Biology for Engineers and Other Non-Biologists
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Week- 02
Lecture - 08
Biomolecules: Enzymes

Welcome. We are discussing biomolecules and their relationship to cell structure and function. We have our own stories through which we pick up these things; we have had a few stories so far. If you, the main messages from the story so far has been that there is there are microorganisms and they (are) they can be categorised into various different domains.

The major biomolecules, I keep mentioning this although I haven't told you everything so far, the major biomolecules are four, we first saw lipids which are water insoluble substances that are soluble in organic solvents, (reasonably) reasonably vague definition but that's what it is, we saw some examples of it. And then we also said that lipids form an important component of the membrane, of the cell membrane, cell membrane is an important cell envelope; some cells have only a cell membrane to distinguish themselves from the environment, some cells have a cell membrane and on top of that a cell wall too, and maybe there are other layers, that we're not talking about now, (we're) let's not get into that.

The nature of the lipid itself makes it possible for lipids to act as or lipid bilayers to act as effective envelopes between the cell and their surroundings. I probably briefly mentioned that in the passing earlier, let me talk a little bit about that and then go further. The, if you look at the way the lipids have organised themselves in the membrane there are let's go here it might be a better picture; there are lipid bilayers.

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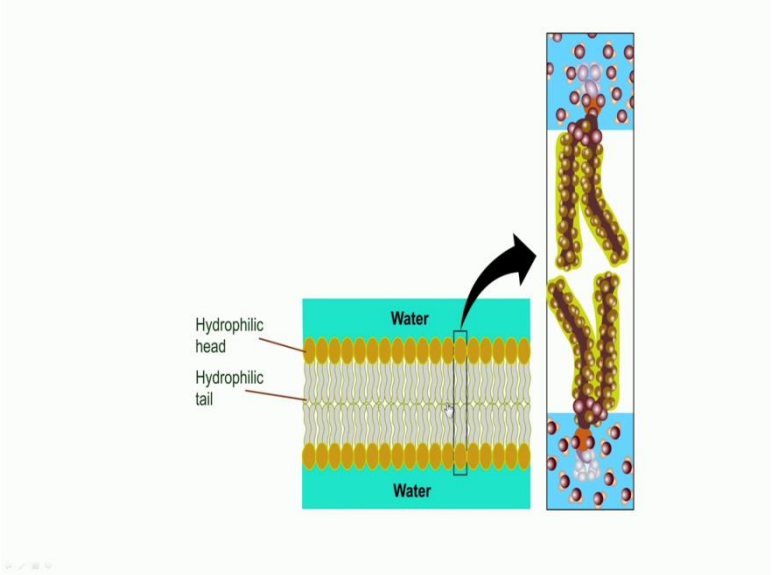

This is water may be extracellular. This is water intracellular, right? And this is membrane in three-dimensions, separates the intracellular the extracellular. If you look at the orientation of the lipids here the hydrophilic heads are oriented towards water naturally because the hydrophilic means water loving and they would like to be oriented towards water. And hydrophobic like likes like therefore they orient towards each other and by their very nature there is a hydrophilic layer here, there is a hydrophobic core here, and a hydrophilic layer here, separating the intracellular from the extracellular parts.

And this bilayer can self-assemble, nobody needs to put in any energy to assemble these layers by the very nature of their molecules, (the the) here is water, here is water and therefore the water loving parts will orient towards water and the water heating parts, the hydrophobic parts, this is hydrophobic here, hydrophobic part will come together here. If we think about it, this forms an effective barrier to the cell from its surroundings. Substances cannot very easily pass through the membrane; molecules cannot very easily pass through the membrane because of the nature of the membrane.

Here you have water loving water loving so that's okay. But when water loving molecule comes here it needs to pass through a water heating core and the rates of pass through the membrane would be slowed down significantly. In a typical cell, there are actually proteins, you know the (the), cell membrane is fluid mosaic of these lipids and proteins, some of those proteins actually function to transfer substances from the outside to the inside, glucose goes from outside the cell to the inside the cell, through some (some) such proteins and many other

things have transporters that make it possible for such substances to move from the outside of the cell to the inside of the cell at reasonable rates.

So by the very nature of these molecules they act as effective barriers to outside components and they protect the cell. So this is one of the important structure function relationships compared to lipid. We have already seen that proteins, amino acids, polymers of amino acids are proteins and they are a large class of important biomolecules in the cell and there is a structure function relationship , one of which we have seen.

By the very nature of amino acids and the polymerisation of amino acids into proteins, different parts of the proteinaceous chain would attract depending on the side groups that are there and their interactions with water, all these combined, there could be interactions between molecules that are on different parts of the chain. Because of that the protein molecule folds, and because of the proper folding the protein molecule is able to act as an enzyme or even carry out its other functions, non-enzymatic functions and so on. So by the very nature of the polymers of amino acids they get their function.

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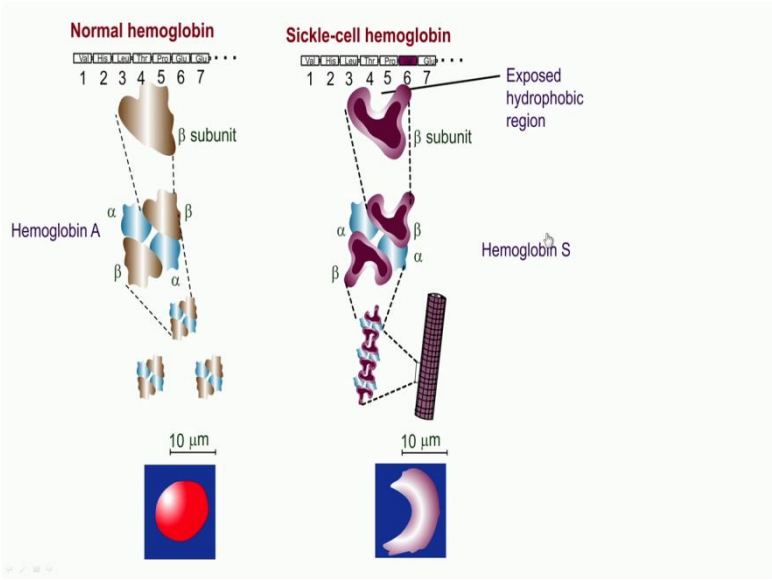

And if the appropriate folding of the haemoglobin chain leads to the haemoglobin protein when it folds properly then it is able to carry oxygen and the red blood cells looks is nice and clean like this disc shaped structure, whereas if in the sixth position the glutamic acid gets changed to valine for some reason, then the structure entirely goes out of proper orientation

and it is no longer able to carry oxygen properly, not just that it makes the shape of the red blood cells sickle shaped, and we get (sickle cell) sickle cell anaemia because of this.

So there is a good structure function relationship between these molecules. We have so far seen lipids, carbohydrates, carbohydrates as you know are intermediates in metabolism, they are a large energy stores and so on so forth. We will look at that in in some detail later in the lectures. And then of course proteins which (are) which form very many different functions in the cell because of the nature of the structure.

Having said these I think in the last class we stopped here the structure function relationship. Let's take things further from here in this lecture. Let us, okay, we will take a side story now, okay? We are still in the major story of curd making, we are asking different questions and trying to answer them. As a part of answering them, we are uncovering very fundamental aspects of biology, biological molecules and so on.

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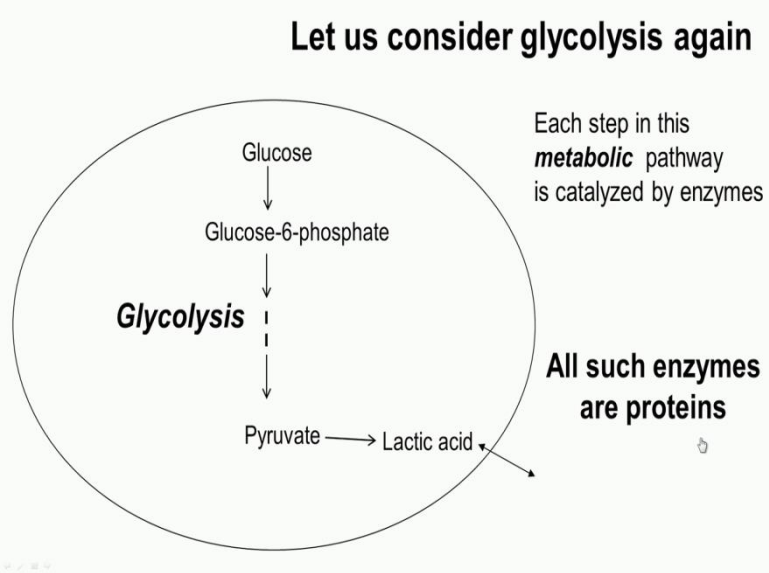

Now our side story here is glycolysis again, you know, we know that glucose going to pyruvate through glucose 6-phosphate (glucose 6-phosphate) 6-phosphate and so on so forth, is called glycolysis, these set of reactions and the curd formation upon because of the acid, the lactic acid that is produced from pyruvate through a couple of steps, gets released into the medium and the pH (we) we will get to that (and) and this causes curd formation, right?

If we look at this glycolysis, focus on glycolysis, all these are carbohydrates, fine. How are the conversion from one carbohydrate to another, glucose to glucose 6-phosphate, glucose 6-phosphate to fructose 6-phosphate and so on so forth. Each step is, of this so called metabolic

pathway, glycolysis, is catalysed by enzymes, okay? Each of these steps is made possible at the temperature of the cell because of (catalyst) catalyst called enzymes, and all such enzymes that we're concerned with, are proteins.

There are other molecules in the cell that have enzymatic activities such as RNA, we will keep that aside for the time being, we'll just focus on the majority of enzymes in the cell in terms of (glycolysis) in terms of metabolic pathways and they are all proteins.

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Proteins play huge roles in every cell and they make the various functions of the human body possible, okay? I would like you to watch this particular video if you look at the number here, it is number 14, role of enzymes in the human body.

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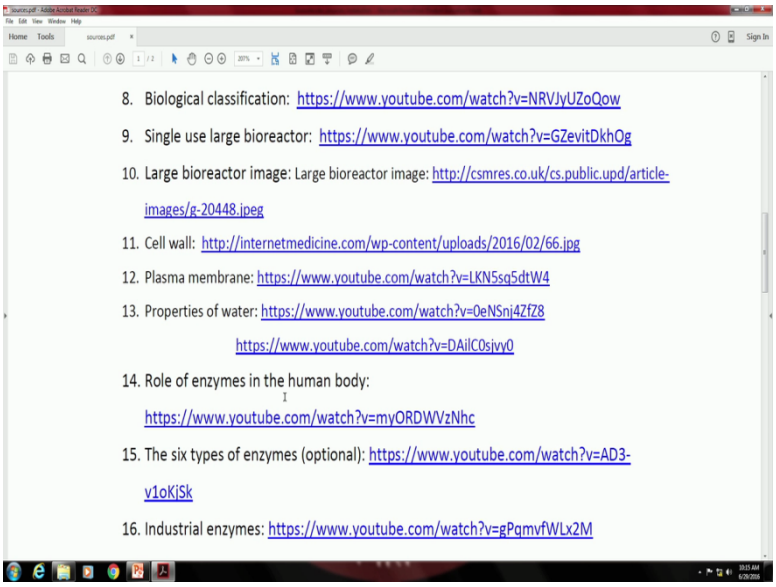

It's a nice shot, let's say 3 to 4 minute video, I think. if you watch that you will get an idea as to the variety of functions that enzymes perform in the human cell and different human cells put together as the human body, and the why humans do what they do, the biochemical basis is all given (is) is all made possible because of the enzymes, biochemical basis is the enzymes. So please take a look at this video.

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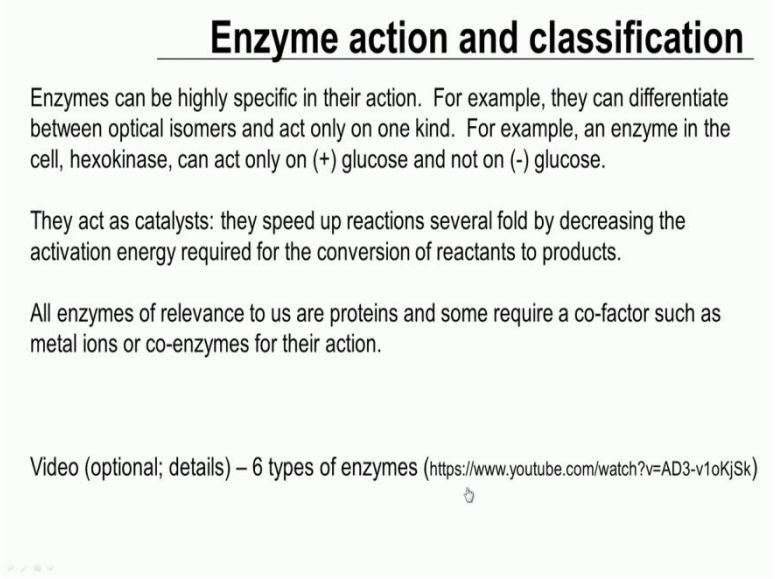

Let us talk a little bit about these enzymes which are specialised proteins or a class of proteins. Enzymes can be highly specific in their action, and that's what gives it its power. For example, they can differentiate between optical isomers and act on only one kind of

optical isomer, okay? the enzyme hexokinase, that's the name of the enzyme, it can act only on (plus) glucose and not on (minus) glucose, you know, D-glucose, L-glucose, dextro (rotary) rotary, leavo rotary, dextro rotary, leavo rotary and so on .

The hexokinase can distinguish between these two, it will act only on (plus) glucose it will not act on (minus) glucose. It can be that specific. And by the way, in nature in the cell are D-carbohydrates and L-amino acids naturally occurring. So L-carbohydrates and D-amino acids are not natural, usually, okay? There are always exceptions, usually you can consider this. So they are very specific in their action.

They act as catalysts, you all know what a catalyst does it speeds up the rate of the reaction several fold at that temperature pressure condition and thereby makes the reaction possible. Here, say, they speed of reactions several fold by decreasing the activation energy required for the conversion of reactants to products, (you know), you all might remember the reaction coordinate here, the (energy) energy coordinate here, the reactants are here, the products are here at a lower energy level, there is an activation energy that needs to be crossed for this to happen.

The enzymes just bring down the activation energy as any catalyst does. All enzymes of relevance to us are proteins and some enzymes require something called cofactor, such as metal ions or what are called coenzymes for their action, okay? You have an ampro-enzyme which is the proteinaceous part and to which you add a cofactor, metal ions or coenzymes as they are called to give the whole enzyme, okay? Just have this in mind as information, so (f) the enzymes may not be active in the absence of these cofactors which could just be a metal ion.

Okay? Manganese one. This video, this is optional clearly this gives you the six types of enzymes that six types that the enzymes are classified into in a standardised fashion. If you want you can take a look at it, it's a lot of detail, if you're really interested you can go and take a look at this. When you have an enzyme a catalyst, it speeds up the reactions, makes reactions possible, make cell reactions possible at the temperature pressure of the cell, which is very moderate, right? How do we quantify the activity of the enzyme, okay?

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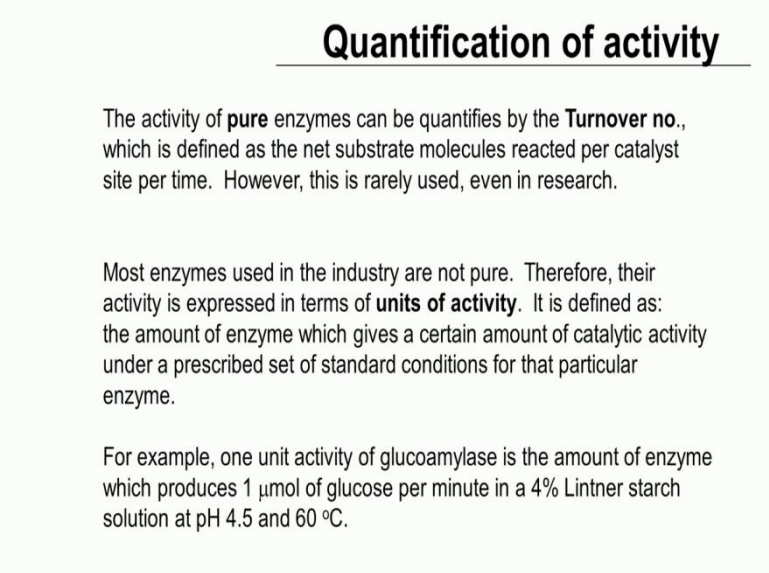

Its quantification is a very important aspect if you want to do something further with it, okay? Knowing information is fine, then we will have to find means of quantifying the information which leads to better analysis and better manipulation application and so on so forth. That's why quantification becomes very important. As engineers, some of you would know this already I just thought I will verbalise this clearly.

The activity of pure enzymes can be quantified by something called a turnover number. Turnover number is defined as the net substrate molecules reacted per catalyst site per time, okay? The way the enzyme action comes in we say it was because of the three-dimensional folding. The three-dimensional folding creates pockets that are very specific to the substrate molecule or the reacting molecule, reactant.

The reactant comes and sits there and then it gets converted to a product by various different means and it is the appropriate fit of the substrate molecule of fold in the enzyme, a part of the enzyme that makes this possible, okay? So it is defined as the net substrate the number of net substrate molecules reacted per catalyst site per time, okay? But it's rather difficult to find out the number of molecules reacted per catalyst site per time through regular investigations and therefore it is rarely used even in most research, okay? None of us use turnover number when we talk of activity of enzymes especially when we are trying to use enzymes.

Most enzymes used in the industry are not pure, that doesn't matter. Their activity is expressed in terms of units of activity, right? And this is one of the reasons why we don't look at turnover number because you need highly pure enzymes to even measure turnover

number (which) which itself is not very straightforward. And on top of that you have the regular enzymes not being very pure, okay?

So we settle for something called units of activity. Units (unit) Unit of activity is defined as the amount of enzyme which gives a certain amount of catalytic activity under a prescribed set of conditions for that particular enzyme, okay? It is highly variable and applicable only for that particular enzyme, okay? And people have, kind of, agreed on this. This is how it is right now. For example, one unit activity of glucoamylase is the amount of enzyme which produces 1 micro mol of enzyme, 1 micro mol of glucose per minute in a 4% Lintner starch solution at pH 4.5 and 60 degree C, okay? (It) it can be as specific as that; amount of enzyme which produces 1 µmol of glucose per minute in a 4% Lintner starch solution at pH 4.5 and 60 degree C. So, this is what we use to (to) quantify the activity of enzymes.

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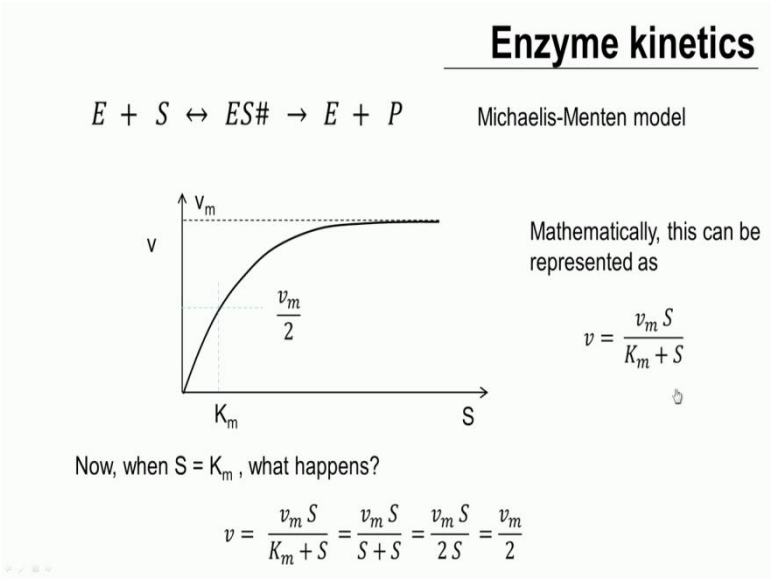

The speeds at which enzymes act are important to know, the kinetics is important to know of especially for design. Suppose we are designing an enzyme reactor to carry out an enzymatic reaction, we need to know how long to wait for the reaction to take place if it is a batch kind of system, or even if it's a continuous kind of a system for design of flow rates and so on and so forth, we need to know the kinetics. So let us look at a simple kinetics, simple enzyme kinetics, there are very many different kinds of kinetics, which are which govern enzyme action; we will just look at one, for example, okay?

This model is as follows; the enzyme reacts with the substrate to reversibly form what is called the enzyme substrate complex, okay? There is a reversible reaction here, forward, there

is a forward reaction here, there is a backward reaction here, there is an equilibrium at certain time and enzymeplus substrate gives you the enzyme substrate complex. And this irreversibly goes to give the enzyme molecule back and the product from the substrate, okay? This model is called the Michaelis-Menten model and it's the simplest model in terms of enzyme kinetics.

If we work out the rates by using this model and material balance considerations, okay, it's a reasonable derivation, about two pages long and if we (plaw) if we look at how the variation is with the substrate concentration, variation of the rate of the reaction with substrate concentration, it will be something like this. It's rectangular hyperbola, okay? It is reasonably linear here, then it goes like this and asymptotically reaches what is called a Vm, Vmax.

Mathematically, this kind of behaviour can be represented as V equals to VmS by Km plus S. This kind of a formulation would yield this behaviour. So VmS by Km plus S is a good rate expression that one can choose as a first approximation for enzyme kinetics, okay? This is (of) of course, this is first approximation, this is called the Michaelis-Menton model.

Now, when the substrate concentration becomes equal to Km, let us see what happens to this expression, right? If it becomes equal to Km, v equals to VmS by Km plus S , so okay Km is S, so let's substitute S for Km, so you get VmS by 2S, S plus S, 2S and S and S can cancel out and you get Vmax by 2 or Vm by 2, okay? What does this mean graphically at S equals Km, the V that you find, would be Vmax by 2, okay?

So Km is called the substrate concentration which gives half maximal rate of the reaction, right, and Km is the Michaelis-Menton constant. So this is a nice expression to know the first approximation to know for enzyme kinetics, and it is useful in many different ways, I just mentioned one.

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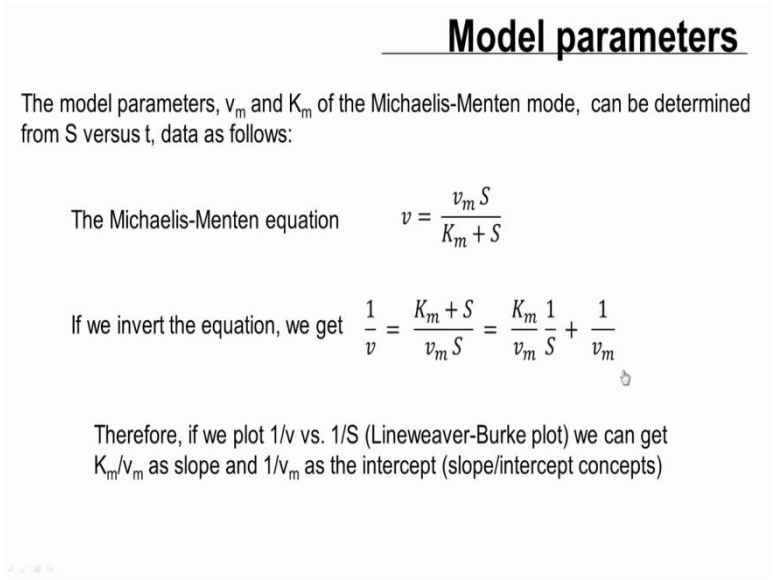

To find out, Vm and Km, as you can realise Vm and Km will be dependent on the enzyme of the substrate and for each combination there will be a Vm and a Km, so how do you find that out, how do you find out these model parameters here, Vm and Km. To find that out, what we do is something like this, one of the things that we can do is something like this because we'll have to find that out from data.

These model parameters can be determined from the substrate versus time, the variation of substrate concentration versus time data, you do an experiment (you) we carry out an enzymatic reaction and then we monitor the substrate concentration with time and that's the data that we have, we have the substrate concentrations at various times. From that data, how do we get the model parameters, is what we are going to look at now, at least a basis of that.

The basis is as follows; the Michaelis-Menton is V equals to VmS by Km plus S. If we invert this, you know, invert the left hand side and therefore we invert the right hand side, we get 1 by V equals to Km plus S by VmS, just inversion, so if we separate out the terms, this is a plus term here, therefore if we separate out the terms, this term would become Km by Vm into 1byS and this term, S and S would cancel out and we get 1byVm, okay?

So this is of the form Y equals to Mx plus C or if we plot 1byV versus 1byS we expect to get a straight line with Km by Vm as a slope and 1 by Vm as the intercept. Such a plot is called the Lineweaver-Burke plot, plot of 1 by V versus 1 by S, you calculate velocity from the substrate concentrations and then plot 1 by V versus 1 by S, you get Km by Vm as a slope, and1 by Vm as the intercept. And I think this audience would know the slope and intercept,

right? There is the equation of a straight line Y equals Mx plus C, and M is the slope, C is the intercept that's we tried to plot it this way, so that from a straight line we can get these parameters.

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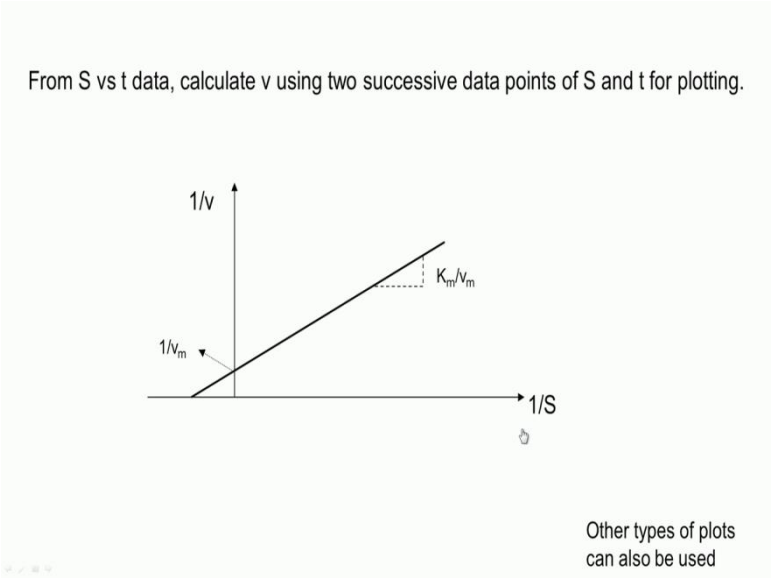

So from S versus t data calculate V using two successive datapoints S, of S and assign it to the midpoint of the time interval there and then 1 by V versus 1 by S, straight line, Km by Vm as a slope and1 by Vm as the intercept. Other types of plots can be used, there are some difficulties with this in the sense that the data here is not very reliable and it will determine the slope and so on. Let's not get into that, this is an introductory course. So this is one way of quantifying enzyme kinetics.

We talked of enzymes in the cell and how to quantify the enzyme activity and how to get how to represent the kinetics mathematically and how to get those parameters, the model parameters. Now let us look at the industrial uses of enzymes, they are very widely used, (you know) if you look at this video, it'll tell you the various ways or some of the ways in which enzymes are used and their usage is billions of dollars per year (kin) kind of level. For example, if you okay let me just point this out to you. (The) the industrial enzyme is video number 16, you might want to click on that and take a look at that.

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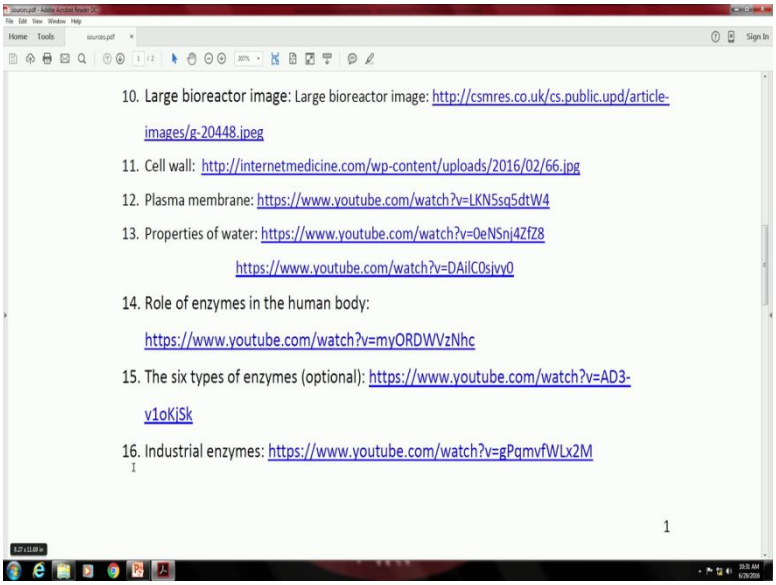

That's this video, okay? Please do that.

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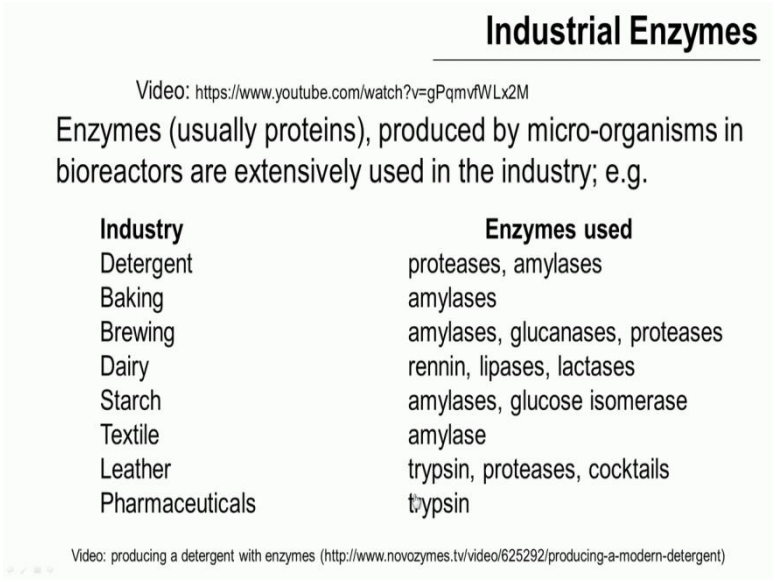

Enzymes, as we said usually proteins, are actually produced by microorganisms in bioreactors and that is pretty much a source of enzymes nowadays, okay? The other sources such as you know earlier there used to be other sources such as animals and so on. They are not very extensively used nowadays, most enzymes produced by microorganisms in bioreactors.

For example, the the detergent industry uses proteases and amylases for better cleaning power. Baking industry uses amylases; brewing industry uses amylases, glucanases and

proteases; dairy industry, rennin, lipases, lactases; starch industry uses amylases, glucose isomerase; textile industry uses amylase; leather industry, trypsin, protease and cocktails of enzymes and so on; pharmaceuticals, trypsin and so on, okay? So, heavily used as I said billions of dollars worth per year is what what is involved, the (am) amount of money involved in enzyme use; enzyme manufacture, enzyme use.

So this video gives you some idea as to how enzymes are used in detergents, please take a look at this video, it's by novozymes but look at the the details in that video. I also (have) have in mind our disclaimer for all these recommendations.

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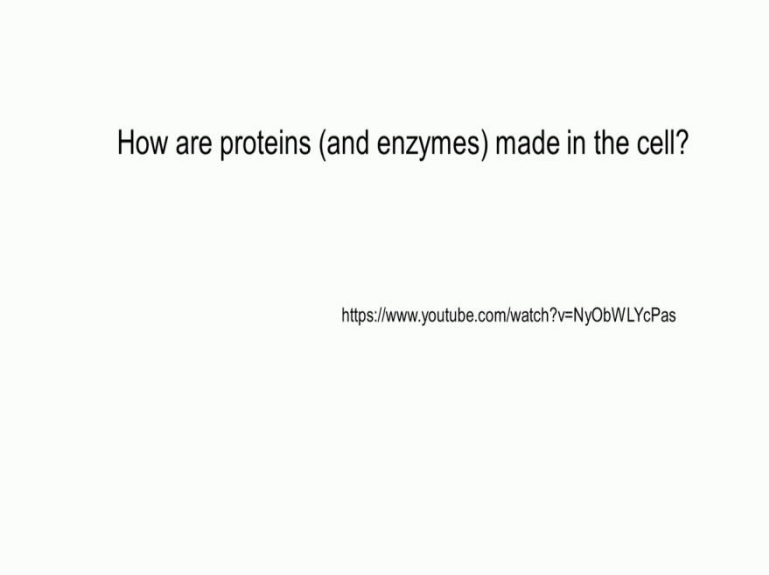

When we meet up next, this is the story that we are going to ask in the larger story of curd making, how are proteins and enzymes made in the cell, okay? We (saw) we saw that enzymes are being (used) heavily used in the cell, for catalysing various different reactions and so on, enzymes produced by microorganisms are used in everyday products and so (I mean) (that's) that's a very large industry, the next story we're going to see how (the) the proteins and the enzymes are actually made in the cell, okay? Let's meet up later. See you then.