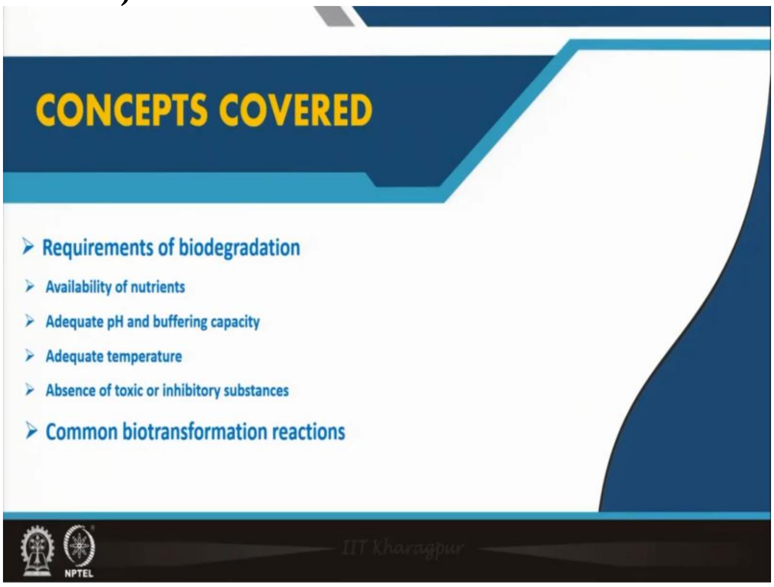
Environmental Biotechnology
Prof. Pinaki Sar
Department of Biotechnology
Indian Institute of Technology, Kharagpur

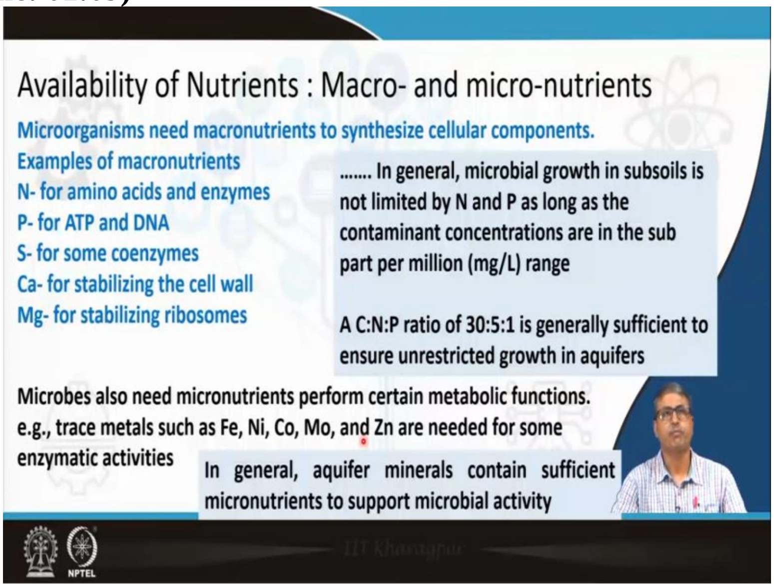
Lecture - 41
Biodegradation (Contd.,)

(Refer Slide Time: 00:31)



Welcome to the next lecture on biodegradation and in this particular lecture, we are going to discuss about some of the very important parameters of the biodegradation which are considered as the requirements of biodegradation and these include availability of nutrients, adequate pH and buffering capacity, temperature and absence of toxic or inhibitory substances and followed by that we are going also to talk about the common transformation or biotransformation reactions.

(Refer Slide Time: 01:09)



Now, in our earlier lectures on the requirement of bioremediation or biodegradation, rather we have discussed about some of the priority parameters. Now, followed by these we are now going to discuss about some of the associated factors which are considered to be very critical for controlling the biodegradation activities of microorganisms present in any environment and among these, the presence of different nutrients.

Both the macro and micronutrients are considered to be very critical. Now microorganisms need these micronutrients and macronutrients to synthesise the different cellular components. For example, the different macronutrients that we have already discussed like carbon, hydrogen, nitrogen, phosphorus, sulphur, magnesium, calcium and sodium etcetera. The nitrogen for example is very essential for amino acid and enzymes.

Phosphorus is for ATP and DNA synthesis sulphur is for some coenzymes, calcium is for stabilising the cell wall and magnesium for stabilising ribosomes. Now, these are important because during the biodegradation of organic pollutants, it is expected that the carbon substrates which is carbon organic pollutant molecules, which are being metabolised or degraded by the organisms.

They generally utilise the carbon that those are derived out of those degradation process along with carbon oxygen and hydrogen are also available and these organic substances are often used as their source of energy in heterotrophic, metabolism and source of electrons as well. However, the organisms growth and organisms activity would be severely impaired if these macronutrients particularly the nitrogen, phosphorus, sulphur, these 3 for example.

If they are not available in our general microbial growth in sub soil environment polluted environment is not limited by nitrogen and phosphorus as long as the contaminant concentration are in the sub part per million level and its carbon nitrogen and phosphorus ratio of 30 is to 5 is to 1 is generally sufficient to ensure unrestricted growth in aquifers. However, in several petroleum hydrocarbon impacted environments.

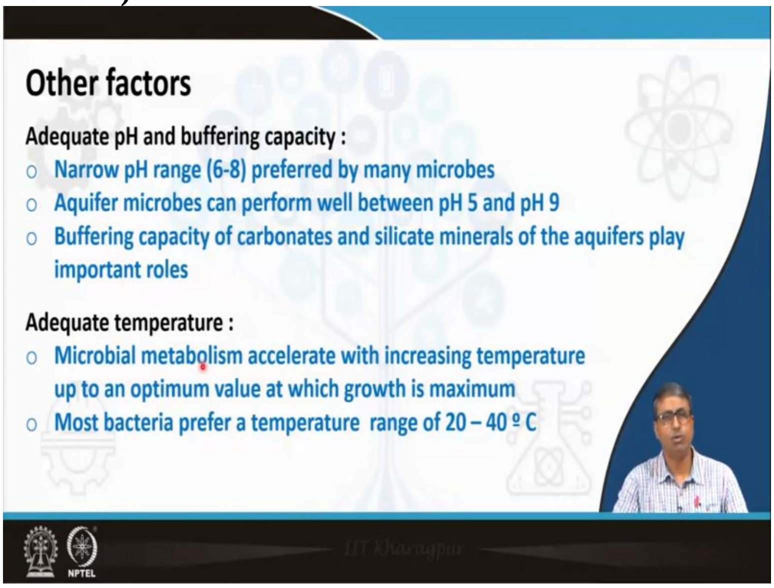
We have noticed that the concentration of carbon is very high and these are mostly the petroleum waste and petroleum spills and the compared to the carbon the concentration of nitrogen in particular, and also sometimes the phosphorus are very low and the natural catabolic abilities of the microorganisms towards the degradation is a severely constrained because of the lack of adequate nitrogen and phosphorus.

And along with these macronutrients microbes also need several micronutrients. So macro and micro both are required and these micronutrients are particularly more useful or critical, because of their role in several metabolic functions. For example, the trace metals of iron are sometimes the higher concentration of iron as well and nickel, cobalt molybdenum, zinc, are needed for enzymatic activities and in soil as well as in aquifer systems often if you see that there are minerals and these minerals are capable of providing these micronutrients.

So, in again in natural environment, often the biodegradation may not be impacted very severely by this due to the lack of these micronutrients, but one has to be take care about this and that is why it is very, very important that in order to facilitate or understand the, the scope for intrinsic bioremediation or engineered bioremediation through biodegradation of hydrocarbon contaminants.

The chemical analysis of the environments are thoroughly done, because once we perform such a chemical analysis, elemental analysis in particular, we came to know about the concentration of different elements presence, and based on those information, we can actually make a decision that way that we need to provide some supplements of these micronutrients. So, that the microbial growth are not restricted just because the supply of the micronutrients for example, our micronutrient is limited.

(Refer Slide Time: 06:17)



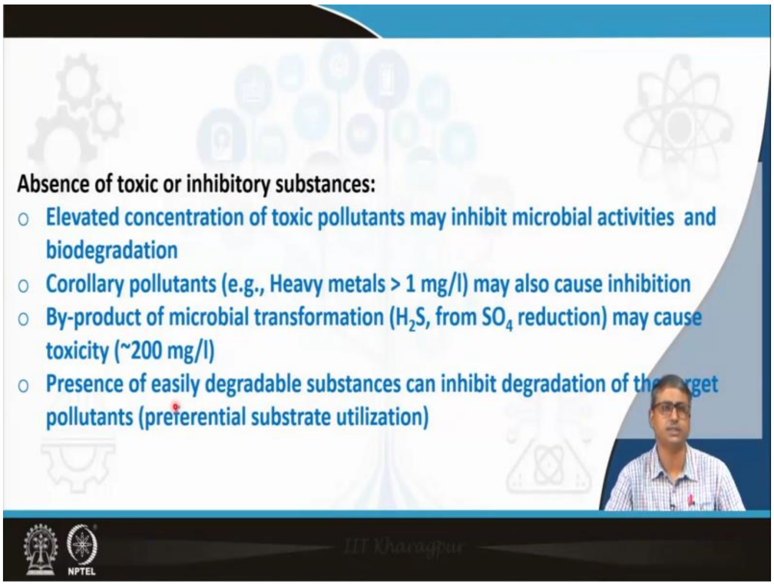
Now, another very important aspect is the presence of the optimum pH and also the buffering capacity of the environment, the contaminated environment where the biodegradation process is expected for environmental biotechnology purpose. So, the adequate pH basically the narrow pH range of 6 to 8 is preferred by many microbes and in contaminated aquifer for example, aquifer microbes can perform well still between pH 5 and pH 9.

And however these aquifers are contaminated environments must be monitored with respect to the pH particularly and with respect to aquifer, we find that the buffering capacity of carbonates and silicate minerals which are present generally in the aquifer as a dominant mineral phase; they play a significant buffering capacity. So, naturally, microbes are not constrained by the pH of the environment in such cases.

However, as I mentioned, the pH has to be measured and monitored periodically. In order to understand that everything is good for the microbes to proliferate and function towards the biodegradation of the hydrocarbon compounds the followed by the pH another important point is the adequate temperature microbial metabolism generally accelerates with increasing temperature and up to an optimum value at which the growth is maximum.

And most of the bacteria they prefer a temperature range between 20 degree to 40 degrees centigrade and estimation of these temperature range is very, very important very much important because if it is less than 20 degree or if it is more than 40 degree, then maybe we need to think of some specialist organism who can survive and act well perform well under those lower temperature or higher temperature.

(Refer Slide Time: 08:24)



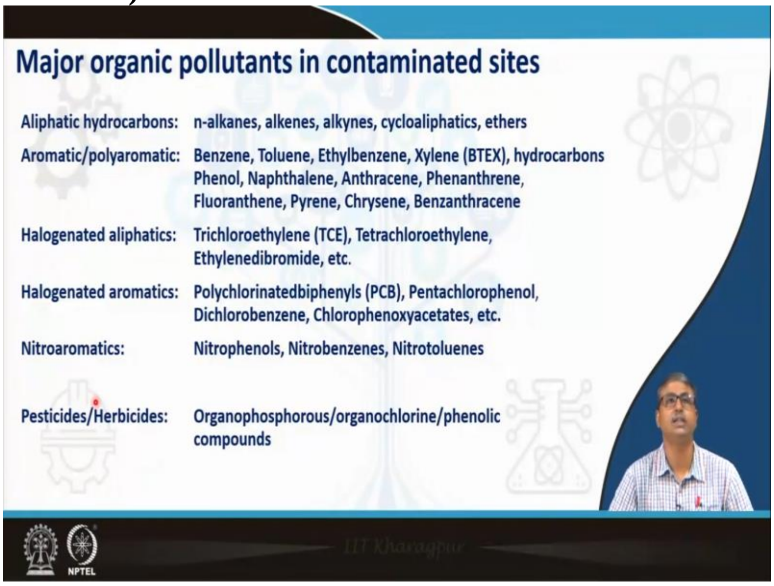
The next parameter is the absence of toxic or inhibitory substances. In many contaminated environments, particularly the hydrocarbon contaminated environments, we see that the elevated concentration of the toxic pollutant itself inhibit the microbial activities and biodegradation. So the concentration of the pollutant molecule is a very important parameter the corollary pollutants.

For example, heavy metals which are often present along with the organic pollutant is a kind of a situation where we see that is a mixture of several pollutants are there. So if in such cases, we see the heavy metals are present, toxic heavy metals like chromium, zinc, cadmium etcetera and if there are concentrations of more than one milligram per litre. Those higher concentrations of heavy metals can impose inhibitory effects on microorganism particularly the catalytically relevant microorganisms.

By-product of microbial transformation for example, the product or by-product of the sulphate reduction that is the hydrogen sulphide may cause toxicity. So, that could be one parameter that we need to monitor in order to again ensure that the microorganisms are not going to suffer. Because of these by-products of different microbial transformation and the last point about these is the presence of easily degradable substances how can inhibit the degradation of the target pollutant.

For example, if we; have acetate or lactate or other readily metabolizable sugar molecules in an environment or in case of pollutants, where the pollutants are basically used as a source of nitrogen or source of phosphorus. If you have a readily metabolizable nitrogen source or phosphorus source then phosphate source then possibly the pollutant molecules are not going to be degraded. So, as efficiently as it is actually expected if such; easily degradable substances are not there.

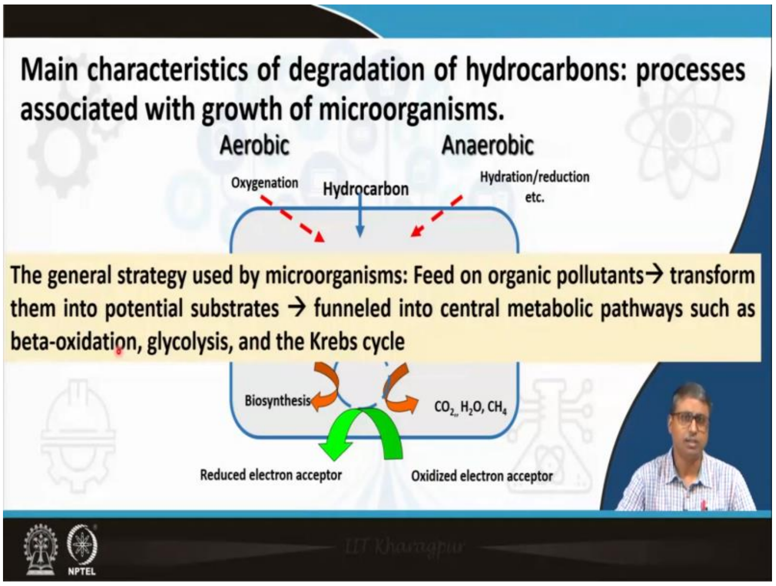
(Refer Slide Time: 10:45)



Now, we will move on to the next part of the session that is the common biotransformation mechanisms. So before we go and discuss about the biotransformation mechanisms, which facilitate the biodegradation of organic pollutants. Here is a kind of a summary of the different type of organic pollutants which are present in the contaminated site and as you can see that it includes a diverse array of hydrocarbon molecules.

Including the aliphatic hydrocarbons, aromatic hydrocarbons, which include both the mono aromatics substituted aromatics and the poly aromatics halogenated aliphatic and halogenated aromatics are the where the halogen groups are added to the either the aliphatic molecules like trichloroethylene or tetrachloroethylene or more halogenated aromatics like polychlorinated biphenyls pentachlorophenol etcetera. Nitroaromatics that were the nitro groups are added to the aromatic compounds and different pesticides, herbicides etcetera. So, these are the common classes of organic pollutants generally present.

(Refer Slide Time: 11:49)



Now, main characteristic of the degradation of hydrocarbon process associated with the growth of microorganisms. So, we will talk a bit detail on that this particular aspect that the biodegradation of hydrocarbons of the organic pollutants that is the topic of main interest, today, a can be considered to be as a part of a growth of the microorganism or they may not be involved in the supporting the growth of the microorganism.

So, we will we see that the biodegradation can favour the growth of the microorganism because as we have seen earlier the biodegradation of organic pollutants they provide a number of resources including the precursor molecules or the ATP or the energy and also the electrons. Now, utilising those resources the microorganisms can grow and profusely. So, that is called the growth associated microorganisms.

So, in this part we are going to discuss these growth associated biodegradation process. As you can see that this is the bacterial cell is a simpler form of cell that is presented here. So, both under aerobic as well as under anaerobic condition, the hydrocarbon molecules are transported inside the cell and a particular type of reaction happened that is either it could be oxygenation or hydrogen or reduction based on the availability of oxygen in the system.

And those types of reactions are called initial attack on the molecule because the hydrocarbon molecules are not available for the central metabolic pathways to be metabolised readily. So, they need to be initially of transformed into some molecules, some forms in which they may be further metabolised or degraded. So, the initial attack facilitates are the readiness or prepare the molecule for the next stage of processes.

And subsequent to those 2 steps these the molecules are converted to smaller molecules, the bigger molecules are converted to smaller molecules and those smaller molecules are then capable of entering into the central carbon metabolism for example, the tricarboxylic acid cycle and based on the availability of the electron acceptor, like the terminal electron acceptor.

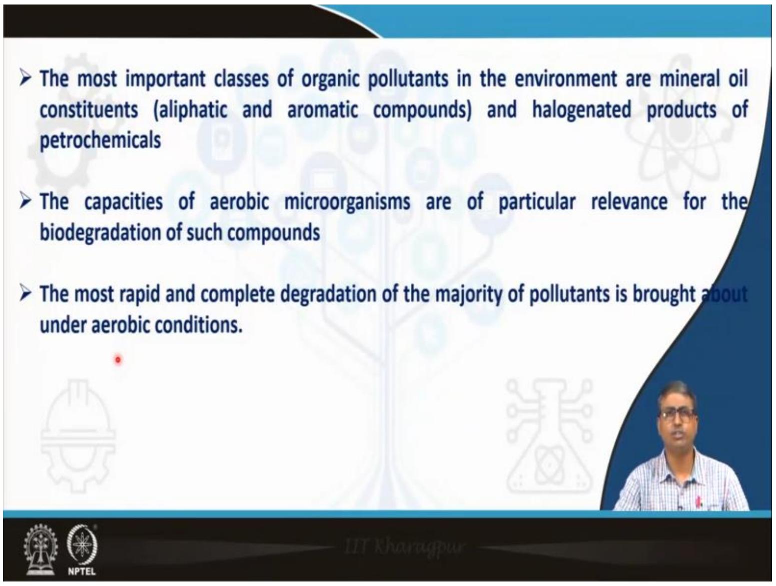
And also the; based on the availability of different other nutrients like nitrogen, sulphur, phosphate etcetera. These metabolic pathways essentially then facilitate the biosynthesis of macromolecules and enable the cell to proliferate and grow more and creating more biomass out of that. So, one way the microorganism or the bacteria is able to grow able to divide, divide and produce new cells using the, carbon and the energy obtained from the hydrocarbon degradation.

And in the other way the hydrocarbon molecules are oxidised, if they are mineralized, they will be broken down or oxidised up to carbon dioxide or methane and water molecules. Now, this general strategy is used by the microorganism is basically is to feed on the organic pollutants. So, these microorganisms they use these hydrocarbons as their food and as their carbon and energy source and they are heterotrophic bacteria mostly.

And they although the hydrocarbons like the poly aromatics, mono aromatics or alkanes are not a regular substrates for many bacteria, but although they know how to deal with it, because earlier lecture we have learned that some of these molecules are present during the course of evolution. So, the microbes or the bacteria they know how to oxidise these molecules.

So, they transform slowly, slowly these, these alkanes and aliphatic and aromatic molecules into some potential substrates, which can then be funnelled into the central metabolism and then the central metabolic pathways will lead to the complete degradation of the molecule like the alkanes converted to fatty acids will be subjected to beta oxidation and followed by the acetyl CoA which is produced acetyl CoA molecules will be oxidised through the Krebs cycle.

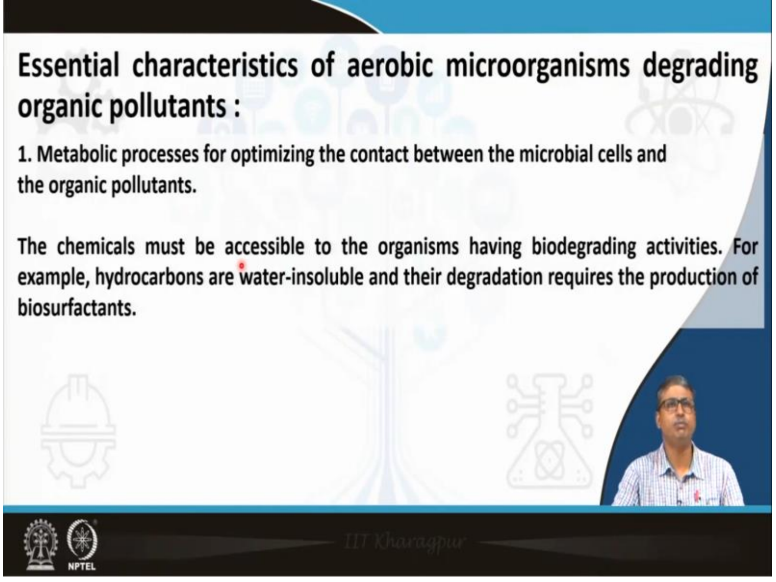
(Refer Slide Time: 16:19)



Now, the most important classes of organic pollutants in the environment are different types of mineral oil constituents like as we presented earlier that the aliphatic and aromatic compounds they are halogenated they have substituted molecules which are derived from petrochemicals and allied industries and the capacities of aerobic microorganisms are of particular relevance for the biodegradation of such compounds. Because these roll of oxygen when the aerobic condition is present prevailed and the aerobic microorganisms are present.

They are capable of utilising oxygen for 2 purposes that we are going to see as the most rapid and complete degradation of these majorities of the pollutants is brought about under these aerobic conditions.

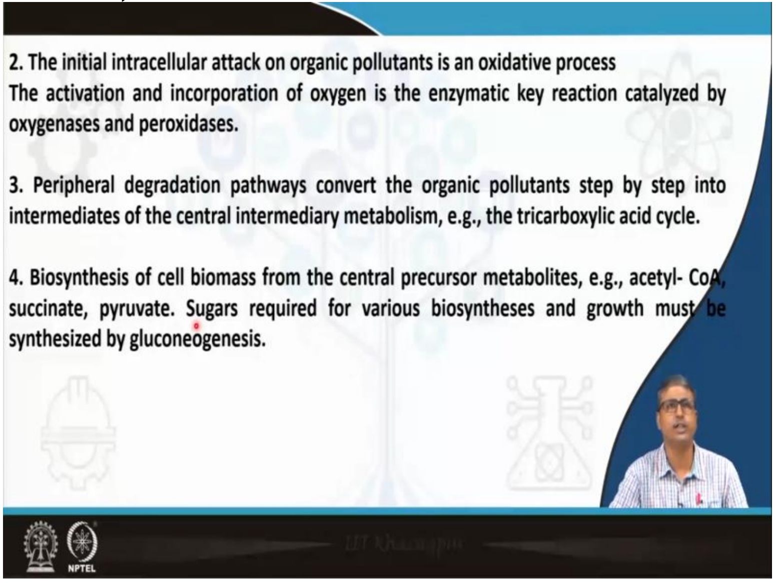
(Refer Slide Time: 17:07)



And some of these essential characteristics of aerobic microorganisms degrading the organic pollutants are the metabolic processes for optimising the contact between the microbial cells and the organic pollutants. So, the organic pollutants are often hydrophobic. So, the microorganisms need to have to arrange some mechanism as to adopt some mechanism through which the contact is established between the hydrocarbon molecule and the microbial cells.

So, the chemicals the hydrocarbon molecules are must be accessible to the organism having the biodegradation activity and for examples the hydrocarbons are water insoluble and their degradation requires the production of biosurfactants. So, what do we have found that many of these biodegrading organisms they are capable of producing biosurfactants and these biosurfactants are actually make these hydrophobic organic pollutants available to the microorganisms.

(Refer Slide Time: 18:04)

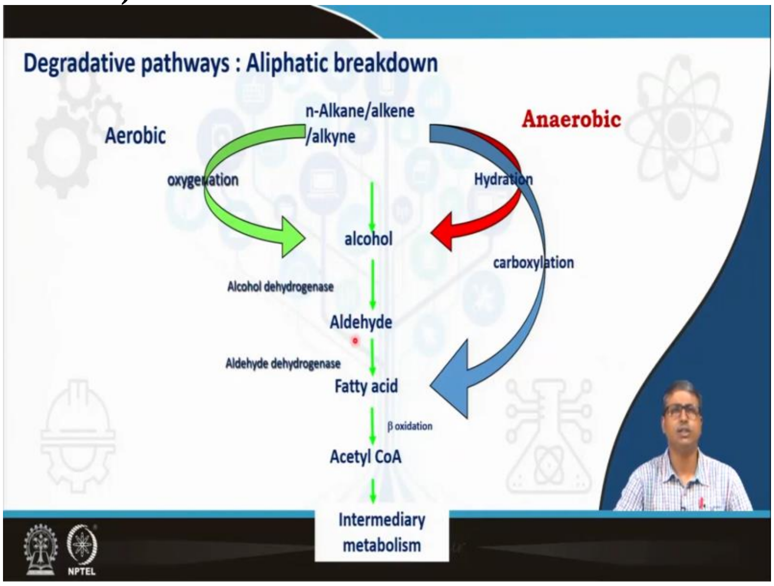


Now, second step is the initial intracellular attack on the organic pollutant molecules. So, as soon as the organic pollutant is taken inside the cell, a series of oxidative reactions oxidative transformations are carried out. This oxidative process includes the activation and incorporation of oxygen within the hydrocarbon molecules whether it is aliphatic or aromatic and it is one of the key enzymatic reactions catalysed by the oxygenases and peroxidases.

Next step would be the peripheral degradation pathway. Once the aromatic or the aliphatic molecule is activated, with the use of oxygen, then the peripheral degradation pathway will convert this organic pollutant into multiple through multiple steps into intermediates of the central intermediary metabolism for example, the metabolites which are which are to be to be processed further through tricarboxylic acid cycle.

And finally, once the TCS cycle is able to the central carbon metabolism is able to metabolise those carbon molecules, the biosynthesis of cell biomass from the central precursor metabolites like acetyl-CoA, succinate, pyruvate etcetera will happen. Now, sugars required for various biosynthesis and growth must be synthesised by gluconeogenesis. So, the biodegradative organisms are capable of performing such reactions as well.

(Refer Slide Time: 19:38)

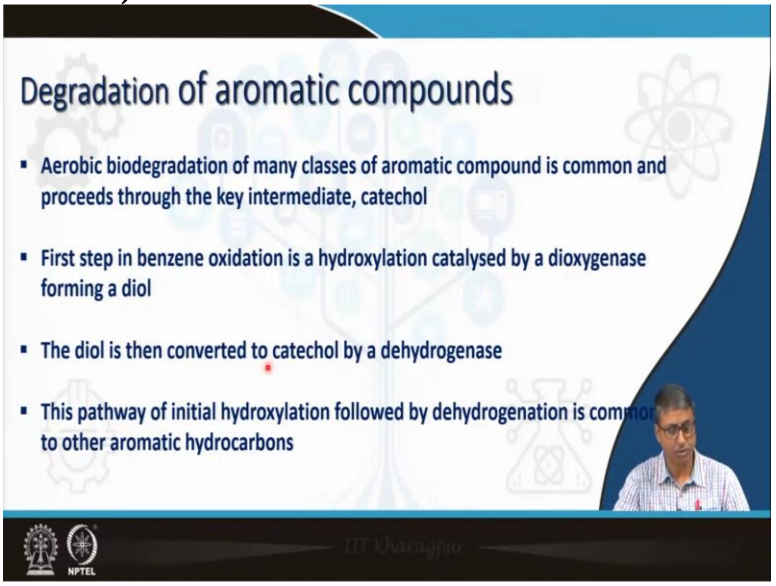


So, the aliphatic degradative pathway or aliphatic breakdown pathway would be something like this, that under the aerobic condition, the alkene and alkyne or alkaline molecules will be oxygenated to predict to form the alcohols because the oxygenation will lead to the formation of the alcohol and from the alcohol molecules the alcohol dehydrogenases will act on them and we will produce the respective aldehydes and the aldehydes will be further oxidised to produce the fatty acids.

And then the fatty acids will be subjected to a beta oxidation which is a part of a regular central carbon metabolism and following this fatty acid beta oxidation, the acetyl CoA molecules will be synthesised and these acetyl CoA molecules will be then participate in the intermediate any or the tricarboxylic acid cycle type central carbon metabolism and will be oxidised further or they will produce different cellular precursors.

Which will be useful for the; cell biosynthesis process. Now, the same process can happen also under anaerobic condition, where hydration and carboxylation will lead to the formation of either the alcohol or it they will produce the fatty acids and then the alcohol or the fatty acid will undergo the similar type of reactions producing the different type of oxidised products up to carbon dioxide and methane.

(Refer Slide Time: 21:02)

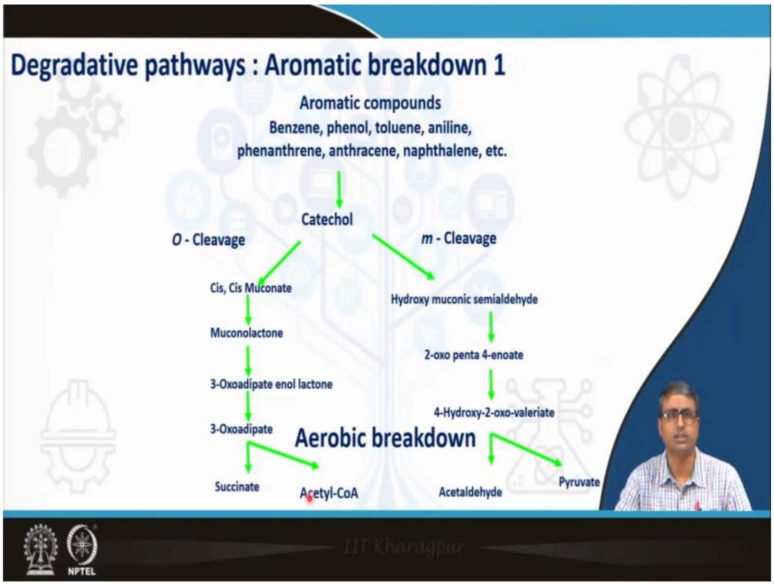


Now, with respect to aromatic compounds, aerobic biodegradation of many classes of aromatic compound is common and process through the key intermediate catechol. So, like for aliphatic and aromatic aliphatic compounds in particular as we have seen that mostly they are processed through fatty acids. So, all the alkyne molecules and aliphatic molecules are oxidised or sometimes they are hydrated or reduced also.

And eventually they are all converted to some form of fatty acids in the fatty acids are metabolised. In case of aromatic compounds or the aromatic compounds under aerobic condition they are all processed or converted oxygenated into other to form a common intermediate that is called catechol and this is considered to be the first step in oxidation of the aromatic compounds like benzene and that is through hydroxylation catalysed by dioxygenase forming a diol of 2 moles of oxygen's are incorporated.

And the diol is formed and then the diol is then converted to catechol by another enzyme which is called the dehydrogenase. So, these enzymes are operating they operate in synergy and they are able to convert the aromatic hydrocarbon molecules into ultimate to catechol. Now, these pathway of initial hydroxylation followed by dehydrogenation by the dehydrogenase and dioxygenase type of enzymes are very common and they are applied to many other aromatic carbons as carbons.

(Refer Slide Time: 22:37)

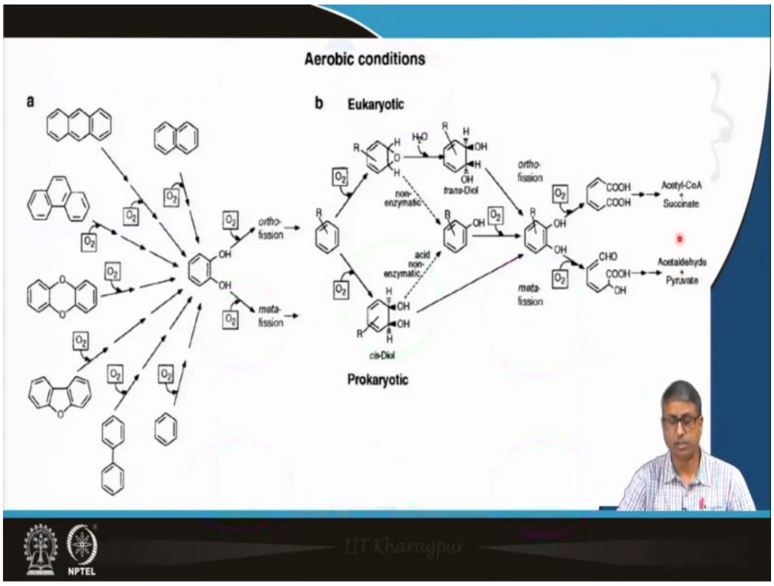


Now, so, as I mentioned that the aromatic different type of aromatic compounds and broad range of aromatic compounds will undergo these dioxygenation sometimes, 2 moles of oxygens are incorporated separately using some monoxiginise otherwise, it is the dioxygenase and process by dioxygenase and the dehydrogenation reaction then eventually the catechol is produced.

These catechol will undergo a 2 types of oxidative or biodegraded process one is called ortho cleavage and other is the meta cleavage it depends on exactly where these catechol ring structure is broken the location of the ring cleavage and accordingly we see that either through ortho cleavage it is the succinate or succinic acid is produced which is along with that acetyl Co is produced or during the meta cleavage pathway.

The acetyl aldehyde and pyruvic acid will be produced. Now, all these products succinic acid and acetyl CoA and acetaldehyde pyruvates they are all intermediates are very important precaution for central carbon metabolism.

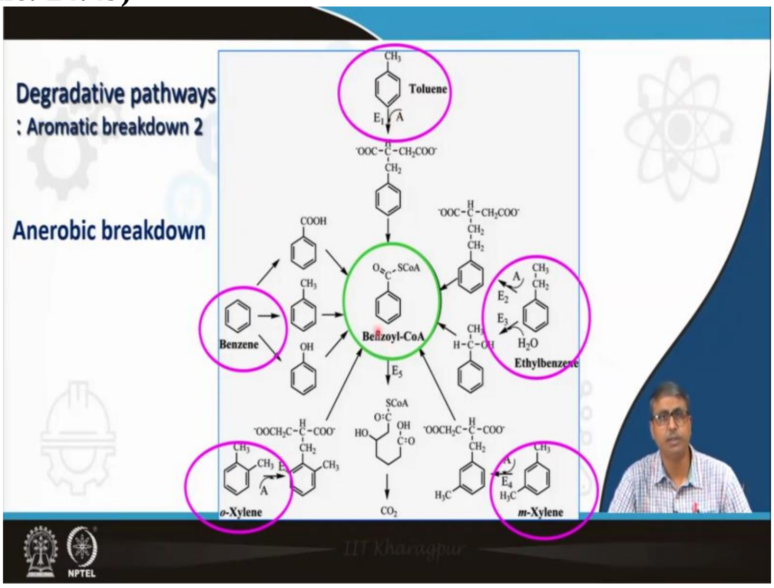
(Refer Slide Time: 23:51)



So, they can straightaway enter into the central carbon metabolism. Now, one very interesting aspect of this aerobic degradation of hydrocarbons, aromatic hydrocarbons is that the funnelling mechanism that all the different type of mono aromatic or poly aromatic hydrocarbons are oxygenated and converted subsequently to a central intermediate which is called catechol.

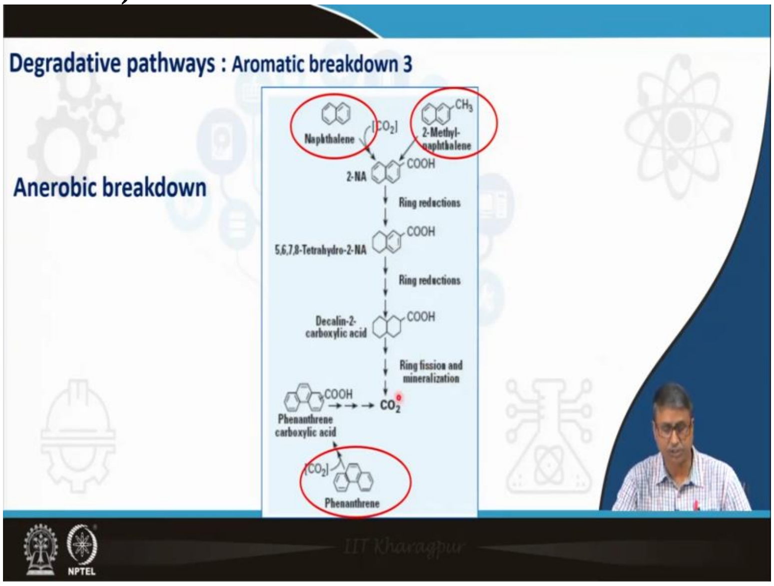
So, that is called a kind of a funnelling reactions at all the different kinds of aromatic, polyaromatic, mono aromatic substituted some kind of other aromatics also, as you can see, these are more complex than simple poly aromatic compounds they are all oxidised and they are converted to this catechol and then this catechol undergoes the further ring cleavage reactions to produce eventually the acetyl CoA succinic acid or the acetaldehyde pyruvic acid and these are then subsequently oxidised through the central carbon metabolism.

(Refer Slide Time: 24:49)



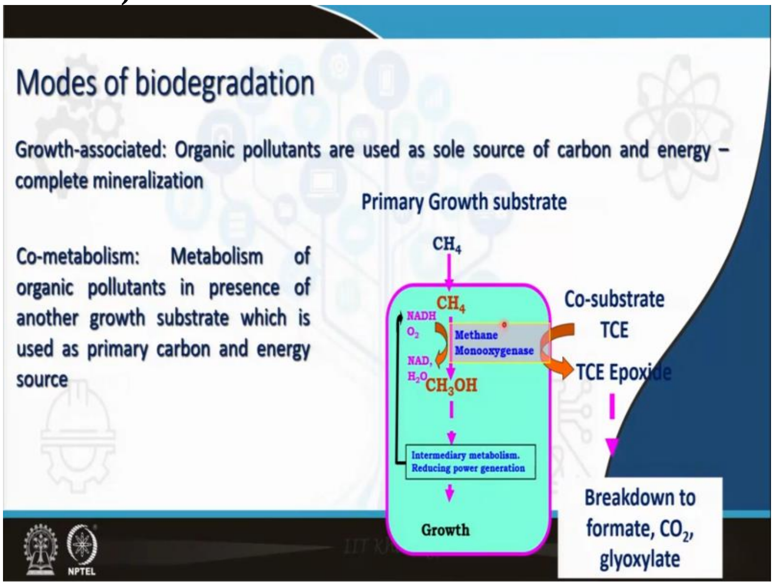
Under anaerobic condition, however, the degradation processes are found to be more diverse type. Sometimes, the hydrocarbon molecules are biodegraded under anaerobic condition through a intermediate called benzoyl CoA.

(Refer Slide Time: 25:06)



Or some other times, we see that the aromatic breakdown under anaerobic condition can occur through a kind of a ring reduction, where we see some kind of acids are produced and then these assets are undergoing ring fission and mineralization to carbon dioxide.

(Refer Slide Time: 25:22)



So, diverse types these aromatic hydrocarbon degradation under anaerobic condition has been reported. Now, the modes of biodegradation there are actually 2 types of modes have available. One is the growth associated that means, the organic pollutants are used as a sole source of carbon and energy and complete mineralization is observed as we have seen earlier that the organic pollutants are used as the source of carbon and electron and also the energy.

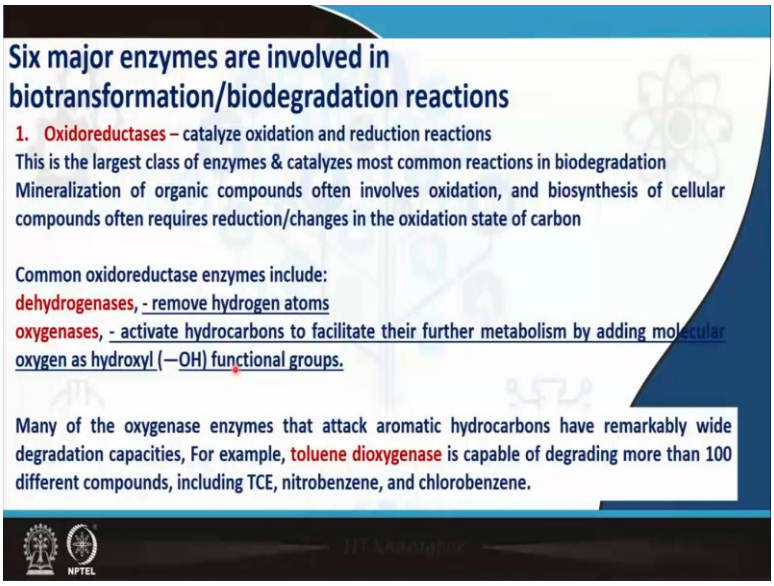
And the microbes are able to grow utilising the hydrocarbon compounds hydrocarbon pollutants as their growth substrate. However, there is another type of mechanism of degradation which is called co metabolism that is the metabolism of the organic pollutants in presence of another growth substrate, which is used as a primary carbon and energy source. That means, the polluted molecule is not actually the target molecule of the biodegradation.

But eventually because of the lack of specificity of the enzyme, the hydrocarbon molecule is actually degraded eventually for example, in this picture as you can see that the methane monooxygenase enzyme which is actually present there in methane oxidising or methane neutralising microbes to oxidise methane to produce these methanol and then the it goes into the intermediary metabolism and then it can produce the electrons and reducing power and facilitates growth.

So, regular methane oxidising bacteria they utilise this path it is nothing special about that only the methane monooxygenase gene, which is very critical. Now, methane monooxygenase gene can also transform TCE to TCE epoxide and then subsequently TCE epoxide can be broken down to format carbon dioxide and glyoxylate. So, TCE is not actually the substrate for growth subsides rather but because of the lack of specificity of this methane monooxygen is along with methane.

This enzyme can also recognise TCE as its substrate and then converts this TCE to TCE epoxide and thereby the facilitating the degradation of TCE.

(Refer Slide Time: 27:35)



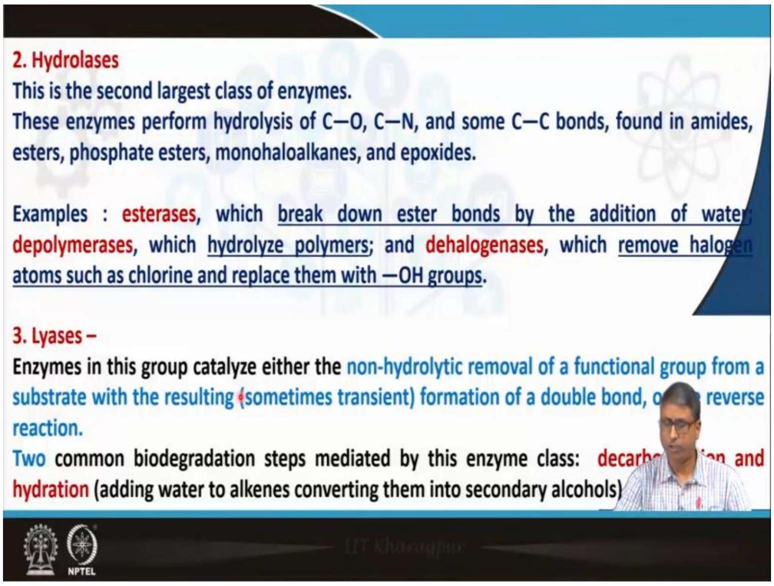
Now, with respect to the different enzymes which are involved in the biotransformation reaction, we have identified there are actually 6 major classes of enzymes which facilitate the bio transformation and biodegradation reactions and the first of the types in the major type is the oxidoreductases which catalyses the oxidation reduction reactions and this is also the largest class of enzyme and catalyses most common reactions in the biodegradation.

Because we possibly understand this fact that most of the cases the organic pollutant biodegradation is an oxidation process. Now, mineralization of the organic compound is a complete degradation of the organic compounds often involves oxidation and biosynthesis of several molecules. Now, common oxidoreductase enzymes include the dehydrogenases, which removes the hydrogen atom or the oxygen is which activated the hydrocarbons to facilitate their further metabolism like dioxygenase or monoxygenase.

As we have not noticed during the hydro aerobic biodegradation of hydrocarbon compounds. Now, many of these oxygen is enzymes that attacked the aromatic hydrocarbon have remarkable of diversity that means, they can actually convert a diverse type of aromatic hydrocarbons under aerobic condition into their respective forms which can be then converted to catechols.

So, that already I have presented one that how the funnelling type of thing is there, so, many diverse types of organic hydrocarbons, aromatic hydrocarbons are actually taken up by these oxygenases and hydrogenases and eventually all of them are converted to catechol.

(Refer Slide Time: 29:22)

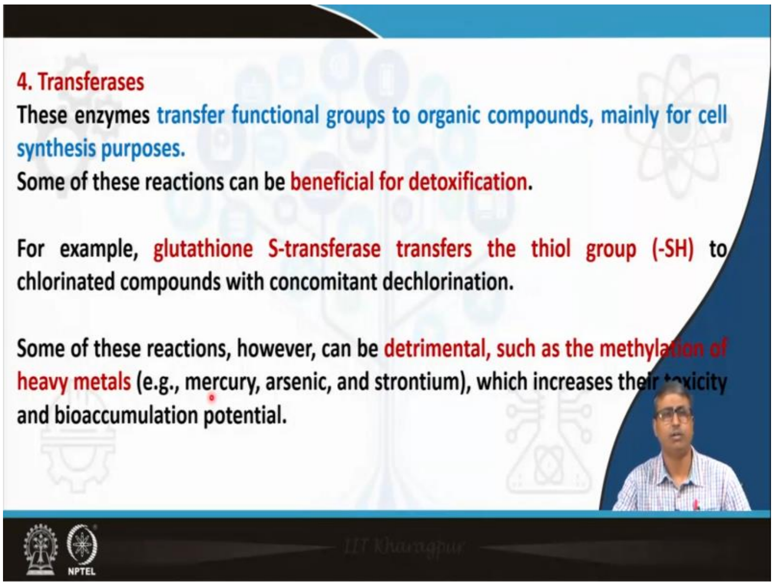


The next enzyme is the hydrolysis. This is the second largest class of enzymes and these enzyme, these enzymes perform the hydrolysis of C-O, C-N and some of the C-C bond breakage and found in amides esters phosphate esters, monohaloakanes and epoxides. For example, the esterases and the polymerase and also the dehalogenases are found to play a very important role in breaking down of different ester bonds or the polymer or remove the halogen atoms such as chlorine and we place them with age groups.

Now, once you are able to remove the halogen atom and replace it with the chlorine atom and replace it with hydroxyl group, the biodegradation through normal mechanism like oxidation and then formation of the intermediates are which are more suitable for central carbon metabolism to process our possible. Lyases are another class of enzymes and this group of enzymes either perform the non-hydrolytic removal of a functional group from its substrate.

With resulting sometimes transient formation of a double bond or the reverse reaction and 2 carbon biodegradation steps mediated by this enzyme classes the decarboxylation and also the hydration.

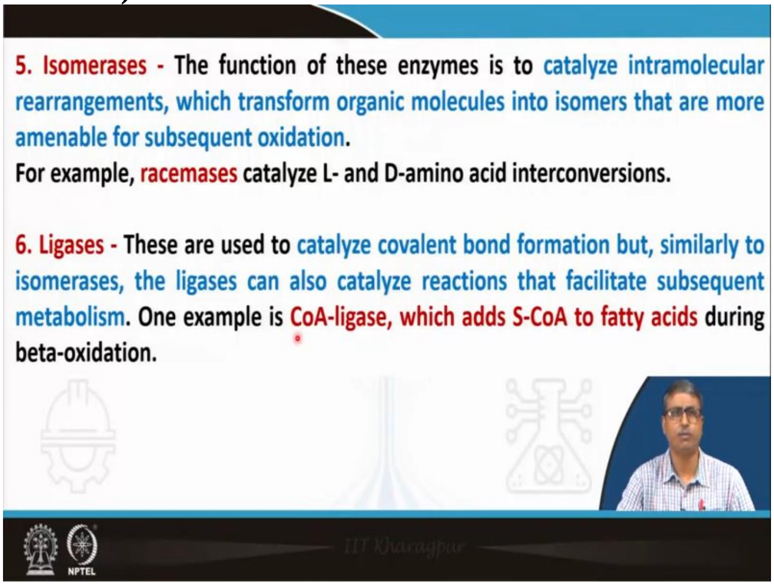
(Refer Slide Time: 30:44)



The fourth one is the transferase these enzymes transfer functional group to organic compound mainly for cell synthesis purposes and some of these reactions can be beneficial for detoxification as well. For example, glutathione S transferase the thiol group to the chlorinated compounds with concomitant dechlorination. So, once you remove or the simultaneous removal of the chlorine group and addition of the sulfhydryl groups are achieved.

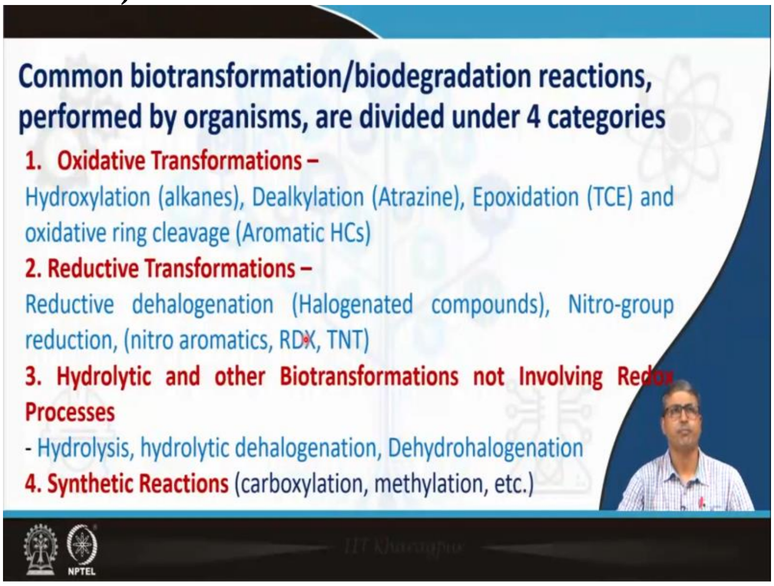
Some of these reactions however, can be detrimental also for the cells particularly for from the biodegradation point of view such as the methylation of heavy metal because once you methylate mercury, arsenic and strontium etcetera. are the toxicity and bioavailability of the heavy metals increase. So, therefore, these are of a significant environmental concern.

(Refer Slide Time: 31:36)



The fifth enzyme is the isomerases are the function of these enzymes is to catalyse intermolecular rearrangements, which transform the organic molecules into isomers and that are more amenable to suitable oxidation. For example, the racemases which catalyse the L and D amino acid inter conversions or the ligases is these are used to catalyse the covalent bond formation, but similar to the isomerases these can also catalyse reactions that facilitate subsequent metabolism. For example; CoA-ligase which adds the S-CoA as the fatty acids during the beta oxidation reaction.

(Refer Slide Time: 32:15)

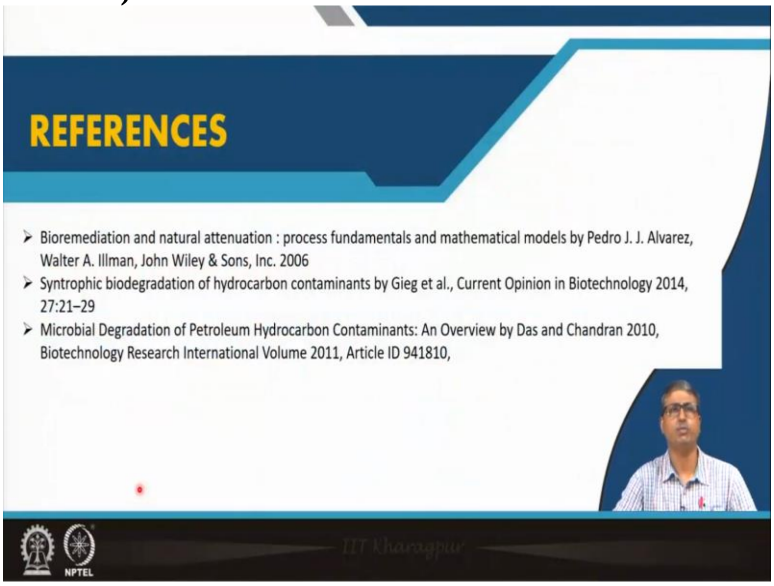


Now, the; 4 common bio the biotransformation or biodegradation reactions performed by the microorganism. So, once we have these different type of enzymatic reactions, these enzyme enzymatic reactions can be arranged under these 4 types one is the oxidative transformation where hydroxylation of alkynes the alkylation of compounds like a hetrogine, epoxidation of TCE type compound and oxidative ring cleavage like aromatic hydrocarbons.

Are all conducted and those types of reactions are called oxidative transformations. Reductive transformations are including the reductive dehalogenation that is halogenated compounds, are all biodegraded nitrile group reduction nitrile, aromatics RDX TNT type of compounds are all biodegraded through reductive transformation hydrolytic and other biotransformations not involving redox processes like hydrolysis hydro climatic dehydrogenation de hydro halogenation are also there.

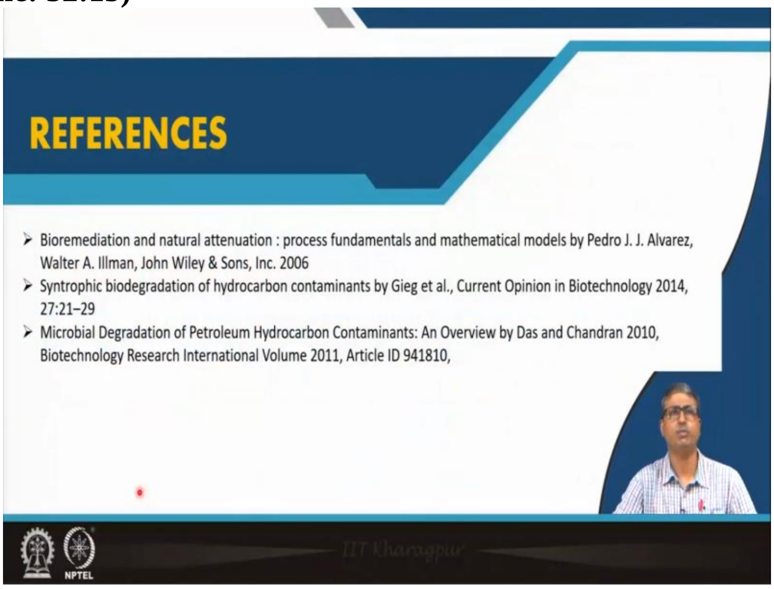
And the last one is the synthetic reactions which includes the carboxylation and methylations, so, that the molecule can be incorporated into some anabolic reactions and then incorporated into the biomass.

(Refer Slide Time: 32:15)



So, for this part of the lecture, mainly these are the following literature and references particularly the book written by the Pedro Alvarez and illaman Walter illman will be most important.

(Refer Slide Time: 32:15)



In conclusion, in this particular lecture and possibly the last lecture on the biodegradation part, the various requirements of biodegradation are discussed and the common biotransformation reactions, enzymes and the basic principles of aliphatic and aromatic compounds under aerobic and under anaerobic conditions particularly for aromatic compounds are discussed in detail. Thank you.