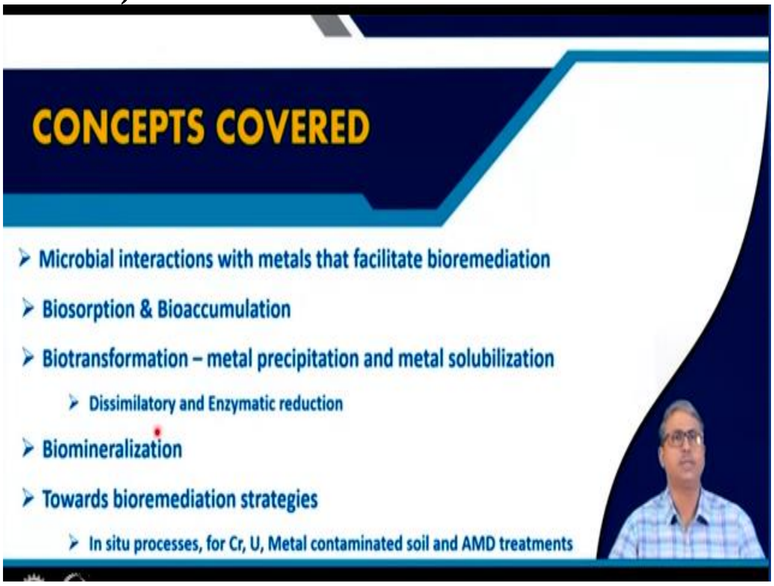
Environmental Biotechnology
Prof. Pinaki Sar
Department of Biotechnology
Indian Institute of Technology - Kharagpur

Lecture - 43
Microbial Interactions with Heavy Metals and Metalloids - Bioremediation

Welcome to the next lecture of our course on environmental biotechnology and in today's lecture, we will be discussing on the microbial interactions with heavy metals and metalloids particularly with reference to bioremediation.

(Refer Slide Time: 00:46)

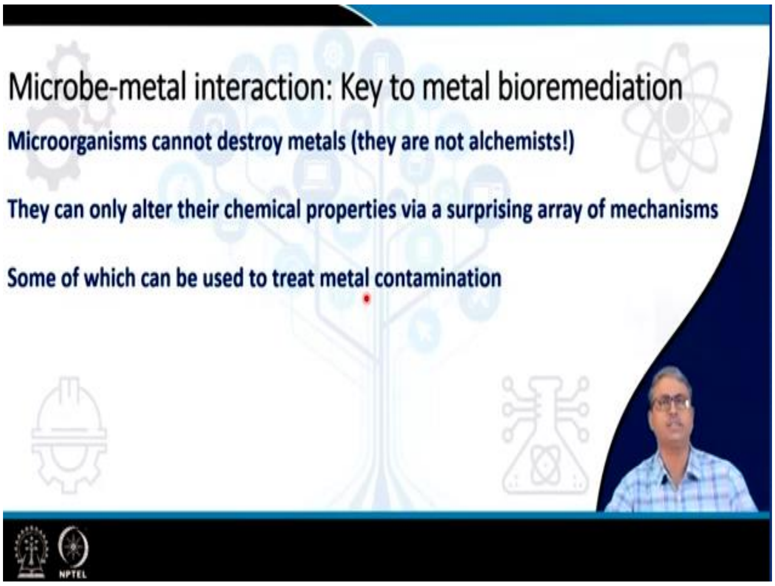


CONCEPTS COVERED

- Microbial interactions with metals that facilitate bioremediation
- Biosorption & Bioaccumulation
- Biotransformation – metal precipitation and metal solubilization
  - Dissimilatory and Enzymatic reduction
- Biomineralization
- Towards bioremediation strategies
  - In situ processes, for Cr, U, Metal contaminated soil and AMD treatments

Now, in this particular class the following concepts will be discussed, we will discuss about microbial interaction with metals that facilitate bioremediation, we will learn about some of the important aspects of biosorption and bioaccumulation. Biotransformation that facilitates metal precipitation and metal solubilization both by dissimilatory as well as enzymatic reduction processes will be discussed. This will be followed by biomineralization and some points about bioremediation strategies.

(Refer Slide Time: 01:23)



Microbe-metal interaction: Key to metal bioremediation

Microorganisms cannot destroy metals (they are not alchemists!)

They can only alter their chemical properties via a surprising array of mechanisms

Some of which can be used to treat metal contamination

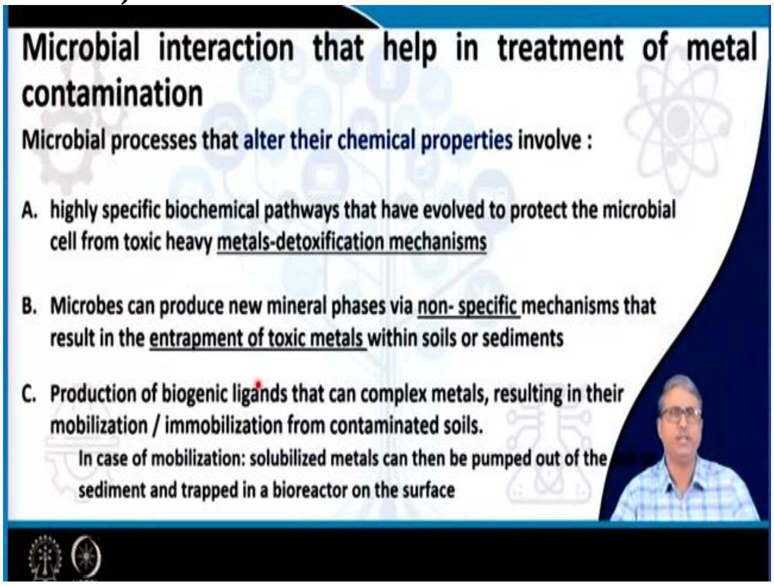
Now microbe metal interaction is considered to be the key to the metal bioremediation process. Now, with respect to bioremediation of heavy metals, including the metalloids and also the radionuclides, microorganisms cannot destroy the metals, they are not alchemists. So, this is a very fundamental point that we all must understand. And this understanding helps us to actually realize that then how microorganisms could be useful in developing bioremediation strategies.

Because metals will remain metals even after the microbes they interact with themselves. So, there must be some kind of very intelligent way of looking into this interaction where our interactions because microbes interact with a number of processes. These processes through which microbes interact with different heavy metals, metalloids and radionuclides it is truly very diverse.

And this diversity of interactions in many cases, what do you see that can only alter the chemical properties, sometimes making them more available to the rest of the ecosystem components, sometimes allow them to precipitate, sometimes allow them to be more solubilized or sometimes allow these metals to be sequestered or absorbed or accumulated within some microbial cells like bacteria or fungal cells. Now some of these processes of interactions can be used to treat the metal contamination.

Now when you say that treat metal contamination, so this could be practically all type of metal contaminations whether it is only the metals present they are or metals present along with hydrocarbons and other pollutants. So, over the periods of time like we see that from the last 30 40 years our understanding on these micro metal interaction has improved to such extent that now we are in a position to better comprehend the entire process of this microbe metal interaction and then developed strategies in which these microbial interactions with metals can be utilized for the bioremediation process development.

(Refer Slide Time: 04:04)



Microbial interaction that help in treatment of metal contamination

Microbial processes that alter their chemical properties involve :

A. highly specific biochemical pathways that have evolved to protect the microbial cell from toxic heavy metals-detoxification mechanisms

B. Microbes can produce new mineral phases via non- specific mechanisms that result in the entrapment of toxic metals within soils or sediments

C. Production of biogenic ligands that can complex metals, resulting in their mobilization / immobilization from contaminated soils.

In case of mobilization: solubilized metals can then be pumped out of the sediment and trapped in a bioreactor on the surface

Now, microbial interaction that helps in treatment of metal contamination could be of 3 basic types. Now microbial processes that eventually alter the chemical properties now, we say chemical properties that mean it also affect the environmental behaviour on environmental fat, environmental toxicity, bioavailability of the heavy metals. So, these are wide ranging processes. So, these are multiple processes, which are involved in altering the fat and chemical properties of the heavy metals.

So, the first and foremost is the highly specific biochemical pathways, which are present in microorganisms that have evolved to protect the microbial cell from toxic heavy metals. Since microorganisms are leaving with these metals from the origin of the life or origin of their systems. So, they know they have learned how to deal with these metals. So, many of these metals like chromium, uranium, cadmium, mercury most of these heavy metals arsenic.

They are toxic either at low concentration or at moderate concentration or even at high concentrations. So, no matter what is the type of metal? Most of the metals are toxic or even all metals are toxic beyond certain threshold limits that we all know even the trace metals which are essential considered to be essential for microbial metabolisms. They are also toxic beyond certain threshold level and also the metals which are not absolutely required by living cell like for example, mercury, arsenic and cadmium.

For example, they are toxic to even at a lower concentration now, for all different types of metals microbes have developed different detoxification mechanisms. These detoxification mechanisms are one of the targets during the bioremediation process development, for example the chromium detoxification, arsenic detoxification, mercury detoxification or even the cadmium or cobalt or copper detoxification system, these are naturally present in microbial cells microbial system.

So, what we are doing? We are learning these detoxification mechanisms and trying to improve it make it more specific and then trying to see that how this detoxification based metal or transformation or metal detoxification processes can be developed, so that they will be suitable for metal bioremediation system. The second one is the fact that microbes can produce new mineral phases by via nonspecific mechanisms that result in the entrapment of the toxic metals within the soil or the sediment.

So, these are mostly nonspecific interaction nonspecific in the sense that they do not discriminate between the exact name or the type of the metals, these interactions are mostly targeted towards the broad categories of metals like divalent cationic, for example so many of the divalent cationic are positively charged. Break down pieces are subject to a specific type of interactions and these interactions with the microbes eventually lead to the entrapment of the toxic metal in some component of the environment.

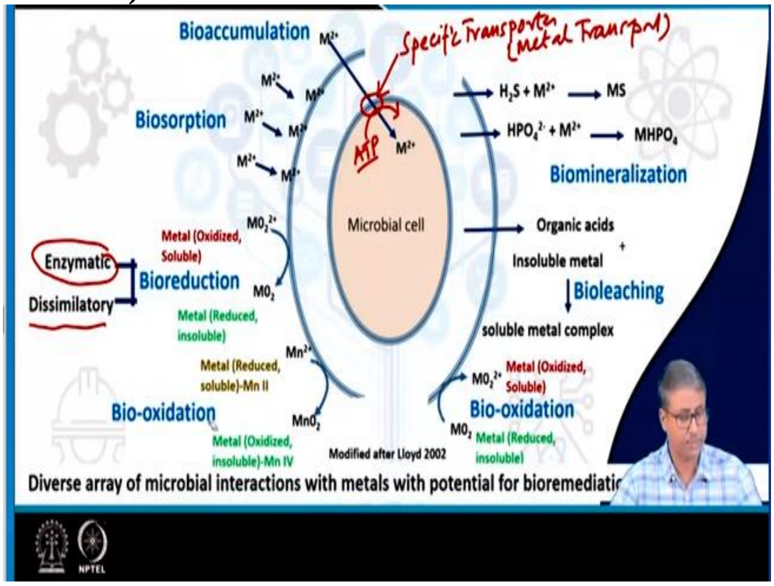
And if it is within the soil or sediment or the mineral phases, then it is good for the bioremediation purpose. The third one is the production of biogenic ligands which are chemical moieties or chemical groups that can complex the toxic metals resulting in their mobilization or sometime immobilisation from the contaminated soil. So, this is the kind of these biogenic ligands like phosphate, carbonate, hydroxyl or sulfide or these ligands which are produced by microbial metabolism.

They interact with the metals which are present in the environment and then alter the nature of the metal, sometimes they make the metal more mobile, more soluble, sometimes they make the metal insoluble if they are insoluble products are insoluble, then the insoluble metals are precipitated and that becomes a direct benefit out of the interaction because precipitation leads to their local immobilisation of the metal whereas the mobilization of the metal.

Sometimes the organic ligands particularly when they are attached to the metals, the metals become more soluble. So, these soluble metals are found to be very useful because these soluble metals can be pumped out of the sediment or leached out of the sediment and then they can be trapped in a bioreactor on the surface and then can be re used in certain other industrial process. So, both these mobilization and immobilisation processes which are facilitated by different biogenic ligands are found to be useful.

So in a nutshell, what do you see that there are 3 distinct there may be many processes that I will discuss, there will be many processes through which the microbes interact with the heavy metals, and metalloids and radionuclides and those processes can be suitable for metal, bioremediation, but all those different processes can be categorized into 3 broad categories. One is the detoxification system based, another is the entrapment based and the third one is the mobilization or immobilisation based through biogenic ligands.

(Refer Slide Time: 09: 50)



Modified after Lloyd 2002

Diverse array of microbial interactions with metals with potential for bioremediatic

Now if we see these particular schematics so here are the diverse array of microbial interactions with metals with potential for bioremediation are discussed. So, this is the microbial cell and I have just enlarged the cell in these 2 other live boundaries in order to accommodate different type of interaction. So, first is as we discussed in our earlier lecture that the divalent metal ions or maybe other metal ions also like arsenate or chromate can also be transported inside the cell.

So, the microbial cells, they are able to accumulate the metals inside the cell so toxic metals can be accumulated inside the cell so this is one way of interaction. Now, since the metal is toxic or many of these metals including the chromate or hexavalent, chromium diplomate or arsenic were arsenic 5 plus all these are toxic even the cadmium, cobalt, zinc could also be cadmium is anyway toxic but the cobalt, zinc, nickel etcetera which are nontoxic at low concentration but could be toxic or are indeed toxic at higher concentration.

So, microbes will be utilizing different detoxification strategies obviously, but before deploying the strategies detoxifying detoxification strategies, the microbes will accumulate metal. So, it is not that all microbes will accumulate all metals of course, but what we see that many microbes are capable of accumulating the metals particularly up to certain concentration levels, the divergent catalysts and even arsenate and chromate they are also accumulated inside the cell this is called bioaccumulation based process.

Which is interestingly driven by the metabolism bioaccumulation is of course, driven by metabolism because, when we look at the bioaccumulation process, we will be able to see that they are involvement of specific transporters. So, these are the specific transporter, so this is for example, a specific transporter which is called metal transport for example and metal transporter often the required supply of energy like ATP because these process are energy driven process.

So, these processes are most of the time we found that these are the characteristics of the metabolically active cells, why metabolically active cells? Because metabolically active cells; will only have sufficient energy for example, in the form of ATP. Now, the second one is the biosorption which is basically the adsorption and adsorption of divalent cationic and on the cell surface mostly and this is a metabolism independent process.

So, low energy is involved in this, so this is almost executed for all the divalent cationic and almost all microbial cells, gram negative, gram positive fungal cells, algal cells all cells they show these properties because of the an ionic nature of the cell membrane and cell envelop widely divalent cationic they found it is a nice place to bind on those the an ionic or negatively charged groups.

The next process is an enzymatic or otherwise, the transformation process which is called a bioreduction process where the soluble metals. Soluble metals could be different types of soluble metals are there for example, uranium the soluble metal which is in oxidized form can be reduced further and following reduction the insoluble species will be produced. So, following reduction the insoluble or species will be the produced and then the insoluble of form will precipitate.

So, this insoluble form will be precipitated at this is catalysed by a specific enzymes, so these enzymes may be very specific to the metal or concerned or they may be coupled with the dissimilatory process, dissimilatory I will discuss these things in little more detail. So, these processes are catalysed in one case by specific enzymes like we will discuss that for example mercury or for arsenic, we can see because these arsenic and mercury are absolutely not required by the cells so but they are toxic.

So, arsenic and mercury type of heavy metals or metalloids can be reduced by bacteria using specific reductases, so these are considered as enzymatic reduction whereas, for example the chromium, uranium, technetium and other several other radionuclides and metals, they are often used as electron acceptor during the anaerobic growth, anaerobic metabolism of the cells. and during this anaerobic reduction of the chromium or uranium or technetium or similar other heavy metals.

The microbes are not interested in the reduced product that is why it is called dissimilatory, dissimilatory in the sense that the reduced products are not utilized by the microbes. Otherwise, if the reduced product is used by the microbes, then it will be considered as dissimilatory process. So, the bioreduction could be enzymatic and dissimilatory and the reduced product is insoluble. So, basically this will precipitate that this will not remain in the solution phase at all.

So, the next process is a very interesting process which is called biomineralization. During biomineralization the microbes they produce different types of ligands, one of the very interesting ligand is the phosphate group, which the microbes often produce because they break down or they utilize the organic phosphates and the phosphate groups are released and these phosphate groups are very efficient in binding the divalent metal ions and they can lead to the precipitation of the metal phosphates.

And all the metal phosphates they precipitate in the surrounding environment so around the cells outside the cells, these metal phosphates are precipitate. So, these biomineralization mostly facilitate the precipitation of the insoluble metal deposits which are either with phosphate or with sulfides or they precipitate the outside the cell. Similarly, the bioreduction is also a process which inside the metal in the outside the cell. So, the insoluble metals will be deposited or precipitated outside the cell.

The cell will not be accumulating those reduced or mineralized product in most of the cases what we see. Similarly, these sulfides which are produced during the sulphate reduction. So, this is another process which is mainly carried out by anaerobic bacteria, anaerobic sulphate reducing bacteria they used sulphate as the terminal electron acceptor and they produce reduced the sulphate as their terminal electron acceptor and sulphate is converted to sulfide and these H 2 S which is basically a gaseous H 2 S.

It has the ability to interact with the divalent cationic which are present in the facility and it is found to be one of the best biomineralization method because the moment is alpha reducing bacteria they are capable of producing the H 2 S. The H 2 S is readily reacts with the divalent cationic and produces the metal sulfides. The metal sulfides are again insoluble and they form the minerals. So, from their small nucleation centers gradually the minerals they grow up and huge deposits of metal precipitates, metal sulfide precipitates or metal phosphate precipitates they deform.

Now, all these mechanisms bioaccumulation, biosorption, bioreduction and biomineralization they have been found to be very useful for bioremediation or for the removal of the metals, because in all these cases, we find that they either remove like biosorption or bioaccumulation, they removed the metals from the aqua space or the bioreduction that allow the basically the precipitation of the insoluble metal or the biomineralization that also facilitates the precipitation of the mineral form of the metals.

However, through the production or use of some ligands which are produced by the bacteria or the fungal cells. Now, here is 2 another interesting way of dealing with the metals which are executed by many bacteria and fungi that is the production of organic acids. Many microbes that produce organic acids, which can react with the insoluble metals and these when the insoluble metals are reacted to the organic acids. So, the metal organic complexes are formed. And those complexes are found to be mostly soluble complex.

And these soluble complexes are more mobile than the insoluble counterpart therefore, the soluble metal complexes they leach out or they mobilize from the soil or the sediment and that process is called bio leaching. During the bio hydrometallurgy, or recovery of different important industrially important metals, these processes of bioleaching have been found to be very important and we will discuss about this bioleaching in a separate lecture.

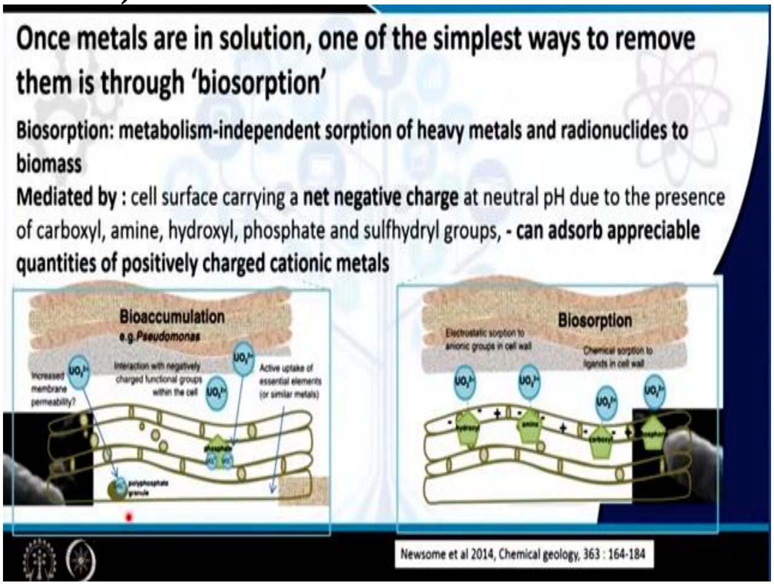
Now, the another mode of the redox transformation is called bio-oxidation, in case of bio-oxidation what happens the metals which are reduced and insoluble, they are oxidized to produce more soluble form of metal and these more soluble forms of metals even they also helping the bioleaching or extraction of the metals from the contaminated soil or contaminated sediments. So, often if we; want to flush out or if we want to recover or remove the metals from the sediment or the soil or other environments.

We can use this bio-oxidation or the organic acid mediated bioleaching process where the metals will be soluble and insoluble metals will be mobilized or will be removed from the solution or the sediment and then they can be either precipitated by some other means and can be recovered and reused. Now, there is another very interesting method of bio-oxidation that has gained considerable attention in recent times that is called bio-oxidation through dissimilatory mode of interactions.

So, metals like manganese for example, which when they are in reduced form, they are more soluble. So, manganese for example, manganese 2 is soluble more soluble when they are reduced form, but if we are able to oxidize them, then it is converted to Mn 4 that is MnO 2 and this MnO 2 is readily precipitate the form actually minerals and one of the concepts of biomineralization could be by bio-oxidation of iron or manganese kinds of things, which can be oxidized through microbial redox transformation.

So, these are again redox mediated transformation that microbes can use these and then they convert these manganese or iron for example into these insoluble forms they precipitate and they can be useful for further bioremediation processes that I will discuss.

(Refer Slide Time: 22:31)



Once metals are in solution, one of the simplest ways to remove them is through 'biosorption'

Biosorption: metabolism-independent sorption of heavy metals and radionuclides to biomass

Mediated by : cell surface carrying a **net negative charge** at neutral pH due to the presence of carboxyl, amine, hydroxyl, phosphate and sulfhydryl groups, **- can adsorb appreciable quantities of positively charged cationic metals**

Newsome et al 2014, Chemical geology, 363 : 164-184

Now, once metals are in solution, one of the simplest ways to remove them is through the biosorption. So, we will now go back to the processes which are just discussed like biosorption bioaccumulation followed by the precipitation based methods and then again the biomineralization based processes. So, the biosorption process has gained considerable research interest at some point of time because it was found to be the simplest way to remove the metals and radionuclides.

Because it is metabolism independent often and the microbial cells are having high surface area to volume ratio, they can interact with these divalent cationic for example very rapidly and they can sequester or they can absorb these divalent cationic very effectively. So, these are metabolism independent adsorption process, and mediated by the cell surface or negative charge groups like the carboxyl, amine, hydroxyl, phosphate, sulfhydryl groups and can adsorb appreciable quantities of positively charged cationic metals.

So, huge amount of metals can be precipitated or can be dissolved rather we do not use the term precipitated in this case, we say it is a dissolved on the surface because it is not an either enzymatically mediated precipitation or ligand based precipitation. So, here as you can see, that the cell in the different types of organisms that have been found to be very useful in having this negatively charged moieties or ligands in the cell membrane or ligand or other capsular structure of the cells, they are able to bind the divalent uranium for example.

In this case, so divalent cationic can be adsorbed through electrostatic adsorption to these anionic groups and then subsequently micro precipitation and ion exchange process can also take place and eventually more amount of the divalent cationic can be deposited in the adsorbed on the cell surface. And since it is a metabolism independent process, it has gained considerable interest.

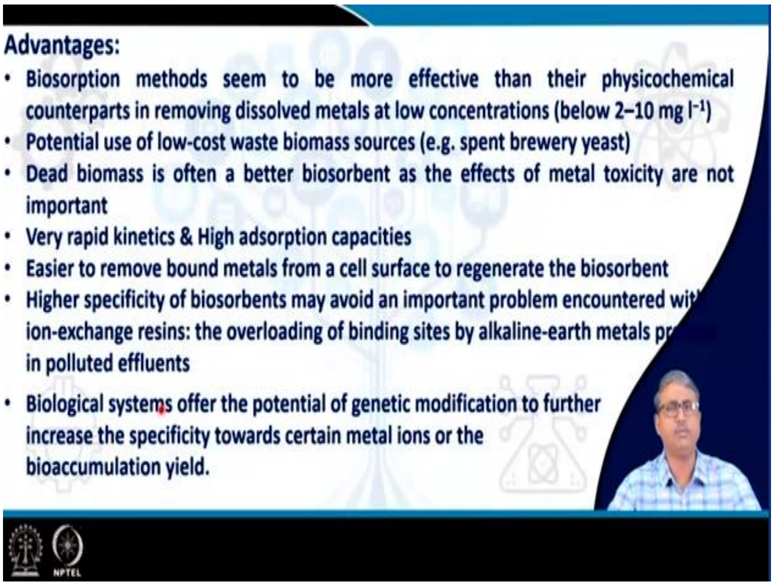
Because the toxicity and all those points are not valid because these cells maybe dead also so basically dead cells can be used for this purpose. On the contradict to this bioaccumulation which is very well studied in case of Pseudomonas and other species have been found to be facilitated by internal transport and through different type of metal they have the property to penetrate the cell like uranium is a very toxic.

And they have the ability to penetrate the membrane and enter inside the cell and there the cells are able to precipitate or sequester these internal uranium UO 2 2+ through polyphosphate granules because the cells they need to have some mechanism to comeback the toxic effect of uranium. So, the bioaccumulation as I mentioned, as long as the concentration is within the tolerable range of the organisms to the organisms will be happy.

But as soon as the concentration of the internal light or the bioaccumulated metals, go beyond the threshold the tolerable limit then the bacteria or the fungi they will express their and they will execute different detoxification strategies. So, we have noticed that for different not only for uranium. But a large number of other heavy metals that microbes produce a significant number of detoxification strategies including influx as we discussed in our earlier lecture or expression of metallothionein.

In eukaryotic cysteine or even in cyanobacteria we see small cysteine called sulfhydryl group containing protein molecules for sequestering this toxic or divalent cationic metals are radionuclides inside the cell.

(Refer Slide Time: 26:31)



Advantages:

- Biosorption methods seem to be more effective than their physicochemical counterparts in removing dissolved metals at low concentrations (below 2–10 mg l^{-1})
- Potential use of low-cost waste biomass sources (e.g. spent brewery yeast)
- Dead biomass is often a better biosorbent as the effects of metal toxicity are not important
- Very rapid kinetics & High adsorption capacities
- Easier to remove bound metals from a cell surface to regenerate the biosorbent
- Higher specificity of biosorbents may avoid an important problem encountered wi ion-exchange resins: the overloading of binding sites by alkaline-earth metals p in polluted effluents
- Biological systems offer the potential of genetic modification to further increase the specificity towards certain metal ions or the bioaccumulation yield.

Now, the advantages of these biosorption are the biosorption methods seem to be more effective than their physicochemical counterparts because for many waste including uranium also a physicochemical methods including other divalent cationic, cadmium, amine etcetera, for industrial waste treatment physicochemical methods including the resins etcetera, ion exchange resin are in use.

So, biosorption process actually was developed they kind of comparable of technology which can offer a solution to the removal of the metallic pollutants. Because it can remove the dissolved metals at very low concentrations that was one of the best advantages that below 2 to 10 milligram per liter, the biosorptions mechanisms can work very effectively. And I must say that ion exchange resins are not suitable for such a low concentration.

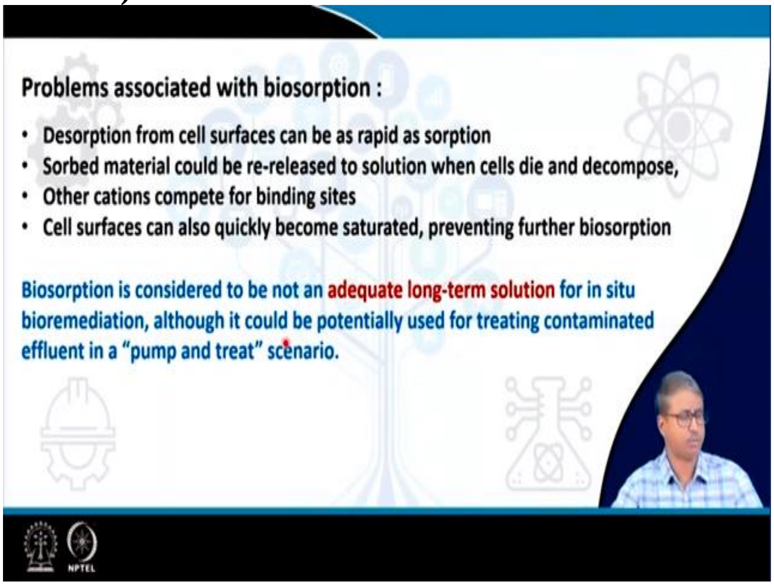
Otherwise the cost will be very high if we want to use some physicochemical methods for very low concentration of waste. Potential use of low cost waste biomass, so many other industries including the biotechnology industries, often they produce the for example, the brewery yeast and other system produce large amount of biomass. So, those biomasses can be utilized for biosorptions processes.

Even the dead biomass can also be used and dead biomass is found to be a better biosorbent because the metabolism is not involved and toxicity problems are not associated with dead biomass. Biosorption shows basically rapid kinetics and high adsorption capacities. It is also possible to remove the bound metals from the cell surface to regenerate the biomass. So, often we have seen that the biosorbent can be used several cycles like we can rejuvenate biosorbents after we remove the metals bound metals from the cell surface.

Higher specificity of biosorbents may avoid an important problem encountered with ion exchange resins and with particularly ion exchange resins. Having the; problem of the overloading of the binding sites by alkaline earth metals in presenting the polluted environment. But in some cases, we have found that device orbits algal and fungal biosorption, bacterial biosorbent are found to be more specific to certain heavy metals and radionuclides. So that is again another advantage against the ion exchange resins.

Further the biological systems since the biosorbent are basically obtained from biological systems they are bacterial or fungal cells or algal cells. So, we can actually engineer the cells are the genetically modified the cells to improve their specificity and affinity.

(Refer Slide Time: 29:33)



Problems associated with biosorption :

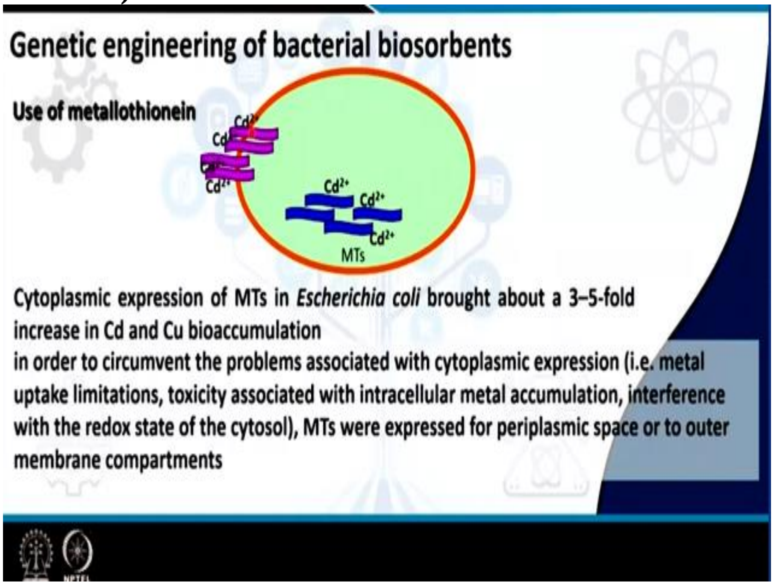
- Desorption from cell surfaces can be as rapid as sorption
- Sorbed material could be re-released to solution when cells die and decompose,
- Other cations compete for binding sites
- Cell surfaces can also quickly become saturated, preventing further biosorption

Biosorption is considered to be not an adequate long-term solution for in situ bioremediation, although it could be potentially used for treating contaminated effluent in a "pump and treat" scenario.

Now there are some significant problems associated with these biosorption like desorption on the cell surface can be as rapid as sorption. Sorbed material could be re-released to solution when cells die and decompose sometimes they disintegrate also releasing the metals into the solution again. Other cationic compete for binding sites so all biosorbent not very specific. So, there may be some competing ions are there and these competing cationic can bind and enforce some kind of competition with the target toxic metals.

Cell surfaces can also quickly become saturated preventing further biosorption. And essentially biosorption is considered to be not an adequate long term solution for in situ bioremediation. So, it may be suitable for some of the pump and treat scenario where you can have a column based process and through that a large number of biosorption based processes have been actually developed.

(Refer Slide Time: 30:35)



Genetic engineering of bacterial biosorbents

Use of metallothionein

Cytoplasmic expression of MTs in *Escherichia coli* brought about a 3–5-fold increase in Cd and Cu bioaccumulation

in order to circumvent the problems associated with cytoplasmic expression (i.e. metal uptake limitations, toxicity associated with intracellular metal accumulation, interference with the redox state of the cytosol), MTs were expressed for periplasmic space or to outer membrane compartments

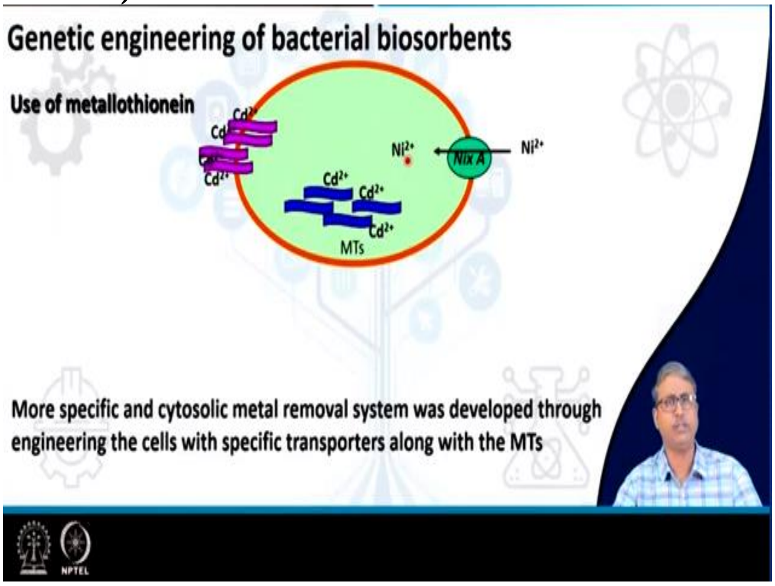
Now, in the next section, we are going to discuss about the genetic engineering of the bacterial biosorbents, particularly using the concept of the metallothionein which are found to be low molecular weight cysteine rich proteins engaged in binding a toxic heavy metals in mostly in eukaryotic system, but also present in cyanobacteria. So, these metallothionein genes are expressed in bacteria for example in experimental way, we express them in E. coli.

And when we express the eukaryotic metallothionein in E. coli through genetic engineering, we found that they can efficiently bind cadmium accumulate cadmium, and so part of bioaccumulation E. coli accumulates the toxic cadmium and E. coli cells can sustain the toxicity because the cadmium ions are bound with the metallothionein which are expressed inside the recombinant E. coli cells. In order to circumvent the problem associated with the cytoplasmic expression that is the metal uptake limitation.

So, if we want to have cytoplasmic like in this case, cytoplasmic expression of the metallothionein the metals need to be transported inside the cell. So, you have other issues also. So, the scientists the thought that instead of expressing the metallothionein in the cytoplasm, if we just express the metallothionein on the periplasmic space on the outer membrane, that might actually relieve the cells in terms of any toxicity issues.

So that was also done successfully where the metallothionein are expressed in such a way that they will be located on the periplasmic space or the outer membrane and or the membrane envelop and the divergent catalysts like cadmium can be effectively bound to this.

(Refer Slide Time: 32:31)



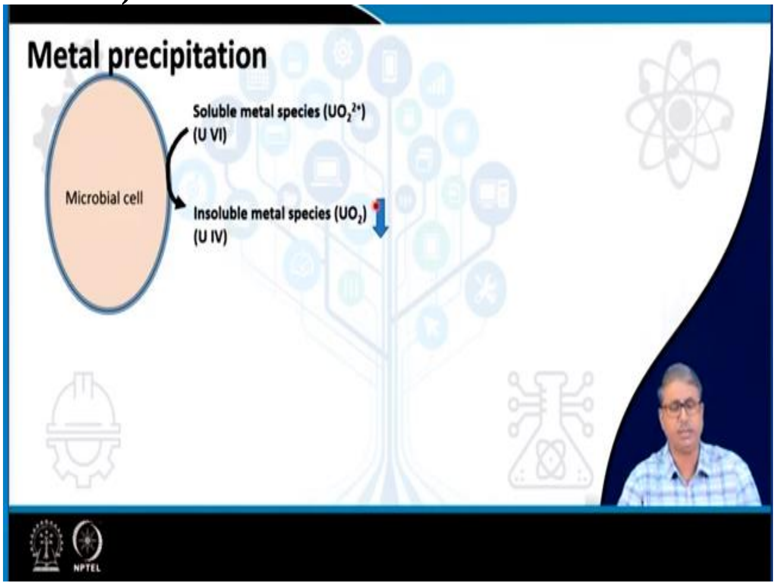
Genetic engineering of bacterial biosorbents

Use of metallothionein

More specific and cytosolic metal removal system was developed through engineering the cells with specific transporters along with the MTs

Now, this is not the end of the road with respect to metallothionein based engineering, more specific and cytosolic metal removal systems are further developed to engineering the transport process. So, we also engineered specific transporter like nickel transporter and the nickel is transported inside the cell divalent nickel ions and then the nickel ions were precipitated or accumulated through the use of the metallothionein.

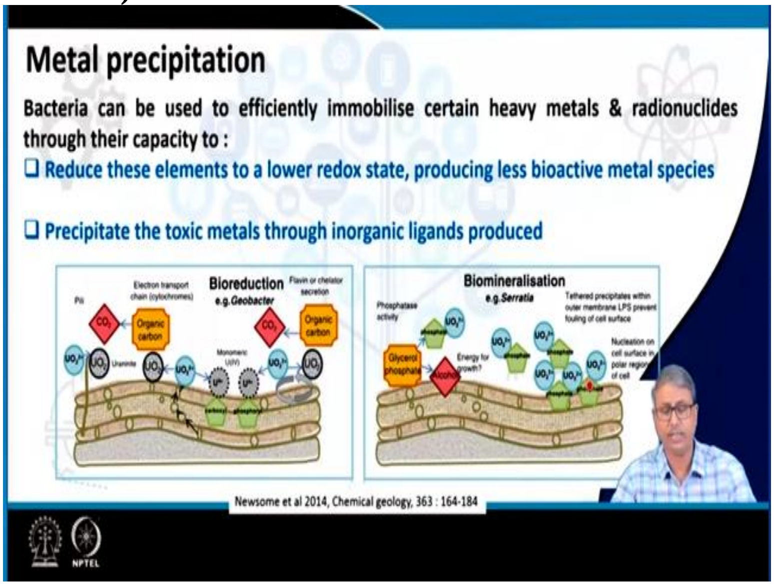
(Refer Slide Time: 32:59)



Metal precipitation

Now, with respect to metal precipitation which has been found to be one of the major means of bioremediation of toxic metals and radionuclides, we will discuss this part in a little more detail. So fundamentally, the microbial cells are capable of precipitating metals like uranium for example, where the soluble metal species like UO 2 2+ is reduced though insoluble metal species like uranium 4. So, uranium 6 to uranium 4 and the uranium 4 this particular form of uranium, it precipitates naturally it precipitates in a aqua’s environment with neon neutral leach.

(Refer Slide Time: 33:42)



Metal precipitation

Bacteria can be used to efficiently immobilise certain heavy metals & radionuclides through their capacity to :

☐ Reduce these elements to a lower redox state, producing less bioactive metal species

☐ Precipitate the toxic metals through inorganic ligands produced

Newsome et al 2014, Chemical geology, 363 : 164-184

Now, this particular process of metal precipitation through redox transformation, basically, we are transforming the oxidation state of the metals or the radionuclides can be achieved by 2 means, so bacteria can be used to efficiently mobilise that is the precipitate the metals or radionuclides because of their capacity to reduce these metals in a lower redox state like in case of uranium with uranium 6 to uranium 4 producing less bioactive metal, so uranium 4 is not at all bioactive because it precipitates.

It this precipitation can also be achieved through inorganic ligands produced that we have learned during our biomineralization discussion that through by producing different inorganic ligands like sulfides, hydrogen sulfide or phosphate or even carbonate, the microbes can also lead to the precipitation of the metals like even uranium can be precipitated by uranium carbonate or uranium phosphate and many other heavy metals can be precipitated through sulfides precipitation.

Now, these are the 2 processes that you can see here that form the bioreduction that is the first one that reduce these elements to a lower redox state like uranium 6 to uranium 4. So, you have the uranium 6 that can be reduced uranium 4 and for this reduction process you need a source of carbon and source of electrons so that can be organic carbon. So, ideally these organisms will be mostly heterotrophic and they can engage either a cytochrome based system or a flavin based system.

So, there are multiple systems discovered for the transfer of the electrons from the electron donor to the uranium UO 2 2+, so that UO 2 2+ can be reduced to different form of uranium which can precipitate which are insoluble. So, effectively this type of reduction is found to be possible for not only for uranium, but also for chromium, for technetium, for selenium and many other similar redox active metals where the reduced forms are insoluble.

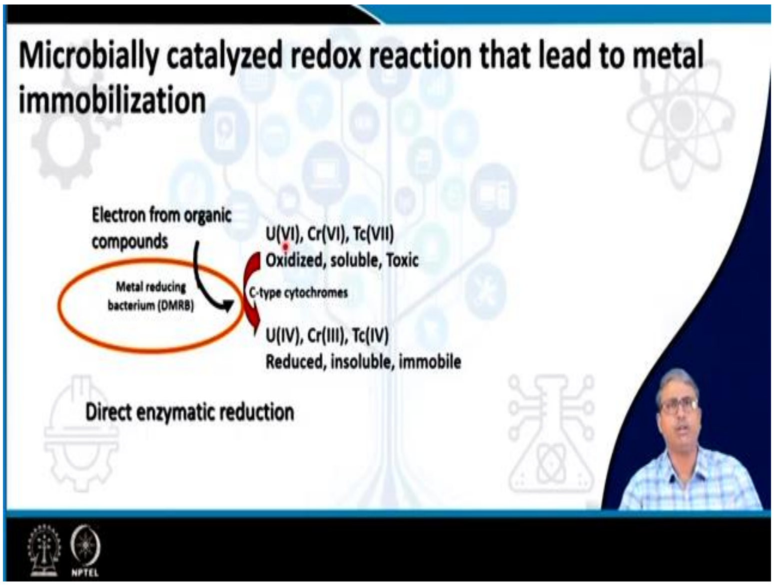
So, this is found to be one of the best way even for in situ bioremediation because it has been found and I will discuss that briefly that by providing suitable carbon source and if you have the appropriate microorganisms who can do these things like use their cytochromes or flavin based systems to transfer the electrons and reduce the uranium species or chromium species will like hexamine chromium to trivalent chromium or uranium 6 to uranium 4.

And then uranium 4 can precipitated or only precipitated in the exterior environment and that will remain in a precipitate found unless and until that is going to be deoxidized. So that part I will also talk briefly. Now, the other part that I mentioned is the precipitation of the metals through inorganic ligands which is a part of biomineralization as well. So, here you can see that uranium for example can be biomineralized.

Because by producing phosphates and these phosphate moieties can be produced by providing glycerol phosphate, this is organic phosphate donor, this organic phosphate molecule can be metabolized can be broken down so that the phosphate groups are released by bacterial phosphatase. So, specific bacterial strains have been isolated and characterized who produce this phosphatase enzyme.

And they can also allow these precipitation a huge amount of uranium phosphate and not only uranium phosphates a large amount of metal phosphates are produced. And when they start depositing nearby the cells they form a nucleation center and as the nucleation of these uranium phosphates or other metal phosphates starts, this nucleation center basically allows the further growth of the minerals and then chemical deposition of these uranium and other metal phosphates are fought.

(Refer Slide Time: 37:50)



Microbially catalyzed redox reaction that lead to metal immobilization

Electron from organic compounds

Metal reducing bacterium (DMRB)

U(VI), Cr(VI), Tc(VII)
Oxidized, soluble, Toxic

C-type cytochromes

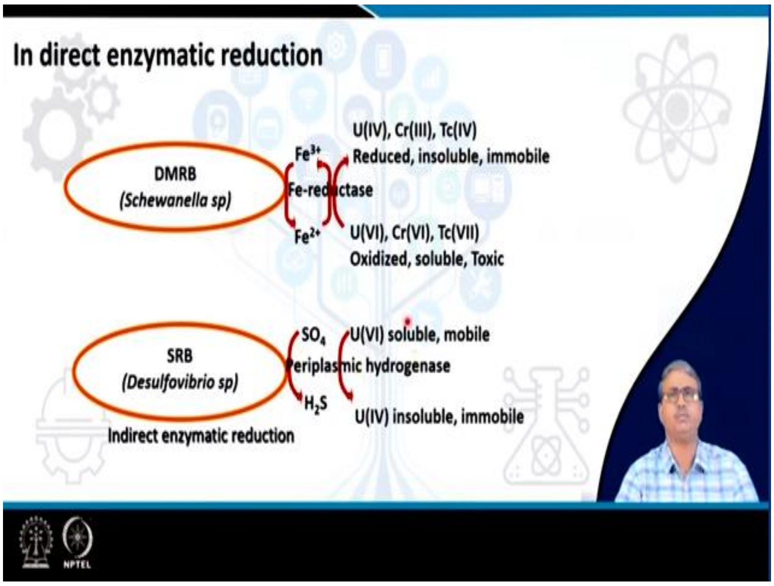
U(IV), Cr(III), Tc(IV)
Reduced, insoluble, immobile

Direct enzymatic reduction

Now, this process of metal reduction, leading to the formation of the insoluble or immobile reduced forms which have been found to be very popular and very effective for heavy metal and radionuclide bioremediation can be achieved by 2 mechanisms. One is called direct enzymatic reduction where the microorganisms involved allow the flow of electrons from the organic compounds and reduce the uranium, chromium, technetium etcetera which are present in the oxidized form.

And in all these oxidized forms are more soluble, more toxic and may be in the groundwater in your soil or sediment if they are there. Then if you are able to provide the suitable electron donor and carbon source and your appropriate microorganisms are there then they will reduce it through this cytochrome or flavin based systems and it is called a direct mechanism. Because direct enzymatic reductions they allow conversion or the reduction of these highly soluble species into insoluble species and these insoluble species they readily precipitate into the surrounding environment.

(Refer Slide Time: 39:01)



In direct enzymatic reduction

DMRB
(Schewanella sp)

Fe^{3+}
Fe-reductase
Fe^{2+}

U(IV), Cr(III), Tc(IV)
Reduced, insoluble, immobile

U(VI), Cr(VI), Tc(VII)
Oxidized, soluble, Toxic

SRB
(Desulfovibrio sp)

SO_4
eriplasmic hydrogenase
H_2S

U(VI) soluble, mobile

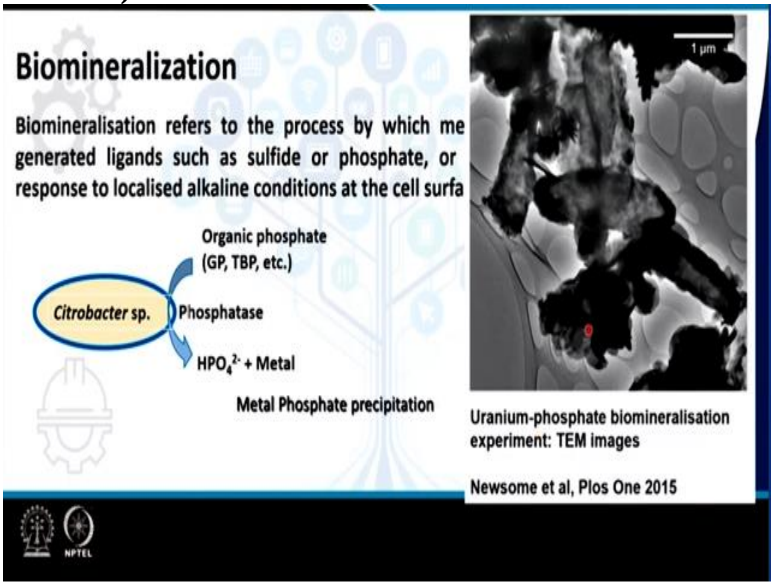
U(IV) insoluble, immobile

Indirect enzymatic reduction

The other process is called the indirect enzymatic reduction like the C one type of organism who can actually reduce the either Fe 3 to Fe 2 by ion reductases and Fe 2 can further reduce the uranium, technetium or chromium 6 in direct reduction by using Fe 2+ as the electron donor, so Fe 2 donor the electron and uranium, chromium or technetium is reduced, so that way it is called in direct enzymatic reduction.

The in direct enzymatic reduction also occurs in case of sulphate reducing bacteria like desulfovibrio and many other species, where the sulfides which are produced these bacteria can also use the like periplasmic hydrogenases and can actually facilitate the precipitation of soluble uranium into insoluble deposits.

(Refer Slide Time: 39:51)



Biomineralization

Biomineralisation refers to the process by which me generated ligands such as sulfide or phosphate, or response to localised alkaline conditions at the cell surfa

Citrobacter sp.

Organic phosphate
(GP, TBP, etc.)

Phosphatase

HPO_4^{2-} + Metal

Metal Phosphate precipitation

Uranium-phosphate biomineralisation experiment: TEM images

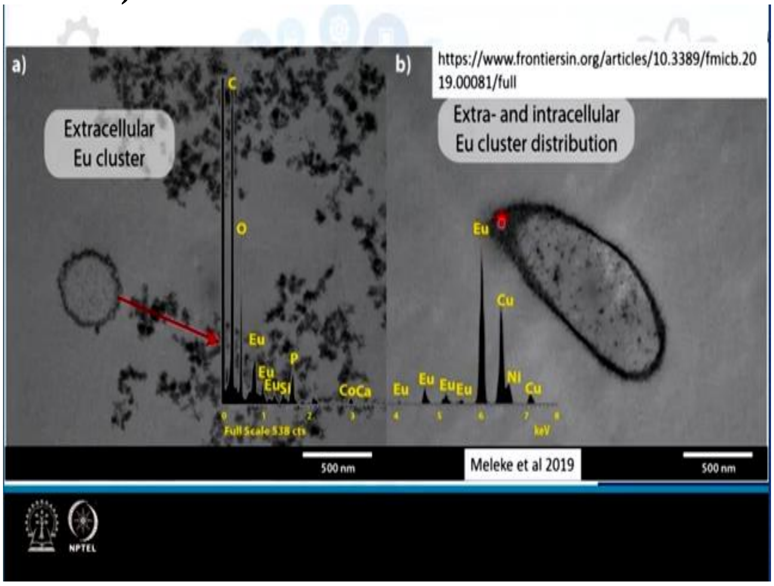
Newsome et al, Plos One 2015

Now, with respect to biomineralization by ligands this process is basically a process in which metals are precipitated with microbially generated ligands like phosphate or sulfides. And these are one of the best examples is the citrobacter species and this particular citrobacter species has been studied very elaborately with respect to its ability to produce these phosphatase enzyme.

And these phosphatase enzyme can precipitate different type of allow the precipitation of different heavy metals including radionuclides also in very large quantities in and around the cells provided an organic phosphate donor is provided. So, the phosphatase enzyme basically catalyses the release of phosphate moiety and the phosphate moiety is able to react to the metal again this is to some extent the indirect mechanism because the microbe is basically dealing with the phosphate.

And the phosphate group with ligands which is produced is reacting with the metals. So, similar things are also there with the sulfide producing sulfate reducing bacteria, the sulfides which are produced, they react with the metals divalent metals is and allow the precipitation of the metal sulfides. For example, in the right side there is a picture of the uranium phosphate biomineralization you can see around the cells large amount of uranium phosphate depositions are reported in a recent paper by Newsome et al.

(Refer Slide Time: 41:23)

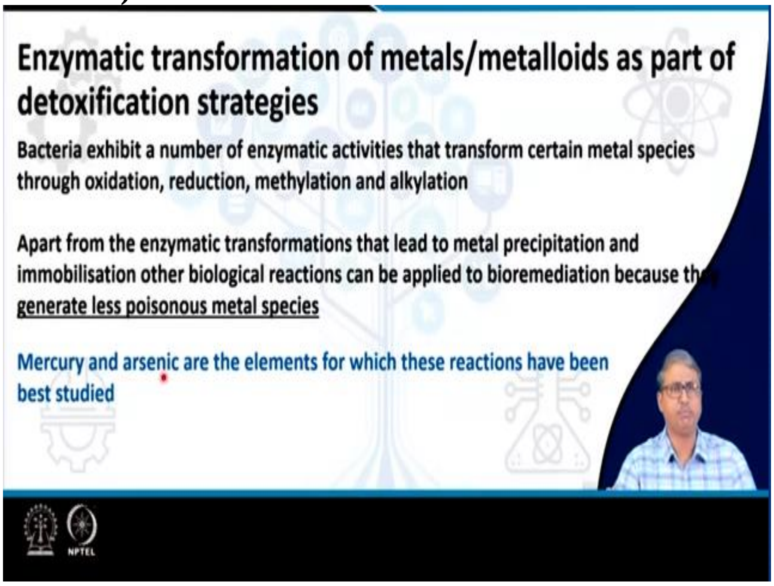


https://www.frontiersin.org/articles/10.3389/fmicb.2019.00081/full

Meleke et al 2019

And the further study we have found that this group of researchers with Melake et al 2019 they have found that an europium another very interesting rare earth element is biomineralized in a similar way in and around the cell. So, you can see this is the bacterial cell a large amount of europium is deposited and to every sensitive method of energy dispersive X-ray analysis, the scientists have confirmed that this is the europium deposits.

(Refer Slide Time: 41:51)



Enzymatic transformation of metals/metalloids as part of detoxification strategies

Bacteria exhibit a number of enzymatic activities that transform certain metal species through oxidation, reduction, methylation and alkylation

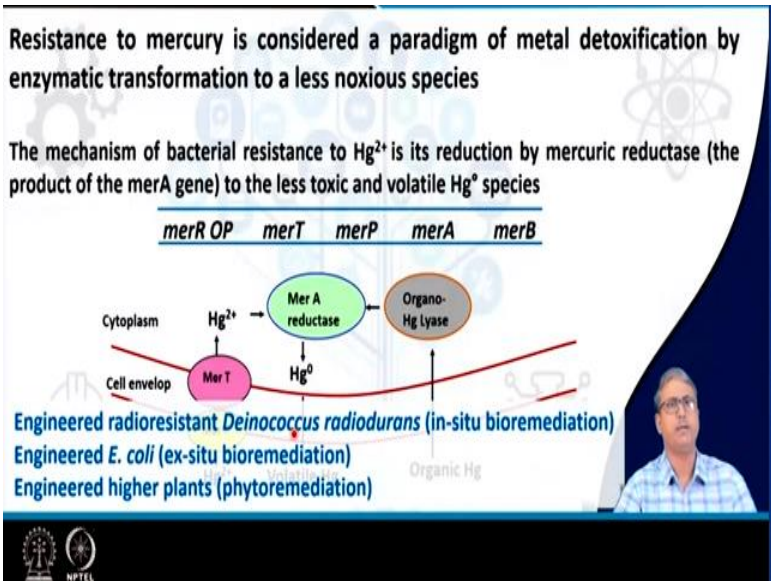
Apart from the enzymatic transformations that lead to metal precipitation and immobilisation other biological reactions can be applied to bioremediation because they generate less poisonous metal species

Mercury and arsenic are the elements for which these reactions have been best studied

Now, enzymatic transformation of metals and metalloids part of the detoxification strategies. So, now these are enzymatic processes which are basically not connected to electron transport or dissimilatory type of reductions like we have learned just before these that uranium, chromium, technetium etcetera are reduced as electron acceptor, but other than that, some other toxic metals or metalloids can also be transformed enzymatically.

These enzymatic transformations also lead to the conversion of the metals or metalloids into less toxic form or an altered form which can be then utilized in the bioremediation process. And mercury and arsenic are the elements for which these reactions are best studied and implemented also for bioremediation processes. Now, we should remember that mercury is not essential anyway for the cell and that is true for the arsenic also. So, it is absolutely clear that mercury enzymatic transformation of mercury or enzymatic transformations of arsenic by microbes are purely detoxification strategies.

(Refer Slide Time: 43:08)



Resistance to mercury is considered a paradigm of metal detoxification by enzymatic transformation to a less noxious species

The mechanism of bacterial resistance to Hg^{2+} is its reduction by mercuric reductase (the product of the merA gene) to the less toxic and volatile Hg° species

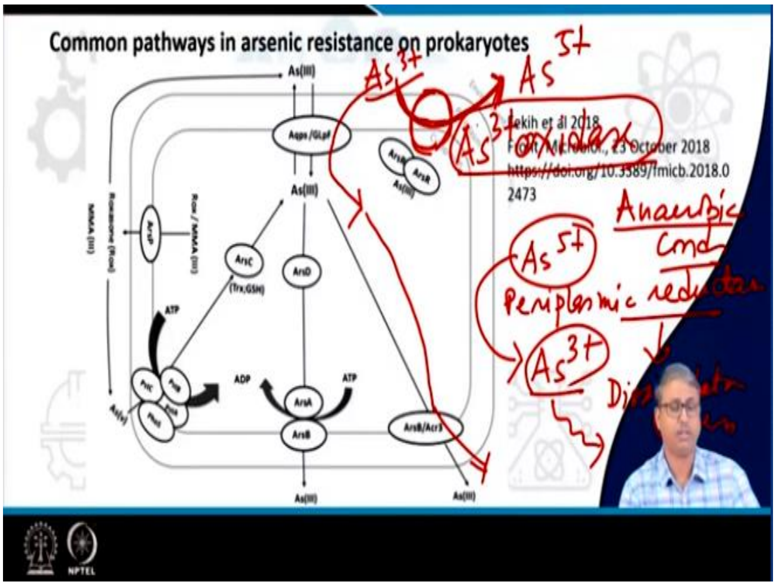
Engineered radioresistant *Deinococcus radiodurans* (in-situ bioremediation)
Engineered *E. coli* (ex-situ bioremediation)
Engineered higher plants (phytoremediation)

So, for example resistance to mercury is very well studied, because this is catalysed by the cytosolic enzyme, mercuric reductase. So, you can see here there is a merA, the name of the protein is merA which is mercuric reductase. And once the mercury is transported inside, so for mercury, most of the mercury reducing bacteria they deposit the complete set of genes that is called operon and they transport to a specific transporter. So, mercury is very toxic. So, divalent Hg 2+ is very, very toxic.

So, you need to have a very specific transporter, so that the Hg 2+ enters inside the cell and then immediately it is reduced to Hg 0 and H 0 is a volatile form and it leaves the system. Similarly, using arsenic also so I will talk about that, but before that, based on this mercury reduction system, one of the pioneering work was done with radioresistant Deinococcus that you do this for in situ bioremediation of highly radioactive environment where lots of mercury is there.

So, there are certain such contaminated sites for which these engineered deinococcus radiodurans was developed and with the mercury detectors ability, so the deinococcus radiodurans is a natural bacterium who can withstand high amount of ionizing radiation and still when it is engineered in terms of mercury reduction, it is capable of reducing mercury as well.

(Refer Slide Time: 44:42)



Common pathways in arsenic resistance on prokaryotes

Fekih et al 2018
Front. Microbiol., 23 October 2018
https://doi.org/10.3389/fmicb.2018.02473

Now, with respect to arsenic, we find that there are a number of strategies, now arsenic is very interesting metalloid for which we have 2 forms one is arsenic 3 which is more toxic, and we have arsenic 5 which is less toxic. Now, arsenic 5 enters inside the cells through phosphate transporter because arsenate resembles phosphate and enters into the cell by mimicking the phosphate and when once it enters into the system of the cell in the cytoplasm this enzyme which is encoded by ArsC that is a cytosolic arsenate reductase, it reduces arsenate to arsenite.

So, AS3 is proteins now AS3 can also be transported inside the cells through a specific proteins which are called aquaporin kind of pumps which have been reported to get involved in arsenic transportation as well, this could be part of the arsenic homeostasis what you called that cells they try to make living with arsenic. So, they do not say no to the arsenic they allow the arsenic to come in and all the arsenic 3 which are accumulated.

Because of either direct entry through these aquaporin kind of pumps or by reduction of the arsenic to arsenite are subsequently flushed out by different influx system like ArsB system or Acr3 system or other systems which actually utilizes different type of energy sources and they throw out or influx out the arsenic as arsenic 3. Now, apart from these microbes are also capable of transforming arsenic like arsenic 5 can be reduced to arsenic 3+ by what you called periplasmic reductars which are often connected to the dissimilatory process also.

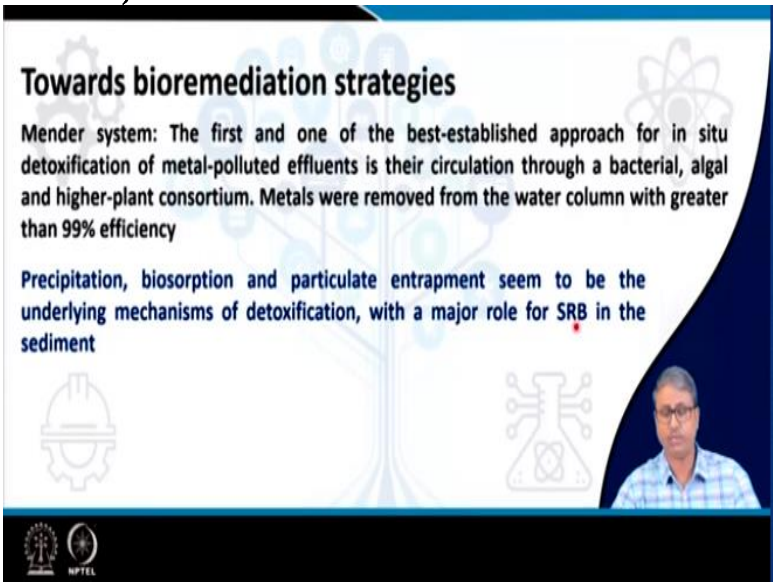
So, recent time we have seen that many bacterial strains they are capable of doing this reduction using the cytochromes and similar flavin based systems which we could notice for uranium as well. So, there are these bacteria under anaerobic condition, they use arsenate and reduce arsenate to arsenite and since arsenite is more soluble, it goes into the aqua's space, but arsenite is more toxic also.

So, the microbes they must utilize some strategy to compete the arsenic 3 toxicity for that they use another periplasmic space or cell envelop bit based enzyme that is called Aio or arsenite oxidase. So, these arsenite oxidase it convert so this is basically we call it AS 3+ oxidase. So, this arsenite oxidase is very specific enzyme it is not a kind of a general cytochrome or flavin based enzyme this is very specific enzyme arsenite oxidase and those bacteria who process these arsenite oxidase in their outer membrane.

They are capable of oxidizing AS 3+ to AS 5+ and this could be considered as a detoxification strategy again because arsenic 3 is very toxic and if you do not oxidized to arsenic 5 then it will enter through these aquaporin and come inside the cell and then within the cell it will execute toxicity because arsenite 3 will interfere with the cellular sensitive macromolecules otherwise you have to use some deflux pump to throw it out.

So, some bacteria they adopt this mechanism that allow it to come in and then influx it otherwise some bacteria they prefer to oxidize H g to AS 3+. So, these are found to be very, very interesting mechanisms which actually allow the cellular homeostasis in terms of arsenic.

(Refer Slide Time: 49:07)



Towards bioremediation strategies

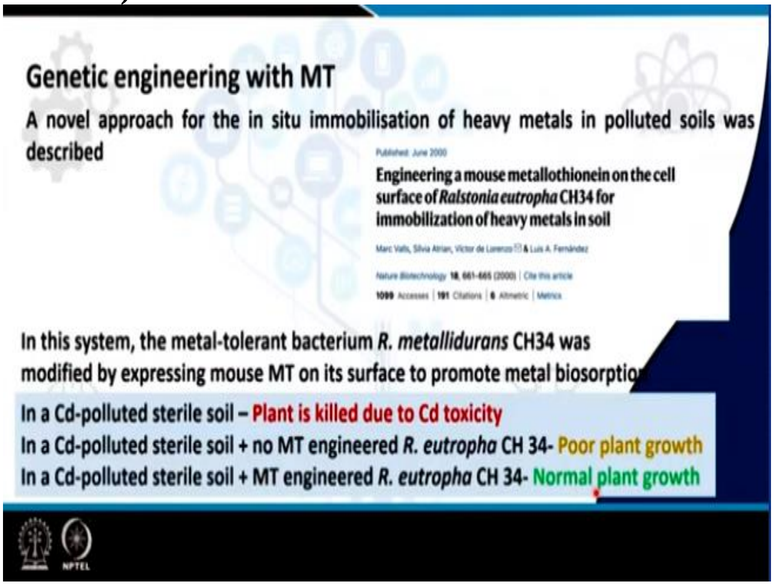
Mender system: The first and one of the best-established approach for in situ detoxification of metal-polluted effluents is their circulation through a bacterial, algal and higher-plant consortium. Metals were removed from the water column with greater than 99% efficiency

Precipitation, biosorption and particulate entrapment seem to be the underlying mechanisms of detoxification, with a major role for SRB in the sediment

Now towards the bioremediation strategies, all these microbe metal interactions, almost all the types that we discussed today, they are tested up to field level also for in situ or ex situ treatments. So, one of the systems was this mender system. That system is basically one of the best established at some point of time for in situ detoxification of metal polluted effluents particularly with bacterial algal and higher plants consortium and the system are able to remove the divalent cationion with 99% efficiency.

Similarly, different consortium based system where multiple microorganisms are there and they are involved in metal removal through multiple processes including precipitation, biosorption and particulate entrapment also allowed the development of different metal bioremediation particularly with the use of or the role of sulfate reducing bacteria in the sediment system.

(Refer Slide Time: 50:15)



Genetic engineering with MT

A novel approach for the in situ immobilisation of heavy metals in polluted soils was described

Published: June 2000

Engineering a mouse metallothionein on the cell surface of *Ralstonia eutropha* CH34 for immobilization of heavy metals in soil

Marc Valls, Silvia Atrian, Víctor de Lorenzo & Luis A. Fernández

Nature Biotechnology **18**, 661–665 (2000) | Cite this article

1099 Accesses | **191** Citations | **6** Altmetric | Metrics

In this system, the metal-tolerant bacterium *R. metallidurans* CH34 was modified by expressing mouse MT on its surface to promote metal biosorptio

In a Cd-polluted sterile soil – Plant is killed due to Cd toxicity

In a Cd-polluted sterile soil + no MT engineered *R. eutropha* CH 34- Poor plant growth

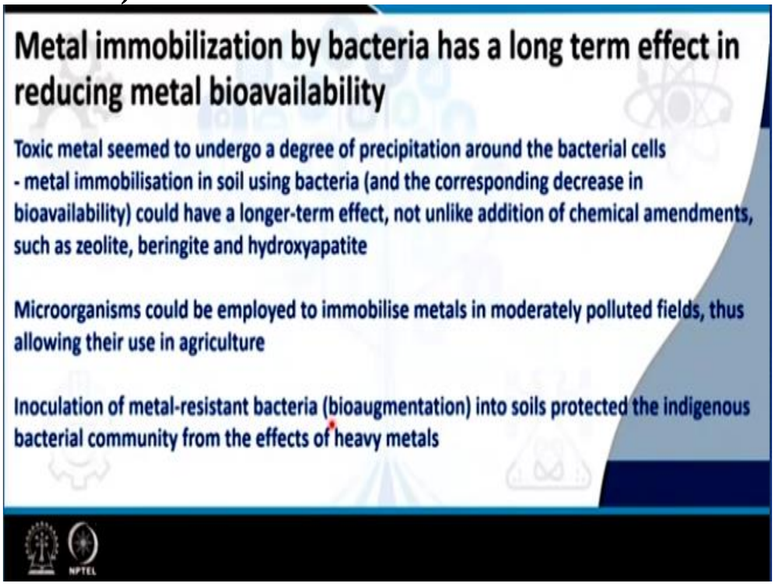
In a Cd-polluted sterile soil + MT engineered *R. eutropha* CH 34- Normal plant growth

Genetically engineered metallothioneins they have been found to be very important in soil remediation. And particularly in one of the cases we found that the Ralstonia eutropha CH34 one of the best known metal resistant bacterium is engineered with respect to these metallothionein gene eukaryotic metallothionein gene and the metal tolerant this is naturally metal tolerant this matallidurans CH34 which is also known as Ralstonia eutropha is modified with the mouse metallothionein to the surface to promote the metal biosorption.

So, the bacterium was engineered so that on the surface of the bacterial cell, the divergent catalysts like cadmium for example, actually the experiment was done with cadmium. So, in cadmium polluted soil, if they tried to grow the tobacco plant and the plant is killed due to the toxic cadmium toxicity, and when normal Ralstonia eutropha CH34 it is not engineered was added bioaugmented rather poor plant growth was observed.

However, when the soil was augmented with Ralstonia eutropha CH34 which is engineered with respect to metallothionein genes, it has been found that the normal plant growth is there.

(Refer Slide Time: 51:40)



Metal immobilization by bacteria has a long term effect in reducing metal bioavailability

Toxic metal seemed to undergo a degree of precipitation around the bacterial cells - metal immobilisation in soil using bacteria (and the corresponding decrease in bioavailability) could have a longer-term effect, not unlike addition of chemical amendments, such as zeolite, beringite and hydroxyapatite

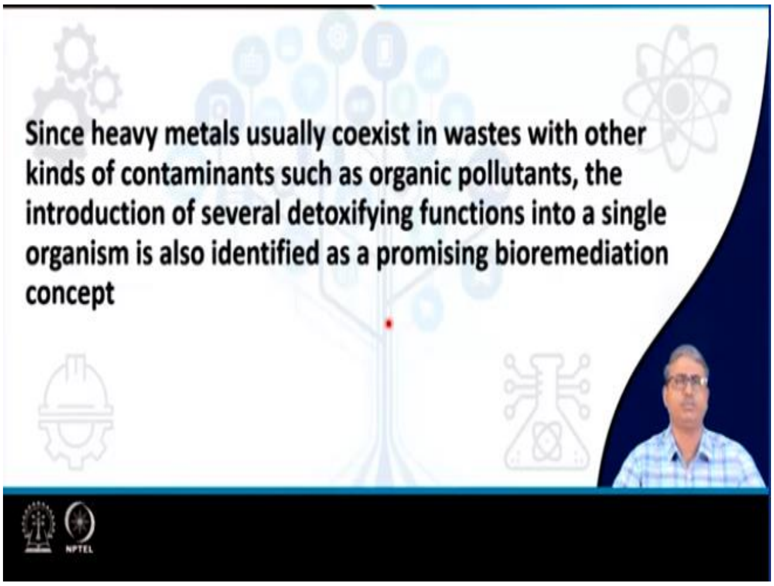
Microorganisms could be employed to immobilise metals in moderately polluted fields, thus allowing their use in agriculture

Inoculation of metal-resistant bacteria (bioaugmentation) into soils protected the indigenous bacterial community from the effects of heavy metals

So that proves that the metal immobilization by bacteria could be having potentially having a long term effect in reducing the metal bioavailability because the metal seem to undergo a degree of precipitation around the bacterial cells particularly these type of cells and metal immobilisation in soil using this type of system would have a long term effect but unlike addition of the chemical amendment like zeolite etcetera.

Microorganisms could be employed to immobilise metals in moderately polluted fields thus allowing their use in agriculture. Inoculation of metal resistant bacteria like bioaugmenting these type of engineered or otherwise naturally present bacteria who can actually sequester or bizarre huge amount of metal externally found to be very, very packages with in terms of protecting the soil which are moderately contaminated and they have been found to protect the indigenous bacterial community from the heavy metal toxicity also.

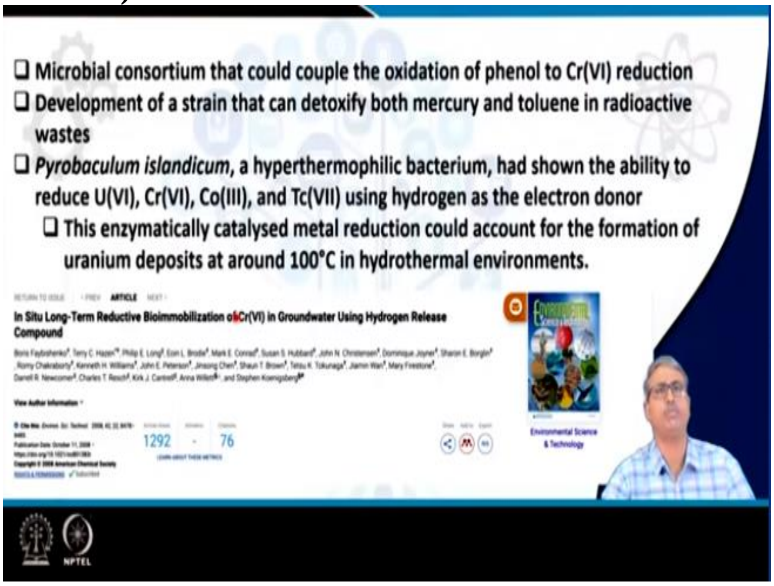
(Refer Slide Time: 52:39)



Since heavy metals usually coexist in wastes with other kinds of contaminants such as organic pollutants, the introduction of several detoxifying functions into a single organism is also identified as a promising bioremediation concept

Now, since heavy metals usually coexist in waste with other kinds of contaminants particularly organic pollutants. So, lots of developments have been done on simultaneous detoxification of the multiple pollutants particularly the organic pollutants, as well as the heavy metal pollutants.

(Refer Slide Time: 52:59)



- ☐ Microbial consortium that could couple the oxidation of phenol to Cr(VI) reduction
- ☐ Development of a strain that can detoxify both mercury and toluene in radioactive wastes
- ☐ *Pyrobaculum islandicum*, a hyperthermophilic bacterium, had shown the ability to reduce U(VI), Cr(VI), Co(III), and Tc(VII) using hydrogen as the electron donor
  - ☐ This enzymatically catalysed metal reduction could account for the formation of uranium deposits at around 100°C in hydrothermal environments.

ARTICLE

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

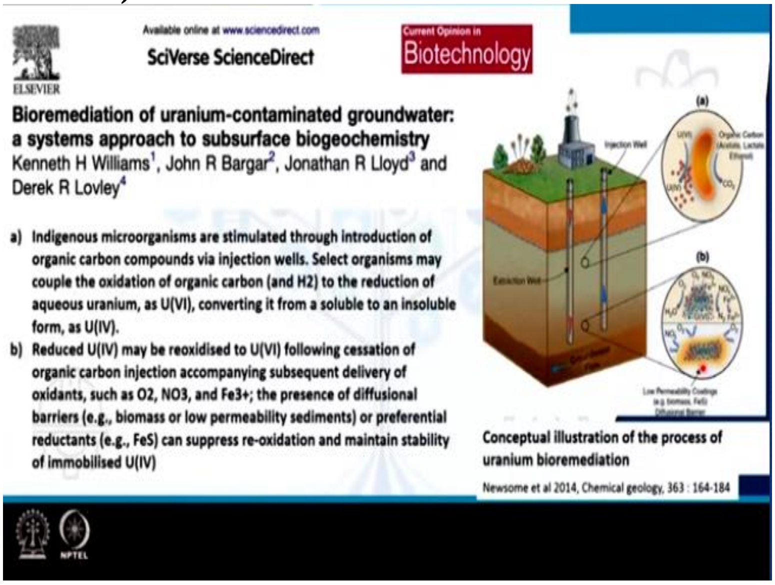
1292 - 76

Environmental Science & Technology

For example, microbial consortium has developed which can oxidize organic pollutants like phenol and utilizing that electrons releasing from the phenol oxidation and using that carbon they can facilitate the chromium reduction. Development of strain that can detoxify both mercury and toluene in a radioactive waste particularly with the deinococcus radiodurans and using the hyperthermophilic bacterium, pyrobaculum which can actually reduce uranium, chromium, cobalt, technetium using hydrogen as the electron donor was found to be very effective.

And we can see that this in situ long term reductive bioimmobilization of chromium in groundwater using hydrogen release compounds, so they use some hydrogen released compounds and they were able to implement on a field level this groundwater by remediation of chromium.

(Refer Slide Time: 53:56)



Available online at www.sciencedirect.com

SciVerse ScienceDirect

Current Opinion in Biotechnology

ELSEVIER

Bioremediation of uranium-contaminated groundwater: a systems approach to subsurface biogeochemistry

Kenneth H Williams[1], John R Bargar[2], Jonathan R Lloyd[3] and Derek R Lovley[4]

a) Indigenous microorganisms are stimulated through introduction of organic carbon compounds via injection wells. Select organisms may couple the oxidation of organic carbon (and H2) to the reduction of aqueous uranium, as U(VI), converting it from a soluble to an insoluble form, as U(IV).

b) Reduced U(IV) may be reoxidised to U(VI) following cessation of organic carbon injection accompanying subsequent delivery of oxidants, such as O2, NO3, and Fe3+; 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)

Conceptual illustration of the process of uranium bioremediation

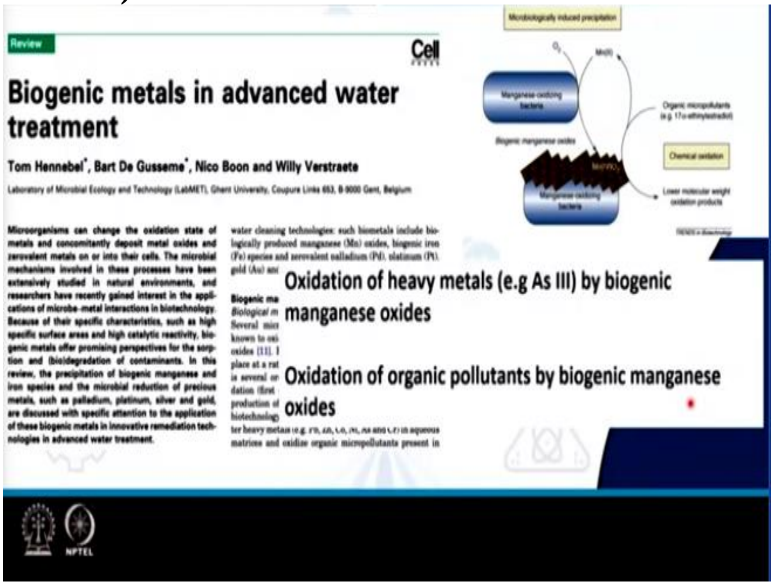
Newsome et al 2014, Chemical geology, 363 : 164-184

Similarly, the uranium reduction ability of the microbes, because I have discussed in detail about uranium reduction that is also used significantly in bioremediation of uranium contaminated groundwater, in different subsurface environments, particularly the USDA sites. And as you can see, there are 2 types of systems in one system the indigenous microorganisms who are they are stimulated to reduce uranium because uranium can be used as electron acceptor and by providing a suitable carbon and electron source like acetate.

They acetate are injected, so you have an injection well, so the injection well basically in the underground environment, these carbon electron donors like acetate is injected and utilizing that the microbes are able to precipitate uranium and these are insoluble uranium. Now, uranium when it is reduced, it will be precipitated but the reduced uranium maybe re oxidized to uranium 6 again following cessation of the organic carbon injection accompanying subsequent delivery of oxidants like oxygen nitrate and either Fe 3+ in the etcetera.

So, in order to reduce the chances of reoxidation of uranium from the reduced from to oxidize form, the scientists they found another innovative way that if they are provided with Fe 3, then Fe 3 might be reduced to Fe 2 and the ability to produce these iron sulfides or iron minerals will effectively actually create a kind of a low permeability conditions or coatings and these low permeability conditions will actually stabilize this uranium allowing it to remain in the solid phase. So, in a sense will immobilise the uranium 4, so otherwise it would have moved out.

(Refer Slide Time: 56:03)



Review

Cell

Biogenic metals in advanced water treatment

Tom Hennebel*, Bart De Gusseme*, Nico Boon and Willy Verstraete

Laboratory of Microbial Ecology and Technology (LabMET), Ghent University, Coupure Links 653, B-9000 Gent, Belgium

Microorganisms can change the oxidation state of metals and concomitantly deposit metal oxides and zerovalent metals on or into their cells. The microbial mechanisms involved in these processes have been extensively studied in natural environments, and researchers have recently gained interest in the applications of microbe-metal interactions in biotechnology. Because of their specific characteristics, such as high specific surface areas and high catalytic reactivity, biogenic metals offer promising perspectives for the sorption and (bio)degradation of contaminants. In this review, the precipitation of biogenic manganese and iron species and the microbial reduction of precious metals, such as palladium, platinum, silver and gold, are discussed with specific attention to the application of these biogenic metals in innovative remediation technologies in advanced water treatment.

water cleaning technologies: such biometals include biologically produced manganese (Mn) oxides, biogenic iron (Fe) species and zerovalent palladium (Pd), platinum (Pt), gold (Au) and

ter heavy metals (e.g. [illegible]) in aqueous matrices and oxidize organic micropollutants present in

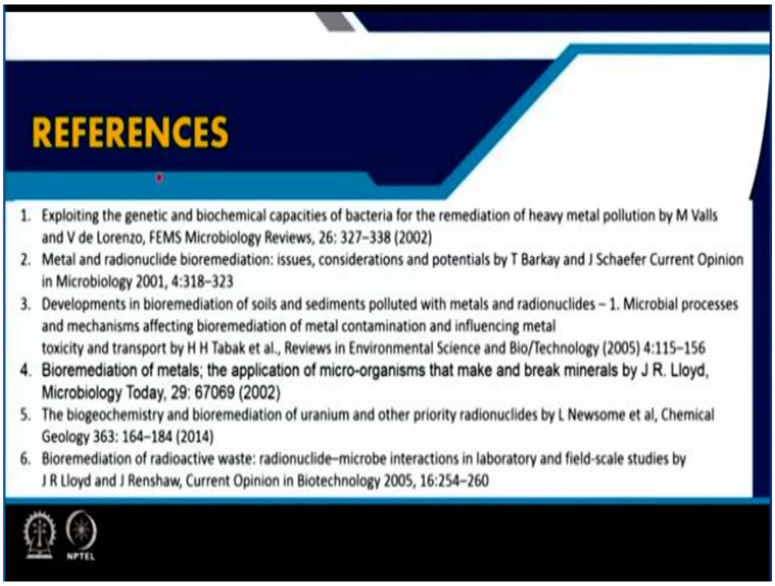
Oxidation of heavy metals (e.g As III) by biogenic manganese oxides

Oxidation of organic pollutants by biogenic manganese oxides

Now, the last point in today's lecture is the biogenic metals because in my summary slide I showed that the microbes can actually oxidize metals like manganese and produce manganese oxide minerals. Now, these manganese oxide minerals which are called biogenic minerals can be utilized in treating waste water and water particularly when they are having emerging pollutants including some of the estradiol or other pollutants and they can also allow removal of arsenic like arsenic 3.

Because oxidation of arsenic 3; can be possible by these biogenic manganese oxides or oxidation of organic pollutants even in micro quantity can be possible by biogenic manganese oxides. So, in recent time we see increase in research interest is shown towards developing bioremediation technologies using these kinds of biogenic minerals for with manganese or even iron.

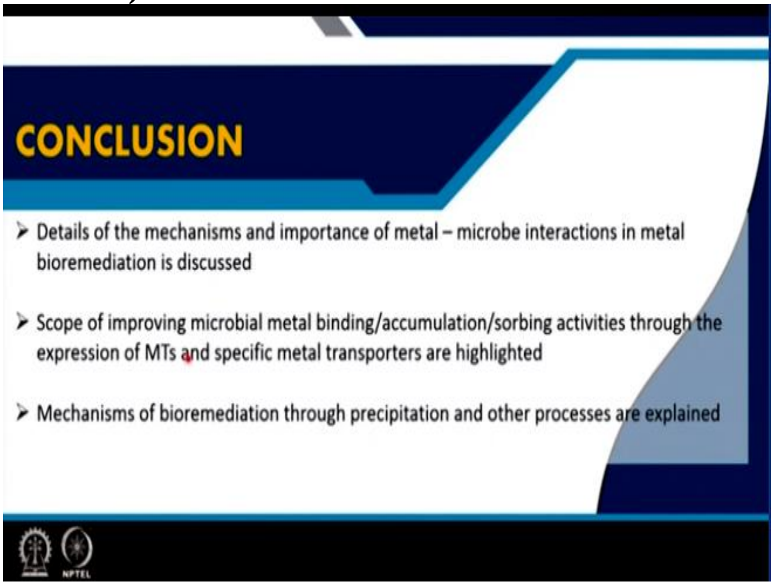
(Refer Slide Time: 57:06)



REFERENCES

1. Exploiting the genetic and biochemical capacities of bacteria for the remediation of heavy metal pollution by M Valls and V de Lorenzo, FEMS Microbiology Reviews, 26: 327–338 (2002)
2. Metal and radionuclide bioremediation: issues, considerations and potentials by T Barkay and J Schaefer Current Opinion in Microbiology 2001, 4:318–323
3. Developments in bioremediation of soils and sediments polluted with metals and radionuclides – 1. Microbial processes and mechanisms affecting bioremediation of metal contamination and influencing metal toxicity and transport by H H Tabak et al., Reviews in Environmental Science and Bio/Technology (2005) 4:115–156
4. Bioremediation of metals; the application of micro-organisms that make and break minerals by J R. Lloyd, Microbiology Today, 29: 67069 (2002)
5. The biogeochemistry and bioremediation of uranium and other priority radionuclides by L Newsome et al, Chemical Geology 363: 164–184 (2014)
6. Bioremediation of radioactive waste: radionuclide–microbe interactions in laboratory and field-scale studies by J R Lloyd and J Renshaw, Current Opinion in Biotechnology 2005, 16:254–260

(Refer Slide Time: 57:11)



CONCLUSION

- Details of the mechanisms and importance of metal – microbe interactions in metal bioremediation is discussed
- Scope of improving microbial metal binding/accumulation/sorbing activities through the expression of MTs and specific metal transporters are highlighted
- Mechanisms of bioremediation through precipitation and other processes are explained

So, for this lecture, these following references can be used. And in conclusion, in todays lecture the details of the mechanisms and importance of metal microbe interactions in metal bio remediation is discussed. Scope of improving microbial metal binding, accumulation, sorbing capacities through the expression of metallothionein and specific metal transporters are highlighted. Mechanisms of bioremediation through precipitation and other process are explained.