Environmental Biotechnology
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Lecture – 52
Bioremediation case studies (Contd.,)

Welcome to the next lecture of our course environmental biotechnology and in today's lecture we will be talking about some of the very important case studies on bioremediation which will demonstrate the capabilities of natural microorganisms rather microbial communities in developing environmental biotechnology processes.

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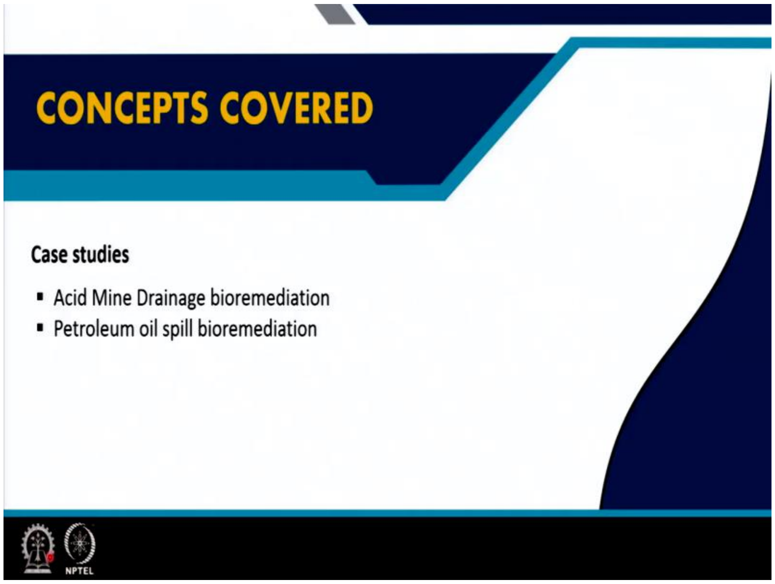

So, we will be discussing about 2 case studies one is based on the acid mine drainage bioremediation and the other one would be on petroleum oil spill bioremediation.

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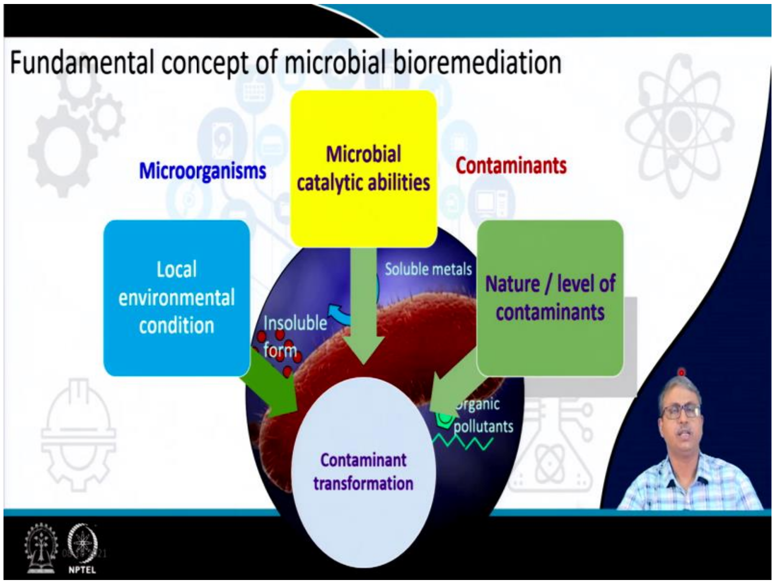

Now, before we go into this two case studies I would just like to revisit the concept of bioremediation where we where we know that the microorganisms they interact with the contaminants and this interaction affects the chemical properties environmental mobility persistent of the compounds and the toxicity of the hazardous compounds. And this could turn a soluble metal into insoluble precipitates or alternatively can oxidize and break down the organic pollutants like the petroleum oil into less toxic and harmful products.

And these contaminant transformations which are catalyzed by microorganisms are indeed controlled by the microbial catalytic abilities but also they are controlled by the local environmental conditions prevailing in the contaminated site and also the nature and level of the contaminants. Because these level of the contaminants and the nature of the contaminants they have profound effects on the on the organisms their catalytic abilities and also they have very significant effect on controlling the local environmental conditions as well.

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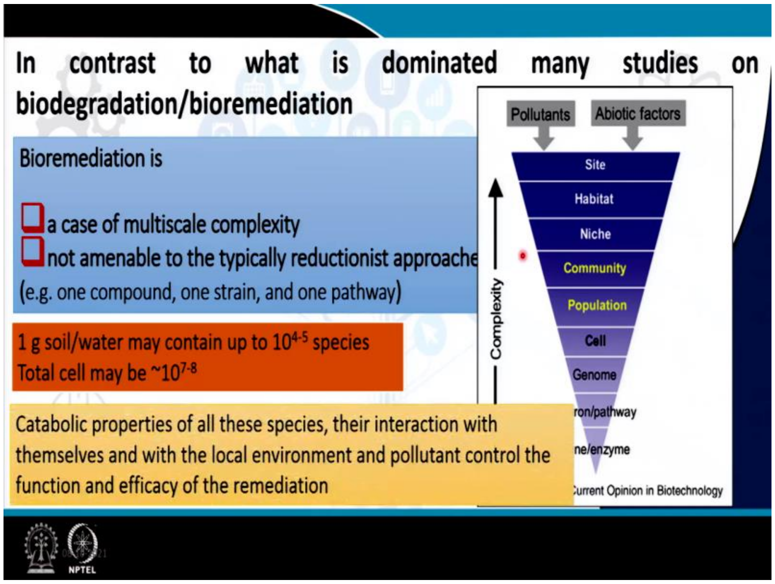

And subsequent to this fact that these bioremediations are basically achieved through microbial interaction with pollutants we must also remember that bioremediation processes are a case of multiscale complexity. So, we have multiple layers which may start with a gene or enzyme and it goes up to the the habitat or the sites and typical reductionist approaches like one only one pollutant one bacteria or one microbial strain and one pathway involved in that is may not be correct particularly when we consider the in situ bioremediation processes.

And this is because the diversity of the species in natural environments are enormous and the catabolic properties of all these organisms their interaction with themselves and with the local environment and the pollutant these control the function and efficacy of the remediation process.

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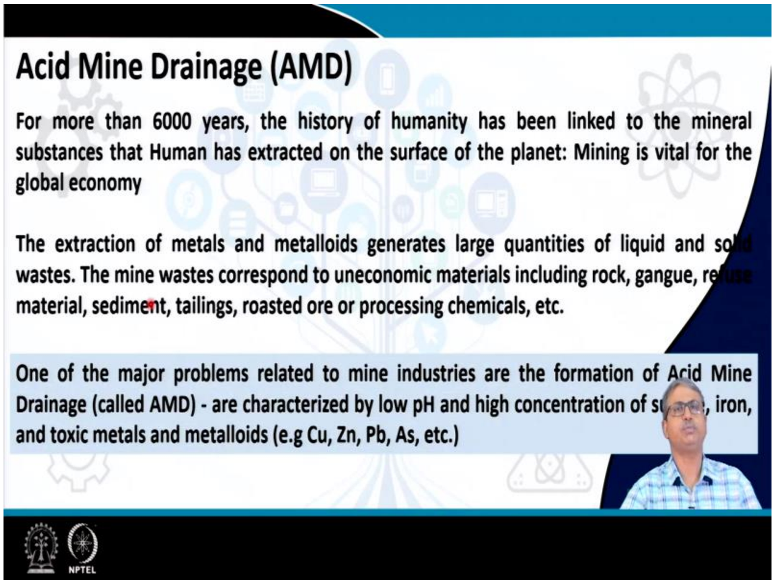

So, during this two important pollutant or remediation scenarios we will see that how these systems level of understanding is being gained important place in bioremediation process development and understanding the role of microorganisms in that. Now, first one is the acid mine drainage. Now, what is acid mine drainage. So, of course it is pollutant from the mine industry and if we look at the mine industries it is it is known that for several thousand years from the history of humanity is linked with the mineral substances.

And continuously we are utilizing this minerals and mining is found to be vital for the global economy. Particularly when we look at the climate change mitigation strategies and increased emphasis on clean energy renewable energy. Enhanced requirement of metals are placed in the forefront because we need more materials in order to capture the energy in different form in order to make use of the renewable sources of energy which are available on our planet.

Now, that means that extraction of the metals and metalloids are going to be more. Now more extraction of metals metalloids generate large quantities of liquid and solid waste. And the mine waste correspond to uneconomic materials including rock different kind of other materials sediment tailings waste rocks roasted ores etcetera and one of the major problems related to the mine industries are the formation of this acid mine drainage or which is called AMD which is basically a liquid waste coming out of the waste rock piles tailings etcetera.

And it is naturally produced in the mine sites and it is characterized by low pH low means very low up to one pH or less than 1 pH very acidic high concentration of sulphate several thousand milligram per liter it could be iron toxic metals and metallites. Different metals are metalloids and present in this acid mine drainage solution.

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So, we have actually tried to work on one of these acid mine drainage producing mine industry and you can see the mine seepage is being discharged the acid toxic acid mine containing waste is naturally seeping out and on the distant places you can see the deposition of the copper which which is actually is being produced by this industry this mine industry and during the wet season you can expect that the whole region will be flowing like a river full of this highly toxic and acidic mine drainage.

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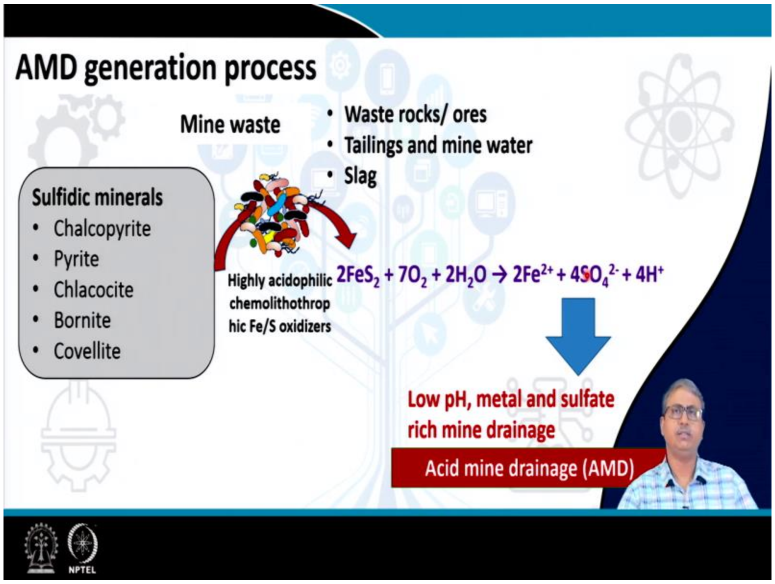

Now, how this acid mines drainage is produced? This acid mine drainage is basically produced naturally from the different type of mine waste materials like the waste rocks ore, tailings, mine water slag and many of these sulfidic minerals are responsible for facilitating the catalytic actions of specific group of bacteria mainly although some fungi and archea are also found to be involved in that and these are highly acidophilic organisms and they are mostly chemolithotropic and they are iron and sulphur oxidizers.

So, this is a very characteristic property of these organisms that they are capable of utilizing this minerals or the metals metal sulphides as the source of electrons and they use most of them are autotroph. So, they can fix the atmospheric carbon dioxide. So, they are extremophilic chemolithotrophic iron and sulphur oxidizing organisms. So, the catalytic events lead to the production of sulphate along with Fe 2+ and other metal ions.

And this sulfate further proliferate the acidification process because it can actually solubilize the metal further and the strong protonation also facilitates the further leaching of metals and metal sulphates into the liquid phase. Resulting into the highly acidic metal and sulphate rich mine drainage which is called acid mine drainage.

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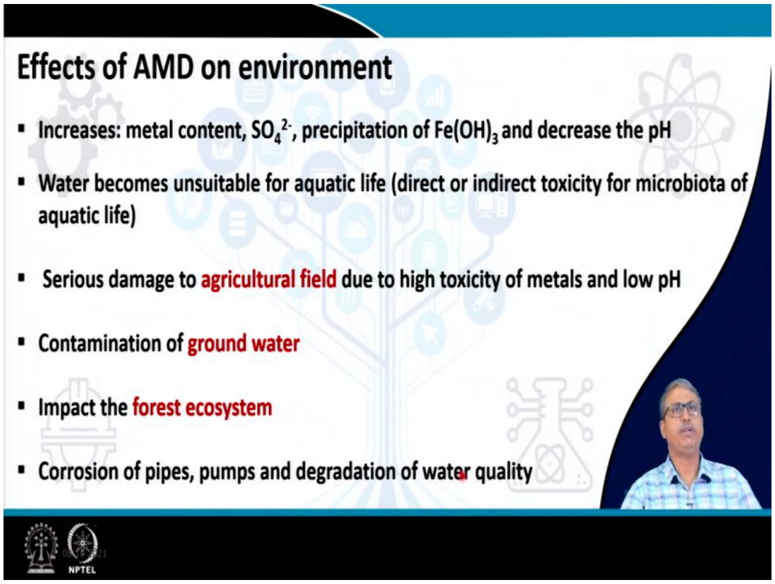

Now, the effect of acid mine drainage are very well studied across the world wherever this leaching of acid mine drainage are there. It increases the metal content sulphate precipitation of iron hydroxide and decrease of the pH is very common problem. Water becomes unsuitable for aquatic life and it is going to invade the aquatic habitats the lake, river, pond etcetera. Directly or indirectly the toxicity will be manifested to the all the biotic components.

And the entire ecosystem of the aquatic habitats agricultural fields the ground water in and the forest ecosystems in the vicinity of the acid mine drainage everything will be damaged destroyed and even in the industrial setup because of the high acidity it will also lead to corrosion of the pipes pumps degradation of the water quality which is being used in the industry. So, it becomes a very very important pollutant and hazard for the overall environment.

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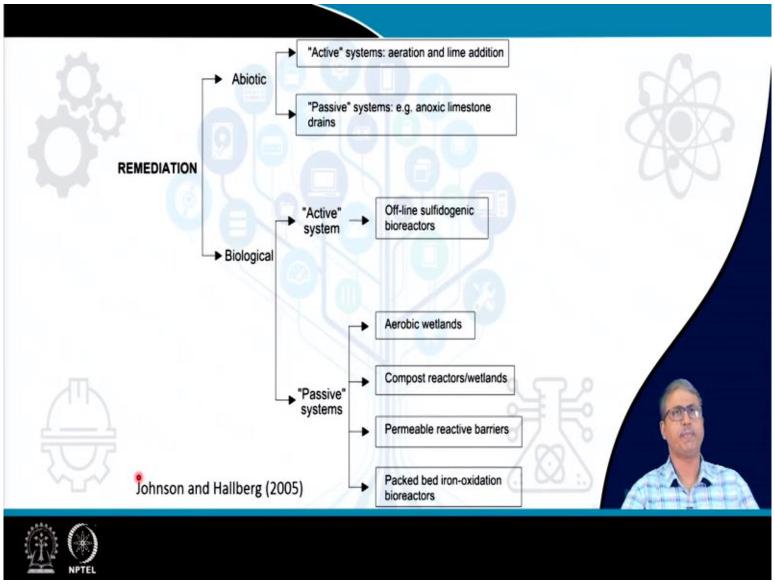

So, remediation processes are being studied and developed and implemented in many places and this could be abiotic and biotic. Within the biological remediation process we see there are active system and passive system. The active systems are basically offline sulphidogenic bioreactors whereas the passive systems are aerobic wetlands compost reactors or wetlands permeable reactive barrier to protect the groundwater from eventual contamination and packed bed iron oxidation bioreactors.

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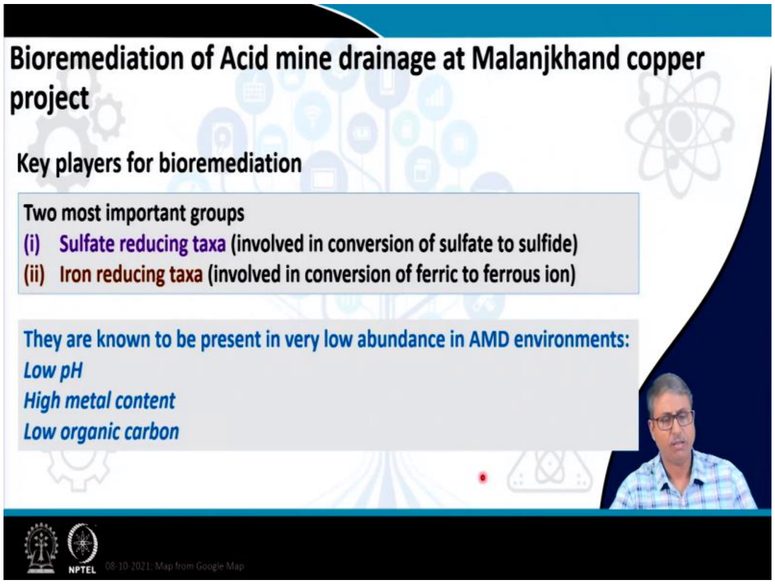

Now, the case study that I am going to present today is on the bioremediation of AMD at the Malanjkhand copper project which is one of the largest copper producing mines in India and the key players for bioremediation which are identified are the two most important groups like sulphate reducing organisms which convert the sulfate to sulfide and then sulphide is able to precipitate either binding with the iron or the metals and iron reducing taxa that involved the conversion of Fe 3 to Fe 2.

Now, they are known to be present these organisms that are the SRB and IRBs sulphate reducing bacteria and iron reducing bacteria they are known to be present in very low abundance in the AMD environments naturally because of the low pH high metal content and low organic carbon. So, as I mentioned earlier the acid mine drainage in environments are highly acidic with lots of toxic heavy metals and organic carbon contents are generally low.

The catalytically relevant organisms which are useful for bioremediation of the AMD that is the sulphate reducing bacteria and iron reducing bacteria they are however heterotrophic. So, they need higher concentration of organic carbon and many of them are unable to withstand the low pH. So, there are problems associated with these organisms which are otherwise required or relevant for the bio remediation.

So, the resultant is that the microbial communities which are present in the AMD sites are naturally having these organisms which are required for by remediation but the abundance of these organisms are generally very low.

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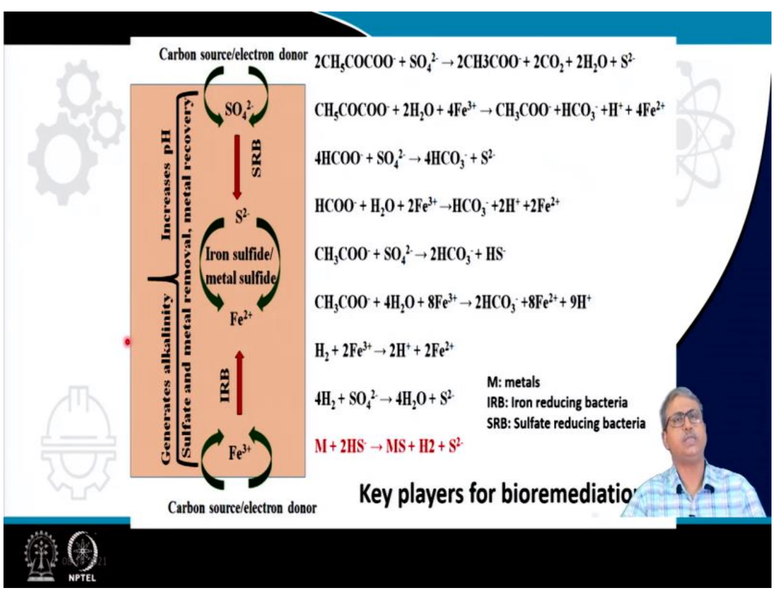

Now, what is the role of these IRBs and SRBs if we look at the functions of these two group of organisms we will understand that both these organisms are heterotrophic. So, they utilize the carbon sources while the SRBs are capable of reducing sulphate as the terminal electron acceptor and they produce sulphides and the sulphides can interact with the other iron and metals present there and precipitate the metal sulphides.

And that facilitates the increase in pH because the sulphates are getting removed. On the other hand the IRBs they are capable of reducing iron Fe 3+ as their electron acceptor and they produce alkalinity and the sulfates and metals are removed and the metals are precipitated that and that facilitates the recovery of the metals further.

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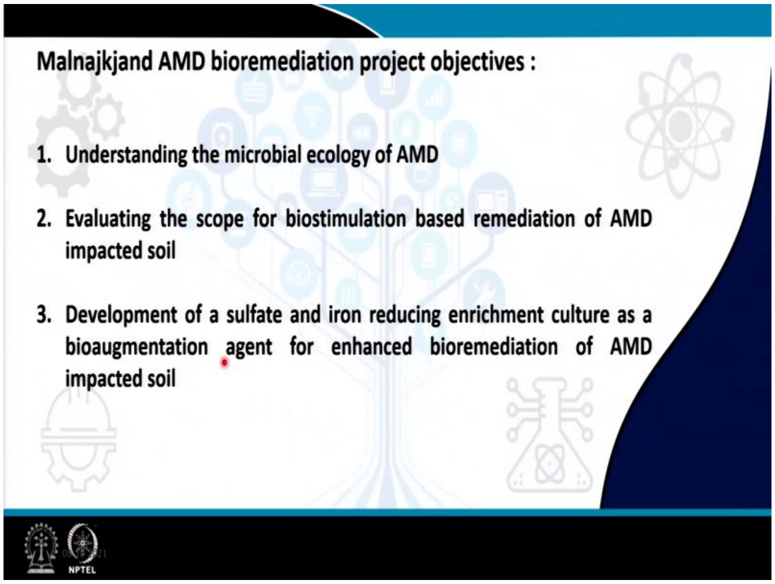

Now, within this particular bioremediation project we try to understand the microbial ecology because we understood that these organisms might be present these refer to the bioremediation relevant organisms. And we wanted to evaluate the scope of the bio stimulation based remediation approach in the impacted environment and developed a suitable bio augmentation agent for the enhanced remediation of the AMD impacted environment.

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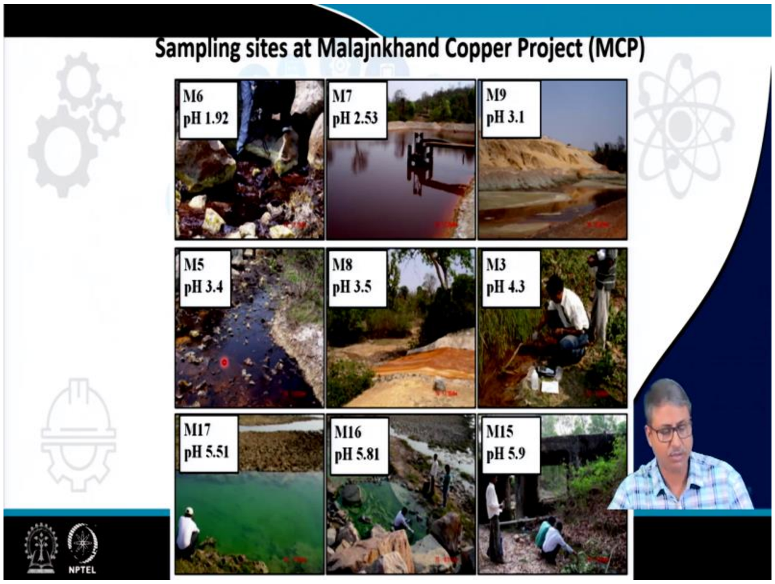

So, these are some of the environments that have been worked with you can see that the naturally the water is coming out from the the rock burdens and this is the water with having a pH of 1.9 and also the water which is acid mine drainage water is stored in tank with very low pH of 2.5 and subsequently you can see that the water are flowing which are all having ph less than four or even so.

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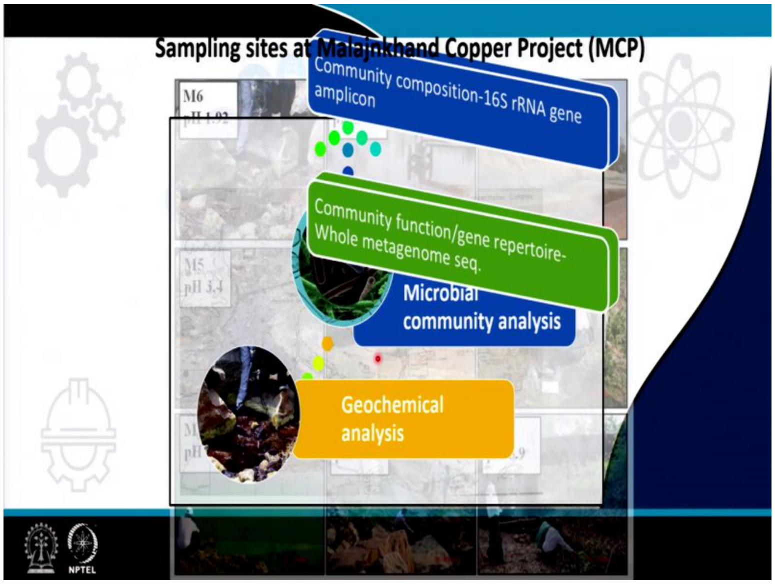

Now, we took the microbial community analysis approach in which first the geochemical analysis of the environments were performed and then the microbial community analysis were done using state of the art techniques and finally the community functions and community compositions were identified and eventually the bio remediation process was developed.

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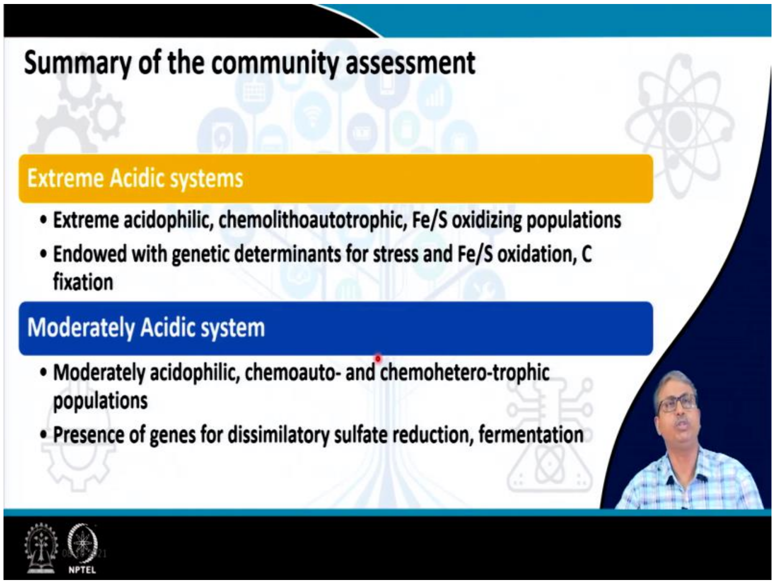

So, what we observed from the community assessment that the extreme acidic systems are enriched with acetophilic chemolithotrophic organisms which are responsible for producing the AMD that is the iron and sulphur oxidizing populations. And they possess the required genetic determinants for withstanding the stress because they are highly these environments are highly acidic and having high concentration of metals and sulfates.

Compared to that the moderately acidic systems are having moderately acidophilic chemoauto and chemoheterotrophic populations and the presence of genes required for dissimilatory sulphate reduction and fermentations are observed. So, this is a very interesting finding because this dissimulatory sulfate reduction is considered to be the important step towards reduction of the sulphate into sulphide.

And the fermentation is also is a desirable property for these organisms who actually rely on the sulphate reduction processes.

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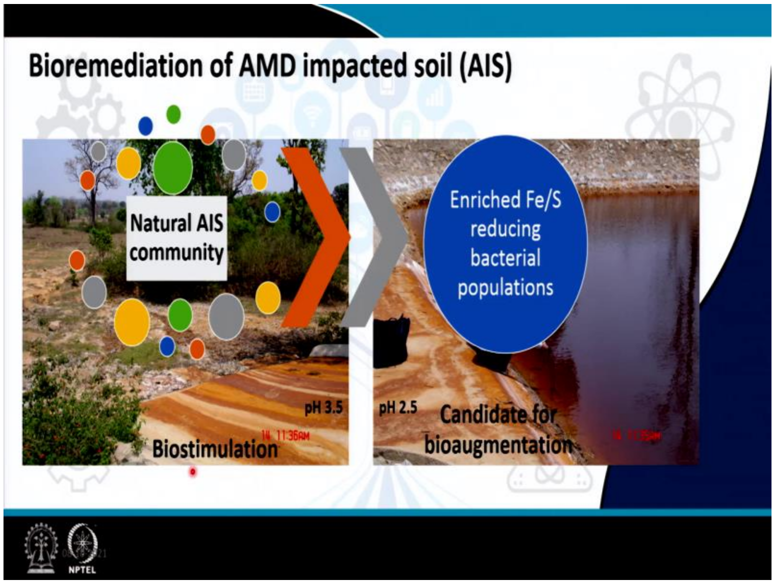

So, this is the pond where the acid mine drainage is actually accumulated and stored and this water is peeled into the nearby area and this is the impacted environment. So, we targeted this acid mine drainage impacted environment by obtaining a natural community through biostimulation and this natural community members were obtained through biostimulation because they are biostimulated in a sense that they are the organisms who are having the desirable iron reducing and sulfate reducing abilities.

And these organisms which were biostream obtained through bio stimulations were further characterized and developed as a candidate for bio augmentation and subsequently these populations were tested for their efficacy in bioremediating the acid mine drainage impact and impacted environment.

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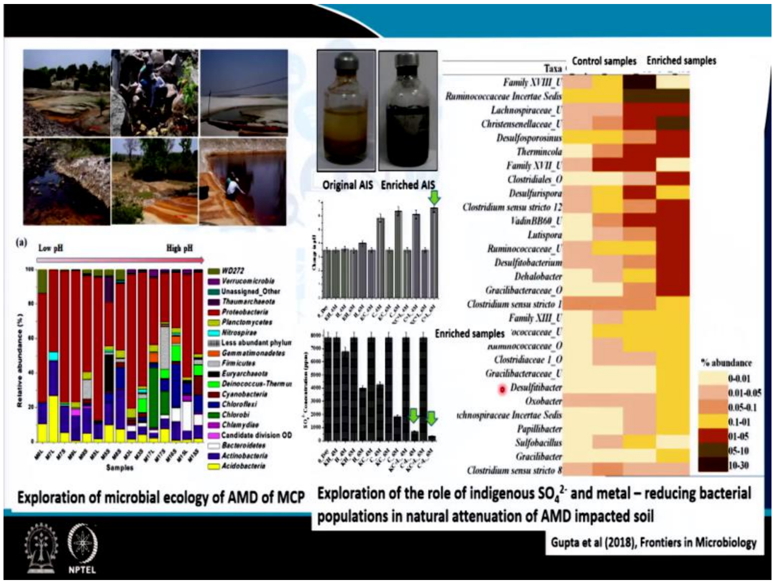

So, here are the samples and the overall community data that can be seen that from the low pH to the high pH the community composition is nearly consistent in terms of some organisms like proteobacteria. However presence of the members like farmicutes which are often connected to the iron and sulphate reduction processes are very less abundant in the low pH environment but they are relatively more abundant in the higher pH environment.

So, fine we took this acid mine drainage impacted in sample and it was amended with nitrogen and carbon source as a bio stimulant and following several months incubation we observed that there is observable change in these bio stimulant added microcosms that the formation of sulfides and the black precipitates are obvious and we look into the p change in the pH and change in the soluble sulphate concentration.

We could see that the pH has come from 3.5 to close to 7 following the addition of the biostimulant and also the concentration of the sulfate has declined significantly. Now, these cultures which are we call them enriched cultures enriched through nitrogen and carbon supplementation were subsequently analyzed for the community profiling through 16s ribosomal RNA gene analysis using a high throughput sequencing technique is a cultivation independent approach.

And what we can observe that compared to the control samples the enriched sample shows significantly high abundance the the darkness of the colour indicates the increase in abundance. So, we can clearly identify that members of unclassified family 18 Ruminococci, Lachinospiraceae, Christianasenellacea, Desufossporonias etc and several member of Cloustriadiales are enriched in the nitrogen and carbon supplemented samples.

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TABLE 1 | Details of physicochemical parameters of the microcosm setup.

Parameters	0_Day	H_4M	H_6M	C_4M	C_6M	C+L_4M	C+L_6M
pH	3.51 ± 0.01	3.60 ± 0.01	4.01 ± 0.01	5.86 ± 0.02	6.37 ± 0.01	6.12 ± 0.01	6.61 ± 0.01
ORP	200.51 ± 0.95	165.7 ± 1.0	140.61 ± 1.55	130.02 ± 1.09	120.23 ± 1.20	125.21 ± 1.15	110.02 ± 1.01
SO_4^{2-}	7838.20 ± 39.64	6780.78 ± 54.08	4005.88 ± 19.15	4282.33 ± 22.72	1860.21 ± 14.75	720.06 ± 11.31	365.58 ± 22.11
Fe^{2+}	130.89 ± 4.72	202.81 ± 3.57	336.64 ± 12.66	980.37 ± 6.45	628.83 ± 9.08	300.64 ± 4.77	79.29 ± 2.1
Fe	179.10 ± 1.55	300.81 ± 1.0	415.75 ± 1.75	1386.31 ± 1.11	735.13 ± 0.89	320.94 ± 1.10	90.01 ± 1.10
Cu	1.84 ± 0.07	0.31 ± 0.11	0.28 ± 0.08	0.13 ± 0.06	0.12 ± 0.07	0.18 ± 0.06	0.16 ± 0.05
Zn	1.79 ± 0.33	0.57 ± 0.21	0.46 ± 0.10	0.39 ± 0.10	0.16 ± 0.06	0.14 ± 0.04	0.13 ± 0.05
Ni	0.39 ± 0.11	0.32 ± 0.03	0.28 ± 0.06	0.16 ± 0.06	0.14 ± 0.07	0.16 ± 0.06	0.16 ± 0.07

All the units are represented in ppm except ORP (mV) and pH (SI unit). H, C and C+L denote unamended, cysteine amended and cysteine and lactate amended microcosms while 4M (120 days incubation) and 6M (180 days incubation) represent the time of incubation. The values are represented mean of three independent experiments with standard deviations.

Subsequent to this we try to monitor the pH of this incubation and compared to the zero day samples the carbon and nitrogen added sample show a significant rise in the pH from 3.5 to 6.6 the sulphate level decreased from 7800 to 365 and the iron concentration was also diminished the total iron concentration diminished and the concentration of the heavy metals were also lowered.

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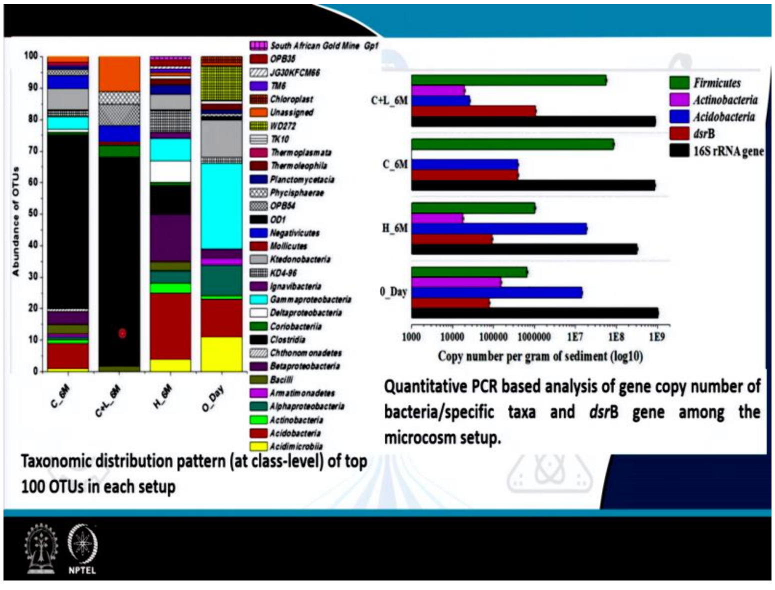

Now, a species level identification of these enriched samples along with the control or the zero day samples clearly indicated that there was a shift in the community composition. This was the original sample and this was the sample original sample added with only water after six months. And the same sample added with both nitrogen and carbon or only nitrogen showed a significant increase in abundance of clustedia members.

This was very noticeable clearly noticeable. Now, we perform the real time PCR based analysis which you know that will give us a quantitative assessment of the gene copy numbers and is being used rigorously in validating the data. So, this quantitative PCR method was used and compared to the zero day the enriched sample you can see the abundance of the firmicutes the green bar the gene copy number indicates a very significant increase.

And compared to these the acidobacteria or the acidobacteria the blue one which was a normally normal abundant population the red colour over here is reduced in the enriched population. So, that means following the nutrient amendment there was a significant change in the community.

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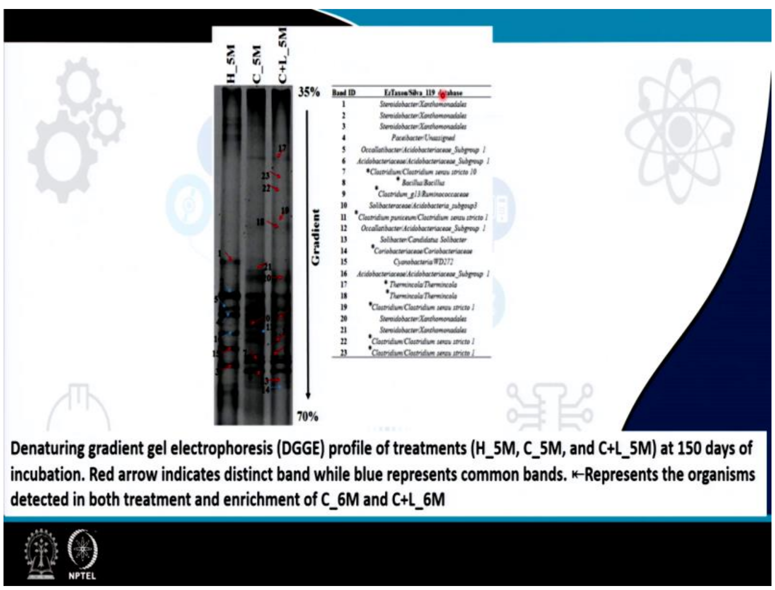

Now, this was further validated through denaturing gradient gel electrophoresis another cultivation independent method as we discussed earlier and compared to the control sample the bands were eluted and cloned and sequenced and we found that in contrast to the control sample which was added only with water then the carbon and nitrogen added sample showed ample presence of clostridia and other members which are known to be sulfate and iron reducers.

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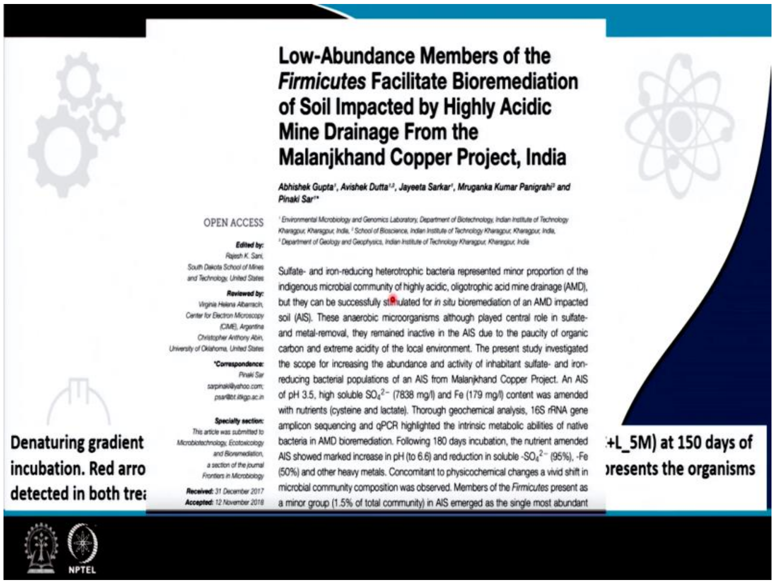

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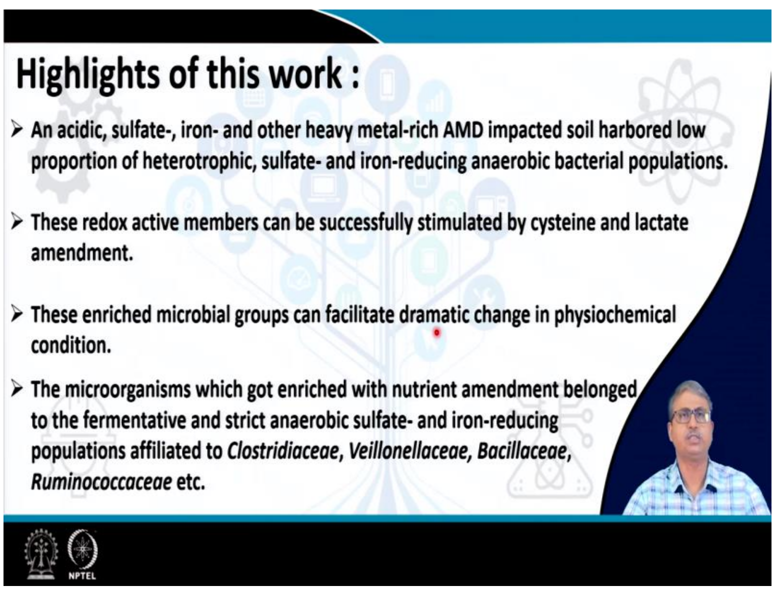

Now, what we have finally obtained from this work is that that an acidic sulphate and iron other heavy metal rich AMD impacted soil harvard low proportion of heterotrophic sulfate and iron reducing anaerobic bacterial populations which can be stimulated through appropriate nitrogen and carbon source. It means intrinsic abilities are there or naturally some organisms are there which are capable of doing the desirable function towards bioremediation.

But supply of the required carbon and nitrogen sources are essential and these organisms which are enriched through the supply of nitrogen and carbon source they can facilitate a dramatic change in the physico chemical conditions which is evident from the change in the pH and sulphate concentration heavy metal concentration etcetera. And these members belong to multiple taxa including the clustedia c belliniolensi bacillacy ruminococcus most of the strict anaerobic or fermentative heterotrophic member and sulfate and iron reducing bacteria.

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Now, subsequent to these this enrichment culture was taken further and we characterize that and we use that culture to as a bio augmentation agent into acid mine drainage contaminated environment with easily metabolizable carbon substrate because as I mentioned these organisms are heterotrophs. So, they need to be provided with suitable carbon sources and when we attempted that that these enriched populations which were represented by the fermicutes and other anaerobic fermentative members.

They were added into the acid mine drainage environment along with cellulose they not only allowed the desirable reduction of sulphate and iron and raising the pH values but also they enhance the indigenous microorganism present in the acid mine drainage impacted environment.

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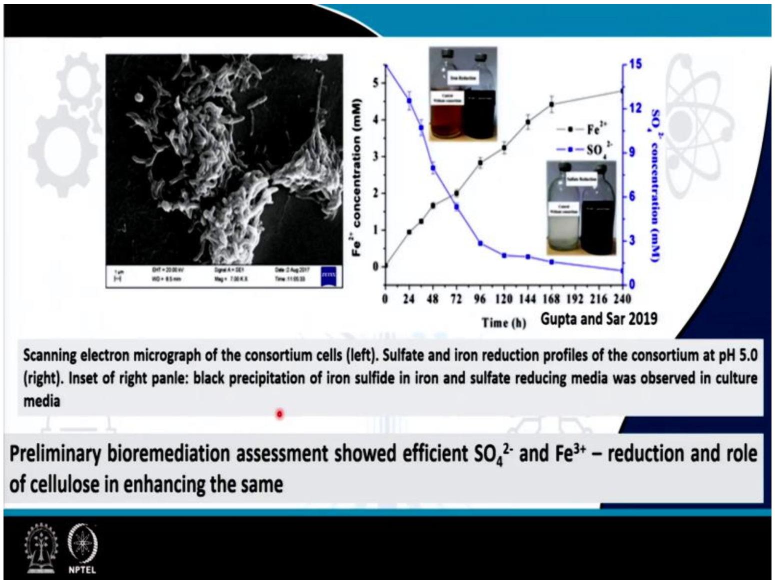

So, this is the community that we actually used as bio augmentation agent and another set of microcosm study indicated that within 240 hours the concentration of the sulfate you can see is significantly lowered and in fact the concentration lowered within 96 hours itself and then it progressed slowly. And during the course of time the iron was also reduced. So, the concentration of Fe 3 increased. So, that means the simultaneous reduction of iron and sulphate occurred.

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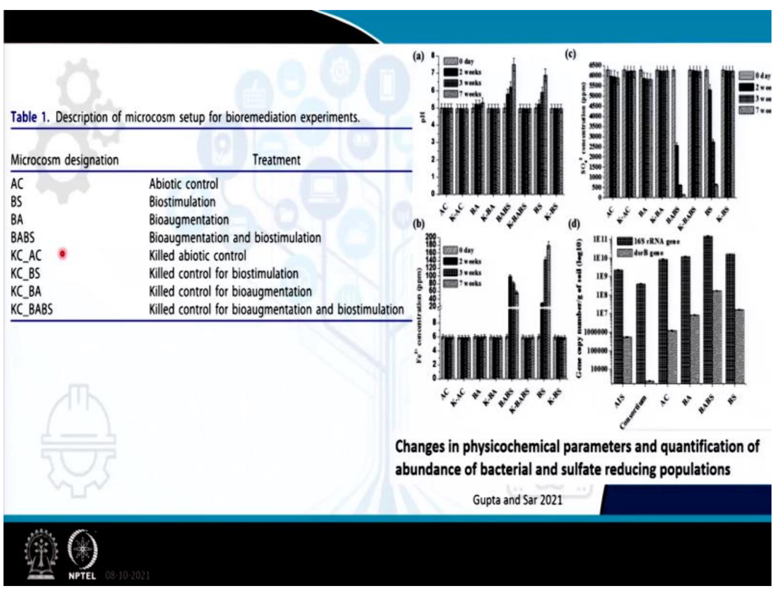

Now, this community which was enriched was used as a bio augmentation agent but we still did some bio stimulation by the addition of cellulosic materials. Because this particular enrichment based community that is used as by augmentation agent they need carbon sources. So, used low cost carbon substrates cellulosic carbon substrate and that actually allowed the indigenous organisms also to be stimulated further.

So, we got this setup which is called bio augmentation and bio stimulation that is abbreviated as BABS. So, what we observed that following seven weeks incubation the pH if you see the BABS set the pH was increased the concentration of sulphate decreased the concentration of iron decreased and also the copy number data the real time PCR data indicated that the dissimulatory sulphide reductase gene or the DSR gene copy number increased significantly.

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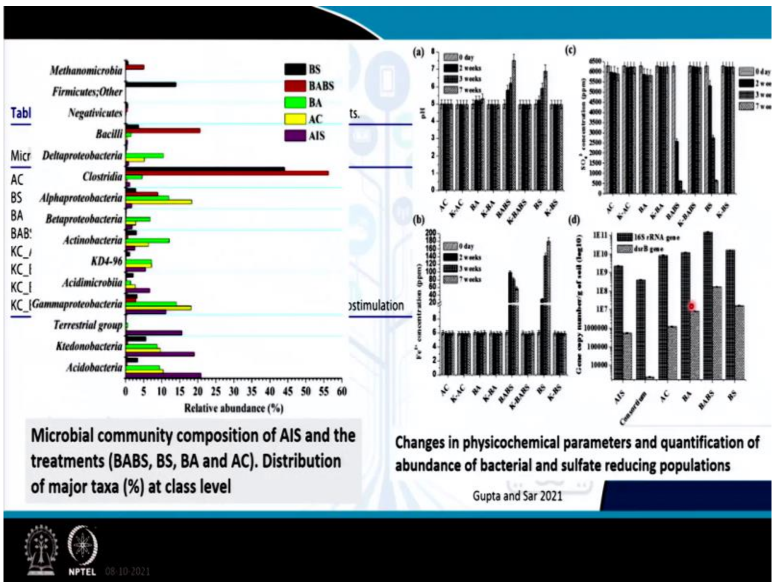

Indicating that that sulphate reduction processes are going on at the same point of time the microbial communities are analyzed and you can see that in a BABS that is the red one the abundance of the clostridium and the delta proteobacteria member which are responsible for the sulphate and iron reduction particularly the class TAC members are profoundly increased.

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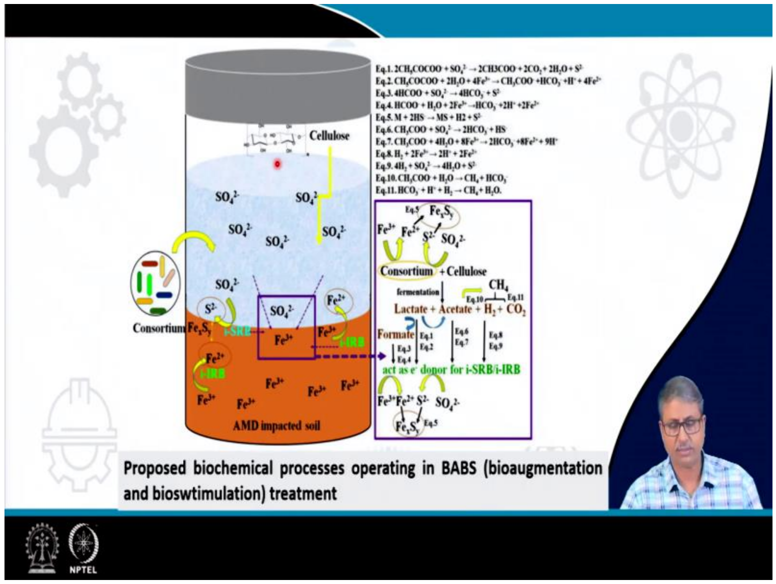

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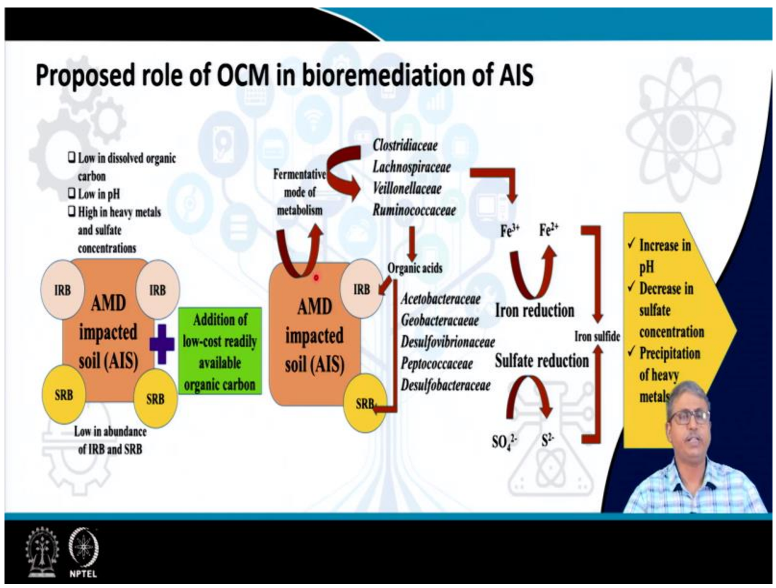

So, based on this we develop the hypothesis that two groups of organisms are actually playing the very important role in achieving the bio remediation that when we are adding the low cost readily available organic already metabolizable carbon substrates the fermentation process is initiated and the fermentation of the carbon substrates are producing organic acids which are basically utilized the utilized by the iron and sulphate reducing bacteria.

For the fermentation process these following members are responsible whereas for the iron and sulphate reductions these other set of organisms are responsible. So, that means the entire process of bioremediation is conducted by multiple organisms which again reminds us about the functional guilts which we studied in microbial community function. And essentially this interaction between the fermentative and iron sulphate reducing populations allowed us to achieve the reduction of iron and reduction of sulfate that led to the increase in pH decrease in sulphate concentration precipitation of heavy metals.

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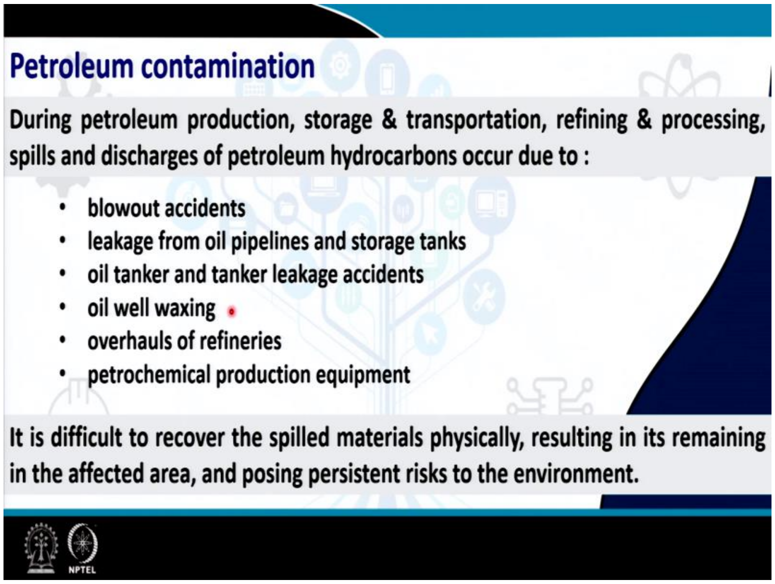

Next we will move into the bioremediation of the petroleum oil spill. Now, petroleum contamination is caused because of the petroleum production activities, storage, transportation, refining and processing and also eventual spills and discharges. Particularly the blow out accidents leakage from oil pipelines and storage tanks oil tankers and tanker leakage oil well waxing overall refineries petrochemical production equipment from different steps the spillage of petroleum oil occurs.

And it is difficult to recover this peeled material physically resulting in its remaining in the affected area and posing a persistent risk to the environment.

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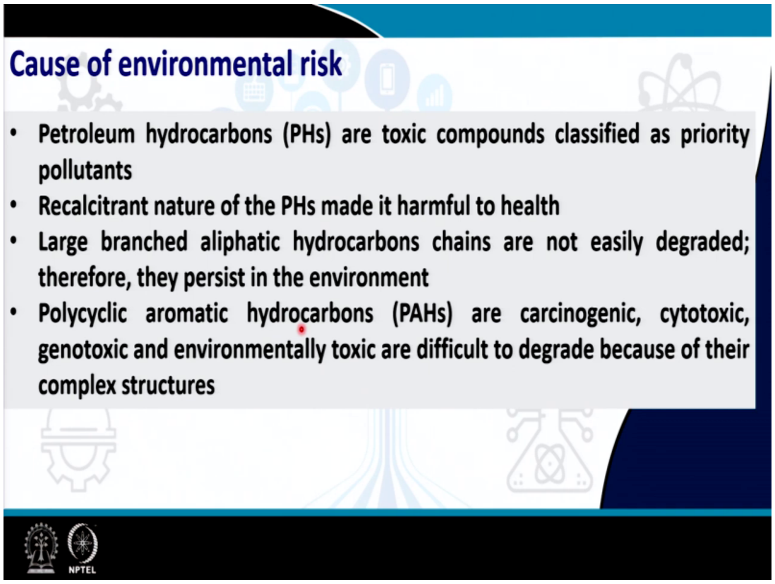

Now, what are the environmental risk the petroleum hydrocarbons which are released during these spills are toxic compounds and classified as the priority pollutants. They are recalcitrant nature many of them very slowly degrade under most of the environments large branched aliphatic hydrocarbons chains are not easily degraded therefore they persist in the environment. And the polycyclic aromatic hydrocarbons like pH are carcinogenic, cytotoxic, genotoxic and environmentally very hazardous and many of them are difficult to degrade because of their complex structures.

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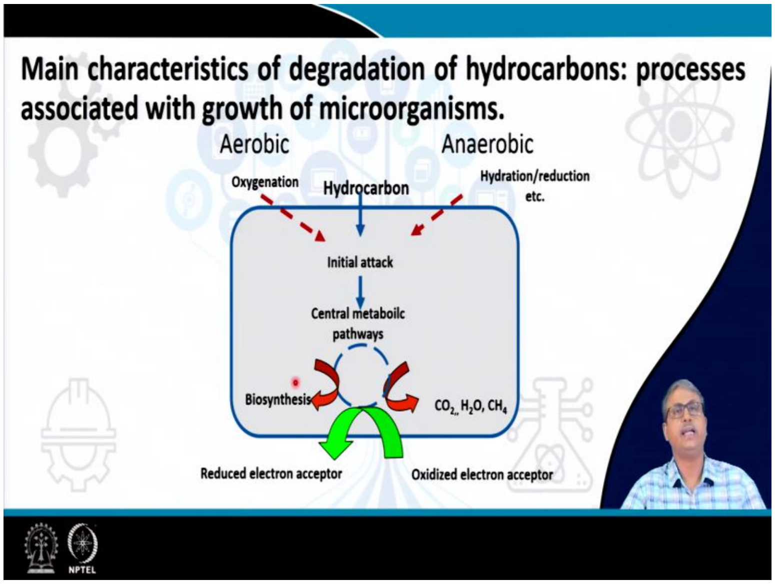

Now, the basic process summary of this petroleum bio remediation is that they are degraded either by aerobically or by anaerobically through initial oxidation or other reactions. And then they are brought into the central metabolic pathway in which they are metabolized through the central carbon metabolism and that allows them to produce the precursor for cellular biosynthesis that is again helping the cells to grow. And they utilize different electron acceptors including oxygen and alternate electron acceptors.

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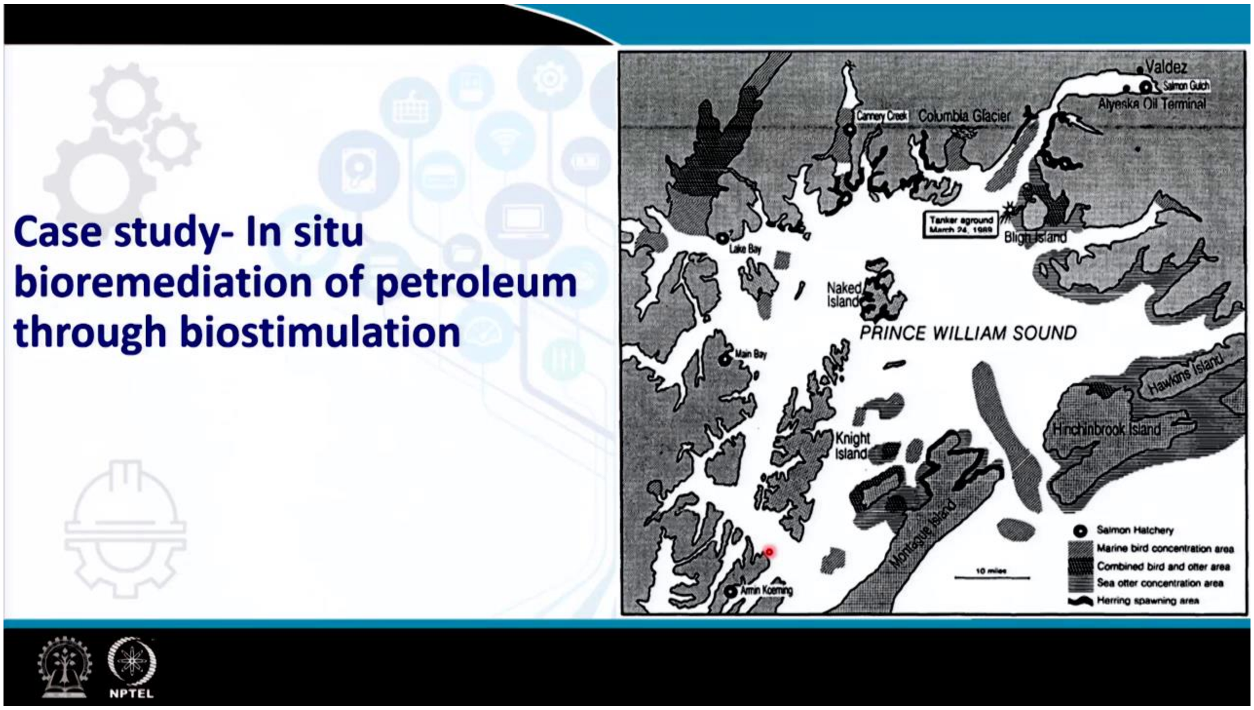

So, we move on to the first case study that is the in situ bioremediation of petroleum through bio stimulation.

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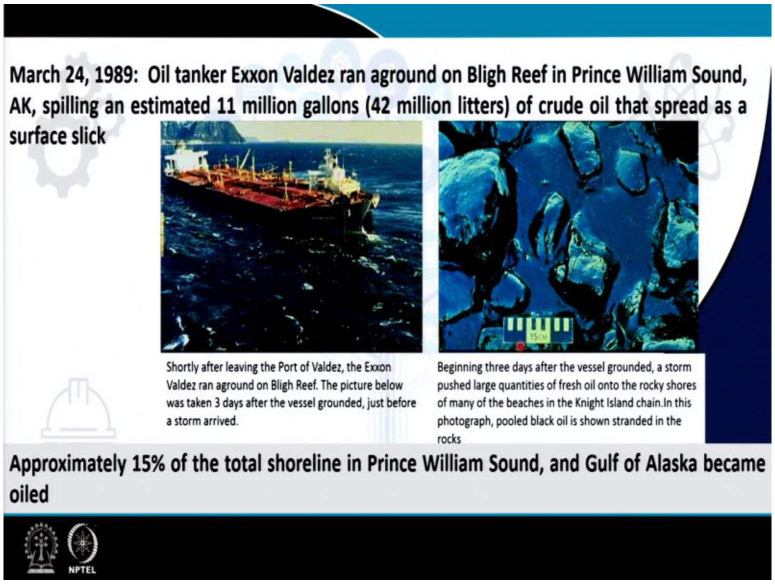

And that is that happened through a accidental spill in March 24 1989 oil tanker Exxon Valdez ran aground on the Bligh Reef in Prince William around Alaska spilling an estimated 11 million gallons that is 42 million liters of crude oil that spread as a surface leak. So, this is the ship the photograph shortly after leaving the Port of Valdez the action voltage ran aground and the picture below was take which is taken three day after the vessel grounded.

And subsequent to this there was a strong and the oil actually moved into the nearby beaches and the coast area and you can see the how the rocks are completely covered by the leaked or spilled oil. And approximately 15% of the total short line in Prince William Sound and Gulf of Alaska become oiled thick coating of petroleum crude oil was there.

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Now, the biomedication strategy was adopted through EPA environmental protection agencies initiative as you can see through the amendment of biostimulation agent or biostimulating agent. So, basically fertilizers were added at the source of NPK and the idea was to enhance the activities of hydrocarbon degradation. Because the hydrocarbon degrading bacteria might be naturally present and since high concentration of hydrocarbons are there the degradation would require sufficient supply of nitrogen phosphorus and potassium because for the cellular growth only hydrocarbon or the carbon source may not be sufficient.

So, you can see in the picture over here that field demonstration of evaluating the bioremediation process is going on where the scientists they have actually spread the fertilizer and following incubation significant amount of decrease in the oil content was observed.

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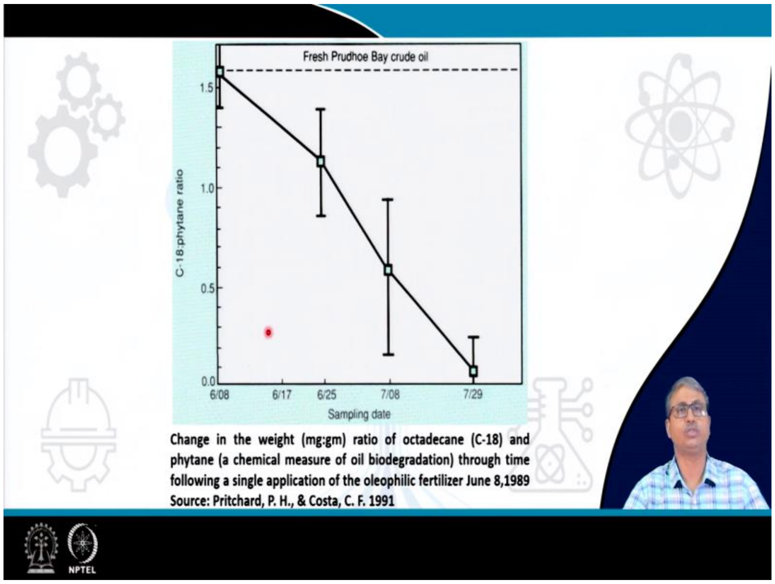

Now, this picture shows the change in the weight ratio of octadecan that is a C18 compound and phyton a chemical measure of the oil biodegradation through time following a single application of the oleophilic fertilizer in June 1989. So, it is a significant decline within one month period we can see a huge decline in the concentration of the major petroleum oil constituents.

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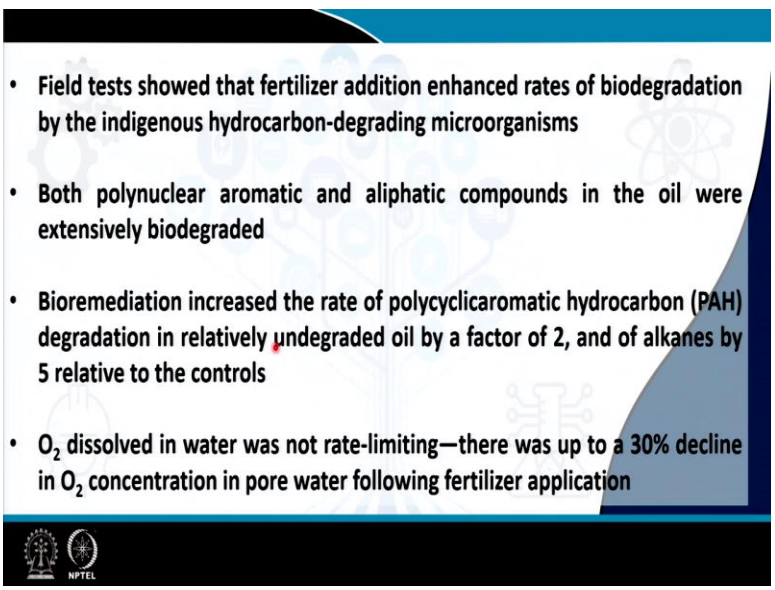

So, field test showed that the fertilizer addition enhanced the rate of bio degradation by the indigenous hydrocarbon degrading microorganism both the polynuclear aromatic and aliphatic compounds in the oil were extensively biodegraded. And the bioremediation increased the rate of polycyclic aromatic hydrocarbon degradation in relatively undegraded oil by a factor of two and the alkanes by five relative to the controls.

And oxygen dissolved in water was not a rate limiting factor there was up to a 30% decline in oxygen concentration in pore water following the fertilizer application which is explainable because the biodegradation process must be utilizing the oxygen as the terminal electron acceptor.

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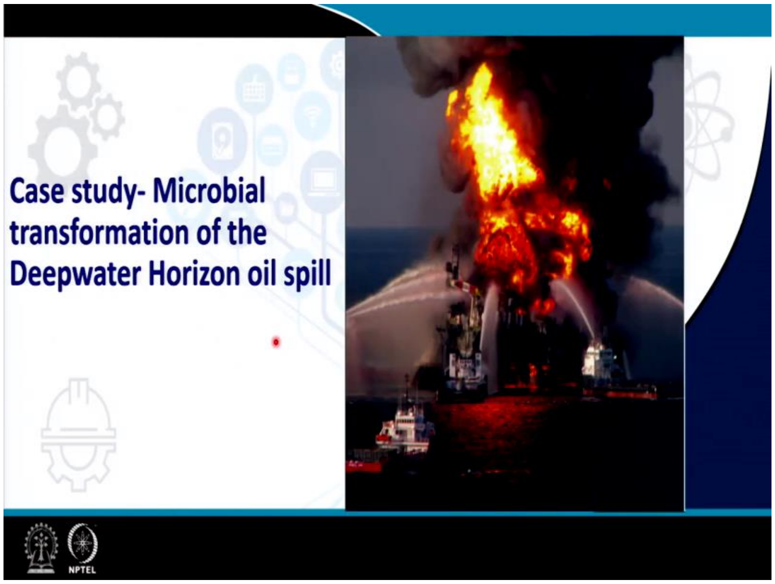

The next one is the case study on the microbial transformation of the deep water horizons oil spill which is considered to be the largest oil spill in the history.

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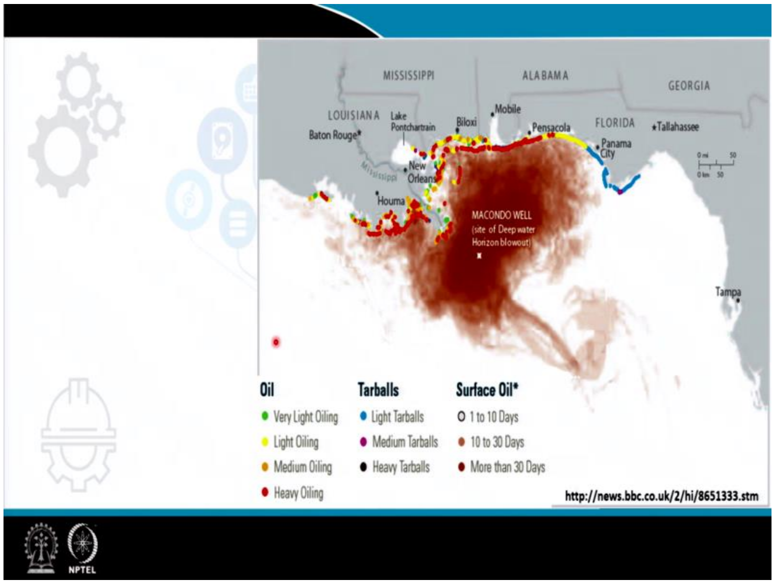

So, as you can see in the picture. So, here is the underground under the sea oil head which is used to produce huge amount of petroleum oil and Macondo well that is called is a site of the deep water horizon blow out we call it.

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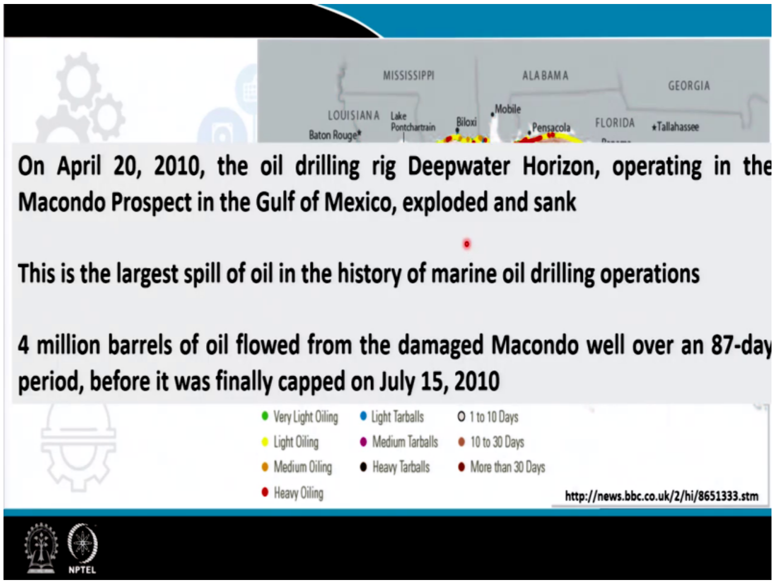

Now, on April 20 to 2010 the oil drilling deep water horizon operating in the Macondo prospect in the gulf of Mexico exploded and sank. So, the oil had actually blown out this is the largest spill of oil in the history of marine oil drilling operations and the x and the complete rig sank into the water 4 million barrels of the oil flowed from the damaged Macondo well over an 87 day period before it was finally capped on july 15 2010. So, the engineers and scientists were successful in capping it but before that a large amount of oil was spread across the sea.

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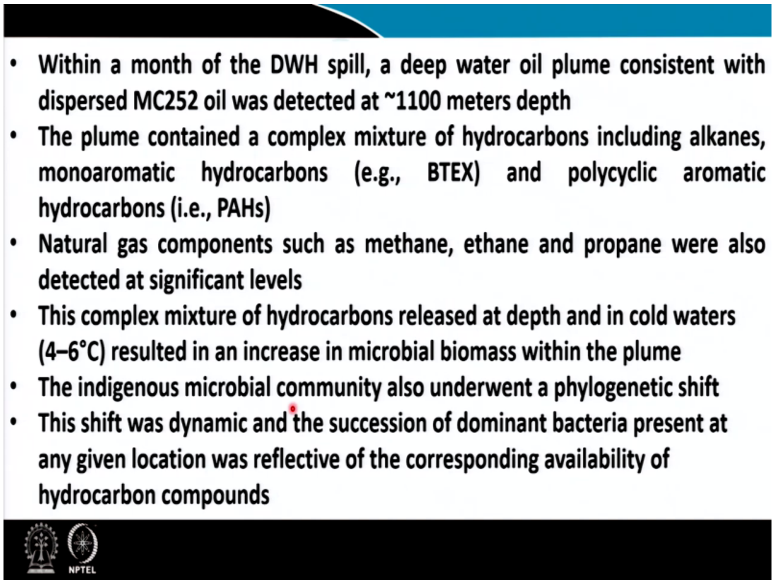

Now within a month of the deep water horizon spill a deep water oil plume consisted of dispersed oil was detected at 1100 meter depth. So, under the surface in the sea water a plume of oil was detected. And the plume contained a complex mixture of hydrocarbons including different alkanes monoaromatic like btx poly cyclic aromatic hydrocarbons etcetera. And natural gas components such as methane, ethane, propane was also detected.

And this complex mixture of hydrocarbon released at depth at more than one kilometer under the water and in cold water that is the water temperature is around 4 to 6 degree centigrade resulted in an increase in microbial biomass within the plume. So, naturally microorganisms started colonizing on that plume and they grew on that. Now the indigenous microbial community also underwent a phylogenetic ship because the sea water was already having a community obviously and that community undergone a significant shift because of this oil spill.

The shift was dynamic with respect to time and the succession of the dominant bacteria present at any given location was reflective of the corresponding availability of the hydrocarbon compounds. So, with respect to the natural degradation abilities of the microorganisms microorganisms they colonized they were impacted they degraded and they disappeared again.

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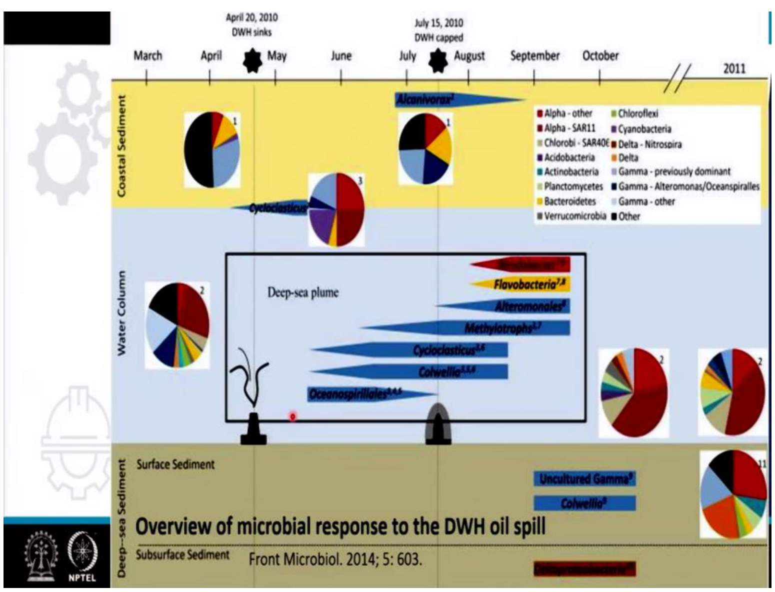

So, this picture represents the shift in the overall community composition and we call it overview of microbial response to the deep water horizon oil spill. And as you can see the samples were collected from deep sea sediment this region the water column where the plume was found and the coastal sediment which was away from the actual spill site. So, on the April the accident happened and following that we can see that within one and half month time the members of these osinopyles which are aerobic and anaerobic hydrocarbon degraders they appear they proliferated hugely.

And they persisted for more than one month in the water and then they gradually decrease their abundance was reduced. Interestingly as the abundance of this ocean of spiralis members declined the abundance of two more important groups Cholula and cycloplasticus they appeared. These members are found to be capable of degrading the oil aerobically aromatic hydrocarbon particularly and cholula is known for in situ degradation of ethane and propane.

And similarly few months later some other groups like alternate monoliths or methylotrophs flavor bacteria all these members emerged in the plume and these methanol drops they oxidized the methane to methanol methanol was further degraded by secondary consumer of C 1 compounds. Similarly we observed that within the coastal region also the cycloplastics which is aerobically aromatic degrading organism was enriched or naturally enriched rather.

And subsequent to that the members of the alkane works which is a potent aliphatic hydrocarbon degrader appeared late after the July and august from April the accident happened and in the surface area these organisms appear little late but they were found to be very important in degrading naturally degrading the the the spilled oil.

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For these case studies the following references are found to be suitable. So, these are the references to be used for the AMD bioremediation.

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And these are the references to be used for the oil spill by remediation.

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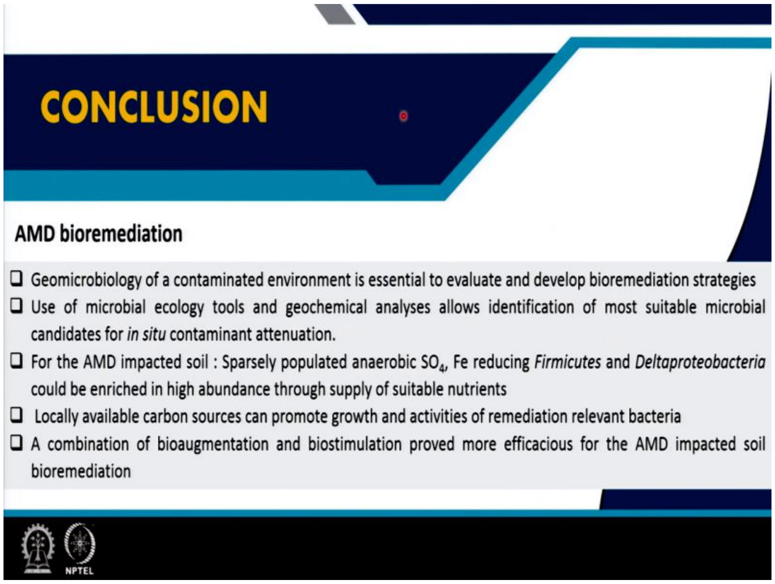

As a conclusion of the AMD bioremediation the geo microbiology experimentation and implementation studies indicated that these kind of work are required essential to evaluate and develop the bioremediation strategy because the intrinsic complexity of the bio remediation process. Use of microbial ecology tools and geochemical analysis allowed the identification of the most suitable microbial candidates for in situ contaminate attenuation.

For the AMD impacted soil the sparsely populated anaerobic sulphate and iron reducing bacteria like formicutes and delta proteobacteria member could be enriched in a high abundance through supply of the suitable nutrients and locally available carbon sources which are utilized by the heterotrophic populations are important resources for the remediating organisms. And a combination of bio augmentation and bio stimulation proved more efficacious for the AMD impacted bioremediation.

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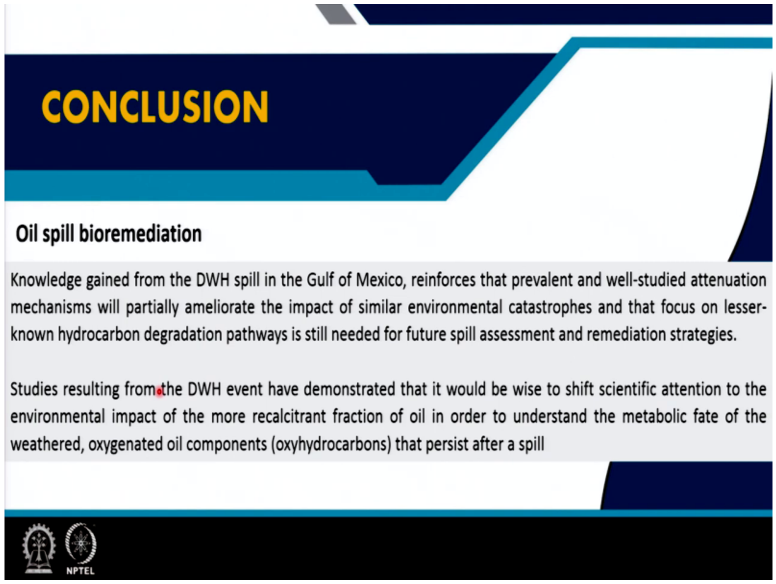

With respect to oil spill by remediation it is understood that the knowledge gained from the deep water horizon spill in particular reinforces that prevalent and well studied attenuation mechanisms will partially ameliorate the impact of the similar environmental catastrophe and that focus on lesser known hydrocarbon degrading organisms and degradation pathway is still needed for future spill assessment and remediation strategies.

Studies resulting from this deep water horizon spill event have demonstrated that it would be of wise to shift scientific attention to the environmental impact of the more recalcitrant fraction of the oil. Because those fractions of the oil will remain for longer period of time in order to understand the metabolic fate of those weathered oxygenated oil components which you called oxyhydrocarbons that persist after a spill, thank you very much.