Environmental Biotechnology
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Lecture – 23
Physiological Ecology and Resource Exploitation by Microorganisms (contd.,)

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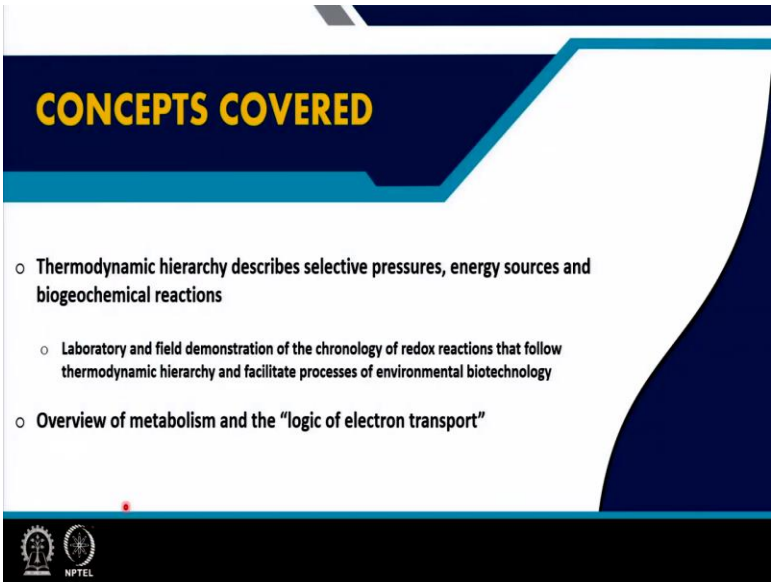

Welcome to the next lecture on the Physiological Ecology and Resource Exploitation by Microorganisms. In this particular lecture we will continue our discussion on the thermodynamic hierarchy that describes the selective pressures energy sources and bio geochemical reactions within different environmental conditions. And in particular in this lecture we are going to discuss with respect to two examples.

One for the laboratory and another for the field demonstration of the chronology of redox-redux reactions that follows the; thermodynamic hierarchy and facilitate the process of in the environmental biotechnology. We will also discuss about the overview of metabolism and the logic of electron transport.

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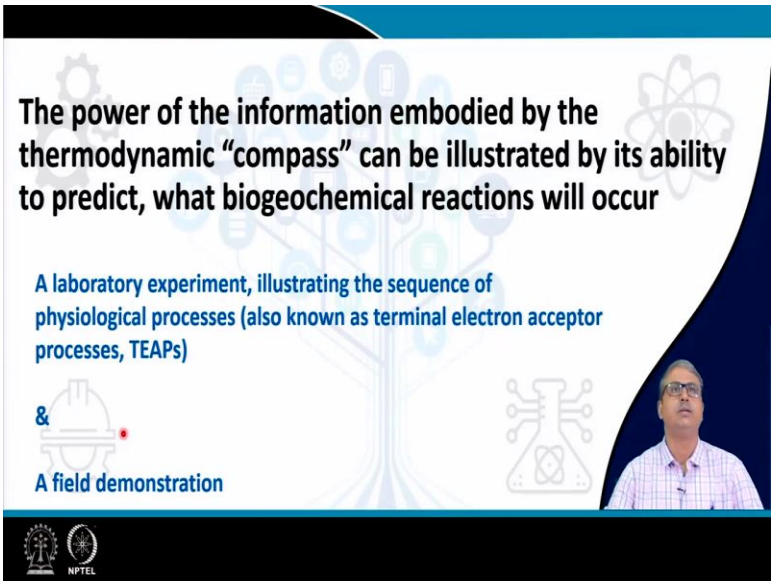

Now, the power of the information embodied by the thermodynamic compass that we have discussed earlier can be illustrated by its ability to predict what biogeochemical reactions will occur. In this regard a laboratory experiment will be illustrated to understand the sequence of physiological processes also known as the terminal electron processes or TEAPs and we will also demonstrate or discuss about a field demonstration that in a real world situation.

How this compass could be useful in predicting the processes which would be relevant for the environmental system or developing the environmental biotechnology process.

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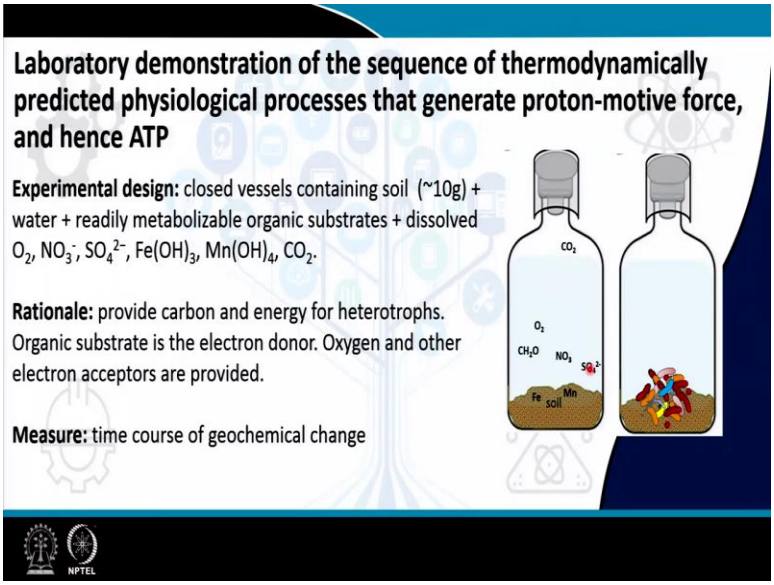

Now, laboratory demonstration of the sequence of thermodynamically predicted physiological

processes based on the compass that we have already talked about would be fine we would be able to find out that which are the processes which are more preferable or which are the processes which are favorable in terms of generating the proton motive force and hence atp within the given environment.

So, for this experiment a closed vessel or a glass vessel basically containing a soil from the contaminated site. For example if we are starting on a on the bioremediation or similar pollutant pollution abutment technology development in a contaminated environment using a landfill or any other petroleum oil or any other contaminated site. So, we can take some amount of that contaminated site sample which is represented here as a contaminated soil and some water.

And then readily metabolizable organic substrates because the microorganisms must be provided with sufficient organic resources reduced organic substance may be the starch or maybe a mixture of low molecular weight organic compounds like glucose, yeast extract, acetate, lactate all these substrates can be provided and very importantly the terminal electron acceptors. So, dissolve oxygen, nitrate, sulphate, iron oxides and manganese oxide and carbon dioxide.

So, these are as you can understand these are all electron acceptors and from the prior discussion on the thermodynamic hierarchy or the chronology of these reduction process we understand that some substrates as electron acceptor will be possibly used first like oxygen because free energy gain is maximum followed by nitrate and then iron or manganese then sulfate and then carbon dioxide.

So, even if we have everything together the microorganisms or the organisms may not be utilizing everything together that rather they will follow a kind of a trend. So, we will experimentally or we can experimentally prove this that how actually this happens within using a particular environment we need a wastewater or a contaminated soil or any other kind of environment.

So, this is kind of an image of the system that we can use that a closed vessel the vessel must be airtight and where we have taken the soil and it I must emphasize here or mention that the soil

contains the microbes present within it because we want to evaluate the performance by this microorganism. And we have added the substrate these are the carbon source the oxygen, carbon dioxide, nitrate, sulphate.

Since the iron oxide and manganese oxides are not soluble they will form precipitates or be added at a solid particle itself within the soil and it they will mix up or you have to mix them and then it is incubated. Now, we need to remember that the soil is already having microbes within it. So, these microbes will show reaction because they will use this organic carbon as a source of carbon and energy and the electrons will be donated to either oxygen and other electron acceptors.

So, what is the ration of this experiment the rational of this behind this experiment is to provide carbon energy for heterotrophic organisms and the organic substances which are provided is the electron donor. And the oxygen and other electron acceptors will be reduced and that will be validated and what we are going to measure over here in this experiment we are going to have a time course of the geochemical changes.

So, what geochemical change a very simple geochemical change that we are going to monitor is the water sample from here can be withdrawn periodically and the water can be used to test the concentration of the electron donors which are provided. Or the electron accept or the reduced electron acceptors which are used like oxygen nitrate carbon dioxide will be converted to methane, sulphate will be converted to sulfide fe three will be converted to fe two etcetera.

So, following an incubation under this closed condition the microorganisms which are already present in the soil will propagate will proliferate will grow. Why we think that they will grow because we have provided them with substrate like the carbon substrate and we also have provided different type of electron acceptors. So, the microorganism present there will grow the time of incubation might vary depending upon the time of other kind of soil or kind of sample that we have used.

Some soils contain microorganisms which are able to grow slowly while some microorganisms

can grow fast. So, it could be within a week we can observe or we can have a month incubation. We can have a year round incubation with samples taken or withdrawn periodically. Now, what is expected it is expected that the cells which are present in the soil will metabolize the carbon substrate which is we have provided ready metal metabolizable carbon substrates organic carbon we have provided them.

So, the electrons will be derived from this reduced organic carbon and this reduced organic carbon will be oxidized the electrons will be settled through electron carriers electron transport system and eventually oxygen will be the preferred electron acceptor followed by nitrate manganese iron sulfate and carbon dioxide. So, ideally all these electron acceptors will get their turn of being reduced following the compass that we have already studied.

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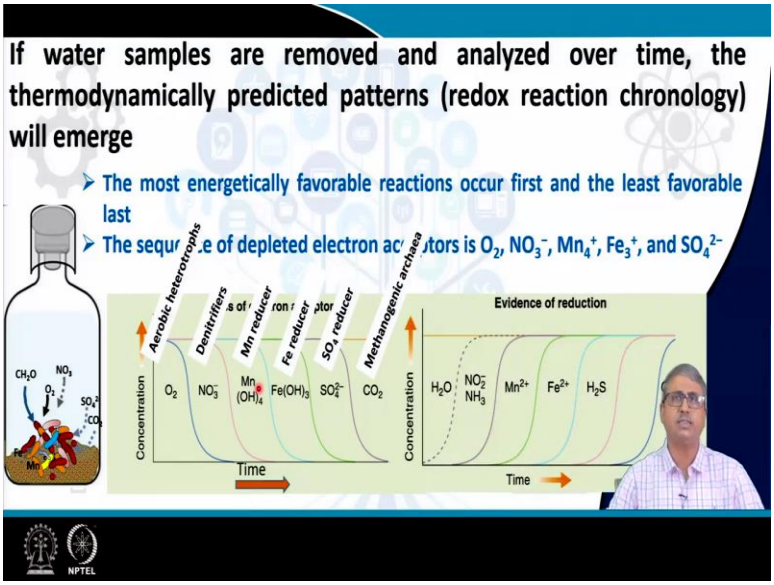

Now, if we take out the water sample from this closed vessel and analyze this water sample for the particular electron acceptors that we have provided or the product of the electron acceptors when they are reduced. Like if the nitrate is reduced to ammonia or right nitrate is reduced to nitrogen or the sulphate is reduced to sulphide or carbon dioxide is reduced to carbon methane we can surely monitor that.

So, if we can do that that is a time scale of sampling and anal analysis of those electron acceptors and their product following reduction. So, we can expect that this will this is going to follow a

thermodynamically predicted pattern which is referred as redox reaction chronology. So, the most energetically favorable reaction as we have already discussed will occur first and that is the use of oxygen.

So, oxygen will possibly use first if there are aerobic organisms. So, oxygen will be used only if aerobic organisms are there and then aerobic bacteria will proliferate. And the least favourable one will be utilized last. So according to our thermodynamic hierarchy we know that carbon dioxide is the least preferred before that the sulphate and then the iron and manganese and nitrate etcetera are there.

So, the sequence of depleted electron acceptors starting from oxygen to the carbon dioxide can also be followed. So, essentially if we are able to collect sample periodically maybe a day interval or a week interval or a month interval depending upon the kind of sample. So, generally a couple of day's interval might give us a good result for a landfill leachate or similar type of sample. So, with respect to time we will be able to see that the oxygen concentration is declining first before the nitrate conditions start declining.

Why oxygen is declining first because the it is the aerobic heterotrophic organisms who are already there in the soil and start utilizing the carbon source which is provided and utilized they utilize the oxygen as terminal electron acceptor because they are aerob. So, they make use of oxygen make use of the substrate and then oxidize the substrate they grow and as a result the oxygen declines as soon as the oxygen declines level of oxygen reduces.

The nitrate will be used by the nitrate reducing organisms or the bacteria who are capable of denitrifying this and then we will see that after some time the nitrate reduction follows. So, if we have a maybe a days or few days or week interval data. So, we will see that the oxygen level declines and by the time the oxygen level drops a significant level like here the nitrate reduction starts and as the nitrate level declines to a significant level the manganese reduction occurs and then iron reduction and then this sulfate reduction occurs.

Now, there may be a question that why manganese reduction cannot takes place at the same time

when nitrate is there. As soon as the oxygen level declines both nitrate and manganese could be reduced. Ideally yes but actually no why? Because earlier we have noticed that first of all manganese reduction yields less energy compared to nitrate reduction. So, if nitrate is present micro organisms would prefer to reduce nitrate to produce sufficient energy.

So, that they make use of it if nitrate is exhausted then only they will look for any other electron acceptor and if manganese is there manganese will be surely reduced. The second point is when nitrate is there manganese reducing catabolic abilities the gene responsible for reduction of manganese for example will be replaced. So, that the; organism who are capable of denitrifying can proceed with nitrate reduction in an undisturbed way.

Now, at the same time if we look at the organisms of the processes involved. So, following aerobic heterotrophic organism that the denitrifiers will proliferate then the manganese reducers then the iron reducers and the sulphate reducers and the methanogenic archaea will eventually proliferate. So, in a in this kind of closed incubation we will we will be able to see that the chronology of redox reactions very clearly.

We can simultaneously also measure the reduced products of this electron acceptor like for example the oxygen is reduced to water. So, if we have a heavy isotope of oxygen used like O 18 is used then perhaps we can monitor the H 2 O with oxygen with O 18 oxygen or we can measure the nitrite or ammonia which are produced because of the nitrite reduction we can we can measure the dissolved Mn con concentration because otherwise the Mn was insoluble Mn hydroxide was insoluble but this Mn 2 plus is soluble.

So, it will be appearing in the water and we can measure it using atomic absorption spectros photometry or a inductively coupled plasma emission spectroscopy that is the ICP, iron Fe 2+ can be measured H 2 S can similarly be measured by different spectrophotometric and other assets. And of course methane can also be measured after some time because of course methane production of methane will take time because methane will be only produced once all the electron acceptors are exhausted used up.

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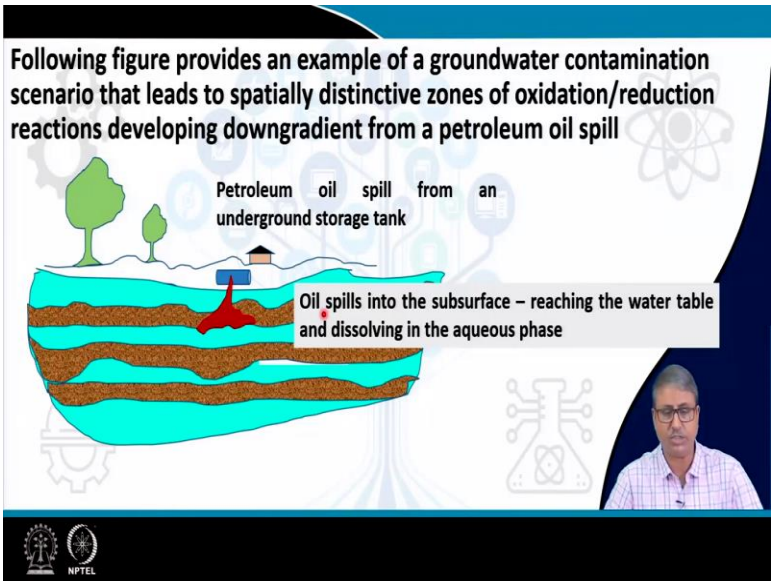

Now, the laboratory demonstration of the redox reaction chronology has been manifest innumerable times in the real world contaminated field sites. So, the example that I have explained is useful is very very useful for testing the electron acceptor regime in any kind of environmental samples. So, before predicting any process it is advised that the sample should be subjected to a kind of a very simple experiment like that.

Now, regarding the field visualization or demonstration of this particular concept we can use the following figure which provides an example of ground water contamination scenario that leads to specially distinctive zones of oxidation reduction reactions developing down gradient from a petroleum oil spill. So in this case we are taking an example the tank a petroleum oil containing tank is there underground and this is a storage tank which is very many a times are seen in the petrol pumps for example or any kind of storage facility.

The underground tank might leak and the spillage of this petroleum oil occurs. So, what happens this oil spills and oil enters into the underground horizons which are might be the ground water table and full of sediments or sands which are rich in iron and manganese etcetera. Now, under normal circumstances this oil spills into the subsurface and gradually it will reach to the water table and then it will be dissolving in the aqueous phase the ingredients of the oil will tend to dissolve as much as possible and their chemical conditions allow them.

So, they will be dissolving. Now, under undisturbed condition this underground environment might have oxygen some amount of oxygen will be there surely at least in the upper layers followed by dissolved nitrate sulfate. And iron and manganese oxides would be there in the sand or the sediment through which this leaked oil will eventually pass through okay. Now, if we look at the chronology of events which will which will follow the chronology of redox reaction the petroleum oil is basically mixture of hydrocarbons the reduced carbon C-C bonds are there.

So, these hydrocarbon molecules are going to be utilized by the microorganisms mostly the aerobic microorganism first because we know that aerobes are thermodynamically this process is favorable. So, the aerobic bacteria will metabolize oxidize the hydrocarbon leading to the production of H 2 O and the metabolite metabolized product of metabolic products of the hydrocarbons. And those hydrocarbon metabolic products might be useful by or used by other microbes present in that.

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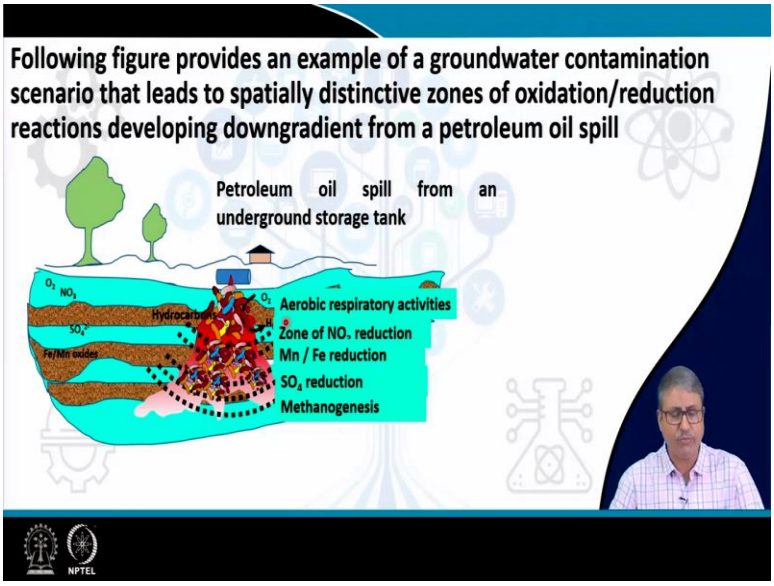

And gradually that process will set up a condition which is called a zone of aerobic respiratory activity. So, in this case close to the leaking or leaking tank we will find aerobic respiratory activity zone of aerobic respiratory activity because the dissolve oxygen is plenty in that zone and naturally the aerobic organism will start acting on them. Now, very soon this oxygen will deplete because it is continuously being used up by the aerobic bacteria and the same time the oil is moving downward.

So, two processes are happening simultaneously one is the oil is moving downward. So, what happens if the oil moves downwards the availability of oxygen decreases because if there are diffusional barrier. The availability of oxygen also declines because of the use of oxygen or the oxygen is consumed by the aerobic bacteria very fast. So, essentially after some depth the availability of oxygen will be. So, low that the; aerobic process won't be able to function there.

So, this is the limit of the aerobic respiratory activity or the zone of aerobic respiratory activity. So, as the oil moves into deeper ground water oxygen depletion will occur and gradually the cells will grow but utilizing the other electron acceptor which are available there. As we may understand that if nitrate is available the nitrate will be used. So, we will have a zone of nitrate reduction then the zone of sulphate sorry Mn or Fe reduction.

Because they are already there in the underground sand of the sediment followed by sulphate reduction and then eventually when all the substrates electron acceptors are exhausted and the oil has percolated deep inside the underground cyst strata where dissolved electron acceptors are all exhausted even the solid Mn and Fe are also exhausted it is going to be the methanogenesis where the carbon dioxides which are produced due to the microbial metabolism will be converted to the methane.

So, this is a kind of a field implementation or field demonstration of the process that we have seen in many many locations where similar kind of petroleum or other hydrocarbon contaminated contamination has happened.

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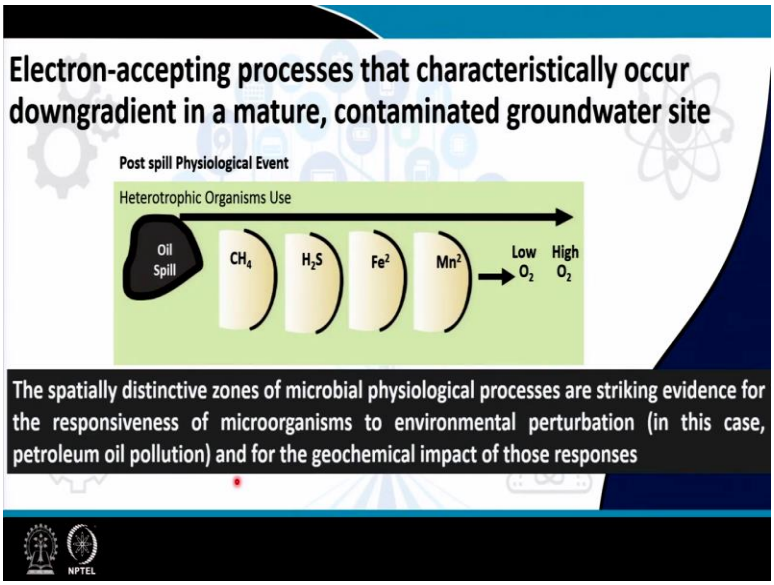

Now this has a separate picture as well. So, in the first way we saw that a leaking tank where aerobic heterotrophic processes or zone of aerobic metabolism is the closest to the these spilled oil. But if we consider a oil spill which occurs deep under the ground deep under the ground. Then we might have a different kind of scenario this is particularly more relevant where there is a downward movement of the oil eventually and oil is actually.

Now, located on the deeper earth crust may be several tens of meters below the ground and it is not exposed to any kind of oxygen rich environment any way. So, we can we can see a different set of processes in this kind of a mature contaminated ground water site. What has been found in such mature contaminated ground water site that the heterotrophic organisms that they are they are arrange them they are arranged according to the chronology of the redox reaction because the closest to the oil spill where the oil is there.

It is so, strongly anaerobic and possibly it is the methanogenic organisms which are prevailed in the closest region closest vicinity it is the methanogens which are going to produce methane. Subsequent to that is a sulphate reducer so, it will produce sulphides then the iron reducers manganese reducers and the nitrifiers and then the aerobic organisms far away from the oil spill side because the oil might be diffusing slowly within this strata.

And as the oil molecules reaches far away positions where there might be having access to some

oxygen the oxygen will be used otherwise to the closest to the point of oil spill it is the methanogenic organisms or the methanogenic archaea those are going to be more active. Now, the specially distinctive zones of microbial physiological processes which are observed a striking evidence for the responsiveness of microorganism to environmental perturbation in this case the petroleum oil pollution and for the geochemical impact of those responses.

So, this particular thing has 2 aspects one is the how the microorganism responds to the environmental perturbations and they try to degrade the environmental contaminant like the petroleum, oil in this case and also the geochemical impact of the response. Geochemical impact in the sense how the electron acceptors are being used and eventually a altered geochemicals environment is created.

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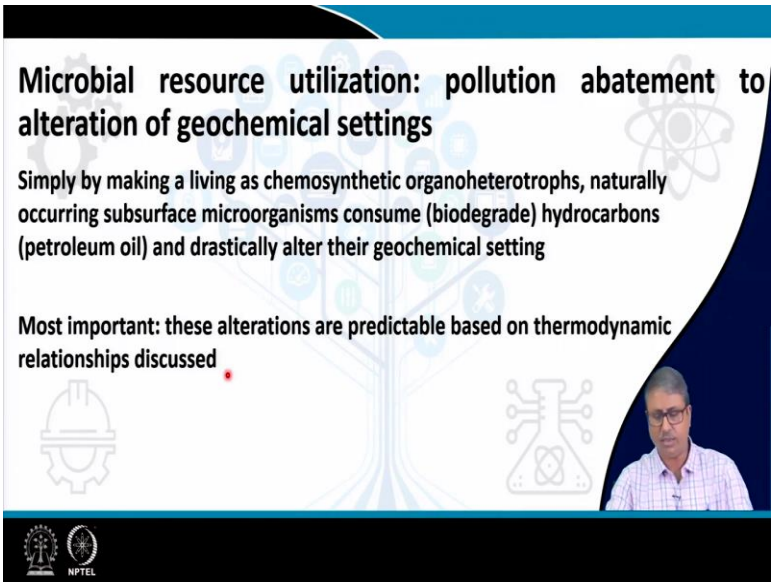

Now, in this case the microbial resource utilization is directed towards both pollution abutment to the alteration of geochemical settings. So, therefore simply by making a living as chemosynthetic organo heterotrophs naturally occurring subs subsurface microorganisms consume or biodegrade the hydrocarbons like petroleum oil in this case and drastically alter their geochemical settings. Most important is this that these alterations are predictable based on the thermodynamic relationship in this. That is the thermodynamic hierarchy or the compass that is discussed.

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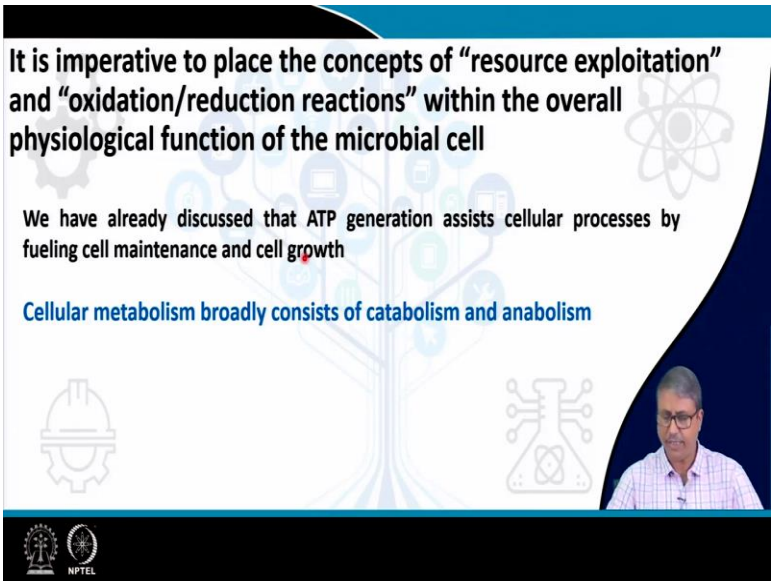

Next we are going to talk briefly about the overview of metabolism and the logic of electron transport. It is imperative to place the concept of resource exploitation that we have been discussing and oxidation reduction reactions within the overall physiological function of the microbial cell. Now, what is that overall physiological function of the microbial cell we have already discussed that ATP generation is a major process or the major physiological function that assists cellular process by fueling cell maintenance and cell growth.

So, eventually everything is based on the fundamental requirement of ATP. So, this because the cells require ATP that is why the cell is doing all the metabolic reactions and this atp is used by all other biosynthetic reactions. Now, cellular metabolism in this case is broadly consists of two type of reactions catabolism and anabolism.

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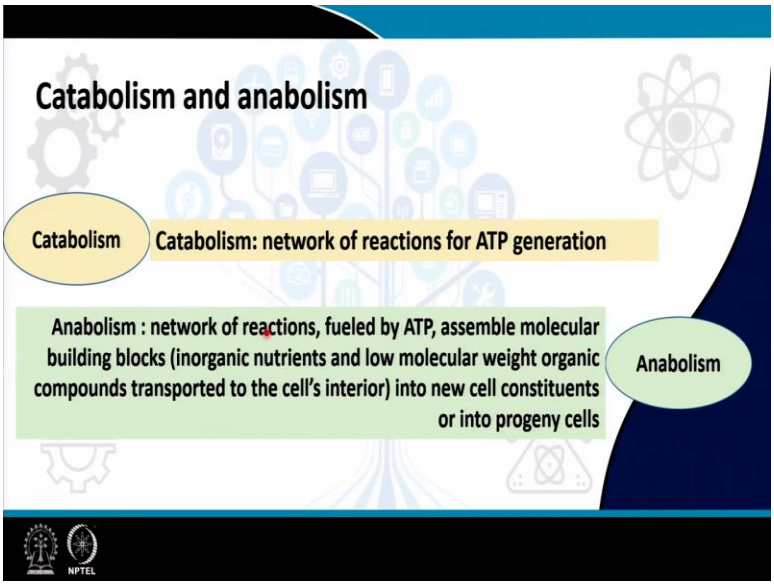

Now, catabolism is a set of network of reactions for ATP generation it is basically the oxidative reactions where in the substrate the reduced molecules organic or inorganic whatever they are oxidized to yield the electrons out of it. And these electrons are funneled through electron acceptors and eventually the electron transport chain operates to produce the protomotive force and the protomotive force yields the ATP molecule.

On the other hand the anabolism refers to a net of network of reactions which are fueled by ATP the ATP which are produced or available in the cell are utilized during this network of reactions which assemble molecular building blocks that is your synthesis of macromolecules using different inorganic nutrients and low molecular weight organic compounds which are transported into the cell interior and formation of new cell constituents like the macromolecules or cell division which allows the formation of progeny cells.

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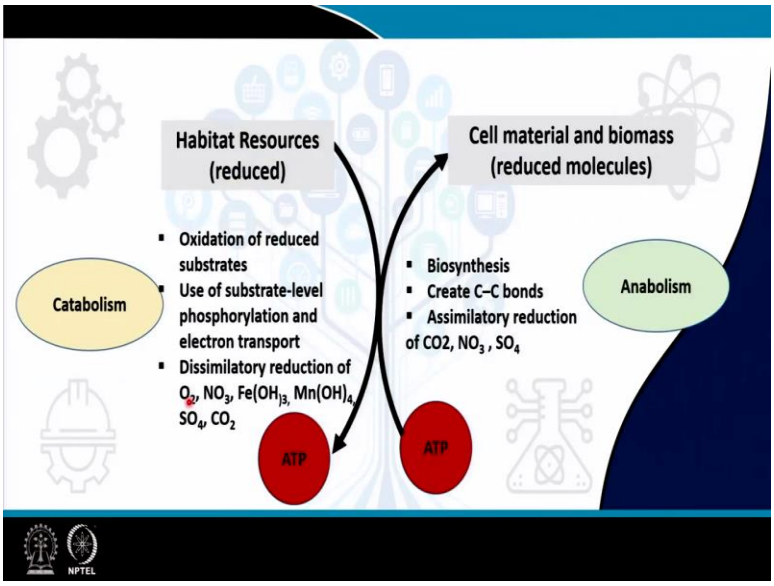

Now, these two processes are actually interlinked on the one hand we can see that in any habitat we have the habited resources which are basically different kind of reduced substances and these reduce reduced substances are subject to oxidation may be the carbon compounds and other compounds which can be oxidized to yield energy. The energy in the form of electrons and this is facilitated by the oxidation of reduced substances like the glucose molecule or the hydrocarbon molecule that we have just seen during a petroleum oil contamination.

And its metabolism through the glycolysis leads to the substrate level phosphorylation that we have also discussed earlier and then electron transport as the electron moves through the electron transport chain producing the protomotive force and the protomotive force leads to the formation of the ATP. In this habitat resource utilization by catabolism one very important aspect is that a number of electron acceptors are used including oxygen nitrate iron manganese sulfate etcetera.

Now, they are reduced as terminal electron acceptor. Now, when nitrate is reduced to ammonia or nitrogen iron is reduced to Fe 2 or Mn is reduced sulphate is reduced to sulphide or carbon dioxide is reduced to methane. None of these reduced products are assimilated inside the cell. These reduced product are of no use for the cell and the they are just discarded that is why this reductive process is called dissimilatory reduction.

So, the catabolism is basically having these two component in one side it is the oxidation of the

reduced substances providing the electron source electrons which will allow the generation of ATP and also the you the reduction of different terminal electron acceptor which will facilitate the disciplinary reduction process. Now, the ATP molecules which are generated as well as different type of precursor molecules which are produced during this oxidation of the organic molecule in catabolism leads to the function of the anabolism.

The network of reactions that basically allow the biosynthesis of the macromolecules, create new carbon-carbon bonds all the molecules like the carbohydrate lipids nucleic acid everything is produced out of the precursor molecules which are generated during this produced during the catabolic reaction. A number of molecules are assimilated like for amino acid synthesis they may require the amino group or they require the sulfur.

So, there could be some reduction reactions. So, nitrate can be reduced to ammonia or sulphate could be reduced to provide the sulphur. So, these reductions are called assimilated reduction because these products of this reduction. Like the ammonia for example ammonia will be utilized if it is utilized by the cells for the synthesis of nitrogen containing nitrogen group containing macromolecule these are called assimilatory reduction.

So, the anabolic reactions that starts with the use of the ATP which are produced during the anabolic the catabolic reaction and the precursor molecules lead to the formation leads to the formation of new cell material cellular macromolecules and the biomass that is the reduced molecules.

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So, this particular section of the lecture is covered from this book our environmental microbiology from genomes to biogeochemistry by medicine.

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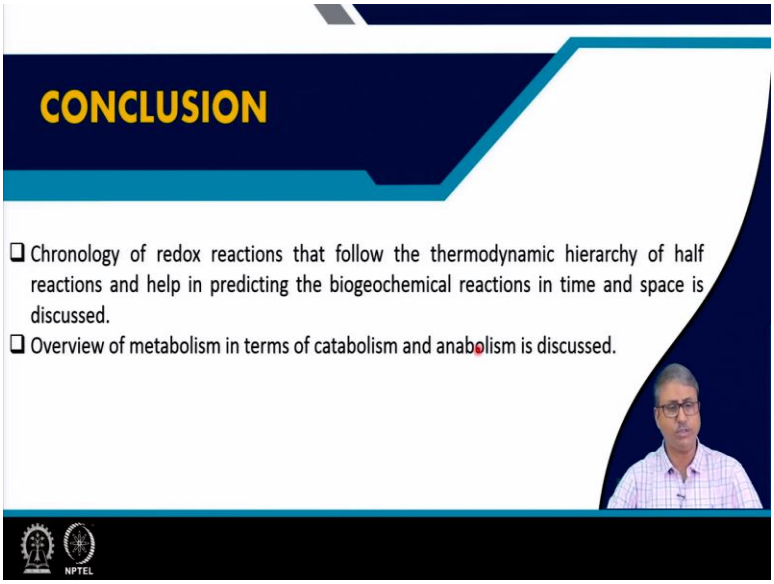

And in conclusion the chronology of redox reactions that follow the thermodynamic hierarchy of half reactions and help in predicting the biogeochemical reactions in time and space is discussed. Overall an overview of metabolism in terms of catabolism anabolism is discussed, thank you.