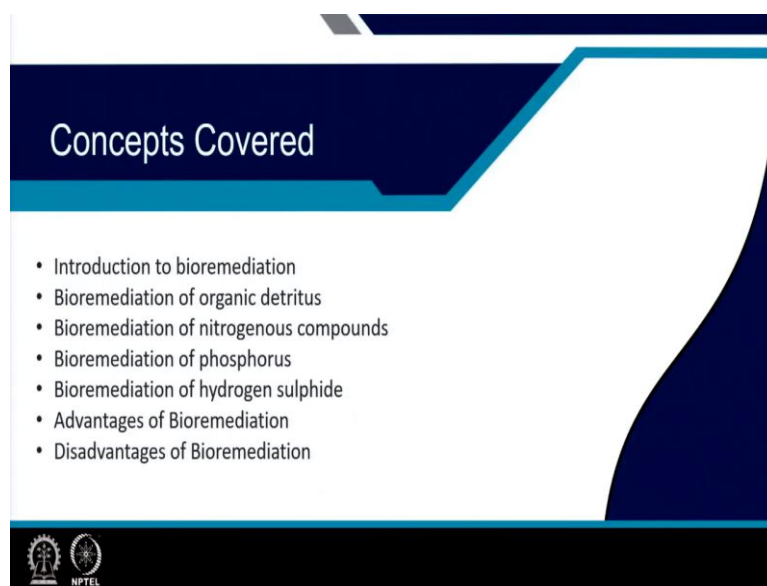


Advanced Aquaculture Technology
Professor Gourav Dhar Bhowmick
Department of Agriculture and Food Engineering
Indian Institute of Technology Kharagpur
Lecture 48
Bioremediation

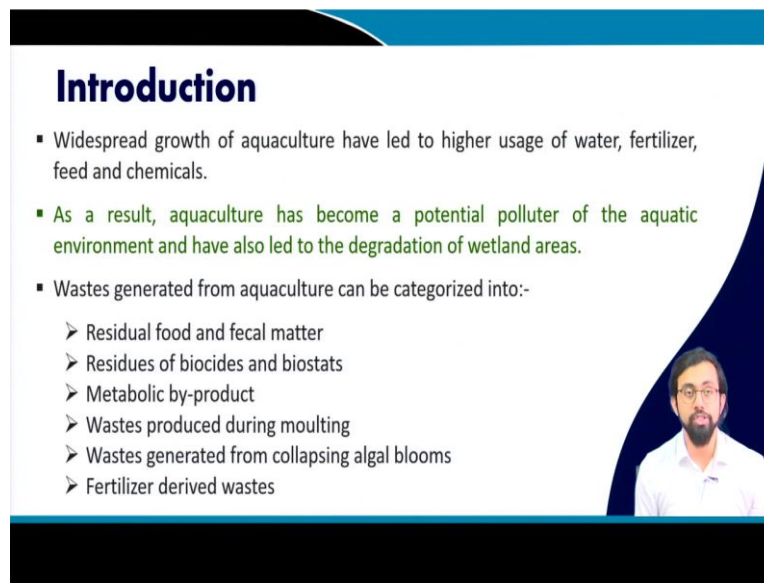
Hello, everyone. Welcome to the third lecture material of the module 10 Technology of Cleaner Production. My name is Professor Gourav Dhar Bhowmick, I am from the Agricultural and Food Engineering Department of IIT, Kharagpur.

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The concepts that I will be covering in this particular lecture material are the introduction to the bioremediation, bioremediation of organic detritus, bioremediation of nitrogenous compounds, bioremediation of the phosphorus compounds and bioremediation of the hydrogen sulphide present in the aquatic regions, and what are the advantages and the disadvantages of bioremediation.

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Introduction

- Widespread growth of aquaculture have led to higher usage of water, fertilizer, feed and chemicals.
- As a result, aquaculture has become a potential polluter of the aquatic environment and have also led to the degradation of wetland areas.
- Wastes generated from aquaculture can be categorized into:-
 - Residual food and fecal matter
 - Residues of biocides and biostats
 - Metabolic by-product
 - Wastes produced during moulting
 - Wastes generated from collapsing algal blooms
 - Fertilizer derived wastes

So, to start with, you know that the agriculture it is like an, the aquaculture is kind of a part of we know like this broader sense of environmental uses of human activities. So, it is more like, we use the water we use the energy source from the environment, and then we use it for some beneficial purpose. So, in this particular case, we use this energy, fertilizer, feed, chemicals, these things we use and we need to provide it to our aquaculture species for them to grow and survive naturally in artificially.

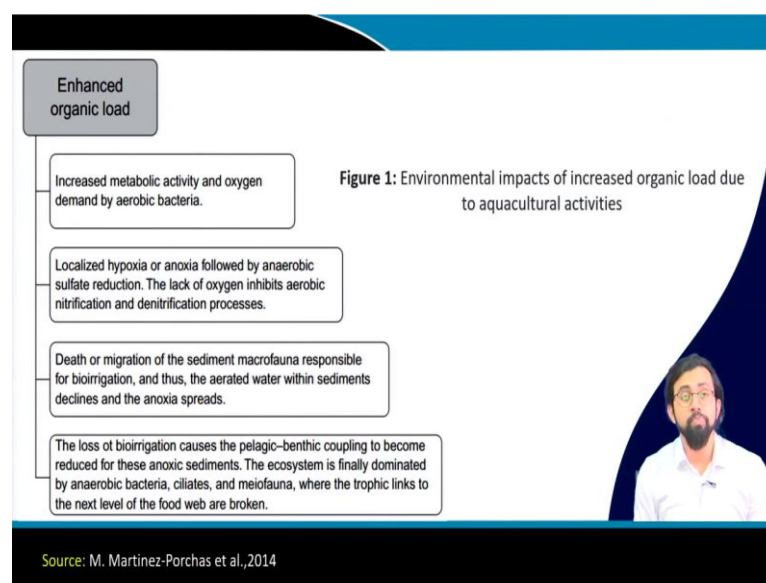
So, as a result, it becomes one of the major pollutants source like major polluter of the environment, because in aquaculture when they use these additional chemicals and when they use these compounds use these molecules, they actually does harm after the uses after they have been used in a form that we want and then the further products byproducts are actually sometimes toxic or can be considered as a pollutant to the systems because those are not naturally present in the aquatic system or even in general in the nearby surrounding environment.

So, that is why aquaculture is considered as one of the major polluters, even you will be shocked to know that it is one of the major sources of greenhouse emission as well. So, what are the wastes? What are the form of waste that is actually formed from this aquaculture industries? So, they can be actually categorized, majorly into the residual food and fecal matter, residues of biocides and the biostats, metabolic byproduct, their wastes produced during their moulting.

I hope you know moulting, moulting is like the crustaceans they get rid of their outer layer and to go ahead with the and when there is the formation of the new layer, and this outer layer actually considers a different kind of organic substances and which is called this moulting phenomenon, which can cause a huge waste generation in this kind of form.

Waste generated from collapsing the algal blooms, and also fertilizer derived waste. When you supply the additional fertilizer, the waste that has been generated due to that it also are one of the major source of pollution in the surrounding society.

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So, what are the environmental impact on the increased organic load due to the aquaculture activity? When there is increment in the load of organic, means like organic load in the aquatic body. It increases the metabolic activity and the oxygen demand of aerobic microorganisms. Localized hypoxia or anoxia happened followed by anaerobic sulfate reduction. This lack of oxygen actually inhibits all the aerobic nitrification process.

This nitrification process actually it needs oxygen as we already mentioned in earlier lectures, right? So, once there is an inhibition in the oxygen whereas there is a laser presence of the dissolved oxygen, it will cause this anaerobic microorganism to die off and because of that these processes will be drastically affected like this nitrification process.

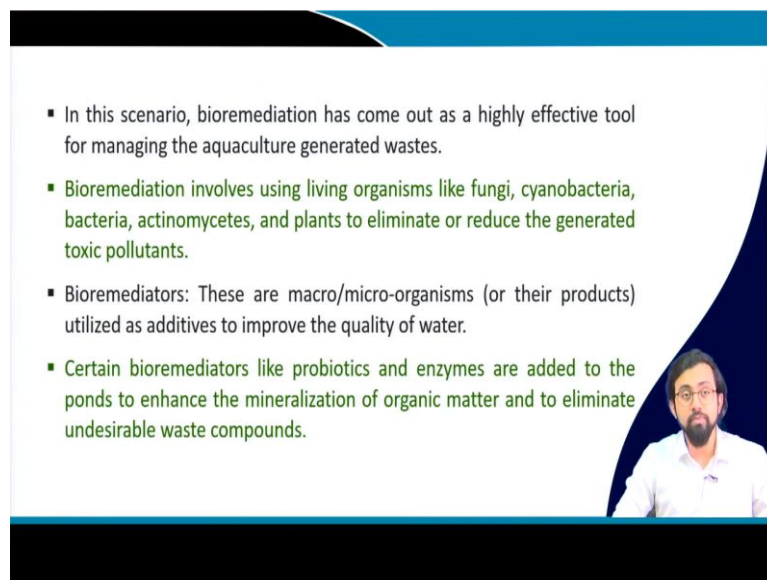
The death or the migration of the macrofauna are responsible for biodegradation is also one of the major reason. This sediment macrofauna they are actually majorly this benthic population are kind of, they can be considered as carbon sink even in the ocean floor, even as

small as even the pond structure. So, this benthic population, the population like the sediment microbiota, they are very much useful in different cells, it is actually helpful for us as well, in like passively they can actually help us as well in different ways.

So, this macrofauna's, they actually, they slowly migrated off, they slowly completely scrapped off from the systems because of this enhanced organic load, because of this enhanced organic load and the presence of anoxic condition, this biodegradation process we can lose and it will, what will happen, at the end, there will be an anoxic sedimentation, this anoxic sedimentation is what like this anoxic microorganism will start prevailing there and they will completely harm the whole food web system the food ecosystem there, because you will not find.

Because in general, this pelagic and benthic coupling which is considered as an pelagic zone and the benthic zone their coupling is very important like whatever is forming in the detritus in the benthic region that can be the food for the just the nearby I mean, like neighboring trophic chain. So, once you get rid of those food chain, once all of a sudden, your food the availability will be drastically changed, it will drastically affect your whole food web in general.

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- In this scenario, bioremediation has come out as a highly effective tool for managing the aquaculture generated wastes.
- Bioremediation involves using living organisms like fungi, cyanobacteria, bacteria, actinomycetes, and plants to eliminate or reduce the generated toxic pollutants.
- Bioremediators: These are macro/micro-organisms (or their products) utilized as additives to improve the quality of water.
- Certain bioremediators like probiotics and enzymes are added to the ponds to enhance the mineralization of organic matter and to eliminate undesirable waste compounds.

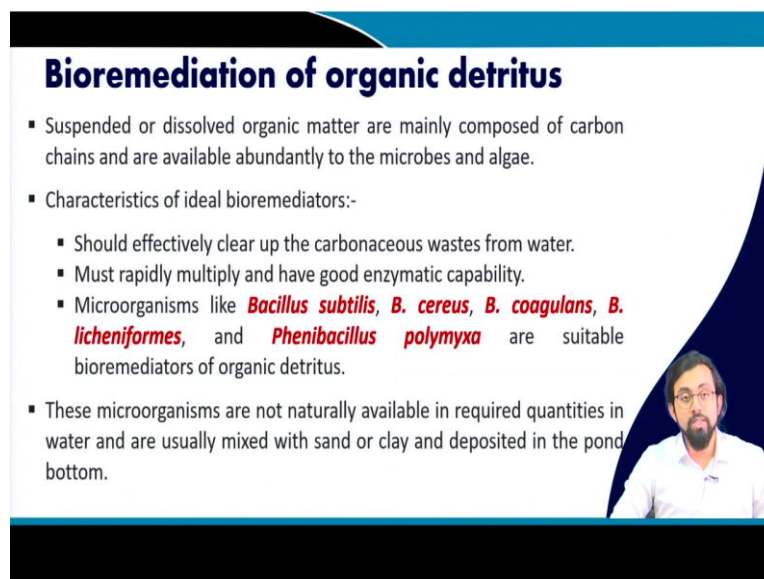
In this scenario, bioremediation actually considered as a very effective tool like for managing this aquaculture generated waste. What are the other way of doing that? Definitely, the different kinds of treatment unit we can install, this treatment unit will treat the water and

after the treatment is done then they again put back the water back to the water pond or water tank and because of that we can get rid of the unwanted pollutants.

Bioremediation is also one of the very highly efficient technique that we normally use it but it involves generally the living organisms like different fungi, cyanobacteria, normal bacteria, actinomyces, plants to eliminate and reduce the generated toxic pollutants. What are the bioremediators? Majorly they are the macro or microorganisms or their product or byproducts which utilized as additives to improve the quality of your pond or pond water or say like tank water where you are culturing your farm water.

Certain bioremediators like probiotics and enzymes are also added to the pond to enhance the mineralization of organic matter and to eliminate the undesirable waste compounds.

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Bioremediation of organic detritus

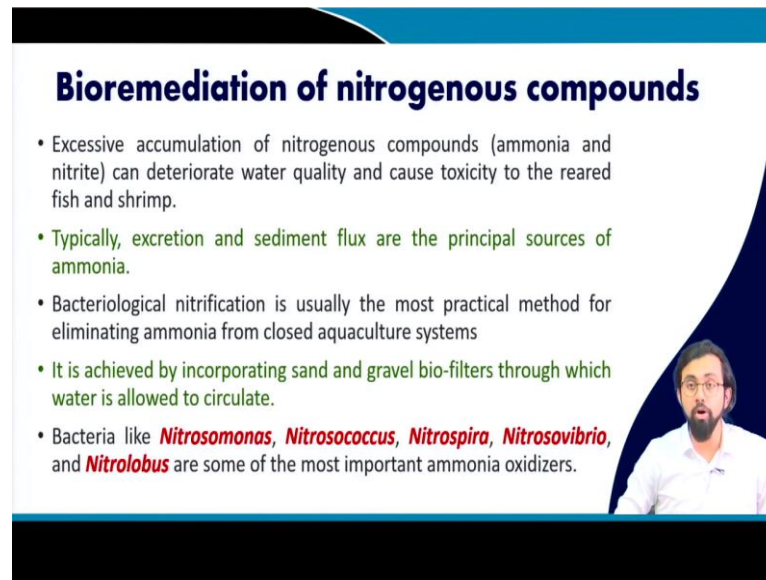
- Suspended or dissolved organic matter are mainly composed of carbon chains and are available abundantly to the microbes and algae.
- Characteristics of ideal bioremediators:-
 - Should effectively clear up the carbonaceous wastes from water.
 - Must rapidly multiply and have good enzymatic capability.
 - Microorganisms like *Bacillus subtilis*, *B. cereus*, *B. coagulans*, *B. licheniformes*, and *Phenibacillus polymyxa* are suitable bioremediators of organic detritus.
- These microorganisms are not naturally available in required quantities in water and are usually mixed with sand or clay and deposited in the pond bottom.

So, how we can bioremediate the organic detritus? What do I mean by the organic detritus? The majorly the suspended and the dissolved organic matter which are majorly composed of the carbon chains, they are abundantly available for the microbes and the algae. What is the characteristics of ideal bioremediators in this particular case?

Bioremediators, that it should effectively clear up the carbonaceous wastes from the water and also it should must rapidly multiply and have very good enzymatic capability, and the microorganisms like bacillus subtilis, bacillus cereus, bacillus coagulans, bacillus licheniformes, phenibacillus polymyxa, these are some of the very well-known and suitable bioremediators of organic detritus present in the benthic zone of pond.

This microorganism, it actually not naturally available, you have to provide them with artificially you have to provide them as along with the food additives. And you can generally mix it with the sand and clay and deposit it in the pond bottom.

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Bioremediation of nitrogenous compounds

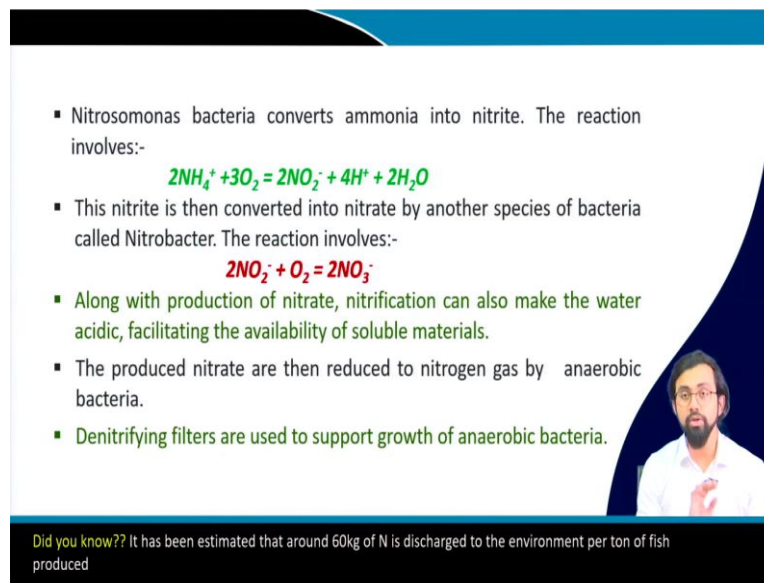
- Excessive accumulation of nitrogenous compounds (ammonia and nitrite) can deteriorate water quality and cause toxicity to the reared fish and shrimp.
- Typically, excretion and sediment flux are the principal sources of ammonia.
- Bacteriological nitrification is usually the most practical method for eliminating ammonia from closed aquaculture systems
- It is achieved by incorporating sand and gravel bio-filters through which water is allowed to circulate.
- Bacteria like *Nitrosomonas*, *Nitrosococcus*, *Nitrospira*, *Nitrosovibrio*, and *Nitrolobus* are some of the most important ammonia oxidizers.

What are the bioremediators for nitrogenous compounds? Excessive accumulation of nitrogen components say like ammonia or nitrate, what will happen, you know they are very much harmful, they can cause high amount of toxicity to your aquatic species rearing fish and streams. Therefore, we go for this bioremediation of this nitrogen compound, and what are the principal source of this ammonia, is the excretion and the sediment flux.

And also, the bacteriological nitrification is usually the most practical method for eliminating ammonia from closed aquaculture systems. In order to achieve this, what we need to do we need to provide the microorganisms like *Nitrosomonas*, *Nitrosococcus*, *Nitrospira*, *Nitrosovibrio*, *Nitrolobus*, so, these kinds of microorganisms they are very much helpful, they are most important ammonia oxidizer and they we can get help us get rid of the presence of ammonia in our farm.

It can also be achieved by sand or gravel by filters through which water is allowed to circulate or you can provide them with some biofilters made of some plastic bio medias as well. So, in general, the bacteriological nitrification is the best method that you should go through to eliminate any amount of ammoniacal nitrogen present in your system.

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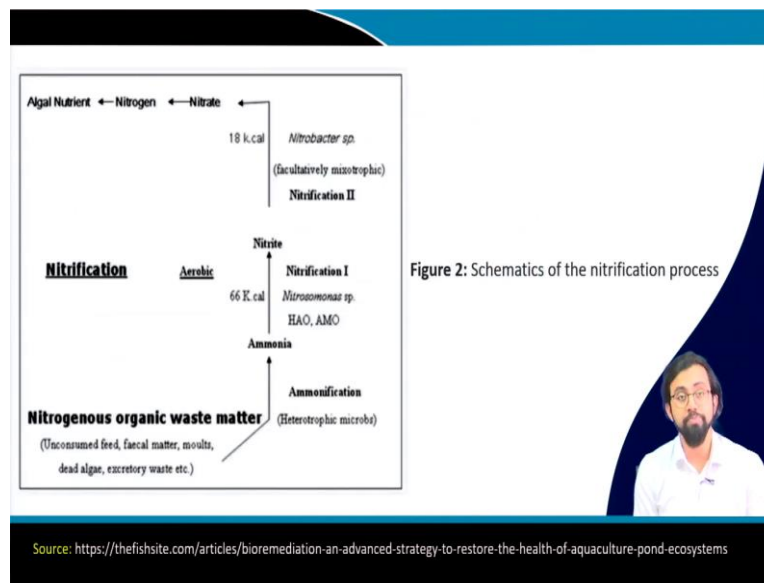
- Nitrosomonas bacteria converts ammonia into nitrite. The reaction involves:-
$$2\text{NH}_4^+ + 3\text{O}_2 = 2\text{NO}_2^- + 4\text{H}^+ + 2\text{H}_2\text{O}$$
- This nitrite is then converted into nitrate by another species of bacteria called Nitrobacter. The reaction involves:-
$$2\text{NO}_2^- + \text{O}_2 = 2\text{NO}_3^-$$
- Along with production of nitrate, nitrification can also make the water acidic, facilitating the availability of soluble materials.
- The produced nitrate are then reduced to nitrogen gas by anaerobic bacteria.
- Denitrifying filters are used to support growth of anaerobic bacteria.

Did you know?? It has been estimated that around 60kg of N is discharged to the environment per ton of fish produced

Further this Nitrosomonas bacteria we know that it converts ammonia into nitrite and this nitrite can be converted into nitrate by the help of Nitrobacter. These all are nitrifying organisms which works as in the presence of oxygen. And along with the production of nitrate, nitrification can also make the water acidic which actually helps the facilitating the availability of different soluble materials.

The produced nitrate are then reduced to nitrogen gas by anaerobic microorganisms, this is called the denitrification phenomena. In this denitrification phenomenon different denitrifiers are used, they actually help reducing the nitrate to nitrogen gas, this is a dinitrogen gas to be precise, and they actually get dissolved to the atmosphere. Denitrifying filters we use which support the growth of anaerobic microorganisms or denitrifiers to help this denitrification process.

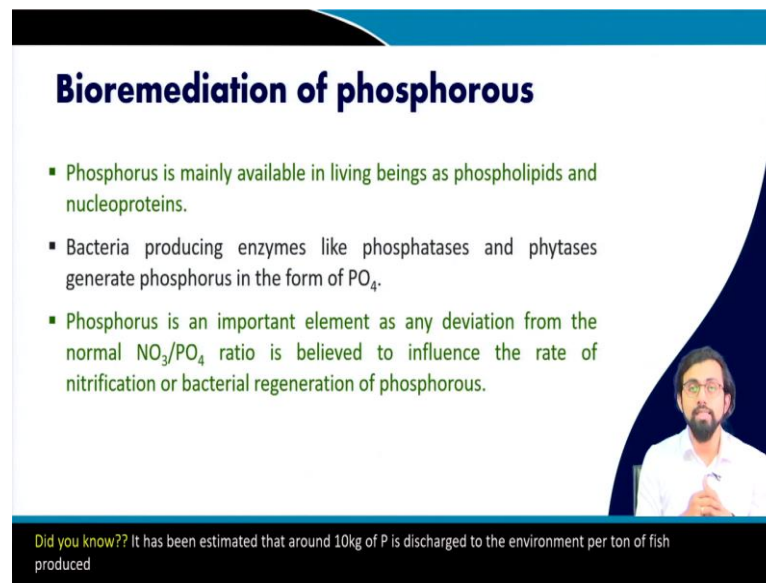
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This is the whole system how it looks like. You see the nitrogenous organic waste matters, the unconsumed feed fecal matter, moults, dead algae, excretory waste, etcetera. Because of the presence of different heterotrophic microorganisms, it converts into the ammonia by the process of ammonification. Then they work with the because of the presence of different nitrifying organisms they convert into nitrate, this is the aerobic process.

Then nitrate to nitrogen, which is anaerobic denitrification process. Then this nitrogen will go to the atmosphere or this nitrate rich waste water you can use it for algal nutrient or even for supplementary for plant purposes, that is what we done in hydroponic systems, like you know aquaponic systems, this nitrogen rich wastewater has been fed to the hydroponic crops.

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Bioremediation of phosphorous

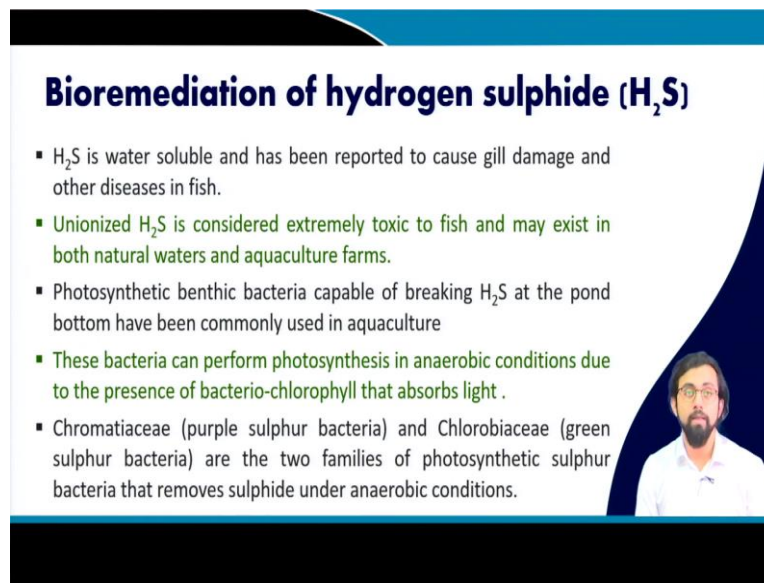
- Phosphorus is mainly available in living beings as phospholipids and nucleoproteins.
- Bacteria producing enzymes like phosphatases and phytases generate phosphorus in the form of PO_4 .
- Phosphorus is an important element as any deviation from the normal NO_3/PO_4 ratio is believed to influence the rate of nitrification or bacterial regeneration of phosphorous.

Did you know?? It has been estimated that around 10kg of P is discharged to the environment per ton of fish produced

So, that is a very standard way of doing that, so get rid of the nitrogen and carbonaceous compound what about the phosphorus, phosphorus is mainly available in the living beings as phospholipid and the nucleoprotein. These bacteria which produced the enzyme like phosphatase and the phytases are actually generate phosphorus in the form of phosphate, PO_4 .

So, phosphorus is an important element in any deviation from the normal nitrate to phosphate ratio is believed to influence the rate of nitrification or bacterial generation of phosphorus. So, this kind of microorganisms, this kind of bacteria which can produce these enzymes are actually very much useful to bioremediate the phosphorus waste from the system.

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Bioremediation of hydrogen sulphide (H₂S)

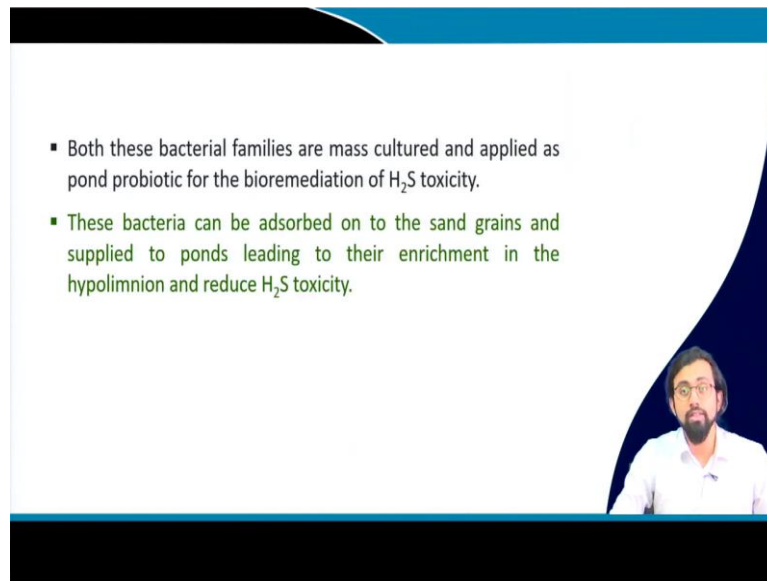
- H₂S is water soluble and has been reported to cause gill damage and other diseases in fish.
- Unionized H₂S is considered extremely toxic to fish and may exist in both natural waters and aquaculture farms.
- Photosynthetic benthic bacteria capable of breaking H₂S at the pond bottom have been commonly used in aquaculture
- These bacteria can perform photosynthesis in anaerobic conditions due to the presence of bacterio-chlorophyll that absorbs light .
- Chromatiaceae (purple sulphur bacteria) and Chlorobiaceae (green sulphur bacteria) are the two families of photosynthetic sulphur bacteria that removes sulphide under anaerobic conditions.

What about the hydrogen sulphide? That is also another one of the nuisance or like we should get rid of this hydrogen sulphide because it can cause gill damage and some other diseases in fish. Unionized H₂S is considered extremely toxic to the fish and may exist in both natural water and aquaculture farms. Photosynthetic benthic bacteria capable of breaking this H₂S at the pond bottom which we normally use it in aquaculture practices.

This bacteria is very special because they can perform photosynthesis in anaerobic condition due to the presence of their bacterio-chlorophyll that can absorb light. So, this Chromatiaceae or this purple sulphur bacteria or the Chlorobiaceae or we call them green circle bacteria, they are the two families of photosynthetic sulfur reducing bacteria that removes the sulphide under the anaerobic conditions.

So, these are the very important, this bioremediation of hydrogen sulphide. This is why we should go ahead and this is one of the major reasons why we should go ahead with this bioremediation of hydrogen sulphide.

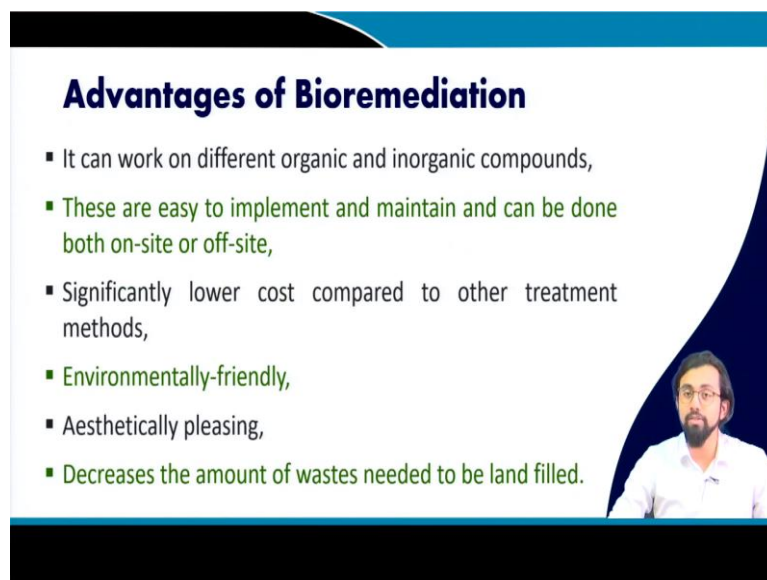
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- Both these bacterial families are mass cultured and applied as pond probiotic for the bioremediation of H₂S toxicity.
- These bacteria can be adsorbed on to the sand grains and supplied to ponds leading to their enrichment in the hypolimnion and reduce H₂S toxicity.

Both this bacterial families are mass cultured and applied as one probiotic for the bioremediation of H₂S toxicity. And these bacteria can be absorbed on the sand grains and supplied to ponds leading to the enrichment in the hypolimnion and it can reduce the H₂S toxicity in general.

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Advantages of Bioremediation

- It can work on different organic and inorganic compounds,
- These are easy to implement and maintain and can be done both on-site or off-site,
- Significantly lower cost compared to other treatment methods,
- Environmentally-friendly,
- Aesthetically pleasing,
- Decreases the amount of wastes needed to be land filled.

So, we discuss about the bioremediation of carbon compounds, nitrogen compounds, phosphorus compounds, and we also discuss about the bioremediation of hydrogen sulphide as well. So, if we talk about what are the advantages and the disadvantages of bioremediation, so it will give us a more clear idea about what is it?

And how these disadvantages of the bioremediation can also be troubleshooting the future, can be considered as future research activity and it has actually been done by experts from all over the world and they are working on it and how they can help us to get rid of this toxicity and help get rid of all these disadvantages of the bioremediation.

I will discuss about it in coming slide. So, the advantages of bioremediation, it can work on different organic and inorganic compounds which is good, this is easy to implement because we need something because first of all this is biological, this is a living thing. We are not dealing here with something which is not living and which will be having some drastical effect in the follow-up procedures or say like in a later in the near future, we are doing everything in biologically here.

Most of the enzymatic extract that we get from this microorganisms they are very much organic in nature, and because of that, it is all because of this reason it is called bioremediation, it is far, far better than all the chemical remediation procedures and all the physical remediation procedures.

It is also easy to maintain and can be done both on-site and off-site. Like suppose if I give you one example of another type of remediation processes like say liming, remember we discuss about liming, why do we need liming, why it is helpful. Liming is you cannot do it on-site, even if you do on-site liming, what will happen it will be very detrimental for your aquatic species that is rearing at that moment. What you need to do?

You need to completely get rid of all the aquatic species, you need to clean the pond, then you have to supply the liming that is the best way of doing liming, that once or before the farm season started and you want to go ahead with the farming of particular area, you have to get it all the water from your pond, lime it, wait for 48 hours or so, and then you do some continuous water exchange so to get rid of any follow up events, follow up effects of this liming, and then you can go ahead with it.

So, it comes with a lot of disadvantages but in case of bioremediation it is possible for on-site as well as off-site application. Definitely it cost much, much, much lesser than other treatment units and all, because you are using some biological system only, biological living beings only which can be cultured and which can be supplied in a very small amount it will definitely once it will find its own way find its own favorable condition it will grow

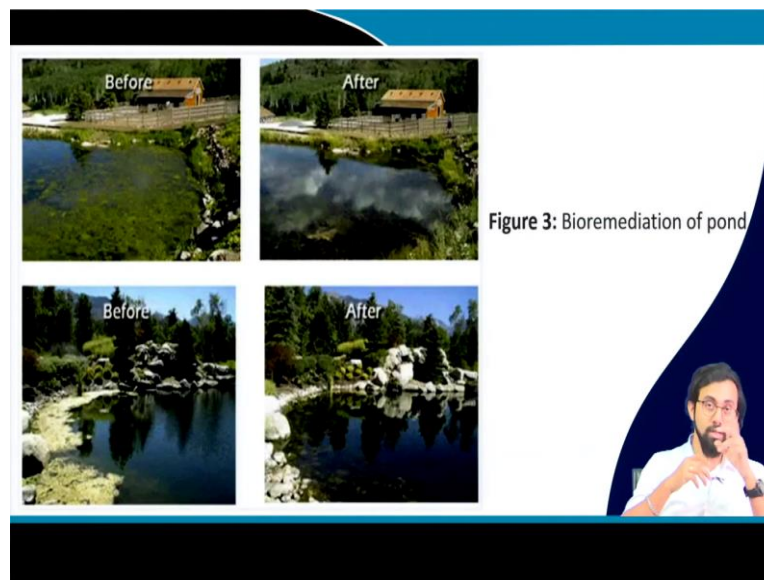
drastically it will clean the zone, it will clean the condition in the natural, that is a unwanted conditions that is prevailing in your system.

It can significantly improve the environment condition, it is actually environment friendly, it is not having any, I would say like drastic effect in your pond system or your pond sediment. It is aesthetically pleasing, because when you do the bioremediation you will get rid of all the unwanted materials which is like say floating condition that is algal blooms, that is like you know this organic material that will keep on increasing.

So, it will cause drastic change in the food wave at the end it will cause the eutrophication. So, this bioremediation helps you to make it aesthetically pleasing that means it is clean enough and good enough for you know, it gives you some aesthetic enhancement, it provides a beautification which actually helps us and so aesthetically pleasing. It decreases the amount of wastes needed for the landfill.

Suppose at the end of a culture period, you need to take the scrap the pond bottom up and you have to throw it somewhere for landfilling, because you know you are going to culture it again. So, this bioremediation will help you get rid of this.

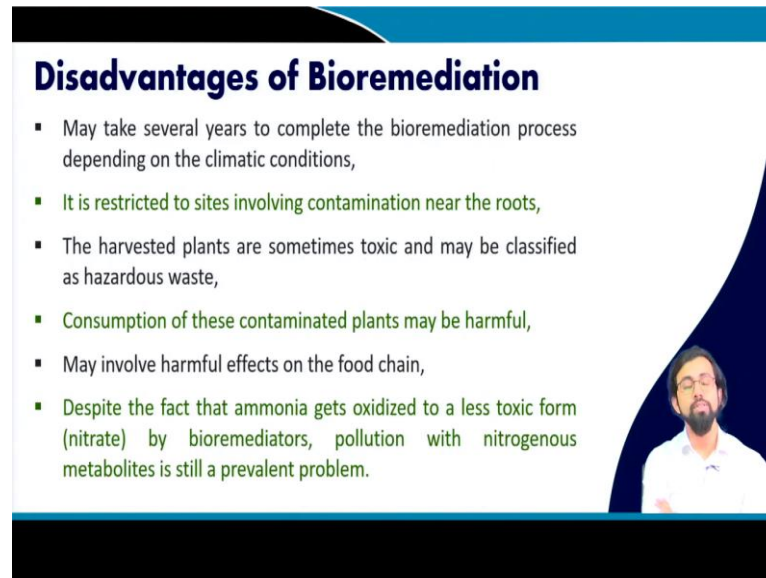
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You see why I have told you it is aesthetically pleasing. See the bioremediation once it is done before and after it is like so greenish in nature at the end you see it looks so good it is like so clean blue water. In the bottom also, you see before and after before it was like having like lot of obnoxious pollutants, this pollutant level is drastically dropped down you can see

in the after picture. So, that is how it looks like after the bioremediation is done that is how it looks like the before and after picture.

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Disadvantages of Bioremediation

- May take several years to complete the bioremediation process depending on the climatic conditions,
- It is restricted to sites involving contamination near the roots,
- The harvested plants are sometimes toxic and may be classified as hazardous waste,
- Consumption of these contaminated plants may be harmful,
- May involve harmful effects on the food chain,
- Despite the fact that ammonia gets oxidized to a less toxic form (nitrate) by bioremediators, pollution with nitrogenous metabolites is still a prevalent problem.

So, what are the disadvantages of bioremediation? Bioremediation, there is always the other side of the coin, it is something like that. In case of bioremediation, it may take several years to complete the bioremediation process depending on the climatic conditions. It is restricted to the sites involving contamination near the roots that is also problem.

The harvested plants are sometimes toxic, may be classified as hazardous waste because of presence of some unwanted biological components. So, sometimes the contaminated plants may be harmful. So, better to treat it properly, boil it properly and have it or go and ask the expert what to do with that, it may involve the harmful effect on the food chain, but still it is better to go ahead with this bioremediation process rather than waiting for your pond to completely die.

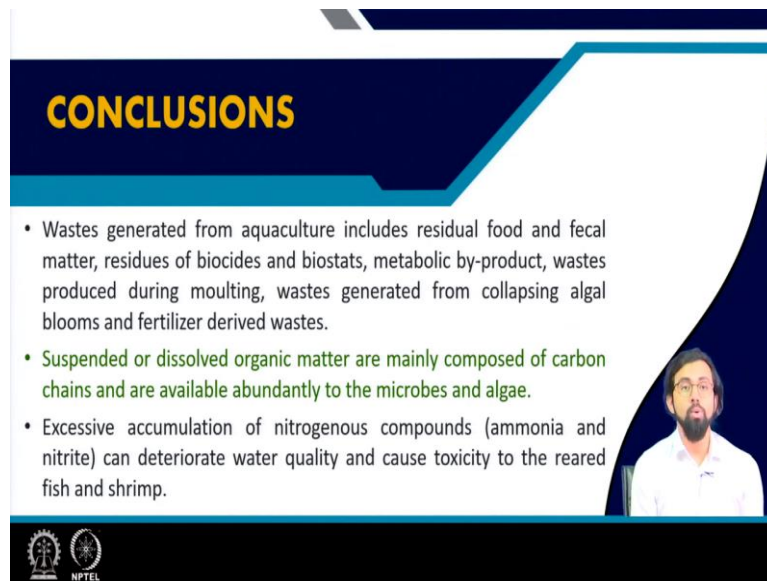
Despite the fact that ammonia gets oxidized to a less toxic form of nitrate by bioremediators, however, the pollution with nitrogenous metabolites is still by prevalent problems. So, why I say so, because see the nitrate, suppose you somehow do the nitrification process and ammonia is converted into nitrate.

But in the middle, if you remember the process, nitrification process correctly, ammonia is converted into nitrite first, then from nitrite, it converts into the nitrate, this nitrite is actually

sometimes it stays in the system. And when it stays in the system, sometimes it can cause drastical harm to the system to the unit, to the unit in general.

And it has a lot of metabolites it forms and these nitrogen metabolites are also not good for our health good for in general, like for any follow up practices or any, it is not, it is not good at all to be frank.

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CONCLUSIONS

- Wastes generated from aquaculture includes residual food and fecal matter, residues of biocides and biostats, metabolic by-product, wastes produced during moulting, wastes generated from collapsing algal blooms and fertilizer derived wastes.
- Suspended or dissolved organic matter are mainly composed of carbon chains and are available abundantly to the microbes and algae.
- Excessive accumulation of nitrogenous compounds (ammonia and nitrite) can deteriorate water quality and cause toxicity to the reared fish and shrimp.

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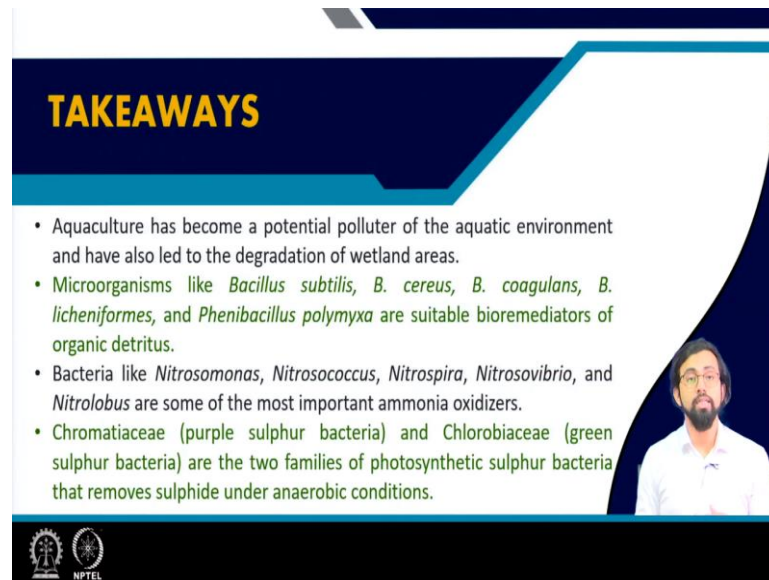
So, what we have learned from this lecture material, the conclusion that we can derive that the waste that is generated from the aquaculture includes the residual food, fecal matter, residues of biocides and the biostats, metabolic byproducts, the waste produced during the moulting of crustaceans, wastes generated from the collapsing algal blooms, the fertilizer derived waste. All these things cause a huge amount of pollution into the river water body and all.

In order to get rid of it, what we can do, we can use some kind of bioremediators. From the name itself, you can see the bio means it is a living organisms, it is like a living being or the extract of a living being, the extract of the byproduct of living, so that we can do and we can utilize this kind of these products for remediation of organic compounds, remediation of nitrogen compounds, phosphorus compounds, hydrogen sulphides and etcetera.

And we also learned the advantages and the disadvantages of bioremediators and how we can troubleshoot those disadvantages also we discussed in detail. We discuss the excessive accumulation of nitrogen compounds, it can deteriorate the water quality, we got to know that

this toxicity can be very harmful, very lethal to our fish and shrimps. So, that is why we need to go ahead with this bioremediators.

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TAKEAWAYS

- Aquaculture has become a potential polluter of the aquatic environment and have also led to the degradation of wetland areas.
- Microorganisms like *Bacillus subtilis*, *B. cereus*, *B. coagulans*, *B. licheniformes*, and *Phenibacillus polymyxa* are suitable bioremediators of organic detritus.
- Bacteria like *Nitrosomonas*, *Nitrosococcus*, *Nitrospira*, *Nitrosovibrio*, and *Nitrolobus* are some of the most important ammonia oxidizers.
- Chromatiaceae (purple sulphur bacteria) and Chlorobiaceae (green sulphur bacteria) are the two families of photosynthetic sulphur bacteria that removes sulphide under anaerobic conditions.

NPTEL

The different kinds of microorganisms like bacillus subtilis, bacillus cereus, bacillus coagulans, they are suitable by bioremediators for organic detritus. Bacteria like Nitrosomonas, Nitrosococcus, Nitrospira, Nitrosovibrio, Nitrolobus, and all these bacteria are very much important for fixing the nitrogen species, especially ammonia deoxidizer there.

On the other hand, this Chromatiaceae and this Chlorobiaceae this two different families of photosynthetic sulfur reducing bacteria are helpful for removing the sulphide under anaerobic conditions. So, all these things that we have learned that it is possible to clean to, how to say like to somehow help get rid of the all the pollutants from the wastewater by using different bioremediation techniques.

So, this bioremediators mainly the living organisms that we use and it can be easily cleaned. What are the other ways of doing that? We know that in the case of recirculating aquaculture systems, we use different treatment units like the primary treatment, secondary treatment, in the secondary treatment also actually it is kind of a biological treatment process there also we introduced some essential microorganisms or helpful microorganisms, which actually treats the waste by consuming it and converting into their biomass.

Then we go for this tertiary treatment units where this can be done by UV or ozonation and all these techniques. So, this is also one way of doing that. And this is also, bioremediation is also another standard way of doing that. So that is it.

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REFERENCES

- <https://thefishsite.com/articles/bioremediation-an-advanced-strategy-to-restore-the-health-of-aquaculture-pond-ecosystems>
- <http://aquafind.com/articles/BioremediationInAquaculture.php>
- M. Martinez-Porchas, L. R. Martinez-Cordova, J. A. Lopez-Elias, and M. A. Porchas-Cornejo, "Bioremediation of Aquaculture Effluents," *Microbial Biodegradation and Bioremediation*, pp. 539–553, Jan. 2014, doi: 10.1016/B978-0-12-800021-2.00024-8

The slide features a dark blue header with the word 'REFERENCES' in yellow. Below the header is a white area containing the references. In the bottom right corner, there is a small video inset showing a man with a beard and glasses speaking. At the bottom left, there are two logos: one for NPTEL (National Programme on Technology Enhanced Learning) and another for a university.

These are the references that you can follow. I really wish you can, like it would be better if you can take a picture and search it in the Google. So, you will get very detailed in-depth details about it and got to know more about the different bioremediation processes, and how it works in general. So, that is it for the day. See you in the coming lecture. Thank you.