Applied Environmental Microbiology
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Lecture - 30
Applied Environmental Microbiology

Dear students, in today's lecture, we will start our venture into the applications of environmental microbes. So far, we have been talking about the chemistry of microbes say metabolism their functions their genomes and different kinds of microbes including the bacterial eukaryotes, prokeca and archaea and also virus viroids and prion in the last lecture. In today's lecture, we will look at the first application of in microbes that we get from environment and the first application, we are interested in is remediating the problems we have created. So, remediation means finding a remedy for them and because microbes are giving us a remedy for our problems we call it bio remediation. So, bio is remediating our problem.

Now, this bio remediation can include many things usually it in it is referred to as when microbes consume by eating or degrade some pollutant. So, there is a pollutant like hydrocarbon petroleum and when microbes eat it up and clean it up we call it bio remediation that is say usual use of the word bio remediation.

But the word in itself is much more, broad and it includes any time, we use microbes to get rid of our problems. So, we will start today by talking about 2 other kinds of microbes that I did not include in the last lecture and then we will jump into bio remediation and we start bioremediation by talking about one of the major pro environmental challenges in the safe this century which is the acid mine drainage and the mining problem alright. So, let us get started ok.

So, as I mentioned we will start first with other-other microbes that I did not talk about in the previous lecture apart from bacteria virus viriod and prion. So, we have protozoan and algae. So, let us look at their applications and the utility. So, a protozoa is a eukaryote, but say a unicellular eukaryotes usually when we talk about eukaryotes the first impression for many is that oh we are talking about multi-cellular life forms such as humans such as moss fungus, but we have unicellular prokaryotes as and if you

remember from your class sixth science you must have learnt about amoeba or class 6 or class 8 science, you must have learned about amoeba that a protozoa.

So, protozoa is unicellular which means that a single cell organism is highly specialized. So, unlike bacteria, it can not only do the base initial functions of life, but it has some very interesting novel features for example, most protozoa can actually engulf bacteria digest them and eat them. So, it actually has an injection digestion and even excretion process. So, in this sense it resembles multi-cellular organisms, but it is; obviously, if they are different from multi-cellular life forms its ecologically diverse.

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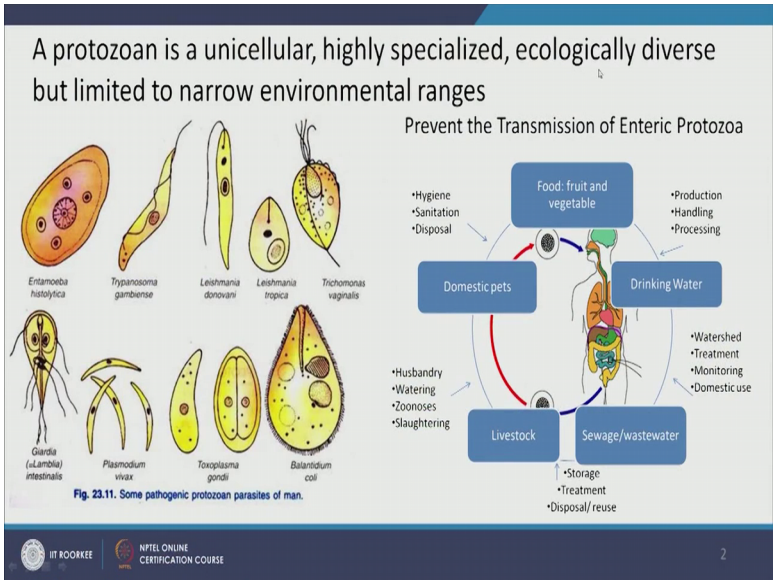

So, you find different kinds of protozoa present in different kinds of environment, but its limited to narrowed environmental range.

Now, please note that the archaea is our quite prevalent in environment and they are usually found in extreme environments like let us say oligotrophic environment high pressure high temperature highly acidic highly alkaline environment, but also in their ubiquitous in nature elsewhere for example, we have archaea growing in soil right we have some archaea in our drinking water distribution system bacteria.

On the other hand are ubiquitous, they present everywhere now protozoa are more limited in where they are found to thrive. So, some protozoa that we have; obviously, we have protozoa they thrive in our body many of them are pathogens. So, very interested in

them and here you have some pathogenic protozoa and look how different their morphology is. So, entamoeba histolytica, it causes do you know what it causes it causes amoebic dysentery and this is endemic in many parts of our country right now at least I know from where I come Uttar Pradesh, we wishes entamoeba, histolytica related infections are and endemic and one of the issues we have with this infection is that this protozoa is getting resistant to the anti microbials, we used to kill it.

And the other problem with protozoan diseases my dear students is that because they are eukaryotic many drugs that kill them. So, let us say I have protozoan infraction let us say entamoeba histolytica infections; however, amebic dysentery and it is not a very pleasant experience, but I take metronome dissolved to in a dissolved to kill myself. Now these drugs did not only hurt the protozoa, but because the protozoa is eukaryote they also affect my system because I am also my human cells are also eukaryotic.

So, we cannot overdose the anti microbials used against protozoa, then you have trypanosome, gambians and then you have leishmanias causes leishmaniasis really bad disease leishmanias tropical and trichomonas vaginalis doctor causes disease in vagina you have giardia causes really bad disease of flow of stomach a plasmodium vivax. Now plasmodium vivax causes malaria your toxoplasma gondii and balantidium coli. So, different morphology is causing different diseases.

Now, how do we prevent this enteric protozoa enteric means the ones that are inside us well they are usually foodborne and they have a very strong oral fecal drought? So, if we can put a stop to oral fecal drought if we can ensure the safety and sterility of our food, then it is not a problem we not get the diseases. So, the way it we in humans get infected is we drink contaminated water producer proliferate in our body and then we excrete them they going sewage or waste water in India, we do not have a very good sewage collection system in most part of the country and wherever we do we have issues with waste water treatment and then the contaminated water contaminates environment which contaminates animals which contaminates our pets which contaminates our food.

So, on a pet from contaminated food, but or the contaminated environment contaminates all of them and we get exposed to all 3 if we eat meat we get from livestock if you are if you have domestic pet. So, you might get diseases from them protozoan diseases and if you eat contaminated food then we get we can get say protozoan diseases from them now

with in case of plasmodium vivax or falciparum either of the malarial parasites this is not; however, the transmission works the transmission is vector borne.

So, it is water related disease; buzz it, but it is vector borne. So, what it implies is that it requires a vector for transmission here it is direct ingestion. So, if I ingest contaminated food then I get sick right.

But in plasmodium it requires the mosquito to breathe in water get the caster sickness and then carry the plasmodium protozoa and. In fact, a healthy animal healthy human being and then carry it forward and the disease spreads. So, for, but for others mostly it is over figured out for this it is plasmodium it is vector borne already.

Now here is the thing if you do not want to fall sick to protozoa it does not mean you stop eating food does not mean you stop having pets does not mean that you stop eating meat what does say what it does mean is that you maintain some sand some sanitation and the place where we really need to focus the sewage and wastewater treatment for most developed countries.

These diseases are not an issue entamoeba histolytica is I do not know if there is an outbreak of entamoeba histolytica in any developed country right now, but in India, it is endemic and what I mean by that is that right; now in India, there are pockets that have severe infestation with entamoeba histolytica because our sewage and wastewater treatment facilities really bad.

So, where we need to focus is here in sewage and wastewater interestingly after I am done with bio remediation I will be picking up wastewater treatment and we will be talking about waste water microbiology, but you will get to understand more about this for a treatment. So, stay alert and keep tuned already.

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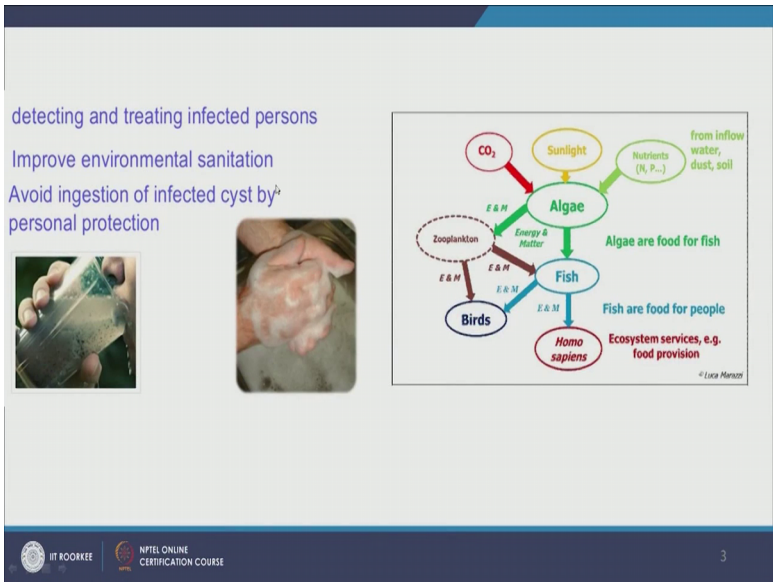

Now, how now again coming back to protozoa diseases how to avoid them, we can first need to detect and treat infected people because remember, if you look at this diagram the. In fact, the infected people if not treated will multiply the copies or protozoa will go through their fecal matter and. In fact, other people we need to improve environmental sanitation and last part of it this wastewater treatment and we need to avoid ingestion of. In fact, is infected cyst by personal production for protection? So, you need to wash your hands before we eat we need to clean drinking water. So, this is all about protozoa for now and then as we go through different environmental challenges if protozoa plays the role in them will talk about protozoa again.

Now, let us look at algae; algae again eukaryote, but it needs to be studied separately because it is a photosynthetic eukaryotes. So, basically it is like a plant, but it is not a plant. So, what algae can do is it takes sunlight produces food doing using photosynthesis and then it is consumed by zooplankton which are consumed by birds and fishes and then fishes are consumed by homo-sapiens some birds are also consumed by homo-sapien. So, eventually the algae contributes to human food in a long way there are some algae in some parts of the world that are eaten. So, we directly consume algae to. So, algae are very intimately linked with our human help ok.

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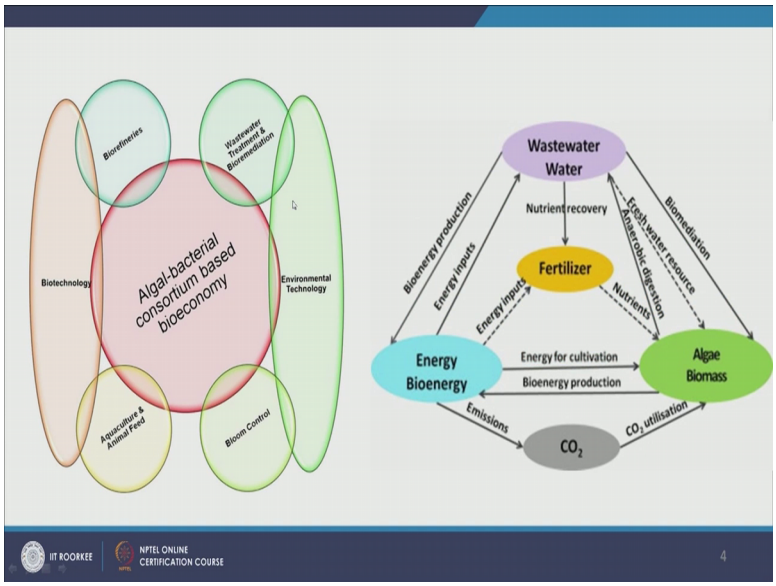

But this is not all about algae there is more about algae to algae in recent decades have proven to be a promising source of energy and a promising source of organic matter for doing different things. So, let us look here algal bacterial consortium based bio-economy. So, we have developed we are developing and we have developed a consortium of algae and bacteria that can do different kinds of jobs for us for example, they can clean our sewage for us they can remediate our contaminants for us. So, we can use them in wastewater treatment and bio remediation we use them in environmental technology when we are trying to get more energy make green energy or other things we use them for bloom control, right.

So, if there is an algal bloom in a lake and we need to control it and if we add the right bacteria if you add the right kind of algae we might be able to restrict the bloom also algae and bacteria depending on how they are growing and how much feed they can generate they can also be used for aquaculture feed and feeding animals there is a lot of application right now in biotechnology in bio refinery and all those fuels remember that because some algae under stress will store lipids in their body and this blip these lipids can be used as fuels.

So, here is another diagram on the right which is showing how wastewater treatment fertilizer energy and carbon dioxide cycle can be matched with algal biomass and this can help us generate more sustainable forms of energy cleaner water more.

Sustainably get good source of fertilizer sustainably.

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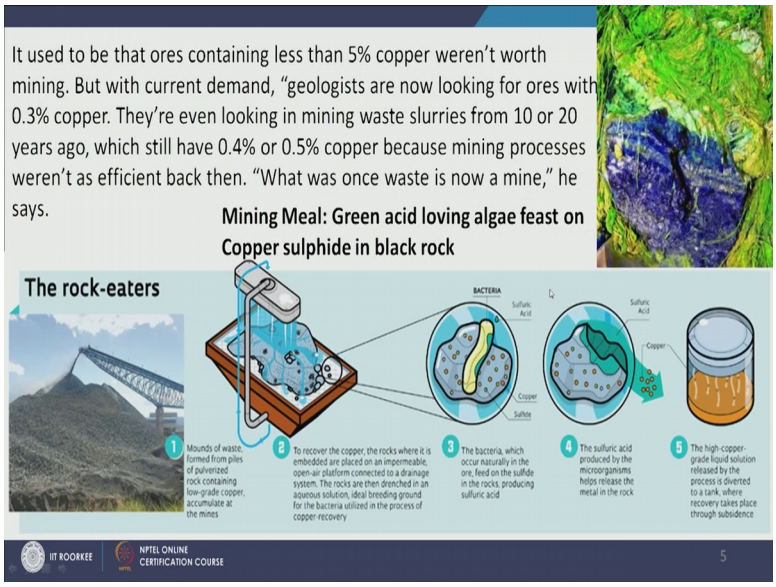

Let us move on now. Now we are moving on to bio remediation. So, there is good really slow and let me tell you about when I talk about bioremediation as I mentioned in the introduction we are using the bio for finding remedy for our problems.

So, I know the popular understanding a bio remediation right now is well then a microbe or microbes are eating pollutants bioremediation, but that is only one form of bioremediation. So, what I want to start with is mining definitely for gold for silver and to some extent for copper we have already went past the peak production. So, these me metals these ores which are highly valuable. So, all our electronic circuits are copper the jewelry is gold.

Gold is also used for medicine gold is also used in research same as with silver it is a very powerful antimicrobial, it is used in many different forms of research and different kinds of activities, it is also precious metal that is used in jewelry. So, gold and silver including copper these are very very important for our human activities and not just them, even iron and other things, but for these metals we know that we have passed the peak production. So, what it implies is that we are not going to produce gold silver copper anytime again in that abundant quantity as we were doing earlier.

So, basically we have exhausted the rich resources the rich mines rich ores on our globe and the ones that are still there will not be able to meet them will not be able to meet the same production rate as we were doing earlier. So, in this case now that our rich ores are almost depleted we have to go back to the poorer ores the ores that have much lesser quantity of the metal in them.

Now it is not economically feasible that if you are mining metals from core that are ore that are less there has less than 5 percent of the metal in them. It is not economically feasible. So, early people used to waste it, it is just waste, but now using microbes we can actually leach out the metal and then concentrate it and not only 5 percent, but we can go to ores that have up 2.3 percent of metal like 0.3 percent copper 0.3 percent gold and then get our precious metal out of it and still make a profit.

So, how does this work well this works very simply metals these metals have a quality that if they are washed with acidic media they will dissolve in the acidic media this is how we concentrate them now how do you make acidic media and how do you dissolve them this is catalyzed and driven by microbes.

So, let us look into it. So, in this slide lets read this out it used to be that ores containing less than 5 percent copper burnt worth mining, but with current demand geologists are now looking for holes with 0.3 percent copper that is very little copper, they are even looking in mining waste slurries from 10 or 20 years ago which still have 0.4 percent or 0.5 percent copper because mining processes were not as efficient back then what was once a waste is now a mine. So, this is from article had left copy and on the right you have a picture which the all algae actually not just bacteria, but algae that is feasting on copper sulphide on a black rock and thus you have these beautiful green and blue formation.

So, how does this mining business work when it comes to microbes how do microbes help us extract metal from such low concentration ores ore said 0.3, 0.4, 0.5 that is very less earlier even 5 percent was less than 5 percent; 5 percent was not economically feasible. So, the way we do it is that we accumulate all the ways the low percent ores and then when they have been isolated they are put in a drainage system when they are drenched with an aqua solution that allows bacteria to grow. So, when bacteria grow in

the aqua solution they do the electron donation electron acceptors acceptance business and they lower the pH when they lower the pH if they allow the dissolution of copper.

So, usually if you know if you remember from your chemistry in eleven twelfth class and from your geography most of the ores are reduced form of metals. So, you will have iron sulphide and many of the ores are actually pyrites. So, remember copper pyrite island pyrite. So, these are pirates and pyrite means their sulphide.

Now, we have sulphur oxidizing bacteria that will oxidize sulphur makes sulphur dioxide and you will get sulphuric acid sulphuric acid which reduce the pH drastically. So, if we can convert sulphide into sulphate also sulphide that will in the presence of aqua it will become sulphuric acid or sulphuric acid and the metal will dissolve and it will leach out.

So, we use metal leaching and we reduce by the dissolve by washing it with less with highly acidic media and we reduce the pH of the aquas media by letting the microbes oxidize the ores. So, I took this waste here I put it in a bath and this bath allows sulphur oxide oxidizing microbes to flourish when they flourish. So, this iron pyrite ore copper pyrite which is CuS or Fe2S this FeS2 for this is done this is oxidized by the bacteria and it produces sulphuric acid now what does sulphuric acid do it washes away the metal. So, you have copper washing away.

So, the sulphuric acid produced by microorganisms help release the metals in the rock, now the high copper grade liquid solution released by process is diverted to a tank where we do a recovery through subsidence ok.

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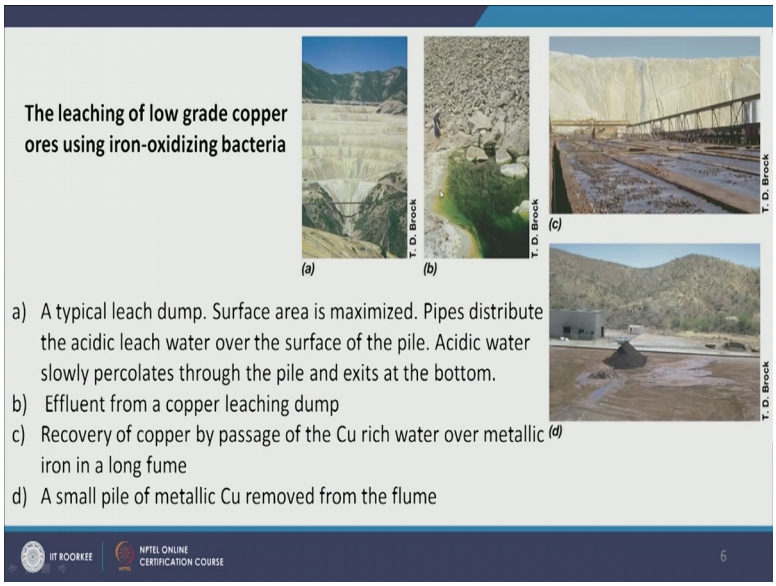

So, these are other pictures from USA and they are from the origin look there by the original Doctor Brock researcher. So, here you have low grade copper ore that is being leached out using iron oxidizing bacteria. So, in many a times copper and iron many copper and iron ores actually co exist. So, this is one of those mines and this is your typical leach dump and here we try to maximize the surface area and thus we have different steps as you see here.

Now we have pipes at pipes that go through and they distribute the acidic leach water over the surface of the pile and acidic water will slowly percolate down and it will wash away from here and when it washes you see this well really dark green this is rich in copper and this is the effluent and here this effluent is the copper rich effluent is being passed over metallic iron and here you have a small pile of copper that you have extracted.

So, the way this copper iron reaction works is very beautiful let us take a look.

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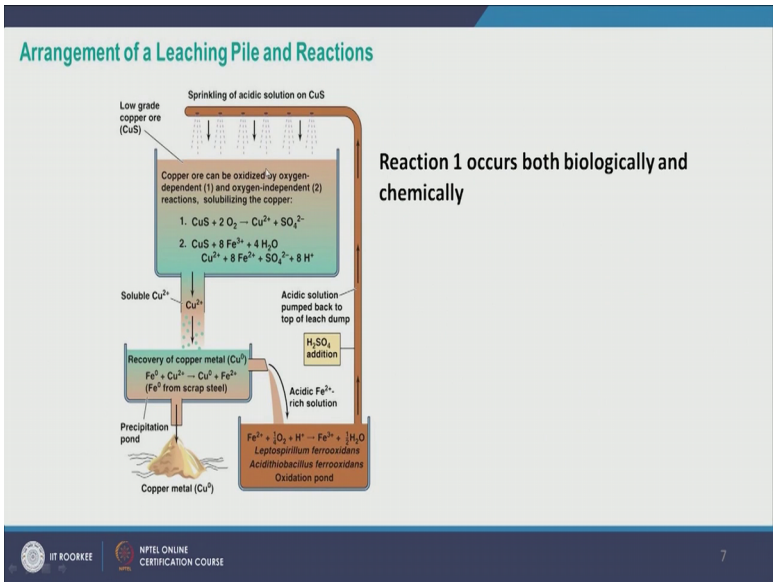

So, remember we arranged the copper the poor quality copper sulphide and you have put the poor quality copper sulphide here and you are sprinkling acidic solution on it and copper they this; this reaction the first reaction oxidation of copper sulphide happens both chemically and biologically. So, you have microbes that are oxidizing couple pyrite and making cupric ion and sulphate ion and then copper pyrite also treats with iron and is present with iron and sulphate and low p H. So, lot of h and because of this the copper gets dissolved and when copper gets dissolved you can request send it to the recovery part. So, in recovery of copper metal you have you have iron ferrous you have cupric and then you reduce a copper oxidize the iron right and then you have copper metal that makes a small pile here which is this.

On the other hand, how do you make the acidic solution now this is this reaction helps make acidic solution and it uses the leftover ferric iron from here it con ferrous iron sorry it converts it into ferric iron in this process it makes and this water is recycled here by and H2So4 is added separately to make it acidic. So, this is again metal catalyzed by microbes. So, this is your ferrous iron it is being oxidized and made ferric iron. So, these are iron oxidizing bacteria. So, not here, we have copper oxidizing bacteria or we can say sulphur oxidizing bacteria and we have iron oxidizing bacteria both together make sure that we separate copper from iron and if you need to just add more acid ok.

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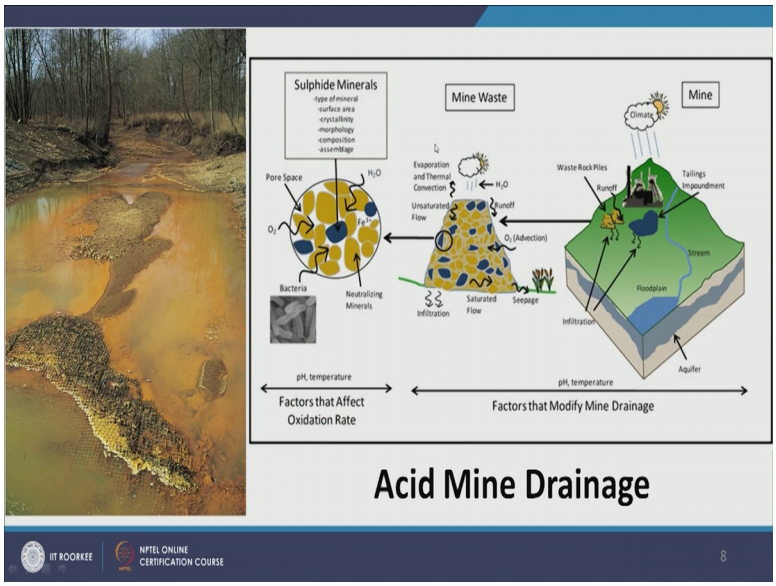

Now, there is the second part of this metal leaching the first part is econ making it making it economically feasible and attractive for us to even mine matters from very poor quality ores by using biologically driven chemical reactions on the other hand when we are mining that say inning gets a big problem in many parts of us, it is a recognized big problem in they; we do not talk much about it though when we mine we are exposing the minerals that are that have been for thousands.

So, in I do not know how many years put under away from oxygen anoxic zone in and another big zone now we are exposing them to oxygen when we do that they start getting oxidized right and the microbes that were oxidized metals they become very happy because not they have oxygen to or to get more energy because oxygen is best electron acceptor for them. So, they also start thriving the metal oxidizing microbes and the metals are chemically also getting oxidized.

So, we have a very fast biochemical oxidation of metals now when they get oxidized they are usually soluble in water. So, if there is a ground water stream if there is an aquifer by its very likely or there is a surface water stream its very likely that the leach it of the mine let us say there is a rain or there is just water passing through the mine or we are washing you know you there is a washing step mean people do mining. So, that gets very rich in metals.

Because now the metals have oxidized and they have their being in acidic media they are getting dissolved first thing because most let me break this down for you all right dear students.

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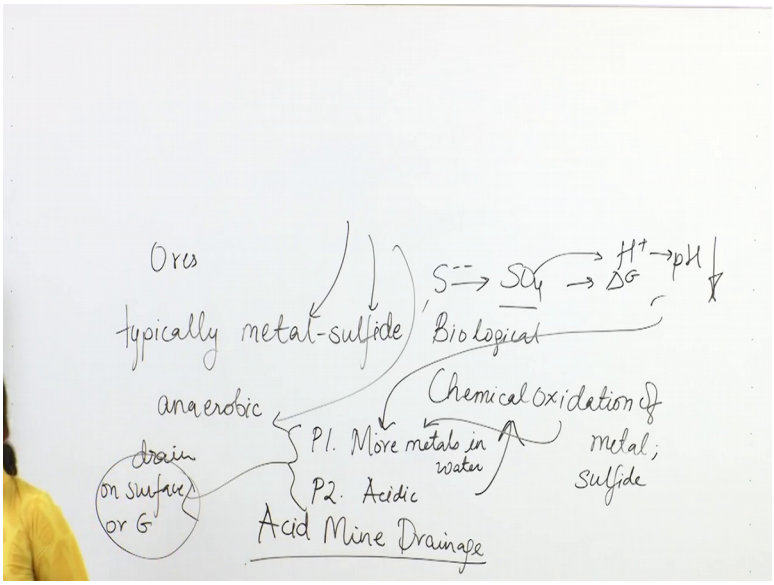

So, in yours you have typically metal sulphides that you are interested in mining and typically it is an anaerobic zone and when you when you start mining it you expose it to oxygen and thus oxygen comes in and what was an anaerobic zone earlier now is now has presence of oxygen. So, 2 beautiful things happen first thing that happens is that there is a biologic the microbes that are very happy to oxidize sulphide they come into picture. So, you have sulphide oxidizing microbes that would love to make sulphate out of it because oxygen is present and they will derive good amount of energy which they can channelize for growth and for sustenance.

So, biological oxidation the other thing that happens is because the metal is now being exposed directly to oxygen metal they automatically undergo oxidation. So, there is a chemical oxidation of metal. Now sulphide in itself is highly reduced. So, it is very vulnerable and susceptible to oxidation. So, sulphide also undergoes oxidation chemical oxidation now you have 2 problems when the sulphide undergoes oxidation you have sulphate now sulphate is a very acidic ion. So, you have lot of h plus that will increase in your water right in the aquifer groundwater or in the surface water when that happens the pH drops. So, here the pH will drop.

Now, in excess of hydrogen ions in a in excess of low pH what happens is we have more dissolution of metal. So, because of pH dropping we have what problems do we have problem number one we have more metals in water more metals dissolved in water and lot of heavy metals and excess metal in water is not good for health is not good for environment the second problem is our surface water or groundwater is acidic and if it is acidic enough and usually with addition of sulphate.

It does get very low in very low in pH it is harmful for life in all forms it corrodes the rocks it corrodes pipes and it destroys the aquatic system. So, it is hazardous both are hazardous. Now this is not just from sulphur oxidation, but even the chemical oxidation of metal will promote further addition of metal in the water because oxidized versions of metal oxidized form. So, metals are usually not for all, but usually more soluble in water.

So, we have higher metals in water not only because of dropping p h, but also because of chemical oxidation of metals. So, there are 2 sources of elevated pH level. So, now, we have water that is where there has very high amount of heavy metals and metals and very low pH.

Now this problem this water it will go and it will drain on surface or underground and either way it is going to damage the geography is going to damage the ecosystems and its going to damage the microbes and if we use this water as source of drinking water it is going to affect the quality of the drinking water that is supplied to the community. So, this problem over all this destruction of the water quality and this deterioration of water quality because of metals leaching into water and because of lowering of pH which help more metal leach into the water is called as acid mine drainage acid mine drainage.

So, let us look into acid mine drainage so, on the left this is a picture of a remediation site where people are trying to reduce acid mine drainage, but if you look carefully this is a beautiful forest area and this is a stream moving here and the colour this is not the colour of mud this is the metals the copper metal that have that are excess in the water now giving an iron on. So, copper and iron both that are giving it this redish and greenish colour, this water is highly acid if you step in you will get burned. So, this river is practically dead despite its levels of oxygen being, but nothing cancer no fishes will survive only acid or filling microbes will survive in it and metal taller and microbes to survive in it how does this acid mine drainage happen.

So, for acid mine drainage usually we have sulphide minerals because remember its acid mine drainage. So, the pH needs to go down and it is not just oxidation of metals that allows metals to dissolve, but it is a low pH that really promotes the dissolution of metals. So, you the best way to lower the pH is have sulphide oxidizing to sulphate or sulphide. So, sulphide minerals we have them here the black ones and the oxidation rate depends upon the type of minerals.

So, if it is iron pyrite or copper pyrite or uranium pyrite it depends on that it depends on the surface area there is more surface area for chemistry the distortion will faster it depends on the crystallinity we are we in one of the research that I was involved in we found also not just the ama percentage crystallinity of the mineral, but it is also the kind of crystal its making the kind of geometry the mineral has that also backs its oxidation rate which is morphology.

It depends on composition it is depends on assemblage how they have assembled together all this determines how much oxygen is present here for metal to get oxidize it and which depends on the pore space it also depends on how much water is present food to dissolve the metal how much ferric ions are being produced how much neutralizing minerals are present its quite possible that we have some alkaline mineral here like lime and that resists dissolution of water just by reducing increasing pH.

So, all in temperature makes a difference the pH makes a difference. So, all these factors will affect the oxidation rate and dissolution of metal into the water. Now, once it has dissolved how will it what are the factors that will affect the drainage of the water you have the rain how much precipitation is received what is the evaporation of precipitation what is the thermal convection, how much is the surface flow, how much is the run of how much is the unsaturated flow, how much is saturated flow, how much oxygen is entering in, what is the c page what is the infiltration of water, what are the aquifer design you know the layout of aquifer the slope, all these factors will affect the slope or the flow off of the drainage of acid mine water acidic mine water and then it.

So, here we have aquifer it depends on the floodplain. So, what floodplain we are in run of climate all these things will affect how much dissolved metals and acidic water will spread to what extent.

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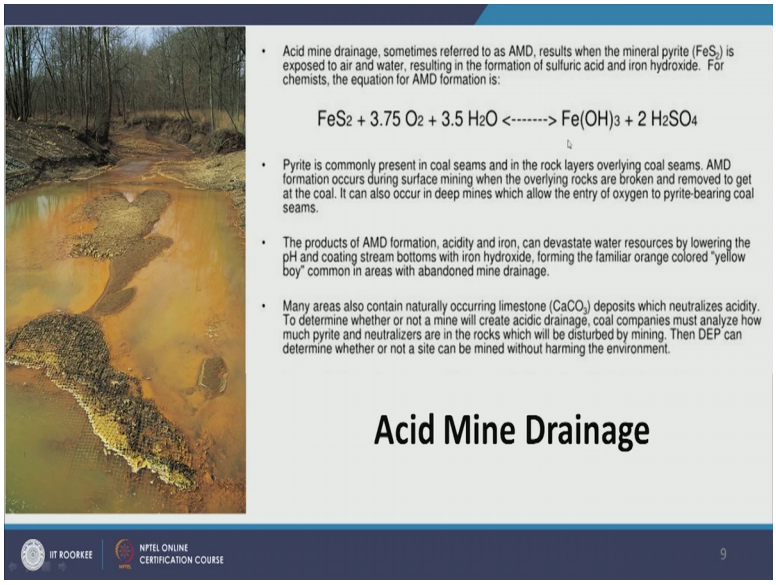

Together this problem is acid mine drainage now how does acid mine drainage work what is its chemistry let us read out some basic things about acid mine drainage acid mine drainage refers sometimes as AMD just abbreviating it results when a mineral pyrite is exposed to an water results in formation of sulphuric acid and the metal oxide and this is usually present in coal.

So, we have often even if you are mining coal we are not mining metal, but because there is iron pyrite present co present with the coal the iron pyrite will oxidize and make sulphur dioxide and sulphuric acid and create problems it occurs when such during surface mining the overlying rocks are broken and removed to get the coal it also occurs in deep mines because it allows now the entry of oxygen to pyrite bearing coal seams the products of AMD are acidity and iron can devastate water resources by lowering the pH coating stream bottoms with iron hydroxide forming the familiar orange coloured yellow boy.

So, this is how to call them in US areas, good abandoned mine drainage many areas also contain naturally occurring limestone which neutralize acidity to determine whether or not a mine will create acidic drainage coal companies must analyze how much pyrite and neutralizers are present in the rock which will be disturbed by mining and then people can determine whether we should go ahead and do mining or not this is applicable in u s, but similar process should be applicable in India too.

Now, before we enter bioremediation my dear students I would like to take pause here and give you what is the biological approach to treating acid mine drainage. So, remember acid mine drainage is happening because our metals are getting oxidized and I metals are getting oxidized because they are getting exposed to they are getting exposed to oxygen and the pH is lowering down as they get oxidized. So, they get further dissolved and just one thing leads to another and then we have heavy metals and water and you have very acidic pH water now in order to remedy this what people have done is that they have added biomass. So, you can add biomass such as bagasse and into the near the mines you can make layers of bagasse and you can introduce or you can increase conditions that will encourage sulphate reducing microbes.

So, one of the problem with acid mine drainage is production of sulphate if we can contain this we will allow the pH not to fall will allow the dissolution to be limited dissolution of metals. So, in order to contain this, what we need to do is in order to contain sulphate levels we need to have some reversing activities something like this.

Now, in order to reverse sulphate and make sulphide out of it we need anaerobic conditions.

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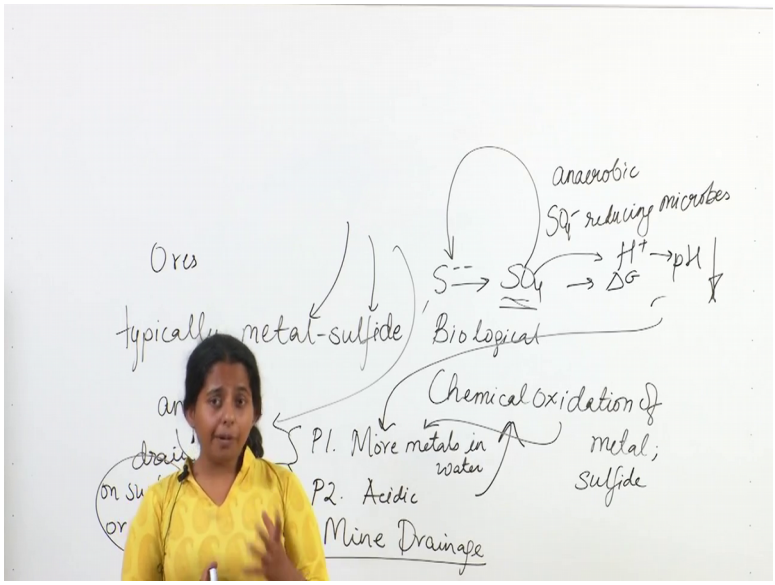

And we need sulphate reducing microbes alright so, anaerobic conditions and sulphate reducing microbes. So, to create anaerobic conditions we doze it with some very good electron donors, it is a lot of biomass and some easy to eat electron donors such as

acetate or lactate and when they initially eat up we have lot of heterotrophic ground and which said the microwave community running and then they can eat the other waste organics that you have put there and then we want to increase encourage sulphate reducing microbes because it is going to anaerobic conditions oxygen will be depleted most oxygen is depleted to go for other electron acceptors eventually it will reach for sulphate and there is plenty of sulphate present.

So, it will also reduce sulphate and make self sulphur or sulphide out of it in when this happens the mineral again stabilizes right its innovative sequestration of metal it stabilizes itself and it is no longer dissolved in water. So, this is one technique in which people have approached biological technique with which people have approached to address acid mine drainage problem.

Now, let us say I tell you that there is an acid mine drainage problem in Bihar and parts of UP,p how are you going to up microbiologically how are you going to approach this problem and solve it. So, think about it and when we upload the next lecture keep your eyes open and I will teach you how we can use the microbiological tools that you have learned in previous lectures to understand what is happening in an acid mine drainage problem.

Understand the effect the toxic effects on ecosystem on public health and then when you are remediating by adding biomass and by adding the electron donor not necessarily biomass, but definitely the electron donors and lot of food for microbes to eat when you remediate how do you track whether the remediation is happening or not. So, the next class I will be teaching you all that. So, stay tuned.

Thank you.