Material and Energy Balances
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Module No # 05
Lecture No # 21
Biochemical Reactions: Enzyme Kinetics

Hello everybody welcome today's lecture on biochemical reactions in the previous lectures we talked about a specialized type of reaction which are called the combustion reaction. Today we will talk about biochemical reaction which are specifically useful for people working on biotechnology and bio – process industries. So the start of the lecture we will discuss what enzyme reaction are and how we can used them for performing materials balances with respect to enzyme reactions.

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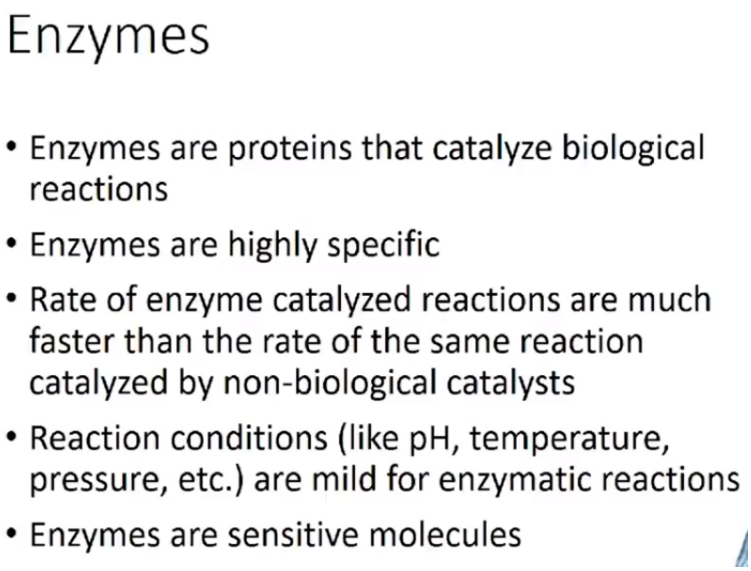

What are enzymes? Enzymes are proteins that catalyze biological reaction these enzymes are very highly specific and these enzymes can actually increase the rate of reaction very significantly the rate of reaction enzyme catalyze reactions are usually much higher than the rate of the same reactions which can be catalyst by non-biological catalyst.

The reaction condition which is used for an enzymatic reaction the condition which is PH temperature are usually mild because you do not want the enzyme to get denatured. The enzyme

are very sensitive because extreme condition and temperature of PH can easily damage or denature of this enzymes and for this reaction most of the times reaction with respect to enzymes happen at mild condition which are closer to natural conditions.

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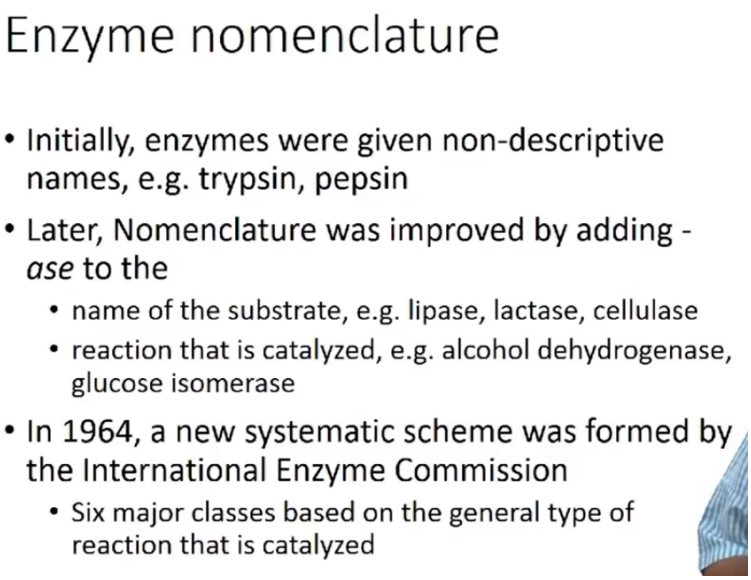

Enzymes are named with the certain specific method so initially enzymes were given non descriptive names so just trypsin, pepsin etc., And over a period of time people realize this is not a useful way of enzymes so they started having new nomenclature where they added ase to the either the substrate or the reaction that is being catalyze by the enzyme.

So this gave the little more clarity on enzyme did and example like lipase, lactase and cellulose you know that it is acting on lipase or lactase or cellulose if the name was in alcohol or dehydrogenase or glucose isomerase. We knew that it perform the dehydrogenase or isomeriase reaction this gave some information about what the enzymes were. This was further improved in 1964 in a new systematic scheme was formed by the international enzyme commission.

So the enzymes where classified under 6 major categories and within that there was sub classifications you will not go into sub classifications but we will give the 6 major classification which are defined based on the reaction they catalyzed.

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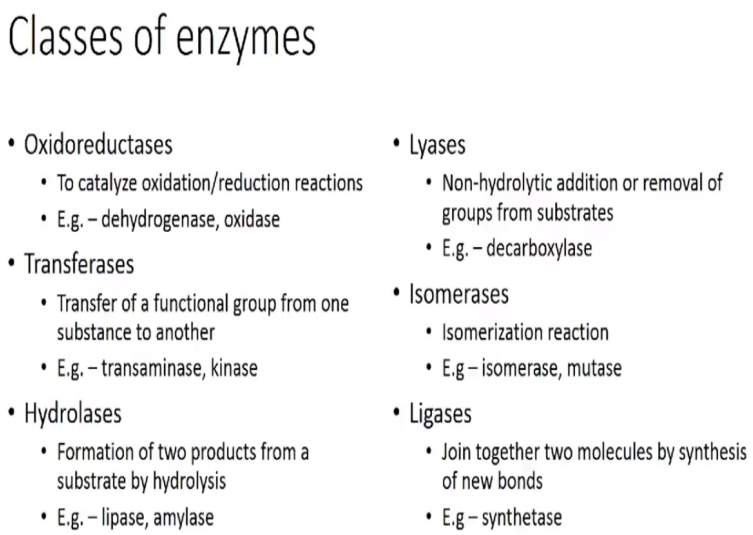

So the first would be oxide-reductases these enzymes catalyzed oxidation and reduction reactions example would be dehydrogenate or oxidase. You also have transferees which are basically enzymes which helps in transferring the functional group from one substance to another example would be kinase and transaminase and so on. You have hydrolases which form to product from a sub state from hydrolases reactions.

Lipase and amylase are examples where hydrolysis happens Lyases or enzymes which basically catalyze non- hydrolytic addition or removal or groups from the substance example would be decarboxylase. You have isomerases which catalyze the isomerization reaction isomerase mutase are examples and you have ligases which catalyze action with join two molecules by formation of new bonds to form a final product example would be a synthetase.

So these are the major six classes of enzymes and within this there are many subdivisions we will not get into the details of that those are covered in basic biochemistry course if you interested please refer to some fundamental bio chemistry book which will give you a better classification and give you all the details about the subclasses for enzyme classification.

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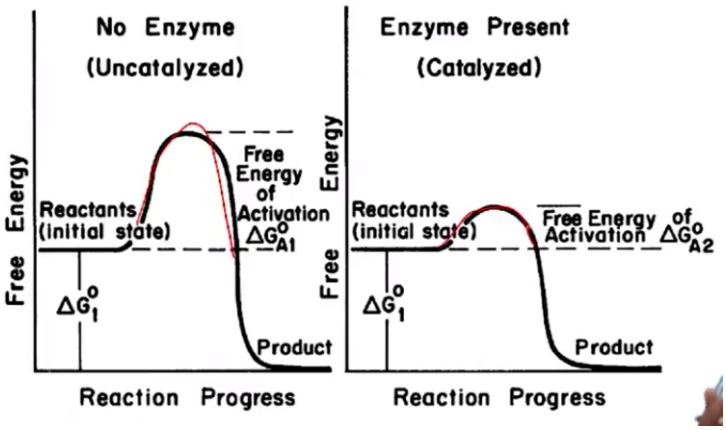
How do enzymes work?



How do enzymes work enzymes are like any other catalyst so what do catalyst go in general you have the free energy of reactants and the free energy of the products for the reactants to be converted from the free energy state to the product free energy state they actually have to go through a hill which is shown here this hill is the activation energy which the reactant have to have so that it get converted to form the product.

So when the enzyme is present or any catalyst is present this activation energy is reduced so you see in the enzyme catalyze reaction the hill is smaller this gives you the lesser activation energy thereby making the reaction more spontaneous than what it would have been if no catalyze or enzyme was present so this is how enzymes work.

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Simple enzyme kinetics

- Substrate (S) is converted to product (P) in the presence of an enzyme (E)

$$S \xrightarrow{E} P$$

- The rate of reaction (v) can be given in terms of change in concentrations of the substrate ([S]) or the product ([P])

$$v = -\frac{d[S]}{dt}$$

$$v = \frac{d[P]}{dt}$$

Now when we perform enzyme based reaction we have to understand how the kinetics of enzyme works. So there are some simple kinetic models which help us to understand how enzymatic reaction happen. So let us assume this particular simple reaction where substrate is converted to a product in the presence of the enzyme so the simplest reaction equation for the would be S gives P in the presence of E and the enzyme.

So the rate of reaction V for this can be given in terms of the change in concentration of the substrate or the product. So that would be given mathematically as V = - DS DT or V = DP DT well – DS DT is the rate of consumption of substrate S which respect to time and with DP DT would be the rate at which the product it is produced with respect to time.

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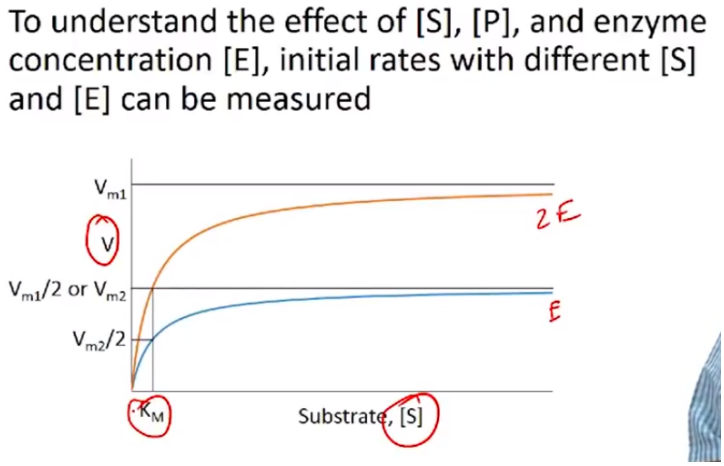

So when you actually try to understand the effect of substrate concentration product concentration of enzyme concentration you get this particular set what you have here is results from multiple experiment where different concentration of substrate and enzyme where used and the initial rate of there was measured. So this was plotted as initial rate V versus substrate concentration S.

So this particular plot follows the saturation kinetics as I can see here you have two different curves one for an enzyme concentration E and other one is for enzyme concentration of twice of E. So what you see here is as the concentration of enzyme increase the maximum rate which can happen as actually increased personally. So let us look at what are the parameters here other than the maximum rate which is VM we also have another term called KM.

KM is a constant which is the dissociation constant so KM gives us an understanding of the affinity of the enzyme to the substrate so lower the KM higher is the affinity.

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Simple enzyme kinetics

- When [S] is low, reaction rate is proportional to [S] (first order)
- When [S] is high, reaction rate does not depend on [S] (zero order)
- Maximum reaction rate (v_{max}) is proportional to [E]
- Enzyme reaction can be written as

$$E + S \underset{k_{-1}}{\overset{k_1}{\rightleftharpoons}} ES \xrightarrow{k_2} E + P$$

If you were to look at this particular setup what we see is when the substrate concentration is low the reaction rate is proportional to the substrate concentration which is what you see in the initial phase where it is almost a straight line in the initial stage when the substrate concentration is low as the substrate concentration increases what you see is the reaction rate does not depend on the substrate concentration any mole which is what you see here.

So what you see here the reaction rate has not changed significantly although the substrate concentration has been increasing tremendously this is because of the 0 order reaction when substrate is excess. And you also see that the maximum reaction rate V max or VM is proportional to the enzyme concentration. So why does the reaction follow this kind of set up so what you usually have is the enzyme reaction has given here.

Enzyme and substrate react to form the enzyme substrate complex using a reversible reaction and this then ends up producing the enzyme from the product. So the product leaves and the enzyme remains in it native state. So this is the enzymatic reaction so when the substrate is present at low concentrations then the number of actives sides which is present in the enzyme becomes the limiting factor.

So you have all the substrates attached to an active side and as you increase the substrate concentration more substrate attaches to the substrate side thereby increasing the rate of the reaction proportionally to the substrate concentration giving you a first order reaction. However

when the substrate concentration exceeds certain number what will happen is all the active sides happen in the enzyme are occupied with substrate molecule.

This means adding more substrate will not hasen the rate of the reaction so that is when rate of the reaction reaches the maximum which is V max or VM. So now to increase the rate of the reaction after all the active sides are filled with the substrate molecule we can add more enzyme thereby increasing the number of active science as the active sides increase you would have the rate of reaction also increasing thereby the maximum velocity or the maximum rate also increases.

So this explains why we see a first order reaction when the substrate concentration is low and a 0 order reaction the substrate concentration is high and we see a increase in V max or VM when we increase the enzyme concentration.

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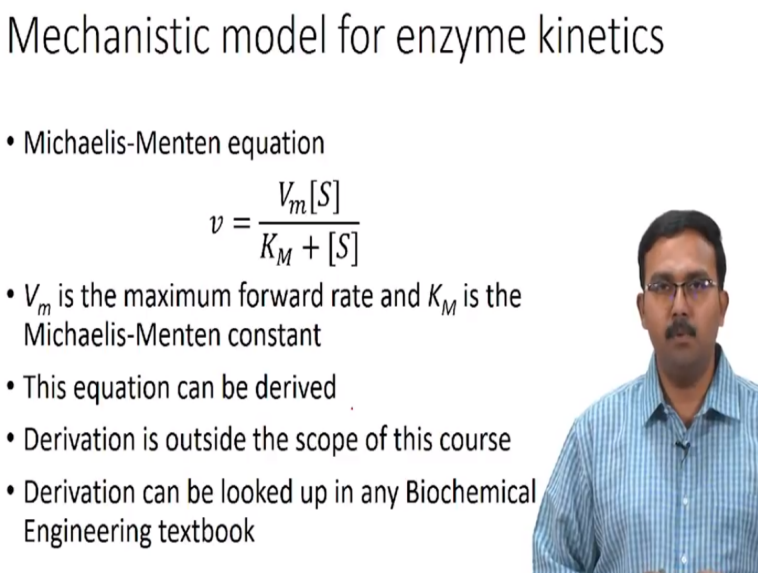

So from this equation we can derive a mathematical model called as the MICHAELICS MENTEN equation this is a mechanistic model for enzyme kinetics and the equations is written down as V = VMS divided by KM + S so the terms V represents the velocity or the rate VM represents the maximum rate it can happen for the reaction and S represents substrate concentration KM is the MICHAELICS MENTEN constant.

So among these things MICHAELICS MENTEN constant as I mentioned the represent the dissociation constant and thereby talks about the affinity of the reaction to the substrate and you also have VM which accounts for the enzyme concentration so VM as enzyme concentration or initial enzyme concentration in builds into it and VM is proportionally varying with respect to enzyme.

So this can actually be derived however the derivation for this equation is outside the scope if this course you can look up any biochemical engineering text book where would be able to see the derivation and you can go through them for your own exercise.

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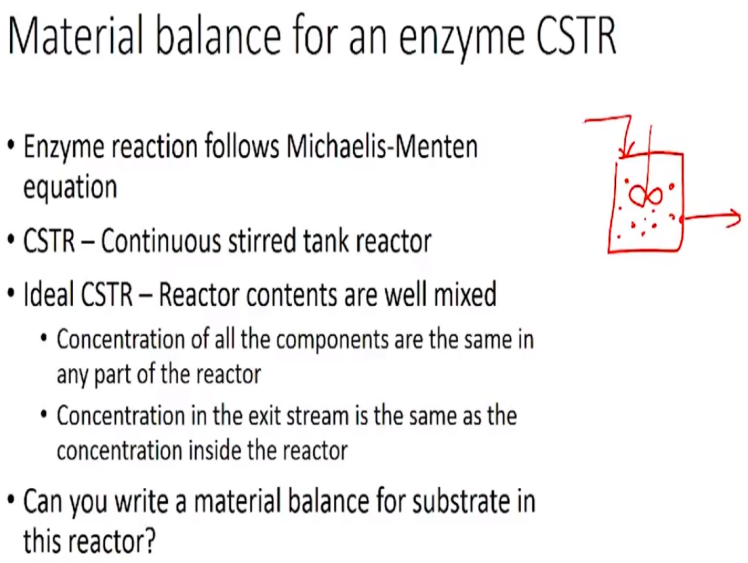

Now from our material balance perspective let us consider a reactor where enzymatic reactor is happening so we will account for CSTR which is a continuous third tank reactor in which an enzyme reaction that follows and MICHAELICS MENTEN and kinetics is happening. So we will assume that it is an ideal CSTR which means the reacting contents are well mixed.

This means the concentration of all the component are the same for the any part of the reactor this would mean that the concentration in the exit stream the same as the concentration inside reactor. So under this ideal condition what will happen is any feed which is coming in will get instantaneously mixed well inside the reactor thereby you will have uniform concentration throughout the reactor as you are taking it out in the exit stream.

The concentration in the exit stream will be equal to the concentration which is present at the exact outlet point inside the reactor which will be the same as the concentration all over the reactor thereby giving you a uniform concentration in your exit stream and your reactor so let me explain to you with the diagram as you see here this would be a simple representation for CSTR.

If I have an ideal CSTR the concentration of a component would be same on all these points we have marked that is because it is a well-mixed reactor. So this would also mean the concentration at this point which is where the exit is being drawn would also have the same concentration as the concentration present anywhere in the reactor as this concentration is same as what you see in any part of the reactor.

The concentration of exit stream will also be the same as the concentration in the inside the reactor. However the concentration in the inlet can be different as soon as it enters into the reactor it will get well mixed and you will have uniform concentration inside the reactor so this is what it is an ideal CSTR represents. Now can you write a material for substrate for this reactor let us try to do that.

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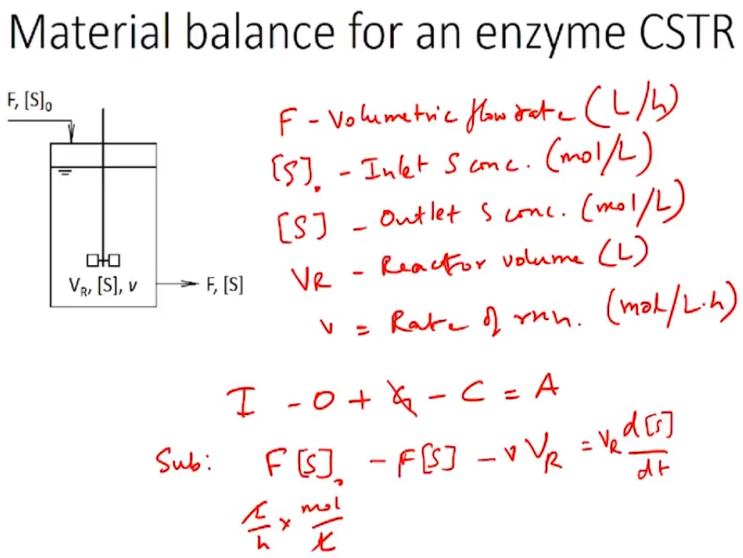

So this represents a CSTR with all the parameters listed now so here you have F which is volumetric flow rate so which would be in terms of liters per hour and you would have S naught which is the inlet substrate concentrate this can be in terms of moles per liter or grams per liter and you have S which is the outlet substrate concentration again in terms of moles per liter and

you have VR which is the reactor volume which would be in terms of liters and V represents the rate of the reactions and the units would be moles per liter per hour.

Now that we have all these parameters let us start writing the balance equation for the substrate we start with input – output + generation – consumption = accumulation for the time being let us not assume steady state will start writing on the equation and then we will ahh cancel out terms which can be removed at steady state conditions. So we are writing for the substrate balance so writing for the reactants which means there is no generation term you only have a consumption term.

So you can have generation term so the input would be F times S naught so this give us the unit of liters per hour times moles per liter giving us moles per hour so the balance equation we are writing is for moles per hour – F times S which is the substrate concentration in outlet stream and these two will be of same units now we have to write the consumption term so we know the rate of consumption which is VE.

So this rate of consumption is given in terms of moles per liter hour and we need the term to be in liters per hour and this rate is with respect to the volume of the reactor. So the number of moles of the reactor which is consumed per liter of the reactor volume per hour is the rate of reaction given so to get the total number of moles would be consumed per hour we have to multiply this velocity or rate with the volume of reactor which is VR.

This would be equal to the accumulation which would be DSDT rate of change of subset concentration times VR which should be the volume giving us the rate of change of substrate amount with respect to time. So this would be the balance equation we start with.

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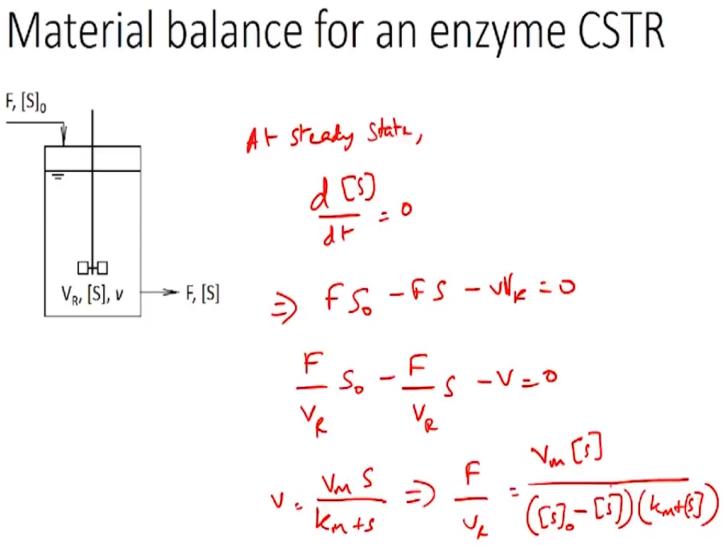

So from here we can assume certain condition such as steady state and thereby at steady state you have DSDT = 0 so this implies the equation becomes S naught – FS – VVR = 0. So dividing the entire equation by volume of the reactor we get F by VR times F naught – F by VR times S – V = 0. From the MICHAELICS MENTEN equation we know that V = VMS divided by KM + S.

So we can substitute this here we get F / VR = VM times S which is substrate concentration and leaving divided by substrate concentration inlet S naught – S times KM + S. So this equation can be derived using material balance equation.

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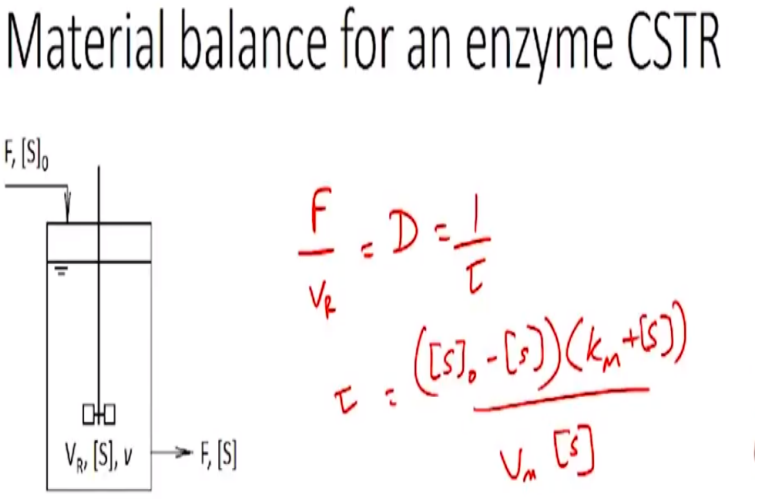

So now we have the definition for F by VR as dilution rate so this is a term which is usually used for understanding and designing reactors so this dilution rate is equal to 1. tou which is the residence time inside the reactor. So we can calculate the residence time Tou as S naught – S times KM + S divided by VM times S. So using this equation we can calculate the residence time for the reactor and also we can calculate the volume of the reactor to design the CSTR for the required operations.

Using this equations we can calculate Tau which is the residence times in the reactor and also VR which is volume of the reactor thereby help in designing the reactor. So the simple material balance can help us in designing the volume of the reactor which would be required for getting a certain level of conversion. Now let us test our understanding using an example problem.

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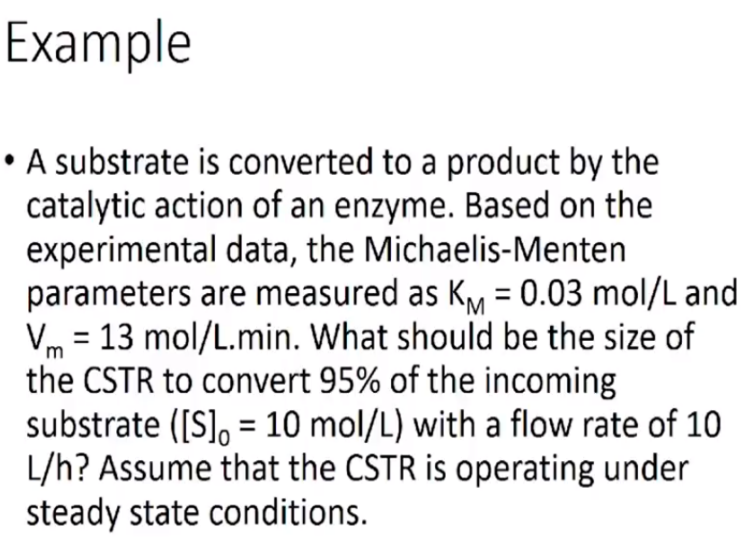

So here is an example problem here is the example problem a substrate is converted to product by the catalytic action of the enzyme. Based on the experimental data the MICHEALIS-MENTEN parameters are measured as KM = 0.30 moles per liter and VM = 13 moles per liter minute. What should be the size of CSTR to convert 95% of the incoming substrate.

So let us assume the inter substrate concentration is 10 moles per liter with a flow rate of 10 liters per hour. Assume that CSTR is operating under steady state conditions let us go and solve this problem as we solve any other material balance problem.

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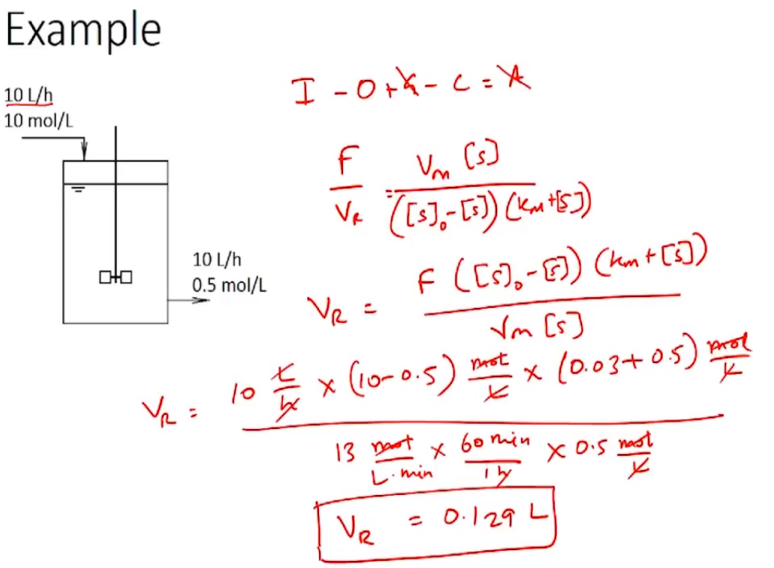

So the first step would be to identify the basis so here we have been 10 liter per hour is the inlet feed of the subset so we will take that as the basis with that we can write material balances which would be input – output + generation – consumption = accumulation at steady state there is no accumulation as we are writing the balance for the substrate the generation would also be 0.

So this equation will come down to the same equation which we have derived thereby we will get F by VR as V max or VM times S divided by S naught – S times KM + S. So now what we have this equation we have that calculation VR so VR or the volume of the reactor would be F times S naught – S times KM + S divided by VM times S. So let us write down all these values VR = 10 liters per hour times 10 – 0.5.

So we have 95% conversion which means only 0.5 moles per liter of the substrate is leaving the system so you have the final substrate concentration of 0.5 moles per liter this would be moles per liter times 0.03 K which is he value for KM which is 0.5 which is the S value which would also be in terms of moles per liter divided by V max which is 13 moles per liter minute so we have the flow rates in terms of hours.

So we will convert this to hours by 60 minutes per hour times 0.5 moles per liter so now these liters cancel these moles cancel off this moles also cancel of and we have these two liters getting cancelled off. So finally we get a dimension of liter which would mean the volume can be calculating from here as ER = 0.129 liters so this would be the volume of the reactor which is required for 95% conversion of this substrate which is being fed.

With this we come to the conclusion of the example on enzymatic reaction so in the next lecture we will talk about another class of bio chemical reactions which is self grow until then thank you and good bye.