Environmental Chemistry and Microbiology
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Module - 9
Lecture - 45
Microbial Metabolism - II

Welcome everyone to lecture number 45 and the second part of Microbial Metabolism.

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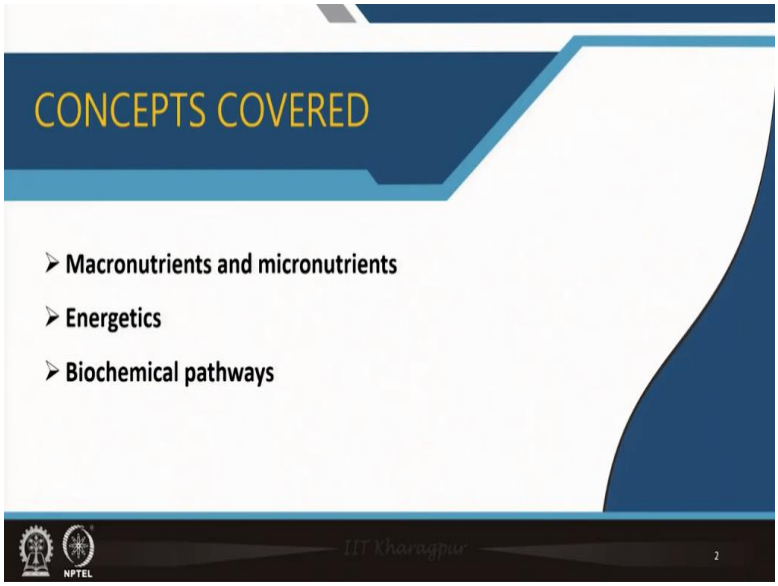

We are going to look at how the cells derive energy from different combinations of electron donors and acceptors.

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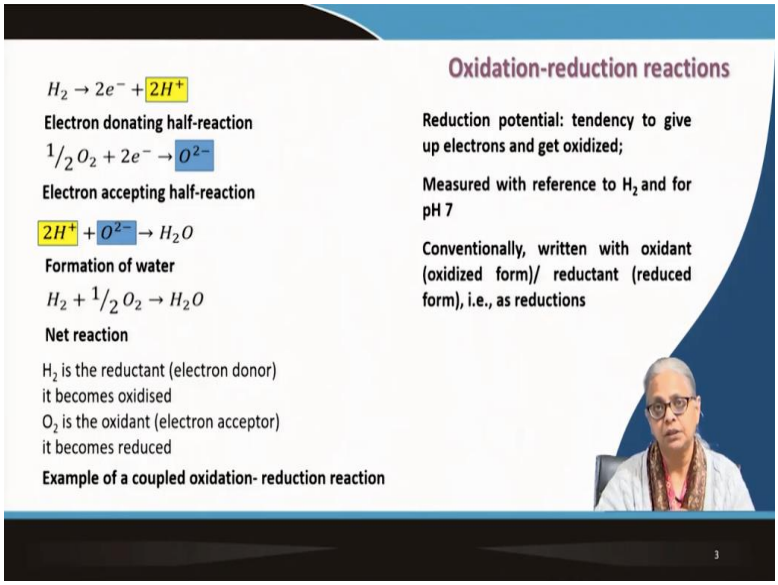

So, let us revise a little bit about oxidation-reduction reactions, just in case you have forgotten. So, here we have hydrogen. When the half-reaction for hydrogen is 2 protons and 2 electrons, so, this is called the electron donating half-reaction. And then we have oxygen. So, when oxygen picks up these electrons, this becomes the electron accepting half-reaction.

It is very important for all of us to keep in mind that for any organism to survive, it is the combination of electron donor and electron acceptor. If that combination of 2 half-reactions is energy yielding, then the organism is going to get the energy it needs for survival and reproduction. So, here is our net reaction for the formation of water. You know that if you were to put these 2 gases together, you will automatically get water. So, this is both exergonic as well as spontaneous reaction. So, H_2 in this case is the reductant or the electron donor. It gets oxidized. And oxygen is the oxidant or the electron acceptor. It gets reduced. Now, this is the simplest oxidation-reduction reaction that we can think about. It also tells us something about how to measure the reduction potential. So, the tendency to give up electrons and get oxidised is basically what we are measuring, when we measure the reduction potential.

It is always measured with reference to hydrogen and for pH 7. Having understood the importance of oxidation reduction reactions, we also need to understand how we generally write them. So, conventionally, we write the oxidant or the oxidized form on the left, and the reductant or the reduced form on the right.

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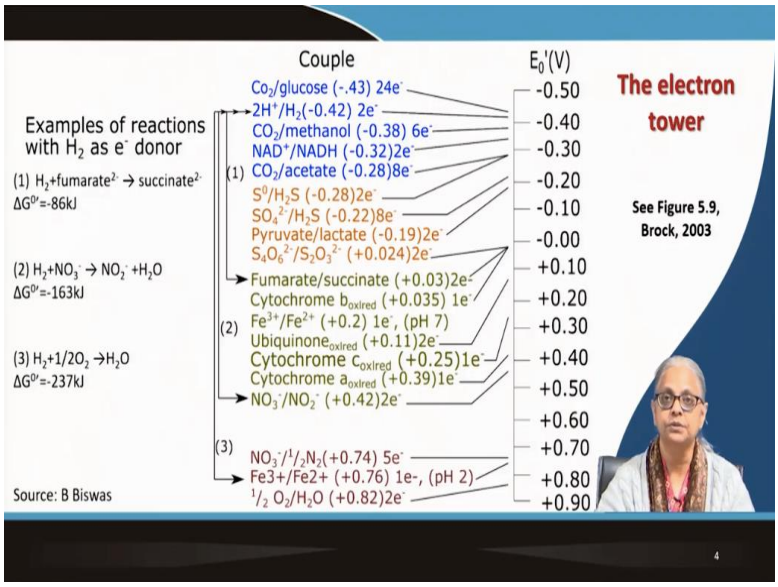

So, these are examples of that. So, here you have carbon dioxide and glucose. Carbon dioxide is converted to glucose in the photosynthesis process, and glucose is converted to CO_2 in the aerobic respiration process. So, no matter which reaction is happening, this is the way we write it.

So, here we have the electron tower. And what you see are the most negative redox values or the electron potential at the top, and the most positive values at the bottom. Now, what is important to note is that electrons released from the top of the tower are caught by the electron acceptors at the bottom of this electron tower. So, the greater the fall of electrons, the greater the amount of energy released in terms of electrical potential, which can then be converted to chemical energy in the form of ATP. So, you can see that the greatest distance is from glucose to oxygen.

So, the top and the bottom; if you combine those 2, that is where the greatest release of energy is going to be. So, if I can; I will just say it instead of writing it. If you have glucose plus oxygen going to CO_2 and water, that is nothing but a combination of these 2 half-reactions. The first one is the top one, and at the bottom you have O_2 going to water. So, the highest release of energy happens in aerobic respiration, when you have a compound that is highly biodegradable like glucose, combining with oxygen and going to CO_2 and water.

So, that is one example. Now, let us take some other examples to make it clearer. Now, let us take number 1 that is shown over here. Here we have hydrogen which will act as the electron donor. So, it is in reverse. So, this is in reverse, hydrogen gas going to protons; and it is being combined with fumarate going to succinate. So, it is not a big fall, it is a small fall. So, you can see the, literally the distance along the electron tower.

So, the ΔG value for this reaction is -86 kilojoules. And then you have hydrogen again combined with nitrate. The fall of electrons is a little bit more, yes. You have $\Delta G^{0\prime}$ as -163 kilojoules. And finally, you have the combination of hydrogen with oxygen going to water. And that is the secondary action going all the way down. This one is, the arrow is not correct, the arrow goes further down to the last reaction; and that releases -237 kilojoules.

Now, these are examples of the two half-reactions being combined to release a lot of energy. So, there are hydrogen utilizing organisms or bacteria that can use hydrogen as the electron

donor, and these 3 – fumarate, nitrate or oxygen as the electron acceptors. So, these are the combinations that you can think about that these are these examples.

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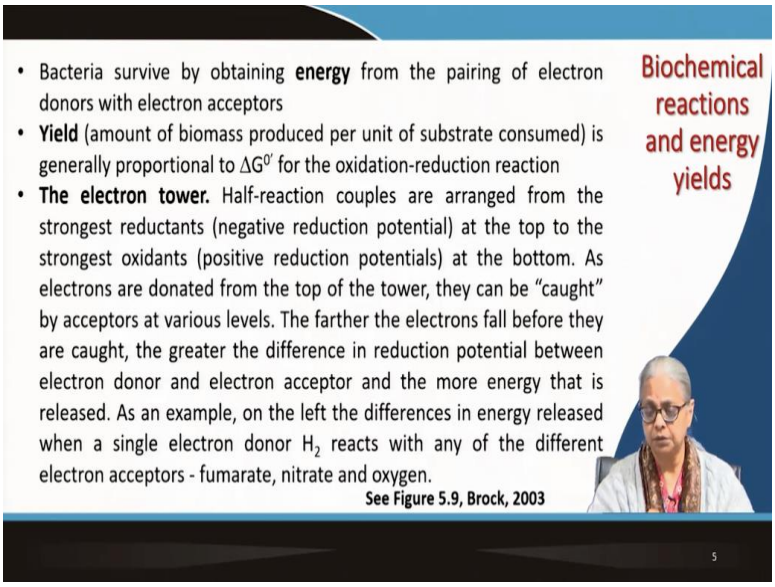

So, I have already mentioned that bacteria survive by obtaining energy from the pairing of electron donors with electron acceptors. And then we come to the yield. The yield in general is assumed to be the yield; let us first take the definition of the yield. Yield is the amount of biomass produced per unit of substrate consumed. And this is generally considered to be proportionate to the ΔG^0 for the oxidation reduction reactions, when you combine the electron donors and the acceptors.

So, like I said, glucose and oxygen is the best example of aerobic respiration. And it is also the proof that no other terminal electron acceptor in combination with any of the other organic compounds is going to give you a higher yield in terms of biomass or in terms of energy. So, we can do some calculations in the next topic when we come to that. So, I have said most of what I wanted to say here.

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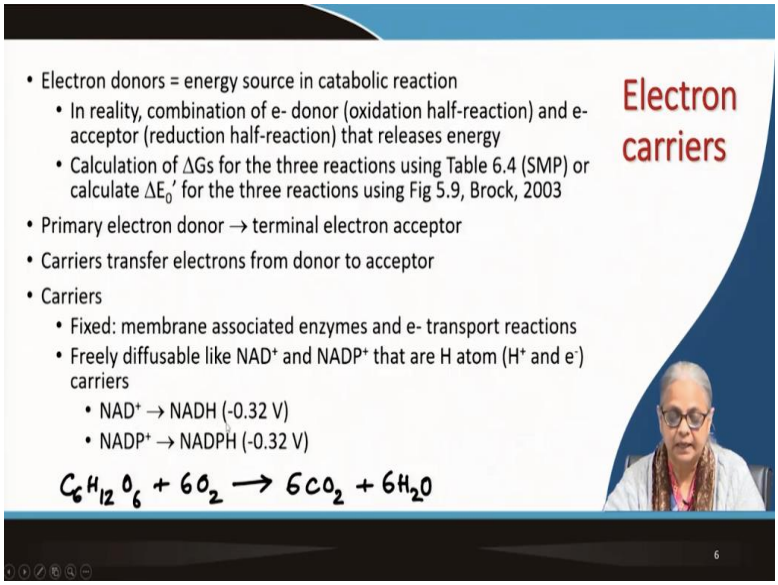

Now, let us come to electron carriers. So, we know that the electron donors are the energy source in the catabolic reactions. In reality, it is the combination. Very often in the literature, you will find statements that the electron donors are the energy source; but it actually is the combination of the 2, that releases energy. There may be sufficient energy in the electron donor by itself, but that is not what we are going to do.

What we are going to do in your assignment is to ΔG for 3 different reactions. You can use 2 different textbooks. So, we have Sawyer, McCarty and Parkin. That is one textbook. And the second textbook that has similar calculations is Brock's biology. So, now, remember what is happening in the entire electron transport process. What is the electron transport process and why is it important?

The first thing is; here we have the example of glucose along with oxygen going to CO_2 and water. Now, glucose, the oxidation state of carbon in glucose is 0. The oxidation state by definition for oxygen gas is also 0. And here in CO_2, carbon has +4, oxygen has -2, same thing in water. So, you can see that the primary electron donor is glucose. So, this is our glucose molecule. Now, how many electrons has glucose donated in going to CO_2?

So here, this is C_6. We have 6 carbon dioxides, and it has gone from 0 to +4. So, 6 times 4 is 24 electrons. So, glucose has donated 24 electrons. What is the compound that has accepted these 24 electrons? After all, they are not floating around, they have been accepted in equal amount. So, here we have oxygen which has an oxidation state of 0; and it has gone to -2 in carbon dioxide, and -2 in water as well.

So, those 24 electrons have been picked up by oxygen in both CO_2 as well as in water. So, that is our terminal electron acceptor. So, oxygen is our terminal electron acceptor and the primary electron donor is glucose. Now, how is this transfer of electrons happening? Is it a direct transfer? Is it a one-reaction transfer? The answer is far from it. The answer to this reaction; this reaction is an extremely simplistic description of what happens in reality. There are a large number of enzyme mediated reactions where there are carriers like NAD^+, FAD^+; all these carriers come into play in transferring electrons from the donor to the acceptor. So, we are going to go through all of it and try to understand what is important in terms of the process, the biochemical process of transferring electrons and generating ATP. Some people would say this is biochemistry, not microbiology; but to understand the bacteria and why they are capable of doing so many different things in so many different environments, you need to understand the basis of it. And this understanding of electron transport is crucial to the understanding of biodiversity within bacteria.

So, I was saying that there are 2 types of carriers, fixed and freely diffusible. So, the fixed carriers are the ones that are membrane-associated enzymes that are part of the electron transport reactions. And you also have freely diffusible carriers like NAD^+ and $NADP^+$, that are capable of donating and accepting both protons and electrons, or the entire hydrogen atom as a whole. So, you can see NAD^+ going to NADH. You can see the electron potential as well as $NADP^+$ and NADPH.

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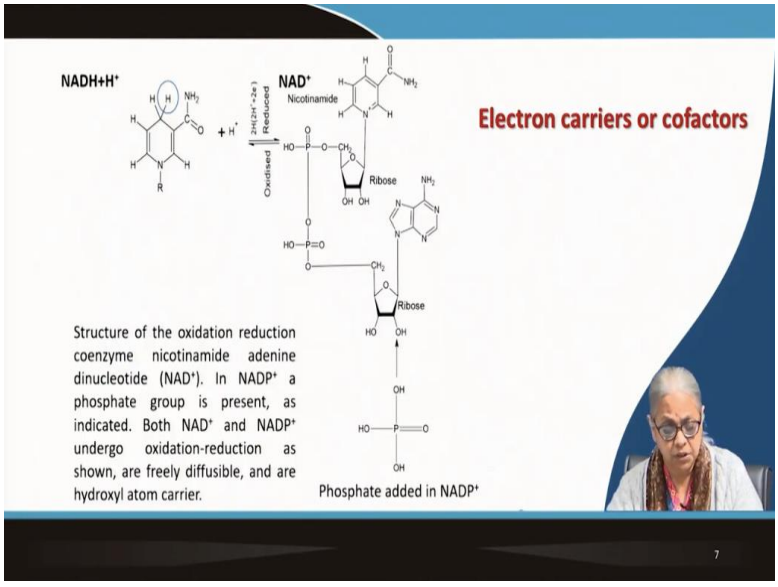

So, here we have the chemical structure of NADH. And when there is a proton, what it will do is; like I said, it reverses between NADH and NAD^+; or if it is associated with a phosphate,

like I have shown over here; so, here at the C2 position, the phosphate can be added here. In that case, it is NADP +, and it can pick up a hydrogen atom and become NADPH. So, these are the 2 forms.

And here, 1 hydrogen and an electron from the nitrogen will be removed along with the proton, and you get 2 hydrogen atoms. So, you can see the plus charge at the nitrogen over here. The loss of a hydrogen at the opposite end of the ring. That is basically how it reverses its oxidation state. And that is how it transfers both protons and electrons in the electron transport chain.

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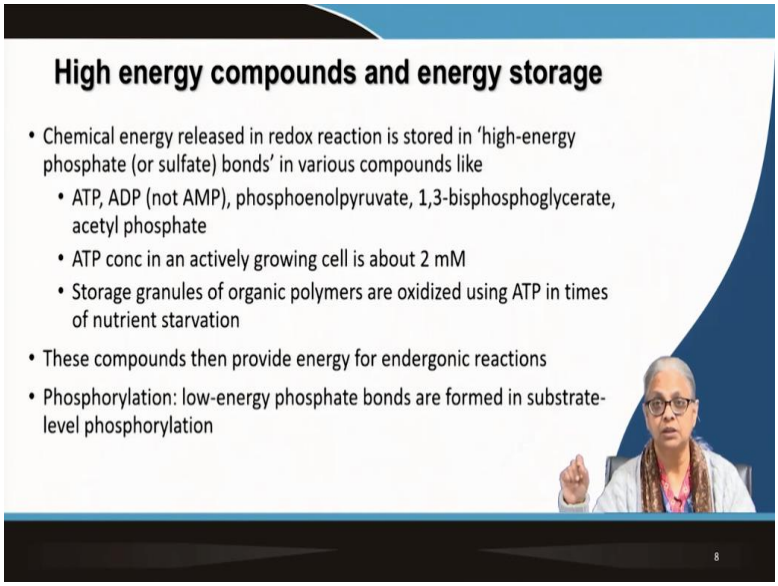

So, now that we know that we have different carriers for transporting electrons, and we also realize that this electron transport process is how energy is going to be generated in the form of ATP. So, chemical energy which is released in the redox reactions has to be stored in high-energy phosphate or in some cases in sulphate bonds. So, we have ATP and ADP. These are both high energy bonds. AMP on the other hand, is a low energy bond. Then we have phosphoenolpyruvate. We have 1,3-bisphosphoglycerate as well as acetyl phosphate. These are all examples of high energy bonds. ATP concentration in an actively growing cell is 2 mM. Storage granules of organic polymers can be oxidized using ATP when there is nutrient starvation in the environment or rather when the environment has low levels of nutrients.

These high-level energy containing compounds can provide energy for endergonic reactions and that is why they are so important. Then finally, we have phosphorylation. So, low energy phosphate bonds are formed in what is called substrate-level phosphorylation. So, you have glucose 6-phosphate. So, that is; I think I have some examples, yes.

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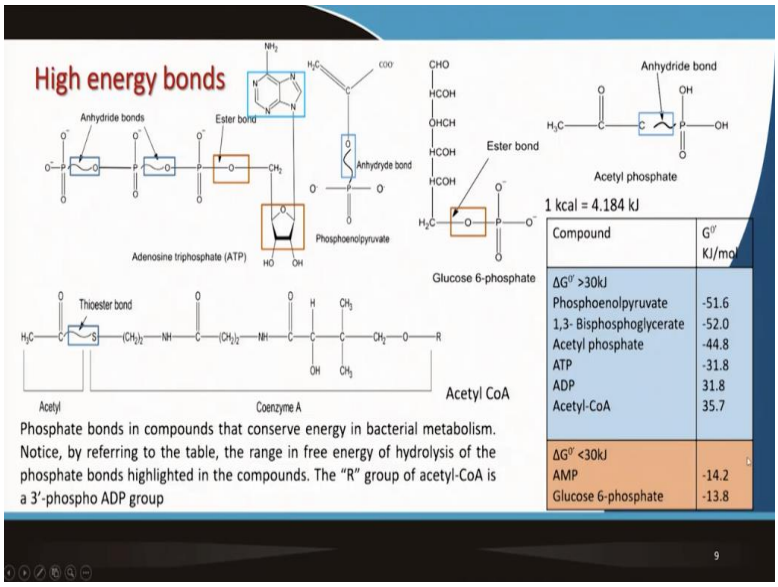

So, in this slide, you can see the low energy bonds. They have ΔG^0values less than 30 kilojoules. So, AMP, adenosine monophosphate and glucose 6-phosphate are low energy bonds. The high energy bonds are all shown over here. These are anhydride bonds. So, you have ATP, you have phosphoenolpyruvate, you have acetyl phosphate and you have acetyl-CoA. So, we will be looking at all of these compounds when we try to understand how energy is generated by the bacteria or other organisms in various redox reactions.

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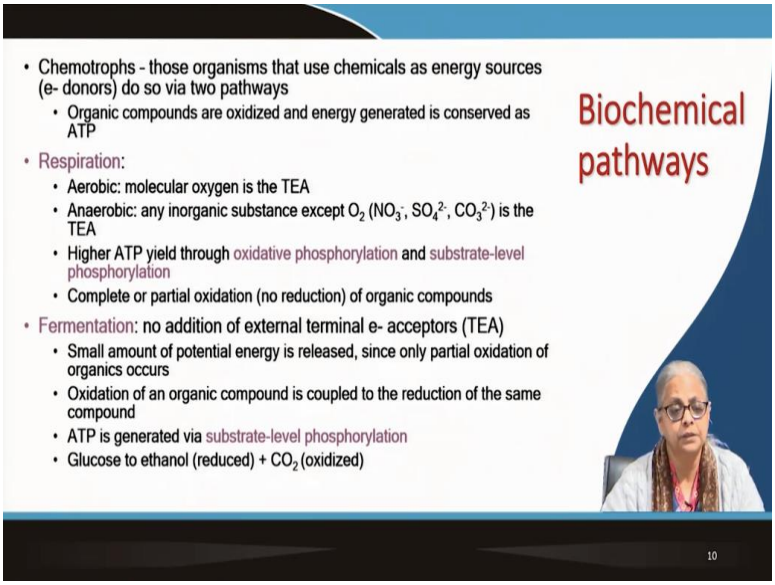

So, for understanding how energy is generated, we need to have some understanding of the biochemical pathways. Now, there are several methods that organisms can use for deriving energy. So, we know that there are chemotrophs. These organisms are the ones that use

chemicals as energy sources or electron donors. Now, they can use it in 2 pathways; you have respiration or fermentation.

Within respiration, you have two pathways, aerobic or anaerobic. Now, organic compounds are oxidized and energy is generated and conserved in the form of ATP; we all know that. Let us take a look at these 2 pathways.

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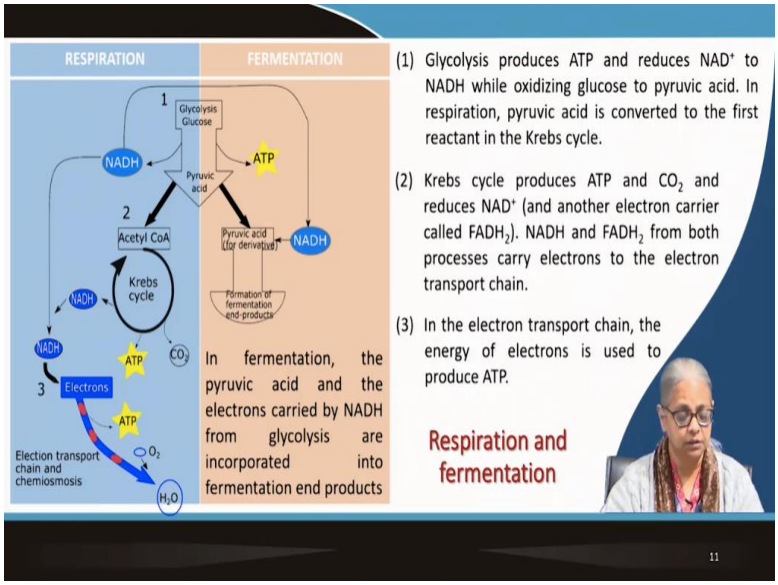

So, these are the 2 basic pathways, respiration and fermentation. Our starting compound remains our favorite; we all like sugar, that is glucose, so let us stick to it. So, here we have glucose as our starting point. This glucose is a C-6 molecule. This C-6 molecule has to be broken down into a C-3 molecule. Pyruvic acid is a C-3 molecule. So, this process of converting glucose from C-6 to C-3 is called glycolysis.

In this process, ATP as well as NADH is being generated. Now, at the point at which pyruvic acid is generated, there are 2 choices that the organism can make, depending on the availability of electron acceptors. So, you have pyruvic acid. Now, if pyruvic acid is directly converted to fermentation products; in fermentation, what happens is that there is no external electron acceptor.

So, if you remember the glucose plus oxygen going to CO_2 and water, there our electron donor is glucose, electron acceptor is oxygen, and CO_2 and water are the end products. In fermentation, pyruvic acid is not combining with any external electron acceptor. So, there is

no oxygen and there is no substitute for oxygen. So, it is just pyruvic acid which is a C-3 compound. It will eventually be converted into other fermentation products.

I will show you some examples of that in a little bit. The other choice is respiration. So now, this pyruvic acid enters what is called the Krebs cycle. So, in Krebs cycle, the first thing that happens is that pyruvic acid before it enters the Krebs cycle, that C-3 molecule is going to be converted to a C-2 molecule called acetyl-CoA – coenzyme A attached to an acetyl group. So, this acetyl-CoA is a C-2 molecule. Now, this C-2 molecule comes into the Krebs cycle.

So, this pyruvic acid which is a C-3 molecule has to be converted first to a C-2 molecule, which is what happens in the first step. So, you have acetyl-CoA, where C-3 is converted to a C-2 compound. This C-2 compound enters this citric acid cycle or Krebs cycle and is converted into two more carbon dioxide molecules. So, the entire C-3 molecule has now been completely oxidised, completely mineralised to 3 CO_2 molecules.

So, whether you include this first step of combining it with acetyl, or generating acetyl-CoA or whatever you want to do. So, this Krebs cycle, in all cases, the first step, you also get NADH and ATP generation; second step, you again get, in the Krebs cycle or citric acid cycle, you will produce ATP and CO_2, and reduce NAD^+ to NADH, yes. NADH and $FADH_2$ from both processes, will carry these electrons through the electron transport chain.

When these electrons and protons are being carried through the electron transport chain, 2 things are happening. Protons are being pumped out of the membrane to generate proton motive force, and the electrons are passed on from one carrier to another to produce ATP. So, this is a very rough and clear schematic of what is happening. And like I said, the level of details, you can keep probing into more and more details to get better understanding of this, but we are, we will go through some amount of that in the subsequent topics.

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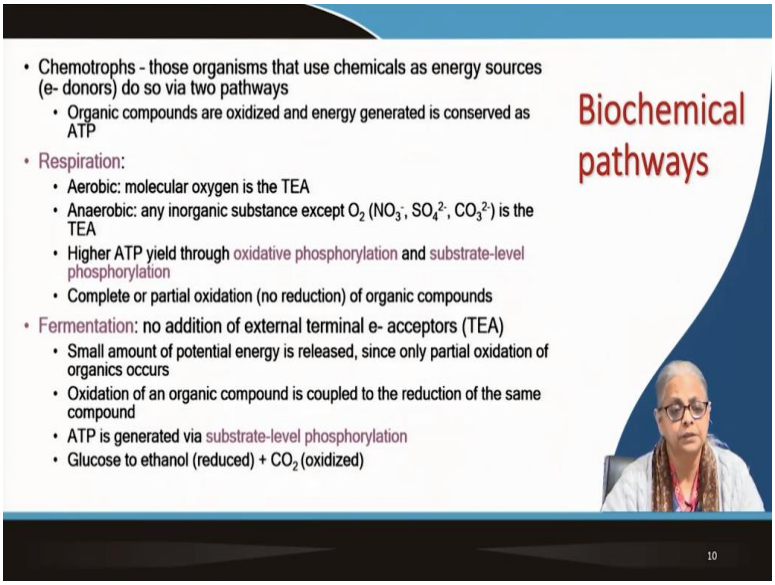

I need to say one more thing. Now, that is respiration. So, the first part is common to both respiration and fermentation. Glucose is converted to pyruvic acid. 2 molecules of pyruvic acid are generated from 1 molecule of glucose. First step, common to both pathways.

Within respiration, there are different terminal electron acceptors that are used. So, if the terminal electron acceptor is molecular oxygen, then it is called aerobic respiration. If the organism uses an inorganic substance other than oxygen like nitrate, sulphate, carbonate; if these are the terminal electron acceptors, then it is called anaerobic respiration. So, in the absence of oxygen, it is called anaerobic respiration. The pathways are the same. The biochemical pathways will be the same, but it is the electron acceptor at the end of the electron transport chain (that) is different.

So, you get a higher ATP yield. I have already shown you the electron tower; glucose at the top, oxygen at the bottom, there is no higher yield. So, as long as there is oxygen in the environment, most of the bacterial species will utilize organic carbon, combine it with oxygen and get the highest possible energy yield. This is done through 2 processes, oxidative phosphorylation and substrate level phosphorylation.

We will take a look at both of them. In aerobic as well as anaerobic respiration, you can get complete oxidation or partial oxidation; both can happen for the organic compound. I have already mentioned that glucose gets completely oxidized to CO_2. So, that is an example of complete oxidation. You can have partial oxidation. So, you can have the organic compound

being partially oxidized. So, it does not go to CO_2; it may stop at some intermediate. However, you do not get any reduction of the organic compound. So, the defining point about respiration is that the organic compound is not reduced, it is either partially or completely oxidized. In fermentation, in contrast to respiration, there is no external terminal electron acceptor. So, the same organic compound, whether it is pyruvate, whether it is acetate, whether it is glucose, whatever it is, it is going to be partially oxidized and partially reduced. So, half of it will be reduced, half of it will be oxidized. Whatever electrons are being generated in the oxidation reaction will be picked up in the reduction reaction. So, small amounts and because there is no combination with an external terminal electron acceptor, so, very small amounts of energy are released, because you get only partial oxidation of the organic compound.

Now, the oxidation is coupled to the reduction reaction of the same compound. ATP is generated in 1 process only, and that is substrate level phosphorylation. There is no oxidative phosphorylation. So, glucose can be converted to ethanol; lactose can be converted to lactic acid; and it basically ends there.

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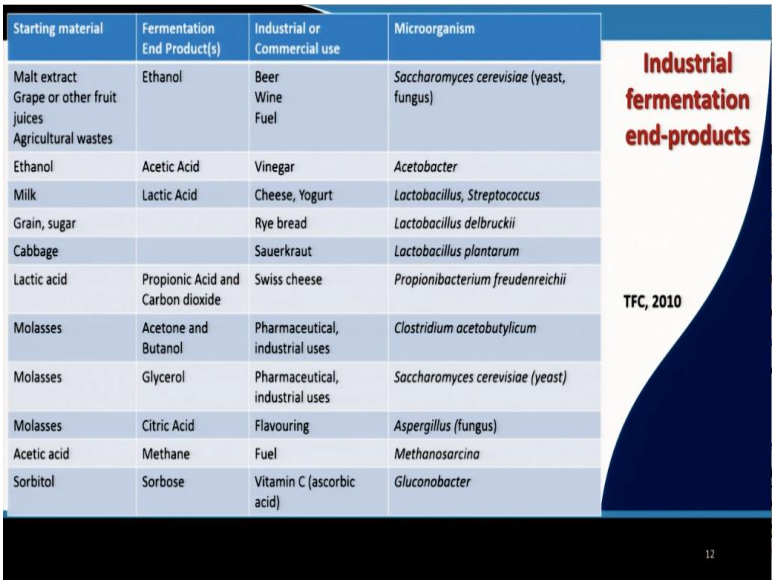

Starting material	Fermentation End Product(s)	Industrial or Commercial use	Microorganism
Malt extract Grape or other fruit juices Agricultural wastes	Ethanol	Beer Wine Fuel	*Saccharomyces cerevisiae* (yeast, fungus)
Ethanol	Acetic Acid	Vinegar	*Acetobacter*
Milk	Lactic Acid	Cheese, Yogurt	*Lactobacillus, Streptococcus*
Grain, sugar		Rye bread	*Lactobacillus delbruckii*
Cabbage		Sauerkraut	*Lactobacillus plantarum*
Lactic acid	Propionic Acid and Carbon dioxide	Swiss cheese	*Propionibacterium freudenreichii*
Molasses	Acetone and Butanol	Pharmaceutical, industrial uses	*Clostridium acetobutylicum*
Molasses	Glycerol	Pharmaceutical, industrial uses	*Saccharomyces cerevisiae (yeast)*
Molasses	Citric Acid	Flavouring	*Aspergillus* (fungus)
Acetic acid	Methane	Fuel	*Methanosarcina*
Sorbitol	Sorbose	Vitamin C (ascorbic acid)	*Gluconobacter*

This slide shows us some examples of industrial or commercial applications of fermentation. So, we have the starting material in the first column; the fermentation end products in the second column; the industrial or commercial application in the third column; and in the last column, we have the microorganism, the name of the microorganism or the species that is responsible for this fermentation reaction.

So, for example, the production of beer is started by using malt extract. The fermentation product is ethanol and the mediating enzyme (organism is yeast) or the enzyme that is

responsible for this reaction is *Saccharomyces cerevisiae*, which is a yeast or a fungus. Then we have grape or other fruit juices which are used for making wine. Again the fermentation end product is ethanol, and the same fungi is used for mediating this reaction.

In the last case, we have agricultural waste which can be converted to fuel. So, we have biodiesel production that has become very popular. It is especially popular in India, Brazil, U.S. All these places, agricultural waste is now not just agricultural waste, but even certain crops are used for converting and generating fuel. Again, these are reactions that are mediated by yeast or fungi.

Then we have; the starting material, when it is ethanol, it can be converted to acetic acid. That is what we call vinegar. Commercially, it is sold as vinegar; and the microorganism responsible for it is *Acetobacter*. Our next example is milk. Milk can be converted by fermentation; the fermentation end product will be lactic acid and the industrial products are cheese and yoghurt. Now, these processes are mediated by microbes like lactobacillus and streptococcus. Then we come to cabbage. Cabbage is converted to sauerkraut which is a very popular food material. That is generated using *Lactobacillus plantarum*. Lactic acid can be converted to propionic acid and carbon dioxide in the production of Swiss cheese. We have the name of the microorganism responsible for this, that is *Propionibacterium sp.*

Molasses, which comes from sugarcane can be converted to acetone and butanol. These are both pharmaceutical as well as industrial applications. The microorganism responsible is *Clostridium acetobutylicum*. Molasses can be converted to glycerol. Again, there are pharmaceutical as well as industrial applications; and the mediating microorganism is *Saccharomyces cerevisiae*.

We have molasses being converted to citric acid, which is used as a flavouring agent; and that is done by another fungi, *Aspergillus*. Then we have acetic acid which is converted to methane, to produce biogas; and that is *Methanosarcina*. Sorbitol is converted to sorbose, which is used in the production of vitamin C, which is (L-)ascorbic acid; and the mediating microbe is *Gluconobacter*.

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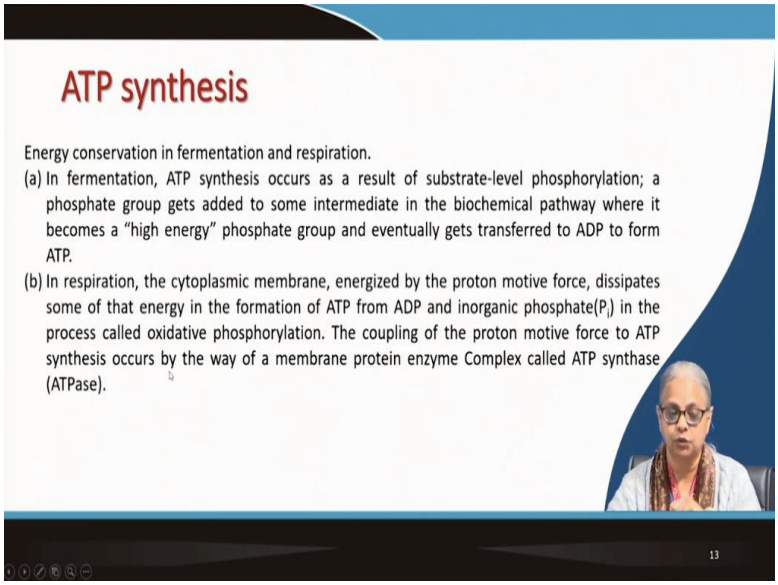

We now come to ATP synthesis. Now, how is ATP generated by the bacteria or any of the other organisms? So, energy that is derived from the combination of electron donors and electron acceptors has to be conserved, whether it is a fermentation pathway or a respiration pathway. So, like I said, in fermentation, ATP synthesis happens only in that first step of glycolysis, and that results from substrate-level phosphorylation.

We will go into that when we go into glycolysis, which is also called the Embden-Meyerhof pathway. A phosphate group is attached to some intermediate in the biochemical pathway to result in a high-energy phosphate group. So, that is called phosphorylation. So, it is called substrate-level phosphorylation. Because the organic compound, whether it is glucose or any other starting organic compound, phosphate will attach itself to that organic compound, and that is why it is called substrate-level phosphorylation.

Now, this is common to both, respiration as well as fermentation. What is not common to both is the next one, and that is called oxidative phosphorylation. So, in respiration, the cytoplasmic membrane is energized by the proton motive force.

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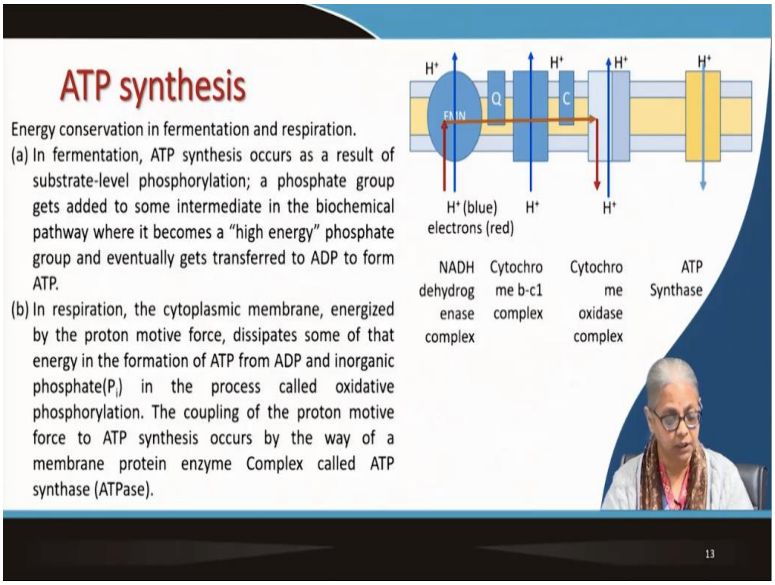

So, in the respiration pathway, the cytoplasmic membrane has to be first energized by the proton motive force. I already explained in the previous topic, in the previous module that the NADH carriers which are capable of accepting and donating both protons and electrons, they will help to pump out protons to the outer side of the cytoplasmic membrane. So, you can see that very clearly over here.

In respiration, the cytoplasmic membrane or the plasma membrane is energized by the proton motive force. We have already seen in the previous topic that this proton motive force is generated by these complexes; 3 of them are shown over here, and the fourth one is ATP synthase. You can see that protons are pumped out from the cytoplasm to the outer side of the plasma membrane. So, that is the first thing. That is the energizing of the membrane.

Now, these electrons that are with NADH, these electrons are transferred from one carrier to another. So, you can see several carriers associated with the plasma membrane are going to transfer electrons from one carrier to the other. In the end, these electrons will come back into the cytoplasm and along with ADP as well as oxygen or whatever else the terminal electron acceptor is, at this stage, ADP will combine with phosphate utilizing the proton motive force, generate ATP using this ATP synthase enzyme; and the terminal electron acceptor will pick up all these electrons through the electron transfer chain. You will get reduction of this, whether it is oxygen or nitrate, sulphate, carbonate, whatever it may be. So, this is the coupling of the proton motive force to ATP synthesis. And we have seen some of that in the previous topic.

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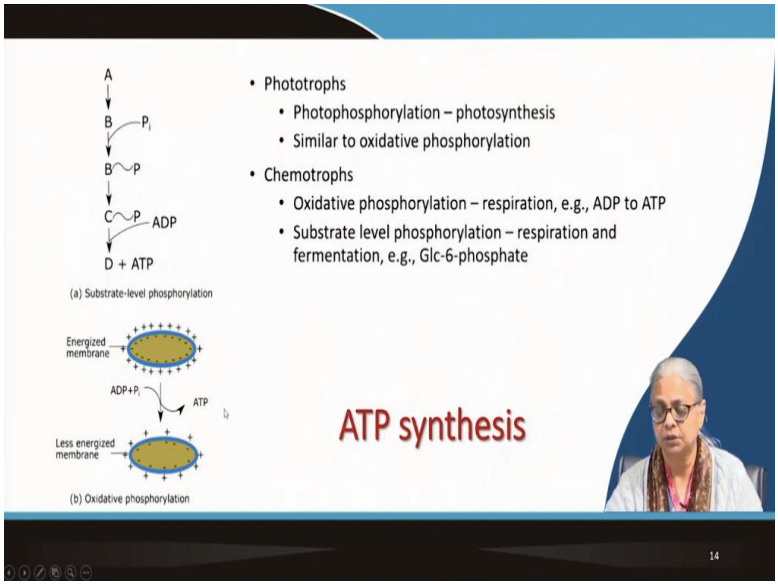

So, we have two pathways. We have respiration and fermentation. And like I said, substrate-level phosphorylation is common to both pathways. So, here we see an example of substrate-level phosphorylation. So, you have your substrate. It can be glucose or any other organic compound. It gets converted. A phosphate is added to that intermediate and a new compound is formed.

For example, glucose 6-phosphate is a low energy bond, so, less energy is utilized here. Actually, I should rephrase that. For example, in glycolysis, glucose is phosphorylated and you get a low energy bond in terms of glucose-6-phosphate utilizing an ATP. So, a glucose 6-phosphate, like I said, is generated in glycolysis. It is a low energy bond. An ATP molecule is utilized in the process. So, that is an example of substrate-level phosphorylation.

Like I said, glycolysis is common to both respiration and fermentation. Now, another ADP molecule is added, another phosphate is attached to it, from these phosphorylated organic compounds. So, this phosphate that is attached to an organic compound, which is an intermediate in the glycolysis pathway, that will be then added to an ADP to generate ATP. So, these are all examples of substrate level phosphorylation. This is the only thing that fermentative bacteria can do.

Then we come to oxidative phosphorylation. So, this is another example of that. So, you can see the proton motive force. There is a higher concentration of protons on the outside of the membrane compared to the inside. Just like your dry cell where you have concentration gradient of electrons that are being generated; so, we call it the electron motive force.

In this case, you have the proton motive force. So, this proton motive force is then channeled back through ATP synthase to generate ATP. So, this is your less energized membrane, where ADP with 1 phosphate is converted to ATP. And that is what I showed in a previous slide. So, here you have phototrophs. Photophosphorylation is used in photosynthesis, and it is similar to oxidative phosphorylation. And like I said, oxidative phosphorylation is used in respiration to convert ADP to ATP.

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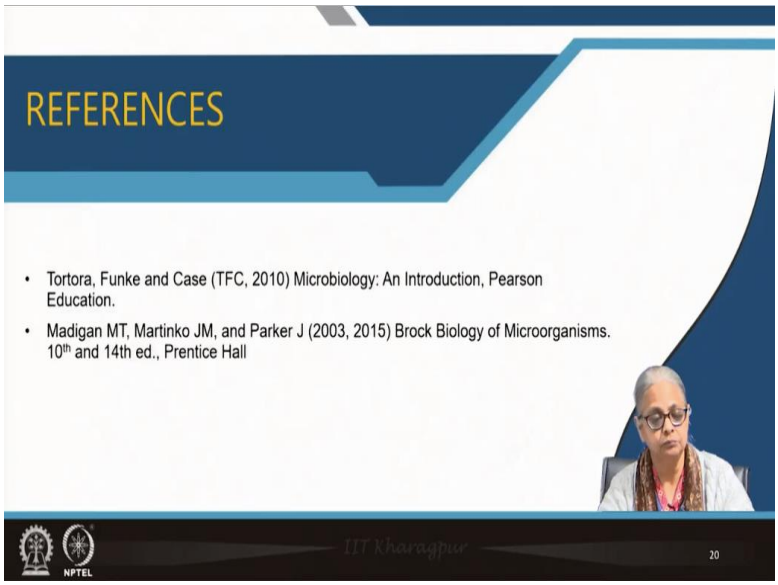

Thank you. We will then complete the next part in the next lecture.