Environmental Biotechnology
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Lecture – 26
Methods in Microbial Ecology with Relevance to Environmental Biotechnology (Contd.,)

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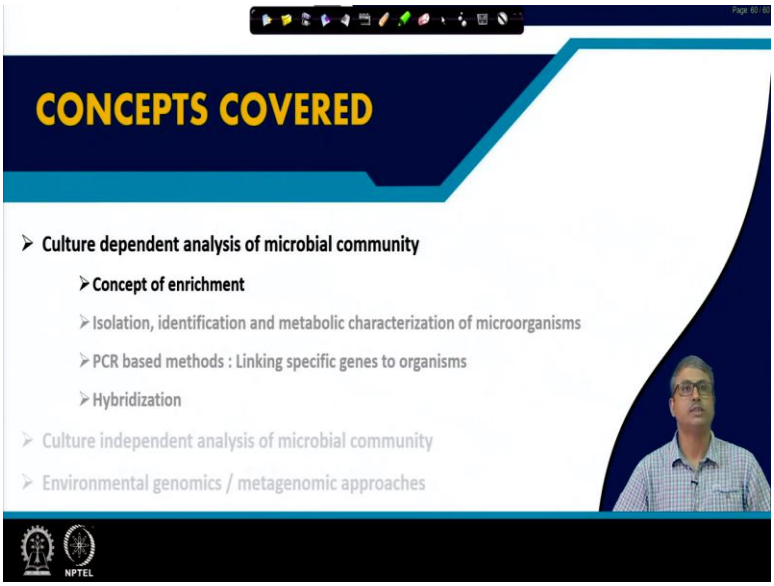

Welcome to the 26th lecture on methods in microbial ecology with relevant relevance to environmental biotechnology. And in this particular lecture we are going to discuss about the culture dependent analysis of microbial community and in particular we will emphasize or discuss on the concept of enrichment based culturing and isolation followed by their characterization.

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So, microbial communities from environmental systems are often analyzed using a large set of tools or methodological approaches but before we go and discuss about this approaches or tools we must clearly understand that what are we going to analyze. If we have an environmental sample whether it is a petroleum oil contaminated soil or a paddy field in either case we have to know very clearly that what are we going to analyze in terms of the microbial communities that they are present.

And once we determine that the parameters that are going to analyze what protocols or what methodology will be adopted that means how are we going to analyze the communities. Now as I discussed earlier almost in all environments whether it is a contaminated environment or a non-contaminated or pristine environment. In every environment we have a large number of bacterial diversity or a significantly high microbial diversity in most of the systems.

So while characterizing these communities keeping in mind that these are going to be useful for developing an environmental biotechnology process we need to be very clear about what are we going to look for.

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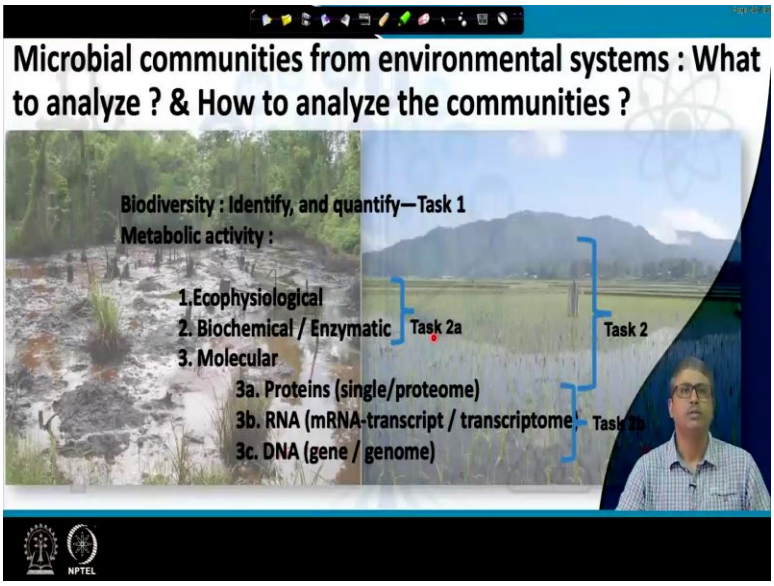

So, there are 2 major tasks to complete with respect to this that means if we want to answer to the first question that what to analyze. The first parameter or the answer to this question that what are you going to analyze the first and foremost important parameter to be analyzed is the biodiversity that means the species which are present are microorganisms which are present there they are identity that means the identification of the species present there and quantification of those species.

Now in this regard I must reiterate that the number of species that are present in any of these environments are significantly high at least few thousand species of bacteria or in some of the cases bacteria and archaea and then along with them there are plenty of fungi and often algal species and sometimes virus and protozoa they are also there. So depending upon the system and depending upon the research question or the broader research question with respect to all the technology that we are trying to develop the diversity could include even the virus.

For example for a drinking water system or a food or some kind of system where clinical or epidemiological or health related parameters will be important there we need to include even the protozoan virus fungi bacteria archaea. So every different types of microorganisms can be included. So, the total number of species or total number of organisms that we are possibly trying to identify could be several thousands.

And it is not only identification of the species alone it is also the quantification of each of these. Now what do I mean by quantification quantification means by quantification means each of the species abundance how abundant are they? So if we have 100 different species we should be able to tell that species number one is 5% that means if we take 100 species or 100 cells 5s will be belonging to a particular species some species might be 10% abundance or some species may be 1% or some species may be 0.1 or less than 0.1% or maybe very less which are often considered as rare microbiome. So, that is the task one.

The second and most important another task is the assessment of the microbial activity and this is the activity which is basically connecting us to the biotechnological or the process parameters that we are trying to set for a given system. So with respect to the microbial communities activity the activities of the organisms in terms of their different metabolic properties metabolism is basically categorized into 2 sub tasks like task 2a and task 2b.

In 2a mostly we perform different type of eco physiological and biochemical or enzymatic assessment. So the selection of methodologies will guide us. So, there was a point earlier I mentioned how to analyze the communities. So even if we have a petroleum contaminated site or we have a agricultural paddy field which appears to be quite pristine and apparently not. So, much contaminated but you do not know about the pesticides and all those things.

So there might be some hazardous contaminants present in the water itself. So anyway,. So, we can take the samples and then implement task one. So if we are implementing task one then possibly we would be using approaches that will allow us to characterize the activity of the organisms present in these environments with respect to their eco-physiological attributes. Eco-physiological attributes means the physiological attributes with ecological significance.

This along with the biochemical parameters,. So, there are a set of biochemical parameters which are often used in order to characterize the microbes in order to understand their electron donor preference their electron acceptor preference their carbon source preference their nutritional status as we discussed earlier heterotrophy, lithotrophy, autotrophy, mixotrophy there could be different type of nutritional categories.

So biochemical assessments followed by enzymatic assays. So, there are many enzymes which are very specific enzyme like catalase, oxidase, urease and couple of more enzymes are there which are very standard set of enzymatic test those are often used to characterize the organisms in terms of their basic metabolic properties. So, that is basically task 2 with respect to the task 2b we generally focus our approaches using molecular methods.

Molecular methods meaning methods which will analyze the proteins, proteins in the sense either a particular protein or multiple proteins or even the proteome, proteome means the all the proteins present in a cell or a community. The analysis could be also targeting the RNA molecules now why RNA molecules within RNA we can actually target all the mRNAs which are present or some specific mRNAs.

Why mRNA? mRNA is targeted because during the process of gene expression when the DNA is transcribing producing the RNA and RNA is translating producing the proteins which are then modified to form different type of enzymes. So, RNA or the mRNAs produce during this entire process of protein synthesis or the gene expression provides us the opportunity to identify the real scenario of gene expression or the metabolic activity.

So, in terms of a petroleum refinery or petroleum contaminated site or any other environments if we want to know what are the enzymes actually being produced or what are the genes encoding those enzymes are expressed there. So, the RNA based method would be one of the most important or most useful method. The next one is the DNA based method which is rather more straightforward.

Because most of the or almost every characteristic properties which are executed metabolic characteristics particularly even taxonomic properties as well are encoded by genes. So, we can analyze individual genes specific genes targeting a specific enzyme or encoding a specific enzyme or en encoding some specific other molecules like the ribosomal RNA which are useful for the taxonomic purposes.

So, the DNA based analysis are done in order to identify these specific genes the genes of interest could be the functional genes that means the genes which are responsible for producing mRNA and then mRNA are responsible for producing enzymes and also we can actually characterize the entire genome of the organism. So, this genome could be for individual bacteria archaea fungi algae present there or it could be the entire set of the genome that is the meta genome that we will be talking in some other lecture that is the task 2b.

So, together the task 2a and 2b allow us to characterize the metabolic activity of the community members.

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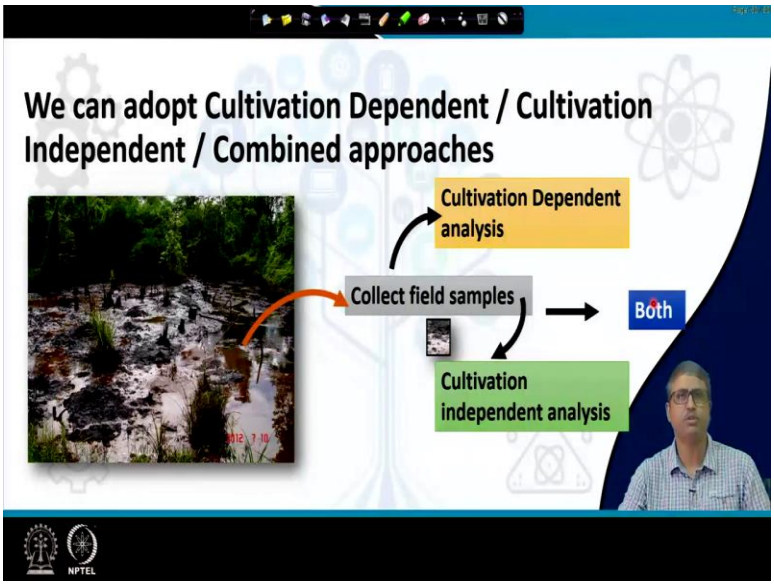

Now as we proceed further suppose we have a sample from a petroleum spilled environment. So, this is a forest area and of course it is an environment within the India itself. So, we have some kind of natural oil spill occurring there or oil is present there in a forest environment. So, to characterize the site or characterize the communities present there in order to reclaim this environment or reclaim the forest or restore the environment that is the remediation or bioremediation.

Or in order to produce some useful products like convert this contaminated waste into a resource for example if we can convert them to some useful bioenergy related products like methane or methanol or something like that that would be very useful for a biotechnology purpose. So, now

with respect to characterizing this community which is present over here, so, there are three options. So, one can adopt either a cultivation dependent method.

In our earlier lecture we have learned that the microorganisms present in environment are basically many of them or most of them are not able to grow or we are not able to grow them in the laboratory. So, they are called uncultivable organisms. So, any method which will allow us to study only the organisms like bacteria who are cultivable that is the 1% or less than 1% of the total organisms or total bacteria present in the environment would be the cultivation dependent method.

So, knowingly that it will only allow us to identify characterize and build strategies or technologies only on that 1% or less than 1% of the total organisms present over there nevertheless this is going to be a very important method because we will get some bacterial strain isolated and there are plenty of examples outstanding technology developments have been achieved where handful of pure culture bacteria were isolated from environment and they have been characterized and the process development have been achieved successfully for environmental processes.

So, there could be cultivation dependent process there could be also cultivation independent process. So, what do we mean by cultivation independent process? Cultivation independent processes are those processes where isolation attempts where we will be avoided that means once we have the samples with us like if collection of the field sample is done. We can actually characterize the community diversity community activity directly by targeting specific molecules like the enzymes proteins RNA or DNA directly from the sample.

So, in this case we are not going to have any kind of isolation direct isolation of bacteria or archaea or any organism. So, rather from the sample we will get the nucleic acids. So, from the sample we will get the nucleic acid extracted and that nucleic acid will be subjected to analysis that will learn in due course. So, this is cultivation dependent analysis where organisms will be isolated most of the time pure culture of bacterial strains will be isolated.

And they will be subjected to this characterization and also the activity assay or in case of the cultivation independent analysis we will avoid or will not engage into any kind of isolation of bacteria. We may do something in the later stage if we feel so, but as a general practice in cultivation independent analysis or approach we do not target isolating any individual bacteria rather we rely on the total population.

Whatever is there we will follow a protocol a method through which the characterization of the community in terms of the species composition quantification diversity metabolic activities all the necessary questions will be answered through cultivation independent analysis. And finally there could be a mixture of both mixture of the both. Mixture of both protocol in a sense that we can set up some specific cultivation method where some organisms together they may grow as we will see today in subsequent part of this lecture that enrichment based methods are there where a specific substrate specific condition is created or provided to a community and the community often responds to that.

So, once the community is able to respond towards those substrates those conditions some organisms are grown. Of course we may not be able to isolate them as pure culture but some organisms will grow as it will the culture broth will term we will be target we will be able to see if we do some kind of other assessment like measurement of the ATP or under the microscope if we see will see the large number of cells are growing.

So, that means some cells are responding favorably towards the substrate that we provided now from those cultures or those broth where mixed cultures very, very often mixed cultures are there we can implement a culture independent approach because we may not be able to isolate everyone as a single or pure culture. So, that is actually considered as the kind of an approach which is a combinatorial approach where both cultivation dependent and independent protocols are used.

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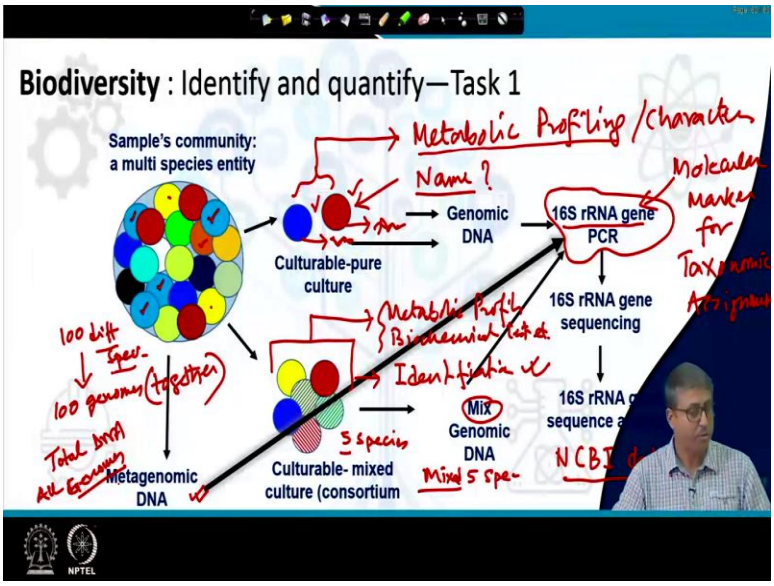

So, now we have a contaminated environment or any kind of environmental sample where we understand that there are multiple species are there of course there will be multiple species. So no matter whether these pieces are cultivable or not cultivable. So, we can have a very straightforward strategy or a set of strategies which will help us to fulfill task one. That is the identify the species or organisms and also quantify them who are there and what is the relative abundance of each of them.

So, one of the very common approach that or common approaches that we have been talking about or will be talking more maybe sometime or even today is the cultivation dependent method where the culturable bacteria that is the pure cultures will be obtained. So, I will talk about the different methods to isolate the pure cultures. So, pure cultures mean the culture of a bacteria which is not mixed with any other organism.

So, it is only one species is there and this particular species is able to grow in the laboratory condition that is called that is why it is called culturable. Now once we have this culturable bacteria we can have a kind of number of strategies and I can help you to understand that what are the strategies we are going to take. So, one of the strategy could be that once you have these organisms you can subject them to a kind of what we called metabolic profiling or metabolic characterization.

So here in all these organisms which we isolate we can isolate even 10 bacterial strain or twenty bacterial strains all these individual strains will be subjected to metabolic profiling or metabolic characterization that may include the biochemical and enzymatic assay and ecophysiological tests etcetera. So, there are very well set methodologies and test parameters are there and we will see in a kind of an example that we will discuss in subsequent classes that how these are done.

So, we can actually characterize each of these. So, if we have 100 such organisms we can we can actually characterize all the 100 organisms very very easily. Now moving forward. So, one part is the metabolic profiling or metabolic characterization of the pure culture bacteria or pure culture archaea that we isolate and subsequent to that isolation we generally try to extract the genomic DNA from those bacterial strains. Why we extract the genomic DNA? We extract the genomic DNA because we want to identify them using the most sensitive and apparently most precise method that is the 16s ribosomal RNA gene based method.

So, we will talk about that this particular gene based method the why do we select this gene and all these things in some other class but to restrict ourselves from those elaborate web discussion. So, we will refer to only the 16s ribosomal RNA gene is a kind of what we called molecular marker. So, it is a kind of molecular marker for taxonomic assignment. So, in order to; identify the taxonomy of the organism?

So, what is the identity. So, what is if we want to know what is the identity what is the name of the organism can anyone tell me what is the name of the organism if I search for this how do I search? What do I search. So, in this case maybe 10 years before or maybe 20 years before when the sequencing technologies and the analysis protocols were not that straightforward we used to analyze the or the identification of the name to find out the name we used to have this metabolic profiling only. So, biochemical tests etcetera.

So, you might have already come across the Burghy's manual. So, using those system very systematic protocols of biochemical assay and other enzymatic test eco physiological test we had a protocol we still have a protocol something like that but nowadays we rely more on the molecular marker like a 16s ribosomal RNA gene. So, although the alone 16s RNA gene may not

be may not be able to provide you a complete comprehensive identification.

So, there may be a requirement of multiple other genes multi locus sequence typing or something often we also use but those are further detailed. So, generally once we have the culturable pure culture bacteria any number of bacteria individually each of the strains will be subjected to this 16s RNA gene analysis. And once the gene sequencing is done we are able to analyze those sequences and then finally we get to the the taxonomy of the organism.

Now there may be a question that from the sequence how do we get the taxonomy of the organism we have the sequence. So, once we have the sequence we can actually search this sequence in a sequence database which is called NCBI very popular database. In NCBI database there are large number of 16 sequences already deposited. So, the moment we put our sequence as a kind of a query there the sequence will be matched.

So, the based on the relative identity of our query sequence to the database sequence sequences rather the results will come and from that result we can actually identify that what is the closest match. Now based on the closest match we can we can we can say that this particular bacteria or pure culture bacteria isolate belongs to this taxonomic category. So, there will be some more requirements to finally validate that whether it really belongs to that particular category.

I may find one bacteria just as a bacillus but whether it is a bacillus megaterium or bacillus subtilis or some other species of bacter bacillus to know that Ii may need to analyze little bit more like some more genes which I said that multi-locus sequence typing or some other metabolic or chemotaxonomic parameters might be required. So, we will discuss partly some something like that in a later time.

So, after this culturable or culture based method we have another very interesting method which is also culturable but here we are expecting a mixed culture. Why we are expecting a mixed culture we are expecting a mixed culture because this is a system. Why we are not intending to isolate pure culture bacteria why we are not intending or trying to isolate pure culture bacteria is fundamentally the because bacterial strains or microbial strains when they live in natural

environment they always engage with multiple interactions with them.

So, if you do not allow them to interact with each other each other means the species among themselves they interact we have already learned about syntrophy for example that made intra species metabolite transfer. So, if we do not allow them to transfer metabolites because when we try to isolate pure culture often we use a technique which is called strict plate technique or some kind of spreading method where we forcibly separate the cells.

Physically cells are placed some at some distance. So, that actually inhibits the interaction close interaction between the cells. So, in order to allow these close interactions to happen between the cells or the species we often encourage the mixed culture that is that is also already will be discussed in detail in enrichment system. So, in during that we can actually allow them to grow still in the laboratory providing a substrate set of substrate or set of condition.

And we do not allow them to or do not do not intend to isolate as pure culture rather we prefer to leave them as mixed culture and then characterize the mixed culture. Now with respect to how do we characterize the mixed culture the characterization could be done in a same way like the metabolic profiling. However in this case the; metabolic profiling the result of metabolic profiling will be providing us the information about the metabolic activity or metabolic property of the entire population or entire group of populations.

Or entire community which is cultivated now this is a mixed community or mixed culture rather I will say. So, the metabolic profiling of this entire enriched culture can be obtained. So, if we just go and may if I just try to write this thing, so, now this entire sample can be taken and we can do again the metabolic analysis or metabolic profiling or for example the same biochemical and other ashes biochemical tests etcetera.

At the same time we can go for their specific identification. So, when we go for the specific identification. What do we do we extract the genomic DNA now unlike the pure culture bacteria where you can extract the DNA from these bacteria separately and the DNA from these bacteria separately because they are growing separately but in this case they are not growing separately.

So, what you extract actually is a mixture of DNA.

So, if we have let us say 1, 2, 3, 4, 5 different organisms. So, each of the seeds for example represent different organism, so, we assume that there are actually 5 species just assume or imagine. So, if we have 5 species if we extract the total DNA out of this mixed DNA will have the DNA of all 5 species. So, it is a mixed. So, we are not go trying to separate it let that be mixed. However within this mixture we will allow the sixteen s gene analysis and eventually from this analysis we can we can make out there are protocols we will discuss those protocols that how we actually differentiate the 5 species who are present there and their relative abundance of course.

So, finally after this analysis we can actually identify through NCBI database and there are couple of other database also that what are the taxonomic identity. So, identification of this mixed population is done. Now the these 2 methods were culturable methods basically. So, culture dependent method.

Now the next method is the meta genome based analysis or meta genomic approach where the total DNA of the community itself extracted now what is the difference between this and this. So, in this case we basically extract the total DNA that is representing possibly the all the genomes which are they are in the system. So, this system might have for example if this system is having 100 different species.

So, we are extracting 100 genomes together but remember they are together we are not able to separate them at this moment. And also please do remember this or try to try to make it clear that these 100 different species are with different abundance. Like if you take this example that this bright yellow is only 2 and the red is only 2 but there could be some blue which are 4 like this blue which are 4.

So, if we extract the total DNA the representation from the blue will be relatively more rather than the representation from the yellow. And if you look at this particular set this representation will be lesser than even the yellow or the red because this is represented by only one. So, if we

extract the entire genome or the meta genome there will be always a kind of if we believe that all the organisms are able to or we are rather able to extract the DNA from all the organisms equally then there would be kind of an reciprocating proportion within this pool of DNA.

So, meta genomic DNA is considered to be a kind of a representative mixture provided all organisms are able to lies equally lysis cell lysis happen for all and all the genomic DNA were extracted. Now once we have this genomic DNA extracted this genomic DNA can be subjected to again the 16s RNA gene base analysis. And once we have that 16s RNA gene based analysis that that will be little complicated because now we have assuming 100 different species were there.

So, 100 different types of sixteen s RNA genes will be there so there must be it will be a requirement that there should be some differentiation technique otherwise; how do we sequence them? Because sequencing all 100 together would be little complicated if particularly if we use a Sanger sequencing technique. So, maybe a next generation sequencing based technique or a another type of methods like a clone library method or some kind of other methods would be useful to segregate the different type of 16s and then finally identify them. So, we will be learning these methods in little more little bit more details in subsequent lectures.

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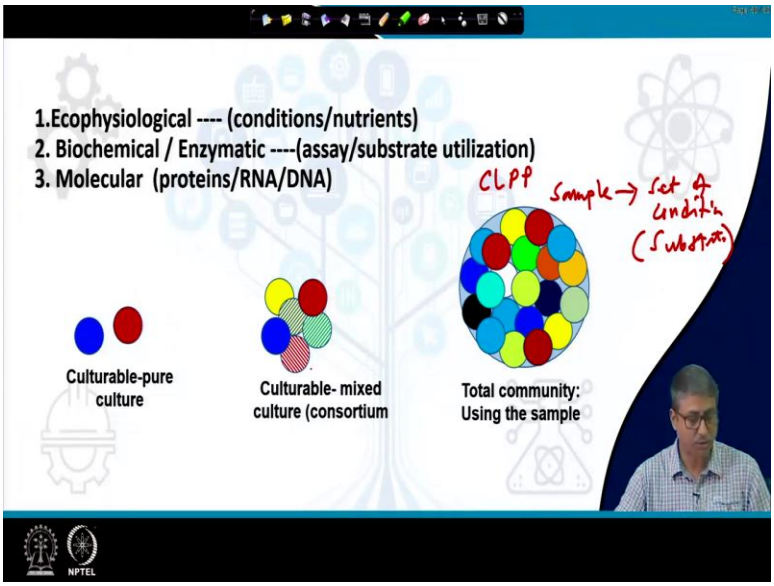

So, now coming to the next point that once we have these organisms with us either the pure

culture bacteria or the mixed culture which are the enrichment or the total community we can perform the eco-physiological test we can perform the biochemical or enzymatic test or even we can perform different type of molecular test. So, that means we can take this individual bacterial strains and put them under different substrate different nutrients different pH temperature redox condition, electron, acceptor, electron donor etcetera.

We can assay the enzymes and similarly we can do the same thing with this enriched population even we can do the same thing with the total community also. So, we can subject the total community itself with different substrate and see how they are going to respond to that. So, there is a very standard protocol which is called community level physiological profiling CLPP. So, when you do that community level physiological profiling often we use so, that is called CLPP community level physiological profiling.

So, the entire community that is the sample itself this small set of sample is subjected to the a set of conditions inside of condition, condition in the in the sense the substrates you can also you can also design a kind of test where you can subject these 2 different ph temperature redox condition and then see how they are responding. So, you can actually make a sense out of this that what is the community's potential.

Similarly the cultural members or the pure culture members all members can be subjected to or different categories can be subjected to similar analysis.

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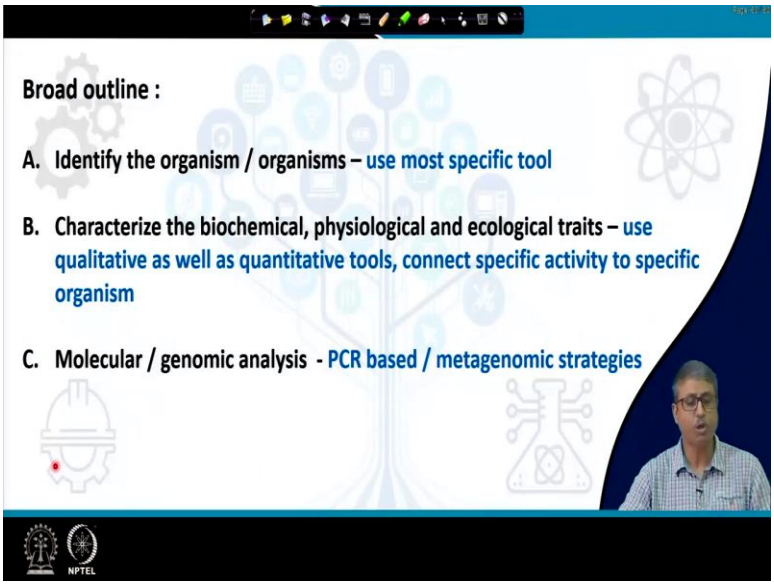

Now the broad outline of this type of analysis would be identify the organism or organisms and it is better to use the most specific tool like the 16s ribosomal RNA as I mentioned. Characterize the biochemical physiological and ecological traits. You use the traits which are qualitative as well as quantitative and try to connect specific activity to specific organisms. So, there would be a kind of a necessity that we should be able to connect the activity to organism.

Otherwise we may find that the particular community is able to do this but who are the organisms who are responsible. For example if you are if we are seeing that there is a production of methane in a particular wastewater or a particular other system then who are the organisms. We may rely on literature or the prior research but those are from other locations other environments exactly on the samples on which you are working or I am working if I want to know exactly what are these species what are the organisms involved in production of the methane from the substrate those are present there.

I need to have some methods which will connect me to or you to the specific activity to the specific organism. The next is the molecular genomic approach where either PCR based approach or metagenomic strategies will be used. And finally the selection of methods could be cultivation dependent independent or selection of methods could give us diversity function or both.

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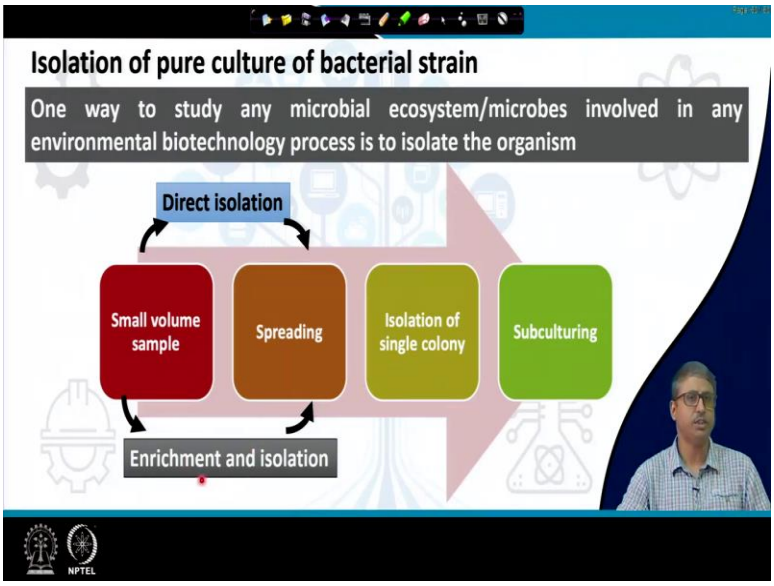

Now we will move forward to have the specific microbiological methods. So, there are a set of specific microwave microbiological methods used in environmental biotechnology particularly with respect to the profiling of the community and that includes the isolation to meta genomic approaches. Now, moving forward isolation of pure culture of bacterial strain. Now when one way to study any microbial ecosystem or microbes involved in any environmental biotechnology process is to isolate the organism.

So, we need to isolate the organisms from the system in order to study that now we generally what we do we take a small volume of sample we try to spread it on a agar medium or a kind of other roll tube method or use some other methods for the anaerobic organisms and try to isolate the single colony and then try to sub culture them confirm the purity of the colony and then identify characterize find out the metabolic abilities and try to see how those organisms could be useful in environmental purposes.

Now here there could be 2 choices one is the direct isolation. So, you take the sample spread it dilute it and spread onto the agar and the other is the enrichment and isolation. So, in case of enrichment that we are going to discuss now we put the sample which is whatever environmental sample we have into a kind of a set of given environment. And allow a specific group of organisms of our interest.

So, for example if it is a petroleum contaminated site we would like to know who are the bacteria or the archaea who are metabolizing specific group of petroleum. So, we may add alkane or aromatic compound polyaromatic compounds or even the crude oil or maybe some other petroleum compounds and then incubate it for some time. Incubate what incubate the sample itself with a medium.

So within the medium if we add the substrate which we want to test that this substrate is utilized by whom then following some incubation time if we just plate them and try to isolate them possibly then we will be isolating some bacterial colony or archaeal colony who are enriched with respect to a particular substrate or particular condition that we have provided.

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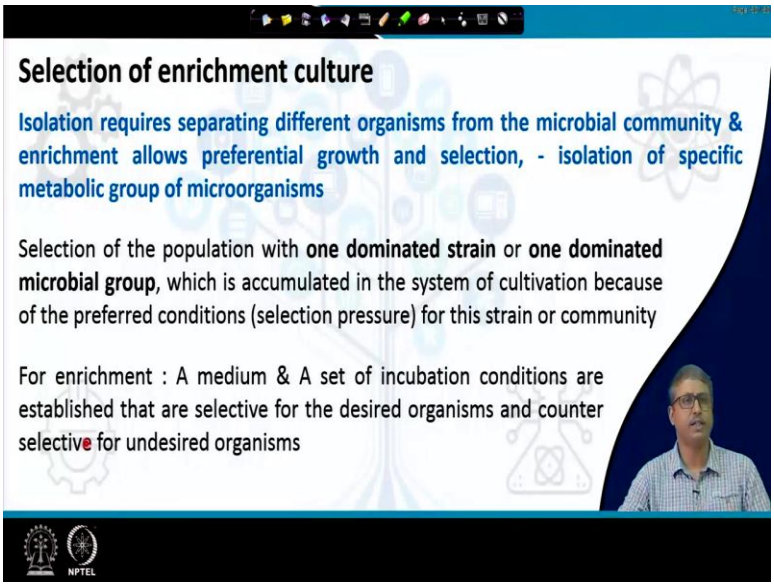

Now the selection of the enrichment culture, so, it isolation requires separating different organisms from microbial community and enrichment allows the preferential growth because in any environment we will have as i said multiple species are there multiple organisms are there. So, each of the organisms might be having their different abilities to utilize the substrates. So, if we want to know that exactly with respect to a particular substrate what are the organisms responsible for be it a kind of a toxic metal like arsenic chromium or it is a pollutant like petroleum hydrocarbon.

So, in any case or is hydrogen or oxidizing or carbon dioxide fixing. So, in any case we can

actually segregate the organisms of our interest from rest others how through enrichments. Now these enrichments are going to be very selective we have to be very very careful and very much careful about providing the substrate selection of the substrate and also the conditions that we provide them.

So following the enrichment procedure, so, selection of a population with one dominated strain or one dominated microbial group, group means couple of species might be there which are accumulated in the system of cultivation because of the preferred condition selection pressure for example if I have a petroleum contaminated sample for example and I want to know who are the hydrogen metabolizing organism or who are the benzene metabolizing organism.

So, what I will do I will take the petroleum contaminated soil and I will put it into a medium and then add either hydrogen in one set or the petroleum oil in other set and after some period of incubation I will see what are the organisms growing there since I have provide. So, if I put continuously this election pressure and create something some other conditions that no other electron donors are there except hydrogen.

So, obviously if no other electron donors are there except hydrogen then after some time I may expect that hydrogen oxidizing organisms will be enriched and i will be able to grow but it is also possible that the kind of organism that I am looking for they are they have a more complex growth requirement and I am unable to provide those. So, my enrichment may fail as well. So, for enrichment we need a medium a set of incubation condition and once these are established a selection procedure will be followed and the desired organisms may be obtained.

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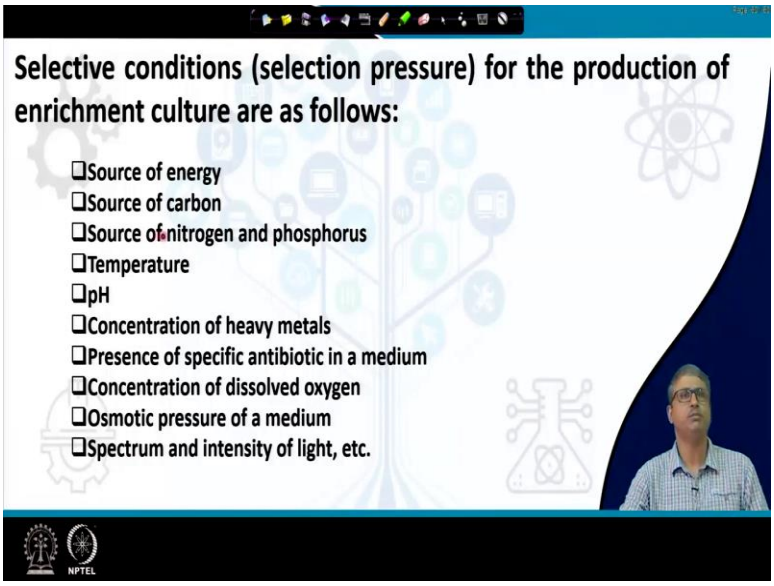

And the selective conditions or the selection pressure could be the broad range of factors like the carbon source energy source nitrogen phosphorous temperature pH etcetera.

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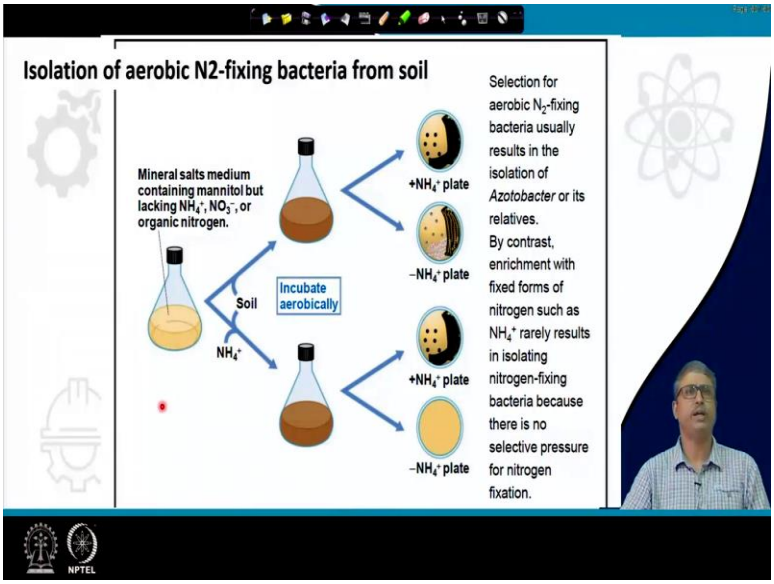

Now we are we will be looking at an example where isolation of aerobic nitrogen fixing bacteria from soil will be tested. So, we are going to have a minimal which is devoid of any ammonia nitrate or any other organic nitrogen. So, this is absolutely and nitrogen minus medium why nitrogen minus because we want to isolate or enrich nitrogen fixing bacteria from soil. So, we want to target those organisms in this enrichment study who are capable of utilizing atmospheric nitrogen only now what are going to do we are going to add the soil into this.

So, we just add the soil into this and following some incubation we plate it onto 2 different types of aggregate plates in one of the agar plates it is the same medium that is there but it is now added with ammonia in the other medium the same medium but no nitrogen source is there. So, we will see that in ammonia containing plate there are colonies which is obvious that there will be bacteria who are present in the soil and capable of utilizing ammonium ion and they will grow but we will also see that in the minus ammonium plate and it is not only minus ammonium there is no other nitrogen source provided in the medium there are some colonies growing.

Now who are these olonies why are they growing how are they growing because i have not provided any nitrogen source there and if you know that nitrogen is one of the most essential component without nitrogen no cell will survive where from this nitrogen is coming. So, this is basically the nitrogen fixing bacteria possibly they have been enriched because I did not allow any nitrogen source to be present there.

So, in a nitrogen minus medium I put my soil and then I allow them to grow in a condition this head space or the empty space must be containing air and this air is having nitrogen and that nitrogen has been assimilated or utilized by nitrogen fixers. So, what happened during this incubation process the nitrogen fixers must have been enriched. So, in a minus end plate those colonies are going to appear. In order to understand these that exactly this the same thing has happened another set is prepared where we have intentionally added ammonium in the same mixture.

So, we have added soil into this minus N medium and then added ammonium into that and when we added ammonium after the plating on the same plus n and minus N plate we do not see any colony appearing on the plus or minus N plate plus N plate is going to have but minus n is not this is negative selection negative selection in the sense that since we have added ammonium over here the microorganism did not feel that they need to fix nitrogen because they are provided with ammonium.

So, whatever nitrogen fixers were there possibly they were they were lost because continuously ammonium ion was there and because of them ammonia the ammonia or the the fixed nitrogen

utilizing bacteria they were they overgrown and eventually the the possibility of getting nitrogen fixers were lost. So, but with this technique the upper tech path we are able to isolate some nitrogen fixing organisms.

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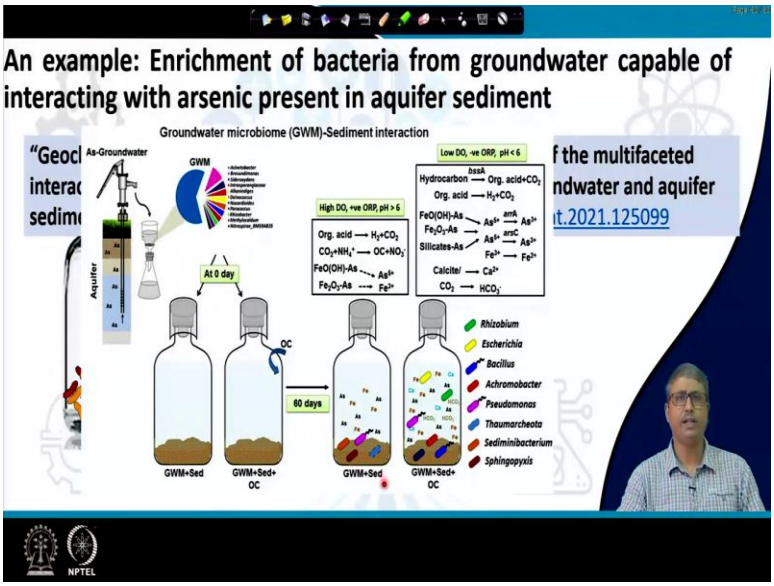

In a recent experiment in our laboratory we were trying to enrich bacteria from ground water particularly arsenic contaminated groundwater. You know that arsenic is a very a deadly contaminant present in ground water system in large part of India and within this arsenic contaminated groundwater we are trying to actually see that who are the bacterial species what are the bacterial species who reacts with or we interacts with arsenic present in the aquifer sediment.

So, what we did? We took a serum vial or is a glass vial which can be closed because we wanted to keep anaerobic condition and then we put the microbes from the ground water. So, we took the ground water and filter it and put the filter membrane over here. So, the microbes from the ground water came here. And then we also add the arsenic and iron containing sediment. So, we want to know that what are the bacteria who can interact with this sediment and then mobilize arsenic.

So, this is the title of the paper that is recently published and what when we did this kind of incubation what we observed that in presence of sediment a number of bacterial strains are

enriched. However if we add little bit of organic carbon into that we could see that entirely different set of organisms are enriched. For example the organisms like rhizobium and the organisms like estrichia and there are certain other organisms also who are enriched definitely in presence of like the bacillus.

These organisms were not present when only groundwater were interacting with sediment but the moment we added organic carbon into this these organisms were enriched. So, it clearly proves that the enrichment ability and all these organisms are capable of interacting with arsenic but it also shows that the ability to isolate or enriched organism through this kind of technique is very much constrained by the substrate which are provided.

In this case there was no organic carbon provided but in this case organic carbon was provided. So, maybe more heterotrophic or organotrophic market microorganisms who are able to metabolize the organic matter provided and transform arsenic or having some intrinsic ability to interact with arsenic where were grown.

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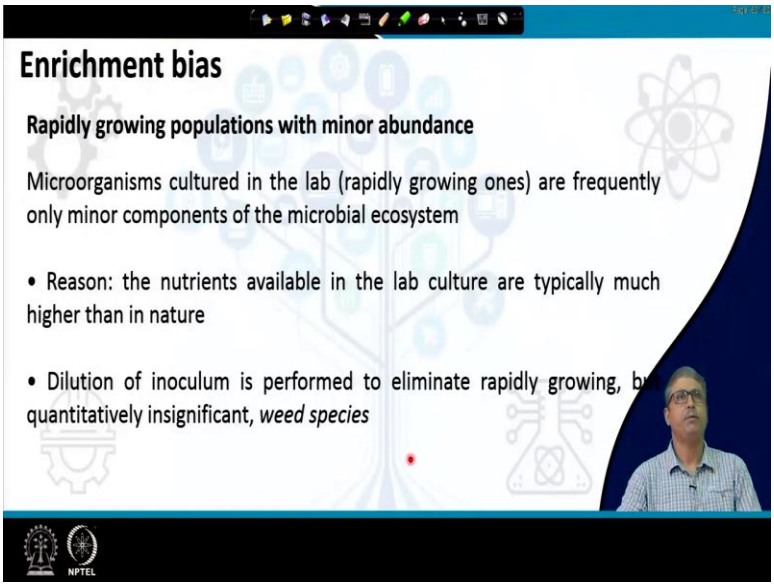

Although the enrichment based methods are found to be very useful they have some bias enrichment bias that is referred as. So, rapidly growing populations with minor abundance often appears. So, microorganisms cultured in the lab which are rapidly growing once are frequently only the minor components and the reasons could be the nutrients available in the lab culture are

typically much higher than the nature. And dilution of inoculum is performed to eliminate rapidly growing but quantitatively in its insignificant weed species.

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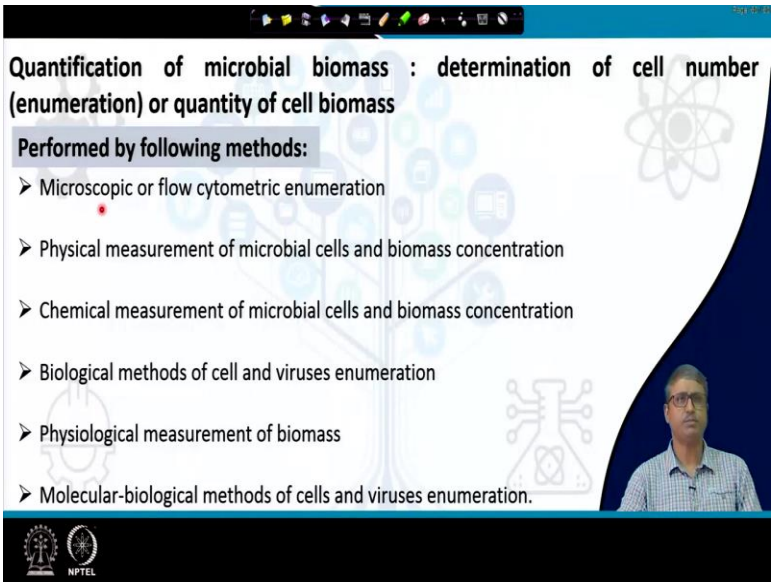

Now finally the quantification of the biomass. So, determination of the cell numbers enumeration or quantification of the biomass can be done by a number of protocols or the methods like the flow cytometric based method, physical measurement of microbial cell and biomass concentration chemical measurement of biomass concentration and microbial cell biological methods of cell and virus enumeration physiological measurement and molecular biology based enumeration is also possible.

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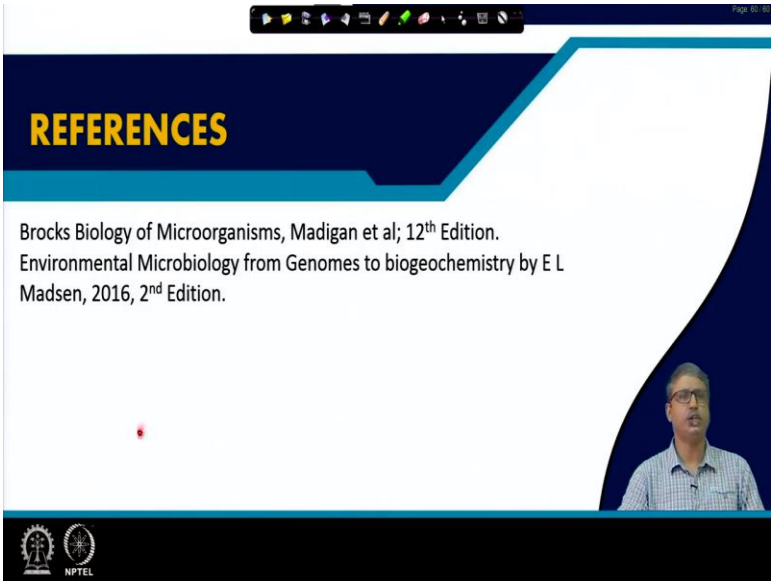

So, for this lecture part of the information is taken from the block biology and partly from the environmental microbiology from genomes to biogeochemistry.

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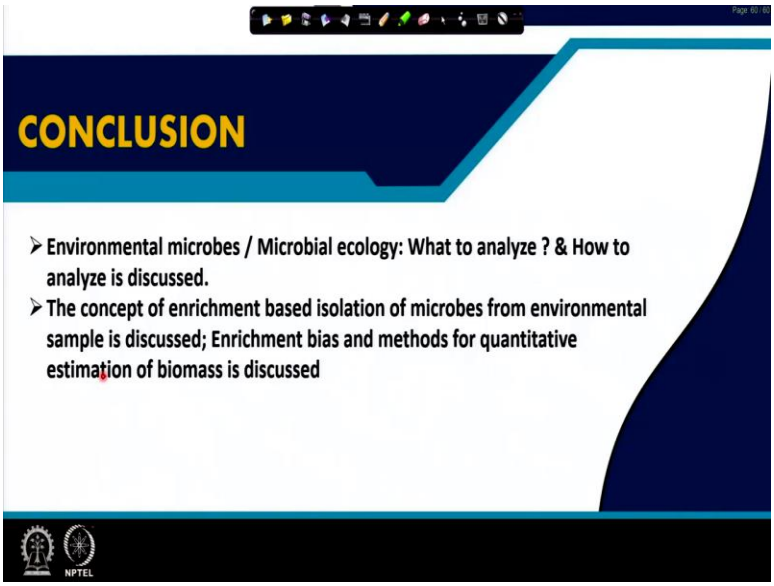

In conclusion the environmental microbes on microbial ecology when it is subjected to the methodological analysis that what are the organisms present we must be very clear about what are we going to analyze and then how do we analyze these parameters. The concept of enrichment based isolation of microbes from environmental sample is discussed enrichment bias and a method for quantitative estimation of biomass is also highlighted, thank you.