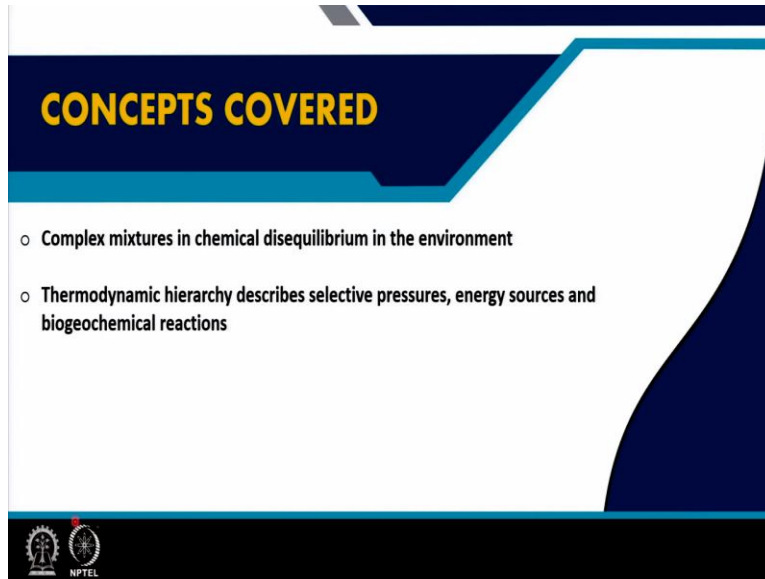


Environmental Biotechnology
Prof. Pinaki Sar
Department of Biotechnology
Indian Institute of Technology, Kharagpur


Lecture – 22
Physiological Ecology and Resource Exploitation by Microorganisms (contd.,)

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CONCEPTS COVERED

- Complex mixtures in chemical disequilibrium in the environment
- Thermodynamic hierarchy describes selective pressures, energy sources and biogeochemical reactions



Welcome to the 22nd lecture of this NPTEL course on environmental biotechnology which is on the Physiological Ecology and Resource Exploitation by Microorganisms. In this particular lecture the complex mixtures in chemical receive equilibrium in the environment will be discussed and this will be followed by the thermodynamic hierarchy that describes the selective pressure energy sources and bio geochemical reactions within different environmental systems.

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Complex mixtures in chemical disequilibrium in the environment

Generation of ATP, the energy currency of the cell

The diagram illustrates the generation of ATP. It features two input boxes: a blue box labeled 'Energy source (Chemical /Light)' and a yellow box labeled 'Carbon source (Sugar)'. Arrows from both boxes point to a central orange box labeled 'ATP'. A dashed arrow also points from the 'Carbon source' box to the 'ATP' box. Below the diagram, text states: 'Without ATP, cell maintenance, cell motility, biosynthetic reactions, replication, cell growth, & heredity would be impossible'. The slide includes a small portrait of a man in the bottom right corner and logos for a university and NPTEL in the bottom left corner.

So to; begin with the complex mixture in chemical disequilibrium in the environment. So, one of the major aspects of cellular metabolism within diverse environments where in environmental biotechnology processes are studied or developed generation of ATP the energy currency of the cell remains one of the very important factor for driving the cellular processes and as we understand that this generation of ATP which is the energy currency of the cell is connected to metabolisms cellular metabolism with respect to deriving the energy from the different energy sources.

Like the; chemical energy or the light energy or it could be from the reduced carbon substrates or carbon compounds or sugar molecules which upon oxidation also produce this energy or the ATP molecules. Now without these ATP molecules cell maintenance cell motility biosynthetic reactions which facilitate the synthesis of all the macromolecules within the cells replication of the DNA or the genetic material cell growth and heredity would be impossible.

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The biochemical mechanism of ATP generation relies upon either substrate-level phosphorylation or membrane-bound electron transport chains

Electron transport chains create the proton (or sodium) motive force that drives ATP synthases embedded in cytoplasmic membranes

Now the biochemical mechanism of ATP generation fundamentally relies upon either the substrate level phosphorylation or it could be through the membrane bound electron transport chain or electron transport system. Now as we possibly understand that the substrate level phosphorylation which occurs during the glycolytic reactions of the metabolism particularly when the sugar molecules are oxidized through embryonic furnace pathway.

And we observe that there are steps through which the ATP molecules are generated and as the sugar molecules are broken down into smaller molecules like the pyruvic acid. For example from the glucose at the same time these catabolic reactions like the embryonic furnace pathway or the glycolysis also produce the reduced electron acceptor that is the NADH or the electron carrier and the pyruvic acid which is produced upon oxidation of the glucose molecule is further metabolized through tricarboxylic acid cycle or TCA cycle and leads to the production of further NADH or reducing powers.

Now while this ATP which is directly produced from substrate level phosphorylation represents the one of the major outcome of this glycolytic reaction. The reduced electron carrier like NADH which are produced in plenty of the number during this glycolytic reaction and subsequent TCA cycles they transfer the electrons into the electron transport system and as you can see in the right panel that the reduced electron carriers like NADH or FADH₂ which are produced during the glycolytic reactions or the TCA cycle.

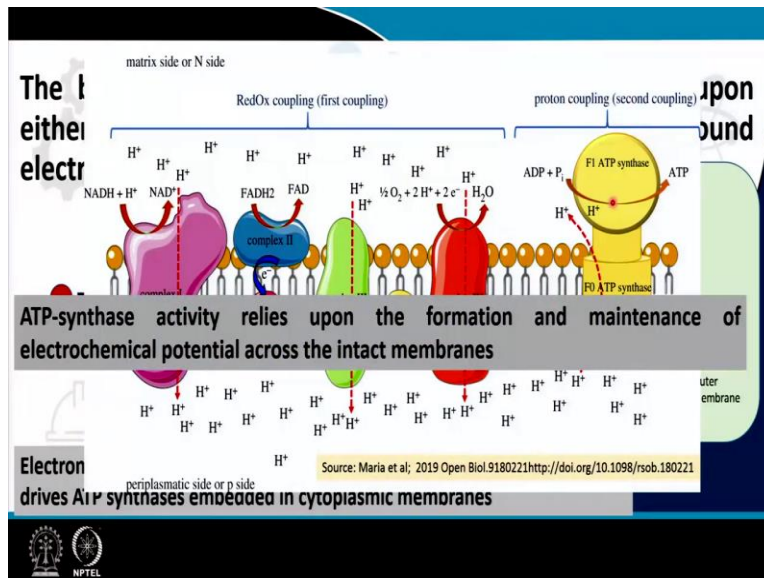
They donate the electrons to the electron carriers which are membrane bound and these membranes bound electron carriers they are able to transfer the electrons to the terminal electron acceptor like the oxygen and thereby producing water molecule. But more interestingly while the electrons are transferred from the reduced electron carrier like NADH to the terminal electron acceptor oxygen. During this electron transport system some of the electron transporting complexes also acts as proton efflux and efflux pumps.

And they reflux out proton into the periplasmic space or the cell exterior. Now these electron transport chains these this is called electron transport chain the trans transportation of the electrons from the reduced electron carrier like the NADH or FADH 2 to the terminal electron acceptor and this represent the electron transport chain which is composed of different electron transporting carrier complexes.

Now due to the functioning of this proton efflux process or proton flux pumps which are integral properties of the sum of the electron carriers carrying electrons during this membrane boundary electron transport chain these create the proton or sodium protein motive force. So, essentially we we see that a large number of protons accumulate within the periplasmic space and thereby creating the proton motive force.

And these protomotive force essentially then drive the synthesis of ATP through the enzyme complex which is known as ATP synthase which is embedded into the inner membrane or the cytoplasmic membrane.

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This picture elaborates this process that the complexes as they carry electrons from NADH to oxygen they export the protons and these protons are allowed to come inside through this ATP synthase complex enzyme complex. And this ATP synthesis complex essentially generates the ATP molecules which are required for the cell. So, essentially what do you understand that the oxidation of the substrates either the energy source or the carbon source which are reduced which can be utilized as both energy and carbon source the electrons are derived.

And those derived electrons are given to the oxidized NAD to produce the NADH and the NADH carries the electron to the electron transport complex which finally leads to the production of the ATP through the proton motive force. Now the ATP synthase enzyme complex which is responsible for the production of ATP the activity of this ATP synthesis enzyme relies upon the formation and maintenance of the electrochemical potential across the membrane.

So, this membrane must be intact. So, within an environmental context we need to understand that the membrane integrity must be maintained all time during the cellular process. Any toxic substance or any toxic condition which might interfere with the cellular membrane constituents or disrupt the membrane the cellular integrity membrane integrity is compromised or lost might or will definitely result into the production of this proton motive force and essentially which will lead to the decreased production of the ATP from those oxidation reduction reactions.

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The ultimate driver of ATP synthesis is the variety of thermodynamically unstable materials that are commingled in the waters, sediments, and soils of the biosphere

Now the ultimate driver of the ATP synthesis is the variety of thermodynamically unstable materials that are commingled in the water sediment and soils within the biosphere or basically any part of that biosphere that is the environment. Now what does this mean we have just seen that the ATP production is actually facilitated by the oxidation of NADH or the reduced electron carrier. Now where from these reduced electron carriers like NADH are produced.

The NADH are produced because of the oxidation of the substrates molecule what type of substrate? Substrate which are energy rich and upon oxidation they give rise to the electrons and those electrons are carried by the reduced electron carrier or the oxidized electron carrier to produce the reduced form that is NAD plus is converted to the reduced form of the NAD plus that is the NADH plus.

Now who are those electron donors the fundamental electron donors from where the source of the electrons from where the electrons are originally obtained and then taken by the NAD plus. Those sources are the thermodynamically unstable materials. For example they reduced carbon compounds or other inorganic materials which can be processed or catalyzed or rather made up metabolized through microbial enzymes to get the electrons out of them and then those electrons can be given to the electron carrier like the NAD plus.

Now these materials for example ammonia or reduced sulphur compounds or iron oxide

compound these all these compounds are mixed in a very active form within diverse components of an any kind of environment be it wastewater or be it a rock or a sediment or a polluted soil or any kind of other materials. So, everywhere these materials which are basically many of them are thermodynamically unstable and could be metabolized by the cells to get the electrons out of them most often. And sometimes donate the electron to them to reduce them and that drives the synthesis of ATP.

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The Earth, its habitats, sub-habitats and microenvironments have always been in a complex state consisting of commingled materials

In a state of heterogeneous mixture of rock, water, gases, and other materials bathed in sunlight

"How do microorganisms make physiological sense of this complexity?"

NPTEL

Now let us look at the different subsets of sub habitats within the planet earth. So, the earth as a whole or its different habitats or sub habitats and their micro environment the enumerable number of micro environments which exist within this landfill dump site or a pile of ore material mineral containing or heavy metal containing rock ores. Or a agricultural site or a activated sludge containing waste water treatment plant.

So, we can have innumerable number of micro environments within them and they constitute a kind of represent a kind of complex state consisting of commingled material. Now this compangled materials are most of them are organic or inorganic substances these organic or inorganic substances are active in term that they can donate electron or they can accept electron. Sometimes they can be oxidized like a carbon source like a alkene chain or a glucose molecule or any other reduced organic carbon which may be oxidized.

Or it may be ammonia or sulfide or heavy metals which can be transformed through redox reactions. So, these materials are all mixed together in any kind of environment. So, wherever we are we try to implement or investigate environmental biotechnology processes or try to develop environmental biotechnology processes be it for increasing the productivity of the crops or handling some of the environmental pollutants or making the water treatment process more effective. Now these all environments are in a state of heterogeneous mixture and most of the time these mixtures represent mixture of rock water gas and other materials but in sunlight.

So, what is the significance of sunlight over here? The sunlight drives the photosynthetic reaction most of the time. Under dark condition also the fixation of carbon is possible by many microorganisms which are chemoautotrophic microorganism. But principally on the surface of the earth or any environment which is exposed to sunlight. This light driven photosynthesis or fixation of the inorganic carbon into reduced carbon material plays a very important role by providing the reduced carbon substrate.

Now this reduced carbon substrate makes a very important position in entire thermodynamic process that drives the metabolic reactions because these are readily oxidizable. They can be oxidized provided the condition is suitable and the appropriate enzyme complexes by the microbes are available and they can produce electrons these electrons can be given to NAD plus to produce NADH plus.

And NADH plus can create the proton motive force and from the proton motive force the ATP molecules can be generated. Now it is of fundamental importance to understand how do microorganism make physiological sense of this complexity because there in every environment or a part or within a micro environment micro habitat there are as I mentioned the innumerable types of substrates are there.

So, this is perfectly a kind of a heterogeneous mixture of different oxidized and reduced substrates. Now microorganism needs to make living out of that. So, we need to understand how they make physiological sense.

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Thermodynamics: One way to achieve a unified and orderly view of the biosphere

- the branch of chemistry that rigorously predicts the chemical reactions that are energetically favourable and ones that are not
- can systematically arrange the types of chemical reactions that occur in complex, heterogeneous mixtures typical of biosphere habitats – we can predict and catalogue the energetically favorable reactions

The slide features a background with faint icons of a hard hat, a beaker, and a molecular structure. In the bottom right corner, there is a small video inset of a man in a checkered shirt speaking. At the bottom left, there are logos for a university and NPTEL.

Now in order to understand that how microorganisms make a physiological sense out of that we; need to follow certain principles of thermodynamics. Thermodynamics which is one way to achieve a unified and orderly view of the biosphere or a part of the environment it is also a branch of the chemistry that rigorously predicts the chemical reactions that are energetically favourable and ones that are not.

So, looking at the thermodynamics we can understand the reactions which reactions are favourable and which reactions are not favourable under certain condition or under standard conditions or bio biochemically or physiological conditions also thermodynamics can systematically arrange the types of chemical reactions. So, if you have a number of chemical reactions which are possible as I mentioned earlier oxidation of water oxidation of ammonia oxidation of nitrite oxidation of sulfide oxidation of glucose and simultaneously reduction of oxygen reduction of nitrate reduction of sulphate reduction of night or iron.

So, many reactions are possible which are I am talking about mostly the oxidative and reductive reactions. So, many reactions are possible in an environment. Now is it possible to arrange the type of reactions that could that could perhaps occur within a complex heterogeneous mixture based on certain criteria? Yes it is possible. So, it is the thermodynamics or thermodynamic criteria or characteristics which help us to arrange the type of reactions particularly the oxidation reduction type reactions that kind that may occur in a complex heterogeneous mixture which are

typical for any environmental system.

And when we have that arrangement that the reactions which are which are more preferable in an oxidizing conditions and reactions which are more preferable within a reduced conditions we can predict and catalog the energetically favourable reactions we can pretty easily identify which type of reactions might be more preferable or energetically preferable or favourable within a given environment.

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Types of reactions

- Changes in state
- Dissolution/precipitation
- Complexation
- Acid/base
- Sorption/desorption
- Oxidation/reduction (key for biological systems to generate ATP)

$\Delta G = \Delta G^0 + RT \ln \frac{[C][D]}{[A][B]}$

ΔG = free-energy change under conditions specified
 ΔG^0 = free-energy change under standard conditions of 1 atm pressure and 1 m concentration
 T = temperature (degrees K)
 R = universal gas constant (8.29 J/mol/K)

So, this is a very common plot that often we use to identify these thermodynamic criteria that the reactants are converted to products and we can calculate the free energy change. And the types of reactions can be of different nature change in state dissolution and precipitation, complexation acid base reactions, absorption, desorption, type of reaction and oxidation reduction reactions which are very relevant for biological systems particularly of environmental relevance to generate ATP.

However absorption desorption kind reactions complexation dissolution precipitation and even the changes in state are also important with reference to change in the different type of environmental pollutants or other compounds which are useful or relevant for microbial metabolism.

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Types of reactions

- Changes in state
- Dissolution/precipitation
- Complexation
- Acid/base
- Sorption/desorption
- Oxidation/reduction (key for biological systems to generate ATP)

Identify resources which are exploited by microorganisms energy sources and facilitate the ATP generation

It is the thermodynamically unstable resources that have provided selective pressure for microbial energy-production strategies throughout evolution

Now with the help of the thermodynamics we can identify resources which are exploited by microorganisms as their energy sources and facilitate the ATP generation. So, if in a kind of an open landfill site if we have certain substance we can identify these substances by some chemical analysis and looking at the redox condition that is the oxidizing or reducing condition of the environment we can very well identify or define which are the substrate which might be or possibly be oxidized by the microorganism under the given environment.

Or what are the compounds or what are the substances which might not be metabolized like oxidized or reduced under this given condition. It is the thermodynamically unstable resources particularly that have provided the selective pressure for microbial energy production strategies throughout the evolution. Like for example if we look at the oxidation of the water the breaking of water the photolysis which you as we know during the photosynthesis reaction.

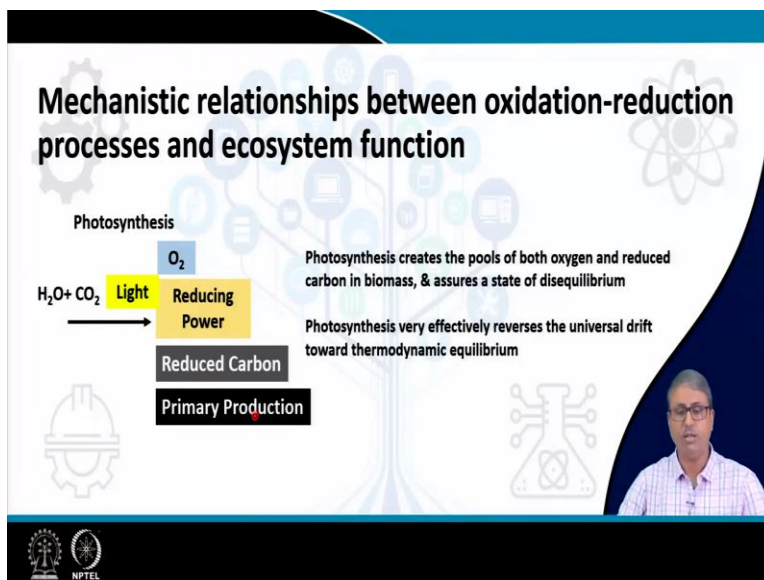
So, why photolysis of water is? So, preferred compared to other perhaps that is more favorable thermodynamically compared to any other reaction why reduction of oxygen to water is considered to be energetically more favorable rather than reduction of sulphate to sulfide or reduction of carbon dioxide to methane. So, in an environment if we; have both carbon dioxide and oxygen for example.

Now are we going to have methane generation from carbon dioxide? Possibly no because the

reduction of carbon dioxide to methane is energetically not favourable thermodynamically it is not favorable the free energy changes the delta G is very very less. So, the microbes will not be benefited. So, if we have oxygen or any other terminal electron acceptor which are having a better free energy provision carbon dioxide reduction to methane may not be adopted by the cells over there.

So, even if you have organisms microbes who are able to carry out meat and generation from carbon dioxide those microorganisms will not be acting appropriately towards the generation of the method.

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Now in the next point we are going to discuss about the mechanistic relationship between oxidation reduction processes and the ecosystem function. As we have understood that photosynthesis is one of the major driving process in this entire biosphere and also in any kind of environment it is not only photosynthesis but it could be chemosynthesis also like chemoautotrophy.

So, photo photosynthesis is very important because the **the** plant biomass that we see or the algae and the phototrophic microorganisms who absorb the light energy and can fix the carbon dioxide into the reduced carbon. So, basically with the help of the light energy and with the available carbon dioxide and water the reducing power like the NADH are produced or NADPH are

produced.

And at the same time the photolysis or the breakdown of the water molecule leads to the production of oxygen. So, this has a very important implication on the overall the metabolism or metabolic scenario. This reducing power is used by the microorganisms and other organisms who are carrying out the photosynthetic reaction to reduce the carbon. So, essentially the carbon dioxide is reduced to reduced form like the for example the glucose or glucose like reduced carbon form.

And these reduced carbon molecules they **they** allow or they represent the primary production within an ecosystem. So, in any kind of ecosystem which are **which are** exposed to solar light we can expect that the primary production is by the **the** photosynthetic reaction. However in environment where sunlight is not received the primary production could be manifested by the chemoautotrophic reactions which are microorganisms which are capable of fixing carbon dioxide the same reaction but without the the involvement of light the energy is obtained from other organic or inorganic substances in that case.

So, we have already seen that there are different type of microorganisms which are may be the litho autotrophic or organo autotrophic organisms who can obtain the electrons from either the chemical forms like the inorganic chemical compound or the organic compound and utilize that electrons to fix the carbon dioxide to reduce carbon and essentially that reduced carbon forms the primary production.

So, no matter whether it is the photosynthesis or it is the chemosynthesis this reduced carbon is going to form the the base of the primary production. Now this synthesis of this reduced carbon in all ecosystems all environments that creates the pool of both oxygen and reduced carbon in the biomass. So, this reaction has a very important bearing on the environment because it produces both the oxygen as well as the reduced carbon.

So if we look carefully reduced carbon could be the substrate for the heterotrophic or the organotrophic organisms. So, any organism who is not capable of fixing carbon dioxide would

rely on this reduced carbon and metabolize this carbon to or oxidize this carbon to produce the necessary ATP molecules and also the precursor for the macromolecular synthesis and other cellular metabolism.

On the other hand this oxygen which is produced out of these photosynthetic or chemosynthetic reactions could actually take part in the electron accepting reaction. some time ago we were looking at the electron transport chain. So, we have also noticed that within the electron transport chain there is a terminal electron acceptor the electrons which are basically subtle to the electron transport chain must be accepted by some acceptor and after that or beyond that that no more electron transportation reaction will occur.

So, that is called the terminal electron acceptor. So, oxygen is the most preferred electrical electron acceptor reason behind that the free energy gain from the by reducing oxygen to water molecule is found to be maximum within the biological context. And this formation of oxygen and reduced carbon in the form of biomass assures a state of disequilibrium because out of the carbon dioxide and water and other inorganic resources it is continuously producing the reduced carbon molecules.

So this formation; of carbon molecule and the oxygen both that assures the state of this equilibrium. Now photosynthesis or the chemosynthesis of the or the fixing of carbon dioxide is very effectively reverses the universal drip to our thermodynamic equilibrium. We are going to talk about that very soon.

Now overall what we have understood that the oxygen molecules which are generated and the reduced carbon which are produced they basically drive the microbial metabolism through a series of oxidation reduction reactions.

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Metabolism by chemosynthetic organotrophic life

Blending of reduced biomass + oxygen + oxidized compounds (e.g. nitrate, Fe oxides, Mn oxides, sulfate, CO₂) sets the stage for metabolism by chemosynthetic organotrophic life

Metabolism by chemosynthetic lithotrophic life

Commingling of reduced inorganic compounds (e.g., H₂, NH₃, H₂S) with oxygen + other oxidized materials sets the stage for metabolism by chemosynthetic lithotrophic life

Nonequilibrium state is created by co-occurring pairs of reduced and oxidized materials in waters, sediments, and soils of the biosphere

IIT Bombay NPTEL

Now the major oxidation reduction reactions which are responsible for the drift are the metabolism by the chemosynthetic organotrophic life form and the other one is the metabolism by chemosynthetic lithotrophic lifeform. So, in the chemosynthetic organotrophic life form blending of reduced biomass reduced biomass the reduced carbon which is produced. We just saw that following carbon fixation the reduced carbon are produced.

So, that reduced carbon when it mixed with the oxygen which is present because oxygen is also produced oxidized compound because in any environment there will be plenty of oxidized compounds including nitrate iron oxides manganese oxide sulphate carbon dioxide etcetera that sets the stage for metabolism by organotrophic life because organotrophic organism would actually oxidize the reduced biomass and use the oxygen as the terminal electron acceptor.

Sometimes these are also used as their other electron acceptor if oxygen is not available. On the other hand the lithotropic life form will rely on the co-mingling of the reduced inorganic substance because there would be some substrates which are always the inorganic substances which are always reduced like the ammonia for example or sulfide for example or even hydrogen. So, these reduced inorganic compounds with oxygen because again oxygen would be there most likely.

And other oxidized materials set the stage for the metabolism by the chemosynthetic lithotrophic

form. So, in any environment or it is a part of the environment we will expect that these organotrophic and lithotropic forms of lives are both possibly running parallelly. Maybe sometimes the organotrophic life will predominate over the tropic line some of the environments we see the lithotropic life is more more abundant or more dominant over the organotrophic form.

But it is highly likely that the reduced biomass eventually produced by even the lithotropic mechanisms would be metabolized by the organotrophic life form. So, overall a non equilibrium state is created by co-occurring pairs of the reduced and oxidized materials in water sediment and soil of the biosphere for example oxygen water nitrate nitrogen MnO_2 to Mn nitrate to ammonia, sulphate to sulphide and carbon dioxide to methane.

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The slide features a central graphic of a tree with various scientific icons (gears, beakers, atoms) as branches. The text is as follows:

Using the thermodynamic hierarchy of half reactions to predict biogeochemical reactions in time and space

Compass for environmental microbiologists : Predicting and interpreting microbially mediated ATP generating biochemical reactions

At the bottom left, there are logos for a university and NPTEL. On the right side, there is a small inset video of a man speaking.

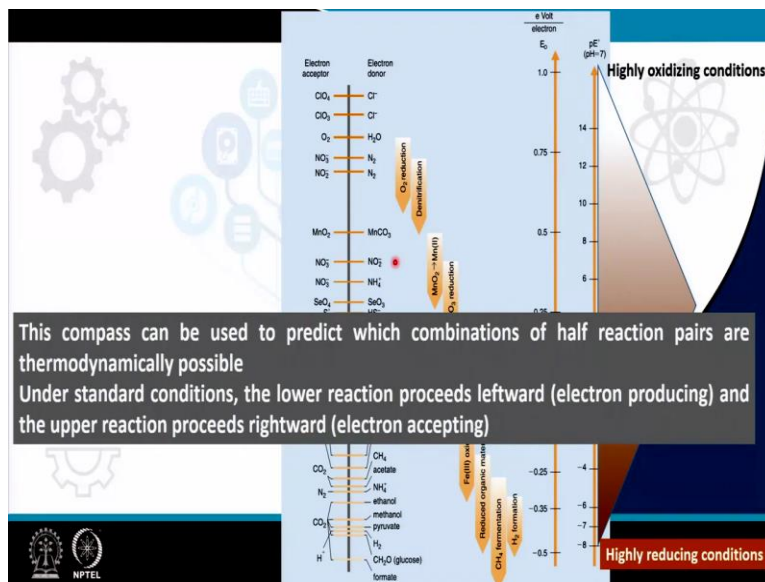
Now next we will study that how the use of this thermodynamic hierarchy can be made particularly with respect to the half reactions to predict bio geochemical reactions in time and space and this particular thermodynamic hierarchy that we will see. Now is considered as a compass for environmental microbiologist and environmental biotechnologist because it helps in predicting and interpreting microbiologically or microbially mediated ATP generating reactions.

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Relationship between reduced and oxidized substrates as a vertically arranged hierarchy of oxidation– reduction half reactions

So, relationship between reduced and oxidized substrates as vertically arranged hierarchy of oxidation reduction half reaction.

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So, you can see there are multiple half reactions are presented over here and the electron volt is mentioned and the pE is also presented as p at pH 7. So, the compounds which are listed on the scale on the left side we have the compounds which are in their oxidized form for example oxygen or the nitrate or the nitride or MnO 2 these are all oxidized and on the right hand side on this particular scale we have the compounds which are in the reduced form basically these compounds can be acted as a electron donor.

So, for example water H_2O can be electron donor similarly if we have ammonia ammonia could be electron donor and so on. Now the transition from this oxidized state to the reduced state depends on the redox status of the system of interest firstly. Now whether oxygen will remain as oxygen or will form water or nitrate will remain as nitrate or form nitrogen dinitrogen or ammonia or for example the selenite will form as a selenate or selenide.

All these things depend on MnO_2 will remain as MnO_2 will form $MnCO_3$ these all will depends on the redox status of the system of interest. Firstly that what is the redox condition because this is a scale which is considered as a compass. Now using this compass if I go to a landfill site and if I go little deep inside the landfill site like I dig maybe one or two meters inside the landfill site I may encounter a reduced condition or oxidized condition.

So, the kind of reactions which would be possible at that state because the redox condition or the redox state of the system is going to control that what type of reactions what whether this these don't acceptors will be accepting electrons and converted to electron donors or not or vice versa. So, if we look at the status of these conditions we will find that some of the reactions are favourable under highly oxidizing conditions.

As you can see from this pE values while some reactions like reduction of carbon dioxide to methane for example or reduction of sulphate to sulfide these are preferable or favourable under highly reducing condition. So, these are the two things one we need to assess what type of half reactions are possible that is the substrates which can be oxidized or reduced by different analytical techniques we can we can take any wastewater or contaminated site or any environment where some biotechnological processes are to be initiated.

So, firstly we need to analyze that what are the substrates which can be which are the unstable substrates which can be oxidized or reduced. So, potential electron acceptors and electron donors are to be identified and then it is necessary to identify whether the environment is a reducing condition or an oxidizing condition because some reactions might be possible under oxidizing condition but some reactions might be only possible under the reducing condition.

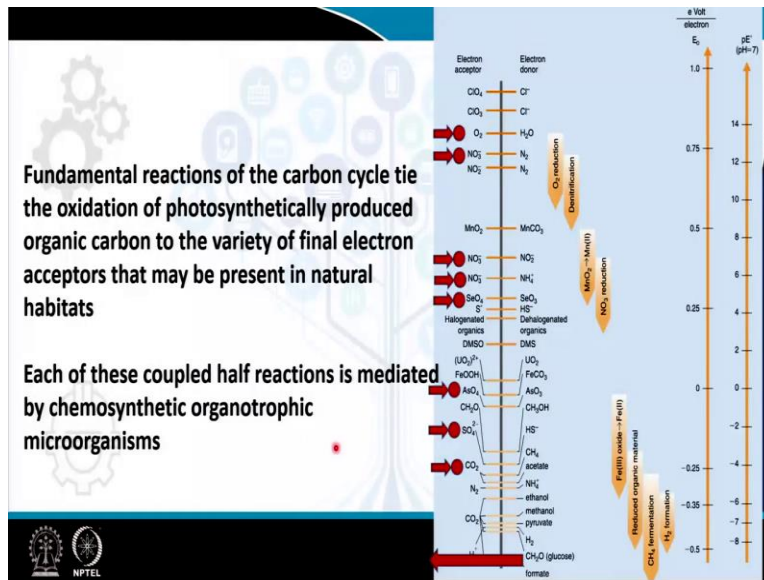
I mentioned about the formation of methane from carbon dioxide some time ago which is possible only under highly reducing condition. So, if we see that high amount of carbon dioxide is there but the condition or the redox state is positive like positive 10 or 5 or 6 then we may not expect any kind of carbon dioxide reduction under the same condition because under that condition oxygen is going to be reduced and to produce water.

Or if oxygen is lacking then eventually nitrate after nitrate Mn or may be iron will be reduced but carbon dioxide reduction would definitely not takes place unless the condition is going to be highly reduced. Now this compass can be used to predict which combinations of the harp reaction pair are thermo dynamically possible. These are all reactions from these thermodynamic sorry the half reactions.

So, the half reaction pair out of them which one are possible in a given environment is only predicted from this compass or the list under standard conditions the lower reactions proceed leftward that is electron producing and the upper reaction proceeds rightward that is electron accepting. So, under normal standard conditions we will see these reactions will prefer like the oxygen would be reduced to water or ammo nitrate will be reduced to nitrogen etcetera.

Whereas under the reduced condition we may see that carbon dioxide is reduced or sulphate is reduced to sulphide.

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Now if we look little more in depth into this particular compass we will be able to find out that this is very well connected to the carbon cycle as well or in any environment where reduced carbon or carbon molecules are cycled in all environments eventually carbons are cycled. So, fundamental reactions to the carbon cycle tie the oxidation of this photosynthetically produced or otherwise produced organic matter to the variety of final electron acceptors that that may be present. Now if we look at this the carbon or the glucose this can be oxidized, so, this can be oxidized to produce the electrons and these electrons will move into different electron acceptors.

Like it will move to oxygen as a terminal electron acceptor the electrons might move to the nitrate or to the sulphate or the selenite or arsenic or even to carbon dioxide to produce methane. Now the free energy change that the free energy gain by this transfer will vary because if the electrons travel from this glucose oxidation to water the free energy gain is maximum compared to that of nitrate or selenite or arsenate or sulfate or carbon dioxide.

So, essentially we can expect energy gain which decrease or decreases very significantly as we go we come down in this particular thermodynamic scale. Now each of these coupled half reactions is mediated by chemosynthetic organotrophic microorganisms. So, the reactions that we are mentioning like the reduction of oxygen as a terminal electron acceptor reduction of nitrate to nitrogen or reduction of nitrate to ammonia or reduction of selenite or reduction of iron oxide arsenic sulphate to sulphide or carbon dioxide to methane are all catalyzed by specific group of

microorganisms.

Sometimes some reactions are catalyzed by more than or a single microorganism can catalyze more than one reaction but often they are very specific to individual group of microorganisms.

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Eight common processes that are recognized to occur in carbon-rich habitats

Process	PE regimes			
	(PE° = log K)	Heterotrophic reactions	ΔG° (kJ/eq.)	
Aerobic respiration	$\frac{1}{4}O_2(g) + H^+ + e = \frac{1}{2}H_2O$	+13.75	$CH_2O + O_2 \rightarrow CO_2 + H_2O$	-125
Denitrification	$\frac{1}{5}NO_3^- + \frac{6}{5}H^+ + e = \frac{1}{10}N_2 + \frac{3}{5}H_2O$	+12.65	$5CH_2O + 4NO_3^- + 4H^+ \rightarrow 5CO_2 + 2N_2 + 7H_2O$	-119
Manganese reduction	$\frac{1}{2}MnO_2(s) + \frac{1}{2}HCO_3^- + \frac{3}{2}H^+ + e = \frac{1}{2}MnCO_3(s) + H_2O$	+8.9	$CH_2O + 2MnO_2 + 4H^+ \rightarrow CO_2 + 2Mn^{2+} + 3H_2O$	-98
Iron reduction	$FeOOH(s) + HCO_3^- + 2H^+ + e = FeCO_3(s) + 2H_2O$	-0.8	$CH_2O + 4FeOOH + 8H^+ \rightarrow CO_2 + 4Fe^{2+} + 7H_2O$	-42
Fermentation	$\frac{1}{2}CH_2O + H^+ + e = \frac{1}{2}CH_3OH$	-3.01	$3CH_2O \rightarrow CO_2 + CH_3CH_2OH$	-27
Sulfate reduction	$\frac{1}{8}SO_4^{2-} + \frac{9}{8}H^+ + e = \frac{1}{8}H_2S(g) + \frac{1}{2}H_2O$	-3.75	$2CH_2O + SO_4^{2-} + 2H^+ \rightarrow 2CO_2 + H_2S + 2H_2O$	-25
Methanogenesis	$\frac{1}{8}CO_2(g) + H^+ + e = \frac{1}{8}CH_4(g) + \frac{1}{4}H_2O$	-4.13	$2CH_2O \rightarrow CO_2 + CH_4$	-23
Acetogenesis	$\frac{1}{8}CO_2(g) + H^+ + e = \frac{1}{8}CH_3COOH + \frac{1}{4}H_2O$	-4.2	$2CH_2O \rightarrow CH_3COOH$	-22

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So, based on this overall process we could find out 8 common processes these are which are recognized to occur in a carbon rich habitats which includes the aerobic respiration to denitrification manganese reduction iron reduction fermentation sulfate reduction methanogenesis and finally acetogenesis.

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Eight common processes that are recognized to occur in carbon-rich habitats

Process	PE regimes			
	(PE° = log K)	Heterotrophic reactions	ΔG° (kJ/eq.)	
Aerobic respiration	$\frac{1}{4}O_2(g) + H^+ + e = \frac{1}{2}H_2O$	+13.75	$CH_2O + O_2 \rightarrow CO_2 + H_2O$	-125
Denitrification	$\frac{1}{5}NO_3^- + \frac{6}{5}H^+ + e = \frac{1}{10}N_2 + \frac{3}{5}H_2O$	+12.65	$5CH_2O + 4NO_3^- + 4H^+ \rightarrow 5CO_2 + 2N_2 + 7H_2O$	-119
Manganese reduction	$\frac{1}{2}MnO_2(s) + \frac{1}{2}HCO_3^- + \frac{3}{2}H^+ + e = \frac{1}{2}MnCO_3(s) + H_2O$	+8.9	$CH_2O + 2MnO_2 + 4H^+ \rightarrow CO_2 + 2Mn^{2+} + 3H_2O$	-98

- **Aerobic respiration occurs at a pE of 13.75 and its free energy yield exceeds that of the other reactions**
- **In a given aquatic or terrestrial habitat rich in carbon, microorganisms endowed with the physiological capacity to carry out aerobic respiration will have an advantage**
- **– their ATP-generating ability exceeds that of the other microbial residents**
- **As long as the supply of oxygen is adequate, aerobic respiration will predominate**

$\frac{1}{8}CH_3COOH + \frac{1}{4}H_2O$ Environmental Microbiology · Madsen EL, 2016

So, as I discussed aerobic respiration will occur at the pE of 13.75 or nearly 4 because it is the most oxidizing condition which prefers or favours the use of the oxygen as a terminal electron acceptor and its free energy yield exceeds that of the other reaction. So, if you have the free energy content is the maximum free energy constant that is the the minus 125 kilojoule per reaction. So, that is the maximum.

So, if we have oxygen in a system oxygen is going to be use a terminal electron acceptor if the condition is oxidizing. If the; we have to look at the pE also if the condition is not oxidizing then perhaps oxygen may not be reduced. Now in a given aquatic or terrestrial habitat rich in carbon microorganisms endowed with the physiological capacity to carry out aerobic respiration will obviously have an advantage. So, it is not only the aerobic respiration which will provide more energy to the system or the cells.

But also the organisms which are capable of performing the aerobic respiration will have an advantage and certainly they will proliferate they will make use of this energy more. Their ATP generating ability exceeds that of the other microbial residents and as long as the supply of oxygen is adequate aerobic respiration will predominate. However as soon as the oxygen level declines because the oxygen level if it is a kind of an environment where the free diffusion of oxygen is not possible then the oxygen level is going to be declined because the organisms are continuously using the oxygen then as the oxygen level declines because oxygen is continuously used as a terminal electron acceptor.

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Eight common processes that are recognized to occur in carbon-rich habitats

PE regimes

➤ Once the supply of oxygen is exhausted, nitrate-respiring organisms will become predominant ... and so on down the hierarchy

Denitrification	$\frac{1}{5}\text{NO}_3^- + \frac{6}{5}\text{H}^+ + \text{e}$ $= \frac{1}{10}\text{N}_2 + \frac{3}{5}\text{H}_2\text{O}$	+12.65	$5\text{CH}_2\text{O} + 4\text{NO}_3^- + 4\text{H}^+$ $\rightarrow 5\text{CO}_2 + 2\text{N}_2 + 7\text{H}_2\text{O}$	-119
Manganese reduction	$\frac{1}{2}\text{MnO}_2(\text{s}) + \frac{1}{2}\text{HCO}_3^- + \frac{1}{2}\text{H}^+ + \text{e}$ $= \frac{1}{2}\text{MnCO}_3(\text{s}) + \text{H}_2\text{O}$	+8.9	$\text{CH}_2\text{O} + 2\text{MnO}_2 + 4\text{H}^+$ $\rightarrow \text{CO}_2 + 2\text{Mn}^{2+} + 3\text{H}_2\text{O}$	-98
Iron reduction	$\text{FeOOH}(\text{s}) + \text{HCO}_3^- + 2\text{H}^+ + \text{e}$ $= \text{FeCO}_3(\text{s}) + 2\text{H}_2\text{O}$	-0.8	$\text{CH}_2\text{O} + 4\text{FeOOH} + 8\text{H}^+$ $\rightarrow \text{CO}_2 + 4\text{Fe}^{2+} + 7\text{H}_2\text{O}$	-42
Fermentation	$\frac{1}{2}\text{CH}_2\text{O} + \text{H}^+ + \text{e} = \frac{1}{2}\text{CH}_3\text{OH}$	-3.01	$3\text{CH}_2\text{O} \rightarrow \text{CO}_2 + \text{CH}_3\text{CH}_2\text{OH}$	-27
Sulfate reduction	$\frac{1}{8}\text{SO}_4^{2-} + \frac{9}{8}\text{H}^+ + \text{e}$ $= \frac{1}{8}\text{H}_2\text{S}(\text{g}) + \frac{1}{2}\text{H}_2\text{O}$	-3.75	$2\text{CH}_2\text{O} + \text{SO}_4 + 2\text{H}^+$ $\rightarrow 2\text{CO}_2 + \text{H}_2\text{S} + 2\text{H}_2\text{O}$	-25
Methanogenesis	$\frac{1}{8}\text{CO}_2(\text{g}) + \text{H}^+ + \text{e}$ $= \frac{1}{8}\text{CH}_4(\text{g}) + \frac{1}{4}\text{H}_2\text{O}$	-4.13	$2\text{CH}_2\text{O} \rightarrow \text{CO}_2 + \text{CH}_4$	-23
Acetogenesis	$\frac{1}{4}\text{CO}_2(\text{g}) + \text{H}^+ + \text{e}$ $= \frac{1}{8}\text{CH}_3\text{COOH} + \frac{1}{4}\text{H}_2\text{O}$	-4.2	$2\text{CH}_2\text{O} \rightarrow \text{CH}_3\text{COOH}$	-22

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The nitrate respiring organism will become predominant why nitrate reduction to denitrification process because its energy yield is next to oxygen and it is higher than the others. And one once the nitrate is also exhausted that is the denitrifying process is done these microorganisms eventually proliferate for some time and then the subsequent processes like manganese iron reduction followed by fermentation and other reactions will follow.

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Eight common processes that are recognized to occur in carbon-rich habitats

PE regimes

➤ Once the supply of oxygen is exhausted, nitrate-respiring organisms will become predominant ... and so on down the hierarchy


Denitrification	$\frac{1}{5}\text{NO}_3^- + \frac{6}{5}\text{H}^+ + \text{e}$ $= \frac{1}{10}\text{N}_2 + \frac{3}{5}\text{H}_2\text{O}$	+12.65	$5\text{CH}_2\text{O} + 4\text{NO}_3^- + 4\text{H}^+$ $\rightarrow 5\text{CO}_2 + 2\text{N}_2 + 7\text{H}_2\text{O}$	-119
Manganese reduction	$\frac{1}{2}\text{MnO}_2(\text{s}) + \frac{1}{2}\text{HCO}_3^- + \frac{1}{2}\text{H}^+ + \text{e}$ $= \frac{1}{2}\text{MnCO}_3(\text{s}) + \text{H}_2\text{O}$	+8.9	$\text{CH}_2\text{O} + 2\text{MnO}_2 + 4\text{H}^+$ $\rightarrow \text{CO}_2 + 2\text{Mn}^{2+} + 3\text{H}_2\text{O}$	-98

➤ This predictable sequence of physiological processes is reinforced at the level of gene regulation within individual microbial cells

➤ Final electron acceptor allowing the highest free energy yield inhibits expression of genes required for utilization of final electron acceptors residing lower in the hierarchy

Methanogenesis	$\frac{1}{8}\text{CO}_2(\text{g}) + \text{H}^+ + \text{e}$ $= \frac{1}{8}\text{CH}_4(\text{g}) + \frac{1}{4}\text{H}_2\text{O}$	-4.13	$2\text{CH}_2\text{O} \rightarrow \text{CO}_2 + \text{CH}_4$	-23
Acetogenesis	$\frac{1}{4}\text{CO}_2(\text{g}) + \text{H}^+ + \text{e}$ $= \frac{1}{8}\text{CH}_3\text{COOH} + \frac{1}{4}\text{H}_2\text{O}$	-4.2	$2\text{CH}_2\text{O} \rightarrow \text{CH}_3\text{COOH}$	-22

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Now this predictable sequence of physiological processes is reinforced at the level of gene regulation within individual microbial cells. So, it has been found that final electron acceptor allowing the highest free energy yield inhibits expression of genes required for utilization of finer electron acceptor residing lower in the hierarchy. For example if a particular organism may

be a pseudomonas strain is having both the genes for aerobic respiration as well as for the denitrification or maybe for manganese reduction.

These genes are different differently controlled. So, in the presence of oxygen the denitrification related gene or the manganese reduction related genes are going to be repressed that is that is very important. Some bacteria as I mentioned can perform more than one electron transport processes in a natural environment many times many bacteria are capable of doing this they are capable of utilizing multiple electron acceptors and many of them might have a branch electron transport system as well.

However it is highly regulated by the genetic background of the cells as I mentioned the genes which are involved in reduction of other terminal electron acceptors which are which are provided with or will lead to the production of less amount of energy will be repressed unless the maximum energy yielding process is exhausted those genes are not going to be expressed.

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So, with this we end this particular lecture and this part of this lecture may be read from the environmental microbiology book from genomes to biogeochemistry by Madsen.

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CONCLUSION

- Any part of our environment represents a state of commingled materials; - How do microorganisms make a physiological sense of this complexity is an important aspect of environmental biotechnology,
- Thermodynamic logic in utilization of resources and generation of ATP is discussed
- Mechanistic relationships between oxidation-reduction processes and ecosystem function is highlighted
- Use the thermodynamic hierarchy of half reactions to predict biogeochemical reactions in time and space is discussed



And in conclusion any parts of our environment represent a state of commingled materials how do microorganisms make a physiological sense of this complexity is an important aspect of environmental biotechnology. Thermodynamic logic in utilizing the resources and generation of ATP is discussed, mechanistic relationship between the oxidation reduction processes and ecosystem function is highlighted.

And finally use of the thermodynamic hierarchy of high reaction to predict biogeochemical reactions in time and space is discussed, thank you.