Environmental Biotechnology
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Lecture – 14
Microbial Ecology and Environmental Biotechnology - Part C (Contd.,)

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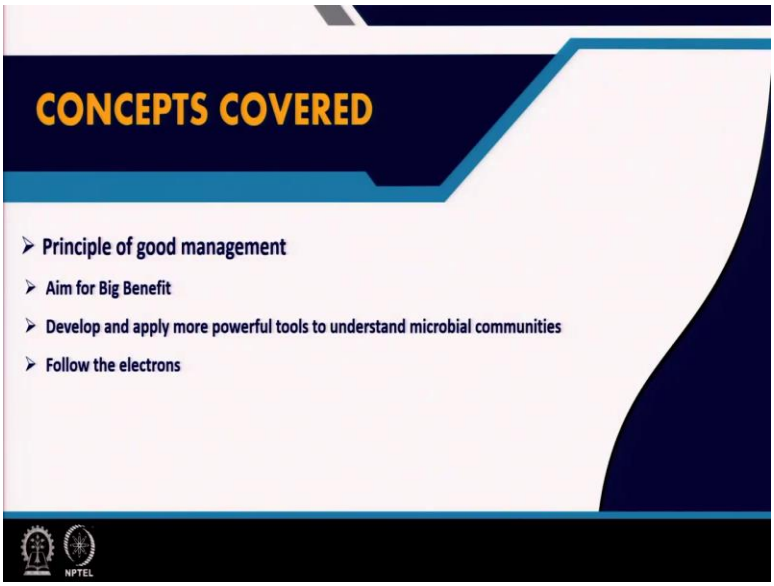

Welcome to this lecture on Microbial Ecology and Environmental Biotechnology and in this lecture the following concepts will be covered. We will discuss on the principle of good management with respect to utilizing the knowledge and understanding of microbial communities in environmental biotechnology. And with this respect we would like to emphasize on couple of points.

The first is the aim for big benefit, the second is develop and apply for more powerful tools to understand microbial communities and follow the electrons.

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Aim for the big benefit: Environmental biotechnology aims for the biggest benefits it can deliver to the society and the biggest benefits will come when environmental biotechnology is directed towards the most daunting challenges of the society that is the water and energy sustainability. These are the two areas which are identified as the also the priority areas to be to be targeted for environmental biotechnology research.

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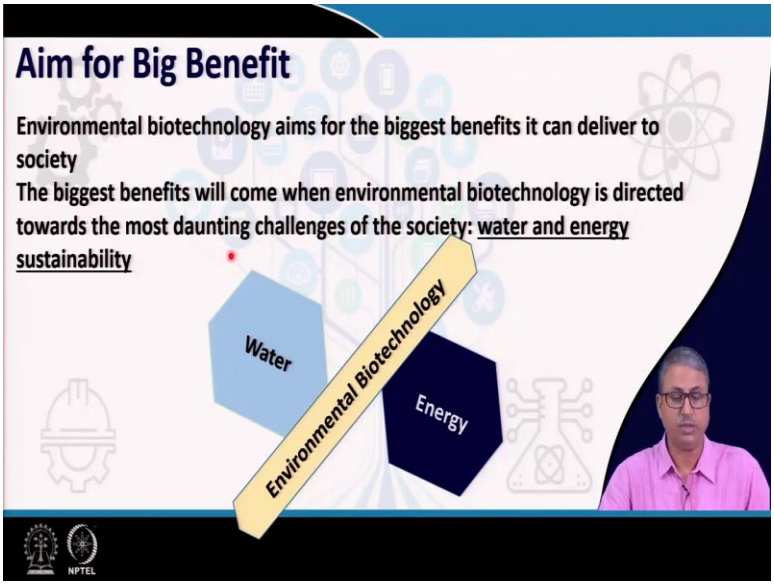

Now with this respect the big benefit is achieved when waste is conceived as a resource. Now the waste waters sludge residues and other waste of today which are actually being generated in millions of tons across the globe must be viewed as resources that can yield renewable water, renewable energy and in some cases other materials of industrial value.

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Now as we understand that these large amounts of waste which are mostly contaminating our water bodies our surface habitats are biodegradable. Most of them are biodegradable. So, with that consideration that most of the waste materials are biodegradable the developing countries are likely to have a double benefit from the application of environmental biotechnology in addressing the issue of waste management at the same time recovering the resources from the waste.

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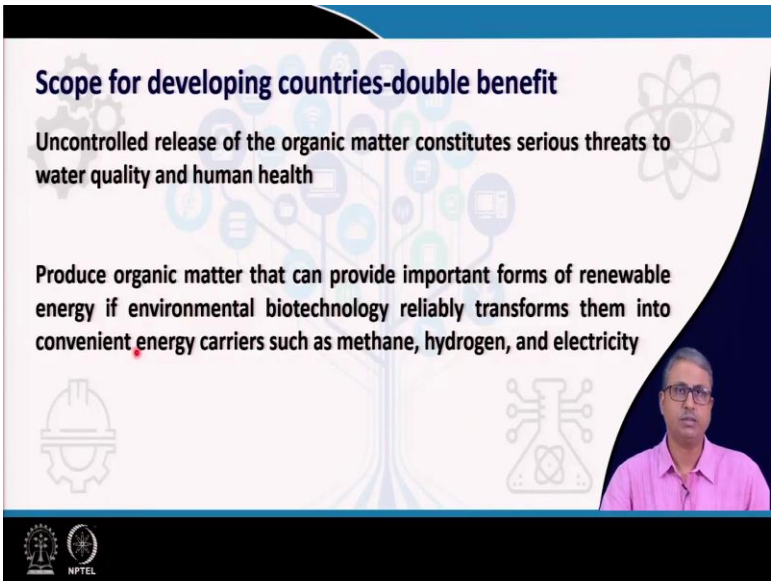

Now, the uncontrolled release of the organic matter different type of organic pollutants constitutes serious threats to water quality and human health and as I said that that is true for the entire globe. And any kind of produce organic matter which is released and he is having no control on its fate mobility within the environment it can provide important forms of renewable energy if environmental biotechnology reliably transforms them into convenient energy carriers like methane hydrogen and electricity for example there could be some more.

So, basically the concept is that the organic matter organic pollutants which are being produced and dumped and released into the environment can be utilized through environmental biotechnology to reliably transform them into different type of energy carriers that is energy carriers like methane hydrogen etcetera.

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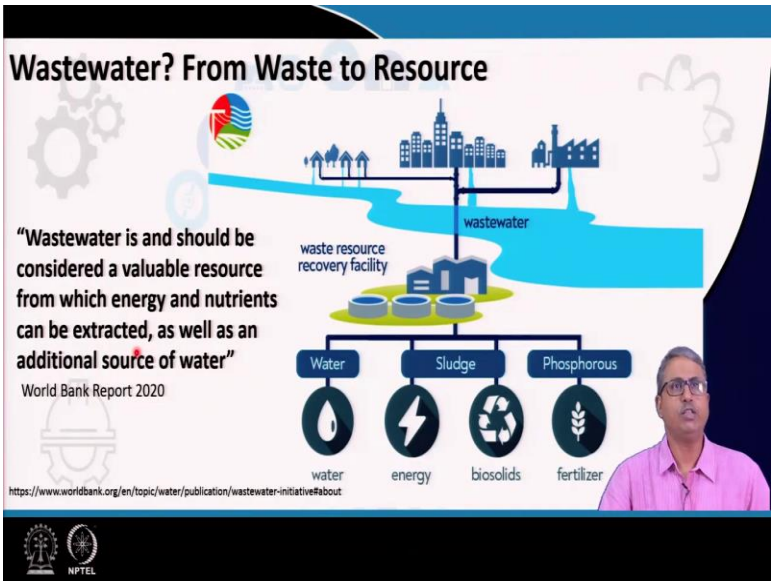

Now according to World Bank report 2020 waste water is basically is not a waste it is basically to be considered as resource. Because waste water is and should be considered a valuable resource from which energy and nutrients can be extracted as well as additional source of water. So, particularly for the developing countries it has been suggested that the waste water can be treated through various specific means and where environmental biotechnology has very specific and very important contribution to meat.

The waste water can be treated to recover the water and recycle it. Waste water can be treated and during the treatment we get the sludge and those sludge can be used to generate further energy or may be converted to different bio solids and those biosolids may be utilized for some other purposes. And also we can recover the important elements like phosphorus for example to be utilized as a fertilizer. So, this list is actually huge.

So, it is only depending on the technology that we are able to develop and implement to treat the wastewater and convert the waste materials into different type of resources. And while we try to do that while we try to aim that there is a very big role or very critical role played by the microorganisms microbial communities because it is the community that is microbial community that is going to work there.

So, the previous point that we were earlier discussing that work for them, so, that they work for

us. So, we need to understand the microorganisms when they work within a waste water treatment or in a kind of a nutrient recovery system. So, that we can make the best use from them we can recover the nutrients we can recover the energy or recover the water by utilizing the suitable microbial community function.

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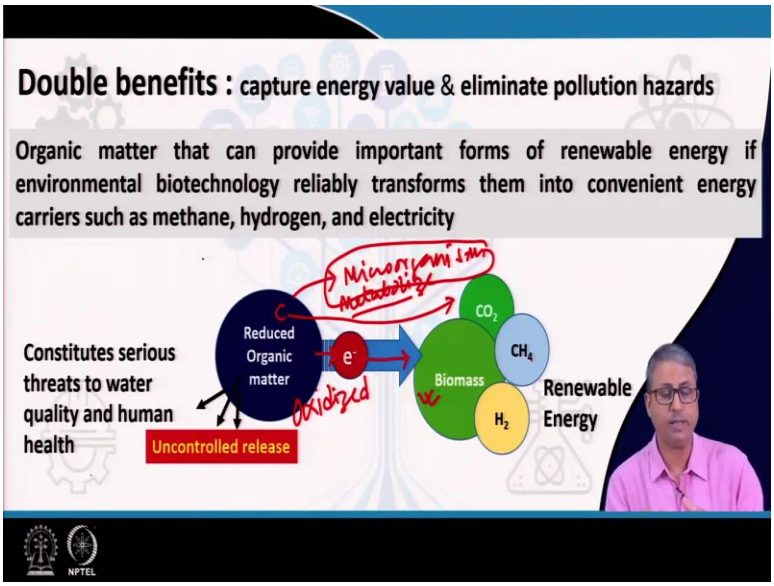

Now as it is mentioned that for the developing countries it is possibly going to offer double benefits. Because it will allow us to capture the energy value and also eliminate the pollution hazard. Now developing countries have the potential to gain a double benefit by investing on environmental biotechnologies that simultaneously capture energy value and eliminate pollution hazard. Now organic matter that; provide important form of renewable energy; if environmental biotechnology can transform them into the different energy carriers.

For example the reduced organic matter which is basically considered as a substrate for microbial metabolism. So, ideally microorganisms can be used can be developed. So, that the microorganism can utilize this particular material and here we have the microorganisms and these microorganisms will metabolize these organic matter. And the during this metabolism the electrons will be extracted from them and as the electrons are extracted because the organic matter are going to be oxidized.

So, they are going to be oxidized during this metabolism this part of the metabolism possibly you

are aware of that these are the called catabolism. So, during this oxidation process the reduced organic material are going to be oxidized and the electrons will be released. Now these electrons will flow and eventually it will allow the organisms to generate energy. Energy in terms of ATP energy in terms of reducing power like NAD pH or NAD HH plus and essentially it will allow huge growth of the cell.

So, that will allow the formation of huge biomass and the reduced organic matter like the reduced carbon like glucose for example will be oxidized to carbon dioxide. So, the carbon which is present as reduced carbon in the glucose will be converting to carbon dioxide. Now if we look at the other part of this particular process that if we are able to redirect some of this electron flow that instead of going towards the biomass formation or instead of being oxidized completely in presence of oxygen.

If we are able to channel channelize these electrons to appropriate electron acceptor or to appropriate metabolic processes we might get energy carrier like methane or hydrogen. Similarly the others other part of this process is that as we are able to develop the microbial system the microbial system which are capable of oxidizing this organic matter and converting them into methane hydrogen or other energy carrier.

As we are able to utilize more and more organic matter for that process which otherwise was basically dumped the reduced organic matter otherwise would have been dumped would have been released in an uncontrolled manner and when they were released on in a in a natural environment whether in a water bodies or in other land landfill sites etcetera they constitute serious health problems.

They constitute serious threats to the animal plant human including human health and well-being. So, this uncontrolled release can be controlled easily as we develop technologies which will be able to convert this organic matter into some electron carriers there may be some biomass produced but methane hydrogen and other electricity kind of things which will allow us to generate huge amount of energy from them.

So, it is going to be a double benefit because in the one way we will gain the energy or energy carriers on the other way we will reduce the uncontrolled release and that uncontrolled release otherwise would have be responsible for deterioration of the environment and also impacting human and animal and plant health very severely.

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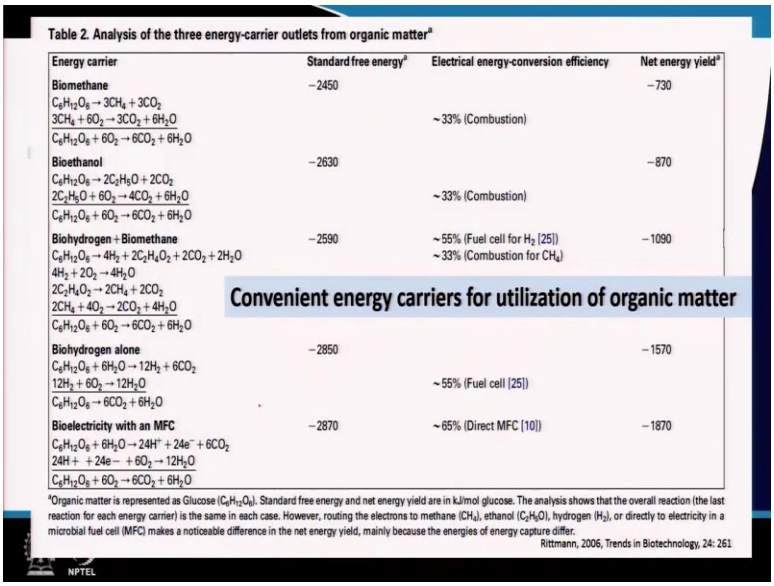

So, convenient energy carriers for utilization of organic matter so, if we see that while utilizing the organic matter through different microbial metabolism. So, a number of energy carrier are possible that that basically includes the bio methane to bio ethanol and bio hydrogen, biomethane mix or bio hydrogen alone and bio electricity through microbial fuel cell. So, there are there are extensive research and there are huge opportunities are being created in this respect.

That how the waste material like the organic waste in particular can be treated can be converted through microbial systems microbial processes where microbial communities are actually involved in most of the cases.

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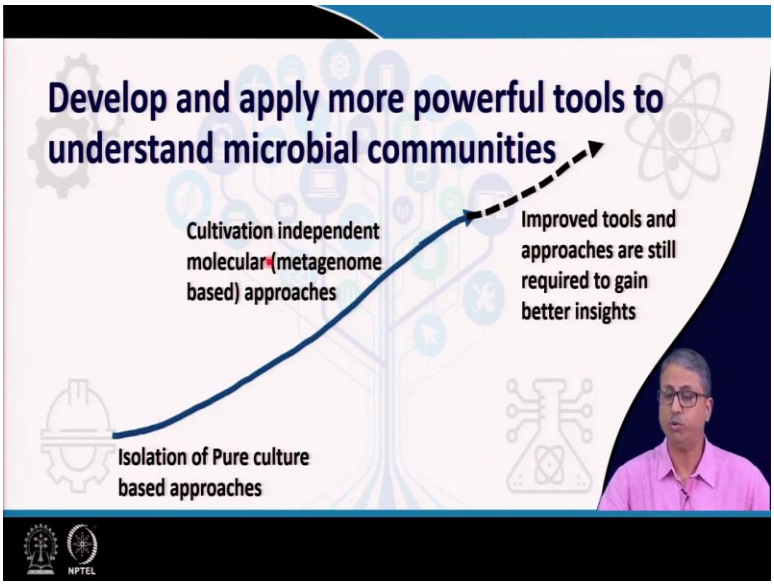

The second point is develop and apply more powerful tools to understand the microbial communities. This is very, very challenging and very important also. Now when it comes to understanding the microbial communities we have been discussing about microbial communities and their how we study them to some extent like we talked about the self assembly etcetera. But we l if we look at the different type of methods which are which are being conventionally used in environmental processes or environmental related processes.

It has been based on different isolation of bacteria which we called or cultivable approach based processes for a long period of time. So, we generally try to isolate the bacterial culture and try to grow them and then we try to investigate their property. So, in almost all cases what we find that we try to basically grow a couple of bacterial cell from any kind of wastewater.

So, suppose we have some waste material waste water if we have some then from this waste water we can have a few bacteria and these bacteria can be tested in terms of their suitability with respect to any environmental processes. Now that practice has been has been changed in last of last few decades because what we have learnt that most of the organisms present in any kind of environment.

So, including wastewater are not cultivable not cultivable what does that mean? That means that if we take the water or the waste and try to isolate bacteria from that out of 100 bacteria present

in the waste if we assume that there are 100 bacterial cells present only one or even sometimes less than one bacteria will grow on laboratory condition. This we have learnt that many organisms are not cultivable that means ninety nine percent organisms are not cultivable.

So, 99% nearly near it may vary to some extent. So, 99% of the bacterial species or bacterial members present organisms present in any kind of environmental sample are not cultivable. So, our efforts to understand these and our efforts to address these issues enabled us to develop methods to some extent and we have we have developed the scientists were able to develop what you called cultivation independent molecular method.

So, where in cultivation independent molecular method we are not we are not using any kind of we are not using any kind of laboratory based culturing method rather what we are trying to do we are trying to use the meta genome which is basically represent in the entire nucleic acid pool and this meta genome what you call meta genome the term the meta genome is coined in order to represent the entirety of the system.

So, basically from the water we extract the meta genome. So, that is collection of all genomes all genome means all species and all species genomic DNA that is to together it is called the meta genome, so, in a very simple term. So, collections of all genomes are obtained when we take kind of an approach where the total nucleic acid is extracted from the sample. So, from a wastewater or a system of environmental relevance we extract the meta genome and then a number of cultivation independent strategies are used.

So, we are not going to discuss these cultivation independent molecular strategies over here but may talk about them at some other point of time. Now, another important aspect is that once we started utilizing this cultivation independent method we have a kind of what is called as a paradigm shift. Paradigm shift in the sense that you say that you know that now that only 1% or less than 1% organism was or were actually cultivable.

So, all our knowledge based on how these organisms actually function with reference to a particular environmental setup particular function was based on few cultivable bacteria which we

were able to isolate. But as soon as we realized that no there could be improved math methods and utilizing those improved method we can actually look into the total microbial community as much as it is possible.

So, we made the great advancement with respect to that. So, we will talk about those advancement and how these things are actually done in some other lectures. But what is realized that we need to have more powerful tools because even after knowing some of the species member or many of the species member which are present in a given environment given environmental setup and with having some idea about how these organisms could be connected to a specific environmental function it is not yet complete.

Because we need to win that we as we said is a win-win technology we know in technology in the sense we need to understand completely the microbial process operating in a particular environment. Maybe its waste water treatment or maybe it is a removal of a particular pollutant or generation of energy or nutrient elements from something else. So, we need to have more improved tools and approaches that still those are required to gain a better insights.

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Name	Target	Goals
Hybridization after RNA extraction based	rRNA	Phylogenetic identity of individual strains or coherent groups
FISH (Fluorescence in situ hybridization)	rRNA	Phylogenetic identity and spatial relationships of individual strains or coherent groups
DGGE	Specific Gene	Fingerprint of community structure (cDNA gene) or phenotypic potential (other genes)
Cloning and sequencing	Specific Gene	Catalog of phenotypic potential for a certain type of gene
Quantitative PCR	Specific Gene	Quantification of community structure (rDNA gene) or [26] phenotypic potential (other genes)
Reverse transcription PCR	Specific Gene	Community function according to expression of a target gene
Metagenome based tools	Total DNA & RNA	Phylogenetic identity of entire community and genomic details

Now these are the some of the methods that already been developed in last decades or so following the discovery of ribosomal RNA molecule as a molecular marker for microbial phylogeny and microbial identification. And also having the DNA based methods where or RNA

based on nucleic acid based methods where the cultivation approach can be totally bypassed and we can straight away head to the question that what are the organisms present what are their functions and how the functions are actually realized.

The fundamental questions that we initially asked with respect to microbial community. Now for example the hybridization with after the DNA RNA extraction particularly targeting different ribosomal RNA taxonomic, group specific ribosomal RNA can provide us information on phylogenetic identity of individual strain or coherent groups. Now for example if you are interested on phototrophic microorganisms with respect to carbon dioxide fixation process.

So, if we know that carbon dioxide fixation is driven by certain type of organisms. So, if we are able to make the probes small oligonucleotide fluorescently leveled molecules which will be useful in hybridization process and then following an RNA extraction we can hybridize with those selected probes which are specific to the autotrophic bacteria for example we will be able to find out that whether these autotrophic bacteria are present they are not.

And by using different types of probes which are targeted towards different taxonomic or phylogenetic groups we will be able to actually decipher the different autotrophic population for example. Similarly fluorescent in situ hybridization is another technique by which the spatial relationship. Because under the microscope we will be able to see that if we have a sludge sample if we have a biofilm sample if we have some other kind of environmental sample where we want to see exactly what type of organisms are located where?

It is to some extent useful for having this self assembly structure as well to some extent not completely but we will be able to have some idea about who is closer to whom because if we have different type of fluorescent probes for different taxonomic group. Or phylogenetic group we will be able to find out under the microscope because here the sample will be treated directly or seen directly under the microscope.

Denaturing gradient gel electrophoresis is one of the methods which was very popular in with respect to the finger printing of the community. And we can use any kind of specific gene

taxonomic marker gene or functional gene and can assess the nature of the community in terms of a kind of a finger printing or we can also assess the phenotypic potential and the variation within the functional genes that indirectly will give us some input about the phenotypic potential.

Because the variation of the finger printing or the variation of the DGG result will help us to understand how the phenotypic potential is varying particularly if we have a treatment different treatments are given to a community to understand under which treatment condition what type of changes are happening in a particular system. Cloning and sequencing of particular gene quantitative PCR will provide the quantization of the community structure community major member.

Reverse transcription picture will provide us the community function according to the expression of a targeting targeted gene and finally the meta genome based tools that we which we just briefly mentioned in our earlier slide that the total nucleic acid based technique where the it is not a principally a PCR may not be a PCR based technique always and which will provide us the phylogenetic identity of the entire community and will also enable us to get the genomic details.

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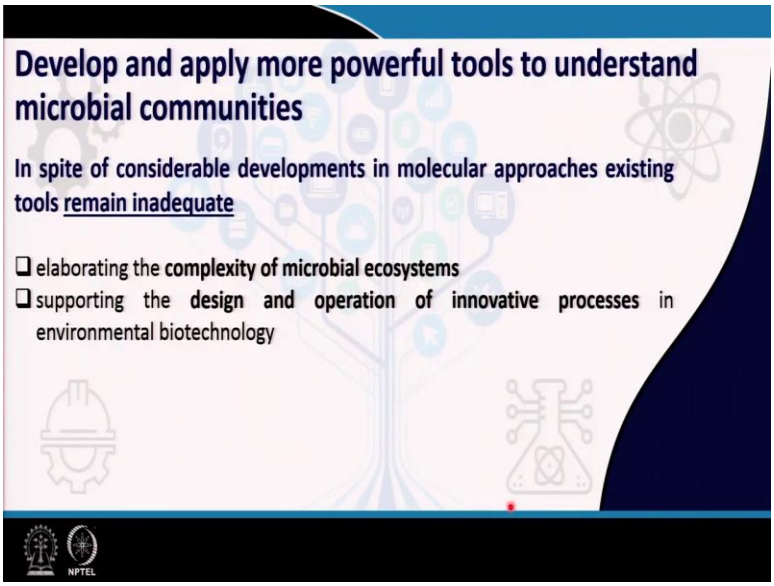

Now as we continue discussing the particular sub topic on develop and apply more powerful tools to understand microbial communities. We need to understand that in spite of considerable developments in molecular approaches the existing tools remain inadequate still it is not

sufficient. Elaborating the complexity of microbial ecosystems is a very big challenge because it is not only the fact that we identify how many species are there and what is the relative abundance of how many species each of the species.

But the actual real complexity the assembly process the clustering the interactions the antagonistic interaction and the synergistic interactions all need to be resolved and with respect to the different type of nutrients different type of changing environmental conditions so, that we can control them and also when you talk about supporting the design and operation of innovative process in environmental biotechnology. So, the existing tools are found to be highly inefficient.

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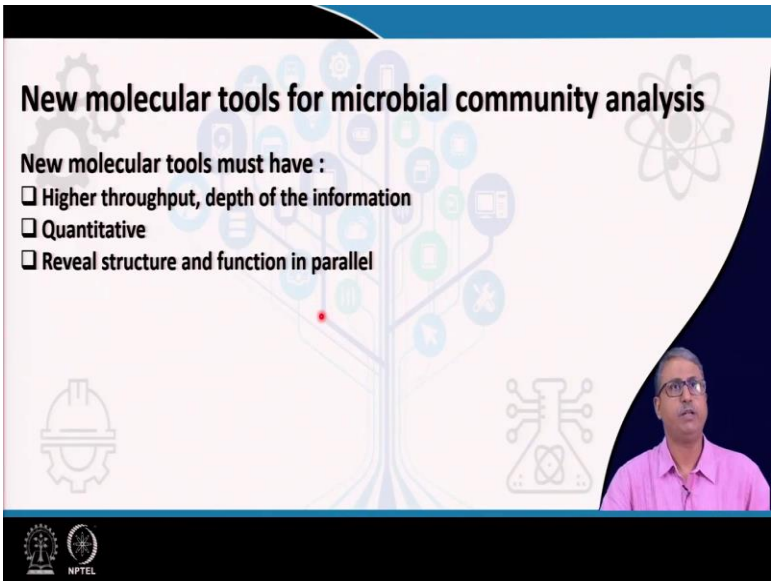

Now new molecular tools for microbial community analysis must have higher throughput. So, that it is it is going to analyze a large number of sample at the same time and also the depth of the information. Because many species will be there and some of the species will be relatively less abundant than the other and it must be quantitative and it should reveal the structure and function parallel.

Because we need to know who is there and at the same time the quantitative evaluation about that and also their function that what type of functions are being carried out by them. So, these are again connected to the four basic questions that we originally asked but it has to be more quantitative with more throughput and with depth of information.

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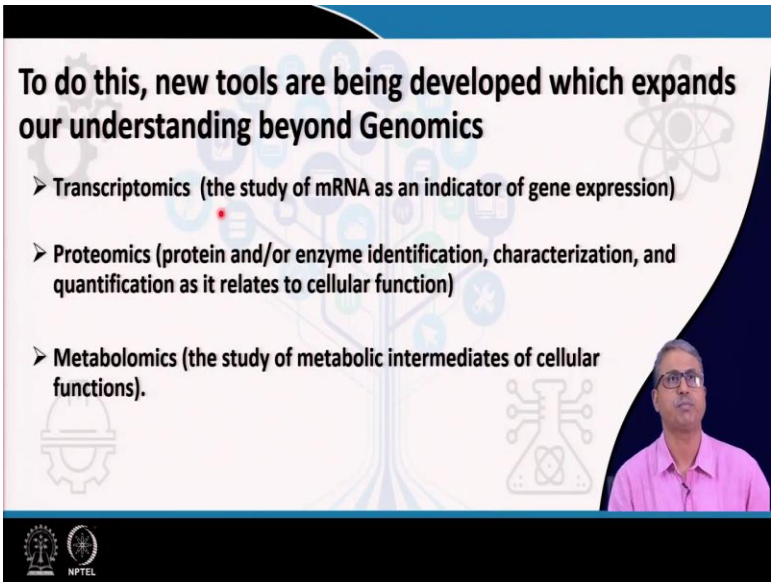

Now to do this new tools are being developed which expands our understanding beyond genomics. So, during our journey from typical laboratory isolation based pure culture based methods to cultivation independent method we had the in invention of the whole genome sequencing technique for microbes and a number of bacterial and alkyl and fungal strains are being sequenced.

And we came to know a lot from those genome sequences and this is a part of a genomics. But genomics is done only based on the organisms which are mostly isolated as pure culture although there are techniques where organisms can be sequenced in terms of their whole genome without culturing them always but those are not regularly available or regularly used in environmental biotechnology setup because of the of the of the lack of the knowledge or maybe access to the technology.

So, the next one was the meta genomics where entire population entire community members all the members of the community were subjected to the analysis that is called the meta genomics using the total DNA we are able to do this and we are able to answer the four questions which are related to the microbial community function. Now beyond meta genomics so, we also have the next couple of points which are basically the meta transcriptomic and metaproteomics and essentially the meta metabo metabolomics.

So, with respect to these so, I will write here, so, we have the meta genomics which will take care about the total community top total community and will answer the question who are they kind of question in the first question that what type of organisms are there. But at the same time transcriptomics will allow us to access the kind of gene expression what type of genes are being expressed over there.

So, we will be able to know what type of genes are being expressed and that will help us essentially to answer the question that what is the actual function being carried out by the organisms because from the kind of genes which are being expressed if we study them and under different conditions that will be immensely useful. The studies of the proteomics which will allow us to identify different proteins, enzymes, characterize them and quantify them and relate them to the different cellular function.

And finally the metabolomics where the; study of the metabolic intermediates or cellular functions can be investigated.

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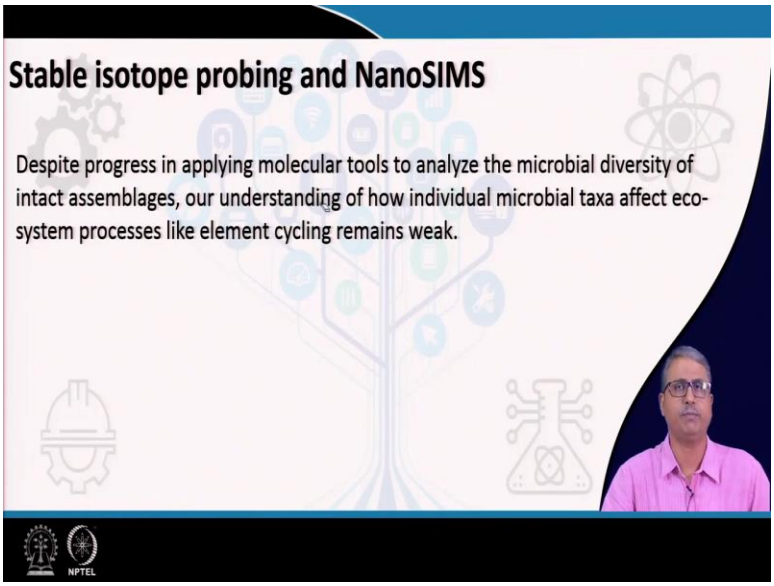

Now in recent years or recent decade we will see I will say that some more advancement. Some very specific an interesting improvements or scientific methods have been developed. Two of them are stable isotope probing and nanosims. So despite progress in applying the molecular

tools like meta genome or meta transcriptome to analyze the microbial diversity of intact assemblage our understanding of how individual microbial taxa affect ecosystem processes like element cycling remain very weak.

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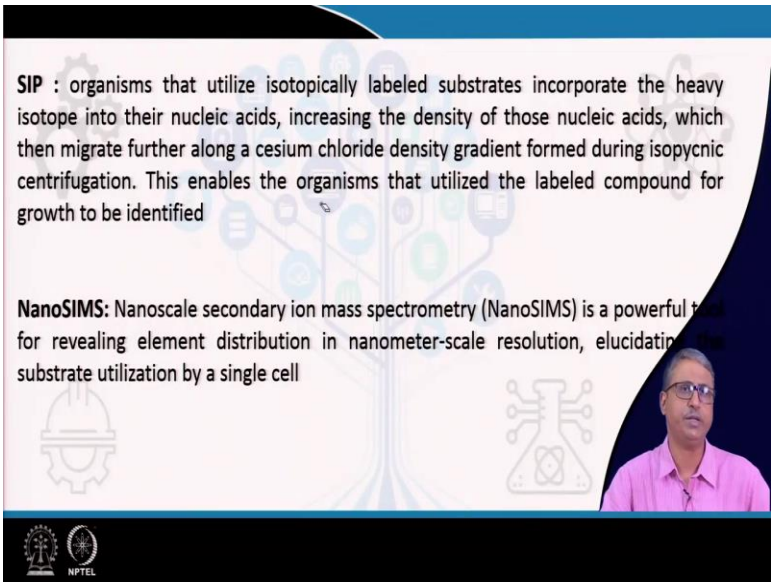

So, stable isotope probing which basically allow the in incorporation of stable isotopes of a specific substrate which is under investigation and the community is fed with that substrate. And later we as the substrate is utilized by the by the community members some community members will be more active towards utilizing that substrate and some may not be. So, some cells will be preferentially loaded with heavier isotope and when you do a DNA structure and a cesium chloride density gradient centrifugation will be able to separate out the heavier fraction of the DNA the g meta genomic fraction.

And when we sequence and analyze that heavier fraction of the meta genome we would be able to identify what are the organisms who are the organisms who are mainly responsible for utilizing the particular substrate. The other one is the nanoSims where nano scale secondary ion mass spectrometry is used which is a powerful tool to for revealing the element distribution in nanometer scale resolution and elucidating the substrate utilization by a particular cell.

So, even a particular cell level in only stable isotope probing we are unable to define whether a particular cell is doing that it will come as total community DNA which is heavy DNA but in

case of a nanoSims we will be able to attribute that whether a particular single cell is performing in terms of utilizing a particular subset which is again of course level with the heavier isotope.

And if we couple this with fluorescent institute hybridization or some other method we will be able to answer even more questions with respect to that.

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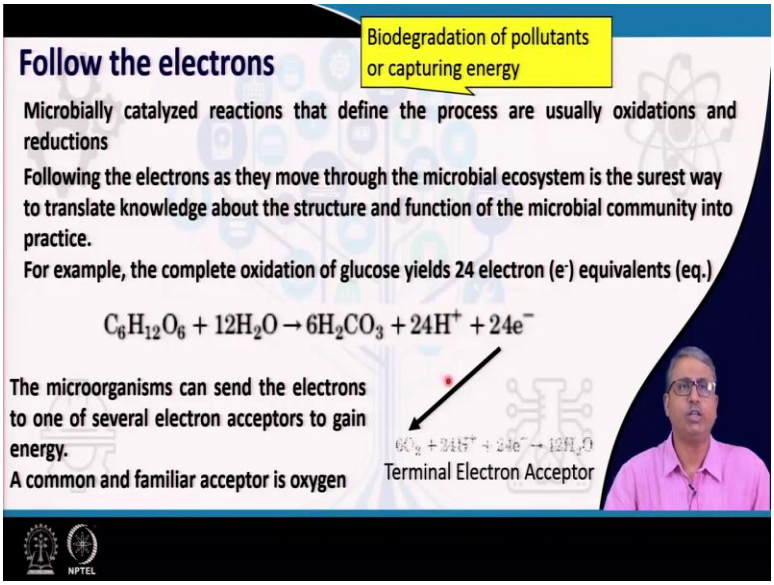

Now the next important point is follow the electrons. Now the microbially catalyzed reactions that define the process are usually oxidations and reactions and as I mentioned earlier when organic compounds are available for microbial metabolism microorganisms oxidize that substance or substances and the electrons are released. These electrons are transferred to electron acceptors if oxygen is there under aerobic condition these electrons will go to the oxygen.

And it will create the proton motive force that will electrochemical gradient and that gradient will allow the cells to generate ATP and also some form of other form of energy during the course of the metabolism which are the reducing equivalent. Now these reactions are basically oxidation reaction and reduction reaction. On the one hand the substrate is oxidized like the glucose or the organic matter is oxidized and on the other hand the electron acceptors are going to be reduced or reduced.

Now often with respect to bio degradation of pollutants or capturing energy we often found that

these oxidation reaction reactions are very, very popular very, very common. Now following the electrons as they move through the microbial ecosystem is the surest way to translate knowledge about the structure and function of the community into practice because it may not be possible or may not be happening that all the community members all these species involved or present in a particular community are involved in the oxidation process. It might be possible that out of 1000 species members only a handful like 50 or 100 or 200 species are actually involved with the oxidation process rest may be involved in some other processes.

So, how the electron is flowing through the community members would be a very important step to translate the knowledge of ecosystem function into a kind of a practice. For example the complete oxidation of glucose yield 24 electrons equivalence and as you can see that electrons are produced during this oxidation. Now the microorganisms who are involved in this oxidation process can send the electrons to one of the several electron acceptors to gain the energy.

And it is known that under the aerobic condition oxygen is going to be the preferred electron acceptor. So, the electrons will be sent to or accepted by the terminal electron acceptor oxygen under anaerobic condition. Whereas under anaerobic condition when we see that anaerobic respiration is happening alternate electron acceptors might accept the electrons and it is it is a very regular is very common event.

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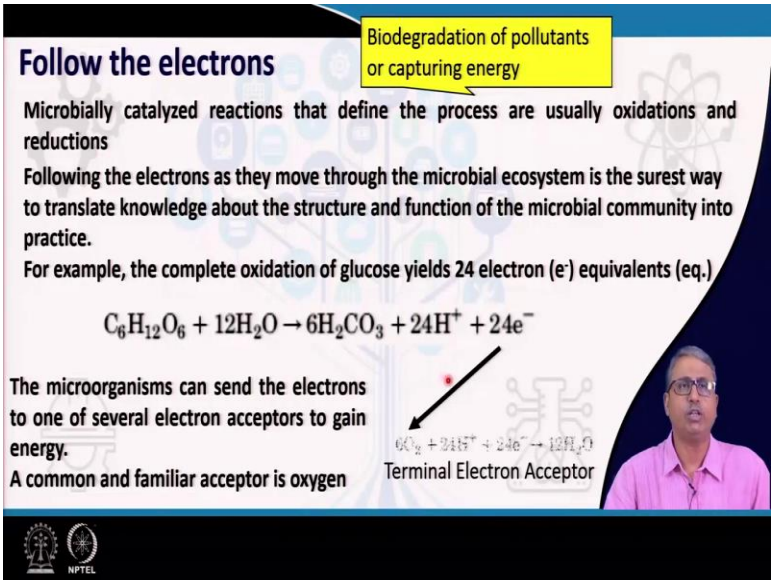

Now the overall reaction would be the sum of the electron donating and electron accepting half reactions which will yield around minus 120 kilo joule of energy which the microorganism can use for synthesis or other energy consuming reactions. So, there will be a huge amount of energy which will be produced that the microorganism will essentially use.

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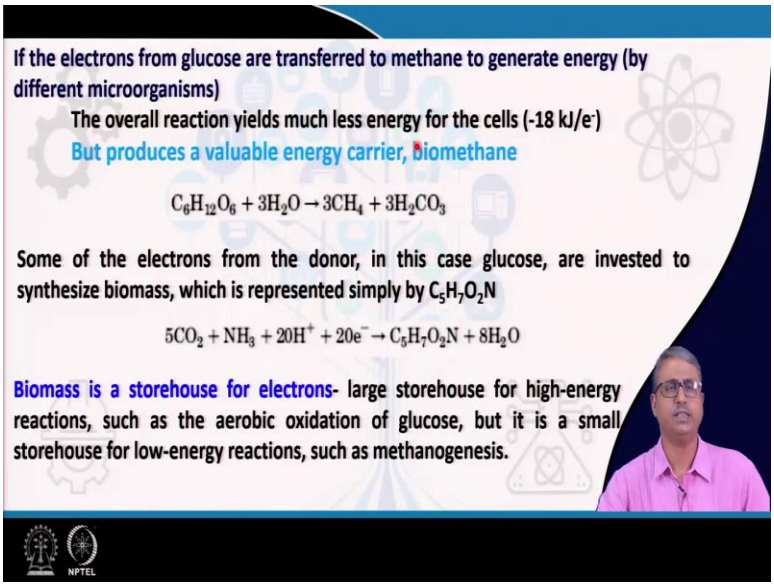

Now if the electrons from the glucose or the reduced carbon compound which is being oxidized are transferred to methane for example. If oxygen is not there for example and carbon dioxide is actually reduced to attack the electrons essentially the methane is going to be produced. And if the electrons are dumped on carbon dioxide to produce the methane and generate energy by different microorganism the overall reaction will yield much less energy.

Compared to the previous case where the electrons are being transferred to oxygen where we see that it is around minus 120 kilo joule per electron equivalent here it is only minus 18 kilo joule per electron equivalent. But this will going to produce a very valuable energy carrier that is biomethane. So, the energy yield might be less for the cells but for us for the environmental biotechnologist we will have the methane which is going to be produced in those cultures where these micro some may be some different microorganisms surely because the aerobic microorganisms are not going to do this.

This is a different set of microorganisms. So this is again the allocations of functions within a

community you will have some members who are going to oxidize the carbon and there are certain other organisms who could be utilizing the electrons and producing methane out of it. Now some of the electrons from the donor in this case glucose are invested to synthesize biomass as well. And so, essentially compared to the previous case where oxygen is available some biomass is going to be produced.

But the amount of the biomass produced will be even lesser than the aerobic aerobically produced biomass. Now nevertheless this biomass which is is produced under this anaerobic condition where methane is produced is a storehouse for electrons. Because it is a large storehouse for high energy reactions such as the aerobic oxidation of glucose but it is a small store house for the low energy reaction such as methanogenesis. But eventually some biomass are also produced.

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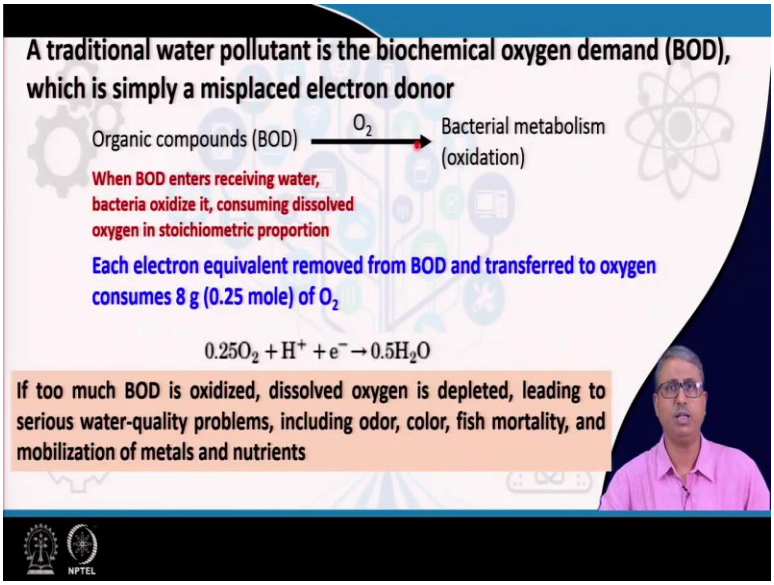

Now if we take the example of BOD which is a very traditional water pollutant. In any wastewater or polluted water bodies we will find organic pollutants are there and we consider BOD biological oxygen demand as simply a misplaced electron donor why we say so? Because organic compounds which are basically responsible for this oxygen demand when it is it is also referred as BOD. So, when this organic matter or biologically oxidizable organic matter enters the receiving water bodies bacteria they say aerobic heterotrophic bacteria they oxidize this.

And when they oxidize it they release the electrons and these electrons are finally dumped on the oxygen because oxygen is going to be the electron acceptor for them. Now that consumes the entire set of dissolved oxygen whatever available in stoichiometric proportion more amount of organic matter is available bacteria act on that and huge amount more the amount of oxygen will be depleted and that will lead to basically bacterial growth.

So, what will happen each electron equivalent removed from BOD and transfer to oxygen consumes eight gram of oxygen. So, oxygen level depletes very, very quickly and this depletion of oxygen level if it is too much depletion of oxygen level then it will lead to serious water quality problems including odour, colour, fish mortality and mobilization of metals and nutrients. And it will change the entire microbial or even macro ecological landscape of a particular water body because the dissolved oxygen is very important.

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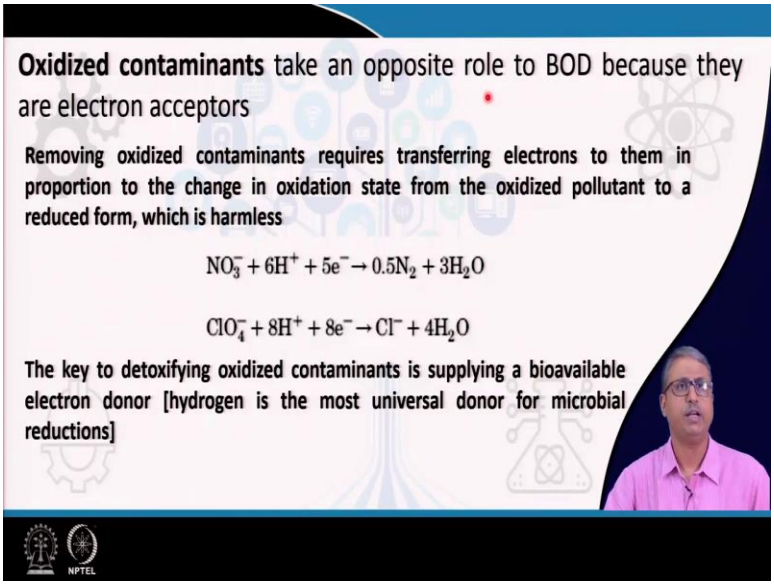

Now oxidized contaminant whatever if present within this ecosystem or this contaminated water take an opposite role to BOD because they are electron acceptor like nitrate or may be the chlorate or may be iron or uranium or chromium, hexavalent chromium these could actually accept the electrons. So removing oxidized contaminants requires the transferring electron to them in proportion to the change in oxidation state from the oxidized pollutant to a reduced form which is harmless.

So, basically nitrate if we can transfer the electron to nitrate instead of oxygen it will be converting nitrate to nitrogen or we can we can manage actually the problem of with the chloride pollution also. Now, the key to detoxifying the oxidized contaminant, so, one way we are trying to manage the BOD and the other way we are also able to achieve the controlling the other pollutants which are basically oxidized pollutant like nitrate for example.

So, now the key to detoxifying the oxidized contaminant is supplying a bioavailable electron donor like hydrogen which is in most of the universal donor for microbial reductions.

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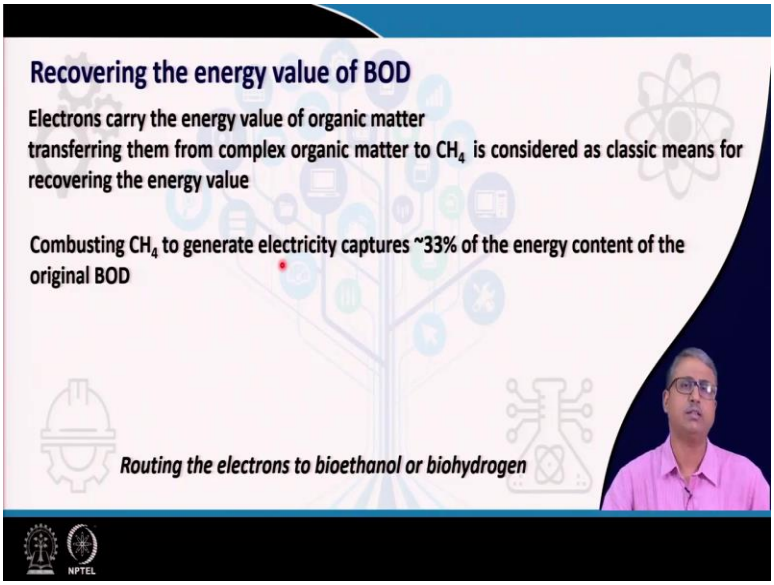

Now recovering the energy value of BOD: Now electrons carry the energy value of organic matter and transferring them to complex organic matter like to methane is considered as classic means for recovering the energy value and the combusting the combat combustion of this methane would generate electricity and that will possibly capture around 33% of the energy content of the original BOD.

Now this can be further elaborated when we route the electrons to not to methane but to bioethanol or bio hydrogen or certain other other products certain other electron carriers that can be discussed in subsequent classes.

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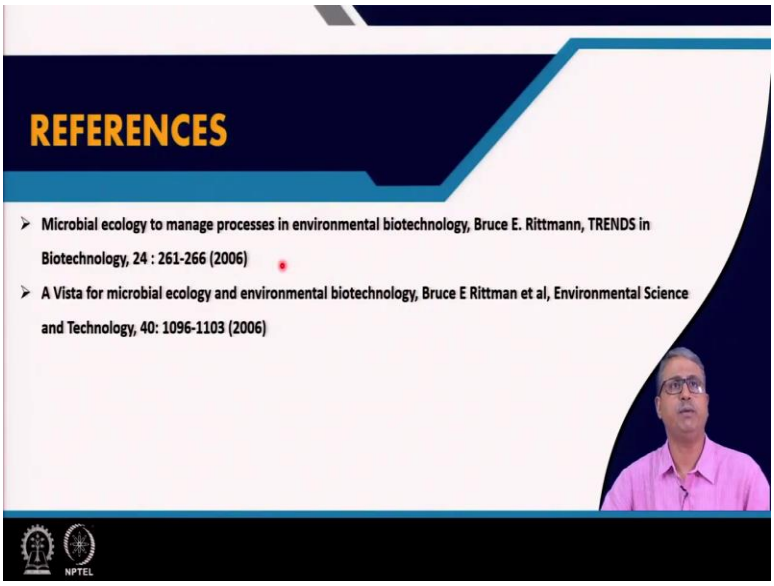

So, for this we have we can follow these two literature Microbial Ecology to Manage Processes in Environmental Biotechnology by Bruce Rittman and A Vista of Microbial Ecology and Environmental Biotechnology by Bruce Rittman.

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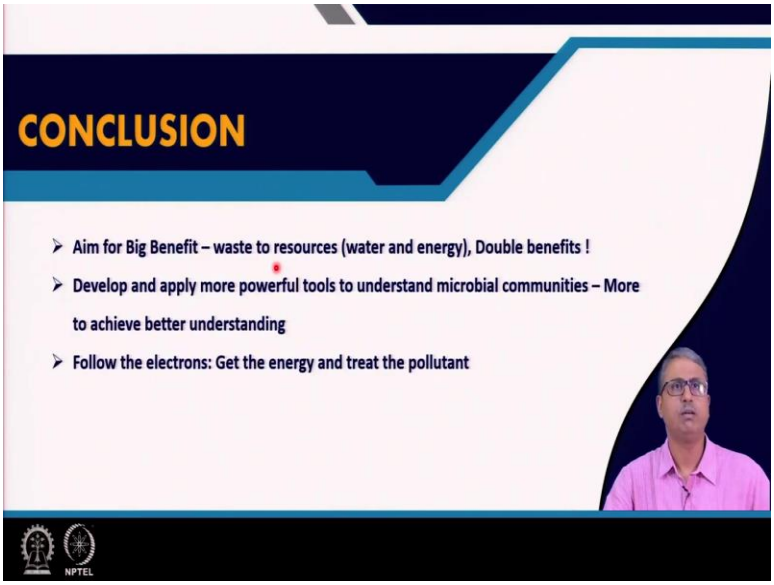

And to conclude the aim for big benefit waste to resources like targeting the major aspects of the recovery from the waste material sustainable water and energy management is discussed. And it is it is also highlighted that this could be a double benefit for many countries including the particularly the developing countries. There is a need to develop and apply more powerful tools to understand microbial communities more to achieve better understanding.

Because better we understand the communities better will be their application and we also discuss the point follow the electrons where get the energy and treat the pollutant. So, how this can be translated it is a big point of interest with this, thank you.