Environmental Biotechnology
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Lecture – 38
Bioremediation Continued

Welcome to the next lecture of our course on environmental biotechnology. In this particular lecture, we will continue our discussion on bioremediation.

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And in particular in this lecture we will move forward from the pregenomics approaches to the whole genome and then subsequently the metagenome based approaches adopted for bioremediation.

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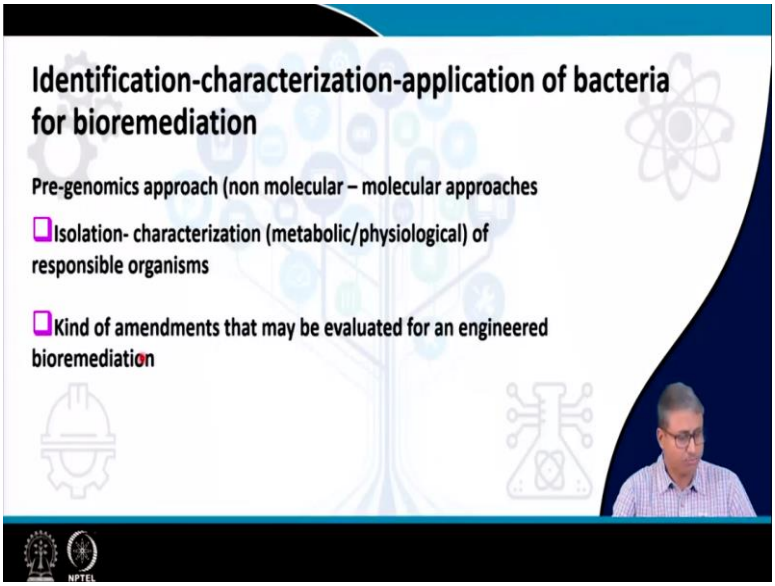

Now, if we look at the early days of bioremediation research as I discussed in my earlier lectures that isolation and characterization of relevant microorganisms particularly bacteria were considered to be the most important step towards bioremediation and as we are able to actually divided these course of development of bioremediation at least into two major phases.

One is considered to be the pregenomics phase or pregenomics approach both including the non-molecular as well as molecular approaches and then post genomics approaches. Here the term genomics basically refers to the whole genome sequencing and high throughput next generation sequencing based approaches which also include the Metagenomics and transcriptomics, Metatranscriptomics etcetera.

So, in the pregenomics phase or when we were actually not doing much of the whole genome sequencing or the meta genome analysis of the samples in order to understand the bioremediation activities or in order to identify the organisms which could be useful for bioremediation. Major emphasis used to be given or is given still in some cases or some places, emphasis is given on isolation and characterization of microorganisms.

Now these isolation and characterization could be done by various ways and the characterization particularly refers to the metabolic or ecophysiological characterization of the responsible organisms, organisms which are identified to be the potential candidate organism. So, here the word responsible refers to the potential candidate organisms and subsequent to this characterization processes we actually were able to identify the major metabolic activities or the catalytic activities of the particular organism or the candidate organism which will make the particular organism suitable for its application in various bioremediation process development.

And this not only includes the isolation and characterization of the organisms which are isolated, but also helped us to identify the kind of nutritional or other amendments which may be required for the particular candidate microorganism in order to survive well within the polluted or contaminated environment and those are considered to be the part of the engineered bioremediation.

Now during these process of time the initial phases were mostly non-molecular because of the lack of the adequate molecular biology tools, but subsequently as the molecular approaches were available like the PCR cloning and sequencing were available.

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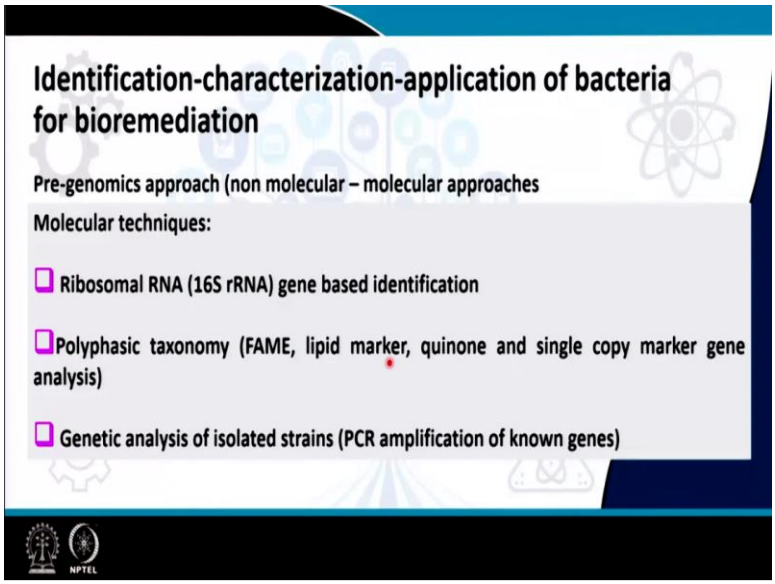

So, we are able to utilize the tremendous capacity of ribosomal RNA based identification of those isolates. So, the isolated bacterial taxa or the isolated organisms they are taxonomic identity were definitely delineated and it is not only the ribosomal RNA gene based taxonomy, but also different type of polyphasic taxonomy basically that includes along with 16S ribosomal rRNA fatty acid, methyl esters analysis, lipid marker, quinone and other single copy marker genes which are good for determining the taxonomic affiliation of the organisms were also included and eventually the genetic analysis of the isolated strains were performed.

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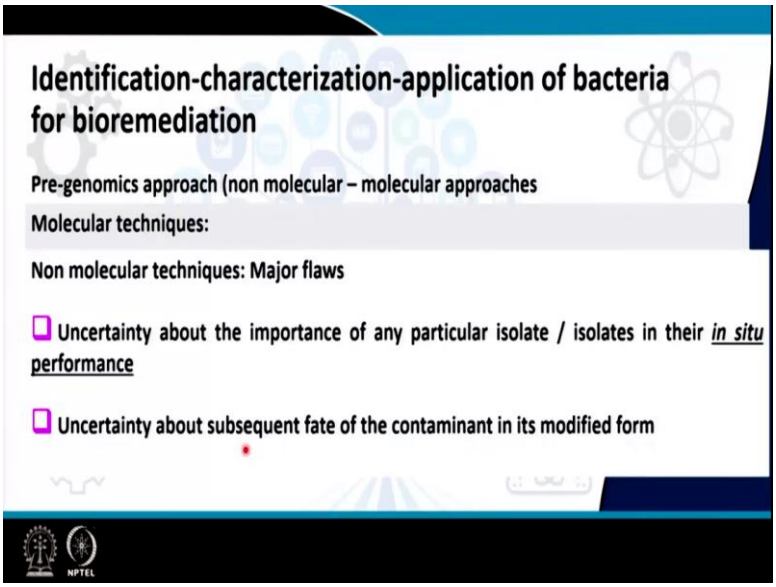

And all these non-molecular to molecular analysis helped us to overcome some of the drawbacks of the non-molecular so called non-molecular approaches particularly the uncertainty about the importance of any particular isolate or isolates in their in situ performance and about the subsequent fate of the contaminant in its modified form. So, these were actually identified to be the major drawbacks of the non-molecular techniques.

But with the molecular techniques we are able to actually delineate that how this target organism the candidate bacterial strains which are isolated and developed and characterize in the laboratory were later exposed to the real environment how they are going to be performing we using the 16S ribosomal rRNA techniques we are able to monitor that. It is also remain a point of concern about the fate of the contaminants in its real environment because in the laboratory treatability studies were often done.

But in real environment how the pollutant molecule is going to be converted to a kind of a totally non-toxic or innocuous form or not determining those things were very important. So, eventually different type of molecular asses not exactly the DNA or rRNA based assess, but also the molecular in the sense the chemical assess including the different type of mass spectrometry based like gas chromatography GC MS or HPLC MS.

And other similar techniques were used to determine the fate of the pollutant including the different other kinds of x-ray based diffraction analysis or maybe the speciation analysis etcetera for the radionuclides or the heavy metal ions.

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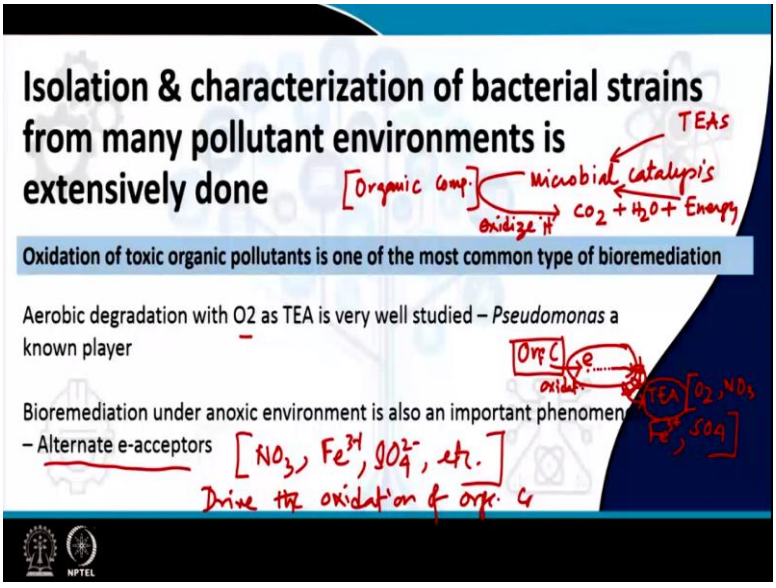

Now one of the major point with reference to these isolation and characterization of bacterial strains remain in this phase when whole genome sequencing based approaches were not implemented because they were not available perhaps. So, is that many organism were isolated from the polluted environment. So, this is particularly true during the 1980s to 1990 early 2000 we see it is a tremendous impetus was given towards isolating, characterizing organisms which are relevant for bioremediation and their possible implementation.

Particularly under the legacy of US DOE Department of Energy United States Department of Energy where bioremediation was attempted and successfully implemented for a large scale of sights, large number of sites were treated using bioremediation microbial bioremediation using specific microbial activities. Now with respect to these isolation and characterization things so today we are going to discuss some of the aspects before we move forward towards the genomic based events.

Now one thing that was possibly discussed in earlier classes that oxidation of toxic pollutant remain one of the most common types of biodegradation. So, if we try to identify these process; so we have the organic pollutant molecule or organic compound which is the pollutant molecule. So, it is basically subjected to the microbial catalysis. So, what microbe will do with this organic compound?

Microorganism will try to oxidize it so they will try to oxidize it. So, following oxidation what will happen eventually the complete degradation is there so carbon dioxide the H2o will produce and lot of energy will be released. Now these energy will be utilized by the microbe itself to grow and to proliferant, but for these process to happen the microorganism require essentially the supply of the terminal electron acceptors.

Why terminal electron acceptors are required in this process because if we look carefully suppose this is a bacterial sale. So, you have the organic carbon over here so these carbon will be oxidizing and electrons will be released. So, these electrons will be eventually transported through the electron careers which are considered to be the most important reducing powers will be generated like Nicotinamide adenine dinucleotide phosphates etcetera NADH or FADH2 etcetera.

And finally these electrons will be donated to the membrane bound electron carriers. So, these membrane bound electron carriers will require the terminal electron acceptor at the end. The electron will subtle from one into another and the finally it will go to TEA for example oxygen or nitrate or sulfate or iron depending upon the redox potential of the environment. So, we have discussed these things possibly in our earlier lectures.

So, one component is very important that the degradation of these organic compound or the oxidation of these organic compound is controlled by the availability of these terminal electron acceptor because if sufficient electron acceptors are available and if the redox potential is favorable then the candidate organism or the selected bacterial strain who is capable of oxidizing the organic compound the target organic compound will be able to oxidize it.

So, the oxidation process is strongly governed by the availability of the terminal electron acceptor which could be in oxygen in case of aerobic bacteria and so in many cases we have found that the aerobic degradation with oxygen as terminal electron acceptor is very well studied in many aerobic habitat or oxic habitat surface contamination wherever it is exposed to air including the petroleum oil and different other hydrocarbon contaminated sites we have found that these pseudomonas type organism.

Even the Burkholderia and pseudomonas and many similar other type of strains were plenty and they were considered to be very, very capable of degrading the organic pollutants using oxygen as the terminal electron acceptor. So, you need to have the electron acceptor available and the microbes must be capable of, they should have the appropriate enzymes coding genes within them and other regulatory system should be favorable.

Now, in case of anoxic environment groundwater the underground systems, subsea habitats wherever we have organic pollutant contamination we see that under this anoxic environments alternate electron acceptor, alternate electron acceptor meaning means these are the electron acceptors like nitrate, iron, sulfate and also the maybe manganese and other selenium etcetera could also be playing a role as alternate electron acceptor.

So, eventually all these thing will drive the oxidation of organic compound which is the pollutant molecule in this case.

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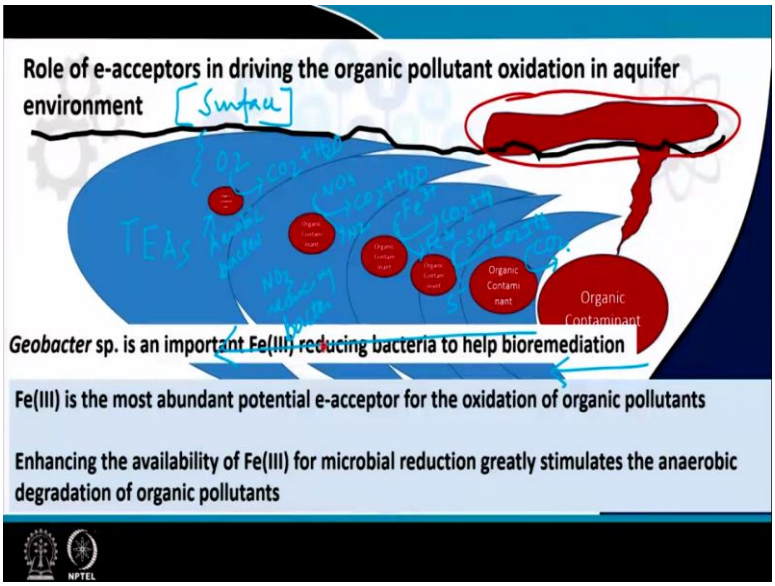

Now, if we look at to some extent a realistic situation where there is a surface spill as you can see over there is a surface spill. So, this is some tank or something which might have leaked and some petroleum or hydrocarbon or organic contaminant is present it maybe a landfill site also where leachates are being produced.

And these leachates are gradually moving into the subsurface. So, these entire part I will write it as this is basically subsurface and the upper part is the surface this part is surface and this part is the subsurface. So, with this the depth is mentioned. Now the organic contaminant whatever has leached might have reached to the certain depth. Now this organic contaminant would diffuse into the underground environment.

And as you can see that they will possibly form a gradient and it will distribute all around depending upon the underground geological strata or the nature of the aquifer or the groundwater flow. So, if we consider that the groundwater is actually flowing in this direction then possibly the entire region will have this fate of organic contaminant. Now, the electron acceptors the EAs or the terminal electron acceptor rather.

So, I will write TES will play a very important roles over here. TES must be present all around, but since the redox condition or all (()) (15:49) may not be present also like in the area which is closer to the surface perhaps the concentration of oxygen will be still better and what we can see that using oxygen as a terminal electron acceptor the degradation of the compounds will be happening.

So, you will have some degradation, but in relatively deeper region where oxygen is not able to penetrate or maybe because of other reasons the dissolve oxygen level is less. So, in these region partly anoxic condition will be started prevailing and possibly the nitrate will help the organic carbon to be decomposed or oxidized because nitrate reducing bacteria. So, these will be the aerobic bacteria in the first level.

In the second level we will have nitrate reducing bacteria and so in the third level we may have the iron reducing bacteria and that will facilitate so nitrate will be converted to nitrogen, Fe 3 will be converted to Fe 2 plus and the organic pollutant will be degraded. In the next region it could be the sulfate which will allow the degradation and the sulfate will be converted to sulfide.

And in the next zone possibly it will be a methanogenic archaea who will use the carbon dioxide itself and will possibly allow the degradation to be happening in a very, very slow rate because those archaea are not thermodynamically is not very efficient in doing these entire business of hydrocarbon degradation, but they will work together with the sulfate reducers as we have earlier discussed during our Syntrophism class possibly.

So, according to the availability of the terminal electron acceptors these different zones could be possible and in reality we have found that naturally since oxygen will always be available closer to the surface wherever the oxygen is able to diffuse and closer to the pollutant itself even if some oxygen is there the aerobic organism would consume that oxygen because they will be trying to decompose or oxidize the organic matter, organic pollutant very quickly.

So, eventually the dissolve oxygen will be depleted. So, even if you have oxygen those oxygen will deplete and then the anoxia will start prevailing and under this anoxic condition this nitrate, iron, sulfate reducing bacteria will start. So, basically it is the game played by the different anaerobic organisms by virtue of the different electron transporting mechanisms that they have.

Now in this regard what we have seen in our earlier research particularly with respect to US DOE research that Fe 3 plus because of its natural occurrence in the geological strata because it is a part of the soil system, rock system often iron is one of the major component along

with silica. So, Fe 3 is one of the most abundant and also potent electron acceptor for the degradation of the organic pollutant because Fe 3 in this case acts as the potentially electron acceptor and facilitated the degradation of the organic compound it has been found.

Enhancing the availability of Fe 3 in contaminated environment organic pollutant contaminated environment is found to be a very promising approach for microbial reduction because that greatly stimulates the anaerobic degradation of organic pollutant. So, if we want to achieve enhanced organic pollutant degradation in anaerobic environment mind that in anaerobic environment where oxygen is not going to play an important role.

Alternate electron acceptors of course nitrate could be there, but it has been observed that iron particularly is preferred by some of the degrading organism. So, I will just take you to one of the best studied case where we see that Geobacter type of bacterial organisms or bacterial strains are found to be very important iron reducing bacteria to help in the bioremediation. Most of the US DOE or many of the US DOE sites we have found that Geobacter was found to be very important.

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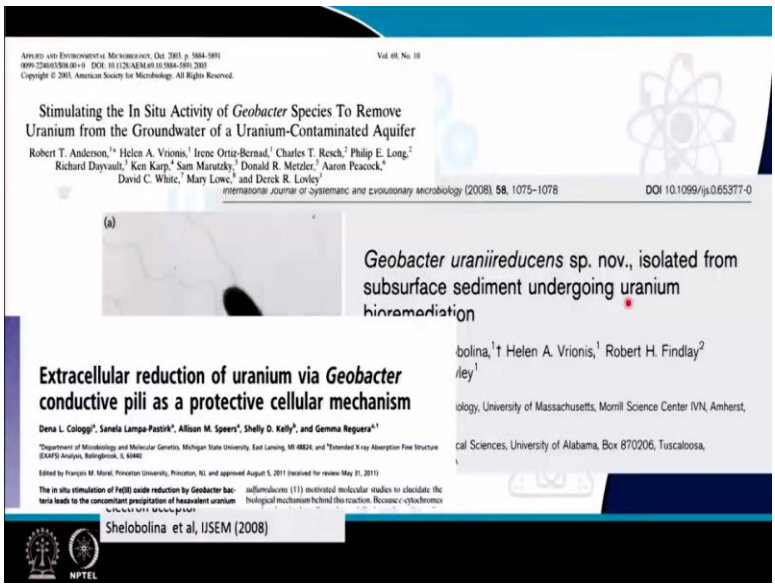

So, initially the presence of the Geobacter was noticed during bioremediation trial where uranium was removed or remediated from the underground contaminated aquifer by simulating the in situ activity of the Geobacter species because Geobactor can reduce uranium 6 to uranium 4 and uranium 4 precipitates under aquifer environment carbonate (()) (21:13) aquifer environment.

So, the groundwater becomes free of soluble uranium. So, from those contaminated bioremediated sites of uranium contaminated aquifers this particular strain of what is refer as Rf4T which is basically a Geobacter strain and later identified or taxonomically designated as uraniireducens because it is capable of reducing uranium very specifically. Now, these bacterium is capable is reducing uranium as a terminal electron acceptor.

So, it can utilize basically uranium as a electron acceptor, uranium 6 and uranium 6 can be reduced to uranium 4 oxidation state and uranium 4 as I said will be precipitated under the carbonate aquifer environment at pH close to neutrality and these bacterium or these strains requires organic substance. So, basically the activities of these type of Geobacter bacteria can be promoted and actually has been promoted through the injection of acetate.

So, acetate is found to be very useful in donating the electrons for the electron transport based processes and later it was found that the extracellular reduction of uranium can be possible or is possible by a conductive pili as a protective cellular mechanism because that enables these bacterium Geobacter uraniireducens to transfer the electrons to insoluble deposits of iron etcetera even.

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And what was further achieved that the anoxic biodegradation of different aromatic hydrocarbon containing systems can also be achieved through injecting different kind of iron oxides. Since these Fe 3 is readily soluble and it remains relatively less bio available. So, this particular work has attempted to fuse these Fe 3 with some ligands and that makes the Fe 3 more soluble.

So, insoluble Fe 3 oxides is transformed into kind of a binding with organic ligand lead to a kind of a solubility and that soluble Fe 3 + is used as the electron acceptor while the aromatic hydrocarbons are degraded by the Geobacter type of bacteria. So, Geobacter is a very smart bacteria it can degrade organic carbon present in the anaerobic environment utilizing iron Fe 3 as electron acceptor.

It can also reduce uranium if some suitable other organic electron donors are there. So, you can think of electron donors like acetate or in case the environment is simultaneously contaminated with both hydrocarbon as well the uranium then perhaps the Geobacter would be the best candidate and indeed it has been found that Geobacter strains are very, very efficient in degrading the organic pollutant in utilizing the organic pollutant as source of carbon and electron and on the other hand utilizing the uranium as terminal electron acceptor.

Thus facilitating the process of simultaneously degrading both the things. So, you can just imagine that this is the Geobacter and you have the hydrocarbon which is the organic compound or you might have acetate these either of these will work. So, they will take the electrons and the carbons from these and they will transfer these two either Fe 3. So, in case of normal cases they like to transfer.

If you have only hydrocarbon then you can think of like hydrocarbon will transfer to these thing so hydrocarbon will be converted to Co2 plus H2o, but in case of uranium you may think of that acetate is injected because these acetate allows the electron because it is not a pollutant at all and microbes like Geobacter are able to utilize it reducing uranium 6 to uranium 4 precipitates.

But in case you have both these as well as this then there is no need to add acetate or no need to think of any iron because then the hydrocarbon itself will act as the electron donor and carbon source and this will eventually go to this. So, that kind of possibilities are also there and that is why the Geobacter type of bacterial strains are found to be enormously useful.

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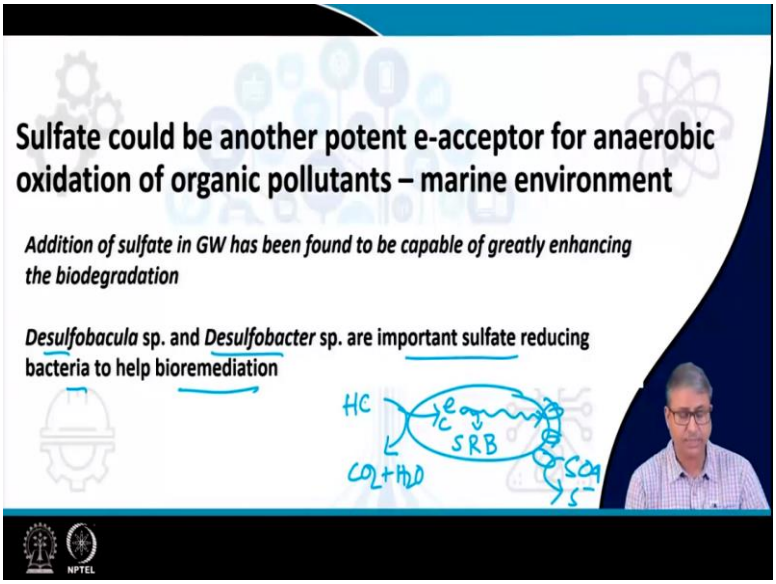

Now, it is not the case that iron is the only suitable electron acceptor. Of course, nitrate could be also another very important electron acceptor, but under strict anaerobic condition nitrate reduction may not be feasible thermodynamically or from the redox point of view as we discussed in our resource utilization lecture. So, sulfate is found to be another potent electron acceptor particularly in the marine environment or the environment where we have sulfate concentration available.

And if you have organic pollutants in those environment similarly the organic pollutants can be degraded using sulfate instead of iron as a terminal electron acceptor. Now it has been also found that addition of sulfate in the groundwater is very, very efficient technique because it can actually greatly enhance the biodegradation of organic compounds because in these case the process will be similar.

So, you have these I will write SRB sulfate reducing bacteria and in this case the hydrocarbon contaminant will be degraded to carbon dioxide plus H20 and the carbon and the electrons which are generated will be utilized by this bacterium and the electrons will finally go to the membrane where you have sulfate now as a terminal electron acceptor and you will have the sulfates produced.

So, these bacterial members like the Desulfobacula or Desulfobacter have been found to be very important. These organisms have been found to be very important sulfate reducing bacteria which helps in bioremediation. So, one thing we must be very clear about that these

degradation or the bioremediation equities based on oxidation process then we should provide electron acceptor.

But if it is based on reduction process like in case of uranium we saw then we must provide them the electron donor or the carbon source.

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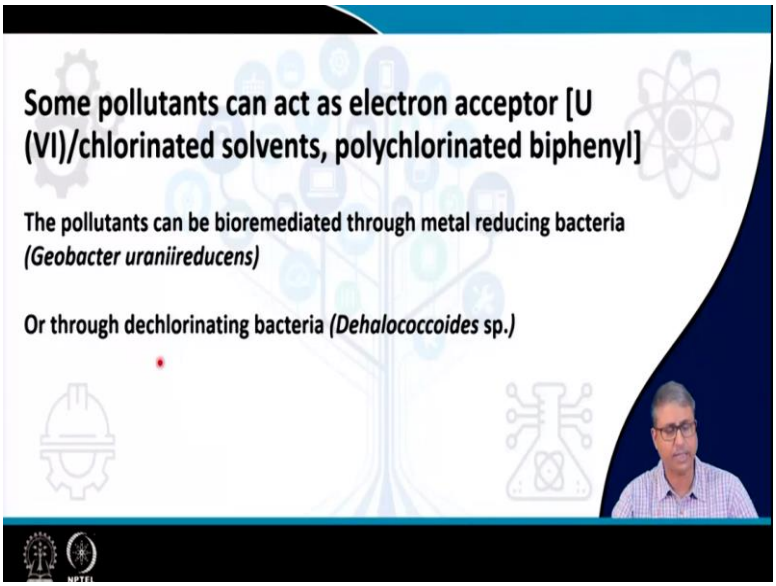

Now, some pollutant can act as electron acceptor as I mentioned uranium or maybe the polychlorinated biphenyl and other chlorinated solvents. So, in that case as I already mentioned that the uranium or these polychlorinated biphenyl they on the chlorate iron they can be utilized as their electron acceptor. So, now the pollutant can be bioremediated through either the metal reducing bacterial like the Geobacter uraniireducens or through a dichlorinating or reductive dechlorination rather by Dehalococcoides.

So, we will learn about the details of the mechanism of these processes both the metal reduction or uranium reduction and the dechlorination reactions in our subsequent lectures. Today we are actually discussing the major progress that we have made on isolation based study. So, all these bacterial strains that I am discussing the Geobacter, the dehalococcoides they have been isolated.

They have been isolated from the contaminated sites, the bioremediated sites and they have been investigated in terms of their abilities that how actually they are capable of functioning and when we were doing all these activities that isolating the bacterial strain like Geobacter

or Dehalococcoides or sulfate reducing bacteria which can promote hydrocarbon degradation in presence of sulfate.

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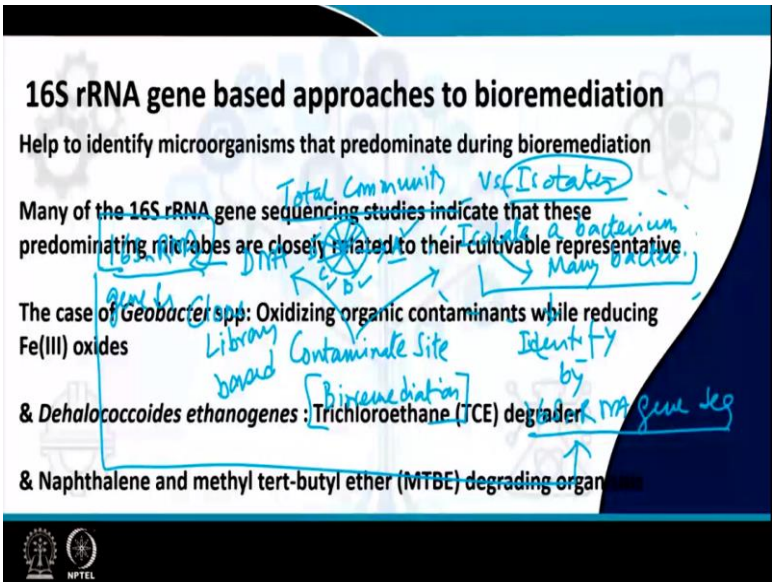

The developments were going on in terms of the 16S ribosomal rRNA gene based analysis. So, large number of sequencing events were taken up and that type of approaches, availability of 16S rRNA based approaches help us to identify the microorganism that predominates during the bioremediation. So, possibly these has actually two implications. One is that we are possibly isolating bacteria that we are already aware of that you have let us say a contaminated site where maybe you are trying to do some bioremediation by some existing knowledge or based on some other information.

So, you can always isolate a bacteria or a bacterium or you can isolate many bacteria and as a part of their identification, identify them through by 16S rRNA gene sequence. So, that is always there that you can isolate as many as bacteria possible which might be useful for your bioremediation studies, bioremediation process development and individually you can identify them by 16S ribosomal rRNA gene sequence that is always there.

But on the other hand you can isolate the DNA as we have already learned, isolate the DNA from the contaminated site and then sequence the 16S. So, in earlier days like in early 2000 BC or maybe before that also in many of these US DOE uranium and hydrocarbon contaminated sites lot of attempts were made successful attempts and that time it was all basically clone library based analysis because the next generation sequencing was not there.

So, ideally we got 16S rRNA gene sequences. Now these sequences can be matched with these sequences you can always do that. So, one way is kind of a total community I will say. So, you now see the progress. So, initially we are isolating like this part we were just isolating many bacteria and identifying them as a potent candidate. We were as soon as the 16S become available to us we tried to implement that and identify them more specifically.

But at the same time as soon as 16S ribosomal rRNA gene based method was available to us we were very clear that this can be applied directly with the contaminated site. We can actually take out the DNA from the environment and analyze. So, total community versus the isolates we started comparing. Then somewhere sometimes we actually tried to find out that organism which were actually being isolated are also there in the total community.

So, many of the 16S rRNA gene based sequence studies indicate that these predominating microbes are closely related to the cultivable that means what we actually got through total community in total community you might have some memory still with you that some members are more abundant than others if we have a relative abundance plot. So, you have some members member A maybe more abundant when you look at the total community relative abundance if you remember.

So, relative abundance wise you may find that species A is more abundant followed by species B and then C. Now you may find that your cultivable species isolates are representing sometimes A itself, sometimes B, sometimes C or sometimes even D. So, they are matching to each other. So, exactly the 16S rRNA gene based analysis allow us to identify that phylogenetically many of the isolated organisms were closely related to what actually predominates and the same thing exactly happened with the Geobacter members.

In case of Geobacter we had already isolated a number of Geobacter species and you can understand that up to the name was given later the Geobacter uraniireducens or Geobacter metallireducens many Geobacter species were isolated and characterized very thoroughly characterized for their ability to reduce metals and oxidize organic pollutants, but when we perform the 16S rRNA based analysis with the community sample we found that they are also having in reality many Geobacter.

And not only it is for the Geobacter, but also for example for the trychloroethane TC another very important contaminant present in many of the DOE sites Dehalococcoides these type of bacteria. So, Dehalococcoides is another pure culture isolates were obtained and it is not only Dehalococcoides even aerobic and anaerobic different contaminated sites like in case of naphthalene.

In case of tert-butyl ether degrading organisms we are isolated pure culture bacteria who are degrading these contaminants and when we recover or retrieve the 16S sequences from the contaminated environment we found the same environment we found that these isolates are actually very closely related to the members of the total community. So, indeed that is a very good report or good indication that the isolation was done in a very nice, nice manner.

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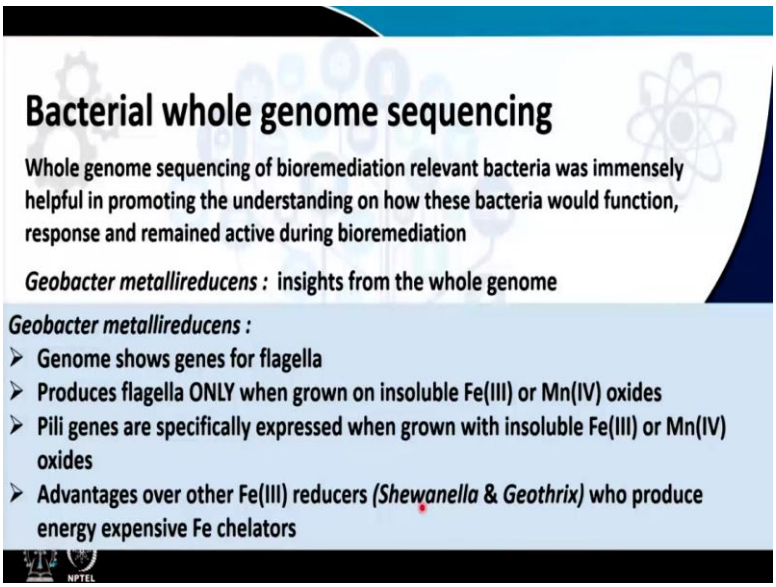

Now as we progressed the whole genome sequencing were now available. Now whole genome sequencing of bioremediation relevant bacteria in particular because by the time many bacterial strains including the Geobacter, Desulfobacula, pseudomonas, Burkholderia, and Dehalococcoides many strains or bacterial isolates were available and those were subjected to whole genome sequencing.

No single gene based PCR the entire genome of those organism were sequenced and when we perform that we found that it was immensely helpful in promoting the understanding on how these bacteria would function, respond and remain active during bioremediation. These was truly fascinating. So, one example I will give you that is the Geobacter metallireducens. So, originally before we name the Geobacter as some of the Geobacter uraniireducens the

initial name was Geobacter metallireducens because it was capable of reducing metal like iron.

So, originally when we isolated or the scientist isolated the Geobacter strain they found that it is actually non motile it is not able to move. It does not produce any flagella, but when we sequence the whole genome we found that the genes for the flagella the flagellin protein are there. So, what does that mean? That means the organism could be able to produce flagella, but the way we are testing it not allowing it to produce the flagella.

Now the implication of the flagella is if the bacterium is having the flagella the bacterium will be able to move towards the nutrient, move towards the electron acceptor, move towards the electron donor. So, the applicability of the functionality of the bacterium selected bacteria will increase. Now at the same time or the little later the scientist investigated that if we provide insoluble electron acceptors.

If we provide insoluble electron acceptor like iron 3 oxides so what was the finding that this is the bacterium let us say this is the Geobacter strain and if it is provided with let us say nitrate it would not produce any flagella. Now nitrate will give you no flagella because nitrate itself is soluble, but if you give Fe 3 for example oxides you see flagella are produced that means they have now produced the flagella.

Why because if they have flagella Fe oxide are insoluble. So, they cannot move Fe 3 oxides cannot move, but these bacterium or the bacterial cells they need Fe 3 why because Fe 3 is the electron acceptor for them. So, these bacteria now will start moving and will reach to the iron dots or iron minerals or iron particles Fe oxide insoluble particles and their it will stay very close to these.

And will be able to reduce these to Fe 2 + and while doing these it will possibly metabolize the hydrocarbon or will metabolize acetate or other carbon sources because it is basically heterotrophic bacteria. So, we came to know that. I have summarized the major findings over here. So, what are these summary events? Summary events are that the genome of the Geobacter metallireducens.

So that it has the flagella gene so it can potentially produce the flagella then later investigations that indicate that it can produce flagella only when grown on insoluble Fe 3 or Mn4 oxides because Mn can also be used as electron acceptors. Pili genes are also specifically expressed when grown with insoluble Fe 3 or Mn. Now pili is a kind of another connectors rather which will facilitate or which facilitates the (()) (40:32) electron transfer for these kind of bacterial strains.

Now in this regard the scientist also observed that these Geobacter metallireducens and other strain Geobacter strains they have specific advantages over other iron reducing organism like Shewanella and Geothrix those are also present, but from a bioremediation point of view we found that Geobacter is more efficient why because this Shewanella and Geothrix they produce energy expensive iron Chelators because Fe 3 if they want to use Fe 3 as terminal electron acceptor they need to solubilize it.

So, Shewanella and Geothrix type of bacteria they are iron reducers they also occupy similar contaminated sites, but they produce the Fe Chelators which chelates the Fe 3 and make it available to the bacterial cells, but those production of those Fe Chelators are energy expensive they need to spend lot of energy. In contrast to that Geobactor metallireducens they do not produce any Chelators rather what they do?

They produce the flagella and they move towards the organism directly or they produce the pili, the pili remains a kind of a nanowire in the later studies showed that the pili acts as a kind of nanowire allowing extracellular electron transfer to iron 3 molecules.

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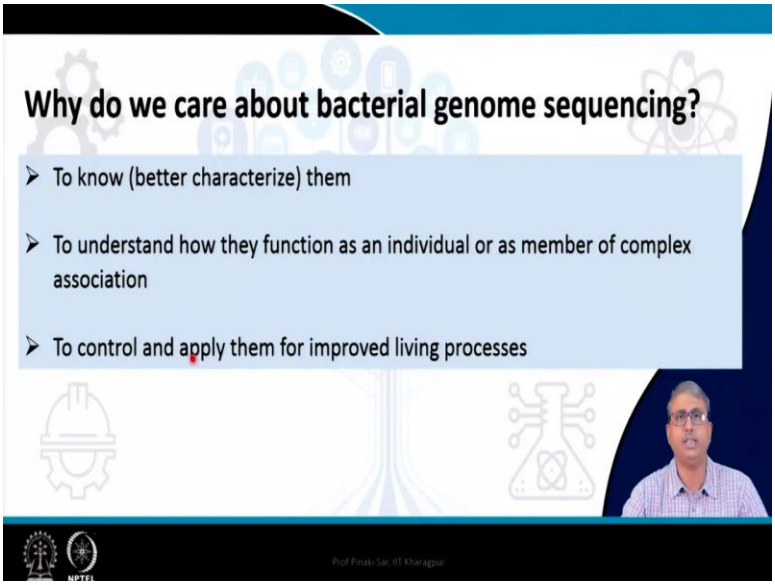

Now, in the subsequent period lot of genome sequencing of the environmental or bioremediation relevant bacterial strains, archaeal strains were performed. Now here I am going to briefly present why do we care about bacterial genome sequencing? It is specifically to know or better characterize these organisms. You have just seen the example with Geobacter metallireducens.

It is also true to understand how they function as an individual or as a member of the complex association. This genomic data will allow us to understand and also to control and apply them in a improved living processes.

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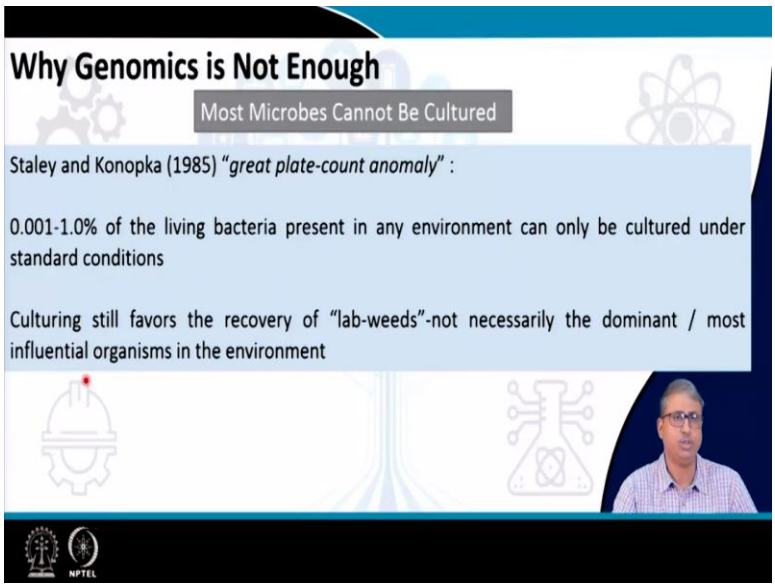

However, the genomics is always not good it is not enough I will say rather because fundamentally most microbes cannot be cultured. So, with whom you will do the genome

sequencing. You should have the isolates it was very lucky event for those people investigating on a US DOE sites because those scientist could isolate the Geobacter strains or Dehalococcoides strain.

But considering that many more bacterial strains could be there who are absolutely not cultivable because Staley and Konopka great plate count anomaly and subsequent studies have indicated that it is close to 1% or less than 1% of the total bacteria populations are actually culturable. And also somehow we have learned over the years that culturing under different lab conditions and if we are slightly I will not say careless.

But we are not taking proper care rather we may have lot of (()) (43:33) which are not necessary the dominant or the most influential organism in their environment. So, during the culturing process we need to be really very careful it is not only the culture medium that are going to be used, but also we should take care about the other environmental conditions which might allow better recovery of cultivable bacteria.

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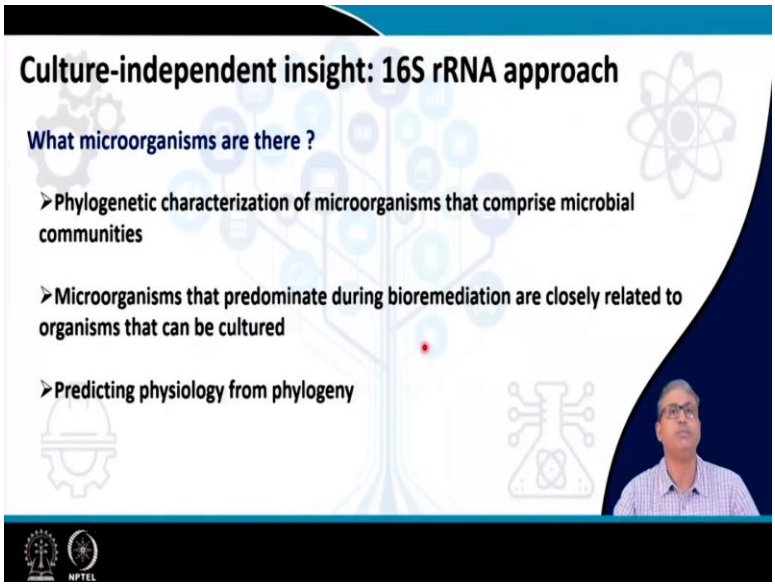

Now as soon as cultivation independent methods and parallel the whole genome sequencing methods were available so we are able to utilize this 16S rRNA gene based approaches because that allowed us to identify the phylogenetic characteristics and what we can surely say that the microorganism that predominate during the bioremediation are closely related to organism that can be cultured.

So, our confidence microbiologist confidence about these bioremediation systems were built up or become stronger and we started predicting the physiology from the phylogeny which is basically phylogenetic interpretation so that converted to the metabolic or physiological interpretations.

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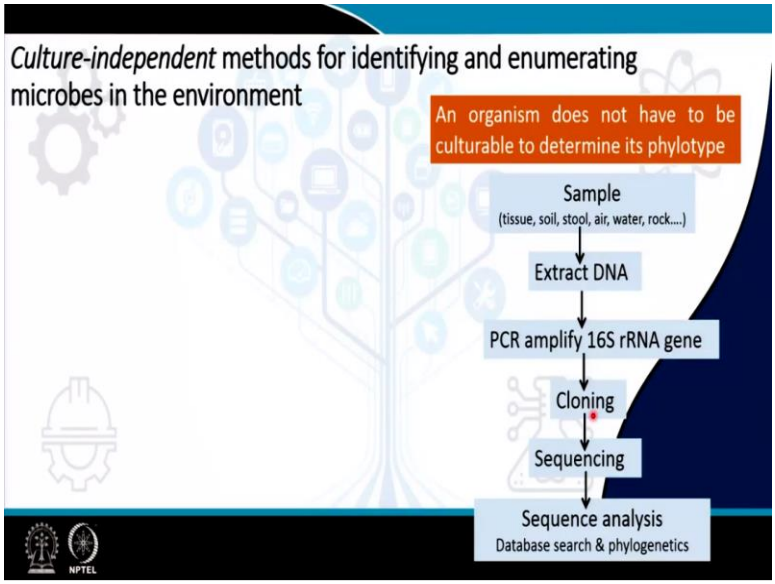

Now the culture independent methods for identifying the enumerating microbes were developed in a very straight forward way because in that case the organisms they are not necessarily to be cultured as we already studied earlier (()) (44:56) DNA amplify the 16S rRNA gene and either we do manual cloning or during the next generation sequencing process this cloning will be done through the sequencing machine or sequencing reaction itself and then we perform the sequence analysis.

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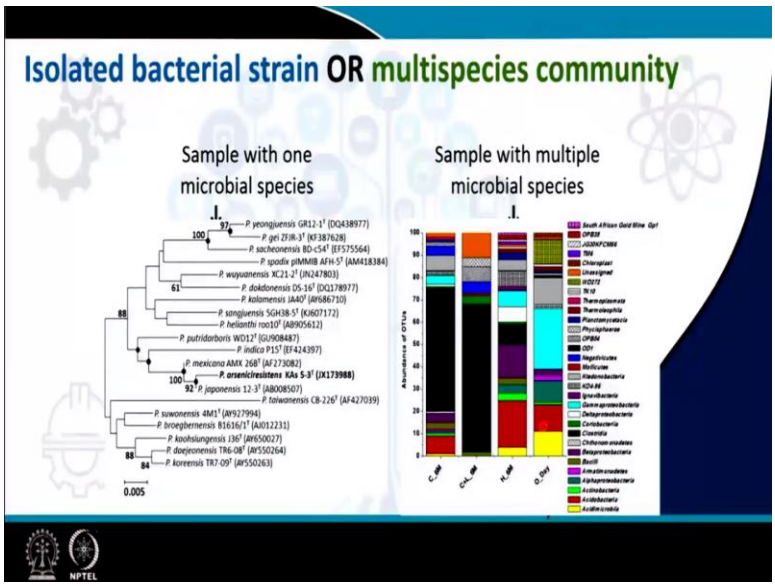

Now, I will show you the basic layout that in case of single bacterial strain we had a kind of only genomic strain only one 16S and sequence match we will establish the identity. In case of multiple species present in a contaminated environment we need to have some kind of bio informatics analysis or the cloning process should be more precise so that we are able to segregate the 16S rRNA gene sequence and analyze them.

So, actually we are expecting results like this that we will have identification at very precise identification of the organism like in this case pseudomonas strain is identified from a arsenic contaminated groundwater and on the other case we are able to actually identify the different type of bacterial taxa which are present in a contaminated environment.

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But that was not the end of the road. Only 16S rRNA genes are having its benefits, advantages, but also having some limitations as we have discussed in some of the earlier lectures. So, eventually the concept of Metagenomics emerged because that was found to be one of the best method to analyze all the organism which are present in the environment, their functional (()) (46:30) as well as possibly analyzing the interaction between the organism.

So, this was perfectly all right when we are trying to isolate few bacterial strains and identifying them, but that was not about everything. We were not able to understand how they interact with themselves, how they interact with their environment, how they respond actually all these things were not very clear to us.

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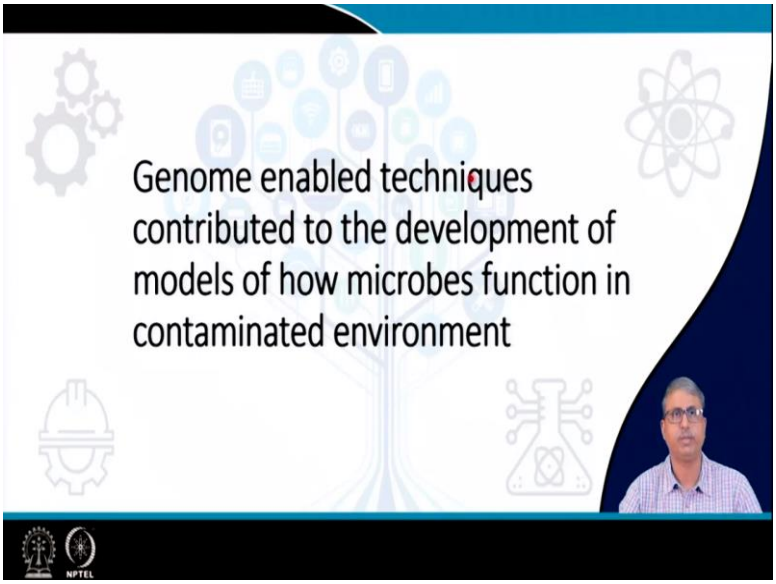

So, essentially the genome enabled techniques contributed to the development of models to how microbe function in the contaminated environment.

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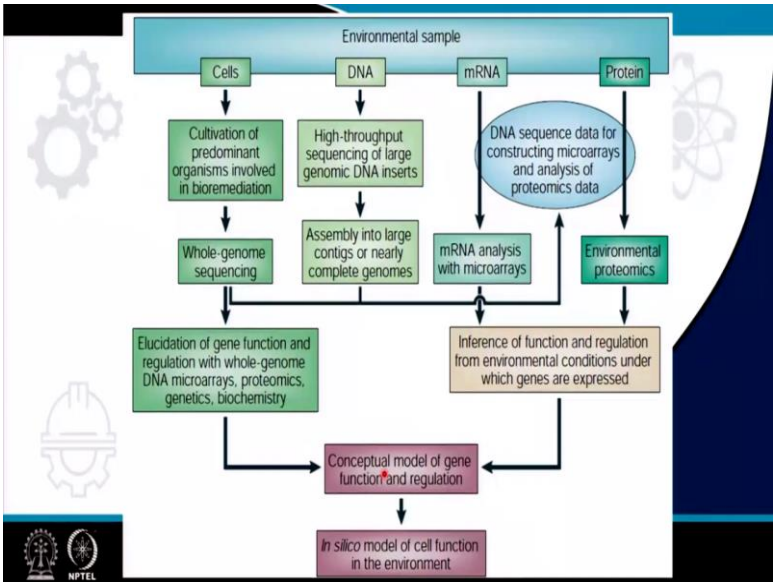

So, essentially we are able to delineate the what you called as a kind of a toolbox that the environmental samples can be subjected to a wide ranging methodology including the isolation of the cells that could be done through the cultivation followed by their characterization and whole genome sequencing and then elucidation of the gene function, regulation etcetera.

And then developing a conceptual model for gene function regulation and then also how they are going to function in the environment one of the best example is the Geobacter uraniireducens or metallireducens where the organism are isolated they sequenced whole

genome and then all transcriptomics and functional gene based analysis were performed to model the functioning of these organism.

The alternate and parallel strategies could be destruction of the total DNA or the total RNA or mRNA or the total protein. And we have possibly discussed part of these approaches in our earlier classes. So, eventually all these DNA RNA these are omics based approaches or we can consider them as the Metagenomics analysis because here in many cases we will not be applying any PCR it is the entire DNA or the entire mRNA pool can be sequenced or it can be Metaproteomics where the entire pool of proteins will be analyzed.

And then we will be able to obtain some inference about the function and regulation of these multiple organisms including their interaction, their ability to interact with other species, the ability to interact with their environmental factors and then developed the conceptual model about their functionalities and their regulations because regulation is very important for the bioremediation process because most of the in situ application sites or the open environments are subjected to variations, climatic variations and other variations.

So, how the organism or the organisms who are the candidate organism who are responsible for the remediation could respond that becomes a very important task.

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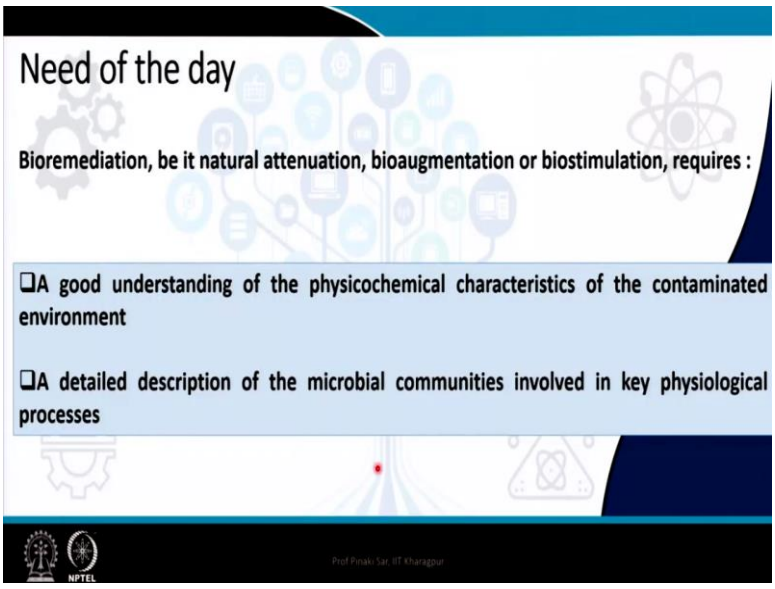

So, what is the need of the day? So, bioremediation whether it is a natural attenuation or engineered process it would require a good understanding of the physicochemical

characteristics of the contaminated environment as well as a detailed description of the microbial community present. So, both are required.

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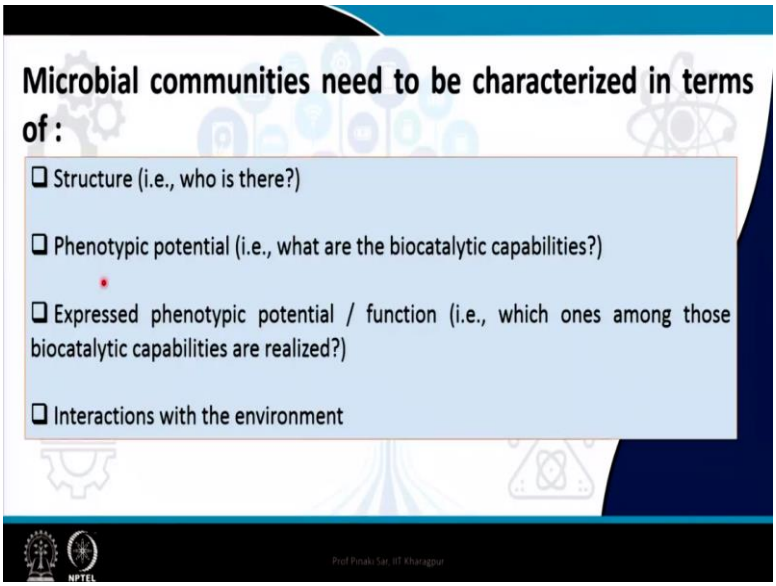

And it is not only true that we have to have answer of the four questions that microbial ecologist often ask.

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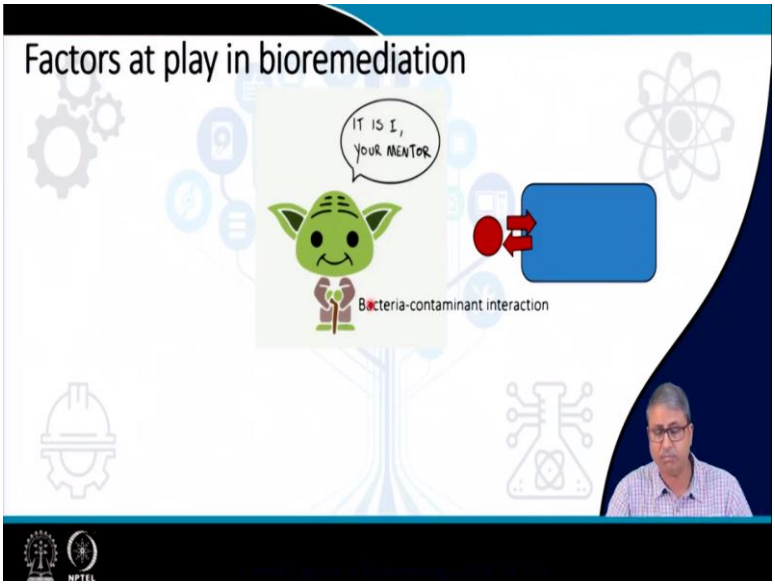

But also we need to understand how different interactions between the contaminant and the organisms are playing a role.

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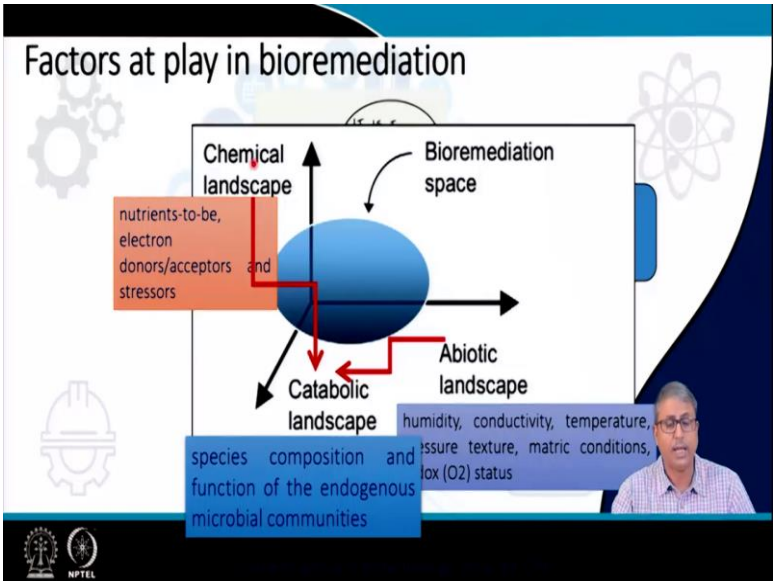

Possibly something like this where we consider this as a bioremediation space and both the catabolic landscape as well as the chemical landscape where we have the nutrients to be or the abiotic landscape like conductivity, temperature, humidity, matric conditions etcetera. They control the species composition they control the species function and on the other hand these also will have a repercussion on that.

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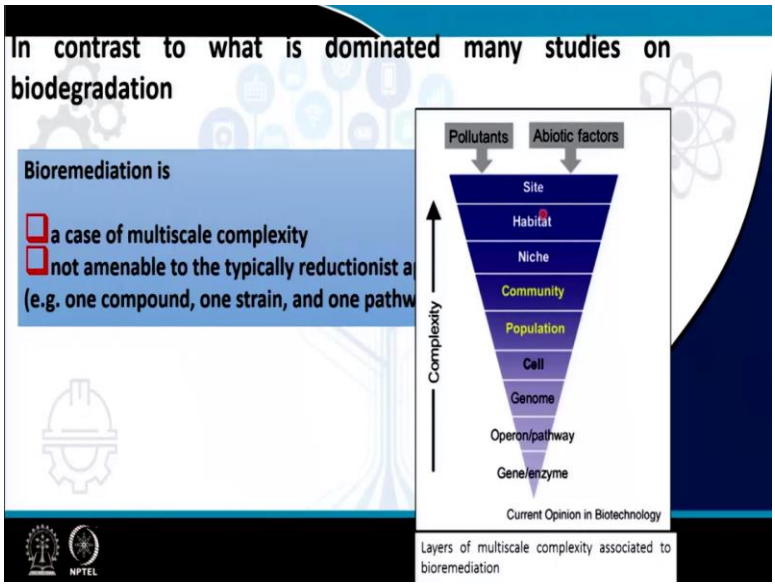

So, in contrast to what is dominated in many studies on bioremediation or biodegradation what you have learnt that it is a case of multi scale complexity. It may not be always true that one bacterial strain, one compound and only one pathway is going to be most important for that it may not be. So, it is said that not amenable to the typical reductionist approach it may not be true always.

So, ideally it is as I mentioned is a multilayered structure which starts from the gene and the gene or enzyme they interact with the pollutants and they also enable the pollutants to degrade or bioremediate it, but they are all organized as operons and pathways. They are part of the genome and the sales then sales are the member of the species they belong to the population, populations belong to the community.

Community functions within the niche and the niche they are in a habitat and microhabitat and then you have the site the polluted site where the bioremediation is to be carried out. So, when you have a pollutant present in a site you have a number of abiotic factors. Both these pollutants abiotic factors will play a roles in controlling all these parameters, all these multilevel response will be received up to the gene expression or the enzymes which are going to be present.

And the enzyme are going to be most important component for carrying out the transformation reaction.

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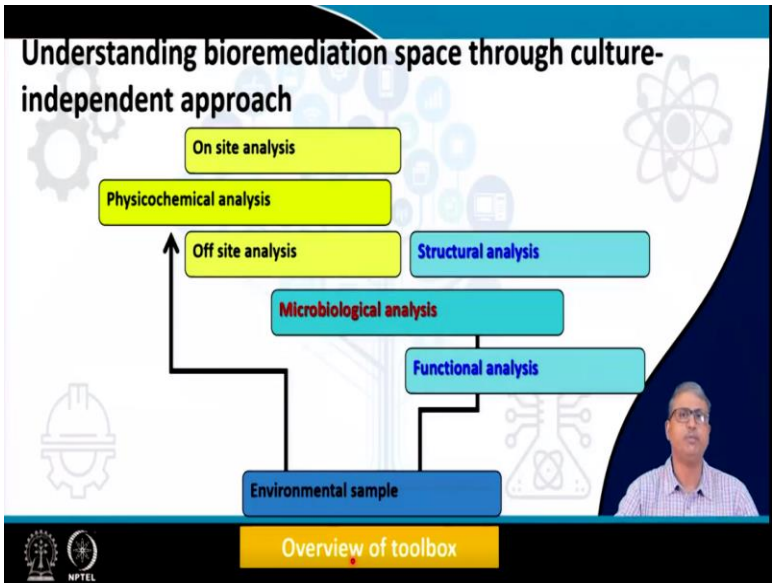

So, essentially we come out with this kind of a bioremediation toolbox what I suggest is that understanding of bioremediation space through culture independent approach would require some kind of concept which will have a bioremediation toolbox that will include the environmental sample to be studied for multiple geochemical or environmental factors as well as the microbiological or microbial ecology analysis which will have both the structural and functional components.

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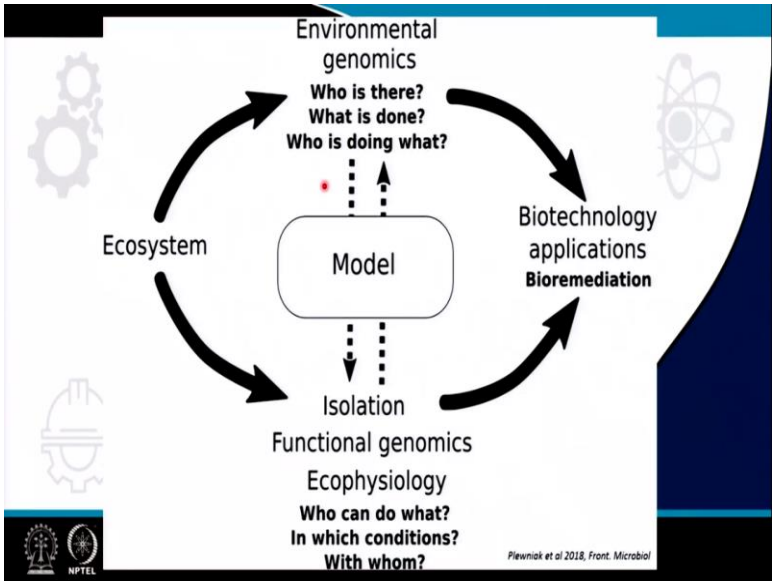

And that would possibly help us to lead us to understand the entire community function and possibly will lead to the environmental genomics which will answer the fundamental questions like who is there, what is done and who is doing what and then apply that knowledge into the biotechnology applications particularly with respect to bioremediation.

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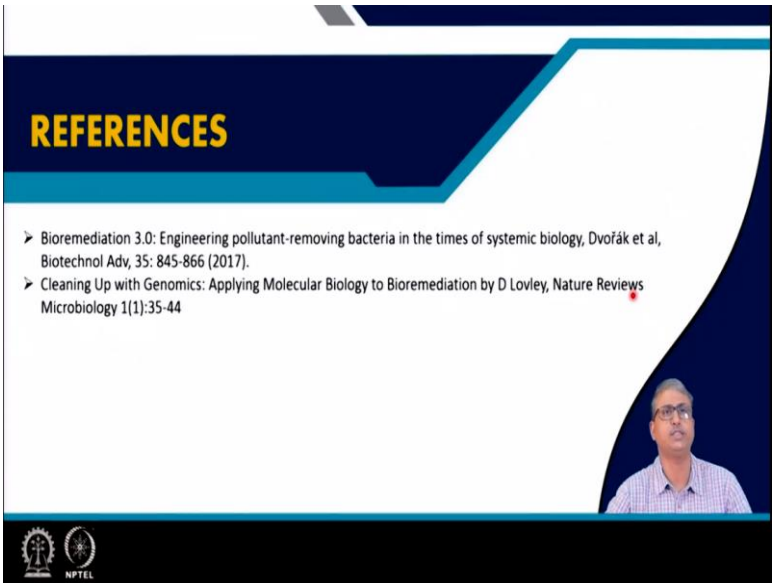

So, for this part of the lecture mainly these two articles will be useful.

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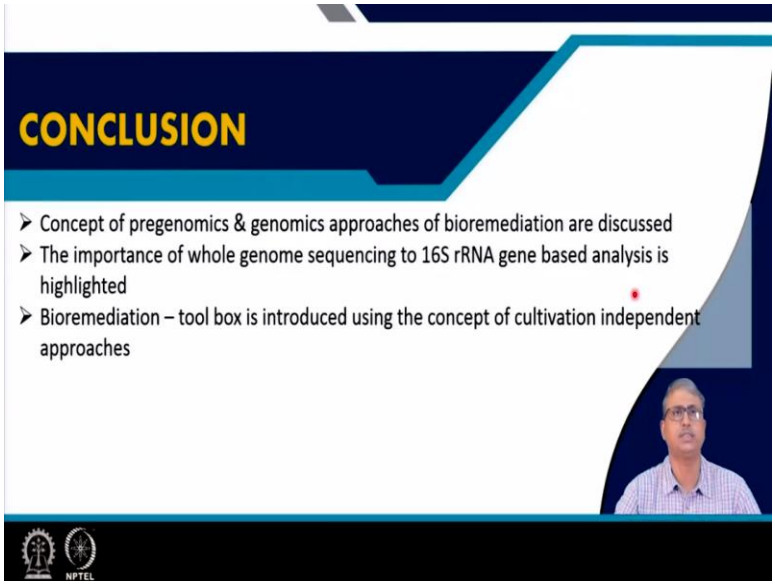

And in conclusion the concept of pregenomics and post genomics and whole genome based approaches in bioremediation are discussed. The importance of whole gene sequencing to 16S rRNA gene based analysis is highlighted and finally we came to the concept of the multilayered complexity is introduced and also the bioremediation toolbox is introduced with cultivation independent approaches. Thank you so much.