Environmental Biotechnology
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Lecture – 15
Microbial Ecology and Environmental Biotechnology - Part C (Contd.,)

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Welcome to this lecture on Microbial Ecology and Environmental Biotechnology wherein the following topics are going to be discussed. We will continue our discussion on principle of good management and in particular we will learn about the importance of slow growing biomass within environmental biotechnology systems. And then we will discuss about the three major themes which have emerged as a kind of documentation from the partnership between the microbial ecology and environmental biotechnology.

And we will also discuss how these three themes or these three peaks are going to have very major contribution in environmental biotechnology.

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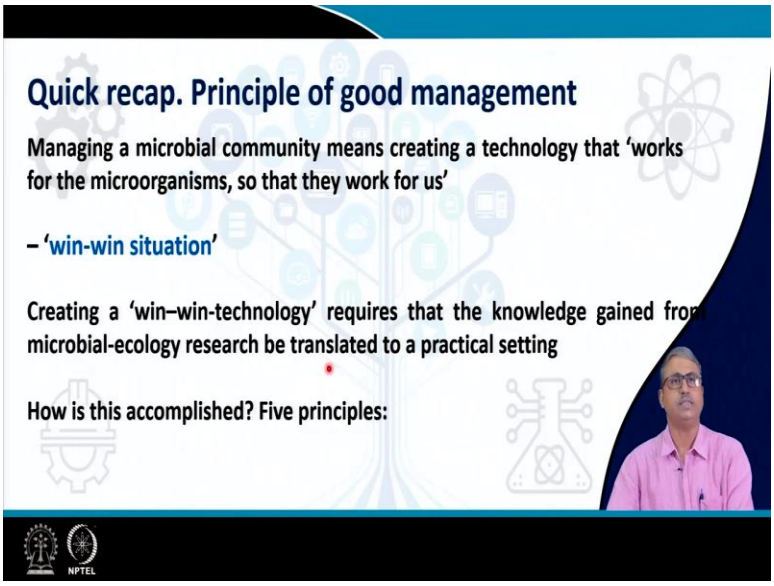

Now before we move forward to understand the slow growing biomass here is a quick recap about the principle of good management that we have already talked about. And we have already learned that managing a microbial community for any biotechnology process means creating a technology that works for the microorganisms. So, that they work for us. And this eventually leads to a win-win situation.

And creating a win-win technology where the microorganisms are allowed to work first for themselves. So, that the microorganism can work for us this concept requires that the knowledge gained from microbial ecology research be translated to a practical setting that means that we need to have a very thorough understanding about the microbial ecology function not only on the microbial community structure.

How these species interact they function and eventually how can we translate that knowledge into environmental biotechnology application.

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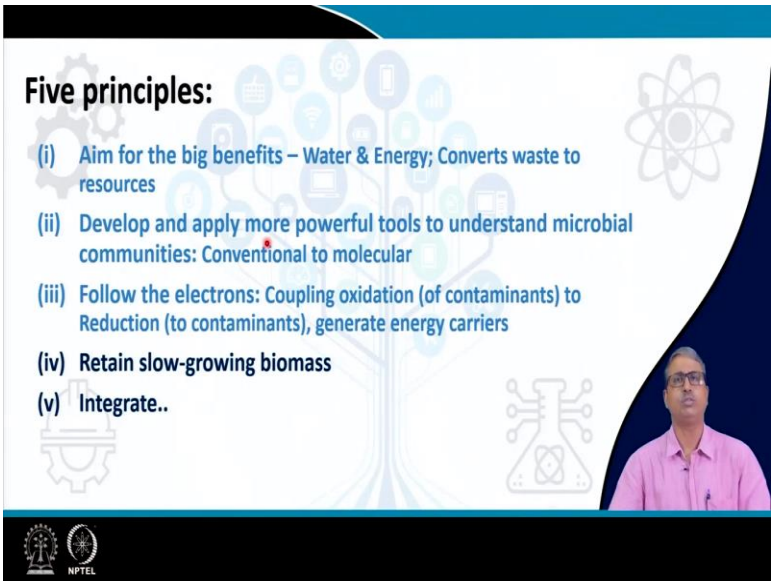

Now with respect to this we have also identified that there are 5 principles and these five principles remain that we should aim for the big benefits as we discussed like water and energy are the most important agenda. And we should also target our environmental biotechnology research towards converting the waste to resources. Develop and apply more powerful tools to understand the microbial communities conventional to molecular approaches because of the immense complexity of the microbial communities.

We have also discussed about the importance of following the electrons within any ecosystem in order to realize the importance of microbial metabolism and its translation into practical settings particularly the coupling of oxidation of contaminants for example the organic pollutants to reduction of some other contaminants like nitrate or other heavy metals or chlorate or perchlorate or generating different energy carriers.

We are going to discuss briefly now importance of the slow growing biomass and following that we will see how to integrate all the information that we generate from this overall discussion.

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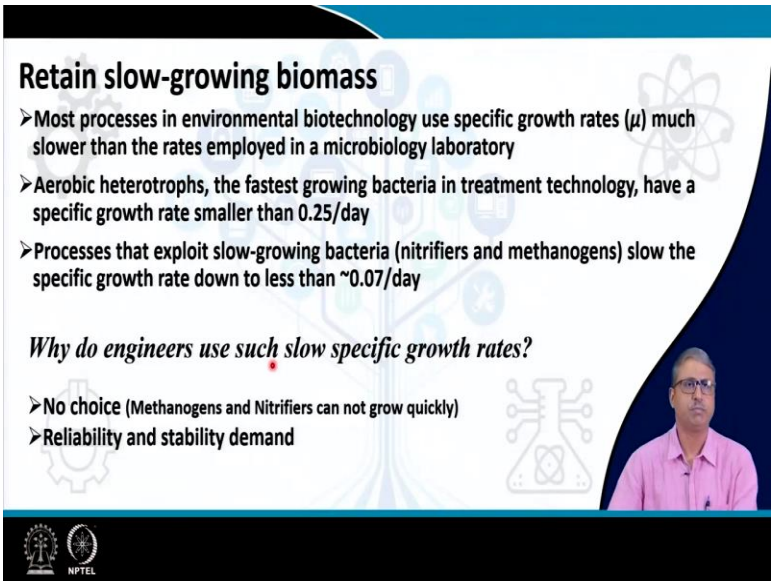

Now we are going to talk about retaining the slow growing biomass. Now most biotechnology processes or environmental biotechnology processes use specific growth rates much lower than the rates employed in the microbiology laboratory. And aerobic heterotrophs for example one of the fastest growing bacteria in different type of treatment technology waste water treatment technology for example have a specific growth rate smaller than 0.25 per day.

Whereas the processes that exploit slow growing bacteria for example the nitrifiers and methanogenic archaea show the specific growth rate or slow down the growth rate to less than approximately 0.07 per day. Now why do environmental engineers use such slow specific growth rates or processes those are relying on such slow specific growth rate having bacteria or archaea. One of the reasons is that there is no choice because the methanogenic archaea or the different type of bacteria and other nitrifiers they cannot grow quickly.

And also the second point or second rational for selecting such slow growing organism is the reliability and stability demand. So, in order to keep the processes of methanogen allowing methanogen to grow or in the sense producing methane out of the materials of the organic material organic waste components from a waste water treatment plants. The reliability and the stability of the entire process demands that we must rely on such organisms which are intrinsically slow growing organism.

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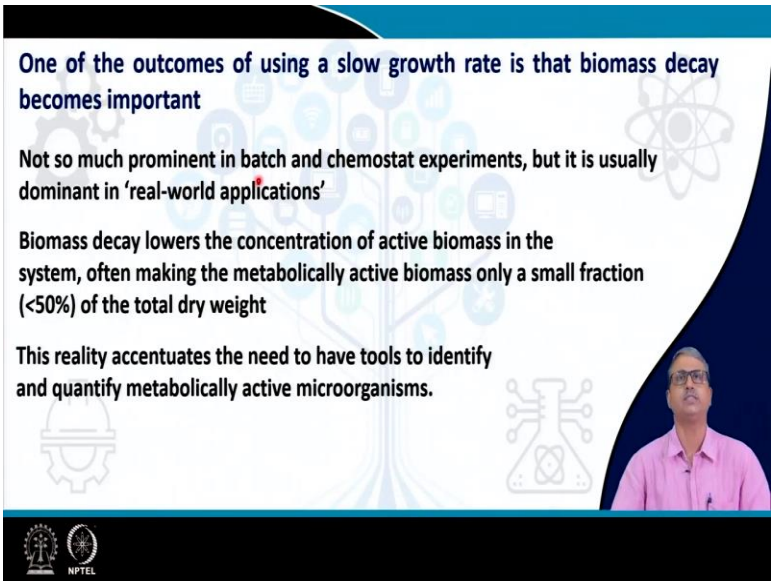

Now interestingly one of the outcomes of using a slow growth rate is that the biomass decay becomes important and not this biomass decay because the organisms are growing very slowly. This biomass decay is not so, much prominent in batch or chemostat experiment which are often carried out in the laboratory or in the control environment setup but it is dominant in real world applications where the wastewater treatment facilities are operating particularly.

Now, biomass decay lowers the concentration of active biomass in the system and often making the metabolically active biomass only a small fraction that is less than 50% of the total dry weight. Now the entire process that the metabolically active biomass is controlled to be on a on a minimal zone compared to the total dry weight of the microbial biomass present is controlled in such a manner that the overall metabolic output that is the outcome of these processes.

For example the methane which is produced from the different organic pollutants which are present in the waste water or waste stream or waste sludge is balanced with the input and the output and this reality action towards the need to have tools to identify and quantify metabolically active microorganism. So, here is the requirement for more precise method towards monitoring and controlling or sometime for other purposes also those organisms which are involved in such slow running processes like the nitrification or the methanogenesis.

So, we need to have we need to develop such tools molecular tools which can actually identify

very precisely or quantify metabolically active microorganisms. So, this is important very important to understand that we are referring to metabolically active microorganism because there could be organisms which are dead organism dead cells. So, we do not want to attribute or do not if we do not want to unnecessarily give weightage to them like when we talk about the total dry mass present in a particular community or the members of the cells which are not metabolically active.

So we should have methods which will identify quantitatively metabolically active microorganisms within such systems where the slow growing biomass is playing important role.

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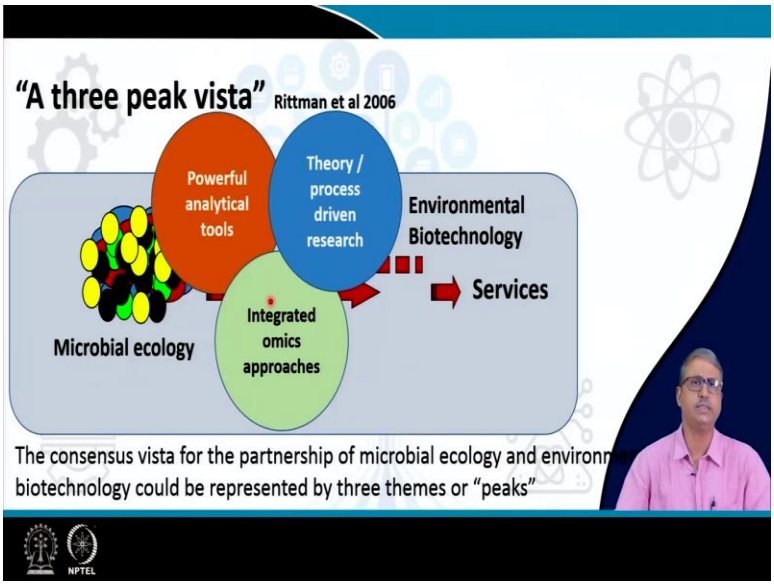

The next point is a very interesting point which is referred as the three peak vista which is originally mentioned in the review By Bruce Rittman in 2006. So, as we try to understand the partnership between the microbial ecology which is basically the science push we say it is a science push on the environmental biotechnology side which provides the pull because it is providing the services and as the services are expanding on the range of services are expanding or the demand for the services are increasing.

We need to look back we need to investigate the different type of microbial systems in order to understand them in order to facilitate their metabolic abilities to and harness those metabolic abilities to achieve the goals or the services which are on demand from metabolic form from the

environmental biotechnology side. Now the consensus vista for the partnership of this microbial ecology and environmental biotechnology could be represented or is very nicely represented by Bruce Rittman with respect to this particular aspect with three themes or peaks.

And these three peaks or these three these three themes are very well defined first and foremost is the powerful analytical tools. So, when we look at this ecology microbial ecology with respect to environmental biotechnology applications within this horizon we have a definite point of powerful analytical tools I will highlight the importance of the powerful analytical tools and in subsequent lectures we will also learn about the about this powerful analytical tools in environmental biotechnology application.

The next one is the integrated omics approaches. Now we have might have some idea about omics approaches that means the all inclusive approaches it could be genomics it could be meta genomics it could be proteomics it could be transcriptomics it could be metabolomics. So, there are many types of omics approaches. So, here we want to emphasize on the integrated omics approach not a single or any any single omics like only genomics.

It is not enough that is that is exactly what Bruce Rittman has identified and his colleagues. And the third one is very, very important which is the theory and process driven research it should not be a tool or just experiment driven research. It should not be a matter of like Rittman said there is a matter of stamp collection like we used to collect stamps different stamps of different countries different time frame stamp collection is.

It should not be the microbial molecular ecology and environmental biotechnology related tools and omics approaches their applications everything should be directed towards developing theory and processes which will eventually allow us to model and then predict the performance build the such process or engineer such process where microbial communities will successfully sustain and perform for a definite reason.

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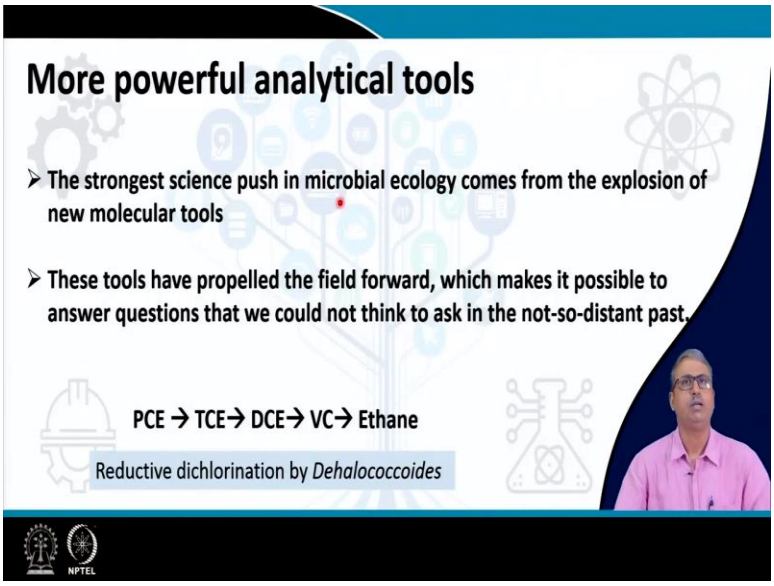

Now with respect to the first point that is the more powerful analytical tools. Now what do we mean by most powerful analytical tools. So, with respect to we are not going to emphasize or highlight or rather discuss about the analytical tools which are used to characterize the environmental as a whole like the the geochemical or the chemical parameters the physical conditions within the environment etcetera.

But rather we are trying to understand the analytical tools are trying to emphasize on the analytical tools which will connect us to this to the microbial activities with respect to a particular chemical event or sometimes more precisely a metabolic event which leads to some kind of environmental impact. For example a degradation of a particular environmental pollutant Now with respect to these in last 30 years or so, there have been consistent development with respect to different such analytical tools it is not the analytical tools with respect to chromatography and mass spectrometry of course those have been developed enormously.

But here as I mentioned we are going to discuss about such tools which will link the microbial metabolism to a particular environmental process for example a degradation of a contaminant or it could be sequestration of carbon dioxide or producing a particular biomineral of interest. Now the strongest science push because as we have learned earlier that the microbial community of microbial ecology provides the science push towards the successful achievements in the environmental biotechnology.

So, one of the strongest science push in microbial ecology comes from the explosion of the new molecular tools. These are relying on mostly the molecular biology concepts using RNA or DNA or sometimes even the proteins or other small molecules targeted tools. Now these tools have propelled the field forward of course which makes it possible to answer question that we could not think to ask in the not so, distant past.

So, if we look at 25 or 30 years 40 years ago we were we were not in a position to ask those questions which we are asking in the past 10, 15 years and we are making very significant progress in the present time as well. So, for example if we took a particular compound a tetrachloroethane for example which is a very very important ground water pollutant? Now this particular tetrachloroethane is degraded under the anaerobic condition through a process which is called reductive dechlorination.

And it has been found that there are many microorganisms which can actually degrade partly or partially this compound to up to ethane which is which is to some extent we can call it benign or it will have a very minimal impact on the on the environment particularly in the ground water system. So, reductive dechlorination by dehalococci this type of microorganism have been found to be a very very prominent one.

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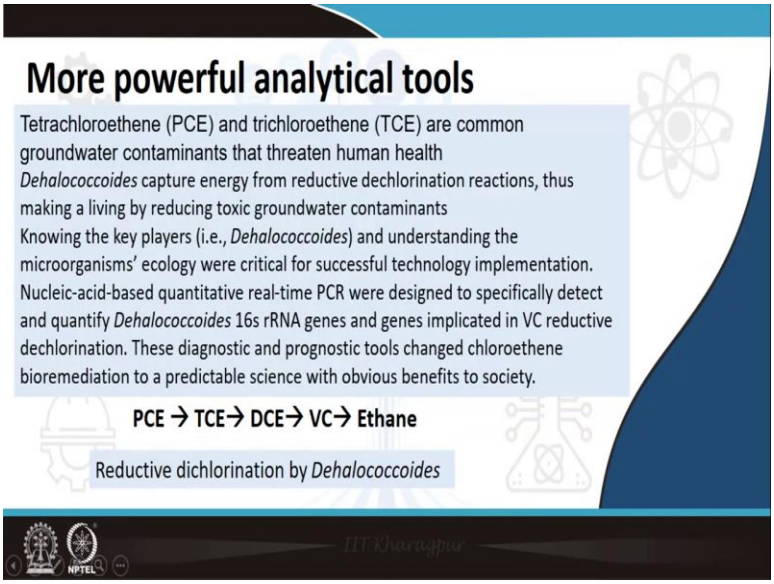

And in this context I would like to share some of the information as I mentioned that these compounds as a tetrachloroethane and its derivatives the trichloroethane which are very notorious and common ground water contaminants. And interestingly the dehalococcoid is type of microbes which are which are able to metabolize these compounds this tetrachloroethane or trichloroethane or even the dichloroethanol vinyl chloride even and they they use very, very interesting process or very important process which is called reductive dechlorination they remove one by one the the chlorine group from the the molecule and they play a key role.

So, dehalococcis types of bacteria have been considered as a key player. And understanding the microorganisms ecology were critical for successful technology implementation. So, across the United States if we see there are many studies successful implementation studies where groundwater contaminated with tetrachloroethane or similar compounds have been treated successfully remediation bioremediation was done successfully with organism like dehalococcidis.

It can be possible that intrinsically a particular com environment might not have dehalococcid. So, often dehalococcid strains which are capable of degrading PCE or TCE completely to ethane are added into those systems which are called bio augmentation. So, there is an important requirement that the nucleic acid based quantitative real time PCR based tools were designed to specifically detect and quantify dehalococcoides 16s ribosomal RNA genes and genes implicated in vinyl chloride reductive dechlorination process.

This diagnostic and prognostic tool changed the chloroethane bioremediation to a predictable science with obvious benefits to the society. So, using such quantitative tools using such quantitative stools, so, around maybe we can say that from say 15 to 18 years ago these were done very successfully. And these kind of research or development in molecular or powerful analytical tools paved the path that how developmental development of molecular analytical tools must be done in order to facilitate the translation of this knowledge of microbial ecology science into a practical application.

Because it is a predictable science now looking into the gene copy numbers of the expression

level of these dialogues we can actually predict that that how this degradation of this toxic tetrachloro ethane is progressing within a community and it has been very very successful.

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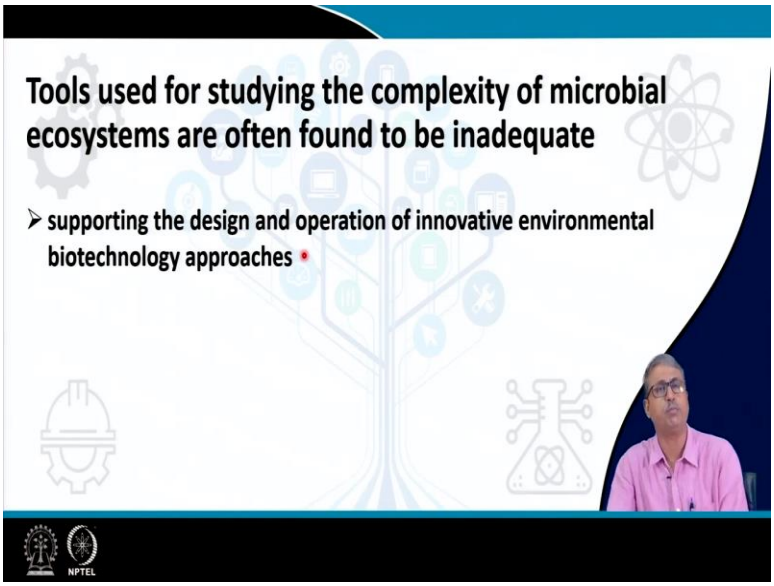

Now this story is not that that developing that such QPCR based or quantitative PCR based methods are going to be enough. Now tools used for studying the complexity of microbial ecosystem are often found to be inadequate. So, one way of saying the examples are citing the examples are good that we have we have a clear cut case of application studies that how a specific organism is involved in a particular biodegradation process.

And we are capable of developing precise methods quantitative methods to predict characterize and predict analyze the entire process of degradation process within a environmental system. On the other hand there are numerous processes which are very complex they are not driven by a single organism like dehalococcoides degrading the tetrachloroethane. So, they are much more complex. Now with respect to studying such complex systems which are often used in different environmental biotechnology processes we found the tools available to us are inadequate.

Particularly in order to support the design and operation of innovative environmental biotechnology approach for routine processes like activated sludge based waste water treatment processes. Detecting some of the major organisms or a microbially enhanced nutrient removal process some of the organisms are very well known. So, for them it might be working very well

but when you talk about some processes which could be more complex.

Then we need to rely on methods which are which are more appropriate to assess the complexity and then predict the performance of the organisms.

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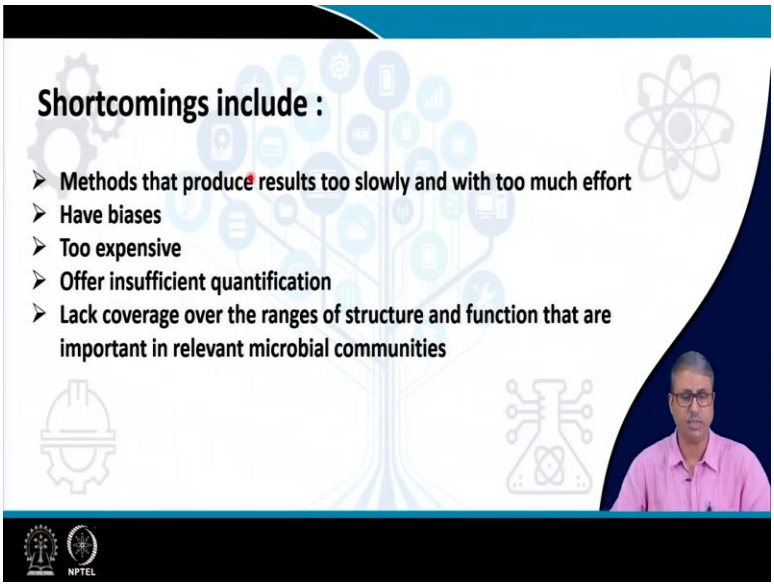

Now here are the shortcomings which are enlisted for the methods and that is why some of these methods which have been developed or being developed are identified. Number one is the method that produce results too slowly and with too much effort. So, some of the methods that are conventionally being utilized by scientists and environmental engineers are capable of producing results very slowly.

It takes week to give you the result and with too much efforts. Some of the or most of these methods they have biases. These are of course not the molecular or advanced methods these are the methods which conventionally people are using in environmental engineering system some of these methods are very expensive and offer insufficient quantification. So, you will not be able to quantify the individual processes very very particularly.

And these processes often lack coverage over the ranges of structure and function that are important in relevant microbial communities.

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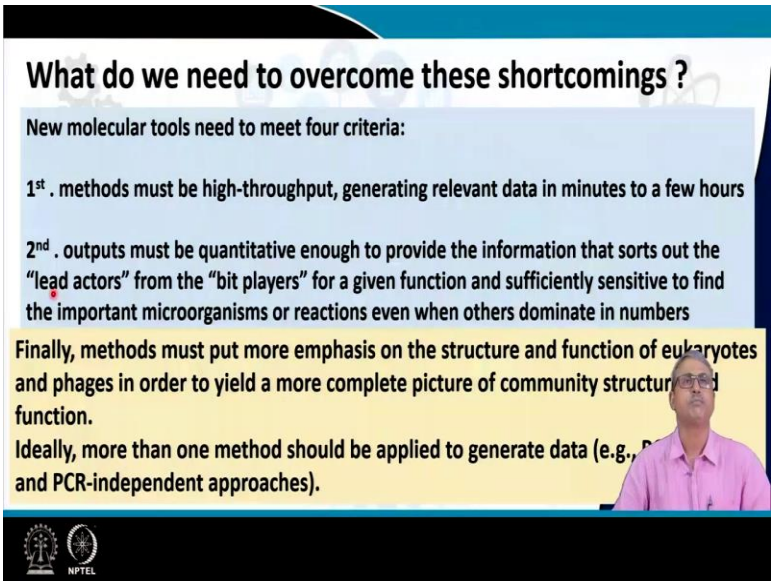

So, what do we need to do to overcome these shortcomings because shortcomings are quite well identified now. So, we should have new molecular tools in order to meet four criteria. If you are able to do that possibly then we will be able to address the shortcomings. So, number one is the methods must be high throughput. So, the slowness that it will take 7 days or. So, it is not there. So, they are high throughput they will be they will be capable of handling 10's or 100's of samples together generating relevant data in minutes to a few hours.

So, it should be high throughput handling numerous samples together and also generating the relevant and quantitative data in within several within few minutes or hours not of course days or weeks. The second is the output must be quantitative it must be quantitative enough to provide the information that shorts out the lead actors from the bit players for a given function and sufficiently sensitive to find the important microorganisms or reactions even when others dominate in numbers.

So, we will talk about this situation which is very, very important and often encountered in any kind of environmental biotechnology processes where we have trillions of cells per liter of volume. So, if you have so, many cells of minimum 10 to the power 4 or 10 to the power 5 different type of species. So, you have ten to the power 4 mean 10000 to 100000 different species. Now out of these one lakh species microbial species bacterial species present in a sample how can you find out so, easily so, precisely who are the lead actors.

Lead actor means carrying out or can be can be found responsible for a particular function from the bit players. Bit players means who are playing less important role with respect to a given function. The given function may be a degradation of a particular pollutant or as I mentioned conversion of hydrogen or acetate to methane or oxidation of methane to alcohol or may be the sequestration of carbon dioxide or degradation of petroleum or something else.

So, differentiating the lead actors from the bit players and it should be sufficiently sensitive still it is to be out of one lakh species present there that it will be able to sensitive enough to identify the important microorganisms or the reactions when others dominate in the member. If you can assume that out of 1 lakh species there could be only 20 species who are very important and playing the important role.

So, you have on the other hand so many other organisms. So, 99980 species are there who are not playing critical role you have only 20 organisms playing critical role out of one lakhs total species still you want to find out those 20 species; who are responsible for the critical function that you are looking for. So, your method should be such. Third is the output must provide the types of information that reveal structure and function parallel.

Because out of so, many species the one lakh species present for example if 20 species are found to be very response very much important and they are critical for the function that you are looking for. So, you should be able to identify their taxonomic affiliation that is the structure of those pieces as well as exactly what are they doing how they are doing it what kind of enzymatic reactions they are catalyzing because they are intimately connected.

And finally the methods must put more emphasis on the structure and function of eukaryotes and phages that is viruses in order to yield a more complete picture of the community structure and function because there will be no such community or hard to find a community where eukaryotes and viruses are not there. So, focusing entirely on microbes prokaryotic microbes like bacteria or archaea would be misleading or incomplete.

So, you need to have methods which will which will successfully delineate the role of eukaryotic organisms and the viruses. Ideally more than one method should be applied to generate the data. For example the PCR based and PCR independent approaches. Because PCR reactions as we have learned that often they are very when they are they are biased to some extent. They are dependent on the selection of the primer conditions PCR reaction conditions and the gene sequences which are used to design the primers. So, we should have methods which are PCR independent methods.

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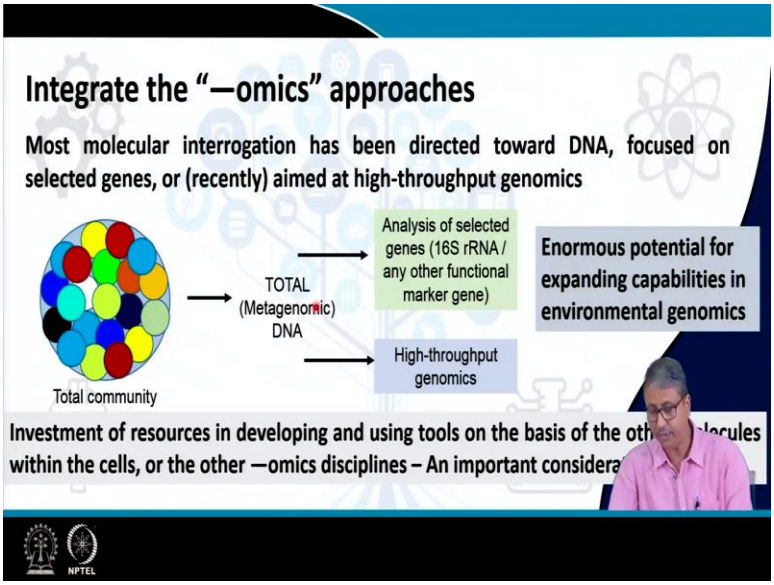

Finally we need to integrate the omics approaches. So, slowly we learn that it is not enough to have a kind of understanding or rationalizing the questions but also we have to rationalize the methods and approaches. So, most molecular interrogation has been directed towards DNA because we often use DNA as a kind of an handling molecule. So, take out the DNA from any sample and identify a target gene most often that have that has been done or sequence the entire genome or meta genome using different high throughput genomic platforms.

So, ideally we have a total community which is represented by this cluster of cells and from any activated sludge to waste water treatment to a petroleum oil contaminated site or methane producing system you can have a total DNA which is called meta genomic DNA. And then you can either analyze the selected gene that is a marker gene like 16 ribosomal RNA or 18s ribosomal RNA or its regions for bacterial or fungal analysis.

Or any kind of functional marker genes for studying the method metabolism to study the carbon fixation or carbon sequestration to nitrate reduction to other compound degradation you can have a range of functional genes and you can analyze those genes using PCR based or PCR independent methods. And finally you can also have a high throughput genomics where a large number of samples can be analyzed simultaneously.

And these analysis these approaches either a gene targeted selected gene targeted methods or high throughput omics methods they have shown enormous potential for expanding capabilities in environmental genomics. But we need to have investment of resources in developing and using tools on the basis of other molecules within the cells as well. Because relying only on DNA may not be a very good idea or may not be an idea which will provide us the comprehensive or holistic picture of the entire community function.

When we are planning to gain in the kind of a win-win situation we want to understand how microorganisms are working for themselves. So, that they can work for us if we have that type of temperament that type of attitude within our self the scientific temperament within our self then relying only on meta genomic DNA or meta genome may not be a very good idea we need to rely on other molecules within the cell that is the other omics disciplines must be brought into and that is found to be an important consideration.

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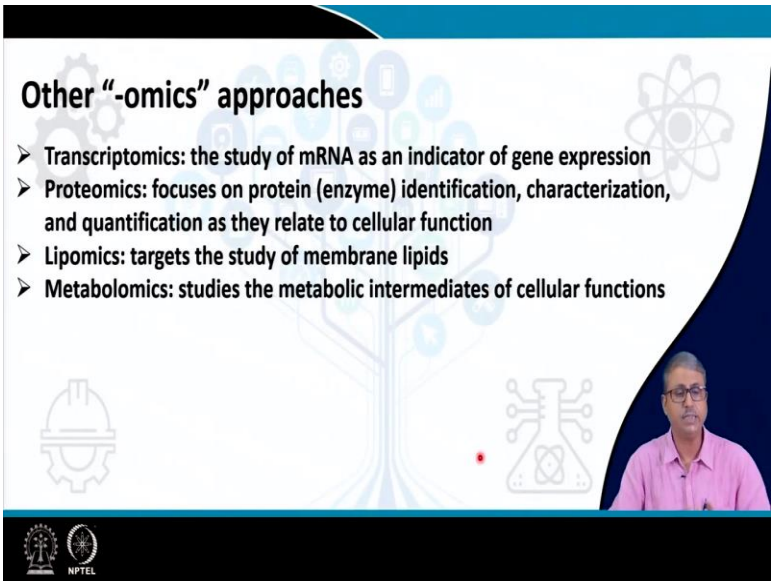

And these other omics approaches include use of the mRNA total mRNA as an indicator of the gene expression that is the transcriptomics or the proteomics which focuses on the protein or enzyme identification all the proteins present in a particular system. Their characterization quantification as they relate to cellular function, lipomix that targets the study of the membrane lipids, metabolomics that is the study of metabolic intermediates of cellular functions.

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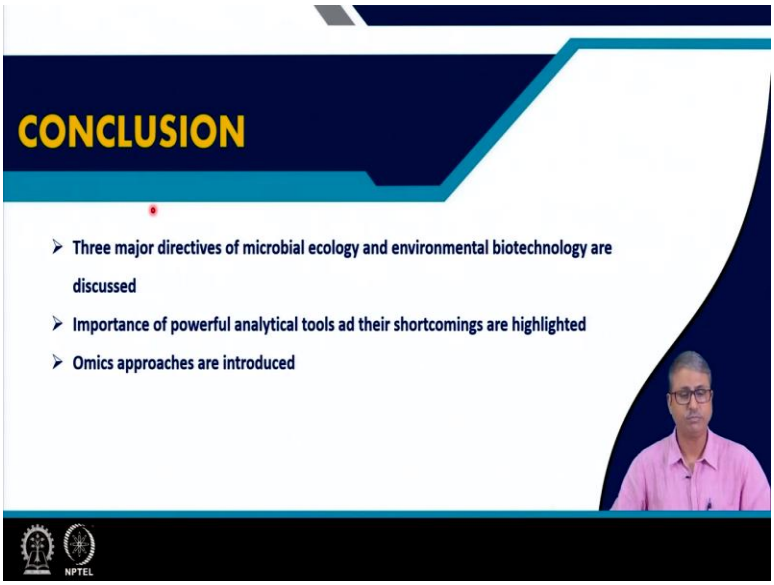

Now for this section we are using the two reviews by Bruce Rittman. And in conclusion in this section we have discussed the three major directives or the themes of microbial ecology and environmental biotechnology we have also discussed shortly about the briefly about the slow growing biomass and their importance. Importance of powerful analytical tools and the shortcomings are highlighted and omics approaches are introduced, thank you so much.