Environmental Biotechnology
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Lecture - 40
Biodegradation (Contd.,)

Welcome to the next lecture of this course environmental biotechnology and this is lecture number 40. And in this particular lecture we are going to discuss further on the topic of biodegradation of organic pollutants. So, in this particular lecture we would be highlighting some of the very important aspects particularly the requirements of biodegradation of organic pollutants.

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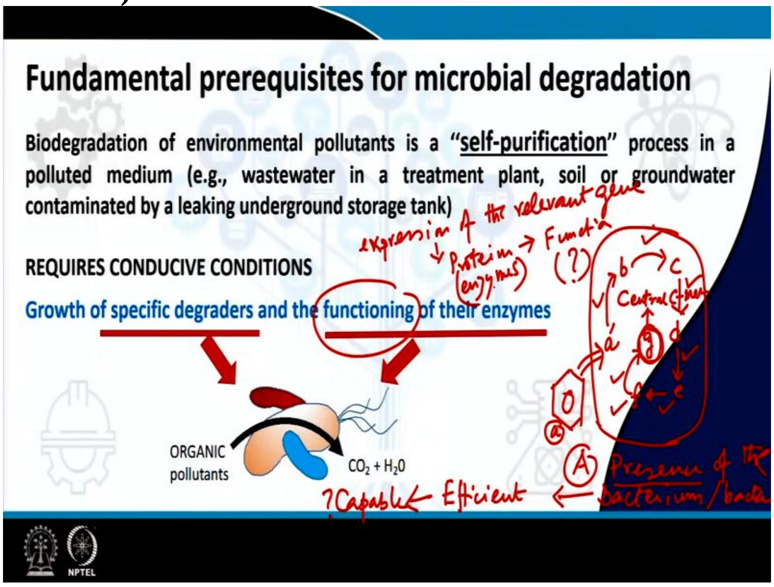

Now fundamental prerequisites for microbial degradation of organic compounds, organic pollutants are very well studied for the last more than maybe 100 years now and particularly as we understand with the advent of different culturing techniques and characterization of microbial metabolic properties their genomes etcetera. We have gained the considerable understanding about what are the factors? What are the requirements?

That actually controls the microbial biodegradation of organic pollutants particularly when it is come to the environmental application of those organisms are towards the degradation of the pollutants. Now one of the fundamental prerequisites for this process is the understanding that biodegradation of environmental pollutants is a self purification process by nature in a polluted medium.

So, any kind of polluted environment will have this self purification process set in by virtue of the microorganisms bacteria, archaea, fungi they will be naturally growing in that or those environments and this could be the wastewater treatment plant as contaminated soil or groundwater contaminated with different kinds of pollutants like organic pollutants like leaking underground storage tanks which have been actually a very common phenomenon for many of the sites which have been successfully bioremediated.

So, this concept of self purification explains that the microorganisms which are there in the natural environment that is the indigenious organisms are present even in the contaminated sites they will gradually acclimatize with the contaminants present there and will try to grow and will grow definitely and they will carry out the biodegradation process. However, this self assembling group of organisms which are having the relevant biocatalytic activities towards the degradation of the organic pollutants.

These particular processes require certain conducive conditions and particularly the growth of specific degraders and the functioning of their enzymes have been identified to be one of the most important criteria for the self purification process. Now we have already learned 2 terminologies one is the engineered bioremediation and another is the natural attenuation. So, if we just try to quickly recap the concept of natural attenuation.

Where the organisms who are there in the contaminated site itself are allowed to work on the pollutant molecules and they lead to the degradation and the remediation to be achieved. Whereas in case of engineered bioremediation we have learned that there could be bio stimulation through adding different nutrients or electron donor, electron acceptors etcetera which actually facilitates the existing microbes to degrade the pollutants or we can add selected efficient biodegrading organisms that is called the bioaugmentation.

Now one point whether we are adopting these natural attenuation or we are adopting a biostimulation or bioaugmentation based engineered approaches one thing is required and that is the presence of the specific degrading organisms. Now these organisms could be a versatile type. So, there is no boundary that these organisms must be of pseudomonas type or bacillus type may not be.

And in many cases what we have seen that the self purification processes are carried out by a diverse type of organisms some of the organisms are individually capable of degrading the pollutants while some other organisms they rely on syntrophic interactions between them and then that syntrophic metabolism favors the degradation of the compounds particularly when we expect mineralization to be happening.

Now the one part is the growth of the specific degrading microorganisms and their growth. So, their growth should be promoted and the functioning of their enzymes so, these are the 2 things. So one is the organisms who are to be present there and the other is the functioning of their enzymes. Now if we consider this simple cartoon that these are the organisms who are capable of degrading or carrying out the complete degradation or biomineralization of organic pollutants to carbon dioxide and water.

Now so one point is that there should be the degrader organism let us say some organisms. So, these are the degraded organisms. Now the degrader organisms must be capable of having the complete expression of all the production of the required enzymes which are responsible for carrying out the degradation process. So, ideally it is not merely the presence or absence of the organism only but also it is the presence of the functional enzymes.

So, ideally if we consider that this is just we imagine that this is a microbial cell and this particular microbial cell let us assume is capable of degrading the aromatic compounds like benzene compound or phenol or toluene or xylene or some other poly aromatic compounds. So, this organic pollutant firstly will be taken up inside the cell and the cells will try to act on that. So, these are the 2 points point number 1 is the presence of the organism.

So, presence of the bacterium let us say in this case it is a particular species bacterium so bacteria means only 1 type of bacteria but real strain one particular type of bacterium or it could be multiple bacteria also. Now, one is the presence of the bacterium who is identified to be an efficient and is capable of what capable of degrading the particular contaminant in this case these aromatic compounds number 1.

Now number 2 is this degradation process if we see we will be able to see if I just identify these compounds as a that a will be taken up inside the cell then a will be gradually converted and it will be converted to a number of intermediates and then something will be formed this g will be a part of central carbon metabolism. So, ideally this compound a transported inside the cell and then a will be subjected to a number of enzymatic steps.

And these enzymatic steps will finally lead to the synthesis of some product, that product will be a part of or the precursor for the central carbon metabolism maybe pyruvic acid or acetyl CoA or acetate or something like that. Now as we understand that presence of these bacteria will be necessary because this bacteria is having all the enzymes who are capable of doing this process.

Now, having said so it is also necessary to understand that in a contaminated environment the mere presence is not sufficient the organism who is capable? Who is efficient? Who is having all these enzymes? All the enzymes must be expressed must be produced and they must be able to carry out the reaction. Now one part is the expression of these genes responsible for all these enzymes.

The other part is that even the enzymes are formed the microbes might be requiring some other factors for the functioning of the enzymes as you know that the functioning of the enzyme is a big aspect because enzymes are sensitive to different other factors including the pH, temperature, heavy metals are organic compounds and many other things. So what I said that the presence of organisms is 1 aspect it could be very specific degrader.

Like benzene degrading bacterium or a toluene degrading species or a phenol degrading bacterial strain something like that as well as the functioning of the relevant enzyme to the functioning needs their expression of the gene. So, these will have basically the expression of the relevant gene expression. So, expression of the relevant genes number 1 and from these the proteins will be formed and these proteins will be allowed to function.

And this is actually subject to many other factors whether appropriate conditions are there require these enzymes protein so these are the enzymes proteins. So, whether these enzyme proteins will be able to function or not so that has to be understood very clearly.

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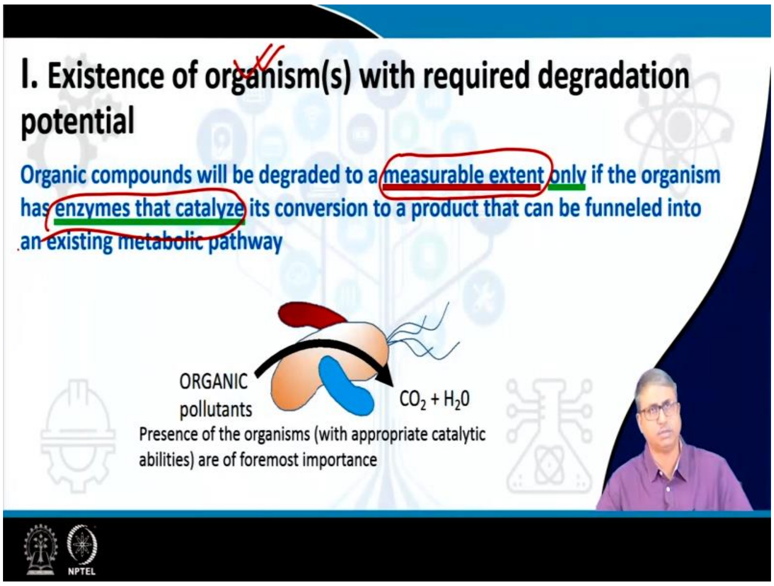

So, with that we proceed to the next major aspect of the requirements for these biodegradation. So, first is the existence of the organism with the required degradation potential. So, in the previous slide itself I have emphasized that the organism that is a capable organisms are to be there. Now microbial ecology experiments are protocols or techniques guide us to answer this question.

That whether the; organisms who are capable of performing a particular degradation is there or not and their relative abundance the weather the organisms are metabolically active etcetera, etcetera. So, we know that how to how to achieve this goal that the existence of the organism so this is for example is done confirmed that the existences of the organism are there. Now with respect to that there are certain other points.

Points are like these that the organic compounds will be degraded to a measurable extent, measurable extent means with which we will be happy our environmental people will be saying that this x concentration x ppm or x gram per kg or gram per liter concentration of a particular substance is now degraded to some microgram or less than microgram per kg or per liter and within a definite period of time so that is very important.

So, they are to be degraded to a measurable extent and that measurable extent of degradation is going to be achieved only if the organism has enzymes that catalyze it conversion to a product that can be funneled into the existing metabolic pathway. So, it is subjected to the expression of the enzyme that I have mentioned in my previous slide. So, the compound has to be converted to some kind of product which can be like in the previous slide I have drawn.

That the product g on the metabolic outcome of all the reactions of the benzene degradation of the phenol degradation like pyruvate or acetate will be funneled into the existing metabolic pathways. Now for example these are the presence of the organisms is required and also the organisms will be capable of producing all the enzymes required for converting the product into converting these target compounds the product molecule. And the product molecule will be fed into the existing metabolic pathway.

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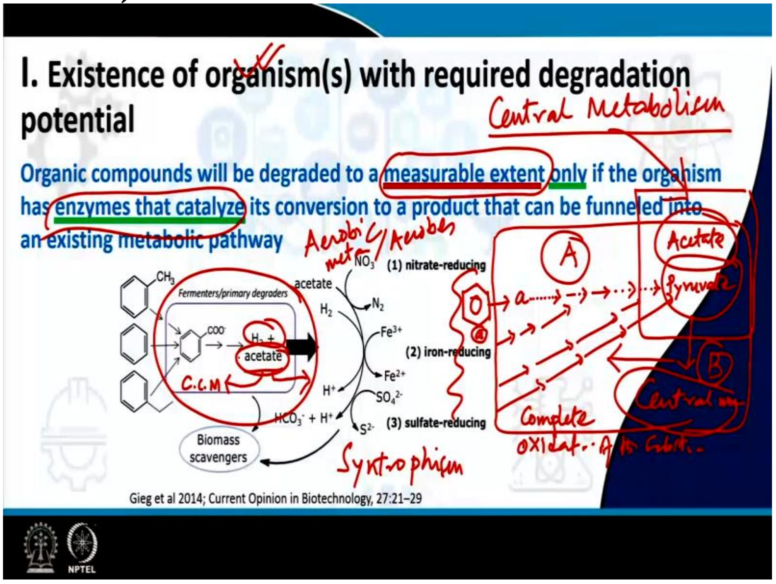

Now in that respect I will also mention before I explain this particular topic or figure that the metabolism has actually 2 steps 1 step for example this is the organic pollutant and here we have the cell. So, this compound is taken up inside the cell which I have indicated as a so a is taken inside the cell and then there are steps there are many steps through which the a is converted gradually one by one to some intermediate let us say the intermediate name is pyruvate.

So, in most of the biodegradation processes we can see that no matter what is the target compound whether it is a poly aromatic compound whether it is a mono aromatic compound whether it is that simple benzene molecule or a phenol or a toluene or it is a alkene molecule of some carbon chain or more than 10 carbon chain or more than 20 carbon chain. So, all these different organic carbons are generally funneled we called funneling.

Because we can have actually here diverse types of organic compounds they will be using maybe different, different steps but eventually they will converge on some reaction some products these products will be something like this pyruvate. Now what is the significance of for example pyruvate it may be acetate also in some cases of pyruvate or acetate. Now what is the significance of having acetate or pyruvate?

Biodegradation of the contaminant organic pollution as I said that goes on in 2 steps. So, this is the first step, step A where the pollutants will be converted to some molecule which is a part of I will write that central metabolism. So, what is central metabolism? In any microbial metabolic process or metabolic landscape we see that there are certain central metabolic processes are going on central carbon metabolism, central nitrogen metabolism, central sulfur metabolism.

So, these are the bunch of pathways bunch of steps bunch of reactions who are facilitating conversion of different substrate to different products and intermediates and these intermediates are feeding into the bulk precursor pool. So that everybody or every other reactions running in the cell can utilize those metabolites. So, this is the purpose of the central metabolism and they are often generating lots of energy.

And reducing power particularly when it comes to the; carbon central carbon metabolism. Now this acetate and pyruvate which are produced as part of the phase A of the biodegradation are actually very important intermediate or starting molecule for the phase B which is the central metabolism. For example you possibly all know about the citric acid cycle or the TCA tricarboxylic acid cycle.

Where the pyruvic acid or the acid molecules can be completely metabolized to carbon dioxide and producing lots of energy as well as other intermediates like succinic acid alpha ketoglutarate acid and all these alpha ketoglutarate etcetera can be utilized in different other cellular biosynthetic reactions. So, the central metabolism is allowing the complete oxidation of the substrates so, both A and B are required.

If we leave it only on A then suddenly the B part because central carbon metabolism is anyway going in the cell all cells they are performing central carbon metabolism. So, eventually it will be running either to TCA cycle or it will go through fermentation process. So, one of the fundamental requirement will be that the organism is capable of degrading this through the phase A as I mentioned to a measurable extent.

So that the organisms will be able to feed these products of these phase A into the central carbon metabolism central carbon metabolism already exists in all cells. All cells all microbial cells bacteria archaea they perform the central carbon metabolism. So, biodegradation will only provide the substrate which will be the starting molecule or some intermediates of the central carbon metabolism so that they will continue.

Now and as we can see in the panel the figure that is presented here now these phase A type of reactions could be basis could be very dedicated reactions like I will discuss about these dedicated reactions. Maybe I will show you some examples and they will produce products like hydrogen acetate etcetera. Now some of the microbial strains they themselves are capable of utilizing them as I mentioned so they will be a straightaway going to the central carbon metabolism.

So, maybe this particular bacteria they will themselves use this acetate for their own central carbon metabolism and they will generate energy out of it. But it may so happen that they release part of this acetate outside and other organisms will be enjoying this acetate or pyruvate as a substrate for their carbon and energy source and they will grow happily on them. So, they could be nitrate reducers they could be iron reducers they could be sulfate reducers.

And they will produce more biomass and some other metabolic products also and this is particularly true when we have an anaerobic condition if it is not an anaerobic condition then possibly some aerobic bacteria. So, some aerobic metabolism by some aerobes will be performing that otherwise this organism will be itself may be capable of degrading this acetate or pyruvate completely.

So, one single bacterium or bacterial strain can be performing the complete degradation or it could be a kind of a joint venture where different other organisms are also associated with the degradation process particularly when we see the process of syntrophism because hydrogen and acetate both are utilized by different methanogenic organisms to facilitate the degradation of the organic pollutants.

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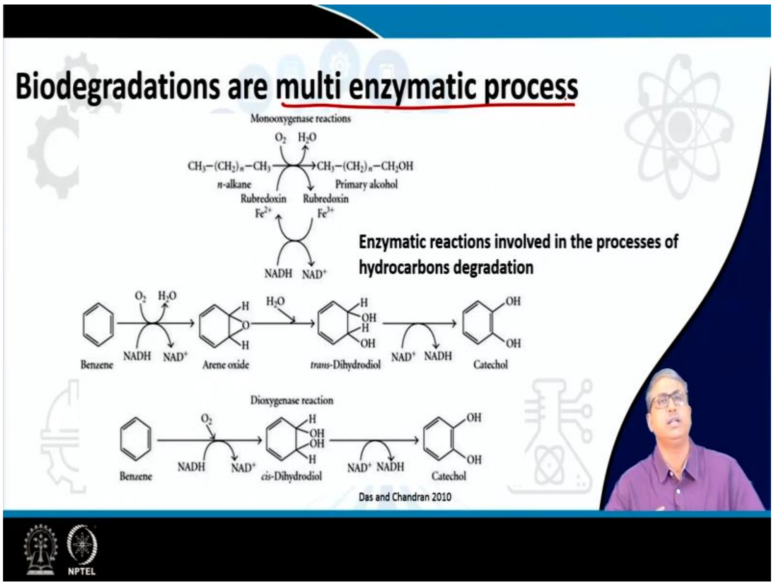

Now the second point with respect to this is, that it is not only that the candidate organism would be there and the candidate organism will be having just the biodegradation steps it is not just about degradation step or biodegradation reaction it is actually a multi enzymatic process. So, in most of the cases we see that you might have noticed that earlier I have drawn A prime A prime to B, B to C, C to D etcetera, etcetera.

Similarly if we look at the biodegradation of most of the compounds these are truly, truly multi step and many step reactions. So, in each of these steps we will have involvement of specific enzymes and these specific enzymes are going to play a role in catalyzing or the converting the one substrate to an intermediate and the intermediate will be converted to another one.

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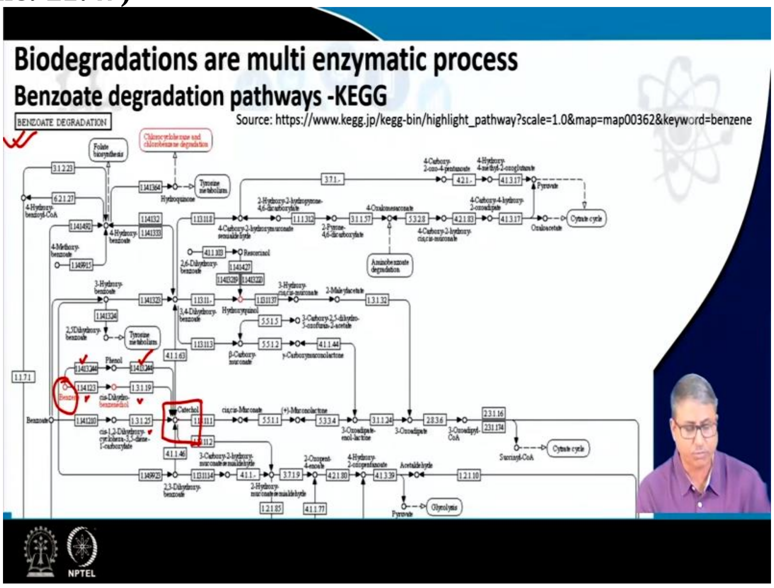

So, let me show you something which is called the benzene or benzoate degradation pathway. So, this pathway is obtained from the KEGG database and the link is provided here. So, you can always use this link to study more and now I will highlight the benzene degradation part. So, here the entire pathway is for benzoate degradation and there are many reactions involved in benzene reaction.

One thing I will show you that will take a part of this and then we will so here you can see that benzoate it is written and this is actually degraded through phenol and finally through catechol because catechol is one of the major intermediates of this degradation process it happens inside the cell. So, the benzene is taken inside the cell and it is metabolism so you can see there are multiple steps.

So, all these numerical values these are actually the EC numbers enzyme classification numbers and in a live form in online form if we just click on that it will show us all the details of these enzymes. So, you can see that there are multiple possibilities through which the benzene or benzoate it can be converted to for instance the catechcol which is found to be a very important metabolite.

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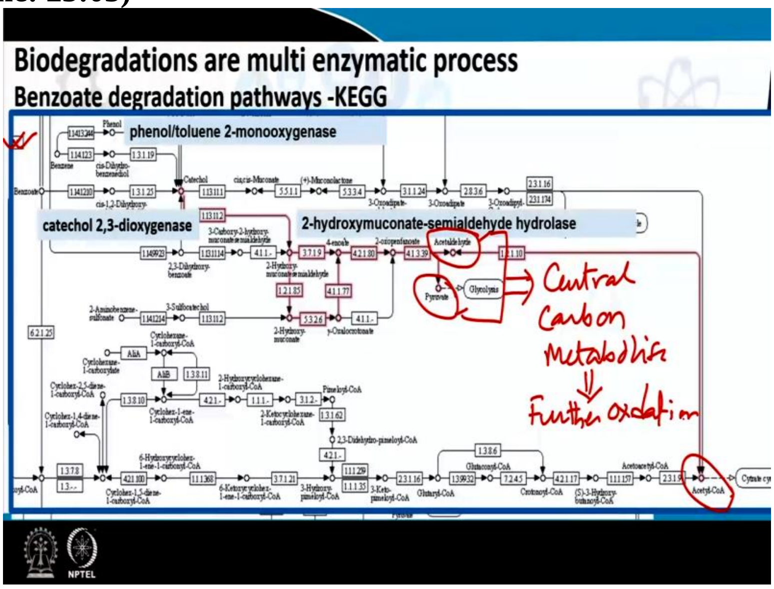

And I will discuss a little bit more on this catehcol. Now I have enlarged this particular part which we wanted to actually discuss in a little more detail. So, this is one of the sections of benzoate degradation where you can see that the benzoate is converted to catechol and this catechol is subsequently catalyzed again through multiple reactions and then all these multiple reactions I will enlarge it further.

So, all these multiple reactions eventually lead to what? Lead to if we consider catechol so 1 line is highlighted here I have marked it for your understanding that you can see that the catechol is converted to some semialdehyde first 2 hydroxy muconate semialdehyde because this is one of the pathways there are many pathways which can so you can see for example there is one pathway running over here there is another pathway which may go like this and that this side.

So, there are multiple paths so we are talking about the red coloured pathway. So, you can identify that the catechol is converted to semialdehyde muconate semialdehyde and then muconate semialdehyde can undergo 2 sets of reactions either in this way or in the this way but essentially whether it goes it will be converted to that oxopentanoic 4 enol and then hydroxy oxopentanoate and then it will be forming the acid aldehyde and pyruvic acid.

And this acetaldehyde and pyruvic acid will be directly fed into the citric acid cycle or TCA cycle because that is a very known pathway of the central metabolic so these acetaldehyde and these pyruvate molecules will straightaway be moving towards the central carbon metabolism which will facilitate the further oxidation and it is not only oxidation along with oxidation they will produce the many intermediates those intermediates will be again acting as a precursor.

Otherwise the complete oxidation will lead to the formation of the energy and the reducing power which the cell will enzyme. So, these are the some of the some of the enzymes I could retrieve.

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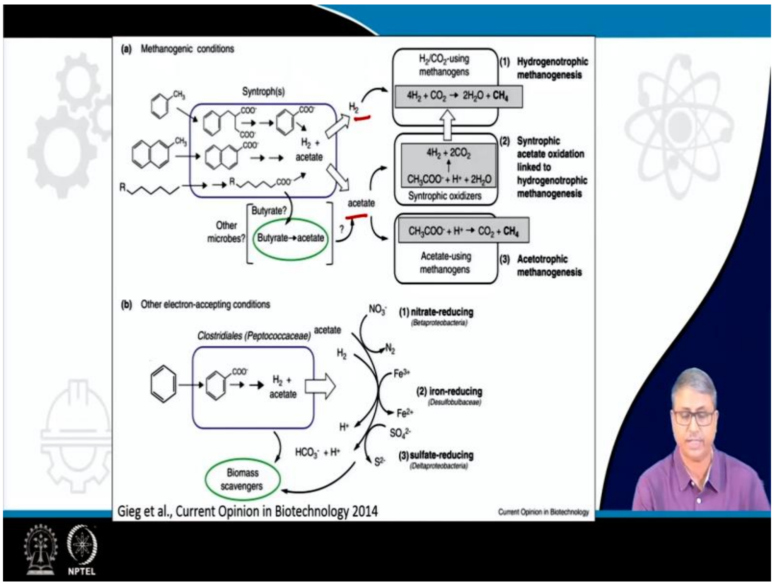

Now as I mentioned that these degradation processes can be happening under both methanogenic conditions where electron acceptors or other electron acceptors are possibly not there. So, directly in the lack of oxygen it is possibly fermented and following fermentation some of the target compounds will be converted to hydrogen and acetate and then different kinds of methanogenic organisms will facilitate the utilization of hydrogen and acetate.

And ultimately they will make the entire process thermodynamically favorable and it is actually an example of the interspecies metabolite transfer like hydrogen and acetate which are produced from the pyruvate and acetate which are produced in this case we have already noticed that we have these acetaldehyde and acetate all are being produced. So, if the oxygen is not available or terminal electron acceptors are not available.

Then pyruvic acid and these acetaldehyde will straightaway be going towards the fermentation and following the fermentation they will produce the hydrogen acetate that will be used by the methanogens. However during the presence of other electron acceptors for example if oxygen is consumed or oxygen is not there in the particular contaminated environment in most of the cases we see the oxygen it used to be there at some concentration level. But eventually the oxygen is used up by the aerobic organism.

So, I will come to that point so then the other electron accepting reactions like the nitrate sulfate iron etcetera they will facilitate the process of degradation.

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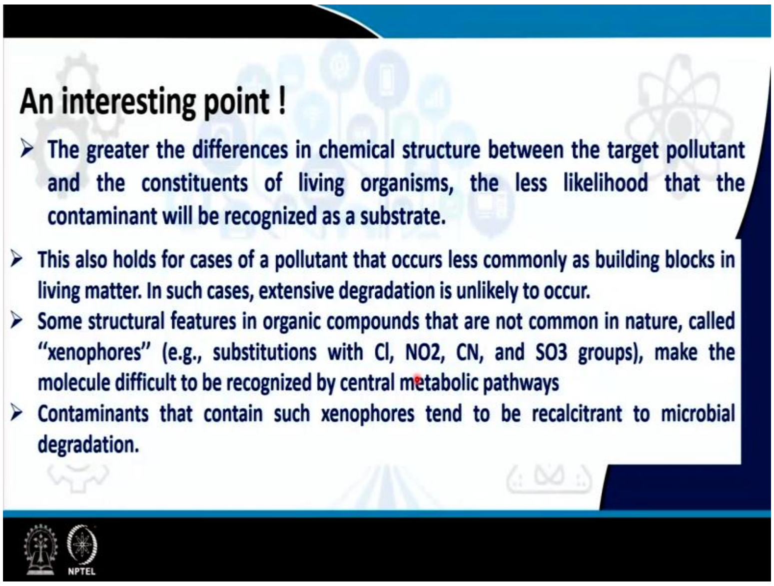

Now with this respect another and very interesting point is the greater the differences in chemical structure between the target pollutant and the constituents of the living organisms the less likelihood that the contaminant will be recognized as a substance. So that means the target compounds we have discussed that the benzene is taken up inside the cell and is degraded up to acetyl CoA or acetate or pyruvate.

Now if the benzene molecule is not identified as a substrate what will happen? Fortunately benzene will never be identified as a not as a substance because microorganisms have evolved with these kinds of petroleum derived hydrocarbon residues or chemical structures for their long, long period of coexistence during the course of evolution but in case some new molecules come out there is a kind of modification in the existing chemical structure of the alkene or maybe in the aromatic rings.

The microorganism if they are unable to recognize them as substrate then they will possibly not be able to facilitate the degradation process this whole for the cases of a pollutant that occurs less commonly particularly if a pollutant is there which is less commonly present as a building block in the living matter like the particular pollutant molecule has never been seen by the microorganisms or part of its products or chemical structures are not there in the central metabolic pathways etcetera.

Then extensive degradation is unlikely to occur because this molecule seems to be new to the cellular system not only from recognition point of view but also the chemical architecture of the entire cell possibly recognizes this molecule as a foreign molecule and as a new molecule for the cell because it is not resembling any of the intermediates present in the cellular system. Now some structural features in organic compounds that are not common in nature are called xenophores.

The xenophores are very common in terms of the when you see the recalcitrance of some compounds are discussed. The presence of the xenophores like the substitution of Cl, NO2, CN, or SO3 groups in a particular organic compounds these are called xenophores and substitution of these groups lead to a kind of a make them kind of unrecognizable for the microbial system.

Now contaminants that contain such as xenophores tend to be recalcitrant to microbial degradation because after this xenophore molecules are present the microbial systems will feel something that this is a kind of an absolutely new molecule to me I do not know how to deal with this.

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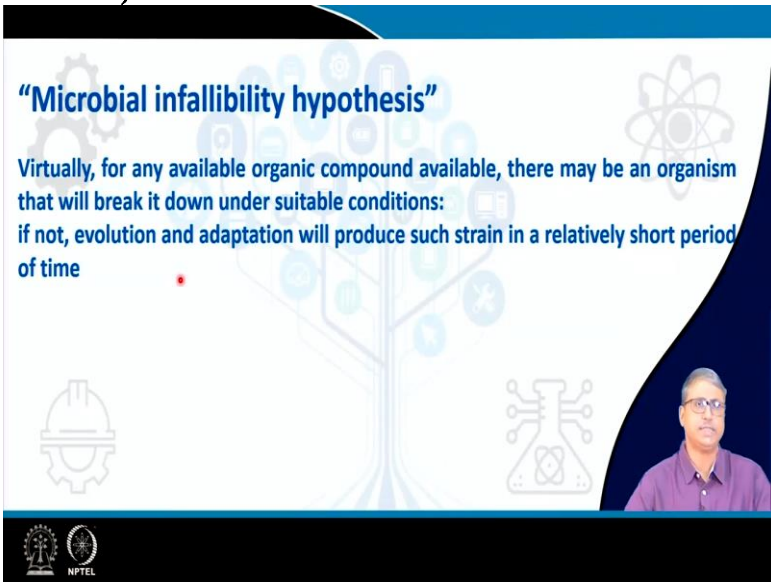

In that respect we should talk briefly about the microbial infallibility hypothesis which is a very well known hypothesis with respect to biodegradation. And it says that virtually for any available organic compound there may be an organism that will break it down under suitable conditions. So, all organic compounds present available to the microorganisms will be definitely degraded at some point of time at some suitable conditions.

If not if the compound is not getting degraded then evolution and adaptation will produce such organisms such bacteria archaea are fungi which will own a relatively short period of time which facilitate this degradation process.

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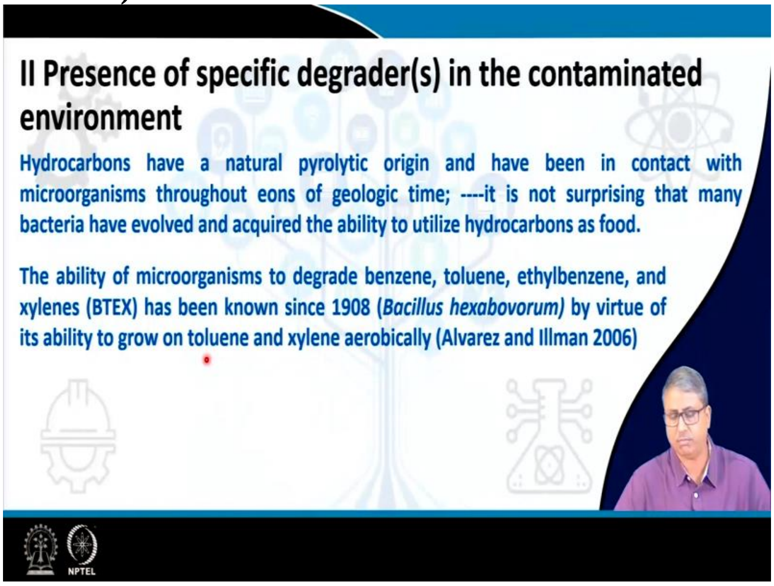

The second prerequisite is the presence of specific degrader in the contaminated environment. Now hydrocarbons have a natural pyrolytic origin that I have discussed and over for the evolutionary origin and have been in contact with microorganism throughout eons of geologic time. So microbes are interacting with hydrocarbons aromatic and aliphatic. So, they for them it is not at all a new molecule.

It is not surprising that many bacteria have evolved and acquired the ability to utilize hydrocarbons as a food. So, when we started working on the hydrocarbon biodegradation scientists were very surprised initially but very soon they have realized that as if it is a regular food for them because there are plenty of natural environments underground aquifers etcetera. where different concentrations of naturally produced hydrocarbons are present.

And microbes are regularly utilizing those hydrocarbons as their food, food in a sense that is a source of carbon and energy from them. The ability of microbes to degrade for example the benzene, toluene, ethylbenzene and xylene which is referred to as the BTEX has been known since the 1908. As you can imagine that 1908 the; Bacillus hexabovorum later was identified by virtue of the ability to grow on toluene and xylene aerobically. So, there have been numerous studies and reports and we have a whole plethora of information and an organism's available for that.

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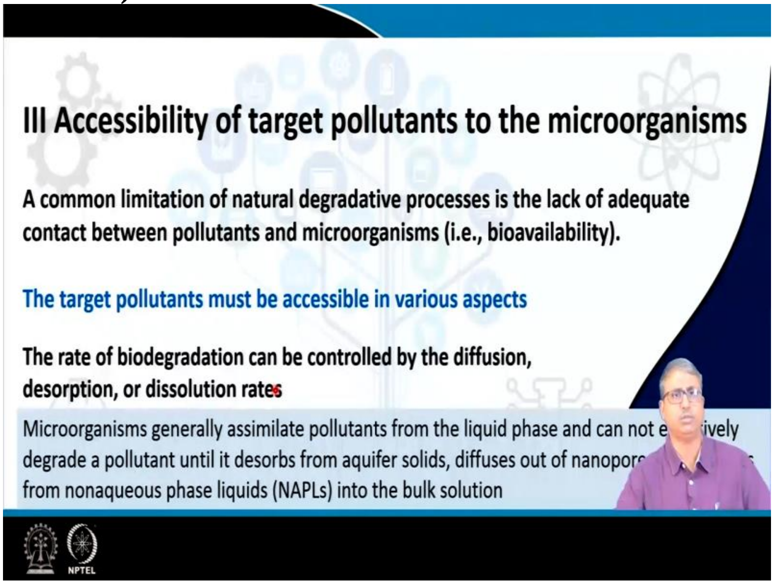

The next requirement would be the accessibility of the target pollutant to the microorganisms the pollutant molecule is there in the environment and the candidate, organism who can degrade the pollutant is also there. And we expect that the pollutant organism is capable of expressing all its genes everything is fine is all set to degrade the compound. But degradation may not proceed with a kind of expected rate or expected extent.

Because there will be some other limitations could be limitations these limitations are considered as the limitation from bio availability point of view and because the lack of adequate contact between the pollutant and the microorganisms these the target pollutant must be accessible to the bacterial strains or the archaea strains or fungal strains. And unless they are in a aqueous phase because microorganisms will work only in the aqueous phase.

So, the pollutant molecule should be available in the aqueous phase from where the microbes will be able to interact. And therefore the rate of biodegradation can be controlled by the diffusion, desorption or dissolution rates which are actually applicable for the contaminant molecule the organic molecule which is present along with the soil particles or the rocks or the micro pores or the nano pores inside the rock or crust or within the aquifer sediments.

So, how quickly or what extent these hydrocarbon molecules are getting diffused out so that the microbes are able to interact with them or they are desorbed out or they are dissolute out. Now microorganisms generally assimilate the pollutant from the liquid phase only and cannot effectively degrade a pollutant until it desorbs from the aquifer solids etcetera.

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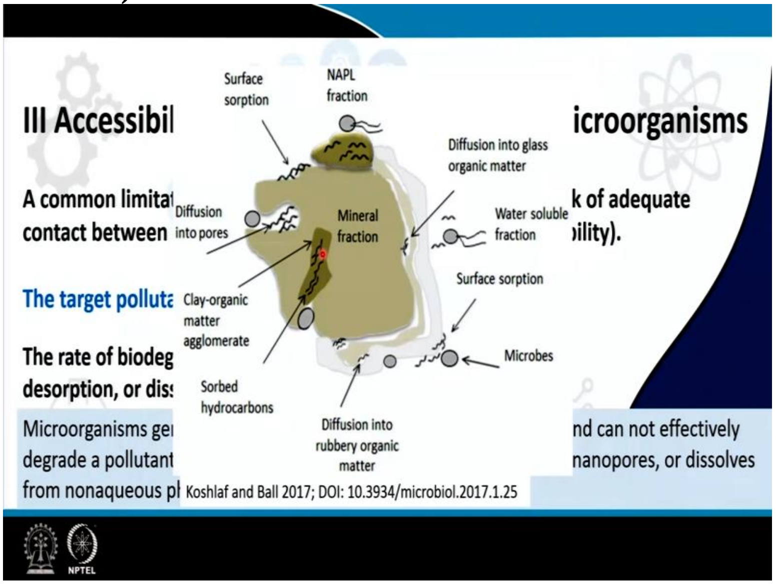

So, one picture that I will show you here this is the published in this paper and you can access and go through this paper with respect to all the details. So, if we consider a mineral fraction mineral fraction means there is a rock or some minerals present in the rock. You can see that there are there are many small, small habitats kinds of things are there habitat for the other zones where the organic pollutant can have their specific interaction with them mineral fraction.

And they remain in that like non aqueous phase liquid fraction the bacteria will not be so if we consider the bacteria, bacteria will not be able to interact or degrade it unless these non aqueous phase liquid fraction is desorbing out and then available in the aqueous phase liquid surface sorption to diffusion into pore. So, the in the mineral phase there is pores in which the organic pollutants are residing and the microbes are unable to react with them.

The spherical structure is for the microbe. So, microbes are unable to reach out there because they are sitting inside and they have a stronger introduction will be mineral fraction. So, ideally we need to consider the fact that how actually the pollutant organic molecule will be available to the bacterial strain. So, the microbial strains so one aspect could be that the production of the availability of different biosurfactants by the microbes. So, microbes often we have found that they produce these biosurfactants molecules to emulsify or solubilize or make these hydrocarbon molecules available to them.

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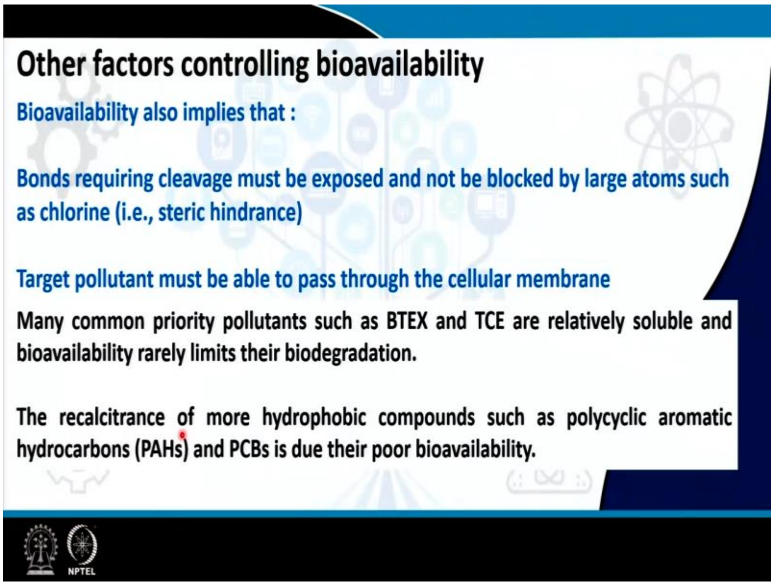

So, other factors which control the bioavailability could be the bonds requiring the cleavage. Cleavage means where exactly the enzymes are going to attack on the organic molecule structure must be exposed and not be blocked by large atoms such as a chlorine molecule or so. So that each of the major bonds which are supposedly the target meridian for the enzymatic catalytic reaction they should not be under some kind of steric hindrance.

And other one is that the target molecule or the target pollutant molecule must be able to pass through the cellular membrane quite easily. Now many common priority pollutants like the BTEX benzene, toluene, ethylbenzene, xylene or trichloro ethyne kind of compound are relatively soluble and bioavailability rarely limits there biodegradation. So, they happily move into the cellular system and cells are able to degrade with them very quickly.

However the polyaromatic hydrocarbons and PCBs for example they are more hydrophobic and therefore the microorganisms they face different levels of challenges in terms of interacting with those.

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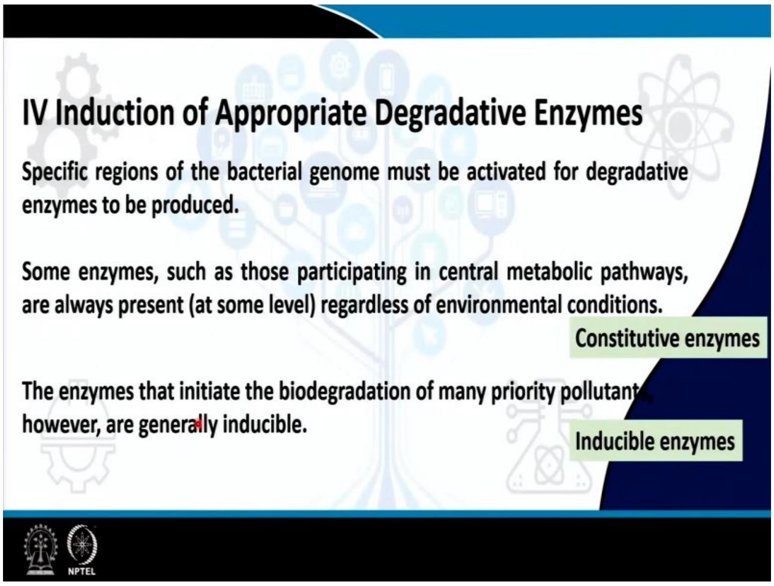

The next one is the induction of appropriate degradative enzymes. So, as we have seen already that the microbes need to actually deploy a set of enzymes to facilitate the degradation process. Some of the enzymes particularly the central carbon metabolism part of the enzymes or constitutive enzymes most of the microbes as I mentioned they are expressing those enzymes but for the specific degradation apart microorganisms need to induce some of the gene some of the enzymes.

And the presence of the inducer and the repressors are very, very relevant because inducible enzymes are induced only when the inducer molecules or maybe a lower concentration of the organic pollutant itself would actually facilitate the induction of those genes into the from their genome or on the other hand the presence of some radially metabolizable other substrates would replace the expression like presence of ethanol.

For example if you have ethanol along with this organic pollutants you will see the ethanol is getting metabolized rather than the organic carbons because the genes are not getting expressed because of the some intrinsic repression which are posed by the other repressor molecules.

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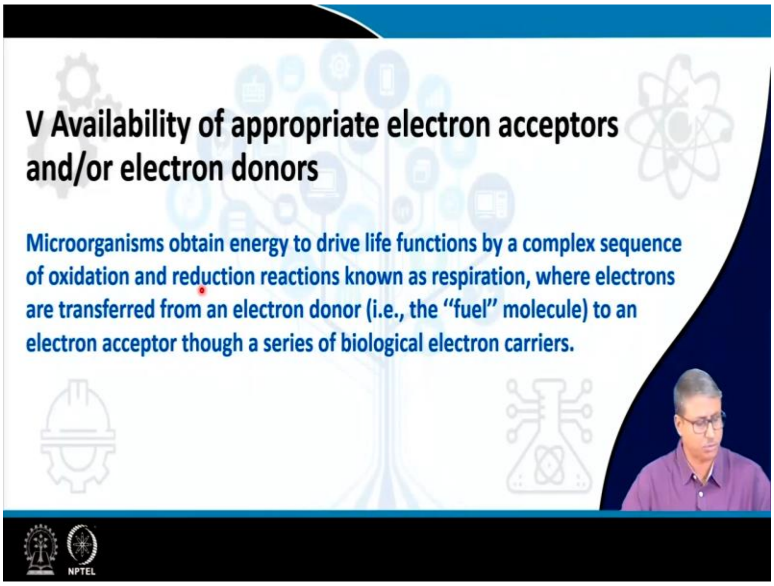

The last point for today is lecture regarding this biodegradation process is the availability of the electron acceptors and electron donors. This is another very important aspect because microorganisms why they are interacting? I said sometimes that it is used the pollutant organic pollutant molecules are biodegradable because they want to use them as food as the source of their carbon and energy.

That means the microbes that try to obtain the energy from these molecules this organic pollutant molecules to drive the life functions either by producing the ATP or the reducing power or the proton motive force and the precursor molecules and these reactions are complex set of reactions very complex set of reaction I have shown you some part of the KEGG pathway for the benzoate reaction you have possibly noted that there are several reactions going on.

And the microorganism they express all these genes and produce the enzymes in order to facilitate the oxidation of the target compounds wherever oxidation process is applicable for the biodegradation. So, it is basically not only oxidation but also a complex sequence of oxidation and also reduction process. Because some where the; electrons must be transported to an electron acceptor.

Because if no electron acceptor is available to accept the electrons then the entire process will stop. So, it is not a merely oxidation process only it is actually oxidation in one hand and reduction on the other hand, other hand in the sense some electron acceptors must be available and must be playing role in accepting the electron so that the flow of electron goes on now where the electrons are transferred from electron donor that is a fuel molecule to the electron acceptor.

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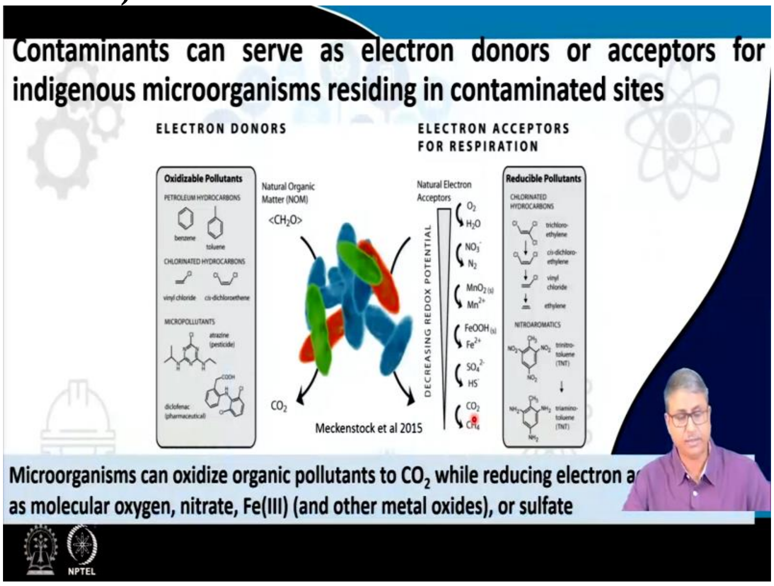

So, we have already discussed this part of this thing that the contaminants can serve as electron donors most of the cases where oxidation is involved and wherever the oxidation based biodegradation is happening all the oxidizable pollutants they are being utilized as a source of electrons and the electrons are being transferred to electron acceptor so these are the electron acceptor. So, under the aerobic condition oxygen is the electron acceptor under anaerobic condition different other electron acceptors they play a role.

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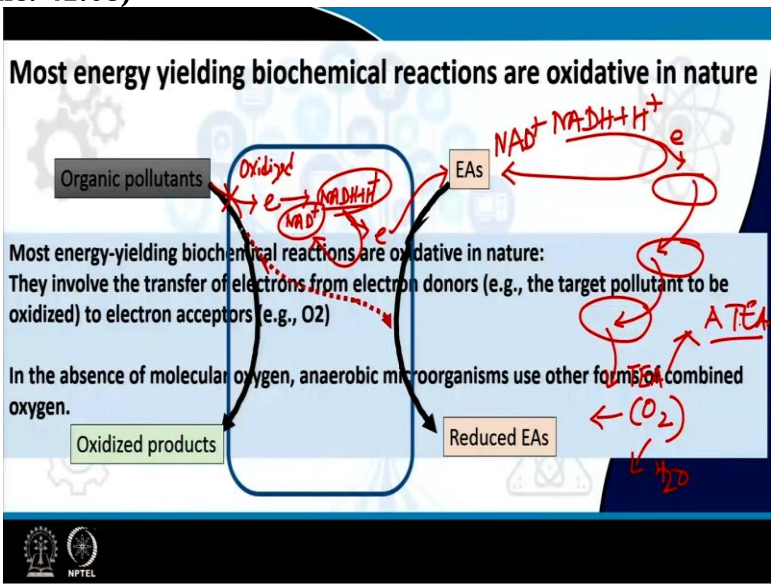

And so basically as the organic pollutants are degraded to oxidize pollutants let us say pyruvate acetate or will be complete degradation the role of these electron acceptors are very critical if electron acceptors are not available then possibly this process will not proceed this process will collapse because where the electrons will go find any because we already have discussed this part and I will just quickly try to remind you that the organic pollutants are actually getting oxidized.

So, the electrons are coming out now these electrons should be taken up by somewhat so who is going to take let us say it is the NAD plus who will take the electron and it will turn into NADH plus H plus now these NADH plus H plus should donate to electron to someone and then turn into NAD plus so these re oxidation NADH H plus must be occur because the cell maintain a definite pool of NAD plus and NADH plus.

If they have more of NADH H plus accumulating in the cell that means the cell will get a signal that we are energy sufficient we have more NADH H plus that means is there carrying electron so do not need to carry out any further oxidation eventually this may be stopped. So, they need to re oxidize in order to facilitated to organic pollutant so when we thing of bioremediation and a field level or a practical process or a practical set up in a real contaminated environment.

We need to assume or we need to consider that where this electron will go finally so electrons will be obviously going to terminal electron acceptors. So, then if we consider that NADH plus H plus is having the electrons and electrons wants to come back to the NAD plus wants to convert to NAD plus that means these electrons must be given to someone so who is these is someone? So, these are the electron carriers as I have already mentioned in one of my classes.

So, these electron carriers the carry the electrons and finally the electrons are given to the terminal electron acceptor that is under aerobic condition or aerobic condition is oxygen so oxygen is getting converted to water but if oxygen is not there or if the oxygen is consumed in most of the contaminants river or lake or other ecosystems we see that the oxygen is consumed very quickly.

Because the aerobic bacteria aerobic microbes they try to utilize the presence of oxygen as a terminal electron acceptor and they degrade the organic pollutant. And therefore the oxygen level decline which is the often connected to the event which is called the BOD the BOD of the water particularly. So, BOD refers to basically that the biological oxygen demand it is indicating the organic pollutant load.

Organic pollutant load in the sense because if you have higher amount of organic pollutant obviously the oxygen will be used consumed more so the oxygen level will fall quickly. Now the drop in oxygen level will give you some idea that how much organic pollutant was there? So, basically what I want to mean or discuss over here that the oxygen is preferred because under aerobic condition.

It is the thermo dynamically most preferable substrate the micro organism will be very happy they will grow utilizing the organic pollutant. And they will grow, grow, grow but very soon the oxygen level might fall down because of the diffusional barrier or many other factors. So, it will switch to the alternate TES and this alternated TES will now play very important role.

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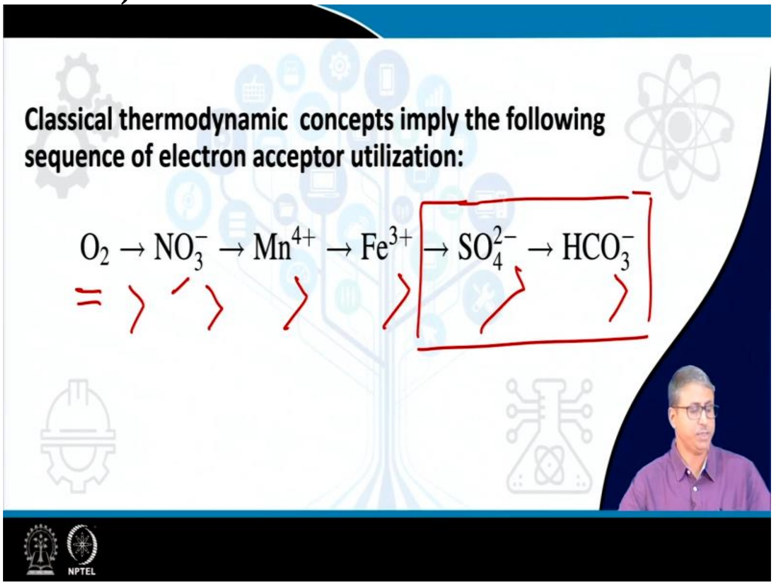

And what we have seen that the classical thermo dynamic concept implies that the following sequence of electron acceptor utilization is mostly pre dominate in organic pollutant contaminated natural environmental particularly under aquifers conditions or underground conditions where the level of oxygen is highly controlled because oxygen is only produced most of the cases minimally and it is actually diffused oxygen form the top of the soil activity.

So, the oxygen level declines naturally the aerobic process will gradually will be playing minor roles and the de nitrification process using nitrate as electron acceptor manganese reducing bacteria then manganese reducing process followed by iron sulphate and eventually the methanogens will come into play where they will try to use the carbon present on the carbonate or carbon dioxide and the electron acceptor and they will produce methane.

And that all this process will drive the biodegradation of the organic compound but based on the thermo dynamic gain that Gibbs free energy which could be produced the extent of the degradation will also vary obviously this will be the producing the maximum Gibbs free energy. So, it will most preferred and the extent of organic pollutant degradation expected or may be expected very high so it will go like the extent of degradation will also follow the same trend.

So, in a sulphate reducing conditions the extent of degradation much lower compare to the nitro reducing conditions and compare to the aerobic conditions and under this methanogenic conditions where no other electron acceptors are there the biodegradations will be highly restricted however in many of the contaminanted we see that the sulphate reducer and this methanogens they play role together that called sintrofic metabolism.

And through which they are facilities somehow the better degradation organic pollutant up to some extend many of the USDY sites for example these application of this and in marine environment what you have notice that the presence of sulphate it prefers the biodegradation of organic pollutant because many pollutant degrading bacterial they use sulphate as terminal electron acceptor.

And convert the organic pollutant it to acetate other secondary fermentation products like hydrogen and those products are utilized by the methanogenic organisms there by allowing this inter species metabolite transfer and over all that process the degradation of the organic pollutant.

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So, for this lecture I advise you to consider these articles so most of the articles are found to be very useful particularly the book written by bioremediation and natural attenuation and also the presentation of some of these articles are found to be very lusit and one can easily understand importance of different aspects of this bioremediation process.

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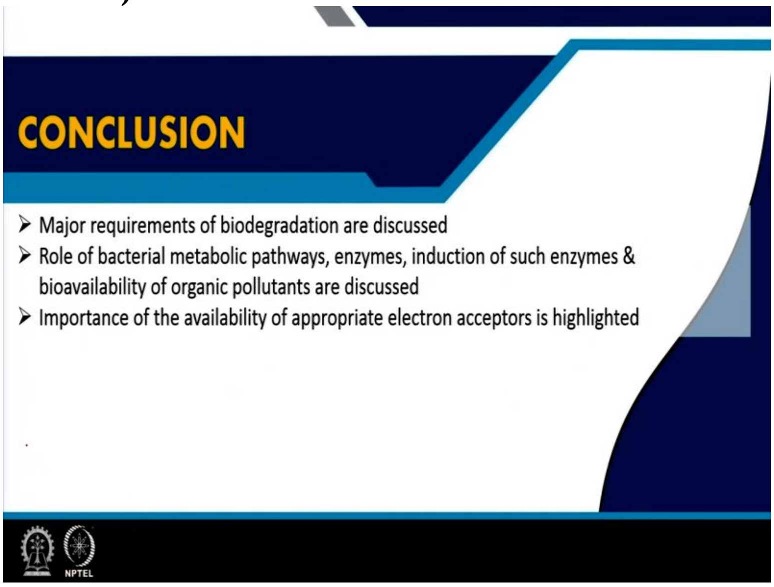

So, in conclusion in this class we have discussed about the major requirements of biodegradation we also discussed about the role of bacterial metabolic path ways enzymes induction of such enzymes bioavailability of organic pollutants etcetera importance of the availability of appropriate electron acceptors are also discussed and thank you so much.