Applied Environmental Microbiology
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Lecture – 34
Bioremediation II

Dear students, in today's lecture, we will continue a; what we were talking about in the last lecture that is bioremediation and we will be focusing mostly on degradation of contaminants. We start with metallic contaminant as we talked about in the last lecture and then we go on to organic contaminants. So, let us gets started.

If you remember in the previous lecture we discussed what is bioremediation and with the most popular definition of our bioremediation is degrading organic compounds using biological activities. However, I did mention very clearly many a times that bioremediation is broader than just degradation of contaminants or sequestering them. So, and we talked about metal leaching in the previous class how we actually use microorganisms in their activity to get more metals out of poor quality ores and we also talked about acid mine drainage, which is what happens when the metals are leached out and because of sulphate oxidation and as a result we have highly acidic and highly acidic surface water groundwater which have lot of heavy metals in them and which is a health and environmental problem.

Now, we will go to uranium bioremediation, in many parts of our country India and definitely across the globe uranium mining holds promises of generating a relatively cleaner and more efficient source of electricity. Now, a problem with uranium is that, when we mind the uranium it gets exposed to oxygen. So, if you remember what we talked about in AMD, that is acid mine drainage that when the mines in the ores get exposed to oxygen based getting oxidized and in case of most metals or certain metals at least the oxidized form of the metal is more soluble in water than the reduced form. So, uranium has 2 valency states that are of concern here, 1 is the uranium 4 and the other is uranium 6, all right.

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So, the more oxidized 1 is the more soluble. So, we want to reduce uranium so that it remains sequestered and it does not dissolve in water and in the sites, this research most of this research has happened in USA because there was a time when they were very eager in mining uranium and generating cleaner energy source from there.

However, now that this research well the uranium mining has diminished for different reasons because of change in policy there, but this remains to be a major environmental concern the presence of uranium in groundwater presence of uranium in soil, wherever uranium mining has happened across the world and definitely in India. So, when we mine uranium it gets oxidized and the wonderful thing the not the wonderful thing the harmful thing that happens is that the oxidized uranium dissolves in groundwater because now it is more soluble and then it is transported over long distances.

So, many a time's people actually can detect uranium in the drinking water and in the irrigation water which is a health hazard. So, here is a picture show in how uranium might transfer from 1 part to another and this is true for any contaminant actually.

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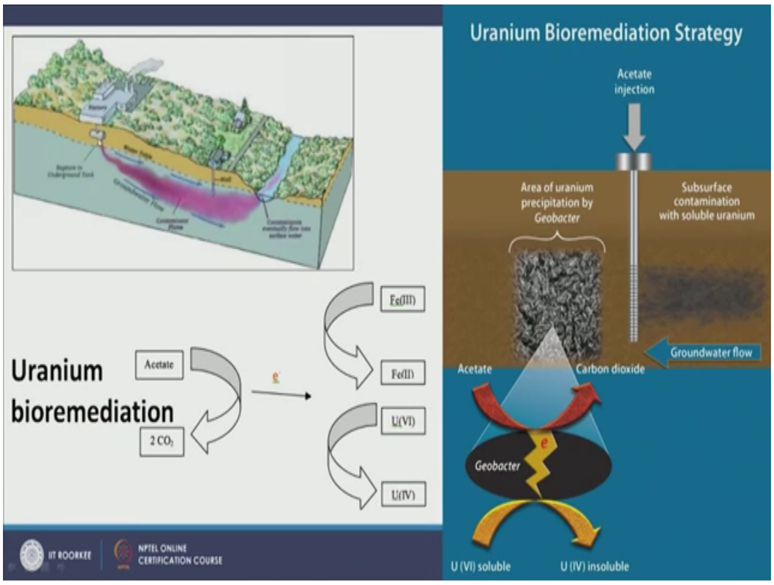

Here, we have a factory it is disposing it is very safe full be mining a factory mining industry that is had that has it spent all that it is disposing or it could be a petroleum factory or it could be any other factory, when they usually maintain keep their waste underground and it is any rupture in the underground tank, the water will take the contaminant along with it and it will become, it will it can be picked up by the wells downstream and by the surface water downstream.

So, if when it comes to uranium as I mentioned it has 2 forms uranium 6 and uranium 4 and uranium 6 is the more oxidized form, obviously, because it has lost 6 electrons. So, what we want to do is convert the uranium 6 into a uranium 4, in presence of oxygen it becomes uranium 6 which is more soluble and thus more mobile, but if using any technique we can convert uranium 6 to a number 4, we will have the less soluble. So, we can write insoluble here to contrast, so comparatively insoluble and immobile.

So, this process of converting uranium 6 to uranium 4th to render it less soluble and immobile is uranium sequestration. So, the earlier approaches for doing uranium sequestration involve chemical reactions where people try to chemically reduce the uranium 6 into uranium 4; however, mister Dr. Derek Lovley realized that there are some microorganisms that will convert uranium 6 to a uranium 4, it is very easy for them to do it, even though they do not use the energy when that they should get when they converted uranium 6 to uranium 4.

So, if you remember we talked about microbial energetic, then if microbes reduced uranium 6 to uranium 4 and they get some energy out of it, then this energy we can use for their sustenance and replication. However, uranium of it we do not know micro if microbes are able to use this energy, we believe that we do not use this energy, that is what evidence we have.

Then, the question is why would microbes then want to reduce uranium, if they do not get any energy from it? And this is what Dr. Derek Lovley's work is really fascinating because he realized that, he observed that and he gave evidence in favour of this that microbes do not metabolize uranium out of their selfish interest, but it is a co metabolism it just happens to be so and why it happens to me so, because uranium reduction gets coupled with iron reduction.

So, usually in uranium mine iron is also present in 2 forms ferrous and ferric. So, ferric is the more oxidized form and ferrous is the less oxidized from. So, the iron reducing bacteria when they reduce ferric to ferrous, they gain energy from this process and then the enzymes and the proteins that they use for carrying out this reduction just by near presence of uranium 6 in their vicinity will also catalyze this reaction.

So, if you remember I have talked about this that metals; heavy metals particularly microbes cannot bring it inside their self, inside their body. So, these enzymes at iron reducing microbes will use to convert ferric into ferrous, these are extra cellular enzyme systems, so these extra cellular enzyme systems just because in their vicinity there is another metal that is oxidized and it so happens that this metal can also be reduced using the same enzymes, even when this energy is not being captured by microbes, this is how we do uranium sequestration.

Now, how can we encourage iron reducing microbes to reduce iron? Now, remember if this is a reduction of iron which means iron is electron acceptor, so we need an electron donor, if you remember electron donor plus electron acceptor you get energy microbes type. So, what we do in order to encourage iron reduction is we add an additional electron donor and the typical 1 that is amended in the soil on the water is acetate because acetate is very easy to digest for iron reducing microbes and most microbes in general; however, acetate does not disappear like glucose does.

So, the effects of acetate are expected to be longer. Now, in this research there were some very other wonderful observations that came into picture which was that not only do microbes drive the chemistry. So, this is biochemistry in sense not chemistry or biology. This is biochemistry in sense that microbes drive the chemistry and then chemistry effects the microbes. So, there is a big relationship between microbes and the chemistry in the soil. Now, because this is happening underground, we notice that when microbes affect the chemistry which is when they reduce the uranium or when they reduced the iron or oxidize the iron, they also not only affect the chemical state of iron and uranium, but they also affect the mineral structure.

So, they affect the kind of minerals we will find, what you know the kind of crystals these minerals will make underground. So, for example, will they make from (Refer Time: 08:59) pirate or will they make some other kind of pirate ones iron has been reduced. Now, that is why this field where we look at how microbes are interacting with the ions, the chemicals in the ground and how they are affecting the structure of earth, this is an entire field in itself and it is called biogeochemistry.

So, biology is determining the earth's structure is determining the chemistry and it is also the other way around, the chemistry affects what kind of microbes will grow and how much and why and same is true with the geology because we certain minerals all up on certain minerals microbes might find it easier to make biofilms and thus easier to survive, this is a very fascinating field right now and again for students who are interested in higher studies I highly encourage you to look up biochemistry and good researchers in this field.

All right, so here we have a bioremediation strategy for uranium which is uranium sequestration, we drill a well and we inject acetate. So, when we inject acetate, the acetate is consumed by is oxidized. So, it acetate is electron donor, it is oxidized into carbon dioxide and that microbe what it does is it reduces iron and co reduces uranium. So, the microbes that ice the detect direct Lovley's lab isolated were geobacter, they belong to this particular species.

So, here is a clearer picture we have uranium bioremediation acetate is being oxidized, the electron is reducing iron, it is also coreducing uranium and because of this reduction now uranium is precipitating. Here, uranium was not precipitated, but the groundwater is flowing from right to left and here the uranium is now precipitated because of acetate edition, all right.

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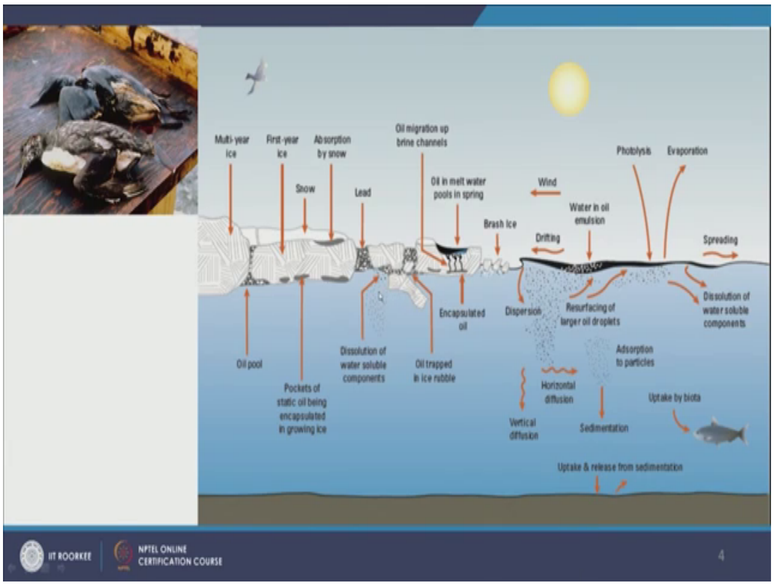

Now, let us move on to oil spills; recently in Chennai there was a major oil spill and that affected the coast, affected and we do not even know the expanse of the oil spill. For example, the data about how much oil spilled? How much area was damaged? What kind of damage has occurred? That data is yet to be a arrived in public and they here to be declared on public domain.

Now, when oil spills happen it is not just that the environment gets dirty, but it seriously affects the aquatic life, the avian life. So, here is a picture on the left of 2 birds that have been drenched oil and they are probably going to die or they are already dead if not already dead and in fact, I encourage you to go and look up the pictures of oil spill in Chennai in 1 of the most recently photographed oil spill was Gulf of Mexico, oil spill in April 2010. So, go ahead and look up the pictures and see the massive damage, it is really beautiful because if you can see the pictures you can get a second hand experience on how much damage does it do to aquatic system, to avian system, how much it damages our coasts and you know many animals such as tort always use coast to lay their eggs. So, when oil spill happens damages of course, there and young once it died. So, we are already in middle of 6 mass extinctions and oil spills are really bad, so we need to have a way of clearing the oil.

Now, if oil is dead in the ocean, how can we get move the oil. So, it does not affect the fishes, the aquatic system or does not affect the birds that feed on these fishes. If the oil is on the coast we need to know what is the best remediation strategy to clean the coast, if the oil is in our groundwater we need to know how to remediate it, how to remove this oil.

So, let us look here, let us in now in across the globe many of the major oil wells and oil fields which serve as a major source of oil spills. So, either the oil spill will happen at the time of taking it from the ground and sending it to refinery or will happen during transportation. So, if it happens when you are taking it from the ground, most likely we are taking it run from under sea, well and if oil spill happens in the in the ocean, this is a picture trying to say what it is multiple fates might be.

It is quite blister from the left side, it is quite possible that the oil let has spilled somewhere will finally, come and get trapped under the land. So, if there is an overhang for example, if there is a solid ice, multiyear ice that is like a permafrost then the oil will be trapped here and we will not notice any oil contamination on the top or from the surface, but it is here.

It is also quite possible that when the ice keeps growing, so every year when the winter comes, this oil that has been pooled in the bottom of the multiyear ice might get trapped. So, you might have ice in inko casing the oil pocket, it is also possible that and well depending on the oil it is more soluble components like aromatic components will dissolve and they will have direct impact on the aquatic system because most of them are very toxic and because they are soluble remember microbes they have by lipid lake. So, they expect that on the both sides of by lipid layer you have a quest mixture, so inside echoes media cytoplasm, outside also echoes mixture.

So, microbes they like to live in water or in moist places. Now, so they cannot enter oil and degrade oil, they can only do it on the surface. So, when the water soluble components get dissolved in the water then microbes can easily come and degrade it. So, these usually degrade pretty quickly, the other is quite possible. So, in different ways it can get trapped, it can make pools sheets and pools on the surface. So, this was observed in Gulf of Mexico, they when after the oil spill in the Gulf of Mexico the oil reached the coast of USA that the oil had either made tar balls or it had made sheets, so they it can make sheets.

Now, sheets are tricky because they do not allow the oxygen to the limit base exposure to oxygen of the oil because remember aerobic degradation is faster than anaerobic degradation. So, the aerobic microbes will be degrading it on the top and anaerobic will be doing at the bottom. Now, this gets tricky because anaerobic will take long time, so these reduce the effective surface area for aerobic degradation. The other thing is the oil, that is spilled up on the top can percolate into the groundwater can percolate into the ground.

The other possibility is that the oil might just form a slick layer on the surface of the water body and again the soluble components will dissolve. It is also possible that because of the movement it might fall for missiles, emulsion and they might go up and down just might room around and then once they are pushed down they might again coalesce with each other make larger droplets and come back up.

It is also possible that they undergo photo degradation, so under because of exposure of light them some of the volatile components might evaporate, they might undergo horizontal and vertical this diffusion, some of it the heavy particles might actually sediment and then might be degraded and released from sediment, a lot of it get gets up taken by private biota li, alive beings aquatic beings and then this might wash on the show or might go from 1 place to another my disperses from missells

So, this is a usual fate of oil if it spilled in near ocean, if it is spilled on ground then this is how it looks like, you spill oil here and if there is groundwater aquifer here it will go, it will make a plume. So, it makes 2 kinds of plume I think I talked about it, but it makes plume and because it is not water, it does not mix with water, it makes an napple plume. So, napple is non aqueous phase liquid pume; plume and now depending on it is characteristics in it is specific gravity it is motion will be different and it might it will eventually be transported in the direction of the groundwater, all right.

So, by Americans to bioremediation if these are matini oil droplets and good news for bacteria.

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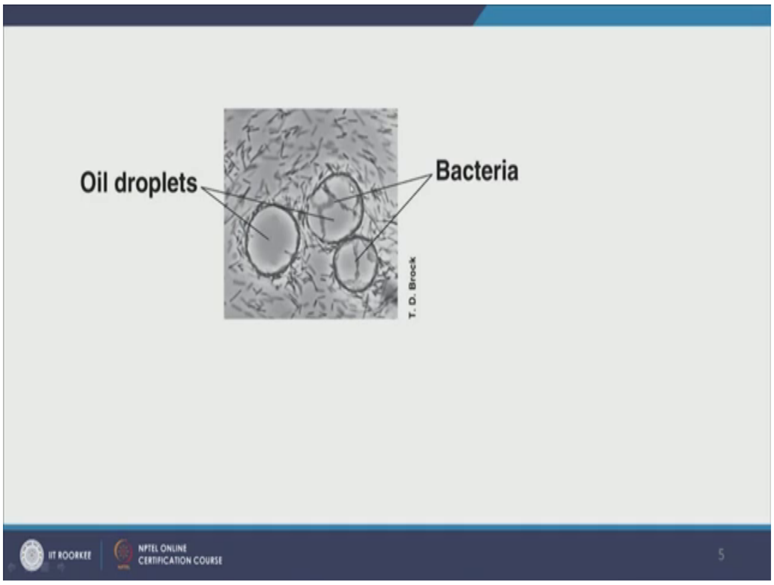

Because, these are really small droplets, they have larger surface area. So, when it comes to petroleum degradation 1 of the limiting feature is that we want the surface area to volume ratio, we want to increase the surface area to volume ratio for the oil droplet for the oil as much as we can because remember, microbes cannot enter the oil and degrade it, they can only eat it on the surface. So, you look here the microbes in this picture are eating these tiny microbes and these are all droplets and they are eating it from the surface.

So, around the rim, some are sitting on the top, but there is no microbe inside the oil droplet, they would not survive, delicate ruptured because remember that the top the outside layer of the microbial cell membrane is water loving. So, if it comes in contact with liquid, it will get repelled, it cannot enter, but even if you thrust it in there it will get disrupted and the middle there which is fat loving will change it is direction and the cell membrane will be disrupted integrity will be destroyed, bacteria will die.

So, we know that the surface area to volume ratio should be maximized. So, what can I do, if I have this slick here the slick layer here floating in the sea. So, when in Gulf of Mexico in 2010 some 7 years ago, when there was an a major oil spill what the government, what the BP did then? The company and the people who are responsible for treating it, they did they injected a lot of this they surfactant, now surfactant is basically a detergent corexit, a now this detergent what it will do is it will make missiles out of this oil, it does the same principle that you have learnt in your high school chemistry and briefly perhaps revisited in 12th class, which is basically it will take the oil and because the detergent has a hydrophobic and hydrophilic and the hydrophobic end will attach to the oil and hydrophilic end will attach to the water will be loving the water and then it will make missiles small droplets with detergent around it.

Now, because it has divided the oil slick into small droplets and what it has actually increased the surface area to volume ratio for microbes to come and attack. The other beauty is many times the surfactant itself is very easily biodegradable. So, in that case the microbes who are not very who do not find it energetically very advantageous to eat oil, may first eat of the detergent and then when they are rich and they are good in number and there they are hitting the exponential phase of growth, then it is easier for them to attack and eat the oil.

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Already and I mentioned in the last class that some important topics in understanding bioremediation gene regulation, metabolism we have already covered metabolism, metabolic control, aerobic and anaerobic pathways of contaminant degradation and transformation, we were talking about them today or in the next lecture and then the enrichment cultures.

So, we have talked about enrichment cultures what are different ways of enriching them. So, we will not be talking about them and then bioremediation technology these are important to know. I have already talked about molecular biological fingerprinting tools and most commonly used and accepted nowadays are the next generation sequencing technology. Now, let us look at some xenobiotic that in which we can apply or bioremediation understanding to degrade them.

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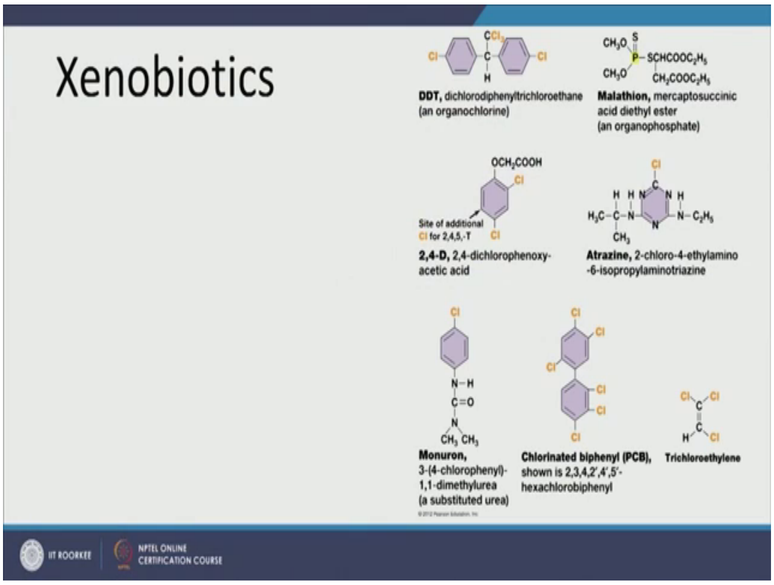

Now, in the last lecture I talked about xenobiotic and I told you these are compounds that us has not seen before until man developed them, invented them and produced them. So, some of the tricky ones that have really affected our environment are DDT and I hope you know why DDT is bad; DDT is used for preventing for killing the bugs that eat the biters like mosquitoes.

Now, the problem by DDT is that it gets into the cycles of the food chain of food cycle of many organisms including humans and then it affects the integrity of eggshells and thus many birds have died out, this is really sad. Then, we have malathion, this is also used for same reason for killing mosquitoes and this also an organophosphate is quite recalcitrant does not degrade very easily and it is very toxic for humans and for many other life forms and it also accumulates in the food cycle then this is 2, 4-D it is a herbicide, not again no very recalcitrant again this is atrazine another pesticide not good for it also accumulates in environment and is not good for life forms, this is monuron, PCBs are a major nuisance because they have destroyed many aquifers in the water quality, water have deteriorated, water quality of many aquifers and this is trichloroethylene very difficult to degrade most of them. Now, we can use our bioremediation techniques, we can use our understanding of microbiology to degrade them.

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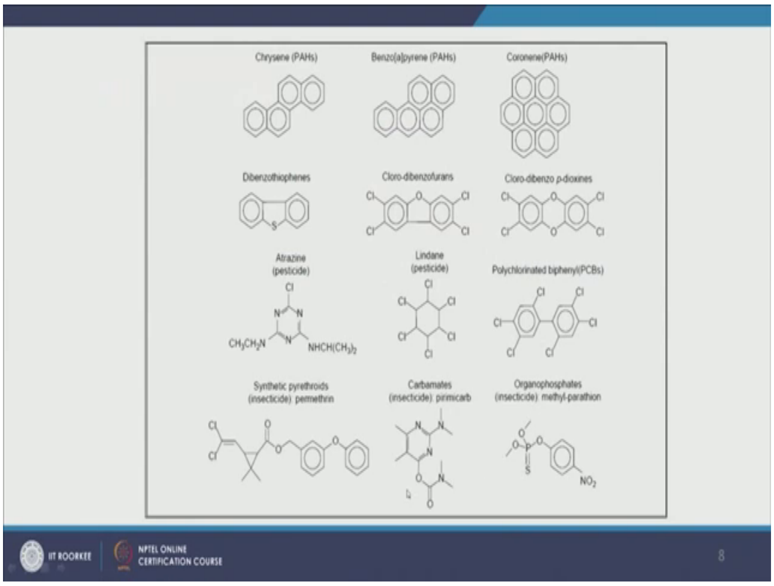

So, now, these are some more xenobiotic for you to look at.

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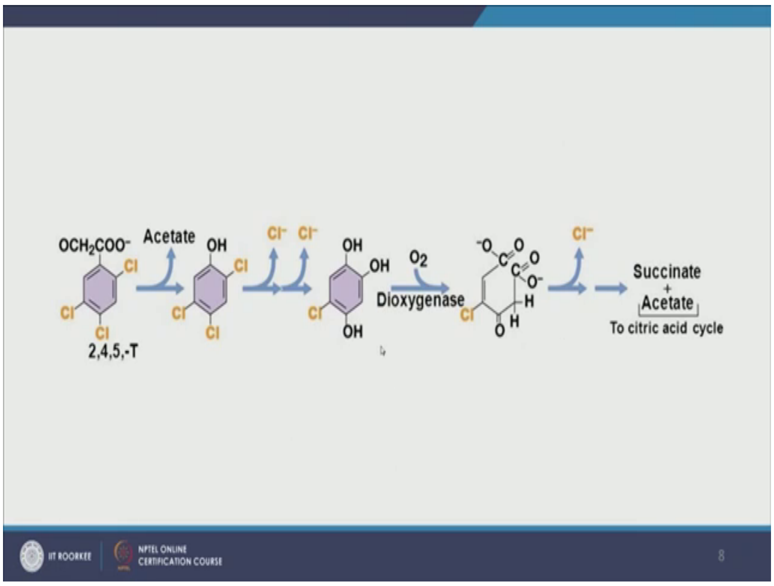

And, but 1 thing known you should notice that malathion and trichloroethylene do not, but most of them have an aromatic structure, aromatic ring or poly aromatic, these are poly aromatic structures, there are multiple aromatic rings.

Now, the aromatic rings give the compound, this particular compound high stability because once the electrons are in resonance their energy level drops and now they are very stable and because they are very stable they are not amenable it is not easy for them to undergo chemical reactions and in order to make them transform them or degrade them, we have to add it in energy. So, we get a very big energy barrier just because of the presence of resonance.

Now, this is your herbicide 2, 4, 5, T and look how degradation of microbial induced microbial driven a degradation of 2, 4, 5, T herbicide looks like. So, 2 4 5 T has acetate here and then has 3 chlorine and the first step is that acetate is removed and replaced by OH this is catalyzed by enzymes and then it loses 2 of it is chlorine which are replaced by OH, then it has a dioxygenase enzyme that comes in, but it does not dioxygenase is as the name suggests it will add to oxygen. So, 1 oxygen is attached here, 1 off scene is attached here and when that happens the ring breaks, so this carbon instead of attaching to this carbon, now we are attached to oxygen.

So, if we have 2 acetate, the ring has broken. Now, this is very important enzyme because this is an enzyme that breaks the ring dioxygenase is, once the ring is broken the aromus, aromaticity is gone and now this can be easily degraded. So, this is the chlorine is lost and it is easily degraded into succinate and acetate, they are further taken to citric acid cycle we have talked about citric acid cycle and then they can be degraded. So, in this way we have oxygen we have degraded 2, 4, 5, T herb, herbicide. So, chemically it is going to be very energetically expensive and environmentally harmful to degrade it, but biologically the microbes will use their enzymes and degrade it.

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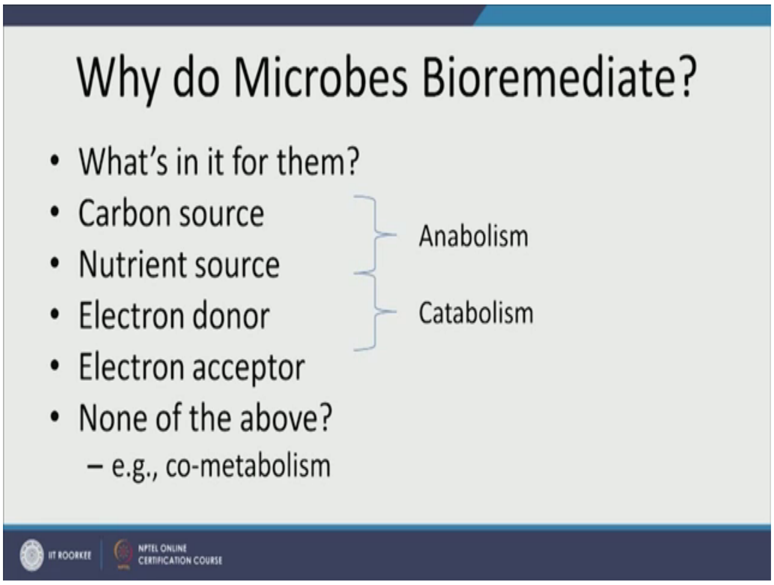

So, why do microbe's bioremediate? Why do the degree these organic compounds well? What do they get it will get from them? They get carbon source. So, here you have, so many in this herbicide you have. So, many carbons and all of them can service carbon source, they also get nutrition, they get electron donor or they get electron acceptor depending on the contaminant and sometimes they get none of the above, then either getting energy nor getting carbon nor getting electrons nor giving electrons. So, why would they do it then?

So, if you remember what we talked about in uranium sequestration, uranium is sequestered in it is co sequestered, it is co reduced because the microbes are trying to reduce iron and when they reduced iron, they get energy from it. So, there it is advantageous for them, but the enzymes that are reducing iron also happen to reduce uranium and their extracellular so, during if uranium is sitting in proximity of iron if you get reduced to and you to get sequester.

So, these are the reasons why microbes will bioremediate, so mostly for their own benefit and sometimes none of the above. So, when they get carbon and nutrients source from the contaminant this is anabolism. So, basically there using the contaminant as a way to increase their biomass, so carbon will help make them more biomass, nutrient will give them elements required, nutrition area required for you know sustaining their life and if they are using it as electron donor or acceptor there using it for catabolism.

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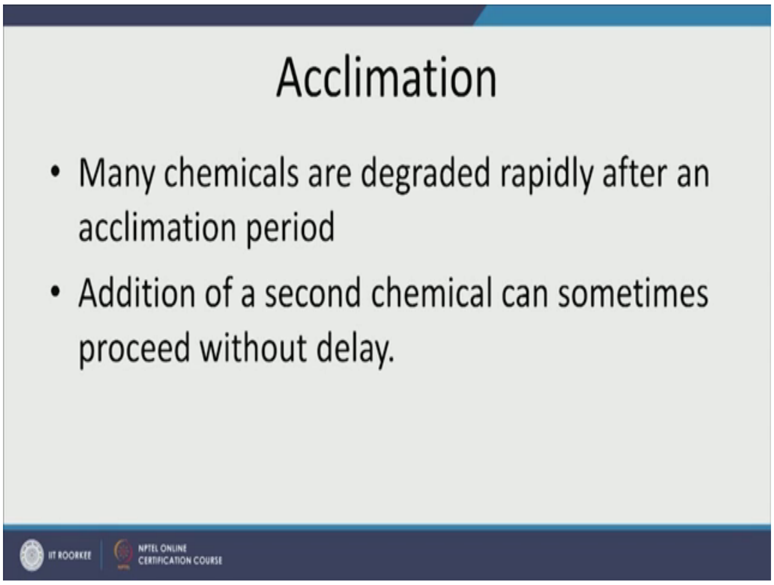

Now, one of the problems that I think I have talked about this in brief earlier acclimation. So, in acclamation the microbes yes I did in the last lecture when you talking about acid mine drainage. If you remember I mentioned that the microbes that are that should be from let us say cow manure microbes, that should be reducing sulphate and thus allowing the metal to be precipitating as sulphide, these microbes when they were put in the field they were not very happy, they did not do a very good job in fact, it was as good as they are not being there, the reason was they were not acclimated to such low PH and such heavy metal load.

So, if the acclamation is adaptation, giving the microbes giving an opportunity to get used to the contaminant because these many of these contaminants are xenobiotics or if there not xenobiotics the microbes that can do a good job at degrading them or removing them or transforming them, they are not used to it they are not familiar with it. So, we need to give acclamation time and once microbes have got their acclamation time they used to it then they can degrade it rapidly.

So, many chemicals are degraded rapidly after an acclamation period and sometimes if I want to skip the acclamation step, I can add a second chemical. So, if you remember I talked about how surfactant might actually help microbes degrade the oil is that it first step it increases surface area to volume ratio by dispersing the oil, once dispersion has happened it also serves as carbon source.

So, microbes that are not used to the oil can first feast on the surfactant, which is not hydrophobic and then they when they are healthy and they are acclimated to the presence of oil they can start creating enzymes, it will help them eat after oil. So, if we add a second chemical like we had a surfactant, it can is a electron donor it can help or acceptor it can help skip the acclamation step or reduce it, why? How does it work? And why do microbes need acclamation time? First of all environmental conditions may not be favourable.

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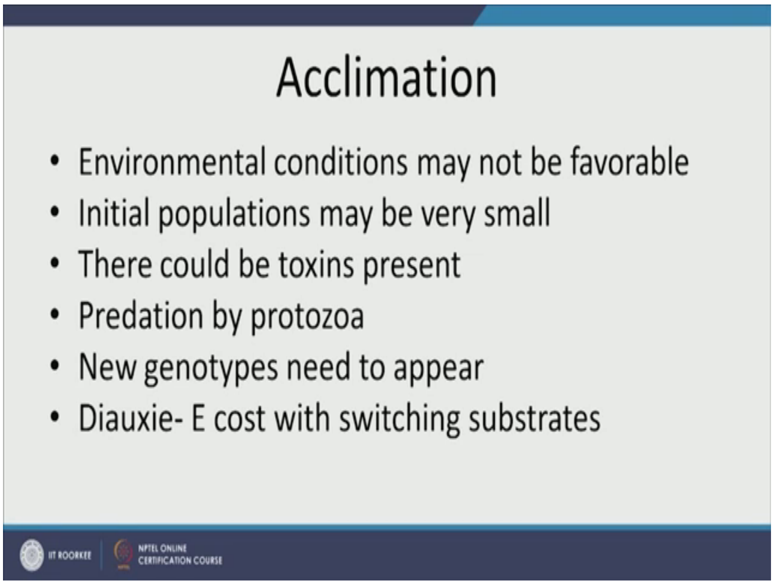

So, microbes that can reduce sulphate, now we are putting them in highly acidic environment with heavy metals present in around them, it is not favourable for them and they will die out and the other reason might be the initial population is very small. So, any I have like a very a small vial of microbes and I have an entire aquifer at treat, it is not going to way. So, I would want to say grow them, acclimate them to then aquifer conditions and make sure that they grow in size, big size they and if I put my small while microbes directly into the aquifer because the initial population is very small, they I may not see substantial results until they reach a population threshold where the activity is substantial enough for me to note, it is also possible that in the environment there are some toxins present, for example, heavy metals in acid mine drainage.

So, the heavy metals I might act as might have toxic effects on microbes and not allow the microbes to do their job. It is also possible that there is some critters were present and they are eating the bacteria and there are microbes are not able to do their job. So, the and then it is also possible that new genotypes need to appear; now this is very important. For example, in case of oil spill let us say there is a lake that has never been exposed microbes that have not been exposed to oil, but now our tanker comes and it disposes some oil in it yeah.

So, now, the microbes there do not know the oil, they need to have need in evolved into new genotypes that can actually degrade the oil and tolerate the oil, so we need to wait for the new genotypes to appear. So, if someone asks well there are microbes in the lake and there is all in the lake wire microbes not eating the oil there might be many reasons maybe that the microbes that can eat the oil are in small number. So, they need to grow up enough before we see any substantial degradation of oil or it may be possible the oil has some toxins in it like aromatic compounds that are toxic to microbes.

So, that is why they are not able to eat it, it is also possible that these microbial community in the lake are waiting for or waiting for new genotypes to appear and these new genotypes will be able to degrade the oil and the other is the energy cost of switching the substrates. So, initially the microbes in the lake were eating other kind of organic matter, natural organic matter, but now they have to switch to eating oil.

So, when did they make the switch, then they have to decide as an energy coaster. So, that is the other reason why there is an exclamation there switching is slow, the other thing is enzyme induction and like phase. So, just converting d n well are making the enzyme activity substantial enough and the microbes that can eat oil you reach a good phase enough they have to undergo the lack phase, exponential phase and then we will see substantial microbes.

So, all of these all these are reasons for acclamation why we need acclamation. Now, I have been talking about this that if a microbe, aromatic compounds are very difficult to degrade because the energy level is very low.

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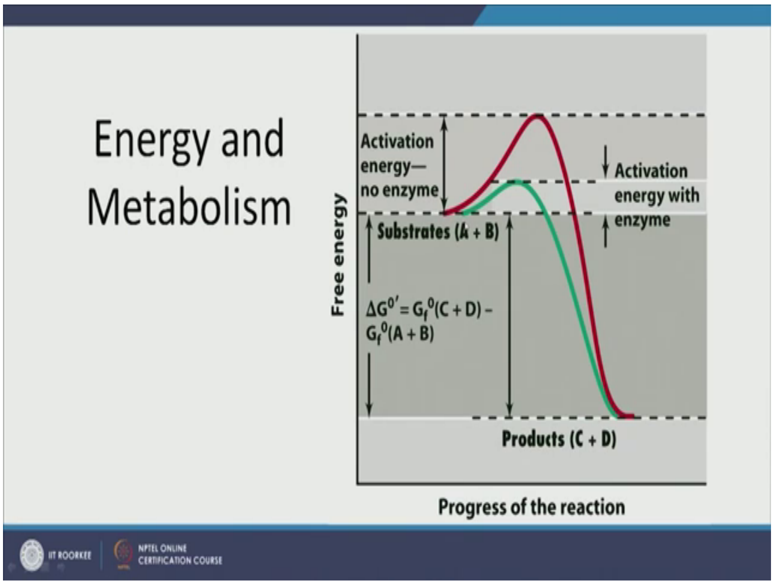

So, let us say this is our substrate A and B, this is what we want to degrade and these are the products C and D they will make you, there is a good amount of energy to be made from this reaction that my groups can benefit from. However, there is often an energy barrier. So, the question here is why if we know that degradation can happen for example, we know 2, 4, 5 T herbicide can be degraded and microbes are already doing it why do we want to use microbes? Why do not we use some chemical reagent and then or we change the chemistry such a way that we cause a degradation, well the reason is usually there is a chemical barrier, this an energy barrier and this is your energy barrier here, this is energy barrier.

Now, if there if microbes they use enzymes to jump energy barriers, without enzyme this is your energy barrier. So, you require more energy, you required more heat more electricity or more whatever for this energy barrier to be crossed and products to be formed, but these microbes very clever little ones they make enzymes that reduce the energy barrier. So, it is easier for microbes to do it in the ambient condition to carry out the same reaction in ambient conditions.

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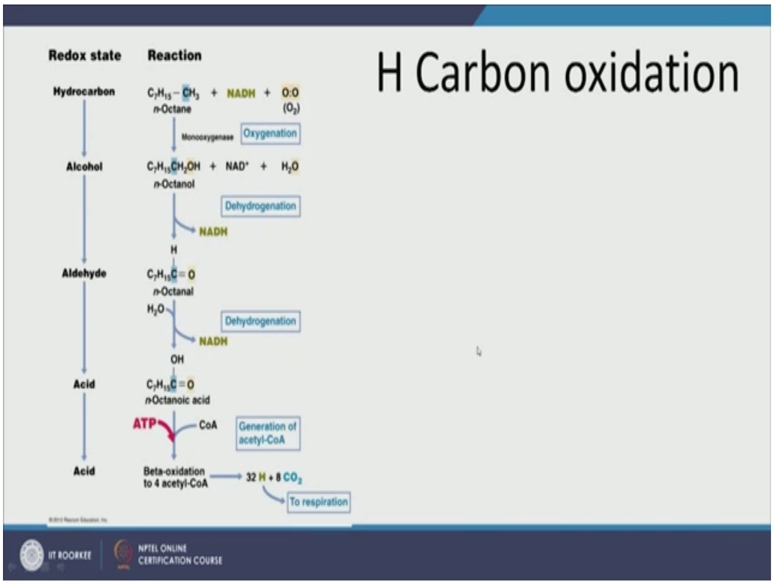

All right, now we will go through oxidation reduction of different kinds of contaminants in the next lecture. So, in the briefly all those go through the hydrocarbon oxidation and the next lecture we will take over other contaminants and other kinds of degradation process. So, let us say you have an octane, it is a 8 carbon, hydrocarbon. Now, the first step would be oxygenates; oxygenation, so mono oxygenase enzyme will catalyze this.

So, basically 1 hydrogen at the tail end would be replaced by a wedge. So, you are adding 1 over, so you 1 also you need mono oxygenase enzyme and then, when you do that you will release your H and when you release the H, now you are releasing NADH which is electron rich, energy rich compound. Now, when you have made a octanoic, which is the oxidized form of an octane it has here carbon CO and then, it will undergo dehydrogenation. So, it will lose another hydrogen this 1 and now we have n octanoic acid, so here another oxygen will come in attach, so now, you have made acid.

Now, once you have made acid, these things are very easy to break. So, you can what you, how will break is that you will join this will break down as acetyl CoA and this CoA will undergo degradation and then you will need an energy input and then you will make you to beta oxidation of acetyl CoA and finally, you will have 8 carbon dioxide and 32 H and this will be respired. So, this is how hydrocarbon degrades.

So, dear students this is all for this lecture, in the next lecture, we will take up the oxidation, reduction, basically degradation and transformation of other organic compounds.

Thank you so much.