Environmental Biotechnology
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Lecture – 51
Bioremediation case studies

Welcome to the next lecture of our course on environmental biotechnology and in this particular lecture we are going to discuss about some of the the important case studies on bioremediation.

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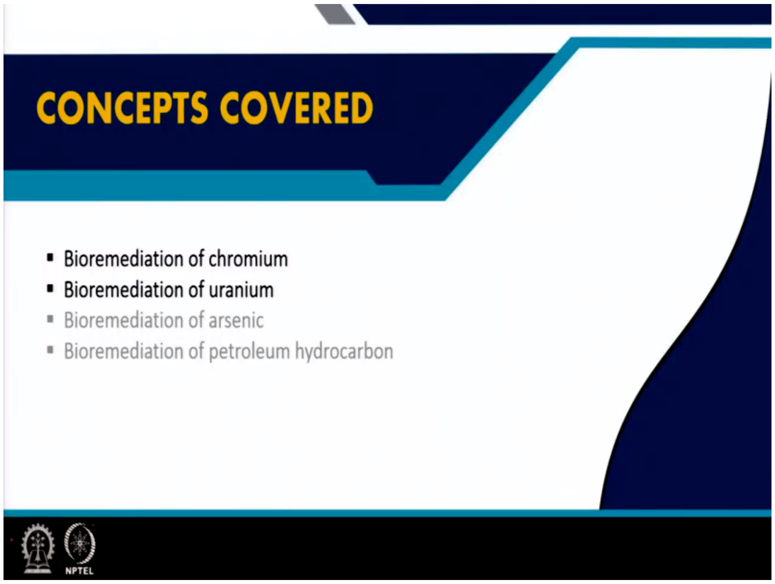

CONCEPTS COVERED

- Bioremediation of chromium
- Bioremediation of uranium
- Bioremediation of arsenic
- Bioremediation of petroleum hydrocarbon

So, in particular these two aspects of bioremediation that is the bioremediation of chromium and by remediation of uranium will be discussed particularly with reference to the the case studies where the bioremediation processes are being implemented in today's lecture.

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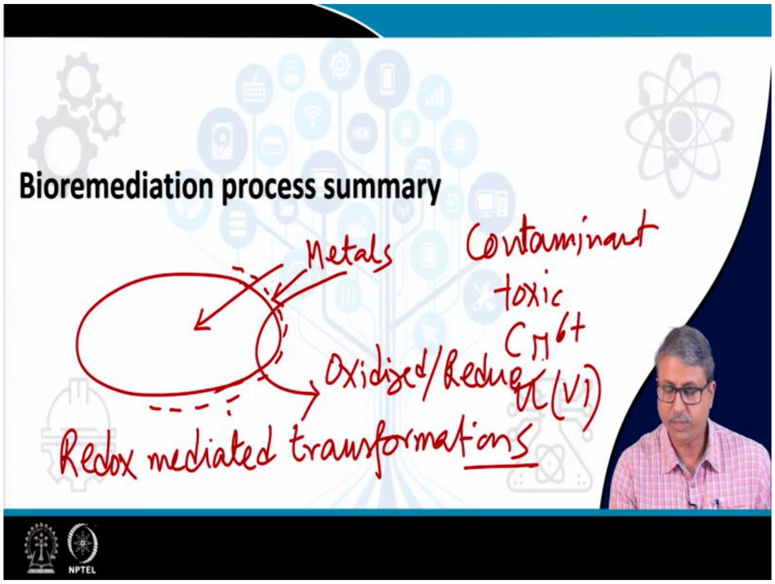

Bioremediation process summary

Now before we go into the details of these two case studies with respect to bioremediation of uranium and chromium. Let us quickly have a kind of a process recap of the by remediation process and as you might recall that we already discussed in detail about these aspects that in most of these all cases the bioremediation processes are actually the catalyzed by the microorganisms. And these microorganisms are capable of transforming different contaminants.

So, we have this contaminant in this case let us say the toxic chromium 6+ or uranium also 6. So, whether it is the contaminant toxic contaminant like chromium or uranium or maybe the petroleum. So, whatever may be the; contaminant or maybe even the plastics also or other hydrocarbon HCs. So, the microorganisms which live in the natural environment they are capable of transforming and interacting these with these toxic contaminants.

So, the basic concept again is quite simple that the microorganisms they interact with these toxic contaminants. So, if we if we try to of keep our focus on the toxic metals and radionuclides like uranium then we might be able to identify that these toxic metals like these metals they are they are getting interacted by these microbes because the microbes they are having a negatively charged surface because of these cell wall and cell membrane components.

And some of these divalent cations etcetera where they are being absorbed or accumulated inside the cells. A major process is the redox mediated transformations. So, these transformation reactions could be oxidation reactions or could be the reduction reaction. So, basically the metals are oxidized or reduced. So, during this case studies scenario we will see that how these processes are actually being implemented.

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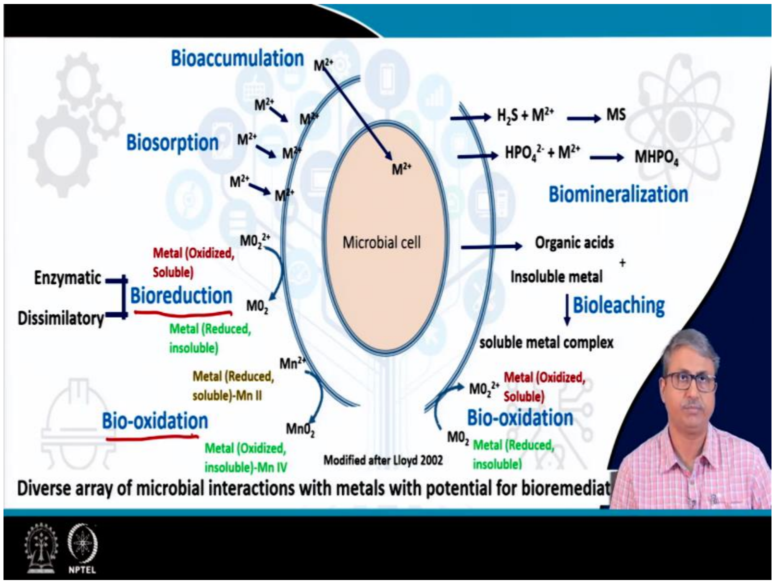

Bioaccumulation M^{2+} → M^{2+}

Biosorption M^{2+} → M^{2+}

Microbial cell

$H_2S + M^{2+} \rightarrow MS$

$HPO_4^{2-} + M^{2+} \rightarrow MHPO_4$

Biomineralization

Organic acids

Insoluble metal +

Bioleaching

soluble metal complex

Enzymatic

Dissimilatory

Bioreduction

MO_2^{2+} Metal (Oxidized, Soluble) → MO_2 Metal (Reduced, insoluble)

Bio-oxidation

Mn^{2+} Metal (Reduced, soluble)-Mn II → MnO_2 Metal (Oxidized, insoluble)-Mn IV

Bio-oxidation

MO_2 Metal (Reduced, insoluble) → MO_2^{2+} Metal (Oxidized, Soluble)

Modified after Lloyd 2002

Diverse array of microbial interactions with metals with potential for bioremediat

So, we just have a quick recap again of this microbial processes where we see that the microbes are able to either accumulate or absorb the different ivalent cations and including some of the the anionic complexes like the chromate or the arsenate etcetera and the another very important aspect of the metal microbe interaction that I was referring to earlier that is the bio transformation or the reduction based processes.

And these processes could be reduction or could be oxidation as well. So, there are processes which are actually bio oxidation or bio reduction the similarity of both these processes that is the bio reduction and bio oxidation the similarity is that in both the cases the oxidation state of the metal like chromium, arsenic, uranium etcetera the redox active metals their oxidation states are altered.

This alteration is basically done because either these metals or metalloids are used as source of electrons. So, they are oxidized or they are used as a as an electron acceptor that means during this process of electron accepting as you know that the microbes are capable of using different metal ions as inorganic electron acceptors. So, they are able to perform their anaerobic respiration using these metal ions.

So, these reductive processes or these oxidative processes are both found to be very very important for the bio remediation purposes. There is another process which is also found to be very interesting and gaining importance is the bio mineralization where the micro organisms they release a number of ligands like the phosphate or H 2 S as you can see the specific group of microorganism for example the sulphate reducing bacteria for example they produce the sulphides and these sulphides could react with the metal ions and the metal sulphides are precipitate.

Alternatively the phosphate ion which are produced by the many bacterial strains using external phosphate organic phosphate donor molecules these phosphate groups are also able to interact with the positively charged metal ions and then they produce the metal phosphate complexes. So, it is basically these two types that is the redox transformation process processes and the bio mineralization based processes.

So, before I go into the details of this process I would like to quickly have a recap further detailing of these processes for example if we want to just see how this redox transformations are actually the catalyzed.

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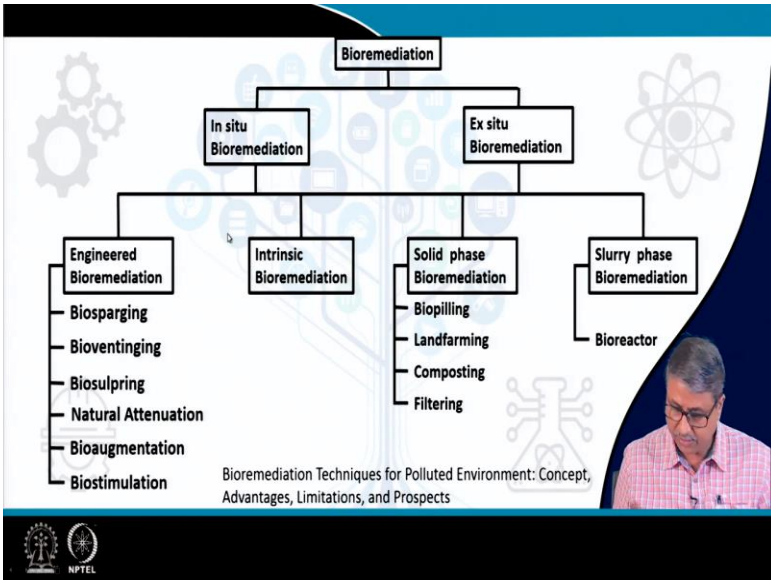

- Bioremediation
  - In situ Bioremediation
    - Engineered Bioremediation
      - Biosparging
      - Bioventinging
      - Biosulpring
      - Natural Attenuation
      - Bioaugmentation
      - Biostimulation
    - Intrinsic Bioremediation
  - Ex situ Bioremediation
    - Solid phase Bioremediation
      - Biopilling
      - Landfarming
      - Composting
      - Filtering
    - Slurry phase Bioremediation
      - Bioreactor

Bioremediation Techniques for Polluted Environment: Concept, Advantages, Limitations, and Prospects

So, we can understand that there are bacterial strains naturally present. So, under any aquifer or contaminated environment. So, microorganisms are naturally present.

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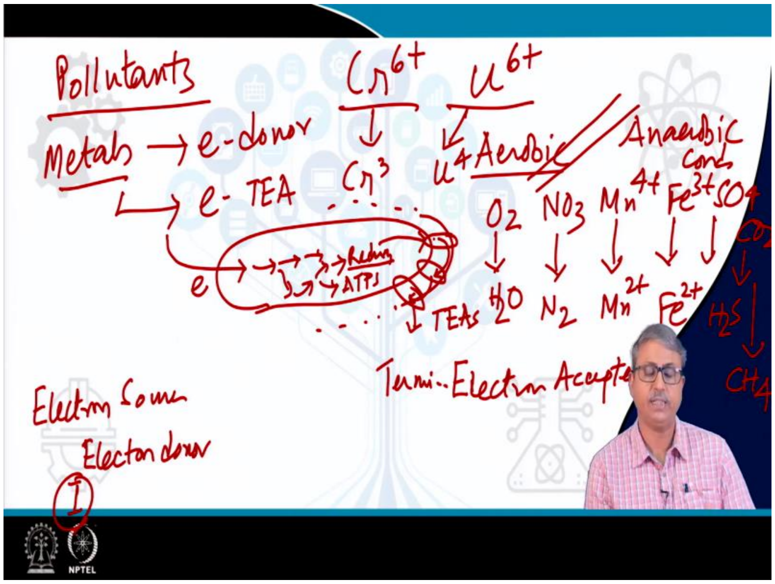

So, there are two important requirements from the microbial point of view one is the electron source, so, you need an electron donor and on the other hand you need an electron acceptor and this is basically we called as terminal electron acceptor. So, one way you have to have some compounds or some inorganic material in this case we are talking about metals and radionuclides. So, you have to have something which will be donating the electrons.

And these electrons will be processed through the diverse metabolic pathways and eventually will help the cell to produce the necessary ATPs and reducing power and ultimately this reducing powers will take the electrons to the electron transport chain which is located in the membrane. Now this is actually the electron transport chain if you remember this is composed of a number of electron carrier complexes and this electrons which are which are coming from this reducing power which is like NADH H plus or FADH 2 donating the electrons to this electron transport chain complexes eventually capable of transferring this electron to the terminal electron acceptor.

So, we call them TEA terminal electron acceptors. So, under the aerobic condition for example it is the oxygen who acts as the terminal electron acceptor and oxygen is reduced to water.

However under anaerobic condition compounds like nitrate Mn 4+ iron sulphate and even carbon dioxide they can also accept the electrons and then they produce the reduced product like nitrate can be converted to nitrogen Mn can be converted to Mn 2+ Fe 3 can be converted to Fe 2 plus sulfate can be converted to sulfide and carbon dioxide can be converted to methane.

Now these are the situations where we have the anaerobic condition. Now depending upon the environmental situation like in a aquifer environment. Now, we know that the aquifer may be aerobic may be partially aerobic it may be having a fluctuating oxygen level or it may be anaerobic. Similarly a contaminated soil or contaminated landfill or any other contaminated sediments where lots of metals and other pollutants are there they might be having the aerobic or anaerobic conditions prevailing in them.

And in between you have always a kind of a transition zones where the concentration of oxygen is gradually declining and the microbes are able to metabolize the alternate electron accept this is number one point number one. Now when we have the pollutants, so, we are talking about the pollutants in this case we have the metals. So, some of the metals can act as e donor a while some of the other metals can be act as terminal electron acceptors obviously under naught all redox conditions both these processes will be applicable.

So, some of the metals will be able to donate electrons under certain redox condition whereas some of the other metals will be able to accept the electrons under the same redox potential condition. So, if we look at these two toxic metals that that is the point of interest in today's class that is the chromium for example. So, we are going to talk about hexavalent chromium for example and the uranium. So, uranium 6+ we are talking about uranium 6.

So, both these are able to accept electrons. So, under the prevailing conditions; so, it has been found that many bacteria it is not the property of one particular group of bacteria but many group of groups of bacteria we see that they are capable of utilizing this chromium or chromate or uranium which is basically uranium 6+ as uranium 6 as electron acceptor and they use diverse mechanisms to reduce this chromium 6 to chromium 3.

So, it will be reduced to chromium 3 and uranium 6 will be reduced to uranium 4. So that is the point number two. So, under anaerobic condition especially; although some bacteria can do these reduction processes under aerobic condition also because they have the enzymes which are capable of doing such functions. So, these are these processes are actually implemented during the bioremediation processes which could be in situ or ex situ.

So, most of the time these in situ processes are preferred because in the in situ process the the waste material like the aquifer the groundwater the sediment the soil is there in place. So, the remediation process will be adopted while the toxic water or the toxic what waste material is in place it is not going to be removed from its original location.

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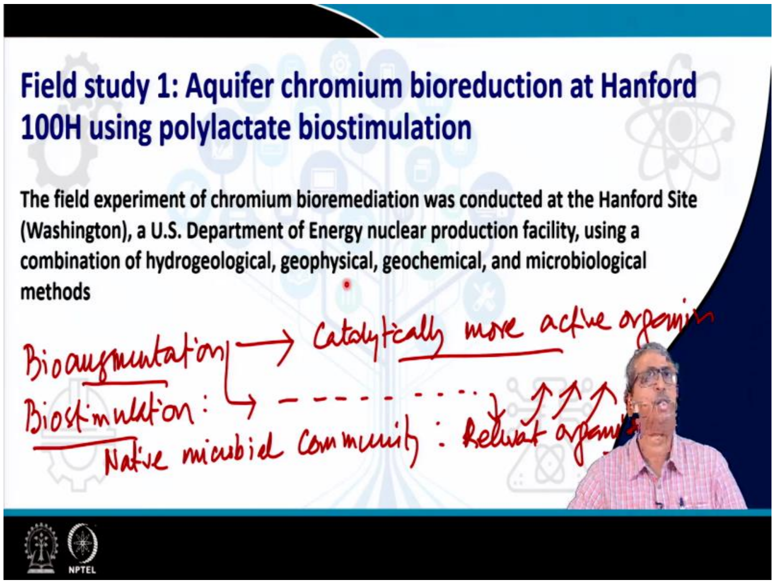

Field study 1: Aquifer chromium bioreduction at Hanford 100H using polylactate biostimulation

The field experiment of chromium bioremediation was conducted at the Hanford Site (Washington), a U.S. Department of Energy nuclear production facility, using a combination of hydrogeological, geophysical, geochemical, and microbiological methods

Bioaugmentation → Catalytically more active organism

Biostimulation : → - - - - - - - . . .

Native microbial community : Relevant organ

Now let us first look at this case study we call it field study one for aquifer chromium bioreduction at hand ford site using a polylactate biostimulation approach. So, this biostimulation approach is a very interesting approach where we see that if you try to recollect some of these bioremediation strategies that indigenous organisms that is the native microbial community often process the relevant organisms these organisms mean multiple species or multiple population.

So, when you may expect that the community itself is having relevant organisms these are different members of different genera members of different populations etcetera. Now, in a contaminated aquifer environment you might have these relevant organisms if you have this relevant organisms then we can take up one strategy that is called the bio stimulation strategy. So, what is biostimulation? So, we add external agents which actually go diffuse and act on these relevant organisms and encourage them.

They allow them to proliferate more they allow them to function more aggressively and in a simple sense a simple sentence that the relevant organisms are enhanced. So, this bio stimulation can be done by adding sometimes electron acceptor sometimes by adding electron donor sometime by carbon sources or even can be by adjusting the pH or the redox conditions also. So, this is found to be one of the the best strategy in case of a engineered remediation.

So, when you say engineered remediation you must be able to remember that there is another process which is called natural attenuation in case of natural attenuation we completely rely on the catabolic abilities of the native bacterial populations and we do not interfere with respect to adding any stimulants like the bio stimulation or something like that. Of course there is another strategy in terms of the engineered approach like bio stimulation we discussed that is the bio augmentation.

So, in case of bio augmentation what we do we add catalytically more active organism. So, often it has been found in the natural community is not having sufficient number of the relevant catabolically relevant organisms in this particular contaminated environment. So, we need to recruit organisms from external resources. So, we might have some cultures which may be mixed culture might have some pure culture bacteria which are not ordinary bacteria they are catalytically found to be more superior or they are more effective efficacious in terms of the particular reaction that is being targeted.

For example if you want to have a chromium bioremediation for example we should have in mind that the chromium should be or must be remediated through reduction that is the hexavalent chromium needs to be reduced to trivalent chromium. So, we should catalytically active organism means the chromate reducing bacteria simply. So, we should have some bacterial culture some bacterial strains and we can adopt that strategy as well that is called the bio augmentation.

In case of bio stimulation we do not add any bacteria directly rather we add some stimulating agent as I mentioned earlier. So, during this field experiments which was done under the united states department of energy bio remediation program. So, a number of radionuclide and metals and metalloid contaminated environments were located sites were located in the USDE program and basically these were all targeted at the different facilities which are called us DOE nuclear production facility using a combination of hydro geological geophysical geochemical and microbiological methods.

So, a large group of scientists were engaged to perform the survey of a large number of sites although we are going to talk about only one particular site in this case study that is the Hanford site and let us go to the site.

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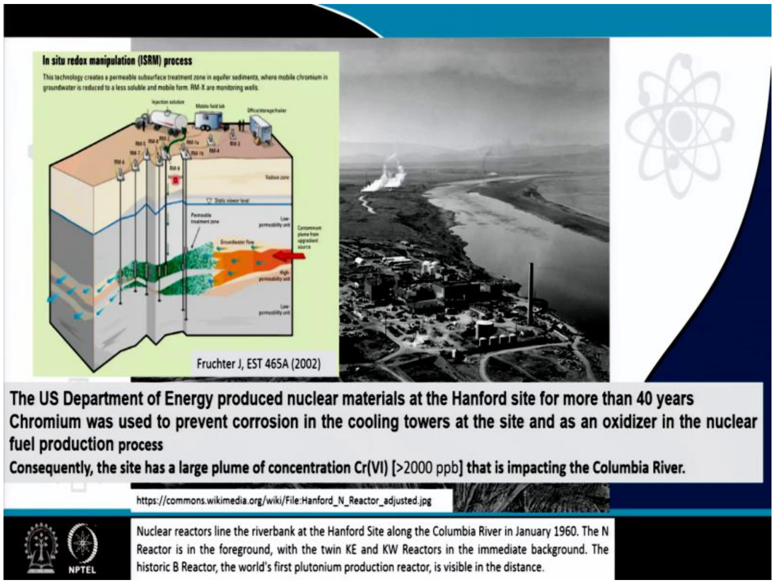

In situ redox manipulation (ISRM) process

This technology creates a permeable subsurface treatment zone in aquifer sediments, where mobile chromium in groundwater is reduced to a less soluble and mobile form. RM-X are monitoring wells.

Fruchter J, EST 465A (2002)

The US Department of Energy produced nuclear materials at the Hanford site for more than 40 years

Chromium was used to prevent corrosion in the cooling towers at the site and as an oxidizer in the nuclear fuel production process

Consequently, the site has a large plume of concentration Cr(VI) [>2000 ppb] that is impacting the Columbia River.

https://commons.wikimedia.org/wiki/File:Hanford_N_Reactor_adjusted.jpg

Nuclear reactors line the riverbank at the Hanford Site along the Columbia River in January 1960. The N Reactor is in the foreground, with the twin KE and KW Reactors in the immediate background. The historic B Reactor, the world's first plutonium production reactor, is visible in the distance.

So, this is the picture of the Hanford site as you can see this this is perhaps the one of the most beautiful picture of this Hanford site nuclear reactor facility which is built in order to produce the weapon grade nuclear material. And I have learnt that actually the atom bombs were actually having the plutonium which was in enrich produced in these reactors this Hanford site reactor. So, historically it has a great importance and this particular picture seems to be taken in 1960.

And the fact that is relevant for environmental aspect is it is just in the bank of the Columbia river and we can see number of nuclear reactors. So, these are not operating anymore and what is important is that these reactors and the associated facilities including the storage of the spent fuel and all those things they produced huge amount of waste. So, if you look at this, this department of energy facility produces nuclear material at the site for more than 40 years.

And chromium was found to be one of the most important toxic material which is later discharged into the environment and this chromium though it is not radioactive and as such the nuclear program might not have a direct connection but it has certain obvious reasons to be there like is to prevent the corrosion in the cooling towers at the site and as an oxidizer in the nuclear fuel production process.

And consequently the site has a large plume of concentration of chromium 6 which is highly toxic more than 2000 ppb and in some studies it is referring to up to maybe 4000 ppb and that is impacting the Columbia river that means they have some underground plume that we can see over here. So, this is a cartoon published in one of the environmental science and technology papers by the group of scientist who was working on in situ redox manipulation a process.

That was another process developed through permeable reactive barrier in order to combat the flow of toxic chromium. So, as you can see that underground. So, at the underground of this there is a plume of chromium contaminated water and this chromium contaminated water is flowing towards the river. So, gradually this chromium contaminated water is coming into this river and contaminating the river.

And putting the entire river ecosystem under severe threat and affecting the public health drinking water system the overall ecosystem balance. So, USDOE has taken severe measure to combat this situation and along with the chemical and physiologic physical and other processes the bioremediation events were also taken up.

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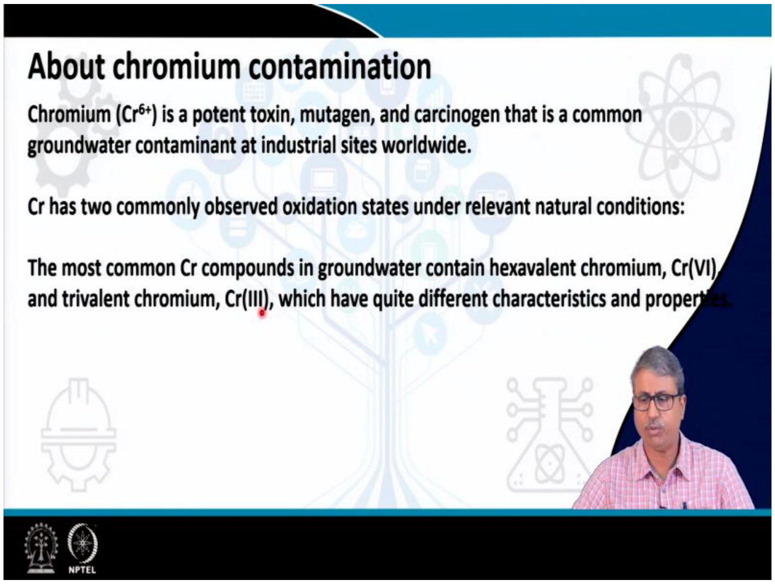

About chromium contamination

Chromium (Cr^{6+}) is a potent toxin, mutagen, and carcinogen that is a common groundwater contaminant at industrial sites worldwide.

Cr has two commonly observed oxidation states under relevant natural conditions:

The most common Cr compounds in groundwater contain hexavalent chromium, Cr(VI), and trivalent chromium, Cr(III), which have quite different characteristics and properties.

Now, little bit more background information about the chromium contamination. The chromium in its hexavalent form is a potent toxin mutagen and carcinogen that is a common ground water contaminant at industrial site worldwide. So, no matter whether it is a nuclear facility or a non nuclear facility. So, chromium hexavalent chromium is a very commonly used industrial chemical and it is often found in many of the industrially industrial waste contaminated sites including India.

Now, it has two commonly observed oxidation states that is the point of interest under relevant natural conditions. So, it has two oxidation states which can be observed under natural conditions. Now, the most common chromium compounds in groundwater contain the hexavalent chromium or chromium 6 and the trivalent chromium that is the chromium 3 which have quite different characteristics and properties. So, this chromium 6 and the chromium 3 they are quite unique.

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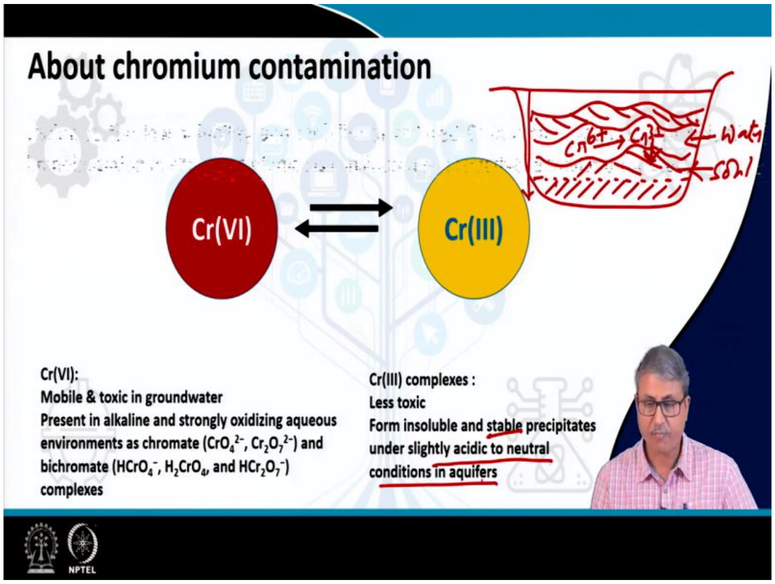

About chromium contamination

Cr(VI):
Mobile & toxic in groundwater
Present in alkaline and strongly oxidizing aqueous environments as chromate (CrO_4^{2-}, $Cr_2O_7^{2-}$) and bichromate ($HCrO_4^-$, H_2CrO_4, and $HCr_2O_7^-$) complexes

Cr(III) complexes :
Less toxic
Form insoluble and stable precipitates under slightly acidic to neutral conditions in aquifers

Unique in the sense they are both chromium and they are interconvertible because chromium 6 can be reduced to chromium 3 and chromium 3 can be oxidized to chromium 6. Now, chromium 6 is a mobile element. So, it can or compound it can chromate ion can move very quickly and its very toxic in when it is in the ground water or in the soil system. So, it is it is more soluble rather in aqueous phase. Present in alkaline and strongly oxidizing aqueous environment as chromate ion mainly and also as bichromate complexes.

So, overall the chromatin bichromate all these are chromium 6 complex and its very mobile present significantly in the in the aqueous medium and also it is bio available and it is very toxic. In contrast to that chromium 3 complexes are less toxic and it forms insoluble and stable precipitates under slightly acidic to neutral conditions in aquifers. So, what does that signify? It signifies that chromium 6 is very much soluble.

So, let us take for example if we try to just quickly look at the scenario that just let us assume that this is underground situation and you have this is the depth. So, you have the water inside and this is the sediment part this is all the sediment and in between you have the this is the aquifer for example and you have otherwise all these are different layers of sediments and rocks.

Now, within this you have the chromium 6+, chromium 6+ therefore will be in the aqueous phase. So, this is the water phase and this is the solid phase or the solid or the rock or salt sand sediment etcetera under the aquifer environment. Now, the moment you convert this chromium 6 to chromium 3. Now, chromium 3 cannot stay in the aqueous phase. So, basically it forms insoluble complex with the with different complex with the present with the available ligands and it precipitates.

And the precipitates are quite stable precipitate that means the precipitates that those are formed will not be or will not readily oxidize back to the chromium 6 not readily. So, particularly under slightly acidic to neutral conditions and if you look at this the most of these aquifers that we are dealing with are mostly circumnuetral conditions.

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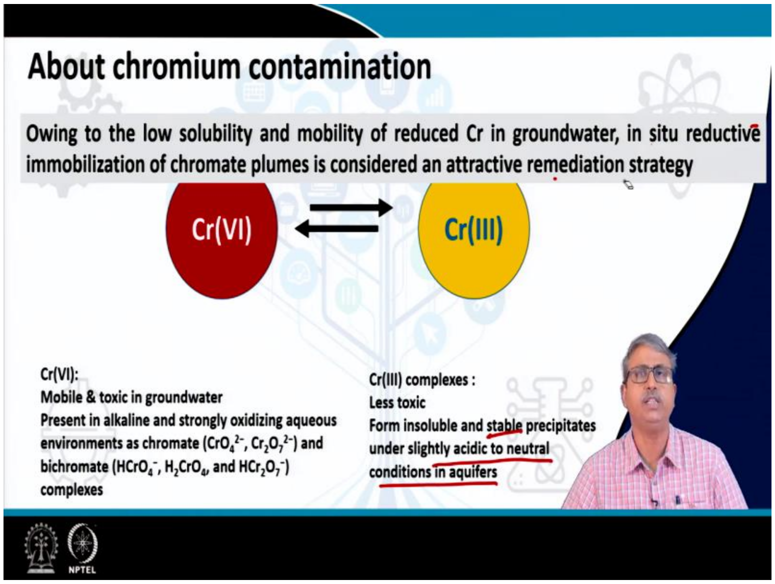

About chromium contamination

Owing to the low solubility and mobility of reduced Cr in groundwater, in situ reductive immobilization of chromate plumes is considered an attractive remediation strategy

Cr(VI):
Mobile & toxic in groundwater
Present in alkaline and strongly oxidizing aqueous environments as chromate (CrO_4^{2-}, $Cr_2O_7^{2-}$) and bichromate ($HCrO_4^-$, H_2CrO_4, and $HCr_2O_7^-$) complexes

Cr(III) complexes :
Less toxic
Form insoluble and stable precipitates under slightly acidic to neutral conditions in aquifers

Now going to the low solubility. Now, going to the low solubility and mobility of this reduced chromium in ground water because as I explained that once the hexavalent chromium is reduced to chromium 3 the chromium 3 is having a low solubility and mobility and it tends to always precipitate and form stable complexes with the this sediment and other solid phases of the environment. Now, in situ reductive immobilization of chromate plumes that chromium containing the ground water system is considered an attractive remediation strategy.

So, in underground system if we; can reduce chromium 6 to chromium 3. The chromium 3 will precipitate and that precipitated chromium 3 will help us to get the water free from the soluble chromium.

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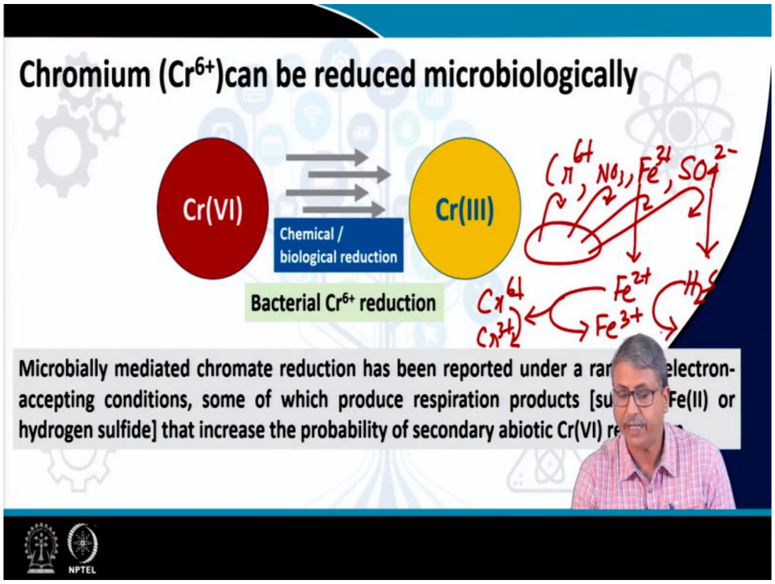

Chromium (Cr^{6+})can be reduced microbiologically

Microbially mediated chromate reduction has been reported under a ran electron-accepting conditions, some of which produce respiration products [su Fe(II) or hydrogen sulfide] that increase the probability of secondary abiotic Cr(VI) re

Now, this chromium 6 can be reduced microbiology of course can be reduced chemically also. So, we can we can think of chemical reduction as well as biological reduction. Now, in environmental biotechnology this reduction of the chromium through the bacteria in particular is highly important. So, microbially mediated chromate reduction has been reported under a range of conditions under a range of electron accepting condition.

Because in underground aquifer we often see a mixture of electron acceptors are there and chromium might need to need to face a competition with respect to accepting the electrons because many other electron acceptors might be there and since other electron acceptors are also there like iron sulphate nitrate etcetera are there some of these which produce the respiration products such as the Fe 2 or hydrogen sulfide that increase the probability of secondary abiotic chromate reduction.

So, what is that about the chromate reduction. So, we have we have already seen that there are microbes who are capable of reducing chromium. Now, in under the aquifer environment chromium is not alone. So, we have the chromium and along with chromium as I mentioned that we have. So, chromium is there but along with chromium there are nitrate particularly iron and sulfate. So, when we have these microbes working on them. So, they may reduce chromium they may reduce nitrate they may reduce iron or they may reduce sulfate.

So, if they reduce iron for example they produce Fe 2+ or they if they reduce sulphide they produce H 2 S. Now, interestingly this Fe 2+ can be re-oxidized under the aquifer environment with reducing chromate 6+ 2 3+. So, instead of direct microbial or bacterial reduction of chromium 6 Fe 2 can be aiding into the reduction of chromium 6 into chromium 3 and similarly H 2 S can also do that. So, H 2 S can add into the reduction of chromium 6 to chromium 3 forming a sulfate. So, these are also possible. So, direct reduction of the chromium is possible as well as the indirect reduction of the chromium is possible.

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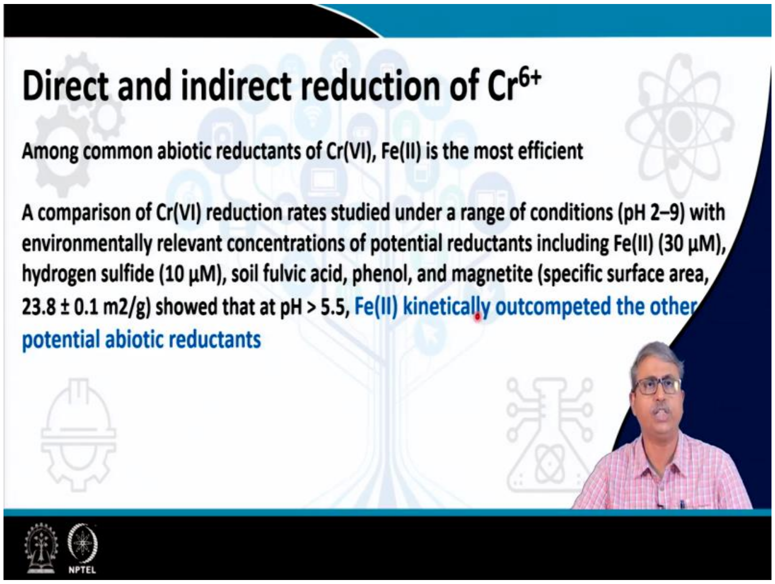

Direct and indirect reduction of Cr^{6+}

Among common abiotic reductants of Cr(VI), Fe(II) is the most efficient

A comparison of Cr(VI) reduction rates studied under a range of conditions (pH 2–9) with environmentally relevant concentrations of potential reductants including Fe(II) (30 μM), hydrogen sulfide (10 μM), soil fulvic acid, phenol, and magnetite (specific surface area, 23.8 ± 0.1 m2/g) showed that at pH > 5.5, Fe(II) kinetically outcompeted the other potential abiotic reductants

Now, what are the direct and indirect? Among the common abiotic reductance of chromate the hexavalent chromium the iron is found to be most efficient Fe 2+ and it is also very interesting that under the aquifer environment Fe 3is abundant as a part of the geological properties. Now, this Fe 3 if it is reduced to Fe 2 by microbial action this Fe 2 can act towards reducing chromium 6 further and in that regard scientists have done a number of studies.

And what they have observed that not only Fe 2 there could be other chemical compounds are produced for example under the range of conditions that is a pH 2 to 9 with environmentally relevant concentration of the potentially reductance including the Fe 2, hydrogen sulphide which can be produced very well by reduction of iron 3 Fe 3+ or the sulfate, fulvic acid, phenol magnetite etcetera. In most of these cases people have scientists have found that Fe 2 which could be easily produced by reducing Fe 3+ is kinetically more efficient.

And it kinetically out competed the other potential abiotic reductant that means even if you have this fulvic acid phenol magnetite or sulfides the Fe 2 will be a potent reductant for reducing the chromate.

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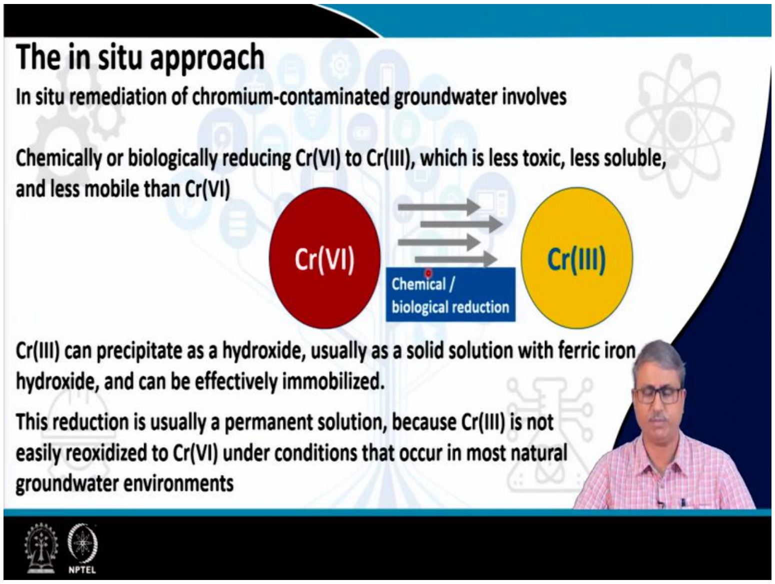

The in situ approach

In situ remediation of chromium-contaminated groundwater involves

Chemically or biologically reducing Cr(VI) to Cr(III), which is less toxic, less soluble, and less mobile than Cr(VI)

Cr(III) can precipitate as a hydroxide, usually as a solid solution with ferric iron hydroxide, and can be effectively immobilized.

This reduction is usually a permanent solution, because Cr(III) is not easily reoxidized to Cr(VI) under conditions that occur in most natural groundwater environments

Now, during this in situ approach for this hand foresight initially the institute remediation of chromium contaminated groundwater was done involving both chemical as well as biological agents to reduce the chromium 6 to chromium 3 which is actually the chromium these obviously as I mentioned is less toxic less soluble and less mobile than chromate. So, the target was to produce or the convert chromium 6 which is very hazardous to less toxic and stable precipitate forming chromium 3.

Now, chromium 3 can precipitate as hydroxide usually as a solid solution with ferric iron oxides and can be effectively immobilized. So, that is a part of nat immobilization that can be achieved particularly when we have an iron in that. Now, this reduction is usually a permanent solution because chromium 3 is not easily deoxidized to chromium 6 under conditions that occur in most natural groundwater environment.

So, the reverse reaction is possible but not feasible under the conditions which are prevailing in the ground water conditions. In most of the cases scientists have studied this thoroughly and come to the conclusion that the under the natural water condition the con reverse reaction that is the oxidation of chromium 3 to chromium 6 is not preferred.

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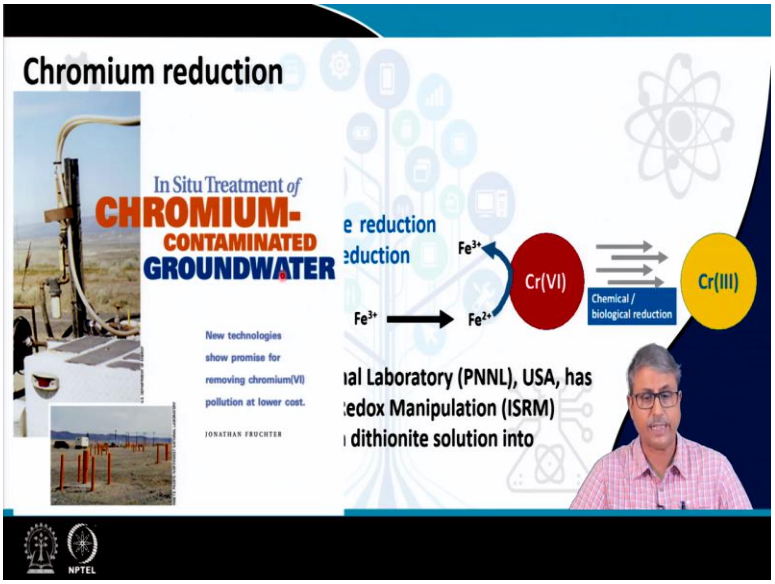

Chromium reduction

In Situ Treatment of CHROMIUM-CONTAMINATED GROUNDWATER

New technologies show promise for removing chromium(VI) pollution at lower cost.

JONATHAN FRUCHTER

e reduction

eduction

al Laboratory (PNNL), USA, has

edox Manipulation (ISRM)

dithionite solution into

Now, the chromium reduction is commonly used chemical reductant for chromium 6 to chromium 3 is chemical reductant with subsequent immobilization two of the chromium 3 is the Fe 2+ as I mentioned because it is found to be kinetically is much superior than other. Now, this particular approach was taken in the Hanford site as well. So, the pacific northwestern national laboratory PNNL, USA has developed and implemented an in-situ redox manipulation technology that is called ISRM technology using the injection of sodium diethyonate solution into the ground water.

Now, what is the job of the sodium diathernic sodium dithionite injection into the aquifer leads to the reduction of Fe 3 releasing Fe 2 which is needed for the chromate reduction. So, diethyanet is producing the electrons and these electrons are capable of reducing the Fe 3 which is present as ferric oxide or hydroxide in the aquifer solid phase and this resulting Fe 2 is capable of reducing chromium 6 to chromium 3 and Fe 3 is getting produced and this sodium diethylate is a not a very stable compound.

So, after some time these actions of the diethane will be diminished into the aquifer system. So, this was the report published in the journal environmental science and technology where in situ treatment of chromium contaminated groundwater was achieved successfully.

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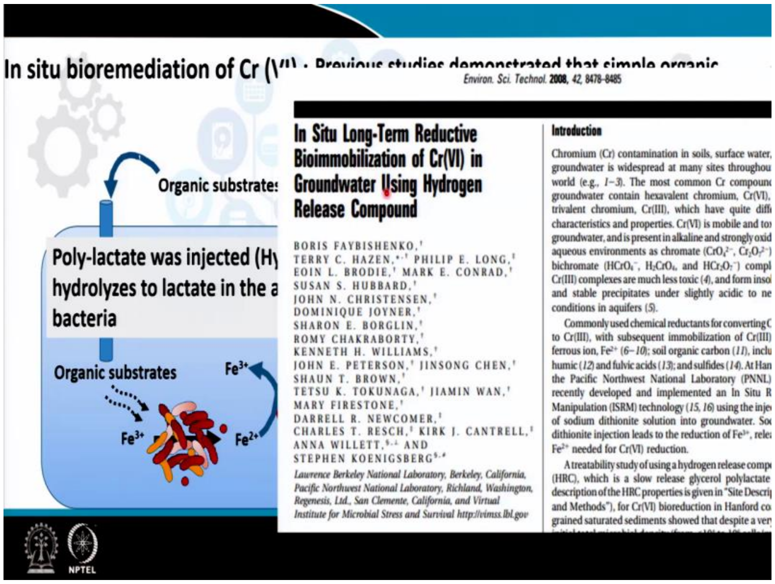

In situ bioremediation of Cr (V

Organic substrates

Poly-lactate was injected (Hy
hydrolyzes to lactate in the a
bacteria

Organic substrates

Environ. Sci. Technol. 2008, 42, 8478–8485

In Situ Long-Term Reductive Bioimmobilization of Cr(VI) in Groundwater Using Hydrogen Release Compound

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TERRY C. HAZEN,[*,†] PHILIP E. LONG,[‡]
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Introduction

Chromium (Cr) contamination in soils, surface water, groundwater is widespread at many sites throughou world (e.g., 1–3). The most common Cr compounc groundwater contain hexavalent chromium, Cr(VI), trivalent chromium, Cr(III), which have quite diff characteristics and properties. Cr(VI) is mobile and to groundwater, and is present in alkaline and strongly oxid aqueous environments as chromate (CrO_4^{2-}, $Cr_2O_7^{2-}$) bichromate ($HCrO_4^-$, H_2CrO_4, and $HCr_2O_7^-$) compl Cr(III) complexes are much less toxic (4), and form inso and stable precipitates under slightly acidic to ne conditions in aquifers (5).

Commonly used chemical reductants for converting C to Cr(III), with subsequent immobilization of Cr(III) ferrous ion, Fe^{2+} (6–10); soil organic carbon (11), inclu humic (12) and fulvic acids (13); and sulfides (14). At Han the Pacific Northwest National Laboratory (PNNL) recently developed and implemented an In Situ R Manipulation (ISRM) technology (15, 16) using the inje of sodium dithionite solution into groundwater. So dithionite injection leads to the reduction of Fe^{3+}, rele Fe^{2+} needed for Cr(VI) reduction.

A treatability study of using a hydrogen release comp (HRC), which is a slow release glycerol polylactate description of the HRC properties is given in "Site Descri and Methods"), for Cr(VI) bioreduction in Hanford co grained saturated sediments showed that despite a ver

Now, along with this and subsequent to this the scientist found that why to inject this. So, dithionite rather in case of stimulating this bacteria if that is the point that you want to actually have Fe 2+ you are otherwise using sodium diathynide but what about the fact that if we can produce this Fe 2+ in situ. So, the in situ bioremediation of chromium 6 was achieved because previous studies demonstrated that simple organic carbon compounds like lactate could stimulate the iron reducing bacteria in the soil to reduce enough Fe 3 to Fe 2 that the Fe 2 could reduce chromium 6 to chromium 3 and precipitate it in the soil.

So, effectively the scientists they injected organic substrates which are called compounds which are easily utilized by this iron reducing bacteria. So, with through different injection wells they put these or injected these lactic acids or polyreactive compounds and these compounds are finally utilized by this iron reducing bacteria which are naturally present. So, these compounds might be available to all organisms of course the iron reducing bacteria will be happy to utilize along with sulfate and nitrate reducing bacteria as well.

But we are more focused on production of F e2. So, F e3 will be reduced to Fe 2 by this iron reducing bacteria and then the Fe 2 will be able to reduce chromium 6 to chromium 3.. Now, poly lactate was finally chosen as a compound to be injected and it is also called hydrogen released compound. The polylactate actually hydrolyzes to lactic acid in the aquifer which is readily utilized by the indigenous bacteria particularly the iron reducing bacteria.

And basically this work was published in the journal environmental science and technology with the title in situ long term reductive bio immobilization of chromium in groundwater using hydrogen release compound.

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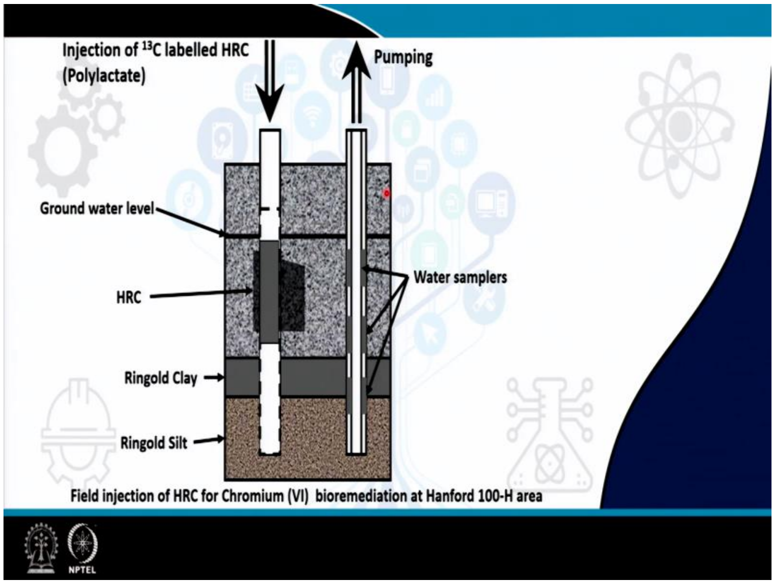

Field injection of HRC for Chromium (VI) bioremediation at Hanford 100-H area

And this was the sampling or the injection well strategy that you can see that you have the injection well as well as the pumping and the monitoring was continuously being done in order to facilitate it the process. So, what was observed that in two weeks time after the injection of this polyacted compound the total bacterial diversity increased from less than 10 to the power 5 cells per ml to more than 10 to the power 7 cells per mm.

So, enhanced bacterial activity as expected the oxygen was depleted fast because in the presence of lactate the aerobic organisms will try to consume the available lactate and very soon the oxygen will be depleted and then nitrate and iron will be reduced. Within 3 to 4 weeks time of injection the chromium 6 came down in the monitoring wells and stayed well below the level of drinking water for several months even after the nineteen oxygen return to the original concentration.

Because as I mentioned that chromium 3 is a very stable form and under the prevailing ground water conditions the chromium 3 is not readily oxidized back to chromium 6.

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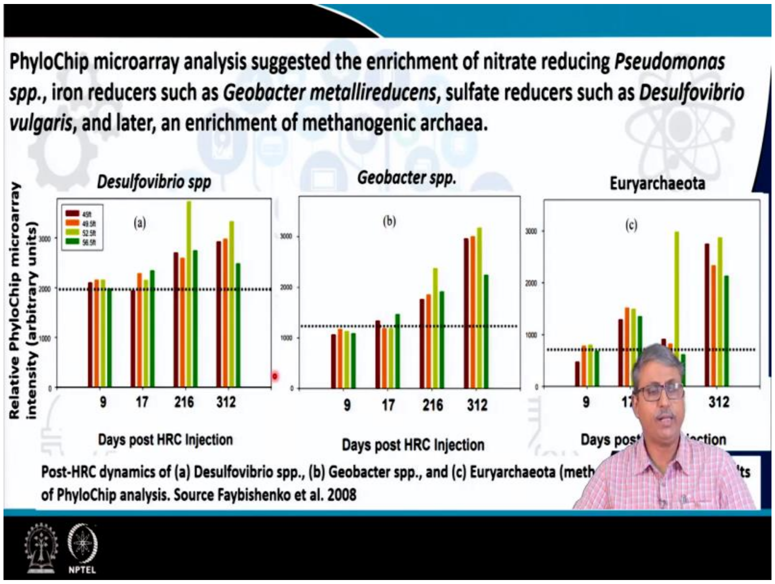

PhyloChip microarray analysis suggested the enrichment of nitrate reducing *Pseudomonas spp.*, iron reducers such as *Geobacter metallireducens*, sulfate reducers such as *Desulfovibrio vulgaris*, and later, an enrichment of methanogenic archaea.

Post-HRC dynamics of (a) Desulfovibrio spp., (b) Geobacter spp., and (c) Euryarchaeota (meth ts of PhyloChip analysis. Source Faybishenko et al. 2008

Subsequent microbial community analysis by microarray data suggested that a large number of organisms including the pseudomonas or geobacter even the dissulfovbrio the sulphate reducers and archaea were developed or improved you can see with respect to time scale how these different organisms are at different depth. So, these are the different colors indicate different depth and at the different time like days post HRC index injection is data is presented.

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Developments in bioremediation of soils and sediments polluted with metals and radionuclides: 2. Field research on bioremediation of metals and radionuclides

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Key words: heavy metal, radionuclide, field test, bioremediation, biotransformation, biodegradation, natural attenuation, treatment train, bioaccumulation, biosorption, volatilization

Abstract

Bioremediation of metals and radionuclides has had many field tests, demonstrations, and full-scale implementations in recent years. Field research in this area has occurred for many different metals and

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Geophysical Monitoring of Hydrological and Biogeochemical Transformations Associated with Cr(VI) Bioremediation

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Understanding how hydrological and biogeochemical properties change over space and time in response to

Our study was motivated by the hypothesis that several of the expected transformations would yield end products that could be detectable using time-lapse geophysical methods. Recent studies have demonstrated how indirect (yet spatially extensive) geophysical data sets can be integrated with direct (yet sparse) hydrological data to provide estimates of subsurface properties in high resolution and in a minimally invasive manner. Two recent books (1, 2) describe the state-of-the-art in this field of hydrogeophysics. Although hydrogeophysical challenges exist (e.g., ref 3), recent synthetic and field studies have illustrated how geophysically obtained hydrological property estimates have improved the prediction of contaminant transport in natural systems over predictions based only on wellbore-based characterization data sets (4, 5).

Several recent studies have also explored the effects of microbial processes on geophysical attributes (6, 7). For the most part, these studies have been performed at the laboratory scale to investigate time-lapse changes in a geophysical attribute corresponding to a particular biogeochemical transformation. Although the studies hint at the potential that these methods have for field-scale monitoring, only a few studies have attempted to use geophysical methods at the field scale to monitor processes that occur in association with in situ contaminant reme-

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Introduction

Chromium (Cr) contamination in soils, surface water, and groundwater is widespread at many sites throughout the world (e.g., 1–3). The most common Cr compounds in groundwater contain hexavalent chromium, Cr(VI), and trivalent chromium, Cr(III), which have quite different characteristics and properties. Cr(VI) is mobile and toxic in groundwater, and is present in alkaline and strongly oxidizing aqueous environments as chromate (CrO_4^{2-}, $Cr_2O_7^{2-}$) and bichromate ($HCrO_4^-$, H_2CrO_4, and $HCr_2O_7^-$) complexes. Cr(III) complexes are much less toxic (4), and form insoluble and stable precipitates under slightly acidic to neutral conditions in aquifers (5).

Commonly used chemical reductants for converting Cr(VI) to Cr(III), with subsequent immobilization of Cr(III), are ferrous ion, Fe^{2+} (6–10); soil organic carbon (11), including humic (12) and fulvic acids (13); and sulfides (14). At Hanford, the Pacific Northwest National Laboratory (PNNL) has

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Use of carbon stable isotopes to monitor biostimulation and electron donor fate in chromium-contaminated groundwater

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HIGHLIGHTS

- In situ microbial Cr(VI) reduction was stimulated via injection into groundwater of sodium lactate solution.
- Transformation and fate of organic electron donors were associated with Cr(VI) reduction.
- Carbon isotope ratios of metabolites were measured to estimate the efficiency of biostimulation.
- Carbon isotope ratios show the microbial metabolites derived from the injected electron donor.
- Carbon isotope ratios of PLFA demonstrates the transfer of carbon from 13C-labelled lactate to a portion of the microbial community.

And here is the some of these screenshot of the good papers which might be of interest.

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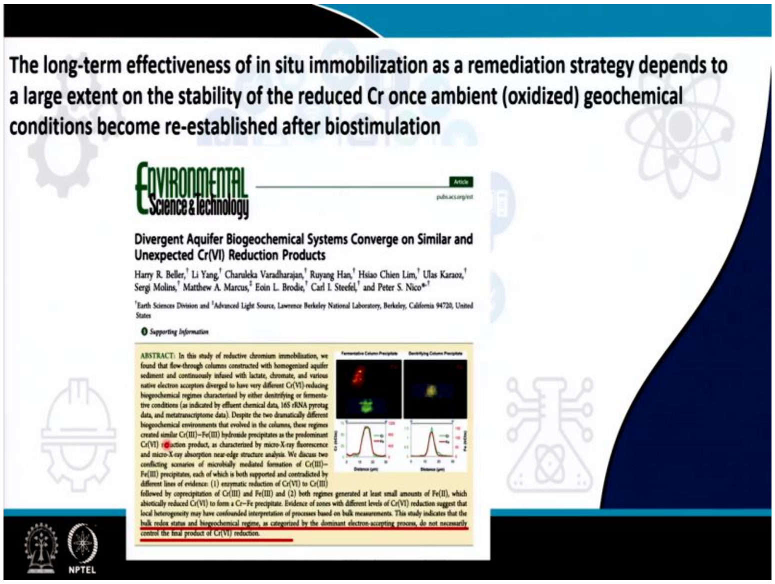

The long-term effectiveness of in situ immobilization as a remediation strategy depends to a large extent on the stability of the reduced Cr once ambient (oxidized) geochemical conditions become re-established after biostimulation

Environmental Science & Technology

Article

pubs.acs.org/est

Divergent Aquifer Biogeochemical Systems Converge on Similar and Unexpected Cr(VI) Reduction Products

Harry R. Beller,† Li Yang,† Charuleka Varadharajan,† Ruyang Han,† Hsiao Chien Lim,† Ulas Karaoz,† Sergi Molins,† Matthew A. Marcus,‡ Eoin L. Brodie,† Carl I. Steefel,† and Peter S. Nico*,†

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Supporting Information

ABSTRACT: In this study of reductive chromium immobilization, we found that flow-through columns constructed with homogenized aquifer sediment and continuously infused with lactate, chromate, and various native electron acceptors diverged to have very different Cr(VI)-reducing biogeochemical regimes characterized by either denitrifying or fermentative conditions (as indicated by effluent chemical data, 16S rRNA pyrotag data, and metatranscriptome data). Despite the two dramatically different biogeochemical environments that evolved in the columns, these regimes created similar Cr(III)–Fe(III) hydroxide precipitates as the predominant Cr(VI) reduction product, as characterized by micro-X-ray fluorescence and micro-X-ray absorption near-edge structure analysis. We discuss two conflicting scenarios of microbially mediated formation of Cr(III)–Fe(III) precipitates, each of which is both supported and contradicted by different lines of evidence: (1) enzymatic reduction of Cr(VI) to Cr(III) followed by coprecipitation of Cr(III) and Fe(III) and (2) both regimes generated at least small amounts of Fe(II), which abiotically reduced Cr(VI) to form a Cr–Fe precipitate. Evidence of zones with different levels of Cr(VI) reduction suggest that local heterogeneity may have confounded interpretation of processes based on bulk measurements. This study indicates that the bulk redox status and biogeochemical regime, as categorized by the dominant electron-accepting process, do not necessarily control the final product of Cr(VI) reduction.

And the subsequently the long term effectiveness of this in situ immobilization as a remediation strategy was studied because it was observed that it could be reversing back like oxidizing back. To the large external stability of the reduced chromium. So, stability of the reduced chromium was also studied and it was found that the bulk redox status of the biogeochemical regime as categorized by the dominant electron accepting process do not necessarily control the final product of chromium 6 reduction.

So, the overall study showed that there is a there is a very high degree of implementability of this concept and they successfully remediated this site.

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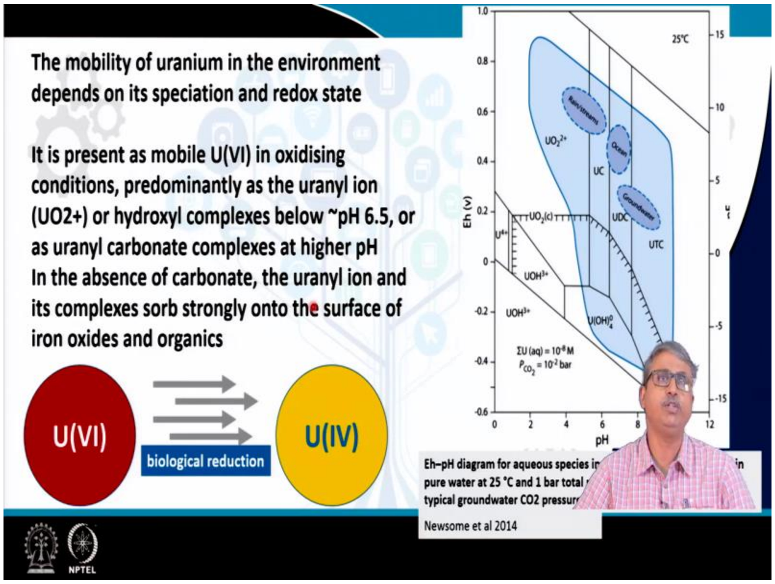

The mobility of uranium in the environment depends on its speciation and redox state

It is present as mobile U(VI) in oxidising conditions, predominantly as the uranyl ion (UO2+) or hydroxyl complexes below ~pH 6.5, or as uranyl carbonate complexes at higher pH
In the absence of carbonate, the uranyl ion and its complexes sorb strongly onto the surface of iron oxides and organics

Eh–pH diagram for aqueous species in … in pure water at 25 °C and 1 bar total … typical groundwater CO2 pressur…

Newsome et al 2014

Now next we will learn about the the uranium bioremediation through the case study. Now, uranium contamination is again linked to these nuclear activities which is considered to be a legacy of the environmental contamination and elevated concentration of uranium and along with uranium different other radionuclides present in the mining and milling area at the sites where uranium ores are processed and where uranium was enriched as a part of the nuclear processing nuclear industries including the nuclear warfare as well as the nuclear energy.

And this contamination potentially represents an uncontrolled source of radiation also and therefore regulatory bodies may require it to remediate the acceptable level. So, that was the understanding from USDOE site. So, if United States Department of Energy they took adequate initiatives to actually control the the problem with uranium and other toxic metal and radionuclide pollution.

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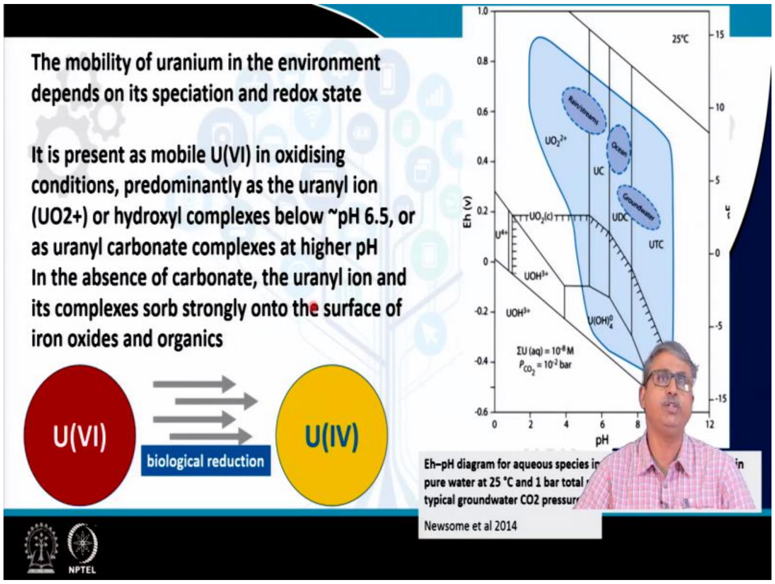

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Eh–pH diagram for aqueous species in … in
pure water at 25 °C and 1 bar total …
typical groundwater CO2 pressure…

Newsome et al 2014

Now, with respect to uranium again as we have seen with respect to chromium the mobility of the uranium in the environment depends on the speciation and the oxidation state. Again it has two very common oxidation states one is a uranium 6 and uranium 4 and in uranium 6 in oxidizing conditions predominantly is a form of urinal ion UO2+ or hydroxyl complexes as you can see. So, with the ph regime close to 6 or below that we can expect that it will be mostly right uranium 6+ UO2+ uranil ion we call it or it may form some urinal carbonate complex at higher pH.

So, as we move into the higher ph it forms the uranium carbonate complexes. So, essentially again the solubility and the toxicity of uranium is highly linked to this oxidation state. So, uranium 6 is highly soluble in aqueous medium is toxic whereas uranium four which can be produced out of reducing the uranium 6 is very less soluble and it forms complexes with the carbonates and other materials present in the aquifer solid phase.

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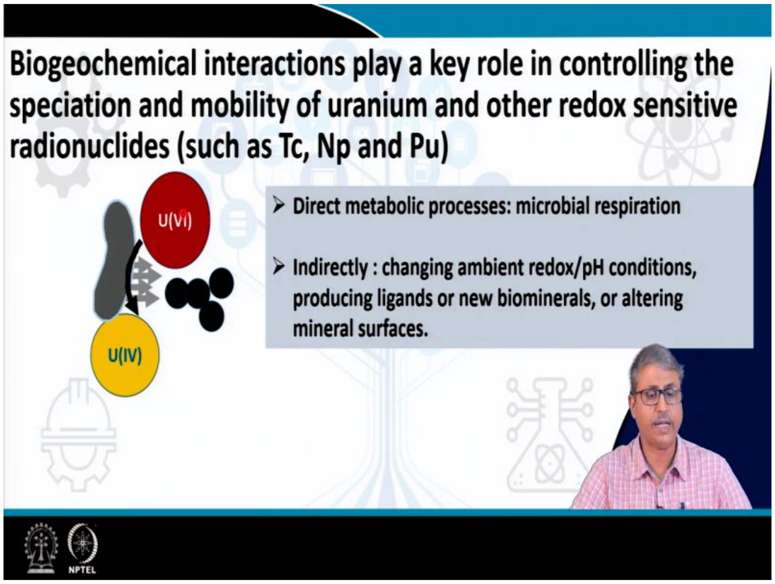

Biogeochemical interactions play a key role in controlling the speciation and mobility of uranium and other redox sensitive radionuclides (such as Tc, Np and Pu)

- Direct metabolic processes: microbial respiration
- Indirectly : changing ambient redox/pH conditions, producing ligands or new biominerals, or altering mineral surfaces.

Now, the biogeochemical interactions been found to be very important and playing a key role in controlling the speciation that is the inter conversion between the 6 and the 4 species oxidation state of uranium and that is actually linked to the mobility of the uranium and it is it is also true not for other toxic redox sensitive radionuclides like technetium neptunium and plutonium also. So, direct these are the two processes are found one is the direct metabolic process that is the microbial respiration.

So, in case of uranium there are increasing number of evidences that the microbes can actually reduce these uranium 6 directly that is called direct respiration. There is another way of reducing it that is called the indirect reduction that is the changing the ambient redox ph conditions producing ligands or new bio minerals or altering the mineral surfaces. So, in geologic geological context we can we can understand that when the microbes are able to produce these ligands or produce different bio minerals or alter the mineral surfaces the uranium 6 can precipitate heavily on those.

So, for example as I mentioned the uranium 6 can be reduced directly or microorganisms can produce certain ligands or certain groups. And these can actually bind to the uranium 6 and lead to the precipitation of the uranium in the solid phase.

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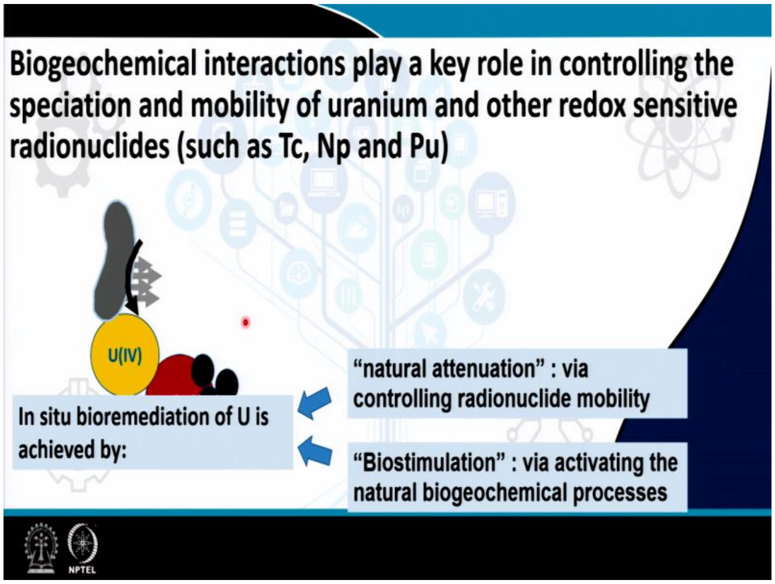

Biogeochemical interactions play a key role in controlling the speciation and mobility of uranium and other redox sensitive radionuclides (such as Tc, Np and Pu)

In situ bioremediation of U is achieved by:

"natural attenuation" : via controlling radionuclide mobility

"Biostimulation" : via activating the natural biogeochemical processes

So, therefore the in situ bioremediation of uranium within the contaminated groundwater sites can be achieved by two processes. One is the natural attenuation as I mentioned earlier via controlling the radionuclide mobility and this natural attenuation is absolutely relying on the abilities of the natural micro organisms microbial communities or else bio stimulation can also be done like via activating the natural bio geo chemical processes the geo chemical process which are going to actually transform the uranium 6 to uranium 4 or will produce the ligands or will bring such changes in the solid phase or produce other molecules which might interfere with the solubility and will make the uranium precipitated into the environment.

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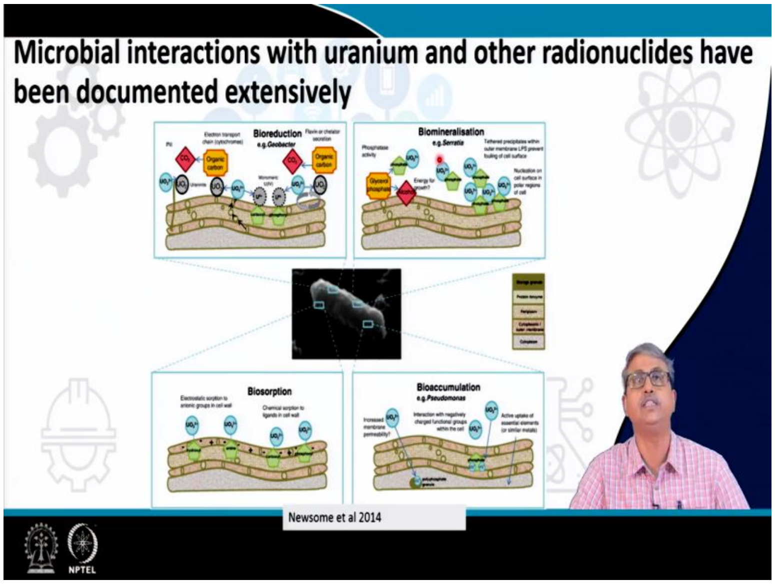

Microbial interactions with uranium and other radionuclides have been documented extensively

Newsome et al 2014

So, we have found that these are the four basic mechanisms with respect to microbial interactions with uranium. These are the uranium bioreduction is on the top of the list it can be reduced by diverse mechanisms and along with that we have the bios option or bio accumulation but also interestingly this process which is bio mineralization. We have already discussed these processes in detail in our earlier lecture on metal micro interaction.

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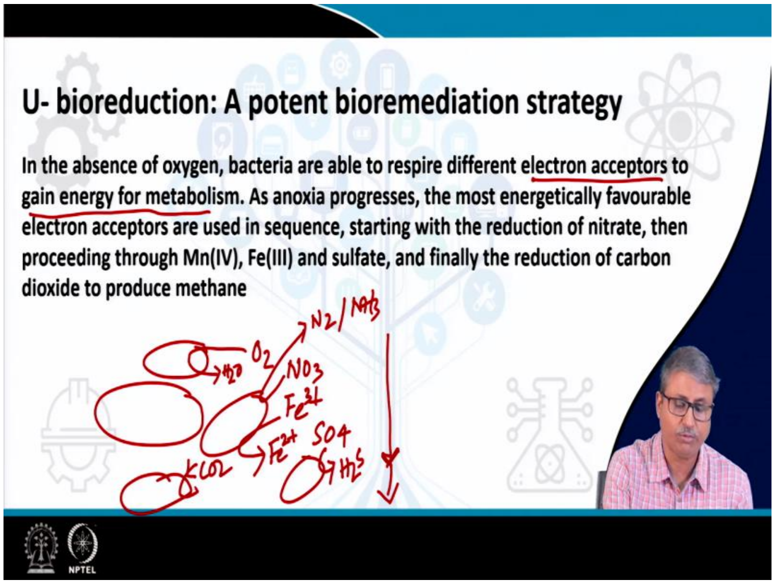

U- bioreduction: A potent bioremediation strategy

In the absence of oxygen, bacteria are able to respire different electron acceptors to gain energy for metabolism. As anoxia progresses, the most energetically favourable electron acceptors are used in sequence, starting with the reduction of nitrate, then proceeding through Mn(IV), Fe(III) and sulfate, and finally the reduction of carbon dioxide to produce methane

With respect to uranium bioreduction if it has been found that this is this could be a potent bioremediation strategy. Now, what happens in the absence of oxygen under the aquifer condition. So, bacteria are able to respire with different other electron acceptors to gain energy for metabolism because they need to utilize different electron acceptor. So, that the energy is gained because these electrons are eventually passed to the terminal electron acceptor and the reducing power like NADH+ or FADH 2 is reoxidized.

So, electron transport chain facilitates this process. Now, as the anoxia progress the most energetically favourable electron acceptor are used in a sequence and that follows the redox chronology of redox reaction. So, if we consider that in aquifer environment we have some amount of oxygen of course some nitrate will be there iron 3 and sulfate will be there. These are the common electron acceptors present in the aquifer environment.

So, microorganisms who are use I am drawing one cell but actually these are the job of multiple different cells different species are active because all organisms may not be active under the anaerobic condition. So I am just drawing one cell here to show that these could be aerobic bacteria and utilizing oxygen under the conditions where oxygen is available. But as we go down and as we have some amount of organic materials the oxygen is getting depleted.

So, either you go down you see there are diffusional barriers and other restrictions. So, the oxygen level also declines. So, as the oxygen level declines the other electron acceptors like nitrate. So, nitrate will be acting as electron acceptor producing back nitrogen or ammonia. So, there are different mechanisms. Similarly Fe 3 could be utilize an electron acceptor and if it will be produced and at strongly reducing condition or strongly negative redox potential condition sulfate will be reduced to produce sulfide or H 2 S.

And in further down eventually we will see that carbon dioxide which might be present due to the activities of these all microbes because they are they are ultimately catalyzing different organic or oxidizing different carbon sources also and the CO 2 are produced. So, these CO 2 molecules will be utilized as electron acceptor and eventually methane will be produced. So, this will be the chronology of electron acceptors which are to be produced.

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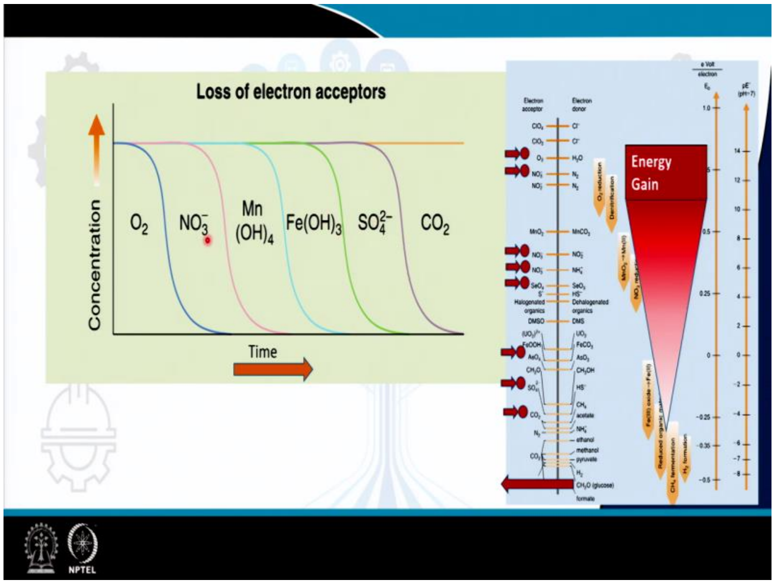

Loss of electron acceptors

So, that will actually follow the diagram that we discussed earlier and we can see that and depending upon this electron acceptors used the energy gain will be different like if they are using oxygen the highest amount of energy will be gained compared to nitrate and then the the manganese or iron reducing bacteria and then sulphate reducing bacteria and finally carbon dioxide reducing bacteria archaea rather.

Now, this could be very easily observed in the aquifer environment if we continuously monitor the dissolve oxygen level followed by the nitrate Mn, iron, sulphate and carbon dioxide or methane will be able to see that they are following a chronology like first the nitrate under anoxic condition then the Mn then the Fe then the sulphate and then the CO 2 because they are guided by the amount of energy which is produced out of this reducing process because unnecessarily they are not going to reduce sulfate if nitrate is available because reducing nitrate is going to give them more energy.

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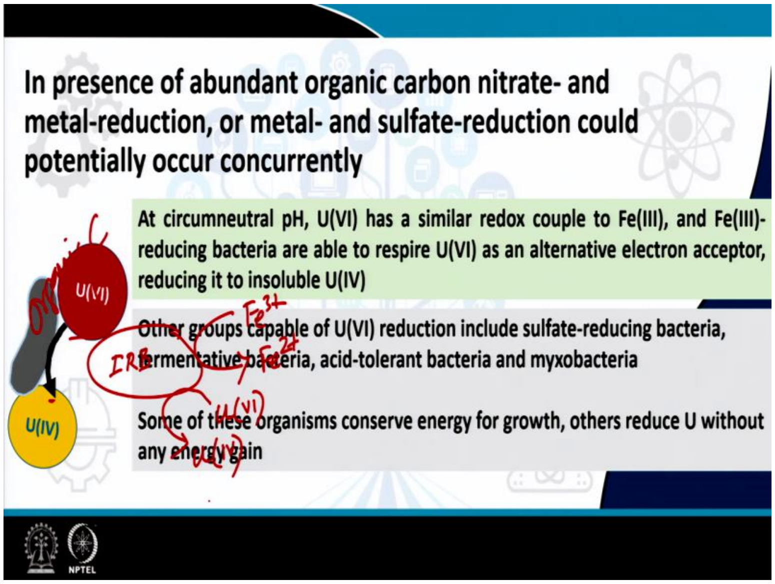

In presence of abundant organic carbon nitrate- and metal-reduction, or metal- and sulfate-reduction could potentially occur concurrently

At circumneutral pH, U(VI) has a similar redox couple to Fe(III), and Fe(III)-reducing bacteria are able to respire U(VI) as an alternative electron acceptor, reducing it to insoluble U(IV)

Other groups capable of U(VI) reduction include sulfate-reducing bacteria, fermentative bacteria, acid-tolerant bacteria and myxobacteria

Some of these organisms conserve energy for growth, others reduce U without any energy gain

Now if there is abundant organic carbon in the or in the aquifer environment which are generally found to be oligotrophic. But if we have some arrangement that excess organic carbon is provided then we may find out that nitrate and metal reduction or metal and sulphate reduction could potentially occur concurrently. So, the chronology will not may not be followed. So, the chronology that we have seen may not be followed.

So, that means under this circumstances we can see that nitrate along with Mn are actually getting reduced simultaneously whereas the iron and sulphate can also be reduced simultaneously. So, this is the beauty of the process. Now, particularly at pH close to neutrality uranium 6 has a similar redox couple to Fe 3 and Fe 3 reducing bacteria are able to respire uranium 6 as an alternative electron acceptor reducing it to insoluble uranium 4.

So, if we go back here will be able to see that we have this redox potential data and with the the the pE values and also the redox couples are shown you can clearly see that the uranium and iron they are redox couples are quite similar. So, the point is emerging over here that if you have sufficient organic carbon for example acetate or lactate something like that. So, if you have sufficient organic carbon.

So, what is the job of organic carbon here that we will see. So, the iron will be reduced. So, Fe 3 will be reduced to Fe 2+ many of these bacteria which we called iron reducing bacteria. So, commonly they are called as IRB iron reducing bacteria they will also reduce uranium. So, uranium 6 will be converted to uranium 4. So this reaction will happen and this is true also for many of the sulfate reducing bacteria also.

So, will be able to see that it is not only that this IRB or Iron Reducing Bacteria they are capable of reducing uranium when particularly we have enough supply of organic carbon but also the sulphate reducing bacteria they are also and some of the fermentative organism they will be also able to join hands and will be reducing uranium to uranium 4 which is going to be readily precipitated.

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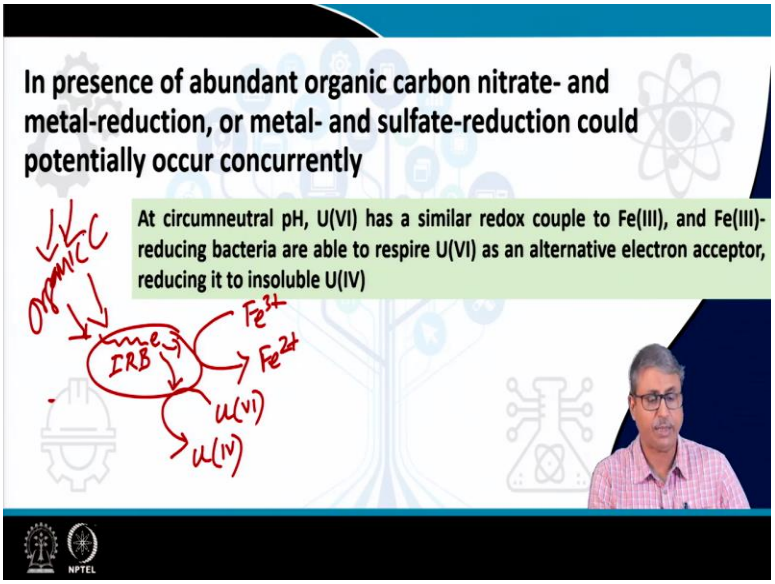

In presence of abundant organic carbon nitrate- and metal-reduction, or metal- and sulfate-reduction could potentially occur concurrently

At circumneutral pH, U(VI) has a similar redox couple to Fe(III), and Fe(III)-reducing bacteria are able to respire U(VI) as an alternative electron acceptor, reducing it to insoluble U(IV)

Now, uranium bio reduction has been proposed as a bio remediation technique stimulated by adding an electron donor to promote the enzymatic reduction of aqueous uranium 6 to uranium 4. Now, in the previous drawing if we look carefully jack what is the function of this organic carbon. So, basically the organic carbon is getting oxidized and it is providing the electrons those electrons which are basically supplied to the electron transport chain via the the electron carriers like NADH+ or Fe DH 2.

So, the more number of more amount of organic carbons which are readily metabolizable and metabolized by this iron reducing bacteria or even the SRBs and some of the fermentative bacteria they are capable of reducing your uranium no matter whether they are SRBs or they are IRBs.

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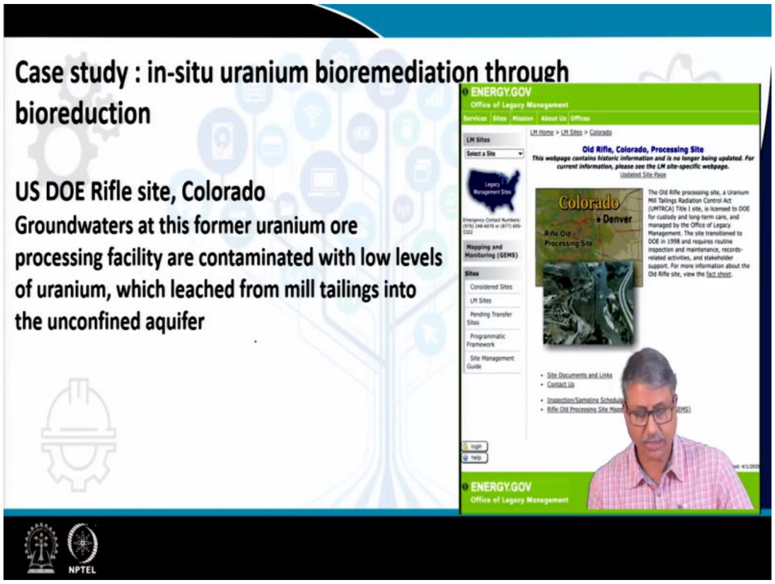

Case study : in-situ uranium bioremediation through bioreduction

US DOE Rifle site, Colorado

Groundwaters at this former uranium ore processing facility are contaminated with low levels of uranium, which leached from mill tailings into the unconfined aquifer

Old Rifle, Colorado, Processing Site

This webpage contains historic information and is no longer being updated. For current information, please see the LM site-specific webpage.

The Old Rifle processing site, a Uranium Mill Tailings Radiation Control Act (UMTRCA) Title I site, is licensed to DOE for custody and long-term care, and managed by the Office of Legacy Management. The site transitioned to DOE in 1998 and requires routine inspection and maintenance, records-related activities, and stakeholder support. For more information about the Old Rifle site, view the fact sheet.

Emergency Contact Numbers: (970) 248-6070 or (877) 695-5322

So, a number of strategies is actually established through different cytochromes different nanowire even extracellular released flamin compounds are found that they facilitate the transfer of electrons to uranium. Now, the case study that we are going to briefly say see here is about the US DOE Rifle site, Colorado and the groundwater as this former uranium ore processing facility.

So, this is basically this was uranium ore processing facility and in this facility we see that low level of uranium is there and which is leach from mill tailings into the unconfined aquifer. So, here you can see again. So, under this facility in the mill tailings where the mill tailings were dumped the the low concentration of uranium leached into the aquifer environment.

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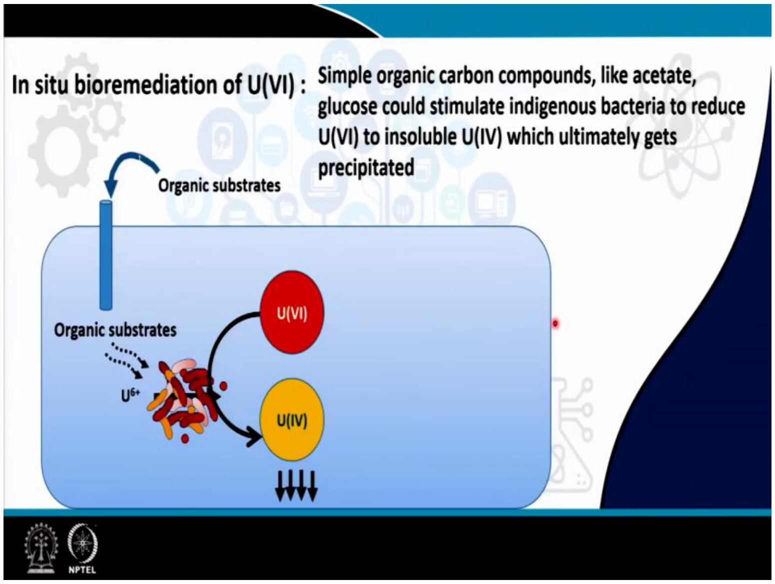

In situ bioremediation of U(VI) : Simple organic carbon compounds, like acetate, glucose could stimulate indigenous bacteria to reduce U(VI) to insoluble U(IV) which ultimately gets precipitated

Now, here also the similar type of strategy was adopted that the organic substances like the the acetate for example along with acidity large good range of compounds like methanol ethanol glucose lactate etcetera tested in order to find their efficacy. And the basic logic was that the to activate this iron and sulphate reducing bacteria who can actually reduce the uranium 6. Parallel studies microbial ecology and microbial diversity studies were conducted in order to identify these organisms that what are these organisms present.

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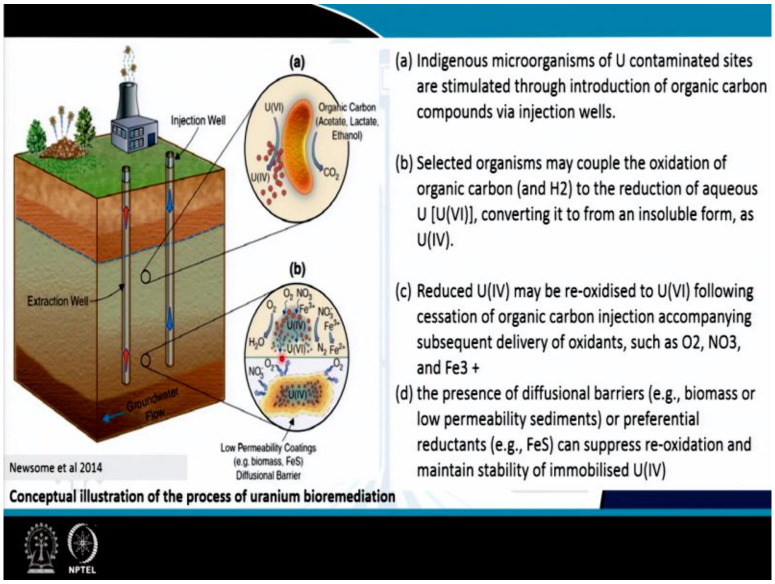

Newsome et al 2014

Conceptual illustration of the process of uranium bioremediation

(a) Indigenous microorganisms of U contaminated sites are stimulated through introduction of organic carbon compounds via injection wells.

(b) Selected organisms may couple the oxidation of organic carbon (and H2) to the reduction of aqueous U [U(VI)], converting it to from an insoluble form, as U(IV).

(c) Reduced U(IV) may be re-oxidised to U(VI) following cessation of organic carbon injection accompanying subsequent delivery of oxidants, such as O2, NO3, and Fe3 +

(d) the presence of diffusional barriers (e.g., biomass or low permeability sediments) or preferential reductants (e.g., FeS) can suppress re-oxidation and maintain stability of immobilised U(IV)

So, it was found that subsequent to this process. So, there could be actually events going on like this that the once you actually inject this your acetate for example through the injection well the indigenous microorganism of the contaminated sites are stimulated and using this organic carbon. And selected organism may couple the oxidation of organic carbon to reduction of uranium because this could be the IRBs and SRBs which are then URB, Uranium Reducing Bacteria.

And the reduced uranium which is uranium 4 may be reoxidized to uranium 6 following cessation of the organic carbon. So, there may be always a chance that uranium 4 can be oxidized back to uranium 6. So, in order to look into that aspect the scientists have two options one is the presence of diffusional barrier like a biomass or low permeability sediments or a preferential reductant like iron sulphide complex.

So, they have found that if we have this iron sulphide complex. So, this iron sulphide complex actually allow the stability of this uranium 4 and maintain the immobilized uranium 4 within the aquifer solid phase.

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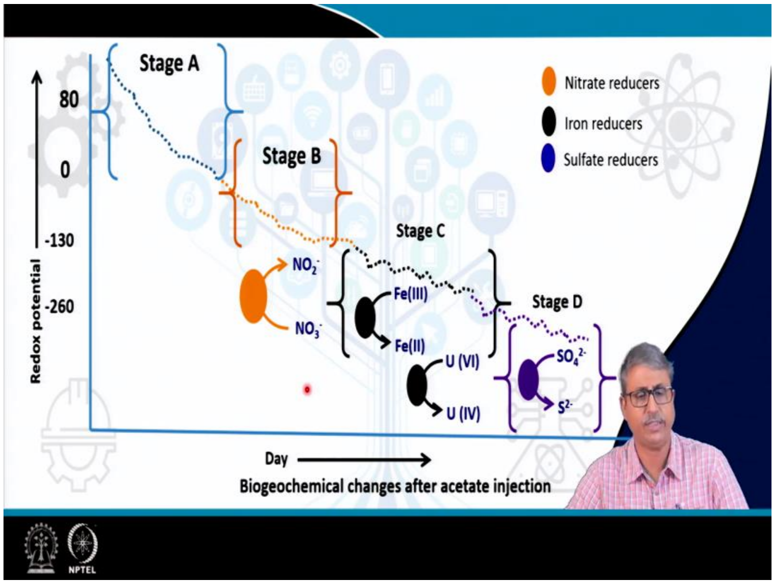

And the sequence of events were also identified that in a stage A stage B stage C stage D how nitrate reducing iron reducing uranium reducing and sulfate reducing organisms a self reducing bacteria are proliferating into the system.

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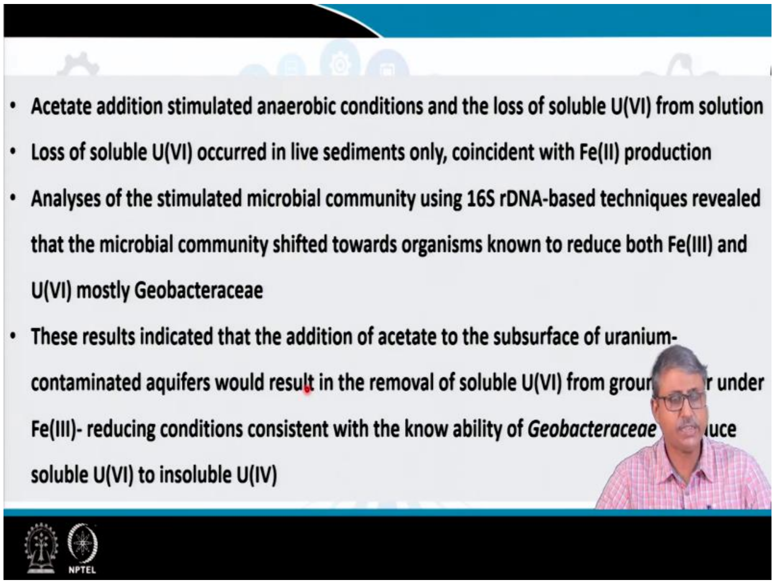

- Acetate addition stimulated anaerobic conditions and the loss of soluble U(VI) from solution
- Loss of soluble U(VI) occurred in live sediments only, coincident with Fe(II) production
- Analyses of the stimulated microbial community using 16S rDNA-based techniques revealed that the microbial community shifted towards organisms known to reduce both Fe(III) and U(VI) mostly Geobacteraceae
- These results indicated that the addition of acetate to the subsurface of uranium-contaminated aquifers would result in the removal of soluble U(VI) from grou r under Fe(III)- reducing conditions consistent with the know ability of *Geobacteraceae* uce soluble U(VI) to insoluble U(IV)

So, essentially this acetate utilization allowing along with that the uranium reduction to uranium 4 was observed and what was very prominent that the growth of or the enrichment natural enrichment of the geo bacteria c member.

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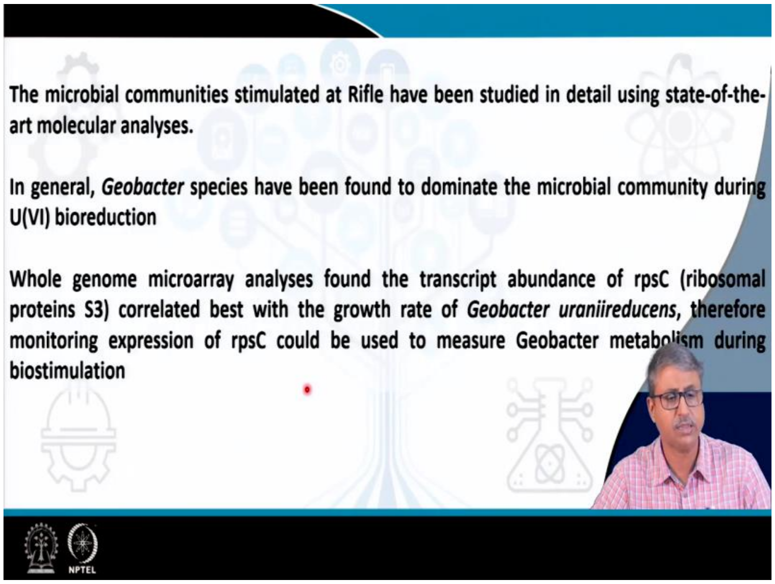

The microbial communities stimulated at Rifle have been studied in detail using state-of-the-art molecular analyses.

In general, *Geobacter* species have been found to dominate the microbial community during U(VI) bioreduction

Whole genome microarray analyses found the transcript abundance of rpsC (ribosomal proteins S3) correlated best with the growth rate of *Geobacter uraniireducens*, therefore monitoring expression of rpsC could be used to measure Geobacter metabolism during biostimulation

And in subsequent studies the microbial communities were monitored with all kind of tools including the culture independent transcriptomics meta transcriptomics and genome sequencing all these all these things were implemented. It was observed that in general geo vector species have been found to dominate the microbial community during this iron reduction bioreduction.

And these many of these members they actually later identified as geobacter uranium reduces were found to have mechanism specific mechanisms through which they are able to reduce uranium. Now, whole genome microarray analysis found that the transcript abundance of rpsC that is a particular ribosomal protein correlated base to the growth rate of geo vector uranium reduces therefore monitoring expression of this could be a kind of a bio marker for monitoring the activities of these organisms.

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APPLIED AND ENVIRONMENTAL MICROBIOLOGY, Oct. 2003, p. 5884–5891 Vol. 69, No. 10
0099-2240/03/$08.00+0 DOI: 10.1128/AEM.69.10.5884–5891.2003
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Stimulating the In Situ Activity of *Geobacter* Species To Remove Uranium from the Groundwater of a Uranium-Contaminated Aquifer

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Received 16 April 2003/Accepted 16 July 2003

The potential for removing uranium from contaminated groundwater by stimulating the in situ activity of dissimilatory metal-reducing microorganisms was evaluated in a uranium-contaminated aquifer located in Rifle, Colo. Acetate (1 to 3 mM) was injected into the subsurface over a 3-month period via an injection gallery composed of 20 injection wells, which was installed upgradient from a series of 15 monitoring wells. U(VI)

APPLIED AND ENVIRONMENTAL MICROBIOLOGY, June 2008, p. 3718–3729 Vol. 74, No. 12
0099-2240/08/$08.00+0 doi:10.1128/AEM.02308-07
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Microbial Communities in Contaminated Sediments, Associated with Bioremediation of Uranium to Submicromolar Levels

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Microbial enumeration, 16S rRNA gene clone libraries, and chemical analysis were used to evaluate the in situ biological reduction and immobilization of uranium(VI) in a long-term experiment (more than 2 years) conducted at a highly uranium-contaminated site (up to 60 mg/liter and 800 mg/kg solids) of the U.S.

Environmental Microbiology (2009) 11(10), 2611–2626 doi:10.1111/j.1462-2920.2009.01986.x

GeoChip-based analysis of functional microbial communities during the reoxidation of a bioreduced uranium-contaminated aquifer

Joy D. Van Nostrand,[1,2] Wei-Min Wu,[3] Liyou Wu,[1] Ye Deng,[1] Jack Carley,[4] Sue Carroll,[4] Zhili He,[1,2] Baohua Gu,[4] Jian Luo,[5] Craig S. Criddle,[3] David B. Watson,[4] Philip M. Jardine,[4] Terence L. Marsh,[6] James M. Tiedje,[6] Terry C. Hazen[2,7] and Jizhong Zhou[1,2*]

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ethanol, showing a substantial change in community structure with increased gene number and diversity after ethanol injections resumed. While gene numbers showed considerable variations, the relative abundance (i.e. percentage of each gene category) of most gene groups changed little. During the reoxidation period, U(VI) increased, suggesting reoxidation of reduced U(IV). However, when introduction of DO was stopped, U(VI) reduction resumed and returned to pre-

APPLIED AND ENVIRONMENTAL MICROBIOLOGY, Sept. 2006, p. 6288–6298 Vol. 72, No. 9
0099-2240/06/$08.00+0 doi:10.1128/AEM.00246-06
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Application of a High-Density Oligonucleotide Microarray Approach To Study Bacterial Population Dynamics during Uranium Reduction and Reoxidation†

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Gary L. Andersen,[2] Terry C. Hazen,[2] Paul M. Richardson,[3] Donald J.
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Received 1 February 2006/Accepted 21 June 2006

**Reduction of soluble uranium U(VI) to less-soluble uranium U(IV) is a pr
migration from contaminated aquifers. It is generally assumed that, under co
is stable and immobile; however, in a previous study, we documented reo
reducing conditions (Wan et al., Environ. Sci. Technol. 2005, 39:6162**

So, here are the screenshots of some of these papers which are related to the the case studies of uranium bioremediation. And it is not only for the Rifle site multiple US DOE sites have been subjected to underground in situ by remediation using similar concept.

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- In situ long-term reductive bioimmobilization of Cr (VI) in groundwater using hydrogen release compound by Faybishenko et al., . *Environmental Science & Technology*, *42*(22), 8478-8485 (2008).
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- Physiological and transcriptional studies of Cr (VI) reduction under aerobic and denitrifying conditions by an -derived pseudomonad by Han et al., *Environmental science & technology*, *44*(19), 7491-7497 (2010)
- https://cumulis.epa.gov/supercpad/SiteProfiles/index.cfm?fuseaction=second.cleanup&id=1001114, acc

So, these are the references for general in general this first review paper seems to be very useful along with other papers related to the chromium bioreduction and bioremediation case studies.

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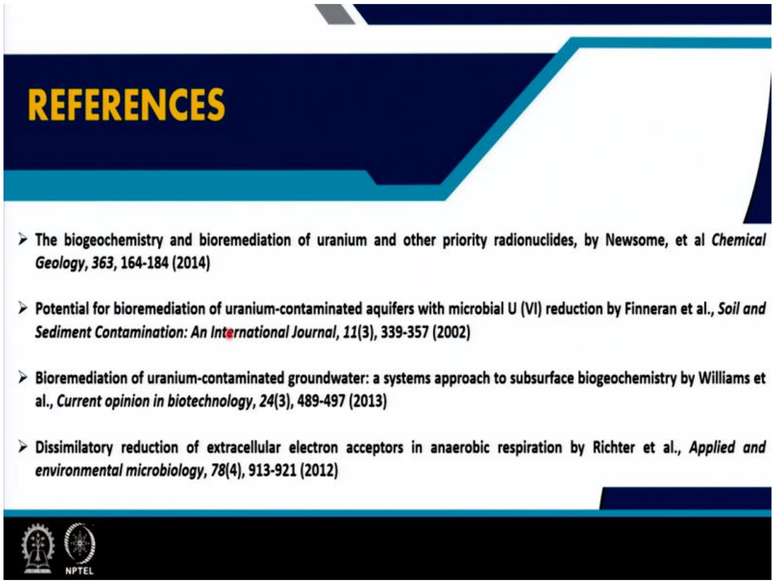

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- Bioremediation of uranium-contaminated groundwater: a systems approach to subsurface biogeochemistry by Williams et al., *Current opinion in biotechnology, 24*(3), 489-497 (2013)
- Dissimilatory reduction of extracellular electron acceptors in anaerobic respiration by Richter et al., *Applied and environmental microbiology, 78*(4), 913-921 (2012)

And also the papers related to uranium bioremediation case studies.

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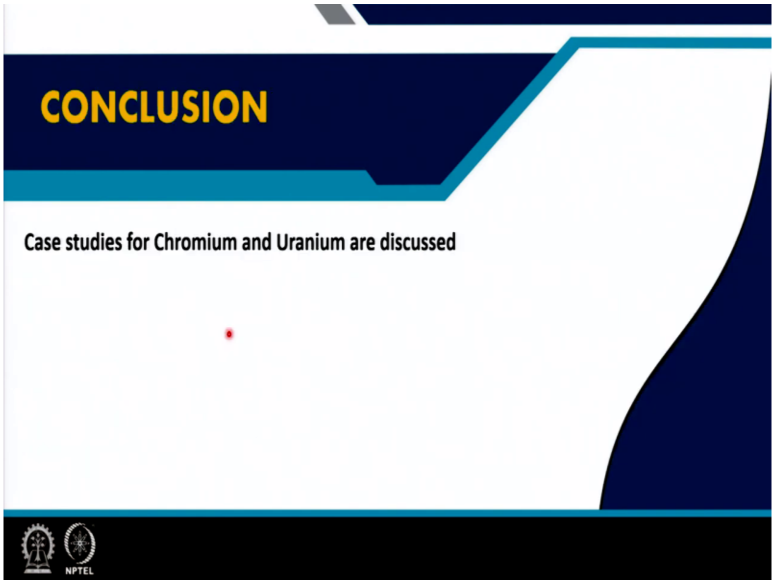

CONCLUSION

Case studies for Chromium and Uranium are discussed

And in conclusion the two case studies related to chromium and uranium remediation by remediation are discussed in detail. In particular we see that how the injection biostimulation actually through to injection of these carbon sources the relevant microorganisms are enhanced or enriched naturally so that they could perform the desirable function. So, thank you very much.