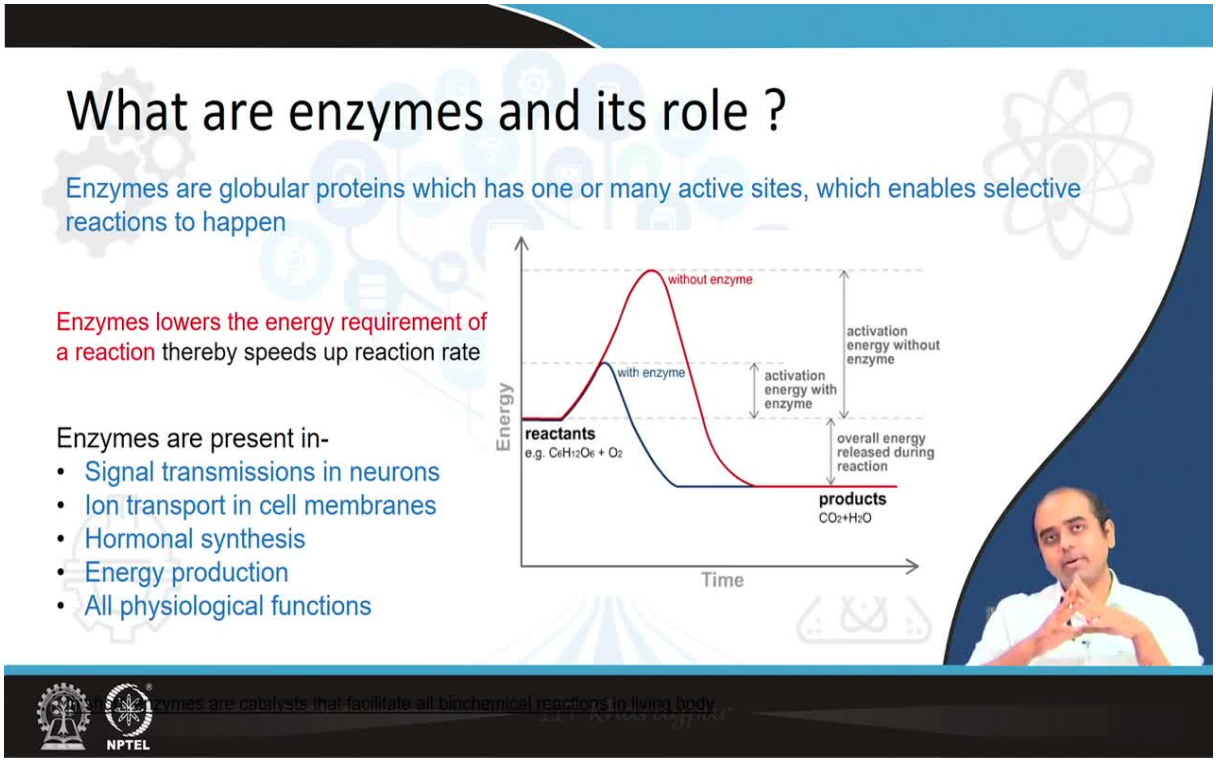

So, enzymes work very similar to the way this catalyst works, it tries to reduce the activation energy or the activation energy barrier and as a result, the reaction does proceed in the desired direction. So, it essentially reduces the energy requirement and, in the process, it increases the rate of reaction also for non-selective reactions or reactions where you want to suppress the byproducts certain enzymes can also, I mean enzymes are generally very selective in nature.

So, if they are a possibility of side reactions, the enzyme will only act for the desired reaction and will try to reduce the energy barrier for that reaction reducing or preventing the possibility of formation of these byproducts that is how enzyme also play a big role in the selectivity or enhancing the selectivity of the final product if there is a possibility of several side reactions, which is generally the case for any bio or biomolecular reactions. Enzymes are present in almost any biological activity or physiological activity for example, transmission or signal transmission in neurons, these are extremely fast enzymatic reactions.

Again, ion transport along the cell wall membranes is a very popular role and is a very popular enzymatic activity and this depends on the sodium and the potassium ion channels across the phospholipid bilayer of the membrane and discovery of these enzyme activities led to the Nobel Prize long back.

Synthesis of different proteins other amino acids as well as hormones and other compounds in the body enzymes play a role it also this famous TCA cycle, this energy production or this conversion of ADP to ATP this energy conversion or the Krebs cycle is the cellular pathway for production of energy there are several enzymes which is presents the different steps or the different pathway of this enzyme.

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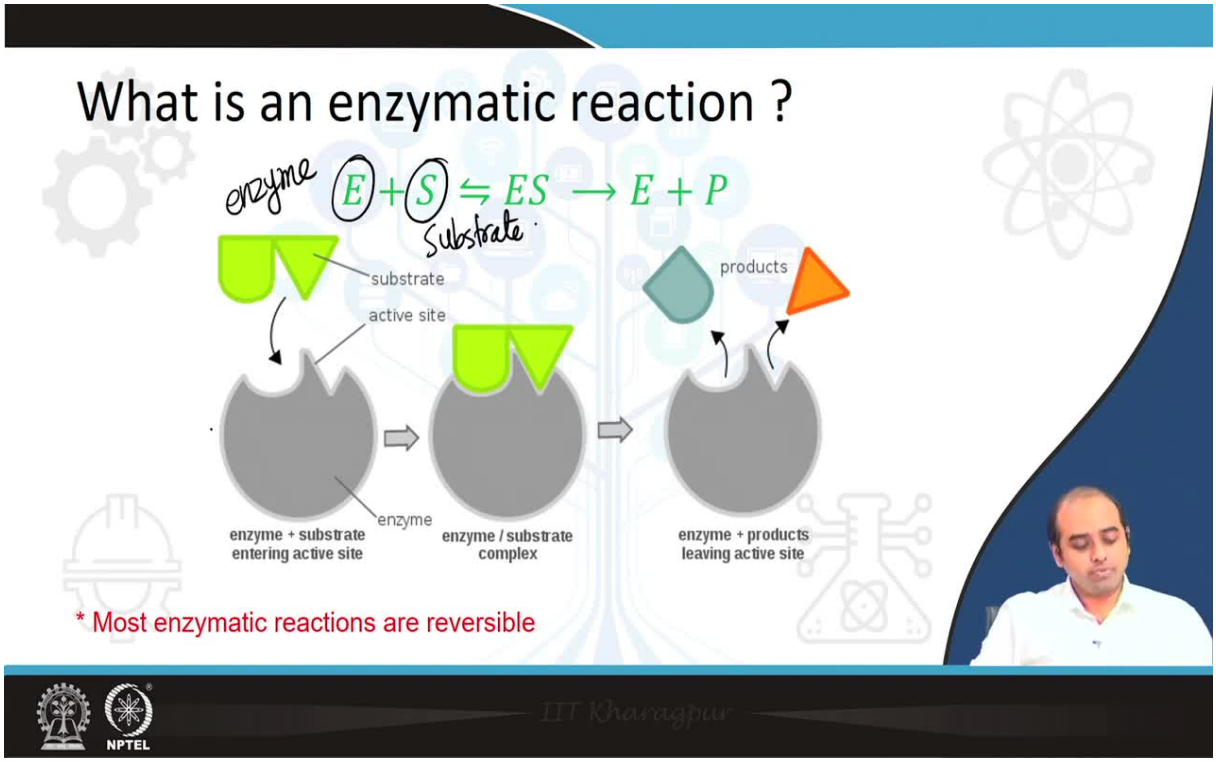

So, how does the enzymatic reaction essentially work? So, you have this enzyme which acts or essentially the substrate which acts or binds to these enzymes. So, this is enzyme s is substrate. So, in this picture you can see this cartoon that how these substrates bind to the enzymes leading to the formation of the enzyme substrate complex and ultimately these enzyme substrate complex leads to the formation of products and byproducts and the enzyme is freed back again for the next available reaction.

So, this formation of the enzyme I mean the substrate or this enzyme substrate complex is a reversible step. So, it may happen that instead of forming the product, the enzyme substrate complex again dissociates back into the enzyme and the substrate. So, that is a reversible step, but the final product is an irreversible step because that part the product does not combine back to the generally does not combine back to the enzyme to get or to form the enzyme substrate complex. So, that is generally an irreversible step. So, these are very site

specific which of course, tells you about their highly selective nature of their function and these enzyme activities is heavily influenced by environmental factors.

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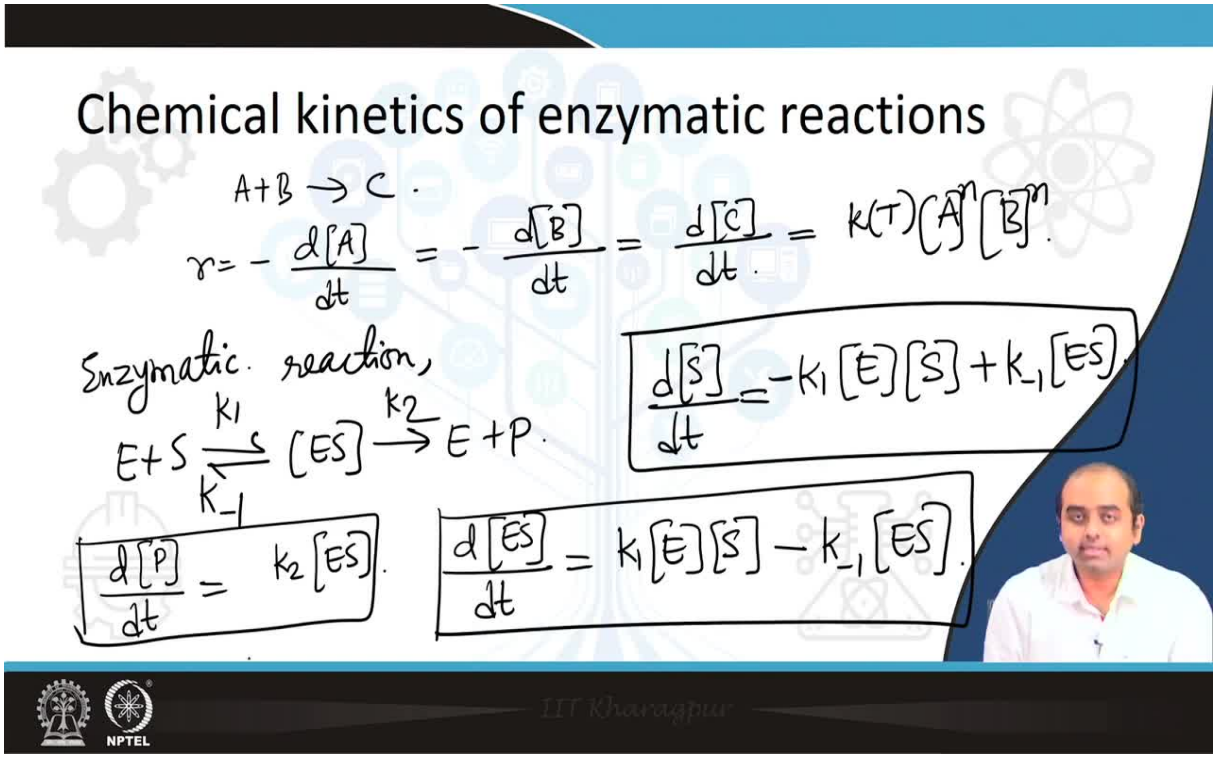

So, before we talk about the influencing factors, let us understand the kinetics of the chemical reaction. So, generally these enzymatic reactions typically, let us say typical reactions we know A plus B which gives C we generally write the rate of reaction as minus dA dt which can be also written down as equal to rate of disappearance of P or rate of production of P, like that.

So, typically for this case, these reaction rates can then be a function of I mean the kinetic constant is a function of temperature and n could be the order of the reaction so, for a typical enzymatic reaction forming. Let us say this product formation rate constant is k2 and the substrate is irreversible so, we write k and k minus 1. So, you can also similarly write down the different this reaction rates for the different steps.

So, for example, you can write dp dt is equal to k2 ES this is something we can write, then you can write d ES as and this is the disappearance these are the reaction the rates that can be written down. So, you have this is one reaction, then you have this is another reaction, this is another reaction and for the enzyme we generally do not write the dynamic equation what we write is the enzyme conservation.

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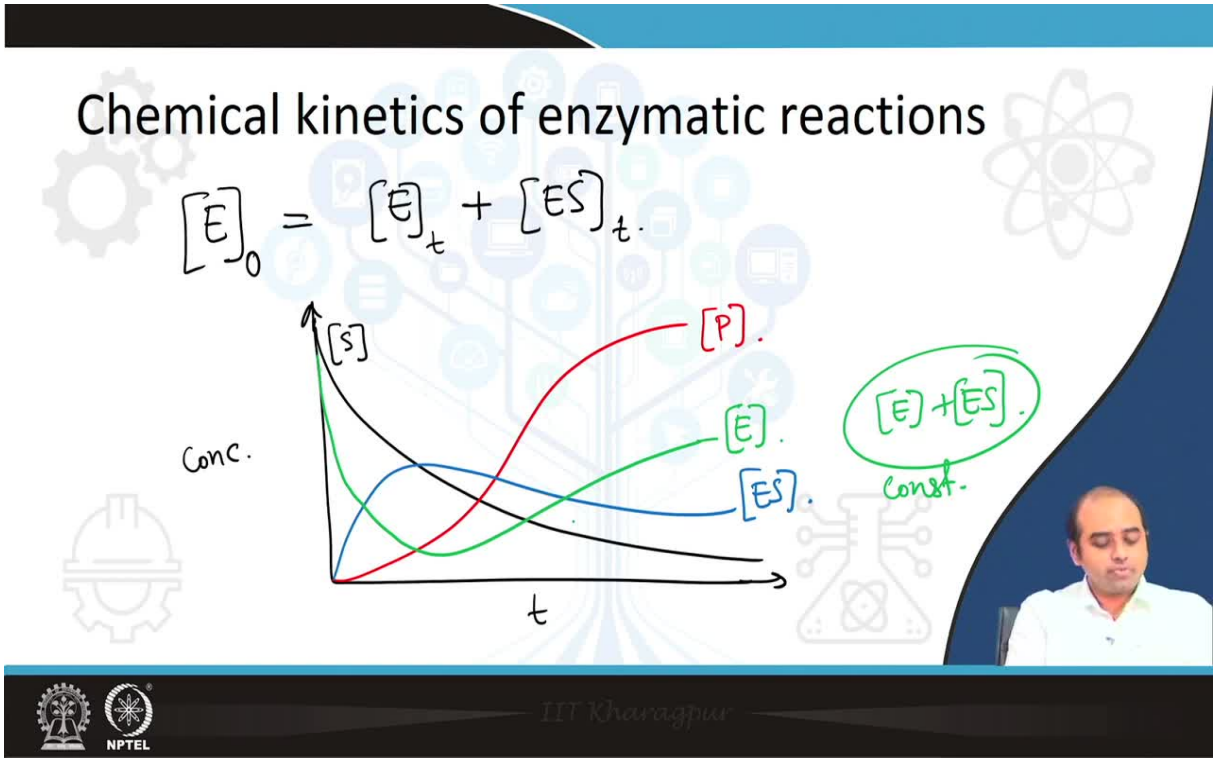

So, this enzyme at any time T which is present in the form of this enzyme in the form of the species in the system as E and E S is conserved. So, E whether it is taking part in the reaction with the substrate or forming after the product is form and this enzyme substrate complex the total enzyme is never consumed. So, at any point of time, this E and E S, I mean they are summation or the total mass of the enzyme is constant.

So, you have 4 unknown species in this problem the ES you have then S you have then T you have and of course the E of the enzyme that is taking part in the reaction you have. So, you have 4 equations and if you try to work out the different their concentration you try to solve them this coupled ODE you will see that the substrate concentration goes down. So, this is the substrate concentration goes down with time you will find that the product concentration goes up with time. Now interesting part about the enzyme substrate complex.

So, there is a local maxima for this enzyme substrate complex and this concentration of the free enzyme in the system is like this. So, please note that the summation of E plus ES at any time, this summation has to be constant because enzymes are conserved that is the reason you see that when E is going down ES is going up.

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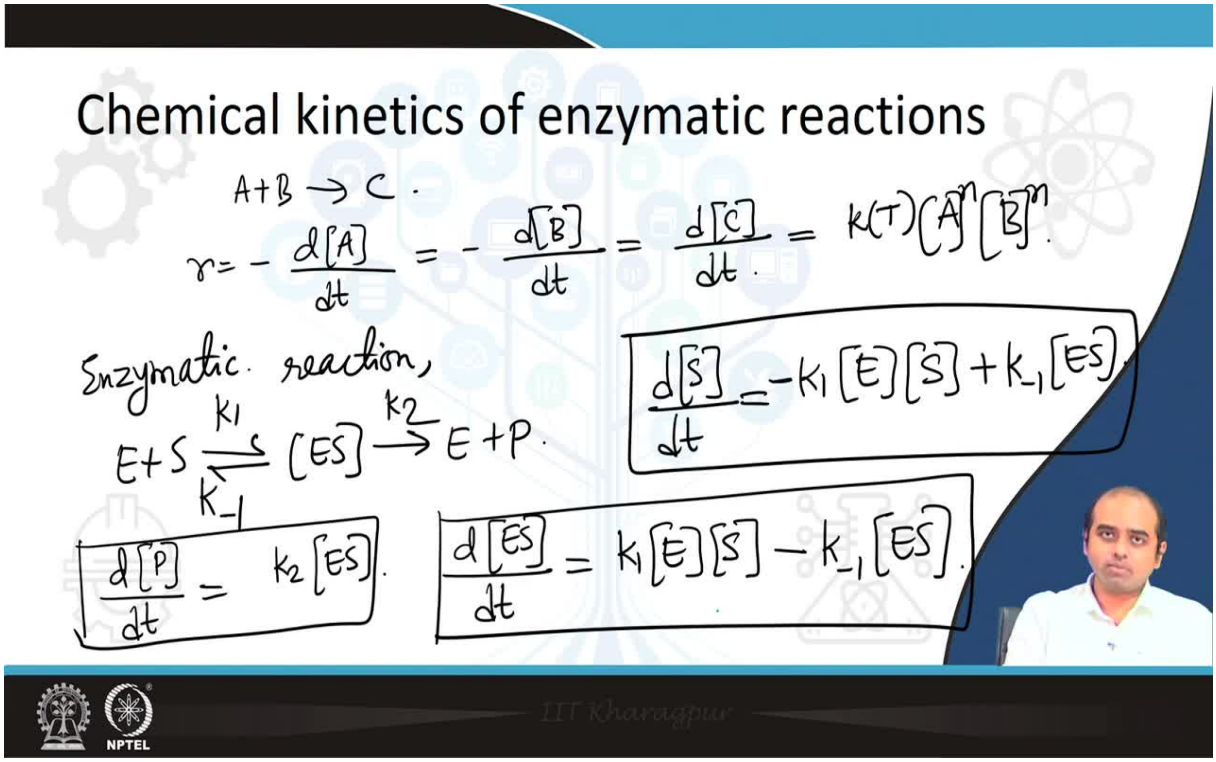

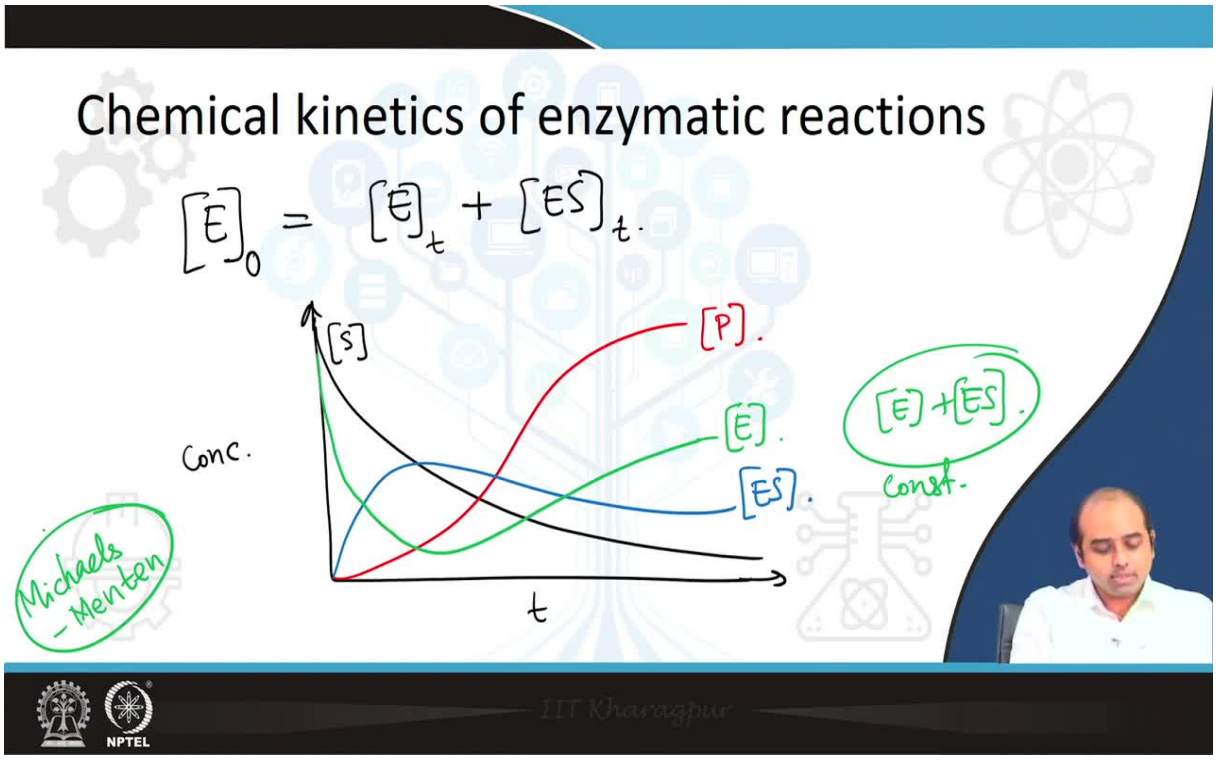

So, from these reaction rates essentially you can if you consider that fast rate of reaction I mean this enzyme substrate complex is at the pseudo steady state and you try to equate that expression of E ES into these equations and from the product you will get you will work out

the Michaelis Menten reaction got this equation, so that is something quite relevant for enzymatic reaction kinetics.

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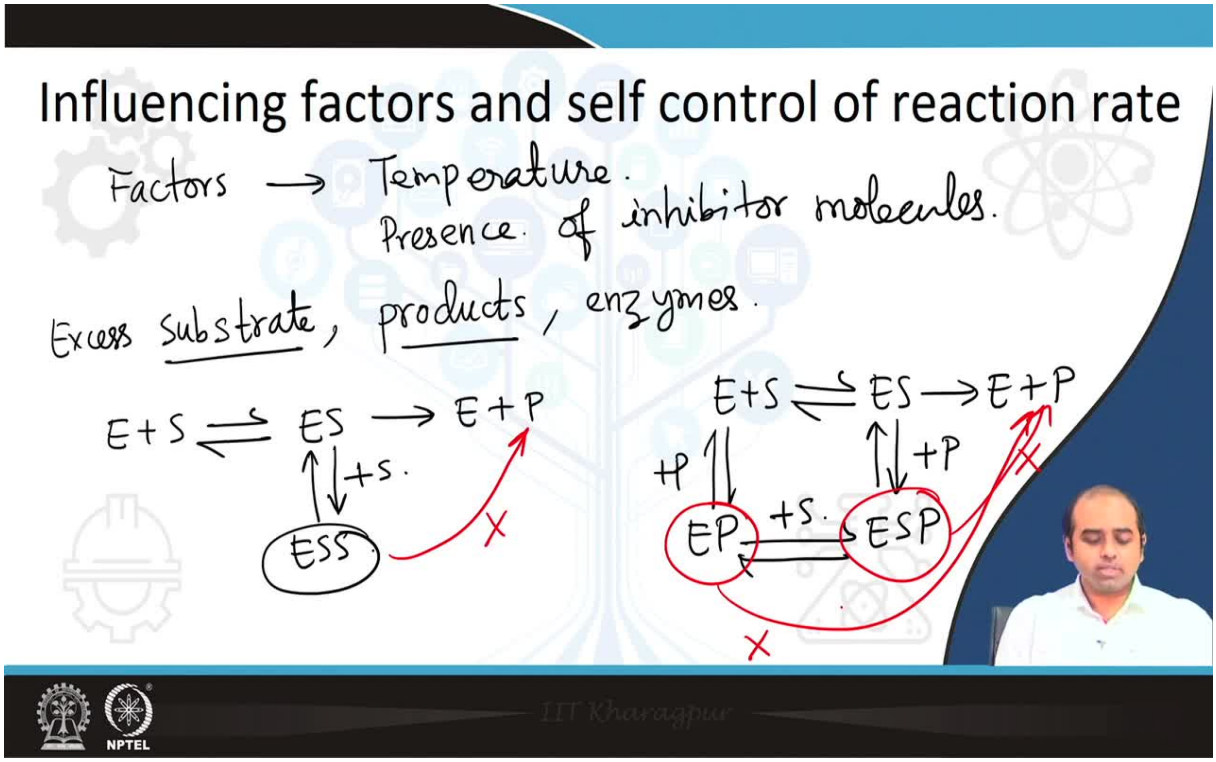

So, now, let us talk about the different influential factors and the control I mean the self-control of the reaction rate. So, the factors which affect our temperature and presence of any inhibitor molecule. So, let me just write them down. So, as far as the factor that is concerned, the factors could be temperature and you know how temperature affects because it affects the rate of the, I mean the reaction kinetic constant as well as presence of inhibitor molecules.

So, all of us know that when you are having food poisoning the toxin present, externally that is ingested is destroys most of the gastrointestinal enzymes and leading to problem gastric problems come into picture or the stomach issues. It is because of these inhibitor molecules that does try to react with these peptides, these enzymes they affect those I mean they destroyed those chemical or this biomolecular reaction.

Temperature when you have fever, you will often encounter that fever is associated with some issues in digestion why because with high temperature these reactions are affected again the enzymatic reactions leading to digestion of the food is again affected so, these are the influencing factors we will talk about the inhibitor molecules in detail, but there are some

self-control of the reaction rate or there are factors which leads to control of the reaction rate and this could be presence of excess substrate.

This is a very nice natural way when there is the excess substrate or if you have excess products or if you have excess enzymes, the reactions are in most cases the reactions are self-control and they do not get out of hand. So, if there is excess substrate does not mean the reaction will happen too fast or there will be a lot of formation of the products.

Similarly, if there is a lot of formation of the products, it does not mean that still new products will be forming. Similarly, if the enzymes are too much, this is not a very common case if the enzymes are too much, then it does not mean that there will be a lot of this reactions will be taking place, but the common ones are the excess substrate and excess products. So, what happens is that in the case of excess substrate, this is the reaction which you have the normal reaction pathway of enzymatic reactions.

Now, if there is excess substrate into the system it generally acts on this enzyme substrate complex and leads to the formation of ESS. And this enzyme substrate complex, there are two substrates will not form the product. So, essentially this is like a natural control, if there is presence of excess substrate in the system, it will bind to this enzyme substrate complex leading to a possibility or I mean leading to a situation where a substrate is consumed, but still the product is not formed. So, excessive substrate may not always produce excessive products, that is a natural control on the process.

Similarly, in the case of the product. So, this is the normal reaction we are having. So, if there is excess product in the system it may happen, I mean it is it is generally the case that this bind to the enzyme and forms this enzyme product complex it can bind here, it can bind here also and it can also bind here. So, if there are excess product in the system, it will again consume the enzyme leading to formation of either enzyme product complex or enzyme substrate product complex. So, in a way that and these complexes will not lead to the formation of the product.

So, that is not possible it is only the enzyme substrate complex will form the product. So, these extra formations or some undesired formations taking place will lead to reduction of the effective product rate. You can also work out the, I mean the associated kinetics I mean the concentration profiles and you will see that if these additional reaction pathways are allowed and which is normally the case this was to have a natural control on the formation of the

product. So, these are generally the likely scenarios which you can encounter which one can encounter when you have excess product or excess substrate in the system.

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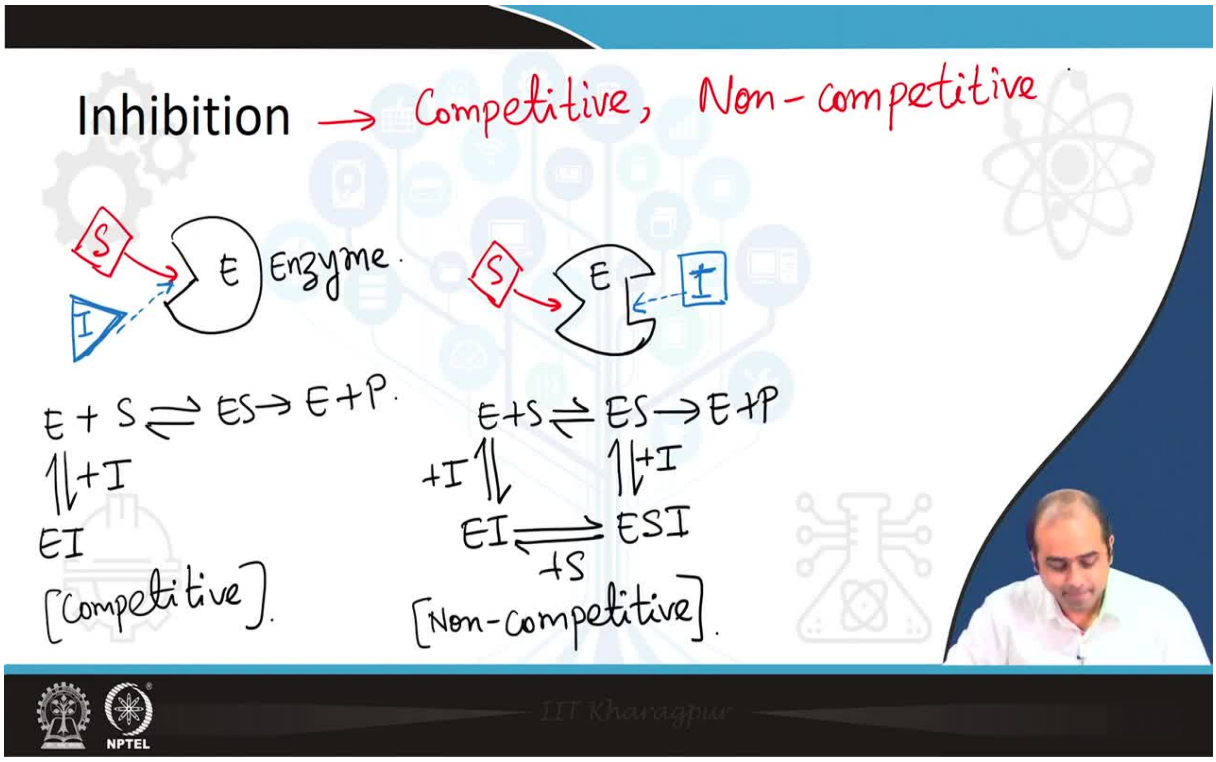

Now, let us talk about the inhibitors so, there are 3 types of inhibition competitive, non-competitive and there is also known as another one is uncompetitive. So, what is competitive reaction I mean inhibition issue so, let us say I have an enzyme something like this, let me draw it properly. Let us say this is the enzyme and to that I have a substrate which can get fixed here but, there is also a possibility of another molecule we call them as inhibitor that can also bind here. So, this is the enzyme and the enzyme can bind with the substrate as well as with the inhibitor and there is only one active site for this attachment.

So, if there is a presence of this inhibitor, so, both the substrate and the inhibitor will compete for the same site. So, the normal reaction whatever we have, there will be competition with this enzyme for the same spot and this will lead to the formation of the enzyme inhibitor complex which of course will not lead to the formation of the product. So, this is competitive inhibition. And this is very common actually.

In the case of noncompetitive mode, let us say this is the enzyme and we have the same substrate which can fit in here but the site there is also another site in the system where

another molecule can also fit in. So, this inhibitor molecule can also fit into another site of this enzyme. So, even though they are fitting in different sites, still this enzyme inhibitor substrate complex if it is formed will not lead to the product. So, the reaction pathway will be altered something like this.

So, this is the normal path so, the enzyme can bind sorry the inhibitors can bind with the enzyme even without the presence of substrate this is a possibility it can also bind with the enzyme substrate complex because they are not competing for the same site, sorry this is ESI. And even after the enzyme inhibitor complex is formed, there is also a possibility that a substrate can join and form enzyme substrate inhibitor complex, but any one of these complex enzyme inhibitors, enzyme substrate inhibitors, these complexes will not lead to the formation of the product. So, this is non-competitive.

So, non-competitive is actually a dangerous situation and this generally happens when there is some structural change to the enzymes I mean the natural way is the competitive enzyme, competitive mode of inhibition where the inhibitor molecules will compete for the same sites, but if there is a possibility of non-competitive inhibition, it does not matter whether substrate is able to bind or not, the inhibitor can bind to the enzyme with or without the presence of the substrate and that will destroy the product formation. Uncompetitive is generally not very common and that is something let us not talk about them because that is not a very common scenario.

So, it is mostly the non-competitive and the competitive modes of inhibition, which plays a big role in the enzymatic reaction pathways. So, of course, presence of these inhibitors will trigger additional reaction pathways and it will also reduce I mean delay the product formation and it will also reduce the product formation a lot of substrate will go to unnecessary or undesirable formation of the undesirable complexes which will not lead to the formation of the product. So, this is generally the mode of inhibitor actions which influences the enzymatic reactions significantly.

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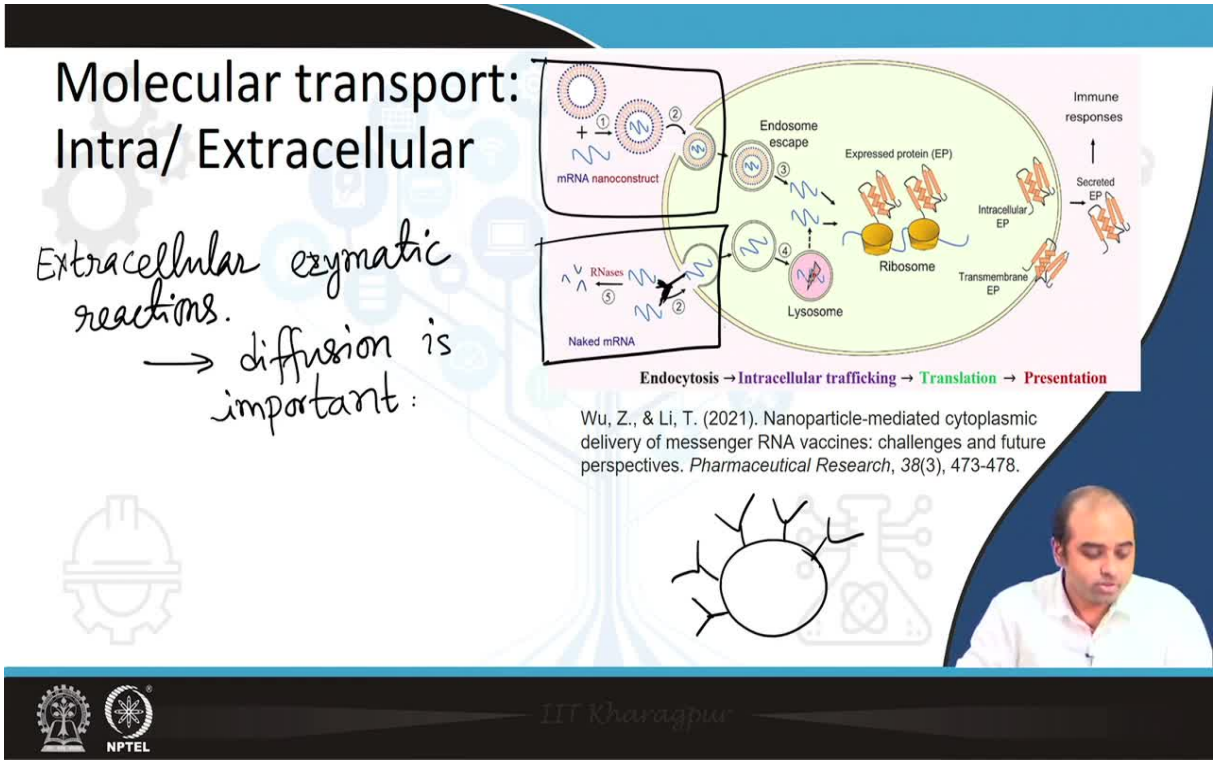

Now, what about intra and extracellular reactions most of these enzymatic reactions as you know take place either inside of the cells or outside of the cells both are quite likely scenarios. So, if the reactions I mean these enzymatic reactions happen inside the cell. So, it is quite likely that these substrates are mostly uniformly distributed inside the cellular space or the cytoplasm of their cells.

But if you are talking about the extracellular reactions, in that case, that diffusion of the substrate to the site of the cell where the enzymes are present for example, if you see this part, so, see this part, so, this whatever this RNA etc., is coming towards the enzyme this is getting introduced into the cell.

So, this process of getting attached to the cell wall, getting attached to the cell wall, this process or this process will only happen when this RNA diffuses close to the cell. So, all extracellular enzymatic processes or enzymatic reactions. So, in this picture, it tries to show that how this RNA tries to penetrate through the cell wall and goes into the cytoplasm and then it is reacting and something else is happening, but it is also, I mean how this RNAs will get attached to the cell wall is also question.

So, generally there are some sort of receptors, which are present on the cell walls which bind to these RNAs or whatever these biomolecules and then they get introduced inside. So, there

is a sort of enzymatic reaction of this attachment of the molecule onto the cell walls or even without that.

Even it is also possible that on the cellular surface you have this what is called these receptors and there is ligand receptor reaction taking place. So, these ligands will react to these receptors or will react on the cell wall where some enzymes are present, this is the extracellular enzymatic reactions. So, in any extracellular enzymatic reactions, diffusion is important.

So, without diffusion it is not possible for these extracellular reactions to happen. For the intracellular case where the substrate may be generally dispersed well or homogeneously in the cytoplasm of the system, the diffusion may not be so significant, but all extracellular reactions does depend on the diffusion.

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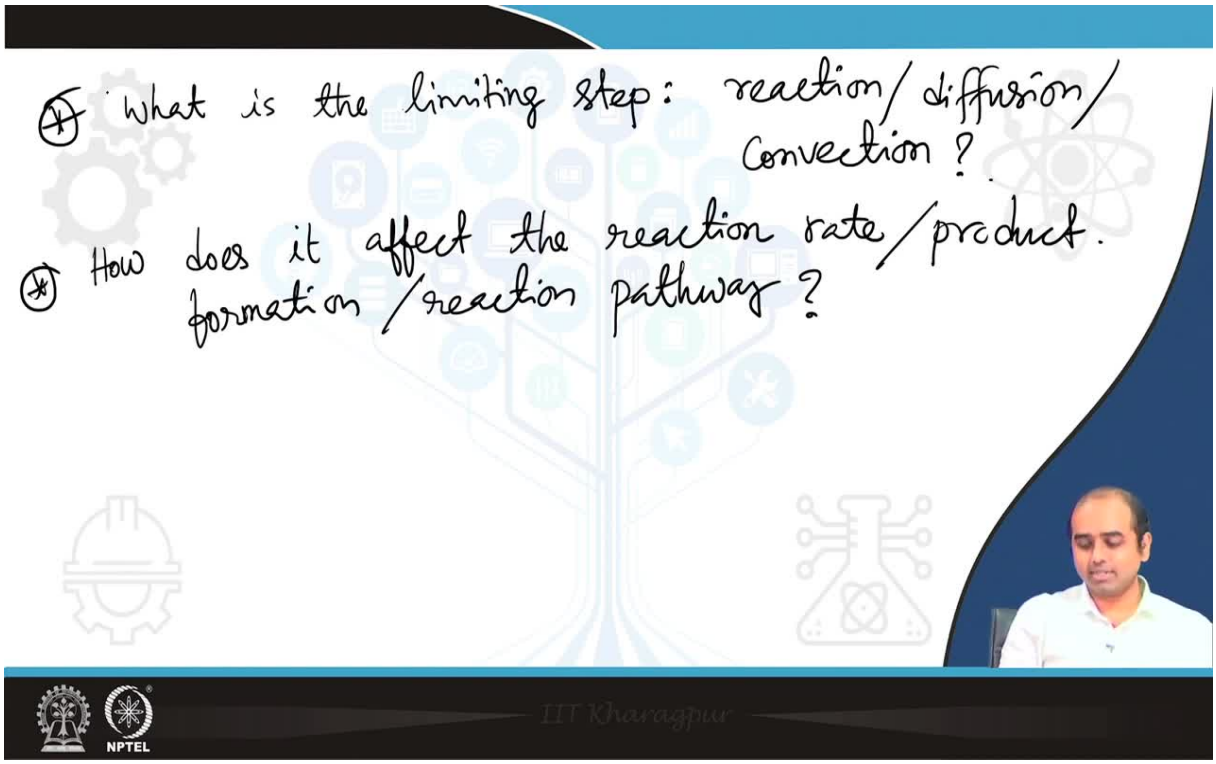

So, the key question to ask in I mean generally the case for extracellular reactions that what is the limiting step even if diffusion is present is the dynamics of diffusion fast or small, I mean fast or slow. So, what is the limiting step? Reaction, diffusion or to some extent outside the cell there could be possibility of the presence of convection and the next question that it affects that how does it affect I mean how does this mass transfer affect the reaction rate or the product formation or the reaction pathway.

By reaction pathway I mean that let us say if there is some inhibitor present in the system, but the diffusion of these inhibitors is very slow or the mass transfer of these inhibitors is very slow. So, even though the inhibitors are present there is quite, I mean there is a unlikely possibility that these inhibitors will influence the enzymatic reactions. So, these are the important questions for which this apart from modeling the reaction steps or the reaction dynamics it is also very important to consider the mass transfer in this process.

So, we will not talk about more into these situations that is more of transport in physiological systems you can see there is this book by there is a transport phenomenon book on physiological system and where you can find several of such situations where you take into account of mass transfer in cellular reactions or enzymatic reactions.

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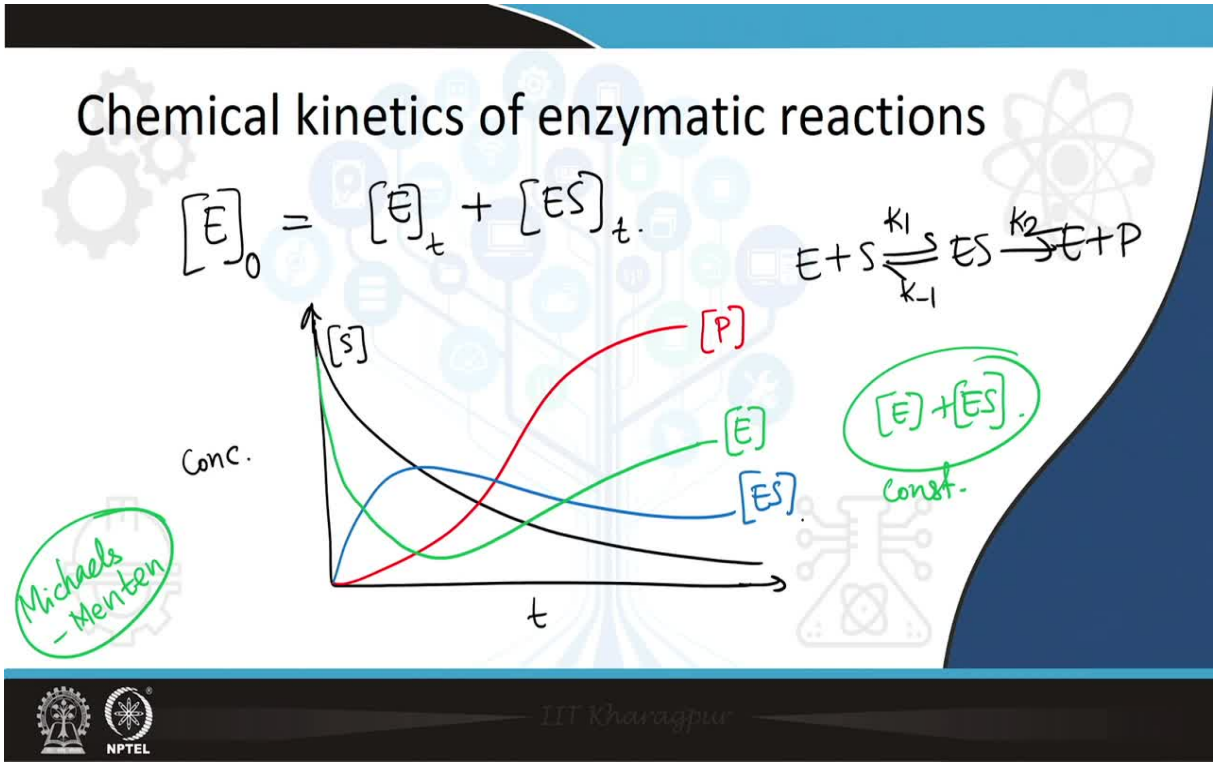

But one of the things that I must also highlight here going back that what is the reaction I mean this profile as I was talking about is something that we generally get by solving the reaction kinetic equations. But it is also possible that from the concentration profiles of the possible species in the system, you can develop the reaction pathway.

So, let us say there is a like multi step reactions, several steps the reactions in happening and you do not know what is the reaction pathway, but you can calculate or you can obtain the

concentration profiles of all most of the involved species, then you can actually fit, you can actually fit these reaction profiles back to the proposed reaction pathway.

So, what I mean is that based on the reaction pathway you can develop the corresponding rate expressions or the rate equations and unknowns would be the kinetic constant. So, from the concentration profile, you can find out what could be the possible values of these kinetic constants for a particular proposed reaction pathway and based on these proposed reaction pathways for which you find that and there is a non-zero value of these rate constant or the rate constant are not small, it suggests that these are the possible reaction steps in the system.

And this is how a biomolecular reaction pathway is often explored or is devised because you do not, so see in a biomolecular reaction a biologist or a biochemist will be able to track the products or the species and how its concentration is changing and he or she may not be able to even calculate most of the species, if there is a complex multi-step bio chemical reaction taking place involving almost like 20 or 25 species, one may not be able to calculate the ultra-low concentration of some of the species but at least the major species can be tracked.

So, from the knowledge of these concentration profiles you have, I mean how do you propose the reaction pathway? So, you consider that these are the possible reactions. Like for example, in this case the possible reaction I mean the reaction that we have considered is E plus S, ES giving E plus P so, this is just 2 step reaction involving 3 kinetic constants.

So, if you know these 4 species profiles, I can write down the dynamic equation or the rate constant for these all of these individual species and then from there I can find out from the reaction profiles what are the values of these kinetic constant and if I find that some of these kinetic constants are not small or if they are small, then we can understand that that reaction is not a likely possibility in this case.

So, in the similar way if the if you track the concentration profiles of several of these species, several species present in the system and then let us say you propose a reaction scheme and then from there you try to work out the kinetic constants. So, from the profile or this data of the concentration versus time of the individual species, you can fit these rate constants and then you see that what is the magnitude of these rate constants which plays, I mean, which has a considerable magnitude and then you consider that particular reaction is definitely happening in the system.

So, like that the reaction, the different reactions schematics or reaction pathways are actually explored and new discoveries are made about the possible reaction pathways. So, generally the tracking of the intermediaries is difficult. So, even you can have multiple intermediates in the system, where at least the major species can easily be tracked and from there you can get a fair idea about the reaction pathway or the possible reaction pathway in the system.

And that is how leads to the discovery of reaction schemes and the presence of the important reaction intermediaries in the system. Thank you. I hope all of you found this enzymatic reaction to be very exciting. And we will definitely have an assignment problem based on this multi-step enzymatic reaction pathway. Thank you.