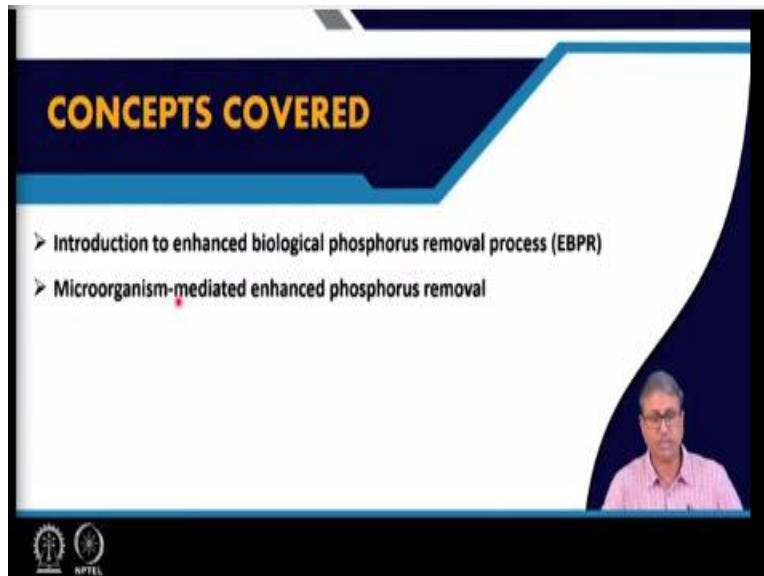


Environmental Biotechnology
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Department of Biotechnology
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Lecture-45
Enhanced Biological Phosphorus Removal Process (EBPR)

Welcome to the next lecture of our course on environmental biotechnology. And in this lecture number 45, we are going to discuss about the enhanced biological phosphorus removal process or EBPR.

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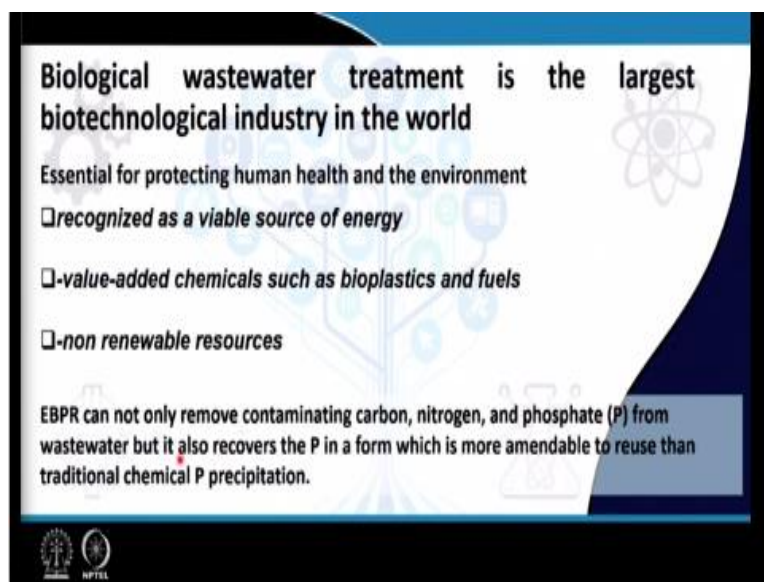
In this particular lecture we will have an introduction to this EBPR process that is the enhanced biological phosphorus removal. And we will also learn about how microorganisms they facilitate these enhanced phosphorus removal. And of course we will also deal with the importance of this phosphorus removal etcetera.

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Now enhanced biological phosphorus removal process or EBPR is considered to be one of the most modern wastewater treatment configurations.

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And in general biological wastewater treatment is considered to be the largest biotechnological industry in the world. And this biological waste treatment processes, these are essential for protecting human health and also the environment. And these waste treatment processes using diverse array of microorganisms are recognized as a viable source of energy because through these waste treatment processes a large number of energy generation opportunities are provided.

It also acts as a source of value added chemicals such as bioplastics and biofuel molecules and also considered to be a source of non-renewable resources. Now EBPR that is enhanced biological phosphorus removal can only remove the contaminating carbon, nitrogen and phosphorous from waste water. But it also recovers the phosphorus in the form in which it is more amendable to reuse than traditional chemical phosphorus precipitation used in the wastewater treatment technologies.

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Biological wastewater treatment is the largest biotechnological industry in the world

Essential for protecting human health and the environment

- recognized as a viable source of energy
- value-added chemicals such as bioplastics and fuels
- non renewable resources

The process has become increasingly viable as a source of phosphate as prices continue rising due to increasing demand and limited global reserves

And the process has become increasingly viable as a source of phosphate as prices continue rising due to the increasing demand and limited global reserve. So, on the one hand we have increasing demand for phosphorus, phosphate rather and declining of natural resources that is the rocks which are considered to be the major source of phosphate in all the industrial processes. And also on the other hand the new opportunities which are emerging because of the microbial activities that lead to not only the removal of the phosphates but also provides us the opportunity to recover that phosphate and provide that phosphate as a kind of for industrial uses.

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Introduction

The increased input of P and N from waste water treatment plants and other sources may lead to a disturbance of the ecological balance in :

- lakes
- slow flowing rivers
- water reservoirs
- shallow regions of marine waters along seacoasts

An overly rich provision of inorganic nutrient

Enhanced cyanobacteria algae ↓ DO

↓ Eutrophication

Eutrophication is a global problem:

Blooms of cyanobacteria and eukaryotic algae occur as a consequence of the breakdown of community homeostasis from continued pollution of oligotrophic aquatic bodies with nutrients like nitrogen (N) and phosphorus (P) at levels which exceed growth-limiting concentrations for these photosynthetic organisms

Now the increase, now what is the background of this phosphorus problem? Now the increased input of phosphorus and nitrogen both from waste water treatment plants and other sources may lead to disturbance in the ecological balance. The reason behind P and N because P and N are released from diverse waste materials and conventional waste treatment plants unless they are categorically designed to handle or to reduce the phosphate and the nitrogen contaminants.

It would generally take care only about the carbons which are present there, like organic carbon mostly in the wastewater and to some extent the nitrogen. But the phosphate which is there in the waste water unless it is this strategy of the wastewater treatment process is developed to handle the phosphates. The phosphates remain as phosphates and they are released into the environment wherever the wastewater treatment plants are discharging their effluents.

And when this phosphate containing effluents from the diverse wastewater treatment plants and other sources are released into the environment, they disturb the ecological balance. Particularly in the lakes which is found to be one of the lakes and the rivers are the most potent sink for this enhanced phosphate, not only for phosphate and also for the nitrogen pollutants.

And slow flowing rivers particularly, water reservoirs and shallow regions of the marine waters along the sea coasts. So, in all these places which actually act as potential sink for the phosphates which are released by wastewater treatment plants if they are not dealing with or they are not

treating the phosphate waste. And also other sources which are producing phosphates and they are releasing these phosphates.

So, now these overly rich provisions of inorganic nutrient like phosphate particularly and also the nitrogen compounds like nitrate and we will discuss about nitrate a little later. So, that leads to a kind of an environmental problem which is called Eutrophication. Now Eutrophication is considered to be a global problem, when you have enhanced amount of inorganic nutrients like phosphate and nitrates for example in your water body.

And generally the water bodies are oligotrophic we call them because they maintain a low organic carbon content unless it is polluted. So, the healthy water bodies which are considered to be oligotrophic or generally low in organic nutrients and also the inorganic nutrients like the phosphate and nitrate. A bloom of cyanobacteria and eukaryotic algae occur.

And this bloom of cyanobacteria and algae occurs as the consequence of the breakdown of the community homeostasis from the continued pollution of the oligotrophic water body. So, if you have oligotrophic water body for instance and for example if we just consider that this is the water body so and if in this water body we have addition of phosphate and nitrogen, nitrate, then the enhanced phosphate and nitrate.

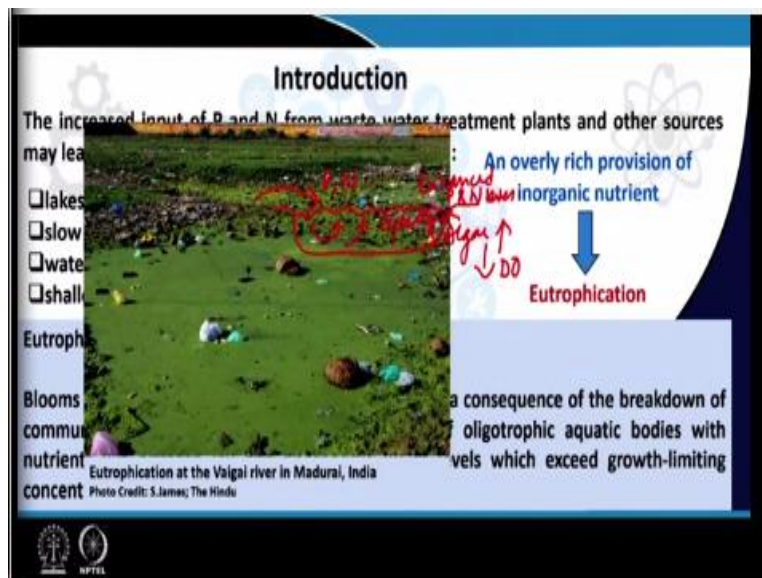
So, the enhanced P and N levels that is the inorganic nutrient levels, what they will do? They will encourage the activities of cyanobacteria and followed by cyanobacteria the algae will come. And this enhanced growth of cyanobacteria, so cyanobacteria and the algae will be promoted. So, the enhanced growth of cyanobacteria and algae will actually disturb the ecological balance, that is called the homeostasis within this lake ecosystem.

And essentially all this algae when initiated by cyanobacteria then algae then the algal cells will die and eventually the all biomass will be depositing as I said part of the sediment leading to the enhanced concentration of organic carbon and nutrients into the lake. And gradually the quality of the water will deteriorate in terms of multiple parameters. The algae and cyanobacteria may produce toxins; they will add the organic biomass.

And the availability of the organic biomass will lead to the decrease in the dissolve oxygen, so the water body will become slowly, anaerobic and as you can understand that if the water body becomes anaerobic then the aquatic life will be of all under threat. And eventually the lake will continue to be converted into a kind of wetland where in a couple of years, if we allow this to happen.

Then gradually all the lake water body will be filled with biomass and eventually we will see that the grasses and all the plants higher plants are growing on that. And eventually the lake will be converted to a wetland, so that is the kind of consequence that we want to deal with. So, basically we need to handle this situation, so Eutrophication is a global problem.

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Now here you can see that one of the eutrophicated lakes of Madurai which was the picture was published. In the early stage where you can see lot of the green algae is growing on them and already the river is seems to be very polluted. And this pollution is adding nutrients further. And these algae as I was explaining will be allowing the deposition of the algal biomass and eventually the lake homeostasis will be totally altered.

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The well known consequences of excessive nutrients

- Foam and slime disturbances on bathing beaches and the formation of toxins by cyanobacteria
- O₂ respiration of the algae at night and especially the later biomass decomposition by bacteria primarily cause an oxygen shortage in the waters and a release of H₂S, among other effects
- O₂ shortage causes a reduction of nitrate to toxic nitrite and release of phosphate from the sediment .
- Processing of drinking water from eutrophied waters additionally demands considerably more effort for purification

Now the well known consequences of the excessive nutrients particularly the phosphate and nitrates is the formation of the foam and slime which disturb the quality of the water bodies. This sea beaches and the formation of toxin by the cyanobacteria, so the water bodies are not suitable for public use. The oxygen respiration of the algae if you have lots of phosphates and nitrates then as I mentioned the cyanobacteria followed by algae will start growing on them.

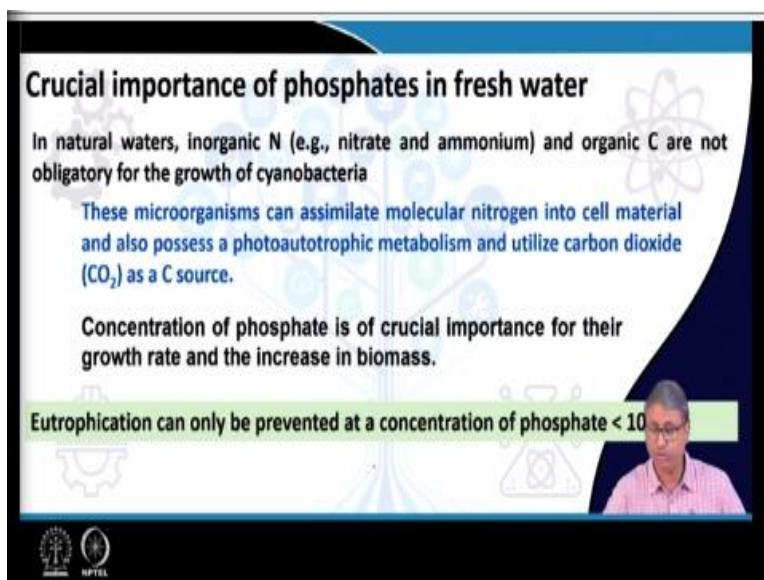
Now the algae will consume the oxygen particularly during the night time. And the biomass decomposition by the bacteria primarily causes oxygen depletion in the water. And that will eventually lead to production of H₂S and because the residual sulfates will be all be reduced as you have more biomass down there. The biomass will provide the organic resources, organic carbon and electrons.

And the sulphate reducing bacteria along with other fomenters will lead to the sulphate reduction and production of H₂S and creating lot of nuisance into that. Because H₂S will precipitate further metals and will stink and everything will be very much deteriorated condition. Now oxygen shortage also causes a reduction of the nitrate to toxic nitrite. Because as you have lower level of oxygen because your algae is consuming oxygen, nitrates are going to be reduced to nitrite and the nitrite is toxic.

And the release of phosphate from the sediment we will take place further. Now processing of drinking water from the eutrophicated water bodies will require additional cost and efforts. Because in many cases the natural water bodies are used for drinking water resources. So, if we pollute with respect to the nutrients like phosphate and nitrate, phosphate is more as a point of focus for today's lecture.

If we pollute our lakes and water bodies which are used for drinking water purpose then possibly we need to spend more money and more facilities to treat those waters to make them of drinking water quality. Because then in that water perhaps more microorganisms have grown because it is not oligotrophic lake anymore or oligotrophic river no more because it is now rich in organic matter and lot of other activities are going on.

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Crucial importance of phosphates in fresh water

In natural waters, inorganic N (e.g., nitrate and ammonium) and organic C are not obligatory for the growth of cyanobacteria

These microorganisms can assimilate molecular nitrogen into cell material and also possess a photoautotrophic metabolism and utilize carbon dioxide (CO_2) as a C source.

Concentration of phosphate is of crucial importance for their growth rate and the increase in biomass.

Eutrophication can only be prevented at a concentration of phosphate < 10

The slide features a background with faint chemical structures and a small inset video of a speaker in the bottom right corner. Logos for IIT Bombay and NPTEL are visible at the bottom left.

Now the crucial importance of phosphates in the fresh water. So, in natural water inorganic nitrogen for example nitrate and ammonium and organic carbon are not obligatory for the growth of cyanobacteria, why? Because under natural condition cyanobacteria they are capable of fixing molecular nitrogen and they can fix the inorganic, carbon dioxide and converts it to organic carbon. Now concentration of phosphate is found to be a crucial importance because that is what in generally is missing in oligotrophic lakes, most importantly.

The moment you provide or we provide phosphates into these ecosystems, water bodies the gap is filled, so the cyanobacteria were just waiting for the phosphates. So, the moment we have a continuous supply of phosphate in the lake or the water bodies this will allow the cyanobacteria to start growing because they are waiting for phosphate only because they can otherwise manage with nitrogen and carbon sources. Now eutrophication can only be prevented as at a concentration of phosphate less than 10 microgram per liter.

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Phosphate recovery
Reducing global P reserves, loss of P as human waste and other wastes

15-20% of world demand for phosphate rock could be satisfied by recovering the P from these waste streams

Now when we try to develop a system because human and the environmental engineers particularly they have worked for a long period of time. So, like more than 50 years or 70 years now we see that the wastewater treatment technologies are developing including the phosphate removal processes using microbes. And eventually we have seen now, we have learnt that it is not as I mentioned earlier not only removal of the phosphate but we can actually recover the phosphate also.

Now reducing the global P reserves or phosphate reserves, loss of P as human waste and other waste. So, basically 15 to 20% of world demand for phosphate rocks could be satisfied by recovering the P from these waste streams. So, all the wastes which are coming from the domestic areas as well as the industrial areas often contain high amount of phosphate. So, if we are able to recover that phosphate, it will be replacing possibly 15 to 20% of the rock phosphate.

So, you can now imagine that actually replacing 15 to 20% of the world demand of the rock phosphates would actually mean that lot of mining activities lot of other expenditures can be minimized.

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Major sources of P in the waste water

- Faecal materials
- Industrial and commercial sources
- Synthetic detergents and other cleaning products
- Point source : wastewater treatment plants

The slide features a background graphic of a tree with various icons (gears, atom, flask) and a presenter's video feed in the bottom right corner. Logos for IIT Bombay and NPTEL are visible at the bottom left.

Now what are the sources of phosphates or phosphorous in the waste water? These are basically the faecal materials, industrial and commercial sources, synthetic detergents and other cleaning products and also there are point sources like different waste water treatment plants.

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P-removal through waste water treatment plants

In the past 50 years many wastewater plants have been designed and built around the world to deliberately reduce not just organic carbon and N levels but also P, using microbially based methods

The slide features a background graphic of a tree with various icons (gears, atom, flask) and a presenter's video feed in the bottom right corner. Logos for IIT Bombay and NPTEL are visible at the bottom left.

Now we will move into the P removal through the wastewater treatment plants. So, as I mentioned over the past 50 years or so, many wastewater treatment plants have been designed

and built around the world to reduce not just the organic carbon and nitrogen level but also the phosphorous level, using microbially based methods. Although the carbon removal methods are often well discussed but the phosphorus removal methods remain missing.

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The slide is titled "Microorganism-mediated enhanced phosphorus removal". It contains the following text:

- Conventional wastewater ($7-12 \text{ mg P L}^{-1}$) treatment can remove 30-40% of the phosphorus (biologically) removed with the primary sludge and due to the growth of the biomass
- But, we need to remove over 90% P (permissible effluent conc. $0.5-1.0 \text{ mg P L}^{-1}$)
- In contrast to the N-compounds, phosphorus can only be removed from wastewater in its solid state after sedimentation

Two boxes at the bottom describe removal methods:

- incorporation into the biomass of the sewage sludge
- chemical precipitation as a phosphate salt

The slide also features a small inset video of a speaker and logos for IIT Bombay and NPTEL.

So, in today's class we are going to talk in detail about that. Now what is microorganism mediated enhance phosphorus removal? So, conventional wastewater treatment which is basically up to having 7 to 12 milligram phosphorous per liter can remove 30 to 40% of the phosphorous biologically. And this phosphate is converted into the sludge and the sludge can be removed and can be utilized for as a source of phosphorus.

But we need to remove over 90% of the phosphorus, removing 30, 40% with normal conventional waste water treatment plant where basically it is carbon removal. And during the carbon removal also the organisms they accumulate some phosphorous and they precipitate as a biomass sludge. So, we need to bring down the phosphate concentration with the permissible limit to 0.5 to 1 milligram phosphorus per liter.

And that sounds to or that that goes to around like 90% of the P present in the waste water treatment. Now this can only be achieved by 2 methods one is the chemical precipitation method as the phosphate salt and of course we are not right now focusing on that. The other alternative is that the incorporation of the phosphorus or phosphate into the biomass of the sewage sludge.

Because compared to nitrogen compound which can be biologically catalyzed to produce nitrogen and nitrogen can leave the system. Phosphorous or phosphates cannot leave the system, so it will be deposited within the biomass.

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The slide features a title "Microorganism-mediated enhanced phosphorus removal" at the top. Below the title is a diagram showing a cluster of red, bean-shaped bacterial cells on the left, with an arrow pointing to a single cell on the right. Inside this cell, several yellow circles labeled 'P' are arranged in a chain, representing polyphosphate granules. A handwritten red label "Polyphosphate body" points to this chain. Below the diagram, a text box explains that this removal is achieved by encouraging the accumulation of P in bacterial cells in the form of polyphosphate (polyP) granules in excess of the levels normally required to satisfy the metabolic demand for growth. It further states that this is a storage process commonly referred to as 'enhanced biological phosphorus removal' (EBPR). A small inset video of a speaker is visible in the bottom right corner of the slide.

So, ideally if we look at the wastewater treatment process. So, this is for example the wastewater where we will allow the specific microorganisms to grow and once these microorganisms are able to encounter the phosphates which are present over there, so they will be able to accumulate the phosphate as polyphosphate body. So, these materials are called polyphosphate body.

So, long chain of poly phosphate groups like for example if we see the ATP molecules where we have only 3 phosphate groups, similar type of anhydride bonds are there but there may be 40, 50, 60, 80, 100 phosphate molecules I mean more than that are tied together. And these are large cellular deposits which we can observe under the microscope the cells which are expert in converting and accumulating phosphorous, phosphate and converting them into polyphosphate bodies are can be seen under the microscope very easily with appropriate strain.

So, now if we allow these bacteria to grow with phosphate, now this water might be having high P. So, we will see after some time that is all these bacteria they will deposit this long chain of polyphosphate bodies within them. And they will grow this bacteria will grow more, so as they

grow more in number, so we have more number of cells appearing. And once you have more number of cells appearing and growing, so they will accumulate more phosphates and what will happen?

The water that will eventually come out of the system will have the phosphate level which is close to the desirable limit, that is 0.5 to 1 unit of per milligram of per liter of water Now this removal is achieved by encouraging this type of bacteria which I have shown here. And these type of bacteria they are called actually of phosphate accumulating organisms or phosphate producing bacteria. So, they accumulate the polyphosphate bodies, so they take the phosphate the soluble phosphate that they transport it inside the cell.

And then they produce the polyphosphate body, so they have the appropriate metabolic mechanisms to produce. This is also a very kind of an exclusive property that all bacteria present in the wastewater will not have this property of producing excessive polyphosphates which will actually meet the industrial waste water treatment level.

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The slide contains the following text and diagrams:

- EBPR uses the capacity of certain bacteria to accumulate polyphosphate intracellularly**
- The key players in this process are :
 - Polyphosphate- accumulating organisms (PAO)
 - Glycogen-accumulating organisms (GAO)
- Both these groups of organisms are selected for during anaerobic-aerobic cycling, an important process of EBPR

Handwritten notes and diagrams include:

- A diagram showing a sequence of four circles representing a cycle, with an arrow labeled "Waste water (P)" pointing to the first circle.
- A diagram showing "PAOs" and "GAOs" with a double-headed arrow between them, and a "P" with an arrow pointing to "PAOs".
- A small inset video of a speaker in the bottom right corner.

Now the EBPR the enhanced biological phosphorous removal process uses the capacity of these bacteria which are known as the polyphosphate accumulating organisms or conventionally also known as the PAOs. So, these PAOs are the organisms who are responsible for accumulating the

phosphates as polyphosphate bodies. Now along with the polyphosphate accumulating organisms or polyphosphate accumulating bacteria they mainly belong to bacteria.

So, we conventionally call them as polyphosphate accumulating organisms. There is another interesting group of organisms which are very closely associated with the growth and activities of the PAOs, these are called glycogen accumulating organisms, GAOs. So, both these groups of organisms are selected for during the wastewater treatment processes which are developed for the removal of the phosphate.

So, now if we look at the wastewater treatment processes which have been developed for phosphate removal or we say enhanced phosphate removal. Why it is enhanced? Because normal wastewater treatment can remove 30 to 40% of the dissolved phosphate but we need 90% removal of the phosphate. So, ideally as you can see over here it is written that the anaerobic-aerobic cycle is there.

So, during the anaerobic-aerobic cycling is there, that means, so your wastewater, so the wastewater which contains the extra or enhanced level of phosphate and we want to remove it. These wastewater must pass through a set of tanks or reactors, so these wastewater will pass through the set of tanks and individually this tanks will have the following designations, like anaerobic anoxic, aerobic.

And then again some sludge will come back here and some water will flow out. So, the inoculum kind of thing will come back and will be added to here and the water will flow through. So, in between the water will be transferred. Now the interesting point is that while under aerobic condition the phosphates will be removed by the PAO, this glycogen accumulation will occur during the anaerobic process.

Now why do we need this glycogen accumulating organism? Actually we do not possibly target this glycogen accumulating organisms initially. Because we were targeting the phosphate removing organisms but we realized that during this process of cycling of the water because that only encourages these PAOs to grow and act properly, they allow this glycogen accumulating

organisms also. Actually this PAOs they have a very intricate and interesting relationship with GAOs, why?

Because PAOs will take up the phosphate and will produce the poly P under the aerobic condition. But we need to have certain kind of cycling in order to facilitate the enhanced phosphate removal. So, the GAOs will actually partly they are more active during the anaerobic process, so during that time they will produce the lipids. So, the glycogen accumulating bacteria they will produce the glycogen deposits which will be then utilized as a source of energy or source of the carbon and electrons for other bacteria like PAO as well.

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EBPR takes advantage of a complex microbial ecosystem to facilitate the process

Integration of modern molecular methods, from single-cell microbiology to -omics approaches, has been used extensively to understand the complexity of EBPR.

Many of the important organisms are identified and their main functions and metabolisms determined - making EBPR one of the best-characterized ecosystems in environmental biotechnology

The slide features a background with a blue and white color scheme, including a stylized tree-like diagram and icons of a gear and a flask. A small inset video of a speaker is visible in the bottom right corner. Logos for IIT Bombay and NPTEL are at the bottom left.

So, we will discuss about that. This intricate relationship between the anaerobic and aerobic conditions are essential for the natural enrichment and performance of this bacteria which are called basically the phosphate removing organisms or phosphate accumulating organism. So, the best performance of these organisms will be achieved only when we have the wastewater treatment system which actually cycles between these aerobic and anaerobic systems.

Now the EBPR takes advantage of the complex microbial ecosystem to facilitate this process. Now what is this complex microbial process? And this complex microbial process was initially not known, initially scientists thought that it might be by some designated group of bacteria or other members fungi etcetera, there they are playing role in phosphate removal. But as soon as

the modern molecular methods, molecular microbial ecology methods from single cell microbiology to omics approach.

That has come in a big way to understand the complexity of the process. And many of the important organisms are identified and their main functions and metabolisms are also determined. So, what we found? That we cannot assign any specific bacteria directly for the function of the phosphate accumulation in this case, so there are some microorganisms. Now the some microorganisms must have some identity.

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Polyphosphate-accumulating organisms (PAOs)

- PAOs: The key organisms in the EBPR process
- remove most of the P from the wastewater
- contain a high fraction of biomass P (up to 12% compared to 3% dry weight in non-PAO) and can be removed with surplus sludge for further processing of the P for recovery

The slide features a background with a stylized tree and icons of a hard hat, a beaker, and a molecular structure. A small video feed of a presenter is visible in the bottom right corner. Logos for IIT Bombay and NPTEL are at the bottom left.

Now these are generally called PAOs, these are the key organisms in this process and remove the most of the P from the wastewater and they contain a high fraction of biomass P. So, they accumulate like up to 12% compared to 3% dry weight in non PAO. So, the PAOs are the catalytically responsible organisms. Now remember, all organisms are not PAO, there are some organisms which are PAOs these organisms can accumulate up to 12% P dry weight basis compared to only 3% a non PAO organisms.

And can be removed with surplus sludge for further processing and P recovery. Now this 12% dry weight P is significant and if we can remove and indeed we do that. So, if we remove that sludge containing the bacteria which are loaded with phosphate, the phosphate can be removed successfully.

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The most well known group of PAOs is '*Candidatus Accumulibacter phosphatis*' (*Accumulibacter*), Betaproteobacteria

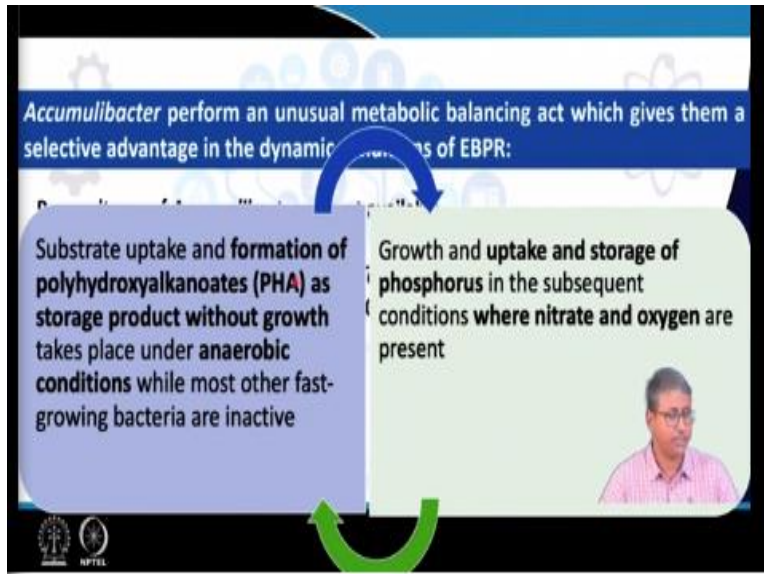
Pure cultures of *Accumulibacter* are not available

Besides *Accumulibacter*, gram-positive *Tetrasphaera* related organisms (*Actinobacteria*) are also regarded as PAOs

Now what are these organisms? This most well known group of PAOs are considered to be '*Candidatus Accumulibacter phosphatis*'. A *Candidatus* means the taxonomic description of this group is not yet complete and they are found to be members of the class Betaproteobacteria. And the major identifiable lowest taxonomic level identity is *Accumulibacter*, so we can designate them like *Accumulibacter*.

And the pure cultures of *Accumulibacter*s are not yet available, they are mostly uncultivable organisms. And besides *Accumulibacter* gram positive *Tetrasphaera* related organisms and certain other *Actinobacteria*, *Streptococcus* like members are also there and they play very important role. However this *Tetrasphaera* is also considered to be which is a member of *Actinobacteria* another PAO. So, as a PAO we can identify the *Accumulibacter* and the *Tetrasphaera*.

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Now the Accumulibacter perform an unusual metabolic balancing act which gives them the selective advantage in the dynamic condition of EBPR. Now if you normally grow Accumulibacter, perhaps it will not execute that function of up to 12% dry weight P accumulation, possibly it will not do that. It has to be cycled between 2 conditions aerobic and anaerobic.

So, let us first see under aerobic condition what happens? Under the aerobic condition growth and uptake and the storage of the phosphate which is in the form of polyphosphate bodies occurs. In the subsequent condition where nitrate and oxygen are present, so ideally using nitrate as electron acceptor and oxygen as electron acceptor. This aerobic mode of metabolism by these bacteria, these Accumulibacter type bacteria, they facilitate the uptake of the P and accumulation and it is conversion into the polyphosphate body.

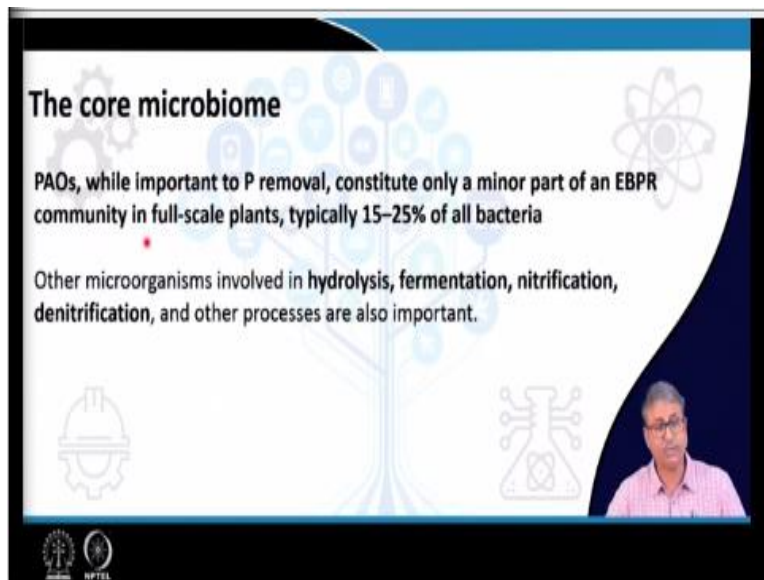
So, ideally they are all aerobic bacteria and they often execute the property of nitrate reductions also. So, here is a point that we can actually deal with the nitrate level also to some extent. Now under anaerobic condition, now the same culture, same water will be transferred through the anaerobic stage. Now under anaerobic condition substrate uptake that is whatever organic carbons are there that will be taken up and the formation of polyhydric hydroxyalkanoates or PHA, which is a potent bioplastic molecule.

As a storage product will be produced without growth taking place under the anaerobic state. So, if we take the same culture under the anaerobic condition, we will see that the bacteria they are actually removing the carbon from the water and converting these carbons into the polyhydroxyalkanoates. And during that time this is anaerobic, they are possibly using the internal ATP generated because for some of the polyphosphates bodies may be hydrolyzed.

Giving them some ATPs to perform these functions where the small organic molecules will be taken up and they will be converted to the internal deposits of PHA. Now interestingly under this anaerobic condition most other fast growing bacteria are inactive because it is a wastewater coming, so the wastewater is already mixed with this PAO containing inoculums.

So, the PAO bacteria they are very smart, so they will survive, they will function not in terms of the P removal but in terms of the formation of the PHA internal lipid within themselves. Now these organisms or this culture when will subsequently transfer to the aerobic condition, they will execute rapid growth under aerobic condition and they will start accumulating phosphate.

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The slide features a central graphic of a tree with circular nodes, set against a dark blue background. The text is white and black. In the bottom right corner, there is a small inset video of a man with glasses speaking. The slide includes several icons: a gear, a chemical structure, a hard hat, and a flask. At the bottom left, there are two small circular logos, one of which is labeled 'NPTEL'.

The core microbiome

PAOs, while important to P removal, constitute only a minor part of an EBPR community in full-scale plants, typically 15–25% of all bacteria

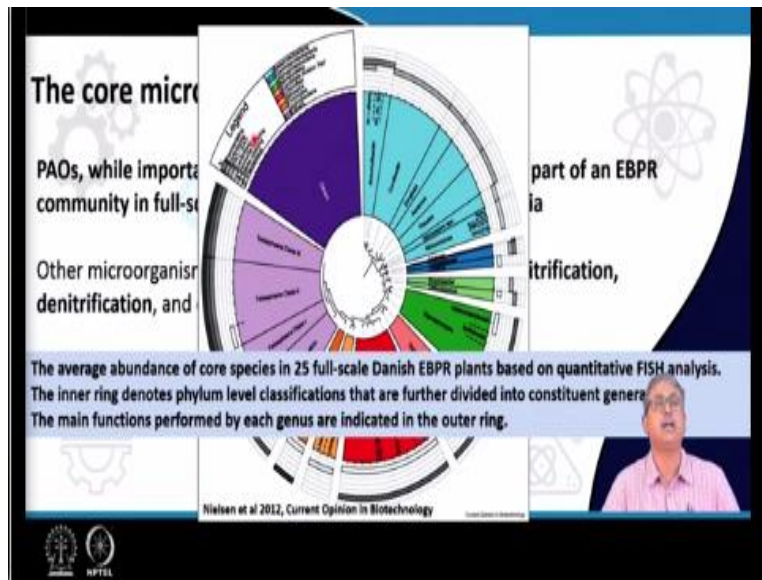
Other microorganisms involved in hydrolysis, fermentation, nitrification, denitrification, and other processes are also important.

Now the scientists they have studied the core microbiome that what are these microorganism because it is a complex system. So, we have found that PAOs while incorporating to P removal, they are important for P removal constitute only a minor part of the EBPR community in full

scale plants. So, across the several countries of Europe, we see there are multiple phosphate removal systems or waste water treatment facilities which use this EBPR system.

So, what we have found that in many cases this known Accumulibacter etcetera, they constitute only a small part, like 15 to 25% of the all bacteria present over there. So, what the other microbes, other bacteria etcetera doing there? The other microorganisms involved there is carrying out the important other functions like hydrolysis, fermentation, nitrification and denitrification and other processes which are possibly important for the growth and activity of the PAOs.

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So, this is the data out of these review paper by Nelson in 2012, which shows that the out of 25 wastewater plants from Denmark. They have reported that the core microbiome is there, so you can actually look at the legend and then find out these are the genera which are given. And the functions carried out by them are provided here, we can see that many of them are fermentative, denitrifier, ammonia oxidizers, nitrite oxidizers.

So, there are many different functions and all these different functions which are some of the functions are may not be directly related to phosphate removal. But when we look into the functioning of the entire system we understand that these are also very, very essential.

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The EBPR as a model system

An in-depth knowledge of the microbial communities in the process and their interactions helps to improve plant design, operation and trouble-shooting by enhancing our understanding of critical aspects of the process

- 1) satisfactory removal of C, N, P, micropollutants, and pathogens
- 2) good solid-liquid separation,
- 3) stable operation
- 4) many fundamental questions regarding microbial ecology of communities can be investigated

NPTEL

Now the EBPR has emerged as a model system. Now an in-depth knowledge of the microbial communities in the process and their interactions help to improve plant design, how this wastewater treatment plants will be designed? How they will be operated? And how will be troubleshooting based on the variations of certain parameters by enhancing our understanding of the critical aspects of the process.

So, these processes are satisfactory removal of carbon, nitrogen, phosphorus, micropollutants and pathogens. Good solid-liquid separation, stable operation and many fundamental questions regarding the microbial ecology of the communities are to be investigated.

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The existence of a full-scale EBPR core microbiome suggests that a limited number of microbes are critical to the process

knowing these microbes and their interactions enables fundamental ecological insights
-----transferable to other plants and perhaps even other similar microbial ecosystems

The key questions we need to address for each core species in the major functional groups are:

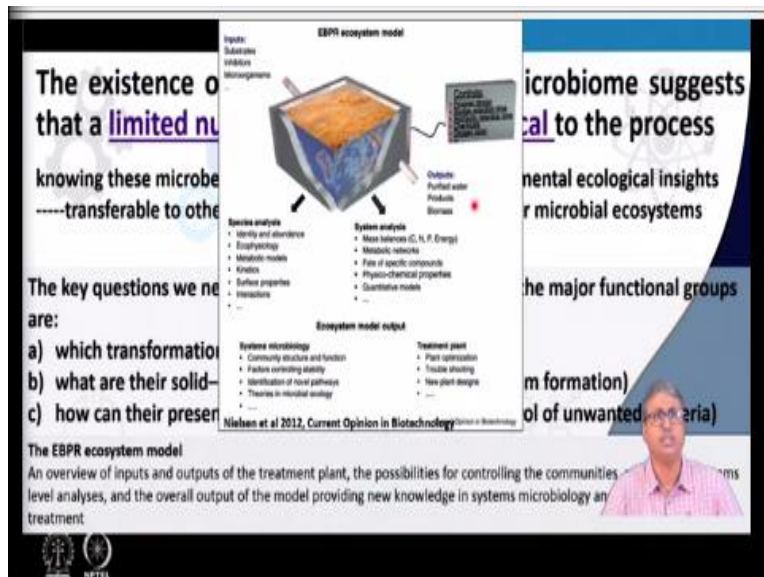
- a) which transformation processes they perform
- b) what are their solid-liquid separation properties (floc/biofilm formation)
- c) how can their presence be manipulated (e.g. removal/control of unwanted bacteria)

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Now the existence of a full scale EBPR core microbiome, which suggests that only 10 to 15% maybe 12 to 20% is the Accumulibacter. So, other organisms are there, so that a limited number of microbes are critical but other microbes are important in order to control this process. Now knowing these microbes that is the selected organisms which are responsible for actual the P removal and others who are supporting them.

So, knowing these organisms and their interactions, how they are interacting with themselves? Enables the fundamental ecological insights that can be transferred to other plants and perhaps even other similar microbial ecosystems and hence the key questions that we need to address for each of these core species in the major functional groups are delineated. And these include the transformation processes which they perform, what are the solid liquid separation properties? Like flocculation or biofilm formation. How can their presence be manipulated? That is removal, control of unwanted bacteria etcetera.

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So, the model has been developed which suggests that if we have the inputs, we can have multiple outputs including the species analysis, the community analysis, the system analysis. And then you have the outputs which are the purified water and you have different products and also the biomass.

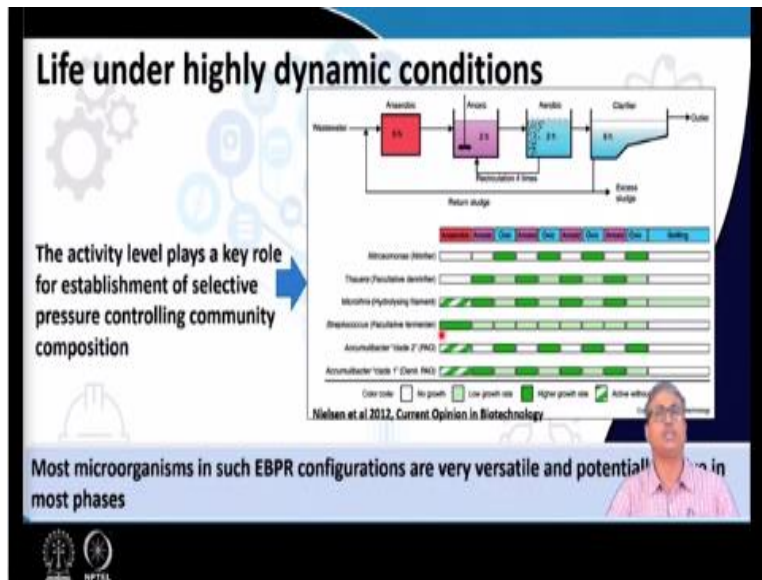
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The slide features a central graphic of a tree with blue nodes and branches, set against a white background with faint icons of a hard hat, a beaker, and a molecular structure. The text on the slide reads: "Life under highly dynamic conditions" and "EBPR exploits rapidly changing environmental conditions that select for the diverse range of organisms (nitrifiers, denitrifiers, etc.) that are able to perform the various transformations required". A small inset video shows a man in a pink shirt speaking. Logos for institutions are visible in the bottom left corner.

Now we will see the life under the highly dynamic condition. It is highly dynamic because we are recirculating the wastewater through these anaerobic and aerobic systems. So, EBPR exploits rapidly changing environmental conditions. So, this is the industrial scale setup where we actually transfer the water from anaerobic to aerobic and again aerobic to anaerobic, all these 2 systems are used.

So, it exploits rapidly changing environmental conditions and these changing conditions are required because that select for the diverse range of organisms. Now again and again that point will be coming in mind that why do we need to recycle between these aerobic-anaerobic conditions? It is required because that shifting the conditions will allow selection of diverse organisms which will be required for the desirable efficiency of the PAOs.

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Now ideally what we see that the system can be operating with the wastewater input? Moving into an anaerobic tank first and then to an anoxic tank and then again to the aerobic tank and then from the aerobic tank the water will move into the clarifier. And from the clarifier the water will further be discharged and from the aerobic tank some of the material will be recirculated and some sludge will come from the clarifier and it will consider as a return sludge.

And the return sludge will come to and mix with the wastewater as possibly as an inoculums. And the left other part of the return sludge will be used as a kind of the product from where the phosphate can be removed. Now if we see the processes which are going on there, there will be processes like anaerobic, anoxic, oxic. Then again as I mentioned that this part will be recirculating at least 4 times. So, these are optimized situations.

So, what Nelson in their paper has actually mentioned? That these are kind of a model situations, model conditions which are being practiced across several wastewater treatment plants. Now down here we have the several organisms, now these organisms are the most prominent organisms and they are relative abundance. Like the colour code here saying that no growth, like the white colour means no growth, light green is low growth rate, green is higher growth rate and the shaded green is active but without growth.

Now the activity level plays a key role for establishment of the selective pressure controlling the community composition and also function. As you can see many organisms including nitrifier, denitrifier, filamentous, hydrolyzing organisms who can produce exozymes and exozymes are involved in hydrolyzing different complex polymers. And then you have the PAOs of 2 phylogenetic clades Accumulibacter clade 1 and clade 2.

So, they are dark green in many and most of the cases they are dark green in oxic conditions but they are also active but they are not growing. For example Accumulibacter if you see Accumulibacter is active but they are not growing here. Similarly if you see Streptococcus very active in anaerobic condition and growing also but it is growing with a low growth rate and same thing is true for the thor and Microthrix as well. So, ideally the most microorganisms in EBPR configuration found to be versatile and potentially active in more than 1 phases.

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A game of four major players could be identified

Denitrifiers : facultative anaerobes, most active under anoxic conditions as the organic substrate concentration is higher than in the aerobic (oxic) tank.

Microthrix: Active in all tanks, takes substrate up under anaerobic conditions, reduce nitrate to nitrite in the anoxic tank, and grow aerobically in the oxic tanks either on stored material or hydrolysis products. Produce exoenzymes in all tanks.

Streptococcus: A versatile fermenter, active under anaerobic conditions, but also able to grow under aerobic conditions.

Accumulibacter : Release orthophosphate under anaerobic conditions and take up substrate and store this as PHA. Under anoxic (clade I) or aerobic conditions (clade I+II), Accumulibacter take up orthophosphate and utilize the stored energy for growth.

The slide includes a small inset image of a man in a pink shirt and glasses in the bottom right corner. At the bottom left, there are logos for IIT Bombay and NPTEL.

And that is possibly one of the major characteristics of this entire process. Now this entire process can be defined as a game of 4 major players, who are the 4 players? Number 1 is the denitrifiers which are the facultative anaerobes, most active under anoxic condition as the organic substrate concentration is high than the aerobic tank. So, they are very active under the anoxic condition because the redox potential under anoxic condition possibly favours them.

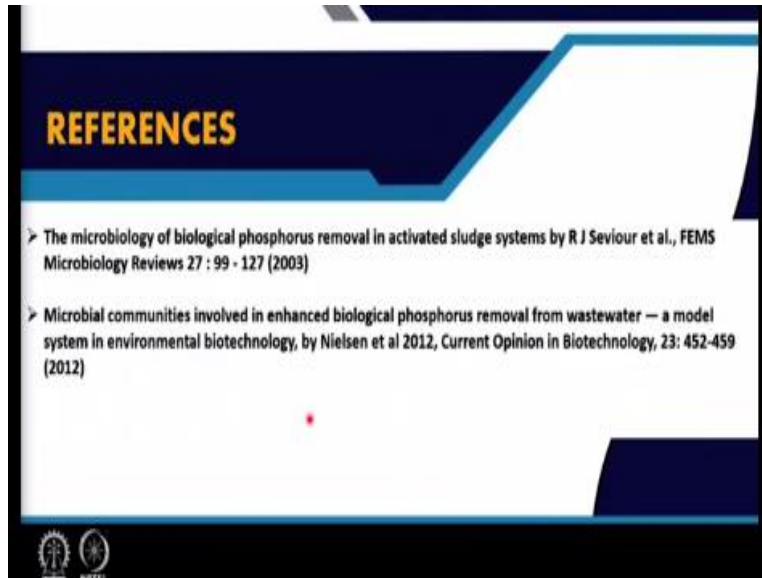
And they utilize the organic compound and they reduce the nitrate. Microthrix active in all tanks, so aerobic, anaerobic, anoxic everywhere Microthrix are there, they take the substrate, use it under anaerobic condition, reduce nitrate to nitrite in the anoxic tank, grow aerobically in the oxic tank either or store material or hydrolyze the products produce the exozymes in all tanks. So, this exozyme production is very critical because this is essential for degrading or hydrolyzing all the large polymers of the proteins and carbohydrates and lipids etcetera which are then making their use in the subsequent steps.

So, Streptococcus is a versatile fermenter, most active under the anaerobic condition but also able to grow under aerobic condition. These ferments produces different metabolites and possibly these metabolites could be of use by other organisms including the Accumulibacter also now Accumulibacter which is most active and able to grow profusely with phosphate accumulation under oxic condition. But it can remain active but they do not grow under anaerobic condition. So, under anaerobic condition, it releases some amount of orthophosphate.

And take up substrate which are present like the volatile fatty acids or small acetate like compounds and store them at the polyhydroxyalkanoids or lipid bodies. So, under anaerobic condition Accumulibacter plays the role of GAOs like glycogen or PHA accumulating organisms. Under anoxic or aerobic condition Accumulibacter take up orthophosphate and utilize the stored energy for the growth.

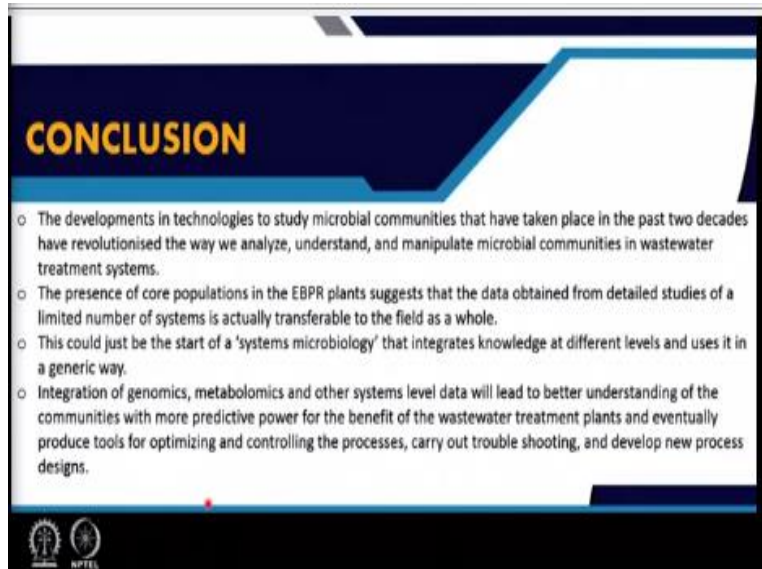
So, this is the beauty of these 2 phylogenetic groups of Accumulibacter clade 1 and clade 2. That one of them is very active during anoxic condition; another is very active during the purely oxic or aerobic condition. So, that means even under complete aerated condition and also in partially aerated or low oxygen concentration level the different Accumulibacter strains are capable of removing the phosphate from the system.

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So, for this lecture these 2 articles will be very useful.

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And finally in conclusion, the developments in technologies to study the microbial communities that have taken place in the past 2 decades have revolutionized the way we analyze, understand and manipulate microbial communities. And we have learned some aspects and a basic concept of that. The presence of the core population in EBPR plants suggest that the data obtained from detailed studies of the limited number of system is actually transferable to the field as a whole.

So, this is a very interesting outcome that we have learnt from EBPR system that other of microbial systems or micro based wastewater treatment and environmental systems can be

developed using a similar concept. This could just be the start up we called system's microbiology that instead of finding 1 species responsible for 1 function. So, here multiple species, multiple functions but helping towards achieving the broader goal that is remove phosphate and allow us to recover the phosphate.

And finally integration of this genomics, metabolomics and other systems level data leads to the better understanding of the communities with more predictive power for the benefit of the waste water treatment plant. And eventually produce tools for optimizing and controlling the processes, carry out troubleshootings and develop new process designing. Thank you.