Encapsulated Cell Bioremediation: Evaluation on the Basis of Particle Tracer Tests

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Abstract

Microencapsulation of degradative organisms enhances microorganism survivability (Stormo and Crawford 1994). The use of encapsulated cell microbeads for in situ biodegradation depends not only on microorganism survival but also on microbead transport characteristics. Two forced-gradient, recirculating-loop tracer experiments were conducted to evaluate the feasibility of encapsulated cell transport and bioremediation on the basis of polystyrene microsphere transport results. The tracer tests were conducted in a shallow, confined, unconsolidated, heterogeneous, sedimentary aquifer using bromide ion and 2 μm, 5 μm, and 15 μm microsphere tracers.

Significant differences were observed in the transport of bromide solute and polystyrene microspheres. Microspheres reached peak concentrations in monitoring wells before bromide, which was thought to reflect the influence of aquifer heterogeneity. Greater decreases in microsphere C/C_0 ratios were observed with distance from the injection wells than in bromide C/C_0 ratios, which was attributed to particle filtration and/or settling.

Several methods might be considered for introducing encapsulated cell microbeads into a subsurface environment, including direct injection into a contaminated aquifer zone, injection through a recirculating ground water flow system, or emplacement in a subsurface microbial curtain in advance of a plume. However, the in situ use of encapsulated cells in an aquifer is probably limited to aquifers containing sufficiently large pore spaces, allowing passage of at least some encapsulated cells. The use of encapsulated cells may also be limited by differences in solute and microbead transport patterns and flowpath clogging by larger encapsulated cell microbeads.

Introduction

In situ biodegradation, the process of using living organisms to degrade contaminants in a subsurface environment, is emerging as a promising method for remediating some contaminated sites. Many contaminants are vulnerable to degradation by indigenous microbial populations. However, some contaminants, such as pentachlorophenol, are not readily degraded by native microbes (Pignatello et al. 1983; Crawford and O'Reilly 1990). Nonindigenous bacteria have been identified that degrade certain resistant contaminants (Crawford et al. 1990).

Organism survival is an important factor in the use of nonindigenous organisms for in situ bioremediation. Introduced microbes may disappear or be reduced to low levels in days or weeks under marginal survival conditions (Stormo and Crawford 1994). Microorganism survival may be limited by contaminant concentrations: local contaminant concentrations may be biocidal, and noncontaminated water (e.g., near a subsurface plume) may contain insufficient carbon/energy sources for biomass maintenance.

Microencapsulation of degradative organisms is a technique that enhances microorganism survivability (Stormo and Crawford 1992, 1994). Microencapsulation allows the inclusion of nutrients and carbon sources in the immediate cell environment, and can provide cells with increased protection in a foreign environment. Preliminary findings showed that encapsulation can increase microbial survivability by more than three orders of magnitude (Stormo and Crawford 1992).

A significant factor influencing in situ bioremediation using encapsulated cells is the transport of encapsulated cell microbeads in the subsurface environment. The encapsulated cell microbeads (or bacteria emanating from the encapsulated cell microbeads) must come into contact with the contaminant for effective degradation. Factors influencing microbead transport in an unconsolidated aquifer include particle filtration and/or adsorption to aquifer sediments. Particle filtration is controlled in part by aquifer grain size (and corresponding connected pore space dimensions) and particle size, and has been described by a number of researchers, including Herzig et al. (1970), Sherard et al. (1984a, 1984b), McDowell-Boyer et al. (1986), and Cheremisinoff and Azbel (1983). Herzig (1970) and McDowell-Boyer et al. (1986) also review physiochemical capture processes.

Aquifer heterogeneity also influences contaminant and microbial transport (Committee on Ground Water Cleanup Alternatives,

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National Research Council 1994). Particle tracers have been used under laboratory and field conditions to evaluate flow in heterogeneous porous media (Borchardt and O'Melia 1961; Maroudas and Eisenklam 1965; Heertjes and Lerk 1967; Herzig et al. 1970; Sakthivadivel et al. 1972; Fitzpatrick and Spielman 1973; Darby and Lawler 1990, Harvey et al. 1989; Toran and Palumbo 1992; McKay et al. 1993; Saiers et al. 1994).

The purpose of this study was to evaluate the feasibility of encapsulated cell bioremediation on the basis of particle transport characteristics. Two intermediate-scale tracer tests were conducted under field conditions using conservative solute (bromide) and polystyrene microsphere tracers. The results of this study were used to speculate on the feasibility of using encapsulated cell microbeads for in situ bioremediation.

Experimental Design and Methodology

Tracer Selection

Tracers were used to identify average ground water flow rates and to represent the transport of encapsulated cell microbeads. Potassium bromide was chosen as a conservative solute tracer because of its low cost and low background concentrations. Fluoresbrite monodispersed polystyrene (2.5% latex) microspheres (Polysciences Inc.), which have neutral surface characteristics and a density of 1.05, were selected to represent the encapsulated cell microbeads. Encapsulated cell microbeads were also injected, but were difficult to count in ground water samples (Petrich et al. 1995).

Plain fluorescent latex microspheres were selected for this study because of their ease of detection, availability in discrete sizes with diameters in a portion of the encapsulated microbead diameter range, and neutral surface characteristics. Polystyrene microspheres with 2, 5, and 15 μm diameters represent the smaller size ranges of encapsulated cell microbead diameters. For comparison, agarose-encapsulated Flavobacterium ATCC 39723 microbeads (Stormo and Crawford 1991, 1992, 1994) ranged in diameter from 2 to 80 μm. Approximately 36% of the microbeads by volume were 10 μm in diameter or less, approximately 99% of the microbeads by number were 10 μm in diameter or less, and approximately 94% of the microbeads by number were 5 μm or less (Stormo and Crawford 1992; Stormo 1993). The 2 and 5 μm microsphere diameters were selected to represent the 94% of the microbeads (by number) that were 5 μm or less in diameter.

Polystyrene microspheres have been used in previous tracer experiments: Harvey et al. (1989) used bacteria-sized (0.2 to 1.3 μm) microspheres having carboxylated, carbonyl, or neutral surfaces in field experiments; Toran and Palumbo (1992) used 1 μm diameter latex microspheres in a series of column experiments. The microspheres used in this study are larger than the bacteria-sized particles used in previous tracer experiments.

Site Selection

A field setting was selected over a laboratory setting because of the potential use of encapsulated cell microbeads in field conditions, and because heterogeneous subsurface conditions are difficult to reproduce in a laboratory setting. Initial borings at the University of Idaho Plant Science Farm, located approximately 3.2 km east of Moscow, Idaho (Figure 1), revealed a confined aquifer consisting of unconsolidated silts, sands, and gravels spanning a depth between approximately 2.5 and 4.0 m below ground surface. This field site was selected because of the presence of a shallow, confined aquifer of relatively uniform thickness consisting of unconsolidated, heterogeneous sediments, and because of its proximity to university laboratories.

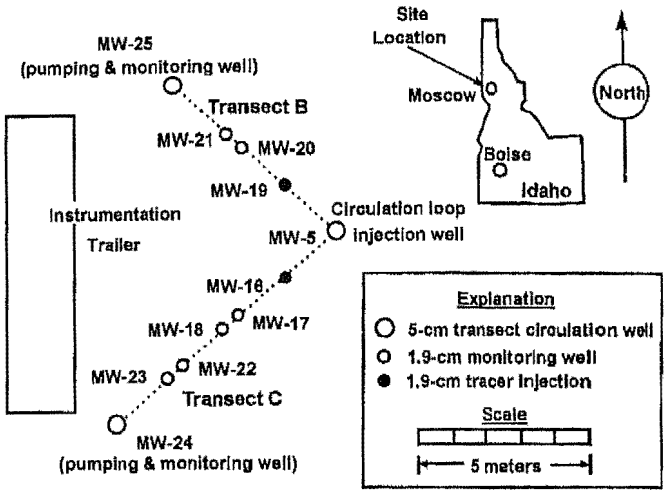

Figure 1. Well locations.

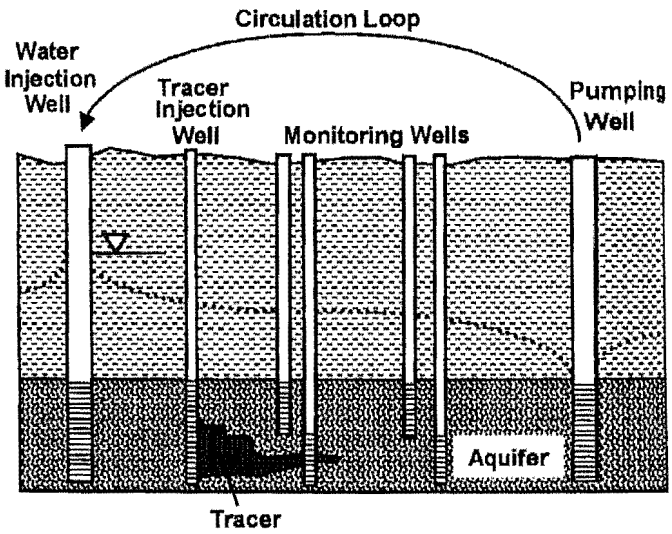

Figure 2. Schematic of recirculating tracer test design.

Tracer Test Design

The tracer experiments were conducted using a forced-gradient, recirculating tracer test configuration. The configuration consisted of a pumping well, a water injection well, a tracer injection well, and monitoring wells (Figure 2). This configuration results in less tracer dilution than might occur in a forced-gradient, single-well radial flow tracer test, and requires less time than a natural-gradient tracer test. This configuration is also a prototype of a possible remediation system design, in which recirculating water allows for repeated contact between encapsulated cells and subsurface contaminants. A disadvantage of the forced-gradient, recirculating-loop system is the difficulty in calculating tracer mass balances, because of eventual tracer recirculation within the loop.

Well Layout

Monitoring wells were installed along two transects (Transects B and C, Figure 1) using a hollow stem auger (other methods, such as direct push, were tried, but were not used because of difficulties

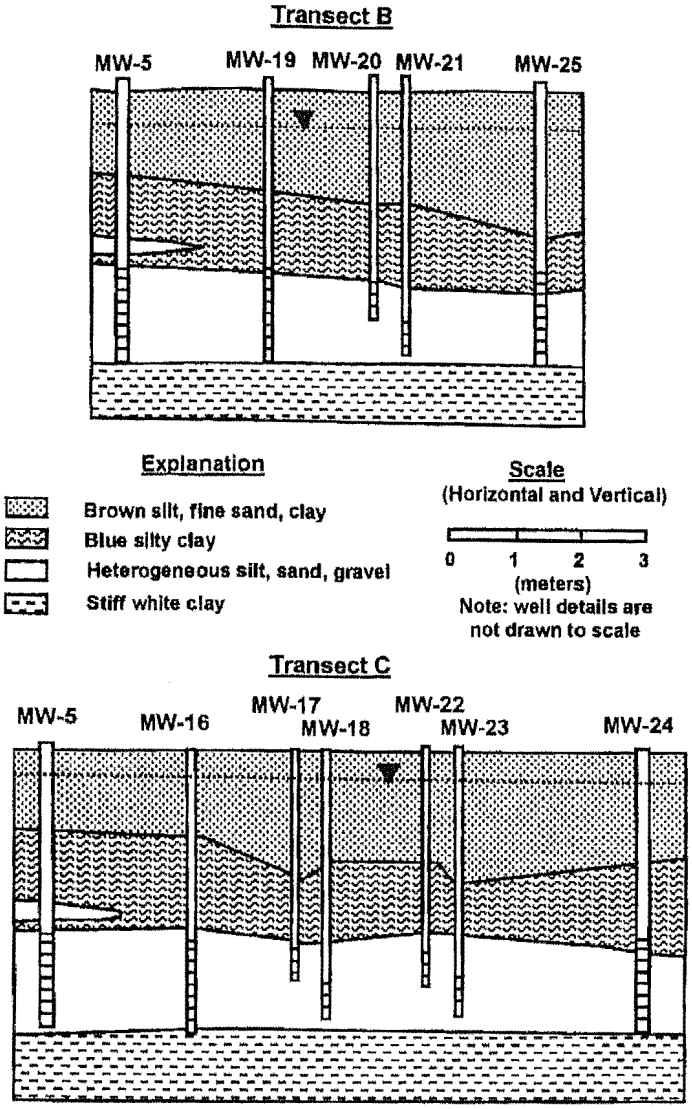

Figure 3. Transect cross sections.

associated with obtaining sediment samples and/or installing adequate seals between multiple screens in coarse-grained portions of this aquifer). Sediment samples were collected with a split spoon sampler and/or from auger flights. A third transect (Transect A) was installed prior to Transects B and C, and was used for hydrologic characterization. Well locations along each transect comprised a two-dimensional monitoring array (monitoring well pairs consisted of an upper aquifer completion and a lower aquifer completion; Figure 2). A two-dimensional monitoring well configuration was selected over a three-dimensional array to minimize well installation, monitoring, and sampling costs, and to minimize flow field disturbances. This configuration was considered adequate for comparing tracer advection rates along a well transect.

Each transect included a 5 cm diameter PVC water injection well, a 1.9 cm diameter tracer injection well, multiple 1.9 cm diameter ground water monitoring wells, and a 5 cm diameter ground water pumping well (Figure 2). The circulation-loop pumping and injection wells and the tracer injection wells were screened across the aquifer zone (152 cm screen length, 0.25 mm screen openings). The monitoring wells were installed in pairs, with 30.5 cm screen lengths (0.25 mm openings). Wells in each pair were spaced approximately 50 cm apart: the first well of each sampling pair was placed in the upper portion of the aquifer zone; the second well was placed in the lower portion. The monitoring wells were not completed as nested pairs (multiple casings in a single borehole) because of concerns about potential vertical leakage between screened intervals. General well completions for both transects are shown in Figure 3.

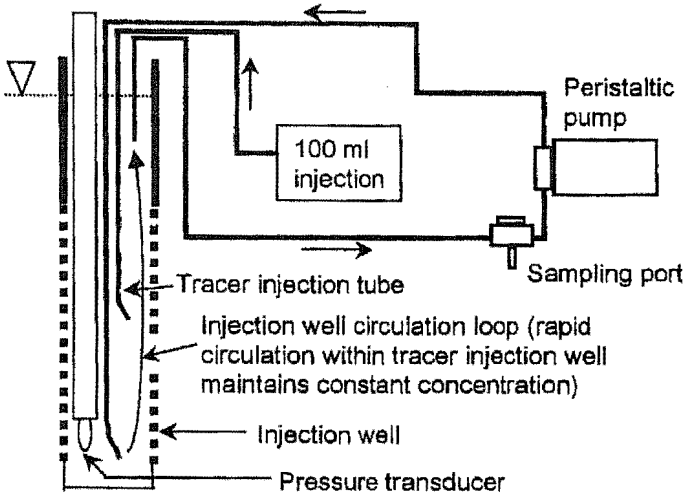

Figure 4. Tracer injection schematic.

Boreholes for the 5 cm and 1.9 cm diameter casings were drilled with 10.1 cm and 6.4 cm (I.D.) hollow stem augers, respectively. Sediment samples were collected with a 3.8 cm diameter split spoon sampler, oven dried, and sieved in a mechanical sieve shaker (ASTM method C 136-84a).

Multiple well aquifer tests were conducted in the wells following development. A complete aquifer test description is provided in Petrich (1995).

Tracer Test and Sampling Methodology

An induced hydraulic gradient was established within each transect prior to tracer injection. Water was pumped from MW-25 and injected in MW-5 in Transect B, and pumped from MW-24 into MW-5 in Transect C (the tracer tests were not run simultaneously). The circulation rates were approximately 2 L/min, which was the approximate maximum rate at which water levels in all transect wells remained above the top of the aquifer (so that the aquifer remained saturated and confined). Average hydraulic gradients over the transect length ranged from 0.14 to 0.15.

Slug injection was selected over continuous injection to minimize particle tracer costs. A tracer injection system with a separate circulation loop was designed to minimize the hydraulic interference from a slug injection in the flow field surrounding the tracer injection well, and the amount of lingering tracer in the injection well above the well screen (Figure 4). Tracer was injected into MW-19 (Transect B) and MW-16 (Transect C). Individual tracers were injected at 20-minute intervals to avoid potential ionic effects between the potassium bromide and microsphere tracers (tracer test times were normalized for comparing results).

Injection well tracer concentrations for the first 100 minutes following injection are shown in Figure 5. The first injection well samples were taken at five minutes after injection; concentrations in the injection wells immediately after injection ($t = 0$) were estimated on the basis of injectant concentrations and injection well fluid volumes.

A continuous sampling system was installed in the monitoring wells to avoid repeated well purging and automate the sampling process. The sampling system consisted of dedicated well samplers,

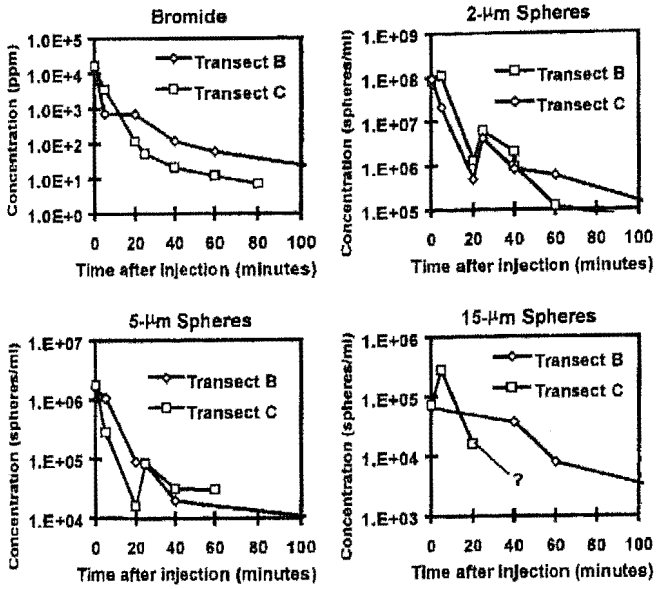

Figure 5. Injection well tracer concentrations (data points at t = 0 are estimated).

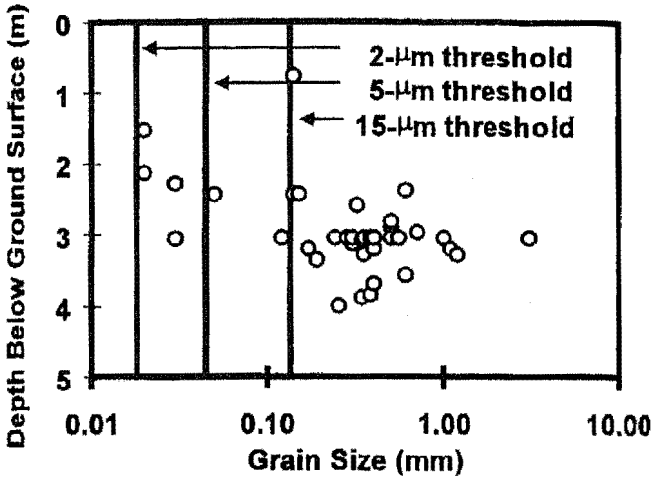

Figure 6. Comparison of aquifer grain size (85% retained) variations with depth and particles size threshold levels. (See "Discussion" section for explanation.)

a sample collection system, and a data collection system. The well samplers consisted of 0.13 cm diameter TFE tubing (Cole Parmer) attached to the outside of a sealed 1.6 cm PVC pipe, which was then placed in each of the 1.9 cm monitoring wells. The purpose of the 1.6 cm pipe was to reduce casing volume, thereby increasing flow velocities in the well casing and reducing particle settling. The volume of water held in each monitoring well with sampler in place was approximately 120 mL per vertical meter of casing length. The pumping wells (MW-24 and MW-25) did not have pipes installed to reduce casing volume (because of their pumping function), but minimal particle settling was expected in these wells because of the induced flow rates.

The TFE sampler tubing was routed from the well samplers through a Cole Parmer Masterflex Digistaltic pump. Flow rates in the continuous samplers ranged from approximately 0.75 to 1.0 mL/min. Experimentation prior to the tracer tests had indicated that the effect of these flow rates on local hydraulic gradients was negligible.

Fluid samples from monitoring wells were collected continuously during 20-minute intervals with Isco fraction collectors, located in an instrumentation trailer. Samples containing 15 to 20 mL were collected in 16 × 150 mm disposable glass tubes. Sample tubes were capped following each tracer test, refrigerated at 5°C, and transported to a University of Idaho laboratory for analysis.

Sediment samples from the bottom of each well were obtained after the last tracer test. Bottom sediments were sampled to determine if tracer particles were settling to the bottom of the well casings (as would be expected if sampling velocities were too low to keep particles in suspension).

Laboratory analyses of bromide concentrations were conducted with a Cole-Parmer bromide electrode. In general, potential error sources for the selective ion electrode measurements may include interference from other ions, temperature variation, and calibration. Selective ion electrodes were considered suitable for rapid estimation of ion constituents in this study.

Polystyrene microsphere analysis was conducted by filtering water samples through 0.2 or 1.0 µm Nuclepore filters and counting microspheres on the filter under a Carl Zeiss microscope equipped with an HBO-100 high-pressure mercury illuminator, a 10x ocular lens, and 4x, 10x, 40x, and 100x (oil) objectives. Water samples with expected high concentrations were diluted with deionized water for easier counting.

Sample tubes were selected for microsphere analysis on the basis of bromide tracer breakthrough. Additional samples were selected to improve the definition of microsphere breakthrough curves. Duplicate slides (approximately 10% of the counted slides) were prepared to monitor variability inherent to slide preparation. Duplicate counts of the same slide were conducted by different technicians to monitor inherent counting variability.

Results

Aquifer Characterization

Subsurface stratigraphy at the site consists of a shallow soil zone, a zone of brown silt containing various amounts of clay and/or fine sand, a bluish-gray zone of silty clay, a confined aquifer zone consisting of silt, sand, pebbles, and gravel, and an underlying zone of stiff, dense, light gray clay (Figure 3). Significant heterogeneities in sorting, layering, grain size, and grain shapes were observed in drill cuttings from the aquifer zone, which ranges in depth from approximately 2.5 to 4.0 m below ground surface. Water levels in wells completed in the silt, sand, and gravel aquifer fluctuated within approximately 0.5 to 1.0 m of the ground surface, indicating confined hydraulic conditions. A summary of grain size distribution data is shown in Figure 6; the number of samples retrieved from the primary aquifer zone was limited by the large diameter of some aquifer materials. The range of sorting, material sizes, degree of sediment rounding, and heterogeneity observed in aquifer sediments appeared consistent with a fluvial depositional environment. Transmissivity values (on the basis of multiple well aquifer test data) ranged from 4.2 to 6.3 cm^2/min; storativity values ranged from 1.4×10^{-4} to 1.5×10^{-3} (Petrich 1995).

Tracer Test Results

Bromide concentrations for tracer tests in Transects B and C are shown in Figure 7. Relative microsphere and bromide concentrations (in the form of C/C_0, where C is the tracer concentration in a monitoring well at any given time and C_0 represents the initial injection well concentration) for Transects B and C are presented in Figures 8 and 9, respectively.

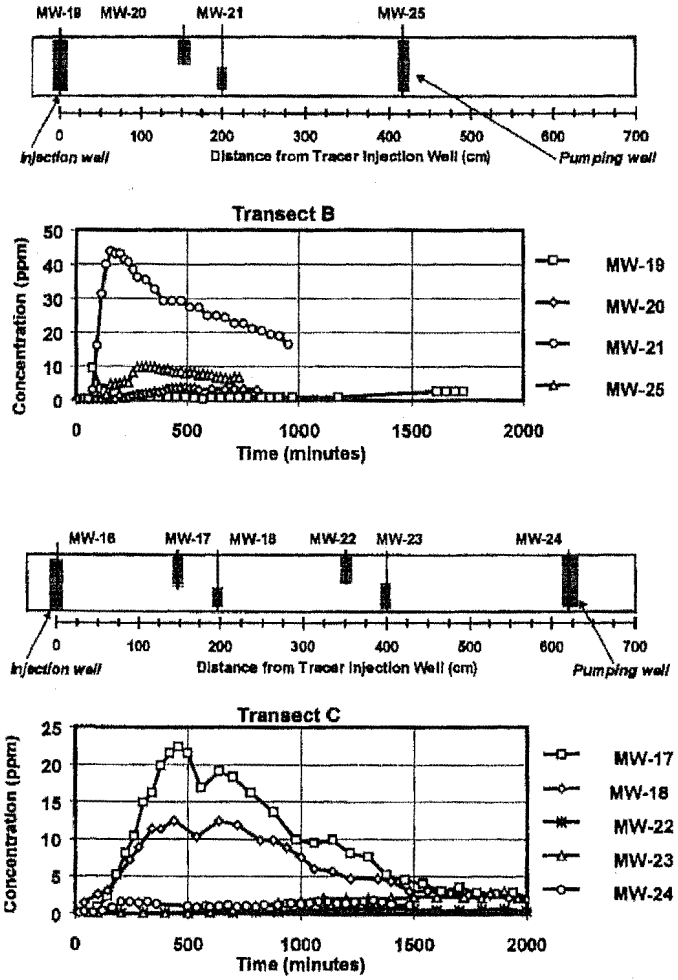

Figure 7. Bromide concentrations in Transects B and C.

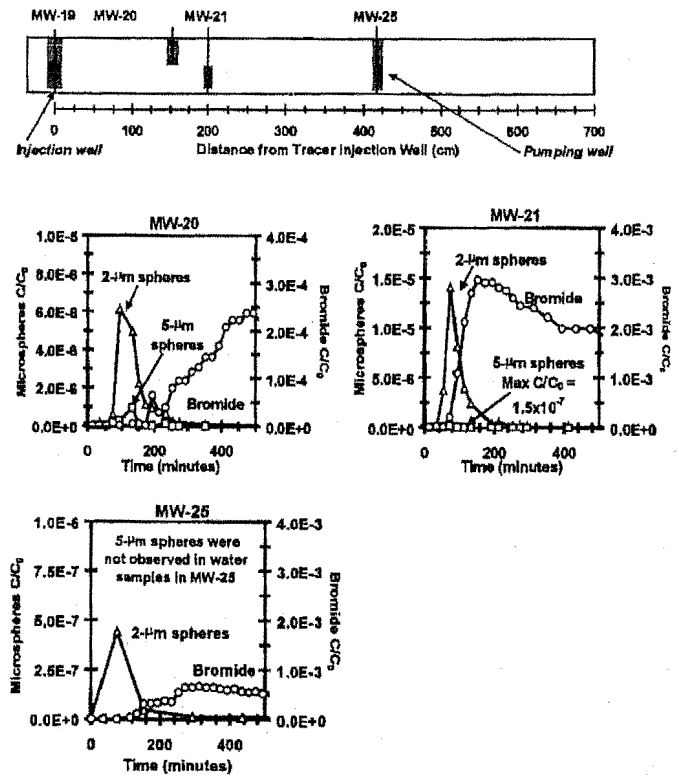

Figure 8. Relative microsphere and bromide concentrations (C/C_0), Transect B.

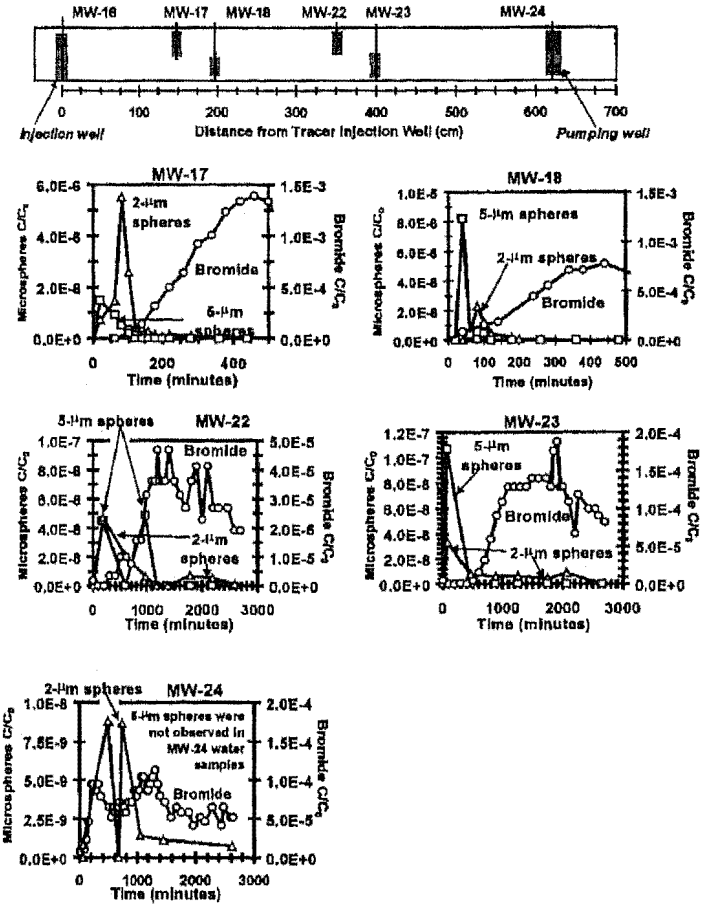

Figure 9. Relative microsphere and bromide concentrations (C/C_0), Transect C.

The following eight observations were made from these data: (1) maximum bromide concentrations varied significantly between wells; (2) the bromide breakthrough curves display asymmetrical shapes; (3) the first detection of bromide and microspheres occurred at approximately the same time in two transect B wells (MW-20 and MW-21) and two Transect C wells (MW-18 and MW-24), and microspheres were detected slightly earlier in the remaining wells; (4) microsphere concentration peaks generally occurred before those of bromide in all wells that had significant concentrations of microspheres (the term "peak" refers to a period of time of elevated concentration, not necessarily a single point on the concentration curve); (5) microsphere concentration peaks were of shorter duration than the bromide peaks; (6) the 5 µm microsphere concentration peaks appear to have occurred before the 2 µm microsphere peaks in some wells but not others; (7) the maximum relative concentrations of bromide were greater than those of the 2 and 5 µm microspheres, generally by two to three orders of magnitude; and (8) the relative microsphere concentrations generally decreased with distance more than the relative bromide concentrations.

Fewer 5 µm diameter microspheres than 2 µm microspheres were observed in monitoring well ground water samples (no 5 µm microspheres were observed in water samples taken from MW-24 and MW-25), and no 15 µm diameter microspheres were observed. However, 2 µm and 5 µm microspheres were observed in all samples taken from monitoring well sediments (accumulated sediments at the well base). Several 15 µm microspheres were observed in sediments removed from the bottom of MW-21, indicating that at least some transport of 15 µm microspheres occurred.

Discussion

The first indication of particle transport potential in the study aquifer was based on the grain size analysis results. Sherard et al. (1984b) observed that particle filtration would occur if $D_{M85}/D_{P15} < 9$, where D_{M85} indicates the 85% retained medium size and D_{P15} represents the 15% retained particle size (Figure 6). On the basis of this relationship and grain-size data, 2 μm particles were expected to migrate through aquifer materials at depths within the entire aquifer zone. Microspheres with 5 and 15 μm diameters were expected to pass through the aquifer at depths between 3.5 and 4.5 m.

Varying bromide concentrations between wells and asymmetrical concentration curves were attributed to aquifer heterogeneity. Aquifer heterogeneity was observed during monitoring well installation and recorded in the sieve analysis data. Higher bromide concentrations were observed in the circulation loop pumping wells, compared to some of the monitoring wells. This was attributed to the presence of preferential flowpaths (flowpaths that did not correspond with the linear transect configuration) through which flow was induced by radial pumping. Extended bromide concentration curve tails were probably caused by bromide moving in lower permeability zones within the aquifer. Bromide concentration increases in MW-19 between 1000 and 1600 minutes after injection appear to indicate tracer recirculation in the transect (several ground water samples were lost during this time period because of sampleline freezing). A dip in the observed bromide concentrations in Transect C at approximately 550 minutes was caused by a temporary fraction collector malfunction.

The initial arrival times for bromide and microspheres were similar in several wells; microspheres were observed slightly earlier than bromide in other wells. Similar initial arrival times for the bromide and particle tracers in some wells indicate that some of the solute moved at the same rate as the microspheres. The apparent earlier arrival of microspheres in some wells is attributed to insufficient sampling and analysis frequency and/or lower detection limits for the particles than for the conservative solute tracer, because microspheres cannot be moving faster than the ground water (as represented by the conservative ion tracer) transporting the microspheres.

Concentration peaks of 2 μm microspheres generally occurred before the bromide concentration peaks, and the peaks were of shorter duration than the bromide peaks. These findings were attributed to the relationship between microsphere diameters and heterogeneous flowpath characteristics. The microspheres are limited to those flowpaths (interconnected pore spaces) large enough to allow passage of the particles. The solute, however, is transported by advection and dispersion through zones of large and small interconnected pore spaces with varying degrees of flow tortuosity. The bromide transport is through a larger aquifer volume, resulting in a larger, broader concentration peak, the bulk of which occurs after that of the microspheres. Significantly higher concentrations of both bromide and 2 μm microspheres in MW-21 than in MW-20 suggest that there was greater particle transport in the lower portion of the aquifer in Transect B; concentrations between upper and lower wells in Transect C were more similar.

Earlier particle concentration peaks with respect to a conservative tracer have been reported for Escherichia coli and particle tracers (Champ and Schroeter 1988), yeasts (Wood and Ehrlich 1978), and iron oxide colloids (Puls and Powell 1992). Toran and Palumbo (1992) observed that 1 μm microspheres arrived ahead of NaCl tracer in a series of laboratory column tests. Harvey et al. (1989) report that "unattenuated bacteria may be transported more quickly than a conservative tracer, simply on the basis of size." Harvey et al. (1993) suggest that bacteria transport peaks can occur earlier than those of conservative tracers when an aquifer is dominated by preferential flowpaths. Toran and Palumbo (1992) also observed that particle concentrations peaked before those of a conservative tracer in column tests, a characteristic which was enhanced with the presence of multiple tubes representing fractures. The phenomenon of earlier particle concentration peaks (with respect to a conservative tracer) has been referred to as the size exclusion effect (Enfield and Bengtsson 1988), in which particles are excluded by size from smaller pores, and, as a result, they travel in larger-pore flowpaths (preferential flowpaths) along which ground water velocities are greater than average.

The 5 μm microsphere concentration peaks occurred earlier in some wells (e.g., MW-17 and MW-18) than those of the 2 μm microsphere peaks. This may be another manifestation of the size exclusion effect. Similar concentration peak times of 2 and 5 μm microspheres in some wells (e.g., MW-20, MW-21, MW-22, MW-23) may indicate that some preferential flowpaths in this transect were able to transmit 2 and 5 μm microspheres in similar quantities.

Some authors (e.g., Toran and Palumbo 1992) have calculated retardation factors to describe earlier concentration peak timing for particle tracers. The retardation factor describes the effects of a solute characteristic, i.e., adsorption. Earlier peak concentration times for particle tracers were described by Toran and Palumbo (1992) with retardation factors of <1. However, for particle tracers with neutral surface characteristics, an earlier occurrence of the concentration peak (with respect to a conservative solute tracer) appears to be a function of particle size and flowpath characteristics (e.g., grain-size diameters, particle packing, flowpath continuity) and not adsorption. Toran and Palumbo (1992) suggest that retardation factor could be used to evaluate aquifer heterogeneity. The term "retardation factor" may be inappropriate, but the development of a "concentration peak factor" consisting of a comparison of concentration peaks for neutral particle and conservative solute tracers could be useful for evaluating relative flowpath (aquifer heterogeneity) characteristics.

Lower relative microsphere concentrations (compared to bromide), and a greater relative decrease of microsphere concentrations with distance, could be caused by chemical interaction, filtration, and/or settling. Toran and Palumbo (1992) attributed retention of microspheres to chemical interaction; Harvey et al. (1989) observed little difference in concentration peak times between conservative solute and uncharged latex microspheres. We hypothesize that lower relative concentrations were caused primarily by particle filtration and/or particle settling. Filtration would be expected in some aquifer zones on the basis of aquifer grain sizes. Greater filtration would be expected for the 5 and 15 μm microspheres than for the 2 μm microspheres. Some particle settling was observed in sediments at the base of all monitoring well casings. Distinct particle concentration peaks prior to the bromide peaks, and similar first detection times, suggest a lack of particle adsorption.

Injection concentrations of the 5 and 15 μm diameter microspheres may have been too low to adequately evaluate transport. Similar microsphere volumes were injected for each size group, but injection concentrations for 5 μm microspheres were approximately two orders of magnitude less than the 2μm injection concentrations; the 15 μm injection concentrations were more than one order of magnitude less than the 5 μm injection concentrations. Injecting similar particle numbers for each microsphere size was

considered to be cost prohibitive. The lower injection concentrations of large particles probably contributed to the lack of 5 μm microspheres observed in MW-24 and MW-25 water samples (in addition to greater possible filtration), although 5 μm microspheres were found in well casing sediments removed from the bottom of these wells following the tracer tests.

Applications for the Use of Encapsulated Cells for In Situ Bioremediation

There are several ways in which encapsulated cells potentially might be used for in situ biodegradation. First, encapsulated cell microbeads might be injected directly into a contaminated aquifer, with a goal of controlled transport and subsequent release and transport of individual microbial cells (as the microbead matrix breaks down). Temporary encapsulation might allow a sufficient acclimation period for newly introduced cells in the subsurface environment. Release of individual cells, or cells resulting from reproduction, might then be transported to portions of the aquifer not accessible by encapsulated cell microbeads. Second, a recirculation system, such as the system used in the tracer tests conducted for this project, would allow for the repeated contact of encapsulated cells and contaminated water, and would enable simultaneous aboveground treatment of other contaminant constituents. Third, encapsulated cells could be injected into the ground to form a "microbial curtain" in advance of a migrating contaminant plume. Contact between the contaminant and the encapsulated cells would occur as the contaminant migrated toward and through the microbial curtain. Nutrient and/or energy sources contained in the encapsulated cell microbeads would help maintain cell survival until the arrival of the contaminant plume.

However, the subsurface use of encapsulated cells may be limited by a number of factors. First, the transport of some encapsulated cell microbeads may occur (as was documented by the transport of 2 and 5 μm microspheres in the study aquifer), but transport may not occur in the same flowpaths containing contaminant. Even individual cells emanating from the encapsulated cell microbeads may not be transported in the same flowpaths as the contaminant. Limited contact between cells introduced via the encapsulated cell microbeads and a contaminant may limit the effectiveness of the remediation strategy, or may increase the time required for contaminant degradation. Second, decreases in relative particle concentrations with distance from the injection wells indicate that particle retention has occurred. Microbead retention would not only limit the transport of the encapsulated cells but may lead to flowpath clogging, possibly resulting in transmissivity reductions. For many aquifers it may be important to preclude injection of larger diameter encapsulated cells. Third, possible interactions between encapsulation matrix and aquifer materials also may limit subsurface transport; these potential interactions were not evaluated in this study.

Conclusions

The purpose of this research was to evaluate the feasibility of encapsulated cell bioremediation on the basis of particle transport characteristics. Conclusions from this study are:

1. Significant differences were observed in the transport of bromide solute and polystyrene microspheres. These differences were attributed to the heterogeneous nature of the aquifer and differences in tracer characteristics.
2. Bromide concentration peaks occurred after those of the microspheres. The bromide peak represents transport along both high and low hydraulic conductivity flowpaths. The microsphere peaks were thought to represent transport primarily along high conductivity flowpaths.
3. Greater decreases in C/C_0 ratios with distance were observed for particle tracers than for bromide. This difference was attributed to particle filtration and/or settling.
4. An absence of 15 μm diameter microspheres and low numbers of 5 μm diameter microspheres in ground water samples were observed relative to 2 μm diameter microspheres.
5. On the basis of these results, the authors speculate that the transport of smaller encapsulated cell microbeads may be induced in some aquifers, but that the use of encapsulated cell microbeads for in situ bioremediation may be limited by heterogeneous aquifer flowpath characteristics and/or particle retention.

Acknowledgments

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