

Lecture – 12

Microbial Metabolism II

So, today we are going to talk about phototrophs, autotrophs, chemolithotrophs and get a generic overview of what their importance is there in life and in different kinds of ecosystems and understand better what are the mechanisms that allow them to use these resources that are present in their environment. So, first of all we will start today by phototrophs phototrophy.

1. Phototrophy

Photosynthesis and Chlorophylls

Oxygenic photosynthesis

$$6\text{CO}_2 + 12\text{H}_2\text{O} + \text{light energy} \rightarrow \text{C}_6\text{H}_{12}\text{O}_6 + 6\text{O}_2 + 6\text{H}_2\text{O}$$

carbon dioxide water glucose oxygen water

Anoxygenic photosynthesis

$$\text{CO}_2 + 2\text{H}_2\text{A} + \text{light energy} \rightarrow [\text{CH}_2\text{O}] + 2\text{A} + \text{H}_2\text{O}$$

carbon dioxide electron donor* carbohydrate water

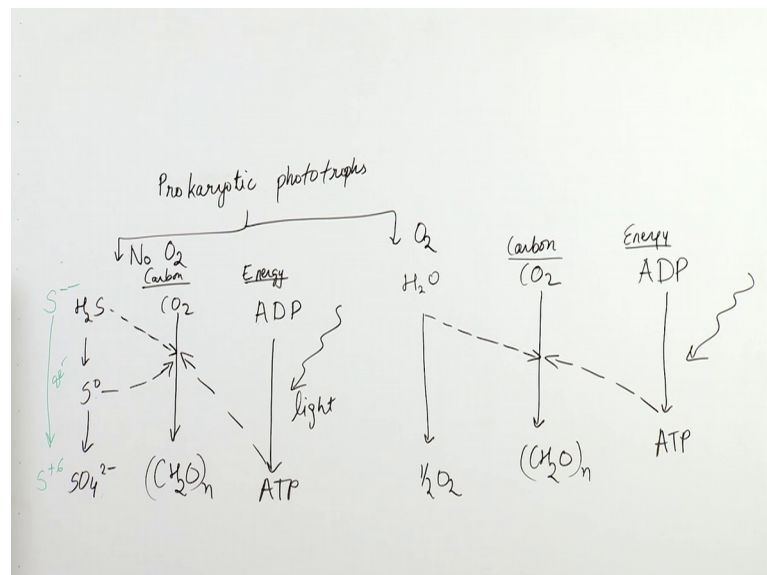
* $\text{H}_2\text{A} = \text{H}_2\text{O}, \text{H}_2\text{S}, \text{H}_2$, or other electron donor

So, as you can notice in this slide we have 2 kinds of phototrophy; Oxygenic photosynthesis and Anoxygenic photosynthesis.

Now, in oxygenic photosynthesis, carbon dioxide is utilized with water and light energy converted into glucose oxygen in water perhaps this is what you have studied in your primary science also, in anoxygenic photosynthesis we have carbon dioxide and then there is some sort of electron donor usually not oxygen because this is anoxygenic. Then there is light source and carbohydrate and water is formed. Now this electron donor can be H_2S hydrogen or some other electron donor and even water.

Now, let us look another let us take another look at Oxygenic and Anoxygenic, Photosynthesis.

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So, if we classify all prokaryotic phototrophs into 2 broad groups one being though the oxygenic phototrophs and the sorry anoxygenic phototrophs and oxygenic phototrophs. Then we notice that they are 3 important integrals for any of these kinds of phototrophs. First I need a reducing source something that is getting oxidized and thus is giving them electrons and then they need a carbon source for making biomass, their biomolecules and they need energy source for driving the synthesis of biomolecules and also the energy for daily sustenance.

So, as we know the name suggests phototrophs. So, the source of energy for converting ADP to ATP in case of both oxygenic and anoxygenic phototrophs would be light source. So, this light excites the ADP allows it to phosphorylate and then it forms ATP the ATPs energy rich molecule. So, it can drive many biochemical reactions one of them is

converting carbon dioxide into glucose or other forms of organic compounds necessary for life.

Now, ATP is not only used for this it is used for other processes as well, but this is the most relevant aspect of ATP utilization when it comes to trophic or the way microbes eat food. Now it is now carbon dioxide to become food or biomolecule it requires energy source well and good, but it also requires some electron source and thus we have this reducing ability, where water turns into oxygen in case of oxygenic phototrophs and supplies electrons to carbon dioxide.

Now, this is a very complex process, but this is a highly simplified picture of what happens in oxygenic phototrophy. So, when you studied in your primary school that photosynthesis is water plus carbon dioxide plus light converting it to food yes that is correct and this is their interrelationship. Now let us take a look at the anoxygenic phototrophs.

Now, anoxygenic phototrophs have similar requirements of something that will supply them electrons they require something that will give them carbon source to make their biomolecules and they require a source of energy. And as the name suggests these are phototrophs thus the source of energy would be light. Similar thing ADP turns into ATP. Now you would be this very important question that you should ask now is we know that ADP turns into ATP throughput or motive force that is generated by tapping to the energy of chemical reaction in the previous lecture for came out organo chemolithotrophs.

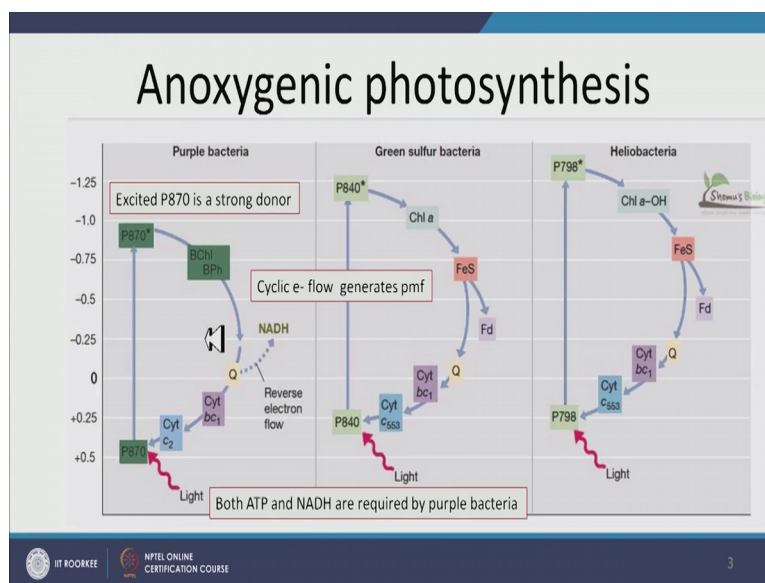
What is happening here the light is falling directly on the cellular membrane, how can it tap the light for this my dear students these phototrophs have specialized either cell or the cell organelles in case of plants and they where they store particular pigments such as chlorophyll or carotenoids which tap into this energy and will very briefly go through them in subsequent slides.

So, this ADP stabbed into ATP carbon dioxide is turned into food very similarly to oxygenic phototrophs; however, in case of the compound at the supply electron here we might have something like H_2S or even hydrogen the hydrogen is a very good supplier of electrons it lots to become water and thus H_2S becomes; Sulphur becomes sulfate now it has supplied lot of electrons. Here if you notice sulphur was in it had minus 2

electrons additional on it here it has 0 and here it has a scarcity of 6 electrons the sulphur has given away 8 electrons thus it is a very good source of electrons all righty.

So, this is an oxygenic phototrophy for you and this is oxygenic phototrophy now let us look at anoxygenic photosynthesis or phototrophy in slight more detail.

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Now when it is an oxygenic we do not have oxygen. So, we do not have a very good electron acceptor or in it is reduced form a very good electron donor available for us and thus we have an additional challenge, which is that we need to ensure that the oxidized form of ATP and NADH have the potential that they have is less than or it matches the potential of the proteins that we have. So, there are protein can reduce them that is what we want we want to proteins to reduce them and how does ATP NADH should have just the perfect potential compared to our protein in this case quinone.

In case of we are talking about 3 different bacteria here purple bacteria, green sulfur bacteria, and helio bacteria. In case of purple bacteria unfortunately the potential of it is primary protein P 8 7 0 is lower than that of NAD plus and thus this cannot reduce NAD plus to NADH and thus give it energy and therefore, it requires activation by light and once light has hit P 8 7 0 it gets excited and makes P 8 7 0 star now excited P 8 7 0 is a very strong donor of electron.

And thus it undergoes a series of biochemical reaction and it finally, reduces NAD plus to NADH and this part where it gives away the electron it is called as reverse electron flow it requires this. So, here notice it is at 0 potential NADH is at minus 0.25 and thus we notice that the electron should flow in this direction, but purple bacteria has to catalyze this reverse electron flow and no other kind of bacteria which is phototrophy needs to do this except for purple bacteria and therefore,. So, purple bacteria does this and then it undergoing a series of other biochemical reaction P 8 7 0 comes back to it is ground state.

Now, this is not the case with green sulfur bacteria and Helio bacteria for each of them the electron flows in the right direction it does not need to go in a reverse direction. Now either of the bacteria the overall picture is that their primary protein gets excited and then as it gets comes back to it is ground state it releases energy and somewhere during the process of releasing energy it produces, compounds, that will reduce ADP or NADH a NAD plus and produce the energy rich compounds in the cell.

So, in case of green sulfur bacteria we have P 8 4 0 that get excited by the light and makes P 8 4 0 star and notice that these compounds that P 8 4 0 makes are very different from the compounds or the intermediates that P 8 7 0 undergoes, and now also different from the intermediates at P 7 9 8 undergoes implying that for each bacteria even though they are doing anoxygenic photosynthesis.

So, the source of energy for each of them is light, but their biochemistry or the proteins that are involved in tapping that energy of light getting excited and then coming back to the ground state and in the process releasing energy for reduction of ADP and NAD plus and other and the compounds it can be energy carriers and energy currency. They are distinct they are very different and you can only imagine for a biochemist how challenging this task is to profile all these proteins in bacteria that behaves.

So, similarly and this brings me back to the first lecture which was basically an introductory lecture for this course where I mentioned how initially we treated microorganisms like a black box we did not know much about them we were only interested in in their overall broad functions. For example, in this case we might be interested in well all of these are phototrophs they use light a source of energy and thus

they are similar and we can say that they have the same growth of growth rate they have same characteristics similar demands and they have they behave in similar ways.

Now; obviously, as the population level a population might have predictable behavior, but on a community level they were very diverse and as we started understanding them better and microbiology grew, we found out that no there are different kinds one is oxygenic one is anoxygenic.

And then even let us say in within anoxygenic we found out that anoxygenic phototrophs can be of very diverse types and each of them not only morphologically different for example, here you will notice in the slide we have purple bacteria, green sulfur bacteria, helio bacteria, these names suggest a certain kind of morphology a certain kind of certain kind of behavior that can be observed visually by human beings such as purple bacteria purple color, green sulfur bacteria, green color utilizes sulfur for it is electron source or something else most probably electron source well in I know it is electron source.

So, this diversity we as we tapped into this diversity we understood that on metabolic level there are microbes are diverse very diverse. Now to give you an example a single sample of an environment for example, let us take soil sediment. A soil sediment may have millions of microbes on it billions of microbes on it and each of these billions of microbes well they are growing a dying, but they belong to certain categories of microbes some could be bacterias some could be protozoa some could be something else you know Archaea.

Now, within a particular domain that is a bacterial kingdom we might have very different kinds of bacteria. Now some could be aerobic bacteria some could be anaerobic bacteria and now each of these aerobic and anaerobic bacteria would have again very different metabolism, some reduce sulfate, some oxidize sulfur, some reduce sulfur, some create methane, some oxidize methane, some nitrogen fixing, some denitrify, some oxidize ammonia.

So, we noticed that within this small sediment sample we might have my groups with very different metabolic pathways and Meta metabolism. And in this in these lectures I am presenting to you one at a time we are talking about phototrophy mentioning you the examples of 3 different kinds of bacteria, undergoing anoxygenic phototrophy, but in experience we know that they are linked with their neighbors.

So, a photogenic of a phototrophic bacteria interacts with some other kind of microbe heterotroph or an autotroph and thus when they combine they talk with each other their chemistry and their behavior on an individual level and even on a community level varies and it becomes very different and thus this all this drive home 2 points. One is that microbial communities are immensely diverse when it comes to metabolism when it comes to their behavior what they consume as food what is the energy sources and what are the proteins that are involved in it.

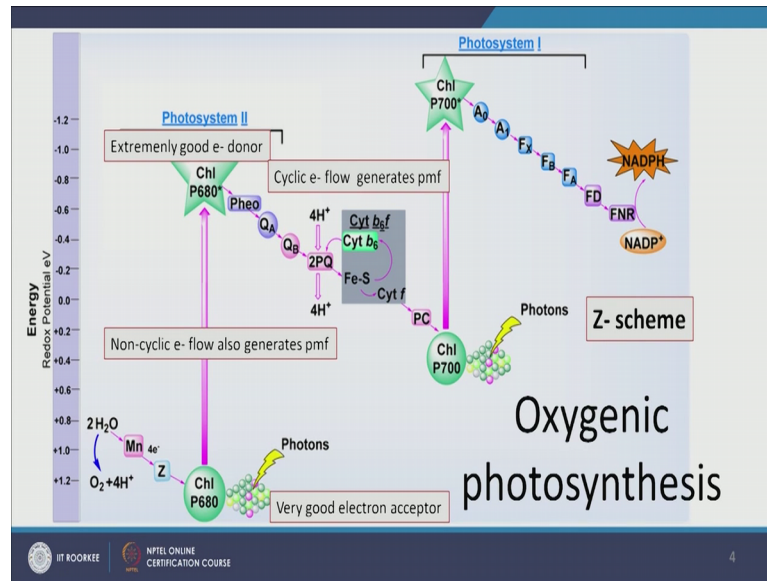
So, the first point is diversity immense diversity and many microbiologists believe that we have barely scratched the surface less than one percent of microbes we really know well. The second point that I want to drive is that when a microbe is studied in isolation it is behavior can be very different from how it behaves in community level.

So, for examples cell was degrading microorganisms some of them can degrade cellulose as single pure culture. So, I have a pure culture of clostridia type of clostridia thermoselam for example, and it will degrade cellulose and anaerobically and perfect and I know the exact proteins that it required I know the cellulose ohm structure for this clostridia,, but I put the same microbe in an environment and things change.

Now, there other settlers recruiters that are competing for their daughter products that after breaking down cellulose into cellulose bios or even further into glucose monomers. So, now, we notice what we studied in isolated culture is different from what we will expect in an environmental sample and thus, but at the same time it is very important to understand what is happening for an isolated culture because that is our foundation, what microbe can do alone is the foundation on which we build our understanding of what the same microbe will do in an environment all rightly. So, let us move ahead.

Now, we have an example of oxygenic photosynthesis here notice that here we had a d shaped cycle. So, protein gets excited returns back to ground state in the series it undergoes series of biochemical reactions, releases energy, you reduces energy, carrier molecules and thus stops in energy.

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Now in oxygenic photosynthesis this is not D shaped it looks like inverted W or as Z shape most scientists believe that this is Z shape. So, they call it Z scheme on Y axis we have the redox potential from plus 1.2 electron volt to minus 1.2 electron volt and on X axis we have their time series or what is happening.

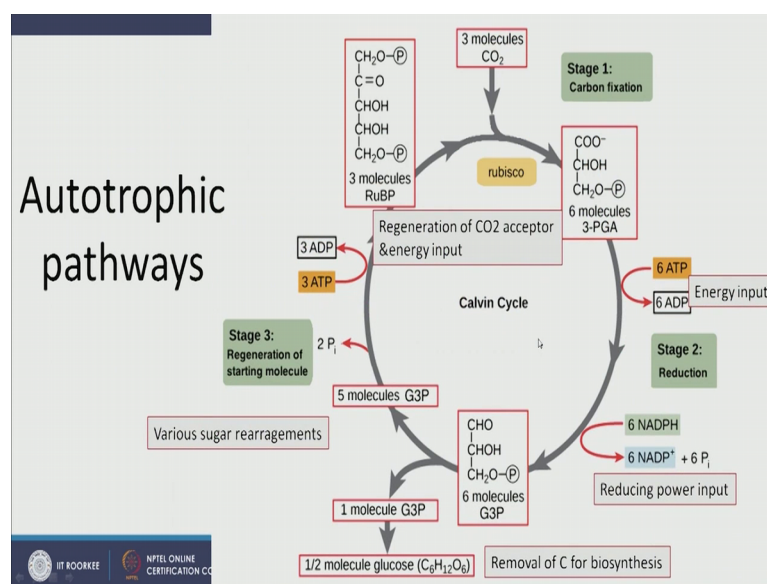
So, first we have this beautiful protein chlorophyll P 6 8 0 it gets hit by photons, gets excited, now as it gets excited, it again wants to come back to ground state, but here is a beauty it never really comes down to ground state it stops here at chlorophyll P 7 0, where it is open to being hit again by photons produces excited version of chlorophyll P 7 0 and undergoes the second phase of photosynthesis and the reduces NADP plus to NADPH energy rich molecule here.

Now, let us look 1 by 1. So, here we have a water molecule and it gets oxidized into oxygen and releases proton undergoes a series of chemical reactions catalyzed by many different proteins and what it does is it reduces chlorophyll P 680 to chlorophyll P 680 in its activated form. Now it is very important to note that chlorophyll P 680 is a very very good electron acceptor, but once it gets to higher energy state and has an extra electron.

Now, this is a very good electron donor and it wants to get rid of its electron and the very good electron acceptor gets excited. Now it is ready to get rid of its electron and as it is trying to get rid of its electron it undergoes this series of biochemical reaction and here we have a cyclic electron flow, which actually generates the proton motive force

which is our food for making ATP as we started earlier in the previous lecture and then it comes back to this chlorophyll P 700 state 700 state and then in photo system one we have another series of biochemical reaction, which reduce NAD plus this overall is referred to as Z scheme.

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Now, let us look at autotrophic pathway now the beauty of autotrophic pathway is that they will actually use carbon dioxide and convert it into glucose. Now this is Calvin cycle which is very popular in popular as in, which is a fundamental understanding that we have autotrophy in microbes and this was one of the first biochemical cycles that we completed and we understood really well when it came to autotrophy.

So, the way it starts its first step is carbon fixation. So, carbon fixation means sequestering carbon from wherever atmosphere or wherever we are water 3 molecules of in this this particular diagram that I have here is actually half of what most of the standard Calvin cycle diagrams where show you. So, in most standard you will have 6 molecules of carbon dioxide coming in it is completely accurate, but just the numbers are halved.

So, do not worry do not be surprised if somewhere else you see 6 molecules of carbon dioxide entering Calvin cycle, it is exactly the same except that everything becomes twice. So, we have 3 molecules of carbon dioxide they come in and they undergo the first stage which is carbon fixation they make 6 molecules 6 carbon molecules 6

molecules of this particular compound and then they consume 6 ATP this is important this is the step of energy input.

So, Calvin cycle we are trying to tap carbon dioxide and make glucose out of it we need energy. So, this is where ATP is consumed in a good quantity and once it has been consumed in a good quantity the next step is reduction. So, it gets reduced and in this process it consumes NADPH. So, NADPH gets oxidized and are bio chemicals here get reduced remember redox reaction one thing gets oxidized other gets reduced they are twins they cannot be separated.

Now, we have this 6 molecules of G 3 P, now one of them is converted directly into glucose note here it says half molecule, but if you multiply everything by 2 this be one molecule of glucose there is no sense when we say half molecule of glucose this is just talking coefficients anyway. So, the rest of them 5 molecules of G 3 P proceed forward and they undergo the third stage which is regeneration of starting molecule.

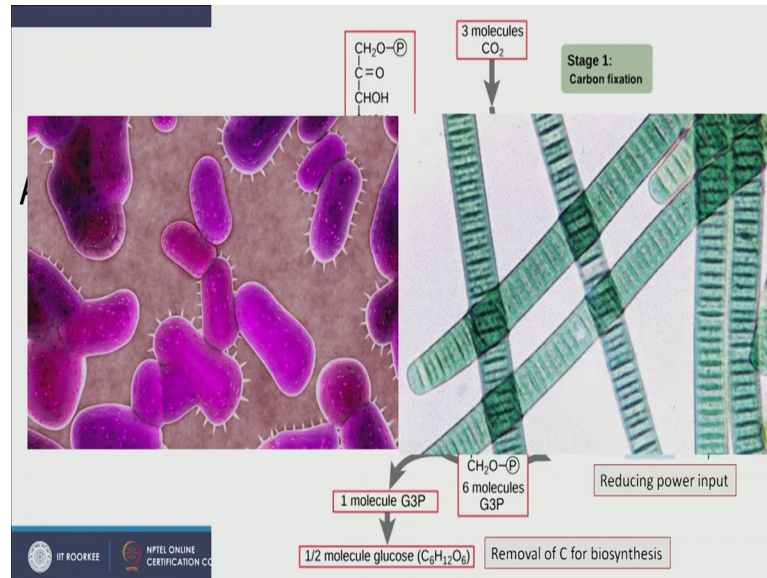
So, we want to regenerate the starting Rubisco molecule. So, here we have generation of 3 molecules of Ribulose 1 5 by phosphate, which have phosphate attached in first carbon and in the fifth carbon and then after this step rubisco is regenerated and the whole point of carbon cycle is continual regeneration of rubisco, which is then in position to accept 3 molecules of carbon dioxide note rubisco is a very big enzyme of which ribulose 1 5 by phosphate is only a portion of it.

And once it is regenerated and it has accepted 3 molecules or 6 molecules of carbon dioxide for a full carbon cycle then it undergoes carbon cycle again and glucose is continually regenerated and thus we note that the cycle is complete. Now note here we had 3 molecules of carbon dioxide coming in and we have half of glucose molecule going on.

So, in a complete Calvin cycle will have 6 molecules of carbon dioxide coming and 6 leaving and then rubisco is regenerated and at a first glance it might see there even rubisco can be broken down into 2 carbon molecules 2 glucose molecules, but no it is a catalyst it is very important for it to continue the Calvin cycle this is autotrophic pathway of how carbon dioxide is converted into glucose molecule.

Now, remember each of these steps are most likely catalyzed by very specialized enzymes which are proteins and which are encoded by DNA FYI and very simple version of it is being presented here now this carbon cycle is.

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Prevent is present throughout the environment and here I have pictures of purple bacteria and green cyano bacteria it is definitely present in these 2 and many more all righty.

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1. Chemolithotrophy

Inorganic Compounds as Electron Donors

- Energy yield is always lower than that for a glucose molecule.
- Much less energy is available from oxidation of inorganic molecules than from the complete oxidation of glucose to CO₂ ($\Delta G = 686 \text{ kcal/mole}$). This is because the NADH that donates electrons to the chain has a more negative reduction potential than most inorganic substrates.

Reaction	$\Delta G'^{\circ}$ (kcal/mole) ^a
$\text{H}_2 + 1/2 \text{O}_2 \longrightarrow \text{H}_2\text{O}$	-56.6
$\text{NO}_2^- + 1/2 \text{O}_2 \longrightarrow \text{NO}_3^-$	-17.4
$\text{NH}_4^+ + 1/2 \text{O}_2 \longrightarrow \text{NO}_2^- + \text{H}_2\text{O} + 2\text{H}^+$	-65.0
$\text{S}^0 + 1/2 \text{O}_2 + \text{H}_2\text{O} \longrightarrow \text{H}_2\text{SO}_4$	-118.5
$\text{S}_2\text{O}_3^{2-} + 2\text{O}_2 + \text{H}_2\text{O} \longrightarrow 2\text{SO}_4^{2-} + 2\text{H}^+$	-223.7
$2\text{Fe}^{2+} + 2\text{H}^+ + 1/2 \text{O}_2 \longrightarrow 2\text{Fe}^{3+} + \text{H}_2\text{O}$	-11.2

- Hydrogen Oxidizers:**
 - Most efficient ($P/O > 1$); $\text{eH}_2 < \text{eNADH}$
 - Hydrogenase may donate electrons to NAD⁺
- Sulfur Oxidizers:**
 - ATP by Substrate level phosphorylation in addition to oxidative phosphorylation
 - Substrate level phosphorylation is via adenosine 5'-phosphosulfate (APS)
- Iron Oxidizers**
 - Acidophilic *Thiobacillus ferrooxidans* $\text{Fe}^{2+} \rightarrow \text{Fe}^{3+}$
 - Acid Mine Drainage if pyrite is exposed to O₂ and H₂O!
 - Circumneutral *Gallionella ferruginea* $\text{Fe}^{2+} \rightarrow \text{Fe}^{3+}$
- Nitrifying Bacteria:**
 - Ammonium Oxidizers ($\text{NH}_4^+ \rightarrow \text{NO}_2^-$)
 - Nitrate Oxidizers ($\text{NO}_2^- \rightarrow \text{NO}_3^-$)
 - Process of "Nitrification" ($\text{NH}_4^+ \rightarrow \text{NO}_3^-$)

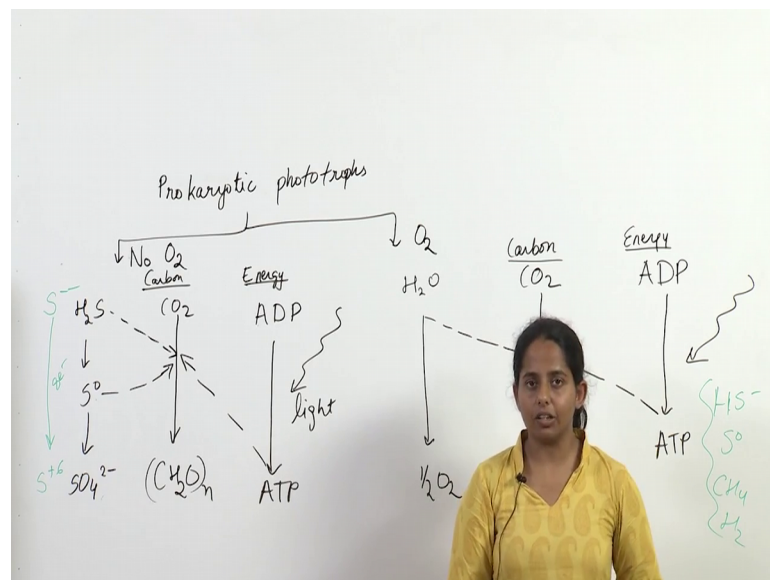
Now, let us look at the other Chemolithotrophy. Now chemolithotrophy as the name suggests we have inorganic compound serving as electron donors. So, for example, in

our an oxygenic phototrophy here we have H_2S serving as electron donor. So, this in one sense is an example of chemolithotrophy in one sense. Now it is very important to understand that when we undergo came only through trophy the in chemolithotrophy the energy that we can tap from in organic molecules is less than the energy that we can tap from glucose.

So, remember as how in previous lectures I mentioned that aerobic microorganisms will have a better bacterial yield and will have faster growth rate then anaerobic microorganisms, because reduction because oxygen's potential to accept electrons is much more than any other electron acceptors, it is the highest ranked electron acceptor and thus the energy gap that it produces. So, let us say this is energy donor this is oxygen electron acceptor.

So, when it accepts electron this ΔG is higher than any other redox reaction can give in life and thus aerobic micro does not grow faster. Similarly in case of chemolithotrophy we notice that when we are oxidizing glucose we generate more energy than we can by oxidizing any of these molecules.

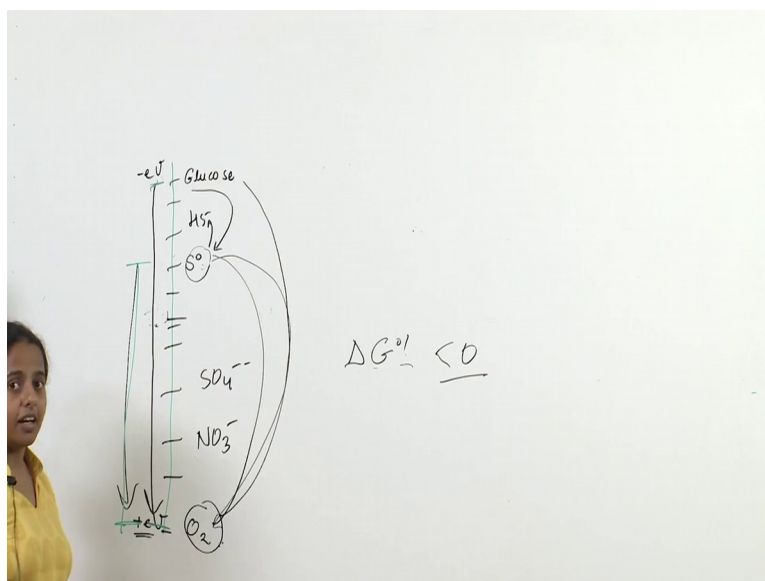
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So, what this should suggest you is that whatever different kinds of electron donors I have whether it is as in this case we have HS^- or we have sulphur or we have methane or even hydrogen more often than not. In fact, always we notice that glucose is the better electron donor than them.

So, we note that if we make our redox tower here.

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We have in a redox tower we have positive electron voltage here and negative electron voltage here. So, we can rank our molecules on this redox tower. Now the more positive or electron hungry molecule is the lower it will be somewhere here we have 0 electron volt. So, the lower it will be on our redox tower.

So, as I mentioned just while ago that oxygen is the best electron acceptor for life you can assume that oxygen will be at the bottom of this redox tower waiting to consume electrons. Now as we write down these different compounds here and here we can write different electron donors let us say here we have sulphur and here somewhere we have glucose all righty.

Now, let us say here we have nitrate here we have sulfate. So, these are these are our electron acceptors and these are our electron donors and we call them electron acceptor an electron donor in always in pairs. So, oxygen in itself is not an electron acceptor it always requires someone to donate it is electron for example, let us say there is sulphur here and it donates it electron then sulfur is electron donor and this is electron acceptor.

On the other hand the same sulfur can also act like an electron acceptor if something really reducing gives it electron and it makes HS^- then sulfur would be electron

acceptor and the more reduced stuff would be electron donor and thus we know that electron donor and electron acceptor are relative terms and they are always in pairs.

Now, when it comes to energy how much energy output will be available to microbes when and when any of these redox reaction happens, we mentioned before we talked about Gibbs free energy and standard procedure is to look at prime not. So, under STP PH 7 and everything is at one molarity both products and reactants.

So, if delta now the bigger the difference there is in delta G the more energy will be available and; obviously, we mentioned that less than 0 is minimum for our life to go on. So, it has to be negative now the more negative it is now for example, if oxygen is oxidizing glucose. Now we have a bigger drop in energy hence delta g would be minus very big number compared to oxygen was oxidizing sulphur.

Similarly, in this chemolithotrophy that we are mentioning we note that when inorganic compounds act as electron donors when they are the ones donating electrons they cannot match the energy that microbes can get when glucose is acting as electron donor now this redox tower must be able to explain this to you why that is. So, here we have we get this much energy whereas, in case of glucose we got more energy and thus oxidizing glucose is more energetically favorable for microbes then oxidizing inorganic electron donors.

And this brings us back here to the first point bullet here in this slide energy yield is always lower than that of a glucose molecule in case of chemolithotrophy and you can imagine chemolithotrops are more often than not slow growing microorganisms compared to heterotrophs. Much less energy is available from oxidation of inorganic molecules than from the complete oxidation of glucose to carbon dioxide which is of enormous Gibbs free energy change of 686 kilocalories per mole this is because the NADH that donates electron to the chain has more negated reduction potential and most inorganic substrates.

So, here is different inorganic substrate and they are getting oxidized and here is their delta g. So, when hydrogen gets oxidized to oxygen with oxygen makes water it is delta G is minus 56.6 for nitrate nitrate to nitrate is minus 17.4 for ammonia to nitrite it is minus 65. So, if we have ammonia to nitrate formation we can add them up and it would be around minus 82.4 if sulfur is getting oxidized to sulfuric acid, we have minus 118.5

and then S_2O_3 to sulfate we are minus 2 to 3.7. So, this is a pretty good electron donor and ferrous to ferric minus 7.2 not big difference.

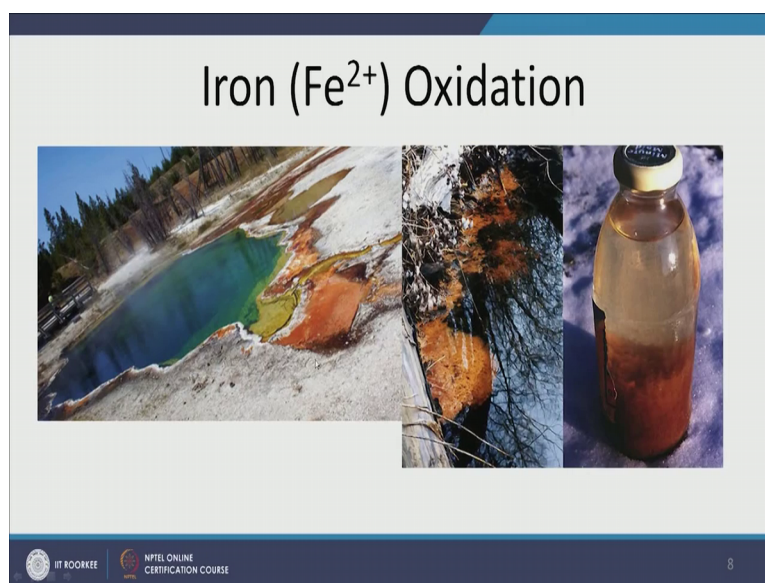
And we can broadly classify them as hydrogen oxidizer sulfur oxidizers iron oxidizers and nitrifying bacteria. So, hydrogen oxidizers are most efficient they and they use utilize this enzyme called hydrogenase, which may or may not donate electron to NAD plus. Now in sulfur oxidizers we have different kinds of sulfur oxidation I can happen it can be sulfur it can be HS minus it can be S minus minus in case of H_2S hydrogen sulfide and here we notice it can be also S_2O_3 .

Now, these undergo substrate level phosphorylation which is fermentation and also oxidative phosphorylation. So, this is the uniqueness of sulphur oxidizers they can undergo fermentation and as well as respiration. Now substrate level phosphorylation which is when they are doing fermentation is through APS. Now in case of iron oxidizers which is the last reaction here in this table we have named (Refer Time: 28:40) microbe here by the way *thiobacillus ferrooxidans* and it is an acidophilic microbe. So, you what does this inform you the word acidophilic philia means love acid means PH is less than 7.

So, this microbe loves when PH is low lower than 7 and then it oxidizes iron to ferric and this must give you information that because ΔG is not very high the conditions in environment must be really suitable to drive this reaction because microbes have very little to gain. So, the environment was pushing the micro well you can always oxidize iron and that is it well.

Now, acidophilic *thiobacillus ferrooxidans* is very interesting it among other acidophiles are very important because these acidophilic iron oxidizers create beautiful sceneries across the globe and here some pictures for you.

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Now, this is the hot volcanic lake and it is very acidic. So, high in protons and here we have iron that has been oxidized to ferric not chemically, but biologically through acidophilic bacteria like thiobacillus.

Similarly, here we have another picture and here we have water from similar lake. Now dear students if you have I encourage you to go ahead and look up on internet something called as blood falls in Antarctica. So, in Antarctica we have blood falls where it looks like a waterfall, but red in color and it looks really beautiful and I will show you pictures. In the next lecture and that is iron oxidizing bacteria at work and it is really beautiful not only are they acidophilic, but they also thermophilic and the microbiology and the chemistry of these blood falls and poles are still being investigated.

So, this is the real challenge and beauty of microbiology specially in environment is that we are still investigating them we are still exploring the frontiers of applied in diameter microbiology and as we will go towards the end of the lectures and I will be talking to you about ha different microbiological tools we have a with us, I will also share about different radiators research that is happening at this time and I hope that will inspire you more to understand this topic all righty.

Acid mine drainage if pyrite is exposed to oxygen and water now what is acid mine drainage this is a real environmental problem. So, as a name suggests we have mine. So, wherever this excessive mining happening now in mining what happens is that elements

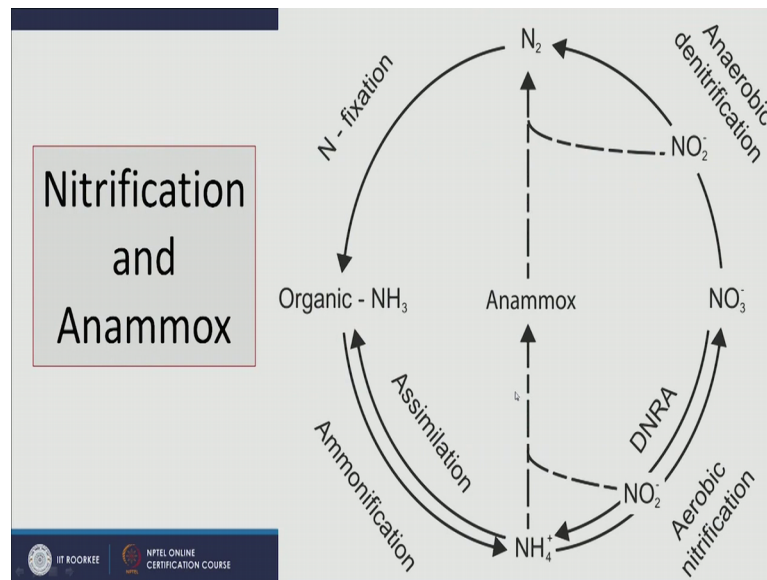
such as metals in general which are not exposed to oxygen usually and are in the reduced form underneath, when they are mined out oxygen seeps into their pores they are exposed to oxygen and then they are oxidized to ferric and this is what happens when pyrite is exposed to O_2 and water.

So, pyrite is what Fe^{2+} was highly reduced form of iron and now it is being oxidized to ferric which is soluble in water and thus we get water that is contaminated with the high amounts of iron this is not only a problem with iron, but it is also a big problem with other things for example, sulfide gets oxidized to sulfate and produces sulfuric acid and sulfurous acid which are not good for our health and which acidify our water and that is why it is called acid mine drainage, because any water that flows through these mines or any river or underground stream that has anything to do with these mines will dissolve these oxidized metals.

So, some metals and their oxidized states are more soluble than in their reduced state such as iron and uranium and thus in these mines that have that are upstream to any water body surface water body or ground water body the pH, usually falls they are very colorful and they are very dangerous for human health this is a major problem across the globe wherever we have mining be it USA be it India in USA they are doing really good job about trying to have mitigate it, but in India we are still working on it.

Then we have circumneutral *Gallionella ferruginea* which oxidizes iron to ferric then we have nitrifying bacteria and we will go a little bit more in detail about what nitrification processes are what are their names briefly, when ammonia gets oxidized to nitrite it is called ammonia oxidation when nitrite can turn into nitrate the nitrate oxidizers and when ammonia goes to nitrate it is called nitrification.

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So, let us look at Nitrification and Anammox cycle this is a picture actually a diagram from a very famous paper in applied environmental microbiology and let us look at the nitrogen cycle as described here. So, anammox you start with them one in middle anammox is a recently discovered feature of nitrogen cycle earlier people did not know it existed they did not know it was possible to bypass the circle and jump directly from ammonia to nitrogen.

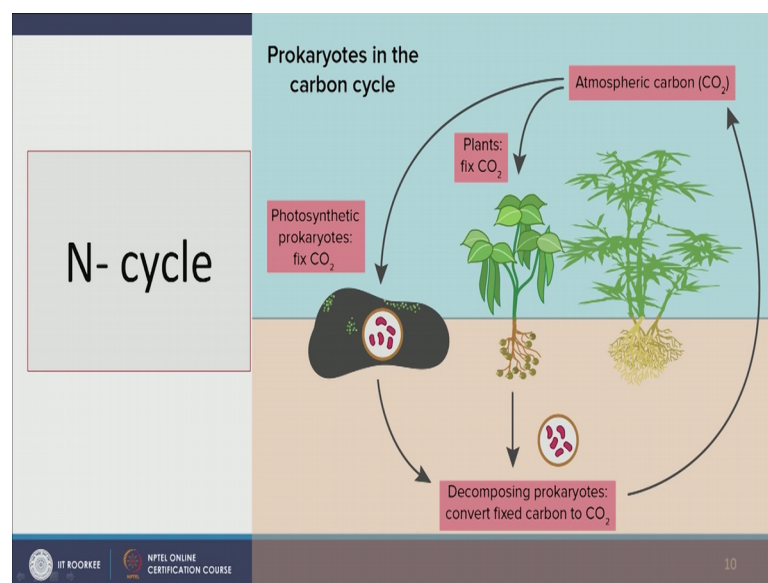
So, this is anaerobic ammonia oxidation, now why nitrogen why not nitrite and nitrate and other highly oxidized forms of nitrogen because it is anaerobic naturally anammox transfer anaerobic ammonia oxidation. So, ammonia directly is oxidized to nitrogen anaerobically. The other way the longer part that people understood better before was well ammonia can convert into organic ammonia by assimilation and when organic matter decays by ammonification it can give back ammonia.

This ammonia can be aerobically undergo nitrification and thus convert into nitrate which can again go to under denitrification and undergo ammonia. Now nitrate can undergo denitrification make nitrite it which can again reduce to nitrogen depending on the environmental condition; under extreme situation nitrogen gets fixed into organic nitrogen ammonia directly and this is called nitrogen fixation nitrogen fixation they are primarily 2 routes one is through thunder and lightning.

So, there is lot of nitrogen present around us in our atmosphere and. In fact, as you sit here and listen to this lecture and as I am delivering. This lecture this nitrogen all around us now when thunder or lightning strikes there is tremendous amount of energy, which directly converts nitrogen into ammonia that is one route the other route is nitrogen fixing bacteria which play immense role in our agriculture, they form these little nodules in our route of nitrogen fixing microbial community that exists in certain roots of plants such as legumes and in this this nitrogen fixation is catalyzed.

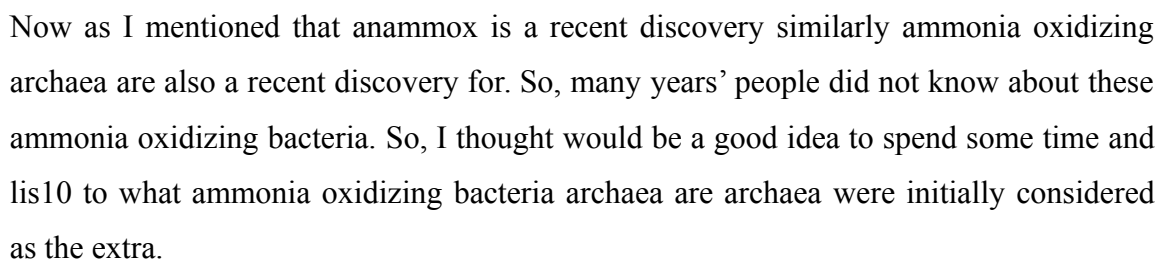
So, basically we have nitrate turning into nitrite. So, this is denitrification nitrite can either turn into ammonia or it can turn into nitrogen nitrogen can be fixed into organic material as organic ammonia, which can be ammonia which on decay can release ammonia which happens a lot in wastewater treatment plant and this is one of the important things that we regulate and this ammonia can also be assimilated into organic matter. So, we have one cycle here and we have another cycle here and this is a recent discovery as I mentioned ammonia directly turning into nitrogen nitride directly turning into nitrogen it is called anammox.

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So, this is a cartoon of a nitrogen cycle and role they prokaryotes play in carbon cycle and this is where this is even though it shows carbon cycle this is where nitrogen fixing bacteria are important. So, in environment again carbon cycle nitrogen cycle water cycle all of them are interlinked they are not independently happening parallel to each other.

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So, most of the microbial processes according to erstwhile scientific community were driven by bacteria, but now we have recognizing that no in certain cases archaea are more important. In fact, the take home message of this particular poster by George (Refer Time: 35:57) and Dan Wilson is that archaea are now recognized as a dominant organism in oxidation of ammonia.

So, oxidation of ammonia especially anaerobically anammox archaea and just archaea that oxidized ammonia archaea might be the major drivers compared to bacteria. So, there are bacteria also who do anammox the archaea also who do anammox and we are noticing archaea drive most of it and look here this tree of life for an archaea for archaea. And we will go through this what this stable suggests in subsequent lectures for now it is important for you to just understand that there is immense diversity in archaea.

Now, in this dendrogram table each line represents a particular branch in this tree of life from archaea, the closer the tumor archaea are according to their genes in the genetic makeup the closer they are on this dendrogram. So, notice here we have 4 4 different kinds of archaea, thaumarchaeota, orange, light blue crenarchaeota, yellow korarchaeota and purple euryarchaeota. And with this I will conclude this lecture and leave you with this that in the next lecture will be talking a lot about the immense diversity and the latest invent discoveries that have happened in microbiology.

So, now we were talking about metabolic diversity in phototrophs autotrophs chemolithotroph heterotrophs chemoorganotrophs and so on and so forth, but now will be talking about taxonomic diversity and noticing how microbes that even are perfectly similar in the metabolism are very different genetically and thus are very different in their behavior characteristics how they interact with the environment how the influence environment and public health us very importantly. So, that is all for today.

Thank you very much.