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Review

Advantages and Limitations of *In Situ* Methods of Bioremediation

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Advantages and Limitations of *In Situ* Methods of Bioremediation

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Abstract
There are two major types of *in situ* bioremediation: intrinsic and enhanced. Both rely on natural processes to degrade contaminants with (enhanced) or without (intrinsic) amendments. In recent years, *in situ* bioremediation concepts have been applied in treating contaminated soil and groundwater. Removal rates and extent vary based on the contaminant of concern and site-specific characteristics. There are a number of factor/variables that affect the rate of removal such as contaminant and co-contaminant distribution as well as concentration; indigenous microbial populations and reaction kinetics; and parameters such as pH, moisture content, nutrient supply, and temperature. Many of these factors are a function of the site and the indigenous microbial community and, thus, are difficult to manipulate. Specific technologies may have the capacity to manipulate some variables and may be affected by other variables as well; these specific issues are discussed with each technology in the following sections.

Keywords: Bioremediation; Pollutants; *In situ* techniques.

1. INTRODUCTION

Naturally, the provision of contaminants from the environment was carried out by indigenous bacteria, which is often called natural bioremediation (natural attenuation). Bioremediation by spatial planning for environmental resource management was called technical bioremediation or often "simply" called bioremediation. Bioremediation in the site depends on the metabolic capacity of indigenous microorganisms and environmental conditions [1]. Indigenous bacteria in many cases could not effectively exclude pollutant compounds, so the alternative that can be carried out was to add bacteria that have the ability to set aside contaminants on contaminated land. This approach was called bioaugmentation [2, 3].

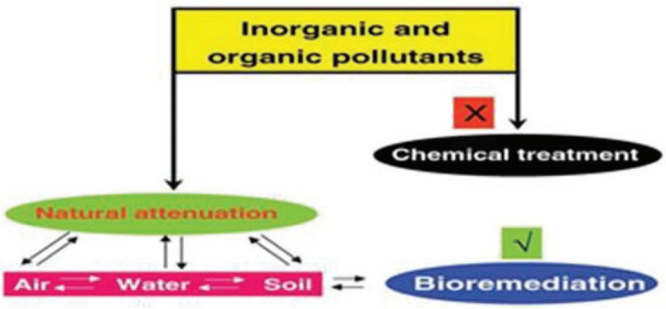
Bioremediation studies in the world have been conducted in the last four decades and include bioremediation purposes research; type of pollutants; aspects of microorganisms as bioremediation agents; bioremediation strategies; and various techniques in bioremediation methods. These studies consist of laboratory and field research.

Several studies were conducted by combining laboratory and field research. Both laboratory and *in situ* research studies had experienced an upward trend in the last 10 years. It means that the increased interest of researchers on *in situ* or field research in general did not reduce the interest of other researchers to keep conducting laboratory research. In comparison to *in situ* bioremediation studies, *ex situ* research had relatively lower growth, since *ex situ* research was mainly directed to contaminated spills of hydrocarbon compounds alone, in relatively small locations, and at relatively high cost in the implementation, especially contaminated soil transport pollutant. *In situ* research has advantage over *ex situ* research in terms of new opportunities for community-based *in situ* bioremediation applications in the recovery of agricultural land from contaminated land [4].

In the period of 2010s, along with the development of *in situ* research, the technique used was not limited only to land farming, but researchers then tried to use other approaches such as composting. Although originally composting has been widely used in *ex situ* research, Mizwar and dan Trihadiningrum [5] have conducted *in situ* bioremediation research of contaminated hydrocarbon land using indigenous bacteria derived from compost; Setiyo *et al.* [6] have also conducted *in situ* bioremediation of contaminated pesticides by using existing microbes in compost. This led to a larger percentage of *in situ* research (73%) of bioremediation research using land farming (57%). Figure 1 shows the existing general methodologies for soil remediation [7].

In general, the physical removal of pollutant from contaminated soil includes both *in situ* and *ex situ* methods. *In situ* remediation is without excavation of contaminated site. It involves destruction or transformation of the contaminant, immobilization to reduce the bioavailability, and separation of the contaminant from the bulk of soil [8]. These techniques are favored over the *ex situ* due to their low cost and reduced impact on the ecosystem.

Figure 1: Natural attenuation and bioremediation are widely accepted for environmental cleanup.



In Latin, *in situ* means "in the original place." Thus, *in situ* bioremediation means bioremediation based on the degradative activities of endogenous microbial populations. In other words, it relies on the indigenous microbial flora of subsurface soils and groundwater. It depends on the premise that the microorganisms already present in a contaminated site have adapted to the organic chemical wastes found there and are able to degrade some or all of the components of these wastes.

The adapted microorganisms continue degradation until some nutrient or electron acceptor attains a limiting concentration. Normally, oxygen level is the limiting factor, but phosphate and nitrate may also become limiting factor. The enhanced *in situ* bioremediation can be carried out by addition of nutrients such as nitrogen (N) and phosphorus (P) to the environment. The addition of nutrients and hydrocarbons to contaminated soils and marine environments has been investigated.

Fertilizers were used to accelerate the removal of oil from the beaches, supplying extra nutrients that were in limiting concentrations. This is one of the best examples of enhanced *in situ* bioremediation, which was carried out to the clean-up of the Exxon Valdez oil spill (1989) in Alaska.

Enhanced *in situ* bioremediation offers several potential advantages in the elimination of hazardous wastes as it speeds up the disappearance of oil by a factor of two to three over its rate of disappearance on untreated site in the samples of oil taken at the end of that time from surfaces.

The biotransformation of this plasmid to various bacteria in natural soil and marine water has been carried out, indicating the horizontal transfer of catabolic genes from one bacterium to another, paving way to create "Superbugs" for bioremediation in differing and metamorphosing ecosystems. Many of the *Pseudomonas* and *Ralstonia* species strains harbored a catabolic plasmid, which encodes the genes for hydrocarbon degradation.

The biosorption of cadmium has been investigated using batch technique. Cd^{2+}-tolerant bacterial strain KTSMBNL 43 was isolated from electroplating industrial soil. The isolated strain KTSMBNL 43 was identified as *Bacillus cereus* based on 16S rDNA sequencing analysis. Various parameters that influence metal removal such as pH, temperature, initial metal concentration, and contact time were investigated, which proved that *B. cereus* can be an interesting alternative, low cost, and environmental friendly biosorbent that may have important application in Cd^{2+} removal from polluted soil [9]. Similar studies show the sorption of aluminum by *Bacillus safensis* isolated from an explosive contaminated site. In a batch system, the optimum pH, temperature, and contact time on biosorption of Al and cell growth were found to be 6.0, 35°C, and 24 h, respectively, with initial metal concentration of 100 mg/L Al. In immobilization studies, sodium alginate was used as a supporting material for cell entrapment under optimized conditions [10].

Bioventing is one of the most widely used methods of remediating soils contaminated by petroleum hydrocarbons. Bio-inventing supplies air to an unsaturated soil zone by using a combination of pumps and blowers that apply a vacuum to the target area while continuously injecting low volumes of air.

In situ bioremediation under anaerobic conditions may also be enhanced by providing electron acceptors such as sulfate or nitrate. However, *in situ* methanogenic bioremediation has not, so far, been investigated thoroughly, although it is recognized that degradation of organic pollutants in anaerobic micro niches in soil, sediment, and groundwater environments contributes significantly to overall *in situ* bioremediation rates.

2. ENVIRONMENTAL FACTORS INFLUENCING *IN SITU* BIOREMEDIATION

Both the rate and the extent of microbial remediation of organic contaminates *in situ* are affected by a number of environmental factors. Some of these may be manipulated, whereas others are difficult to modify within the contaminated site. Some of the important environmental factors that affect *in situ* bioremediation are as follows.

2.1. pH

As the majority of bacteria show optimal growth at neutral pH range, most laboratory-based bioremediation studies have been carried out in about neutral pH. In many studies, adjustment of pH enhances the rate of biodegradation.

2.2. Temperature

The vast majority of *in situ* bioremediation applications have been carried out under mesophilic conditions (between 20 and 40°C). Temperature directly affects the metabolism and growth of bacteria. All studies indicated that even the modest increase in temperature may significantly increase bioremediation rates.

The thermophilic or thermotrophic species are capable of degrading a diverse range of organic compounds in wastes or wastewaters at higher temperature (60–70°C). A variety of techniques have been used to increase the temperature in *in situ* soil remediation applications.

2.3. Water Content

Microbes generally require water activity values (aw) of 0.9–1.0 in order to metabolize and grow. The majority of bacteria grow optimally at aw values in the upper limit of this range. Water content in soils or sediments is an important factor affecting bioremediation rates.

2.4. Soil Type

In general, *in situ* degradation rates are slowed down under unfavorable geological characteristics that include low permeability of soil, fractured rock, and water-logged or arid conditions. *In situ* bioremediation rates are enhanced when the soil is granular or porous.

2.5. Nutrient Availability

Nutrient supplementation (NP) significantly increases bioremediation rates. Good results are seen in hydrocarbon bioremediation of soils and groundwaters when N and P levels have been shown to be limiting.

2.6. External Electron Availability

Oxygen supply enhances aerobic respiratory breakdown of organic contaminates. Addition of hydrogen peroxide is used to introduce oxygen. Hydrogen peroxide (H_2O_2) is about seven times more soluble in water than oxygen and its decomposition in soil yields 0.5 mol of O_2 per mol of H_2O_2 introduced to contaminated site ($2H_2O_2$ ® $2H_2O + O_2$).

2.7. Bioavailability of Organic Pollutants

Improvements in bioremediation rates have been achieved by the addition of biosurfactants or synthetic detergents to the contaminated zone. This is an important factor governing the rate of *in situ* bioremediation. Addition of biodegradable solvents, which assist in desorption and dissolution rates, e.g., adsorption of PAHs (polycyclic aromatic hydrocarbons), by soil particles shows increase in the biodegradation of the adsorbed pollutants.

2.8. Cometabolism

Cometabolic transformation of organic pollutants is an important process in both aerobic and anaerobic environments. Bacterial transformation of DDT (dichloro-diphenyl-trichloroethane) and PCBs (para chloro benzene) provides examples of both aerobic and anaerobic cometabolic biodegradation. Provision of a readily metabolizable substrate may also promote pollutant transformation by enhancing the growth of associated microbes involved in the overall microbial activity.

It is a process whereby microbes involved in the metabolism of a growth promoting substrate also transform other organic contaminants (co-substrates) that are not growth supporting if supplied as sole carbon and energy source.

2.9. Gene Expression

The ability of indigenous microorganisms to degrade organic pollutants is dependent on expression of the genes encoding the required uptake and degradative enzyme systems. If the bioavailable concentration of a pollutant is too low, the expression of inducible operons may not occur.

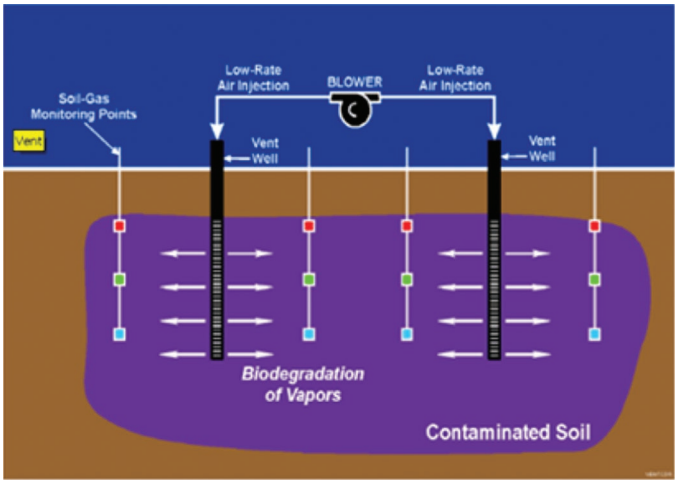
3. ENHANCED *IN SITU* BIOREMEDIATION

The primary *in situ* biological technology applicable to the unsaturated zone is *bioventing*, which is categorized as either aerobic, cometabolic, or anaerobic depending on the amendments used.

3.1. Aerobic Bioventing

In aerobic bioventing, contaminated unsaturated soils having low-oxygen concentrations are treated by supplying oxygen to facilitate aerobic microbial biodegradation. In this technique, oxygen is introduced by air injection wells that push air into the subsurface (Figure 2). Bioventing has a robust track record in treating fuels aerobically from degradable contaminants. Vacuum extraction wells, which draw air through the subsurface, may also be used. When building foundations or similar structures are

Figure 2: Aerobic bioventing.



close to the site, the extraction mode may be used to avoid the buildup of contaminated, and possibly explosive, vapors in the building basements. Extracted gases may require treatment since volatile compounds may be removed from the ground. Bioventing employs lower air flow rates that provide only the amount of oxygen required to enhance removal as compared with soil vapor extraction (SVE). Although operated properly, the injection of air does not result in the release of the contaminants to the atmosphere through volatilization because of these low flow rates [11-13].

Biodegradable contaminants that can be treated primarily by aerobically bioventing are non-chlorinated VOCs (volatile organic compounds) and SVOCs (semi-volatile organic compounds) that are located in the vadose zone or capillary fringe [4, 5, 12, 13]. The U.S. Air Force Bioventing Initiative and the EPA Bioremediation Field Initiative showed that bioventing was effective under a wide variety of site conditions at about 125 sites.

In addition to fuel treatment, aerobic bioventing has treated a variety of other contaminants like lightly halogenated solvents such as 1,2-dichloroethane, dichloromethane and chlorobenzene; and non-halogenated solvents such as benzene, acetone, toluene, and phenol; and SVOCs such as low-molecular-weight PAHs. Since the experience with these other types of contaminants is more limited, laboratory- and pilot-scale studies may be needed to evaluate effectiveness, design the bioventing system, and estimate treatment times. Bioventing has proven to be a useful technology at many sites under a variety of conditions, but like all technologies, bioventing has some limitations.

3.1.1. Limitations

1. Low-permeability soils also may pose some difficulties for bioventing because of a limited ability to distribute air through the subsurface as there are limitations in the ability to deliver oxygen to the contaminated soil.
2. Difficulty in developing a system design that can minimize environmental release and achieve sufficient aeration, and therefore sites with shallow contamination can pose a challenge to bioventing because operating the system in the extraction mode may circumvent the difficulty [11, 13].
3. Bioventing will not stimulate contaminant bioremediation if the contaminated zone is aerobic. If a soil gas survey measures soil oxygen levels consistently above 2–5%, then the soil is sufficiently aerated for biodegradation to occur and oxygen is not limiting degradation. Bioventing will not enhance bioremediation in this situation. This situation is unusual and, if encountered, may indicate that some other contaminants, such as metals, are inhibiting degradation [11, 14].

Although bioventing is relatively inexpensive, bioventing can take a few years to clean up a site depending on contaminant concentrations and site-specific removal rates. If a quicker cleanup is needed, more intensive *ex situ* technologies may be more appropriate [11-13].

3.2. Cometabolic Bioventing

Chlorinated solvents such as TCE, trichloroethane (TCA), and dichloroethene (DCE) can be treated by cometabolic bioventing at a few sites. The equipment used in cometabolic bioventing is similar to aerobic bioventing, but cometabolic bioventing exploits a different biological mechanism. Similar to bioventing, cometabolic bioventing involves the injection of gases into the subsurface; however, cometabolic bioventing injects both air and a volatile organic substrate, such as propane. The concentrations in this gas mixture should be well below the lower explosive limit (LEL) and should be monitored in soil gas [11-14].

Cometabolic bioventing exploits competitive reactions mediated by monooxygenase enzymes [4, 12]. Monooxygenases catalyze the oxidation of hydrocarbons, often through epoxide intermediates, but these enzymes can also catalyze the dechlorination of chlorinated hydrocarbons. Thus, by supplying an appropriate organic substrate and air, cometabolic bioventing can elicit the production of monooxygenases, which consume the organic substrate and facilitate contaminant degradation [11, 14].

Cometabolic bioventing has been used to treat lightly chlorinated compounds in the vadose zone or capillary fringe. Many factors including soil gas permeability, organic substrate concentration, type of organic substrate selected, and oxygen supply and radius of influence the degradation rate and design of cometabolic bioventing systems. Unlike many variables that are determined by site conditions, the selection and concentration of the organic substrate are controllable and can be important to the removal rate.

Treatability or bench-scale testing can be useful in selecting the organic substrate and concentration for a site. In addition, small-scale testing can show that full dechlorination is observed at a site [11-14].

Establishing cometabolic bioventing as the primary mechanism of removal in the field is challenging. Unlike aerobic bioventing, the oxygen use and chlorinated solvent removal are not related stoichiometrically because the metabolism of added organic substrates also consumes oxygen. As a result, measurements of oxygen use, carbon dioxide generation, and contaminant removal cannot be linked stoichiometrically.

3.2.1. Limitations

1. As with aerobic bioventing, difficulty in distributing gases in the subsurface may make the application of cometabolic bioventing more complicated. In some cases such as soils with high-moisture content or low-gas permeability, the design of the cometabolic system may compensate for poor permeability. In the case of shallow contamination, designing a cometabolic bioventing system that minimizes environmental release and achieves sufficient aeration and organic substrate distribution may be difficult [11-14].
2. Another limitation to cometabolic bioventing is the lack of experience with the technology. Although cometabolic bioventing has been shown at a limited number of sites, the technology is not as well understood as aerobic bioventing. Researchers are still studying which contaminants are amenable to this type of biodegradation and what removal rates can be expected. Establishing that biological processes are the primary mechanism for contaminant removal is also more difficult. Finally, regulatory and public acceptance is not as strong for cometabolic bioventing as for aerobic bioventing. However, treatability testing of samples from the contaminated site and pilot-scale testing may alleviate many of these limitations and concerns [15]. As more sites are remediated using cometabolic bioventing, these limitations may ease.

3.3. Anaerobic Bioventing

While aerobic and cometabolic bioventing are useful for degrading many hydrocarbons and lightly chlorinated compounds, some chlorinated species are not effectively treated aerobically. Microbes may degrade these contaminants directly via anaerobic reductive dechlorination or through anaerobic cometabolic pathways. Anaerobic reductive dechlorination is a biological mechanism typically marked by sequential removal of chlorine from a molecule.

Microbes possessing this pathway do not gain energy from this process. Anaerobic cometabolism is similar to aerobic cometabolism in that microbes fortuitously degrade contaminants while reducing other compounds (cometabolites). Anaerobic bioventing may use both biological mechanisms to destroy the contaminants of concern.

The gas delivery system in anaerobic bioventing is similar to other bioventing technologies, but it injects nitrogen and an electron donor, instead of air to maintain reductive anaerobic conditions. The nitrogen injected displaces the soil oxygen, and small amounts of an electron donor gas (such as hydrogen and carbon dioxide) produce reducing conditions in the subsurface, thereby facilitating microbial dechlorination. Volatile and semi-volatile compounds may be produced during anaerobic bioventing. Some of these compounds may be slow to degrade under anaerobic conditions. These compounds may be treated in two ways. Volatile compounds may diffuse into the soils surrounding the treatment zone, where aerobic degradation may occur. SVOCs and VOCs remaining in the treatment zone may be treated by following anaerobic bioventing with aerobic bioventing. Since aerobic and anaerobic bioventing share similar gas delivery systems, the switch can be made by simply changing the injected gas.

Anaerobic bioventing is an emerging technology that has been demonstrated in several laboratory and field studies. This process may be useful in treating highly chlorinated compounds such as tetrachloroethene (PCE), TCE (trichloroethene), RDX (research department explosive, pentachlorophenol, and pesticides such as linden and dichlorodiphenyltrichloroethane (DDT). Due to the limited experience with this technique, laboratory, pilot, and field demonstrations are recommended to confidently apply this technology to remediate a site. As with the other bioventing technologies, the ability to deliver gases to the subsurface is important. Soils with high-moisture content or low-gas permeability may require careful system design to deliver appropriate levels of nitrogen and the electron donor. Sites with shallow contamination or nearby buildings are also a challenge since this technology is operated by injecting gases. In addition, anaerobic bioventing can take a few years to clean up a site depending on the contaminant concentrations and site-specific removal rates. If a quicker cleanup is needed, other technologies may be more appropriate.

4. SURFICIAL SOIL REMEDIATION

This process is similar to the land farming and composting (*ex situ* method). Variations of these technologies involve tilling shallow soils and adding amendments to improve aeration and bioremediation. Since these treatments do not include an impermeable sublayer, migration of contaminant may be a concern depending on the contaminants of concern and treatment amendments. A more prudent approach would be to excavate soils and treat them in lined beds.

This technology will generally require special permission from the applicable regulatory agency. Some types of monitoring for contaminant migration are required frequently.

5. GROUNDWATER AND SATURATED SOIL REMEDIATION

In situ bioremediation techniques are applicable to groundwater and saturated soil, which include dechlorination, enhanced aerobic treatment, biological reactive barriers that create active remediation zones, and bioslurping/biosparging techniques that promote aerobic degradation.

5.1. Anaerobic Reductive Dechlorination

Anaerobic reductive dechlorination has been used at many sites where the groundwater has been contaminated with chlorinated solvents, such as TCE or PCE (trichloroethane or perchloroethane). In this treatment, organic substrates are delivered to the subsurface where they are fermented. The fermentation creates an anaerobic environment in the area to be remediated and generates hydrogen as a fermentation byproduct. The hydrogen is used by a second microbial cometabolic bioventing.

The hydrogen is used by a second microbial population to sequentially remove chlorine atoms from chlorinated solvents [8, 16]. If PCE were degraded via reductive dechlorination, the following sequential dechlorination would be observed: PCE would be converted to TCE, then to DCE (dichloroethane), vinyl chloride (VC), and/or dichloroethane [14].

Anaerobic dechlorination may also occur via cometabolism where the dechlorination is incidental to the metabolic activities of the organisms. In this case, contaminants are degraded by microbial enzymes that are metabolizing other organic substrates. Cometabolic dechlorination does not appear to produce energy for the organism. At pilot- or full-scale treatment, cometabolic and direct dechlorination may be indistinguishable, and both processes may contribute to contaminant removal. The microbial processes may be distinguished in the more controlled environment of a bench-scale system [14].

Anaerobic reductive dechlorination is primarily used to treat halogenated organic contaminants, such as chlorinated solvents. In addition to the variables discussed initially, the treatment rate and system design are dependent on several factors including site hydrology and geology, type and concentration of organic substrates, and site history. As with cometabolic bioventing, the selection of organic substrate and the concentration used are controllable and can be important to the removal rate. Treatability or bench-scale testing can be useful in selecting the best organic substrate and concentration for a site. In addition, small-scale testing can show that full dechlorination is possible at a site. In some cases, dechlorination may stall at DCE despite the presence of sufficient electron donors. If a site does not show full dechlorination (either as part of site assessment or in microcosm testing), a combined treatment strategy, such as anaerobic treatment followed by aerobic treatment, may be successful. Alternatively, bioaugmentation may improve the dechlorination rate [11-14].

5.2. Aerobic Treatment

Enhanced *in situ* aerobic groundwater bioremediation processes are used where aerobically degradable contaminants, such as fuels, are present in anaerobic portions of an aquifer. Air or other oxygen sources are injected into the aquifer near the contamination (see Figure 3). The indigenous bacteria are able to degrade the contaminants [12, 14] as the oxygenated water migrates through the zone of contamination.

Aerobic treatment may also be used to directly or cometabolically degrade lightly chlorinated species, such as DCE or VC. In the direct aerobic pathway, air is injected into the aquifer. The microbes appear to generate energy by oxidizing the hydrocarbon backbone of these contaminants, resulting in the release of chloride [12]. This process has been used to complete contaminant removal following anaerobic treatment at several sites [12, 14].

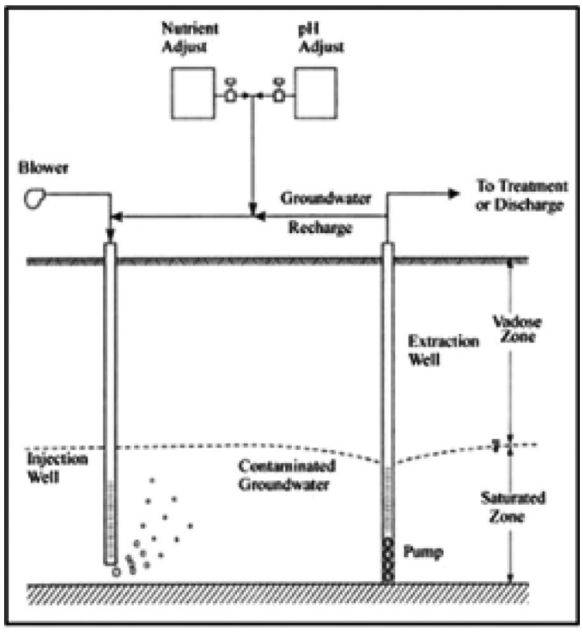
Cometabolic aerobic treatment is founded on the same biological principles as cometabolic bioventing and involves the addition of oxygen and organic substrates, such as methane, to the aquifer. As with other cometabolic processes, these organic substrates are metabolized by enzymes that incidentally degrade the contaminant. In this treatment, sufficient oxygen must be present to fuel the oxidation of both the substrate and contaminant [11, 14].

5.3. Amendment Delivery

In situ groundwater treatment, either aerobic or anaerobic, may be configured as direct injection of air or aqueous streams or as groundwater recirculation. In direct injection, amendments, such as organic substrates, oxygen sources, or nutrients, are directly injected into the aquifer.

For example, oxygen may be sparged into the aquifer as a gas. Lactate or hydrogen peroxide may be injected as a liquid stream; when using hydrogen peroxide, caution should be used as it may act as a disinfectant. In some cases, both liquids

Figure 3: Aerobic treatment (adapted from [5]).



and gases are added. The groundwater recirculation configuration involves extracting groundwater, amending it as needed, and then re-injecting it back into the aquifer.

Recirculation may also be conducted below the ground surface by extracting groundwater at one elevation, amending it in the ground, and re-injecting it into another elevation [12, 14].

In addition to the variables discussed initially, the treatment rates and system design are the result of several factors including site hydrology and geology, amendment to be added, solubility of air or oxygen sources, and site history. The low solubility of air in water often limits reaction rates and may make this process impractical if cleanup time is short [11-13, 15].

Careful attention also should be given to co-contaminants, especially metals. When an aquifer environment is converted from an aerobic to an anaerobic environment, a variety of chemical species may become soluble. Therefore, it is important to check for changes in co-contaminants such as arsenic, which may be solubilized during the treatment process [11–13, 15].

5.4. Biosparging and Bioslurping

Biosparging is most often recommended at sites impacted with mid-weight petroleum hydrocarbon contaminants, such as diesel and jet fuels. Lighter contaminants, such as gasoline, tend to be easily mobilized into the unsaturated zone and physically removed. Heavier contaminants, such as oils, require longer remedial intervals because of reduced microbial bioavailability with increasing carbon chain length [17].

A number of contaminants have been successfully addressed with biosparging technology, including gasoline components such as benzene, toluene, ethylbenzene, and xylenes (BTEX) and SVOCs.

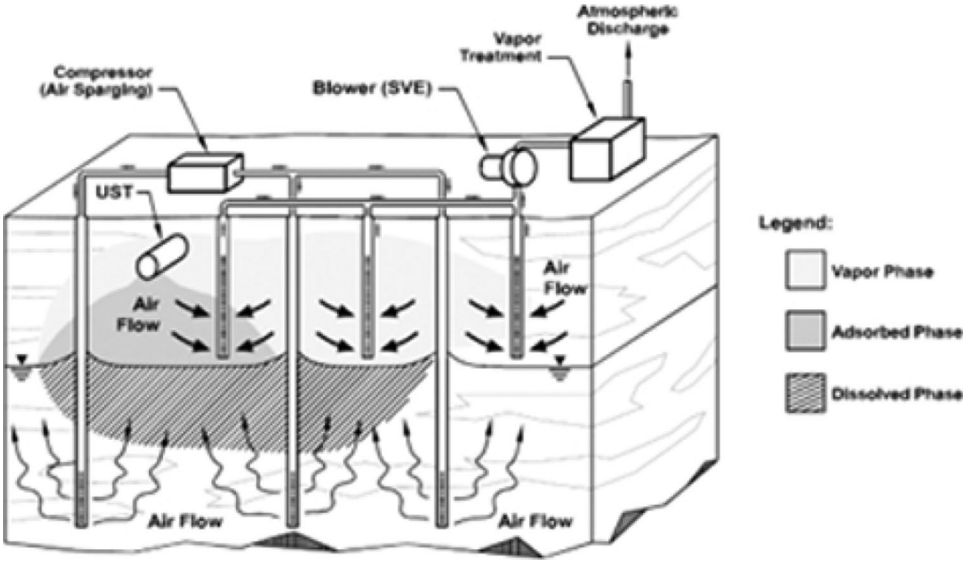
Biosparging is typically achieved by injecting air into a contaminated subsurface formation through a specially designed series of injection wells. The air creates an inverted cone of partially aerated soils surrounding the injection point. The air displaces pore water, volatilizes contaminants, and exits the saturated zone into the unsaturated zone. While in contact with groundwater, oxygen dissolution from the air into the groundwater is facilitated and supports aerobic biodegradation (Figure 4). Biosparging (similar to air sparging) involves the injection of a gas (usually air or oxygen) and occasionally gas-phase nutrients, under pressure, into the saturated zone to promote aerobic biodegradation. In air sparging, volatile contaminants can also be removed from the saturated zone by desorption and volatilization into the air stream. The emphasis on the biological degradation rate over physical removal, as well as lower rates of air injection, is what distinguishes this technology from air sparging.

Care must be taken to determine whether contaminant concentrations in soil gas and released vapors resulting from biosparging require treatment. For this purpose, biosparging may be implemented along with SVE or bioventing as a remedy for increased contaminant concentrations in the unsaturated zone.

One specialized form of biosparging involves the injection of organic gases into the saturated zone to induce cometabolic biodegradation of chlorinated aliphatic hydrocarbons, and this is analogous to cometabolic bioventing. The injection of gases below the water table distinguishes biosparging from bioventing.

In contrast to cometabolic bioventing, the solubility of organic gases in water limits delivery of the primary substrate during cometabolic biosparging applications. This solubility limitation affects the economics of cometabolic biosparging

Figure 4: Biosparging system (used with soil vapor extraction).



applications since the interaction between bacterial cometabolite consumption and cometabolite water solubility directly determines the number of methane biosparging injection wells required at a given site. Safety precautions similar to those required for cometabolic bioventing apply to cometabolic biosparging [15, 18].

Bioslurping is limited to 25 feet below ground surface as contaminants cannot be lifted more than 25 feet by this method. This is an effective way in removing free product that is floating on the water table [19].

Bioslurping, also known as multi-phase extraction, combines the following two remedial approaches:

1. Bioventing stimulates aerobic bioremediation of contaminated soils *in situ*.
2. Vacuum-enhanced free-product recovery extracts light, no aqueous-phase liquids (LNAPLs) from the capillary fringe and the water table [20].

A vacuum is applied to the bioslurping tube and free product is "slurped" up the tube into a trap or oil-water separator for further treatment. Removal of the LNAPL results in a decline in the LNAPL elevation, which in turn promotes LNAPL flow from outlying areas toward the bioslurping well. As the fluid level in the bioslurping well declines in response to vacuum extraction of LNAPL, the bioslurping tube also begins to extract vapors from the unsaturated zone. This vapor extraction promotes soil gas movement, which in turn increases aeration and enhances aerobic biodegradation [21].

6. CONCLUSION

Each of the methods presented in this review article possesses a number of advantages and disadvantages. Phytoremediation by *in situ* method uses plants and their associated microorganisms to degrade, contain, or render harmless contaminants in soil or groundwater. In essence, it employs human initiative to enhance the natural attenuation of contaminated sites and, as such, is a process that is intermediate between engineering and natural attenuation. As the technique depends on natural, synergistic relationships among plants, microorganisms, and the environment, it does not require intensive engineering techniques or excavation. Human intervention may, however, be required to establish an appropriate plant-microbe community at the site or apply agronomic techniques (such as tillage and fertilizer application) to enhance natural degradation or containment processes. *In situ* technique has been used effectively to remediate inorganic and organic contaminants in soil and groundwater.

There was a large gap between laboratory research and field research in land bioremediation research in the world. Bioremediation studies of polluted land of various pollutants, especially heavy metals and hydrocarbon, are mostly carried out in the laboratory to see their microbial potential and examine bioremediation strategies for the best bioremediation performance. But in the last 10 years, they already started conducting field research, especially with *in situ* method.

The bioremediation strategy on hydrocarbons contaminated land in the last decade has been trending topic at bioremediation research in Asia, Europe, and America.

Bioremediation methods were increasingly believed to solve various contamination cases today. Yet, there are not much researchers who have performed any field research and then conducted laboratory testing in order to support and provide information about the phenomenon that occurred in the site during research.

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Conflict of Interest
None.

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