ENVIRONMENTAL MICROBIOLOGY

Bioremediation

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Introduction

As is clear from the word itself 'bioremediation' (bio + remediation) should involve two components the bio i.e. the live component and remediation i.e. the treatment of the contaminant. The term denotes the existence of some contaminant in the matrix which is to be remediated. Therefore, before going into the classical definitions of bioremediation let us discuss what do we need to remediate and why. The answer we will soon find once we begin to analyze the present state of the environment.

Present state of our environment

Rapid industrialization and urbanization over the past many decades has resulted in contamination of all the components of the environment that is the air, the water, the soils and even our food. The process began with the generation of a plethora of synthetic organic compounds for use as solvents, pesticides, refrigerants and chemical intermediates etc. The contamination of the environment has also occurred through a variety of industrial operations like fugitive emissions, accidental spills and leaks, discharge of effluents or dumping of waste. Over the years indiscriminate use of the synthetic chemicals has released several such organic contaminants which are recalcitrant to natural degradation and may also turn hazardous or toxic. Even if released in the sewers, the compounds come back to soil system and the food chain through contaminated sludge. Nevertheless, the next question is why we should be worried about such pollutants.

Potential hazards

The hazards posed by the contaminants depend upon the type of contaminant, chemical species, spatial distribution, the concentration and the route of exposure etc. Apart from this, the nature of matrix (for example, hydrogeological characteristics in case of soil) also affect the potential hazards. Broadly the effect could be summarized in two categories

Short-term hazard: Direct contacts, inhalation of toxic dust/fumes or immediate risk of fire/explosion.

Long-term hazard: Resulting by movement to other components of the environment or generation of secondary toxic products. For example, transportation of contaminants through rain water followed by percolation to ground water and contamination of the drinking water source. The situation that we face today is thus alarming and calls for urgent action for ensuring environmental and health safety.

Solutions

As far as the solution to this problem is concerned, there can be two options i.e. prevention or cure. As the common saying insists that "prevention is always better than cure", the former should always be practiced well. The strategy collectively termed "Cleaner Production" also states that prevention of pollution is a better and more effective answer to the current environmental problems. Thus the challenge today is to develop technologies that consume fewer resources, incorporate recycling and reuse of components thereby reducing the production of wastes while maintaining/improving the efficiency. Even if the switching to "cleaner processes" is by and large accepted, we still face the toxicity and environmental persistence of xenobiotic compounds in the transitional phase. Therefore, there is a need to

develop a wide variety of physico-chemical and biological techniques that can remediate the hazardous contaminants from the environment without causing further damage. The conventional techniques of incineration/land-fills etc. basically transfer pollutants from one medium to another, are expensive and energy demanding. Further, these techniques are often inefficient for handling voluminous effluents containing complexing organic matter and low concentration of contaminants. Biotechnological approaches that are designed to cover such niches have, therefore, received great deal of attention in the recent years.

Bioremediation is an attractive and potential alternative for treatment of contaminated sites. Let us now examine in detail what exactly the term bioremediation implies and the host of technologies that this term encompasses.

Principles of Bioremediation

Bioremediation

The word "bioremediation" was coined by scientists in the early 1980s as a term to describe the use of microorganisms to clean polluted soils and waters. The prefix *bio* defined the process as biological that is, carried out by living organisms. The noun remediation defined the process as one that resulted in the cleaning, or remediation, of the environment, via complete degradation, sequestration, or removal of the toxic pollutants as the result of microbial activity. Degradation means that the microorganisms decompose the pollutants to harmless natural products such as carbon dioxide (CO_2), water (H_2O), or other nontoxic naturally occurring compounds. Sequestration means that the pollutant is trapped or changed in a way that makes it nontoxic or unavailable to biological systems. Removal means that while the pollutant is not necessarily degraded, the microbes physically remove it from the soil or water so that it can be collected and disposed of safely.

Thus bioremediation can be defined as the process of using specific microorganisms to transform hazardous contaminants in soil/water to nonhazardous waste products. However, some definitions that give a broader outlook define bioremediation as biological treatment systems to destroy, or reduce the concentration of hazardous waste from contaminated site. Thus some definitions restrict to the use of microbes only while others seem to incorporate all the biological entities such as plants (phtoremediation). Whatever barriers we define, in fact in nature the process of biological remediation involves both plants and microbes and rather the plant-microbe interaction in root zone has a very important role. Nevertheless, in this chapter we will focus on microbial remediation only.

Bioremediation technologies

There are different treatment technologies under bioremediation and before we begin exploring the basics of bioremediation, let us get acquainted with these terms

Intrinsic bioremediation

This is a process whereby the natural microflora and environmental conditions exist for natural attenuation of a pollutant to safe levels within acceptable time frame. Here natural subsurface processes such as dilution, volatilization, biodegradation, adsorption, and chemical reactions with subsurface materials are allowed to reduce contaminant concentrations to acceptable levels. This requires no intervention but just monitoring of the natural process of biodegradation. However, it should not be perceived as “no action” plan as

long term monitoring must be conducted throughout the process to confirm that degradation is proceeding at rates consistent with meeting cleanup objectives.

Compared with other remediation technologies, natural attenuation has several advantages such as less generation or transfer of remediation wastes; less intrusive (as few surface structures are required) and may be applied to all or part of a given site, depending on site conditions and cleanup objectives. Further it may be used in conjunction with, or as a follow-up to, other (active) remedial measures and the overall cost will likely be lower than active remediation.

Biostimulation

This involves injection of specific nutrients at the site (soil/ground water) to stimulate the activity of indigenous microorganisms. Fertilizers and growth supplements are common stimulants. Presence of small amount of pollutant can also act as stimulant by turning on the operons for bioremediation enzymes. Biostimulation can be done *in situ* or *ex situ*.

Bioventing

Similar to biostimulation but it involves venting of oxygen through soil to stimulate growth of natural or introduced microorganisms. Thus bioventing may complement biostimulation as well as bioaugmentation. It is a promising technology that stimulates the natural in situ biodegradation of any aerobically degradable compounds in soil by providing oxygen to existing soil microorganisms. Bioventing typically uses low air flow rates to provide only enough oxygen to sustain microbial activity. Oxygen is most commonly supplied through direct air injection into residual contamination in soil. In addition to degradation of adsorbed fuel residuals, volatile compounds are biodegraded as vapors move slowly through biologically active soil. Bioventing techniques have been successfully used to remediate soils contaminated by petroleum hydrocarbons, nonchlorinated solvents, some pesticides, wood preservatives, and other organic chemicals. This technique shows considerable promise of stabilizing or removing inorganics from soil as it can induce changes in the valence state of inorganics and cause adsorption, uptake, accumulation, and concentration of inorganics in micro or macroorganisms. However, several factors may limit the applicability and effectiveness of the process for example highly saturated soils, extremely low moisture content or low permeability soils negatively affect the bioventing performance. Build up of vapours needs to be avoided by extracting the air followed by monitoring of off-gases at the soil surface. The biggest limitation is that aerobic biodegradation of many chlorinated compounds may not be effective unless a co-metabolite or anaerobic cycle is present.

Bioaugmentation

Addition of pollutant-degrading microorganisms (natural/exotic/acclimatized/genetically engineered) to augment the biodegradative capacity of indigenous microbial populations is termed as bioaugmentation. Sometimes microorganisms from the remediation site are collected, separately cultured, and returned to the site as a means of rapidly increasing the microorganism population at the site. Usually an attempt is made to isolate and accelerate the growth of the population of natural microorganisms that preferentially feed on the contaminants at the site. In some situations different microorganisms may be added at different stages of the remediation process because the contaminants change in abundance as

the degradation proceeds. However there is no evidence to suggest that the use of non-native microorganisms is beneficial in the situations tested.

Biofilters

Use of microbial stripping columns (containing microorganism enriched compost/soil) is to treat organic gases (volatile organic compounds).

Bioreactors

Biodegradation of contaminants in a large tank or reactor. Bioreactors can be used to treat liquid effluents/slurries or contaminated solid waste/soil.

Composting

Composting is aerobic, thermophilic treatment process in which contaminated material is mixed with a bulking agent (compost rich in bioremediation microorganisms). This is a controlled biological process by which organic contaminants (e.g., PAHs) are converted by microorganisms to safe, stabilized byproducts. Typically, thermophilic conditions (54 to 65°C) must be maintained to properly compost soil contaminated with hazardous organic contaminants and in most cases, this is achieved by the use of indigenous microorganisms.

Soils are excavated and mixed with bulking agents and organic amendments, such as wood chips, animal, and vegetative wastes etc. to enhance the porosity of the mixture to be decomposed. Maximum degradation efficiency is achieved through maintaining aeration and moisture as necessary, and closely monitoring moisture content, and temperature.

Basically three different process designs are used in composting:

- Aerated static pile composting where compost is formed into piles and aerated with blowers or vacuum pumps
- Mechanically agitated in-vessel composting where compost is placed in a reactor vessel, mixed and aerated
- Windrow composting where compost is placed in long piles known as windrows and periodically mixed with mobile equipment

Windrow composting is usually considered to be the most cost-effective composting alternative but it may also have the highest fugitive emissions. Pilot and full-scale projects have demonstrated that aerobic, thermophilic composting is able to reduce the concentration of explosives (TNT, RDX, and HMX), ammonium picrate (or yellow-D), and associated toxicity to acceptable levels and is also applicable to PAH-contaminated soil. The substantial requirement of space and aeration coupled with the need for excavation of contaminated soil limit the application of composting. If VOC (volatile organic compounds) or SVOC (semi-volatile organic compounds) contaminants are present in soils, off-gas control may be required. Lastly, this method cannot treat metals and due to addition of amendments ultimately leads to volumetric increase in the amount.

Landfarming

It is a solid phase treatment system for contaminated soil where tilling and soil amendment techniques are used to encourage the growth of beneficial microorganisms in contaminated area. Different conditions that are controlled during land farming are:

- Moisture content (usually by irrigation or spraying).
- Aeration (by tilling the soil with a predetermined frequency)
- pH (buffered near neutral pH by adding crushed limestone or agricultural lime).
- Other amendments (e.g., Soil bulking agents, nutrients, etc.).

It may be done *in situ* or in a treatment cell and has been successfully used to remove large petroleum spills, wood-preserving wastes (PCP and creosote), coke wastes, and certain pesticides in the soil. The large requirement of space, proper management of leachates and prevention of volatile gases are some of the limitations associated with landfarming. Table 1 summarizes the advantages and disadvantages of different types of bioremediation strategies.

Table 1: Advantages and disadvantages of different types of bioremediation strategies

Techniques	**Examples**	**Advantages**	**Limitations**
In situ	*In situ* bioremediation Bioventing Biostimulation Bioaugmentation	Most cost efficient Noninvasive Relatively passive Natural attenuation Treats soil and water	Environmental constraints Extended treatment times Monitoring difficulties
Ex situ	Land farming Composting Biopiles/Biocells	Cost-efficient Can be done on site	Extended treatment time Need to control abiotic loss Mass transfer problem Bioavailability limitation
Bioreactors	Slurry/aqueous reactors	Rapid degradation kinetics Optimized environmental parameters Enhances mass transfer Effective use of inoculants and surfactants	Requires excavation Relatively high capital cost & operating cost

(Source: M. Vidali Bioremediation. An overview Pure Appl. Chem., 73, 1163–1172, 2001)

Is it a new concept?

Around the world bioremediation technologies are categorized as the "innovative technologies". However, this does not mean that it is a novel phenomenon. Actually bioremediation has been going on since the life began on this planet. It is a relatively slow process, but eventually nature has healed itself of all the disturbances. As a contaminant is introduced into the environment, the microbes of the surrounding area get gradually adapted to this changed environment. They begin to elaborate the process of degrading the

contaminant by evolving the ability to use it as a carbon or energy source. During this natural process, the nature caters to the nutritional and physiological needs of the bacteria and the overall process is thus quite slow (Fig. 1). With our fast development we have introduced the contaminants at a staggering high rate and now we need instant cleanup solution that is not within the scope of the natural processes. The natural process can however be speeded up by man-assisted interventions (Fig. 1) as discussed in the earlier section i.e. either by providing favourable conditions or by increasing the number of efficient microorganisms at the contaminated site. However, the things are not as simple as they appear in Fig. 1. After taking a bird eye view of the different bioremediation technologies let us take an insight into what kind of contaminants can be degraded and how does microbe metabolism handle them.

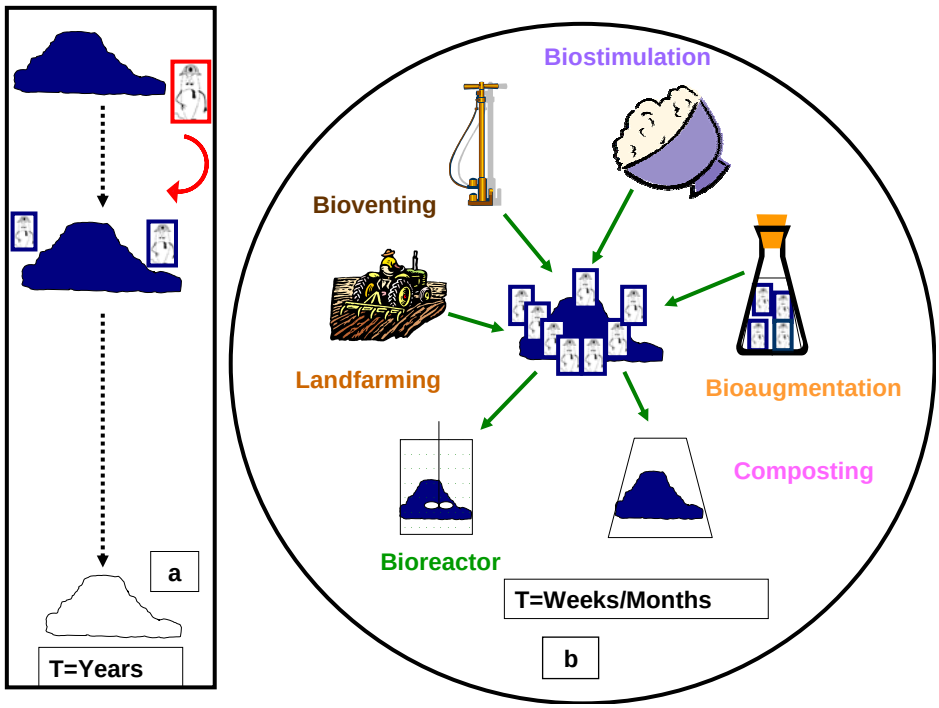


Fig. 1: (a) Natural and (b) Man-Assisted bioremediation

Mechanism of Bioremediation

Broad categories of contaminants

As noticed above, if favourable nutritional and environmental conditions occur, the bacteria are able to readily incorporate the simple organic substances into their cells and oxidize them. However, degradation of complex organic compounds with longer molecular structures is slower. Some compounds are so complex that they cannot be degraded at all, which are termed as recalcitrant or refractory compounds. Still other may be toxic and thus inhibit the growth of microorganisms and their metabolic activity. Such compounds need special techniques or integration of physico-chemical and biological techniques for effective remediation (Fig. 2).

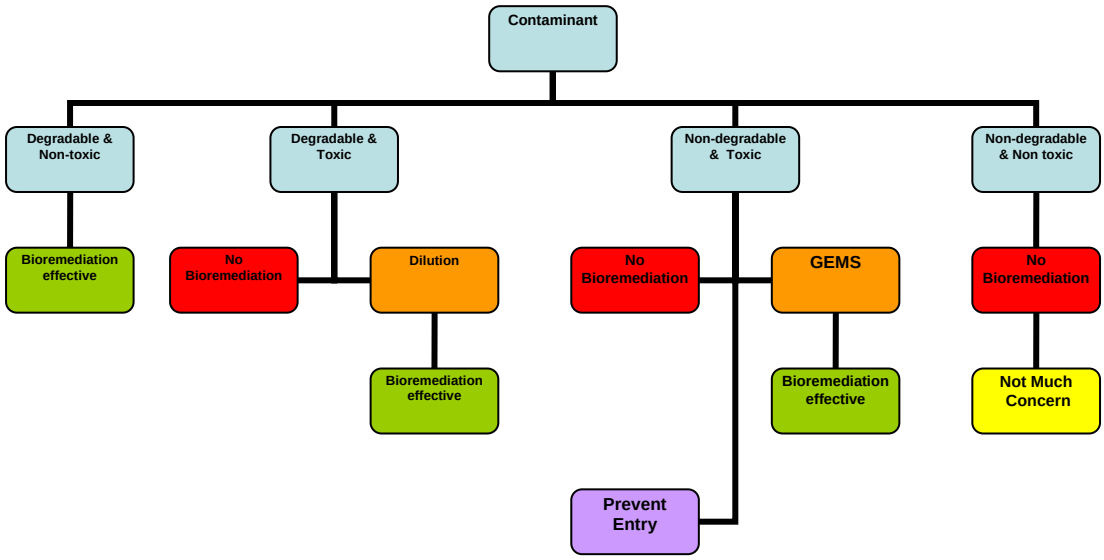


Fig. 2: Bioremediation: Influence of contaminant type

Mechanism of biodegradation

Microbes are the key players in bioremediation as they generate the enzymes that catalyze the degradative reactions. Why the microbes carry out degradative reactions? It is because the microbes use organic substances as a source of carbon and energy. Thus while transforming the contaminant microbes gain energy and raw material for their multiplication and maintenance. Based on the mechanism by which microbes gain energy, they are broadly categorized into three categories (Table 2). However, several xenobiotic contaminants might not be amenable to one of the above described categories and other mechanisms are employed by the microorganisms for degradation of such compounds.

Table 2: Mechanisms of energy generation by the microbes

Mechanism	**Electron donor**	**Electron acceptor**	**Product**
Aerobic respiration	Organic compound	Oxygen	CO_2, H_2O
Anaerobic respiration	Organic compound	NO_3, SO_4, Fe^{3+}, Mn^{4+}, CO_2	N_2, H_2S, CH_4, Reduced metals
Fermentation	Organic compound	Organic compound	Organic acids, alcohols, H_2 & CO_2

Reductive dehalogenation: It plays very important role in the detoxification of halogenated organic contaminants. Microorganisms catalyze a reaction in which halogen atom of contaminant is replaced by hydrogen atom. Thus the reaction adds 2 electrons to contaminant and reduces it. It yields no energy but seems to be detoxification mechanism as dehalogenated derivatives are less toxic and susceptible to further microbial decay.

Cometabolism: In this case also the conversion reaction yields no benefit to the cell. This non-beneficial transformation is often termed as secondary utilization, cometabolism or gratuitous metabolism. Co-metabolism is one form of secondary substrate transformation in which enzymes produced for primary substrate oxidation are capable of degrading the secondary substrate fortuitously, even though the secondary substrates do not afford sufficient energy to sustain the microbial population. It is thus defined as degradation of a compound only in presence of other organic compound that serves as a primary energy source. Several contaminants such as Polynuclear Aromatic Hydrocarbons (PAHs) and pesticides are degraded by this mechanism. An emerging application involves the injection of water containing dissolved primary substrate (e.g. methane, toluene) and oxygen into ground water to support the co-metabolic breakdown of targeted organic contaminants. The addition of methane or methanol supports methanotrophic activity, which has been demonstrated effective to degrade chlorinated solvents, such as vinyl chloride and Trichloroethane (TCE), by co-metabolism (Fig. 3).

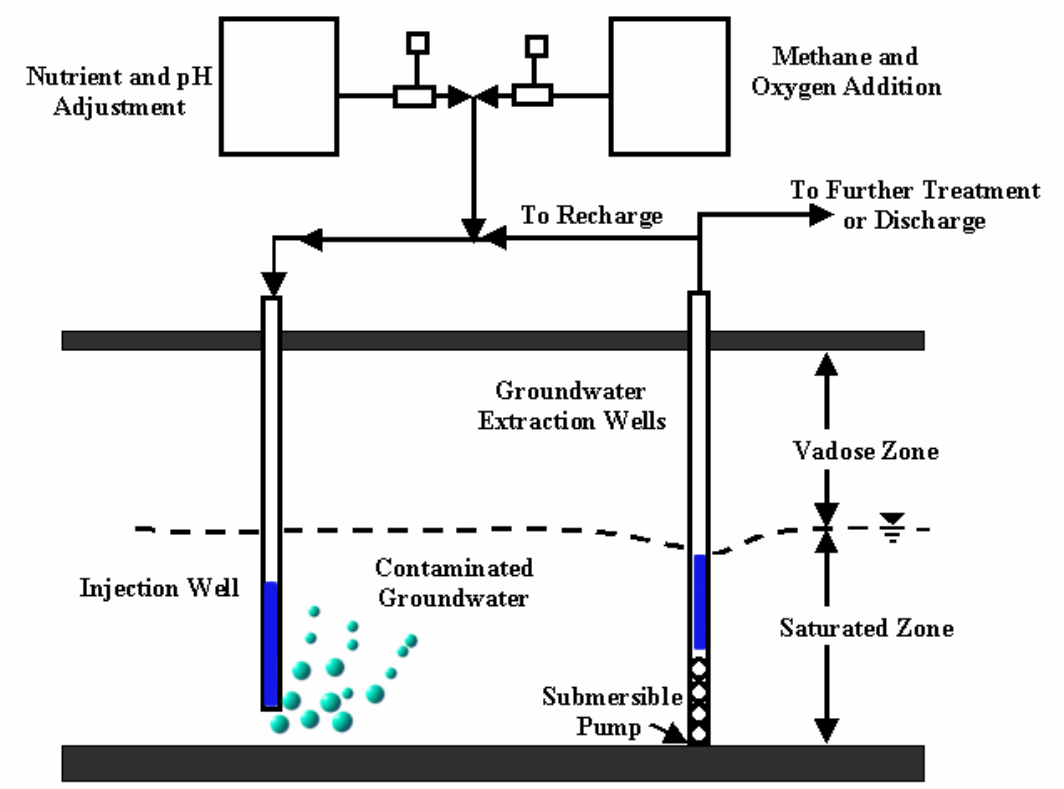


Fig. 3: Schematic diagram of the In-situ ground water remediation via cometabolic activity

(Source: EPA, 1993. In Situ Bioremediation: Biodegradation of Trichloroethylene and Tetrachloroethylene by Injection of Air and Methane, Innovative Remedial Technology Information Request Guide)

Contaminant structure Vs biodegradability relation

Biodegradability is essential for bioremediation of organic pollutants. Chemical structure of pollutant governs the ability of microorganisms to metabolize them, especially the rate and extent of biodegradation. As we discussed above, some compounds are readily biodegradable whereas others are not. Let us look at the general rules that determine this.

- Low to mid molecular weight hydrocarbons and alcohols represent readily biodegradable chemicals.
- Branched and polynuclear compounds are more difficult to degrade that the straight-chain and simple non-aromatic compounds.
- Halocarbons are resistant to biodegradation and are often termed as xenobiotic compounds. Dioxns are very difficult to degrade. Increasing degree of halogenation decreases the rate of biodegradation. For example Dichloromethane as well as Monochloro biphenyls are degraded fast as these are used as carbon and energy source by the microbes. On the other hand Trichloroethane or TCE (widely used solvent) and Polychlorinated biphenyls (widely used in transformers/pesticides) are not used as carbon/energy source and are degraded slowly through cometabolic route.

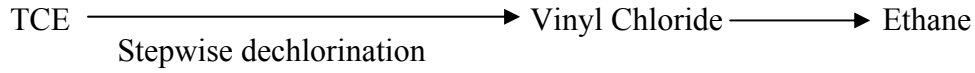
Such rules have been more clearly defined for broad structural categories of the pollutants as below:

(A) Petroleum hydrocarbons

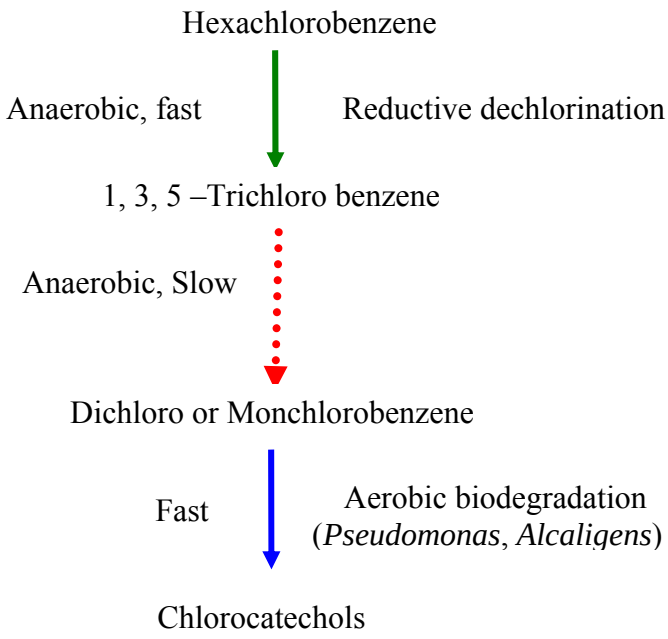
- Short chain alkanes are more toxic and relatively difficult to biodegrade
- N-alkanes of intermediate chain length (C_{10}-C_{24}) degraded most rapidly
- Very long chain alkanes are increasingly resistant to degradation
- When molecular weight is greater than 500, such long chain alkanes are no longer feasible as carbon source
- Aromatic hydrocarbons (especially condensed polynuclear aromatic compounds) degrade more slowly than alkanes
- Some aromatic compounds (benzene, toluene) metabolized under anaerobic conditions but the rates are slower than aerobic reactions
- Alicyclic compounds cannot serve as carbon source unless they have long aliphatic side chains, hence often degraded by cometabolism

(B) Halocarbons

- Most common mechanism is stepwise reductive dehalogenation under anerobic conditions e.g.



- Cometabolic degradation by methanogenic consortium also reported
- Aerobic degradation of TCE by methane utilizing consortium is also possible
- Microcosm studies report both aerobic/anaerobic transformations
- In case of haloaromatics such as chlorobenzenes, aerobic biodegradability decreases with number of halosubstituents
- Extensively chlorinated haloaromatics degraded by stepwise anaerobic dechlorination. The consecutive dechlorination steps are more difficult, slow and often incomplete but the mono and dichloro substitutes can be aerobically degraded for example



(C) Nitroaromatics

- Nitrosubstitution makes the compounds less biodegradable
- Excessively nitrosubstituted aromatics transformed under anaerobic conditions by stepwise conversion of nitro groups to amino groups

Bioremediation Microorganisms/Agents

Different microorganisms are able to degrade different contaminants depending upon the nature and coencentration of contaminant and the metabolic needs of the microorganisms. Scientists around the world are continuously working to find out novel and more efficient biodegraders. Let us discuss the important bioremediation microorganisms under the following heads

Pure Cultures

Since the conventional techniques of microbiology are designed for pure culture studies and also it is easy to work out the efficiency of microbes in such systems, most of the laboratory studies focus on biodegradative capacity of pure cultures. Table 3 shows the famous bioremediation microorganisms. *Pseudomonas* species are most widely detected microbes in the contaminated site due to their extensive biodegradation capacities (Table 4). Among the fugal groups, phanerochaete has been proposed for the biodegradation of various pollutants such as DDT, TNT, high molecular weight polynuclear aromatics like benzo (a) pyrene and plastics such as polyethylene.

Acclimatized Microorganisms & Molecular Breeding

The metabolic range of naturally occurring microbiota may not be capable of degrading certain compounds or certain classes of compounds. There it may be necessary to supplement with the specialized microbes. One way of developing such specialized microbes is by repeatedly exposing them to higher concentration of contaminants. Often the microorganisms with specialized degradation ability can also be enriched from the contaminated site

Table 3: Microorganisms (Pure cultures) helpful in bioremediation

Pollutant	Microorganism(s)	Reference
Atrazine	Acinetobacter species	Singh *et al*., 2004
	Pseudomonas sp. strain ADP	Shapir and R.T. Mandelbaum, 1997
Chlorpyrifos	*Aspergillus niger* *Trichoderma viride*	Mukherjee and Gopal, 1996
	Bacterium strain B-14	Singh *et al*., 2004
2,4,6-Trichlorophenol	*Alcaligenes eutrophus* TCP	Andreoni *et al.*, 2003
2,4-Dichlorophenoxyacetic acid	*Ralstonia eutropha* (pJP4)	Daane and Häggblom, 1999
	Ralstonia eutropha JMP134	Roane *et al.*, 2001
Carbon tetrachloride	*Pseudomonas stutzeri* KC	Dybas et al., 2002
Methyl *tert*-butyl ether	*Hydrogenophaga flava* ENV735	Streger et al., 2002
	β-proteobacterium strain PM1	Smith et al., 2005
BTEX	B. sp. Strain JS150	Kahng et al.,(2001)
	B. Cepacia G4	Shields et al.,(1995)
	R. Pickettii PKO1	Byrne et al., (1995)
	S. yanoikuyae B1	Kim and Zylstra, (1999)
Orange 3, 4-(4-nitrophenylazo) aniline	Pleurotus ostreatus	Xueheng Zhao et.al.,(2006)

Table 4: Pseudomonas strains with proven ability for bioremediation of BTEX

P. putida MT15	Keil et al., (1985)
P. putida F1	Zylstra and Gibson, (1989)
P. putida mt-2 (PaW1)	Burlage et al., (1989)
P. aeruginosa JI104	Kitayama et al., (1996)
P. stutzeri OX1	Bertoni et al., (1998)
P. putida MT53	Kok et al., (1999)
Pseudomonas putida, P. fluorescens	Hojae Shim (2002)

To achieve a complete catabolic pathway for a xenobiotic compound is the ultimate objective for its biodegradative clean up. Biochemical pathways are under constant evolution and this process can be accelerated by plasmid mediated genetic exchange, recombination. A

specially constructed *Pseudomonas* strain in this way (as shown in Fig. 4) has been found to be very effective for degradation of chlorobenzoate and chlorophenols. It has been introduced into the waste streams of chemical manufacturing plant where it could bring about the removal of haloaromatic hydrocarbons.

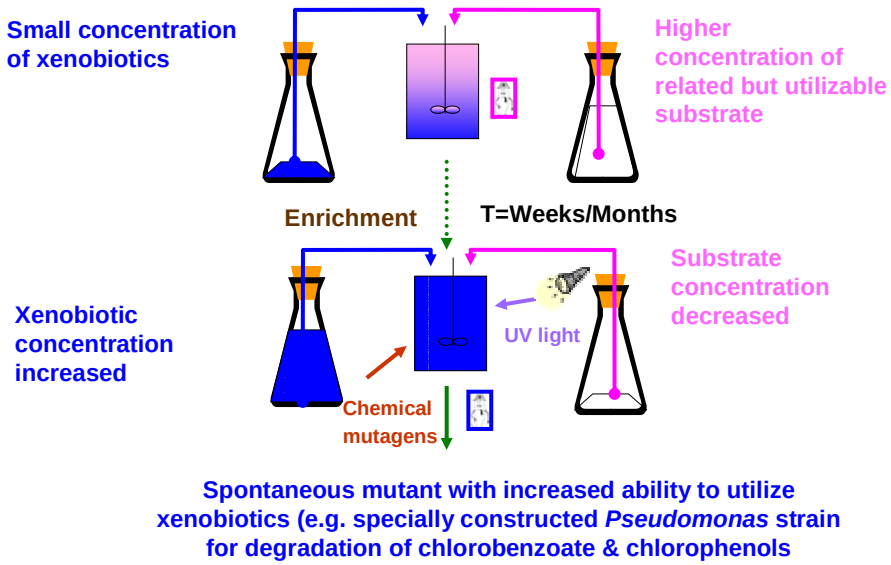


Fig. 4: Molecular Breeding

Genetically Engineered Microorganisms (GEMS)

The microorganisms can be genetically engineered to enhance its enzymatic capability to degrade wide range of compounds. GEMS have the advantage of possessing high growth affinity, rapid growth rate and resistance to toxicity. With such solutions several shortcomings of the bioremediation can be overcome. However, practically the potential results of release of such GEMS into the environment cannot be predicted because suboptimal conditions exist in the field and the GEMS might also face tough competition from the native communities. The artificially introduced genes can persist in environment for example phenol-degrading plasmid has been found in soil 6 years after addition of GEM. A lot of debate exists concerning safety, persistence, containment, and potential ecological damage associated with the release of GEMS in the environment. Due to these constraints it is unlikely that GEMS could be utilized for large-scale bioaugmentation in the coming days.

To overcome the problem of persistence and long-term ecological damage suicidal microorganisms have been designed. These cells have suicide mechanisms (e.g. hok/sok system) so that the cells or the recipients of plasmid die in absence of pollutant.

Adhesion deficient microorganisms: Natural adhesive properties of native bacteria limit their penetration through soil and rock matrix. A specialized adhesion deficient TCE-degrading strain *B. cepacia* has been developed (which could rapidly disperse 25 feet depth) and used successfully for *in-situ* bioremediation of TCE-contaminated aquifer.

Consortium

In nature there is a diversity of types of microorganisms and energy sources. This diversity makes it possible to break-down a large number of different organic chemicals. Even in the most commonly applied activated sludge processes uncharacterized consortia of microorganisms accomplish the task of waste water treatment. Actually microorganisms individually cannot mineralize most hazardous substances. At times the target pollutant is a complex molecule or a mixture of compounds that can only be broken down by a very specific combination of microorganisms (a 'consortium') and pathways (Table 5). Typical pollutants of this type include polyaromatic hydrocarbons, halogenated organics (both aliphatic and aromatic), polychlorinated biphenyls, multi-nitrated arenes (such as the explosive 2, 4, 6-trinitrotoluene [TNT]), organophosphorous or triazinic pesticides and herbicides. In such cases, the solution for successful bioremediation might be inoculation of the polluted biotope with specific populations of microorganisms — bioaugmentation. Complete mineralization can be accomplished through a sequential degradation by a consortium of microorganisms and involves synergism and cometabolic actions (Fig. 5).

Table 5: Consortium for degradation of recalcitrant compounds

Compound	Organism	Reference
Polychlorinated biphenyl (soil)	*Arthrobacter* sp. B1B and *Ralstonia eutrophus* H850	Singer *et al.*, 2000
BTEX	Methanogenic consortia	Da Silva and Alvarez, 2004
Chloroethenes	Consortium that contains *Dehalococcoides*	Lendvay et al., 2003
	Consortium that contains *Dehalococcoides*	Adamson et al., 2003
	Consortium that contains *Dehalococcoides*	Major et al., 2002
Chlorobenzenes	*P. putida* GJ31, *P. aeruginosa* RHO1 and *P. putida* F1ΔCC	Wenderoth et al., 2003
1,1,1-Trichloroethane	Butane-utilizing enrichment culture	Jitnuyanont et al., 2001
Atrazine	Consortia degrading atrazine	Goux et al., 2003
Toluene	nitrate-reducing genera Azoarcus and Thauera, iron-reducing Geobacter metallireducens	Harwood, C.S et.al.,(1997)
Tolune	Pseudomonas putida strain mt-2 Thauera aromatica strain K172, Geobacter metallireducens	Meckenstock et al., (1999)

Microbial mats are laminated, cohesive microbial communities, composed of a consortium of bacteria dominated by photoautotrophic cyanobacteria (also referred to blue–green algae). Mats generally include anoxygenic photoautotrophs (purple bacteria) and sulfur-reducing bacteria. They are embedded in a negatively charged polymeric matrix of gel. Ecological success of microbial mats and their broad array of microbial activities suggest that these microbial ecosystems might be useful to bioremediation of environmental pollutants and

biogeneration of useful products. Microbial mats sequester heavy metals, metalloids, radionuclides and oxyanions. Although these contaminants have complex and contrasting chemistries, mats display a wide variety of mechanisms for removal which occur at the cellular level of the constituent microorganisms and at the community level of the entire consortium. Microbial mats are capable of degrading various organic compounds also and often these compounds are completely mineralized. These include petroleum distillates, trichloroethylene (TCE), tetrachloroethylene (PCE), 2,2′-4,4′-5,5′-hexachlorobiphenyl (PCB), octachlorocyclopentadine (chlordane), 2,4,6-trinitrotoluene (TNT), 2,4-dinitrotoluene (DNT), the pesticides (carbofuran, paraquat and prophos) and absorbable organochlorine compounds (AOX) as effluents from the pulp and paper kraft mill industries.

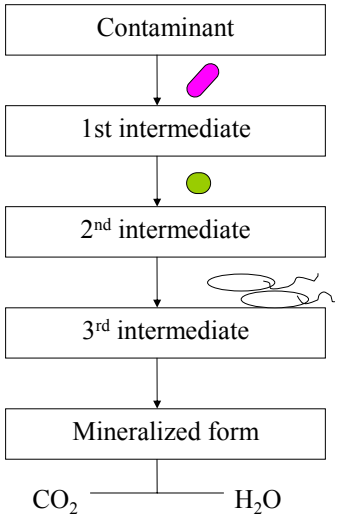


Fig. 5: Mineralization of contaminant by microbial consortia

Enzymes

An appealing alternative to the bioremediation of polluted sites could be that of utilizing cell-free enzymes isolated from their originating cells. Extra cellular enzymes include a large range of oxidoreductases and hydrolases which may explicate a degradative function and transform polymeric substances into partially degraded or oxidized products that can be easily up-taken by cells. For instance, partial oxidation of recalcitrant pollutants such as PAHs by extra cellular oxidative enzymes give rise to products of increased polarity and water solubility and thus with a higher biodegradability. Pesticides of different chemical nature, very recalcitrant compounds like asphaltenes and PCBs, polychlorophenols, PAHs and others toxic pollutants were successfully transformed (in lab-scale studies) by oxidoreductases and hydrolases isolated by fungal, bacterial and plant cells.

Cell-free enzymes can offer several advantages over the use of microbial cells. The most significant features of cell-free enzymes are their unique substrate-specificity and catalytic power; their capability to act in the presence of many toxic, even recalcitrant, substances, and/or under a wide range of environmental conditions, often unfavourable to active

microbial cells (i.e. relatively wide temperature, pH and salinity ranges, high and low concentrations of contaminants); and their low sensitivity or susceptibility to the presence of predators, inhibitors of microbial metabolism, and drastic changes in contaminant concentrations. However, in a natural environment such as soil, several drawbacks or disadvantages may hinder or diminish the catalytic potential of these enzymatic catalysts. The disadvantages of in situ application of either extra cellular, cell-associated or cell-free enzymes, may depend on both the pollutants and the enzymes. For instance, the simultaneous presence of several polluting substances in a contaminated site with synergistic, often negative, effects on the enzyme efficiency, the high costs associated with the isolation and purification of free enzymes, the low stability of enzymes to the harsh conditions of soil all concur to restrict the wide use of enzymes as remediating agents of polluted soils. However, exploration of extreme environments, exploitation of genome using advanced technologies, and protein engineering might open new frontiers for the production and application of enzymes.

Implementing Bioremediation: The Engineering Concepts

Till now we have discussed the fundamentals on which the technology of bioremediation stands. It is very essential to realize and understand these principles before we think of the practical implementation where the main objectives are:

- To enhance the rate and extent of biodegradation of the pollutant
- To utilize or develop microorganisms that can survive the toxic effect of pollutants
- To utilize microorganisms in such a way that no toxic products are produced

As the following discussion on engineering aspects of bioremediation proceeds, we will realize that translating these fundamentals into field applications is not an easy task. It requires a judicious exchange of skills among professionals from various disciplines as shown in Fig. 6.

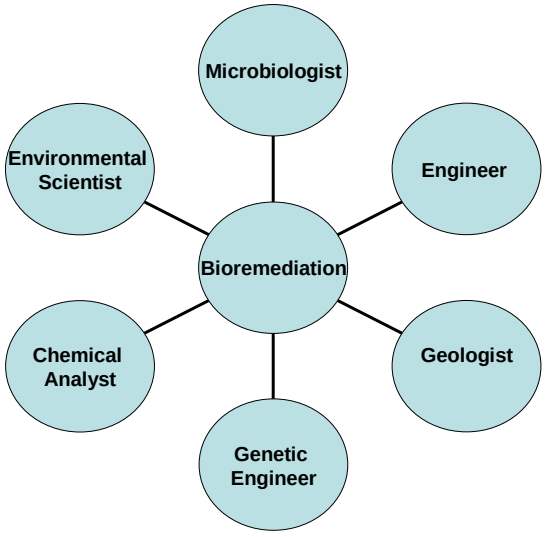


Fig. 6: Bioremediation: An Interdisciplinary Activity

Bioremediation can be performed *in-situ* or off-site. The decision often depends upon the nature of contaminant, objective of the remedial action, availability of funds/time and viewpoints of the local people and authorities. Whenever the pollution effects shallow soil layers, excavation and subsequent treatment at other sites (off-site bioremediation) is recommended either in a biopile (contaminant material is piled on the ground surface) or biocell (contaminant material is deposited and treated underground at a clean site) both of which must be lined to confine the contaminant and leachates.

In-situ Bioremediation (ISB)

Fig. 7 shows the design of the *in-situ* bioremediation (ISB) system. At first the physically removable fraction of the unaltered phase of contaminant (e.g. oil) is removed. It is called as Free Product Recovery. The remaining fraction that cannot be easily removed in this way is then subjected to bioremediation. Free product recovery prevents the microorganisms from the toxicity effects of high concentration of contaminant and makes the bioremediation more feasible. After free product recovery, water is pumped from the recovery well and passed through a filter and then above ground treatment system such as bioreactor/air-stripper. The water is then supplemented with nutrients/electron acceptors and returned to the aquifer close to the source of contamination through injection wells. Oxygen is supplied by sparging air, pure oxygen and sometimes by addition of hydrogen peroxide. First inorganic nutrients are added which is followed by oxygen addition because simultaneous additions may cause plug formation. Gravity and pumping actions pull nutrient/microbe rich water into saturated zone where it comes in contact with the contaminant and eventually treats it.

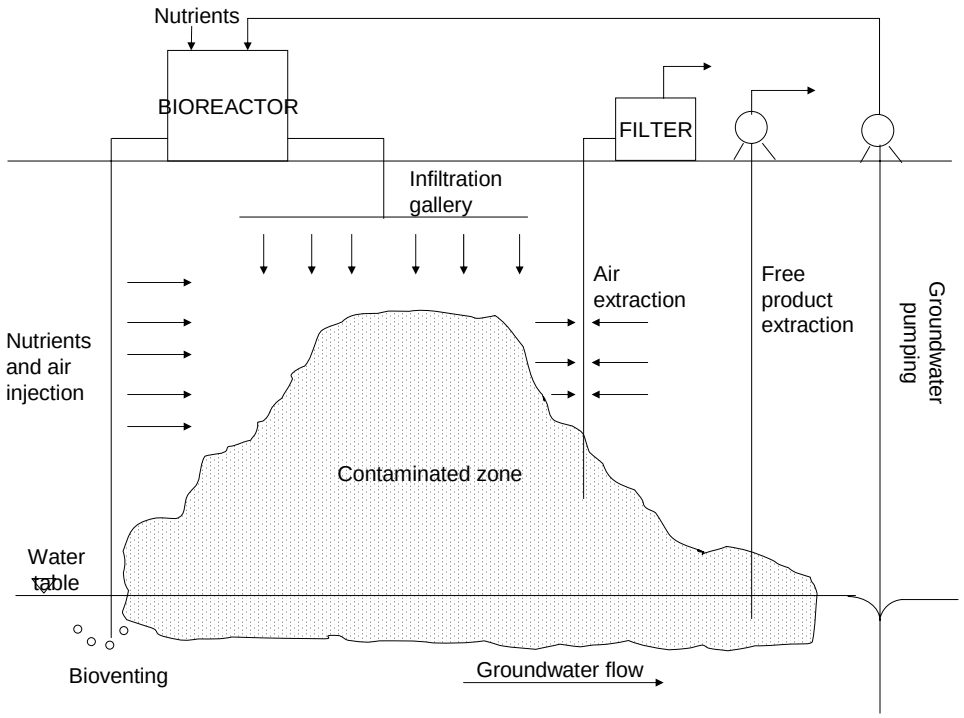


Fig. 7: In-situ Bioremediation technique

(Redrawn from S. Saval, Bioremediation: clean-up Biotechnologies for soils and aquifers in Environmental Biotechnology and cleaner bioprocesses (Eds EJ Olguin, G. Sanchez and E Hernandez, 2000. pp 155—166)

Parameters affecting ISB

The important parameters that affect ISB are discussed below:

Oxygen level

The rate of bioremediation of organic contaminants by microbes is enhanced by increasing the concentration of electron acceptors and nutrients in ground water, surface water, and leachate. Oxygen is the main electron acceptor for aerobic bioremediation. Nitrate serves as an alternative electron acceptor under anoxic conditions. Oxygen level in the soil is increased by avoiding saturation of the soil with water, the presence of sandy and loamy soil as opposed to clay soil, avoiding compaction, avoiding high redox potential, and low concentrations of degradable materials. To ensure that oxygen is supplied at a rate sufficient to maintain aerobic conditions, forced air/oxygen or hydrogen peroxide injection can be used. The use of hydrogen peroxide is limited because at high concentrations it is toxic to microorganisms. Also, hydrogen peroxide tends to decompose into water and oxygen rapidly in the presence of some soil constituents. Anaerobic conditions may be used to degrade highly chlorinated contaminants, although at a very slow rate. This can be followed by aerobic treatment to complete biodegradation of the partially dechlorinated compounds as well as the other contaminants.

Water Content

Water serves as the transport medium through which nutrients and organic constituents pass into the microbial cell and metabolic waste products pass out of the cell. However, too much water can be detrimental, however, because it may inhibit the passage of oxygen through the soil (unless anaerobic conditions are desired).

Nutrients

Nutrients required for cell growth are nitrogen, phosphorous, potassium, sulfur, magnesium, calcium, manganese, iron, zinc, copper, and trace elements. If nutrients are not available in sufficient amounts, microbial activity will become limited. Nitrogen and phosphorous are the nutrients most likely to be deficient in the contaminated environment. These are usually added to the bioremediation system in a useable form (e.g., as ammonium for nitrogen and as phosphate for phosphorous). Phosphates can cause soil plugging as a result of their reaction with minerals, such as iron and calcium, to form stable precipitates that fill the pores in the soil and aquifer.

pH

pH affects the solubility, and consequently the availability, of many constituents of soil, which can affect biological activity. Many metals that are potentially toxic to microorganisms are insoluble at elevated pH; therefore, elevating the pH of the treatment system can reduce the risk of poisoning the microorganisms.

Temperature

Temperature affects microbial activity in the environment. The biodegradation rate will slow with decreasing temperature; thus, in cold climates bioremediation may be ineffective during part of the year unless it is carried out in a climate-controlled facility. The microorganisms remain viable at temperatures below freezing but will resume activity when the temperature rises. Heating the bioremediation site, such as by use of warm air injection, may speed up the remediation process. At Eielson AFB, Alaska, passive solar warming by incubation tanks (ex

situ) or the application of heated water below the ground surface to the contaminated vadose zone is being investigated. On the other hand, too high a temperature can also be detrimental to some microorganisms, essentially sterilizing the soil.

Temperature also affects nonbiological losses of contaminants mainly through the increased volatilization of contaminants at high temperatures. The solubility of contaminants typically increases with increasing temperature; however, some hydrocarbons are more soluble at low temperatures than at high temperatures. Additionally, oxygen solubility decreases with increasing temperature.
It is important to take precautions and prevent the following during ISB

- Flow of pumped water into unwanted area
- Excessive use of nutrients
- Enhancement of micrrorganisms in the unwanted area
- Unwanted interaction of soil/ground water matrix with added nutrients for e.g. reduced Fe and Mn can get precipitated upon oxidation to cause plug formation

Therefore, it is quite clear that a lot of investigations and meticulous planning is required prior to the full-scale ISB. To begin with it is very essential to know whether the site is suitable for bioremediation or not.

Steps in ISB

Information about the site characteristics should be examined beforehand to evaluate the viability of bioremediation technology, which should be followed by confirmation of the biodegradative activity at microcosm level and then at larger scale.

Site characterization

Site characterizations must be done in a logical sequence to be able to respond to the following major questions:

- What chemical compounds are present as contaminants and whether the contamination is superficial or has reached the subsoil?
- What is the depth and extension of contaminant plume and the water table?
- Is the contaminant biodegradable and whether the microorganisms present at the site can degrade it? Are the environmental conditions conducive to biodegradation?
- Is it possible to build bioreactor at the site?

The answers to these questions are obtained by the following characterizations:

(a) Pollutant and geohydrochemical characterization
Pollutant characterization involves determination of composition, concentration, toxicity, bioavailability, solubility, sorption and volatilization of all the pollutants. The examination of hydrological characteristics of the site is essential to understand the mobility of the contaminant. The following geotechnical tests should be conducted to determine:

- Hydraulic conductivity and/or permeability of soil to determine how rapidly water and nutrients can move through the saturated/vadose zone
- Specific yield and storage coefficient of the aquifer
- Zone of influence of recovery and injection wells and the ground water flow direction
- Cation exchange capacity of soils to estimate the nutrient sorption on the soil particles
- Anionic/cationic composition of the soils and ground water.

The favourable and unfavourable conditions have been identified (Table 6) for implementation of the bioremediation. Apart from this location of underground objects like electricity cables, water pipes, sewers and above ground topographical evaluations including the buildings, roads and parking lots etc. should also be conducted.

Table 6: Favorable and unfavorable conditions for implementation of the Bioremediation

S.No.	Favorable	Unfavorable
Chemical Characteristics		
1.	Few organic contaminants	Numerous contaminants; complex mixture of inorganic and organic contaminants
2.	Non-toxic concentration	Toxic concentrations
3.	Diverse microbial groups	Sparse microbial activity
4.	Suitable electron acceptors	Absence of suitable electron acceptors
4.	Ph 6-8	pH extremes
Hydrogeological characteristics		
1.	Granular porous media	Fractured rock
2.	High permeability	Low permeability
3.	Uniform mineralogy	Complex mineralogy
4.	Homogenous saturated medium	Heterogeneous medium, unsaturated-saturated conditions

(Source: Trejo M and Quintero R (2000) Bioremediation of contaminated soils. In Olguin EJ, Sanchez G and Hernandez E (eds) Environmental Biotechnology and Cleaner Bioprocesses. Taylor and Francis, pp 179-188)

(b) Microbiological characterization

The three important prerequisites for successful ISB are:

- Appropriate microorganism should be present on the subsurface
- These should be adapted to the contaminants
- All necessary nutrients should be present or must be added

Interestingly even till mid 1970's the general belief was that there are extremely low number of microorganisms in deep soil layers/ground water as all of them shall be retained by natural filtration through the upper soil columns. However, later on it was shown that naturally occurring species of *Pseudomonas* and *Arthrobacter* were involved in the disappearance of subsurface gasoline. Since then several hydrocarbon degrading microorganisms have been reported. It is desirable to analyze the native microbial flora with respect to its degradative capacity and size of the population with degradative potential.

Biotreatability studies

These tests are normally performed at a mesocosm level and efforts are made to simulate/reproduce the environmental conditions that prevail in the field. For example if a

shallow stratum is to be remediated, large trays or jars filled with soil layers can be used. On the other hand if contamination is present at the ground water table, column packed with contaminated soil can be used as for biotreatability studies. The main purpose of biotreatability studies is to determine the nutritional requirement of microorganisms to perform biodegradation for which following parameters are monitored

- Oxygen consumption
- Carbon dioxide generation
- Exhaustion of added nutrients (Nitrogen and phosphorus sources)
- Contaminant removal

Biotic and abiotic controls should be included to ensure that the contaminant is being removed by microbial activity. When the tests are properly conducted, it is possible to predict the behaviour of bioremediation and the time for large scale applications.

Advantages of ISB

1. Bioremediation is a natural process and is therefore perceived by the public as an acceptable waste treatment process for contaminated material such as soil. Microbes able to degrade the contaminant increase in numbers when the contaminant is present; when the contaminant is degraded, the biodegradative population declines. The residues for the treatment are usually harmless products and include carbon dioxide, water, and cell biomass.
2. Bioremediation is useful for the complete destruction of a wide variety of contaminants. Many hazardous compounds can be transformed to harmless products. This eliminates the chance of future liability associated with treatment and disposal of contaminated material.
3. Instead of transferring contaminants from one environmental medium to another, for example, from land to water or air, the complete destruction of target pollutants is possible.
4. Bioremediation can often be carried out on site, often without causing a major disruption of normal activities. This also eliminates the need to transport quantities of waste off site and the potential threats to human health and the environment that can arise during transportation.
5. ISB is almost always faster than baseline pump-and-treat remediation.
6. ISB may be used in both short and long term timeframes, either by itself or following a more aggressive source zone treatment technology.
7. Bioremediation can prove less expensive than other technologies that are used for clean-up of hazardous waste.

Limitations to ISB

Factors that may limit the applicability and effectiveness of the process include:

- Bioremediation is limited to those compounds that are biodegradable. There are some concerns that in some cases the products of biodegradation may be more persistent or toxic than the parent compound.
- Biological processes are often highly specific. Important site factors required for success include the presence of metabolically capable microbial populations, suitable environmental growth conditions, and appropriate levels of nutrients and contaminants. Cleanup goals may not be attained if the soil matrix prohibits contaminant-microorganism

contact. A surface treatment system, such as air stripping or carbon adsorption, may be required to treat extracted groundwater prior to re-injection or disposal.

- It is difficult to extrapolate from bench and pilot-scale studies to full-scale field operations.
- The circulation of water-based solutions through the soil may increase contaminant mobility and necessitate treatment of underlying ground water. Preferential colonization by microbes may occur causing clogging of nutrient and water injection wells.
- Bioremediation often takes longer than other treatment options, such as excavation and removal of soil or incineration. Bioremediation slows at low temperatures.
- High concentrations of heavy metals, highly chlorinated organics, long chain hydrocarbons, or inorganic salts are likely to be toxic to micro organisms. Concentrations of hydrogen peroxide greater than 100 to 200 ppm in groundwater inhibit the activity of micro organisms.
- Research is needed to develop and engineer bioremediation technologies that are appropriate for sites with complex mixtures of contaminants that are not evenly dispersed in the environment.
- Regulatory uncertainty remains regarding acceptable performance criteria for bioremediation. There is no accepted definition of "clean", evaluating performance of bioremediation is difficult, and there are no acceptable endpoints for bioremediation treatments.

Many of the above factors can be controlled with proper attention to good engineering practice. The length of time required for treatment can range from 6 months to 5 years and is dependent on many site-specific factors.

Microbes in Reclamation of Wastelands including Oil Spills

Petroleum hydrocarbons, although not xenobiotics, are one of the main potential sources of environmental contamination due to their large-scale use. Soil and groundwater are often contaminated with gasoline or diesel fuel from leaking underground storage tanks and because of accidental spills and leakage from pipelines. Due to their mobility, these compounds may cause considerable damage not only in soils but also in water intakes or groundwater reservoirs. As a consequence, cost-effective bioremediation techniques have been developed during this period, especially to clean up oil- and gas-polluted sites. Petroleum contaminated soils and aquifers constitute about 60% of the sites where bioremediation is being used in filed demonstrations or full-scale operations.

Steps in Bioremediation of oil contaminated site

The basic steps involved in the development of such bioremediation strategies are (Fig. 8).

(A) Microbe Isolation and identification
(B) Selection of petroleum degrading bacteria
(C) Generation of biomass
(D) Application of biomass to the contaminated site
(E) Maintenance of the selected bacterial population at the site

(A) Microbe Isolation and identification

Microbe is usually isolated by inoculating trypticase soya broth with the contaminated soil. To enrich the petroleum degrading bacteria a two layered medium comprising lower layer of

minimal nitrogen medium (nitrogen source) covered with petroleum layer (carbon source) can be used. After 48 hours of incubation, the petroleum degrading bacteria can be streaked on trypticase soy agar plate and incubated for 2-3 days. The developed colonies are picked and again inoculated on two layered medium till the pure bacterial cultures are obtained. The bacterial identification can be done by standard biochemical tests, fatty acid analysis or by 16 S rDNA analysis. Care should be taken that the selected organisms are non-pathogenic. It is better to conduct the screening and isolation at around 20^0C temperatures as most of the pathogens best survive around 37^0C. However, further confirmation must be done to ensure the non-pathogenic nature before proceeding with the bioremediation experiments.

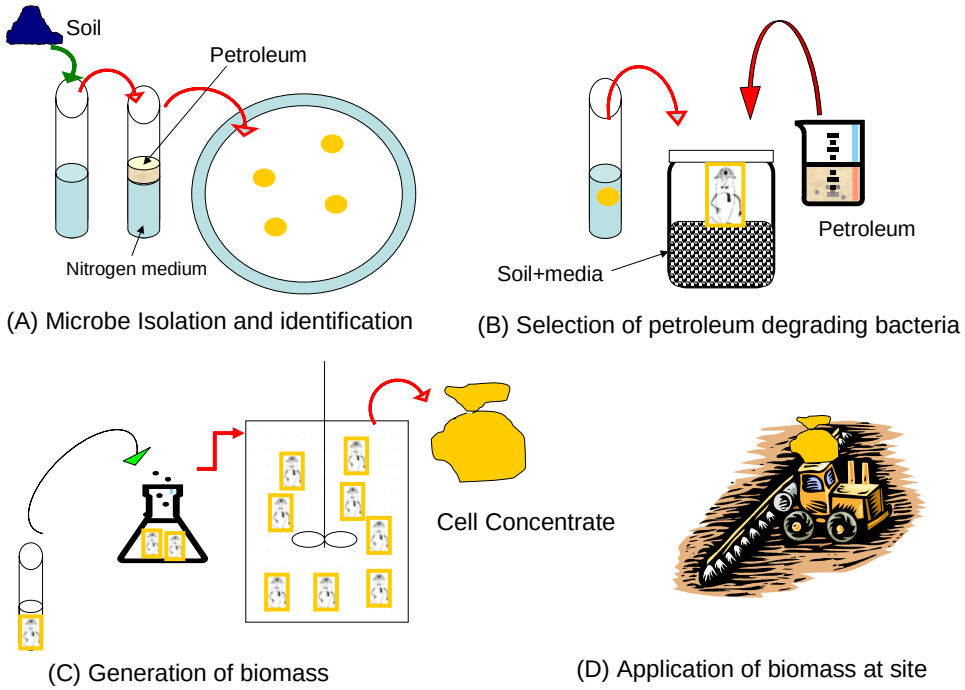


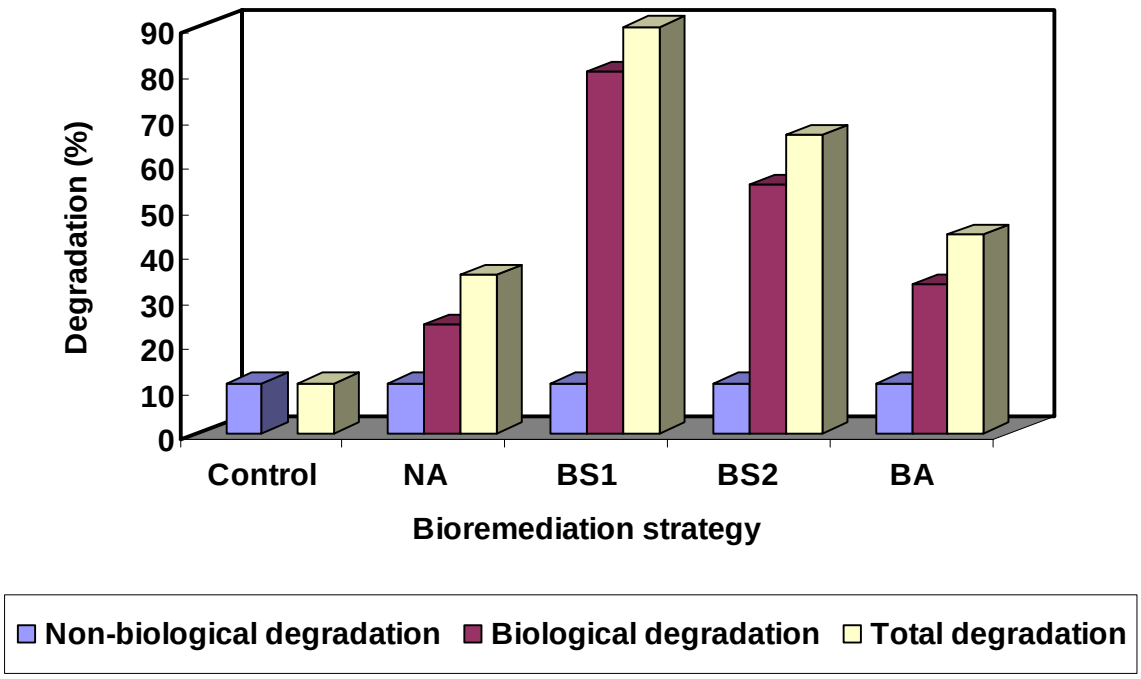
Fig. 8: Steps in bioremediation of petroleum contaminated site

(B) Selection of petroleum degrading bacteria

As shown in Fig. 9, further confirmation of the petroleum biodegradation ability of the selected strains should be done by inoculating petroleum laden sterile soil with the selected culture and incubating it for about 15 days. Total bacterial count and total petroleum hydrocarbon (TPH) of the samples can be monitored to select the most efficient strains.

(C) Generation of biomass

After the microorganism has been identified and its ability to degrade petroleum oil has been tested in the laboratory, the next step is to increase the number of microorganisms for field application. Biomass production is done in large fermentors (Fig. 8) and the subsequent culture is concentrated (by high speed centrifugation) to produce mud like precipitated cells. The concentrated biomass is packed in plastic, tight sealing bags and transported to the contaminated site on ice.



NA: Natural attenuation (No amendment)
BS1: Biostimulation with inorganic N & P source
BS2: Biostimulation with sterilized sewage sludge
BA: Bioaugmentation with live/active sewage sludge

Fig. 9: Diesel biodegradation after 45 days
(Source: Gallego JLR, Loredo J, Llamas JF, V´azquez F & S´anchez J (2001) Biodegradation 12: 325–335)

(D) Application of biomass to the contaminated site

Biomass should be applied to the contaminated soil as soon as possible but before 48 hours of production. The amount of biomass to be applied depends on the total area of the contaminated site as well as the degree of contamination. The biomass is appropriately diluted and applied to the contaminated soil by injection or spray technique. If the contamination is in the upper layers (2-3 feet of surface soil), spraying of the diluted biomass after agitation of the soil (Fig. 8) may suffice but if the contamination has reached lower layers, injection of the biomass to the deeper soil layers is required. Since the untraviolet rays from sun are lethal to the microorganisms, the application of biomass to soil should be best made in the early morning hours before sunrise.

(E) Maintenance of the selected bacterial population at the site

Since the soil has been augmented with more than natural microbial load of that particular soil, special attention needs to be paid to monitor and maintain the oxygen levels, nutrients, water content and pH so that the selected population can effectively degrade the contaminants.

Petroleum degrading microbes

Several petroleum degrading microbes have been identified till date. Austin et al. 1977 identified 99 strains of petroleum-degrading bacteria isolated from Chesapeake Bay water and sediment. These groups were identified as actinomycetes (mycelial forms, four clusters),

coryneforms, Enterobacteriaceae, *Klebsiella aerogenes, Micrococcus* spp. (two clusters), *Nocardia* species (two clusters), *Pseudomonas* spp. (two clusters), and *Sphaerotilus natans* which indicated that degradation of petroleum is accomplished by a diverse range of bacterial taxa. A survey of petroleum-degrading bacteria was carried out in the Indian part of deltaic Sunderbans to evaluate the distribution of the naturally occurring petroleum-degrading aerobic bacteria. Depending on the location, 0.08–2.0% of the heterotrophic bacteria culturable in marine agar medium could degrade crude petroleum hydrocarbons as the sole source of carbon. There was a maximum number of petroleum-degrading bacteria in the waters of Haldia Port and its surrounding areas, where the water is highly polluted by hydrocarbon discharges from a nearby oil refinery and from the ships docking at the port. *Pseudomonas*, *Mycobacterium*, *Klebsiella*, *Acinetobacter*, *Micrococcus*, and *Nocardia* were the most common petroleum degraders. Selected strains belonging to *Pseudomonas* (two strains), *Mycobacterium* (two strains), and *Nocardia* (one strain) degraded 47–78% of Arab-Mix crude oil over a period of 20 days.

The Energy Research Institute (TERI), India has developed the Oilzapper which is crude oil and oily sludge degrading bacterial consortium. This microbial consortium, developed from five bacterial isolates (obtained from hydrocarbon-contaminated sites), was immobilized with a suitable carrier material (powdered corncob). The immobilized culture (oilzapper), which has the survivability of three months at ambient temperature, can be sealed aseptically into sterile polythene bags and transported to the contaminated site. It has been successfully used for clean up of crude oil spills and treatment of oily sludge. More than 40,000 tonnes of oily sludge/oil contaminated soil and drill cuttings have been treated at various locations. More than 30,000 tonnes of oily sludge/oil contaminated soil is under treatment at different locations in India and the Middle East countries.

Further research targets more effective translation of lab results into the real field situations. Poor performance of in situ treatments involving the addition of bacteria have been due to the unknown effects of site conditions on the ability of bacteria to degrade contaminants. Specialized indicator strains of bacteria that produce light in response to the presence of bioavailable polycyclic aromatic hydrocarbons (PAH) are being developed. The indicator strains would enable us to predetermine, whether or not the appropriate nutrient and environmental conditions exist at a site. Microencapsulated bacteria for low-cost bioremediation of petroleum products that are poorly degraded by naturally-occurring bacteria are also being examined.

Case studies

A recent case study demonstrated bioremediation of diesel contaminated soil in laboratory conditions as well as the *in-situ* bioremediation of a natural diesel spill (400,000 l). Diesel fuel is a complex mixture of normal, branched and cyclic alkanes, and aromatic compounds obtained from the middle-distillate, gas-oil fraction during petroleum separation. In this article the different approaches of bioremediation were tested under laboratory conditions. The experimental set comprised of Control experiment (Heat-sterilised soil), Natural attenuation (No amendement), Biostimulation (with a. inorganic N & P source, b. Sterilized sewage sludge), Bioaugmentation (live/active sewage sludge). The authors found best diesel biodegradation when biostimulation with inorganic nutrients was done (Fig. 9). In both lab. and field scale studies the hydrocarbon degraders (*Acientobacter* & *Pesudomonas* species) were found abundant. Therefore, based on the lab results, for the real oil spill an integrated approach of physical removal of diesel (free product recovery) followed by tilling and fertilizing biostimulation treatment was recommended.

Oil contaminated soil in Kuwait could be successfully bioremediated by land-farming within 12 months. Land-farming could be enhanced by adjusting the C:N ratio to 50:1, inoculating soil with HUB (hydrocarbon utilizing bacteria), and adding vegetation (alfa-alfa inoculated with rhizobium cultures) after partial TPH (total petroleum hydrocarbon) reduction. Such enhanced land-farming resulted in degradation of 90% of TPH from light contaminated soil. McBean (1995) suggested an *ex-situ* two-stage process for remediation of semi-volatile organic compounds. The first stage comprised of excavation, heap piling and washing of contaminated soil in presence of surfactants, while in the second stage the collected leachate was biologically treated with removal efficiency of 90% or more. Such a process with decoupled stages has dual advantage that is quick leaching and soil replacement as well as differential optimization of each step.

Role of biosurfactants

Biosurfactants are surfactants which are biologically produced by yeast or bacteria from various substrates including sugars, oils, alkanes and wastes. Examples include rhamnolipids (produced by *Pseudomonas aeruginosa*), sophorolipids (produced by *Candida bombicola*) and surfactin (produced by *Bacillus subtilis*). Biosurfactants have shown their potential for remediation of contaminated soil and water. Both organic and inorganic contaminants can be treated through desorption or biodegradation processes.

Addition of rhamnolipid produced by *Pseudomonas* sp. DS10-129 along with poultry litter and coir pith has been found to enhance ex situ bioremediation of a gasoline-contaminated soil. Rhamnolipids from *P. aeruginosa* UG2 were also able to effectively remove a hydrocarbon mixture from a sandy loam soil and that the degree of removal (from 23 to 59%) was dependent on the type of hydrocarbon removed and the concentration of the surfactant used. Surfactant produced by a strain of *Bacillus subtilis* at was able to remove 62% of the oil in a sand pack saturated with kerosene and thus could be used for in situ oil removal and cleaning sludge from sludge tanks. The use of the biosurfactants in cleaning oil from coastal sand, and from the feathers and furs of marine birds and animals has also been examined. The biourfactant (PS-17) produced by strain *Pseudomonas* PS-17 could efficiently remove oil from sand (95% removal) as well as from feathers (85%) and furs (82%) of marine birds and animals contaminated by oil as opposed to 1-2% oil removal in the absence of PS-17 biosurfactant. The biosurfactants seem to enhance biodegradation by influencing the bioavailability of the contaminant. Due to their biodegradability and low toxicity they are very promising for use in remediation technologies. However, further research regarding prediction of their behaviour in the fate and transport of contaminants, decreasing production costs, in situ production, among other issues will be required.

Conclusions

Bioremediation, which is the nature's way of dealing with the environmental pollution, is gaining significant attention these days. Different approaches to accelerate the intrinsic bioremediation have been developed and used at a number of sites worldwide with varying degrees of success. Techniques are improving as greater knowledge and experience are gained, and bioremediation has gained triumph for dealing with certain types of site contamination. Unfortunately, the principles, techniques, advantages, and disadvantages of bioremediation are not widely known or understood, especially among those who will have to deal directly with bioremediation proposals, such as site owners and regulators. Therefore, apart from the sound fundamental knowledge about these techniques there is a need for

providing a straightforward, pragmatic view of the processes involved in bioremediation, the pros and cons of the technique, and the issues to be considered when dealing with a proposal for bioremediation.

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