Environmental Biotechnology
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Lecture – 25
Methods in Microbial Ecology with Relevance to Environmental Biotechnology

Welcome to the 25th lecture on environmental biotechnology. This particular lecture will be on methods in microbial ecology with reference to environmental biotechnology.

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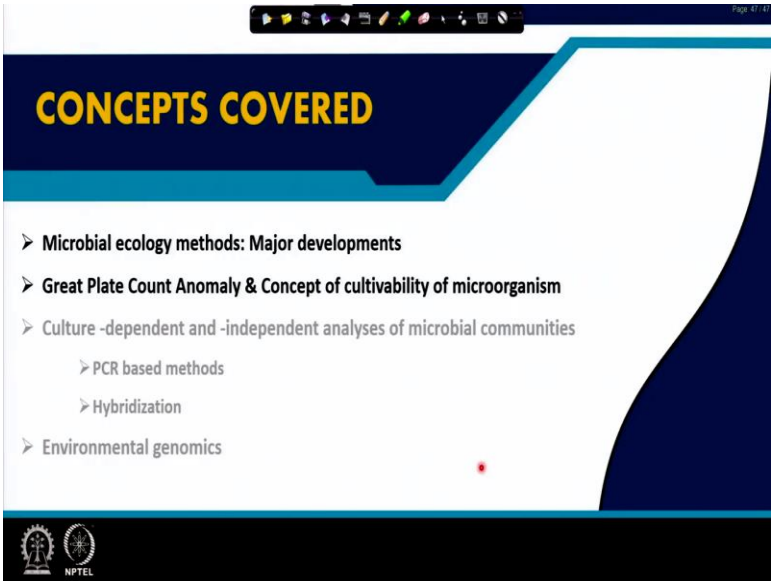

In the particular lecture we would be discussing about the microbial ecology related methods and the major developments that occurred in the past 50 years or so. We will discuss in detail about the uncultivability of organisms and will begin with the great plate count anomaly and different categories of uncultivable or cultivable organisms which are present in any environment will be discussed.

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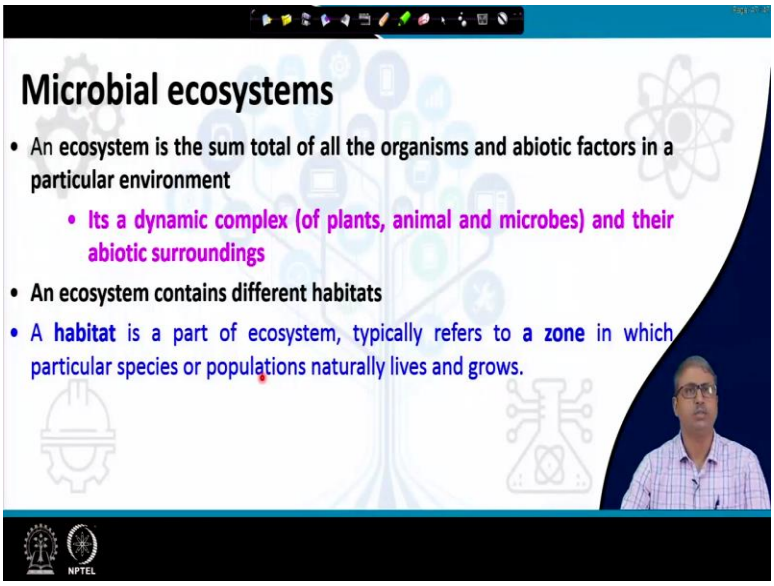

So, to begin with the long term goal of microbial ecology is to gain a better understanding of the ecology of important microorganisms in environmental samples. And as we realize that for any successful environmental biotechnology process development understanding the microorganisms with respect to their ecosystems is a critical component. Microbial ecosystems are a sum total of all the organisms and different type of physical and chemical factors present in a particular environment.

So, it is a basically dynamic complex of not only microorganisms but also plants animals their products and the abiotic surroundings. And ecosystem therefore contains different habitats a particular habitat is a part of the ecosystem typically refers to a zone in which a particular species or populations naturally leaves and grows. So, with reference to methods those are applied to understand characterize and make use of these microorganisms present in any environment towards sustainable process development this basic information about the ecosystem concept and habitat is very important.

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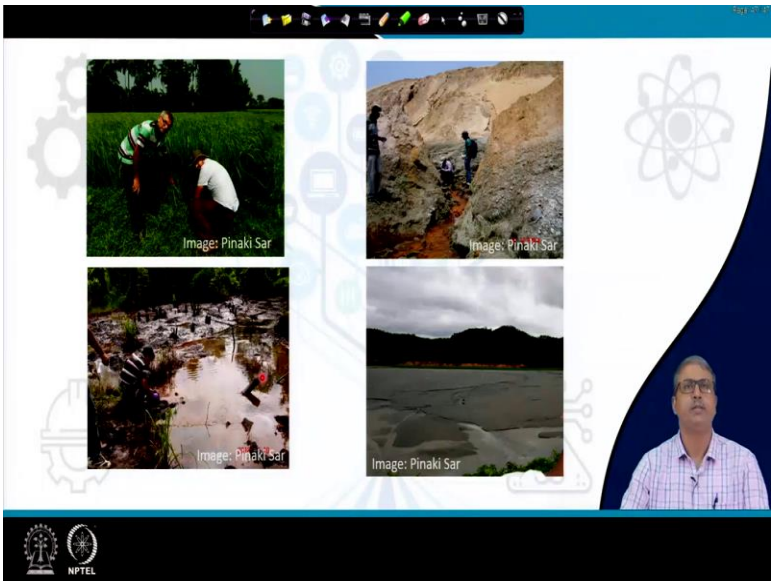

So, here you can see a couple of interesting ecosystems or the zones which are very relevant for environmental biotechnology related process to begin with the agricultural paddy system and naturally petroleum oil spilled environment within a forest area an acid mine drainage producing system within a mine dump site where highly acidic and sulphate mine drainage is flowing through from the acid mine waste dumps.

And below here is another very interesting ecosystem which is basically showing a mind telling damp rather and all these ecosystems are subjected to biotechnological interventions for either crop productivity or bioremediation or sustainable recovery of metals and minerals or mining activities etcetera.

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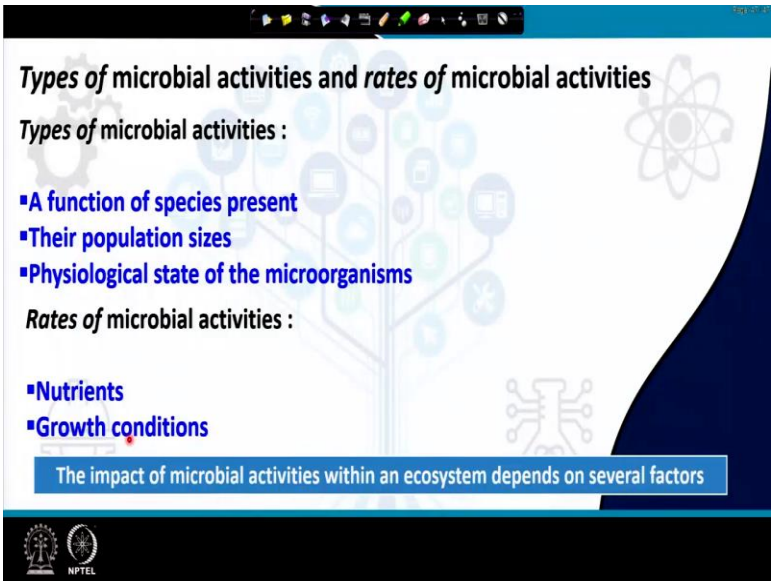

So within all these different kinds of environments or ecosystems what you observe that the types of microbial activities and the rates of the microbial activities are of high significance because those are the processes which are controlling the biotechnological or going to control the biotechnology related process. So, now when we plan for environmental microbial ecology methods the methods should be selected in such a way.

So, that they are able to identify the different type of microbes and also help us to elucidate the rates of individual microbial activities. So, the type of the microbial activities could be delineated as a function of the species present their population sizes and the physiological state of the microorganisms to elaborate this particular point briefly. So, in any environment whatever microbial activities are expected that is basically an outcome of the function of the individual species present just few minutes ago we were looking at a petroleum oil spilled site within a forest area.

So, within that highly petroleum oil contaminated environment what type of microbial activities are expected or whatever type of microbial activities are predominant can be attributed to different type of functions which are carried out by individual species member. So, in a nutshell the community function or the ecosystem function whatever is obtained for any kind of environment is basically an outcome of the individual functions of the species and also the their interaction with the abiotic surroundings.

Now the individual species function is of course a very important component but at the same time the size of those individual populations are very important for example in case of a petroleum oil contaminated site there may be some species who are capable of degrading the long chain alkane or petroleum hydro more complex hydrocarbon molecules but those species let us assume there are certain burkholderia or pseudomonas species who are capable of degrading the very long chain alkenes and complex aromatic compounds.

But the size of those population size of those burkholderia and pseudomonas are of a great concern because there may be few pseudomonas or burkholderia who are capable of degrading those long alkene chains or the complex aromatic compounds or there could be a high population size of those organisms. So, if the population size of those burkholderia and pseudomonas are higher we can expect that within that particular petroleum oil contaminated environment an enhanced activity towards the degradation of the long chain alkanes or the complex aromatic compounds will be achieved.

However if the population sizes of those burkholderia and pseudomonas species are low then it may be found that although such organisms are present because of their abundance which is very low the actual degradation of the long chain alkenes and polyaromatic compounds are not up to the mark. The third point is also very important which is the; physiological state of the microorganisms.

Again if we take the same example that in a petroleum oil contaminated environment we may have the burkholderia species who are very much capable of degrading the long chain polymeric or long chain alkane compounds. But due to some restrictions or some constraints the physiological state of the micro those burkholderia species who are capable of degrading the oil molecules or the alkene chains are not appropriate.

So, they are not in a favorable physiological state. So that they; can express the genes or the enzymes which might facilitate the degradation of the; compounds. So, therefore the activities that we are expecting in any kind of environment must be understood with respect to these three

important parameters. The second important aspect is the rate of the activities. So, there may be in this case that we are discussing the petroleum contaminated site with burkholderia species capable of degrading the long chain alkane molecules.

They are of significant population size and their physiological states are also well maintained however if they are not provided with the required nutrients for example the nitrogen or phosphorus or the phosphate molecules or there are other growth conditions. For example the temperature or the pH are not optimum or not within the favorable regime for those specific group of microorganisms.

Then even if the microorganisms specific microorganism is present their population size is significant and their physiological state is apparently perfect but they are not going to function adequately because they need other nutrients and the growth conditions which are not available. So, therefore when we develop any particular method we should be careful that our method should be able to delineate or decipher both these types of the activities as well as the rate of the activities.

Now the impact of the microbial activities within an ecosystem depends on several other factors that we might discuss in or might have discussed already in some other lectures.

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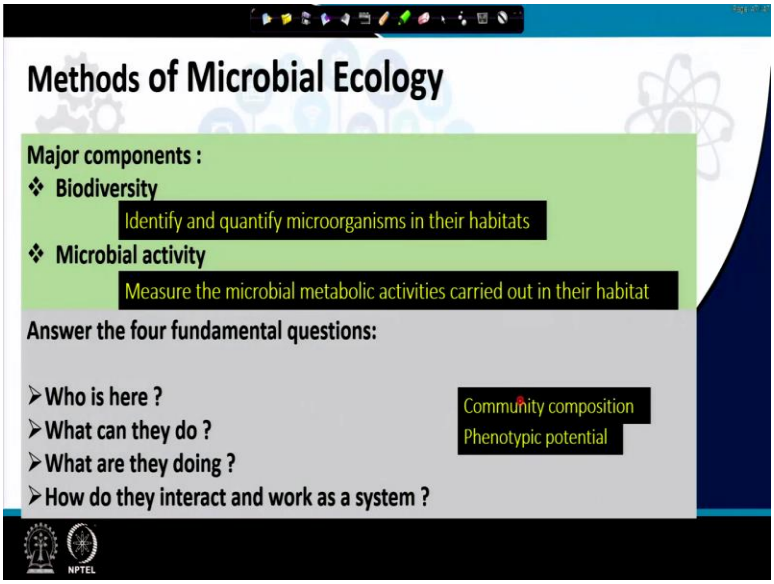

Now coming to the original idea of talking about the methods of microbial ecology which are very relevant for understanding any ecosystem or environment where biotechnological processes are to be implemented or developed. So, there are two components within these methods. So, the methods could be targeted towards understanding the biodiversity that is the kind of organisms which are present over there.

So, in a simple word we can say that that this is basically the method which will set up methods which will allow us to understand the biodiversity of an of an environment of a particular system maybe a wastewater may be a contaminated lake maybe a underground system where some carbon dioxide is going to be sequestered or in any kind of other environments. So, biodiversity will give us a general broad picture about what are the organisms present.

But with respect to the role of or delineating the role or importance of the significance of the microbes which are present there the identification of those microorganisms all microorganisms present in a particular environment and quantification of those organisms. So, identification and quantification are those terms which are very often used and are very important with respect to microbial ecology and environmental biotechnology.

So, if we understand that in a given environment or a subset of the environment or within a habitat of an environment we have 100 different species then each of these 100 different species need to be identified. Identified means their taxonomic identification must be obtained and quantification means that each of those 100 organisms or whatever may be the number. Once we identify the organisms who are these organisms.

We must be able to tell what is the quantitative abundance of each of these species? There may be pseudomonas basilas microbacterium burkholderia and rhodococcus and many other bacterial species archaeal species fungal species everything is there. So, we should be able to tell how much of the pseudomonas and how much of burkholderia and how many are the methanogenic archaea individual species are there.

So, this is this is not so, simple because microbes are. So, tiny organisms that under the

microscope even if you see them it would be hard to actually precisely identify you can count the total number of cells but it is hard to count numbers of a specific type of cells or specific organism or a specific taxonomic group. So, microbial ecology methods have been developed which help us to not only to identify the organisms.

And but to quantify each of the type of the organism even if there are many more organisms like exceeding few 100's different organisms it is possible to quantify each of the species member within any environmental sample. So, this is the part of the biodiversity part on the other hand with respect to microbial activity that is another important aspect of microbial ecology methods with respect to microbial activity.

As I have mentioned earlier that the activity type of activity rate of activity there are many other parameters within this microbial activity. So, with respect to microbial activity the microbial ecology methods try to measure the microbial metabolic activities that means the functions carried out in their habitat. So, if we consider that in a particular acid mine drainage environment there are certain sulphate reducing bacteria who could be useful for precipitating the sulphate because once the sulphate is reduced to sulfide the sulfide precipitates.

So, if we want to assess or monitor the activity of those sulfate reducing bacteria. So, we need to actually assess the enzymes which are involved in sulphate reduction or the other properties which actually will connect us to the metabolic activities metabolic processes of sulphate reducing bacteria within that mining environment possibly if you are able to do that with suitable methods we will be able to utilize such microbial activity for the remediation or reclamation of a contaminated mine site.

Now in this case with respect to the methods of microbial ecology there are 4 fundamental questions of microbial ecology that we already discussed earlier these four questions are to be answered. So, our microbial ecology methods should be able to answer who is here that means who are the organisms who are present here what can they do this is related to the question that we earlier framed as what is the potential of this microorganism that they can possibly do and then what are they doing.

So, these second and third questions are interrelated but they address or they provide answers which help us to understand the role of the microbes at the different level what they can do is the kind of a potential that provided something happen or something not happen they will be doing this but what they are doing is actually a real time phenomenon that actually what they are doing when we collected the sample.

And lastly how do they interact and work as a system. So, there may be as I said 100 or 1000 or few 1000 species member in a particular environment and any many of them are capable of or doing multiple functions. So these functions and their presence of the organisms they must be interconnected with each other now when they are interconnected to each other they must be working as a system. So, how we understand that system? So, that we can we can exploit them to the best of our possibility.

So, the first question that is the who is here or what are the organisms present there would lead to the deciphering the particular point which is called community composition. So, when we develop a method or use a method microbial ecology method with respect to a particular sample to answer the question that who are there or who is here we basically get the answer about the microbial community composition.

In subsequent lectures I might use the term microbial community composition or microbial community structure. So, that refers to what are the organisms present there. So it includes both the identity of the organisms as I mentioned earlier and also the quantitative abundance. So, relative abundance of the species or the taxonomic groups who are present there the second is the phenotypic potential which is connected to what they can do.

So, phenotypic potential can be assessed by a number of methods enzymatic assay or providing different substrates under laboratory conditions. So, phenotypic potential is basically a kind of assessment that these organism could potentially be able to do this but we should keep in mind that these assessment are done under laboratory condition. So, under laboratory condition a particular microbe may respond to substrate differently compared to a real situation because in a

real environment there could be lots of other parameters.

Because that is not a control laboratory environment and also there could be innumerable number of other species other organisms and other physical and chemical factors which will be interacting with those species. So, the real activity or the metabolic activity or metabolic function of the organism we might be different. So, we need to actually address that that what are they doing. So, when we address this question that what are they doing we basically try to refer community function.

So, it is not an individual individual's role it is the community role because even if there is a very important or major bacterial species or archaeal species or a fungal member in a real environment this particular fungal species or bacterial species would be interacting with many other species and also with in presence of many other organisms. So, when we try to answer this question using an appropriate method that what are they doing we basically derive the community function.

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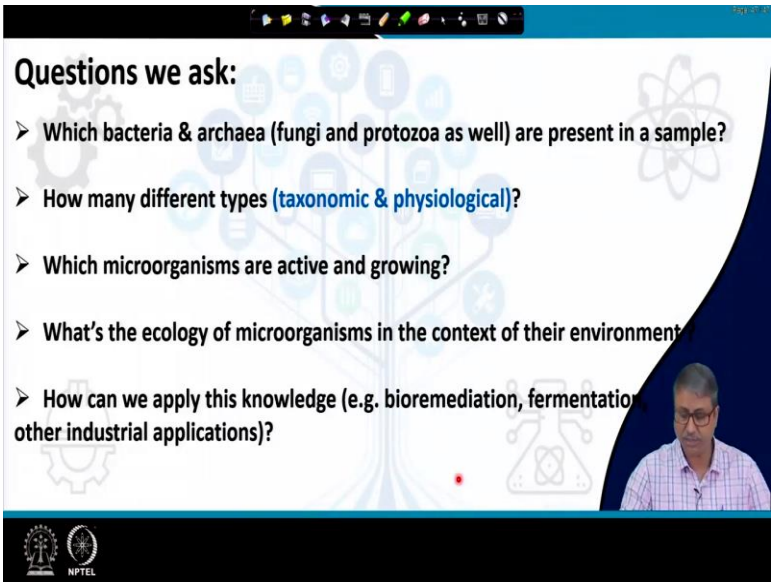

And finally the interaction among the organisms which refers to the systems level understanding. So, the questions that we ask that which bacteria and archaea the microorganisms in this case and including the fungi and protozoa wherever it is applicable like a wastewater treatment perhaps we have to even include virus also. So, for present in the sample, so, if you are

dealing with an activated sludge or a waste water treatment or a we are looking into a drinking water system we might have to consider all possible type of microorganisms who are present there.

So, our method should be able to identify them and also find out the quantitative abundance. So, how many types? So, when we say how many different types of there are there. So, possibly that answer is obtained when we say that the identification of these pieces are already done. So, when you have identification of the species members are done then naturally the taxonomic types are obtained but there could be another categorization which is called a physiological characterization.

So, earlier we have studied the different carbon and nitric energy source requirement. So, a broad categorization of the physiology of the microorganisms are with us either they may be heterotroph or organotroph or they may be autotroph they may be chemoautotroph they may be photoautotroph they may be lithotroph. So, those are the different kinds of physiological categories or types of organisms present there.

The third question which microorganisms are active and growing because often whether it is a drinking water system whether it is a wastewater treatment system whether it is a contaminated lake or river or any kind of other environment where we are trying to do environmental biotechnology process we collect sample bring the sample in the laboratory and test them. So, in this case the testing is with respect to different organisms who are present.

So, we may identify the different organisms and we can also identify their physiological types by appropriate methods that we will discuss. But this is these are all done under the laboratory condition. Actually in the real waste water or real drinking water which are the microorganism or what are the microorganisms which are actually active and growing. Because I may bring the water from the from the field and when I test I may find out organism a is more active.

And growing very fast but actually it is other way around it is it may be organism c or d is actually more active in a real situation because as I already mentioned and you perhaps

understood that the real environment are different from the laboratory environment. So, whenever we develop method we implement method we must be very careful about this that we have laboratory constraints.

So, when we incubate a cell or a microbial culture under 30 degree or 37 degree and use a particular medium with a definite carbon and nitrogen source pH of the medium is also very much controlled these are not always replicating the exact scenarios or exact situation in the real environment. So environmental biotechnology processes must rely on real situation. So, methods must be selected methods must be developed which will address the issue like which microorganisms are actually active and growing.

The next question what is the ecology of microorganism in the context of their environment? It is a very difficult or very complex question because you might be able to identify 10 or 20 or 100 or maybe 500 different organisms. You may be also able to physiologically categorize them that there are 10 different physiological groups of organisms are there. You may also apply certain methods we will talk about those methods that these out of the 100 organisms present there 100 bacterial species present there only 20 are active and growing in the real environment.

Yes we can do that but what is the ecology of the microorganism in the context of the environment? In order to understand that we might need to understand the environment itself because it is more complex than then we perceive and then we need to actually implement such methods which will help us to understand the ecological significance of organisms. Because in most of the environmental biotechnology processes are multi-organismic that means there are more than one microbes involved in the environmental biotechnology process.

So when we have more than one microorganisms for example conversion of a waste to alcohol or may be methane or other useful compounds which are industrially or energetically very useful products. So, these as we already discussed these are all by network of organisms network of metabolic processes both the primary fermenter secondary fermenter sulfate reducers methanogens many different types of organisms are there.

So, we need to understand the complex process of microbial interaction within the environment itself. And then finally answering this last question that how can we apply this knowledge in terms of bioremediation in terms of fermentation in terms of other industrial application that is also a very important aspect for the development or implementation of the method.

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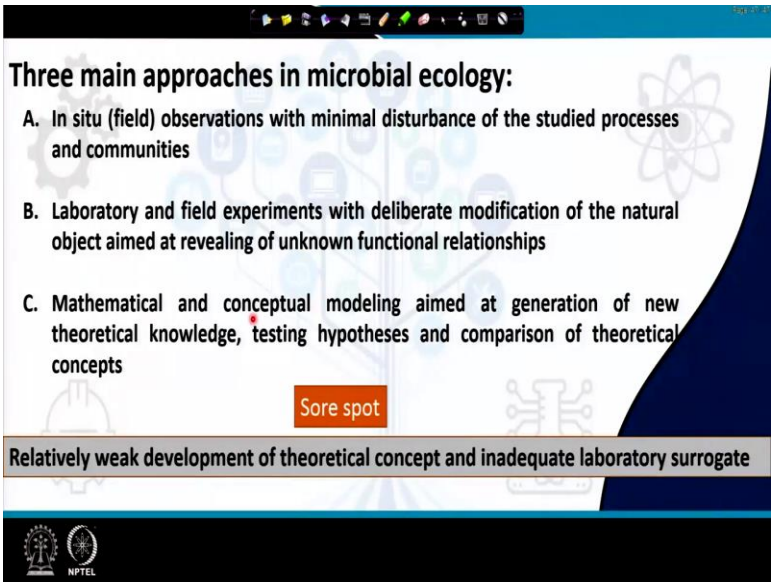

Now with respect to microbial ecology methods there are three main approaches first is the in situ or the field observation. So, field observations are we can go to a contaminated site or we can go to a lake or a river or a site where some kind of environmental biotechnology process is going to take place or is is possibly working there naturally. So, we need to observe considering the in situ conditions with minimal disturbance to the studied processes and communities.

So, we need to implement certain strategies that that will provide us information which are in situ. The second is the laboratory and field experiments with deliberate modification of the natural object aimed at revealing of unknown functional relationship. So, in this case we bring the sample into the lab and then expose the sample or incubate the sample under different conditions that is the modification.

So, deliberate modification means we try to provide different carbon source different nitrogen source different pH different redox condition in earlier lectures we have seen how the redox condition affects the electron acceptor domains. So, we can provide actually different electron

acceptors by creating different redox conditions and then see how the system the soil and its or the contaminated waste or the environmental sample is reacting to this changing redox regime and with respect to the electron acceptors provided.

And from that the unknown functional relationship can be obtained. The third approach is the mathematical and conceptual modeling based approach which aimed at generation of new theoretical knowledge based on the interaction between the species the functional properties the enzymes etcetera testing different hypotheses and comparison of the theoretical concepts. And actually this last aspect that is the methods which will help us to understand and develop conceptual model is called considered as a source spot. Relatively weak development of theoretical concepts and inadequate laboratory surrogates are provided.

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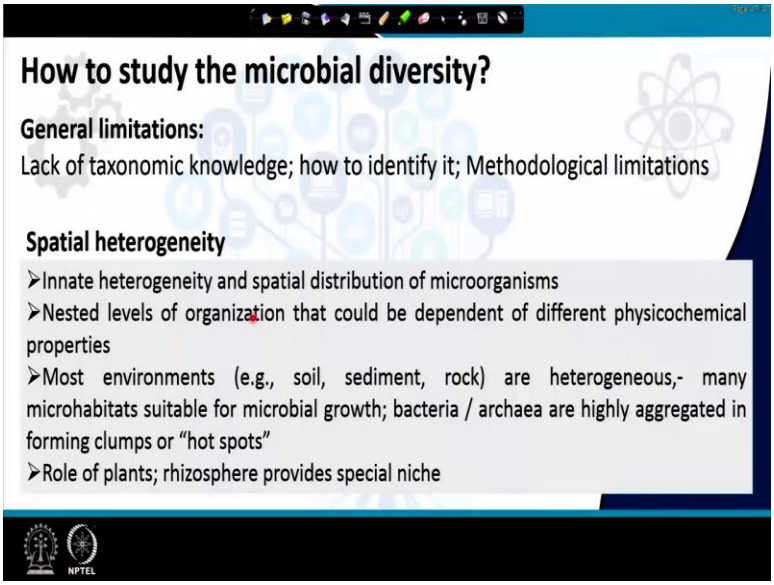

Now, how to study the microbial diversity within a particular environment? So, we will proceed into that that how microbial communities are actually deciphered with respect to answering the taxonomy the diversity the quantitative abundance and their activities particularly four questions that we have framed but there are certain general limitations with this respect. So, one of the most important limitation is that the lack of taxonomic knowledge how to identify it and that is a kind of methodological limitation.

Because microbes are so tiny they are so small that by looking at the microscope through a

microscope you cannot identify exactly what is this organism whether this is a streptococcus staphylococcus what type of organism it is it is not always. So, simple pseudomonas and bacillus sometimes they appear almost same morphologically under the microscope. So, it will be hard to identify them. So, there should be method which will help us to identify the organisms and if we consider that the diversity of the microorganisms are very, very high.

A gram of soil might have several thousand different species. So, if we have several thousand different species within a gram of soil sample or a milliliter of water, one milliliter of water having 5000 different species. So, it is it is very challenging to identify them 5000 different species then there is a point of special heterogeneity. It is kind of a considered as a innate heterogeneity and special distribution of microbes particularly in soil sediments etcetera.

These environments are highly heterogeneous in each millimeter or even less than that there are small micro habitats and these micro habitats are allowing growth and proliferation of specific group of organisms including bacteria, archaea, fungi even protozoa also. So, these micro habitats vary over a small to broad range of length and space and time also. So, especially and temporarily this heterogeneity varies.

And also there is a nested level of organization that could be dependent on different physiochemical properties. A small micro habitat having a set of organisms proliferating when the pH is low but the same micro environment will have a different microorganisms or the activity of the microorganisms will change as soon as the pH is altered. The product of this small micro environment the kind of metabolites produced the kind of changes they bring into the iron and sulphur and other redox active elements these also affect the activities of other organisms.

So, therefore the entire organization is highly nested one micro habitat or micro zone is connected to another one because of the microbial activities one of one produce certain things and these products are utilized by the other organisms.

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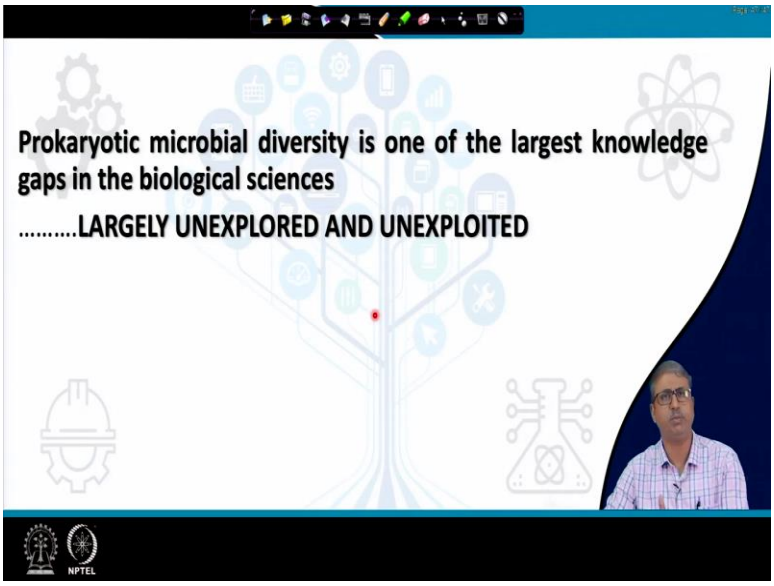

So, after considering all these things we have realized that the prokaryotic microbial diversity is one of the largest knowledge gaps in the biological sciences. So, on the one hand we have large number of questions in front of us. We have understood the complexity of the environments be it a soil system sediment system river system. In most of the systems we have very very much heterogeneity and this heterogeneity is both special and temporal and also nested in nature.

And so, overall the deciphering the microbial community with respect to the diversity and its activity which are very very important for developing and implementing any environmental biotechnology process is identified to be largely unexplored and unexploited.

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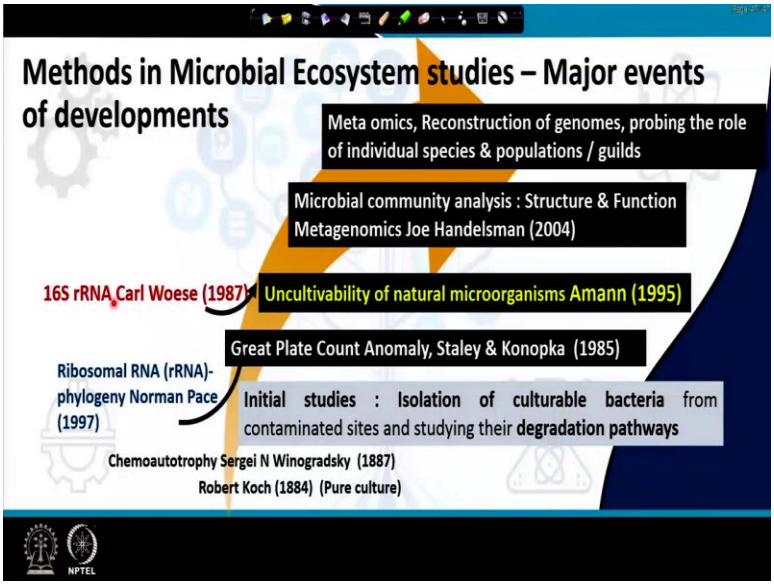

Now why it is so? So, if we look at the methods in microbial ecosystem studies and the major events of developments and we begin with the very well known phenomenon of postulates by Robert Koch in 1884 who put forward the concept of pure culture organism which is unadulterated is not mixed with any other organisms is a pure bacteria, archaea or fungal culture. Only one particular strain or species is present there.

So, that was long time ago 1884 the importance of pure culture was established but the pure culture was not enough because in real environment there will be 1000s of different species together. So, very soon Winogredsky deciphered the concept of chemoautotrophy and how microorganisms behave in a more complex way than possibly we perceived and then subsequently next I will say 100 years or so.

There are many reports many studies with respect to environmental biotechnology and environmental processes most of these studies were targeted towards isolation of cultivable bacteria, cultivable bacteria meaning the bacterial strains which are capable of growth under laboratory conditions. So, you can grow them under laboratory condition that is on agar plate on a broth medium you can grow them you can see the tiny colony growing on the agar plate from different contaminated sites and studying their degradation and other metabolic processes.

So, large number of studies in that particular period large; last one 100 years or maybe little less than that. So, huge number; of studies have been done. So, many bacterial and archaeal species were isolated from different environmentally relevant sites and characterized. While we were isolating or the scientists working on the environmental biotechnology or environmental microbiology or environmental engineering were isolating organism identifying them.

In order to understand the quality of a drinking water or the performance of a activated sludge treatment or a wastewater treatment process there were some parallel developments happening. So, one of the major development that was happening in the corner it was the ribosomal RNA based phylogeny. So, we will talk about this phylogenetic analysis little later but these are the small stretches of DNA within the genome or the chromosome of the microbes which code for the ribosomal RNA.

And ribosomal RNA's are integral part of ribosomes and ribosomes you know they are the machinery is useful for the translation. So without ribosomes translation will not happen and the ribosomes are made up of ribosomal RNA and ribosomal proteins. So, this ribosomal RNA along with ribosomal protein sequences both these sequences were used to understand the molecular evolution of microorganisms during the time of 1997 and on so.

At the same time or subsequent to that or even before that we had a very very well defined concept of 16s ribosomal RNA that is one of the ribosomal RNA molecules that is a 16s ribosomal RNA molecules was id entified as a phylogenetic marker that this particular gene can be used. So, this 16s ribosomal RNA is a part of ribosome but it is encoded by a gene in the chromosome. So, that particular gene is referred as 16s ribosomal RNA gene.

So, that particular gene can be useful for identifying an organism. So, earlier i was talking about identifying a microorganism by looking at the microscope. So, looking through microscope it is very difficult because microbes are all or almost all cases they are either basilly or cocky or streptococcy or something like that. So, it is very difficult to discriminate the different types of microorganisms particularly when we consider that there are more than few thousand species present there.

So, 16s ribosomal RNA was found to be very useful in identifying the microorganisms we will talk and learn about this little later however during this time 1985 or so. There was a very interesting discovery I will say or an interesting report came which refers to great plate count anomaly by Staley and Kanopka. Now what is this great plate count anomaly that we are we are going to talk today.

But this great plate count anomaly refers to the anomaly with respect to number of microbes that we count through a simple agar plate method where we spread a sample on agar plate and try to count how many cells are there or colonies are there. At the same time when we see this sample under the microscope and try to see how many cells are there. So, these two numbers never match the number that we obtained from direct microscopy count is found to be always higher.

At least 100 to 1000 times higher than the number that we get as colony count from the same sample that is called the great plate count anomaly. So, from the great plate count anomaly the concept of uncultivability or unculturability of natural microorganisms evolved. So, that means we will discuss this but to have a very, very preliminary idea that microscopic count is providing more number of cells but the agar plate based method or the colony count method is not providing.

So, many cells and the number of cells are at least two orders of magnitude less like if one cell is coming in a few colony forming unit microscope will fall provide you at least 100 cells. So, what is going wrong. So finally the scientists that they found that it is the uncultivability of the natural microorganisms. So, when we look at the sample with microscope. So, there are 100 cells most of these cells almost 99% of the cells are not capable of growth under the laboratory condition.

So, they are uncultivable and this is a property or this is a kind of a manifestation of a property which is called anti uncultivability. So, many organisms are uncultivable they do not grow under laboratory condition. So, as soon as the 16s ribosomal RNA based methods appear and they are streamlined because of the sequencing development in PCR and sequencing techniques. So, microbial community analysis structure and function both were streamlined initially using the 16s ribosomal RNA gene followed by the function different functional genes that we will discuss in lat in detail in the later lectures.

Then the meta genomics the concept of meta genome came where all the organisms genome can be studied together and finally it is not only the meta genome that is the collection of all the genomes present of all the organisms present in a sample but also the total protein, protein that is called the proteome or the total RNA that is the transcriptome or the meta transcriptome or even the total pool of metabolites produced by a particular community that is called metabata metabolum.

And even the reconstructions of the genomes of the uncultivable organisms were possible and probing the role of individual species and populations or guilds were possible in today's time. So,

there is a kind of an huge or we called a paradigm shift in microbial ecology or environmental biotechnology methods starting from the pure culture bacteria isolating or looking for few known organisms in your sample to a whole lot of omics approaches and a kind of all inclusive studies where every organisms every possible processes can be identified and studied.

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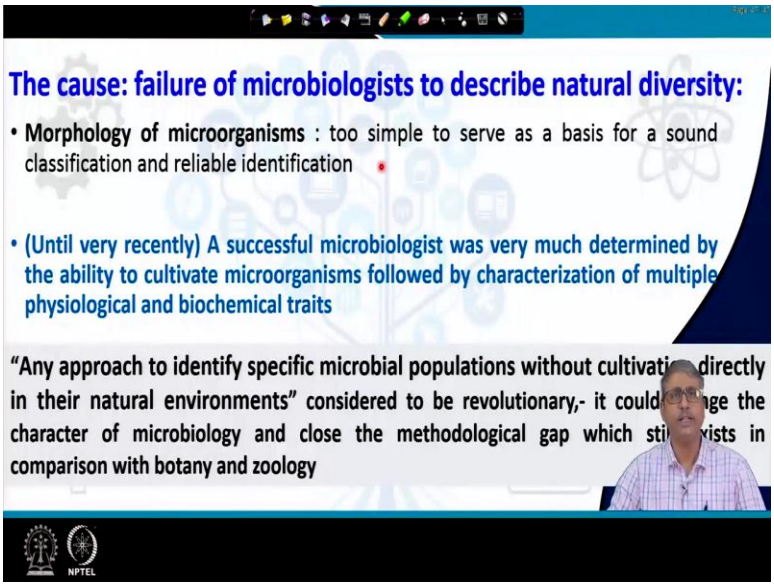

Now if we look at this uncultivability one of the major point is the cause is the failure of microbiologist to describe natural diversity. So, because the morphology as I mentioned it is too simple to serve as the basis for classification system and until very recently a successful microbiologist was very much determined by the ability to cultivate microorganisms followed by characterization of multiple physiological and biochemical traits.

So, it became a kind of a routine practice that in order to decipher the microbial diversity or microbial property of a particular environmental sample any microbial ecologist or environmental biotechnologist used to rely on isolation of few cultivable bacteria or archaea or fungi followed by their characterization and their further analysis. Now it is it is understood that any approach to identify the specific microbial populations without cultivation.

Because now we have realized that 99% of the cells or the species present in an environment are not cultivable. So, any approach that will identify the specific microbial populations without cultivation directly from their natural environment is therefore considered to be a revolutionary

process. It would actually change and it has actually changed the character of environmental microbiology and it has tried to close the methodological gaps which still exist in comparison to other branches of biology particularly the botany and geology.

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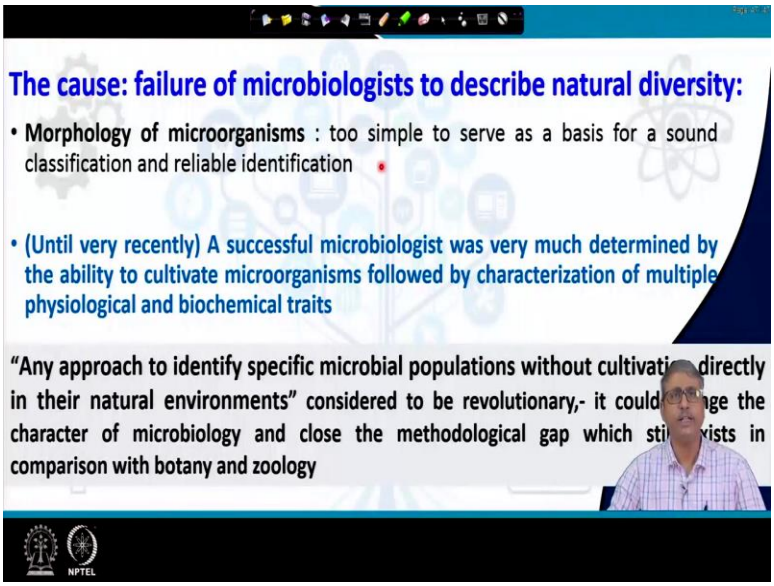

Now this revolution of microbiological thoughts over the past 30 years if we see one of the best examples in the great plate count anomaly as I mentioned earlier that is the discrepancy between the size of the populations estimated by dilution plating and the microscopy. So, in one hand the samples are taken diluted and seen under microscope and the direct microscopic counts are obtained. So, we observed that the direct microscopic counts exceed the viable cell counts by several orders of magnitude.

And the majority of the microscopically visualized cells are viable because we have methods to identify whether those cells are viable we find that they are actually viable but they do not form visible colonies on the plate.

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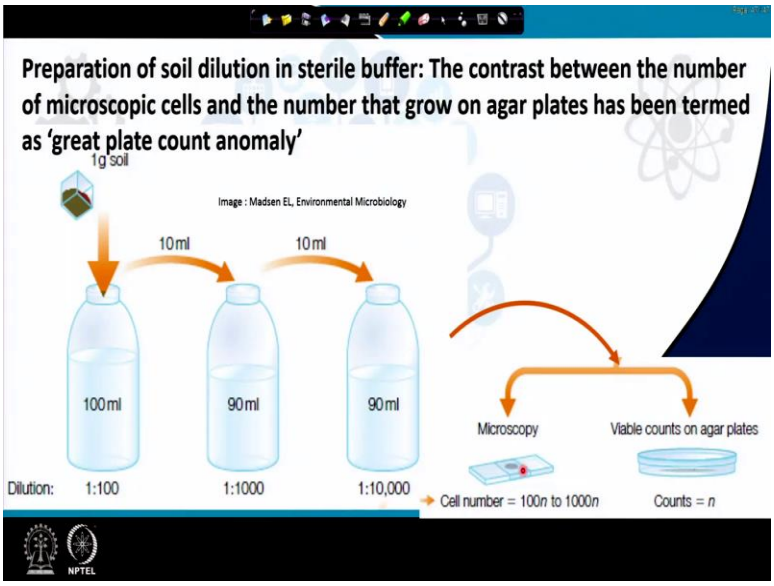

So, for example in this simple example we can see that preparation of a soil dilution in a sterile buffer if we dilute it take a soil one gram of soil and dilute it serially up to 10000 time and then plate this dilute maybe 100 micro liter if we sprayed on to this as well as 100 microliter or a subset of that we can see under the microscope we will see that if under the microscope we are able to count maybe 100 cells or 1000 cells in agar plate only one cell is going to appear.

So, what I was referring earlier that one out of 100 cells will actually grow on a on aggregate plate in a parts or give a viable count.

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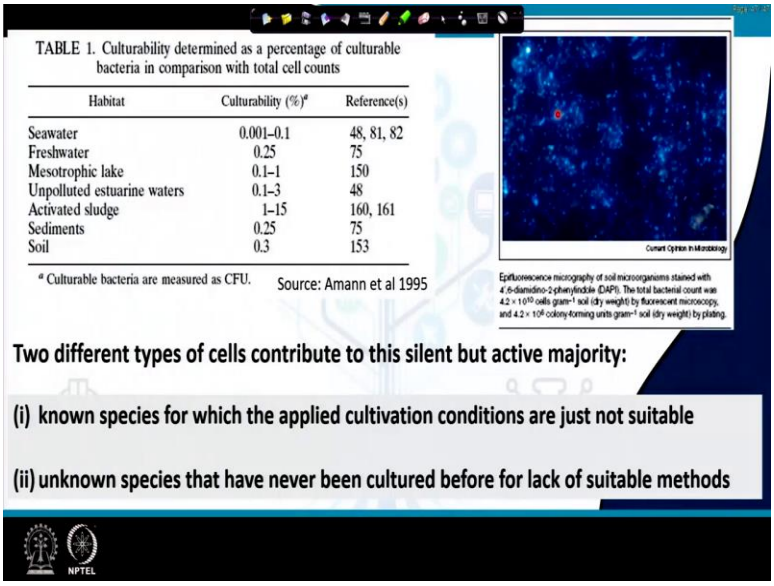

So, in a subsequent study among it all reviewed and found that in different environment

including sea water, fresh water, lake unpolluted water sediment soil even activated sludge the cultivability varies but it almost in all cases remain close to 1% or less than that except the activated sludge. Because in activated sludge you know that we use a particular inoculum or a particular set of organisms who are only growing there continuously and they are they are cultured under that condition only.

So, the cultivability of the organisms are little higher in activated sludge but in rest others it is close to one or even less than one. So, two different types of cells are found to contribute to these silent but active organisms because one out of 100 is able to grow as a viable cell. So, rest 99 cells they are viable but they are not growing the picture on my right side shows basically the Epifluorescent microscopy of a soil microorganism stained with drapey and you can see the numerous cells which are which are present in the sample.

Any time anybody can take a sample from a soil or a water and stain with drapey or similar flows and dyes DNA staining dye and see under microscope and you will be able to wash you will be able to count how many cells are there but if you take a same sample and dilute and plate on agar plate you will not be getting so, many counts, the cultivability will be definitely within this range. So, it is considered that two types of organisms must be contributing.

One is a known species for which the applied cultivation conditions are not just suitable. So, whatever medium or media we have used or the pH or temperature etcetera we have provided it is not suitable. The other could be that these organisms are actually unknown species that have never been cultivated or cultured before and the lack of suitable methods.

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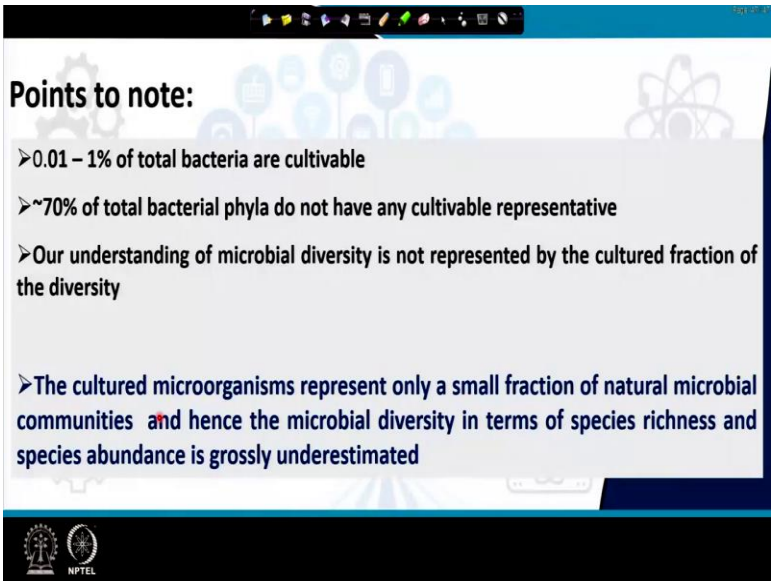

Now the major points to note with this respect are in this respect that 0.01 to 1% of the total bacteria in particular are cultivable, 70% of total bacterial phyla they do not have any cultivable representatives and our understanding of microbial diversity is not represented by the cultured fraction of the diversity. So, the cultured microorganisms represent only a small fraction of the natural microbial communities. And hence the microbial diversity in terms of species richness and species abundance is grossly underestimated.

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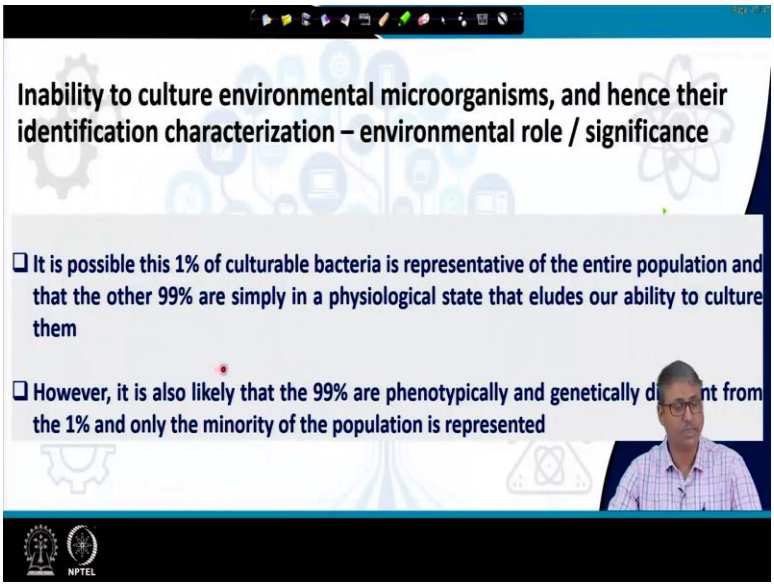

Now with respect to inability to culture these environmental microbes and hence their identification characterization environmental role and significance, it is possible that this 1% of cultivable organisms they are representative of the entire population and that the other 99% are

simply in a physiological state that eludes our ability to culture them. But at the same time it could also be possible that the 99% of the cells are phenotypically and genetically different from the 1% and only the minority of the population is represented.

In most of the cases in the later studies using different environmental genomics tools and we will talk about that that it is found the second one is more true.

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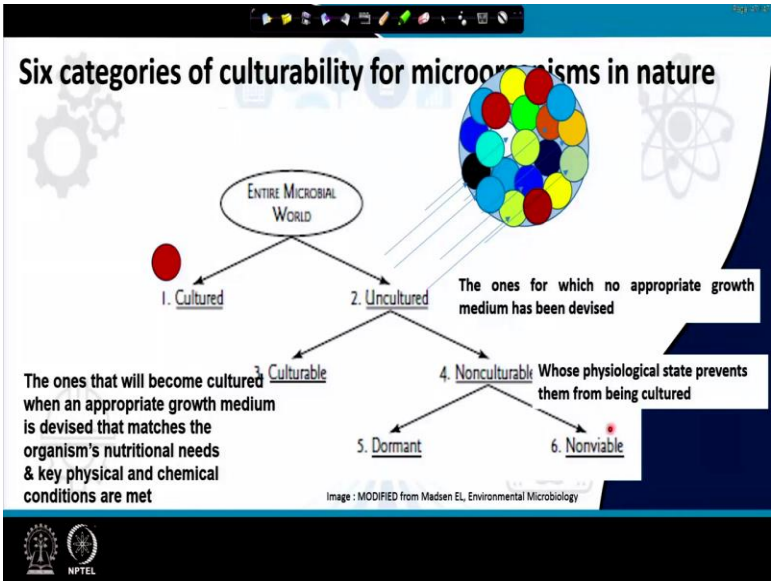

So, the six categories of cultivability of microbes are generally observed in the nature. If we take a sample any kind of sample we take we found that there are some organisms which are cultured and some organisms which are not cultured, cultured means they appear readily on agar plate. If we have a suitable medium we will find out that some colonies are coming out and if we take a kind of an cartoon for that we will see that out of a total community only maybe one out of one 100 will be cultured.

So, culture's are those organisms that have been successfully isolated and purified in the laboratory. So, these organisms if we take up this cell or the colony or the strain and try to identify this you will find that this has been previously identified as a cultivable member most of the time and these are represented in culture collections. So, we have several international culture collections where these organisms are deposited and they are maintained and these are often considered some of them are as a model organism for the basis and 99% of what is known about

the microbiology and environmental microbiology.

So, whatever science we know about the scientific basis of our environmental biotechnology the microbial part of that is basically most of the cases relied on that 99 cases on those few cultivable bacteria because it is the only in the recent past like only 10 years or so, we have methods through which the uncultured organisms are also being characterized. So, who are these uncultured? Who are not appearing on the agar plates or growing on the agar plates.

These organisms could be ones for which no appropriate growth medium has been devised or possibly the pH and temperature etcetera were not appropriate but this uncultured organism could be cultured provided that we have proper medium or proper nutrients provided and also the physical and chemical conditions are provided. So, if you are curious if we are interested we can culture some of the uncultured members.

So in recent past we see there is a lot is a great effort in culturing many of the so, called uncultured organism. Because once we know that many organisms are uncultured we are trying to culture more organisms. But so, within the uncultured organism there could be two category one is culture culturable, culturable means provided that you have a proper medium and growth condition etcetera some of them will grow.

But some of them even after that will not grow those are the non-culturable whose physiological state prevents them from being cultured. So, these kinds of physiological restrictions that these cells or these organisms they have and within this category we may have two types one is dormant. So, like kind of endospores for bacillus or many actinobacteria they are dormant. So, they are they are their metabolic activities are on a very low level and they are not willing to go grow or respond or reactivate themselves and the last category could be non-viable.

So, if we have the entire microbial world in any kind of sample drinking water, ground water, lake water any sample soil sediment anything we kind we may think of having all these six kind of categories. So, among these 1, 2, 3, 4 are definitely the viable cells but the one the last component could be the or are the non viable components.

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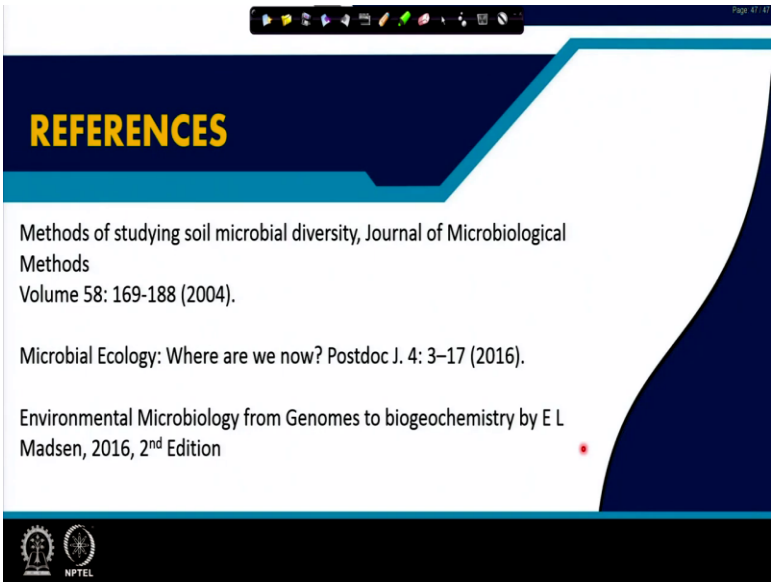

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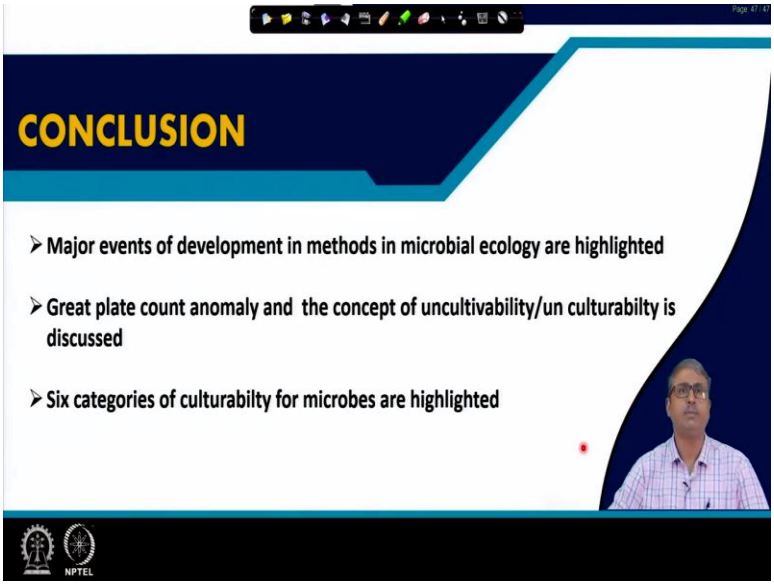

So, for this part of the lecture the following references can be used. And in conclusion some of the major events of the of development in methods in microbial ecology are highlighted we have discussed about the great plate count anomaly and the concept of unculturability and the different kinds of culturability like the six different type of organisms microorganisms which are encountered in any kind of environmental samples are highlighted, thank you.