Environmental Chemistry and Microbiology

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Module - 11
Lecture - 58
Metabolic Diversity - III

Welcome everyone. This is the third and final part of this module. This is lecture 58 of module 11.

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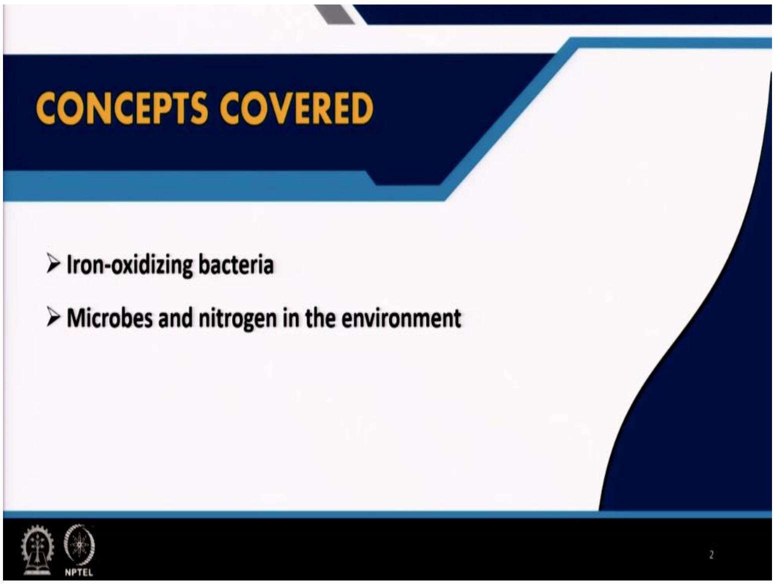

CONCEPTS COVERED

- Iron-oxidizing bacteria
- Microbes and nitrogen in the environment

So, in the last lecture of module 11, we are going to take a look at two other groups of bacteria. The first one is iron-oxidising bacteria and the second one is the group of bacteria that are involved in the nitrogen cycle.

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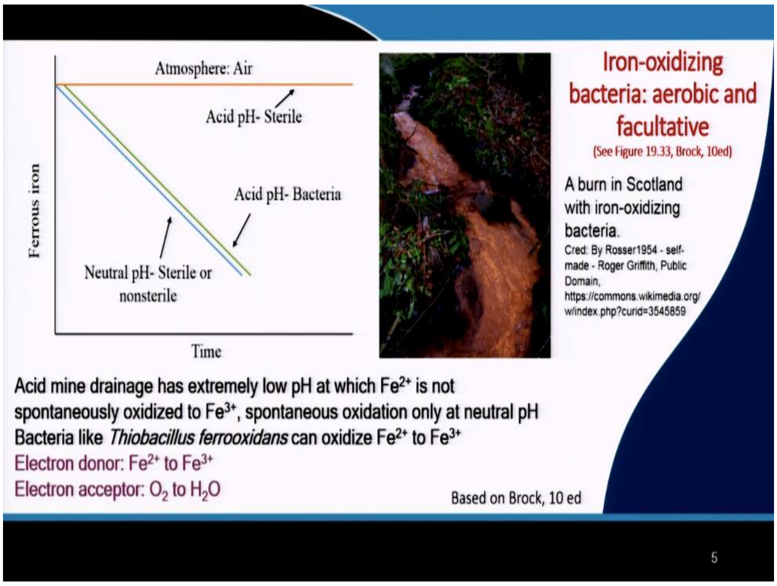

Iron-oxidizing bacteria: aerobic and facultative

(See Figure 19.33, Brock, 10ed)

A burn in Scotland with iron-oxidizing bacteria.

Cred: By Rosser1954 - self-made - Roger Griffith, Public Domain, https://commons.wikimedia.org/w/index.php?curid=3545859

Acid mine drainage has extremely low pH at which Fe^{2+} is not spontaneously oxidized to Fe^{3+}, spontaneous oxidation only at neutral pH

Bacteria like *Thiobacillus ferrooxidans* can oxidize Fe^{2+} to Fe^{3+}

Electron donor: Fe^{2+} to Fe^{3+}

Electron acceptor: O_2 to H_2O

Based on Brock, 10 ed

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We now come to another group of bacteria which is called iron-oxidising bacteria. Now, these iron-oxidising bacteria can be either aerobic or facultative and you can refer to the text, the Brock text, figure 19.33. What you see over here in this slide is acid mine drainage. Now, acid mine drainage comes out of these mining operations and it tends to have extremely low pH. So, very often you will find that acid mine drainage has pH around 2 or even lower than that. Now, in general, this kind of drainage has very high iron content. And we are used to thinking that in the normal conditions around us, when the pH of water is close to neutral, it is close to 7, under those conditions, any iron that is present in the water in elemental form will get spontaneously oxidised to Fe^{2+} - ferrous iron, and Fe^{2+} will get converted to Fe^{3+}which is ferric iron. So, this happens spontaneously at neutral pH.

Now, acid mine drainage is a whole different story, because we have pH that is close to 2. It is no longer a spontaneous chemical reaction. Instead, it is a biologically mediated reaction. And this particular reaction cannot be mediated by any bacteria. There are specific species of bacteria in this example that is shown. It is *Thiobacillus ferrooxidans* and that are responsible for oxidizing Fe^{2+} to Fe^{3+}.

So, this graphic shows us a conceptual way of showing us that the oxidation of ferrous iron to ferric iron is spontaneous only under neutral conditions. So, you can see these 2 lines which show the decreasing ferrous iron concentration under neutral pH, whether the bacteria are present or not, it does not matter, it is a spontaneous chemical reaction. Under acidic conditions, you can see under sterile conditions, there is no change in the concentration of ferrous iron. (There is no conversion of ferrous to ferric iron).

It is only when bacteria are present under acidic conditions, only then is ferrous iron (Fe^{2+}) oxidised to ferric iron (Fe^{3+}). So, this is a very interesting example. It is a very interesting example of a particular group of bacteria which is capable of oxidising iron under acidic conditions. So, by definition, these bacteria are acidophilic bacteria and like I said, they can be aerobic as well as facultative.

There have been several reports in the literature which I will come to in a little bit. The electron donor in these reactions is ferrous iron Fe^{2+} and it is converted to ferric iron Fe^{3+}, and the electron acceptor is oxygen. So, it remains; it is an autotrophic reaction.

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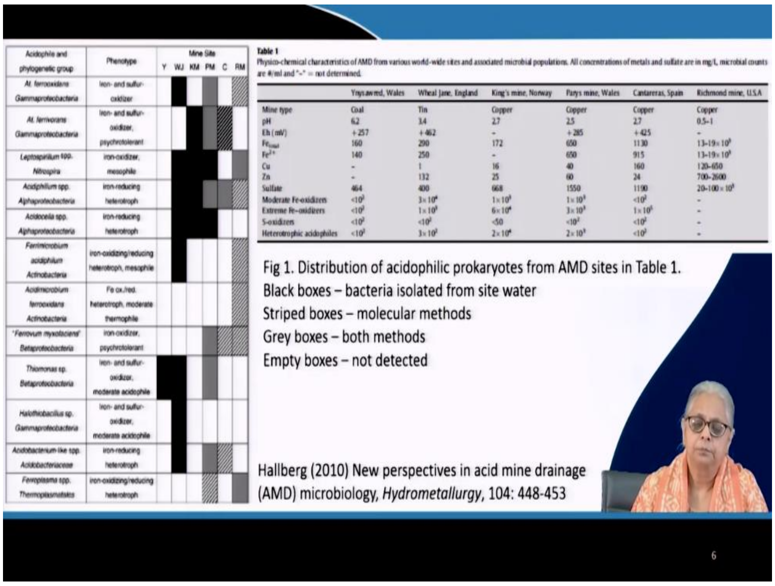

Acidophile and phylogenetic group	Phenotype	Mine Site Y	WJ	KM	PM	C	RM
At. ferrooxidans Gammaproteobacteria	Iron- and sulfur-oxidizer		■		▒		▨
At. ferrivorans Gammaproteobacteria	Iron- and sulfur-oxidizer, psychrotolerant			■	▒	▨	
Leptospirillum spp. Nitrospira	iron-oxidizer, mesophile		■	■	■		▒
Acidiphilium spp. Alphaproteobacteria	iron-reducing heterotroph		■	■	▒	▒	▨
Acidocella spp. Alphaproteobacteria	iron-reducing heterotroph		■	■	■		
Ferrimicrobium acidiphilum Actinobacteria	iron-oxidizing/reducing heterotroph, mesophile		■				▨
Acidimicrobium ferrooxidans Actinobacteria	Fe ox./red. heterotroph, moderate thermophile						▨
'Ferrovum myxofaciens' Betaproteobacteria	iron-oxidizer, psychrotolerant				▒	▨	▨
Thiomonas sp. Betaproteobacteria	iron- and sulfur-oxidizer, moderate acidophile	■	■		▒		
Halothiobacillus sp. Gammaproteobacteria	iron- and sulfur-oxidizer, moderate acidophile		■				
Acidobacterium-like spp. Acidobacteriaceae	iron-reducing heterotroph		■		▒	▨	
Ferroplasma spp. Thermoplasmatales	iron-oxidizing/reducing heterotroph				▨		▒

Table 1
Physico-chemical characteristics of AMD from various world-wide sites and associated microbial populations. All concentrations of metals and sulfate are in mg/L, microbial counts are #/ml and "-" = not determined.

	Ynysarwed, Wales	Wheal Jane, England	King's mine, Norway	Parys mine, Wales	Cantareras, Spain	Richmond mine, U.S.A
Mine type	Coal	Tin	Copper	Copper	Copper	Copper
pH	6.2	3.4	2.7	2.5	2.7	0.5–1
Eh (mV)	+257	+462	-	+285	+425	-
Fe_{total}	160	290	172	650	1130	$13–19 \times 10^3$
Fe^{2+}	140	250	-	650	915	$13–19 \times 10^3$
Cu	-	1	16	40	160	120–650
Zn	-	132	25	60	24	700–2600
Sulfate	464	400	668	1550	1190	$20–100 \times 10^3$
Moderate Fe-oxidizers	$<10^2$	3×10^4	1×10^3	1×10^3	$<10^2$	-
Extreme Fe-oxidizers	$<10^2$	1×10^3	6×10^4	3×10^3	1×10^5	-
S-oxidizers	$<10^2$	$<10^2$	<50	$<10^2$	$<10^2$	-
Heterotrophic acidophiles	$<10^2$	3×10^2	2×10^4	2×10^3	$<10^2$	-

Fig 1. Distribution of acidophilic prokaryotes from AMD sites in Table 1.
Black boxes – bacteria isolated from site water
Striped boxes – molecular methods
Grey boxes – both methods
Empty boxes – not detected

Hallberg (2010) New perspectives in acid mine drainage (AMD) microbiology, *Hydrometallurgy*, 104: 448-453

So, here we have a review paper which talks about acid mine drainage and the microbes that are associated with acid mine drainage. So, there is a whole long list of acidophilic bacteria that have been identified in acid mine drainage in various sites around the world. Most of them are in Wales and one in Spain, one in the U.S. and what you see is that all of these black boxes indicate the bacteria that were isolated from the acid mine drainage water from the site itself. The striped boxes are based on molecular methods; the grey boxes include both identification of individual species as well as molecular methods used for identification; and the empty boxes indicate there was no detection of those particular groups of microorganisms. So, you can see a large number of microorganisms that are iron-oxidising bacteria. So, you can see their concentrations listed in the table.

Other than the first site, all of them have extremely low pH. So, you have pH of 3.4, 2.7, 2.5, 2.7 and less than 1. So, these are all examples and there are both autotrophic as well as heterotrophic bacteria. The concentrations of these bacteria are not very high. They are slightly less than 10^2, which is the detection limit of many of these methods I think. And you also have 10^3, 10^4, 10^5 in one case. So, these are some of the iron-oxidising bacteria that have been identified in acid mine drainage.

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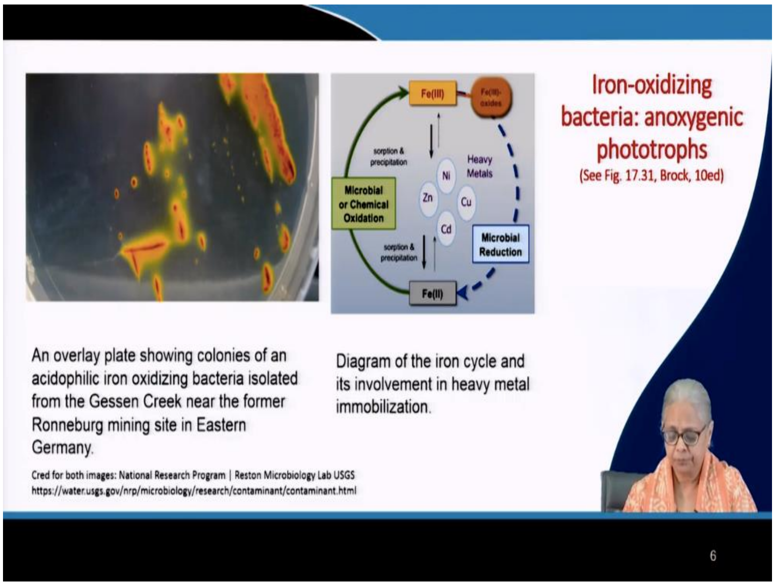

Iron-oxidizing bacteria: anoxygenic phototrophs

(See Fig. 17.31, Brock, 10ed)

An overlay plate showing colonies of an acidophilic iron oxidizing bacteria isolated from the Gessen Creek near the former Ronneburg mining site in Eastern Germany.

Diagram of the iron cycle and its involvement in heavy metal immobilization.

Cred for both images: National Research Program | Reston Microbiology Lab USGS
https://water.usgs.gov/nrp/microbiology/research/contaminant/contaminant.html

Here are more examples of the colonies of acidophilic iron-oxidising bacteria isolated from another mining site in Germany. So, these are just examples of the same. And this is also an example of how iron is recycled in nature, based on both chemical as well as microbial oxidation. So, we know that under neutral pH conditions, elemental iron will be converted to *Fe(II)* and then to *Fe(III)* in the presence of oxygen; that is what rust is all about. So, whenever you have any metal containing iron around you, you know that it gets converted to *Fe(II)* and then *Fe(III)*. Now, *Fe(II)* is fairly soluble compared to *Fe(III)*. *Fe(III)* is more or less insoluble; it forms dark black precipitates and that is generally insoluble. We consider that almost insoluble. So, these are the *Fe(III)* oxides (Not all ferric oxides are black in colour; they have several different colours). Microbial reduction will bring them back to *Fe(II)* and elemental iron.

And the oxidation that I just showed you is also possible. So, this is how iron is recycled literally within the environment by microbes; all of it is by microbes. And you can also have anoxygenic phototrophic iron-oxidising bacteria. So, all of them do exist in the environment.

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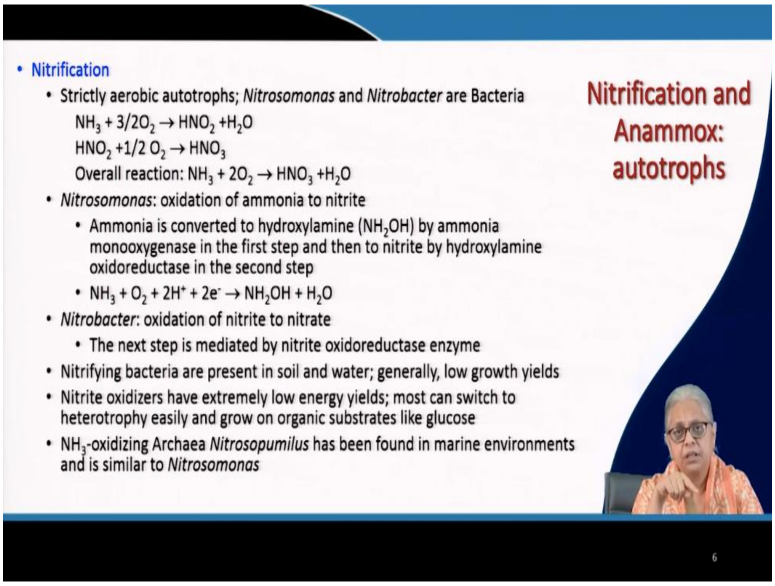

Nitrification and Anammox: autotrophs

- Nitrification
  - Strictly aerobic autotrophs; *Nitrosomonas* and *Nitrobacter* are Bacteria

    $NH_3 + 3/2O_2 \rightarrow HNO_2 + H_2O$

    $HNO_2 + 1/2\ O_2 \rightarrow HNO_3$

    Overall reaction: $NH_3 + 2O_2 \rightarrow HNO_3 + H_2O$
  - *Nitrosomonas*: oxidation of ammonia to nitrite
    - Ammonia is converted to hydroxylamine (NH_2OH) by ammonia monooxygenase in the first step and then to nitrite by hydroxylamine oxidoreductase in the second step
    - $NH_3 + O_2 + 2H^+ + 2e^- \rightarrow NH_2OH + H_2O$
  - *Nitrobacter*: oxidation of nitrite to nitrate
    - The next step is mediated by nitrite oxidoreductase enzyme
  - Nitrifying bacteria are present in soil and water; generally, low growth yields
  - Nitrite oxidizers have extremely low energy yields; most can switch to heterotrophy easily and grow on organic substrates like glucose
  - NH_3-oxidizing Archaea *Nitrosopumilus* has been found in marine environments and is similar to *Nitrosomonas*

Let us now look at another element; the next element is nitrogen. Now, nitrogen is very interesting because it comes in several different forms. You have different oxidation states for nitrogen. It exists in many different oxidation states. So, you have inert nitrogen gas in the atmosphere that is not bioavailable. And you have ammonia NH_3, nitrite NO_2^- and nitrate NO_3^- along with organic nitrogen, which is available; these are all bioavailable forms of nitrogen. So, we are going to look at 2 major processes associated with nitrogen. One is nitrification and the other is anammox. Both of these groups of microbes that are involved in nitrification and anammox are autotrophic bacteria.

So, let us start with nitrification. So, we have strictly aerobic autotrophic bacteria that are involved in the nitrification process. What is nitrification? Let us first look at that; and then some of the species that are involved in this process.

So, the first thing to remember is that nitrification is the conversion of ammonia, inorganic ammonia to nitrite and nitrate. Where is the inorganic ammonia coming from? It is basically coming from organic amino, amines or amino acids and so on.

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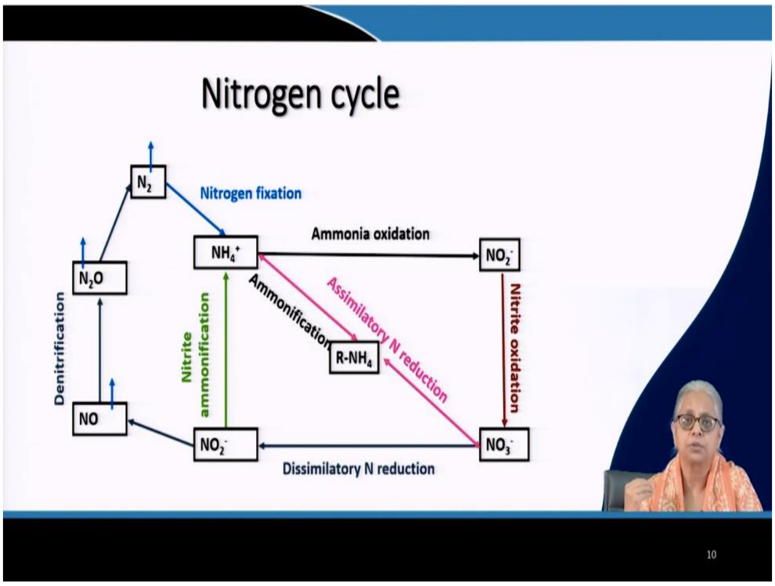

Nitrogen cycle

N_2

Nitrogen fixation

NH_4^+

Ammonia oxidation

NO_2^-

N_2O

Denitrification

Nitrite ammonification

Ammonification

Assimilatory N reduction

R-NH_4

Nitrite oxidation

NO

NO_2^-

NO_3^-

Dissimilatory N reduction

So, when you think about wastewater, one of the biggest things that wastewater contains is organic nitrogen. So, this is shown as R-NH_4. So, this R-NH_4 is organic nitrogen. The first thing that happens is that it becomes ammonia, free ammonia and this free ammonia is then converted by oxidation to nitrite (NO_2^-) and nitrate (NO_3^-) and that is what we are going to look at over here.

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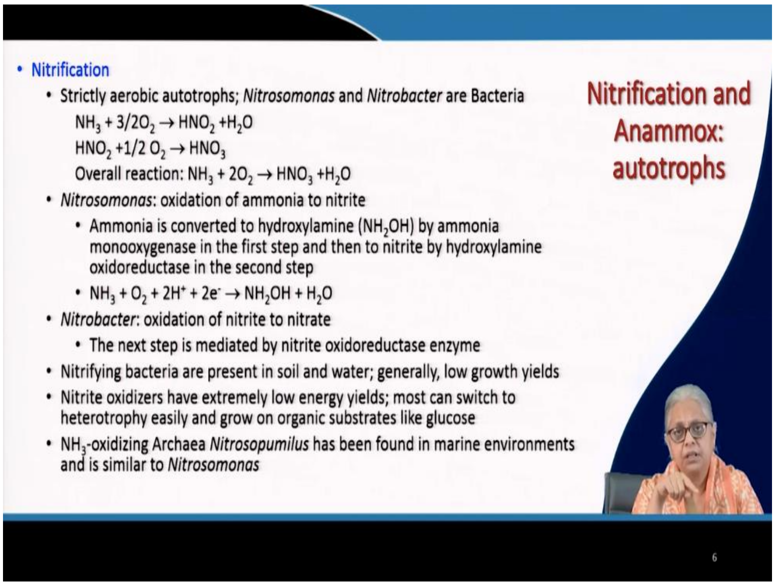

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Now, this ammonia conversion to nitrite NO_2^- and nitrate NO_3^- is mediated by *Nitrosomonas* and *Nitrobacter*. So, these are specific species of aerobic autotrophic bacteria that are responsible for oxidising ammonia NH_3 to NO_2^- and NO_3^-. And the first step; *Nitrosomonas* is the species that will oxidise ammonia to nitrite. In this case, this 1-step process which we generally talk about as ammonia to nitrite is actually a 2-step process.

Ammonia is first converted to hydroxylamine, so, that is NH_2OH, by a particular enzyme ammonia monooxygenase which is present only in *Nitrosomonas* species. That is the first step. This hydroxylamine is then converted by another enzyme hydroxylamine oxidoreductase in the second step. So, the first step is shown over here: $NH_3 + O_2 + 2H^+ + 2e^- \rightarrow NH_2OH + H_2O$.

This NH_2OH is then converted by the hydroxylamine oxidoreductase enzyme to NO_2^-. Then we come to nitrite. Nitrite (NO_2^-) has to be converted to nitrate (NO_3^-), and that is done by *Nitrobacter*. So, this second step in the entire conversion of ammonia NH_3 , ammonia being the most reduced form of nitrogen and nitrate being the most oxidised form of nitrogen; that is the entire gamut of the nitrogen species, so to speak.

The next step is mediated by nitrite oxidoreductase enzyme. Now, these nitrifying bacteria together are responsible for completing the complete oxidation of nitrogen containing compounds. So, these nitrifying bacteria are present in soil, in water and several other environments. They generally have very low growth yields, which is typical of autotrophic bacteria. Because autotrophic bacteria do not rely on organic carbon, they are relying on CO_2 as their carbon source. So, generally, their growth yields are very low. And because of that, their energy yields are also low. I have shown you a correlation between energy and biomass. So, that is typical. Now, in many cases, it has been observed that these autotrophic bacteria can switch to heterotrophy if organic substrates like glucose are available.

That is one point that has been mentioned in the textbook and another thing that has been mentioned in the textbook is that ammonia-oxidising bacteria, these 2 species are part of the (out of the 3 domains: Bacteria, Archaea and Eukarya), out of the 3 domains, *Nitrosomonas* and *Nitrobacter* are part of Bacteria, which means they are modern bacteria. One species of ammonia-oxidising archaebacteria has been found in marine environments. And it is very similar in its mechanism of utilising ammonia. It is very similar to *Nitrosomonas* and the archaebacteria is *Nitrosopumilus*; that is something that seems to have been found recently.

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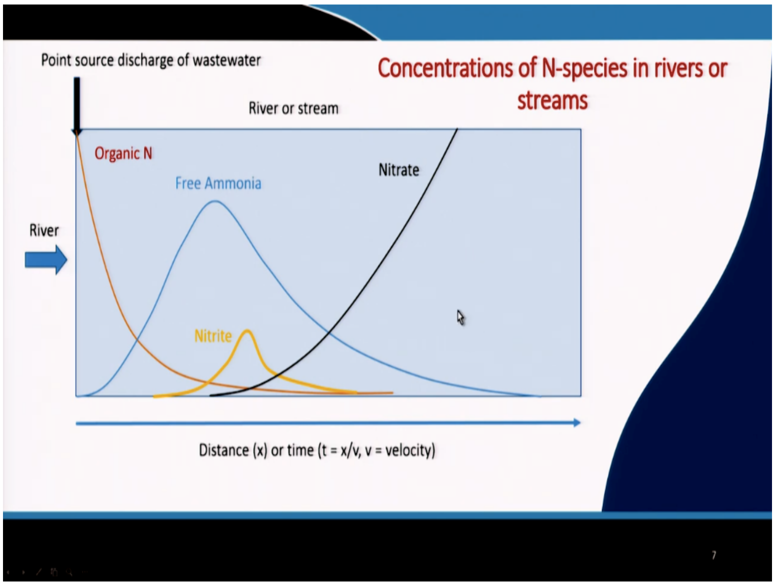

Concentrations of N-species in rivers or streams

Having discussed nitrification in terms of the individual bacterial species that are involved in converting one nitrogen species to another, let us also take a holistic view of how it happens in the environment, in the natural environment. So, this is a very simple and very interesting example of how nitrogen species in rivers or streams are converted from one form to another. So, let us take a look at this river or a stream, and let us imagine that there is a single point source discharge of sewage or wastewater, municipal wastewater.

Now, municipal wastewater has a very high concentration of organic nitrogen. This organic nitrogen, in the beginning is; almost the entire nitrogen that is coming in wastewater is in the form of organic nitrogen; there may be a little bit ammonia as well. So, this organic nitrogen is going to decline rapidly in terms of its concentration, as the water flows from the point at which the wastewater has been released, and as it mixes with the river water and flows further away from the point of release.

Now, this organic nitrogen is going to be first converted to free ammonia. So, here we have ammonium ions. Now, I need to clarify one point, and that is that when you have organic nitrogen, it can be converted to ammonium (NH_4^+) or ammonia (NH_3). So, those are the first 2 inorganic forms that are formed when organic nitrogen is converted by the process of ammonification to 2 forms. Given the pH of most natural waters, they are around 6.5 to 8, 8.5; in that range, you are going to find ammonium, so NH_4^+. But if the pH were higher, then NH_3 would also exist. And there is; both of them coexist, but the dominant species tends to be ammonium ion; and ammonia gas is also present and it can off-gas, and that is what we smell.

So, all that happens in the process of ammonification, where organic nitrogen is converted to either ammonia or ammonium ions.

And this free ammonia will then be converted to nitrite ($NH_3 \rightarrow NO_2{}^-$). You can see 2 curves that show you the increase and then the subsequent decrease. So, the first increase followed by a subsequent decrease is indicative of the fact that organic nitrogen is first being converted to ammonia. This ammonia is being converted to nitrite and nitrate by bacterial species *Nitrosomonas* and *Nitrobacter*. So, you can see this peak. The peak is indicative of the fact that there is a rapid release of free ammonia, which is then picked up by the nitrifying bacteria and these nitrifying bacteria are relatively slow growers. These nitrite forming bacteria will then, this nitrite will be converted to nitrate and that is the most oxidised form of nitrogen. So, there is no further change in the oxidation state of nitrogen. So, you get complete conversion of organic nitrogen to nitrate in a flowing stream.

And you can look at the x-axis in terms of distance from the point of discharge, or you can look at it as time, where time…..; if you know the velocity of the water in the river, then you can convert it to time and/or distance based on the flow velocity of the water.

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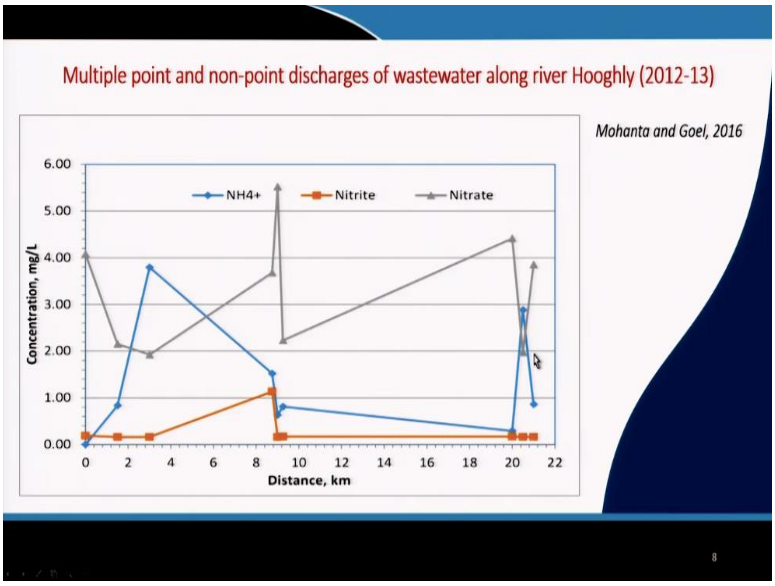

Multiple point and non-point discharges of wastewater along river Hooghly (2012-13)

Mohanta and Goel, 2016

Let me also show you what happens in an actual situation. So, the previous slide is about a hypothetical situation just to illustrate this conversion of organic nitrogen to nitrate, with a series of intermediate nitrogen species.

This is actual data from a project that we did. And here, what you see is the concentration of the 3 major nitrogen species: $NH_4{}^{+}, NO_2{}^{-}$ and $NO_3{}^{-}$ along the river Hooghly and this data was collected in 2012-13. It has been published in a paper. And what it shows us is the same rise and fall, but this rise and fall in concentration is a function of the multiple points and non-point discharges of wastewater. So, if you think about any river that flows by a large city, what you will find is that there are multiple naalahs, drains, etcetera that are discharging either treated or untreated wastewater into these rivers. This is the same story with Hooghly river. So, those are the point discharges. We also have surface runoff from the land area, coming into the river. So, that is nonpoint discharges. In this kind of complex situation, what is the situation with respect to the nitrogen species? So, ammonia nitrogen; you can see, this is the north end from where we started sampling. And the area above distance 0 is agricultural area. So, it is mostly villages with fields and so on. (Added: Directly north of Point zero are suburbs and further upstream are agricultural fields).

From 0 to 22 is the length of the river in km that passes through the 2 cities of Howrah on one side and Kolkata on the other side. So, initially, our ammonium concentration was 0. There was a rapid increase (followed by) a decline; the decline went on all the way until the south end of the part of the river that we were sampling. And there was an increase (spike) followed by a decline again. Now, as I said, the increase and the decrease, this is indicative of the fact that ammonia is being released, ammonium or ammonia are being released; it all depends on the pH. These 2 forms of ammonia are going to be present in water when you have discharges of organic nitrogen. And organic nitrogen can be discharged mainly from municipal sewage. So, that is the biggest one; and that is very clear in this case.

Ammonium or ammonia are converted to nitrite and nitrate by nitrifying bacteria. So, these nitrite forming bacteria are present. You can see, the nitrite peak is coming up much later. And I have already mentioned that nitrifying bacteria are slow growers. So, the peak is formed far after the peak for ammonia is formed and there is no further production of nitrite, because this nitrite is being converted to nitrate. So, here, you can see for nitrate. Now, nitrate is a very interesting case on this particular graph, because you have a fairly high nitrate concentration to begin with. *Now, if nitrate were coming only from municipal wastewater discharges, then it should have been 0, but it is not. And that is indicative of the fact that the fertilizer application to the fields north of these 2 cities has probably contributed to the high level of nitrate, right in the beginning.* That has declined, probably by sedimentation and precipitation and so on *(Another possible reason for the observed decline in soluble nitrate concentrations is uptake* *by phytoplankton).* And there is another peak followed by the ammonia peak. Long after the ammonia peak, you see a peak in nitrate and that corresponds also to the peak in nitrite. So, this is again an indicator of the fact that ammonium has been converted to nitrite and nitrate. And these, then you see a further decline in nitrate concentrations. Again, I am speculating that most of this nitrate may be either part of biomass uptake or sedimentation and precipitation. So, these are possible processes by which nitrate would have sunk or lost from (water), in terms of the concentration. Then you see another peak at the end, towards the southern end. There was a large amount of wastewater discharged at the southern end and the nitrate peak may be another one associated with these ammonia concentrations as well as with nitrite concentrations. It is not very clear. This one is not very clear, but the first two, this set of data is very clear.

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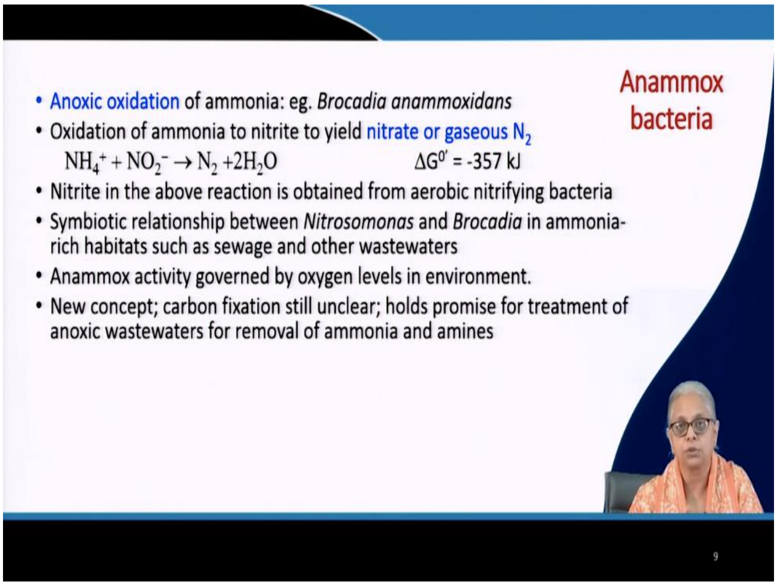
Note: Nitrate levels in river water may be high at point zero for two reasons: fertilizer application and/or municipal wastewater discharges as explained above.



Anammox bacteria

- Anoxic oxidation of ammonia: eg. *Brocadia anammoxidans*
- Oxidation of ammonia to nitrite to yield nitrate or gaseous N_2

$NH_4^+ + NO_2^- \rightarrow N_2 + 2H_2O$ $\Delta G^{0'} = -357$ kJ

- Nitrite in the above reaction is obtained from aerobic nitrifying bacteria
- Symbiotic relationship between *Nitrosomonas* and *Brocadia* in ammonia-rich habitats such as sewage and other wastewaters
- Anammox activity governed by oxygen levels in environment.
- New concept; carbon fixation still unclear; holds promise for treatment of anoxic wastewaters for removal of ammonia and amines

Then we come to anammox. What is anammox? Anammox is a very interesting way of oxidising ammonia in the absence of oxygen. So, in nitrification, we are utilising oxygen, but in this case, what happens is that organic nitrogen has been converted to ammonium form. And this ammonium, along with nitrite will result in the formation of inert nitrogen gas along with water.

Now, this, from an agricultural perspective is definitely not a desirable reaction. However, this is a very important part of the biogeochemical cycle about nitrogen, because it completely….., it basically results from the combination of a reduced nitrogen species and an oxidised nitrogen species, and puts nitrogen back in the atmosphere. So, this is a very important point. And you can see that the $\Delta G^{0\prime}$ for this reaction is fairly high. So, these anammox bacteria are capable not just of surviving but of thriving in an environment where ammonia and nitrite are in plenty. And the best example of that environment is municipal sewage or wastewater. So, you generally have lots of ammonia and lots of nitrite in these environments. So, nitrite in the above reaction can be obtained from aerobic nitrifying bacteria.

There is a symbiotic relationship between *Nitrosomonas* and *Brocadia* in these ammonia rich habitats, like sewage and wastewater. Anammox activity is considered to be governed by the oxygen levels in the environment. Even though they are obligately anaerobic, they themselves will not use oxygen, but the activity level is governed by the availability or the presence of oxygen in their environment.

I have mentioned here that the carbon fixation mechanism is still unclear. It is possible that there are publications, more recent publications with some amount of information about this particular mechanism. And another major application for this type of bacteria, the anammox bacteria is that it can be used for the removal of ammonia and amines, and sending nitrogen back into the atmosphere in inert form. So, this is something that people have been working on in the last few years to quite a large extent. There have been quite a few publications in this area.

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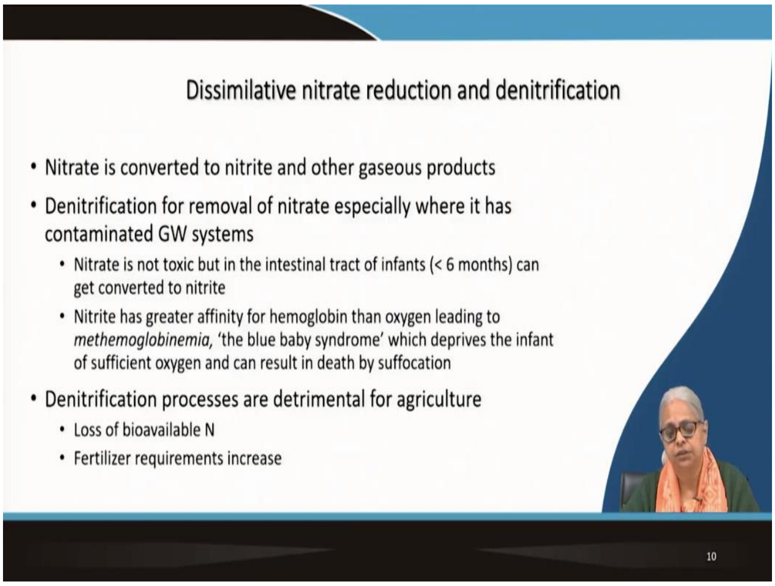

Dissimilative nitrate reduction and denitrification

- Nitrate is converted to nitrite and other gaseous products
- Denitrification for removal of nitrate especially where it has contaminated GW systems
  - Nitrate is not toxic but in the intestinal tract of infants (< 6 months) can get converted to nitrite
  - Nitrite has greater affinity for hemoglobin than oxygen leading to *methemoglobinemia*, 'the blue baby syndrome' which deprives the infant of sufficient oxygen and can result in death by suffocation
- Denitrification processes are detrimental for agriculture
  - Loss of bioavailable N
  - Fertilizer requirements increase

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So, let us come to dissimilative nitrate reduction and denitrification. In this particular process, nitrate is being converted to nitrite and other gaseous products. So, that is what I have shown over here. So, here we have the nitrogen cycle in terms of different oxidation states of nitrogen. So, we have ammonia. It can be oxidised in the presence of oxygen to nitrite and nitrate. All these forms are considered bioavailable forms of nitrogen. And these bioavailable forms can be taken up into the organic biomass, and this is what we call assimilatory nitrogen reduction. So, the oxidation state of nitrogen comes down when it becomes part of the organic material. And that is also called ammonification, because basically nitrogen in organic form is amines. That is the main form. So, here we have our ${NH_4}^+$.

Now, there is dissimilatory nitrogen reduction, which is what we are going to look at. So, we have ${NO_3}^- \rightarrow {NO_2}^- \rightarrow {NH_4}^+$. This nitrite can also be converted under anaerobic conditions to nitric oxide NO, nitrous oxide N_2O and inert nitrogen gas N_2. And all these are gaseous end products which go back into the atmosphere, and that is why it is called dissimilatory nitrogen reduction. So, nitrate in this case is converted to nitrite and other gaseous products.

Now, denitrification for the removal of nitrate happens. It can be used for treating contaminated groundwater. Some of you may be aware of the fact that nitrate is applied in high amounts to fields, mainly for agricultural purposes; because, after all, it is an essential plant nutrient. And this excessive use of fertilizer results in the contamination of groundwater. Because, the excessive nitrate that is present in the fields and the soil will percolate into the groundwater systems and result in high nitrate concentrations.

Nitrate by itself is not toxic, but infants who are less than 6 months old can have a problem because nitrate can get converted to nitrite. Nitrite has a greater affinity for haemoglobin compared to oxygen. So, these children do not have the crucial enzyme that is required for preventing this reaction. And because of the higher affinity of nitrite to haemoglobin, it results in what is called methemoglobinemia or the blue baby syndrome. And this causes the child to suffer from a lack of oxygen and it can cause death by suffocation.

Denitrification processes are detrimental for agriculture because you have a loss of bioavailable nitrogen. And I will just go back to the previous slide (on page 6 of this transcript). These 4 forms are the bioavailable forms of nitrogen. The other 3 forms are not bioavailable nitrogen. So, they are lost from the system. And when you lose bioavailable nitrogen, then your fertilizer requirement increases. So, this is a very delicate balance to maintain from an agricultural point of view and it requires a good amount of management.

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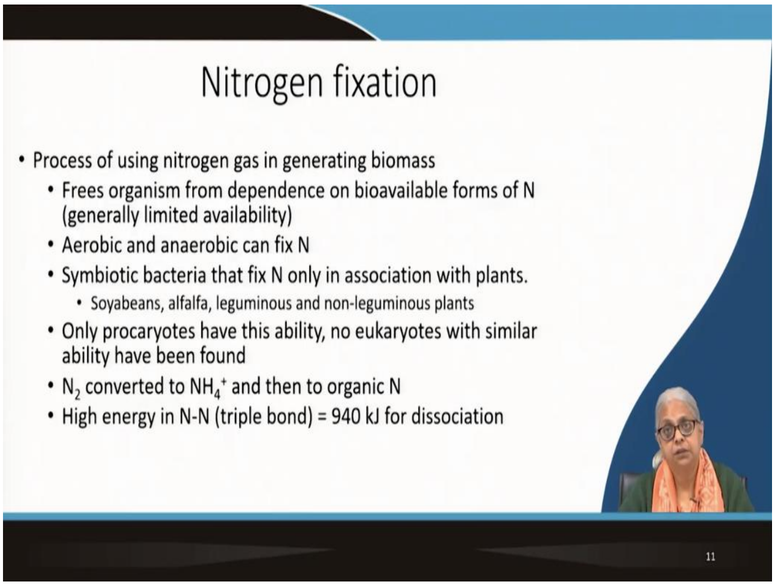

Nitrogen fixation

- Process of using nitrogen gas in generating biomass
  - Frees organism from dependence on bioavailable forms of N (generally limited availability)
  - Aerobic and anaerobic can fix N
  - Symbiotic bacteria that fix N only in association with plants.
    - Soyabeans, alfalfa, leguminous and non-leguminous plants
  - Only procaryotes have this ability, no eukaryotes with similar ability have been found
  - N_2 converted to NH_4^+ and then to organic N
  - High energy in N-N (triple bond) = 940 kJ for dissociation

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Then we come to nitrogen fixation. I have already mentioned that inert nitrogen gas which is abundant in our atmosphere is not bioavailable and for agricultural productivity, for primary productivity on the planet, it is essential to convert this gas to bioavailable forms. So, the bioavailable forms are limited. And if you remember what I said about limiting nutrient, often, the limiting nutrient for the growth or primary productivity of any ecosystem is because of either nitrogen or phosphorus. These are the 2 most common limiting nutrients in the environment. And you can have aerobic as well as anaerobic bacteria that are capable of fixing nitrogen. You can also have symbiotic bacteria that fix nitrogen only when they are associated with certain plants. So, soyabeans, alfalfa, leguminous as well as non-leguminous plants are known to have symbiotic relationships with nitrogen-fixing bacteria.

Only prokaryotic or bacterial species have this ability. As of now, no eukaryotes have been found with a similar ability. Nitrogen can be converted to ammonia, ammonium and then to organic nitrogen. So, you have high energy, in the triple bond, that is available.

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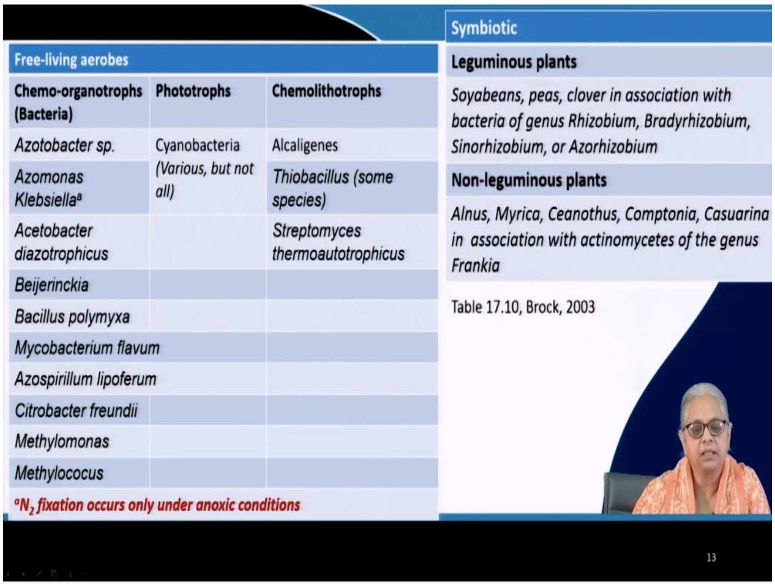

Free-living aerobes

Chemo-organotrophs (Bacteria)	Phototrophs	Chemolithotrophs
Azotobacter sp.	Cyanobacteria *(Various, but not all)*	Alcaligenes
Azomonas *Klebsiella*[a]		*Thiobacillus (some species)*
Acetobacter diazotrophicus		*Streptomyces thermoautotrophicus*
Beijerinckia		
Bacillus polymyxa		
Mycobacterium flavum		
Azospirillum lipoferum		
Citrobacter freundii		
Methylomonas		
Methylococus		

[a] *N_2 fixation occurs only under anoxic conditions*

Symbiotic

Leguminous plants

Soyabeans, peas, clover in association with bacteria of genus Rhizobium, Bradyrhizobium, Sinorhizobium, or Azorhizobium

Non-leguminous plants

Alnus, Myrica, Ceanothus, Comptonia, Casuarina in association with actinomycetes of the genus Frankia

Table 17.10, Brock, 2003

So, in this slide and in the next, I have quite a few examples from the textbook, of different types of species that are responsible for nitrogen fixation. So, we have some free-living aerobic species of bacteria. And within them, we have chemoorganotrophs, we have phototrophs and chemolithotrophs. Now, within the chemoorganotrophs, they all belong to the domain Bacteria in the classification of organisms.

So, here we have *Azotobacter*, *Azomonas* and *Klebsiella*. Now, *Klebsiella* is known to be capable of nitrogen fixation only under anoxic conditions. It is a facultative bacterium. Under oxic conditions, it is a completely aerobic, I think heterotrophic bacteria. It does not even go into the nitrogen-fixing mechanism. So, that happens only under anoxic conditions. That is something that is very peculiar about *Klebsiella*.

Other examples are *Acetobacter diazotrophicus*, *Beijerinckia*, *Bacillus*, *Polymyxa*, *Mycobacterium*, *Azospirillum*, *Citrobacter*, *Methylomonas* and *Methylococcus*. So, these are some of the chemoorganotrophs. Then you have phototrophs; cyanobacteria. Many of the cyanobacteria are capable of nitrogen fixation. We have chemolithotrophs like *Alcaligenes*; we have *Thiobacillus* and *Streptomyces*. All of them are chemolithotrophs.

Now, so we have nitrogen fixing bacteria that are capable of having symbiotic relations with either leguminous plants or non-leguminous plants. A few examples were mentioned in the previous slide and there are a few others here. So, you have bacteria of the genus *Rhizobium, Bradyrhizobium, Sinorhizobium* or *Azorhizobium*. All of these rhizobacteria are capable of fixing nitrogen in association with plants like soyabeans, peas, clover and so on. There are non-leguminous plants as well. *Alnus, Myrica, Casuarina*; all of them in association with *actinomycetes* of the genus *Frankia* can be used for nitrogen fixation.

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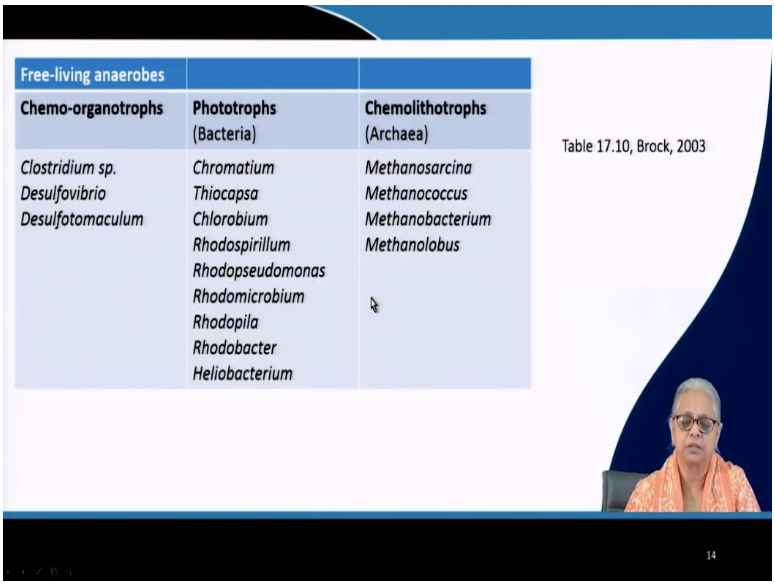

Free-living anaerobes		
Chemo-organotrophs	**Phototrophs** (Bacteria)	**Chemolithotrophs** (Archaea)
Clostridium sp.	*Chromatium*	*Methanosarcina*
Desulfovibrio	*Thiocapsa*	*Methanococcus*
Desulfotomaculum	*Chlorobium*	*Methanobacterium*
	Rhodospirillum	*Methanolobus*
	Rhodopseudomonas	
	Rhodomicrobium	
	Rhodopila	
	Rhodobacter	
	Heliobacterium	

Table 17.10, Brock, 2003

There are some examples of free-living anaerobic nitrogen fixing bacteria here. So, again in the 3 groups of chemoorganotrophs, phototrophs, chemolithotrophs, we have *Clostridium, Desulphovibrio*. In the phototrophs, we have so many other species, *Chromatium, Chlorobium, Rhodospirillum*. And in the chemolithotrophic group, these are all archaebacteria. So, *Methanosarcina, Methanococcus, Methanobacterium*; they are all capable of nitrogen fixation.

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REFERENCES

- Tortora, Funke and Case (TFC, 2010) Microbiology: An Introduction, Pearson Education.
- Madigan MT, Martinko JM, and Parker J (2003, 2015) Brock Biology of Microorganisms. 10th and 14th ed., Prentice Hall
- Mohanta, T and S Goel [2016] Statistical analysis of water quality and antibiotic-resistant bacteria in three aquatic environments over three seasons. *Pollution Research*, 35(1): 107-122.

Thank you. And that is the end of this particular part.