Environmental Chemistry and Microbiology
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Module - 9
Lecture - 44
Microbial Metabolism - I

Welcome everyone, we will now start a new topic. This is module 9, lecture 44 and we are starting a new topic called Microbial Metabolism. So, we are going to look at how microbes are able to derive energy from various electron donors and acceptors. This particular topic is divided into 3 parts. So, we will start with part 1 here.

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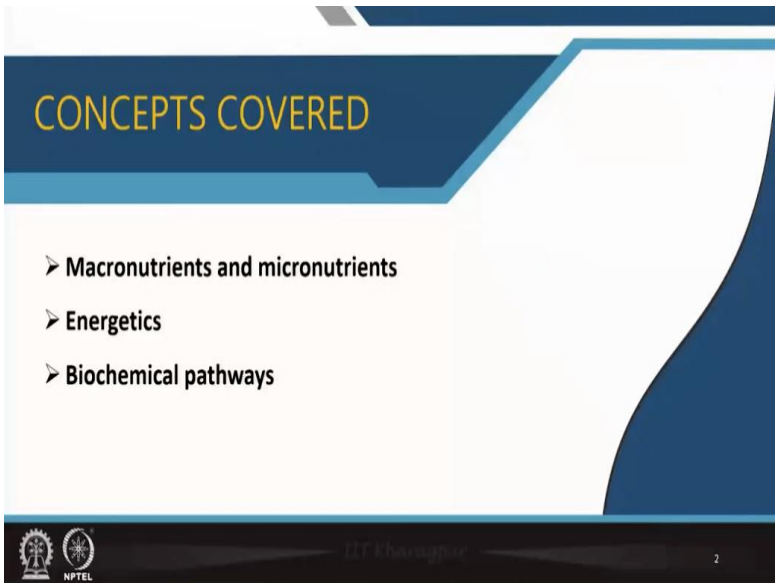

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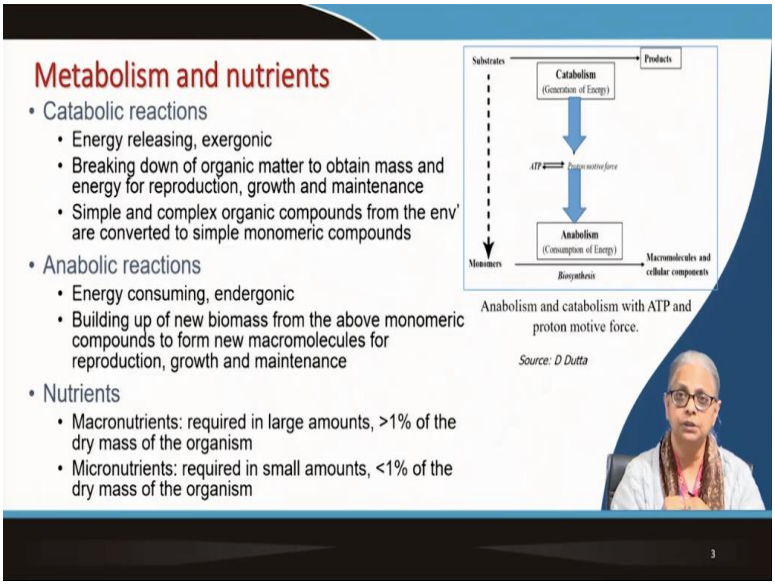

So, let us take a look at what we mean by metabolism. So, metabolism has 2 parts to it, catabolism and anabolic reactions or anabolism. So, here we have our substrates. We know that substrates are food, food for the bacteria; and they take up these substrates. So, when an organism takes up a particular food or a substrate, that substrate has to be broken down. These are macromolecules; they are polymers; they are biological polymers; they are macromolecules; they have to be broken down first into their monomeric units.

So, this process of breaking down large molecules into monomeric units is an energy releasing reaction. So, these are catabolic reactions, as shown in the figure over here. Energy is released and therefore they are called exergonic reactions. Now, this energy that is released in catabolic reactions is trapped in the form of ATP. So, that is why we say ATP is the cell's currency of energy. So, this energy that is released, it first can be trapped. In the case of prokaryotes, it is trapped in the form of proton motive force, which is then converted to ATP. Now, it is not just sufficient for us to take in food and derive energy from it. We are just like bacteria in that sense. We do take in food, we do break it down into monomeric units, we do get energy released from these catabolic reactions.

All of that is common to us as well as the bacteria. What we also need and so do the bacteria is that we need to create new biomass, whether that new biomass is for repairing whatever has gone bad or wrong; and it can be for reproduction. So, in either case, new biomass has to be generated. That is done in anabolic reactions. Now, anabolic reactions consume energy, where the monomeric units that are generated in catabolic reactions are then put together again into new macromolecules.

Now, these new macromolecules will replace damaged ones or they will be used for reproducing and creating new biomass, new cells and so on. So, catabolic reactions by definition are energy releasing and energy generating and that is the source of ATP. So, here is ATP generation. This ATP is then utilised in anabolic reactions to go from monomeric units to polymeric units.

Like I said, breaking down is necessary to obtain mass and energy for reproduction, growth and maintenance. Anabolic reactions are used for building new biomass from the monomeric units that were generated in the catabolic reactions. So, metabolism is the combination of catabolic reactions and anabolic reactions. The 2 together is the metabolism of a particular organism.

Now, different organisms obtain their energy in different ways. The food is different. So, all organisms do not eat the same food, they do not utilize it in the same way. And we are all going to look at how different organisms derive energy from different substrates. So, we use the word substrate. We stopped using the word food. And when we talk about bacteria, we generally talk in terms of substrate.

I have already mentioned that food is really nutrients and these nutrients are of two types. This is a little bit of a repeat of what I have mentioned in the past, but it is important enough for us to keep it in mind. Therefore, it has been repeated here to some extent. So, here we have macronutrients and micronutrients. Earlier, I had defined the Big-6. So, the big-6 are carbon, hydrogen, oxygen, nitrogen, phosphorus and sulphur. These are the 6 elements that are required; and they constitute more than 1% of the dry mass of the organism. There are a few others, depending on the organism. But these big-6 are common to almost all living organisms. Micronutrients are required in lesser amounts, because they constitute less than 1% of the dry mass of the organism.

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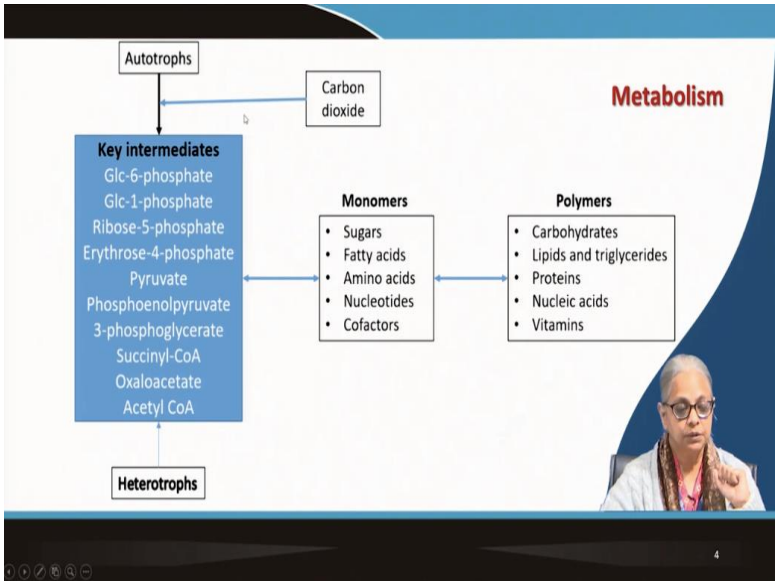

Now, as I mentioned, in catabolic and anabolic reactions, there is a breaking down. In the catabolic part, the large molecules are broken down into monomeric units. There are also key intermediates. So, nature does not spend too much time on creating everything from ground zero; it does not do that. There are key intermediates that are involved in both catabolic as well as anabolic reactions. So, they do not have to go down to CO_2 all the time. These key intermediates are directly taken into the anabolic reactions after they are being generated in catabolic reactions. So, these are the key intermediates that are common. Autotrophic organisms, heterotrophic organisms, all of them utilize these key intermediates, and they utilize them along with the other monomers, that is utilized to create polymers.

So, here I have shown the arrows in both directions. You know the monomers, you know the polymers; we have gone through all of this in the previous topics, in the previous module. We will move on, but it is important for everyone to remember what the monomeric units of the large macromolecules are.

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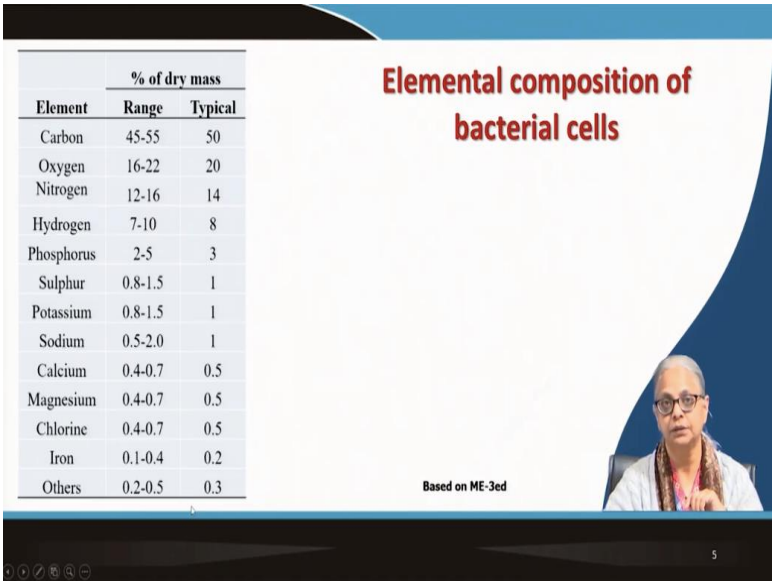

I have also shown you this slide in the previous module and I am not going to repeat too much except to say that it is very important for everyone to remember the key macronutrients. So, you will see that by the definition that we have here, more than 1% of the biomass; if any of these elements constitutes more than 1% of the total biomass of the organism by dry weight, then it is a macronutrient. So, along with the big-6, we also have potassium and sodium over here for bacterial cells. So, here we have the micronutrients calcium, magnesium, chlorine, iron and other nutrients. So, these are the elements that constitute less than 1% of the dry weight of bacteria.

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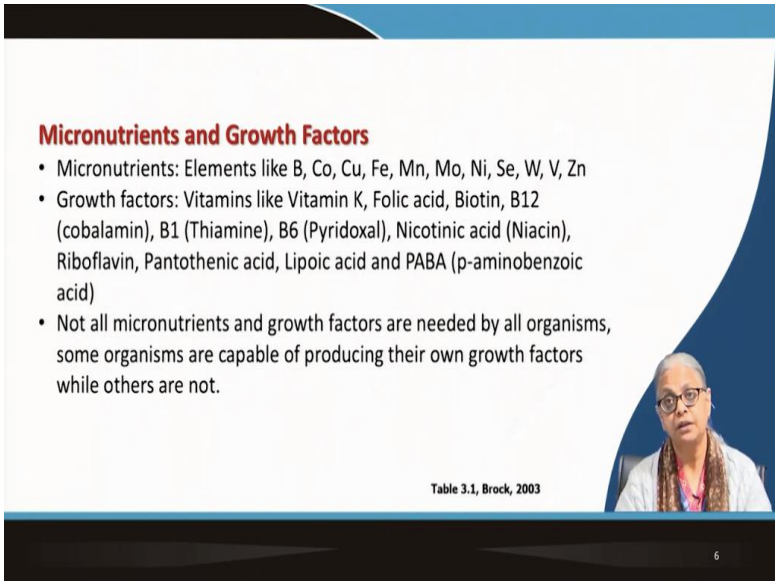

Let us take a look at some micronutrients. So, here we have micronutrients. These micronutrients have elements, include elements like boron, cobalt, copper, iron, manganese, molybdenum, nickel, selenium, tungsten, vanadium and zinc. Let me just briefly explain the

roles that each of these elements plays in the nutrition of microorganisms. So, let us take boron. Boron is an autoinducer of quorum sensing in bacteria.

Cobalt is part of vitamin B12. Copper is used in respiration as cytochrome c oxidase; it is part of cytochrome c oxidase. In photosynthesis, it is used in plastocyanin; and it is also part of superoxide dismutase. Iron is part of the cytochromes; it is part of catalyzes; it is part of peroxidases; it is part of the iron-sulphur proteins; the oxygenases and all nitrogenases. Then we come to manganese. Manganese is an activator of many different enzymes. It is also part of superoxide dismutase. It is also part of the water splitting enzyme in oxygen photosynthesis, as part of photosynthesis photosystem II. Then we come to molybdenum. Molybdenum is a part of flavin-containing enzymes. It is part of nitrogenases; nitrate reductases; sulphite oxidases; the DMSO, TMAO reductases. If you are wondering what all these are, in subsequent lectures, within this module, we will be going through some of these details. So, do not worry about it right now, but just keep in mind that you can refer right back here to where all these words and names and enzymes are coming from. So, molybdenum is also a part of formate dehydrogenase. Nickel is part of some most hydrogenases. It is part of coenzyme F 430 in methanogens. It is part of carbon monoxide dehydrogenase as well as urease.

Selenium is also important. It is part of formate dehydrogenase, some hydrogenases, the amino acid selenocysteine. Then we come to tungsten, where you have; tungsten is part of formate dehydrogenases and oxotransferases of hyperthermophilic bacteria. Vanadium is part of vanadium nitrogenase and bromoperoxidase. Zinc is part of carbonic anhydrase, nucleic acid polymerase and many of the DNA binding proteins.

Then we come to growth factors. So, we know that these growth factors are also very important in the ability of bacteria as well as other microorganisms to both grow and reproduce. So, vitamins like vitamin K which is used for electron transport. Then we have folic acid which is required when you have the utilization of C-1 compounds. So, it is also part of the methyl transfers, very crucial for that. Then you have biotin. Biotin is used for fatty acid biosynthesis as well as carbon dioxide fixation reactions. Then we come to vitamin B12. We know vitamin B12 is often prescribed. It is cobalamin and it is required for one-carbon metabolism and for the synthesis of deoxyribose. Then we come to thiamine. Thiamine is vitamin B1. That is used for decarboxylation reactions.

Vitamin B6 is pyridoxal; and that is used for amino acid and keto acid transformations. Nicotinic acid is a precursor for NAD^+ and we will be seeing a lot about NAD^+ in the subsequent lectures. Then we come to riboflavin which is a precursor for FMN and FAD and we have pantothenic acid which is a precursor for coenzyme A. Again, that is a very important growth factor. And then we have lipoic acid which is used in the decarboxylation of pyruvate and alpha-ketoglutarate. So, these are some of the most important micronutrients. You can see how important they are, even though they are required in extremely small amounts. Now, it is also important to remember that all micronutrients and all growth factors are not needed by all microorganisms.

Some organisms are capable of producing their own growth factors, while other organisms produce different growth factors. So, there are various differences between the organisms in terms of both their need and their ability to create their own vitamins and so on.

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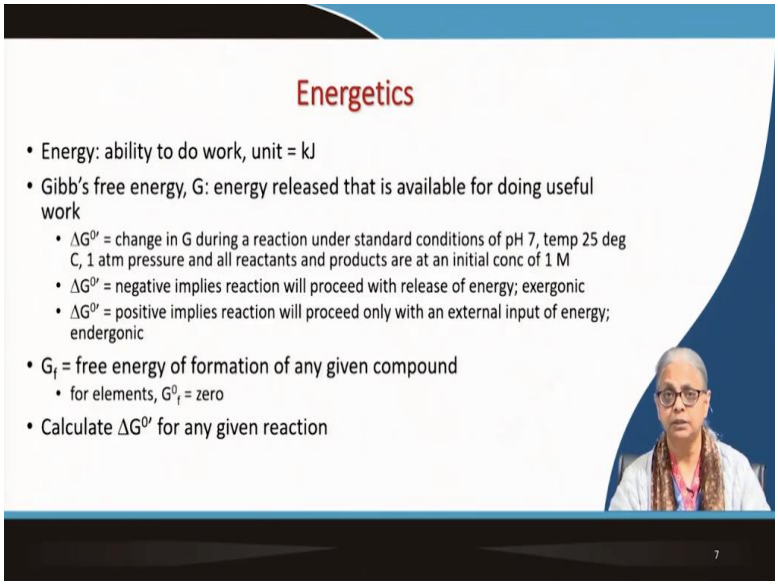

Let us now come to another aspect, and that is energy. So, how does the organism derive energy? What is the form of this energy? So, let us revise something that you already know; and that is what is energy. Energy is the ability to do work. We measure it in units of kilojoules. In the older textbooks, you will find kilocalories. You know how to convert kilocalories to kilojoules.

Gibb's free energy, G, which is abbreviated as G, is the energy released that is available for doing useful work. So, we have $\Delta G^{0\prime}$. This is the change in the free energy during reaction under standard conditions of pH 7, temperature 25℃, 1 atm pressure and assuming that all

reactants and all products are at an initial concentration of 1 molar concentration; so, 1 moles/litre for each of them.

If the value of $\Delta G^{0\prime}$ is negative, it means that the reaction will proceed with release of energy. So, we call it an exergonic reaction. If the value is positive, it means, an external input of energy will be required; and the reaction is endergonic. So remember, catabolic reactions are exergonic, anabolic reactions are endergonic reactions. In terms of determining ΔG^0 for any reaction, you need to know the free energies of formation for all the reactants and products.

So, $G0_f$ is the free energy of formation for any compound. For elements, we assume that the $\Delta\, G^0{}_f$ is zero. So, given this information, you can refer to any standard chemical chemistry textbook and you might be able to find these values and calculate $\Delta G^{0\prime}$ for any given reaction.

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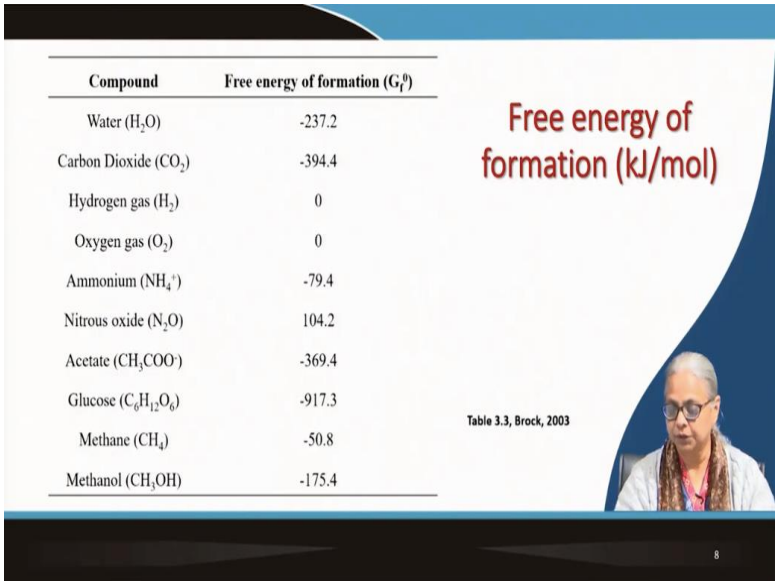

So, here we have some values for the various compounds that we encounter in microbiology all the time. So, you can see the values; they are all shown over here. We have water, carbon dioxide. You can see for the elements hydrogen gas and oxygen gas, it is 0; all the others have negative ΔG values of formation. And this will be important in subsequent parts of this topic.

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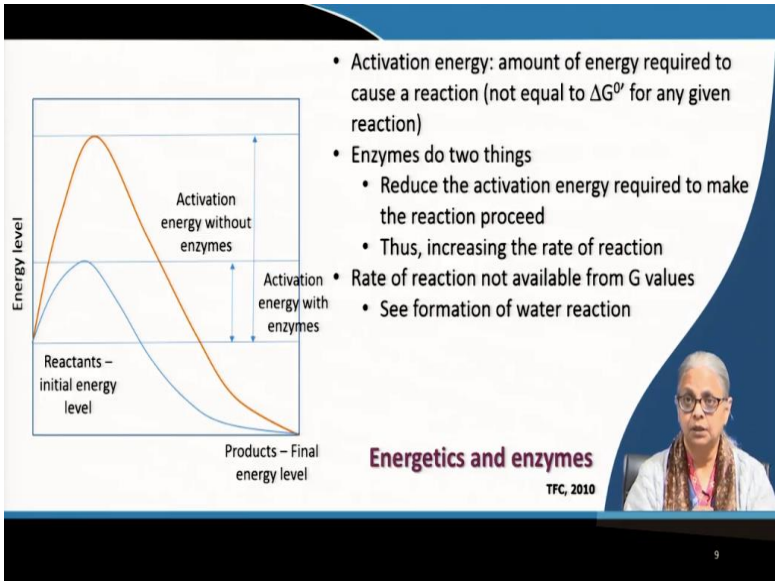

Before we go too far, let us also take a look at what is required to make a reaction happen. So, if you have reactants A and B, these reactants are at a particular energy level. They have their $\Delta G^0$for the formation of these reactants, these compounds. Now, the reaction that we are looking at, we want to look at AB is a compound; it is a single compound. Let us assume it is glucose. Now, glucose has to be converted to CO_2 and water. So, it is being converted into A and B. So, AB is the initial compound. It is being converted to 2 different products. What is required to make this reaction happen? This reaction, without any help is not going to happen spontaneously. It is an exergonic reaction. Let us say glucose and water; you add sugar to water; nothing will happen.

It is the ΔG^0 of the reaction of glucose and oxygen in water is not going to go to CO_2 and water directly. It needs something more. So, I can start heating it and I can combust the entire thing. What will happen? Yes, I will get CO_2 and water at the end of it. But that is not what we normally do. So, that is the activation energy required to make the reaction happen and then get some energy out of it.

So, $\Delta G^0$is one part of the story and the activation energy is a whole different part of what is required to make the reaction happen. So, this is the additional energy required to push this reaction to make it happen. It does not happen spontaneously. It is not a spontaneous reaction. Now, how does the body of human beings or of bacteria derive energy from glucose (and) oxygen and convert it to CO_2 and water?

That happens. It is a biologically mediated reaction; it is an enzyme mediated reaction; both for the bacteria as well as for human beings. And what do these enzymes do. So, for the bacteria as well as for human beings, these enzymes serve 2 purposes. They reduce the activation energy required to make the reaction proceed. So, without the enzyme, you can see the activation energy that is required. I have already mentioned to you. You can add sugar to water; leave it for weeks, months, years. If you add no enzyme, no catalyst; even though the ΔG value of the reaction is negative, it will never happen. It is not spontaneous. It is exergonic, but not spontaneous. So, to make it happen, you need a catalyst. You can either increase or provide activation energy to get it over this barrier. That is the activation energy to make, to activate the reaction. That is one part of the reaction.

And the second thing is, you can use an enzyme. So, that is what our body does; that is what the body of the bacteria do. They have the enzymes to convert glucose and oxygen in a series of biochemical reactions, until the end point is CO_2 and water. So, that is what these enzymes do. They reduce the amount of activation energy required to make the reaction happen and thereby they also increase the rate of reaction.

So, like I said, we know that when we are feeling tired, we take some sugary beverage. So, whether it is tea or coffee or some other beverage, we know that the sugar in it is helping us to get over the slump. So, that is an energy releasing reaction. We are all aware of it, we may not be conscious of all these details, but we all know that if I take something which has sugar in it, I will get an immediate boost in terms of my energy level.

So, these are the same things, same reactions that are happening. Now, the rate of reaction is not something you can derive from the ΔG or the G values and we will come to more about these issues later. The ΔG value simply tells you how much energy can be derived from that reaction. The rate of reaction is a whole another story. We are not going to go there.

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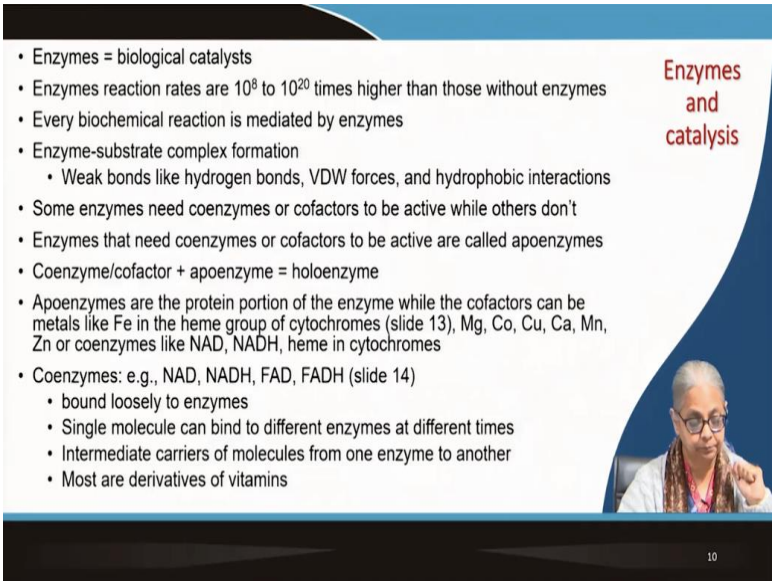

Now, let us focus on the enzymes to a greater extent, because they are the crux of all biochemical reactions. You will find that; in fact, I do not know any example of any biochemical reaction which is not mediated by enzymes. They are all mediated by very specific enzymes. And I remember, way back, they used to be; the enzyme-substrate reactions used to be called lock and key theory; because, just like a specific key will fit into only one lock, in the same way, a specific combination of substrate and enzyme is the only one that will work and allow the reaction to proceed. So, the level of specificity of these enzymes is extremely high. So, enzymes as we know are biological catalysts. The reaction rates are 10^8 to 10^{20} times higher than the reaction that may happen without these enzymes. I can tell you for sure that many of these reactions will not happen at all. Like I said, every biochemical reaction that we know of today is mediated by enzymes.

For these enzyme mediated reactions to happen, there is something that is necessary before that and that is the (formation of the) enzyme-substrate complex. So, when the enzyme and substrate form a complex, only then can the reaction proceed. These enzyme-substrate complexes are formed by weak bonds. Hydrogen bonds, Van der Waals forces, hydrophobic interactions, all these are crucial for the enzyme-substrate complex to form and the enzyme mediated reactions to happen.

So, some of these enzymes can work on their own, while others need coenzymes or cofactors to be active. So, there are different types of enzymes. Now, if there is an enzyme that needs a coenzyme or a cofactor, then that part is obviously called coenzyme or cofactor. And there is

another part that is called the apoenzyme. And the two together are then called holoenzyme; holo meaning whole and apo meaning part of the enzyme.

So, these apoenzymes can be the protein portion of the enzyme; and the cofactors can be metals, they can be other elements. So, you have iron in the heme group. You have magnesium, cobalt, copper, calcium, manganese, zinc, and you have coenzymes like NAD, NADH and the heme group in the cytochromes. These are all examples of the cofactors. Coenzymes, there is some issue about coenzymes or cofactors and their nomenclature. I will refer you to the textbook for that. So, coenzymes, we have NAD, $NAD^{+,}$ NADH, FAD, FAD^{+}, FADH; and we will be going through more of this in the next few slides. They all bind loosely to the enzyme. A single molecule can bind to different enzymes at different times. They are intermediate molecules. They are carriers of molecules (protons and electrons) from one enzyme to another.

They are very crucial in the respiration process, because the electron transport chain is basically based on these carriers or coenzymes. And they are the ones that transfer protons and electrons. And when we go through the entire course, you will be able to understand how crucial their role is. They are almost all derivatives of vitamins.

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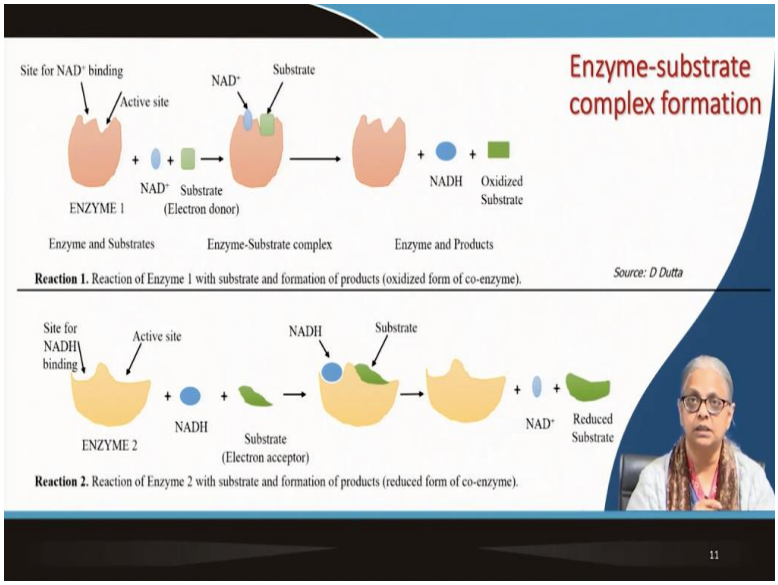

So, here is one example. We have the first enzyme-substrate complex. So, you have the first enzyme. It has 2 sites. One site is where the NAD^{+} is binding, and the second site is for the substrate. So, two things have to happen. I have not spoken about yet; I will be talking about in the next part, and that is oxidation-reduction reactions. Almost all biochemical reactions, not 100% perhaps, but most of the biochemical reactions are oxidation reduction reactions, which

means there is one compound which serves as an electron donor and another compound that serves as an electron acceptor.

So, for ATP to be formed, for the proton motive force to be generated, the electron donors and acceptors; there has to be transport and transfer both. Transfer and transport of electrons has to happen between the compound that is donating electrons and the compound that is accepting electrons. So, it is a long series of biochemical reactions mediated by enzymes.

Here we have NAD^+. This NAD^+ is positively charged. When it binds to the enzyme complex, enzyme-1, along with the substrate, this entire complex gets activated. The substrate is the electron donor. Two things will happen. NADH will, NADH picks up a proton and an electron. So, one of the things is that the electrons will be picked up from the substrate, the protons from the proton motive force and NADH will be formed. And then you have your oxidized substrate. So, NAD^+ has been reduced to NADH and the substrate has been oxidized in the first example. In the second example, the reverse is happening. You have another enzyme which has 2 sites, one for the substrate and one for NADH. This NADH, remember it is a coenzyme. So, now it has to be returned back to NAD^+. So, it has a proton and an electron. It will lose its proton and electron.

The substrate will pick up the electrons and it will get reduced. So, it is an electron acceptor. The protons will be released. They will help in generating the proton motive force and you get this particular form. So, we have gone full circle. NAD^+ has been returned to its original form. In the meantime, proton motive force has been utilized in another series of reactions to generate ATP. We will come to all those details later.

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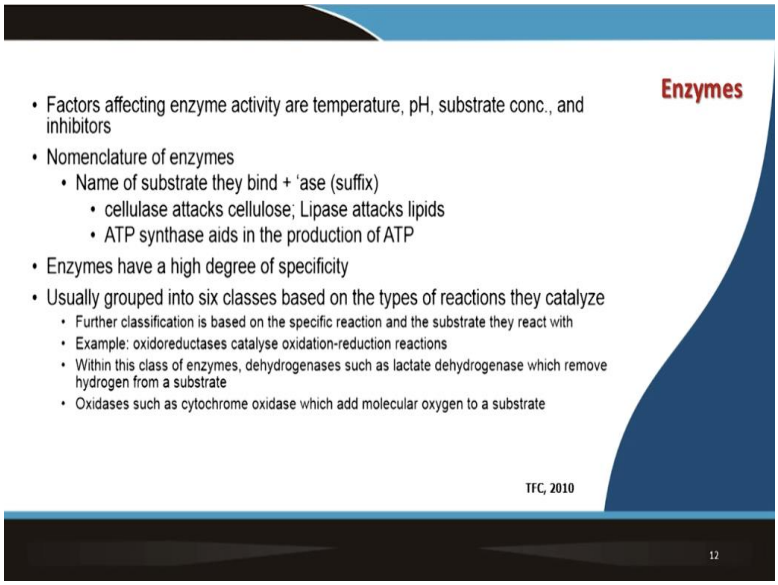

So, here we have some of the factors that affect enzyme activity. Enzyme activity is basically influenced by temperature, pH, substrate concentration and the presence of inhibitors. Let us also understand the basic logic behind the nomenclature of enzymes. The name of the substrate they bind is then suffixed with "-ase". So, simply putting an "a-s-e" at the end of the name of the substrate will give you the name of the enzyme.

So, cellulase is something that attacks cellulose. Lipase is something that attacks lipids. ATP synthase means that it helps in the generation or production of ATP. It is also important to know that enzymes have a very high degree of specificity. They are usually grouped into 6 classes based on the types of reactions they catalyze. So, let us just come to that directly.

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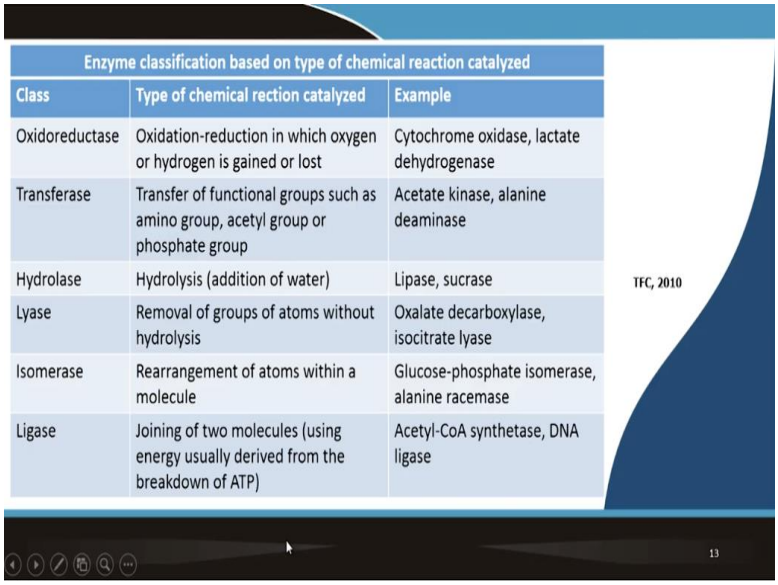

So, here we have 6 classes. The first one is oxidoreductase. It participates in oxidation-reduction reactions in which either an oxygen or hydrogen is gained or lost and 2 examples are shown over here, cytochrome oxidase and lactate dehydrogenase. Now, as the names suggest, cytochrome oxidase helps in adding an oxygen to the substrate, and lactase dehydrogenase helps in removing a hydrogen from the lactate molecule. So, that is lactate dehydrogenase.

Then we come to transferase. Transferase is responsible for transferring functional groups like amino groups, acetyl groups, phosphate groups. These can be transferred using transferase enzyme. So, you have examples like acetate kinase, alanine deaminase.

The third group is hydrolases; and they are responsible for catalysing the hydrolysis, which means addition of water. So, you have lipase, sucrase and so on.

Then you have lyase. Lyase is a group of enzymes. Lyases are groups of, or enzymes that are responsible for the removal of different atoms without hydrolysis as part of the reaction. So, you have oxalate decarboxylase and isocitrate lyase.

Another group of enzymes is isomerase. Isomerase literally means, it is going to catalyse the rearrangement of atoms within the same molecule. So, you have glucose-phosphate isomerase. You have alanine racemase.

Then you have the last one which is ligase. Ligase means joining of 2 molecules using energy derived from the breakdown of ATP. So, you have acetyl-CoA synthase and DNA ligase. These are some of the enzyme groups that are responsible for most of the enzyme mediated reactions.

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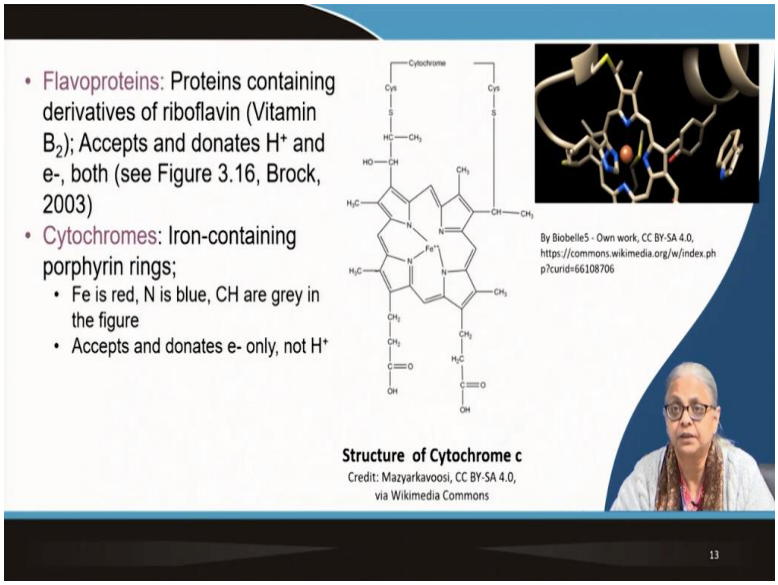

Then we come to flavoproteins. Now, flavoproteins are proteins that contain derivatives of riboflavin, which is vitamin B2. These flavoproteins are capable of accepting and donating both protons and electrons. I am referring you to the textbook again to see the structure of the compound, and how and why protons and electrons can be accepted or donated. And these are all very crucial, because the entire electron transport chain depends on these carriers of protons and electrons for basically transferring electrons all the way from the substrate to the terminal electron acceptor, and in the process generating ATP.

So, these are crucial carriers. Then we come to the cytochromes. Cytochromes are iron containing porphyrin rings. So, you can see the iron at the centre of this porphyrin ring. This is the porphyrin ring which has nitrogens at 4 points at which it is bound to iron. And this is a schematic, again from Wikipedia, yes. You have the fact that these cytochromes can accept and donate electrons; only they do not do anything with protons. So, these are 2 different types of carriers. They are both very crucial, because one set of them can both accept protons and electrons, and the other one only electrons.

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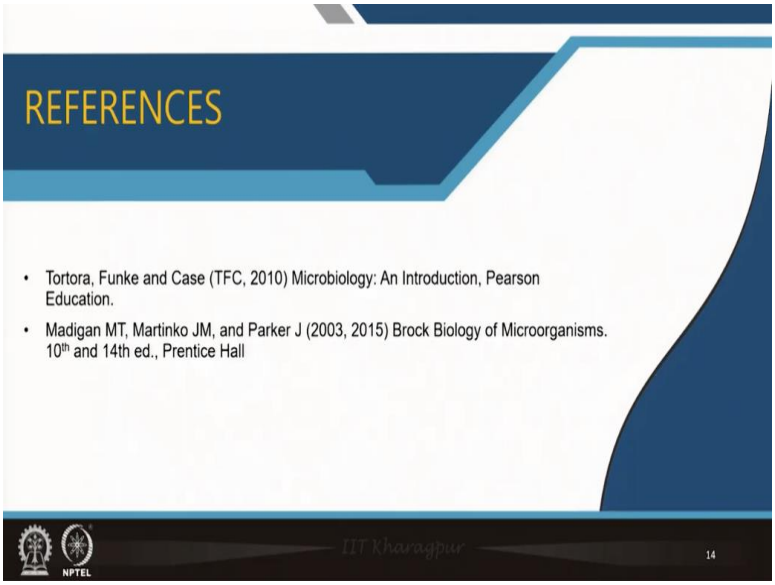

That brings me to the end of part 1 and we will go with some of the other parts of this topic in the next part.