Environmental Biotechnology
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Lecture – 39
Biodegradation

Welcome to the next lecture of our course on environmental biotechnology. In this particular lecture which is of lecture 39 we are going to discuss about biodegradation which is of course a very important part of or the component of bioremediation itself.

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In this particular lecture we are going to have the following concepts to be covered. The concepts of biodegradation of organic compounds will be discussed followed by the role of microbial particularly the bacterial metabolism that is considered as the bacterial engine in biodegradation process and the definition of biodegradation terms will also be presented.

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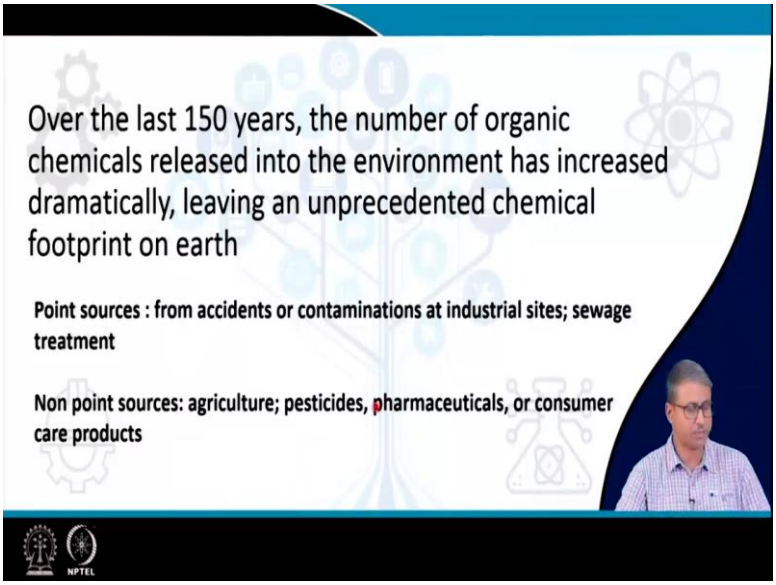

Now, with respect to organic compound pollution or organic pollutants are present in our environment. It is observed that over the last 150 years the number of different organic chemicals released into the environment and also the quantities of the organic chemicals released into the environment which are mostly highly toxic and hazardous and due to the toxicity and hazardousness of these organic chemicals they are released into the environment has increased kind of a footprint of this kind of chemical toxic substances on the Earth.

And when we consider these different type of organic chemicals or organic pollutants released into our environment we find that there are point sources as well as non point sources of emissions. The point sources include the organic pollutants or organic chemicals released from different accidents or contaminations at industrial sites, some of the sewage treatment plants are also responsible for releasing untreated pollutants.

Whereas the non-point sources are identified to be mostly the agricultural activities, agricultural sites where the pesticides and fertilizers and different other pollutants are insecticides are being used, herbicides are being used as well as different pharmaceuticals or consumer care products.

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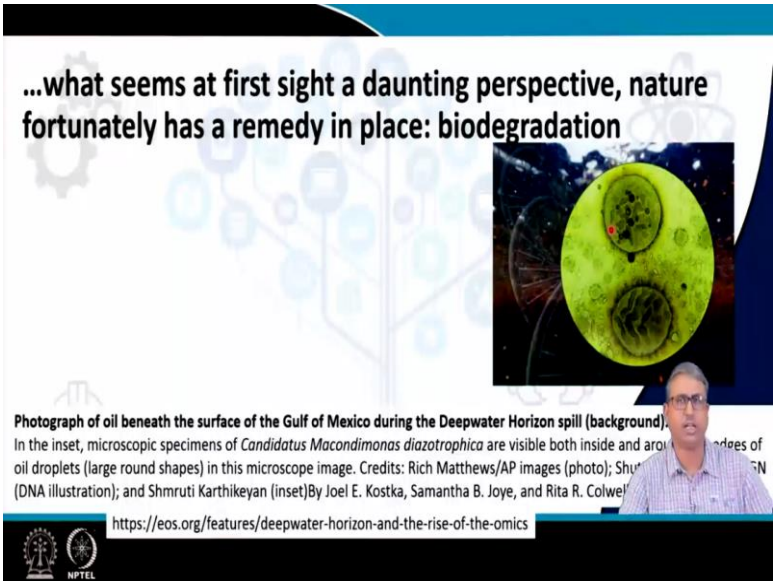

Now, what seems at the first sight a daunting perspective nature fortunately has a remedy in place and this mode of remedy or the remedial process which is natural is called biodegradation. Now, I will show you some of the very pictures of the accidents which led to the spill of huge amount of oil in the marine environment and that happened in the year of 2010 that is called the deep horizon water spill (()) (03:47) horizon spill in the Gulf of Mexico.

And this is a photograph taken from the some airborne vehicle and this is the sea surface which is found to be coated thickly with oil and you can also see some of the ships which are working here in order to combat or control the spread of the oil into the rest of the areas of the seawater. Now, when we try to highlight that in spite of that huge amount of oils being spilled into the natural environment.

Microorganisms are always there and these microorganisms are naturally present in the contaminated sites. So, one of the pictures basically this is the photograph of oil both the surface of the Gulf of Mexico, the background; the background is basically representing the contamination which has happened and also the foreground the greenish yellow color circle this is the representing the microscopic specimen of some bacterial groups that is called or identified as Candidatus Macondimonas diazotrophica.

These are visible both the inside and outside of the oil droplets. So, these circular structure the inner circle is basically the oil droplet and one can identify that the bacterial strain which are identified through some molecular technique or belonging to this Candidatus

Macondimonas both present inside the oil droplet as well as adhering to the oil droplet. So, that confirms that these bacteria or these microorganisms are naturally present.

And they have some interest with the petroleum or the hydrocarbon or the different other types of organic pollutants which are present because of a part of the contamination events.

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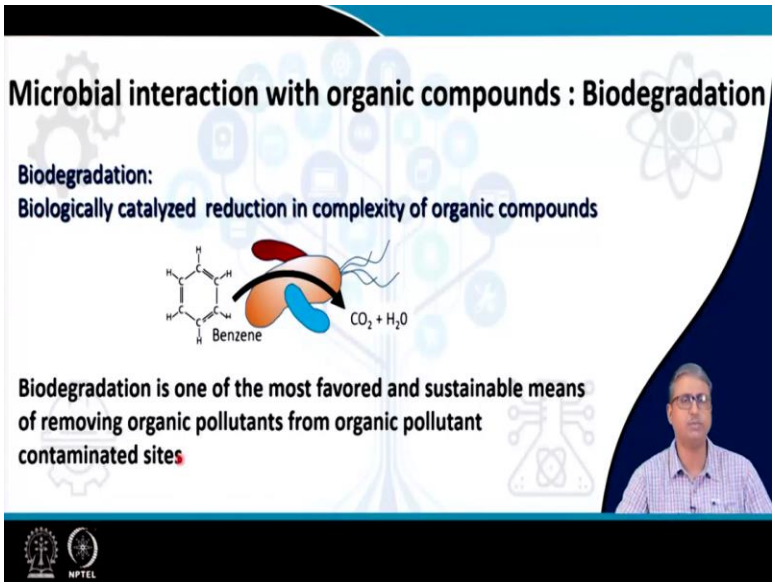

Now, these type of processes where we see that the microorganisms are interacting with the organic compounds is a natural way or natural manner because in most of the cases we see that organic pollutant rich sites environment are full of such microorganisms bacteria especially who are capable of interacting with these organic pollutants, toxic organic compounds.

So, that type of interaction where microbes are interacting with the toxic organic chemicals referred as the biodegradation. So, it is especially defined as a biologically catalyzed reduction in the complexity of the organic compounds. So, biodegradation is basically overall is a microbial interaction with the organic compounds. For example here I have given the Benzene structure as representative of a aromatic organic compound.

Now you can consider the aliphatic compounds as well and polyaromatic compounds and other substituted and more complex organic compounds as well. So, this is just an example that the organic compounds like benzene is used by these microorganisms or microorganism or microbial cells and these microbes they are capable of catalyzing these the benzene structure basically.

And resulting into the breakdown of the complete structure of the benzene molecule in this case and producing carbon dioxide and water that is a kind of a representative picture. The idea is to highlight that the biodegradation process so the process is called biodegradation where microorganisms are interacting with the organic chemicals and these interaction is leading towards reduction in the complexity of the organic compound.

So, reduction in the complexity means these complex structure maybe having couple of double bonds or single bond or some other substitution sometimes. All these chemical complexities will be reduced, reduced in the sense they will be broken down. So, they will be most of the cases oxidized in order to have the electrons released from these organic compounds and the microorganisms will be capable of getting some benefit or gaining some advantages out of these reactions.

So, in a summary the biodegradations are referred as the biologically catalyzed reduction in the complexity of the organic compounds. So, in this case the benzene molecule is converted to carbon dioxide and water. In some cases we may find that the benzene molecule is converted to only some intermediates like Pyruvic acid or acetate which are the important metabolite or intermediates of the central carbon metabolism.

So, in that case it is also a biodegradation because the complexity of the original target molecule is diminished. Now these biodegradation process which is a natural process and where microorganism interact with the organic compounds naturally and they diminish or reduce the complexity of the molecular structure is one of the most favored and sustainable means of removing organic pollutants from organic pollutant contaminated site.

So, we have been practicing this; practicing in the sense the microorganism are being utilized to remove organic pollutants from diverse waste materials for several decades now and we are able to actually decipher most of the cases, the mechanism, the biocatalytic mechanism through which microorganisms are capable of interacting with these organic pollutants and is facilitating the reduction in the complexity of these structures.

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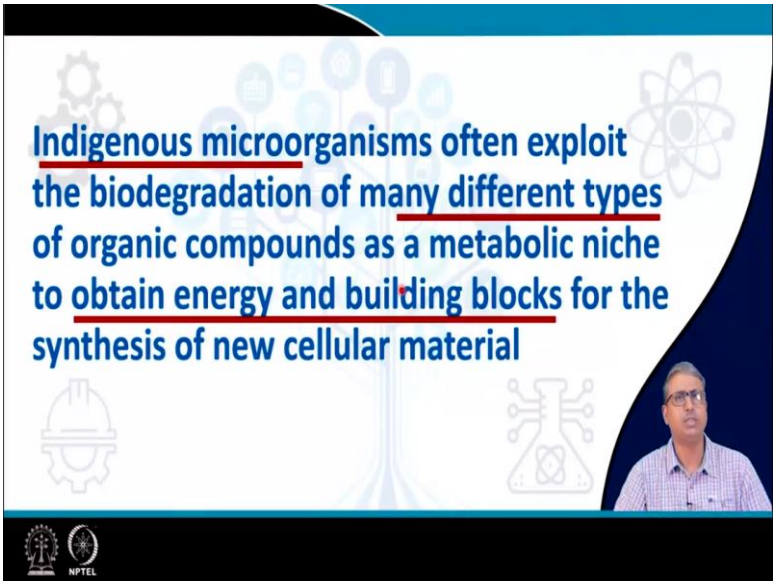

Now, indigenous microorganisms as I mentioned earlier that these are natural process. So, these microorganism the biodegradation is a natural process. So, indigenous microorganisms present within a contaminated site or otherwise present in the natural environment often exploit the biodegradation of many different types of organic compounds as a metabolic niche to obtain energy and building blocks for the synthesis of new cellular material.

So, this is a kind of a spontaneous process it is catalyzed by the enzymes which are present within this microorganisms or the bacteria who are capable of taking part in the biodegradation process and they are also beneficial for the microorganisms who are involved in this process as you can see it has couple of information within it. Number one, it is the indigenous microorganisms that means the microorganisms which are already present in the environment.

For example in Gulf of Mexico oil spill I have shown you the photograph where the scientist have noted that the organism the Candidatus group of organisms are naturally there and they are binding to the oil molecules and they are trying to metabolize the oil molecules. So, eventually possibly they will degrade the oil molecules so these are the natural microorganisms number one.

Number two that these organism are capable of exploiting different types of organic, they exploit this organic compound as they are nutrient resource and they have the suitable enzymes within themselves and they utilize as a part of their metabolic activities within their niche. And as they utilize these compounds diverse array of compounds of the particular

sometimes the particular category like in a aromatic group you may have a particular category aliphatic are category, poly aromatic hydrocarbons are of other categories and so on and so forth.

So, these compounds are used by this microorganism as a resource to obtain the energy and also the building blocks for the synthesis of new cellular material. So, I will elaborate this in subsequent slides.

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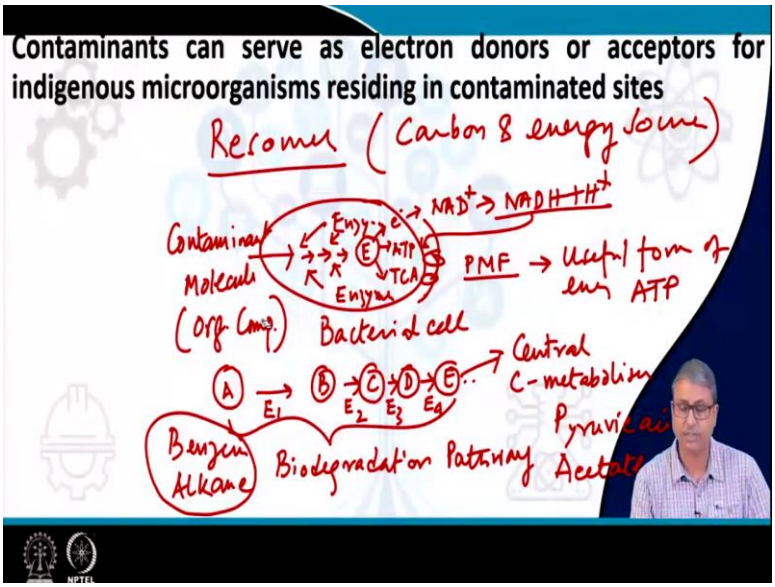

Now the contaminants as I mentioned can serve as electron donors for all acceptors for indigenous microorganism residing in a contaminated site. So, this is one of the most fundamental fact that we are able to identify maybe several decades ago that the microorganism who are present there. So, the natural microorganisms so these are the bacterial cell.

So, when you have a contaminant molecule which is basically the organic compound in this case we are discussing. So, these molecule is taken up by the cell in its different form they activate and they take it and then they try to deploy a number of enzymes; a number of enzymes are deployed so that the large compound A for example is broken down into smaller intermediate.

And some of these intermediates or the end products of these this is you may consider as the biodegradation pathway which is all catalyzed by individual enzyme. So, we can name them like E 1, E 2, E 3 and E 4 and so on. Now these products or products of these biodegradation

pathways for example E in this case. So this E could be an important resource for the cell itself and often it has been found that these are part of the component of what we call central carbon metabolism.

So, how about this example that if we start with let us say the benzene molecule and we end up with Pyruvic acid or sometimes maybe acetate. So, we start with some alkane molecule and we end up with acetate. So, that means the organic compound which is considered to be a pollutant by us maybe for the microbes also, but microbes are considering them as a resource as a nutrient resource as a nutrient molecule. What type of nutrient as a carbon and energy source.

So, this pollutant organic compound is taken up or the microorganisms are utilizing or interacting with this as a nutrient resource it could actually produce give the microbes the carbon and energy and they are facilitating its degradation by virtue of different enzymes these enzymes are produced by this organism and they are producing some end product. These end products or often intermediate products these intermediates products are part of the central metabolism.

So, they are actually precursor for the other metabolic activities. So, these maybe going towards I can write TCA or tricarboxylic acid cycle. So, some kind of cycle like (()) (15:53) that. So, those kind of cycle they are going to move towards. Also there are certain additional benefits like ATPs are produced and also you generate the electrons. Now these electrons are transferred to the electron carrier like NAD + transferred to NADH + H +.

Now this reducing power which is now having the electrons can eventually donate the electron to the membrane bound carriers. So, all these events are happening inside the cell only. So, they donate the electrons to the electron carrier and thus generating what you call Proton-motive force and by virtue of this Proton-motive force they are capable of generating some useful form of energy that is either ATP or transsmembrane gradient which can drive possibly some cellular process including the Flagellar movement to nutrient transport etcetera.

So, in a summary the contaminants are basically utilized as a resource. Now in this case I have given the example that the contaminants are used as potential electron donors or

acceptor that means these process of utilizing the contaminant as carbon or energy source must have oxidized it. So, most of these process that I mentioned here they are basically in that case oxidative process.

However, there are also processes where reduction or reductive reactions are involved in a sense that the microorganisms are capable of utilizing the organic pollutants like most of the time the chlorinated compounds as electron acceptor and they reduce the compound. So, bacterial cells they interact with them and they reduce the compound in that case also they utilize these pollutant as a nutrient source in a sense that they utilize electron acceptor and when they use it as electron acceptor they reduce it and therefore they gain some energy out of that.

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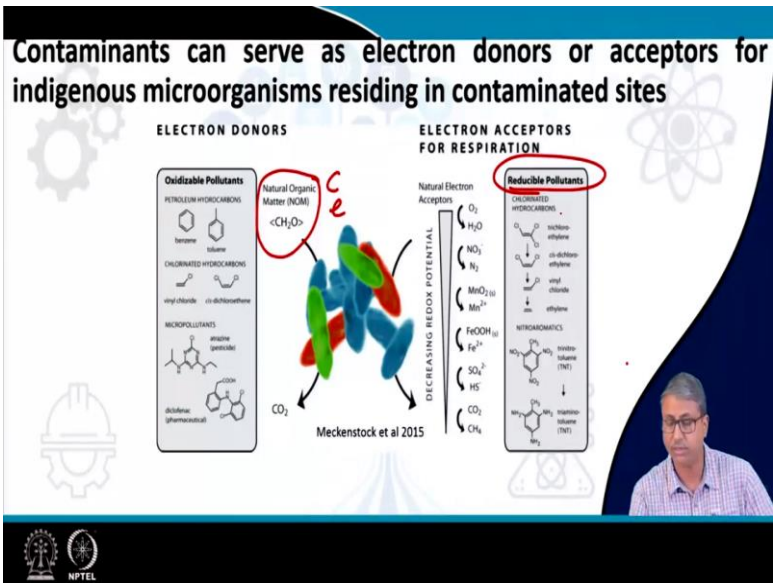

Now moving forward these process can continue for a wide variety of organic compounds. Now, you can see over here that a large number of organic pollutants these are called oxidizable pollutants the pollutants which can be oxidized. So, you can see there are petroleum hydrocarbon like benzene, toluene, xylene, Ethylbenzene there are chlorinated hydrocarbons.

There could be different type of pesticide molecule or pharmaceutical products and different (()) (18:40) of fatty acids molecules, alkane and other molecules. All these compounds can be utilized as organic source. So, they are considered as source of electron. So they are all utilized as source of electron and carbon. So, if they want to use them as a source of electron or carbon they must be requiring some electron acceptor.

So, in that case the microorganism will deploy a natural electron acceptors which are available in the environment. For example in an aerobic environment oxygen will be there, in a anoxic condition as it goes to the subsurface aquifers and subsurface sediments we have found that nitrate followed by manganese or iron oxides or sulfate and eventually nothing else is there and it is highly reducing than carbon dioxide itself will be used as a electron acceptors and the microbes will be able to carry out the degradation.

So, the process essentially will go like this. So, they will carry out the oxidation so they will take out the electrons and these electrons will be transferred to the terminal electron acceptor which is called TEA and the process will continue. So, if oxygen is there it will be aerobic if oxygen is not there they will try to use nitrate or iron or sulfate or manganese or carbon dioxide as they are terminal electron acceptors.

Thereby they will be achieving the oxidation. So, basically the oxidation process of the hydrocarbon molecule will be achieved. Now there is also possibility that some organic pollutants are actually called reducible pollutants like here. They are called reducible pollutants. Reducible pollutants what we observed that the microorganisms are capable of or they intend to reduce them as I mentioned earlier.

And although they may not gain any carbon out of that, but what they gain, they gain the degradation of the compound and they are able to transfer the energy or the electrons to them. So, in that case these chlorinated or halogenated compounds will be reduced and when they reduce these chlorinated hydrocarbons they eventually produce or the Nitroaromatic compounds also eventually we produce the less toxic products or often the compounds which will enter into further biodegradation pathways like if we have ethylene or something like that then that will enter into further oxidative processes.

But also we will be able to gain some energy out of this because in that case they will be utilizing the natural organic matter maybe acetate which is available in the environment or will be provided in the environment in most cases these organic natural organic matters are available. So, using the natural organic matter as a source of carbon and energy they then try to reduce these chlorinated hydrocarbons or the nitroaromatic compounds.

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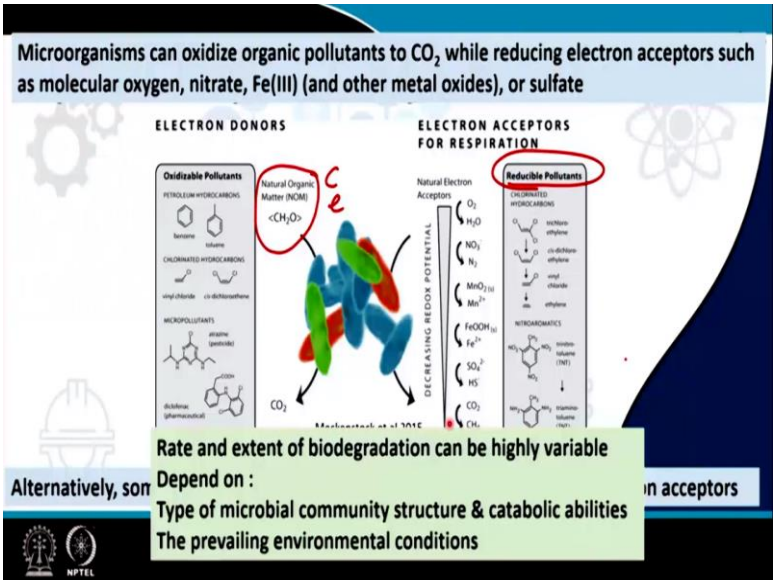

So, essentially these two types of process will happen parallel. In one of the processes what we see that they can oxidize the organic pollutants that is the first process that I explained so they oxidize the organic pollutants to carbon dioxide or certain other intermediates sometimes while reducing the electron acceptor such as oxygen nitrate etcetera as per their availability and the redox conditions.

Alternatively, some pollutants such as the chlorinated solvents or the nitro aromatic compounds can be used as electron acceptor and they can be also detoxified. So, the microorganism they are naturally capable of using these wide variety of processes. So, basically these two processes or these processes can be categorized as either oxidative process or reductive process.

Some most of the cases we see these are oxidative process for certain compounds we see the reductive processes are also beneficial particularly in terms of bioremediation or in terms of removal of the contaminants from the environment. However, naturally these microorganisms they are present they will colonize as soon as the pollution event occurs and we will be seeing that the relative abundance of these organism will increase.

They will associate themselves with the pollutant molecule as we have seen with the photograph that the oil droplets containing the Candidatus organism. However the rate and extend of the degradation is dependent on certain factors and it is actually highly variable. It is variable because fundamentally it is microbially catalyzed process. So, the types of microbial community composition or the members present there.

And their catabolic activities catabolic abilities number one we will control the rate and extend degradation may happen or degradation possibly will happen, but how fast and up to what extent, up to what extent means whether the oxidizable pollutant like toluene or benzene will be degraded up to carbon dioxide or they will be left somewhere in some intermediate like Catechol or some other intermediates which are very common intermediates of these aromatic hydrocarbon degradation.

It depends upon the catabolic ability of the organism anyway naturally these organism will populate and they will be having their increase abundance, but it is a kind of subject to variability. So, sometimes we can see that the suitable community members are already present and they are able to start or initiate the process of biodegradation very rapidly, but sometimes we may need to give some more time so that the organisms naturally occupy those habits and they start interacting with the pollutant molecules in appreciable rate.

So that we see that the pollutant molecules are indeed decreasing and the extent of degradation like the ideal condition would be the carbon dioxide or water if not some intermediates which are the part of central carbon metabolism like Pyruvic acid or acetate which the microbes themselves or other microorganism present in the environment would be happily using.

Second factor would be the prevailing environmental condition. This is one of the very challenging condition factor because there maybe the opportunity through microbes, but because of the prevailing condition like for example the availability of the terminal electron acceptor and also the availability of other nutrients like nitrogen and phosphorous and trace elements and different other requirements by the microorganism.

That is also another important factor. Along with that the temperature pH redox condition of the given environment are given to be very critical for this.

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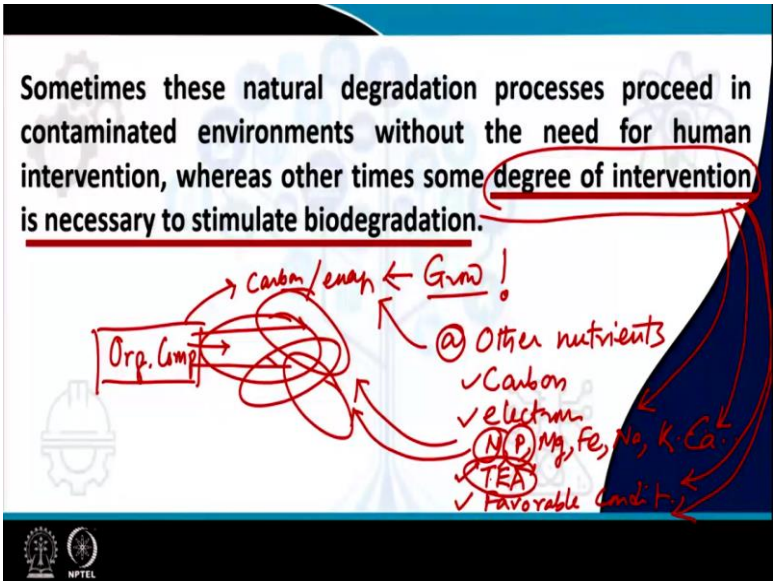

Now sometimes these natural degradation processes proceed in contaminated environments without the need for human intervention where the microorganisms are very efficient, they are capable of interacting with the pollutant molecules and they are capable of providing the sufficient rate of degradation and possibly the extent of the degradation is also expected. What is expected basically (()) (26:47) carbon dioxide or water.

Whereas in other times some degree of intervention is necessary to simulate the biodegradation. Now why do we think that these simulate the biodegradation is required because as I mentioned few minutes ago that the microorganisms are interested so there are many microbes who would be interested to degrade the organic pollutants. So, here you have the organic compound which is to be degraded.

So, there are many microbes who are interested, but if we look at this degradation process, the degradation process organic compound degradation will give them carbon and also give them energy. So, they will be interested to utilize this and will grow, but this growth of the microorganism will require number one other nutrients. Of course, carbon is one of the major nutrients.

So along with carbon and electrons they need other things like nitrogen, phosphorus, magnesium, iron, sodium, potassium many calcium and many other things and also some of them could be electron acceptor. So, terminal electron acceptor would be required. They will also need a kind of favorable condition as I mentioned earlier. So, the degree of intervention refers to arranging these factors the nutrients.

The condition, the terminal electron acceptor and give them the favorable conditions like the pH or the temperature or certain other factors are not favorable then we may need to think of that how do we provide those things so that the pH is actually raised or pH is reduced to the optimum level where this microorganisms are capable of functioning. In general, what we have found that the supply of TEA terminal electron acceptor.

Like the nitrate or iron for example as we discussed yesterday for acetate and other aromatic degradation we found that Geobacter or these bacteria they require iron and similarly the nitrogen and phosphate because any cellular activities would require the macro elements as possibly you know. So, the supply of all these N, P magnesium, iron, sodium, potassium calcium all the essential macro elements.

And as well as the micro elements including those which are considered as terminally electron acceptors are essential. So, the intervention will be in terms of providing these factors which will be kind of a supporting factor which will support the degradation.

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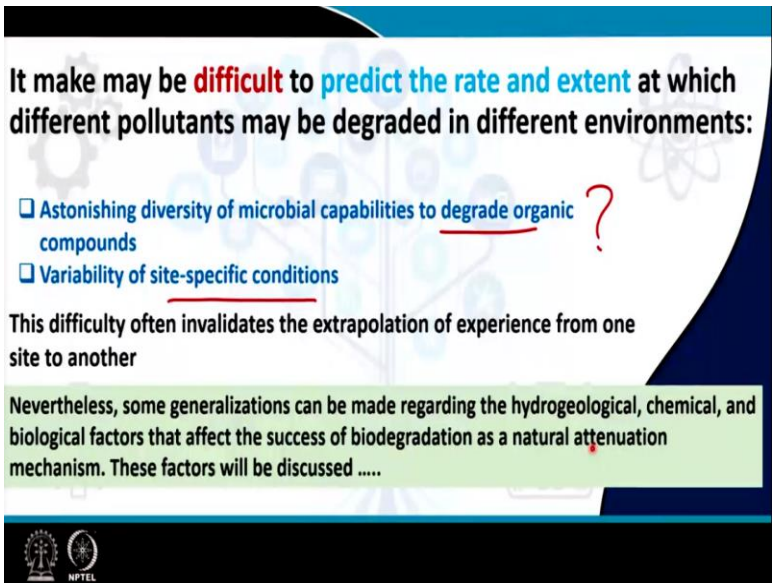

Now, it maybe difficult sometimes to predict the rate and extent at which the different pollutants are actually degraded in different environment. Astonishing diversity of microbial capabilities to degrade organic compounds and also the variability of site specific conditions. So, that is something which makes the thing little difficult to predict the rate and extent so easily.

So, there is a spill or there is a kind of a accidental release of some organic pollutant it is not always so easy to actually predict how and when these contaminant molecules will be totally degraded in that particular environment why so because possibly the diversity of the organism existing in that particular site is often is not known so often we do not know them and also these site specific condition.

Somebody can argue that suppose there is a marine spill somewhere in Arabian Sea or somewhere in Bay of Bengal so we may say that something is already known in Gulf of Mexico. So cannot we replicate that exactly how bioremediation and biodegradations were encouraged in Gulf of Mexico site cannot we replicate that and allow the biodegradation to happen in the Coast of Bay of Bengal or Coast of Arabian Sea.

Perhaps that is not always possible because the variability and the site specific conditions because all the environments are not exactly they maybe saline sea water, but the sea water might have different numerous other factors which are mostly local factors and these local factors actually control the biodegradation process and also the microbial community composition etcetera.

Now this difficulty often invalidates the extrapolation of experience from one site to another the example that I gave for the Gulf of Mexico experience. Nevertheless, some generalization can be made regarding the Hydrogeological chemical and biological factors that affect the success of the biodegradation as a natural attenuation mechanism and during my subsequent slides I am going to highlights some of these points.

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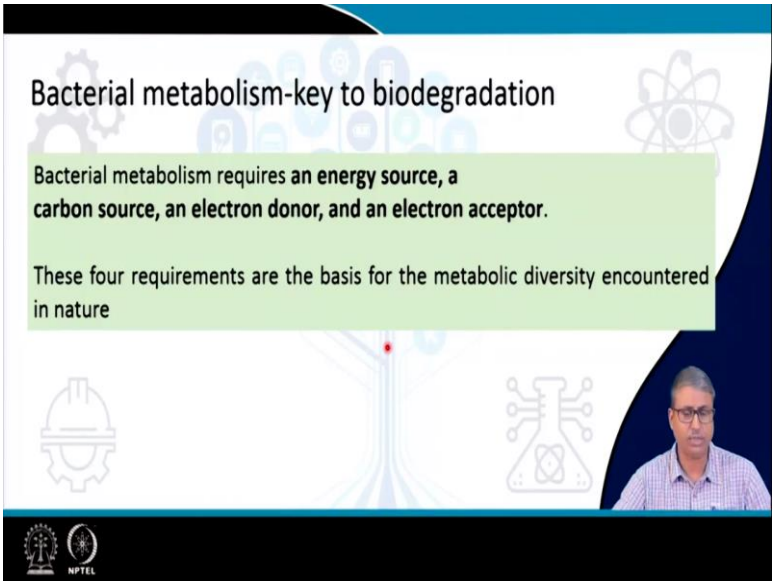

Now one very important point that I am going to talk next is the bacterial metabolism which is key to the biodegradation process. I have already explained part of this, but certain other points need to be explained. So, the bacterial metabolism requires an energy source, a carbon source and electron donor and an electron acceptor. So, these are the four fundamental requirements from bacterial metabolism point of view along with these there will be requirements of the other nutrients like nitrogen, phosphorus and sodium, potassium, calcium, magnesium etcetera.

Now these fundamental requirements the priority requirements are the basis of the metabolic diversity encountered in nature. The other nitrogen phosphorus etcetera are not that critical compared to these four components.

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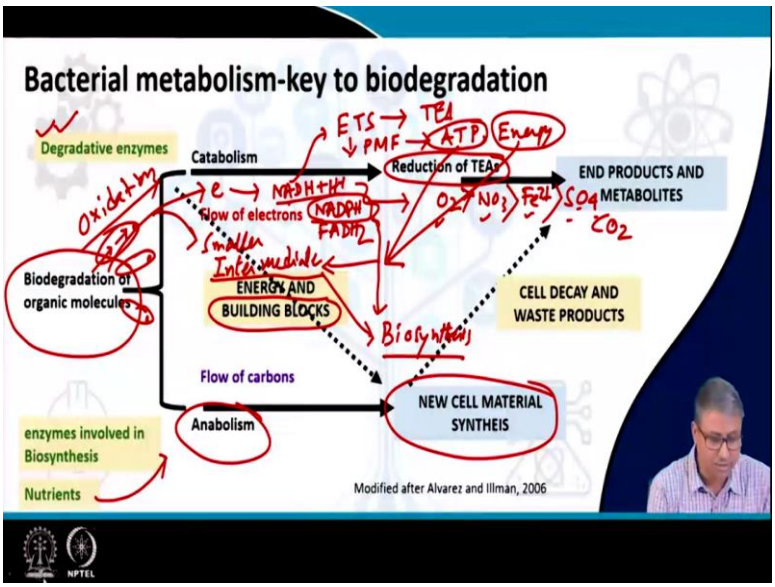

Now when we look at these biodegradation process where the organic pollutants particularly are found to be degraded in a very critical manner. So, we see that you have the organic pollutants. The first set of reactions that the microbes will be able to carry out. So, the microbes will interact with these organic pollutants and they will as I mentioned earlier they will produce the enzymes and they will carry out the oxidation events.

These oxidation events are basically catalyzed by the degradative enzymes. So, the different degradative enzymes produced by this microorganism. So, all these microorganisms will be able to produce the degradative enzymes those who are involved and they will carry out the oxidation of these compound. So, these oxidation of these compounds so it is basically oxidation of these compound will be happening.

And that will lead to a set of biochemical reactions which we called the catabolism. So, the molecule will be broken down it will be oxidized. So, during these oxidation process the electrons will be taken out. Now as the electrons are going to be taken out so the electrons will be taken out and these electrons will be given to the electron carrier like NAD + will take the electron and produce NADH + H + or they will produce NADPH or they will produce FADH2.

Now some of these electron carrier will transfer these electrons to the terminal electron acceptors which are most preferably oxygen if it is aerobic. If not then nitrate will be there, if not then iron will be there if not then sulfate will be there and so on and carbon dioxide will be there either of these will be surely there preferably oxygen followed by nitrate, iron, sulfate and then carbon dioxide.

So, these reduction these terminal electron acceptors will be reduced and the organisms will gain some energy out of this. This will gain energy in the form because as I mentioned earlier as these electron carriers are capable of transferring the electrons of TEA through the membrane bound what you called electron transport system. So, we can have the electron transport system ETS playing a very important role in this.

So, the electrons are transported to TES and when the electrons are transported to TES one obvious outcome is the Proton-motive force and these Proton-motive force is capable of helping the cells to produce some ATP or other forms of energy which the cell can utilize in

terms of transporting ions etcetera. Now these energy or the other transmembrane energy as well as the different small intermediates which are produced during the breakdown process because when the oxidation process is happening the electrons are taken out.

But on the other side we have the molecular breakdown happening. So, for example, in case of a glucose molecule as we have learned earlier possibly in our general biochemistry class the glucose is broken down into smaller intermediates like phosphoenolpyruvate or pyruvate or Phosphoglyceraldehyde etcetera. So, these small molecules will be like for example Pyruvic acid or Phosphoenolpyruvic acid or acetate which are produced they are the building blocks, building blocks for whom?

Building blocks for synthesis of the macro molecules. Now that means the electrons which are released and the small molecules; so smaller intermediates. For example if we have a alkane chain being biodegraded. So, you will have the acetate molecules produced. So, these smaller intermediates maybe two carbon or three carbon molecules along with this energy ATP they will facilitate the biosynthesis reaction.

Now when they combine together allow the biosynthesis reaction that becomes the part of anabolism because the anabolic reactions are those reactions where we have the enzymatically driven biosynthesis reaction and as you know these anabolic reactions are the energy requiring reactions. So, they need energy; so these energy will come either from directly from the ATP or the energy which is transiently with these NADPH for example.

So, these may also give the energy to the biosynthesis. So, you have the readily metabolizable form of energy that is the ATP or the reducing equivalent who is carrying the energy that is NADPH you have the building blocks produced during the oxidation process all things putting together you will be having the anabolism process going on. So, the microbes who are degrading the pollutant molecule basically deploying the degradative enzymes and facilitating the catabolic reactions.

Eventually they are facilitating anabolic reactions also where the other sets of enzymes which are involved in the biosynthesis reactions are there. One important point is here that other than the organic molecule which is going to provide or giving the energy and the small carbon intermediates to facilitate the biosynthetic reactions the cells must be provided with a

nutrients because at some point of time the cells will require the phosphorus or the nitrogen or the magnesium etcetera for different cellular biosynthetic reaction.

And all these nutrient and anabolic reactions putting together you will have the new cell materials synthesized. So, these biodegradation will lead to the synthesis of the new cellular material. Now there is another thing also there that it is not only about the biosynthesis of the new cell material that means the cell growth which is facilitated by this organic pollutant biodegradation.

But it is also true that this cell material itself will undergo decay after sometime because it a natural in the microbial system the growth and decay are coupled. So, after time the cells will start decaying so as the cells are going to decay or following their death the cell mask will be decomposed by different hydrolyzing enzymes. So, that decay and hydrolysis of the cell macro molecules will produce different end products.

And those products are also including some of the metabolites might be which are residual metabolites stored inside the cells. So, eventually you will be able to get the different end product and the metabolites. Now in a complex set up when many microbes are there. So, some microbes might be utilizing the organic compounds as the nutrient resource and growing and making cell mass, but after their death their cell mass is broken down, hydrolyzed and it is given rise to many small, small molecules and a pool of molecules.

Now these small molecules and the pool of metabolites will encourage other microbes, others to grow there. So, we started with the biodegradation of organic pollutants which are facilitated by some key microbial species who are catabolically very active and proficient, but eventually they will promote the entire community function because a flow of nutrient, flow of carbon and flow of energy will establish and that will allow other microorganisms to enjoy the benefit of this biodegradation.

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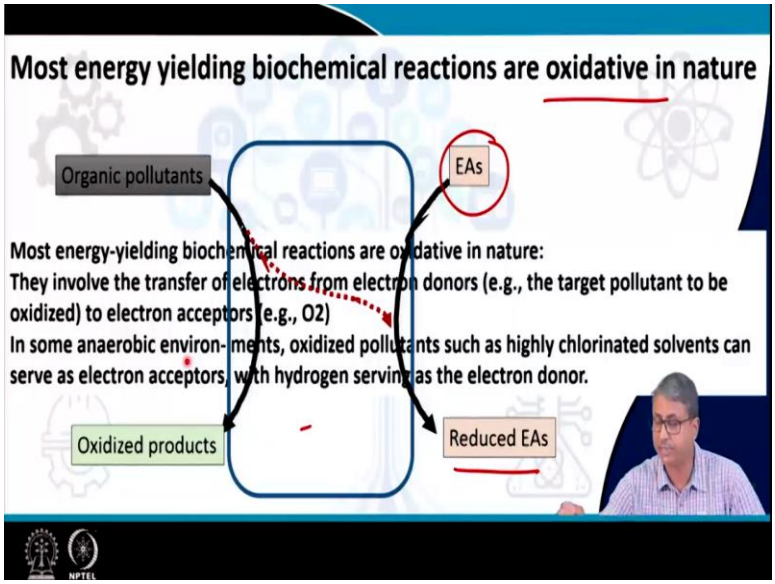

Now, another important aspect is that the most energy yielding biochemical reactions what we have seen just now are basically oxidative in nature because in this case the organic pollutant which is reduced molecule basically they are getting oxidized and diverse array of enzymes are being deployed. These are called oxidative enzymes with initial few hydrolyzing enzymes, the oxidative reactions will begin and we will learn about detail of these oxidative reactions in our subsequent lectures.

So, ultimately we will have the oxidized product. These maybe Pyruvic acid, these maybe acetate or it may be carbon dioxide, but during these oxidation process. So, this is one major event from environmental point of view we need this that my pollutant molecule maybe the benzene molecule is converted to carbon dioxide. So, I am very happy, but at the same time what is the reason that microbes will be interested in this.

Microbes are interested because they are getting electrons out of it and they are getting the carbon out of it and these electrons when they pass to the electron acceptors these maybe transient electron acceptor which are the reducing powers like Nicotinamide adenine dinucleotide phosphate or FAD etcetera. Thereby, generating the reduced electron acceptor or they maybe terminal electron acceptor.

So, they generate huge amount of energy in the cell. Now using that energy the cells will basically grow and as they grow they allow the growth of the other members of the community as well because the metabolites released by these cells either during their own

degradation or during their death and decay these will facilitate the growth and activities of the other microorganisms.

Now the most energy yielding biochemical reaction that we see here are oxidative in nature and they involve the transfer of electrons from the electron donors like the organic pollutants as I explained are acting as electron donor and they are able to transfer the electrons to the electron acceptor terminal electron acceptor for example like oxygen or other electron acceptors if oxygen is not available or under anoxic condition.

Therefore in some anaerobic environment the oxidized pollutant including the chlorinated solvents or nitro aromatics can also be acted as the electron acceptor and therefore they may facilitate the biodegradation of those compound as well.

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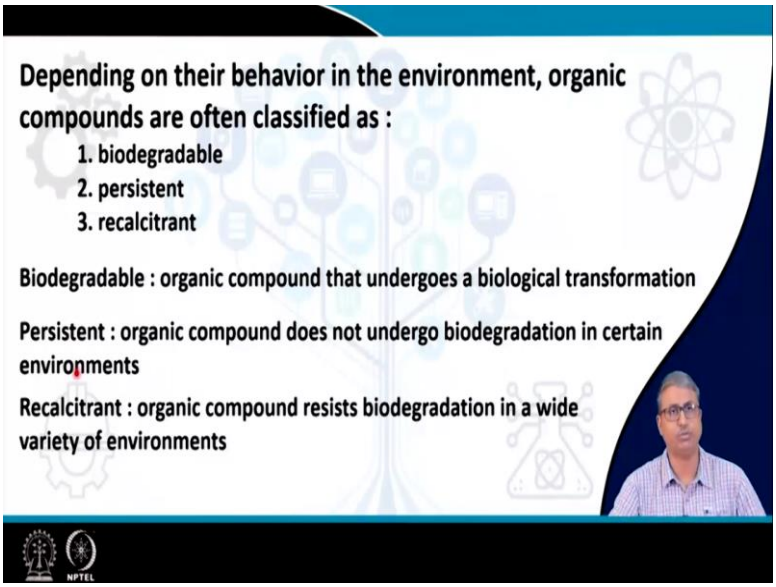

Now depending upon the behavior in the environment the organic pollutants or organic compounds are classified as biodegradable persistent and recalcitrant. Biodegradable are the compounds which undergoes a biological transformation, any organic compound which undergoes biological transformation will be considered as a biodegradable, persistent or the organic compound which does not undergo biodegradation in certain environments.

But might be undergoing biodegradation under certain other environments, recalcitrant are those compounds. Organic compounds which resist biodegradation in a very variety of environment.

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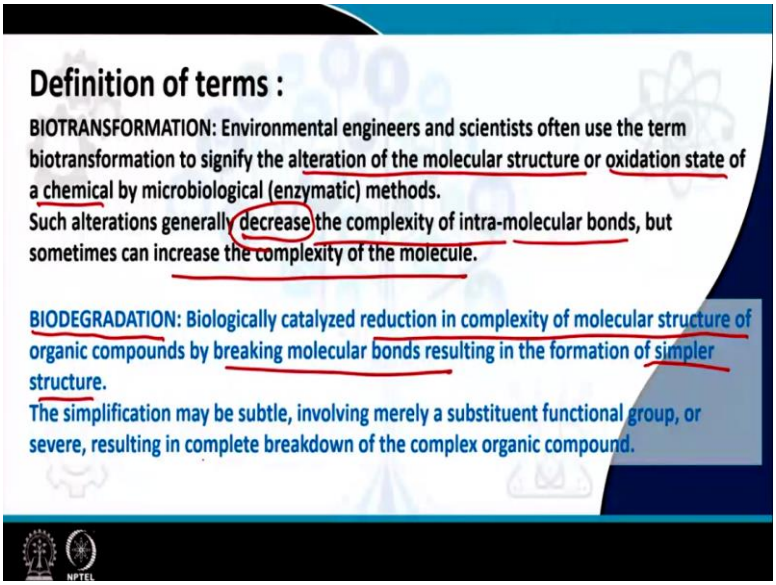

Now with respect to the definition of the terms that we are going to use or commonly use in biodegradation as well as in bioremediation field. Number one biotransformation environment engineers and the scientist often use these term bio transformation to signify the alternation of the molecular structure or oxidation state of a chemical by microbiological or enzymatic method.

So, it must be noted that there are two aspects included within it. So, one is basically these alternation of the molecular structure or oxidation state of the chemical so both could be biotransformation in a broad sense. So, in case of organic molecule possibly it is more suited that we change the molecular structure like a long chain of alkane is converted to small acetate molecule or a benzene ring is broken down to Catechol.

And finally we get some kind of intermediates which are very close to the Pyruvic acid and other glycolytic intermediates or TCA cycle intermediates or in case of some heavy metals etcetera the oxidation sates like the arsenic, uranium, chromium etcetera though oxidation states are altered. So, both can be biotransformation (()) (46:26). These alterations generally decrease the complexity of the intra molecular bonds particularly in terms of the organic molecules which we are discussing today the biotransformation includes such alteration of molecular structure which actually decrease the complexity of the intra molecular bond.

For example if we look at the benzene ring the benzene ring which is having a multiple double bonds which is broken down eventually up to the intermediates like as I mentioned Catechol and other intermediates and finally to the semialdehyde and Muconic acid to

Pyruvic acid or some molecules like that those are actually central carbon metabolites, but sometimes they can increase the complexity of the molecules.

There are some cases where we see that biodegradation actually leads to the complexity of the molecule itself. So, we will discuss about them in due time. The other process is called biodegradation very specific term. So, biologically catalyzed reduction in the complexity of the molecular structure it is a very specific term for organic pollutants where the biologically catalyzed reduction in the complexity of the molecular structure is achieved by breaking the molecular bond and resulting into the formation of simpler structure.

Now these simplification maybe subtle involving nearly a substituent functional group or severe like resulting a complete breakdown of the complex organic compounds. So, if sometimes organic compounds are having nitro groups or other groups like hydroxyl group attached to it. So, removal or the substitution of those functional groups might help in biodegradation like replacing a hydroxyl group with some group or a nitro group with a hydroxyl.

And then hydroxyl group with a hydrogen would actually facilitate the degradation process or it could also lead to the complete breakdown. So, both are included in the biodegradation.

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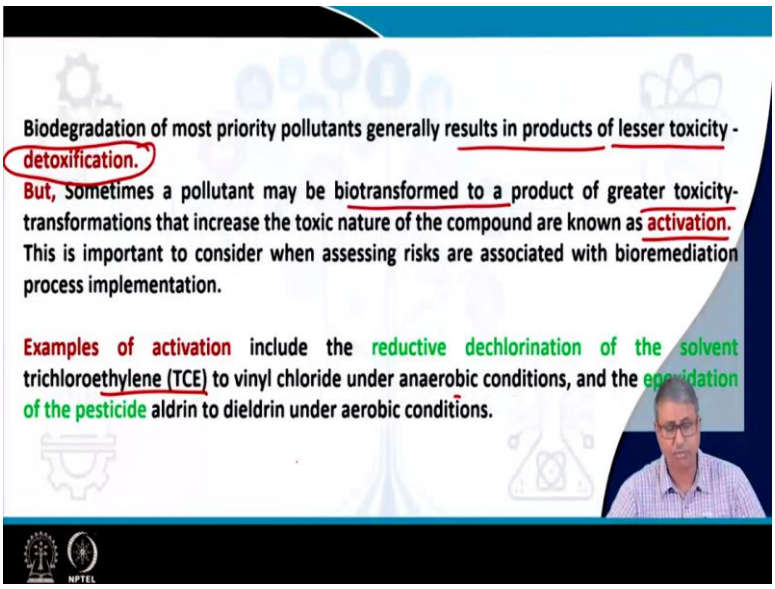

Now biodegradation of most of the priority pollutants which are commonly present in our environment the results into the products which are of lesser toxicity and in that case we called these biodegradation as detoxification also. So, in that case sometimes the

biodegradation is synonymous to detoxification, but not necessary in all cases that the biodegradation will lead to detoxification necessarily no not that.

In some cases we see that the bio transformed product are having more toxicity maybe an intermediate, but it is actually having more toxicity and in that case we call it activation. So, biodegradation in these case is leading to the activation of the molecule. It may be possible that these activated molecule will be now or the next round it will be subjected to another round of microbial attack and it will lead to further degradation of the molecule.

Now this is important to consider when assessing the risk, associated with the bioremediation process implementation because many a times what we see that in a mixed waste environment or where the contaminated site is not clearly a characterized. There might be some compounds who will be actually activated and leading to the toxicity or the hazard of the remediation process eventually.

One the bioremediation engineer or the environmental biotechnologist must be careful about these process. Now example of the activation include the Reductive dechlorination of the solvent trichloroethane or trichloroethylene to Vinyl chloride under anaerobic condition and the epoxidation of the pesticide to diallyl under the aerobic conditions. So, these are considered to be some of the activation reactions.

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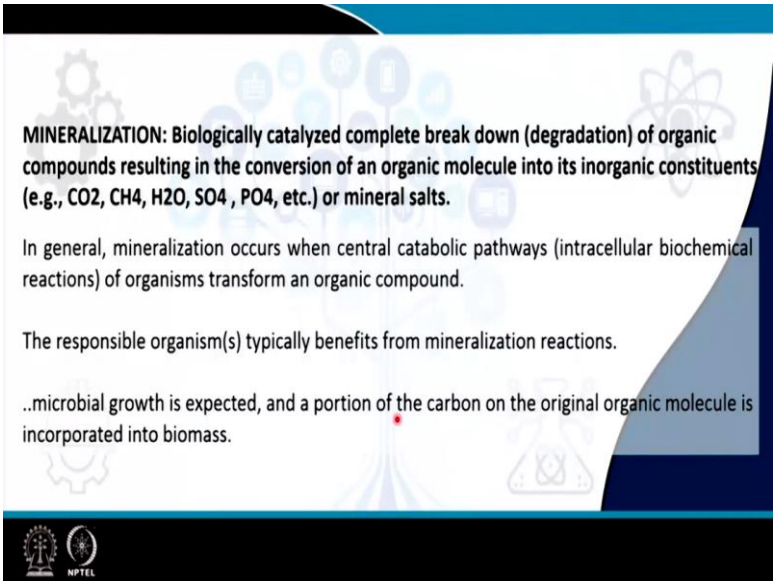

The next and the last one for the today's class is the mineralization. This is biologically catalyzed complete breakdown of the organic compound. Here in biodegradation context the

mineralization is the word is used to refer complete breakdown of the organic molecule, resulting in the conversion of the organic molecule into its inorganic constituents like carbon dioxide or methane water sometimes if it is having sulfur or phosphate attach to it.

So those groups will be released and these will be actually then used up by other cellular activities as their nutrient source or their mineral forms. In general mineralization occurs when central catabolic pathway that is the intracellular biochemical reactions of organism transform an organic compound. Many a times it happens actually so most of the cases we have seen these aromatic hydrocarbons aliphatic hydrocarbons they are mineralized.

That means with the involvement of the central carbon metabolism sometimes ago I was mentioning that the biodegradation might lead to intermediates which are actually the precursor or the part of the central carbon metabolism. So, that means it goes to the central metabolic pathway. Therefore, in that case it will be leading to complete breakdown of the molecule and that is actually mineralization that is the organic compound will be converted to carbon dioxide, water, methane etcetera.

The responsible organisms typically benefits from mineralization because it is a complete breakdown means the oxidation event is allowed completely so lot of electrons and lot of carbon are gained and because it is having so much of energy electrons are being released as I discussed earlier because of the availability of so many electrons and the carbon molecules the organism will be able to metabolize them.

And they will be growing so microbial growth will be expected and a portion of the carbon on the original organic molecule will be incorporated into the biomass. So, directly the organic pollutants if we are able to level isotopically those carbon molecule in a laboratory experiment we will be able to find out that the biomass which is formed out of the biodegradation process is actually having those carbons in their cell itself.

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So, for this lecture these are the following references particularly the biodegradation and natural attenuation book by J.J Alvarez and Walter Illman will be very useful and also there are certain other information available in this other two articles.

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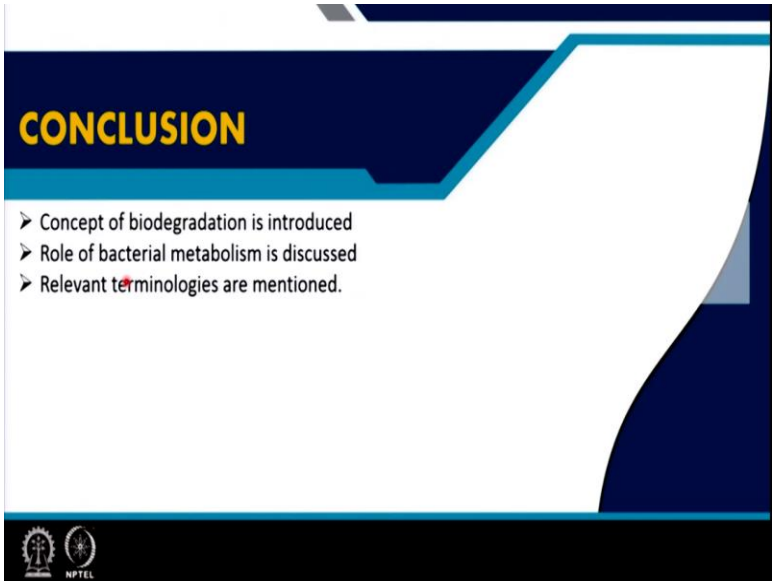

In conclusion the concept of biodegradation is introduced, role of bacterial metabolism and the concept of the bacterial engine in terms of microbial biodegradation of organic pollutant is discussed in detail and relevant terminologies are also mentioned. Thank you so much.