**Environmental Chemistry and Microbiology**
**Dr. Anjali Pal**
**Dr. Sudha Goel**
**Department of Civil Engineering**
**Indian Institute of Technology - Kharagpur**

**Module - 12**
**Lecture - 59**
**Metabolic Diversity - IV**

Welcome everyone to the last and final module. This is module 12 and we will cover the remaining part of Metabolic Diversity. So, this is part 4 of Metabolic Diversity, lecture 59 of module 12.

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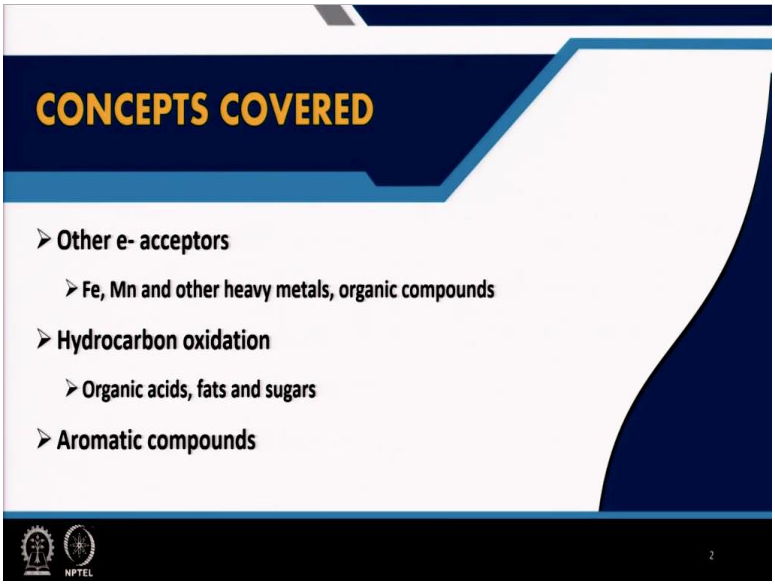


So, in this lecture, we are going to look at some other alternative electron acceptors. We will take a look at iron, manganese, several other heavy metals, organic compounds; all of these can serve as electron acceptors; and we will look at the bacteria that are capable of utilising them. In terms of hydrocarbon oxidation, we have different metabolic pathways for organic acids, for fats and sugars; so, we will take a look at that as well. And finally, we will end this particular lecture with aromatic compounds, how are they biodegraded under aerobic as well as anaerobic conditions.

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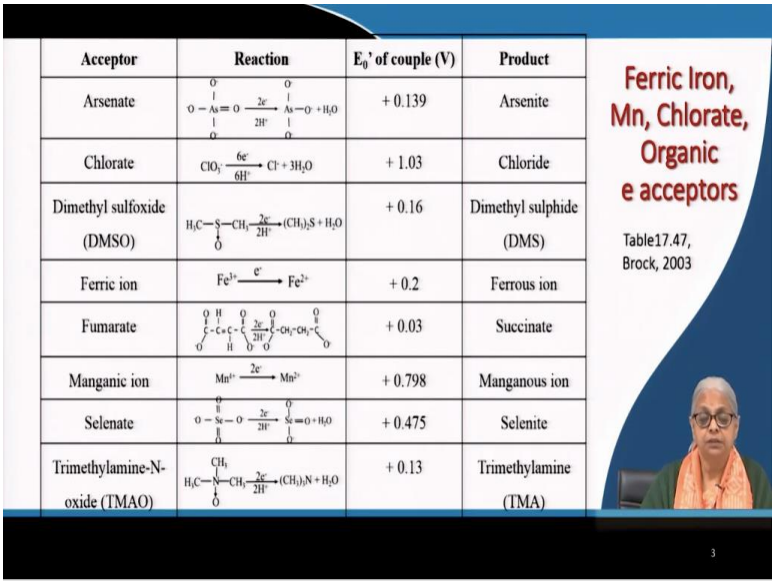


So, here we have a table which shows several other electron acceptors. If you remember back from the 3 biochemical pathways, we have either fermentation or respiration and within respiration, we have aerobic respiration which means the terminal electron acceptor is oxygen. And we have anaerobic respiration which means that the terminal electron acceptor is any other compound other than oxygen.

So, here we have chlorate. Chlorate is formed when chlorine gas or hypochlorite ion are added to water. Then you get chlorate. It can serve as an electron acceptor. You may have manganese in the environment. If it is present as $Mn^{4+}$, which it often is, it can be reduced to $Mn^{2+}$. Then you have selenate. It can be reduced and it can serve as an electron acceptor. Ferric iron, $Fe^{3+}$ can be reduced to $Fe^{2+}$, which happens under anaerobic conditions.

Dimethyl sulphoxide, DMSO can be converted to dimethyl sulphide. And arsenate can be converted to arsenite. TMAO, trimethylamine N-oxide can be converted to trimethylamine. Fumarate can be converted to succinate.

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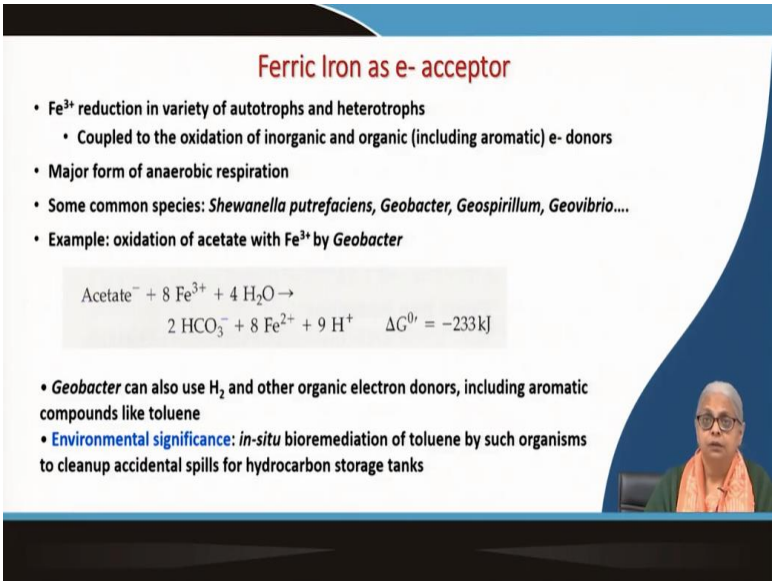


Let us take a look at some specific reactions and the bacteria that are involved in these reactions. So, we have ferric iron as the electron acceptor in this case. Ferric iron can be reduced by a large number of autotrophic as well as heterotrophic bacteria. It can be coupled to the oxidation of inorganic as well as organic compounds. Even aromatic compounds can serve as electron donors. It is one of the major forms of anaerobic respiration.

Some of the common species are *Shewanella putrefaciens, Geobacter, Geospirillum, Geovibrio*. And this is one example of the oxidation of acetate in combination with the reduction of ferric iron by *Geobacter*.

So, $CH_3COO^- + Fe^{3+} + H_2O$; Acetate ($CH_3COO^-$) will be converted to bicarbonate ($HCO_3{}^-$), and ferric iron ($Fe^{3+}$) will be converted to ferrous iron ($Fe^{2+}$). This is an energy-yielding reaction. The ΔG value is fairly high, which means the cell yield is also likely to be fairly high. *Geobacter* can also use hydrogen and other organic electron donors including aromatic compounds like toluene. Toluene, benzene, toluene, xylene, these are very common petroleum-based compounds that are found around petrol pumps and other petroleum-utilising areas where the tanks may have leaks. What is the environmental significance of this?

So, *in situ* bioremediation of toluene is possible by these organisms to clean up accidental spills. So, let us say you have oil refineries, you have petrol pumps, you have several other areas where petroleum is stored in tanks. If these tanks start leaking, either because of an accident or just slow corrosion and so on, that can be remediated by using these particular organisms.

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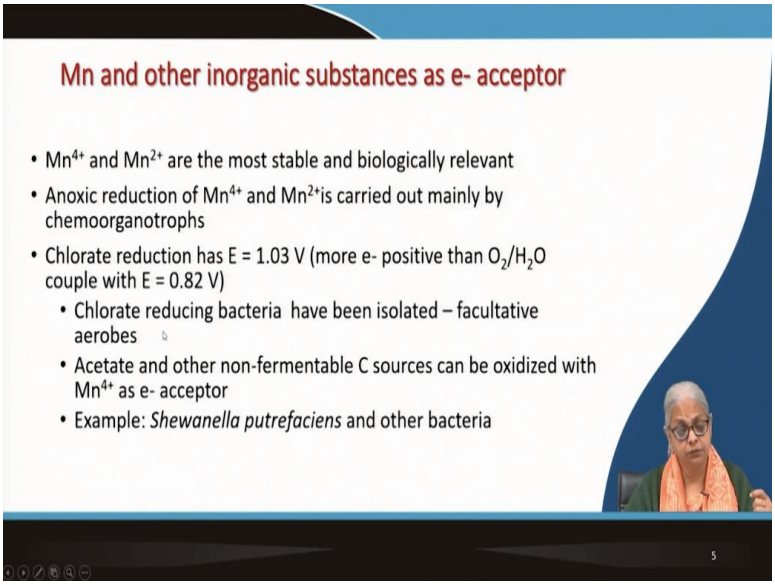


We have manganese and other inorganic substances as electron acceptors. So, you have $Mn^{4+}$ and $Mn^{2+}$. These are stable and biologically relevant nutrients. The anoxic reduction of $Mn^{4+}$ is possible; $Mn^{4+}$ and $Mn^{2+}$is carried out by chemoorganotrophs.

So, chlorate is another example; I have already shown you that. So, it is a far superior electron acceptor (than oxygen). Chlorate reducing bacteria have been isolated. They are facultative aerobes. There may be an issue about toxicity, which I am not sure about. Acetate and other non-fermentable carbon sources can be oxidised with $Mn^{4+}$ as the electron acceptor. So, these are examples of bacteria that are capable of mediating both these reactions with manganese as well as chlorate. So, *Shewanella putrefaciens* and other bacteria can mediate these reactions.

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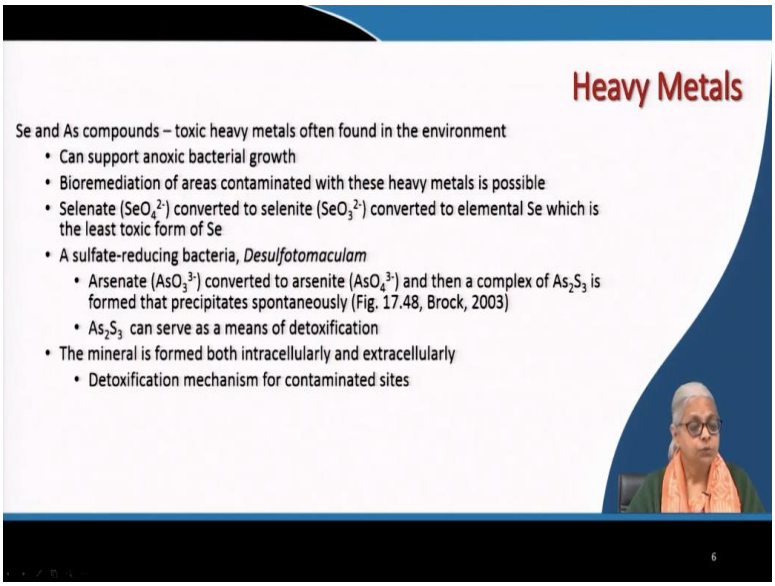


Let us take a look at heavy metals like selenium and arsenic. Arsenic is considered to be a toxic heavy metal. Some people think that selenium is also a toxic heavy metal, but there is some

evidence in the literature that it may be a trace nutrient as well. So, regardless of whether it is completely toxic or toxic only at certain concentrations, let us just take a look at these two common toxic heavy metals that are found in the environment.

Both of them are capable of supporting anoxic bacterial growth. Now, if they are capable of supporting anoxic bacterial growth, that means, if you have contamination of water, soil with these elements, then bioremediation is a possibility. So, if you have selenate, it can be converted to selenite or to elemental selenium which is the least toxic form of selenium.

And you also have a sulphate reducing bacteria *Desulfotomaculum*. So, arsenate can be converted to arsenite, and then a complex of $As_2S_3$ and this $As_2S_3$ precipitates spontaneously. So, this is what we have; arsenic trisulphide which is the reduction of arsenate by a sulphate reducing bacterium. So, you can see, this is the culture after it has been incubated and inoculated with this particular bacterium, *Desulfotomaculum* and the yellow materials are precipitates of $As_2S_3$, arsenic trisulphide.

The process is called biomineralisation or biotransformation. We do not use the word biodegradation for the transformation of metals from one oxidation state to another. Please refer to figure 17.48 in Brock's Biology of Microorganisms, tenth edition. So, coming back to this conversion of arsenate ($AsO_3{}^{3-}$) to arsenite ($AsO_4{}^{3-}$), $As_2S_3$ can be used as a method of detoxification. The mineral can be formed both intracellularly as well as extracellularly. Detoxification is possible for contaminated sites including water as well as soil.

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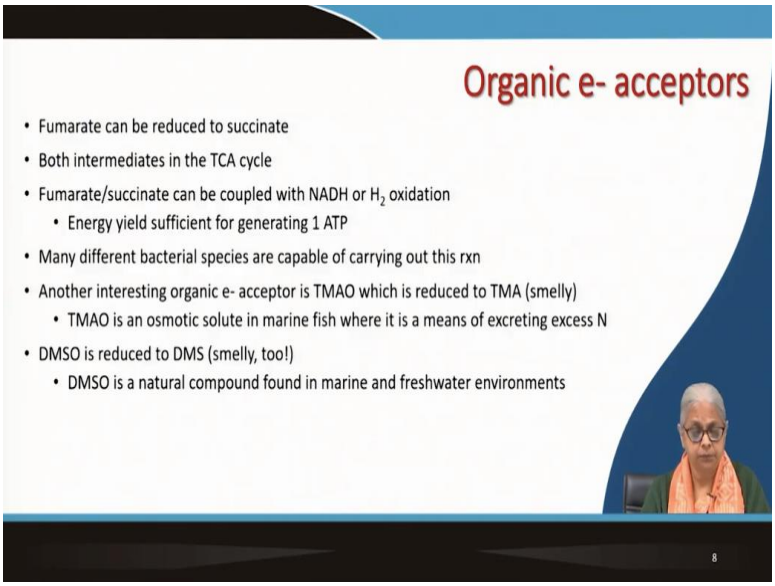


Then we come to organic electron acceptors. So, organic electron acceptors can be fumarate or some of the others that are shown in the table that I showed you previously. Fumarate and succinate are intermediates in the tricarboxylic acid cycle and the fumarate/ succinate half

reaction can be coupled with NADH or hydrogen oxidation. Energy yield is sufficient for generating 1 ATP. Many different bacterial species are capable of utilising them, and this is also the basis of fermentation reactions. Many species like I said are capable of carrying out this reaction.

There are other organic electron acceptors like DMSO, TMAO. And I think I mentioned in previous lectures that when you go to the sea side, there is a typical fishy smell; and that fishy smell is associated with DMSO and DMS. Both of them are natural compounds in marine and freshwater environments.

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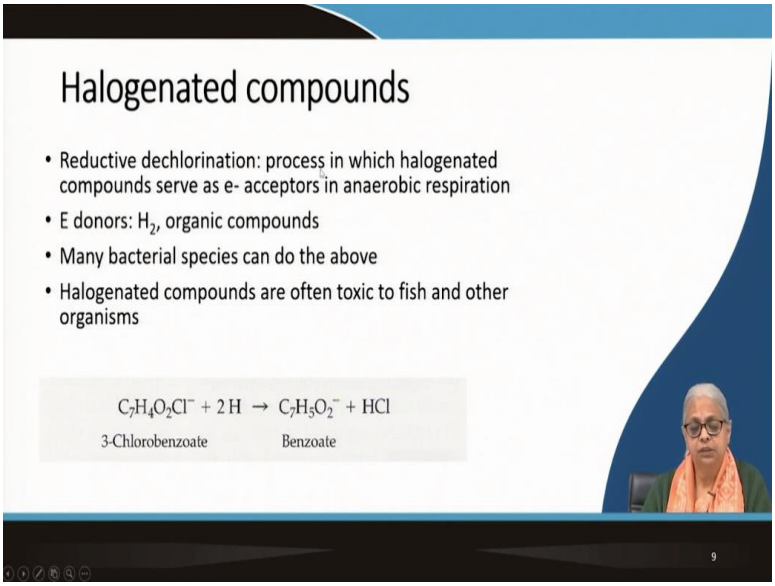


You can also have halogenated compounds. Now, if you have halogenated compounds, one of the biggest problem with these compounds is that, when an organic compound is associated with a halogen, it is typically highly resistant to bacterial degradation. The first step that is necessary for biodegradation to happen is reductive dechlorination. So, in the first case, these halogenated compounds can serve as an electron acceptor in anaerobic respiration. Electron donors like hydrogen and other organic compounds can be coupled with this reaction. So, here you have chlorobenzoate. So, the first thing for further degradation to happen is that chlorobenzoate is converted to benzoate ($C_7H_4O_2Cl^- + 2H \rightarrow C_7H_5O_2^- + HCl$) and HCl is removed. So, once this first step is taken care of, then benzoate can be degraded like any other organic compound. Most bacterial species can do the above. They need acclimation, and halogenated compounds are generally toxic to fish and other organisms.

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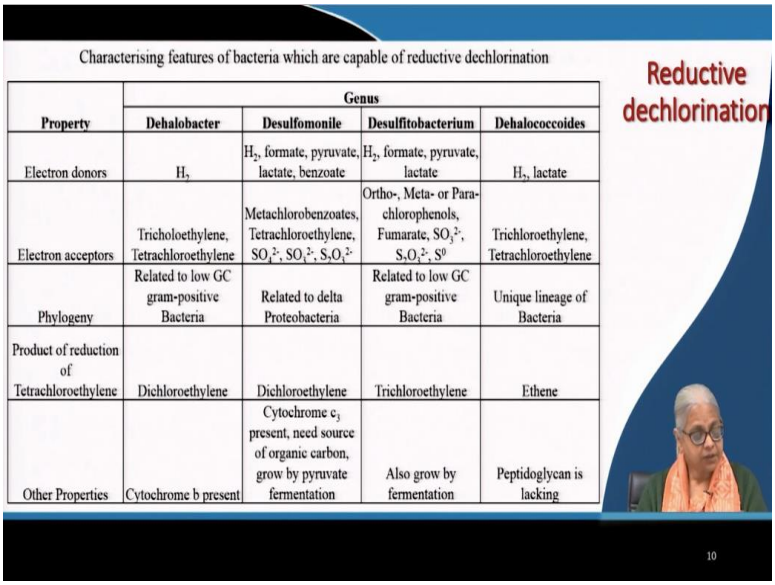


| Property | Genus | | | |
|---|---|---|---|---|
| | Dehalobacter | Desulfomonile | Desulfitobacterium | Dehalococcoides |
| Electron donors | $H_2$ | $H_2$, formate, pyruvate, lactate, benzoate | $H_2$, formate, pyruvate, lactate | $H_2$, lactate |
| Electron acceptors | Tricholoethylene, Tetrachloroethylene | Metachlorobenzoates, Tetrachloroethylene, $SO_4^{2-}$, $SO_3^{2-}$, $S_2O_3^{2-}$ | Ortho-, Meta- or Para-chlorophenols, Fumarate, $SO_3^{2-}$, $S_2O_3^{2-}$, $S^0$ | Trichloroethylene, Tetrachloroethylene |
| Phylogeny | Related to low GC gram-positive Bacteria | Related to delta Proteobacteria | Related to low GC gram-positive Bacteria | Unique lineage of Bacteria |
| Product of reduction of Tetrachloroethylene | Dichloroethylene | Dichloroethylene | Trichloroethylene | Ethene |
| Other Properties | Cytochrome b present | Cytochrome $c_3$ present, need source of organic carbon, grow by pyruvate fermentation | Also grow by fermentation | Peptidoglycan is lacking |

So, we have several examples of reductive dechlorination over here. You can have several different electron donors. These are examples from the literature, where you have different combinations of electron donors and acceptors. So, you have hydrogen coupled with trichloroethylene, tetrachloroethylene. You have hydrogen, formate, pyruvate, lactate, benzoate in combination with different organic compounds.

And you can see here that there are specific species that can mediate a specific coupling of the electron donors and acceptors. Then, what are the products? What are the end products of these reactions? The end products, for example, of the reduction of tetrachloroethylene is dichloroethylene or trichloroethylene or all the way to ethene. There are several other points that are mentioned here. I am just going to allow you to go through them.

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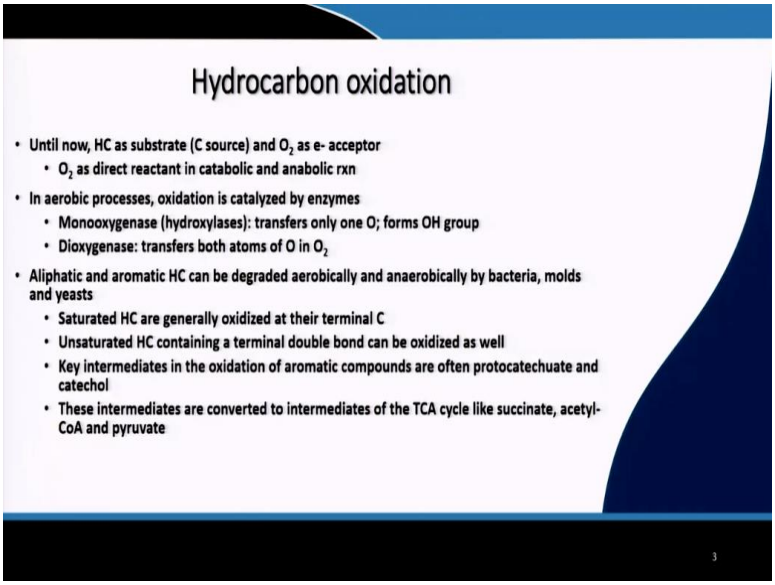

One thing that we have already realized is that oxygen can serve as the terminal electron acceptor, and it can be combined with electron donors like hydrocarbon. So, any organic compound can suffice, and hydrocarbons are just as good a carbon source. So, in this case, oxygen becomes a direct reactant in catabolic as well as anabolic reactions.

In aerobic processes, this oxidation is catalysed by various enzymes. So, there are 2 categories of enzymes, monooxygenases or hydroxylases which transfer only 1 oxygen or they form an OH group, and you have dioxygenase which transfers both atoms of oxygen in molecular oxygen. So, these are the 2 groups of enzymes that are responsible for the oxidation of hydrocarbons. You can have oxidation of hydrocarbons of both aliphatic as well as aromatic compounds. And both of them can be degraded aerobically as well as anaerobically by bacteria, molds and yeasts. Saturated hydrocarbons are generally oxidised at their terminal carbon.

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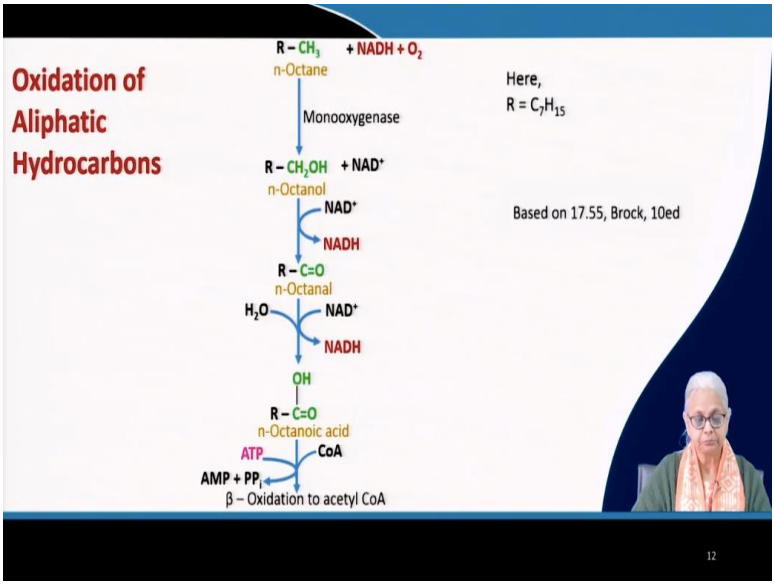


Let me just show you an example of that. So, here we have n-Octane which is an aliphatic hydrocarbon, and you have a monooxygenase enzyme. So, this monooxygenase enzyme in the presence of oxygen as the terminal electron acceptor will convert n-Octane to n-Octanol. And NADH will be converted to $NAD^+$, and water is formed. This $NAD^+$ is converted back to NADH and you get n-Octanal.

And then you have various other steps in the process going to the β-oxidation of n-Octanoic acid, where this particular compound will combine with acetyl-CoA. So, and in this process, 1 ATP is going to be utilised; and 2 NADH have been generated, while 1 NADH was invested.

So, this is saturated hydrocarbons in the presence of oxygen. Unsaturated hydrocarbons contain a terminal double bond. They can also be oxidised.

So, supposing the starting point were an unsaturated hydrocarbon, that can also be oxidised. There are key intermediates in the oxidation of aromatic compounds. These are generally protocatechuate and catechol. So, these intermediates have to be converted to intermediates of the tricarboxylic acid (TCA) cycle, like succinate, acetyl-CoA and pyruvate.

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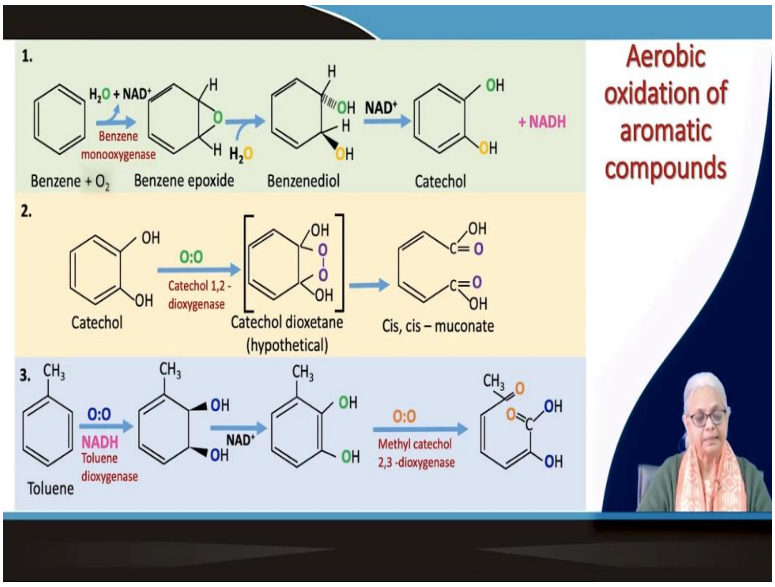


Benzene in the presence of benzene monooxygenase, will be converted to benzene epoxide, benzenediol, and then catechol. Now, this catechol is a key intermediate. It will be further converted by a dioxygenase. And remember, one of the first things for an aromatic compound to be further biodegraded is that the ring has to be broken. So, if we were to put it in a very simplistic way, benzene gets converted to catechol, and then the ring has to be broken. And that ring is broken at this stage. Dioxygenase is converted to *cis,cis*-muconate. You can have toluene as the starting compound. This toluene is first converted by dioxygenase, toluene dioxygenase is going to convert it in a series of reactions. And eventually, you will get methyl catechol. And that will be further catalysed to the same compound. This is methyl-*cis*-muconate.

So, these intermediates, like I said, have to be converted to intermediates of the TCA cycle, like succinate, acetyl-CoA and pyruvate. So, that is the whole point about all these key intermediates is that no matter what your starting compound is, first it gets converted to the

key intermediate, and then through the normal reaction series, it will be either completely mineralised or converted to other intermediates.

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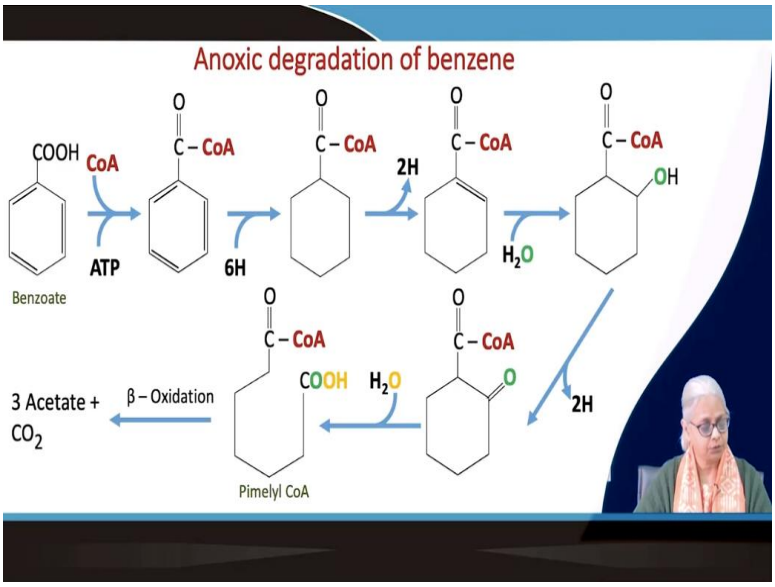


So, here we have the example of benzoate. It has been joined by CoA enzyme; 1 ATP has been utilised; benzoyl-CoA has been formed; protons have been picked up; and in the entire process, Pimelyl-CoA has been formed. And then, that enters the β-oxidation pathway, where the end point is going to be $CH_3COO^- + CO_2$.

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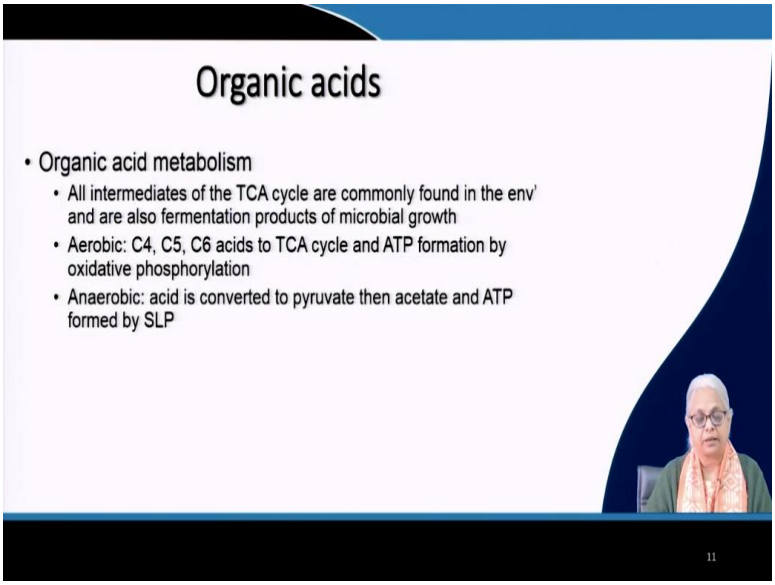


Then we come to organic acids. Organic acid metabolism: All intermediates of the tricarboxylic acid (TCA) cycle are found in the environment. That is why we know that they are common intermediates. They are also fermentation products of microbial growth. So, under aerobic

conditions, C4, C5, C6 acids can be brought into the TCA cycle. Remember that all the TCA intermediates are C4, C5 and C6.

And ATP can be formed by oxidative phosphorylation. Under anaerobic conditions, these acids C4, C5, C6 acids can be converted to pyruvate and then to acetate, and ATP is formed by substrate level phosphorylation; SLP stands for substrate level phosphorylation.

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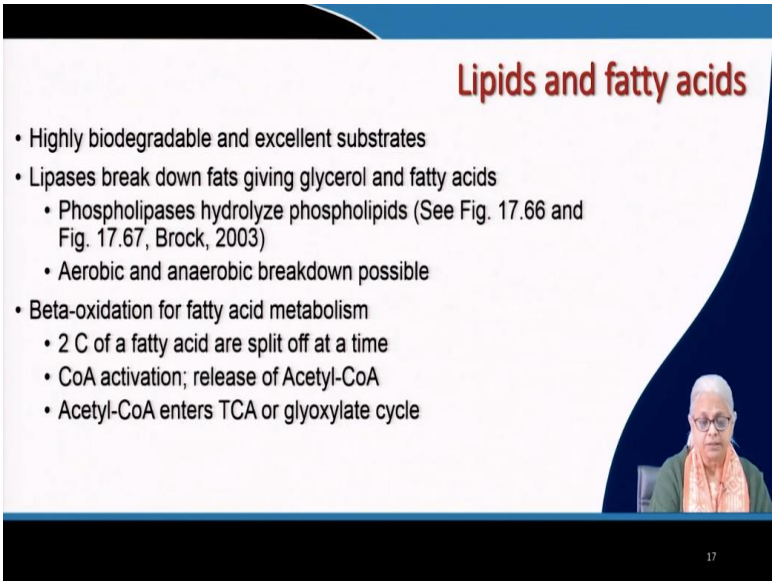


In terms of lipids and fatty acids, we know we all like eating fatty foods and we all know that they are a good source of energy. And even though they may be a problem in terms of diet control and so on, but they are excellent substrates. They are highly biodegradable and serve as good substrates, not just for human beings, but for microorganisms as well. So, lipases can break down fats into their components of glycerol and fatty acids. So, we have phospholipases which hydrolyse phospholipids.

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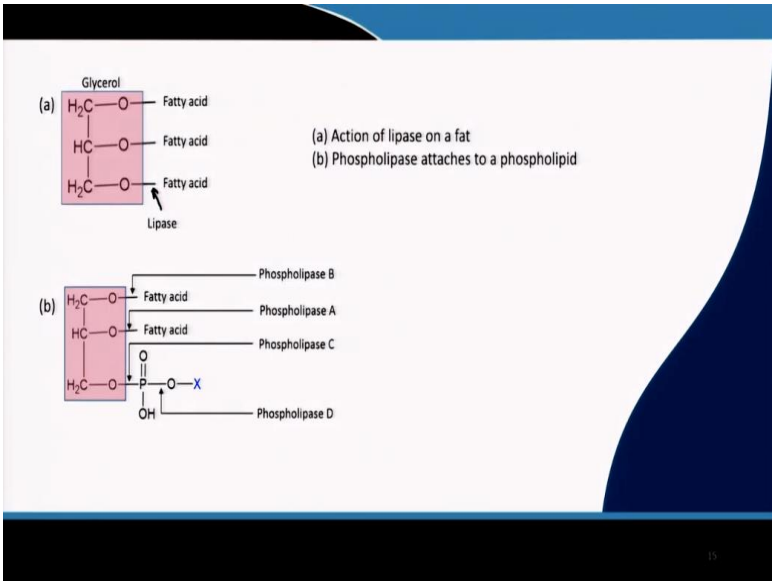


So, what we see here in the first case is the action of a lipase enzyme on a simple lipid or a triglyceride. So, you have the triglyceride molecule. And the enzyme can cut the bond over here where the arrow is shown, at all 3 positions. So, you can turn to figure 17.66 and 17.67 in Brock's Biology, tenth edition, for a photograph that shows you, or rather a micrograph that shows you the action, the impact of the action of phospholipase on egg yolk.

So, this photograph shows you an example of the action of phospholipase. So, when an inhibitor was added along with the presence of phospholipase which is present in egg yolk, because of the presence of phospholipase, the fatty acids of the egg yolk precipitated, which you can see over here. And in the other case, because an inhibitor was added, so there was no phospholipase reaction, and therefore no precipitation of the egg yolk.

So, in aerobic as well as anaerobic conditions, these lipases will break down lipids and fatty acids. Now, how are fatty acids metabolised? So, there is a particular pathway for the metabolism of fatty acids and it is called the β-oxidation pathway. In this particular pathway, 2 carbons of the fatty acid are split in a series of reactions. So, the process keeps repeating itself until the fatty acid is completely metabolised. So, it is a step by step, 2 carbons at a time. Now, remember, acetyl is a 2 carbon, C2 compound. So, these 2 carbons of the first step in the fatty acid metabolism will associate with coenzyme A. That is the activation of the compound with coenzyme A and that will release acetyl-CoA.

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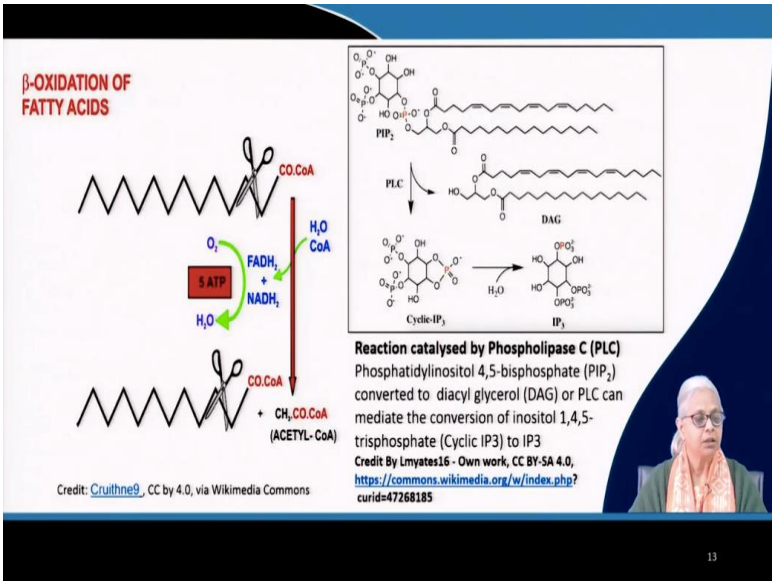


So, here you have a fatty acid. And you can see, coenzyme A is being added at the terminal end and 2 carbons are taken up with the CoA and in that process, ATP is being utilised. And in the next step, you have the formation of the double bond, addition of the hydroxyl and so on. I am not, like I said, going to go into any of these details; I think it is far beyond the scope of our course. So, this acetyl-CoA, remember, is a key intermediate in the TCA cycle, the tricarboxylic cycle or the glyoxylate cycle.

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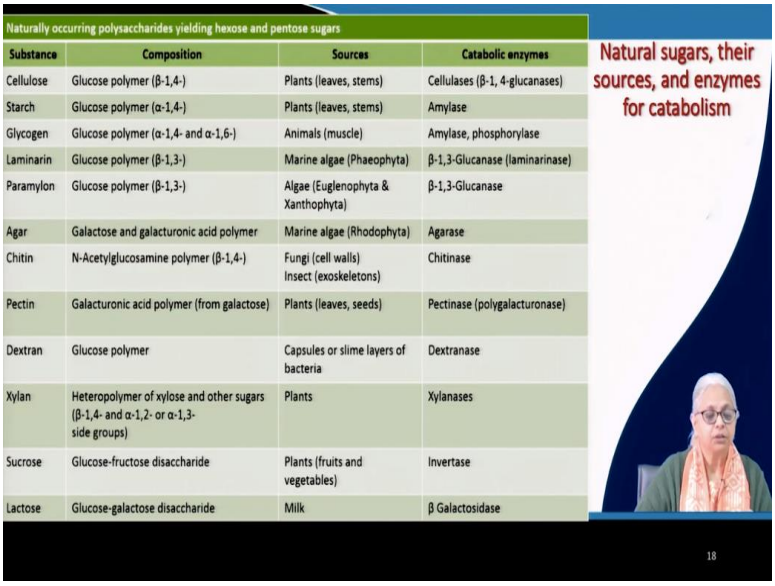


These are examples of C5 and C6 sugars that can be used by various enzymes and serve as energy as well as carbon sources. So, you have cellulose, starch, glycogen, agar, chitin, pectin, dextran, sucrose, lactose; the list just goes on and on, there is no end to it. And you can see the specific catabolic enzymes that are needed to break down these polymeric molecules into smaller and simpler forms.

So, cellulose; I have already given you examples of starch versus cellulose and glycogen; these examples were shown in a previous topic. And then you have so many other examples. And all enzymes tend to be specific for the substrate that they are breaking down.

So, here you can see agar which comes from marine algae, Rhodophyta. The catabolic enzymes are agarase. Then you have chitin. Chitin comes from the cell walls of certain specific fungi or it is part of the exoskeletons of insects and the enzyme that helps to break down chitin is chitinase. In case of pectin, pectin comes from plants, the leaves and seeds of plants and the enzyme that breaks it down is called pectinase. Dextran which is a polymer of sugar or glucose, and is part of the capsules or slime layers of the bacteria, can be broken down by an enzyme called dextranase.

Xylan which is present in xylose and other sugars, it comes from plants; and the enzymes that break it down are called xylanases. Sucrose is a very common sugar. It is a disaccharide, it is a glucose-fructose disaccharide; it comes from plants, basically fruits and vegetables and the enzyme that breaks it down is called invertase. So, lactose which is another disaccharide, it comes from milk, it is made of glucose and galactose; and the enzyme that breaks it down is called β-galactosidase.

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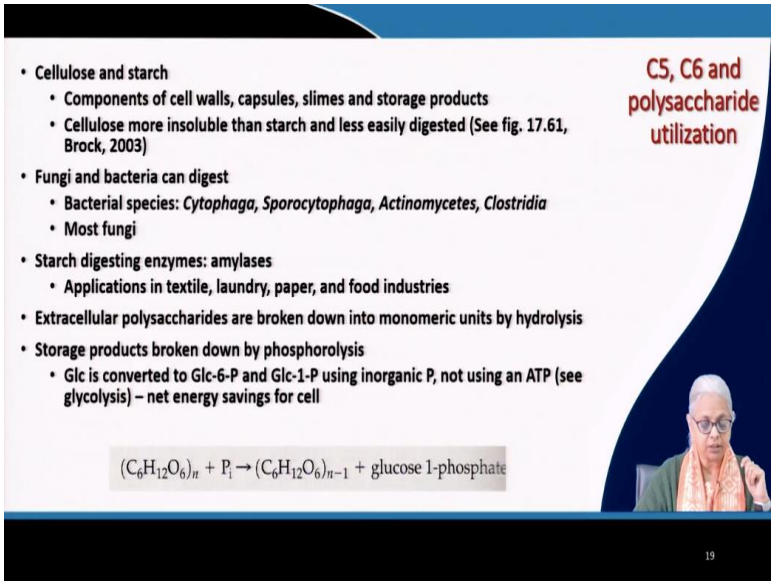


So, if we look at C5, C6 and polysaccharide utilization, we know that cellulose and starch are major components of cell walls, capsules, slimes and storage products. Cellulose tends to be less soluble. Starch is completely soluble in water, cellulose is not as soluble in water as starch,

and it is much more difficult to digest. Fungi and bacteria, both are capable of digesting both cellulose as well as starch. I have shown you examples of that in the past.

Bacterial species like *Cytophaga*, *Sporocytophaga*, *Actinomycetes*, *Clostridia*; all these are examples of bacterial species and fungal species that can digest both cellulose and starch. Starch digesting enzymes include amylases. So, they have several applications in textile, laundry, paper and food industry. You can see a cellulose-digesting bacterium that has attached itself to a cellulose fibre in this scanning electron micrograph (*Refer to book*).

So, *Sporocytophaga* is the species, and that is what you see over here. These are the fibres. These extracellular polysaccharides can be broken down into their monomeric units by hydrolysis, and these enzymes are mediating that reaction. The storage products can be further broken down by phosphorolysis. So, if you have substrate level phosphorylation, glucose is converted to glucose 6-phosphate and glucose-1-phosphate using inorganic phosphorus, not using an ATP, unlike the glycolysis step. So, this is energy savings reaction for the cell.

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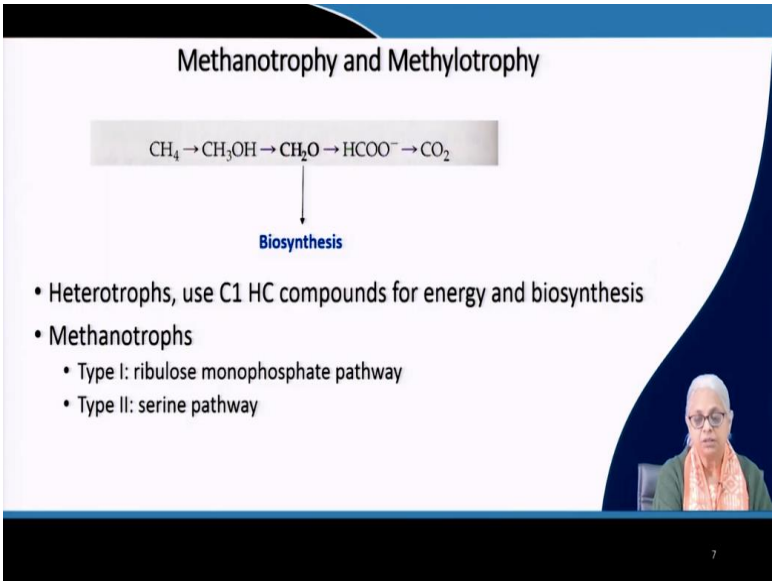


We also have another set of reactions that are possible. Now, we know that in the environment you can have C-1 to C-n organic compounds. So, let us start with C1. Now, if you have C1 compounds; C1 compounds can be methane, methanol or any other compound, formaldehyde, carbon dioxide and so on. So, methanotrophy and methylotrophy has been observed by (in) various species.

I will not go into any details. I think it is beyond the scope of what we are doing here. But there are heterotrophic species which can utilize C1 hydrocarbons like methane, methanol and methyl groups, for both energy as well as biosynthesis. And type I and type II; these are the 2 pathways by which methanotrophs; methanotrophs means bacteria that are utilizing methane or methanol as their substrate. So, ribulose monophosphate pathway (type I) and the serine pathway (type II) are utilizable.

Thank you, and I will stop at this point.