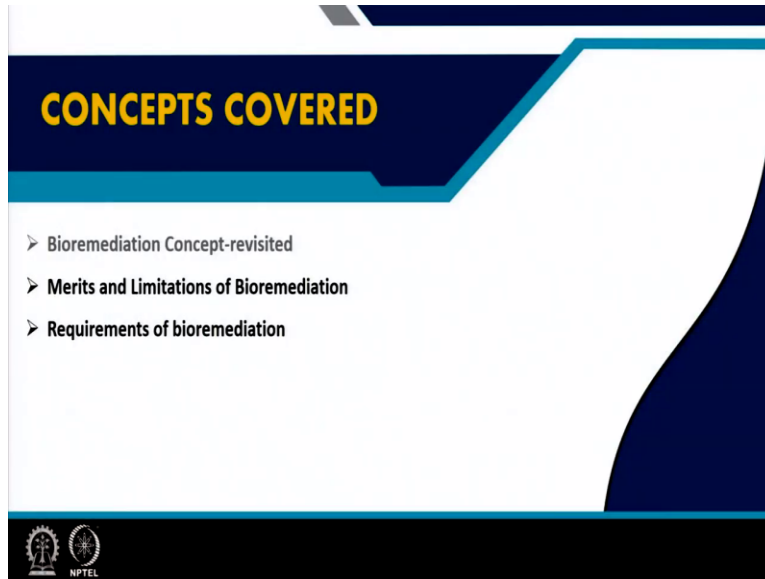


Environmental Biotechnology
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Lecture – 36
Bioremediation (Contd.,)

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Welcome to the next lecture of this course environmental biotechnology and in this particular lecture we are going to discuss about bioremediation. These following concepts are going to be covered in this particular lecture we will have a quick recap on the bioremediation concept followed by the merits and limitations of the bioremediation processes so far practiced and you just investigated will be discussed and then the different requirements of bioremediation will be highlighted.

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Bioremediation: Pollution treatment technology that uses microbial metabolic processes to reduce, eliminate, contain, or transform various contaminants present in soils, sediments, water, or air to benign products

Metabolic strategies; Detoxification mechanisms

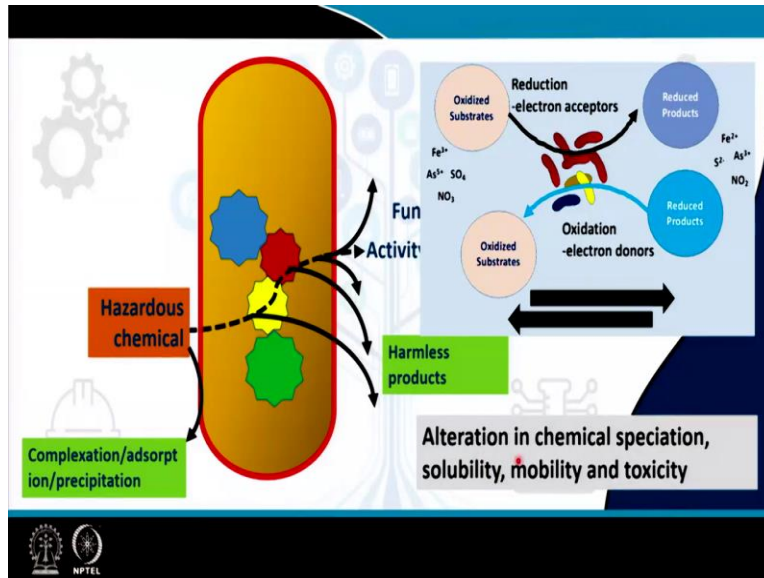
- Organic compounds : C and e donor
- Heavy metals : e donor, acceptor, nutrient
- Other inorganic (N, P, S) : nutrient, e donor, acceptor
- Detoxification strategies
- Co metabolism

The slide features a presenter in a blue and white checkered shirt in the bottom right corner. The background includes faint icons of a microscope, a beaker, and a gear. The NPTEL logo is visible in the bottom left corner.

So, before we begin the definition of bioremediation once more it is the pollution abatement technology or pollution treatment technology that uses microbial metabolic processes to reduce eliminate content or transforms various contaminants present in soil sediments water or air to benign products. And since microbial metabolic processes are the integral part of this bioremediation process.

The metabolic strategies as well as the detoxification strategies or detoxification mechanisms which are involved in bioremediation processes are as follows like the organic compounds are often used as the carbon electron donor heavy metals electron donor electron acceptor and various nutrients other inorganic compounds like nitrogen phosphorus sulfur etcetera are for nutrients or may be for electron donor and acceptor. Various detoxification strategies and co-metabolism related processes are also involved.

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So, as we look into these diagram different hazardous chemicals which are the contaminants or the pollutants of concern are used by microorganisms and they are processed through their different metabolic pathways or metabolic processes. And that metabolic or collection of the metabolic processes will eventually facilitate the bacterial or the microbial activity and microbial growth and there will be different functional outcome with respect to the environmental fate and environmental toxicity of the hazardous chemicals.

Now what we observed that most of the cases the pollutant molecule is utilized or metabolized by the microorganisms and they are converted into different harmless products. Otherwise some chemical compounds are not metabolized directly by the cells because cell might not be able to extract any benefit from them and they are also converted or they are also transferred to some other form through different complexation and adsorption precipitation like mechanisms.

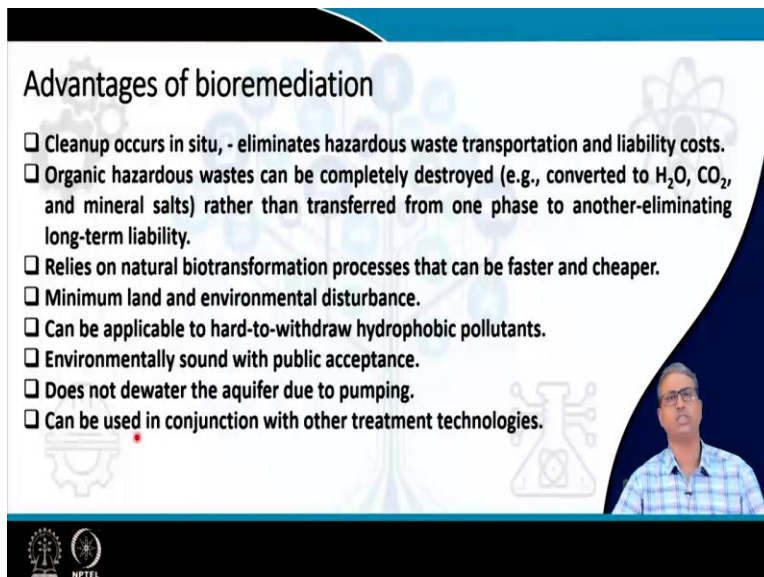
Eventually both these processes like the metabolic transformation of the compounds or the complexation type of reactions or external precipitation of the chemical compounds both facilitate the concentration the chemical structure or the toxicity or environmental mobility of the hazardous compounds which are which are of concern from the bioremediation point of view and therefore all these processes are of very, very much importance.

Now if we look into this processes we are going to discuss today in little bit more detail that

most of these metabolic processes that we are trying to implicate or trying to link while the hazardous chemicals are converted to harmless products are basically oxidation reduction type reactions. And when we consider these oxidation reduction type reactions we often understand that many of these oxidized substrates are used as electron acceptors particularly during the terminal electron acceptor process which is mediated by the electron transport chain.

And at the same time some compounds or some elements are also used as electron donors and they are oxidized and resulting into the different oxidized forms of the same substrate. So both these redox mediated processes basically lead to the alteration in chemical speciation their chemical structure their solubility mobility and environmental toxicity.

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Advantages of bioremediation

- Cleanup occurs in situ, - eliminates hazardous waste transportation and liability costs.
- Organic hazardous wastes can be completely destroyed (e.g., converted to H₂O, CO₂, and mineral salts) rather than transferred from one phase to another-eliminating long-term liability.
- Relies on natural biotransformation processes that can be faster and cheaper.
- Minimum land and environmental disturbance.
- Can be applicable to hard-to-withdraw hydrophobic pollutants.
- Environmentally sound with public acceptance.
- Does not dewater the aquifer due to pumping.
- Can be used in conjunction with other treatment technologies.

The slide features a blue header and footer, a white background with faint chemical icons, and a small inset video of a man in a blue plaid shirt in the bottom right corner. The NPTEL logo is visible in the bottom left corner.

Now before we go into the details of these processes particularly the metabolic engagements which are found to be very critical for achieving the bioremediation some of the major advantages of bioremediation are discussed. So one of the first and foremost advantage is the cleanup or the remediation process when it is used bioremediation is used it can be done in situ that eliminates the hazardous waste transportation and liability cost and also reduces the dispersion of the polluted materials or the pollutants.

Organic compounds organic pollutants can be completely destroyed that is to convert it to they can be converted to water or carbon dioxide and mineral salts rather than transferring from one

phase to another phase and therefore eliminating the long term reliability of those compounds. In today's time we also see that there is a great interest in converting this hazardous waste into some metabolites rather than only to convert into water and carbon dioxide.

Because those metabolites which are produced by the microbes during their degradation of the conversion of the pollutant molecules add to the product portfolio that can be that can be enriched through these processes and for example the biorefinery concept which where we where we convert the pollutants into molecules or the chemical compounds useful within the bioenergy market.

Similarly different kind of other molecules which; are useful for bioplastic and different other materials of industrial significance. Bioremediation has another very important advantage which is the it relies on natural biotransformation processes and many a cases the it is basically the natural catalytic ability of the organisms which are faster and also often found to be more cheaper and minimum land and environmental disturbance because either it is in situ. So, if that in the top of the soil we have some waste contaminated site it can be applied directly or if it is underground then the appropriate methodology can be adopted so, that the above ground activities will continue.

It can be applicable to hard to withdraw hydrophobic pollutants present in the underground system and it has very sound application and public acceptance does not de-water the aquifer due to pumping when it is applied to subsurface contamination for compared to physical or chemical treatments the bioremediation processes they do not require dewatering of the aquifer. And finally it can be used in conjunction with other treatment technologies.

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Disadvantages

- Certain wastes, such as heavy metals, are not eliminated by biological processes (although many metals can be bioreduced or biooxidized to less toxic and less mobile forms).
- It may require extensive monitoring.
- Requirements for success and removal efficiency may vary considerably from one site to another.
- Some contaminants can be present at high concentrations that inhibit microorganisms.
- Can be a scientifically intensive technique.
- There is a risk for accumulation of toxic biodegradation products.

The slide features a blue header and footer. The footer contains the NPTEL logo and a small inset video of a man in a blue plaid shirt speaking. The background of the slide has faint icons of a gear, a beaker, and a molecular structure.

Now some of the major disadvantages are also identified which starts with it the fact that the contaminants like heavy metals cannot be eliminated by biological process. But many metals can be bio reduced that is biologically reduced or biologically oxidized to less toxic and less mobile forms. Bioremediation process that they may require extensive monitoring requirements for success and removal efficiency may vary considerably from one side to another.

So, which is actually considered to be a very important constraint for the application and widespread application of the bioremediation process it is it is more like kind of a local locally optimized process because it is largely dependent on microbial activity. So, microbial activities are highly controlled by local environmental conditions. Some contaminants can be present at high concentration that inhabits innovative microorganisms.

So, contaminant concentration overdosing can be a matter of concern and also can be a scientifically intensive process. So, we need to have a very strong scientific understanding on the process. There is a risk for accumulation of toxic biodegradation products in case of some pollutants.

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Biocatalysis/Biodegradation Database

Lists of

- 219 pathways
- 1495 reactions
- 1386 compounds
- 984 enzymes
- 534 microorganism entries
- 250 biotransformation rules
- 50 organic functional groups
- 76 reactions of naphthalene 1,2-dioxygenase
- 109 reactions of toluene dioxygenase

<http://eawag-bbd.ethz.ch/index.html>

The screenshot shows the website interface with a navigation menu on the left and a main content area on the right. The main content area includes a search bar, a list of statistics, and a section for 'Microbial biocatalytic reactions and biodegradation pathways'. A small video inset shows a man speaking.

Nevertheless in the past two to three decades extensive research and development have been reported and we can identify certain other aspects apart from the organisms or systems developed; that is this kind of database where the required information about the catalysis or the biodegradation processes are documented very well. So, here is the database detail for one of those databases.

So, we can see that a large number of pathways reactions compounds microorganisms involved in them different bio transformation rules etc are all available publicly. So, so this is this is one of the very good things.

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UM Biocatalysis/Biodegradation Database

Group 1 2 3 9 10 11 12 13 14 15 16 17 18

Period

Period	1	2	3	9	10	11	12	13	14	15	16	17	18					
1	H												He					
2	Li	Be						B	C	N	O	F	Ne					
3	Na	Mg						Al	Si	P	S	Cl	Ar					
4	K	Ca	Sc	Ti	V	Cr	Mn	Fe	Co	Ni	Cu	Zn	Ga	Ge	As	Se	Br	Kr
5	Rb	Sr	Y	Zr	Nb	Mo	Tc	Ru	Rh	Pd	Ag	Cd	In	Sn	Sb	Te	I	Xe
6	Cs	Ba	Lu	Hf	Ta	W	Re	Os	Ir	Pt	Au	Hg	Tl	Pb	Bi	Po	At	Rn
7	Fr	Ra																
Lanthanoids				La	Ce	Pr	Nd	Pm	Sm	Eu	Gd	Tb	Dy	Ho	Er	Tm	Yb	
Actinoids				Ac	Th	Pa	U	Np	Pu	Am	Cm	Bk	Cf	Es	Fm	Md	No	

Key | Biologically relevant elements colored

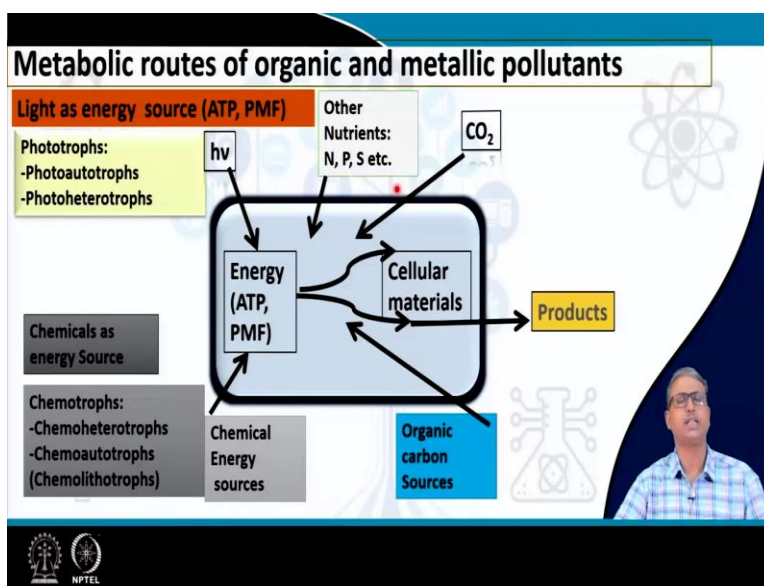
- Major, essential, all life
- Major among, all life
- Major cations, all life
- Essential, trace, all life
- Major biological transition metals
- Specialized uses, some life
- May be bound, transported, reduced, and/or methylated
- Smart or unknown biological function

The screenshot shows a periodic table where elements are color-coded according to their biological relevance. A key explains the color coding: yellow for major essential elements, orange for major cations, green for essential trace elements, blue for major biological transition metals, and brown for specialized uses. The periodic table includes elements from Hydrogen to Oganesson, with Lanthanoids and Actinoids shown separately at the bottom.

In this database there is also very interesting document which suggests that the how the different elements present in the periodic table are can be identified or categorized with respect to their ability to interact with microorganisms. Like you can see that some of the elements highlighted with yellow are the major for essential life form. The pink and red are major cations for all life forms and the orange one is essential as a trace like form.

So, it is established fact that microorganisms in particular they have known mechanisms with respect to enzymes with respect to genes to interact with these elements.

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Now with respect to the metabolic roots applied with respect to different organic and metallic pollutants we can we can explain the entire process through the energy and other nutrient requirement systems. So, every microbial cells require energy and along with energy they need carbon and other nutrients. So, this energy which can be in the form of ATP, so, energy will lead to the formation of ATP our will generate proton motive force when it is transferred to the the membranes. So, it can be obtained through different chemicals and when we have different chemicals as energy sources there could be chemotrophs or there could be heterotrophic organisms or there could be autotrophic organisms.

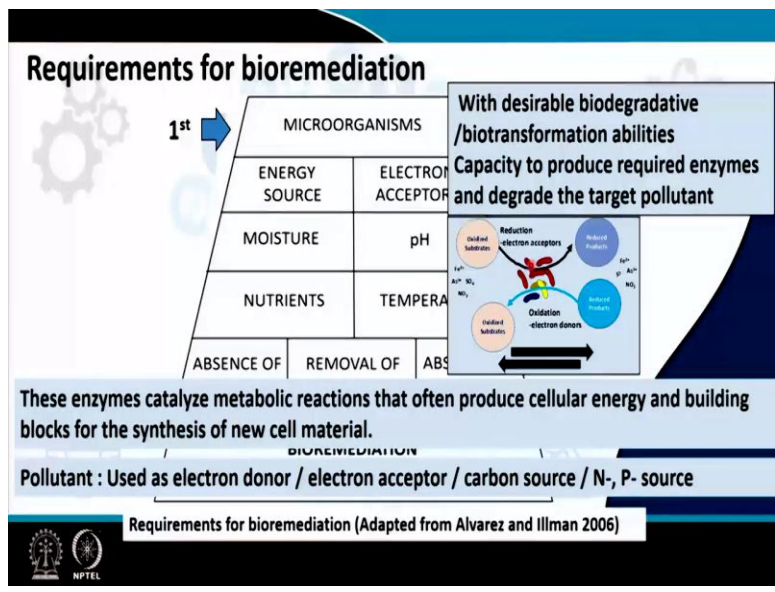
Alternatively light can also be used as energy source there are many organisms bacteria and algae microalgae who are were very efficient in trapping the light energy and therefore they gain

the energy from different sources chemical source of energy or the light source of energy. Several other forms of nutrients other nutrients nitrogen phosphorous sulphur they are also required. Carbon source is another very important requirement for the organisms. Now carbon can be supplied or obtained as organic carbon or it can be inorganic carbon particularly the carbon dioxide or the bicarbonate.

Now when all these required things are satisfied like the energy like the nitrogen and phosphorous, sulphur other trace elements carbon particularly then the cells start utilizing them and cell grow as the cells they start growth. So, basically through the production of the experienced cellular macromolecules different products are released into the environment. So, during the process of bioremediation we see these microbial energy and particularly energy and carbon metabolism reactions but also nitrogen and phosphorous sulphur metabolism reactions.

They play a central role in converting the toxic or hazardous compounds into the harmless or relatively less toxic compounds.

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Now with respect to the requirements for bioremediation we can identify a series of factors the first and foremost factor is the presence of the microorganisms because they are the catalyst this is these organisms. So, the any contaminated site or any bioremediation program should have the microorganisms in its priorities we must have microorganisms. Now these microorganisms

should be capable of taking care of the desirable biochemical or biodegradable transformation.

And they have the capacity to produce the required enzymes that degrade or that transform the target pollutant. Now with respect to this we have already seen this kind of transformation reactions. Now the enzymes involved in these transformation processes are one of the most essential criteria. Now the enzymes which catalyze the metabolic reactions that often produce cellular energy and building blocks for the synthesis of new material.

Now the enzymes which are present by in the in microorganisms they utilize these substrates like the pollutant molecules and either oxidize them or often reduce them also and they try to make use of them as their source of energy and that source of energy is used further to produce the ATP molecule which is useful for the cell function and also for the precursor molecules which are required for the synthesis of different macromolecules.

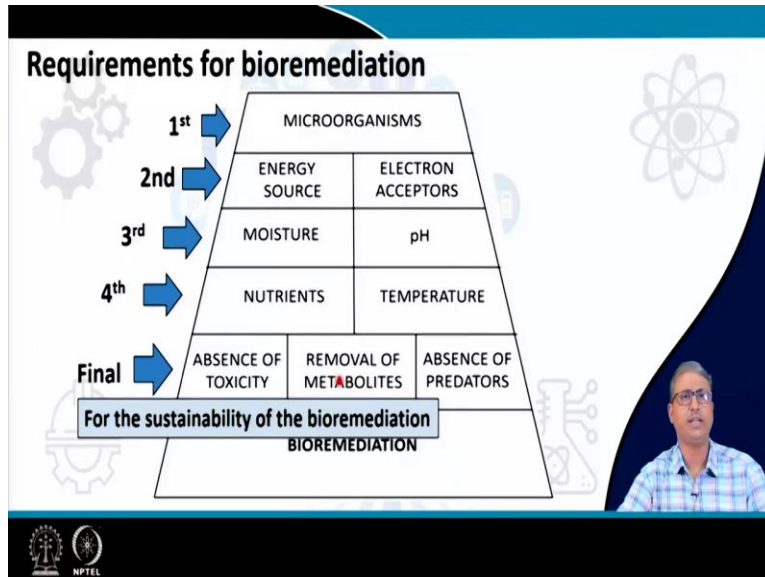
Now in that sense the pollutant molecules are basically used as metabolic resource because these are or multiple pollutants are used as multiple resources. So, for example different ions which are or the or the elements which are toxic at the same time but they can be used as electron donor for example the arsenic or the the ammonia or the nitrite they can be used as electron donor sulfides can be used as electron donors.

Whereas sulphate, arsenide, nitrate they can be used as electron acceptor and different type of organic compounds particularly the whole range of organic polymers the aromatic compounds they are and also the non aromatic compounds or aliphatic compounds the carbohydrate pollutants are also used as carbon source however for that the required enzymes are essential. So, if the microorganisms they contain the required enzymes.

So, they will be able to either oxidize this pollutant molecule con considering the pollutant molecule as a carbon source as a electron donor or they may reduce the pollutant molecule considering them as terminal electron acceptor sometimes the pollutants are also used as nitrogen and phosphorous sources. However, all these processes will be carried out by only the microorganisms who are capable of doing that. So, presences of such microorganisms are going

to be one of the first criteria.

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So, the second criteria would be the energy source and electron acceptor. As we have already seen many times the pollutants themselves they act as energy source or they may act as a electron acceptor but it may not be true for all the cases like organic carbon may be polluted like petroleum hydrocarbons could be excellent electron donors they can be energy source also but they cannot be electron acceptor.

So, you need to have supply of electron acceptor. Similarly there are situations where we have the pollutant itself is electron acceptor like the sulfate in a low pH acid mine drainage environment sulphate is the target pollutant. So, sulphate is acting would be acting as electron acceptor but who will be the carbon source who what will be the electron donor or the energy source, so, that those things need to be satisfied.

Now all these processes where the microorganisms survive and microorganisms produce the enzymes and make use of the electron source energy source electron etc. They need the appropriate pH, moisture and other conditions which are essential for them. Now apart from these three the fourth criteria would be the supply of different other nutrients. Because apart from the electron donor electron acceptor and carbon energy sources the microorganism would be requiring other essential nutrients like carbon like nitrogen phosphorous sulphur different trace

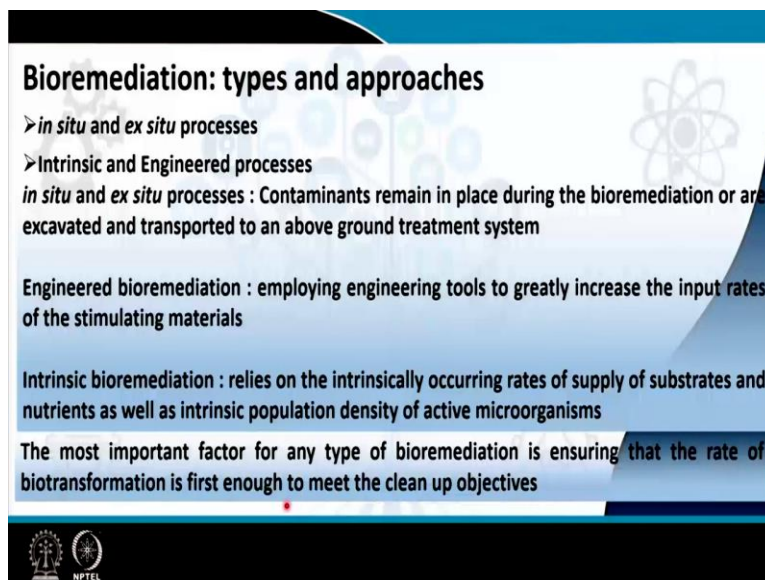
elements and other macro elements.

So, those supply of those essential nutrients must be there and also the temperature regime should not be exceeding the tolerance or the or the limit that the that is that is good enough for the microorganism if the temperature is extreme then possibly the organisms would not be able to function properly finally the absence of toxicity of the compounds. So, the level that the concentration of the particular pollutants which are present or the pollutants who are present there is a matter of great concern.

Absence of toxicity is required removal of metabolites that means during the biotransformation or metabolic transformation of different pollutants different metabolites would be produced. These metabolites might interfere with the microbial community microbial abilities and microbial behaviour. So, those metabolites should be consumed and also the absence of predators. Useful microorganisms may not be attacked by other predating microorganisms.

So, essentially this final layer helps us to achieve the sustainability of the bioremediation process.

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Bioremediation: types and approaches


- *in situ* and *ex situ* processes
- Intrinsic and Engineered processes

in situ and *ex situ* processes : Contaminants remain in place during the bioremediation or are excavated and transported to an above ground treatment system

Engineered bioremediation : employing engineering tools to greatly increase the input rates of the stimulating materials

Intrinsic bioremediation : relies on the intrinsically occurring rates of supply of substrates and nutrients as well as intrinsic population density of active microorganisms

The most important factor for any type of bioremediation is ensuring that the rate of biotransformation is first enough to meet the clean up objectives



Now we will discuss briefly about the types and approaches of bioremediation. So, there are generally two types of bioremediation processes one is called in situ and other is the ex situ. In

case of in situ the contaminants remain in place wherever it is and the bioremediation processes are implemented there whereas during the ex situ process the pollutants are excavated transported to a different facility and they are only they are treated. There are also two types of processes based on the based on the system that are being adopted these are intrinsic and engineered process.

So, engineered by remediation I will talk first where we employ engineering tools which greatly increase the input rates of the stimulating material many a times we see that the bioremediation process requires some supply of the nutrients supply of the electron donor or supply of the electron acceptor. So, in those case cases how we are managing to supply these things these essential nutrients etc through different engineering approaches those are called engineered bioremediation.

The intrinsic bioremediation basically relies on the intrinsically occurring rates of supply of substrate and nutrients. In many cases we see that the aquifers or other contaminated environments are naturally capable of supplying the necessary energy sources necessary nutrient sources and the rates are appreciable with respect to the desirable degradation efficiencies. So, in that case we just rely on the native microbial activities only we have the monitoring system implemented there.

Now the most important factor for any type of bioremediation is ensuring that the rate of biotransformation is first enough to meet the cleanup objectives.

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Bioremediation strategies

in situ and *ex situ* processes :

Contaminants remain in place during the bioremediation

or

are excavated and transported to an above ground treatment system

Fig. 23.2 Flow diagram of bioremediation strategies Sar and Islam, 2013

Now as I was mentioning about the ex situ and in situ processes.
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Bioremediation strategies

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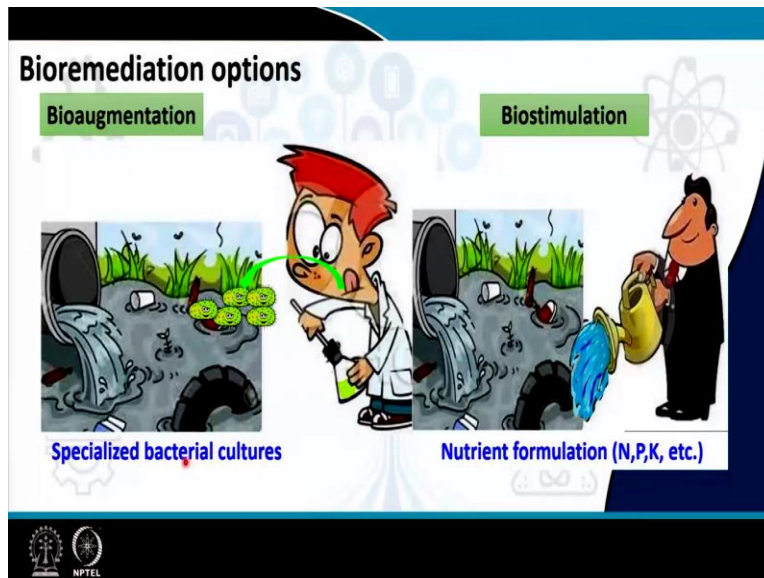
graph TD
    Bioremediation --> In_situ[In situ]
    Bioremediation --> Ex_situ[Ex situ]
    In_situ --> Intrinsic
    In_situ --> Engineered_In_situ[Engineered]
    Ex_situ --> Engineered_Ex_situ[Engineered]
    Intrinsic --> Biostimulation
    Intrinsic --> Bioaugmentation
    Intrinsic --> Biobarrier
    Engineered_In_situ --> Biostimulation
    Engineered_In_situ --> Bioaugmentation
    Engineered_In_situ --> Biobarrier
    Engineered_Ex_situ --> Composting
    Engineered_Ex_situ --> Bioreactor
    Engineered_Ex_situ --> Land_farming[Land farming]
    
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Fig. 23.2 Flow diagram of bioremediation strategies Sar and Islam, 2013

Engineered bioremediation : employing engineering tools to greatly increase the input rates of the stimulating materials

Intrinsic bioremediation : relies on the intrinsically occurring rates of supply of substrates and nutrients as well as intrinsic population density of active microorganisms

So, there are very well characterized plan for this ex situ in situ system like for in situ process we have the intrinsic or engineered. For engineered we have different type of approaches and for ex situ engineered also we have different type of approaches.
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Now, coming to this two important approaches which are the bio augmentation and bio stimulation, bio augmentation is a very specific approach where specialized bacterial cultures which are identified often they are isolated or enriched as a mixed culture or a very pure culture they are grown in the laboratory they are characterized they are very efficient organisms they are grown in bulk and then added into the contaminated site.

So, these are specialized bacterial cultures. So, they can be pure culture of individual bacterial strains or they can be mixed culture but they are a very, very potent degrader or transformer. So, they are most suitable for the bioremediation process and the other alternative is that the different type of nutrient formulations nutrient compounds like nitrogen phosphorous potassium etcetera are added into the contaminated site.

Now how we are going to take a decision that whether the site has to be bio augmented or biostimulated that depends upon the information that we have about the particular site in a particular waste site or effluent if we see that the microorganisms are deficient there are not enough capable microorganisms. Of course through a microbial ecology study we try to try to find the answer of this question.

If the answer of the question is negative that no there is no enough capable microorganisms then we need to actually bioaugment. Otherwise if we see that microorganisms are already present

there but the performance of those bio microorganisms in terms of bio transforming the pollutants are low possibly because of the lack of nutrients essential nutrients like nitrogen phosphorus etcetera. Then we adopt the biostimulation process.

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Bioremediation options

Bioaugmentation **Biostimulation**

Biostimulation :
The intentional stimulation of resident bacteria to remediate the target chemicals by the addition of nutrients, water, electron donors and acceptors, etc.

Bioaugmentation : •
Introduction of microorganisms that have appropriate degradative abilities

The slide features two cartoon characters: a boy with red hair on the left and a man in a suit on the right. They are standing in front of two identical scenes of a polluted area with a large green plant growing from a hole in the ground. The boy is pointing towards the plant, and the man is holding a yellow bag, possibly representing the addition of nutrients or microorganisms. The background is a light blue gradient with faint icons of a tree, a gear, and a chemical structure.

So biostimulation is the intentional stimulation of the resident bacteria to remediate the target chemical by addition of nutrients like water electron donor electron acceptor. Whereas the bio augmentation is the introduction of microorganisms that have appropriate degradative ability.

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Intrinsic Bioremediation

- Intrinsic bioremediation occurs *in situ* and relies on naturally occurring biological processes carried out by indigenous microorganisms
- This is a component of natural attenuation, which includes physical and chemical processes
- Cleanup activities that rely on natural attenuation to reduce contaminant levels and monitoring to determine the remedial effectiveness are referred to as “monitored natural attenuation.”

The slide features a small inset video of a man in a blue and white checkered shirt speaking. The background is a light blue gradient with faint icons of a tree, a gear, and a chemical structure.

Intrinsic bioremediation which occurs in situ and relies on naturally occurring biological processes carried out by indigenous micron. In case of intrinsic remediation we do not add any

kind of neither microorganism nor different chemicals this is a component of the natural attenuation which includes physical and chemical processes are a natural process. Cleanup activities that rely on natural attenuation to reduce the contaminant levels monitoring is a very determinative stage.

So, in order to have this intrinsic bioremediation monitoring of the sites is considered to be very very important.

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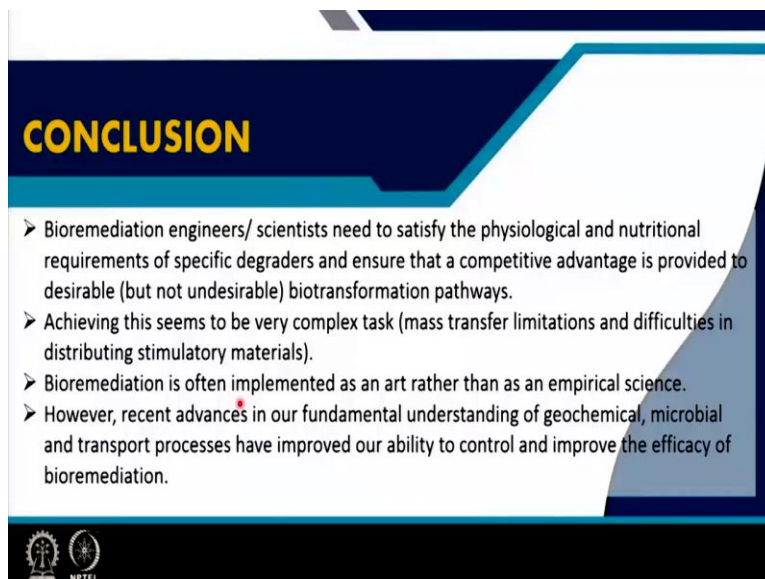


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CONCLUSION

- Bioremediation engineers/ scientists need to satisfy the physiological and nutritional requirements of specific degraders and ensure that a competitive advantage is provided to desirable (but not undesirable) biotransformation pathways.
- Achieving this seems to be very complex task (mass transfer limitations and difficulties in distributing stimulatory materials).
- Bioremediation is often implemented as an art rather than as an empirical science.
- However, recent advances in our fundamental understanding of geochemical, microbial and transport processes have improved our ability to control and improve the efficacy of bioremediation.

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So, for this lecture the following references are to be used and in conclusion we have we have

discussed some of the important aspects of bioremediation including the advantages disadvantages and how the bioremediation process is linked to the microbial metabolism. Essentially the bioremediation engineers and scientists need to satisfy the physiological and nutritional requirement of specific degraders or degrading organisms.

And ensure that the competitive advantage is provided to desirable biotransformation pathway achieving this that we allow the of candidate organisms to flourish seems to be very complex task not only because of microbial community composition which remains many a times not properly known. But even after knowing that also mass transfer limitations and difficulties in distributing stimulatory material sometimes are of concern.

Bioremediation is often implemented as an art rather than an empirical science. However recent advances in our fundamental understanding of multiple parameters including the geochemistry and microbial transport processes enzymatic processes their genetics have improved our ability to control and improve the efficacy of bioremediation, thank you very much.