Applied Environmental Microbiology
Dr. Gargi Singh
Department of Civil Engineering
Indian Institute of Technology, Roorkee

Lecture – 08
Microbial Energetics II

Hello students. In this lecture of applied environmental microbiology, I want to share with you some various fundamental understanding of microbial energetics. And microbial energetics is about how microbes find the energy to sustain life into grow, and how they complete the energetic balance of the environment in the community. And this is very important to understand, I mean you do not have to get into physics of thermodynamics of microbial sustenance and growth. But it is the basics are very important to understand, because as the environment changes the kind of microbial communities we expect with change.

I will give a very simple example, let us talk about ground water plume.

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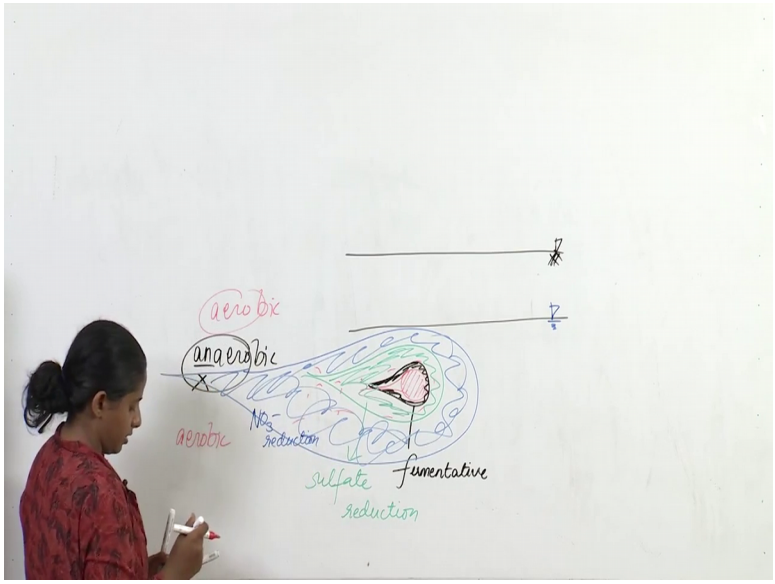

So, let us say this is earth, and this is ground water. And because of an oil spill, now we have a non-aqueous phase liquid plume here; which is a fancy way of saying we have an oil plume here. So, this is the oil spill that happened. And the oil seeped through the soil and came to the water and now it is here as a plume.

Now, when there is food in the environment the microbes will grow and find a way to eat up the food. If not microbe a and microbe B will. So, they or more often than not we notice that if there is a plume, microbes around will start eating it. Now as microbes around it start eating, it initially there is some dissolved oxygen present in the groundwater. So, the microbes eating it are aerobic in nature. Aerobic means aerobic has a word air in it. And thus, these are microbes that consume air they need air via aerobic a living beings, we need air to survive, but not all living beings need air to survive.

So, microbes around with around it will consume the air that they have in their environment, and then once they are done consuming the air, the re aeration rate is very slow; obviously, because there is a nice layer of land or to prevent re aeration and also there is a boundary of the water. So, re aeration is slow and then the water air around this plume gets depleted. So, we generate anaerobic condition. And then if anaerobic condition has been generated that is no air. So, anaerobic is written as an aerobic so, an means no, no air.

So, if there is no air then the microbial communities that can use other electron acceptors apart from oxygen. Now electron acceptor is something that takes electron, such as sulfate or nitrate or phosphate. They will see if sulfate nitrate phosphate are present around. Let us see there are plenty of sulfates then these microbial communities what they will do is they will reduce the sulfate make hydrogen sulfide gas or whatever, and then they will consume this plume they will consume the food. And one sulfates has been depleted when they will move on to night or the other electron acceptors.

So, but there is another phenomena happening here to the plume is also slowly undergoing this solution. Because it is non-aqueous it is hydrophobic it would not dissolve, but it undergoes dissolution to some extent. So, plume does not just stay here stationary it moves around as it moves around, it creates a tail more often than not and there is also some dissolution that happens around it. So, it becomes more like a continuum than a very defined boundary of the plume.

So, what we notice here is that just right around the plume, just right around the plume and maybe even inside the plume to some extent, we have anaerobic condition. There is no electron acceptor left sulfate, nitrate, phosphate, oxygen; obviously, all of them have been consumed. And what we have here is fermentative conditions. So, when there is no

oxygen left, we can expect that this fermentation happening; which is things that do not require electron acceptors to produce energy, those chemical reactions are happening that is what microbes are using to consume this food, make energy out of it and survive. So, after right outside this fermentative zone, we will have some other electron acceptor that will be still acting and kicking.

Now, let us assume in this particular groundwater, we have plenty of sulfate. So, around this there will be an nice boundary of sulfate reduction. So, this is the area. So, in this area, we have microbes that are reducing sulfate producing hydrogen sulfide or hydrogen sulfite. And creating energy by consuming it is all it is the particles of from the plume. And thus, the microbial community in the green region will be very different from microbial community in the black region.

Now, outside sulfate reduction let us say there is some other electron acceptor like nitrate which is a very strong electron acceptor. And we can represent it by blue color. So, there is nitrate present, and then we will have a big nice volume where nitrate reducing microbes will be prevalent and abundant.

So, in this blue portion of the groundwater, we have we still have nitrate left. Now notice this thing. Once the sulfate is depleted this will become fermentative. Once this nitrate is depleted, then the microbes will start using sulfate. And when sulfate is depleted they will start using fermentation techniques. And outside this we have aerobic. And this is nitrate reducing, all righty.

So now the question is why do they follow this particular pathway, why is aerobic as far as possible from plume followed by nitrate reduction followed by sulfate reduction followed by fermentation?

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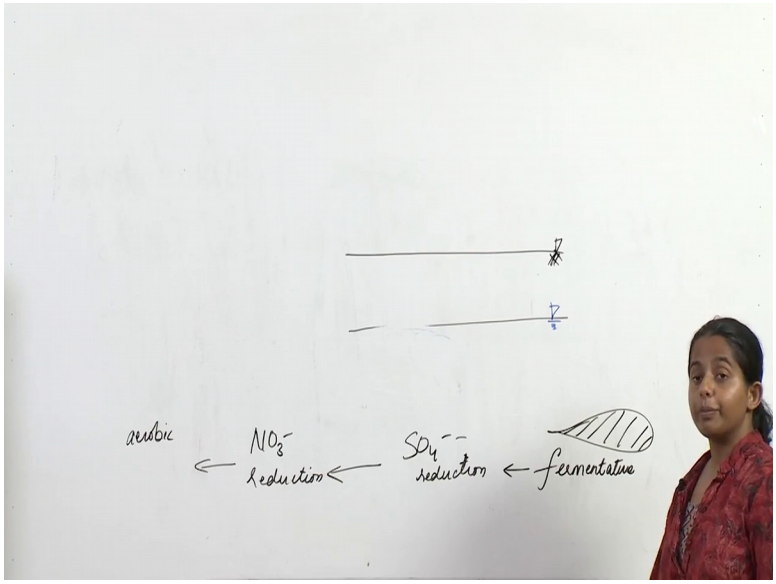

In other words, if a plume is here, if plume of oil is here, then the conditions right next to it are likely to be fermentative, at any given instance. Because other electron acceptors have been very quickly consumed by microbes. Little away from it where we will find let us say sulfate reduction. So, this is where sulfate is being reduced and food is being consumed. Little further away from it we will find microbes that actually reduced nitrate. So, we have nitrate reduction going on. And away from it we will find microbes that are aerobic in nature.

Now this sequence fermentation followed by sulfate reduction, followed by nitrate reduction, followed by aerobic or oxygen reduction; the sequence is determined by energetics. It which is determined by how much energy will microbes get by reducing a particular electron acceptor, as they oxidize the food. And when everything is consumed the enters phenomena called fermentation. So, this is microbial respiration, and that is what energetics is about and that is what we are going to discuss today.

In order to understand microbial energetics, it is very important to understand what 2 microbes required to survive.

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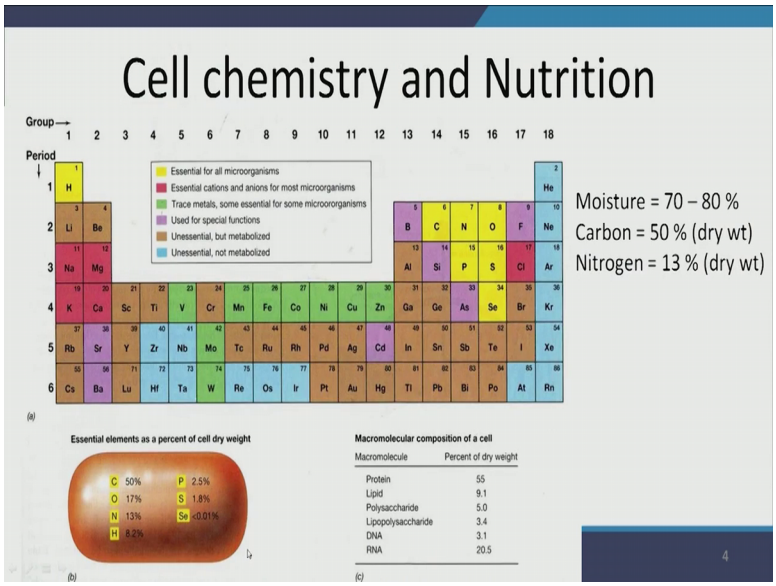

For microbial cell to exist there are certain essential elements that are required, and in this particular periodic table they are highlighted by yellow color. So, look here hydrogen, carbon, nitrogen, oxygen, phosphorus, sulfur and selenium. Most of the soil nearly 70 to 80 percent is water. I have written moisture. 50 percent of dry weight is carbon 13 percent of drive it is nitrogen. Phosphorus, sulfur, oxygen, and selenium are at lesser numbers.

Then the ones in pink here, sodium, magnesium, potassium, calcium, and chlorine, they are essential cations and anions for most microorganisms. Not all, but most. So, some microbes can actually do without them. Then the ones in green are trace metals that are essential for some microorganisms we need them. For example, iron and cobalt are necessary for a hemoglobin to work, but we are not microorganism. But for many of microorganisms they require cobalt. So, when we try to grow them in lab, we need to make sure we give them these trace metals, otherwise they will die out.

Then the ones in purple, boron, silicon, arsenic, fluoride, cadmium, strontium, and (Refer Time: 09:14) these are useful some specialized functions are not all of them required. And not the same microbe may not always need them; the brown ones are unessential, but metabolize. What this implies that these are not required the microbes, but they might metabolize them. That is, they might reduce oxidized them, and they might even assimilate it inside their body, and then eventually kick it out or die. But they are not

required. And in the blue ones microbes have nothing to do with them. And notice that this does not even include actinide in lanthanide series.

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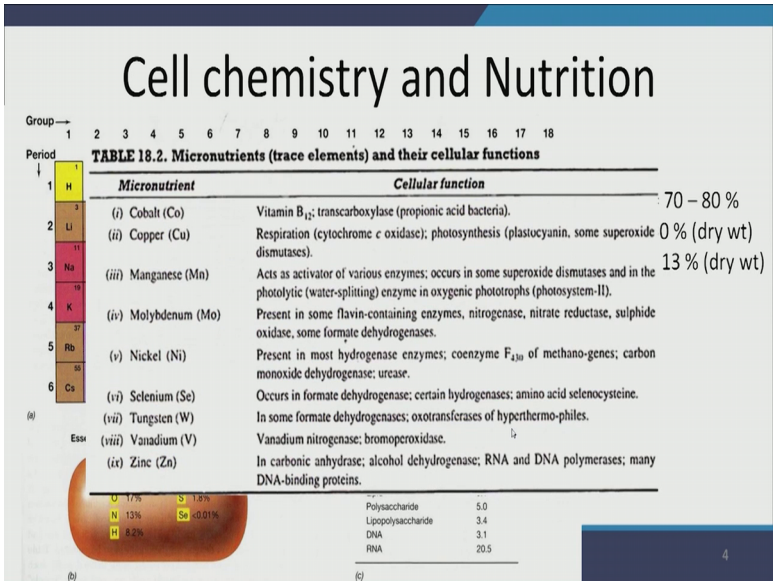

So, these are the trace elements that I talked about. Cobalt is a very important part of it. I am in B 12 propionic acid back later he needs it to copper, manganese, molybdenum, nickel, selenium, tungsten, vanadium and zinc. So, if you work in lab, and you are try to grow microbes for any good length of time. We need to give them dosage of these micronutrients, all righty.

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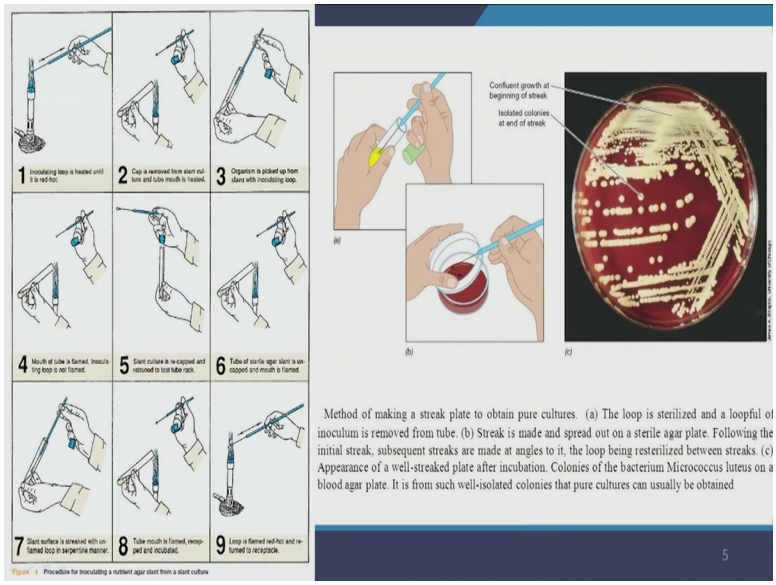

Now, how would we grow these microbes in lab? Usually we plate them, that is the very normal procedure that is being demonstrated here also. Now in order to plate them and create such beautiful colonies, now in this red broth; we have this beautiful colonies. And I must say that each of these is perfectly circular colonies have in theory come from a single cell. Now how do I ensure that I do not contaminate this when I am plating it? How do I not introduce microbes from my body? Because remember our skin has a very rich microbial flora. So, in order not to contaminate this plate as we are trying to grow a particular microbe, it is a important to undergo a procedure of disinfection and sterilization very strictly.

So, the first step is we use an inoculating loop. And here is the if you see there is at any loop here. There is there is a wire here connecting it to this spatula. So, we take this loop and we in sterilize it, it a on burns in burner. So, you flame so to sterilize it in this particular panel. We notice that they are saying inoculating loop is heated until it is red hot to sterilize it.

Now it is we do not need to do that we just make it catch fire by dipping it first in ethanol, and then putting it over the burner. And then what we do is we remove the cap from our media where things are growing. We pick up the organism. And then we sterilize again using flame the tip of the test tube where microbes were growing. We close the stopper, and then we inoculate this particular tip into the test tube where we want it to grow. Again, we sterilize the tip, close it, and then we are done.

If you want to plate we first realized the inoculating loop. Pick up the microbes and then streak them. Look there is a black arrow here showing the direction of streaking and we streak them. And as we streak them we inoculate the play it with microbes. And I will very likely very quickly I would like to tell you, one of the very simple streaking mechanism.

So, let us say this is our plate, perfectly circular. And I have my inoculating loop, and let us make this my inoculating loop. Now what I have done, I have flame sterilized it. By first dipping it in ethanol and then burning it over burns in burner or directly burning it on fme as it is shown in the slide. And then I dip it in the media where a good microbial community that I want to grow, if in it is exponential phase ideally. And then I will pick that up. So, have some microbes sticking on my inoculating loop.

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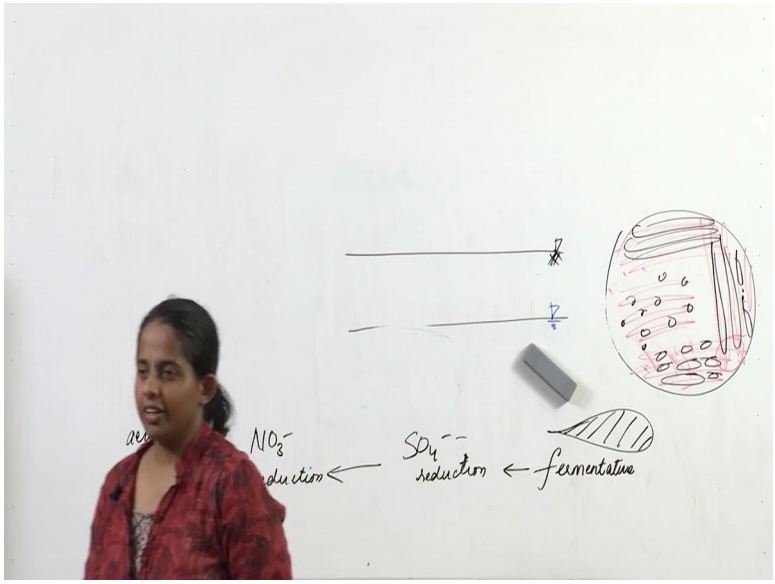

And then I usually streak it like this in one corner of this plate and here because I have just picked the microbial cells from the inoculum. I expect to have a very high density of cells.

So, they will almost grow continuously and follow my streaking like a line. And then, same streak has lost a good percentage of it is initial microbes, same inoculating loop. So, I use this to do it this way. So now, here I will have a lesser concentration of microbial population form of the microbes that streak it here will get swiped here and spread here. And thus, we have much lesser concentration than microbes right here. But we still have a pretty decent concentration. And then without doing anything to my inoculating loop I will again streak it in this direction.

So now basically I am spreading the microbes that are present here, and microbes that are still left on my inoculating loop and spreading them here. So, here I expect even lesser concentration. And finally, I will streak it here making sure that I do not contaminate with this. And why do we do this at least 4 step streaking. We do this at least or maybe I will just instead of instead of streaking it like this. Another possibility is, I whatever is left in my inoculating loop, I will streak it here.

Now, why do we do this? Because when we pick microbial cells that are in exponential growth or in good growth rate from my inoculum media, inoculating media, then there is very high chances that the population of microbes will be very high. So, if I just spread

them in the entire plate, I will get a thick mass that I cannot count and I cannot make sense off. So, it would not be very useful. So, I would rather contain my thick mass in one portion of the plate.

So, this is where you will get like long lines of microbes cannot make sense out of it. And then I spread a portion of it to another part of plate; where again I am very likely to get long chains, but these long chains are likely to have some separations between. Them and then again, I spread a portion of this now here is where separation will become more clear between the colonies that will grow, but some of them would be still elongated. Which is multiple cells have been inoculated and in the 4th when I expect. That I will have individual colonies, that I can count and use for further studies. And let us take a look at the picture.

So, if you look at the picture, this is where the streak streaking process was started. So, we notice near continuous growth, in the second very beautifully streaks were made in this direction. So, we have lines we have some separation here, and then the third one here, 4th one here, and by the fifth one we started getting isolated colonies at the end of streak.

Now, let us come to the energetic. So now, we know how to grow microbes. And we know the nutrient requirement of the microbes.

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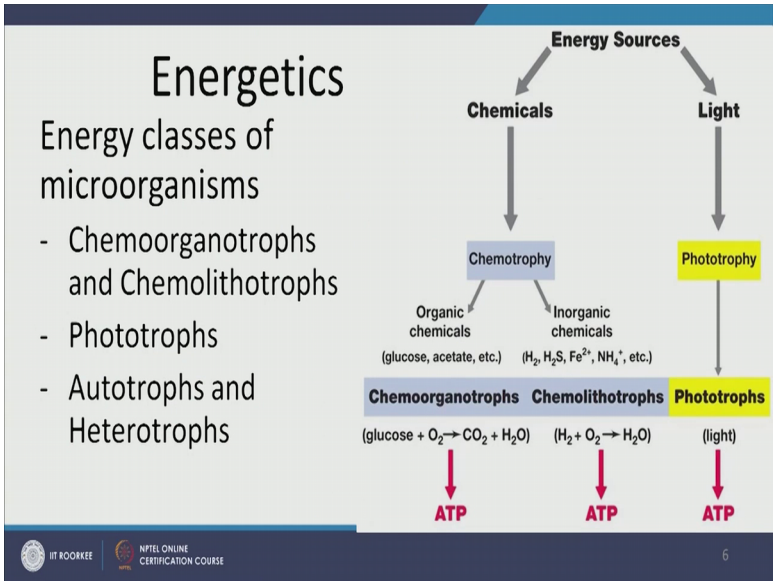

So now, let us try to understand the energetics of microbe, which was which I was talking earlier. Now in order to understand energetics of microbes or the respiration of microbes, we it is helpful for us to look at the classifications we have made of our microbial population based on the energy sources. So, if the energy source of microbe is a light like, plants and trees they used light to make their food, then this kind of microbes are called as phototrophs. Photo means light, trough means food. So, photo trophy oh and the microbes are called phototroph. They used light to make a ATP which is a major energy currency for the cell.

The microbes that use chemicals for producing food, which the humans use chemicals to our food in a way is a an energy rich organic chemical. So, who use chemical, they are together collectively known as chemo trophs. Now if I use organic chemicals just, sugar, acetate or food like, we use food which is more complex organic material, then we are known as chemo organo trophs, chemo means chemical organo means organic chemical, trophs means eaters. So, we basically use all our complex organics they break down eventually into glucose and other simple compounds. We oxidize them using something may be oxygen may not be oxygen. In this example, it is oxygen. Convert them into byproducts and in that process, we generate ATP. This is energy currency of the cell.

There are some microbes that do not use these to produce energy. In fact, they use other reduced microbe other reduced chemicals such a hydrogen sulfide, iron, NH plus 2 form ammonium. These are called chemolithotrophs. Camo means again chemicals. So, their energy source and chemical; letho means rock. So, they are not using organic material they are using minerals troph food. So, they oxidize their inorganic chemicals to produce ATP. So now, we have camoorganotrophs and camolithotrophs.

So, chemical organic chemical eating microbes camo camoorganotrophs. In organic chemical eating microbes camolithotrophs, now I must mention camolithotrophs are more common underneath the earth surface, and in parts of earth that still resemble the ancient earth. So, this would be undersea volcanoes, or volcanic lakes. And then we have phototrophs which is cyanobacteria. Then we have autotrophs and heterotrophs. Now this is another general classification autotroph or microbes that make their own food. Heterotrophs our everybody also. So, here we can say autotroph. And all these are heterotrophs.

Now, here is the thing about heterotrophs. They can more often than not almost always. They can metabolize more than one food source. Now it is important to mention some equal I will only process lactose. Some will only process glucose. Some will process both. So, heterotrophs by definition consume more than one source of food. For example, human beings, we are perfect example of heterotrophs. We consume things other than sugar we can take complex sugars of many different kinds; we can consume protein and fat and still convert them into energy. That is what hetrotrophs 2; and in most environmental situations. We are very interested in heterotrophic phenomena.

Now when we talk about bioenergetics it is very important to look at Gibbs free energy. In 11th and twelfth you must have come across Gibbs free energy, which gives us an idea amount about the amount of work that can be done how much energy is there in the system for doing work. Now in by energetics we use standard notation of Gibbs energy which is del G, with del G naught prime.

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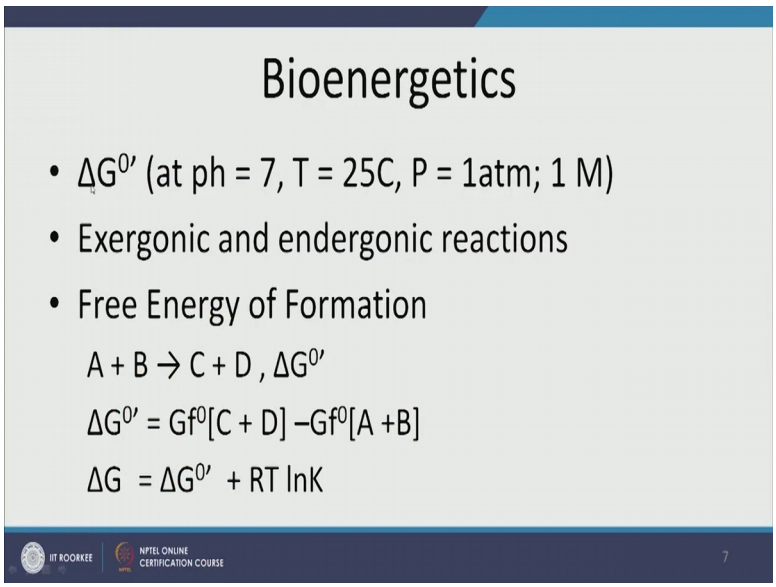

Now del here and change in free energy. So, if there is any reaction or any process happening change in the energy would be final Gibbs free energy minus initial Gibbs free energy. The naught here or the 0 at superscript represents the idea the standard conditions which are pH 7, temperature 25 Celsius atmospheric pressure. The prime represents that everything is at one molarity.

So, if you look here towards the bottom half of the slide, we have a chemical reaction A plus B creates C plus D. So, if we assume all of these chemicals are at one molarity. And the temperature is 25 C pressure is one atmosphere pH is 7, then the del G prime naught would be the Gibbs free energy for C and D, when they are at standard condition, minus Gibbs free energy, when they are standard condition for A and B.

However interestingly del G prime naught is not is very important or relevant for bioenergetics. And the reason for that is the rarely do we have really do we have our chemicals present in one molarity. A very simple example would be if A plus B are producing C plus D. It is very improbable that all of them end up with one molarity concentration. So, we need to account for the individual concentration. Maybe C and D are very sparse, maybe they are more abundant and A and B. And thus, we have an additional term that we add to del G in prime naught. This is RT log k where k is your constant of equilibrium constant for this reaction. Now just to revise this I like to write it down.

So, let us say our chemical reaction that we will studying is A plus B making C plus D.

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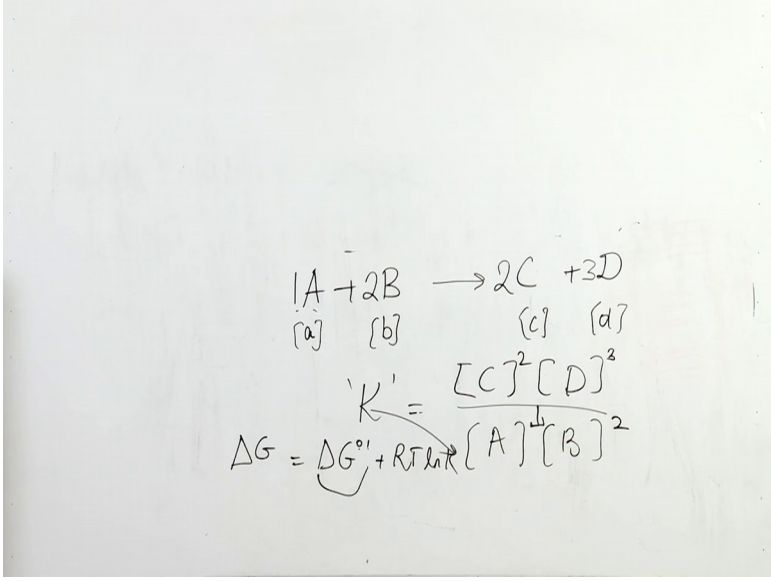

And let us say we start with a moles of a molarity concentration of A B of B and C of C and D of D. And once the chemistry starts we know that things will jumble up, and they will no longer be at this concentration. What we will have left is new values of A B C D in terms of molarity. And thus, in chemistry, very often we use equilibrium constant

represented more often than not by capital k; now equilibrium constant the way to calculate if multiply the molarity of all the products divided by molarity of all the reactants. So, basically, we have C D A B. In a more generic version, each of these have an exponent that correlates to their coefficient in the reaction.

So, let us say I have one mole one a combining with 2 B producing 2 C and 3 D right. So, in this equilibrium constant, the exponent for a would be 1, exponent for B would be 2. For C it would be 2 and for D it would be 3. Now when I calculate this what I get is equilibrium constant. And as it is name defines it, it is a constant. So, no matter what my starting concentrations are abcd. I will always end up at a final concentration of products and reactant that are in agreement with the equilibrium constant.

So, even before doing the experiment, you can actually know what your you know what your equilibrium constant would be. So, when we are looking at del G from microbial perspective, we know that is very improbable that the reactants. And the product would finally, end up at one molarity concentration h, and thus the equilibrium constant would be one which until my career until now I have not heard of.

So, we are more interested in del G which we calculate as del G prime naught prime plus RT log k. So, this k. R is your constant from ideal gas flow. T is your temperature, and del G you can calculate. And how do you calculate del G naught? You look at Gibbs free energy under stp standard temperature pressure and ph. And then everything is one molarity for the product minus it from that of the reactant. You know this, when you add them up you get del G. Now del G will determine whether the reaction is going to be exothermic or endothermic. And in this case, we are preferring to them as exergonic or endergonic which is basically whether the release energy or give take energy.

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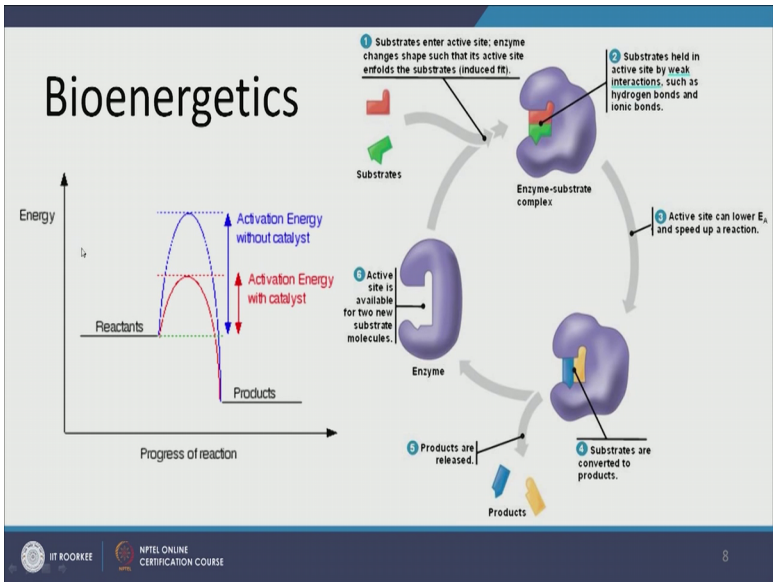

Now, in a Gibbs free energy level, let us say their reactant here, and the products are here. So, products will be at lower energy than reactant. So, that when the reaction happens the generate energy; however, usually there is an activation barrier. The reaction will not start until there is an extra amount of energy provided. And the activation energy required without catalyst here is represented by blue. And once we add a catalyst, then we have a reduction in activation energy barrier represented by red. And so, it takes less energy and less effort by microbes to make products.

This is very important to talk about the effect of enzymes effect of catalysts. In biology because, if you have noticed the chemical reactions that happen in life naturally at our standard room temperature. And are happening in your body right now, do not require you to be standing on fire or being put under higher pressure. When we do these same reactions in lab, you often need to put them put the reactants under high pressure, high temperature or specialized conditions, because the energy barrier is very high. But thanks to enzymes which act like catalysts, the energy barrier is reduced. And the same process can happen nearly at room temperature, or whatever the ambient temperature is.

Now how do these enzymes and catalysts work? One of the key features of catalysis or enzymatic action is that, at the end of the reaction, the catalyst and the enzyme will be recovered completely. And thus, let us say we have this enzyme substrate on the right side. And this is it is shape. So, when it is done catalyzing we should come back to this

exact straight. So, that it can catalyze another reaction. And as a result, very little amount of enzymes or catalysis catalysts are required in life for these reactions to happen. And most of these enzymes by the way in in microbes and in higher order of life are proteins, most of them.

So now these enzymes they usually have some space, some stereo arrangement a configuration that will perfectly hold the substrates. So, A and B let us say the A is red one and B is a green one. They will perfectly fit into the enzyme and form what is referred to as enzyme substrate complex. And when they fit perfectly like lock and key, then the enzyme substrate complex will have a different configuration. You can see does not look like this anymore. And once this has happened, usually the enzyme substrate they fit perfectly and that. So, they are more stable, but their presence there creates stress on the enzyme and thus it forces them their stress is used to convert them into products.

So, when that happens, this this is by the way called active site where the substrates attach. The activation energy is lowered. And then they are converted into products. So, what was A and B now is C and D. And once they have converted the enzyme kicks them out the products are released. And the enzyme is ready to catalyze another reaction. This process goes on as long as it is important. So now, that we know how to make life easy, by using enzymes. Let us look at the redox reaction, and the redox tower.

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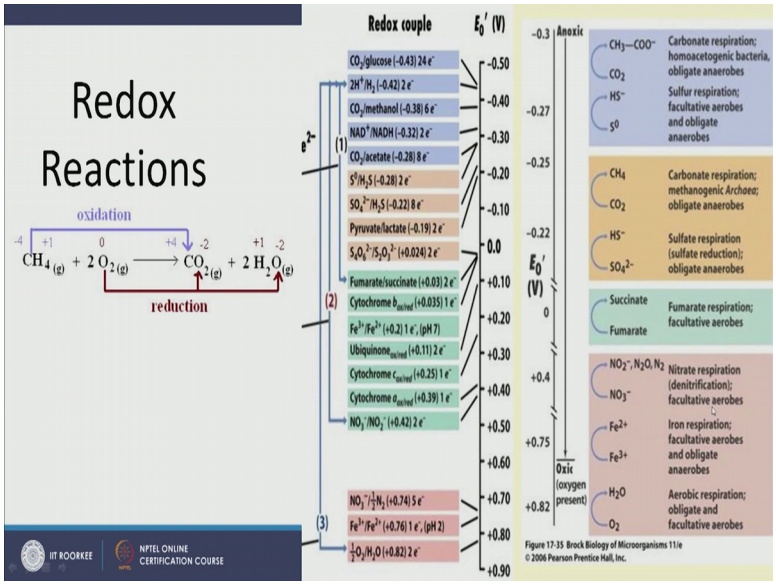

Now they are redox reaction is basically any reaction where we have reduction in oxidation taking place. Red in redox is reduction ox is oxidation. So, reduction is giving electrons oxidation is taking electrons. Here we have a very simple chemist chemical equation, which is very relevant to life. We have methane plus oxygen making carbon dioxide and water. Methane here has hydrogen sort of lending it for electrons when the reaction is complete. This carbon in methane is now giving 4 electrons way to oxygen. So, it in overall losses 8 electrons. So, when there is a loss of electron we call it oxidation. Oxygen here is in covalent bond it has no extra electron, but here it has extra electron and here it is taking electron from hydrogen.

So, 2 it has plus negative 2 and negative 2 because electron is negative charge. So, when you gain electron we say minus 2. So, overall the electrons that carbon has lost in methane, oxygen will gain in carbon dioxide and water. So, oxygen is getting reduced, and methane is getting oxidized. In other words, methane is an electron donor. It will give electron and oxygen is a electron acceptor. And this processes methane's oxygen oxidation, which many microbes I should be used to make energy.

Now what is a redox tower? Redox tower is a tower in which we have arranged many compounds and chemicals that are important for life according to the Gibbs free energy. So, we know that which in which compound is more likely to take electron which is more likely to give electron. In this particular example on the right side, the lower the lower compound or iron cation or anion is on this redox tower. The less likely it is to give electron, it wants to snatch electron. And on the bottom we have low and we hold oxygen in water. It wants to snatch electron. And here we have glucose, it wants to give electron.

Now, this this particular tower is mentioned. So, here we have methane with carbonate. They want to they are perfect electron donors. Here we have oxygen that wants to take electron. Now this is very a beautiful tower, because they have coupled them with their respective electron donor or acceptor for example, the oxygen once it has gained electron it will make water. And it will release this much energy when Fe 3 becomes a Fe 2 to reduce this much energy. Similarly, for nitrate reduction to nitrate reduction to nitrogen gas and then we have so and so forth depending on now notice here at pH 2 iron 3 to iron 2 has a very different energetic balance then when at pH 7 and so on and so forth.

So, this is a very beautiful diagram, and we will revisit it when we when we talk about microbial energetics later. Now if you notice here my dear students, on the right side the extreme right of this panel. We have another picture from the referred textbook of this course proper energy of microorganism. We are noticing that under not only their energy level or oxidation potential, but we have information on the oxygen and object nature of the environment. So, if oxygen is present in high quantity when oxygen wants to reduce into water or into another reduced form. And thus, here we have microbes that undergo aerobic respiration. They are either obligate aerobes or facultative aerobes.

Obligate aerobes are those microbes that require oxygen to survive without oxygen they will die it is obligatory for them. Facultative are those who can if oxygen is present there utilize oxygen. They will reduce it to make energy, but if oxygen is not present they will shift to something else. So, they are facultative they opportune opportunists in microbial world. And then we have iron reduction. So, we have microbes that will reduce iron. Here we have we can have facultative aerobes or obligate anaerobes, you can have both kind of microbes. Because there is no oxygen here we can not have obligate aerobes. We will die only obligate anaerobes and facultative anaerobes.

So, this is where aerobic environment begins and ends. Everything above this is anaerobic. But here notice in the pink box we still have reduction of some electron acceptor happening such as some ionic non-organic electron acceptor. In the blue box we have fumarate respiration. So, and then we have sulfate reduction, carbon dioxide reduction or it is also called methanogensis. Then sulfur reduces to hydrogen and sulfide and carbon dioxide reduces to CH 3 0 carbonate respiration.

Now, remember the plume example that I gave if there is a plume the first step the microbes that would grow in the first stage. Would be aerobic microbes they will consume whatever oxygen is present to degrade the plume. After that if iron 3 is present then we will have iron respiration, if nitrate is present with high nitrate respiration. Once these have been consumed, only then fumarate respiration will begin.

Once all this has been consumed, only then sulfated respiration will begin followed by carbonate respiration. When this is over only then sulphur respiration and carbonate respiration will begun. And this happens so because microbes that will reduce oxygen to head reporter or other things will get mores energy. So, anything that can consume most

energy and create most energy out of the chemical reaction will grow fastest, and will out compete all other microbes.

So, let us say we have facultative aerobes that can also do iron respiration, and that can also use oxygen as an electron acceptor, because oxygen is the best electron acceptor for life. And we use it by the way, of course. So, microbes that will use oxygen will out compete the one that can dead want to do iron desperation. And only when this competition has died out will this began. And that is why we often notice a gradient. Near the plumed oxygen is depleted.

So, some microbes have already shifted here. Once iron has depleted near it microbes shift to nitrate. And after some time, we will notice that near the plume everything has been consumed, and we have either carbonate respiration methanogenesis. And then sulfur respiration, sulfate respiration, fumarate, nitrate, iron respiration and the end we have aerobic respiration.

All right friends so, after now we know that this respiration happens, now let us take a look here. What happens on a very individual microbial scale?

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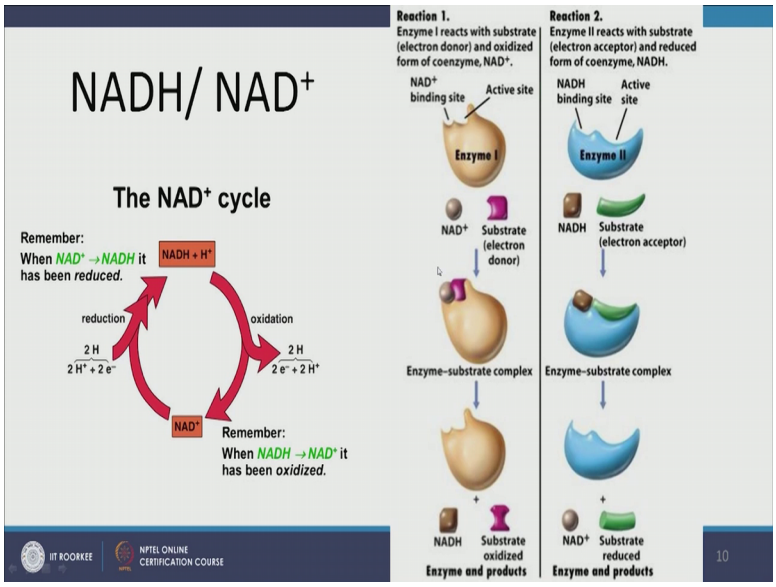

Not just necessarily on a population scale. How does microbe use this energy that is released here they do they use it in form of heat to the use in form of light, or in form of

electron. We know now from our studies in biochemistry. That microbes utilized electron and the way they bring in the energy and make ATP and other things is by using NADH.

This is a wonderful molecule is present in the cell. And the way it works is that NADH gets oxidized, and then it reduces. So, when it is converting from NAD plus to NADH it is getting reduced. And when it is converting back to NAD plus it is getting oxidized and in the process, it releases 2 electrons or gets 2 electrons. So, the way it works is that we have an enzyme. Enzyme one, it has one active site and it has NAD plus binding site. So, NAD plus the oxidized form of NADH will attach to it, and then whatever the food material is, the food material here is in pink in color and is referred to as substrate. Substrate is food when microbiology we are talking about substrate we talking about food it is also the electron donor.

So, the substrate the or the thing that is being operated upon the electron donor in this case will attach here. NADH where attach here. So now, we have enzyme substrate complex. Now NAD plus will take electron from the food, the food will be oxidized. And NAD will become NAD plus. So now, it has 2 extra electrons. Now it can go it can gave this 2 electrons to ADP or whatever needs electron and allow the cell to do business. So, any D an NAD plus NAD plus and NADH are bank, where these transactions happen directly from food.

Now, we have NADH, how will it convert into NAD plus again? So, we have another enzyme for it. Enzyme to the way it would work is NADH will bind here. And here we will have an electron acceptor bind. So, the electron acceptor of the snatch electron from NADH and it will become NAD plus and the substrate will be reduced. And thus, the chemical reaction is completed. So, let us take an example here.

Here we have methane getting oxidized, and carbon oxygen getting reduced. So, in enzyme one who will attach here; something that wants to be oxidized. So, methane will attach here. And a well it is a more complicated than this, I am just making it very simple to give you an example methane here. And NAD plus will snatch it is electron, and it will be oxidized. Now oxygen needs to get this electron. So, NADH would attach here, and here oxygen will come and take electron oxidize NAD plus. And thus, NADH and NAD plus, act like the electron carriers between the electron donor and electron acceptor. So, in biology more often than not, the reactants and products do not come in direct contact

with each other, but they use this messenger NAD plus NADH which transfers electrons between them.

Now what will serve as a perfect electron donor? Usually these are phosphorylated organic compounds. So, phosphate bonds can be very strong depending on whether the ester or iron hydrate bond.

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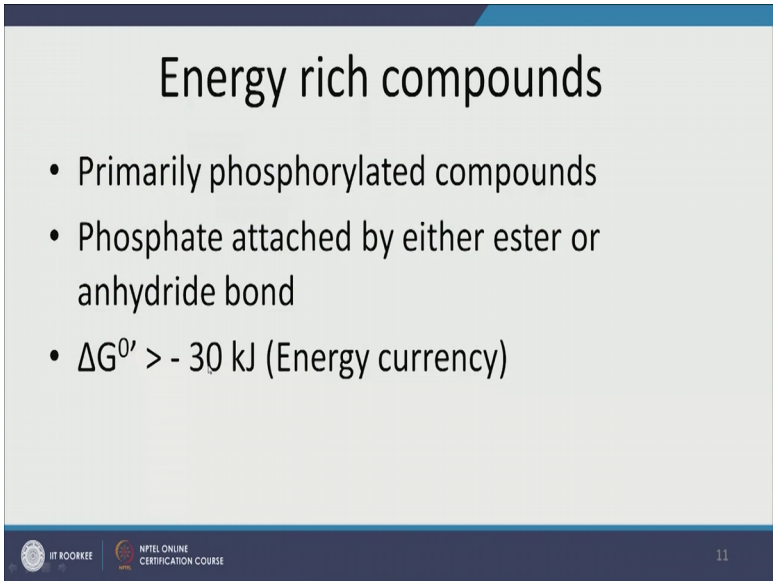

In fact, any compound that has del G prime not greater than minus 30 kilo joule can be used as energy currency by the microbial cell. So, microbes can use them for creating energy.

And in next class I will go through in detail about different kinds of energy currencies, and the most popular one that I think you must have heard about adenosine triphosphate ATP.

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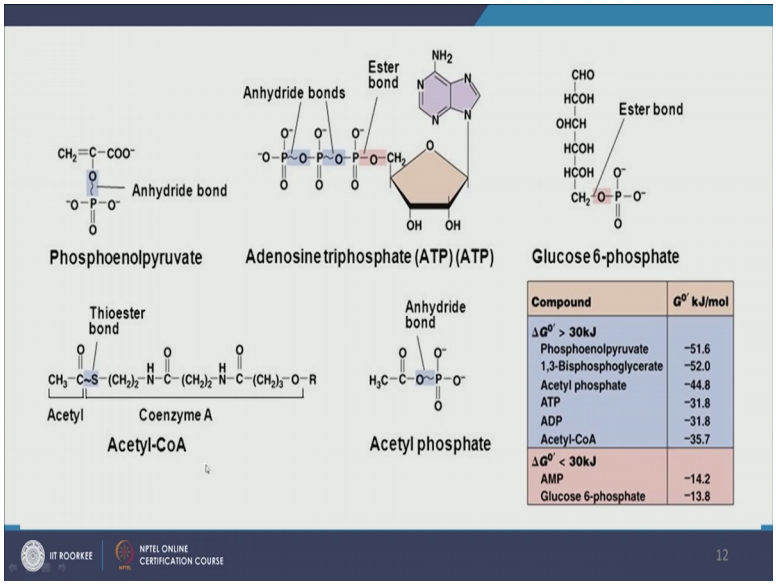

And then we will talk more in detail about acetyl CoA coenzyme a which is very, very important. We will talk about it is detail. Briefly brush through a settle phosphate, glucose phosphate and phosphoenolpyruvate which is very important as we have mentioned before for transporting, substances from outside cell to inside. That is all for today. And I will see you in the next lecture.

Thank you.