



# A bioengineering system for *in situ* bioremediation of contaminated groundwater

RB Knapp<sup>1</sup> and BD Faison<sup>2,3</sup>

<sup>1</sup>L206, Lawrence Livermore National Laboratory, Livermore, PO Box 808, CA 94551-9900; <sup>2</sup>Oak Ridge National Laboratory, Oak Ridge, TN 37831-6194, USA

**Much of the past and current focus of bioremediation has been on laboratory studies of microbial processes. By necessity, early studies have ignored important field properties, parameters, and processes that control the ultimate success of *in situ* bioremediation of contaminated groundwater. This paper presents a bioengineering systems approach that examines the impact of some of these field variables on common bioremediation practices. Using simple systems, the niche of biostimulation is shown to be aquifers with high contaminant sorption. A novel gas-phase biostimulation filter and a novel resting-state bioaugmentation/biofilter approach which show promise for effective field implementation are discussed.**

**Keywords:** *in situ* bioremediation; biostimulation; bioaugmentation; bioengineering; subsurface systems

## Introduction

Typically, the focus of attention of bioremediation is on bacteria as catalysts or enablers; however, fungi, protozoa, microalgae, macroscopic plants, and some lower but macroscopic animals (eg, earthworms) are also implicated in remediation processes. Although a biological form may catalyze site remediation, successful deployment of the catalyst requires understanding the basic principles of chemistry, biology, geology, soil science, hydrology, and chemical engineering. Currently, much of the focus for *in situ* bioremediation is on the biological aspects. The aforementioned principles, and the efforts based on them, may be organized into a complete bioremediation system. This systems approach is critical to the success of any bioremediation plan as many effective degradation processes developed in the laboratory can be made ineffectual by unforeseen subsurface processes.

This paper will focus on the development of systems for the restoration of contaminated groundwater. The microstructure and microecology of soil and groundwater have been reviewed extensively [1,5]. Here, it will suffice to say that the subsurface is a complex system. It is important that bioremediation processes be designed with the understanding that materials introduced to subsurface systems undergo the same transformations as they do during physicochemical separation processes on the laboratory bench-top, including chemical reactions, sorption, phase partitioning, liquid–liquid phase separation, leaching from soil into water or non-aqueous liquids, dissolution of gas into water or non-aqueous liquids, volatilization, phase separation, and precipitation. A major difference between lab and field is the almost complete lack of control over these processes in

the field. It is here that subsurface bioengineering must enter to make bioremediation effective.

Successful *in situ* bioremediation of contaminated groundwater requires engineering a set of subsurface processes. The precise elements in the set depend on the chosen remediation method but control in the field is exercised through well locations, well patterns, injection and withdrawal rates, injection and withdrawal intervals in the wells, and the composition of the injected fluid. Prerequisites for the selection of these which result in the optimal remediation performance are a well characterized site with respect to the groundwater flow field, the contaminant sources, the contaminant suite, the aqueous geochemistry, and, most importantly, heterogeneities in permeability. It is increasingly recognized that permeability heterogeneities control the ultimate cost and success of *any* groundwater remediation scheme. The most successful approaches work with these constraints most creatively. In most cases, the remediation engineer is severely limited by a lack of characterization information due to financial constraints. The challenge is to create a remediation scheme which meets targets despite great uncertainty in many of the fundamental variables which control its effectiveness. Under these circumstances it is even more important that a simple and robust remediation approach be employed to compensate for the lack of engineering control over *in situ* processes.

Even with these constraints, there are some evaluations that can be made with limited site knowledge to assist in the selection of an appropriate *in situ* bioremediation scheme. For biostimulation, which is the injection of growth substrates, co-substrates, and electron acceptors which are limiting the biodegradation reaction, the evaluations involve contaminant retardation and substrate/co-substrate/electron acceptor utilization rates. For bioaugmentation, which is the injection of bacteria to increase the subsurface population, the evaluations are related to natural contaminant fluxes and intrinsic biodegradation properties of the injected cells. In the following, we will attempt to

Correspondence: RB Knapp, L206, Lawrence Livermore National Laboratory, Livermore, PO Box 808, CA 94551-9900, USA

<sup>3</sup>Present address: Department of Biological Sciences, Wellesley College, Wellesley, MA 02181, USA

Received 8 December 1995; accepted 30 July 1996

provide a technical framework for using these variables to select a particular approach. This framework arises from simplified analyses of the subsurface processes related to each approach. We will begin with the most commonly employed method of *in situ* bioremediation—biostimulation—and finish with bioaugmentation, which has yet to be fully explored as a potential method.

### Biostimulation

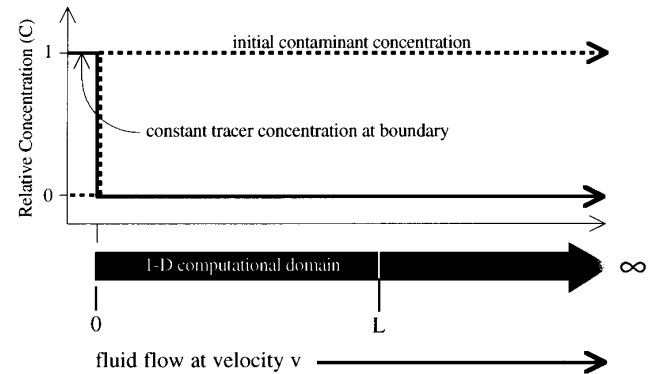
Biostimulation requires that the bacterial species or consortia required to degrade dissolved contaminants are indigenous and it assumes that reactions are limited by either low population densities or by the absence of key electron acceptors. Biostimulation attempts to accelerate pre-existing biodegradation rates by injecting either growth substrates/co-substrates, which increase the population density, or electron acceptors (eg,  $O_2$ ), which limit the reaction. Field experiments [eg, 11,20,21] have demonstrated that cessation of injection was followed, in a matter of hours, by the cessation of biodegradation. To sustain the biodegradation reactions it is required that injection be continuous. Thus, just as in any laboratory column study, the challenge for biostimulation is to have the stimulated bacterial population and contaminated groundwater in the same pore space at the same time. The ability to achieve this mixing requirement is dependent on the medium (liquid or gas) used to inject substrates, co-substrates, and electron acceptors. The conditions under which each of these operates most efficiently will be examined in the following paragraphs.

#### Liquid injection medium

A liquid medium is the most common carrier for substrates, co-substrates, and electron acceptors in biostimulation [7,27]. Water that has been saturated with the key compound(s) is injected into the subsurface to either increase the attached population or to provide a limiting reactant, such as oxygen. The key for its effectiveness is to obtain contact among the injected fluid, the contaminated groundwater and the indigenous population. This is a challenge since injection largely displaces the contaminated groundwater.

**Details of calculations:** The mixing difficulty can be demonstrated by a simple one-dimensional calculation. Water carrying a conservative tracer but devoid of contaminant is injected into a homogeneous porous medium which is initially saturated with water carrying a contaminant but devoid of the injected tracer (Figure 1). The calculations permit advection, dispersion, and equilibrium sorption of contaminant but not injected compounds and preclude any irreversible sources or sinks caused, for example, by chemical precipitation. Analytical solutions for all examples can be found in [26].

We use dimensionless quantities for generality. Dimensionless concentration,  $C$ , for the tracer is with respect to the injected concentration;  $C$  for the contaminant is relative to its initial concentration in groundwater. The dimensionless distance,  $X$ , is the ratio of the distance from the injection point to the observation point to the overall col-



**Figure 1** Computational domain and boundary conditions used in analysis of mixing between injected liquid and contaminated groundwater. The injection point is at 0 and the observation point is at  $L$ . Tracer and contaminant concentration profiles are at a time of 0.

umn length. Contaminant retardation,  $R$ , is caused by equilibrium surface reactions among dissolved compounds and soil minerals. Retardation reduces the rate of transport without changing the shape of the response curve and is defined as [9]:

$$R = 1 + \frac{\rho K_d}{\phi} \quad (1)$$

where  $K_d$  is the solid-aqueous distribution coefficient,  $\phi$  is the medium porosity, and  $\rho$  is the bulk density of the porous medium. For many aquifers and contaminants of interest,  $R \leq 1.5$ ; however,  $R$  can exceed 10 as the organic content of the aquifer increases [19].

The Peclet number,  $N_p$ , is a measure of the relative importance of advective transport to dispersion,  $D$ .

$$N_p = \frac{vL}{D} \quad (2)$$

where  $v$  is the pore fluid velocity and  $L$  the length scale of interest. Advection is the transport of compounds by fluid flow, such as a leaf along a stream, where dispersion is a combination of aqueous diffusion at the molecular scale and spreading due to small-scale variations in velocity. Our scale of interest is at the pore scale since it is here that there has to be actual physical contact between contaminant and bacteria. There is an increasing body of evidence [4,17] that values of aqueous diffusion coefficients are better approximations of  $D$  than are field-measured dispersion coefficients. As such, typical values for  $N_p$  during injection are on the order of 500; advection usually dominates dispersive transport in the subsurface.

We also use a dimensionless time,  $\tau$ , defined as:

$$\tau = \frac{vt}{L} \quad (3)$$

where  $t$  is normal time. Thus a  $\tau = 1$  is the time required for the tracer to be transported from the injection point at  $X = 0$  to the observation point at  $X = 1$ . Most calculations will be done for the half-way point, that is for  $\tau = 0.5$ .

**Nonreactive tracer cases:** The first case examined is the injection of a chemical tracer into a contaminated aquifer where neither the injected tracer nor the contaminants sorb ( $R \equiv 0$ ). This case serves to set the foundation for the more complicated case of injecting a substrate that is consumed by indigenous bacteria. In this simple situation, the injected fluid largely displaces—mechanically pushes away—the contaminated groundwater and mixing between the two is limited to a relatively thin zone at the displacement front (Figure 2a). This mixing zone is created solely by the interdiffusion of contaminant and injected chemicals. It is only within this thin zone that bacteria stimulated by the injected chemicals and groundwater contaminant can come in contact and accelerated biodegradation can occur.

For the case of nonsorbing injectate ( $R \equiv 0$ ) but significant contaminant sorption ( $R > 0$ ), the size of the mixing zone dramatically increases (Figure 2b). For large retardation coefficients (eg,  $R = 5$ ), a large part of the contaminant mass is sorbed onto the soil material. This acts as a contaminant source as injected fluid flows past and leads to increased contact among injected compounds, stimulated bacteria, and contaminants. This is the optimal situation for biostimulation using a liquid medium.

Mixing efficiency can be estimated by the size of the mixing zone,  $\Delta X$ , relative to the volume of fluid injected as represented by the mean location of the nonsorbing

tracer front,  $X_f^{\text{tracer}}$ . If this ratio is small, then a large volume of fluid needs to be injected to mix with a small volume of contaminated groundwater and remediation will not be cost-effective. Efficiency is also a function of time since  $\Delta X$  and  $X_f^{\text{tracer}}$  increase at different rates.

Contaminant retardation is a critical parameter for the effectiveness of biostimulation and should be measured early in the evaluation process. Mixing efficiency is always greater in contaminant plumes that have greater  $R$  (Figure 2c). At the lower limit,  $R = 1$ , the mixing efficiency is about 10% except at very short times. For large contaminant sorption (eg,  $R \geq 5$ ), the mixing zone is of the same order as the injection zone and efficiency approaches 100%.

It can be expected that mixing among injected fluids and groundwater is much greater in contaminant plumes which have a larger degree of sorption than in other plumes. Plumes with high  $R$  would therefore seem to be better targets for biostimulation. It is very difficult and expensive to change an aquifer sorption characteristics. Therefore, the aquifer's characteristics solely determine whether it is a viable candidate for biostimulation. No matter how fast and effective a biodegradation reaction, an efficient bioremediation system will not occur if the bacteria cannot contact the contaminants, which is the case for low sorbing aquifers.

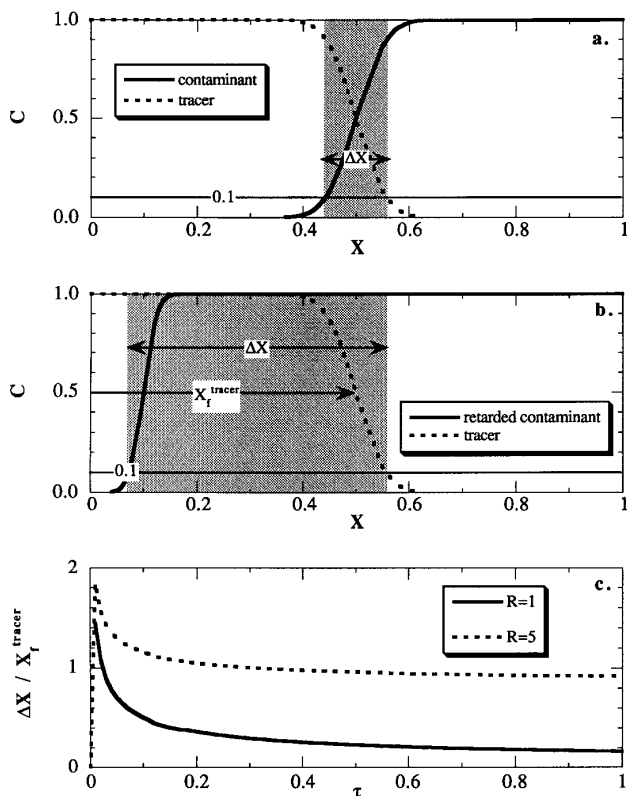
This simplified analysis also shows that it is important to conduct pilot-scale field tests at least 25% of the scale of the expected field operations (Figure 2c). Mixing efficiency decreases with time and distance away from the injection point. Tests for short periods of time bias results by artificially amplifying the degree of mixing, and hence the degree of contaminant destruction, relative to that to be expected at the larger scale and for longer term operations.

**Consumable substrate cases:** The tracer-contaminant system discussed above provides a foundation for an analogous analysis of the substrate-tracer-contaminant system. For this, we superimpose the evolution of substrate concentration when it is kept at a constant concentration at the injection point and is consumed at a constant rate in the calculational domain. Physically, this means that, where the substrate exists in the domain, the indigenous population instantly rises to a steady-state value. This is a simplification but serves to elucidate the principle of mixing, substrate consumption, and contaminant degradation.

The Damkohler number ( $N_D$ ) is the dimensionless parameter grouping which relates substrate consumption rates to substrate supply rates caused by advective transport

$$N_D = \frac{kL}{v} \quad (4)$$

where  $k$  is the consumption rate. For an attached population of  $10^6$  cells per g aquifer medium,  $k$  is typically on the order of  $10^{-3}$  per h. Typical length scales for field operations are on the order of 100 m and average pore velocities during injection are about  $2 \text{ m day}^{-1}$ , which yields  $N_D = 1$ ; at this value, the growth substrate consumption rate equals the advective transport rate and the zone of stimulated bacteria will be confined to a relatively narrow region around the injection point.

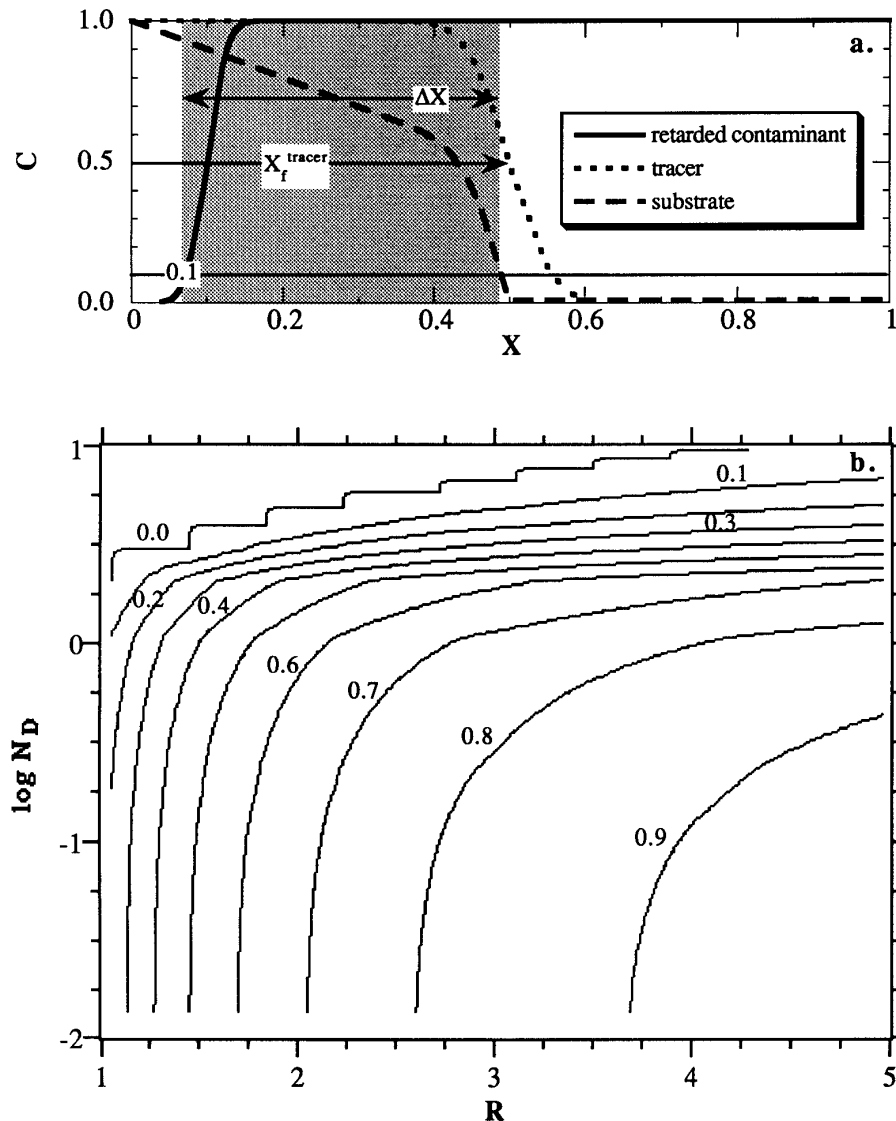


**Figure 2** Concept of zone where injected liquids, as marked by a tracer, and contaminated groundwater mix at the displacement front. The concentration limit defining the mixing zone is arbitrarily chosen to be 0.1.  $\Delta X/X_f^{\text{tracer}}$  is the mixing zone width relative to the injection length. For all plots,  $N_p = 500$ . For (a)  $R = 1$  and  $t = 0.5$ ; for (b)  $R = 5$  and  $t = 0.5$ .

Steady consumption of injected growth substrate, co-substrate, or electron acceptor by an attached population both retards the substrate front and reduces its magnitude relative to an injected tracer (Figure 3a). This leads to a reduction in the size of the mixing zone where contaminant can come in contact with the stimulated bacterial population and be biodegraded. As with the tracer, zone width is directly proportional to contaminant retardation. However, mixing zone width is inversely proportional to  $N_D$  (Figure 3b). At constant  $R$ , relative mixing zone widths always decrease as  $N_D$  increases, ie, mixing decreases as substrate consumption increases relative to fluid velocity. Bacteria consume the substrate before it can be transported upstream to where the contaminants are located. The rate of decrease is very small when  $N_D \leq 0.1$  and is very large when  $N_D \geq 1.5$ . Many contaminant plumes lie in this intermediate range. It can also be seen that, for these, the mixing

efficiency is less than 50%. This is consistent with field observations on the efficiency of biostimulation using methane-saturated water to stimulate methanotrophs to co-metabolize chlorinated ethenes. Biostimulation using a primary growth substrate, as is the rule for non-BTEX plumes, seems to be inherently inefficient except for contaminated aquifers with a high degree of sorption ( $R > 2$ ).

Biostimulation with an electron acceptor can be expected to be more efficient than biostimulation with a primary growth substrate in some circumstances. Growth substrates, as well as some co-substrates, will be consumed wherever there is a bacterial population. If the electron acceptor is only consumed in biodegrading the contaminant, then the electron acceptor front will be less retarded than the growth substrate front. This will increase the mixing zone width and the efficiency. A possible example is a field experiment conducted at a US Coast Guard Air Station at Traverse



**Figure 3** Concept of degradation zone where contaminated groundwater comes in contact with stimulated bacteria. The model assumes an instantaneous steady-state attached bacterial population that consumes the injected substrate at a constant rate; we do not allow contaminant biodegradation so that we may calculate the width of the zone where biodegradation could occur. For all plots,  $N_p = 500$  and  $t = 0.5$ . For (a)  $R = 5$ ; (b) is a contour plot of the relative degradation zone width ( $\Delta X/X_f^{tracer}$ ) as a function of retardation ( $R$ ) and the Damkohler number ( $N_D$ ).

City, MI [27]. Oxygenated water was injected into a shallow BTEX plume in an effort to stimulate biodegradation. Values for  $R$ , extracted from published data, appear to exceed 1000. While  $N_D$  values are not known, this magnitude of  $R$  certainly suggests an efficient mixing process since much of the contaminant mass is initially sorbed onto the solid aquifer medium; it is expected that the contaminant front would lag about 1000 days past the tracer front in the absence of biodegradation for the test conditions. Field observations show the BTEX front lagging on the order of 100–500 days, suggesting that the observed loss of contaminant mass was indeed caused by biodegradation.

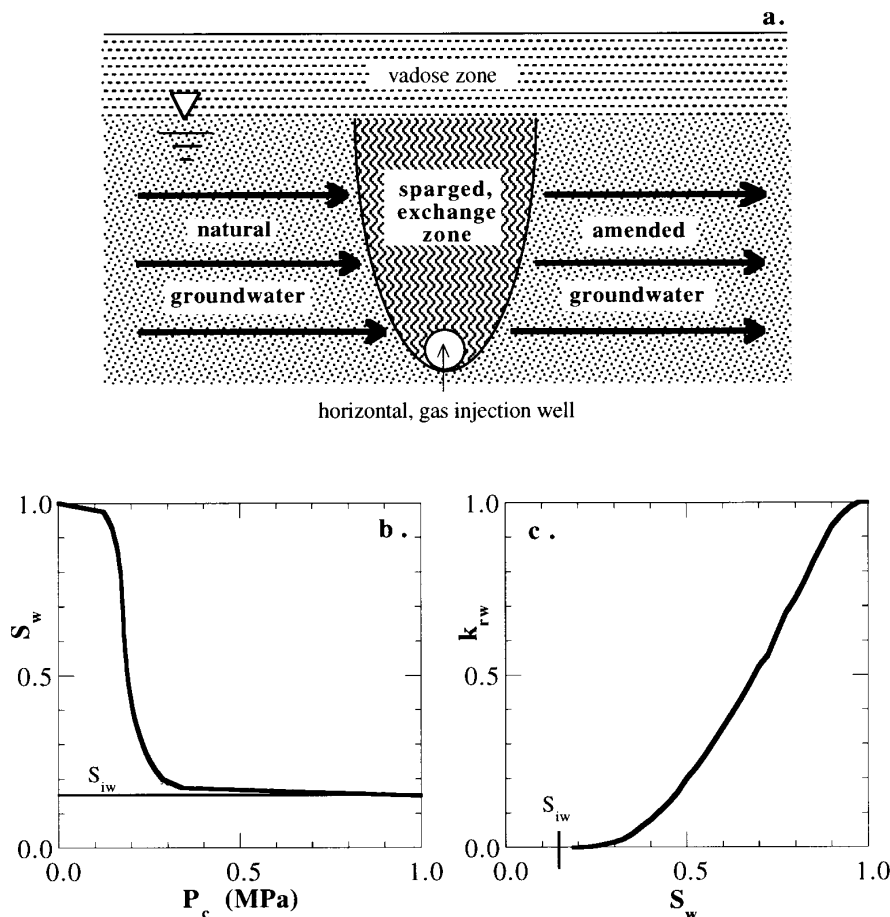
### Gas injection medium

New concepts have recently been proposed to inject a gas phase into contaminated groundwater to increase to the dissolved concentrations of volatile substrate, co-substrates or electron acceptors which may be limiting *in situ* bioremediation [10,12]. The idea is to create a relatively thin zone transverse to a migrating plume (Figure 4a). A gas phase containing the compounds of interest is injected into this zone, thereby creating a separate gas phase *in situ*. As groundwater flows through the zone, mass transfer from the

gas phase to the groundwater occurs until equilibrium levels are reached. As this amended groundwater flows downstream, an indigenous bacterial population utilizes the injected reactants to biodegrade the dissolved contaminants. Istok and others [12] have called this the bubble wall concept.

This concept avoids the mixing problem discussed above by avoiding displacement of contaminated groundwater by injected water. Instead, engineering design reduces to obtaining an adequate degree of mixing in the gas injection zone. Some of the most important engineering considerations for this new technology will be addressed in the following paragraphs.

Buscheck and Nitao [2] have elucidated the physics of gas injection into groundwater which are required to engineer the mixing zone described above. When gas is injected into groundwater, a two-phase fluid system develops in a relatively narrow zone about the injection interval. Gas rapidly rises due to buoyancy forces and breaks through to the vadose zone if there is no overlying permeability barrier, such as thick clay units. The width of the two-phase (coexisting gas plus groundwater) zone and the gas pressure in it depend linearly on the injection pressure. Gas pressure



**Figure 4** Concept (a) of gas phase biostimulation, or bubble wall. The sparged, exchange zone is where an *in situ* gas phase and groundwater coexist and volatiles are exchanged between the two phases. Groundwater exits this region amended with substrates and/or co-substrates from the injected gas phase. Capillary pressure curve (b) and relative permeability curve (c) for a sand [16].  $P_c = P_{\text{gas}} - P_{\text{water}}$ ; the relative permeability of the sand to water is  $k_{rw} = k_w(S_w)/k_w(S_w = 1)$ ;  $S_{iw}$  is the irreducible water saturation or the water saturation below which water loses its continuity and will not flow and  $k_{rw} = 0$ .  $P_c$  increases from injection point to the watertable in the sparged, exchange zone; this requires  $S_w$  and  $k_{rw}$  to decrease.

at the injection point is slightly above the water pressure value and decreases to atmospheric at the vadose zone. The two-phase zone consists of a continuous water phase occupying the smaller pores of the aquifer and a continuous gas phase occupying the larger pores. The fraction of the pore system is that occupied by water at any point, the water saturation ( $S_w$ ), depends on the capillary pressure ( $P_c$ ) at that point. Capillary pressure is the difference between gas pressure and water pressure, and its relationship to  $S_w$  depends on the particular aquifer material (Figure 4b). Consequently, the water saturation is a function of the injection rate. It is expected that an equilibrium water saturation will be established in the two-phase zone with water saturation greatest at the injection point and least at the watertable. Water saturation will not decrease below its irreducible level ( $S_{iw}$ ) unless dry gas is injected which will eventually desiccate the two-phase region.

The volumetric distribution of water in the two-phase zone will control both the rate of mass transfer from the gas phase to the liquid phase and the rate of fluid flow through the two-phase zone. The idea is to maximize the transfer rate of the injected substrates into the liquid phase while minimizing the gas saturation. Experimental studies in laboratory columns indicate that equilibrium among the phase can be rapidly attained [12], minimizing the required thickness of the two-phase region in the groundwater flow direction. In the field, however, it can be expected that transfer will be more efficient near the watertable due to the lower water saturation.

Permeability of an aquifer to the water phase ( $k_w$ ) also is directly dependent on the water phase saturations (Figure 4c).  $k_w$  is a maximum when the aquifer is saturated and is zero at  $S_{iw}$ . Thus, the water permeability of the two-phase mixing zone will be decreased relative to the surroundings. This could cause some divergence of flow around the two-phase zone, unless of course an alternative material is emplaced. In addition, vertical decreases in  $S_w$  will cause decreases in  $k_w$ . Thus, while mass transfer rates are greater in the near vadose zone region, groundwater flux through it is less. It is not clear how this will affect the overall process. But it is clear that field operations will need to be carefully engineered and monitored to accurately interpret results.

In summary, the new concept of two-phase mixing zones to increase substrate or co-substrate concentrations in flowing groundwater appears to have promise. Substantial increases in, for example, both  $O_2$  and  $CH_4$  can be effected by use of the pure phases as the injectate. This reaction zone eliminates the displacement problems encountered in liquid biostimulation and could be an inexpensive alternative. Further laboratory and field work is required to establish the technical foundation and the range of its niche in field situations.

### Bioaugmentation

Bioaugmentation is the addition of bacteria, which are known to degrade the target contaminants, to the subsurface. The particular species injected may or may not already exist in the subsurface. There are two basic types of bioaugmentation: with substrates or co-substrates and in

the absence of any growth substrates (resting-state). Bioaugmentation has received attention in the oil industry both for microbial-enhanced oil recovery [6] and as a permeability control [14]. It has only been partially discussed in the literature with regards to contaminated groundwater [18] but recent work with resting-state cells shows promise. The subsurface processes which control the engineering of each approach are similar to those already discussed, with the addition of cell transport issues, and will be briefly reviewed below.

#### *Augmentation with substrates*

Bioaugmentation with substrates refers to the addition of bacteria to a contaminated aquifer in a liquid which contains primary growth substrates [15]. The concept is that the indigenous population is either of inadequate population density or composition to achieve desired degradation rates and that growth substrates are required to sustain the contaminant biodegradability of both the inoculum and the indigenous species. Engineering considerations for this concept are similar to biostimulation: a liquid is injected, displaces contaminated groundwater, and develops a limited mixing zone. Continuous injection seems to be required since the salubrious effects caused by the injectate have been found to rapidly decay at the cessation of injection. It is in the mixing zone that contamination will contact injected cells and biodegradation can occur.

The overall efficiency of bioaugmentation with substrate injection approaches that of simple substrate injection (Figure 3) with some important additional considerations. As cells are transported in the suspended state, some attach to the aquifer material and form a fixed-bed population [22]. This population will consume injected growth substrates, the stimulated bacteria are available for biodegradation, and performance can be expected to be analogous to biostimulation. Again, injection of any liquid results in displacement of contaminated groundwater away from the injected fluid except for uncommonly high values of the retardation coefficient. In addition, a portion of the injected cells remain suspended, consuming dissolved growth substrates, and are available for biodegradation in the mixing zone. Since cell transport can be slightly accelerated from solute transport, the mixing zone size can slightly exceed that given in Figure 3.

The limitation of this process appears to be the rate of growth substrate consumption. The suspended cells will eventually consume injected substrates and their contaminant-degrading capabilities will terminate. The exact rate of this depends on relative concentrations of cells and substrate. The Mahaffey [15] field experiment obtained degradation efficiencies in the 20% range suggesting that, in this particular case, substrate consumption by suspended cells was rapid and the procedure reduced to a simple biostimulation experiment (Figure 3). Even in the best of circumstances, this approach would appear to provide only marginal benefits to straight biostimulation and is probably not worth the additional costs of cells.

#### *Resting-state*

The injection of cells into an aquifer without nutrients offers a radical departure from previous bioremediation

approaches. It is a more engineered approach as it attempts to control each aspect of the *in situ* bioremediation process. As described by Taylor *et al* [23], Jackson *et al* [13], and Duba *et al* [8], a pure strain or suite of bacteria is selected that has known, optimal contaminant degradational characteristics. These are grown in surface bioreactors under conditions that permit a degree of control over important characteristics of the bacterial population, such as the proper form of the enzyme responsible for contaminant degradation. This control is unique to surface bioreactor operations and cannot be achieved by injecting nutrients into a subsurface characterized by uncontrollable aqueous chemistry and a mixed bacterial population.

In this approach, cells are harvested from the bioreactors, separated from the growth medium, and resuspended in an aqueous solution, such as clean site-ground-water, that is devoid of their required growth substrates. This microbial suspension is injected into the subsurface where a portion of the suspended population attaches to the subsurface media forming a fixed-bed biofilter. The injection process terminates once the biofilter is established, which is on the order of several hours for most situations. This approach uses the microorganisms only to attach to the subsurface solid media and to carry the enzymes and biochemicals necessary to catalyze the biodegradation reaction. No bacterial population growth is expected, required, or occurs in contaminant plumes that do not naturally have growth substrates once the attached population has been established. Even in plumes where growth substrates are present, increases in population density are quite small.

Injection of resting-state cells is conducted through a pattern of wells and creates an *in situ*, fixed-bed, continuous-feed, biochemical reactor as the cells attach to soil particles. After injection has terminated, contact between the attached bacterial population and the contaminated ground-water is achieved by either the resumption of natural ground-water flow or by extracting ground-water through the fixed-bed biofilter. Contaminated water enters the biofilter region, the contaminants are biodegraded as they are transported through the filter, and the ground-water exits at controlled concentrations. This concept is analogous to that used in biofilters for odor control [24]. An expected aspect is that eventually the biofilter will lose its biodegradational capabilities and will need to be replenished; the frequency of replenishment will control the economic viability of the approach.

The resting-state biofilter approach is easily amenable to engineering design. The establishment of an attached bacterial population in the injected zone simply establishes an *in situ* biochemical reactor and chemical engineering principles can be applied to the design. Four key parameters are cell attachment and entrainment rates, contaminant residence times in the biofilter, biofilter degradation capacity, and biofilter longevity. Recent advances begin to give some predictability in the attached population through time in a dynamic system [22], a key for the remaining engineering variables. Residence time is a function of contaminant flux through the biofilter, degradation rates, and the attached population. Laboratory experiments and analytical considerations demonstrate that for a wide range of  $N_D$ , residence time is not an issue, as very thin biofilters will result

in decreasing contaminant concentrations to acceptable levels (Figure 5).

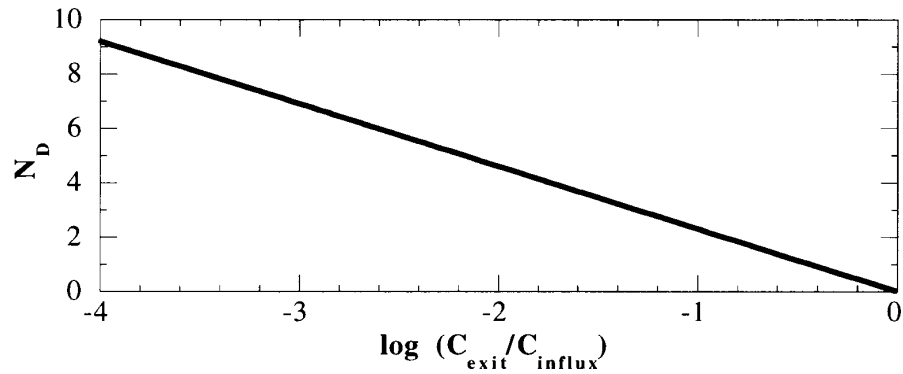
Biofilter degradation capacity and biofilter longevity are the two engineering parameters that control the viability of this approach. Contaminant biodegradation in the resting-state consumes intracellular compounds and can inactivate key enzymes [3]. Therefore, a given population density has a maximum amount of contaminants it can degrade—biofilter degradation capacity; once this is exceeded then the biofilter needs to be replenished. The replenishment interval (Figure 6) then becomes highly dependent on the contaminant flux into the biofilter, the intrinsic degradation capacity (ie, the maximum contaminant mass degradable per unit mass of cells), and the attached population density [25].

The key to the approach is the ability of the injected bacteria to sustain contaminant-degrading capabilities for extended periods of time in the resting-state. This parameter is called longevity by Taylor *et al* [23]. If longevity is a matter of hours as reported in most biostimulation field experiments, then bacteria will have to be injected continuously and the expense of growing bacteria in surface bioreactors will be prohibitive. But Taylor *et al* [23] have shown that longevity can be extended to a month or more, meaning that reinjections will be on that frequency if the biofilter is not capacity limited.

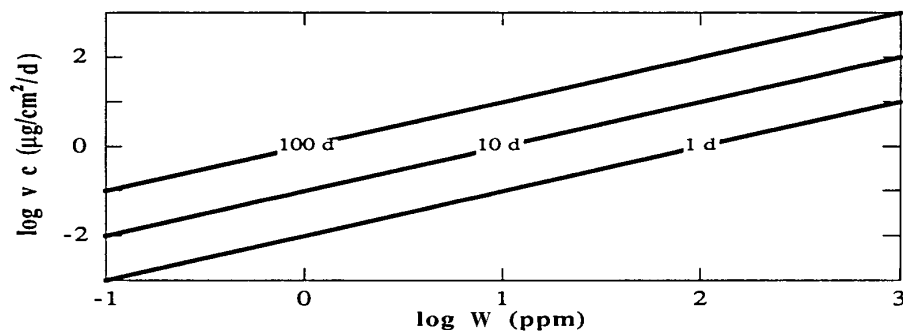
A field test recently conducted of this method [8] shows very promising results (Figure 7). It demonstrates that a resting-state biofilter can be emplaced *in situ*, that substantially complete biodegradation can be achieved, and that biodegradation can occur over extended periods of time, up to 40 days in this experiment. However, further field experiments are required to demonstrate long-term filter performance, the ability to replenish, and the ability to obtain adequate coverage across a contaminant plume.

## Conclusions

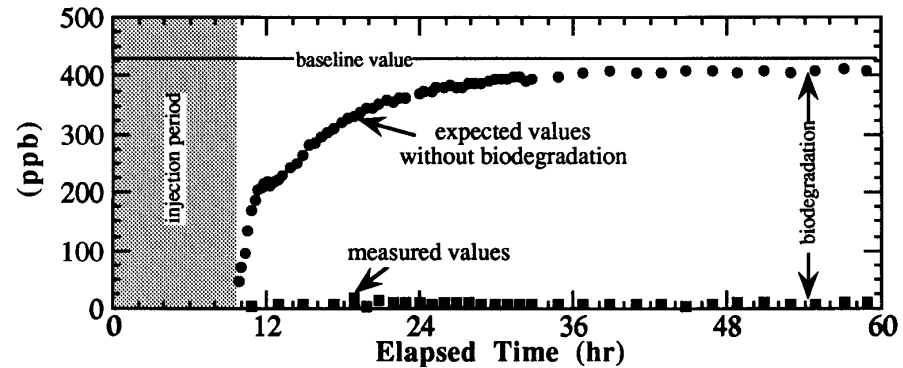
*In situ* bioremediation has been touted as a potential low-cost and efficient means for improving contaminated groundwater. The predominance of past and current work has focused on laboratory studies of particular biochemical and microbial problems, often in isolation from issues which can thwart effective field implementation of a concept. This paper has shown through simple analyses, that the niche for biostimulation using a liquid injection medium is aquifers with high contaminant sorption properties. The reported poor performance of many biostimulation field experiments may be attributable to their application outside this niche. Biostimulation using a gaseous injection medium is difficult to engineer and has not been field tested sufficiently to draw firm conclusions. In contrast, bioaugmentation with resting-state cells is most promising in aquifers characterized by fast moving groundwater and low to moderate contaminant sorption. Insufficient data exist to evaluate the economics of this approach. These simple analyses seem to provide compelling motivation for an interdisciplinary approach in bioremediation research. Ideally, the ultimate deployment scheme and intended field environment are incorporated at the initial stages so that



**Figure 5** Estimates of  $N_D$  for an *in situ* biofilter to reduce contaminated groundwater from a concentration of  $C_{influx}$  to  $C_{exit}$  upon its exit from the biofilter. For bioaugmentation,  $N_D > 10$  are quite accessible.



**Figure 6** Estimates of replenishment interval of an *in situ* microbial filter when it is only limited by degradation capacity.  $W$  is the biofilter contaminant degradation capacity,  $v$  is the pore fluid velocity, and  $c$  is the contaminant concentration entering the biofilter. Contours are replenishment times in days.



**Figure 7** Performance of resting-state biofilter at a field test in a TCE plume in Chico, CA. There was substantially complete biodegradation for the first 60 h of the experiment; biofilter performance decreased thereafter and degradation stopped after 40 days.

the basic laboratory research can include all critical field parameters.

### Acknowledgements

Work of RB Knapp was performed under the auspices of the Department of Energy by Lawrence Livermore National Laboratory under Contract W-7405-Eng-48. This document was prepared as an account of work sponsored by an agency of the United States Government. Neither the United States Government nor the University of California nor any of their employees makes any warranty, express or implied, or assumes any legal liability or responsibility for

the accuracy, completeness, or usefulness of any information, apparatus, product, or process disclosed, or represents that its use would not infringe privately owned rights. Reference herein to any specific commercial products, process, or service by trade name, trademark, manufacturer, or otherwise, does not necessarily constitute or imply its endorsement, recommendation, or favoring by the United States Government or the University of California. The views and opinions of authors expressed herein do not necessarily state or reflect those of the United States Government or the University of California and shall not be used for advertising or product endorsement purposes.





## References

- 1 Bengtsson G. 1989. Growth and metabolic flexibility in groundwater bacteria. *Microb Ecol* 18: 235–248.
- 2 Buscheck TA and JJ Nitao. 1992. Feasibility of *in situ* stripping of the volatile organic compounds at the Savannah River Site: preliminary modeling of a pair of horizontal wells. UCRL-LR-104540, Lawrence Livermore National Laboratory.
- 3 Chang H-L and L Alvarez-Cohen. 1995. Model for the co-metabolic biodegradation of chlorinated organics. *Environ Sci Technol* 29: 2357–2367.
- 4 Chesnut DA. 1994. Heterogeneity and vapor extraction performance. In: *Amer Chem Soc Symposium on Emerging Technologies in Hazardous Waste Management IV*, Sept 21–23, 1992. ACS Symposium Series 554, Amer Chem Soc, Washington DC.
- 5 Cullimore DR. 1993. *Practical Manual of Groundwater Microbiology*. Lewis Publishers, Boca Raton.
- 6 Donaldson EC, GV Chilingarian and TF Yen. 1989. *Microbial Enhanced Oil Recovery*. Elsevier, Amsterdam, The Netherlands.
- 7 Downs WC, SR Hutchins, JT Wilson, RH Douglass and DJ Hendrix. 1993. Nitrate-mediated biodegradation of BTEX in JP-4-contaminated soil and groundwater: a field pilot-scale demonstration project. In: *Bioremediation Field Experience* (Flathman PE, DE Jerger and JH Exner, eds), pp 361–382, Lewis Publishers, Boca Raton, FL.
- 8 Duba AG, KJ Jackson, MC Jovanovich, RB Knapp, NN Shah and RT Taylor. 1996. TCE bioremediation using *in situ*, resting-state bioaugmentation. *Environ Sci Technol* 30: 1982–1989.
- 9 Freeze RA and JA Cherry. 1979. *Groundwater*. Prentice-Hall, Englewood Cliffs, New Jersey.
- 10 Fry VA, JD Istok, L Semprini, KT O'Reilly and TE Buscheck. 1995. Retardation of dissolved oxygen due to a trapped gas phase in porous media. *Ground Water* 33: 391–398.
- 11 Hopkins GD, J Munakata, L Semprini and PL McCarty. 1993. Trichloroethylene concentration effects on pilot field-scale *in-situ* groundwater bioremediation by phenol-oxidizing microorganisms. *Environ Sci Technol* 27: 2542–2547.
- 12 Istok JD, MD Humphrey, JH Donaldson, KT O'Reilly and TE Buscheck. 1995. The 'bubble wall': a passive *in situ* treatment technology for contaminated ground water. *Ground Water* (submitted).
- 13 Jackson KJ, AG Duba, ML Hanna, MC Jovanovich, RB Knapp, NN Shah and RT Taylor. 1995. Field treatability test of the *in situ* microbial filters concept. *Ground Water* (in press).
- 14 MacLeod FA, HM Lappin-Scott and JW Costerton. 1988. Plugging of a model rock system by using starved bacteria. *Appl Environ Microbiol* 54: 1365–1372.
- 15 Mahaffey WR, G Compeau, M Nelson and J Kinsella. 1992. TCE bioremediation. *Water Environ Technol* 4: 48–51.
- 16 Mualem Y. 1976. A catalogue of the hydraulic properties of unsaturated soils. Res Proj 442, Technion Israel Inst Tech.
- 17 Neuman SP. 1995. On advective transport in fractal permeability and velocity fields. *Water Resources Res* 31: 1455–1460.
- 18 Norris RD, RE Hinchee, R Brown, PL McCarty, L Semprini, JT Wilson, DH Kampbell, M Reinhard, EJ Bouwer, RC Borden, TM Vogel, JM Thomas, and CH Ward (Robert S Kerr Environmental Research Laboratory). 1994. *Handbook of Bioremediation*. Lewis Publishers, Boca Raton, FL.
- 19 Schwarzenbach RP, PM Gschwend and DM Imboden. 1993. *Environmental Organic Chemistry*. John Wiley and Sons, New York, NY.
- 20 Semprini L, PV Roberts, GD Hopkins and PL McCarty. 1990. A field evaluation *in-situ* biodegradation of chlorinated ethenes: Part 2. Results of biostimulation and biotransformation experiments. *Ground Water* 28: 715–727.
- 21 Semprini L, GD Hopkins, PV Roberts, D Grbic-Galic and PL McCarty. 1991. A field evaluation of *in-situ* biodegradation of chlorinated ethenes: Part 3. Studies of competitive inhibition. *Ground Water* 29: 239–250.
- 22 Tan Y, JT Gannon, P Baveye and M Alexander. 1994. Transport of bacteria in an aquifer sand: experiments and model simulations. *Water Resources Res* 30: 3243–3252.
- 23 Taylor RT, ML Hanna, NN Shah, DR Shonnard, AG Duba, WB Durham, KJ Jackson, RB Knapp, AM Wijesinghe, JP Knezovich and MC Jovanovich. 1993. *In situ* bioremediation of trichloroethylene-contaminated water by a resting-cell methanotrophic microbial filter. *Hydrological Sci J* 38: 323–342.
- 24 Togna AP and M Singh. 1994. Biological vapor-phase treatment using biofilter and biotrickling filter reactors: practical operating regimes. *Environ Prog* 13: 94–97.
- 25 Tompson AFB, RB Knapp, ML Hanna and RT Taylor. 1994. Simulation of TCE migration and biodegradation in a porous medium under conditions of finite degradation capacity. *Adv Water Resources* 17: 241–249.
- 26 van Genuchten M Th and WJ Alves. 1982. Analytical solutions of the one-dimensional convective-dispersive solution transport equation. US Dept Agriculture Tech Bull No. 1661.
- 27 Wilson JT, JH Armstrong and HS Rafai. 1993. A full-scale field demonstration on the use of hydrogen peroxide for *in situ* bioremediation of an aviation gasoline-contaminated aquifer. In: *Bioremediation Field Experience* (Flathman PE, DE Jerger and JH Exner, eds), Lewis Publishers, Boca Raton, FL.