BIOREMEDIATION FOR SOIL RECLAMATION

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Keywords: bioremediation, biodegradation, soil contamination, groundwater contamination, remediation technologies, bioventing, monitored natural attenuation, organic contaminants, BTEX, PAHs, chlorinated hydrocarbons, cometabolism, sorption, activation

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Summary

Before deciding on an alternative treatment technology, such as bioremediation, as much information as possible should be collected about the contaminated site. This is important because it appears that no single alternative treatment technology has completely eliminated the need to use a conventional treatment technology. In this regard, it may be beneficial to combine *in situ* bioremediation, both sequentially and simultaneously, with other treatment technologies as part of a treatment strategy for the cleanup of a contaminated site.

Clearly, the use of microorganisms to convert or transform harmful contaminants in the subsurface to safe compounds is a complex process. Nevertheless, the many advantages of bioremediation, especially when coupled with other processes in monitored natural attenuation, make it an attractive alternative treatment technology. Accordingly, numerous studies have been conducted to examine many factors influencing bioremediation. Since the ultimate goal in engineered bioremediation is to enhance the degradation rate of contaminants, the focus of many of these studies has included investigating the factors that limit the degradation rate. Each of these factors needs to be addressed during site assessment when considering bioremediation as the treatment technology. Therefore, it is quite evident a serious literature review should be performed and experience on the biodegradability of the target compound should exist – in addition to conducting a thorough site assessment – before proceeding with a field project.

Bioremediation is a technology whose full potential has not been realized yet. The combination of the intricacies of microbial processes and the physical challenge of monitoring both microorganisms and contaminants in the subsurface makes bioremediation difficult to understand. Monitoring the performance of bioremediation is a key to this. The full potential of bioremediation to treat a wide range of contaminants cannot be realized as long as its use is clouded by controversy over what it does and how well it works.

1. Introduction

Soil remediation has been more successful in meeting regulatory standards than soil-groundwater and aquatic sediment remediations. However, conventional soil cleanup methods generally require extreme actions to physically remove contaminants and consequently, may transfer contaminants to the air. This poses risks that are not always acceptable to workers or residents near the contaminated site. Investigations into alternative cleanup technologies continue to gain interest as a result of the limitations of conventional cleanup technologies and the hazards of conventional treatment methods.

In situ bioremediation is an alternative cleanup technology to conventional *ex situ* remediation technologies (e.g. excavation and incineration) and their corresponding high costs, and technical and safety problems. *In situ* bioremediation is the engineered or intrinsic use of microorganisms to control and destroy contaminants in place by biodegradation. It is an especially attractive alternative because it is potentially less costly and can result in the destruction or transformation of hazardous contaminants, which precludes contaminant transfer to other media, a characteristic problem of many conventional cleanup methods. The resulting cost savings and increased safety could make *in situ* bioremediation a preferred alternative cleanup-up technology for responsible parties and regulators alike.

Although cost and safety are primary considerations, *in situ* bioremediation has additional advantages. For instance, engineered *in situ* bioremediation may meet cleanup-up goals more quickly than other conventional methods, such as pump-and-treat. It has the potential to accelerate contaminant desorption and dissolution, limiting factors for many conventional methods, by treating contaminants close to their source, such as contaminants within soil aggregates and micropores, non-aqueous phase liquids (NAPLs), etc. Additionally, treatment is done in place, thereby eliminating transportation costs and liabilities, keeping site disruption to a minimum, and allowing existing use of the site to continue. In place treatment also precludes the need for contaminant long-term storage and its associated liability.

The use of microorganisms to mineralize or transform contaminants to less harmful by-products is what significantly differentiates this treatment technology from other *in situ* treatment technologies. In fact, biodegradation most likely already plays a role in the fate of contaminants as long as appropriate microorganisms and a number of substances to support cellular energy generation and synthesis are present. Owing to their ubiquitous distribution throughout the environment, relatively rapid growth and metabolism, ability to facilitate genetic manipulation, capability to utilize a wide range of organic and inorganic contaminants as substrates, and ability to quickly adapt to a variety of conditions, bacteria are generally considered the most successful group of microorganisms for bioremediation. These qualities also add versatility to the technology.

Despite mounting evidence in support of *in situ* bioremediation, this treatment technology is neither generally understood nor trusted by many who would benefit from its use or who must approve its use. This is because the combination of microbial processes and contaminants in aqueous and terrestrial environments is complex and makes *in situ* bioremediation difficult to apply and assess. However, *in situ* bioremediation may be considered for many sites with differing conditions.

2. Principles of Biodegradation

Degradation of contaminants in natural environments is mediated primarily by two groups of microorganisms: bacteria and fungi (see *Chemistry of Organic Pollutants*). Because the majority of bioremediation systems currently in use are bacterial, emphasis will be placed on this group of microorganisms. Figure 1 shows the major components of biodegradation. Microorganisms require nutrients and a terminal electron acceptor to utilize and degrade a substrate, or contaminant. Oxygen is the required electron acceptor for aerobic microorganisms, while anaerobic microorganisms use other electron acceptors besides oxygen. A standard microbiology text can be referred to for information on microbial physiology, metabolism, genetics, and ecology (see *Biochemical Oxygen Demand*).

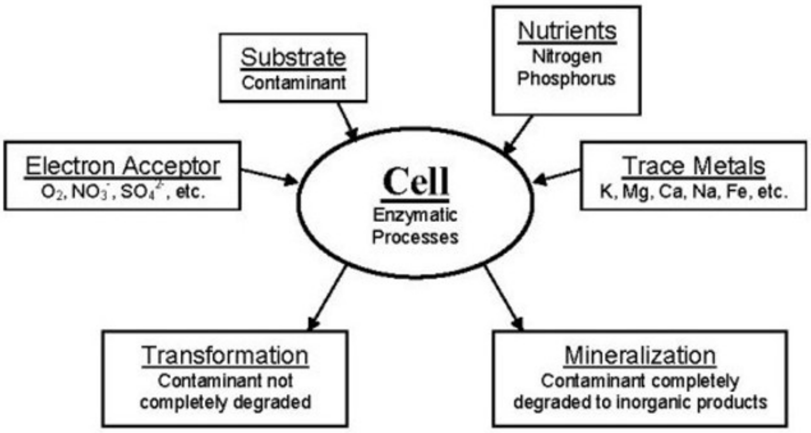

Figure 1. A conceptual view of the process of microbial degradation

Several conditions must be satisfied for biodegradation to take place in an environment. First, an organism must exist that has the necessary enzymes to bring about biodegradation. Second, the organism must be present in the environment containing the chemical contaminant. Third, the contaminant must be accessible to the organism. Fourth, if the initial enzyme bringing about the degradation is extra-cellular, the chemical's bonds acted upon by the enzyme must be exposed for the catalyst to function. The products must then penetrate the cell for the transformation to proceed further. Should the initial enzyme be intra-cellular, the chemical must penetrate the surface of the cell to the internal sites where the enzyme acts. Fifth, because the population of microorganisms degrading the chemical is initially small, conditions in the environment must be conducive to allow proliferation of the active microorganisms.

Because microorganisms are frequently the major and occasionally the sole means for degradation of particular chemical contaminants, the absence of a microorganism, or its inability to function, frequently means that the contaminant disappears very slowly or may not be destroyed at all. It is not certain at the present time how many compounds persist at a particular site because of the absence of microorganisms, the occurrence of conditions not conducive for microbial degradation, or the complete absence in nature of microbial species having the capacity to bring about the transformation.

2.1. Contaminants

The U.S. Office of Technology Assessment has reported that over 200 substances have been detected in U.S. groundwater. These substances can be naturally occurring (such as the arsenic found from installation of wells in Bangladesh and in West Bengal in India) or can be from anthropogenic inputs that include industrial and agricultural organic chemicals, metals, and radionuclides (see *Speciation of Heavy Metals and Radioisotopes*, *Chemistry of Organic Pollutants*). The occurrence of anthropogenic chemicals in U.S. groundwater is not an isolated geographical phenomenon. For example, Europe's groundwater is endangered in many ways with significant pollution observed from nitrate, pesticides, heavy metals and hydrocarbons. Ukraine's Ministry of Nature Protection reports that in eastern industrial areas, the contamination of groundwater by heavy metals, mainly related to mining and chemical industries, is so serious that many wells can no longer be used as a source of drinking water.

Although site contamination is generally reported as groundwater contamination, soil contamination is just as prevalent. Groundwater contamination typically occurs by the initial release of chemicals onto the ground surface. Moreover, many sites are contaminated with more than one hazardous chemical or toxic compound. Therefore, any remediation method or combination of methods selected should be capable of cleaning up all contaminants in the groundwater and attached to the soil.

Additionally, the source of contamination varies. The Ministry of Nature Protection of the Russian Federation reports that 36 percent of their contaminated groundwater is related to industry, 20 percent is related to agriculture, 10 percent is related to municipal landfills, and the remaining 12 percent is from mixed sources. Prior to evaluating any remediation method, the source of the contaminant must be addressed.

2.1.1. Contaminant Types and Biodegradation Potential

Table 1 lists the twenty most abundant organic chemicals reported in groundwater at solid and hazardous waste disposal sites in the U.S. and their chemical formula. Of these 20 chemicals, all but 7 are chlorinated aliphatic hydrocarbons. Some of the others include benzene, toluene, and ethylbenzene, which are components of fuel products (e.g. gasoline, diesel fuel, and jet fuel) and also serve as feedstock for production of other chemicals. Other examples of common organic chemicals that have contaminated soil and groundwater include multiple ring aromatic hydrocarbons (e.g. polycyclic aromatic hydrocarbons (PAHs)), oxygenated hydrocarbons (e.g. acetone, phenol, methyl-tert-butyl ether (MTBE)), nitroaromatics (e.g. trinitrotoluene (TNT)), and aromatic and non-aromatic hydrocarbons that contain nitrogen, phosphorous, sulfur, chlorine and bromine substituent groups.

Rank	Chemical	Structure
1	Dichloromethane	CH_2Cl_2
2	Trichloroethene	C_2Cl_3H
3	Tetrachloroethene	C_2Cl_4
4	Trans-1,2-dichloroethene	$C_2H_2Cl_2$

5	Chloroform	$CHCl_3$
6	1,1-Dichloroethane	$C_2Cl_2H_4$
7	1,1-Dichloroethene	$C_2Cl_2H_2$
8	1,1,1-Trichloroethane	$C_2Cl_3H_3$
9	Toluene	C_7H_8
10	1,2-Dichloroethane	$C_2Cl_2H_4$
11	Benzene	C_6H_6
12	Ethylbenzene	C_8H_{10}
13	Phenol	C_6H_5OH
14	Chlorobenzene	C_6H_5Cl
15	Vinyl chloride	C_2ClH_3
16	Carbon tetrachloride	CCl_4
17	Bis(2-ethylhexyl)phthalate	$C_{24}H_{38}O_4$
18	Naphthalene	$C_{10}H_8$
19	1,1,2-Trichloroethane	$C_2Cl_3H_3$
20	Chloroethane	C_2ClH_5

Table 1. Twenty most abundant organic constituents identified in groundwater by occurrence at 479 U.S. waste disposal sites

Bioremediation, depending on site-specific characteristics, may be used on a variety of contaminants, such as pesticides, fertilizers, metals, and radionuclides. However, not all compounds in one chemical class are biodegradable. For instance, petroleum-based compounds like benzene, toluene, ethylbenzene, xylenes (BTEX) are biodegradable under certain environmental conditions, whereas other petroleum-based compounds such as methyl tertiary butyl ether (MTBE) are not readily biodegradable. The U.S. National Research Council (USNRC) has reported that bioremediation is an established technology for remediation of hydrocarbons and derivatives found in gasoline and other fuel products, alcohols, ketones, esters. They have also reported that bioremediation is an emerging remediation strategy for PAHs, creosote, ethers, nitroaromatics, metals, and halogenated aliphatics. Although a wide range of contaminants is potentially biodegradable, bioremediation systems have been most successfully applied to petroleum hydrocarbons (fuels and refinery wastes) (see *Oil Pollution and Microbial Regulation*), wood preserving wastes (creosote), and chlorinated solvents (PCE and TCE). However, it should be noted that in some instances contaminants might be transformed into more harmful products, such as the case with perchloroethylene (PCE) and trichloroethylene (TCE), which degrade to create vinyl chloride, a human carcinogen (see *Chemistry of Organic Pollutants*).

2.1.2. Effect of Chemical Structure on Biodegradation

Many compounds are considered resistant to biodegradation and persist in the environment. One reason for this is related to chemical hydrophobicity. In general, the more hydrophobic, the less the chemical is bioavailable. This becomes evident with increased molecular weight in a series of similarly structured chemicals, such as PAHs. For instance, biodegradation rates generally decrease going from benzene, to naphthalene, to anthracene, etc. as a result of their increasing hydrophobicity and corresponding decrease in water solubility. Another reason is that not all chemical structures are amendable to biodegradation. For example, the addition of a halogen (e.g. Cl, Br), nitro group (NO_2) or sulfo group (SO_3H) to a readily degradable compound is thought to decrease the compound's susceptibility to biodegradation. The position of the additional substituents is also important. However, incorporation of oxygen into the compound in the form of hydroxyl (OH) and carboxyl (COOH) substituents has been shown to increase biodegradability. Branching of hydrocarbons also results in a lower biodegradation potential. This is shown by the straight chain octadecane (18 carbons), which has a much greater potential to biodegrade than branched phytane (18 carbons).

Competitive and noncompetitive inhibition can also affect bioremediation. A molecule that resembles the contaminant substrate molecule might bind to the active site of the enzyme. This would decrease overall enzyme activity, which would cause competitive inhibition. Noncompetitive inhibition occurs when a molecule binds to a non-active site that results in changing the enzyme’s shape. If the change in shape is sufficient, the enzyme may be inactivated.

2.2. Environmental Factors Affecting Biodegradation

The applicability and success of *in situ* bioremediation processes are primarily determined by the geology and hydrology of the contaminated site. For instance, both play critical roles in determining contaminant distribution. In addition, the hydraulic conductivity is often a limiting factor in applying bioremediation. Contaminated sites with high porosity and low hydraulic conductivities are poor candidates for bioremediation because delivering nutrients and an electron acceptor to the contaminated zone becomes difficult. Therefore, there are a number of environmental factors that must be considered in evaluating the application of bioremediation.

2.2.1. Subsurface Heterogeneity and Abiotic Factors

Soil properties can vary greatly from one region to another. They also can vary greatly within the same region spatially and with depth. The characteristics that define a soil type are the same abiotic factors that influence biodegradation, such as cation exchange capacity, clay type, gradation, intrinsic permeability, liquid limit, organic matter content, particle size, pH, porosity, and soil texture. Each of these factors influences the occurrence, rate, and products of biodegradation. Microorganisms have a range of tolerances to these factors, which affect their growth and activity. Consideration of these factors is necessary, because if they are outside the tolerable limits of the active microorganisms, no biodegradation will occur.

Since groundwater and vapors follow the path of least resistance, regions in the subsurface with high permeability will become preferential flowpaths. Regions with low permeability, such as clays and silts, will remain contaminated. These subsurface heterogeneities play critical roles in contaminant transport and the delivery of nutrients and electron acceptors in engineered systems, since water is the primary delivery mechanism. Contaminated sites with a high degree of geological complexity are often poor candidates for *in situ* bioremediation.

2.2.2. Sorption and Bioavailability

Chemical transport, reactivity, and toxicity can be strongly influenced by the compound's interactions with solid surfaces that exist in the environment. Sorption is defined as the "uptake of a solute by a sorbent". Natural sorbents include soils, sediments, and microorganisms. Sorption includes the processes of adsorption of the solute onto surface or interior voids and the partitioning of the solute into an organic medium, usually organic coatings found on soils, sediments, and clays (see *Soil Chemistry*, *Ecological Chemistry*). Because these organic coatings tend to accumulate on charged surfaces, solids such as clays (or the "fines") typically contain a disproportional amount of these coatings. A chemical's partition coefficient (K_p) is commonly used to determine the movement of an organic chemical in the subsurface (the retardation factor used widely in hydrogeology requires input of K_p). For natural systems, the extent of sorption is primarily determined by the magnitude of the soil-water partition coefficient that is normalized to organic carbon (K_oc) and the fraction of organic carbon that is present in the soil-sediment system (f_oc).

K_oc has been found to be linearly related to the degree of hydrophobicity that is usually determined by the magnitude of a chemical's octanol-water partition coefficient, which is typically reported in log units (common notation is either $\log P$ or $\log K_\text{ow}$). For instance, a set of 94 critically evaluated K_oc's was used to develop a relationship, which estimates K_oc by knowing a chemical's K_ow.

$$\log K_\text{oc} = 0.903 \times \log K_\text{ow} + 0.094 \qquad (1)$$

K_p for a specific aqueous-solid system is then determined by knowing the solid's f_oc where one percent of organic carbon would result in f_oc of 0.01.

$$K_\text{p} = K_\text{oc} \times f_\text{oc} \qquad (2)$$

K_p is also the equilibrium constant for a chemical that has partitioned between the aqueous and solid phases

$$K_\text{p} = [C_\text{sorbed}]/[C_\text{aqueous}] \qquad (3)$$

where $[C_\text{sorbed}]$ is the solid phase concentration of the solute at equilibrium and $[C_\text{aqueous}]$ is the aqueous concentration of solute.

Chemicals that strongly sorb to soil may not transport to a great extent in the unsaturated zone. Therefore, they are more likely to accumulate in surface soils and sediments. Examples of such contaminants include polychlorinated biphenyls (PCBs) and polycyclic aromatic hydrocarbons (PAHs) (see *Persistent Organic Wastes*, *Ecological Chemistry*).

Because sorption strongly influences the bioavailability of an organic chemical, the sorption intensity influences whether the chemical is readily accessible to microorganisms for biodegradation. In addition, chemicals that have partitioned into a separate phase or are present themselves as a separate phase, such as oil, non-aqueous phased liquids (NAPLs), or dense non-aqueous phase liquids (DNAPLs) may also not be bioavailable. However, some microorganisms have developed the ability to change the hydrophobicity of their outer membrane so they can directly contact the separate phase, or they secrete chemicals with surfactant-like properties (i.e., biosurfactants) that can solubilize separate phase chemicals, which can increase bioavailability.

2.2.3. Moisture Content

The microorganisms carrying out metabolic transformation require adequate moisture for their growth and activity. Therefore, the drying of surface soils can severely restrict biodegradation. For instance, decreasing the moisture content will decrease the rate of degradation. The optimum moisture level will depend on the properties of the soil and contaminant. It will also depend on whether degradation is targeted under aerobic or anaerobic conditions, because an abundance of water may cause anaerobic conditions if there is an active enough microbial community.

2.3. Growth-Linked Bioremediation

Mineralization of organic compounds is characteristic of growth-linked biodegradation; where the microbial population grows by utilizing the contaminant substrate and an electron acceptor to produce carbon dioxide, cell components, energy and other products typical of the usual catabolic and anabolic pathways. Generally, the subsurface already contains a relatively large number of microorganisms. Surface soil systems typically contain 10^5 to 10^8 viable bacterial cells per gram of dry soil and 10^5 to 10^6 fungi per gram of dry soil. Subsurface systems typically contain 10^2 to 10^5 bacterial cells per gram of dry soil. Approximately 60-90 percent of isolates in groundwater are gram-negative bacteria and a significant number of the total viable cells found in the environment can degrade unsubstituted hydrocarbons.

2.3.1. Substrate Threshold Concentrations

Sorption also directly influences biodegradation when a chemical partitions strongly to the solid phase and lowers the aqueous phase concentration. The lower aqueous concentration may fall below a threshold concentration where the number of reactions is not significant enough to supply the microbial population with energy for net growth. This concentration is typically referred to as S_{min} in engineered reactor systems. For example, it is well known that more highly chlorinated PCBs may undergo significant dechlorination at concentrations in the 50-100 mg kg^{-1} (ppm) range but no dechlorination is observed near the commonly observed target clean up levels of 1-10 mg kg^{-1} and lower.

Biodegradation may also be limited when there is not sufficient substrate to activate needed enzymes, or there is not a sufficient concentration gradient to transport the chemical across a cellular membrane when diffusion is the primary uptake mechanism. At this low concentration, the substrate may be providing just enough energy to keep the cell alive. Although the substrate is being degraded, the cells are not growing, and the population size is not increasing. When the concentration of the substrate is high, the appropriate metabolic enzymes are activated and diffusion occurs to provide enough of the carbon source to satisfy the needs for maintenance energy and for the processes that lead to increases in cell size, growth, and multiplication. However, threshold concentrations may not always exist. Many organic chemicals have been mineralized at levels below which the substrates fail to support growth, but in these instances, the rate of degradation will decrease when it is directly correlated with concentration.

2.3.2. Nutrients and Trace Metals

An adequate amount of nutrients containing the elements nitrogen (N) and phosphorous (P) are necessary for microbial metabolism and growth. These nutrients must be available to the microorganisms in a usable form, appropriate concentrations, and the proper ratios. In many instances, nitrogen and phosphorous are growth rate limiting. Consequently, biodegradation of contaminants will slow or will not proceed when available nitrogen and phosphorous are depleted or are no longer present. Metals, which are also required by microorganisms, but in trace amounts, are usually present in the subsurface in sufficient quantities (see *Trace Elements, Soil Chemistry*).

Several approaches have been used to supply nutrients by applying various commercial agricultural fertilizers either in solid form or mixed with irrigation water. Care should be taken in determining the nutrient application rates. If there is an over-abundance of nutrients added, they can migrate off site and cause biofouling. The appropriate application rates can be determined based on the amount of organic carbon present and utilization of a carbon/nitrogen/phosphorous ratio. This approach relies on experience obtained under varying conditions and information from published accounts. Although this ratio approximates 100:10:1, the literature reports significant variations, depending on a number of site-specific conditions. For instance, if some of the carbon is mineralized to carbon dioxide and water, nutrient requirements may be less. Another approach to determine the nutrient application rate is by experiment during treatability studies. Because this approach provides site-specific results, it is recommended.

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Biographical Sketches

Dan L. McNally is Chair and an Associate Professor of Science and Technology at Bryant College, Smithfield, Rhode Island. He received a B.S. in Architecture from The University of Detroit, a M.S. in Business Administration and Computer Information Systems from Webster University, a M.S. in Civil Engineering and a Ph.D. in Environmental Engineering from Michigan Technological University. He serves as an advisor and lead environmental industry consultant at the Rhode Island Export Assistance Center and is a co-founder of the Center for Sustainable Business Practices. This has involved environmental technology transfer from private industry to foreign countries in Central and Latin America, Eastern Europe, and China. Teaching responsibilities include undergraduate environmental science courses. Research interests include bioremediation of PAHs in subsurface environments, pollution prevention, and sustainable business practices.

James R. Mihelcic is a Professor of Civil and Environmental Engineering at Michigan Technological University, Houghton, Michigan. He received a B.S. in Environmental Engineering form The Pennsylvania State University and an M.S. and Ph.D. in Civil Engineering from Carnegie Mellon University. He co-directs Michigan Tech's Master's International Program in Civil and Environmental Engineering that allows students to combine graduate coursework and research with two years of service in the U.S. Peace Corps. He is a recipient of the Association of Environmental Engineering and Science Professors-Wiley Interscience Award for Outstanding Contributions to Environmental Engineering and Science and is also lead author for the textbook "Fundamentals of Environmental Engineering." His research and teaching interests are focused on biological processes in natural and engineered environments, industrial ecology and sustainability, and engineering in the developing world.

J. Mark Stapleton, PE, AEE, is a Senior Environmental Engineer and is currently assisting the National Aeronautic and Space Administration (NASA), Johnson Space Center with groundwater remediation using In-Situ Advanced Oxidative Processes on the Center Operations Support Services contract. Dr. Stapleton received his B.S. in Chemical Engineering from the University of Maryland, College Park and an M.S. in Civil Engineering and Ph.D. in Environmental Engineering from Michigan Technological University. Dr. Stapleton has authored and co-authored various research papers concerning bioremediation of soils and sediments. His most recent publication is titled "Darcy's Law and Hydraulic Conductivity," In: AEESP Environmental Engineering Processes Laboratory Manual (Eds: SE Powers, J Bisogni, J Burken, K Pagilla), Association of Environmental Engineering and Science Professors, Champaign IL, 2001. During a faculty appointment to Texas A&M University, Dr. Stapleton worked for United States Air Force Center for Environmental Excellence in San Antonio, TX. His research interests include microbial processes relevant to fate and transport of both organic and inorganic chemicals in the environment, microbial catabolic pathways that are applicable to biodegradation and anaerobic and aerobic *in situ* bioremediations techniques.