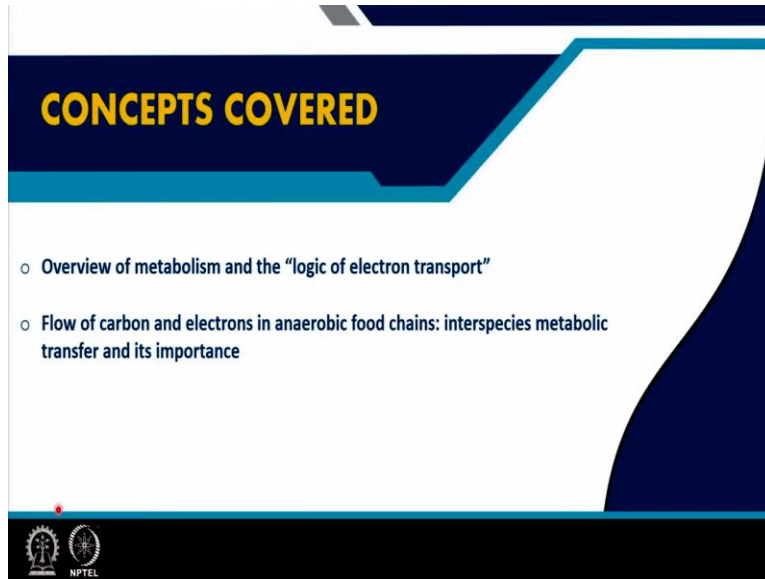


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**Lecture – 24**  
**Physiological Ecology and Resource Exploitation by Microorganisms (contd.,)**

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Welcome to the 24th lecture of this course on Physiological Ecology and Resource Exploitation by Microorganisms will be the major topic of this series of lectures and in this particular lecture we are going to discuss about the overview of metabolism and the logic of electron transport. We are also going to discuss about the flow of carbon and electrons in anaerobic food chains interspecies metabolic or metabolite transfer and its importance.

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Re-cap from the previous session

**Concepts of Resource utilization and Oxidation-Reduction reaction in understanding the overall physiological function**

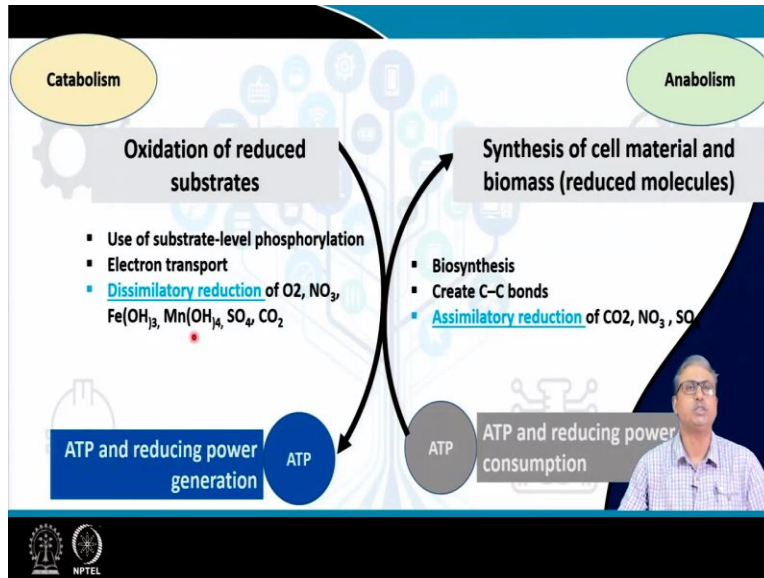
**Catabolism and Anabolism**

The slide features a central graphic of a tree with various icons (gears, a smartphone, a lightbulb, a flask, a microscope, a gear, a leaf, a person, a gear, a leaf, a person) on its branches. To the right, there is a small inset video of a man with glasses speaking. At the bottom left, there are logos for IIT Bombay and NPTEL.

Now, before we proceed into the details of these anaerobic reactions that facilitate metabolism of complex organic materials within different environments by microorganisms a quick recap from the previous session is here. In our previous lectures we have learnt that the learn the concept of resource utilization and oxidation reduction reaction which are found to be very important in understanding the overall physiological function of the organisms present in any environment.

And in terms of environmental biotechnology where we want to exploit these organisms and their different metabolic attributes often utilizing the resources which are available within the environment. So, be it the carbon containing materials or the inorganic resources. So, in this reference the importance of catabolism and anabolism were particularly highlighted.

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So, as we possibly understand that the catabolism is a set of reactions which are highly networked among themselves and that facilitates the oxidation of reduced substrate. So, any kind of reduced substrates present in the environment are subjected to oxidation. And during this oxidation process the molecular structure of the compound is broken down and the electrons and energy are liberated.

Often the carbon molecules which are released during this oxidation process are used to produce the precursor molecules and these precursor molecules are used by the cells for its subsequent biosynthetic reaction. Now, during this course of oxidation of reduced substrates we identify that there is a very important process of substrate level phosphorylation through which ATP molecules are directly generated.

There is also an event of electron transport connected to this catabolic oxidation or catabolic reactions the reducing power like  $NADH + H^+$  or  $FADH_2$  generated during this oxidation process eventually transfer the electrons to the electron carrier complexes present over the membranes and facilitated the electron transport. Through the electron transport process the proton motive force is generated.

And the proton motive force eventually leads to useful function or may be converted to the generation of ATP. One of the very important aspect of this electron transport mediated

catabolism of substances present in any environment is the dissimilatory reduction of different electron acceptors which are available and used by these microorganisms. As we understand that aerobic organisms they utilize oxygen as their terminal electron acceptor and facilitate the operation of the electron transport process and thereby they generate ATP or the protomotive force.

In absence of oxygen, nitrate, iron, manganese, sulfate and several other inorganic compounds or even carbon dioxide can be used as the terminal electron acceptor. We call it terminal electron acceptor because this is the final electron acceptor in the electron accepting or electron transport process which is already discussed in our earlier lectures. Now, the main point of interest over this is that during the catabolic reactions of the catabolism of substances these terminal electron acceptor molecules are reduced.

And they are reduced in a form like nitrate is reduced to ammonia or nitrate is reduced to nitrogen iron is reduced to  $Fe^{2+}$  or Mn is reduced sulphate is reduced to sulphide or carbon dioxide is reduced to methane. Interestingly these reduced products are not assimilated or not utilized by the cells in its own metabolism or growth or maintenance. These reduced products are produced only outside the cell and are of no use.

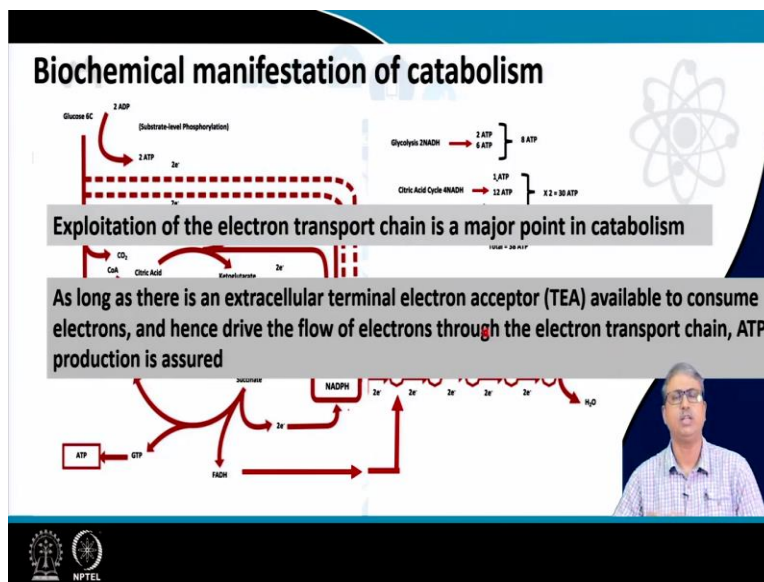
These type of reduction reactions are therefore called as dissimilatory reduction process which are a very characteristic property of the catabolic events or catabolism. Now, on the con in contrast to this catabolism the anabolism reactions facilitate the synthesis of cell materials and biomass that is basically the reduced molecules the macromolecules are produced through this anabolic reactions or anabolism.

Again this is a set of highly networked or interconnected reactions and these reactions are most of the time energy consuming and they consume the energy in the term of ATP and this ATP is the same ATP which is produced during the catabolism reaction. And the ATP along with reducing powers which are produced during catabolism are utilized the precursor molecules which I referred earlier were utilized and eventually the biosynthetic reactions are achieved.

Now, during this biosynthesis reaction C-C bonds carbon-carbon bonds are created these are the synthesis of different molecules macromolecules which eventually produce the cell material or cell biomass. During this anabolism we also find that the reduction of several compounds like nitrate, carbon dioxide, sulfate etcetera happens. Now, this reduction process leads to the reduced material synthesis of the reduced materials like nitrate could be reduced to ammonia.

Now, this ammonia production of the ammonia is different from the production of the ammonia that we found in during catabolism reaction. During this anabolic production of ammonia through reduction of nitrate the produced material like the ammonia for example is produced inside the cell or utilized by the cell for its own function therefore this type of reactions are or the reduction processes are called assimilatory reduction process.

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Now, there is a very interesting network of events as I mentioned of the biochemical reactions and this is referred as biochemical manifestation of catabolism. As we understand that the catabolism of the glucose like molecule facilitates by its oxidation. So, as it gets oxidized the backbone of the glucose the six carbon glucose molecule is oxidized or broken down into two three carbon molecules that is the pyruvic acid.

Now, this pyruvic acid further undergoes the oxidation process to produce this two carbon acetyl coa molecule. And this acetyl coa molecule is subjected to an intense series of reactions through

the biochemical processes what is very important here is that the glucose to pyruvate production facilitates the substrate level phosphorylation that I mentioned earlier and leads to the production of ATP molecule.

Whereas it also produces a number of reducing power that is the electrons are released during the oxidation of glucose to pyruvic acid and these electrons are carried by the NADH or NADPH molecule. So, essentially following this oxidation of the glucose we produce. Now, the microbes they produce ATP molecule through substrate level phosphorylation and a number of reduced electron acceptor or electron carrier that is the nicotinamide added in dinucleotide or nicotinamide adenine dinucleotide phosphates which are in the reduced form.

More number of these forms are produced also during the further metabolism or the degradation of the acetyl coa through the well-known citric acid cycle or TCA cycle. During the TCA cycle also some molecules of ATP or ATP equivalent molecules are generated. Now what is the eventual fate of these reducing powers which are produced. These reducing powers which are produced by the oxidation of the glucose with a series of reactions both including the; the first phase of the glycolysis as well as the TCA cycle.

This NADH H plus or FNAD PH both carrying the electrons that donate the electrons to electron transport chain and a series of electron transfer reactions occur unless and until the electrons they reach to the final or the terminal electron acceptor that is the oxygen eventually water molecule is produced we have already learned that. And during this entire electron transport process proton motive force is generated and that protomotive force leads to the production of ATP through the membrane bound ATPs.

Now, the major point of interest over here is the number of ATP molecules that we generate. Theoretically we can or the cells can generate up to 38 molecules of ATP from the oxidation of one mole of glucose through this aerobic respiration. Now, it is important to understand also that this is subjected to the availability of oxygen because if oxygen is available then only the electron transport chain will be able to operate in such a way.

So, that the; maximum free energy change can be obtained. If oxygen is not available the operation of this electron transport chain will be different as possibly we have already discussed this in our earlier lectures that this will follow the chronology of redox reactions. So, accordingly these reactions will proceed. Now, the exploitation of the electron transport chain is considered to be a major point in catabolism.

How well the cells present in a particular environment are capable of exploiting their electron transport chain. Often with respect to environmental biotechnology processes no matter whether it is bioremediation or carbon sequestration or some other processes petroleum oil recovery or petroleum contamination remediation. In all systems this role of electron transport chain is found to be very, very critical because it is one of the major reactions or set of processes which controls the the catabolism eventually the process of the catabolism.

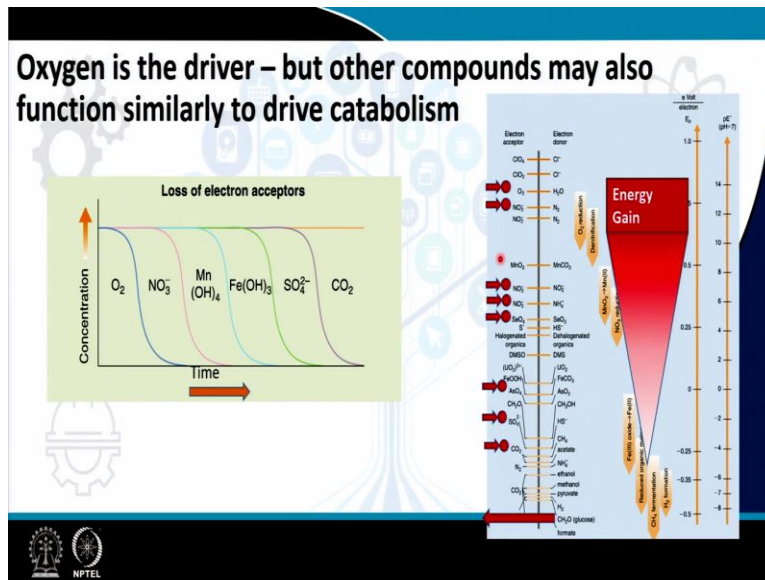
Now, as long as there is an extracellular terminal electron acceptor that is called the TEA for example oxygen that oxygen is going to consume the electrons. So, as long as that is available this process will continue and this will drive the flow of electrons through the electron transport chain and ATP molecules will be produced. So, the assurance that enough; ATP molecules could be produced for the cells to function or cells are benefited.

So, thermodynamically the overall process becomes favorable for the cells and when we assume that the process becomes favorable for the cells the cell will be enjoying that process and possibly that will be for that will be of our benefit as well. But there is a catch, the catch is the electron acceptor must be available. If electron acceptors are not available in the environment then this electron transport system is not going to operate.

So, as long as electron transports molecules or terminal electron acceptor molecules are available the available according to the thermodynamic hierarchy the process will continue and the cells will be able to generate energy again to remind you that all organisms are not capable of deriving energy using different electron acceptors as aerobic organisms can utilize oxygen as terminal electron acceptor nitrate reducing bacteria can use nitrate as their electron acceptor.

Iron reducing bacteria can utilize iron as terminal electron acceptor and sulphate reducing bacteria can utilize sulfate as electron acceptor. There are some examples where a particular bacterial strain or bacterial species can metabolize multiple electron acceptors as well but again as we discussed earlier this is entirely controlled by the genetic mechanism of the cells. The presence of electron acceptors which are going to provide them higher energy will inhibit the expression of the genes which possibly require for utilization of the electron acceptors which will produce lesser amount of energy.

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So, basically this is going to be the in the same format following the chronology of redox reactions. So, basically in aerobic environment as long as oxygen is available in the environment oxygen is the driver, driver of what driver of the catabolic reactions. But other compounds may also function similarly to drive the catabolism and of course not to the similar extent why not to the similar extent because the answer lies here because the energy gain is highly constrained by who is accepting electrons.

If the electrons are accepted by oxygen the gain of energy is maximum but the if the electron is accepted by sulphate or by selenite the gain of energy is nominal or minimal or reduced compared to that of oxygen. So, eventually this process that the oxygen is the driver but in absence of oxygen other compounds which are capable of accepting electrons under the prevailing condition because we need to follow the scale of PE as well that who can take up



electrons under which environment will high with highly constrained by the redox condition of the environment as well.

So, we have already seen through our different laboratory experiments or other studies which are field based studies or field based demonstration that this process follows a chronology of events like the oxygen is the preferred electron acceptor followed by nitrate manganese iron sulphate carbon dioxide etcetera and obviously it is not only that the electron is being taken up by these reactions by this electron acceptors and they are driving the catabolism.

They are driving the catabolism but the essentially the energy output from those catabolic reactions will be less. For example if we have some hydrocarbon residues and we expect that the hydrocarbon residues are going to be catabolized and the cells are going to have energy produced under the environment if oxygen is available the amount of energy produced would be highest and and also the alkene or the hydrocarbon degradation will also be highest.

But if sulphate or carbon dioxide is the electron acceptor obviously the catabolism will proceed but the **the** degradation of the alkane molecule or the hydrocarbon molecule will be less because the the microbial processes are gaining less amount of energy. So, thermodynamically they are less efficient compared to the aerobic metabolism.

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In general, microorganisms carrying out catabolism use fine-tuned enzymatic pathways:

- to oxidize reduced substrates (inorganic compounds for chemolithotrophs; organic compounds for chemoorganotrophs)
- to deliver electrons to the respiratory chains of electron transport systems

Reduced inorganic substance → Oxidation → Reduced organic substance

Glycolysis & TCA cycle by aerobes produce higher ATP

In absence of  $O_2$  anaerobic respiration or fermentative pathways operate through different genes, other TEAs are used

Free energy gain under such conditions follow the thermodynamic hierarchy

$NAD^+ / FAD$ ,  $H_2O$ ,  $O_2$ ,  $H^+$ ,  $H^+$

Inner membrane, Outer membrane

The diagram illustrates a cell membrane with an inner and outer membrane. It shows the flow of electrons from reduced inorganic and organic substances through oxidation to various electron carriers like  $NAD^+ / FAD$ ,  $H_2O$ , and  $O_2$ . Protons ( $H^+$ ) are also shown moving across the membranes. A small inset video of a speaker is visible in the bottom right corner of the slide.

Now in general microorganisms carrying out; catabolism use very fine tuned enzymatic pathways. So, these enzymatic pathways are pretty well understood and to oxidize the reduced substrates which are available may be the inorganic compounds for chemolithotropic mode of nutrition or organic compounds for chemo organotrophic metabolisms. So, one of the very purposes of these fine tuned enzymatic pathways which are involved in the catabolism of different reduced compound is to oxidize the reduced substrates number one.

So, one end the oxidation of the reduced compounds whether it is organic compound or inorganic compound they are going to be oxidized. So, that is one major function of the oxidation process the second is to deliver the electrons to the respiratory chains of electron transport system. So, one part of these entire catabolic reactions as I mentioned fine tuned in order to achieve the most efficient oxidation of the substrates both the organic substance as well as the inorganic substances.

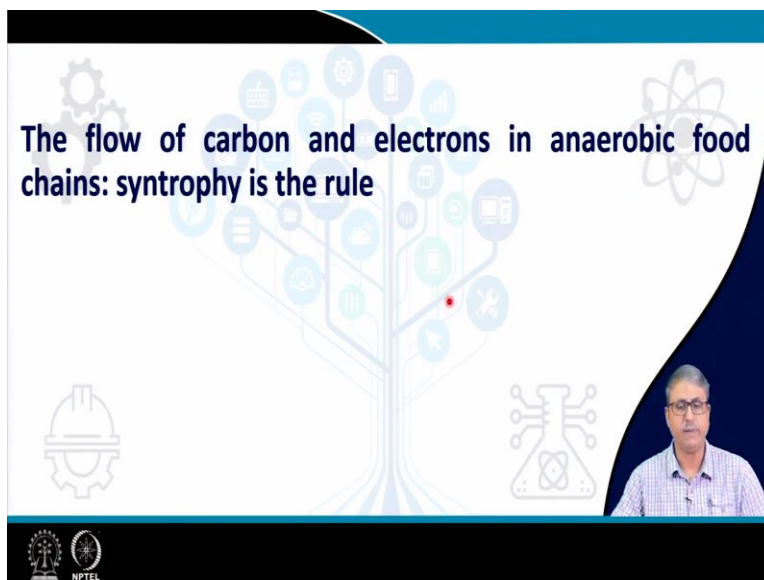
Eventually the electrons which are released from this oxidation process are going to be transferred to the electron transport chain. So, another very important enzymatic pathway is the transfer of the electrons to the terminal electron acceptor through the electron transport chain. Now, the glycolysis and TCA cycles by the aerobic bacteria produce the higher number of ATPs because the the free energy change is maximum from the glucose to the oxygen the free energy change is maximum.

So, the highest number of ATP could be produced but in absence of oxygen the anaerobic respiration or fermentation pathways operate through different genes other TES are used. So, a number of separate pathways are operating. So, these pathways are basically operated encoded by different genes and these genes as I mentioned earlier also these genes are tightly regulated. So, that as soon as oxygen is depleted perhaps then only the genes involved in denitrification or genes involved in iron reduction or sulphate reduction will be expressed.

And they will allow the utilization of sulfate and other electron acceptor as per the redox condition of the environment and eventually that will allow free energy gain. Now, the free energy gain under such conditions because such conditions means in when the oxygen is not

there we follow the thermodynamic hierarchy that we have we have seen just in the previous slide.

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The slide features a central graphic of a tree where the trunk and branches are composed of various icons representing different scientific and technological fields, such as gears, a lightbulb, a computer monitor, a microscope, and a chemical flask. The text "The flow of carbon and electrons in anaerobic food chains: syntrophy is the rule" is prominently displayed in the upper left quadrant of the slide. In the bottom right corner, there is a small inset video of a man with glasses speaking. The slide also includes logos for NPTEL and other institutions at the bottom left.

Now, the next very important point and possibly the last point of this series of lecture is the flow of carbon and electrons in anaerobic food chains that is the syntrophy is the rule. So, we will discuss this keeping in mind that most of the environment may not be aerobic. So, oxygen may not be available. So, when oxygen is not there the anaerobic reactions are going to occur and why we are saying that it is anaerobic food chain.

Because any kind of environment where oxygen is not there we will see that compared to the aerobic mechanism it is more complex and its facilitated by participation of a number of organisms. So, interconnected metabolic reactions are there and that leads to a chain of events that is why it is called anaerobic food chain.

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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	$\frac{1}{4}\text{O}_2(\text{g}) + \text{H}^+ + \text{e} = \frac{1}{2}\text{H}_2\text{O}$	+13.75	$\text{CH}_2\text{O} + \text{O}_2 \rightarrow \text{CO}_2 + \text{H}_2\text{O}$	-125
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	$\frac{1}{2}\text{MnO}_2(\text{s}) + \frac{1}{2}\text{HCO}_3^- + \frac{3}{2}\text{H}^+ + \text{e} = \frac{1}{2}\text{MnCO}_3(\text{s}) + \text{H}_2\text{O}$	+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

Now we have already discussed about the eight common processes that are recognized in the carbon rich habitat. So, any environment will have the aerobic respiration followed by denitrification manganese reduction iron reduction sulphur reduction and finally acetogenesis and methanogenesis. Now, the glycolysis and the TCA cycle the cytic acid cycle and the electron transport chain using oxygen as terminal electron acceptor allow the aerobic microorganism to exploit a prevalent resource in the biosphere or in the any kind of part thereof any kind of environment where oxygen will be there.


The glycolysis TCA cycle etcetera and electron transport chain will occur using oxygen as terminal electron acceptor. So, we will see that the aerobic respiration will follow and a large number of largest and the highest free energy change is there. So, the maximum amount of energy will be produced. So, any kind of contaminated site for example a landfill site where we can see that if oxygen is available the substances present like different kind of organic and inorganic materials will be utilized as a carbon energy source and the cell will generate ATP and that ATP will be utilized for its growth and other metabolism.

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


➤ Glycolysis, the citric acid cycle, and the electron transport chain allow aerobic microorganisms to exploit a prevalent resource in the biosphere: glucose


Aerobic respiration	$\frac{1}{4}\text{O}_2 (\text{g}) + \text{H}^+ + \text{e} = \frac{1}{2}\text{H}_2\text{O}$	+13.75	$\text{CH}_2\text{O} + \text{O}_2 \rightarrow \text{CO}_2 + \text{H}_2\text{O}$	-125
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Many modes of catabolism carried out by several model aerobic microorganisms within diverse environments have been reported: e.g, EMP-, PP-, ED-, Wood-Ljungdahl pathways

ATP → Cell metabolism & growth





Now, many mode of catabolic events are identified many pure culture bacteria and single organisms are identified who are capable of performing the degradation or utilization or the catabolic reactions of the catabolism of the substance completely. It is taking starting from the utilization of glucose or similar organic compound to complex organic compound even the hydrocarbons and different other compounds as well.

So, it is identified the participation of many pathway like Emdent maher partners pathway, pentose phosphate pathway, EDy pathway, Wood-Ljungdahl pathway all the different pathways are operating and many a single bacterial involvement in such processes aerobic processes are formed. Now, compared to aerobic process where single bacterial species or single microbial species could be involved in catabolic utilization of the reduced substrate and making these making the substrate decomposed oxidized and the cell growth to occur. Anaerobic mode of catabolism is more complex and involves multiple groups of microorganism.

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## Compared to aerobic process, anaerobic mode of catabolism is more complex and involves multiple groups of microorganisms

Process	(PE° = log K)	Heterotrophic reactions	ΔG° (kJ/eq.)
Iron reduction	$\text{FeOOH(s)} + \text{HCO}_3^- + 2\text{H}^+ + \text{e}^- = \text{FeCO}_3(\text{s}) + 2\text{H}_2\text{O}$	-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	$\frac{1}{8}\text{SO}_4^{2-} + \frac{9}{8}\text{H}^+ + \text{e}^- = \frac{1}{8}\text{H}_2\text{S(g)} + \frac{1}{2}\text{H}_2\text{O}$	-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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So, anaerobic metabolism of reduced substrates cannot be catalyzed by pure culture of single microorganism. So, this is very important and it is very, very contrasting point with respect to the aerobic degradation many compounds are degraded or converted or utilized by microorganisms single handedly. So, one single bacterial taxa or single bacterial species or single bacteria can like pseudomonas strains barcode area strains then there are many rotococcus strains there are many bacterial strains who can actually metabolize the substrate completely and in presence of oxygen.

But during the absence of oxygen the same bacteria will be performing differently you will found that or will find that the anaerobic metabolism is not possible complete anaerobic metabolism may be part of that is possible. But complete metabolic anaerobic metabolism is not possible with the pure culture of the organism or if it is not cultured if it is uncultured organism even then with the involvement of a single microorganism.

So, what is the fact regarding the anaerobic metabolism of different reduced substrates they are in that case where anaerobic metabolism or reduced substrates are going on there are participation of a number of bacterial species or archaea along with them. So, these anaerobic metabolism of reduced substrates are only carried out by cooperating population. So, what is cooperating populations cooperating populations are populations which are cooperating with each other.

So, they are co-operating one another of physiologically distinctive microorganisms. So, there are two terms one is the cooperating populations of physiologically distinctive physiologically distinctive means you can categorize them very distinctly with their physiological attributes. So, they are different group of organisms with having different physiological and metabolic properties and taxonomically also they are different.

These 1, 2, 3, 4, 5 group of organisms will cooperate they will join their hands; hands in the sense join their catabolic machinery together to degrade the compound or achieve the anaerobic metabolism of the reduced substance.

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**Syntrophy: a situation in which two different organisms cooperate to degrade a substance that neither can degrade alone**

Syntrophic associations of microorganisms are often critical for organic carbon-driven iron reduction, manganese reduction, sulfate reduction, acetogenesis, and methanogenesis

Most syntrophic reactions are secondary fermentations in which organisms ferment the fermentation products of other anaerobes

The slide features a background with faint icons of a gear, a tree, and a flask. A small video inset in the bottom right corner shows a man speaking. The NPTEL logo is visible in the bottom left corner.

That joining hands together by different populations is called syntrophy. Syntrophy is a situation in which two different organisms or even more than that they cooperate to degrade a substance that neither can degrade alone. So, any single bacteria cannot degrade the completely the particular compound but when they are joined with other members other taxa with physiological attributes different physiological attributes the degradation is achieved and the complete degradation is achieved.

So, syntrophic associations of microorganisms are critical for organic carbon driven iron reduction manganese reduction sulphate reduction acidogenesis and methanogenesis. And in large number

of environmental settings or almost in all environmental settings we find that during the anaerobic process it is the syntrophic association which basically drives the metabolism of the substances. Now most syntropic reactions are secondary fermentation.

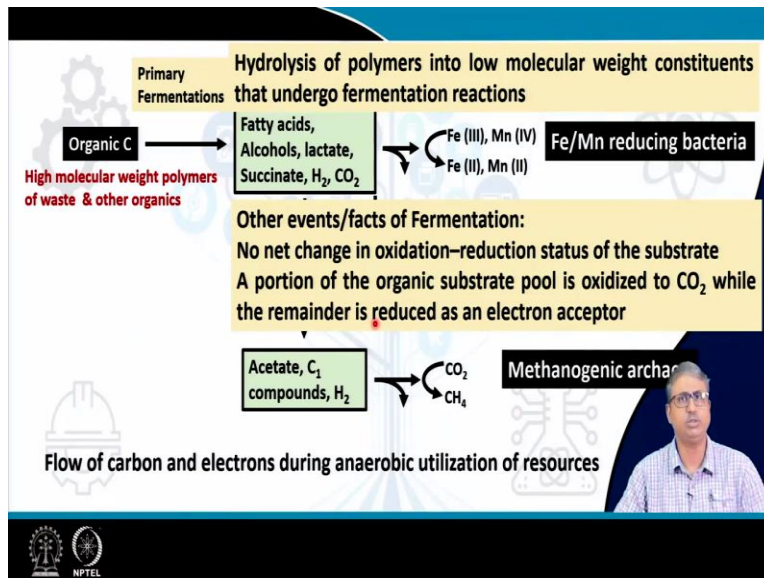
Now, here another important aspect. So, first we understood that during anaerobic metabolism participation of more than one organisms are involved. Now, we are seeing another terminology which is called secondary fermentation what is secondary fermentation. Secondary fermentation is a fermentation reaction which is basically operating utilizing the products of the primary fermentation.

Then what is primary fermentation primary fermentation is the fermentation reactions that we already know for example the glucose is for metabolism to pyruvate and pyruvate is fermented to ethanol for example or to acetate. Now, what do I do with the acetate as can that be fermented again like ethanol can we ferment ethanol again yes there are microorganisms who can ferment ethanol. So, first you ferment ethanol from pyruvic acid. So, pyruvic acid to ethanol fermentation is the primary fermentation and ethanol to acid acetate and hydrogen could be the secondary fermentation.

So, we will discuss this in some detail very soon. So, most syntropic reactions are secondary fermentations in which organisms ferment the fermentation product of other anaerobic bacteria.

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For example if you look at this picture. So, we have the organic carbon which are reduced polymeric material often in natural environment they are more complex than we perceive like they may not be simple glucose or simple phenol like compounds they are more complex. So, primary fermentation allows the oxidation or the catabolism of these two fatty acid alcohol lactate succinate etcetera.

And this eventually is utilized by the secondary fermenters as their substrate. So, secondary fermenters they cannot use organic carbon directly they rely on these compounds which are produced by the primary fermenters and they metabolize these compounds and then produce further products which are again utilized by other organisms. So, it is an interesting set of reactions that we observe.

Now, it is important to understand that the products of this primary fermentation is the substrate for the secondary fermentation this is option one option means the products of the primary fermentation can be utilized by the secondary fermenting organisms who can metabolize the fatty acid alcohol lactate etcetera. Else there could be also anaerobic bacteria like as we have learned earlier iron or manganese reducing bacteria.

Under anaerobic condition these bacteria often they utilize the products of the primary fermenters they generally do not utilize the original reduced substrate directly. So, again it is an

evidence of the coupled process. So, primary fermentation will oxidize the organic carbon complex organic carbon to relatively smaller molecules or easily metabolizable substance like the fatty acid alcohol etcetera.

Now, these are going to be again the substrate for the iron or manganese reducing bacteria or even the sulphate reducing bacteria. So unless the primary fermenters they ferment the organic carbon which are available to these compounds the sulfate and iron manganese reducing bacteria will not be able to act on them. And when they use these substances they use the iron manganese or sulfate as their terminal electron acceptor.

Now, the organic carbon that we see in a natural environment or in a waste water or a waste sludge or refinery waste or different kind of petroleum oil spill and other environments are often high molecular weight polymers which are naturally abundant or they are present there as a part of the contaminants. Now, the fermentation reaction basically allows the hydrolysis of this polymer.

So, one function of the primary fermenters or the bacteria who are involved in primary fermentation process includes the hydrolysis of this polymeric compound into low molecular weight constituents and these low molecular weight constituents then undergo the fermentation reaction. So, apart from the fermentation reaction like we; study the reaction that glucose to pyruvic acid and pyruvic acid to ethanol or lactic acid but the reaction is not.

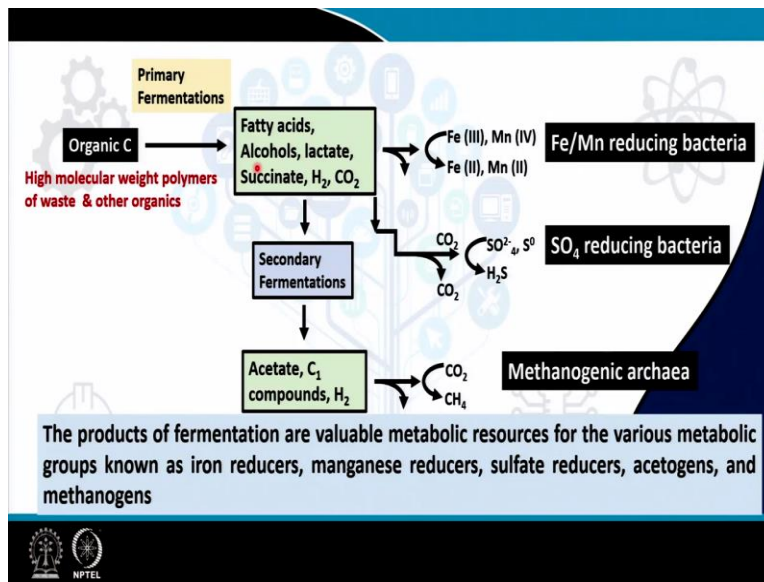
So, so simple where is glucose? Glucose must be produced from the hydrolysis of the polymers which are present there. So, in the natural environment many a times readily available glucose molecules are not there. So, it is the function of the primary fermenters or primary fermentation process which will produce or the hydrolyzed polymers and produce the low molecular weight constituents.

These low molecular constituents will undergo the fermentation reaction and will produce the fatty acid, alcohol lactic, acid succinic acid, hydrogen, carbon dioxide and many other things. Now, some other events of importance in fermentation are that there is no net change in

oxidation reduction status of the substrate. Because part of the organic substance which is used as the substrate molecule is oxidized to carbon dioxide while a remainder is reduced as an electron acceptor.

For example if you see glucose, glucose is oxy broken down to pyruvic acid. So, 6 molecule 6 carbon containing glucose is broken down into 2 2 3 carbon molecule by 2 pyruvic acid molecules. Now, pyruvic acid molecule is again used as an electron acceptor when it is reduced to lactic acid or ethanol. So, that itself is a kind of an electron acceptor is an endogenous electron acceptor. So, that is why we called that a portion of the organic substrate is oxidized to CO<sub>2</sub> while the remainder is reduced an electron acceptor because part of the pyruvic acid can enter into the different kind of oxidative reaction.

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Now, the products of the fermentation, so, we have the product of fermentation including a bunch of compounds low molecular weight often readily metabolizable substrates. These are produced by the primary fermentation. Now, these products of the fermentation are valuable metabolic resources for the various other metabolic groups of bacteria who are the iron reducer sulphate reducer and even the acidogenic and methanogenic bacteria or archaea respectively.

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> In syntrophic associations, an anaerobic food web is established in which metabolic byproducts of one group of microorganisms are essential substrates for another.

> The flow of carbon, electrons and energy occurs among cooperating populations of anaerobic microorganisms.

The diagram illustrates the flow of organic carbon through an anaerobic food web. It starts with 'Organic C' entering 'Primary Fermentations', represented by a cluster of colorful microbes. An arrow points to 'Secondary Fermentations', shown as a smaller cluster of microbes. From the secondary fermentations, three arrows point to different groups of organisms: 'Fe/Mn reducing bacteria' (represented by a cluster of black microbes), 'SO<sub>4</sub> reducing bacteria' (represented by a cluster of orange microbes), and 'Methanogenic archaea' (represented by a cluster of blue microbes). The diagram is set against a background of a gear and a lightbulb, symbolizing industrial and biological processes. A small inset video of a speaker is visible in the bottom right corner of the slide.

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Now in syntrophic association an anaerobic food wave is established because we saw the primary fermentation secondary fermentation at least do two steps or two groups of organisms are there and they are relevant mechanism of working with the carbon molecules are there. As well as the iron reducer, sulphate reducer, manganese reducer and methanogenic archaea are also there because they are going to utilize the products and drive the reaction towards a kind of a favorable condition.

So, an anaerobic food wave or anaerobic network is established in which metabolic byproducts of one group of microorganisms are essential substrate for the other. The flow of carbon electrons and energy occurs among cooperating populations of the anaerobic organisms. Now, this picture explains the same fact but in a more illustrative way that the organic carbon which are there are utilized by the primary fermenters producing a large number of metabolites including the fatty acid, lactic acid, succinic acid, hydrogen etc.

These are then utilized by specific group of microorganisms like the iron manganese reducing bacteria they will rely only on the secondary fermentative products they will not or the primary fermentative products they will not be able to use the organic carbon directly sulphate reducing bacteria or the the methanogenic archaea.

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**Interspecies H<sub>2</sub> transfer during syntrophy**


Hydrogen (H<sub>2</sub>) plays a critical role in interacting populations of syntrophy because it is an important electron donor used by most of these groups

The heart of syntrophic reactions is interspecies H<sub>2</sub> transfer:

H<sub>2</sub> production by one partner, the syntroph, linked to H<sub>2</sub> consumption by the other.

The H<sub>2</sub> consumer can be any one of a number of physiologically distinct organisms: denitrifying bacteria, fer-ric iron-reducing bacteria, sulfate-reducing bacteria, acetogens, or methanogens





Now, with this respect there is a very interesting or important event which is called interspecies hydrogen transfer. This interspecies hydrogen transfer is an example of the metabolic linkage between the cooperating groups which are present in the anaerobic environment. Now, one of the metabolites of these syntrophic reactions is hydrogen. Now, hydrogen plays a critical role in interacting populations of syntrophic because it is an important electron donor used by most of this group.

So, hydrogen is produced one in on the one hand through the primary fermentation reaction or maybe by the secondary fermentation reaction and then hydrogen could be utilized as a readily metabolizable electron donor for a large number of groups including the nitrate reducers, the sulphate reducers, the iron manganese reducers or even the the methanogens or the archaea. Now, the heart of this entropic reaction is basically considered to be the interspecies hydrogen transfer.

Now, hydrogen production by one partner this interop linked to hydrogen consumption by other. So, primarily it could be the primary fermenter who is producing hydrogen or it could be the secondary fermenter who is producing hydrogen and then the methanogen or a nitrate reducer who is utilizing the hydrogen. The hydrogen consumer can be one of the number of different physiologically distinct organisms as I mentioned earlier.

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**Ethanol fermentation to acetate plus H<sub>2</sub> by the syntroph *Pelotomaculum* coupled to the production of methane**

**Ethanol fermentation:**

$$2 \text{CH}_3\text{CH}_2\text{OH} + 2 \text{H}_2\text{O} \rightarrow 4 \text{H}_2 + 2 \text{CH}_3\text{COO}^- + 2 \text{H}^+$$

$\Delta G^{\circ} = +19.4 \text{ kJ/reaction}$

**Unfavorable reaction  
Cant be carried out by the fermenting bacterium alone**

**H<sub>2</sub> produced by *Pelotomaculum* can be used as an electron donor by a methanogen to produce methane, an exergonic reaction.**

**Methanogenesis:**

$$4 \text{H}_2 + \text{CO}_2 \rightarrow \text{CH}_4 + 2 \text{H}_2\text{O}$$

$\Delta G^{\circ} = -130.7 \text{ kJ/reaction}$

For examples if we take a take this example where a syntrophic bacteria *pelotomaculum* is coupling the hydrogen production or the oxidation of the alcohol or the ethanol to the formation of the methane. Now, if we see this particular reaction the ethanol fermentation is normally done by this particular bacterium *pelotomaculum*. Now, the overall reaction if we look carefully is not favorable because the free stand free energy change for this  $\Delta G$  zero prime is positive.

And since it is positive it is basically an unfavourable reaction and cannot be carried out by the fermenting bacterium alone. So, in an in contrast to my earlier discussion we had or I had that the aerobic processes are more often carried out by single bacteria or the pure culture bacteria or single organism. I mentioned that anaerobic metabolism is is not possible to carried out by one species one member why?

Here is the example because the oxidation of one part of the reaction is actually unfavourable. So, it cannot be carried out by the fermenting bacterium alone unless and until we make this  $\Delta G$  negative. Now, consider this reaction more critically here is a product which is called hydrogen. Now, this hydrogen which is produced by this *pelodomeculum* can be used as an electron donor by a methane methanogen methane producing to produce methane which is an exargonic reaction exergonic reaction meaning the  $\Delta G$  of the reaction will be negative.

Now, if you look at this reaction this methanogenesis by different hydrogen utilizing

hydrogenotrophic archaea this is the reaction that the hydrogen reacts with the carbon dioxide to produce methane and the  $\Delta G$  of this reaction the free energy change of this reaction is minus 130.7 kilojoule per reaction.

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**Coupled reaction**

**Favorable reaction**  
Can be carried out by the combined participation of more than one member

**Coupled reaction:**  

$$2 \text{CH}_3\text{CH}_2\text{OH} + \text{CO}_2 \rightarrow \text{CH}_4 + 2 \text{CH}_3\text{COO}^- + 2 \text{H}^+$$

$$\Delta G^\circ = -111.3 \text{ kJ/reaction}$$

(a) Reactions

**Ethanol fermenter**      **Methanogen**

2 Ethanol → 2 Acetate

Interspecies hydrogen transfer:  $4 \text{H}_2$

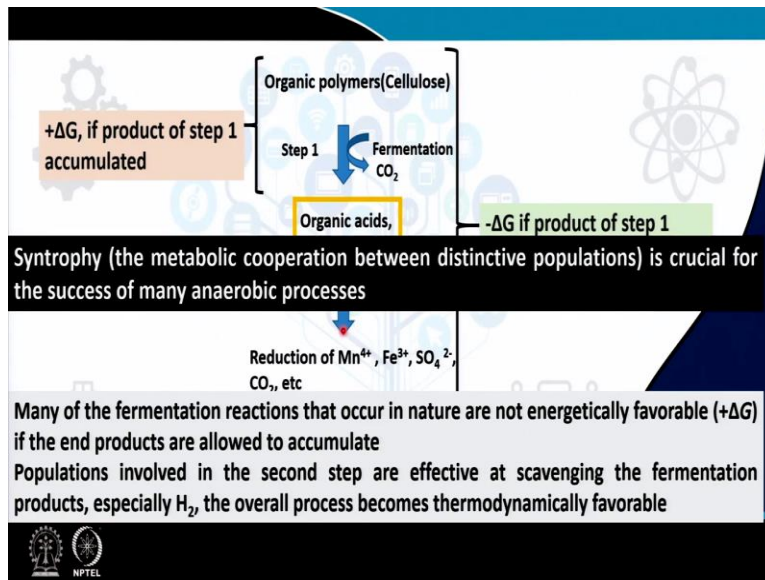
$\text{CO}_2 \rightarrow \text{CH}_4$

(b) Syntrophic transfer of  $\text{H}_2$

So, overall if we consider the coupling of these two processes. So, *Pelotoba macula* along with the methanogenic archaea if they are present together they will be able to successfully allow or facilitate oxidation of alcohol to the production of acetate and the methane both. So, if we want to plan a methane production from the alcohol or production of acetate from the alcohol this is going to be the mechanism or this type of syntrophic association where interspecies hydrogen transfer mainly in this case hydrogen transfer is going to play a role.

So, if we look at this reaction we will find that overall reaction becomes highly favourable and it can be carried out by the combined participation of more than one member that is the peloton meculum and the methanogenic archaea both took part in this reaction and facilitate the fermentation of ethanol to acetate and also the methanogenic production of the methane through the interspecies hydrogen transfer.

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Now, here the one of the major lesson or important lesson is that the delta G or the free energy change of a product of particular step can be positive particularly if the product accumulates. Like if the products of the first step like hydrogen in this case or the sometimes the organic acids if they keep accumulating it becomes the delta G of the reaction becomes positive. But if there is a second step in this case in the previous example it was the methanogenic archaea who helped the first step bacteria like the pellet maculum to make the overall reaction del G negative.

So, if we have a second step the second step can be catalyzed or the achieved by participation of a diverse type of organism may not be only archaea but also bacteria like manganese or iron or sulphate reducing bacteria as well. And then the delta G of the overall reaction becomes negative now many of the fermentation reactions that occur in nature are not energetically favorable actually. So, if the end products are allowed to accumulate.

So, the syntrophic mechanism of syntrophy has evolved to manage this accumulation of the products some accumulation of the products of the first set of reaction are taken into consideration and those products are utilized by a second set of reaction in this entropy. So, essentially the accumulation of products is handled very very very categorically in order to reduce the accumulation the products are immediately used up. So, the reaction flows continue.

So, populations involved in the second step are effective at scavenging the fermentation products



especially hydrogen and overall process becomes thermodynamically highly favourable. So, overall the syntrophy metabolic cooperation between distinctive populations is crucial for the success of many anaerobic processes.

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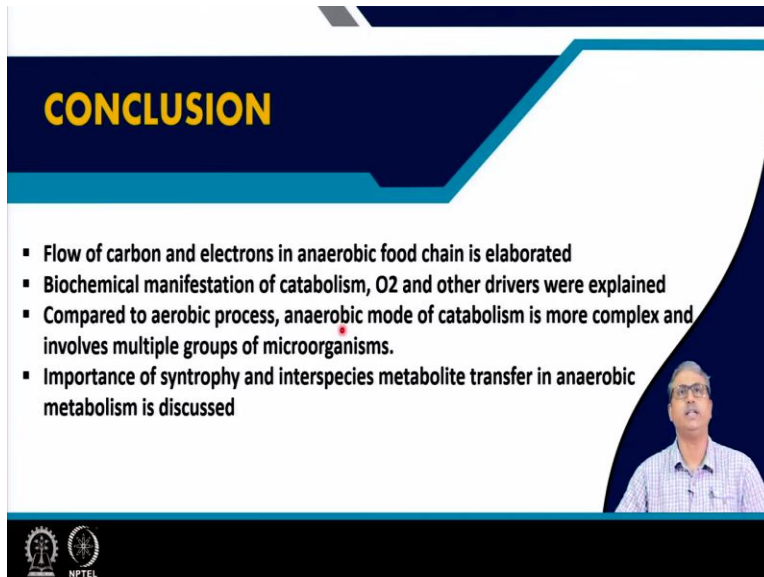


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- Brock biology of Microorganisms by Madigan, et al 12<sup>th</sup> Edition, Pearson, 2009

The slide features a dark blue header with the word 'REFERENCES' in yellow. Below the header is a white area containing a list of two references. A small video inset of a man in a blue shirt is visible in the bottom right corner. At the bottom left, there are logos for IIT Bombay and NPTEL.

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**CONCLUSION**

- Flow of carbon and electrons in anaerobic food chain is elaborated
- Biochemical manifestation of catabolism, O<sub>2</sub> and other drivers were explained
- Compared to aerobic process, anaerobic mode of catabolism is more complex and involves multiple groups of microorganisms.
- Importance of syntrophy and interspecies metabolite transfer in anaerobic metabolism is discussed

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For this lecture these following references are going to be useful. And in conclusion the flow of carbon and electrons in anaerobic food chain is elaborated biochemical manifestation of catabolism oxygen and other drivers were explained. Compared to aerobic process anaerobic mode of catabolism is more complex. And involves multiple groups of microorganisms and finally the importance of syntrophy and interspecies metabolite transfer in anaerobic

metabolism is discussed, thank you.