Chapter 7

Use of Additives in Bioremediation of Contaminated Groundwater and Soil

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Additional information is available at the end of the chapter

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Abstract

This chapter reviews the application of additives used in bioremediation of chlorinated solvents and fuels for groundwater and soil remediation. Soluble carbon substrates are applicable to most site conditions except aquifers with very high or very low groundwater flow. Slow-release and solid substrates are intended to be long-lasting in supplying carbon for microbial growth thereby minimizing operation and maintenance requirements. Microbes as special additives can be used to enhance bioremediation (bioaugmentation) where such microbes are lacking. Oxygen gas can be added to increase aerobic biodegradation, and nutrients addition may be needed to stimulate and maintain sufficient microbial population. pH modifiers to control acidity for optimal microbial growth and degradation can also be added. Delivery of additives to the subsurface can be accomplished through permanent injection wells, direct-push methods, or permeable reactive barriers (biowall). Potential issues with additive use include biofouling, stalling, short circuiting, displacement, reduced hydraulic conductivity, and secondary water quality deterioration. Methods and techniques to deal with these issues are provided and future research needs are identified.

Keywords: Organic contaminants, carbon substrates, additives, bioremediation implementation, potential issues

1. Introduction

Bioremediation is an important type of remediation technology that uses microorganisms (mainly bacteria) to destroy hazardous contaminants or transform them to less harmful forms.

Microorganisms, through their enzymatic pathways, act as biocatalysts and facilitate the progress of biochemical reactions that degrade the targeted contaminants. Bioremediation has been used in the cleanup of organic (e.g., chlorinated solvents, petroleum hydrocarbons, and pesticides), inorganic (e.g., perchlorate and nitrate), metals, and radionuclides contaminated sites [1-3].

Because of the role microorganisms play in bioremediation, any factors impacting survival and growth of these organisms will impact bioremediation. Although microorganisms have been isolated even under extreme conditions, most of them grow optimally over a narrow physical/chemical/biological range. Thus, to effectively and efficiently cleanup a contaminated site through bioremediation, it is important to achieve optimal biogeochemical conditions for microbial communities [4]. The conditions to consider include site hydrogeological characteristics, contaminant concentration, pH, redox potential, nutrients, moisture, and temperature [5-7].

In some cases, natural conditions at a contaminated site can provide all essential materials in large enough quantities that bioremediation can occur without human intervention. This process is often referred as intrinsic bioremediation. This is the primary degradation mechanism behind natural attenuation. However, under the natural conditions of most sites, microorganisms that degrade contaminants may be naturally present in the subsurface, but may not necessarily be there in sufficient quantities required for optimal bioremediation of the site. In these cases, engineered bioremediation (also termed as biostimulation) is needed. Engineered bioremediation relies on accelerating desired biodegradation reactions by encouraging growth of target microorganisms, as well as by optimizing the environment in which the organisms must carry out the detoxification reactions [8]. Engineered bioremediation involves the addition of substrates (electron donors), nutrients, and/or other materials (e.g., pH buffers) into the subsurface to stimulate microbial growth and activity or establish supportive geochemical conditions.

This chapter focuses on the use of additives to stimulate bioremediation of organic contaminants (e.g., chlorinated solvents, petroleum hydrocarbons, and pesticides). Following the introduction, the second section of the chapter discusses major reaction pathways in bioremediation. The third section focuses on the major types of additives and the fourth section discusses in detail how the additives are implemented. The fifth section deals with potential issues associated with additives use in bioremediation. The final section summarizes the chapter and identifies future research needs.

2. Fundamental reactions in bioremediation

Bioremediation can be generally classified into two major types: bioaugmentation and biostimulation. Bioaugmentation is a type of bioremediation that adds microorganisms to enhance degradation of contaminants at contaminated sites. Bioaugmentation involves the delivery of selective and enriched microbial cultures into the subsurface to accelerate biodegradation reactions to achieve rapid and complete degradation of contaminants. The objective

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of bioaugmentation is to increase the overall degradation rate in cases where the indigenous microbial populations cannot completely degrade a contaminant or degradation rates are too low to meet the remedial goals in an acceptable time frame. The introduced microorganisms augment, but do not replace, the resident microbial population [9-10].

Biostimulation, the other type of bioremediation, involves stimulating growth of existing bacteria at sites to enhance degradation of contaminants. This can be done by adding various forms of rate limiting nutrients (such as phosphorus and nitrogen) and electron acceptors (organic carbon), and geochemical conditions modifiers (e.g., oxygen, pH modifiers).

Fundamentally, contaminants at bioremediation sites are degraded through a number of biochemical reactions that include aerobic reactions, anaerobic oxidative reactions, anaerobic reductive reactions, and cometabolic reactions. Aerobic bioremediation uses molecular oxygen (O_2) as an electron acceptor in remediation through direct microbial metabolic oxidation of a contaminant. Aerobic bioremediation is most effective in degrading non-halogenated organic compounds (e.g., BTEX, diesel) to carbon dioxide and water [3, 11]. Similar to aerobic bioremediation, anaerobic oxidative bioremediation also relies on direct microbial metabolic oxidation of a contaminant and is an alternative to aerobic bioremediation in anaerobic aquifers [3]. Contaminants that can be anaerobically oxidized include aromatic hydrocarbons, BTEX, fuels, and some chloroethenes [12].

Oxygen needs to be depleted before anaerobic reductive bioremediation can proceed. Anaerobic reductive dechlorination may be the most important type of anaerobic reductive bioremediation at contaminated sites. In this reaction, bacteria gain energy and grow as one or more chlorine atoms on chlorinated aliphatic hydrocarbons (CAH) molecule are replaced with hydrogen in an anaerobic environment [13-14]. The chlorinated compound serves as electron acceptor and hydrogen serves as the direct electron donor. Hydrogen in the form of H_2 gas used in this reaction is typically supplied by fermentation of organic substrates [15-17].

Cometabolic bioremediation is a reaction in which contaminants are reduced by a non-specific enzyme produced during microbial metabolism of another compound (i.e., the primary substrate) in an anaerobic environment. Cometabolism occurs when microorganisms using one compound as an energy source fortuitously produce an enzyme that chemically transforms another compound. Organisms thus can degrade a contaminant without gaining any energy from the reaction [3, 15, 18].

3. Common substrates and additives used for bioremediation

To stimulate microbial growth and degradation of contaminants, supplemental amendments including those that directly support microbiological growth (C, N, P) and those that maintain or create favorable geochemistry (pH buffering, dissolved O_2) are used. In some cases, surfactants are also used to enhance solubility and bioavailability of contaminants from soil and sediments in order to improve treatment efficiency [19-21].

3.1. Organic carbon substrates

As the major building block for microorganisms, organic carbon may be the most important and prominent additive used in bioremediation. Under anaerobic conditions, many microorganisms are capable of fermentation of organic matter, and some bacteria can produce hydrogen gas. Thus, almost any fermentable substrate can be a potential source of carbon and hydrogen to stimulate bioremediation. These include naturally occurring dissolved organic carbon (DOC), accidental releases of anthropogenic carbon (e.g., fuels), carbohydrates (sugars), alcohols, oils, solids (e.g., bark mulch, chitin), and complex compounds (e.g., whey and cellulose) [22-23]. Table 1 summarizes the attributes of several common substrate types. These substrates are generally classified into three types (soluble, slow release, and solid substrates), and each type will be discussed in more detail in the following sections.

Substrate	Typical delivery techniques	Form of application	Frequency of application
Soluble substrate			
Methanol, ethanol, sodium benzoate	Injection wells or recirculation systems	Dilute in water	Continuous to monthly
Lactate and butyrate	Injection wells or recirculation systems	Dilute acids or salts in water	Continuous to monthly
Molasses, high fructose corn syrup	Injection wells	Dissolved in water	Continuous to monthly
Whey	Direct injection or injection wells	Dissolved in water or slurry	Monthly to annually
Slow release substrates			
HRC® or HRC-X®	Direct injection	Straight injection	Annually to biennially for HRC®, every 3-4 years for HRC-X®, potential for one-time application
Vegetable oils	Direct injection or injection wells	Straight oil injection with water push or high oil/water content (>20%) emulsions	Typically one-time application
Vegetable oil emulsions	Direct injection or injection wells	Low oil/water content (>10%) microemulsions suspended in water	Typically every 2-3 years
Solid substrates			
Mulch and compost	Trenching or excavation	Trenches, excavations, or surface amendments	One-time application
Chitin (solid)	Trenching or injection of a chitin slurry	Solid or slurry	Annually or biennially, potential for one-time application

Table 1. Substrates used in bioremediation (modified from [24-25])

3.1.1. Soluble carbon substrates

As shown in Table 1, sodium lactate, molasses, ethanol, methanol, butyrate, and sodium benzoate have been used as soluble substrates, and sodium lactate and molasses are among the most widely used in bioremediation. Soluble substrates are applicable to most site conditions with the exception of aquifers with very high (> 30 cm per day) or very low (<30 cm per year) groundwater velocities. Soluble substrates applied as dissolved or "aqueous" phase offer the greatest potential for uniform distribution throughout the aquifer matrix relative to other substrates. Soluble substrates are easy to handle, mix, and inject. Advection helps soluble substrate distribution in the subsurface. As a result, it is possible to increase the radius of influence (ROI) and reduce the number of injection points, as a larger volume of substrate can be dispersed from a single injection point. Soluble substrates are best suited for remediation of deep aquifers where drilling costs are high.

The following disadvantages associated with the use of soluble substrates need to be recognized:

1. In order to achieve target total organic carbon (TOC) levels, and at the same time avoid adverse impacts to pH and maximize ROI, it is often necessary to test and adjust substrate loading rates and mixing ratios during the initial phase of injection. The need for optimization of loading rates increases costs of operation and maintenance (O&M) during startup. In general, the life-cycle cost of O&M for soluble substrate systems is high relative to other substrates [23].

2. In high-flow aquifers, soluble substrates readily mix with groundwater. And also because of rapid replenishment of competing electron acceptors from the groundwater, soluble substrates are rapidly degraded and it is difficult to maintain sufficient reducing conditions [24] for bioremediation. Thus, soluble substrates may not be suitable for these conditions.

3. For low flow groundwater, insufficient mixing and contact time of the substrate with the groundwater plume is also an issue. Degradation of substrate can occur before the processes of advection and dispersion allow for distribution of the dissolved organic carbon. Biofouling is also a concern if too much substrate is injected into low flow aquifer without adequate dispersion.

4. Due to the rapid degradation of soluble substrate, repetitive injection may be required, and frequent high concentration injections could lead to biofouling and low pH in groundwater.

3.1.2. Slow-release substrates

The common slow-release carbon substrates used to stimulate anaerobic bioremediation include HRC® (Hydrogen release compounds) and vegetable (edible) oils. These substrates are intended to be long-lasting in their ability to supply carbon for microbial growth. They are relatively immobile in the subsurface, and rely on advection and dispersion of soluble

compounds from the slow-release substrates (e.g., lactic acid for HRC®) for effective delivery throughout the aquifer matrix.

The primary benefit of slow-release substrates is that they require infrequent injection (often only once) with no O&M requirements other than performance monitoring; however, uneven distribution may be an issue for slow-release substrates because of the viscous characteristics of these fluid substrates.

To improve the distribution of slow-release substrates in the subsurface, while still providing a long-lasting source of organic carbon, vegetable oil emulsions have been developed. Microemulsions consisting of 5% to 10% vegetable oil in water (by volume) are relatively low-viscosity mixtures. The use of microemulsions is the result of lessons learned in early vegetable oil field trials. In earlier tests using coarse viscous emulsions or neat vegetable oil, high injection back pressures limited ROI, and reductions in hydraulic conductivity were observed [24-25].

3.1.3. Solid substrates

Solid substrates that have been used in bioremediation include tree mulch, compost, as well as other agricultural byproducts such as cottonseed hulls. Mulch used in bioremediation is usually obtained from shredding and chipping tree and shrub trimmings. To provide a source of nitrogen for microbial growth and also provide a source of more readily degradable organic carbon, green plant material or compost is often incorporated into solid substrates in these applications. Degradation of the solid substrates by microbial processes in the subsurface provides a number of breakdown products (e.g., humic acids). Solid substrates are intended to be long-term sources of organic carbon, with anticipated lifespans exceeding 5 to 10 years [26-27]. The drawback with the solid substrates also lies in the fact that it is hard to be degraded and used by the microbes as readily as the soluble substrates.

3.2. Other additives used in bioremediation

3.2.1. Oxygen gas

In aerobic reactions, microorganisms extract energy via electron transfer during oxidation of contaminants and reduction of oxygen gas. Electrons are removed from contaminants and transferred to oxygen during the process. The major kinetic limitation on aerobic bioremediation is often the availability of molecular oxygen due to low solubility of oxygen gas in water. In the absence of any external supply of oxygen, concentration of dissolved oxygen in water quickly decreases to very low levels, resulting in anoxic conditions and disruption of aerobic metabolism.

To promote aerobic biodegradation, air, oxygen, or other oxygen sources (e.g., hydrogen peroxide, ozone, sodium nitrate, and perchlorate) may need to be added in some systems. Depending on their physical properties, site hydrogeology, and the desired delivery efficiency, oxygen and oxygen-releasing compounds can be delivered to groundwater via different methods. There are two methods to introduce oxygen to aquifers: one is direct supply of air into groundwater through aeration wells; the other is through addition of hydrogen peroxide.

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Dissolved oxygen is released from hydrogen peroxide as the hydrogen peroxide rapidly degrades into water and oxygen gas through hydrolysis [28].

3.2.2. Nutrients

An aquifer normally contains sufficient amounts of nutrients for microbial growth. In engineered bioremediation, however, due to the addition of organic substrate, the nutritional demand imposed by rapid microbial growth may exceed the capacity of the aquifer system [29]. In addition to a readily degradable carbon source, microorganisms also require nutrients such as nitrogen, phosphorous, and potassium (N, P, and K) for cellular metabolism and therefore successful growth [28, 30].

Commonly used nutrients include mineral salts (e.g., KNO_3, $NaNO_3$, $Ca(NO_3)_2$, NH_4NO_3, $(NH_4)_2SO_4$, K_2HPO_4, $(NH_4)_2HPO_4$, $MgNH_4PO_4$,), anhydrous ammonia (NH_3), urea $(NH_2)_2CO$, and many commercial inorganic fertilizers [8]. In practice, nitrogen and phosphorus requirements are often estimated by calculating a carbon to nitrogen to phosphorus ratio C/N/P close to 100/(10 to 5)/1. Many authors report optimum experimental results with a C/N/P of ~70/3/0.6 [31], 8/1/0.07 [32] for crude oil bioremediation.

3.2.3. pH modifiers

The pH range within which bioremediation processes operate most efficiently is approximately 5.5 to 8 [8], as this is also the optimal pH range for many heterotrophic bacteria, the major microorganisms active in most bioremediation technologies; however, the optimal pH range for a particular situation is site-specific.

At a field site, pH is influenced by a complex relationship between organisms, contaminant chemistry, and physical and chemical properties of the local subsurface environment. For example, in low-alkalinity systems, fermentation of complex substrates generates acids, and hydrochloric acid (HCl) is formed during anaerobic dechlorination. These processes may significantly decrease groundwater pH. Reducing groundwater pH to below 5 will likely inhibit microbial growth (e.g., sulfate reducers, methanogens, and some dechlorinating microbes) [33]. Normally, the natural buffering capacity of the aquifer matrix is adequate to prevent the development of acidic groundwater pH; however, at some sites, pH buffer amendments such as sodium bicarbonate may be required to maintain near-neutral pH in groundwater systems with insufficient natural buffering capacity. The maintenance of near-neutral groundwater pH is not only important for microbial growth, but also for secondary groundwater geochemistry.

4. Additives implementation in bioremediation

4.1. Organic carbon substrate selection

The choice of electron donor (substrate) and the delivery methods are essential components of bioremediation. Substrates differ in rates at which they are degraded and become available

for biodegradation. They also differ in the complexity of their composition, in their physical form, and in their cost [24, 34].

The selected organic substrate should be suitable for the biogeochemical and hydrodynamic characteristics of the aquifer to be treated. Selection of an appropriate substrate should take into account expected performance in developing appropriate anaerobic reactive zones, the rate at which the substrate is used (efficiency of use), site infrastructure or land use, substrate availability, application configuration, delivery and distribution requirements, system O&M requirements, and cost of implementation (life-cycle cost, including cost of O&M).

The most commonly added substrates in bioremediation include lactate, molasses, hydrogen release compound (HRC®), and vegetable oils. Ethanol, methanol, benzoate, butyrate, high-fructose corn syrup (HFCS), whey, mulch, compost, chitin, and gaseous hydrogen are less frequently used. The physical and chemical characteristics of a substrate (e.g., phase and solubility) may make certain substrates more suitable than others in a particular application. Sometimes a single substrate is not sufficient, and in fact, combinations of various substrates are becoming more common in contaminated site remediation. For example, an easily distributed and rapidly degraded soluble substrate, such as lactate, may be combined with a slow-release substrate, such as vegetable oil, as it provides longer term supply of organic carbon. In aquifer systems that are naturally aerobic, an easily distributed and highly degradable soluble substrate (e.g., ethanol or lactate) can be used to rapidly induce anaerobic, reducing conditions, thus reduce lag phase of anaerobic bacteria. Then a longer lasting, "slow-release" substrate (e.g., vegetable oil, chitin, or whey) is used to sustain the reaction zone. The combination use of these two types of substrates will minimize the cost of maintaining the treatment system [23-24].

In practice, however, selection of a substrate is often based on contractor experience or familiarity, or as a result of commercial marketing. Substrates are usually selected from a wide variety of available low cost food-grade products such as molasses, vegetable oils, and whey. The potential pitfall of this strategy lies in that ineffective, even inappropriate substrate, may have been selected in some cases, leading to poor treatment results.

4.2. Organic carbon dosage

The underlying principle in determining substrate loading rate is maintaining the balance in a remediation system so that native electron acceptors are fully utilized, while at the same time sufficient electron donor is left in the system to degrade the contaminant mass. To calculate substrate mass required to deplete available electron acceptors flux, substrate composition, stoichiometry, and utilization efficiency of the anticipated degradation reactions need to be known. However, the exact stoichiometric reactions and electron acceptor flux that occur in a site is difficult, if not impractical, to determine [34]. Calculations for substrate demand can be obtained from theoretical hydrogen equivalents resulted from a known mass of substrate versus estimated electron acceptor demand at a site. The electron acceptor demand typically comprises the following three components:

1. Contaminant electron acceptor demand. During anaerobic dechlorination, the contaminant is the electron acceptor, and there is a stoichiometric relationship between electron donor (e.g., hydrogen) and electron acceptor (contaminant mass). The stoichiometric relationships for direct anaerobic dechlorination of CAHs are favorable. For example, on a mass basis, 1 mg of H_2 will dechlorinate PCE (21 mg), TCE (22 mg), DCE (24 mg), and VC (31 mg), assuming 100% use of H_2 by the dechlorinating microorganisms [25, 35].

2. Native electron acceptor demand. The flux of groundwater and minerals in aquifer matrix include electron acceptors that in many cases are preferentially used over target contaminants. In general, bacteria using oxygen, iron, and sulfate generally outcompete *Dehalococcoides* for available hydrogen gas. Therefore, their presence exerts a demand on the electron donor required to satisfy removal of more energetically favorable electron acceptors, these alternate electron acceptors must be depleted before efficient reductive dechlorination to ethene will occur [23, 25].

3. Non-specific demand. In reality, a large percentage of injected substrate, resultant organic acids, hydrogen gas, and other byproducts will be used by opportunistic microbes for different life processes including cell growth, instead of being used by targeting microorganism for remediation. In addition, other factors such as groundwater flow rate, and concentrations of electron acceptors introduced as part of prior remediation efforts (e.g., dissolved oxygen gas, persulfate from in situ chemical oxidation (ISCO)) also impact dosage [25].

Therefore, it is not easy to have a good estimate on the substrate dosage for a remediation site. On one hand, sufficient carbon is required for contaminant degradation; on the other hand, excessive substrate will not only increase cost, but also may cause secondary problems. In practice, a safety factor of 5 to 20 times of pre-tests may be used to account for the uncertainties at a specific site and to provide for a design contingency. The uncertainties may arise from estimating substrate utilization for alternate electron accepting processes (e.g., methanogenesis or solid-phase alternate electron acceptors), and it may also be caused by the presence of DNAPL or sorbed contaminant mass. Nevertheless, care should be taken to use a loading rate that is not excessive (i.e., use of excessive safety factors) to avoid the creation of low pH conditions or secondary impacts to groundwater quality [24, 34].

Alternatively, the substrate loading rate for soluble substrates can be based on achieving an empirical TOC concentration in groundwater. The volume and strength of substrate are estimated to achieve a particular target level in the treatment area after mixing and dilution. For example, Suthersan et al. [36] suggested that a loading rate between 0.1 and 1 gram of organic carbon per liter of groundwater flux per day is sufficient to create and maintain a reducing reactive zone.

4.3. Additives implementation techniques in bioremediation

There are a number of system configurations and delivery strategies that can be used to distribute organic substrates in the subsurface. The appropriate technique depends not only on application goal (mass removal or plume containment) but also on the substrates used. The

physical nature of substrates dictates the addition technique and potential system configurations. The frequency of addition is determined by the longevity of the substrate and its ability to supply required substrate and nutrients.

Liquid substrates can be deployed through direct-push or permanent injection wells. Solid substrates are typically placed in trenches or in excavations as backfill in a one-time event using conventional construction techniques. In addition, groundwater recirculation systems, infiltration galleries, and trenches may also be used to deliver substrates to impacted aquifers.

4.3.1. Injection

Direct injection refers to the process of adding substrates, microorganisms, nutrients, oxidants, or reductants directly into the aquifer at injection points. Installed injection wells or direct-push well points are commonly used injection methods to deliver liquid substrates (Figure 1). Injection wells and injection point locations and spacing depend on site geology and hydrogeology, aquifer and plume characteristics, and volume of substrates or additives to be injected.

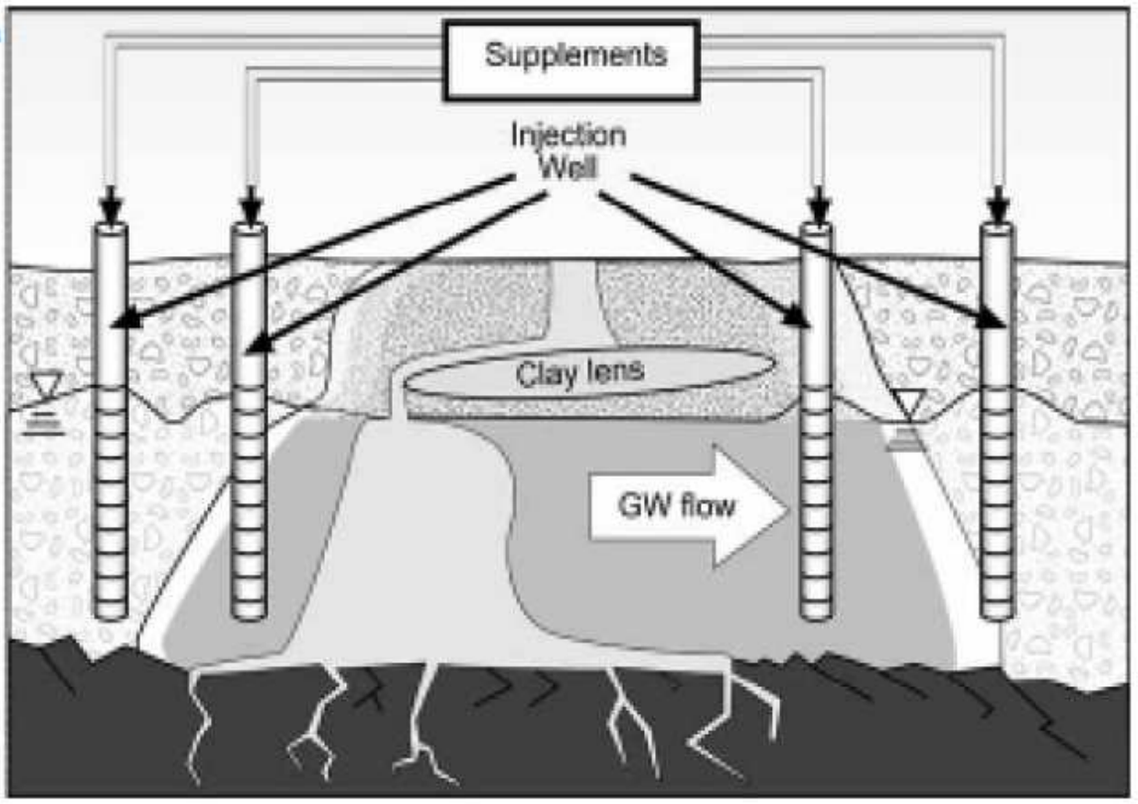

Figure 1. Schematic of the use of direct injection system in bioremediation (Adapted from [15])

Direct-push injection is suitable for both soluble and viscous liquid substrates, and is commonly used for shallow groundwater remediation in unconsolidated formations. The application of this technique is constrained by soil characteristics such as grain size or degree of

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cementation. Gravel and cobbles in sediment inhibit use of direct-push technology. Direct-push (e.g., Geoprobe®) injection does not leave well points in place after injection (as opposed to permanent injection wells), and is only practical for slow-release substrates such as HRC®, vegetable oil emulsions, or whey slurries. Direct-push methods have also been used to install semi-permanent well points for short-term injections [23-24].

Permanent injection wells are generally used for soluble substrates where continuous or multiple injections of substrate or recirculation are required. Permanent injection wells are also necessary for sites that cannot use direct-push technology due to depth or soil lithology issues. If screened at appropriate depths and located within appropriate portions of the plume, existing monitoring or extraction wells from previous investigation or remediation activities can also be used for injection [23-24].

Direct injection may be performed through frequent, single-well injections or less-frequent, multiple-well injections with properly spaced injection points. Direct injection is effective at sites with moderate groundwater flow. Sites with very high groundwater flow can be problematic due to low cross-gradient distribution of the substrate within the plume. Direct-injection approaches are not suitable for highly heterogeneous aquifers because the substrate is not distributed evenly around individual injection points and the contaminant plume.

4.3.2. Recirculation

For sites with low groundwater flow rate, recirculation techniques may be required to obtain effective mixing of substrates and contaminated groundwater. Recirculation systems consist of a closed network of extraction wells and injection wells (Figure 2). The recirculation system is designed to hydraulically control substrate transport through the treatment zone. The distance between injection and extraction wells is dictated by groundwater flow velocities, plume size, and bioremediation process kinetics. Excess amendment that is not consumed is extracted at the recovery well and recycled to the injection well. Recirculation approaches may be the only effective method to achieve more uniform distribution of substrates and amendments at sites that lack significant natural hydraulic gradients [37-38].

Recirculation increases retention time of contaminated groundwater in the treatment zone. Substrate and amendments applied in recirculation systems are more readily controlled and they are distributed through the treatment zone. Recirculation systems can influence a much greater volume of the aquifer, and allow much greater distances between injection and extraction wells; however, continuous operation of a recirculation system requires dedicated equipment and maintenance and thus can be very expensive. In addition, continuous operation creates a potential for biofouling. Alternatively, a recirculation system can be operated in an intermittent fashion, i.e., recirculate for a short time (days to weeks), then shut off for several months, during which time electron donor is consumed and used for contaminant degradation. Intermittent operation of a groundwater recirculation system may be considerably less expensive than continuous recirculation. Periodic operation of a recirculation system will also result in less biofouling of injection wells compared to systems that require continuous recirculation of groundwater and injection of substrates [25].

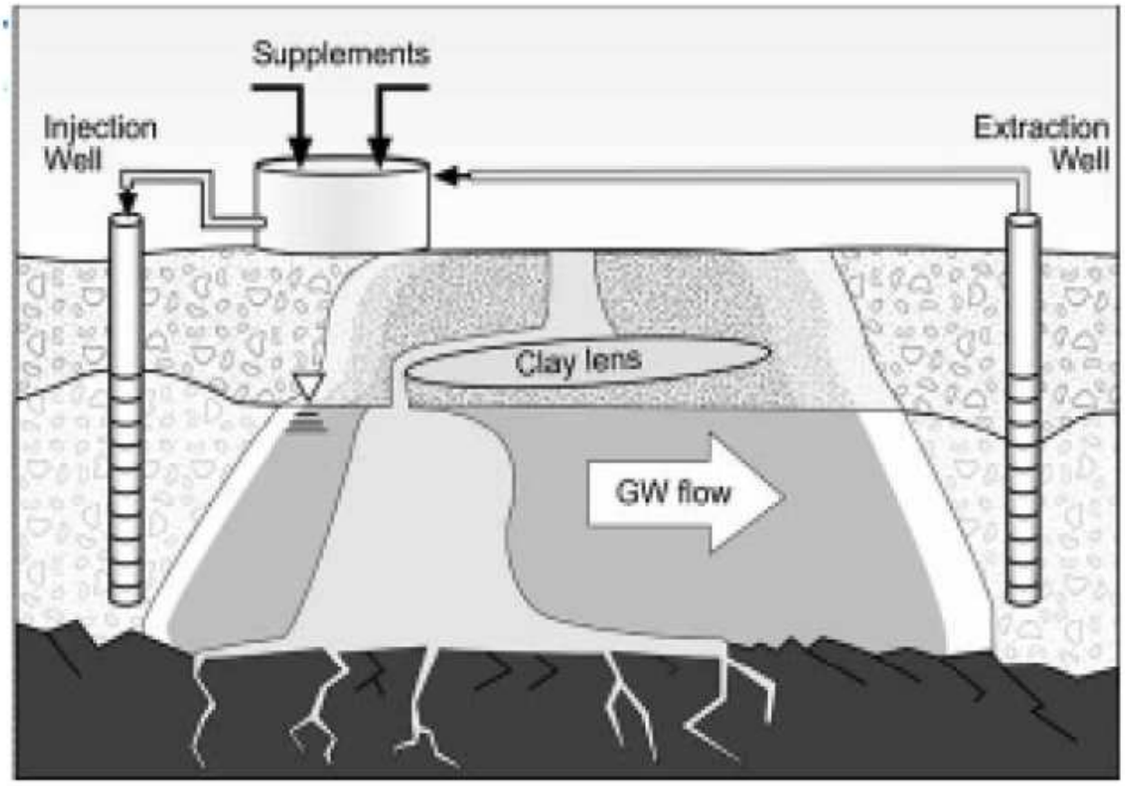

Figure 2. Schematic of a recirculation system in bioremediation (Adapted from [15])

4.3.3. Trenching

Solid substrate treatment systems are typically deployed in an excavated trench in the form of a permeable reactive barrier (PRB, e.g., biowall) (Figure 3). This treatment method relies on the natural flow of groundwater through solid substrates within the PRB to promote contact with slowly released soluble organic matter. This method is particularly suitable for sites with low permeability. Sometimes, perforated pipes can be laid on the top or bottom of the organic fill material to amend the organic fill material with liquid substrates or other amendments to improve performance [23-24].

Trenches may be installed using either continuous one-pass trenchers designed for installing subsurface utilities or hydraulic excavators. Trench depths are limited by type of equipment used, stability of the formation, and ability of the equipment to excavate the formation. Continuous trenching is not practical in hard and consolidated bedrock [23, 27].

5. Potential issues with additives use in bioremediation

Bioremediation has been demonstrated to be an effective technology for degrading chlorinated solvents and fuel hydrocarbons and has been used widely in groundwater and soil remedia-

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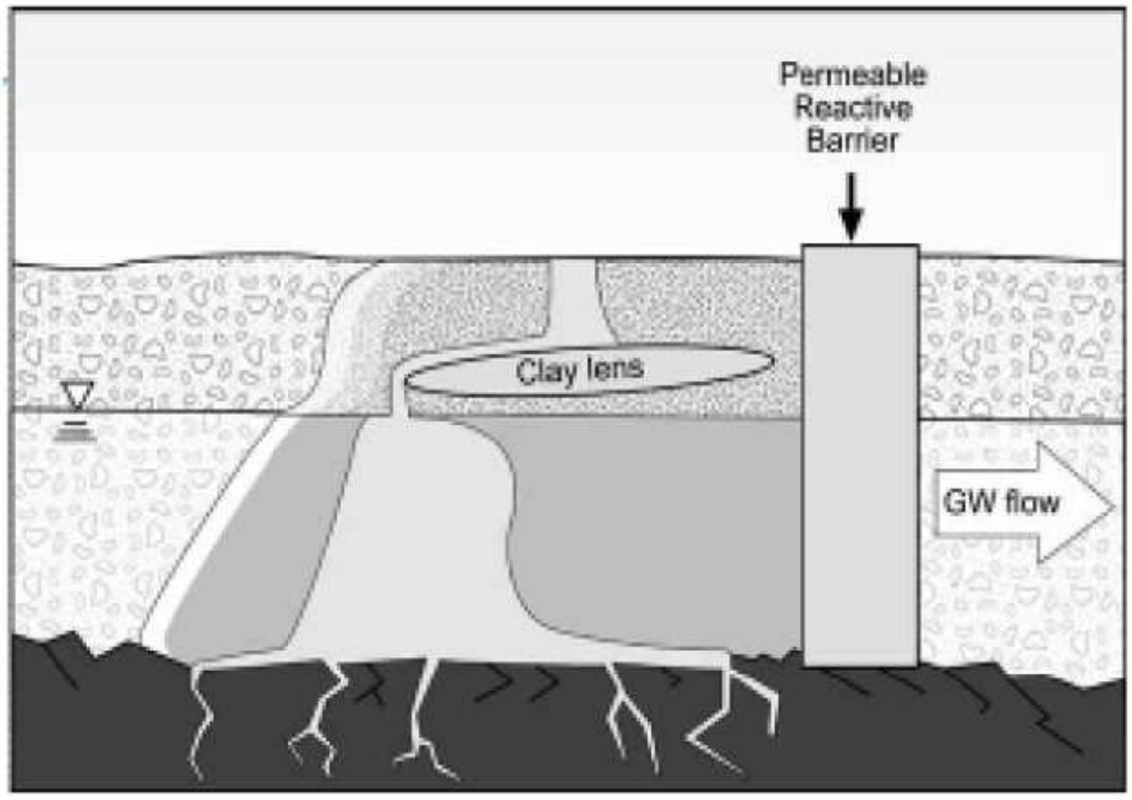

Figure 3. Schematic of a permeable reactive barrier (PRB) composed of organic carbon based materials (biowall) for bioremediation of contaminated groundwater (Adapted from [15])

tion. A number of issues associated with additives use in bioremediation have been encountered and need to be taken into consideration when considering bioremediation at a site.

5.1. Biofouling

Biofouling refers to failing or decreasing effectiveness of a remediation system due to excessive growth of biomass of non-targeting microorganisms. Biomass buildup in wells could decrease fluid injection rates. Biofouling of injection or recirculation wells has been observed at several sites due to growth of biomass or biofilms within well screens and surrounding sand packs. Biofouling in injection wells may impact the ability to effectively inject and distribute substrate.

Several approaches have been used to prevent or mitigate the biofouling issue. Preventive measures typically include well rehabilitation techniques such as surging and pumping, high pressure jetting, pulsed injection, injection of carbon dioxide under pressure, use of a clean water push to remove substrate residue, chemical methods such as surging and scrubbing with hydrogen peroxide or use of non-oxidizing biocides (e.g., Tolcide®) [16,25,39].

5.2. Stalling

Stalling in bioremediation refers to the incomplete degradation of targeted compounds due to accumulation of degradation daughter products. Anaerobic reductive bioremediation of PCE

and TCE, for example, may undergo incomplete degradation to DCE or VC resulting in the accumulation of these daughter products. Stalling has been observed in a number of scenarios:

1. Reductive dechlorination is most effective and efficient under sulfate-reducing to methanogenic conditions. In attempts to increase substrate utilization and reduce competition for hydrogen (e.g., by methanogenesis), hydrogen concentrations are controlled in some remediation systems. This may result in significant portions of the treatment zone remaining insufficiently reducing for complete dechlorination to occur, leading to "stalling" at intermediate degradation products such as *cis*-DCE or VC [24, 40].

2. Microorganisms generally gain more energy from dechlorination of more highly chlorinated contaminants such as PCE and TCE. Dechlorination of daughter products such as DCE and VC may not start until after parent products are sufficiently depleted [23].

3. *Dehalococcoides mccartyi* is the only known bacteria that can degrade chlorinated ethenes completely [41-42]. However, this bacterium may not be present at populations required to achieve complete dechlorination. Limitations in microbial populations create the potential for incomplete degradation and the buildup of daughter products such as *cis*-DCE or VC.

4. Co-contaminants or other geochemical conditions such as low pH, inadequate electron donor availability, or unfavorable geochemical conditions may inhibit microbial populations and lead to incomplete degradation of chlorinated solvents.

5.3. System bypassing or short circuiting

System bypassing or short circuiting is a common issue with in situ remedial technologies that rely on injection and distribution of amendments within the subsurface. Aquifer permeability and preferential pathways are two major factors that may impact distribution of substrates throughout a DNAPL source zone.

Short circuiting of substrates in the vadose zone may occur during the injection of liquid substrates. Whether injections are conducted through permanent wells or by direct-push methods, injection pressures must remain relatively low to avoid unintentionally fracturing the formation. Injection pressures greater than the overburden pressure may cause fracturing of the aquifer formation. This may lead to preferential flow of substrates along open fractures resulting in nonuniform distribution and short circuiting.

Another factor that can result in substrate bypassing is aquifer heterogeneity. Any liquid substrate, including aqueous substrate mixtures, will migrate along the pathway of least resistance (highest permeability). In heterogeneous systems, substrate distribution may bypass lower permeability aquifer zones. In these situations, multiple well points screened in each lithologic layer or zone may be required to avoid short circuiting of substrate to higher permeability zones.

5.4. Reduction of hydraulic conductivity

Reduction in hydraulic conductivity may cause contaminated groundwater to flow around the treatment zone, impacting the ability of the targeted compound(s) to come into contact with the organic substrate and also impacting the ability to effectively further distribute soluble organic substrates. During enhanced bioremediation, hydraulic conductivity may be adversely impacted by several factors:

1. Biofouling of the aquifer due to excessive biomass growth, especially in low flow aquifers.
2. Gas clogging from generation of excessive amounts of dissolved gases including carbon dioxide, methane, and hydrogen sulfide. Gas clogging in the formation may occur when excessive amounts of gases are produced by biological activity. The formation of gas bubbles in the aquifer matrix lowers the aquifer permeability, reducing hydraulic conductivity [24].
3. Physical reduction in hydraulic conductivity and permeability due to the presence of viscous or non-aqueous substrates (e.g., vegetable oils). A significant reduction in hydraulic conductivity has been observed at saturations as low as 10% to 15% of viscous substrate. When reduction in hydraulic conductivity is a concern, oil-in-water saturations of 10% or less are typically recommended [24].

In the case of solid substrates used in trenches, permeability of solid substrate mixtures must remain equal to or higher than that of the surrounding formation. In practice, coarse sand and pea gravel are usually mixed with the substrate to maintain a sufficiently high permeability.

5.5. Displacement and dilution

In groundwater remediation, some mixing of injected substrate with contaminated groundwater will occur due to advection and dispersion. In order to achieve widespread distribution of substrates, injecting large volumes of soluble substrate mixed with potable water is necessary in some cases. However, injection of large volumes of substrate may cause significant displacement and dilution of the contaminant plume. One way to address the displacement and dilution issue is to inject a low volume/high concentration substrate mixture and to rely on advection and dispersion for mixing, although this requires relatively high rates of advection and dispersion to occur. The use of recirculation systems can aid in avoiding this issue.

5.6. pH and secondary water quality issues

When applying organic carbon in bioremediation, caution is required to not supply too much substrate to the subsurface because excess organic substrate generates organic acids and causes decreases in the pH of groundwater. In addition, anaerobic reductive dechlorination generates HCl that could also decrease groundwater pH.

A decrease in pH to the acidic range could potentially mobilize metals (notably iron, manganese) and metalloids (arsenic), creating secondary water quality issues at a site. The release of

these metals is also impacted by the prevailing redox conditions at a site. A decrease in pH could also inhibit growth of bacteria communities such as *Dehalococcoides*, thereby stopping the bioremediation process.

Changes in redox conditions can also enhance solubilization of metals and promote the formation of the following undesirable products (e.g., hydrogen sulfide and methane gases):

1. If nitrate is used, byproducts including nitrite, nitric oxide, nitrous oxide, and nitrogen gas could be generated. The predominant byproduct depends on the enzymes possessed by the microbes present.
2. Iron(II) is more soluble than iron(III), so iron reduction could lead to exceedance of iron water quality criteria.
3. Sulfate is reduced to sulfide under anaerobic conditions. If there are not enough dissolved metals to precipitate metal sulfides, free sulfide and hydrogen sulfide will be generated. Sulfide is toxic to microbial communities and could inhibit degradation of contaminants.

6. Conclusions

1. Bioremediation is widely used in groundwater and soil remediation of organic contaminants such as chlorinated solvents and petroleum hydrocarbons. Additives play an essential role in stimulating microorganism growth and in accelerating contaminant degradation in engineered bioremediation systems.
2. Common additives used in engineered bioremediation include organic carbon, oxygen, nutrients, and pH modifiers. Organic carbon substrate is the most important and widely used additives. A wide-range of materials can be used as carbon substrates. These substrates can be generally categorized into soluble, slow-release, and solid substrate subgroups.
3. The use of additives in engineered bioremediation systems depends on the physical properties of additives, site characteristics, treatment goals, and other factors. Additives are usually added to the subsurface environment through direct injection, recirculation, and trenching.
4. Despite the success of bioremediation technology applications in groundwater remediation, a number of issues have been identified with the use of additives in bioremediation. These include biofouling, stalling, system bypassing or short circuiting, reduction in hydraulic conductivity, contaminant plume displacement and dilution, and pH and secondary water quality issues. Taking these issues into consideration during remediation design and properly addressing these issues during implementation is essential for efficient and cost-effective site cleanup.
5. To enhance the efficiency and promote the application of bioremediation technology in contaminant remediation at contaminated sites, a number of research needs related to

additive use have been identified. These include: (I) Developing new and improved additives to allow for better distribution, less background consumption, and increased effectiveness of target organisms; (II) Developing new technologies for cost-effective delivery of additives, especially for low permeability aquifer systems; (III) Developing innovative ways to address issues encountered in additive use, such as secondary water quality issues and reduction in hydraulic conductivity.

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