Environmental Biotechnology
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Lecture-46
Biological Nitrogen Removal

Welcome to the next lecture of our course on environmental biotechnology and in today's lecture we are going to learn about biological nitrogen removal.

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And in particular in this lecture we are going to discuss about the environmental problem with nitrogen and we will also talk that a large part of this biologically available nitrogen remains untreated and ends up in the wastewater and the concept of biological nitrogen removal and the possibility of recovering that nitrogen particularly the conventional nitrogen removal and limitation of the conventional nitrogen removal will be discussed. And finally the new paradigm of nitrogen removal will be explained.

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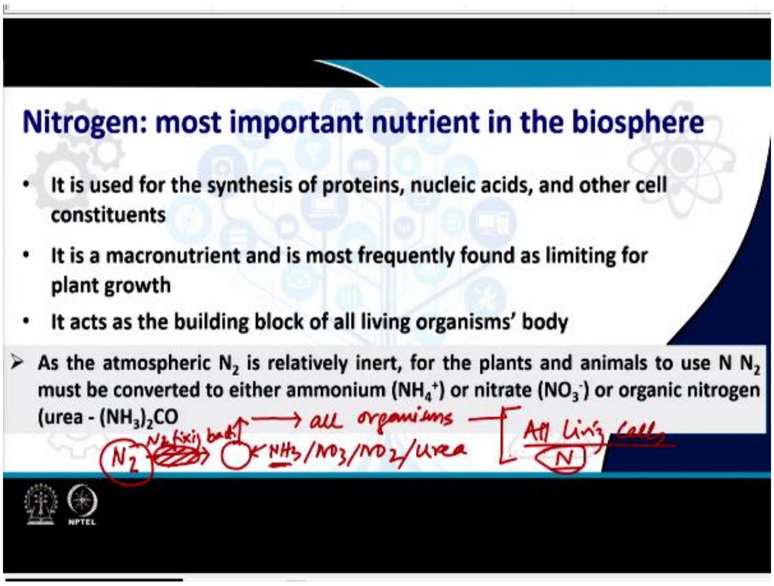

So, nitrogen is one of the most important nutrients in the biosphere, because it is used in the biosynthesis of proteins, nucleic acids and all other cell constituents. It is therefore considered as a macronutrient and is most frequently found as limiting for plant growth. So, that is a very important aspect of the nitrogen because unless it is available to the plants in the form of oxidized or reduced species like ammonia or nitrate or nitrite the plants in particular are unable to utilize the nitrogen because nitrogen which is available in our planet is mostly the gaseous nitrogen, dinitrogen which is considered to be mostly inert.

And this nitrogen which is so important for all the major biomolecules, it actually responsible for the building blocks of all living organisms, so be it the nucleic acid or is the protein so wherever we see these important molecules it is all nitrogen. So, therefore all cells must acquire nitrogen. Now as the atmospheric nitrogen I mentioned is relatively inert it does not react. So, readily with any kind of things those are biologically relevant and can produce biologically utilizable nitrogen forms.

For most of the plants and animals tonight to utilize N nitrogen must be converted to either ammonium or nitrate or the organic nitrogen that is the urea. So, ideally so this is the major role over here that we need to understand that nitrogen which is present in the atmospheric gases needs to be converted to some form which can be utilized by the plants. And from the plants this will be distributed to all organisms because plants are considered to be the producers of the all the fixed carbon and along with the carbon the nitrogen will also be assimilated and through that it will enter into the food chain and therefore the nitrogen will move on.

So, this nitrogen can be organic nitrogen, inorganic nitrogen like it could be ammonia, it could be nitrate, it could be nitrite, it could be urea. So, either or it can assimilate as amino acids or protein molecules of course. So, the responsibility of microorganisms who are capable of transforming this gaseous or so called inert nitrogen to useful for mostly the ammonia nitrate, nitride and perhaps also urea is coming next. So, these are the nitrogen fixing bacteria.

So, basically the nitrogen fixing bacteria they converts this atmospheric nitrogen into reduced nitrogen and then the reduced nitrogen is assimilated utilized by all organisms and eventually it ends up into all living cells. So, all living cells on this planet they get the nitrogen. So, from the atmospheric nitrogen to the nitrogen present in all the living cells there is a very, very important part of nitrogen fixing bacteria and followed by the carbon fixing autotrophic organisms, they allow the flow of the nitrogen.

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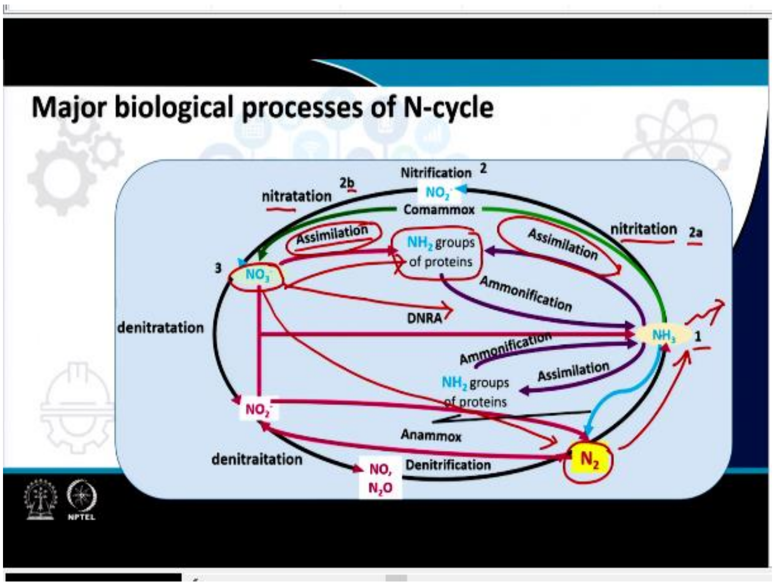

Now if we look at the major biochemical processes of nitrogen cycle which are taking place in the environment and this is important because by utilizing some of these processes we are able to manage the nitrogen which is emitted or released as a nitrogen waste. Because we already learnt that too much of nitrogen is bad for the environment, it is a responsible factor for what you called nutrient pollution.

So, along with phosphate excess nitrogen be it ammoniacal nitrogen or nitrate nitrogen or nitrite nitrogen or organic nitrogen; too much of nitrogen is bad for the environment or any kind of ecosystem because in most of the ecosystem it is the nitrogen which is another limiting factor. So, once we have that arrangement that extra nitrogen will be available to the biotic components maybe through waste influence or contaminated waste materials or by other discharges in which nitrogen is there.

Then that will lead to eutrophication possibly that we have already discussed in some of our earlier classes. Now in order to come back to that situation we are in environmental biotechnology we are trying to take the advantage of the natural nitrogen cycling processes or natural processes in which microorganisms play the important roles in transferring or transforming nitrogen from one form to the other.

So, now let us first see that what are the different transformation reactions which are carried out by the microorganism mostly? So, first we start with the dinitrogen, so the dinitrogen in step 1 is reduced by ammonium. That is the function of the nitrogen fixing organisms which are naturally present in soil and other ecosystem. Now this ammonia or ammoniacal nitrogen which I marked as step 1 undergoes some series of reactions and eventually it ends up into nitrate.

Now this nitrate formation from ammonia as you can understand that this is a kind of an oxidation process it might be a 2 step process and many a times it is a 2 step process like nitritation in which the ammonia is first oxidized to nitrite and then a nitration step in which the nitrite is converted to nitrate. So, eventually we get nitrogen to ammonia and then ammonia to nitrate.

In between the system also produces nitrite and nitrite can be subsequent. So, there are multiple microorganisms who are capable of carrying out these functions. So, sometimes it is a only one organism might function for both the steps or multiple organisms are involved because these are 2 separate steps like 2a and 2b as I mentioned. So, ultimately ammonia is converted to nitrate.

Now this nitrate which is soluble form again is present in the soil and water system can have multiple fates. Fate number 1 that it is reduced into a kind of a process which is called assimilatory nitrate reduction and it is entering into the cellular system as ammoniacal group or NH 2 groups of the proteins or the amino acids. So, it enters into the cellular system we call it assimilatory process or assimilation of nitrogen takes place which actually utilizes the nitrate reduces the nitrate into ammonia and ammonium NH 2 groups or amino groups and it is utilized.

Alternatively it could be also reduced to ammonia which is basically the dissimilatory nitrate reducing process in which the nitrate will be reduced by dissimilatory nitrate reducing or expiring organisms which will use it just as an terminal electron acceptor in absence of oxygen and will reduce it to ammonia and this ammonia will be produced outside the cell. So, cell will not utilize this reduced ammonia.

So, these are the 2 things that one is the assimilatory nitrate reduction to ammonia and another is the dissimilatory nitrate reduction to ammonia. This ammonia leaves the system. There could be another way of reducing nitrate via NO 2 and N 2 O and NO which will finally lead to the production of nitrogen or dinitrogen. So, nitrate can be completely we say mineralized to nitrogen or dinitrogen which can also leave the system.

So, now if we want to think that we have a waste water containing high amount of ammonia possibly we can now understand that there are some microbial processes which might be useful in facilitating converting this ammonia into nitrate and then nitrate to nitrogen and the nitrogen can leave the system. Alternatively some form of the ammonia can be taken up directly by the cells that is called the assimilatory process which can assimilate the ammonia directly or they can reduce the nitrate to ammonia and then use this nitrogen into the cell.

So, in either of the ways the nitrogen will be assimilated inside the cell. So, some fraction of the nitrogen which is available will be ultimately assimilated but most of the nitrogen will potentially be converted to either ammonia or to nitrogen. And both this ammonia and nitrogen can be released outside and so from a wastewater point of view the waste will have reduced nitrogen under that circumstances.

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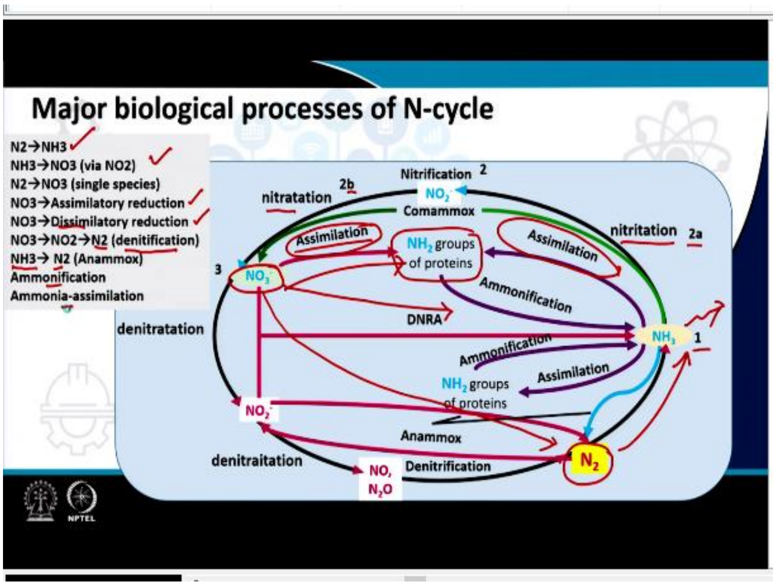

Now these are the following are the major processes that possibly are we found that and regarding the denitrification and anammox and ammonification and ammonia assimilation we will briefly discuss about them in subsequent slides. So, essentially these are the processes what we found that nitrogen to ammonia which is the job of the nitrogen fixers or nitrogen fixing bacteria then ammonia is oxidized to nitrate via nitrite.

So, these are what you called ammonia oxidizing bacteria. Then we have nitrogen to nitrate it could be single species or it could be multiple species then you have the nitrate assimilatory reduction to ammonia and this ammoniacal nitrogen will be taken up by the cells or nitrate could be reduced to ammonia which will be released outside this is called dissimilatory reduction.

Alternatively nitrate could be reduced completely to nitrogen which is called denitrification, for soil for example denitrification is not a good one because that will reduce the soil nitrogen, but for a wastewater treatment process this denitrification could be very useful because there will be able to reduce or diminish the nitrogen content of the water. There could be another process also converting ammonia to nitrogen through NO 2 that is called the anammox process. We can have also the ammonification and ammonia assimilation by the cells.

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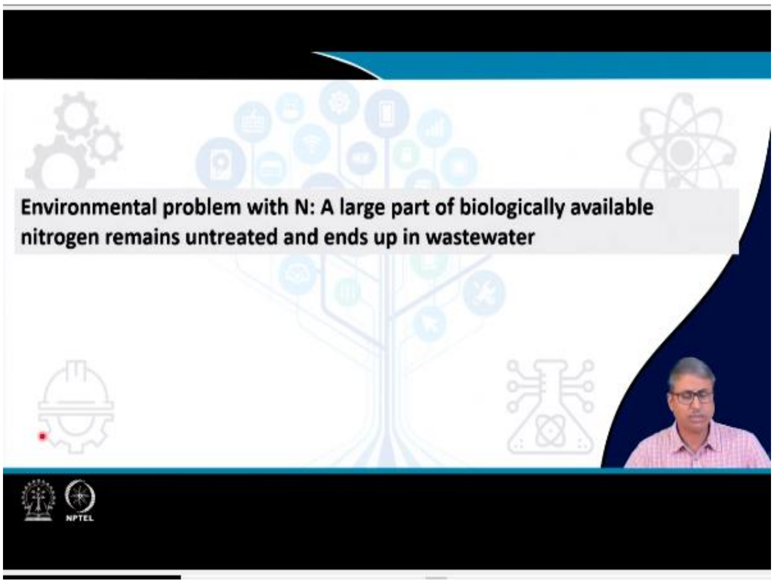

Now the environmental problem with nitrogen; why nitrogen is a concern? I mentioned earlier that nitrogen could be a typical nutrient pollution; it can lead to nutrient pollution because a large part of the biologically available nitrogen remains untreated and ends up in the waste water. So, unless we have some treatment processes nitrogen is released through the all kind of waste through the fecal materials and all other organismic waste.

So, all organisms that they discharge these different forms of nitrogen and this nitrogen ends up along with the industrial processes into the waste water and our river and all these things. In addition to that there are agricultural activities which are also responsible because in agricultural sector we often use huge amount of nitrogen fertilizer and those fertilizer etcetera remain unused by the soil microbiota and the plants. And during the runoff with excess water or during the normal agricultural processes also this nitrogen ends up into the ground water or the nearby water bodies.

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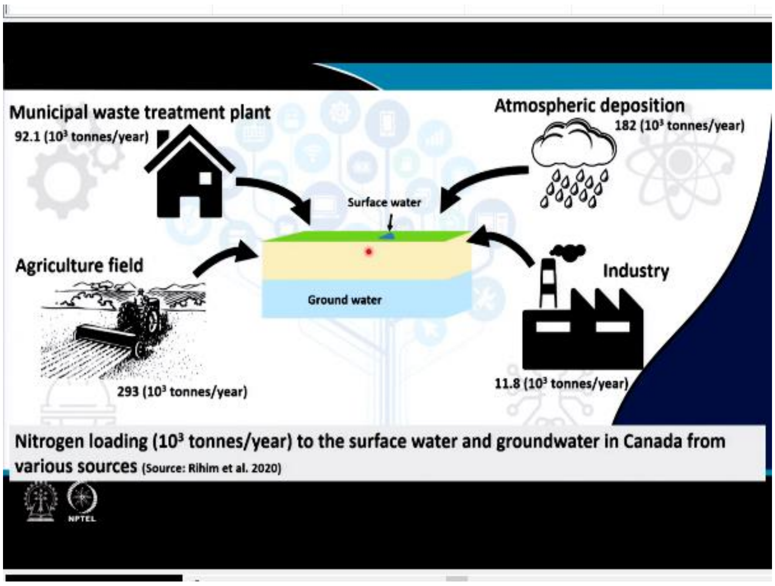

So, there is a recent part, recent study from canadian systems that the nitrogen loading in terms of 10 to the power 3 tons per year is presented and that nitrogen which is amounted to like 1000 tons per year you need to the surface water and ground water and you can see that the contribution from different components are there but the most significant is the agricultural field it is around 300 100 tons per year nitrogen being unnecessarily added into the system.

That is entering into the system and then that is moving into the ground water, moving into the water bodies and moving into all around the system along with the atmospheric deposition industrial activities and municipal waste treatment plants. So, all around this activities we have sufficient or very high amount of nitrogen which is added into the environment and when it ends up into some sinks like ground water system or in aquatic habitats more importantly it leads to severe environmental deterioration.

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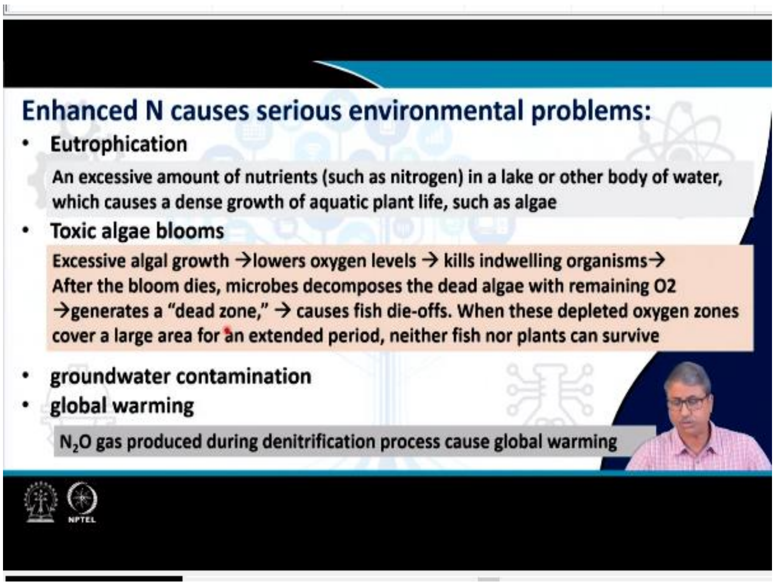

Now enhanced nitrogen causes serious environmental problems, what are these problems? One of the most severe consequences is the eutrophication where we see that excessive amount of nitrogen for example the nutrient the nitrogen as well as the phosphate lake or other water bodies causes a dense growth of aquatic plant life like algae followed by other higher macrophytes and other organisms.

Toxic algal bloom is another severe problem because when you have this extra nitrate along with phosphate that promotes the growth of the algae reduces the oxygen because during the night time the algae respires using the dissolve oxygen. So, as the oxygen level drops the indwelling organisms fish and other aquatic animals they start dying and including the other organisms the microbes which require the oxygen.

After the bloom dies also the algal bloom when it is only dead the microbes decomposes the dead algae with remaining oxygen being consumed that generates basically a dead zone and formation of the dead zone in the sedimentary region or in the below water region causes all the fish and other aquatic animals to die off and when these depleted oxygen zones cover a large area for an extended period neither fish nor plants can survive in that.

So, those ecosystems are completely degraded and deteriorated eventually and many other undesirable natural activities start occurring in those places. So, it actually spoils the entire ecosystem of the aquatic bodies mainly. In ground water also it contaminates and it actually perturbs with the normal ground water microbial and bio geochemical activities and it also leads to the global warming by N 2 O gas for example which is produced during denitrification process and is responsible for the global warming.

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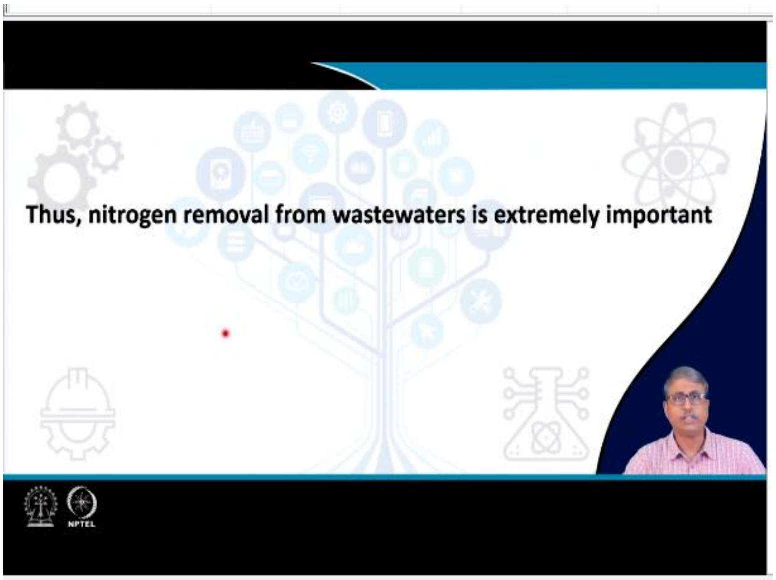

So, overall nitrogen is a problematic nutrient, it is nutrient at low concentration but when it is there is a higher concentration unnecessarily it causes severe problem. So, therefore nitrogen removal from waste water is found to be extremely important because within the wastewater the things are under our control. So, like a enhanced phosphate removal process or phosphorus removal process scientists have developed the enhanced nitrogen removal process.

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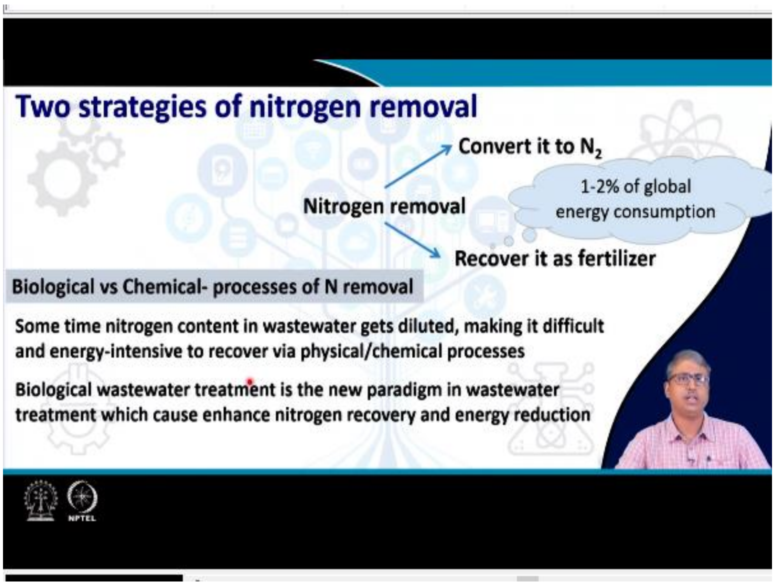

Now 2 strategies of nitrogen removal can be envisaged. One is as I mentioned earlier that ammoniacal nitrogen or nitrate or nitrite nitrogen can convert to dinitrogen and dinitrogen is a gaseous form. So, it leaves the system. So, any kind of nitrogen which is there in your waste water can be converted to N 2 and N 2 will leave the system. The other one is you convert the nitrogen into fertilizer and then reuse it. Try to use that as a nitrogen fertilizer.

And nitrogen fertilizer however is a energy intensive process and it is considered to be 1 to 2% of global energy is consumed during the production of fertilizer from different nitrogen compounds. Now here if we look at the biological and the chemical processes of nitrogen removal chemically you can convert the nitrogen into fertilizer, you can convert the nitrogen also to dinitrogen chemically, you can also convert biologically. Now sometime the nitrogen content in the waste water gets diluted, so if it is very much diluted then it would be difficult and energy intensive to recover via the physical or chemical processes.

Sometimes we adopt physicochemical processes for recovering nitrogen when it is cost effective. But in that case the nitrogen concentration should be higher, but if the nitrogen concentration is low because the waste water is diluted then it is not very efficient process. Also the biological waste treatment is the new paradigm in wastewater treatment which causes the enhanced nitrogen recovery and also the reduction in the energy cost particularly the energy expenditure for the entire process.

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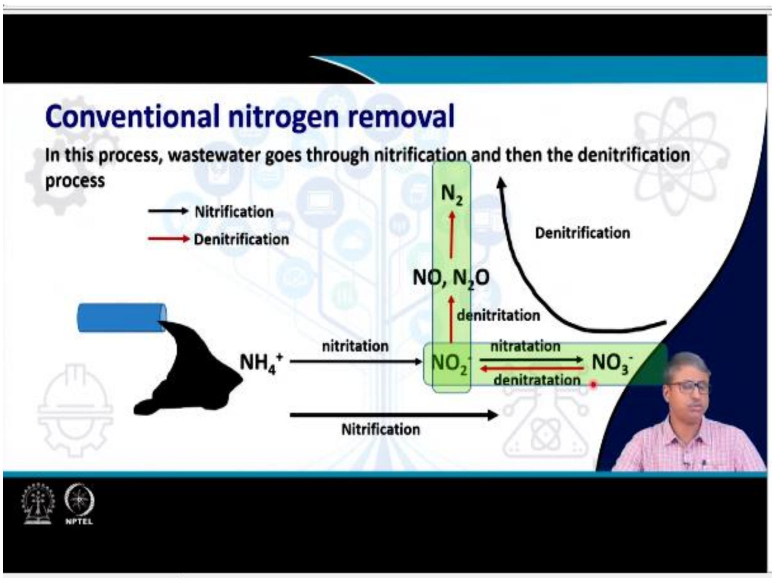

Now what happens if we think of a nitrogen removal from waste water using microbial process or biological process? So, here biological processes refers to mainly the microbiological processes. Now the conventional nitrogen removal is presented over here and in this process you can see the wastewater goes through 2 processes which is one is called the nitrification and then the denitrification process.

So, I will explain them step by step. So, you have the waste water full of ammonia, this is one of the most prominent form of reduced nitrogen present in the waste water roasted fluid, so you can convert this ammonia into nitrate. That is called nitrification. It is a 2 step process in which first the nitration occur that is an ammonia is converted to nitrite and then the nitrite is further oxidized to nitrate.

So, first we oxidize the ammonia to nitrate and then we allow the nitrate to be reduced to nitrogen. That is called the denitrification, again via the nitride only. So, it is actually having a common intermediate that is called the nitrite. In one side we are using subject the ammonia to the ammonia oxidizing bacteria and producing the nitrite through nitritation and then the nitrite which is produced is further oxidized to nitrate.

And the other way we are converting the back the nitrate to nitrogen via the nitrite again. So, these are the 2 prominent processes which are coupled. So, because first the ammonia has to be converted to nitrate and the nitrate will be denitrified because most of the microbes which actually converts this ammonia to nitrite some of them or they live along with the organisms which are able to perform this nitration and nitritation will allow the nitrite to be further oxidized to nitrate.

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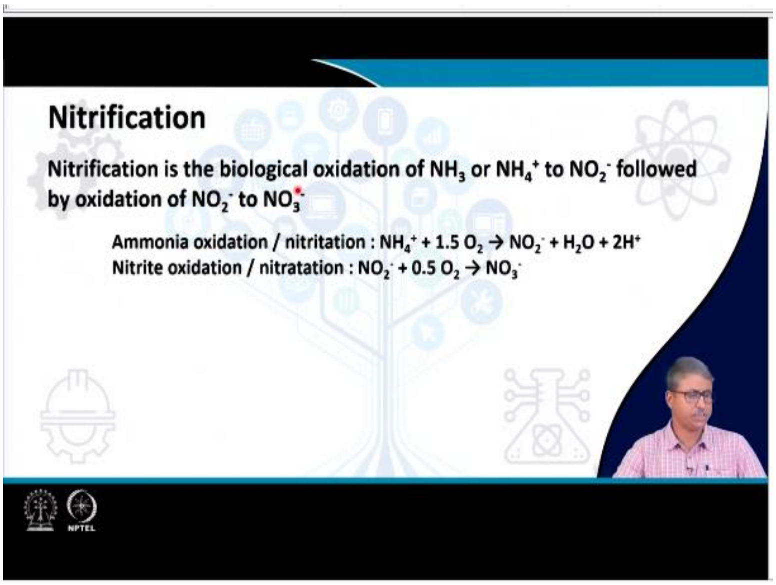

Now nitrification is a biological oxidation rather is a microbiological process of oxidizing ammonia or ammoniacal nitrogen to nitrite followed by oxidation of nitrite to nitrate. So, it is basically 2 step reaction.

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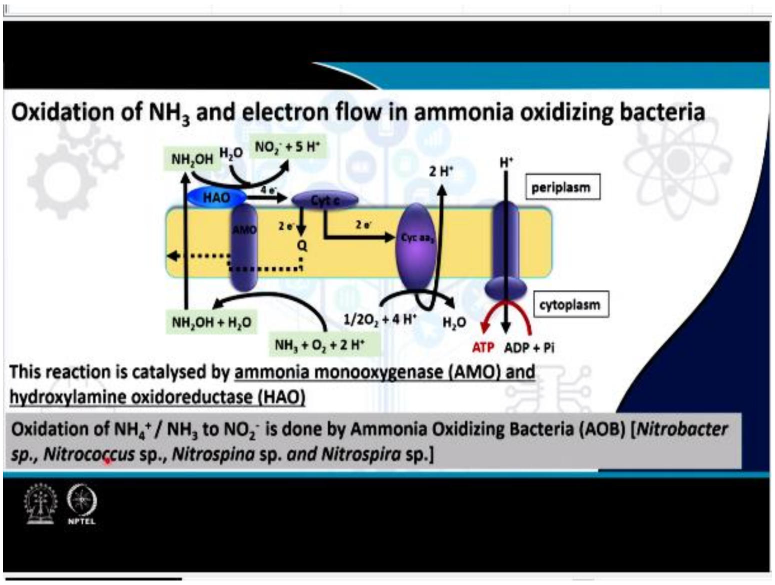

And if we look at the detail of the process of the electron transfer we see that it has actually very interesting step in which the ammonia reacts with the oxygen and it is converted to this hydroxyl amine. The hydroxyl amine actually enters into the periplasmic space where this hydroxylamine oxidoreductase enzyme which is responsible for oxidizing these further to the NO 2. And then the NO 2 will be further subjected to transformation reaction.

Now the oxidation of ammonia or the ammoniacal nitrogen to NO 2 is done by the ammonia oxidizing bacteria or AOB. So, this is the first phase of the reaction where ammonia is converted by the ammonia monooxygenase enzyme AMO or and the hydroxylamine oxidoreductase HAO. So, 2 enzymes are involved in these ammonia oxidizing microbes. They have these 2 enzymes and they are able to convert this ammonia to nitride. And these bacteria are mostly identified as nitrobacter species Nitrococcus, Nitrospina and Nitrospira like members.

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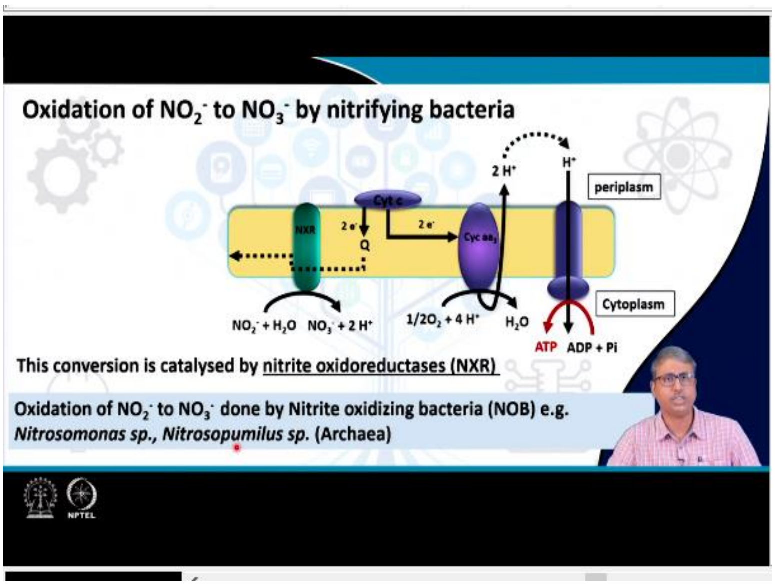

Now in the second step the nitrite is converted to nitrate by the nitrifying bacteria in which we can see again that the nitrite is further converted to nitrate with the use of the electron transport system and we can use this some of the protons which are actually generated out of the proton motive force can be can be utilized in terms of the proton motive force and it can generate ATP out of it.

So, the microorganisms who are responsible for this get some energy benefit out of this. So, they are capable of doing this. Now the enzyme which is responsible for this process is called nitrite oxidoreductase and you can see this nitrite oxidoreductase is capable of oxidizing the nitrite to nitrate and the organisms like nitrozomonas and the archaea nitroso cumulus are found to be responsible for carrying out specifically this step of the nitrite oxidation to nitrate.

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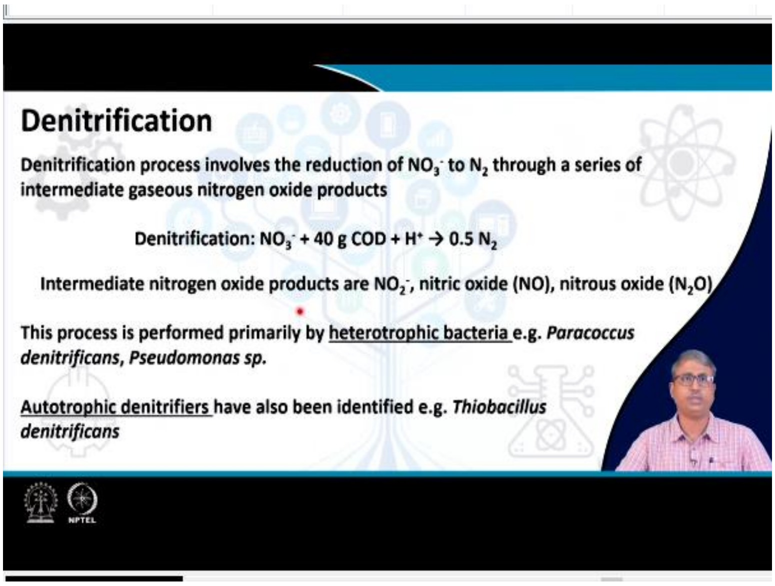

Now there is also another process which is called denitrification. So, the nitrate which is produced will be converted to nitrogen. That is the final step where we want to remove the nitrogen. So, ammonia to nitrate and then nitrate will be denitrified to nitrogen. Now nitrification process involves the reduction of the nitrate to nitrogen through a series of intermediate gaseous nitrogen oxide products.

And the intermediate nitrogen oxide products are for example NO 2 nitride oxides like NO and nitrous oxide into O, some of them are important for as an environmental pollutants as well. Now this process is performed primarily by heterotrophic bacteria but autotrophic bacteria like the thiobacillus denitrificans etcetera are also found to be involved. Among the heterotrophic microorganisms capable of denitrifying that is converting this nitrate to nitrogen which is ultimately providing the solution to this nitrogen problem is catalyzed by the organisms like paracoccus denitrificans or pseudomonas like organisms.

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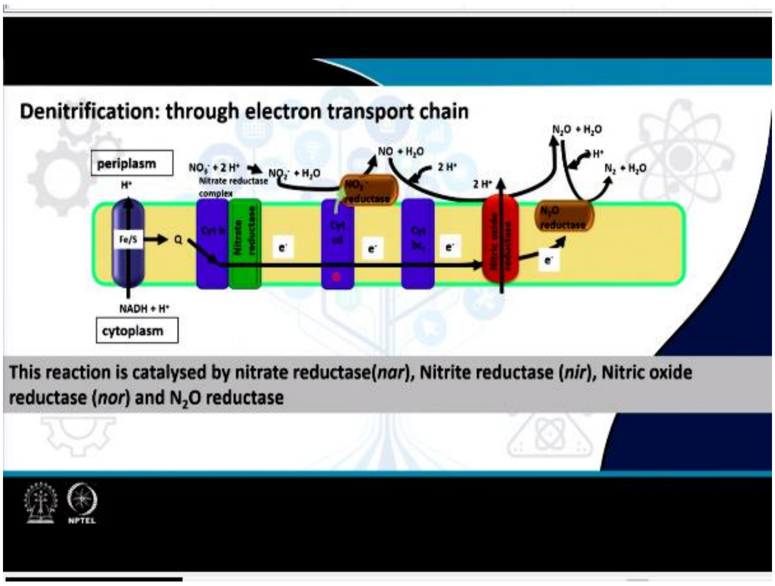

o, here is the process of denitrification through electron transport chain and you can see that the nitrate which is eventually been reduced in a multiple steps by the electron carrier complexes which are also acting as the oxidoreductase enzymes and they are all located on the membrane where electron transport process are going on and they are highly dependent on the supply of the reducing power or the electrons from NADH + H +. Now this reaction is catalyzed by the primarily by nitrate reductase nor nitrite reductase nir nitride oxidoreductase nor and N 2 O reductase.

So, number of reductases are there because I it is a multi step process where nitrate will be eventually reduced to nitrogens, we cannot reduce directly a nitrate to nitrogen, it has to be done in multiple steps. So, multiple oxidoreductase enzymes are used who are located distinctly within the electron transport chain and the organisms like paracoccus or pseudomonas who are capable of performing this reactions they have all these enzymes through which they utilize this nitrate as electron acceptor often during this denitrification process.

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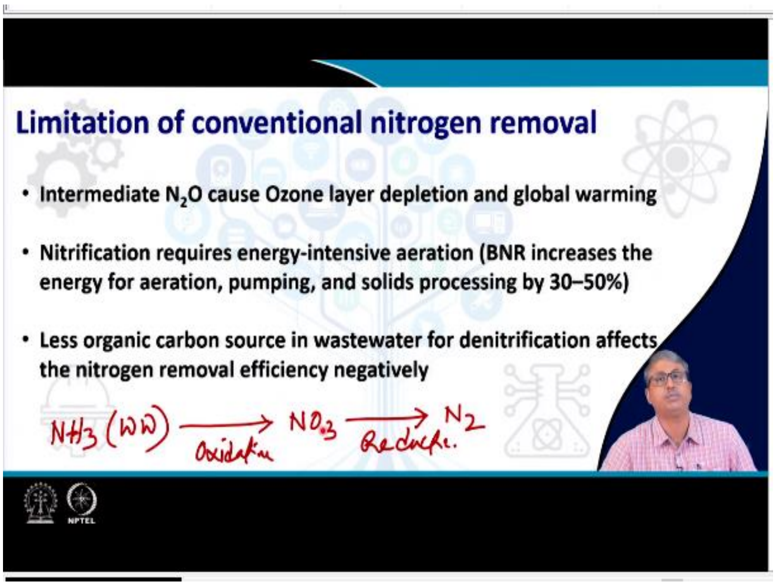

Now so we have understood that possibly this is one of the means that through which the nitrogen can potentially be removed that you have this ammonia which is present in the waste water. So, your waste water must be having lots of ammonia, so that will be initially converted to nitrate and then the nitrate will be converted to nitrogen. So, here first you have the nitrification process and then you have the denitrification process.

So, ultimately the denitrification, so first part is oxidative process and the second part is reductive process and a separate set of microorganisms are involved for these 2 distinct phases of the reactions. Now the intermediates for example the N 2 O which is responsible for causing a ozone layer depletion has been reported. So, these are some of the important limitations that we are right now highlighting that the N 2 O causes the ozone layer depletion and responsible for global warming.

So, that is the intermediate somewhere here in the nitrate reduction. So, nitrification requires energy intensive aeration like the oxidation of ammonia to nitrate in between we have the nitrite as well, so we need supply of oxygen aeration. So, BNR biological nitrogen removal increases the energy for aeration, pumping and solid processing because lot of biomass growth will be there and those needs to be separated out. Less organic carbon source in wastewater for denitrification affects the nitrogen removal efficiency negatively.

So, basically nitrate reducing bacteria most of them they are heterotrophs. So, they need the supply of electrons because here we are trying to reduce nitrate and this reduction of nitrate would essentially means that there will be supply of electron donor and this electron and carbon donors are the heterotrophic organisms or through the heterotrophic organisms they utilize the organic carbon which is present. So, if the organic carbon concentration of the waste water is low then possibly the denitrification process will suffer.

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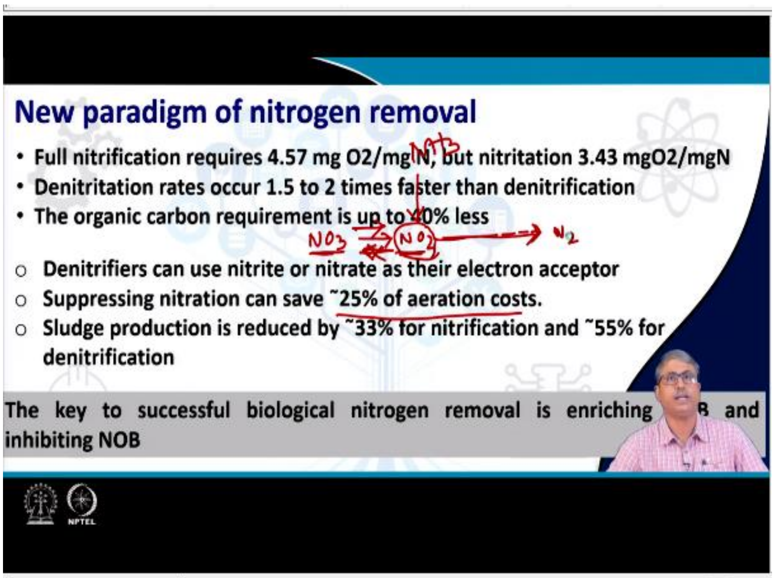

Now we will talk about the new paradigm of nitrogen removal. So, we identified that this could be ammonia to nitrate and nitrate to nitrogen could be a affordable process but there are certain technical issues and issues related to the energy expenditure in particular. Also the presence of N 2 O which is considered to be a major concern of from the global warming point of view as a is a greenhouse considered to be an important component.

Now the full nitrification requires 4.57 milligram oxygen per milligram nitrogen but nitritation requires only 3.43 milligram because it goes via nitrite. So, denitritation rates occur 1.5 to 2 times faster than the denitrification and the organic carbon requirement is up to 40% less, so there could be some strategies. So, denitrifiers can use nitrite or nitrate as their electron acceptor.

That means the organisms who are considered as denitrifier, so the denitrifiers are those who are reducing this nitrate to NO 2 and then possibly 2 nitrogen. So, most of them who are reducing nitrate can reduce nitrite also. Now why we are focusing on nitrite or we are thinking of focusing on nitrite because nitrite can directly be produced from ammonia. Because if we look carefully when ammonia is oxidized by AOB ammonium oxidizing bacteria nitrite is produced and then the nitrite is further oxidized to nitrate.

So, one of the alternatives coming up that instead of oxidizing it up to nitrate if we just oxidize up to nitrite and then allow the nitrite to be converted to nitrogen then that could actually help us, because suppressing the nitration can save 20% of the aeration cost because this part would otherwise require lot of oxygen because nitrite to nitrate is an oxidation process. So, if we stop this lot of savings will come in terms of the aeration cost, also this large production is reduced by 33% for the nitration and 55% for denitrification.

So, the entire this way and this way 2 processes are actually reduced. So, one of the strategies that was planned and developed is that allow the conversion of ammonia to nitrite and then nitrite be converted to nitrogen. Now the key to this successful biological nitrogen removal is basically enriching AOB ammonia oxidizing bacteria and inhibiting the nitride oxidizing bacteria because we do not want nitrite to be oxidized to nitrate.

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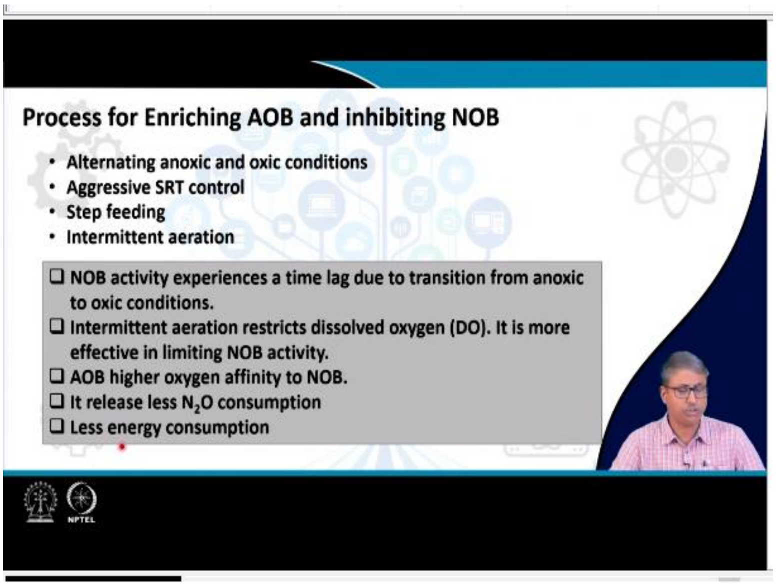

Now process for enriching AOB ammonia oxidizing bacteria, so that ammonia will be converted to nitrite and inhibiting the NOB's that is the nitride oxidizing bacteria, so that we will stop the production of nitrate. Now alternating the anoxic and oxygen condition that might help aggressive SRT control, step feeding and intermittent aeration. These were found to be useful. Now NOB that is the nitrite oxidizing bacterial activity experiences a time lag due to transition from the anoxic to oxygen conditions.

Intermittent direction restricts the dissolved oxygen, it is more effective to limit the NOB activity and AOB higher oxygen affinity for rather than to NOB and it releases less N 2 O from the system and also less energy consuming process.

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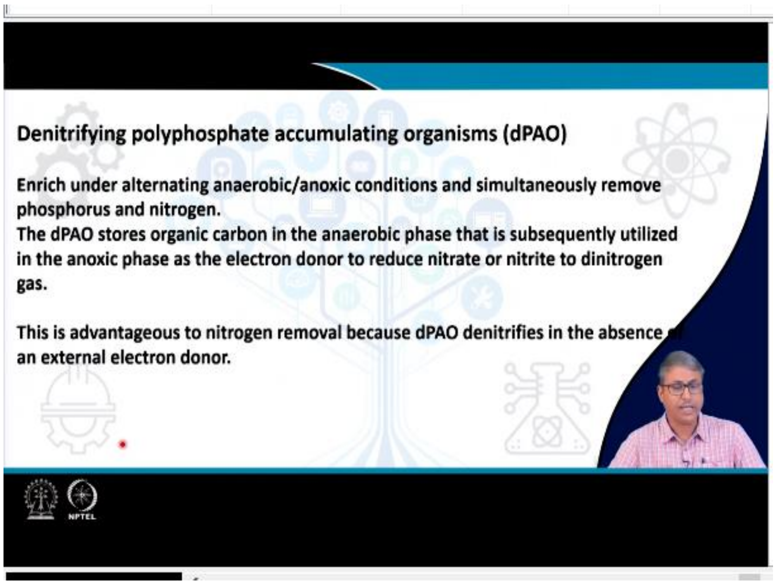

The other aspect of this is the denitrifying polyphosphate accumulating organisms. During our enhanced biological or microbiological phosphate removal lecture we have already learned that how this phosphate removing bacteria like acumuli vector can actually accumulate large number of the polyphosphate bodies. Now during that polyphosphate accumulating process many of these bacteria they under the anoxic growth, they actually can use nitrate as their electron acceptor if the oxygen level is slightly lower.

And they actually consume the nitrate and they also accumulate the phosphate which is present there as a polyphosphate bodies. So, that is another option; that during the process of the enhanced phosphorus removal we can actually integrate the process of denitrification also or encouraging those organisms including accumuli vector for example which can serve both the purposes of removing nitrogen as well as the phosphate.

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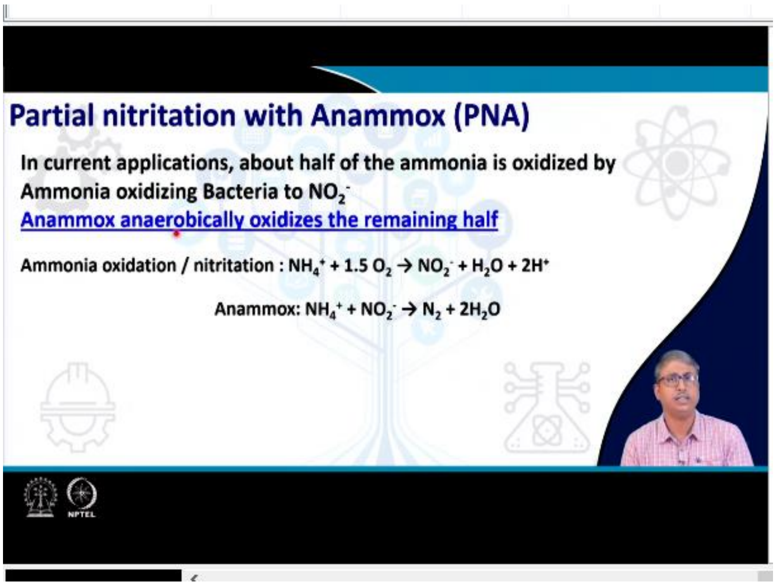

The second one is the partial nitration with anammo that is abbreviated as PNA partial nitration with anammox. Now in current applications about half of the ammonia is oxidized by ammonia oxidizing bacteria to NO 2. Anammox is a system or a type of organisms which catalyze the oxidation of the rest of the ammonia which is their anaerobically, so that the complete ammonia is oxidized. So, half of the ammonia which is present there is oxidized by the aerobic ammonia oxidizing bacteria or AOB to nitrite.

So, remaining half of the ammonia can be oxidized under anaerobic condition and that oxidation takes the advantage of having nitrite in the system because nitrite would anyway be there because ammonia when you oxidize aerobically ammonia is converted to NO 2 and then that NO 2 can be utilized by this ammonia oxidizing anaerobic bacteria to produce nitrogen.

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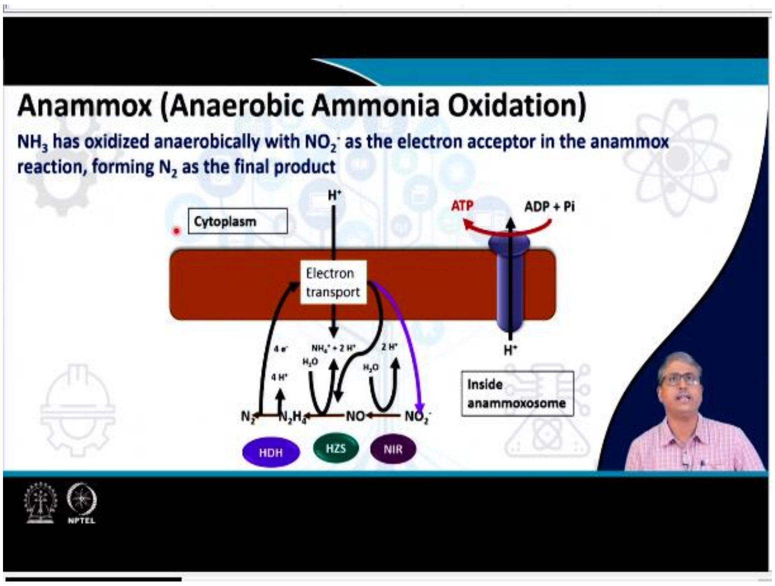

So, here is the anammox process; you can see that ammonia is oxidized anaerobically with nitrite as the electron acceptor in the anammox reaction forming nitrogen as the final product.

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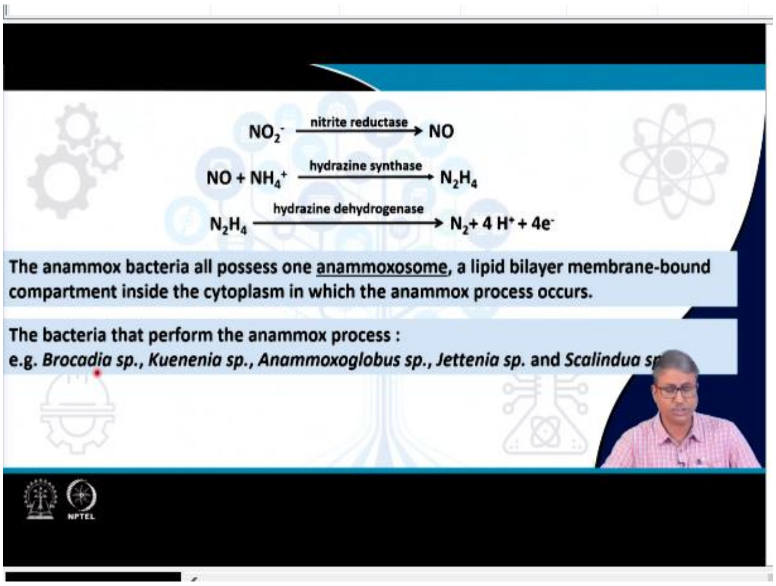

And these are the reaction steps, so it has multiple intermediates through hydrogen and you can see the name of the enzymes also like nitrites reductase hydrazine, synthesis and hydrazine dehydrogenase and the bacteria that perform the anammox process are of versatile or types but most of the time these are the species which we found to be most frequent. The anammox bacteria all posses one anammoxosome, a lipid bilayer membrane bound compartment inside the cytoplasm in which this anammox process generally takes place.

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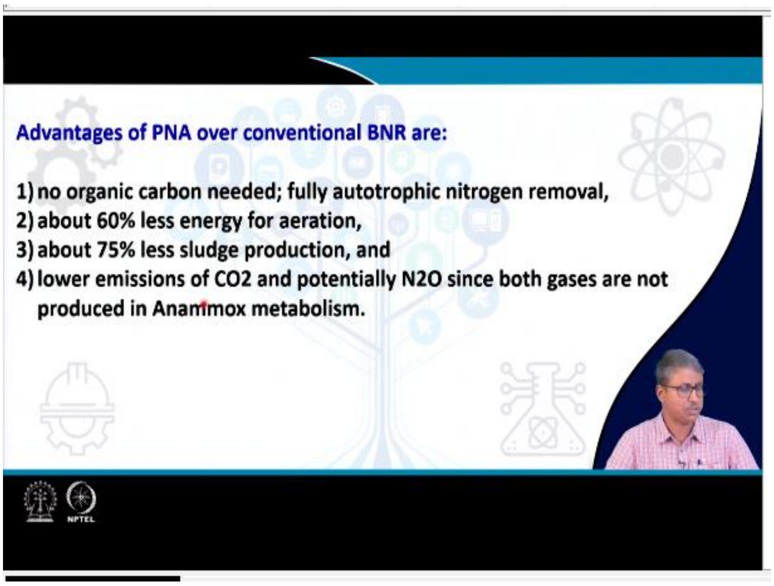

Now the advantage of this PNA or over the conventional BNR processes are no organic carbon is required; fully autotrophic nitrogen removal, because it is distributed into 2 segments, about 60% less energy for aeration because we are avoiding the synthesis of nitrate out of nitrite. About 75% less sludge production and lower emission of CO 2 and potentially the hazard has N 2 O since both gases are not produced in anammox metabolism.

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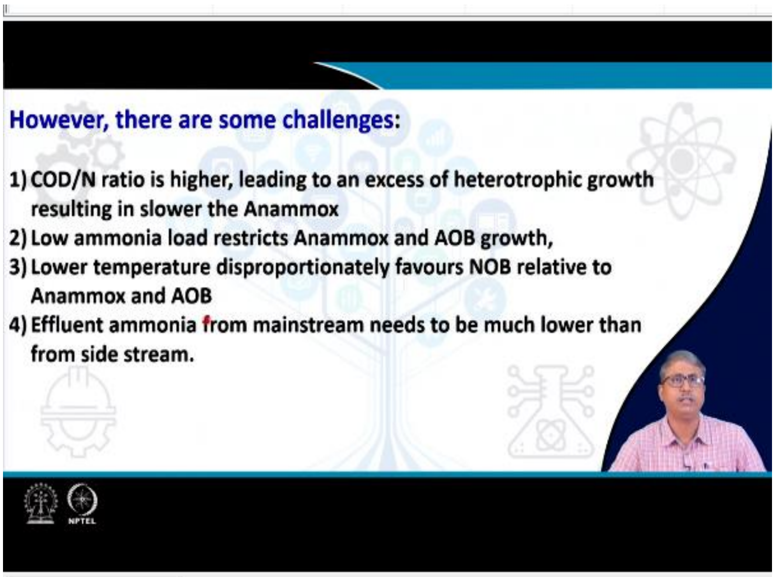

However, there are some challenges also associated with these processes like COD to N ratio which is important criteria, this might lead to an excess of heterotrophic growth resulting in slowing down the anammox process, low ammonia load restrict the anammox and AOB growth, low temperature, effluent ammonia from mainstream needs to be much lower than from side stream. So, these are certain technical aspect and also certain important parameters for the microbiological and catalytic processes present there.

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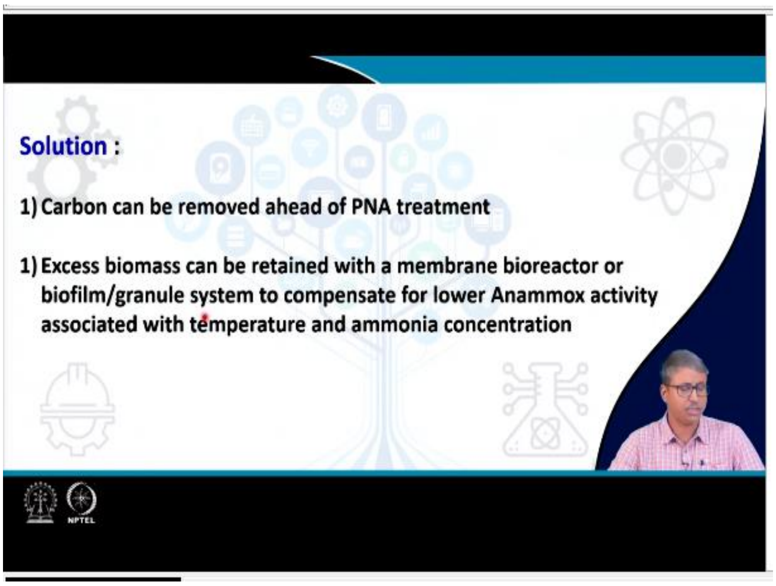

And for that some solutions have been developed by the environmental engineers and environmental biotechnologist like the carbon can be removed ahead of PNA treatment like hydrocarbon biodegradation or how activated sludge treatment or using the aerobic bacteria, heterotrophic bacteria to aerobic mostly to metabolize the carbon in presence of oxygen. Excess biomass also can be retained with a membrane bioreactor or biofilm granule system to compensate for lower anammox activity associated with temperature and ammonia concentration.

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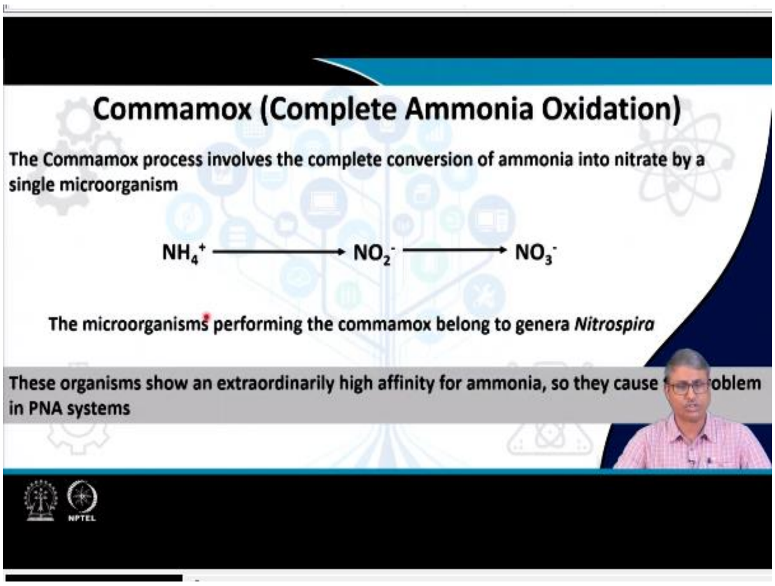

There is another process which is actually of concern to these engineers and environmental biotechnologist working towards biologically enhanced or biologically catalyzed nitrogen removal is the Commamox or complete ammonia oxidation. The Commamox process involves the complete conversion of ammonia into nitrate by a single microorganism. So, ammonia is directly converted to nitrate instead of nitrite. So, if you do not have this step then possibly the scope of denitrification is also gone, so you have to again come back all the way down here and then get into this nitrogen.

So, the microorganisms who are performing the Commamox belong to the mostly the general Nitrospira and these organisms they show an extraordinary high affinity for ammonia. So, they cause the problem in PNA system because they do not allow any ammonia to be left for any other activities and this part oxidation then is not possible, it consumes more oxygen and it is enabling the cells to converting ammonia to nitrate.

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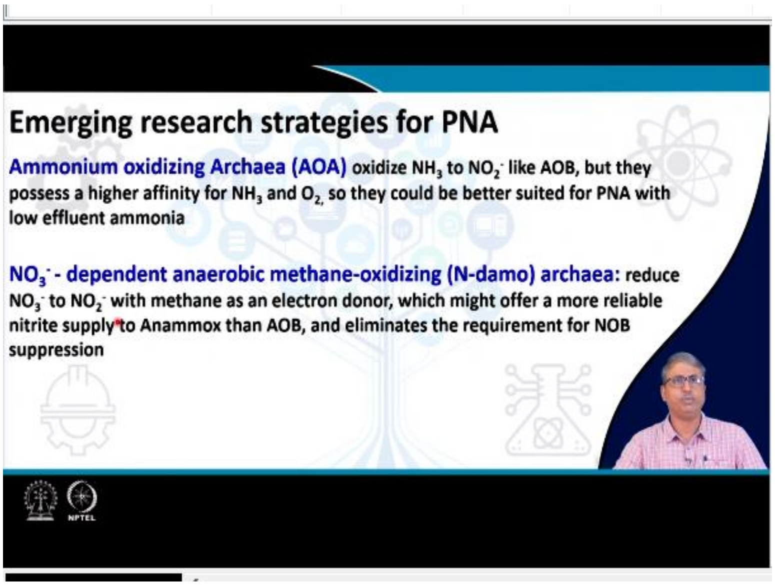

And then subsequently the nitrate will be denitrified. Now emerging research strategies for PNAs will be discussed. One aspect is the ammonia oxidizing archaea we call them AOA which basically oxidize ammonia to NO 2 like AOB but they possess a higher affinity for ammonia and oxygen. So, they could be better suited for PNA with low effluent ammonia and also nitrate dependent anaerobic methane oxidizing archaea that reduce the nitrate to NO 2 with methane as an electron donor which might offer a more reliable nitride supply to anammox than AOB and eliminates the requirement for NOB suppression.

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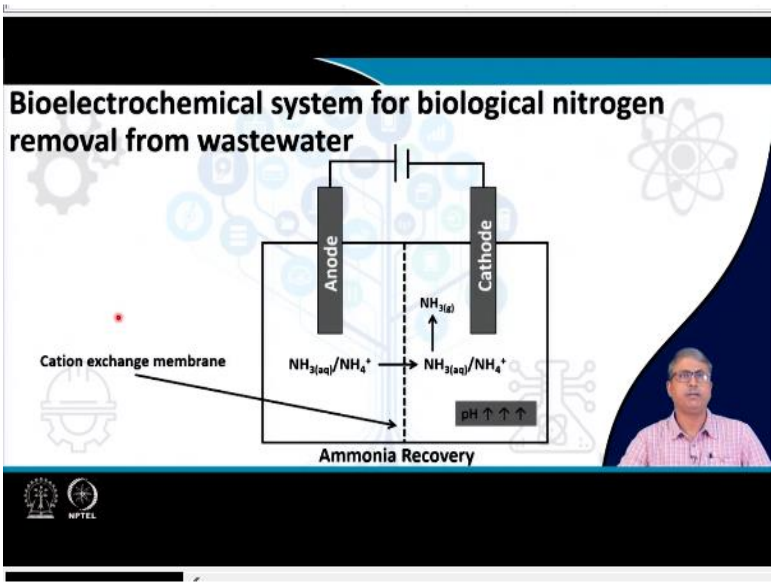

Recently we see that there are developments with respect to bioelectrochemical systems for biological nitrogen removal from the waste water; where we can see that the use of the anode and cathode and we have the cation exchange membrane which can allow the the ammonia which is produced or present there can be moved into this cathodic chamber.

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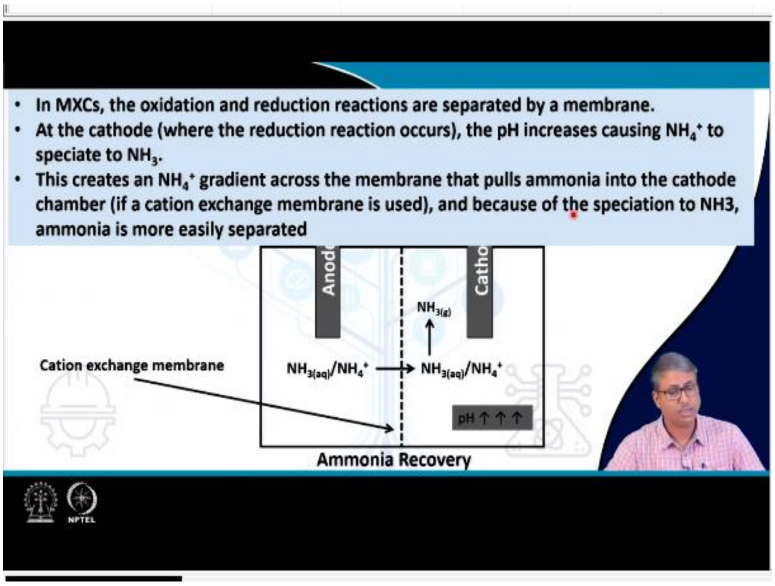

And then if we raise the pH for example then most of the ammonia can be recovered from this system and having this kind of cation exchange membrane creates the ammonia gradient across the membrane and the ammonia can be put in place into the cathode chamber and it can be speciated into a from the ammonium to ammonia and the ammonia can be separated out easily.

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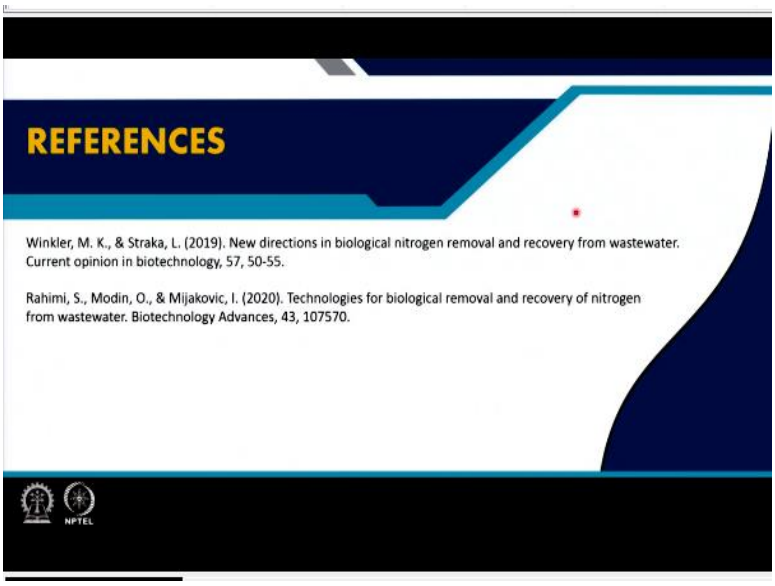

So, there are a couple of similar recent developments are also there. So, overall for this lecture we will be using these 2 references.

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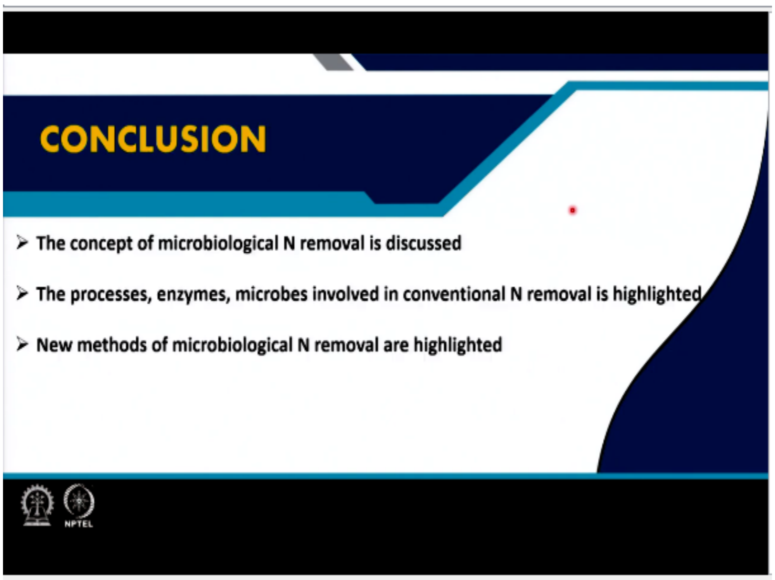

And in conclusion the concept of microbiological nitrogen removal is discussed, the processes enzymes, microbes involved in the conventional nitrogen removal is highlighted and also we have discussed about the new methods of the microbiological nitrogen removal, thank you.