Environmental Biotechnology
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Lecture – 37
Bioremediation Continued

Welcome to the next lecture of our environmental biotechnology course and today's lecture would be on bioremediation.

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In this particular topic or lecture of these bioremediation we are going to discuss about the goals and the progress of bioremediation. We will also discuss the challenges of bioremediation and we will highlight some of the important aspects of bioremediation approaches considered to be very important with respect to the pre-genomics approaches particularly.

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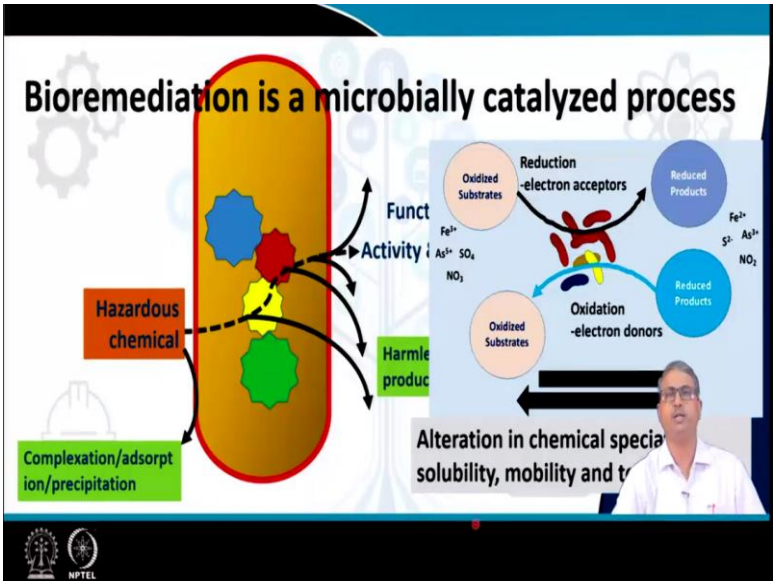

Now as we have discussed already bioremediation is a microbially catalyzed process where the microorganisms are capable of transforming different types of hazardous chemical compounds through different arrays of processes or metabolic processes which includes different type of oxidative reductive reactions as well as reactions involving complexation, adsorption and precipitation etcetera.

And what we have also noticed that during most of these bioremediation relevant reactions the microorganisms they catalyze the oxidative or reductive reactions thereby transforming the contaminated compounds or the hazardous chemical compounds in terms of their altered chemical properties, chemical speciation, their solubility, their environmental mobility and eventually toxicity also.

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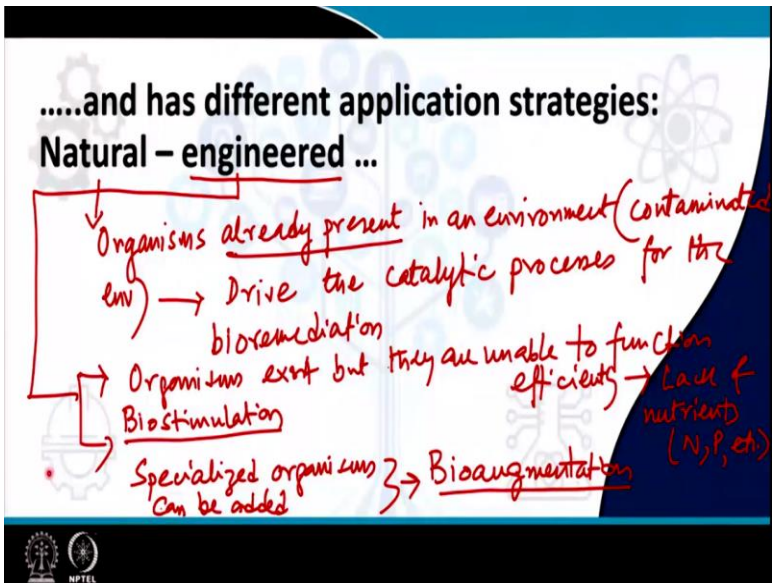

In context to the application of bioremediation we have also learnt earlier that bioremediation has different application strategies and these could be natural and engineered. As we discussed earlier possibly that in the natural bioremediation process we mostly rely on the natural or native organism. So, organisms which are already present there. So, here we organisms already present in an environment particularly the contaminated environment.

So, organism which are already present in a particular environment which is contaminated and which actually drive these organism they drive the catalytic processes for the bioremediation this is one process. However, for the engineered process there could be two types. As we discussed earlier there could be one type where we find that the organisms exist, but they are unable to function efficiently.

Why they cannot function efficiently because most of the time it is we have found that they have a lack of nutrients like nitrogen, phosphorous, etcetera or maybe the ambient condition. So, in this case where organism exists, but they are unable to function efficiently because there is a lack of nutrient. So, we need to add nutrient, for example, if they are deficient in nutrient then add the nutrient or provide the suitable condition like for example the pH or sometimes the terminal electron acceptor etcetera.

This particular approach is referred as biostimulation this we have already learned. Alternatively specialized organism can be added. So, when we add specialized microorganism in a particular environment then we refer to that as bioaugmentation because there we are augmenting the particular environment which is contaminated with a particular type of bacteria so that is referred as bioaugmentation.

So, one is bioaugmentation where specialized microorganisms are introduced who are capable of performing the desirable the catalytic reaction which facilitates the particular bioremediation related event and on the other hand we have the biostimulation approach where organisms exist, but they are unable to function adequately because there is lack of important nutrients or lack of the proper condition in the system.

So, in either of the cases we take appropriate measure and then these are considered as parts of the engineered bioremediation.

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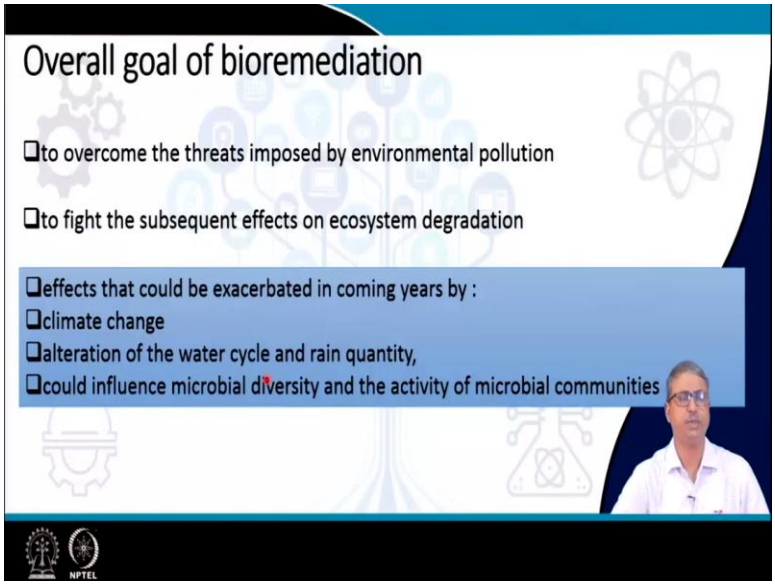

Well now we move forward that in any case that whether it is a biostimulation or whether it is a bioaugmentation that is the engineered bioremediation or it is part of a natural attenuation process the overall goal of bioremediation remains very, very clear. The goal is broadly maybe categorized into two parts one is to overcome the threats imposed by environmental pollution.

So, it is an environmental threat imposed due to the presence of this toxic hazardous chemical compounds in the environment and the other one could be to fight the subsequent effects on the ecosystem degradation. So, one could be the direct damage caused by the pollutant itself so that would possibly address the issues related to the pollutants itself and the second one is to fight the subsequent effect on an ecosystem degradation.

So, once we have the pollutant chemical present in the environment the environment would experience lot of damage and due to these damages the environment will suffer and now the bioremediation could be taken up on those sides where along with the pollutant degradation the recovery of the environment reclamation and recovery of the environment will be taken care off.

Now these effects caused by the pollutants could be exacerbated in coming years because of the climate change because of the alteration of the water cycle and the rain quantity irregular rain quantity and the abstraction or removal of huge quantities of groundwater particularly and the other uses of the surface water which are found to be very important resource for our water demand.

And these all could influence the microbial diversity and activity of the communities which are present in the natural system and therefore they are supposedly helping us to maintain the biogeochemical cycles and to reclaim the natural conditions within the contaminated environment.

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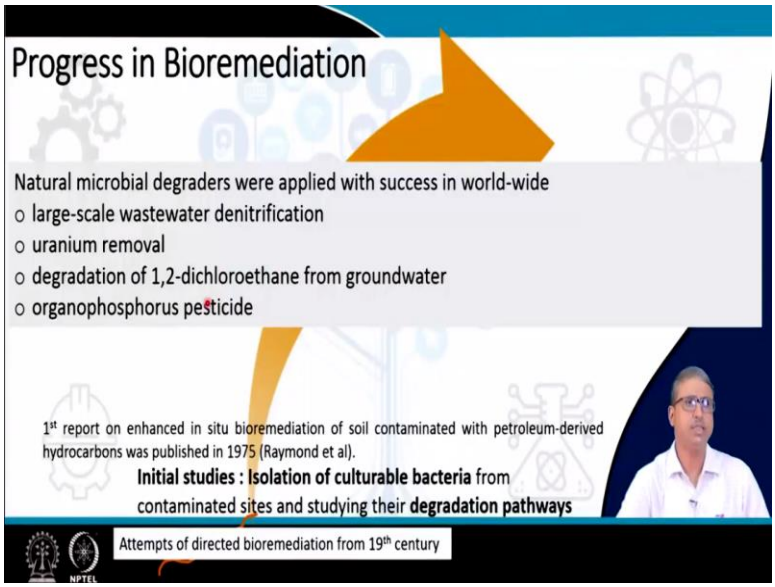

Now with these two broad goals particularly the first goal was very clear to ask for a long period of time and we have been working so human is always trying to actually make a substantial progress in terms of how to remove these contaminants, how to deal with these contaminants. So, if we want to just look into the aspect of how the bioremediation as a process as a part of our understanding has actually progressed over the period of centuries I will say.

So, we see that even in 19th century there were attempts of directed bioremediation those are basically dealing with the waste water treatment and different type of other water treatment processes to cater the water supply to the industry as well as to the societies. Now, initial studies if we see the very systematic studies using bacterial strain to be particular were done mostly using the cultivable bacterial species of course because during the early days the uncultivable bacterial species or the concept was not there at all.

So, the initial studies were mainly focused on isolation of culturable bacteria from different contaminated site and studying their biodegradation pathway. So, large number of studies has been done and if we see that the first report on enhanced in situ bioremediation of soil

contaminated with petroleum derived hydrocarbon. So, one of the most hazardous compounds was published 1975 itself.

And then subsequently the natural microbial degraders or microbial species or bacterial strains in particular were isolated, characterized and particularly they are isolated because the cultivation independent methods were not known were applied with a success in worldwide sites. And what we see that large scale waste water denitrification in particular because the waste water recycling and removal of the huge nitrate load in the wastewater remains one of the challenging task for the environmental engineers.

Removal of uranium from different contaminated sites; degradation of 1, 2 dichloroethane from groundwater and also the organophosphorus pesticides removal. There are other hazardous compounds which were targeted as well and huge amount of success was obviously obtained, but these are some of the major achievements where were observed.

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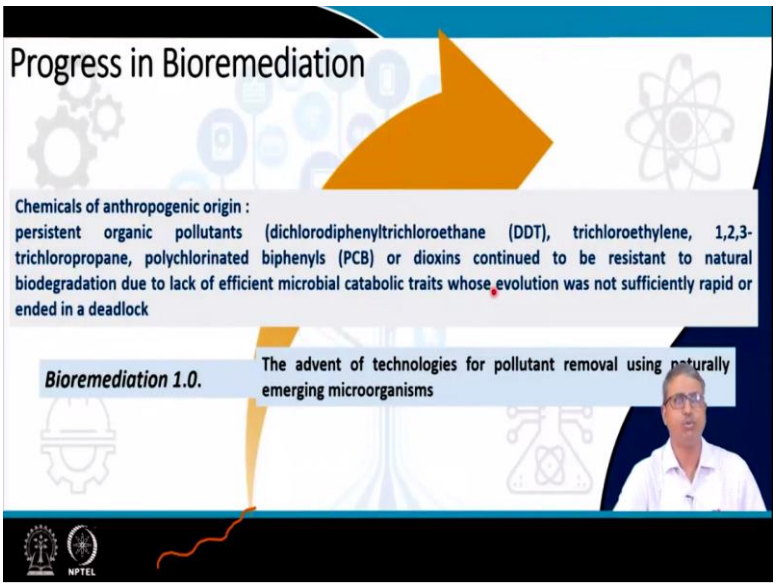

Now, these phase of bioremediation attempt the phase which constitutes mainly the isolation of bacterial strains and their characterization and their deployment for removal of the contaminant whether organic pollutant or inorganic pollutant that set the stage for the advanced bioremediation processes and the bioremediation 1.0 is basically referred to the advent of technologies for pollutant removal using naturally emerging microorganism.

So, the organisms which are naturally present we tried to isolate or scientist they tried to isolate, characterize and develop systems based on those organisms. However, what we found

that with respect to some chemical compounds particularly the chemicals of anthropogenic origin or what we call them as xenobiotics compounds as well as the different type of persistent organic compounds those are found to be resistant to natural biodegradation due to the lack of efficient microbial catabolic traits because they are manmade compound.

And microorganisms they have never been exposed in their evolutionary history with these type of manmade compounds so they possibly have not developed adequate genetic system or the enzymes to catalyze those compounds for whom these xenobiotics compounds are those compounds basically and there is lack of efficient microbial catabolic traits whose evolution was not sufficiently rapid or ended in a deadlock situation.

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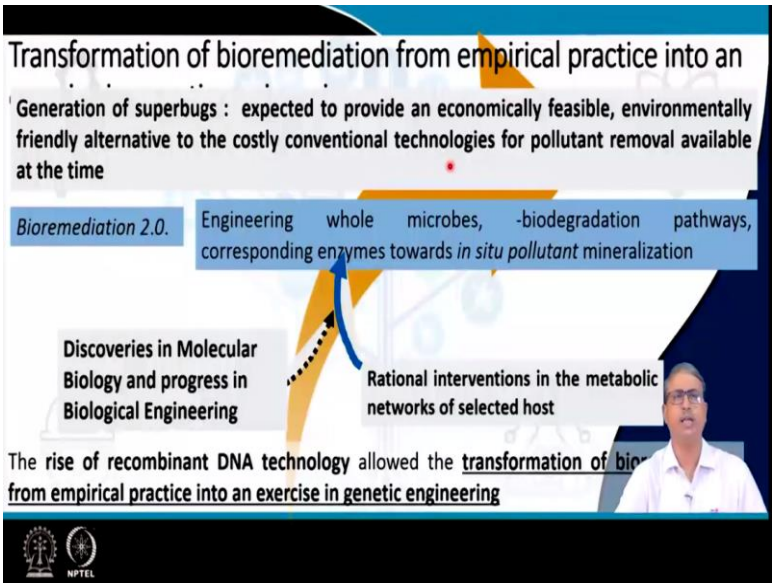

So, rise of the recombinant DNA technology allowed the transformation of the bioremediation from empirical practice into an exercise of genetic engineering. So, we see that during 80s and then 90s we see that the advent of bioremediation was basically through the molecular biology tools because by this time the different molecular biology tools and molecular biology protocols, instrumentations were available or becoming available.

So, many discoveries were made and it helps in not only in deciphering the characteristics of the microorganism in terms of the genes, in terms of the enzymes, their expression etcetera, but also in progressing towards biological engineering of these processes and rational intervention in the metabolic networks of selected host were also attempted in subsequent time.

So, what we see that all those things like the advent of the molecular biology tools and approaches, the incorporation of metabolic network based concepts led to the development of what we call bioremediation 2.0. These bioremediation 2.0 is considered to be kind of an engineered process where microorganisms are engineered. So, whole microorganism bacteria for example they are engineered with respect to their biodegradation pathways because of the large amount of molecular biology tools available.

And availability of those tools make that things possible so many recombinant organisms are created and these biodegradation pathways were well elucidated perhaps some of them were elucidated earlier itself, but now with the incorporation of the genetic methods molecular biology tools they were very well elucidated, their regulation etcetera, genes etcetera and they were particularly use because the corresponding enzymes were found to be useful for their in situ pollutant mineralization or degradation.

And that bioremediation 2.0 were the engineering microbes were actually major point of interest, the generation of superbugs particularly happened where we see that these organisms these superbugs are bacteria which are genetically engineered and they are expected to provide an economically feasible, environmentally friendly alternative to the costly conventional technologies for pollutant removal available at that time. So, if I look at these discoveries we will see that compared to the conventional technology available.

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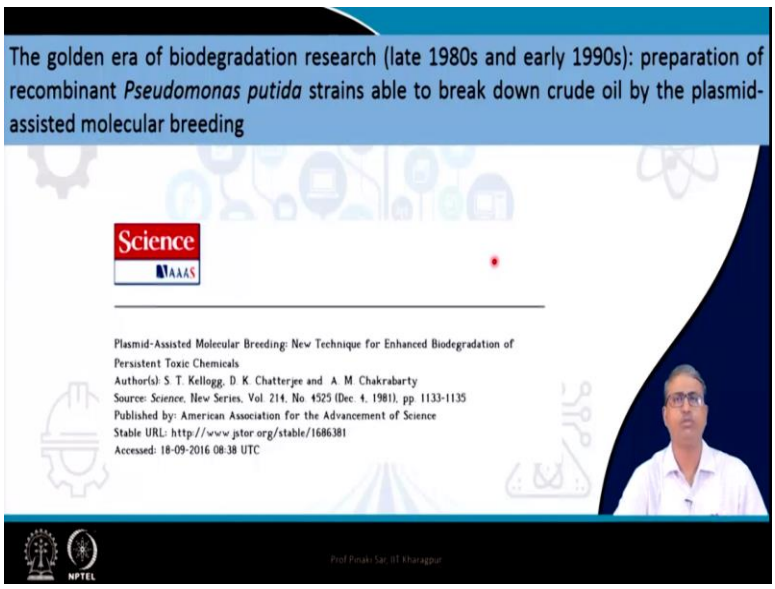

For example the 1980s and early 90s which is considered as the golden era of biodegradation research and the recombinant strains were engineered rather I will say strains were developed

in the laboratory and were successfully applied into the field contaminated sites and which is referred as actually the golden era of biodegradation research. These helped us to develop the strains like pseudomonas putida.

And you can see that this is basically plasmid assisted molecular breeding and it is considered to be a new technique for enhanced biodegradation of persistent toxic chemicals including the crude oil and crude oil derivative and it is a plasmid associated event and part of the molecular breeding.

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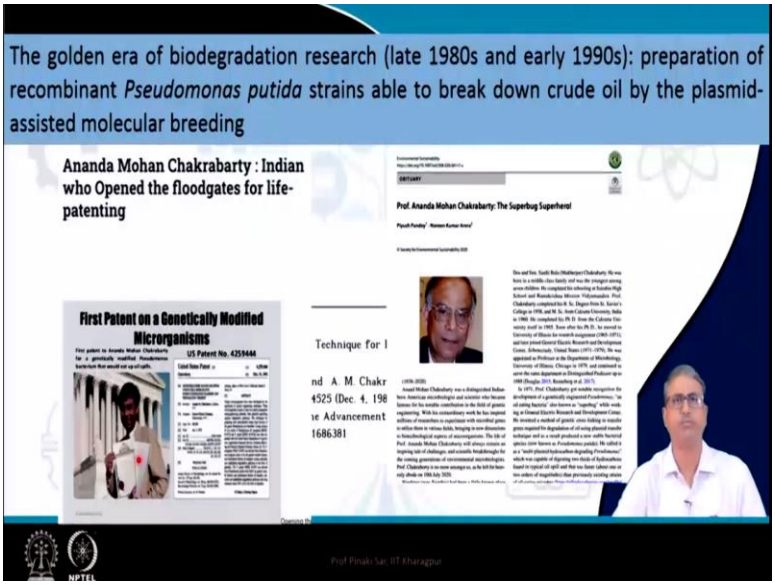

Now based on these concepts of engineering the; organisms Late Professor Ananda Mohan Chakrabarty the Indian microbiologist who opened up the flood gates for life patenting. So, first patent on the genetically modified microorganisms was rewarded to Professor. Ananda Mohan Chakrabarty due to his contribution and discovery of these engineered organisms.

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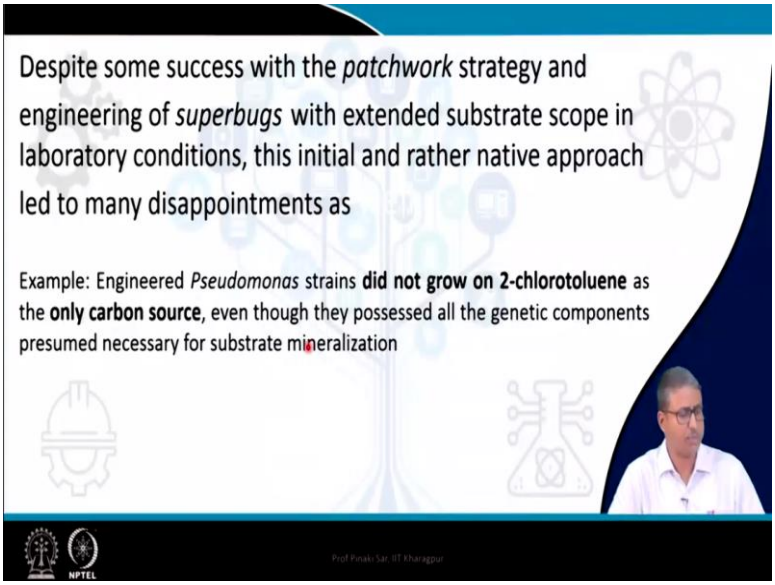

And what we found that subsequent to these phase of what you call golden era of biodegradation and developing the engineered strain part of bioremediation 2.0. So, large number of strains were developed engineer strains were developed and they were tested with respect to decontamination of different pollutants emerging pollutant during that time we are talking about late 80s to 90s.

We found that despite some success with the patchwork strategy was a later strategy it is not nearly based on plasmid, but it is also based on identifying the best genetic modules from different organisms and then bring them together in a single plasmid and then put them into the host organism and these kind of engineering strategies towards the development of superbugs with extended substrate means it is actually the strains were having a broad substrate specificity.

So, in number of environmental pollutants can be degraded by these organisms supposedly they are targeted to be like that, extended he substrate scope in laboratory conditions. This initial and rather native approach led to many disappointments as well because initially it was thought that these engineered microorganisms will be very successful in broad application of bioremediation because they are engineered very intelligently.

However, in many cases it was observed that they are not performing up to the mark. One of the examples remain this one this engineered pseudomonas strains which were extensively studied an engineer did not grow on 2 chlorotoluene which was found to be a potent

contaminant as the only carbon source even though they possessed all the genetic components presumed necessary for substrate mineralization.

That perhaps provided some very interesting insight into the microbial biodegradation abilities. It is not only having the genes because at some point of time the scientist were thinking that putting the genes relevant genes which will make the organism most efficient perhaps genetically we will solve the purpose or solve the problem and these superbugs having all the required genes will be able to degrade the target contaminants.

But eventually we observe that it is not actually growing very happily on the target compounds even although it has many genes. So, that possibly means that there are more complex regulators or more complex requirements which are responsible for controlling the performance of the organism in the environment.

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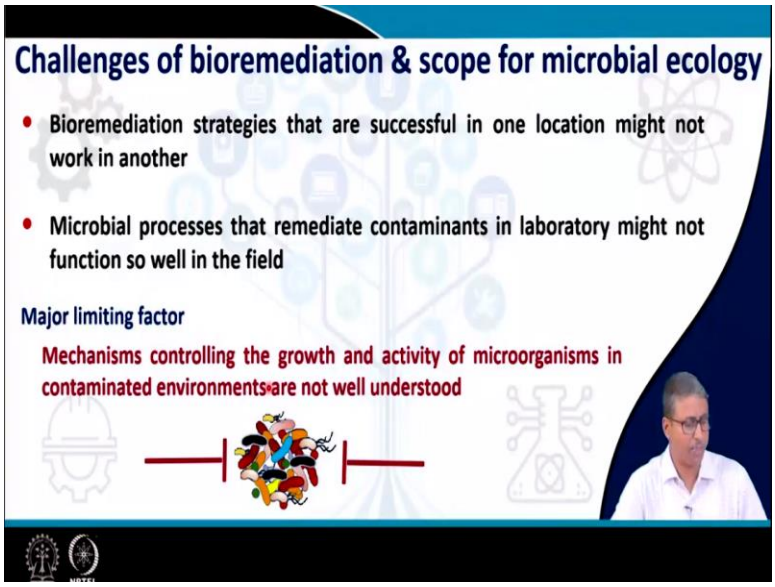

Now in the gradual period of time like 1990s onwards to 2000 when we have lot of molecular biology protocols being developed, implemented, lot of sites contaminated with uranium. Different other radionuclides, chromium and other heavy metals hydrocarbons were bioremediated across United States particularly if we see that US DOE Department of Energy they have done one of the best and most rigorous experiment as well as improve the understanding and implemented the understanding.

But at some point of time the scientist and engineers working on bioremediation using microorganisms isolated grown in the laboratory and then implemented into the field. They

realize that there are certain very important or critical challenges and those challenges are not only challenges and those challenges actually throw very important light on the scope of microbial ecology because from a isolation centric bioremediation process.

The bioremediation engineers and bioremediation scientist started looking into the community itself. So, bioremediation the challenges that we faced or the scientist they faced can be highlighted or can be broadly categorized into two. The one is the bioremediation strategy that are successful in one location might not work in another location which was very evident that process based on certain cultivable bacterial strains working very well in one particular location.

But in another location with a similar contamination types or type of contamination the similar approach, similar strains might not be working very well. The second one was that the microbial processes that remediate contaminants in laboratory might not function so well in the field so that means when we try to develop the processes in the lot of laboratory experiments are done.

So many a times what have been found that during the laboratory studies these bioremediation process remain very successful, very promising, but the moment we want to transfer them into the field many of these laboratory based studies were found to be not good enough to be performing in the real environment. So, one of the major limiting factors identified was that the mechanisms controlling the growth and activity of microorganisms in the contaminated environment are not well understand.

So, that means if we have the microorganisms which are the candidate organism capable to transforming the pollutants, degrading the pollutants or redox while oxidizing the reductive process they are capable of converting at toxic heavy metal to another form or so, but exactly what controls their growth and activity. You may have microorganism or microorganisms in your environment or you might have added in a bioaugmentation study or bioaugmentation based approach.

But having the organism with appropriate genes and enzymes may not be sufficient because there could be many other factors which will eventually control the growth and the activities of those microorganisms who are the key players in the particular environment for the

transformation of the degradation of the pollutants. So, these factors so the two sided I have put some marking that the factors which actually interfere or try to control the growth and activity of the organisms are often not well understood.

And this fact actually connects us to the importance of the microbial ecology because in microbial ecology we have learned the importance of the multispecies, multi-orgasmic concept and where we have also learnt the self-assemblages and the functional guilds and the interaction among the species and the interaction of the species with multiple environmental factors.

Now, when we have the isolates the very, very promising isolates like the engineered pseudomonas strains and many other similar strains ready to be deployed in an environment contaminated environment many a times they are failed to perform up to the mark. One reason could be because the factors which actually will control their growth and activity were not properly understood during that time.

But when we look into the literature scientist were very careful about identifying the challenges and the limiting factors. So, when scientist they found that these could be the challenging factor they try to address these issue. So, gradually in my lectures we will find that how the bioremediation concept has been changed over the time.

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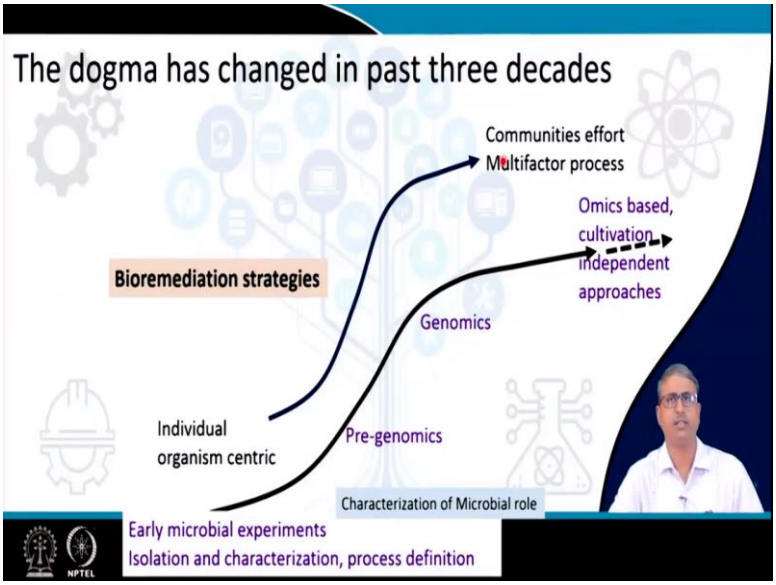

Now the dogma that is followed like you have a pollutant let us say is a toluene and you want to have a bacterial strain who can degrade toluene so for that you might have isolated a

bacterial strain who can degrade the toluene and the organism that particular bacterial strain is having the required genes and the required pathways. So, you are all set to utilize that bacterial strain for toluene degradation in a toluene contaminated site.

So, initially those type of approaches or that type of approaches were very lucrative and very lot of interest, lot of enthusiasm were there. So, these are called early microbial experiments isolation and characterization the process definition. So, we try to find out how these for example a toluene degrading bacteria would perform in the real environment. So, gradually in the early days we started with implementing them.

And exactly during that time the whole genome sequencing concept was not available. So, we term these phase a pre genomics approaches so lot of isolation lot of new isolates, but it is you can see the slope is rising because it is not only a fact that the genome sequence was not available for a routine use. However, we had the notion that many bacteria are uncultivable. So, perhaps we try to isolate as many as bacteria possible with using different culture media, different growth conditions etcetera.

So, numerous bacterial strains were isolated and these isolates were tested and we try to actually characterize the microbial role. So, microbial roles were characterized through individual gene sequencing not the genome sequencing as well as their ecophysiological properties that we have already discussed in our earlier lectures. Now, as soon as the genomics came then we are able to sequence the entire genome.

As soon as we sequence the entire genome many unknown things were explored in front of us or they were in front of us because based on the ecophysiological astudies or metabolic characterization or single gene or one or couple of gene based analysis we were unable to understand the true role or true importance or true capabilities of that particular isolate or isolates.

Genomics particularly the whole genome we are referring here whole genome sequencing of the bacterial strains, the candidate organisms allowed us to know the entire genetic repertoire of the organism and thereafter we have the Metagenomics and Metatranscriptomics and Metaproteomics and all other omics based approaches which are called cultivation independent approaches.

So, till genomics we can consider partly it was cultivation dependent because still we were isolating bacteria and isolates were subjected to whole genome sequencing, but later when the high throughput next generation sequencing become so popular and we are aware about the huge uncultivable bacterial population present in any environment. So, lot of omics based cultivation independent approaches were implemented.

Now, if we look at the development with respect to the strategies that we are adopting. With respect to bioremediation strategies there is also a similar change, but rather is a kind of more rapid change happened that in early days even in the pregenomics or parts of the genomics era also when we were sequencing the genomes of individual organism which we were able to isolate.

So, it was most like individual organism centric approach. It was dependent on our capability to isolate bacterial strain or archaeal strain from different contaminated or otherwise important environment and then characterize them in terms of their bioremediation potential, but as soon as these omics based cultivation independent approaches were available to us. So, the entire strategy or the approaches towards bioremediation was shifted absolutely it was shifted because from individual organism centric view of the bioremediation process.

We landed down to a community centric effort because by the time the importance of microbial communities in function in particularly in executing the function within a particular environment was very clearly as soon as the next generation sequencing based methods were available to us. So, community effect and its multifactor process that was also very clear to us.

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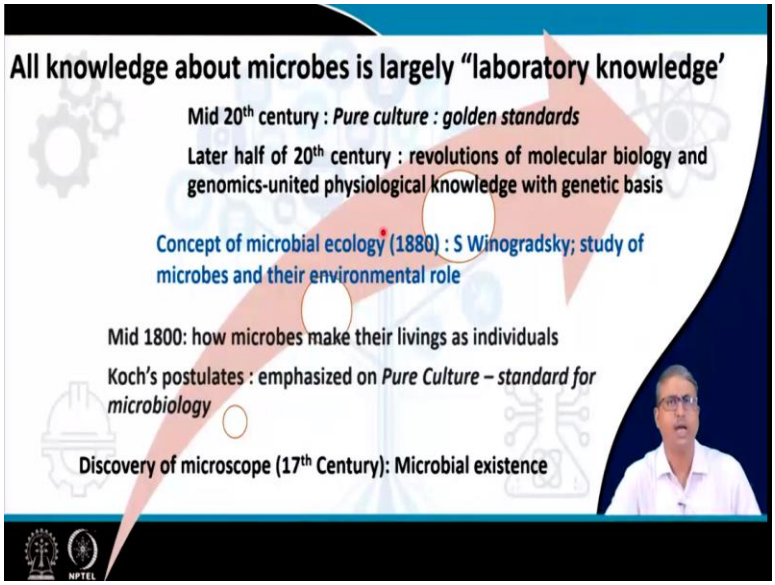

Now, if we look into the knowledge that helped us to actually come up to this level it was not what we are talking the cultivation independent bacteria and all those things because at some point of time what we see that all the bacterial knowledge or bacterial or microbial ability to degrade the pollutants because the microbes or bacteria are the key players in the biodegradation process.

So, when we look into these basically all these knowledge that we have gained maybe till the use of next generation sequencing or Metagenomics cultivation independent approaches were used was basically laboratory knowledge because we started with the microscopic observation and then pure culture without pure culture the culture maybe contaminated something like that.

And then the evolution of the concept of microbial ecology to the application of different molecular biology tools etcetera all were centered around the isolations and few isolated bacteria only once we try to incorporate the advent of the next generation sequencing based methods or conceptually we were ready to accept that there are more bacteria in the environment.

And many of the bacterial species are actually not cultivable they will not appear as pure culture. So, the concept or these ideas of pure culture must be obtained and they should be studied, they should be sequence in terms of their genomes in order to understand the bioremediation potential etcetera, were put under the question mark.

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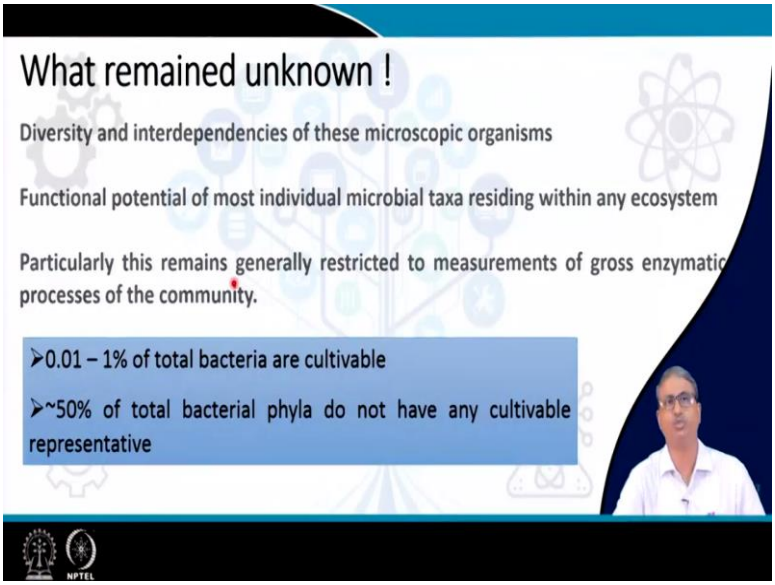

So, now with all these knowledge gained and realization that perhaps we are somehow biased towards cultivation dependent methods for a long period of time, but now we are aware and we are trying to gain some real understanding about the microbial potential, about the community level potential. However, it was also very clear to us that there are certain things which remained clearly unknown.

Diversity and interdependence of these microscopic organism of the bacteria or the archaea or sometimes the Fungi also which are present in a particular contaminated environment. Functional potential of most of the individual microbial taxa residing within an ecosystem. So; neither the diversity nor the interdependencies of the organism as well as their functional potential of the individual taxa residing in the community were not properly understood.

Particularly this remains generally restricted to measurement of the gross enzymatic process of the community like in functional potential when we try to asses it is not true that we are not assessing functional potential of the community, but we were more bothered about gross enzymatic processes of the community in most of the cases. Now considering a fact that 99% of the; total bacteria present in any environment contaminated environment as well remain uncultivable.

Then how do we assess, how do we ascertain which microorganisms are relevant for us and how they are actually dependent on other species which are present in the environment, they may not be the key players, but they are dependent on the other organisms.

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So, eventually these remain a very important task in front of us that is the role of indigenous microorganisms in bioremediation.

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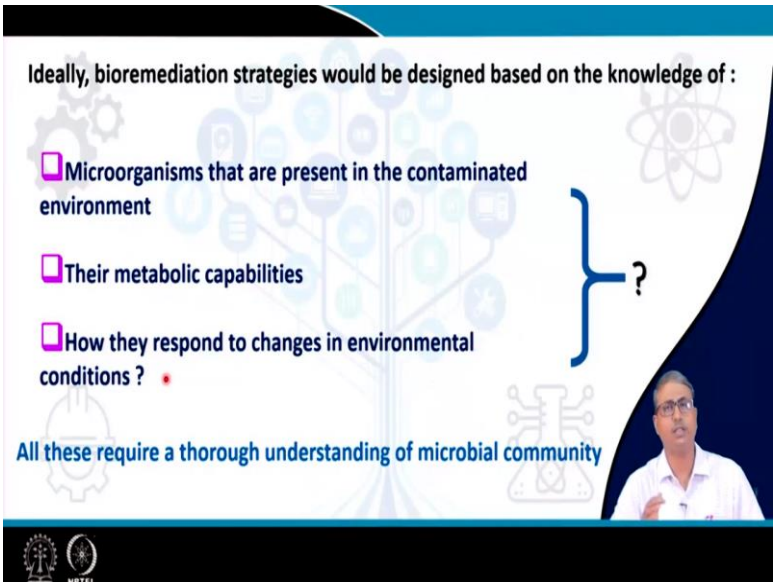

Now it has been very clearly observed or found that the bioremediation strategies ideally would be designed based on the knowledge of something, knowledge of what? Knowledge of the microorganisms that are present in the contaminated environment it is not that one or two isolated species. It is the microorganisms in their entirely that means the community level. With their metabolic capabilities what they can actually do it is not what a handful of isolates can do.

What actually the community can do and next they respond to changes in environmental conditions those question remains very, very important with respect to the bioremediation

strategy development and ideally these answering these questions would be immensely important and critical in order to develop an appropriate strategy for the bioremediation of a particular contaminant or a group of contaminants in a particular environment.

Now, all these require a thorough understanding of the microbial community and then that means we need to go back and sit down with our sample and perform the community analysis kind of stuff.

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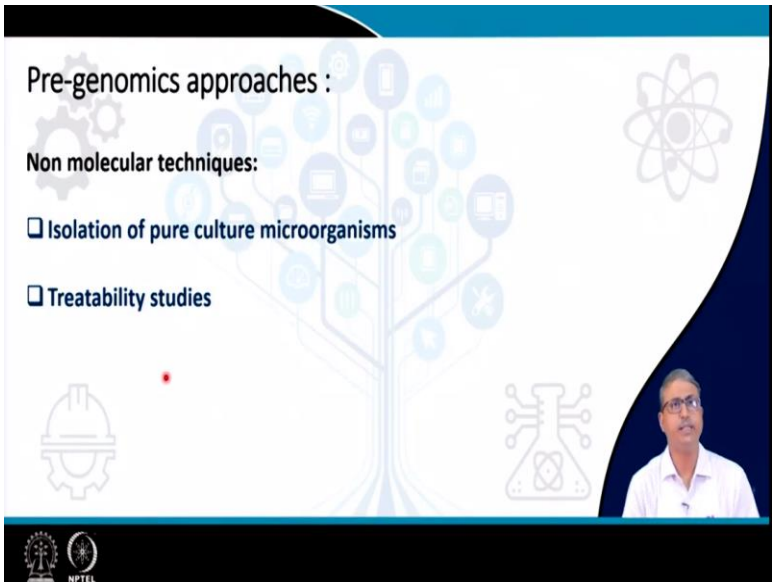

So, in the pregenomics when the; genomics as such was not implemented in a regular bioremediation research. So, you were dealing with the isolation of pure culture bacteria and treatability study. So, I will introduce with you in the treatability studies.

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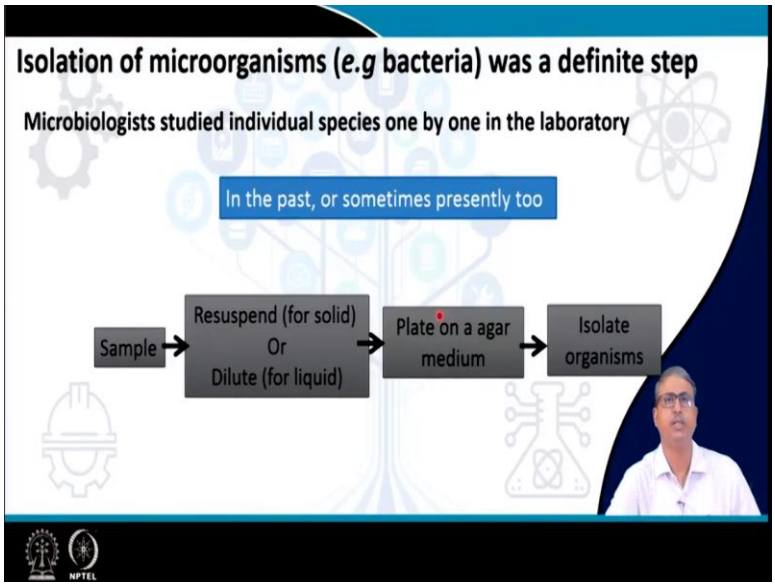

And isolation of bacteria remains a definite step you need to isolate the bacteria who can actually perform the function. So, isolation remain a very straightforward event so sample to the plate based method either aerobic and anaerobic or a tube based method where we used to have the anaerobic role tube or other tube methods anaerobic agar based methods. So, isolate the organism and then these pure culture isolates are subjected to remediation through treatability studies.

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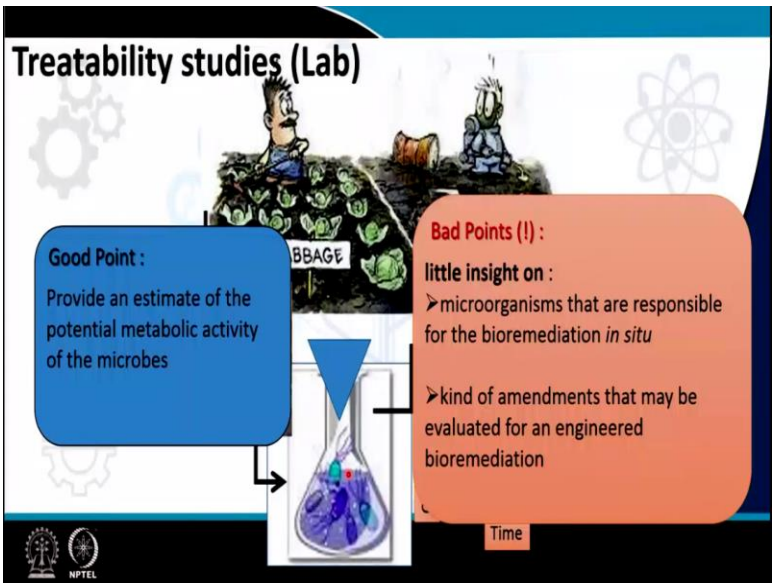

So, in the laboratory we used to have a kind of a contaminated environment and the organisms which are isolated from those contaminated environments or supposedly grown in a laboratory condition in a conical flask and then the substances which are the target compound it maybe toluene, it maybe phenol, it maybe chromium, it may be uranium whatever it may be.

We tried to see that overtime how the particular contaminant is transformed by the target bacteria or the bacterial strains which the scientist were able to isolate in the laboratory like the pure culture bacteria. So, these graph is representing that is typical treatability study that with time how the graph is leading us to isolation of some strains who are capable of performing better than other.

So, that means possibly some bacteria who can actually drive the graph like this that with a minimum time the contaminant concentration will decline something that will be very much desirable and then kinetic modeling etcetera, etcetera were performed. These were all good as long as we try to confined our study in the laboratory and try to have some kind of idea that

okay some contaminated sites or a particular contaminated site is having some bacteria who can degrade or transform the contaminant so that is the good point.

Good point is that this provides an estimate of the potential metabolic activity of the microbe. So, we have isolated the microbe, some of the bacteria can surely be isolated when we isolate the bacteria then they will be tested for their ecological and metabolic activities and analysis of certain functional gene from that we can have some assessment of the organism, but what we are unable to know is basically we gain very little insight on microorganism that are responsible for bioremediation in situ.

In actual contaminated site we might have isolated some bacteria and grown it in the laboratory in the laboratory, within the conical flask or in the tube or in the bioreactor vessel the bacteria might be transforming the pollutant that does not mean that these set of bacteria will transform the pollutant in the same rate in the real contaminated environment because environment is more complex than the culture vessel.

Number two the kind of amendments that maybe evaluated for an engineered bioremediation. So, that was also not very clear to all of us.

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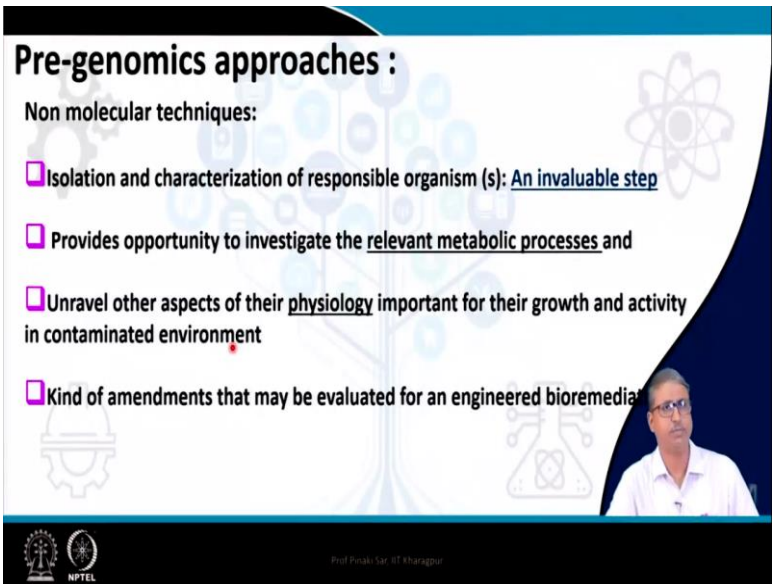

Now this pre genomics approaches we are talking about the pre genomics approaches. So, one thing was very clear that it was dependent on isolation and characterization of the responsible organism. This responsible organism is kind of a manmade term like we made the

term responsible organisms as per our capability. Now who is actually responsible will be identified only when we perform a study or some kind of analysis at a community level.

But nevertheless we try to identify some potential candidates or potent candidates who could be responsible and that was found to be an invaluable step. Indeed, it could be an invaluable step because many of these organisms could be real key player. Although we know that they are representing less than 1% of the total populations. These provide opportunity to investigate the relevant metabolic process through these isolates.

And unravel other aspects of the physiology, importance for their growth, activity in contaminated environment and perhaps if some scientist is really interested or he or she is taking care of this part then he or she could be able to identify the kind of amendments that maybe evaluated for the engineer because if we are isolating some bacteria then characterizing them.

And then finding out that some of them are potent then we can think of that what kind of amendments will encourage them to perform in the real soil environment; soil or in situ conditions. So, those are the kind of advanced thought process that actually helped the scientist or environmental engineers to even develop some of the elegant strategies with respect to the bioremediation.

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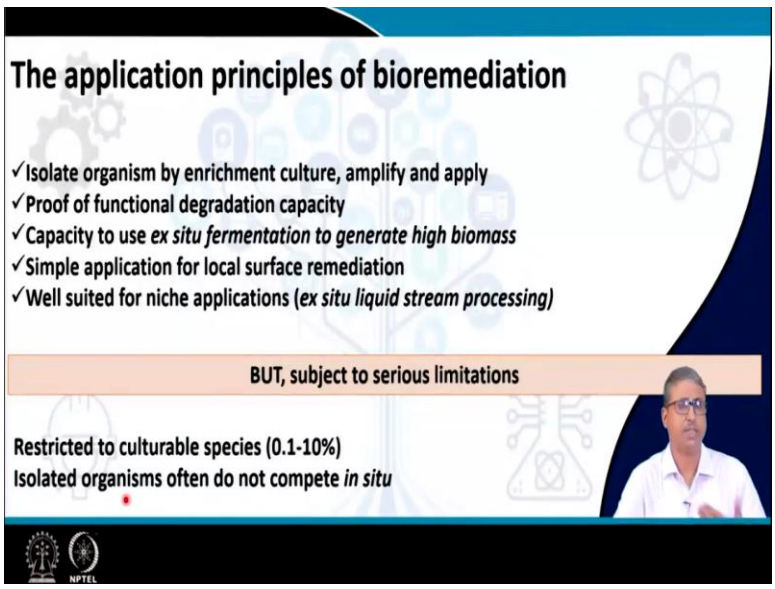

Now the application principles of bioremediation point of view these isolate organisms could be isolated by enrichment culture, there could be amplified and we apply. Proof of functional

degradation capacity, capacity to use ex situ fermentation to generate high biomass, simple application for local surface remediation, well suited for niche application ex situ liquid stream processing, but all these isolation based processes developed for bioremediation they are subjected to serious limitation because they have restricted to culturable species only.

So, the option that we will have real candidate organism will be very less 10% level is only for the activated sludge, but not for the normal soil contaminated with any kind of petroleum or chromium or heavy metal or any other things and often it has been found that isolated organisms often do not compete in situ. They may be very good when they are allowed to perform alone.

But when you leave them to compete with other organisms because other organisms there will be lot of interactions how they survive. So, now after knowing all these things now scientist are and the environmental engineers are developing strategies where the isolated organisms are also investigated with a similar tune.

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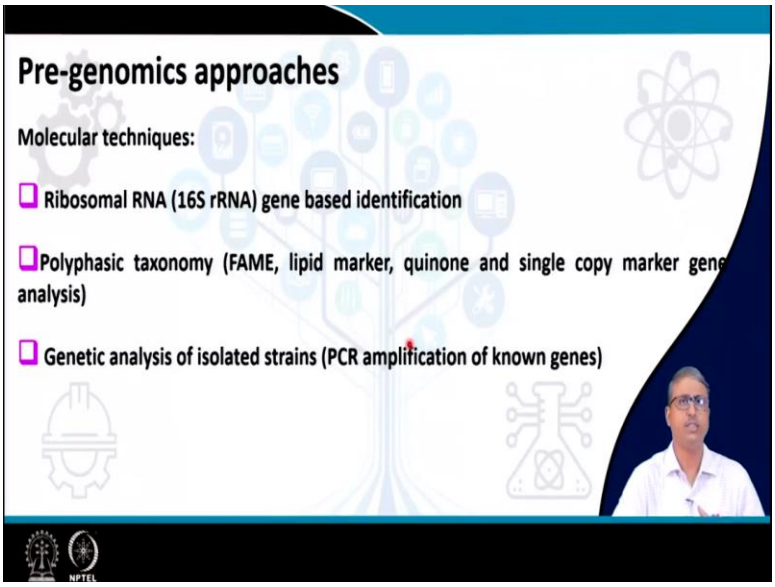

Now one of the major flaws of these some of the pregenomic non-molecular techniques, non molecular technique where we have not deployed any kind of gene based etcetera. Number one uncertainty; about the importance of any particular isolate or isolates in their in situ performance. So, unless you adopt some molecular techniques; particularly the gene PCR and gene based method.

Uncertainty about subsequent fate of the contaminant in the; modified form. So, it may be possible that in the real environment the candidate bacterial strain which is characterized, identified would convert the target pollutant A to intermediate B. Now what will happen to B whether B will be subsequently degraded to C and D and then possibly carbon dioxide and water or the B will be taken up by another organism and B will convert it to something else which will be found to be another toxic substance.

Now when we; have the molecular techniques with us. So, we are able to actually implement the Ribosomal RNA 16S rRNA based technique. Polyphasic taxonomic approaches and genetic analysis of the isolated strains including many of the functional gene.

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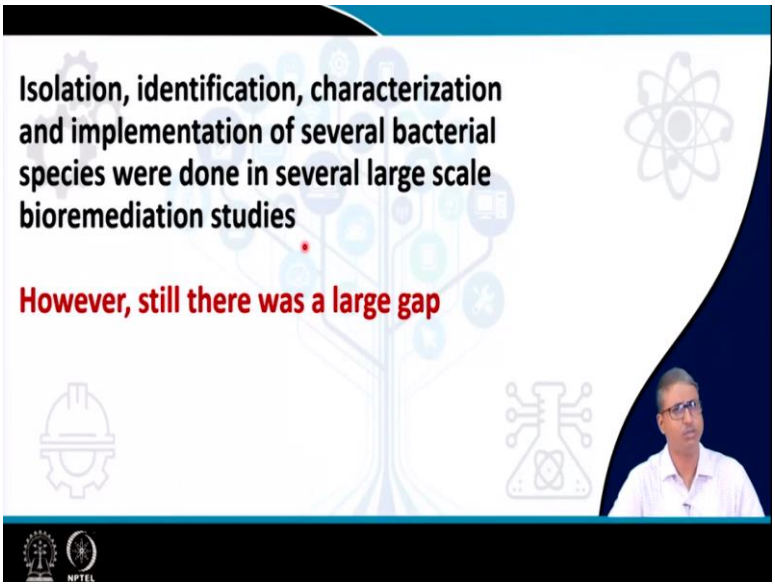

Isolation, identification, characterization and implementation of several bacterial species were done in several large scale bioremediation studies. These studies actually they include all the pregenomics, but molecular level studies as well. So, they try to assess the organisms in terms of their genes, enzymes activities etcetera, but what has been found that still there is a large gap.

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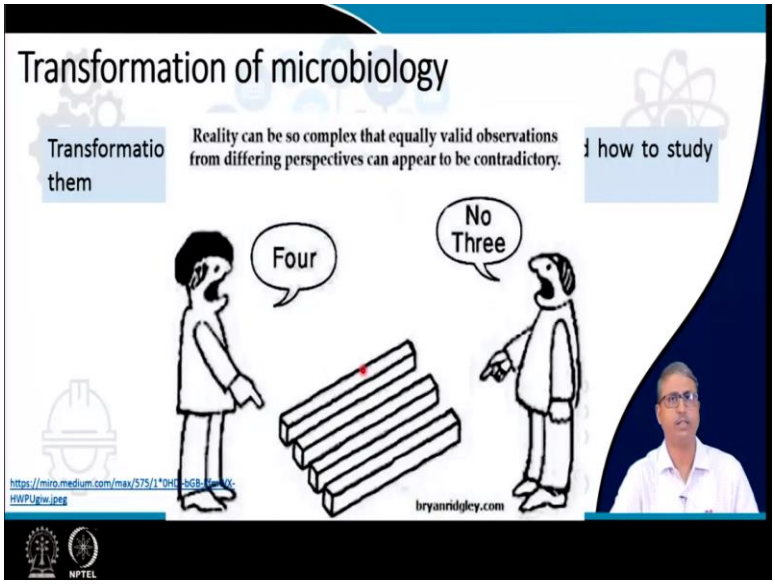

And this large gap is basically connected to the transformation of classical microbiology. It in the transformation in microbiologist view of microorganism and how to study them. I will show you a very interesting cartoon. Now how many bars are here? Now reality can be so complex that equally valid observations from different or differing perspective can appear to be contradictory like whether four bars or whether three bars are there.

So, how many organisms are there whether a particular type of degrading organisms are there or not there, how do you count it? It basically depends on the methods that you are adopting. So, it goes along like a indigenous or classical approaches with the advent of the molecular tools and the cultivation independent approaches.

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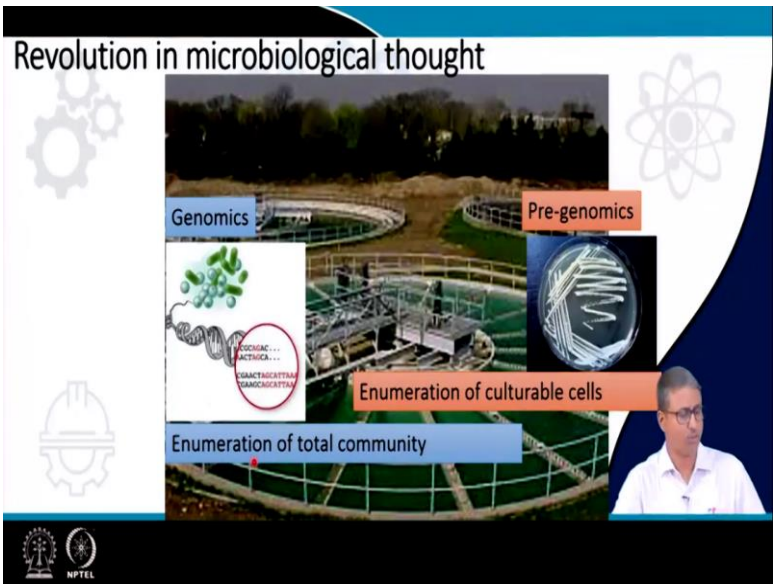

That has led to a kind of revolution in microbiological thoughts. So, we take this wastewater treatment tank and then implement a process of pregenomic non-molecular technique even molecular technique, but pre-genomics no genomics or metagenomics or cultivation independent assessment. So, you just enumerate how many cultivable cells are there and also when we implement a genomics technique we are able to enumerate the entire combinator.

Here the genomics basically refers to the cultivation independent approaches. So, enumerate the total community. So, instead of only the cultivable bacterial strains or archaeal or fungal strains we are capable of enumerating the entire community present in the site. So, it is something like that we are able to view these or you are able to view this. So, perhaps we want to view the community or the environment like this.

That the entirety should be available then we understand the process and then try to implement it. This is basically achieved that actually the picture is like this, this picture is not like that. So, if I go back like this actually the Earth is not like this the Earth is rather like this that came the early credit must be given to the for the great concept of plate count anomaly.

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And the plate count anomaly coupled with the development of ribosomal RNA based methods; high throughput sequencing methods including the omics based tools etcetera helped us to achieve a paradigm shift. Paradigm shift means there is no confrontation between the classical microbiological approach and the molecular microbiological approach, but it is the upliftment of the understanding.

How microorganisms actually perform in an environment that is the bigger question we are trying to answer and then subsequently we are going higher level how these organismic roles can be utilized to achieve the functions that would possibly lead us to a successful bioremediation.

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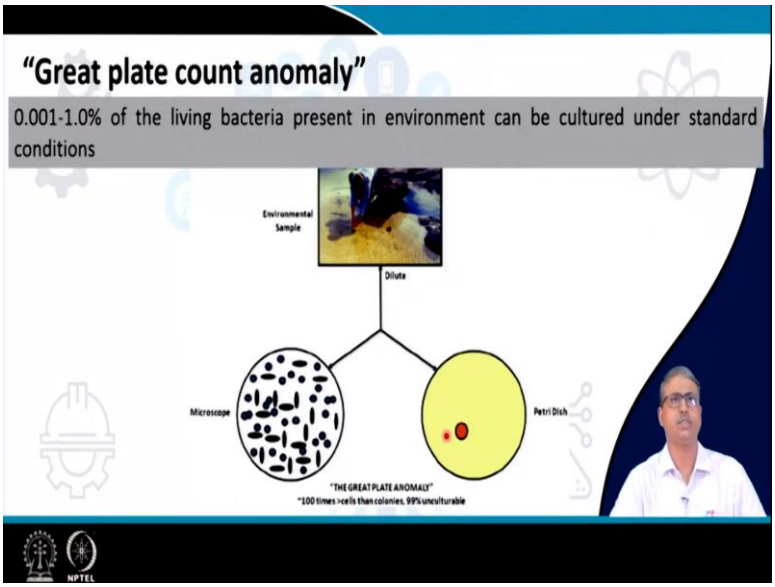

So, like in a plate count anomaly which we have already learned that any environmental sample might have actually significantly higher number of cells or the species when we look at them through a microscope rather than a petri dish or a culture based method. So, essentially when this great plate count anomaly showed us the first sign of light that there could be more microorganisms rather than which are growing on the plates followed by the application of the ribosomal RNA based methods which not necessarily required isolation.

And then development of sequencing technologies and all other arrays of technologies which helped us to have these cultivation independent approaches led us to fill the gap. The gap which possibly has differed the environmental engineers to implement the bioremediation process. So, we will continue our discussion in our subsequent lectures as well as there are many other topics to be covered within this.

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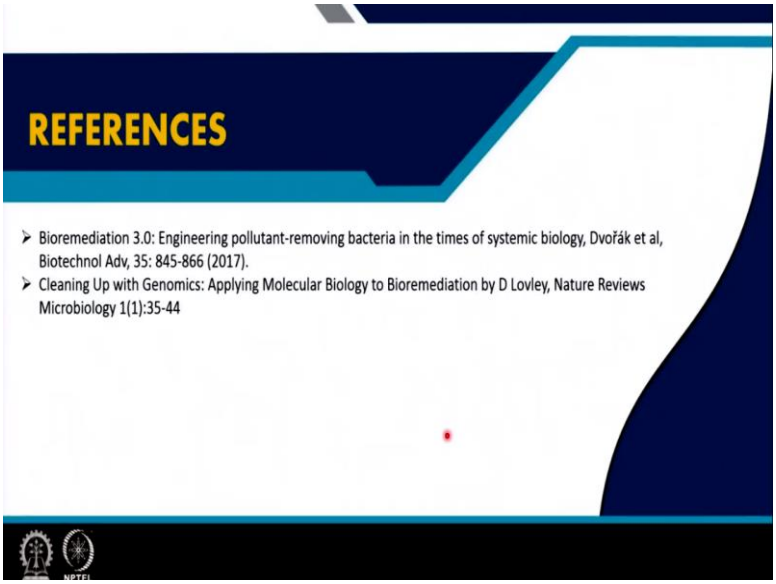

So, as for reference we are going to have these two major articles for this part of my lecture.

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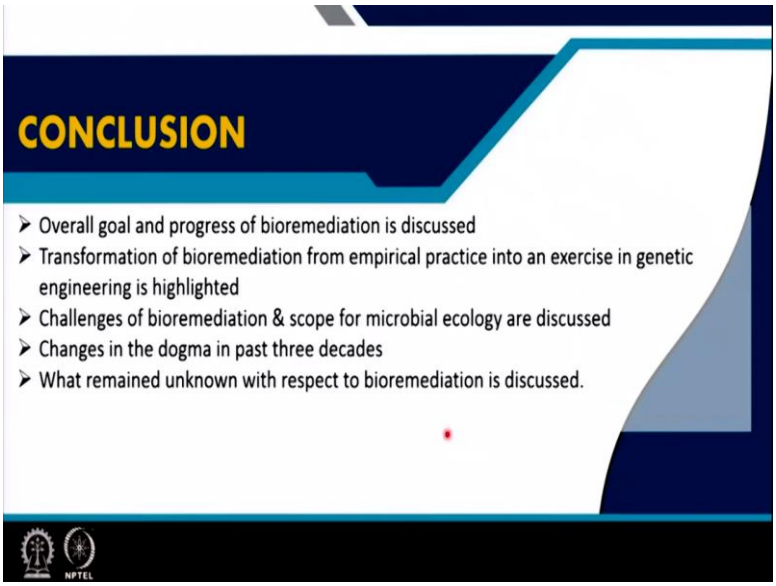

And in conclusion the overall goal and the progress of bioremediation is discussed, transformation of bioremediation from empirical practice into an exercise in genetic engineering is highlighted, challenges of bioremediation and scope of microbial ecology are discussed, changes in the dogma in the past three decades particularly are emphasized and what remained unknown with respect to the bioremediation process is also discussed. Thank you so much.