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CHAPTER 8

Bioremediation with Bacteria and Enzymes

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8.1 Introduction

In the past decades, significant amounts of chemicals have been released into the environment by agricultural, industrial, commercial and other human activities. These chemicals have caused harm to the ecosystem and human health. These pollutants are mainly released into the environment as metal, non-metal, metalloid, inorganic and organic compounds (Cluis, 2004). Organic contaminants include aliphatic, alicyclic, aromatic and polycyclic aromatic hydrocarbons comprising halogenated and non-halogenated compounds, pesticides and explosives. Inorganic pollutants may be metals such as Ag, Al, As, Be, Cd, Cr, Cu, Hg, Fe, Ni, Pb, Sb, Se, Zn, and radioactive elements (Meagher, 2000).

Most remediation techniques involving physical and chemical methods are expensive and produce secondary pollutants in the environment. In order to overcome these problems, biological degradation of pollutants is favored because of its cost effective and eco–friendly approach (Hattan et al., 2003). Bioremediation is a process in which degradation of toxic compounds results in their conversion into non–toxic substances such as CO_2 and H_2O. This process can be facilitated either at contaminated sites (*in-situ* bioremediation) or in bioreactors (*ex-situ* bioremediation) using microorganisms to achieve complete detoxification of toxic compounds (Hwang and Cutright, 2002).

Microorganisms are ubiquitous in nature and have tremendous metabolic ability to degrade and utilize most toxic compounds as sources of energy and growth. They possess characteristic degradative enzymes for biodegradation of respective contaminants through aerobic or anaerobic processes. Bacteria can be classified as aerobic and anaerobic based on their requirement for oxygen for growth. Aerobic bioremediation has 10 to 100 times higher degradation efficiency than anaerobic processes and is, therefore, a commonly used practice (Ahlet, et al., 2001). The rate of degradation can be further enhanced by dispersing adapted bacteria at contaminated sites through a process know as bioaugmentation (Quan, et al., 2004) or by adding required nutrients to stimulate the growth of indigenous microorganisms through biostimulation (Trindade et al. 2005).

In general, biodegradation follows microbial metabolic pathways such as aerobic respiration, anaerobic respiration, fermentation and co-metabolism. Bioremediation of toxic compounds also depends upon the bioavailability of contaminant to microbes, environmental factors and site conditions such as temperature, pH, nutrients, electron acceptor(s), redox potential, water activity, osmotic pressure and concentration of contaminants (Evans 2003; Thakur 2004).

This chapter gives descriptions and presents case studies associated with common soil and groundwater bioremediation techniques. These techniques include biosparging, bioventing, biostimulation, bioaugmentation, bioleaching, anaerobic and aerobic biotransformation, biological fixation, enzyme-catalyzed treatment, biological reactors and natural attenuation.

8.2 Biosparging

Biosparging is an *in-situ* remediation technology that utilizes naturally occurring microorganisms to degrade organic contaminants of concern (COCs) within the saturated zone. The rate of bioremediation is enhanced by inducing air (or oxygen) flow using air injection wells, and if necessary, by the addition of air to the saturated zone (Weston 1988).

A schematic of a biosparging system is illustrated in Figure 8.1. The process is similar to an *in-situ* air sparging system (IAS), except that a lower air flow rate is used to enhance biotransformation and minimize volatilization (primary mechanism of IAS). The air flow rate is controlled by the metabolic demand of microorganisms to successfully remediate the saturated zone. Biosparging has proven most effective in reducing petroleum products at leaky underground storage tank sites (Norris, et al. 1994). Although constituents adsorbed to aquifer material can also be treated to a certain extent, the technique is not suitably effective for highly volatile contaminants.

8.2.1 Factors Affecting Biosparging Processes

There are various factors which are responsible for effective *in-situ* bioremediation of ground water contaminated with petroleum hydrocarbons (EPA-542-R-00-008). These can be divided into two categories: site characteristics and constituent characteristics.

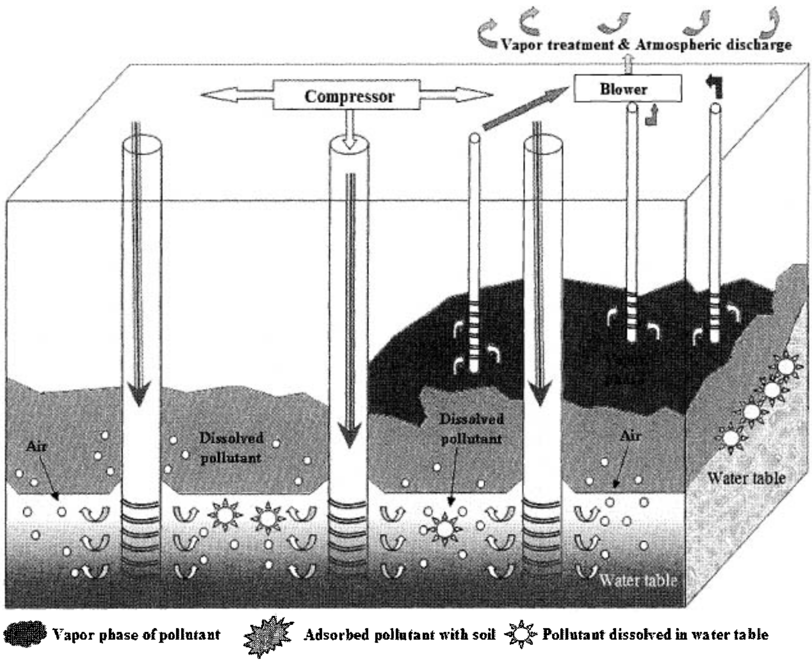

Figure 8.1 Schematic representation of *in-situ* bioremediation of organic contaminant of concern (COCs) using biosparging system combined with soil vapor extraction for ground water treatment.

8.2.1.1 Site Characteristics

Various soil characteristics play a pivotal role in controlling biosparging processes. These include intrinsic soil permeability, soil structure and stratification, temperature, pH, oxygen concentration, nutrients, microbial density and iron content.

Intrinsic Soil Permeability

The intrinsic permeability of a soil is a measure of its ability to transmit fluids which determines the rate at which oxygen can be supplied to the hydrocarbon degrading bacteria. At least 3 to 3 ½ pounds of oxygen is generally needed to degrade one pound of petroleum hydrocarbons. The coarse grained soils (sands) have greater intrinsic permeability compared to fine grained soils (clays and silts) and should be greater than 10^{-9} for effective bioremediation (EPA 542-R-00-006).

The intrinsic permeability of a saturated zone can be calculated using the following equation (Aelion et al. 1995):

$$k = K\,(\mu/\rho g) \qquad \text{(Eq. 8.1)}$$

where k = intrinsic permeability (cm^2)
K = hydraulic conductivity (cm/sec)
μ = water viscosity (g/cm · sec)
ρ = water density (g/cm^3)
g = acceleration due to gravity (cm/sec^2)

Soil Structure and Stratification

These characteristics include the type of soil present and its micro and macro structure. Soil structure and stratification can control the biosparging pressure and distribution of oxygen and nutrients in the saturated zone (Clayton et al. 1995).

Temperature

The optimal bacterial growth is found to be in the range of 10 to 45° C. The rate of microbial activity typically doubles for every 10° C rise in temperature within this range. The rate of degradation decreases below and above the mentioned range of temperature (Filler 1997; Webb and Phelan 1997). In most areas of the U.S., the average groundwater temperature is about 13°C, but groundwater temperatures may be somewhat lower or higher in the extreme northern and southern states. In most cases, subsurface microbial activity has been found to decrease significantly at temperatures below 10°C and distinctly bring to an end below 5°C. Biosparging is an *in-situ* technology, the bacteria are likely to experience stable groundwater temperatures with only slight seasonal variations.

pH

Values of pH between 6 and 8 are most suitable for bacterial growth. The pH of groundwater can be adjusted prior to and or during biosparging process (McCray and Falta 1997). However, pH adjustment is expensive approach due to natural buffering capacity of the groundwater system which requires continuous adjustment and monitoring throughout the operation. In addition, during pH adjustment, it may lead to rapid changes in pH and lead to unfavourable conditions for microbial activity.

Oxygen Concentration

The rate of biodegradation greatly depends on the availability and supply of oxygen at a contaminated site. Oxygen serves as a terminal electron acceptor in aerobic metabolic processes. In the absence or low availability of dissolved oxygen microbes can utilize other electron acceptors (such as nitrate or sulfate) for degradation of contaminants (McCray and Falta 1996). However, this occurs at significantly reduced rates of transformation.

Nutrients

Nutrients play a major role in bacterial growth and metabolism. Frequent addition of nutrients is necessary to maintain the required bacterial populations at contaminated sites. However, over addition of nutrients at the polluted sites may inhibit the rate of metabolism (Norris, et al. 1993). Nitrogen addition can lower pH, depending on the amount and type of nitrogen added.

Microbial Density

Microbial density is an important factor for effective biosparging with a typical range from 10^4 to 10^7 CFU/g of soil. The minimum plate count of heterotrophic bacteria in a biosparging zone should not be less than 10^3 CFU/g (Sleep 1998). Otherwise, the rate of remediation will be too slow.

Iron

Ferrous iron [Fe^{+2}] present in soil precipitates to iron oxide [Fe^{+3}] by oxidation reaction. Ferric iron precipitates can block the soil pore spaces and reduce soil permeability (Mesania and Jennings 2000). Hence, care should be exercised in such soils or groundwater systems.

8.2.1.2 Constituent Characteristics

Success of biosparging process also depends on certain features of chemical constituents present:

Chemical Structures

It is a chief rate determining parameter of biodegradation in biosparging processes. Low molecular weight (nine atoms or less) aliphatic and mono aromatic compounds biodegrade faster than higher molecular weight and complex compounds as summarized in table 6.1 (Nakhla and Niaz 2002).

Table 8.1 Types of constituents and their rates of biodegradation.

	Chemical Contaminants	Sources	Rate of Degradation
Alkanes and mono-aromatics	n-butane, l-pentane, n-octane, nonane, methyl butane, dimethylpentenes, methyloctanes, benzene, toluene, ethylbenzene, xylenes, propylbenzenes, decanes, dodecanes, tridecanes and tetradecanes.	Gasoline, diesel, kerosene, heating fuels, heating oil lubricating oils	Easy for microbial degradation (faster rate)
Poly aromatics	Naphthalenes, fluoranthenes, pyrenes, acenaphthenes	Diesel, kerosene, heating oil, lubricating oils	Complex microbial degradation (slow rate)

Concentration and Toxicity

The presence of very high concentration of petroleum compounds (> 50,000 ppm) or soluble heavy metals (> 2,500 ppm) at contaminated sites can be toxic and tend to retard the growth and reproduction of bacteria responsible for biodegradation. Very low concentrations of contaminants also diminish bacterial activity towards initiation of biodegradation processes. Therefore, an optimum level of pollutant concentration is required (Chapelle 1999). Pollutant concentration below 0.1 ppm is not generally treatable using biological process. Similarly > 95 % degradation of total petroleum hydrocarbons (TPH) is also very difficult to biodegrade due to presence of recalcitrant or non-biodegradable petroleum hydrocarbons.

Vapor Pressure

Vapor pressure plays an important role in evaluating bioremediation rates. Constituents with higher vapor pressures are generally volatilized and not biodegraded. Typically, constituents with vapor pressures > 0.5 mm Hg are likely to be volatilized by induced air stream and those with vapor pressures < 0.5mm Hg undergo *in-situ* biodegradation mediated by soil bacteria (Widdowson et al. 1997).

Product Composition and Boiling Point

Both of these parameters control constituent volatility. Compounds of higher molecular weight and higher boiling points require longer duration for microbial degradation (Leahy and Colwell 1990). Petroleum products are often classified by their boiling point (rather than vapor pressures) and generally all petroleum-derived organic compounds are biodegradable. Products which have boiling points of ≤ 250°C to 300°C will volatilize to some extent and can be removed by a combination of volatilization and biodegradation in a biosparging system. For example in biosparging, biodegradation of petroleum hydrocarbons such as gasoline (40-225°C), kerosene (180-300°C), diesel fuel (200-338°C) and heating oil (> 275°C) requires lesser time than the lubricating oil which is non volatile.

Henry's Law Constant

As has been already discussed in chapter 2, Henry's Law constant is used for the quantitative measurement of volatility of a constituent. It is an important factor that quantifies the relative tendency of a dissolved constituent to convert into vapor phase.

8.2.2 System Design

Laboratory treatability experiments followed by field pilot scale studies are often carried out for successful evaluation of the potential and effectiveness of biosparging for a given contaminated site (Aelion et al. 1996). Commonly, microbial screening and biodegradation studies at the laboratory level and biosparging treatability tests at the field level are conducted to determine, verify and quantify the potential effectiveness of the approach and provide necessary data to design a system.

The essential goals in designing an air sparging system are to configure the wells and monitoring points in order to optimize influence of air on the plume for maximum removal of toxic contaminants. There is also a need to provide optimum monitoring and vapor extraction points ensuring minimal migration of the vapor plume (Johnson et al 1993). The placement and number of air sparge points can affect the sparging pressure and distribution of air in the saturated zone. These air sparging points are required to aerate the dissolved phase plume determined primarily by permeability and structure of soil. The bubble radius primarily depends on hydraulic conductivity of the aquifer material in which sparging takes place and should be determined based on the pilot scale studies. Other factors which affect sparging are soil heterogeneities and differences between lateral and vertical permeability of the soils (Flathman and Jerger 1994). General guidelines for developing a biosparging pilot test plan are summarized in Table 8.2.

8.2.3 Advantages and Disadvantages

Biosparging is an enhanced *in-situ* bioremediation technology widely used for degradation of organic pollutants and petroleum hydrocarbons at contaminated sites in ground water. Various advantages and disadvantages are presented in Table 8.3.