Soil Science and Technology

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Lecture – 33
Biological N Fixation

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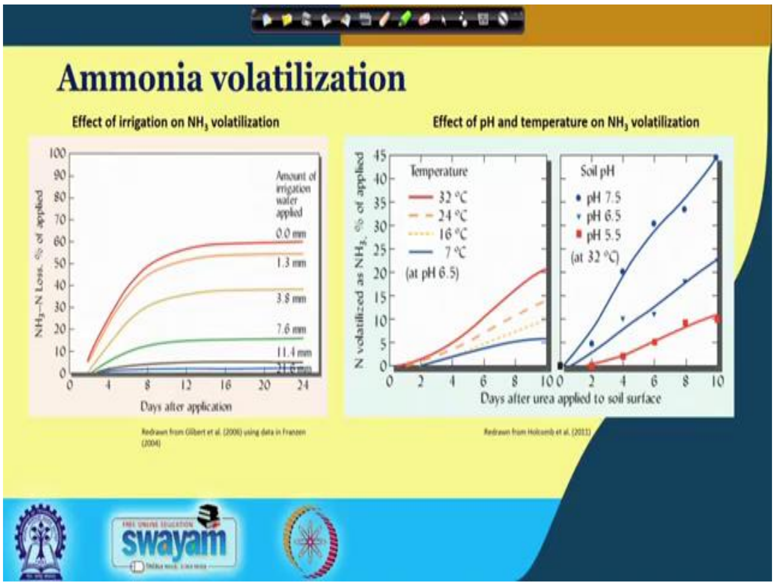

Welcome friends to this new lecture of Soil Science and Technology. And in this lecture, we will be trying to finish the nitrogen cycle. And then we will go and discuss about biological nitrogen fixation, which is one of the most important nitrogen you know transformation process.

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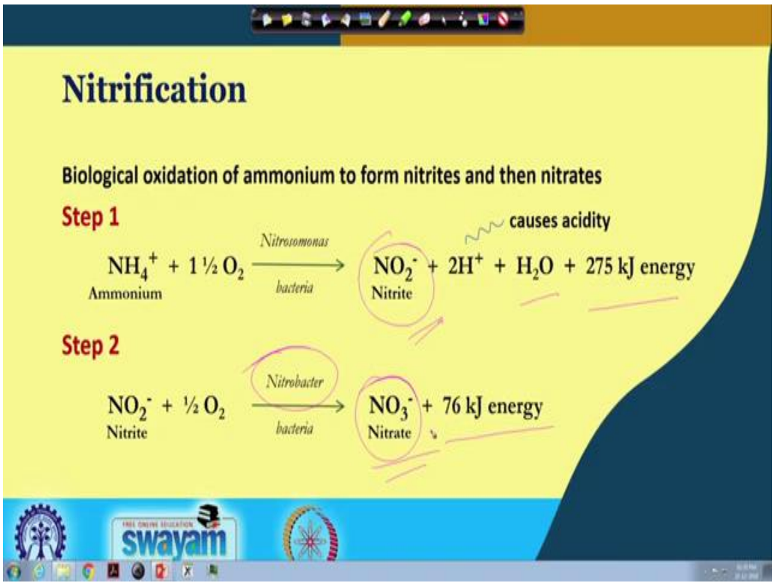

So, in our last lecture, we covered up to ammonia volatilisation. So, let us go ahead, and see what is nitrification? Now, nitrification you know that it is the conversion of ammonium nitrate into ammonium nitrogen into nitrate nitrogen, and it is basically executed in two in a you know consecutive steps.

In the first step, you can see it is basically you know you know oxidation of ammonium with oxygen, and it is mediated by a bacteria called nitrosomonas and ultimately it produces nitrite. And then you know it produces protons, which basically causes the acidity. And also it produces water and 275 kilo joule of energy.

And in second step, these nitrite is very very short lived and very very, it is very toxic to the plants. So, this nitrite is very very short lived; and again, it you know oxidized by the help of bacteria called nitrobacter. And ultimately, it produces nitrate and 76 kilo joule of energy. And this nitrate is the major you know major species available species of nitrogen that is being up take by the plant. So, you know this you know so this conversion of ammonium form of nitrate to nitrate form ammonium form of nitrogen to nitrate form of nitrogen is termed as nitrification. And it is basically oxidation process, where nitrosomonas, and nitrobacter bacteria you know plays a very important role.

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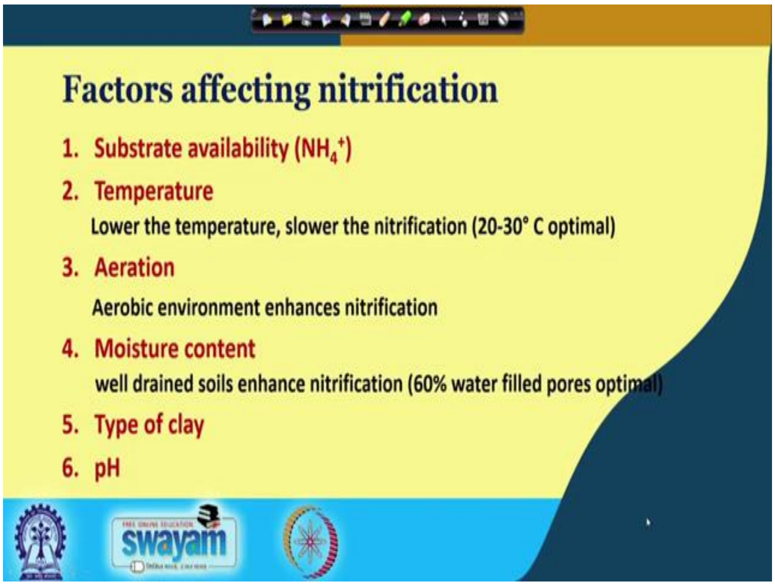

So, let us go ahead, and see what are the different factors which effect nitrification? Obviously, the substrate availability is the major issue. If there is no ammonium availability of obviously, there will not be there will not be any nitrification; because ammonia is a starting point ammonium is a starting point of nitrification process. And then obviously, the temperature lower the temperature, and slower the nitrification. So, we have found that the 20 to 30 degree centigrade temperature is optimal for you know nitrification process.

Aeration is required, because the aerobic environment enhances nitrification is in oxidation process. And moisture content we take as well drained soils to enhance the nitrification, and generally around 60 percent of you know of the water filled pores are optimal for curve you know for nitrification process. And also it depends on the pH and as well as the type of clay.

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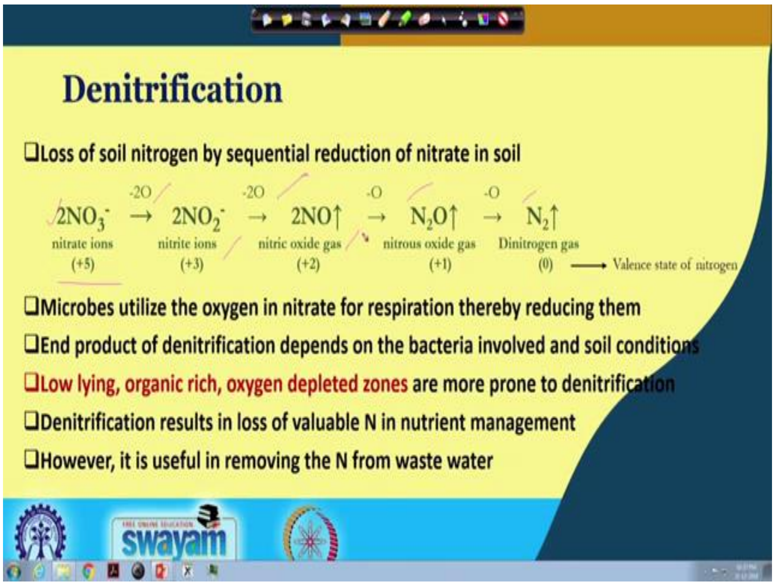

So, what is denitrification? Denitrification is just opposite of nitrification, so it is a you know it is a reduction process, where nitrate nitrogen through a successive steps of reduction, ultimately produces these dinitrogen gas, which ultimately escapes to the atmosphere. So, if you can see here, it is a basically loss of nitrogen by sequential reduction of nitrate in soil. So, the starting point obviously, here is a nitrate, and here you can see the valence state of nitrogen here is plus 5 in case of nitrate.

And as a result of oxygen in a continuous sequential reduction, there is a continuous loss of oxygen as you can see here. And ultimately the second steps, it forms the nitrate ions. And third step is nitric oxide gas. And then the nitrous oxide gas, which is very important you know green house gas. And finally, it produces the dinitrogen gas. And these dinitrogen or nitrogen you know along with nitrous oxide, and these nitrogen escapes into the atmosphere.

So, this is you know this is denitrification process. And you know it is also mediated by the different microbes, and because you know the microbes basically, and it basically occurs in the anaerobic condition, because microbes utilise oxide in the nitrate for respiration there by reducing them. So, in the reducing condition, when there is no oxygen available for these microorganism they uses these nitrate for their oxygen. And ultimately, they reduce this nitrate to dinitrogen which ultimately escapes into the atmosphere.

So, end product of denitrification depends on the bacteria involved and soil conditions obviously. And low lying, organic rich, and oxygen depleted zones are more prone to denitrification. And denitrification results in loss of valuable nitrogen in nutrient management obviously, you can see the nitrogen the final the end product is nitrogen which is being escaped to the atmosphere. So, you know it is useful in removing the nitrogen from waste water. So, this is the only the plus point, which this denitrification process has. So, guys we have you know we have completed this denitrification process also.

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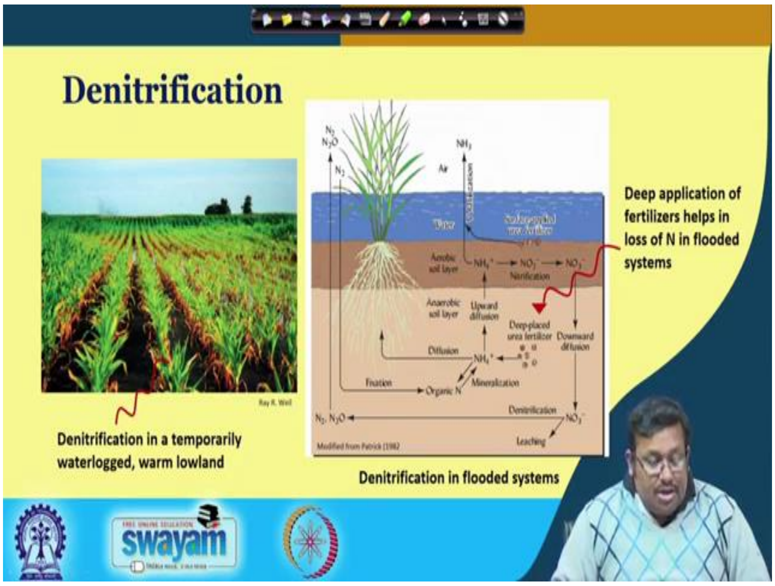

So, let us go ahead, and see no we have to you know there are some pictures of denitrification, you can see these are temporarily waterlogged soil obviously. In the waterlogged soil, all the pore space will be filled up with water. So, there will be no there is there is very little amount of oxygen, which is present in the pores in the pores all the oxygen all the or in other words all the air will be removed by the water.

And as a result of that all the microbes you know the microbes basically, they are facultative anaerobes. These facultative anaerobes basically use this nitrate as a terminal electron acceptor, and then it converts this nitrate into dinitrogen. So, also you can see here the schematic diagram of denitrification in flooded systems, and obviously please remember that deep application of fertiliser helps in loss of nitrogen in flooded systems. So, this is you know this basically to give you an idea about denitrification.

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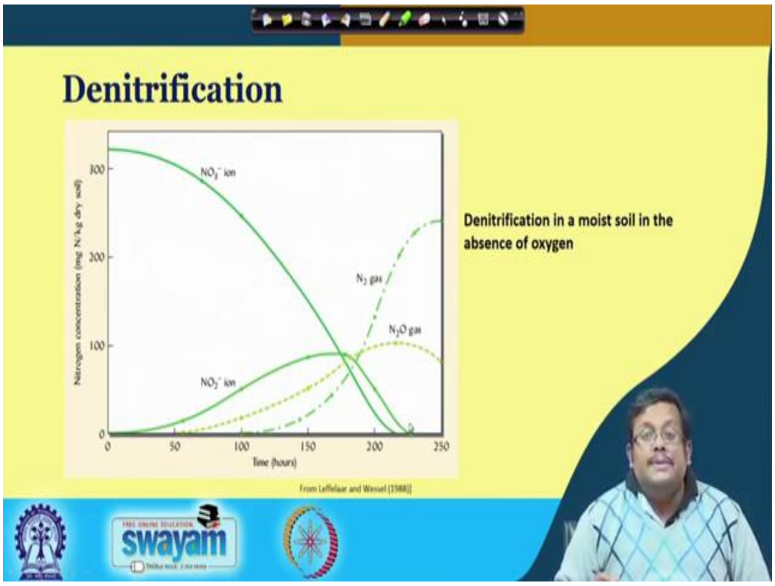

And remember that denitrification has the time passes, the nitrogen concentration. And if you see, the time in hours the relation between the time in hours and the nitrogen concentration, you can see here the nitrous oxide gas, and these nitrogen gas you know forms. And obviously, these are the times after the sub-merges, so after keeping the soil moist. So, you can see continue you know both nitrous oxide as well as nitrogen you know gas their concentration is increasing. And obviously, due to the absence of oxygen, the concentration of nitrate is getting decrease. So, this is basically the denitrification process.

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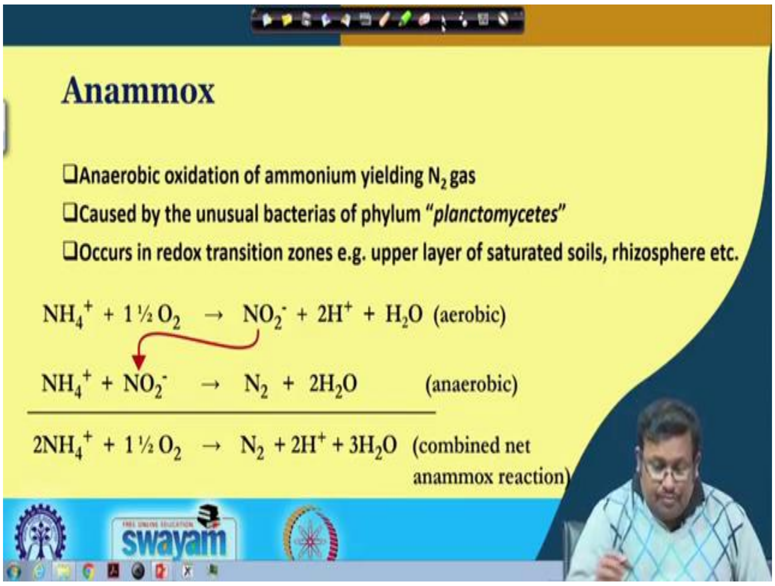

And let us see, what is anammox process. The anammox process is basically the anaerobic oxidation of ammonium yielding to nitrogen gas. So, it is anaerobic oxidation of ammonium, so that is the anammox process. And these basically caused by unusual bacteria of phylum “planctomycetes”. And it occurs in redox transition zones like upper layer of saturated soils, rhizosphere etcetera.

Because, you know that I have told you in our submerge soil lecture that there is a very thin, you know oxidised layer which is present in the saturated soil, especially in submerged soil. And these anammox process basically occurs in that redox transition zone, because it is just these zones basically lies above the reduced zone, which is present below. And so basically there are two steps, one is aerobic, and another one is anaerobic. So, in the first step you can see this ammonium is getting oxidised to form nitrite, and you know it is creating the protons, and water it is in aerobic oxidation process.

In the second step, obviously these nitrite which is denoted in the first step will react with ammonium to produce dinitrogen as well as water. So, if we combine both these both these, we can see this ammonium plus oxygen. So, this is an oxygen, this is an oxidation of ammonium, and ultimately it produces nitrogen, and then protons and water. So, it is again the oxidation of ammonium in anaerobic condition, and ultimately the production of dinitrogen or nitrogen gas. So, this is called the anammox process.

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So, nitrogen loss and environment; obviously, nitrogen loss by denitrification. And anammox obviously, in case of anammox also nitrogen dinitrogen forms, and which ultimately escapes to that you know atmosphere. So, nitrogen loss by denitrification, and anammox is detrimental to plant growth in soils, whereas it is beneficial in waste water management. Obviously, we want to that nitrogen which is present in the waste water getting to be removed.

So, nitrous oxide and you know nitric oxide are greenhouse gases, and you know they cause acid rain, and ozone depletion, etcetera. Because, these nitrous oxide when they went to atmosphere, they also create this acid rain and all this things. So, slow release nitrogen fertilizers and deep placement of fertiliser also can reduce the loss of nitrogen, because the slow release nitrogen fertiliser they control the release of nitrogen, because nitrogen is highly soluble in water specially the nitrate. And this deep placement basically, it helps them to reduce the reaction with atmosphere, and ultimately control the nitrogen you know nitrogen mobility. So, this how we can you know, we can reduce the loss of nitrogen.

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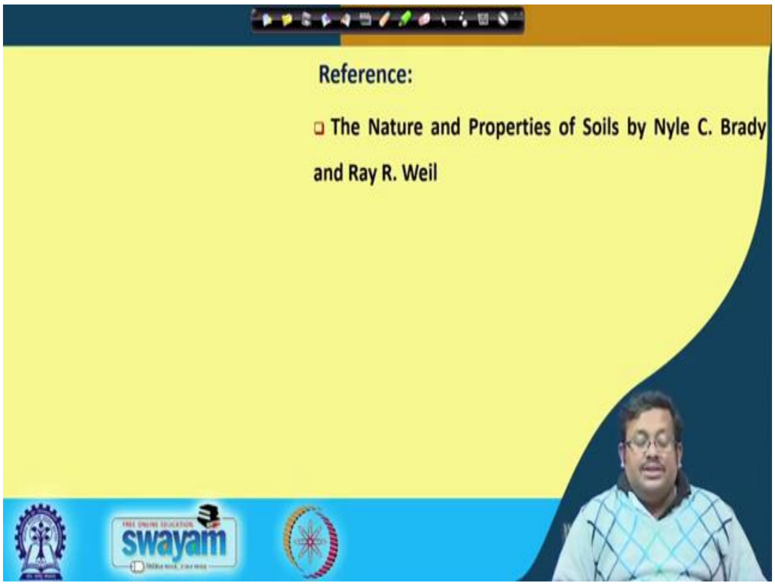

And so guys we have finished this nitrogen cycle. And let us go to a new topic that is biological nitrogen fixation, it is very important you know important topic of nitrogen, so that is why we will discussed this slowly as a topic.

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So, let us see what are the different concepts, which will covered in this lecture in this in this topic that is what is a biological nitrogen fixation, what is the importance of biological nitrogen fixation, then the mechanism of nitrogen fixation, then different fixation systems will discuss different fixation system, and also we will be discussing different factors, which are affecting the nitrogen fixation.

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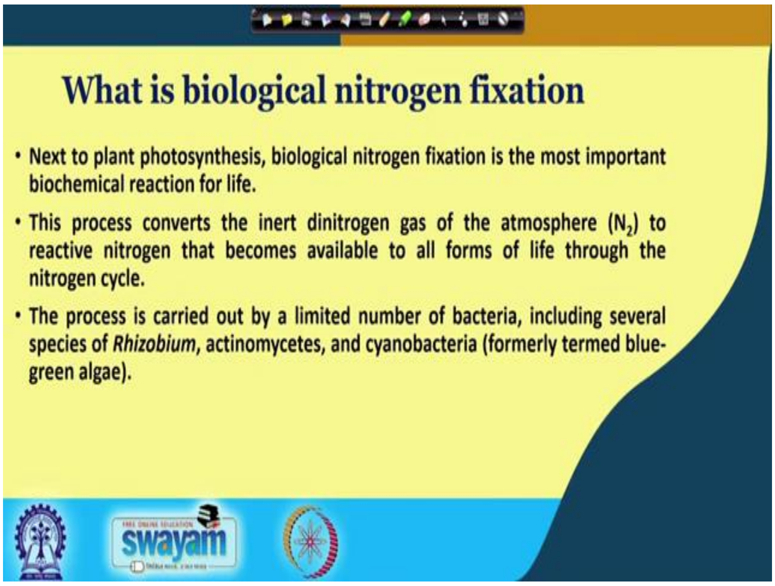

So, what is the biological nitrogen fixation? Now, it is basically next to plant photosynthesis, biological nitrogen fixation remember is the most important biochemical reaction for life. Because, this you know in the biological nitrogen fixation converts the inert dinitrogen gas of the atmosphere to reactive nitrogen that becomes available to all forms of life through the nitrogen cycle.

So, basically it is a conversion of nitrogen gas which is present in the atmosphere to rid some reduce form or organic forms of nitrogen. And ultimately, so by you know and these processes mediated by different microorganisms, so that is why, it is called biological nitrogen fixation. We are fixing the atmospheric nitrogen into the soil, and into the body of different microorganism. So, this I how this is why it is called biological nitrogen fixation. And the process is again carried out by a limited number of bacteria, including several species of rhizobium, actinomycetes, and cyanobacteria formally termed as blue green algae, and we will discuss that later on.

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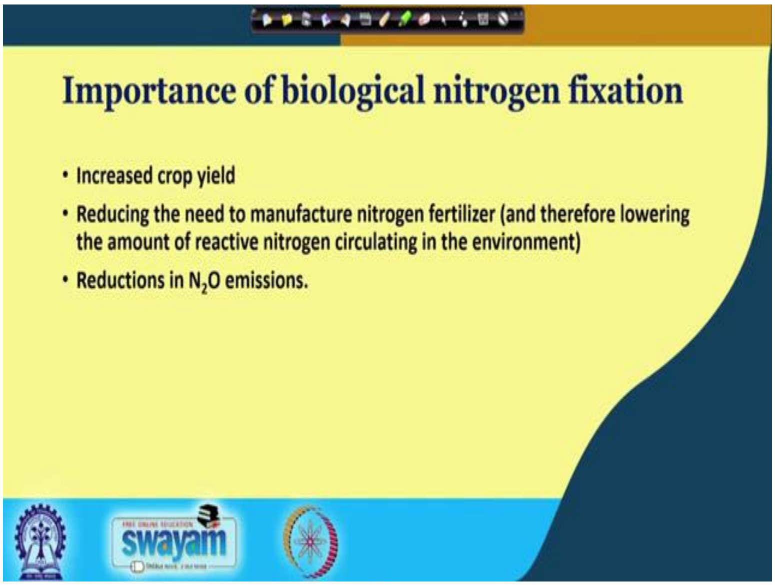

And what is the importance of biological nitrogen fixation. Well basically due to the biological nitrogen fixation, it helps to increase the yield of the crop, and it reduce the need of manufacturing the nitrogenous fertiliser, and you know because going there is a formation of biological. When there is a biological nitrogen fixation obviously, the need for adding supplemental inorganic fertiliser nitrogenous fertiliser is reused.

And therefore, lowering the amount of reactive nitrogen circulating into the environment, and ultimately due to these there is reduction is nitrous oxide emission, which is an important or you know very very dangerous greenhouse gas. So, this is the basic importance of biological nitrogen fixation.

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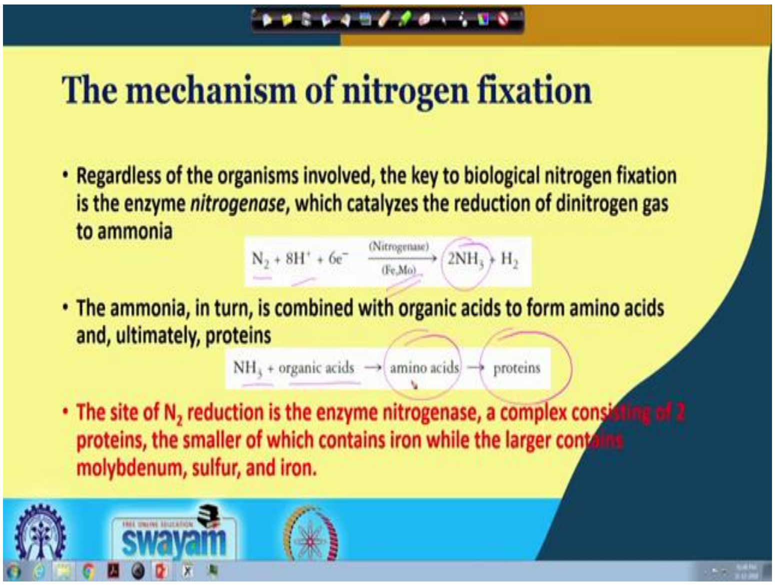

And let us see the mechanism of nitrogen fixation. Obviously, the regardless of organisms involved, the key to biological nitrogen fixation is the enzyme nitrogenase. So, biological nitrogen fixation is mediated through enzymes called nitrogenous, which catalyses which catalyse the reduction of dinitrogen gas to ammonia. And you can see here, you know here nitrogen is getting you know due to the nitrogenous enzyme complex, which basically contains iron and molybdenum, and they are basically reduced to form ammonia.

The ammonia, in turn combine with organic acids to form amino acids, and ultimately proteins. So, these ammonia when reacts with organic acids ultimately forms the amino acids, which in turn you know develop proteins, and because proteins is basically polymer of different you know it is a it is made of these amino acids. And the site of nitrogen reduction is the enzyme nitrogenase, a complex constituting protein a complex consisting of two proteins. The smaller of which contains iron, while the larger who you know unit contains the molybdenum sulphur and iron, we will see that in the next slide.

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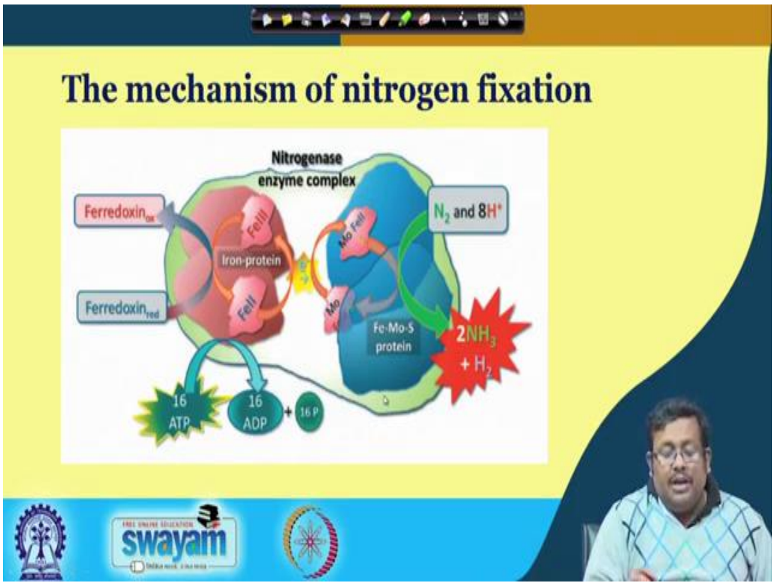

So, if we go to the next slide, you will see there is a nitrogenous enzyme complex. Obviously, there are two units the first the smaller units is basically, we can see here. And the larger unit basically contain the smaller unit basically contain iron contain iron, whereas the larger unit is basically contains iron, molybdenum, and sulphur. So, let us see how this nitrogenous enzyme complex, you know takes part in nitrogen biological nitrogen fixation.

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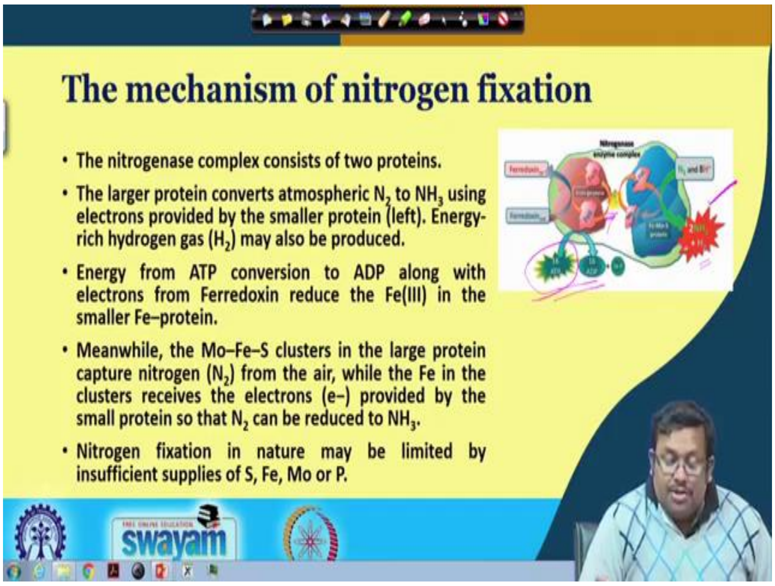

So, you can see these two proteins or these two units of these nitrogenous complex. The larger protein converts the ammonia to the larger protein converts atmospheric nitrogen to ammonia; you can see here the atmospheric nitrogen is getting converted to ammonia here using the electrons, which is provided by the smaller protein. You can see electron is moving from this smaller protein to this bigger protein. And also as a result of this conversion hydrogen you know gas is produced and energy from ATP conversion to ADP.

Obviously, you can see energy from ATP conversion to ADP along with electrons from ferredoxin reduces the Fe^{3} in the smaller Fe-protein. So, in the smaller Fe-protein you can see the Fe^{3} is getting reduced to Fe^{2}. And these reduction of Fe^{2} is basically mediated through this conversion of ATP to ADP or adenosine tri phosphate to adhesion di phosphate. And also from this conversion of ferredoxin reduced to you know reduced version of ferredoxin to oxidised version of ferredoxin. And ultimately, you know iron 3 that is ferric is getting reduced to ferrous, and ultimately there is a movement of you know of this electron to the larger protein, which contains the molybdenum iron and sulphur.

So, the molybdenum-iron-sulphur cluster in the large protein capture nitrogen from the air, where while the iron in the clusters receives the electron provided by the small protein, so that nitrogen can be reduced. Further reduction of nitrogen, you recur electron. And these electron is basically moving from this smaller protein to this larger protein, and ultimately it is reducing the nitrogen to ammonia.

And this nitrogen fixation in nature may be limited by the insufficient supply of sulphur, iron, molybdenum, and phosphorous, it is quite natural, because this nitrogenous complex is made of these all these compounds and also phosphorous is an important element of ATP. So, any you know insufficiency or deficiency of any of these element will cause you know nitrogen fixation process to you know to stop. So, these are some important factors for nitrogen important factors for nitrogen fixation, and I have told you the mechanism of nitrogen fixation.

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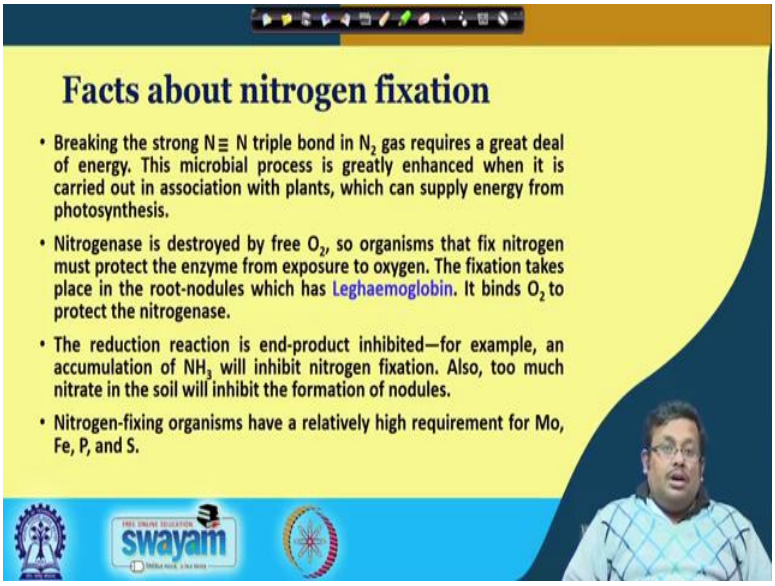

So, let us go ahead and see, what are the other important and exciting facts about nitrogen fixation? Obviously, you remember that the dinitrogen has to be converted to ammonium. So, this triple bond between this nitrogen-nitrogen has to be braked has to be broken, and these you know these requires a great deal of energy. And obviously, the microbial process is greatly enhanced, when it is carried out in a association with the plants, which can supply energy from the photosynthesis.

So, nitrogenase is obviously is getting destroyed by free oxygen. And so the organism that is fixed nitrogen must protect the enzyme from exposure to oxygen. And the fixation takes place in the root-nodules which has Leghaemoglobin. And this Leghaemoglobin basically binds the oxygen to protect the nitrogenase. So, if the oxygen is coming you know if the oxygen comes in contact with the nitrogenase, it will get destroy, and this biological nitrogen fixation will not occur. So, these Leghaemoglobin basically binds oxygen to prevent it to you know prevent it to you know oxidise in nitrogenase.

And the reduction reaction is end-product inhibited obviously, and accumulation of ammonia which is the end-product will inhibited the inhibit nitrogen fixation. Also, too much nitrate in the soil will inhibit the formation of nodules. Because, when there is an abundant amount of nitrate which is already present in the soil. So, there will not be further nitrogen fixation. And obviously, the accumulation of ammonia will also you know reduce the rate of production for end products. So, nitrogen fixing organisms have a relatively high requirement for molybdenum, iron, phosphorous, and sulphur, this quite obvious.

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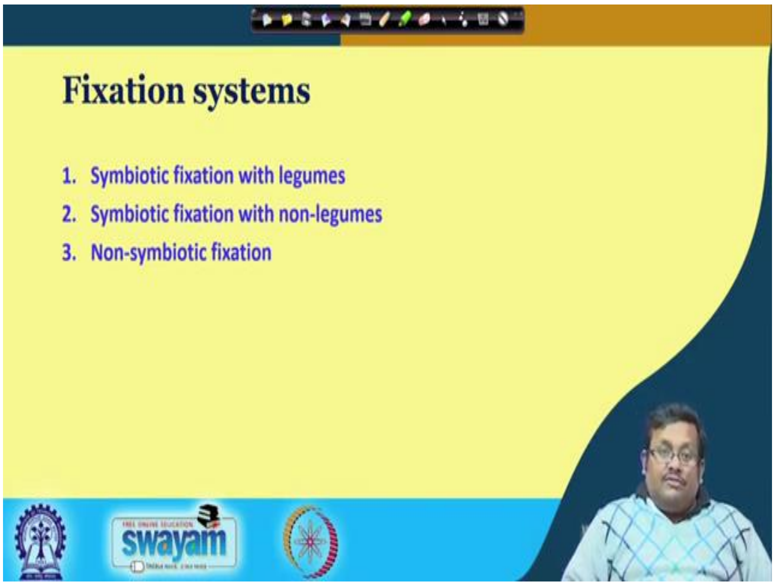

And let us see what are three different fixation system. The three different fixation systems are symbiotic fixation systems with legumes, then symbiotic fixation system with non-legumes, and thirdly it is non-symbiotic fixation.

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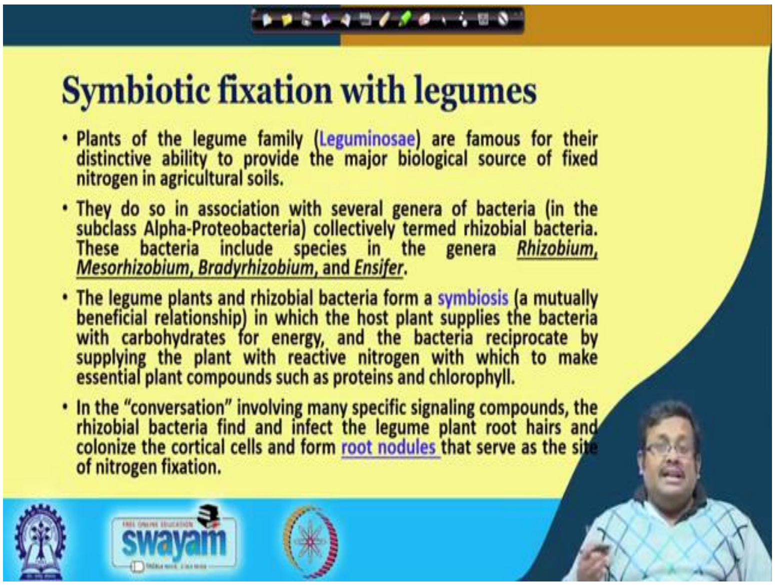

So, what is symbiotic fixation with legumes? Plants of legume family that is Leguminosae family are famous for their distinctive ability to provide the major biological sources of fixed nitrogen in agricultural soils. So, they are basically known for their biological nitrogen fixation ability. And they basically do that, because their association with the several genera of bacteria, you know in the in the sub class of alpha-proteobacteria collectively termed as rhizobial bacteria. And these bacteria includes species in the genera basically rhizobium, then mesorhizobium, bradyrhizobium, and ensifer.

So, the legume plants and rhizobial basically forms the relationship that is symbiotic relationship, it is the mutual benefit relationship in which the host plants supplies the bacteria with carbohydrate for energy, and the bacteria reciprocate by supplying the plant with reactive nitrogen with which to make the essential plant compound such as protein and chlorophyll. So, basically this is a symbiotic relationship, the plant supplies the bacteria with carbohydrates for their energy, which is required to which is required for breaking the dinitrogen. And also this bacteria will give them the plant, this reactive nitrogen which is required for their production of their proteins and as well as chlorophylls.

So, in the conversion involving many specific signalling these conversion is a you know it is a complex procedure, and it involves many specific signalling compounds. And the rhizobial bacteria find and infect the legume plant root hairs and colonize in the cortical cells and form root-nodules. So, these bacteria basically forms root-nodules in the in the roots of the Leguminosae plant, and that serve as the site of nitrogen fixation. So, these root nodules are basically the site for nitrogen fixation remember that.

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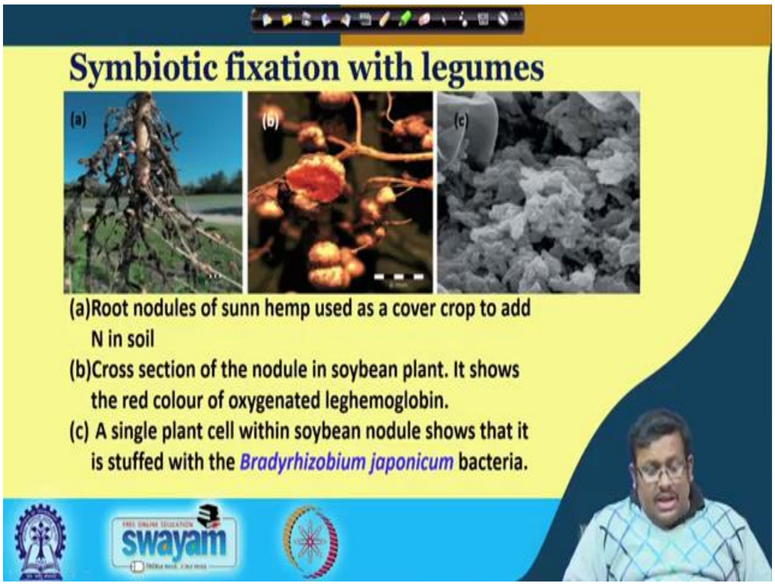

See you can see you know first in the first picture, you can see root nodules of sunhemp or dhaincha you know you know used as a cover crop or for adding nitrogen into the soil. So, root nodules of sunhemp as a cover crop to add nitrogen in soil. And the second photograph, you can see cross section of nodule in a soybean plant. And it basically shows a red colour of the oxygenated Leghaemoglobin.

And finally, you can see in the third picture you can see this single plant cell within soybean nodules which you know shows that it is stuffed with the Bradyrhizobium japonicum bacteria. And remember Bradyrhizobium japonicum responsible fixing nitrogen in association with soybean plant and you can see, these are the numerous Bradyrhizobium japonicum bacteria.

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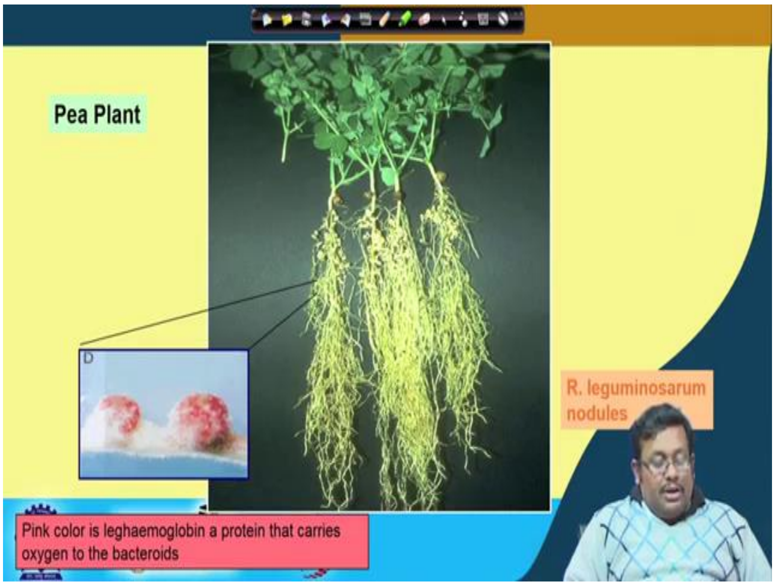

So, you can see some example these again the pea plant and you can see root nodules. And this rhizobium leguminosarum nodules. So, pink colour in the leghaemoglobin a protein that carries pink colour basically suggest the presence of leghaemoglobin, which is a proteins that carries oxygen to the bacteroides.

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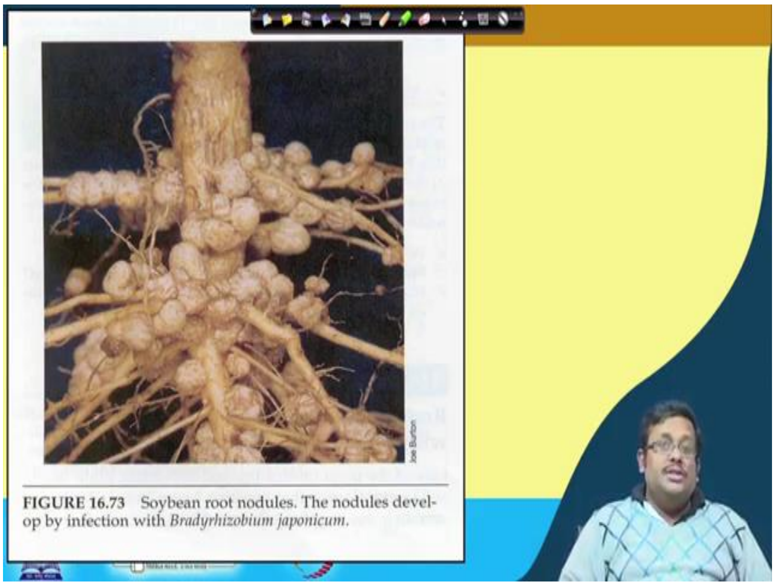

And again this is again the soybean root nodules. So, these root nodules are the basically the sides, where defer you know this nitrogen fixation basically takes place.

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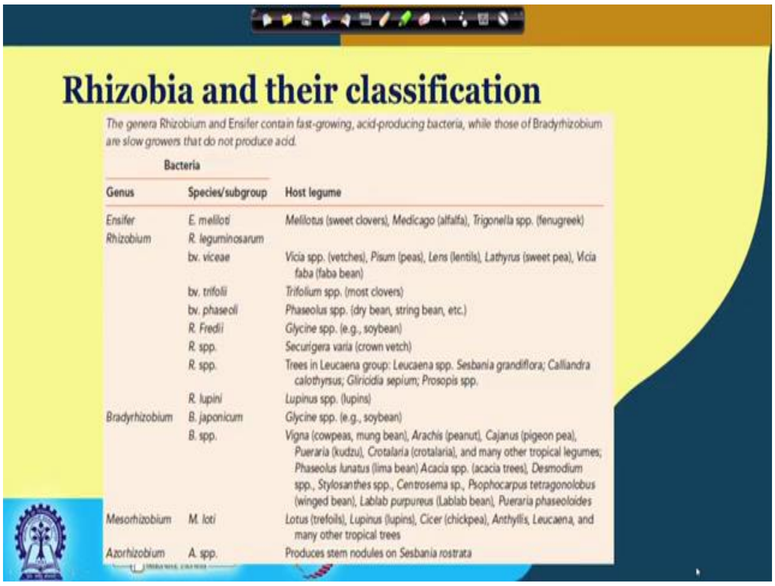

So, remember that specific micro-organism can be associated with specific group; specific plant for fixing these atmospheric nitrogen that means, these rhizobium, and legumes symbiotic relationship is very very specific. A specific organisms can you know form symbiotic relationship with a specific species. So, you can see here, just to give you some example you know rhizobium lupine, it can produce the root-nodules in lupinus or lupinis.

And as a rhizobium species, they can form produces stem nodules of sesbania rostrata, these sesbania rostrata is actually the dhaincha. And you know mesorhizobium loti, they forms this symbiotic relationship with lotus. And also you can see Bradyrhizobium japonicum. So, Bradyrhizobium japonicum basically you know helps in fixing the nitrogen in soybean or glycine max.

And so this you know rhizobium leguminosarum is responsible for fixing nitrogen in case of peas and lentils. So, so bv phaseoli is responsible for fixing the nitrogen in case of phaseolus spp, which is basically dry bean or string bean. And bv. viciae is responsible for you know peas and lentils. So, you can see that specific micro-organism is responsible for fixing the nitrogen in specific host legume. So, specific host legume.

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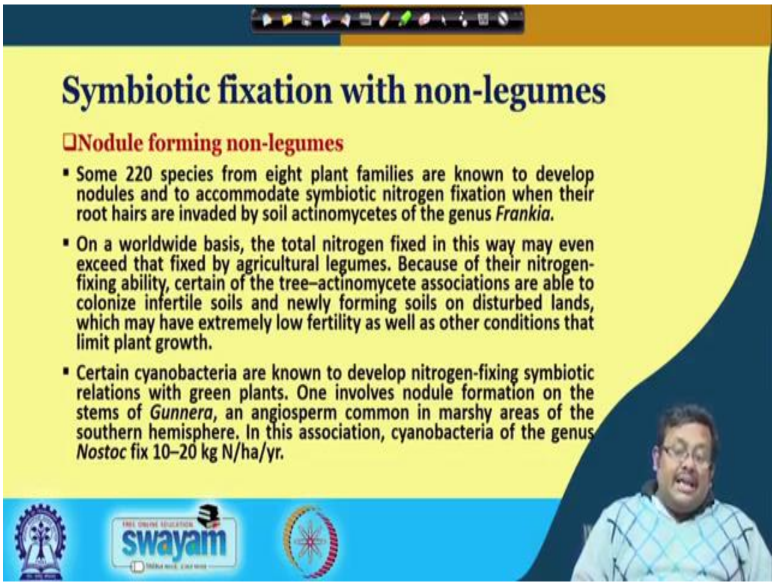

So, let us move ahead, and see what is a symbiotic fixation with non-legumes. Obviously, there are some you know there are some nodule forming non-legumes, obviously there are 270 species of eight plant families that are known to develop nodules and to accommodate symbiotic nitrogen fixation, when their root hairs are invaded by soil actinomycetes. And these soil actinomycetes is basically from the genus Frankia.

And on a worldwide basis, the total nitrogen fixed in this way may even exceed that fixed by agricultural legumes. Because, because of their nitrogen- fixing ability of certain tree-actinomycetes associations are able to colonize infertile soils as well as newly formed disturbed lands, so which may have extremely low fertility as well as their condition that limit the plant growth.

And remember that you know certain cyanobacteria are also knows to develop the nitrogen-fixing symbiotic relationship with green plants. And one involves nodule formation on the stem of Gunnera, and angiosperm common in marshy areas of the southern hemisphere and in this association, cyanobacteria of the genus Nostoc can fix 10 to 20 kg of nitrogen per hectare per year.

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And so let us see some nodule forming non-legumes. Some examples are given, you know genus alnus, the myrica, then casuarina, and all these are nodule forming non legumes ah. So, they form you know the nodules for fixing the nitrogen.

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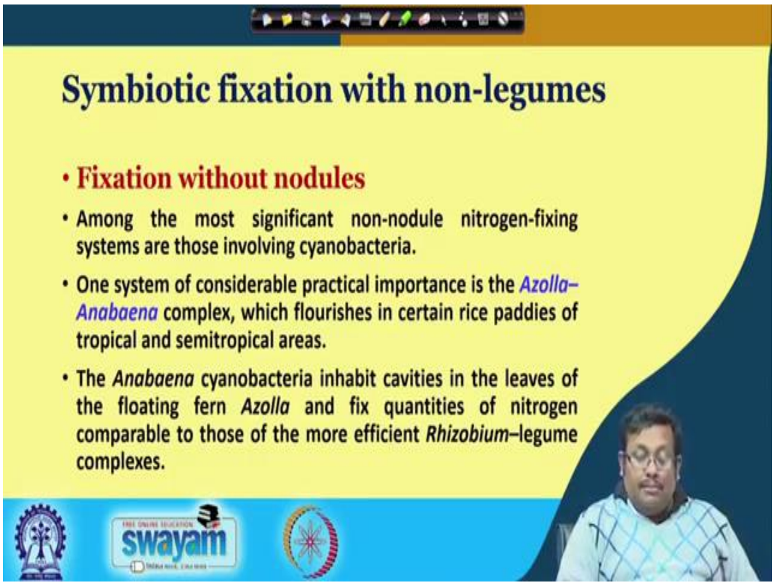

And also symbiotic fixation with non-legumes, so fixation without legumes. So, let us see fixation without legumes. So, among this most significant non-legume nitrogen fixation systems are those involving the cyanobacteria, among all the non-legumes. The most important nitrogen fixation systems is basically occur basically occur through cyanobacteria.

And one system of considerable practical importance is Azolla, and Anabaena complex. Now, azolla is a water fall, and anabaena azolla is basically a algae which and this azolla and anabaena complex or you know symbiotic relationship, basically produces this biological nitrogen fixation. And it flourishes in certain rice paddies of tropical, and semitropical areas. And the anabaena cyanobacteria inhabits cavities inhabit cavities in the leaves of the floating fern azolla, and fix quantities of nitrogen which is comparable to those of more efficient rhizobium legume complexes. So, it is very important.

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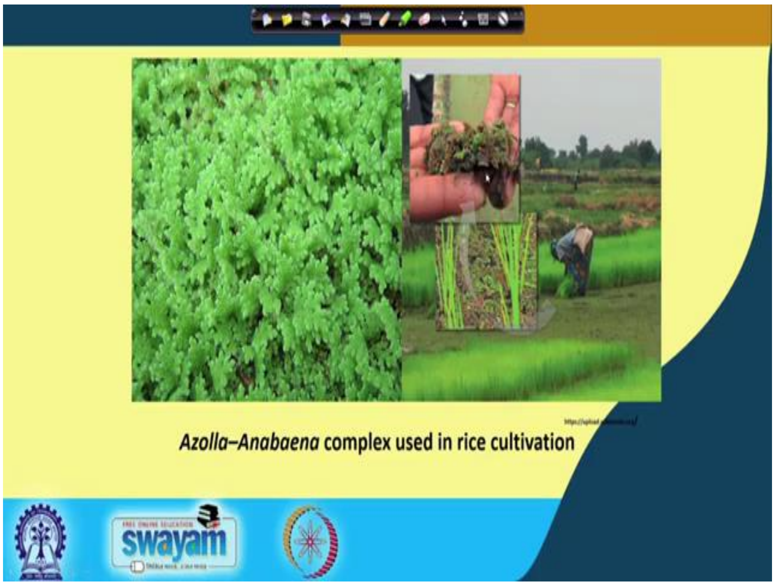

And you can see here the Azolla-Anabaena complex, this in Azolla fern. And obviously, at the bottom of the Azolla, you know these anabaena you know colonies can be found. And basically this type of symbiotic relationship can be found in you know paddy soils, and which is also responsible for fixing atmospheric nitrogen in this type of soils.

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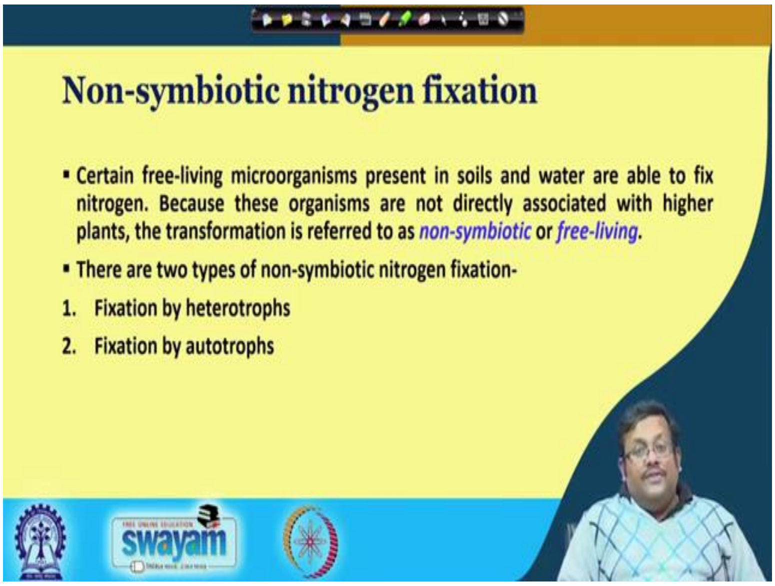

So, non-symbiotic nitrogen fixation, certain free-living microorganisms also present in soils and water, and also they are able to fix nitrogen. Because, this organisms are not directly associated with higher plants, these transformation is referred to as the non-symbiotic or free-living. And there are two types of non-symbiotic nitrogen fixation. One is fixation by heterotrophs, and another is fixation by autotrophs.

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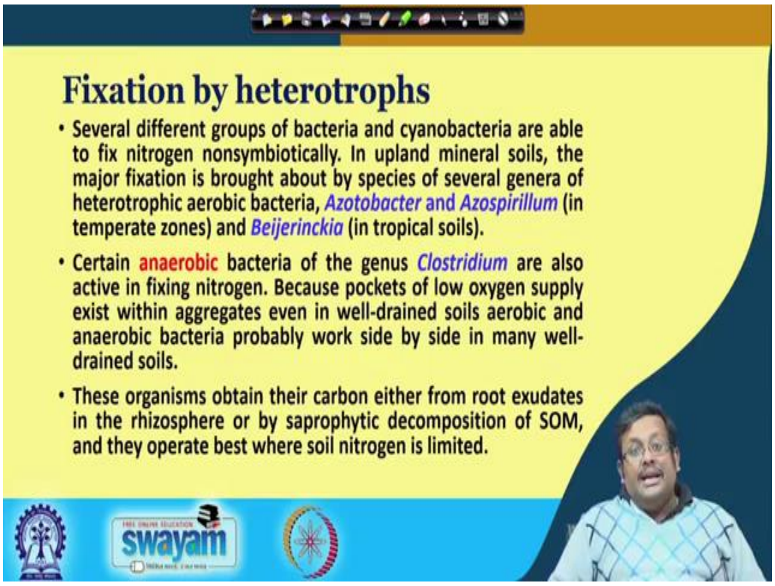

So, several different groups of bacteria in case of heterotrophs. Several groups of bacteria are known for non you know non-symbiotic nitrogen fixation examples are azotobacter, azospirillum, and beijerinckia. And in case of anaerobic condition also the clostridium bacteria also known to fix the nitrogen. So, these organisms obtain there, you know carbon either from root exudates or in the rhizosphere or by saprophytic decomposition of soil organic matter, and they operate best where the nitrogen is limited obviously. When the nitrogen is abundant why, they should fix the atmospheric nitrogen.

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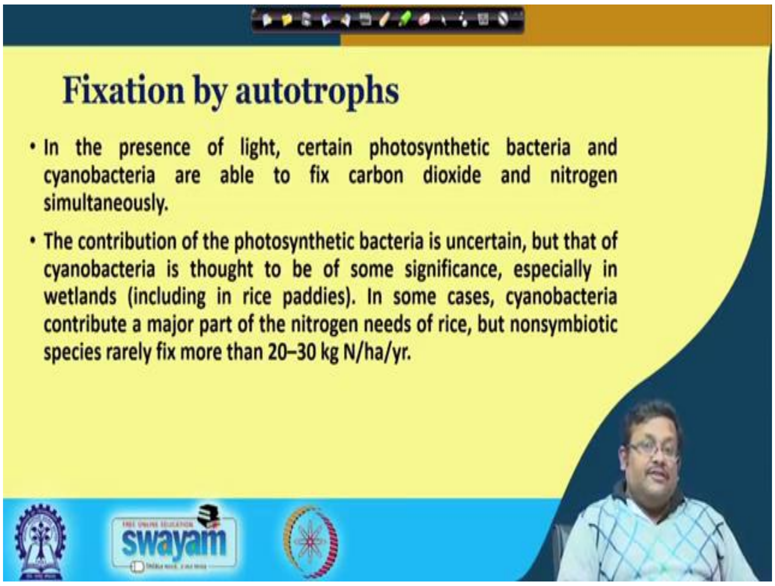

And fixation by autotrophs. Obviously, if the presence of light, certain photosynthetic bacteria and cyanobacteria are able to fix carbon dioxide and nitrogen simultaneously. And the contribution of photosynthetic bacteria is obviously uncertain, but that of cyanobacteria is thought to be some significant, especially in wet land in case of rice paddies I have already told you. So, in some cases, cyanobacteria contribute a major part of the nitrogen needs by the rice, but non-symbiotic species rarely fix more than 20 to 30 kg of nitrogen per hectare per year.

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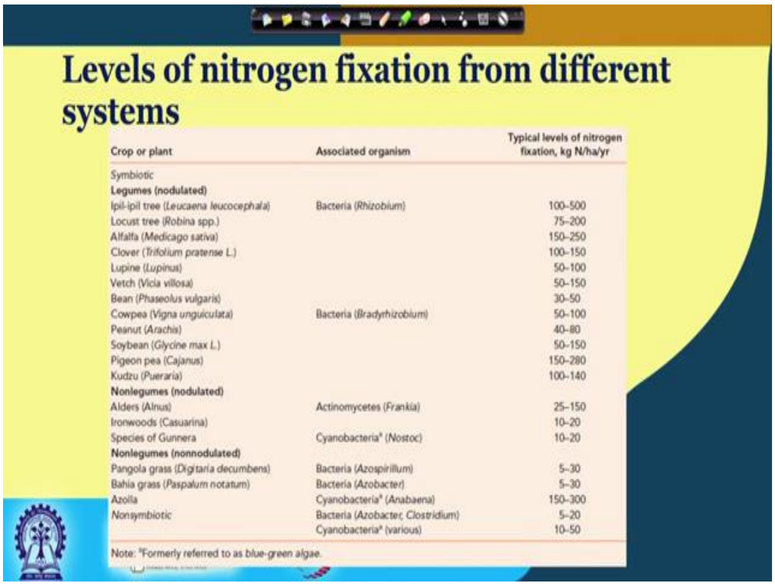

Crop or plant	Associated organism	Typical levels of nitrogen fixation, kg N/ha/yr
Symbiotic		
Legumes (nodulated)		
Ipil-ipil tree (*Leucaena leucocephala*)	Bacteria (*Rhizobium*)	100–500
Locust tree (*Robina* spp.)		75–200
Alfalfa (*Medicago sativa*)		150–250
Clover (*Trifolium pratense* L.)		100–150
Lupine (*Lupinus*)		50–100
Vetch (*Vicia villosa*)		50–150
Bean (*Phaseolus vulgaris*)		30–50
Cowpea (*Vigna unguiculata*)	Bacteria (*Bradyrhizobium*)	50–100
Peanut (*Arachis*)		40–80
Soybean (*Glycine max* L.)		50–150
Pigeon pea (*Cajanus*)		150–280
Kudzu (*Pueraria*)		100–140
Nonlegumes (nodulated)		
Alders (*Alnus*)	Actinomycetes (*Frankia*)	25–150
Ironwoods (*Casuarina*)		10–20
Species of Gunnera	Cyanobacteria[a] (*Nostoc*)	10–20
Nonlegumes (nonnodulated)		
Pangola grass (*Digitaria decumbens*)	Bacteria (*Azospirillum*)	5–30
Bahia grass (*Paspalum notatum*)	Bacteria (*Azobacter*)	5–30
Azolla	Cyanobacteria[a] (*Anabaena*)	150–300
Nonsymbiotic	Bacteria (*Azobacter, Clostridium*)	5–20
	Cyanobacteria[a] (various)	10–50

Note: [a]Formerly referred to as blue-green algae.

So, here you can see the levels of nitrogen fixation from different systems, obviously symbiotic you can see different symbiotic nitrogen-fixation, obviously they varies from hundred to 500 kg of nitrogen per hectares per year. And you know in case of alpha alpha you can see you 150 to 250.

So, the symbiotic nitrogen fixation is known to produce high amount of nitrogen through symbiotic relationship. In case of non-legumes, obviously some little bit amount you can see casuarina, there is producing 10 to 20 kg of nitrogen. And in case of azotobacter, and azospirillum, they are producing obviously, less amount that is 5 to 30 kg of nitrogen per hectare as well as cyanobacteria relationship they produce huge amount 150 to 300 kg of nitrogen per hectare. And some in case of non-symbiotic bacteria like clostridium, they produce 5 to they can fix 5 to 20 kg of nitrogen per hectare per year.

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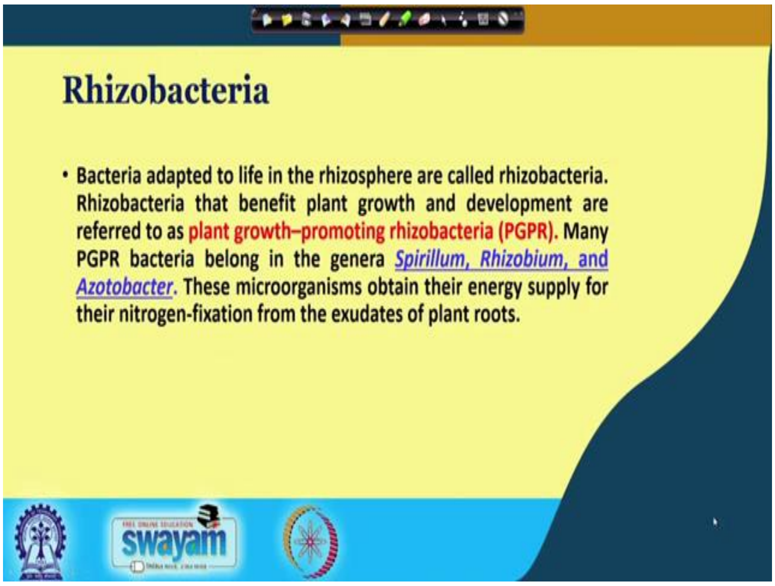

Now, rhizobacteria, rhizobacteria are bacteria adapted to the life in the rhizosphere are called rhizobacteria. And rhizobacteria that benefits the plant growth and developmental known as the plant growth-promoting rhizobacteria PGPR. These are very very important. And many PGPR bacteria belong to the genera of spirillum, rhizobium, and azotobacter. And these microorganisms obtain their energy supply for their nitrogen fixation from the exudates of plant roots. These microorganisms are nowadays a being utilized in different formulation for application into the field, for you know for several beneficial action, for the growth of the plant.

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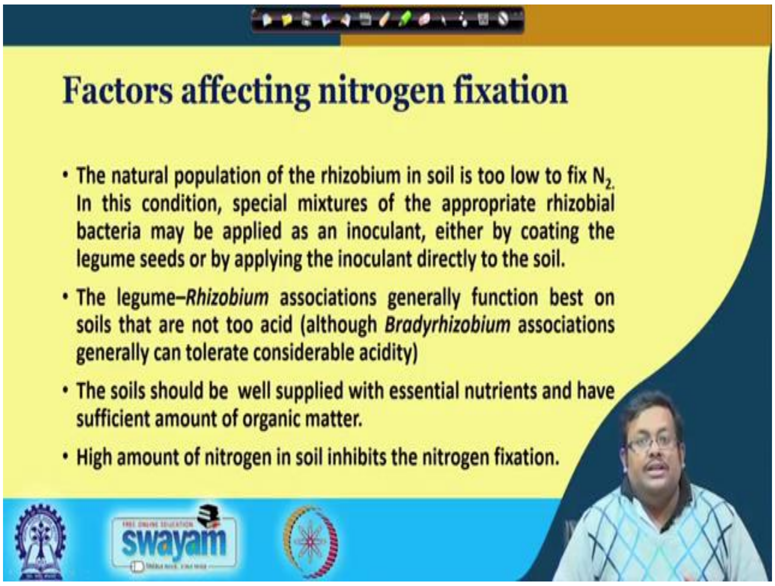

And this might be you know couple of slides. So, the factors affecting nitrogen fixation, obviously the natural population of the rhizobium is soil is too low to fix nitrogen. So, in this condition, special mixture of the appropriate rhizobial bacteria may be applied in the as an inoculant, either by coating the legume seed or by applying the inoculant directly in to the soil. And the legume-rhizobium association generally function best on soil that are not too acid. And the soil should be well supplied with essential nutrients, and have sufficient amount of organic matter. And high amount of nitrogen sometimes in soil inhibits the nitrogen fixation.

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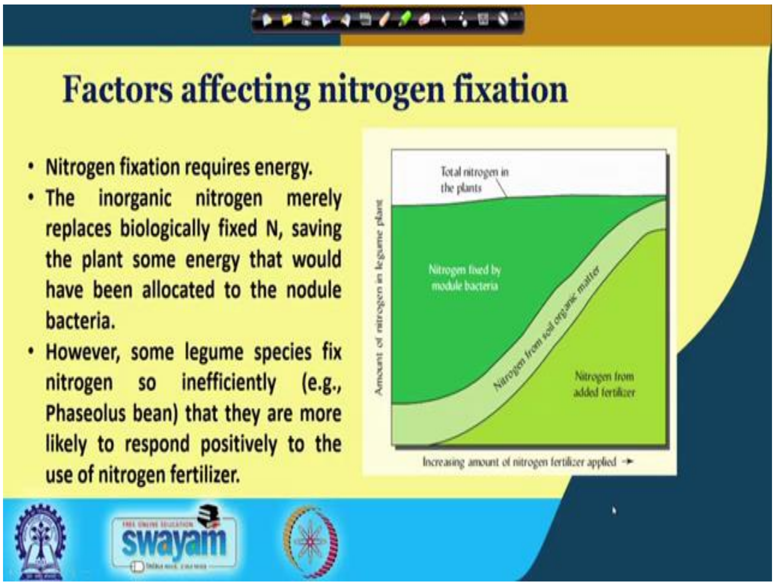

And remember that one thing that increasing amount of nitrogen, when you are increasing the nitrogen fertilizer application, obviously the nitrogen fixed by nodulated bacteria getting reduced. Although, the total nitrogen total nitrogen in the plants may remain fixed. However, you know there is a decrease in the nitrogen fixed by nodulated bacteria, when we increase the nitrogen fertilizer.

So, guys we have covered the one of the major processes of nitrogen cycle that is biological nitrogen fixation. Obviously, we cannot we cannot complete all the important details the nodulation processes, which is very much intricate. And you know require some complex signalling process, we did not have time to discuss all of these.

But, I hope that you got a basic idea about the biological nitrogen fixation, and I will encourage you to go ahead, and you know you know consult several books to get a better knowledge of in its of in depth knowledge of biological nitrogen fixation, because it is very very important for soil and plant growth. So, guys thank you. And let us meet in our next lecture with when we will start soil phosphorous and potassium.

Thank you.