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Final Report
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Stabilization of Microorganisms for in Situ Degradation of Toxic Chemicals

University of Idaho Center for Hazardous Waste Remediation Research
R. L. Crawford and D. R. Ralston, Principal Investigators

General Background

In situ biological degradation of pollutants is a promising method for restoring contaminated subsurface environments. Subsurface bioremediation may rely on native microorganisms, or it may require the introduction of non-native microorganisms for contaminants not readily degraded by native microorganisms (1).

Successful subsurface biodegradation depends on various factors. Aquifer heterogeneity affects contaminant migration at differing scales in almost every geological setting. Furthermore, contaminants with physical and chemical properties different from those of water may be affected differently by aquifer heterogeneities. Aquifer heterogeneity also has a strong influence on native microbial populations and on the subsurface migration of non-native microbial populations.

Bioremediation with introduced microorganisms partly depends on microbial survivability. Many obstacles may interfere with the survivability of microorganisms introduced into a subsurface environment. Microbes must be kept intact and active until contaminated zones are penetrated; introduced bacteria may rapidly disappear or become severely reduced under marginal survival conditions. Varying contaminant concentrations also affect microorganism survivability. Local concentrations may be biocidal, or low concentrations may provide an insufficient carbon/energy source to maintain biomass.

Microencapsulation of degradative organisms is a technique that enhances microorganism survivability (2). Research on microencapsulation techniques using a variety of encapsulation materials, particularly polysaccharides and polyurethanes, is currently being conducted at the University of Idaho. Preliminary findings show that encapsulation increases microbial survivability by more than threefold. Other work by our group indicates that certain microencapsulated microbial enzymes may also be used for the degradation of xenobiotic compounds, without the need for whole microbial cells.

Encapsulated-cell technology could be applied to aquifer contamination in two ways. First, encapsulated cells could be injected into a contaminant plume to begin the degradation process from within the plume. Second, encapsulated cells could be injected in large numbers (or cultivated to large numbers) to form an *in situ* "microbial curtain" in anticipation of the arrival of a plume. Such microbial curtains may be appropriate for use in either unconsolidated aquifers or fractured-rock environments. For both methods, nutrients could be added during the encapsulation process before injection, or into the subsurface environment following injection, to enhance microbial survival and reproduction until a plume arrives.

The use of introduced free or encapsulated cells for subsurface bioremediation depends on a comprehensive understanding of microbial and encapsulated-cell transport characteristics in heterogeneous environments. The experiments carried out under this grant contributed to a greater understanding of the relationships between contaminant, microbial, and encapsulated-cell transport within a heterogeneous aquifer. Future experiments will focus on a controlled field trial for measuring the survival and degradation rates of encapsulated cells.

DISCLAIMER

We have developed methods for large-scale microencapsulation of bacteria and nutrients into microbeads with small enough diameters to travel through aquifers (2). In experiments described in detail elsewhere (3), we have introduced both free and immobilized cells of *Flavobacterium* ATCC 39723, a gram-negative aerobe that degrades various chlorinated phenols, into aquifer microcosms, through which pentachlorophenol (PCP)-contaminated groundwater flowed at *in situ* flow rates. Aquifer samples were collected with an auger, at depths of up to 5 meters, from three wells at the University of Idaho Ground Water Research Site, and packed into 24 columns. Some sterile columns were also prepared, by irradiation at the Washington State University Radiation Center. In some of the columns the free *Flavobacterium* cells were mixed with the aquifer material before packing the columns at a density of $5x10^7$ cells per gram of aquifer material. In others, agarose-microimmobilized *Flavobacterium* were mixed into the aquifer material at the same cell density as above. The majority of columns received identical numbers of either free cells or the agarose-microimmobilized *Flavobacterium*, injected at a flow rate of 10 ml day^{-1}.

The effluent from each column was collected daily for 170 days and analyzed by UV spectroscopy or HPLC for remaining PCP. Selected samples were also analyzed by a chloride electrode and ion chromatography for chloride concentration to confirm PCP degradation (4). There were no statistically significant differences between the degradation rates of free or encapsulated *Flavobacterium* in sterile or native aquifer material as tested in these experiments.

After degradation studies in the columns containing aquifer materials were completed, the PCP-contaminated groundwater flow into the columns was stopped and the columns were stored at 20°C. Selected columns were sacrificed after 13 and 24 months of storage to determine the survivability of the introduced *Flavobacterium* under conditions of starvation, predation, and dehydration. Thirteen months after water flow stopped, replicate enrichment flasks were made with 30 g of aquifer material from selected columns. Aquifer material was removed from storage and placed in 50 mM HEPES buffer at 7.2 pH and supplemented with 0.5 g/L of glutamate and 50 mg/L of PCP. In columns sacrificed after 13 months of storage, the only enrichments that showed PCP degradation were those from columns that had been inoculated with agarose-immobilized *Flavobacterium* or injected with agarose-immobilized *Flavobacterium*. Similar enrichments were also made after 24 months' storage, except that 30 g, 3.0 g, 0.3 g, and 0.03 g enrichments were made from a larger selection of treatments, and the PCP concentration was 75 mg/L. The only additional treatment that showed enrichment for PCP degradation after 24 months was from free *Flavobacterium* injected into sterile columns. The agarose-immobilized *Flavobacterium* columns that could be enriched for PCP degradation at 12 months could also be enriched at 24 months by 30-g inoculations. The other inoculation amounts and treatments did not yield positive enrichments for PCP degradation at 24 months.

This work has shown at the lab scale that encapsulated PCP-degrading *Flavobacterium* were able to survive under conditions of starvation, predation, and lack of water. Agarose-immobilized *Flavobacterium* were able to degrade PCP even after 24 months under severe conditions. Free cells, however, survived only under sterile conditions, rather than the simulated field conditions. These results have encouraged us to test encapsulated cell survival and biodegradation activity under actual field conditions.

Field Site Development

Field experiments were conducted at a field site on the 157-acre University of Idaho Plant Science Farm, located approximately three kilometers east of Moscow, Idaho. The farm is used primarily for agriculture and forestry research (Figure 1). Twenty-four ground water monitoring wells have been installed to date in the three-acre site. Wells were drilled with a SIMCO 2800 HS drill rig equipped with 10.1-cm and 6.4-cm (internal diameter) hollow- stem augers. Wells were completed with 5-cm and 2-cm diameter PVC casing and screened in 152-cm or 30-cm intervals.

Nineteen of these wells have been installed in a cluster along three transects (Figure 2 and Table 1). The wells have been sited along multidirectional transects so that the site anisotropy and heterogeneity can be evaluated. Sediment and/or split spoon samples have been collected from each borehole for sediment-size analysis. Monitoring well logs indicate a horizontally continuous, confined, heterogeneous aquifer ranging from approximately 1 to 1.5 meters in thickness and consisting of interbedded silt, sand, and gravel zones (Figure 3). Current hydraulic testing indicates hydraulic conductivity values ranging from 10^{-2} to 10^{-4} cm/sec; preliminary storativity estimates range from 10^{-3} to 10^{-4}. The aquifer is overlain and confined by approximately 2 meters of relatively impermeable clay, and is underlain by a very stiff, impermeable clay. The site differs significantly, in sediment size, depositional regime, particle sizes, and aquifer heterogeneity, from other sites where extensive tracer testing has been conducted (6-9). This site will be used in future studies of immobilized cell transport within heterogeneous aquifers.

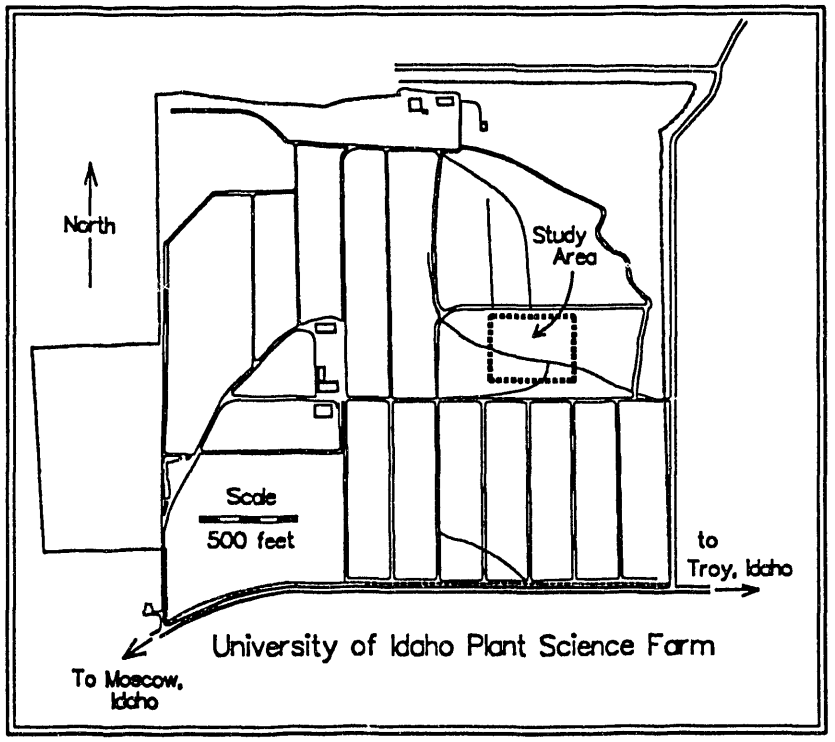

Figure 1. Site Map

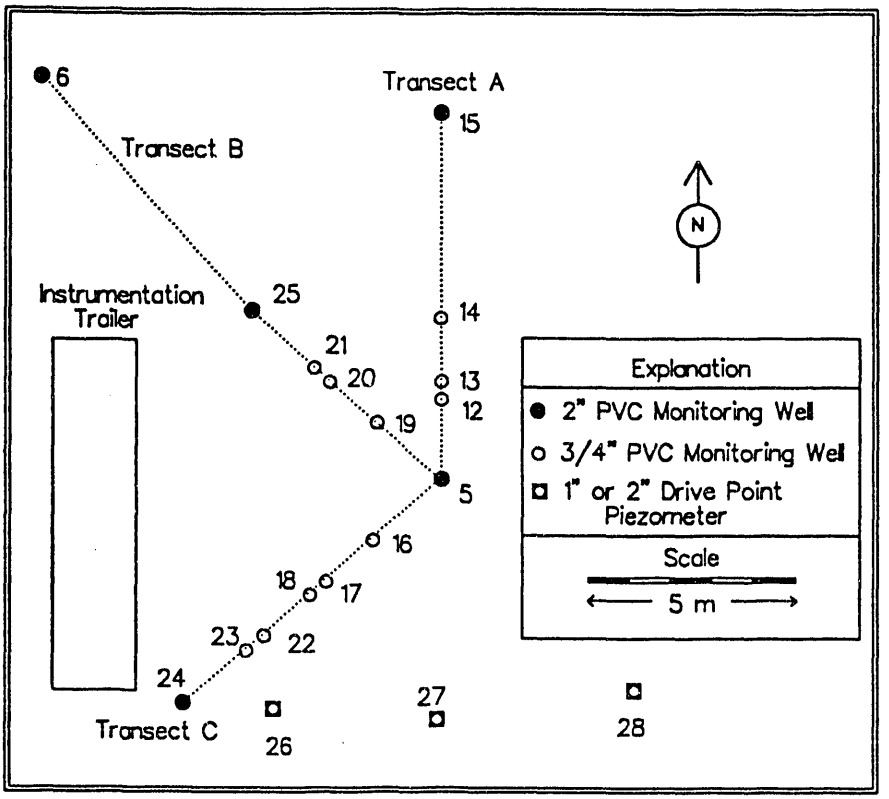

Figure 2. Monitoring Well Cluster 1

Table 1. Monitoring Well Summary

Monitoring Well	Type	Screen length
5	5-cm PVC	152-cm
6	5-cm PVC	152-cm
12	1.9-cm PCV	30.5-cm
13	1.9-cm PVC	30.5-cm
14	1.9-cm PVC	152-cm
15	5-cm PVC	152-cm
16	1.9-cm PVC	152-cm
17	1.9-cm PVC	30.5-cm
18	1.9-cm PVC	30.5-cm
19	1.9-cm PVC	30.5-cm
20	1.9-cm PVC	30.5-cm
21	1.9-cm PVC	30.5-cm
22	1.9-cm PVC	30.5-cm
23	1.9-cm PVC	30.5-cm
24	5-cm PVC	152-cm
25	5-cm PVC	152-cm
26	3.2-cm steel drive point	61-cm
27	5-cm steel drive point	61-cm
28	3.2-cm steel drive point	61-cm

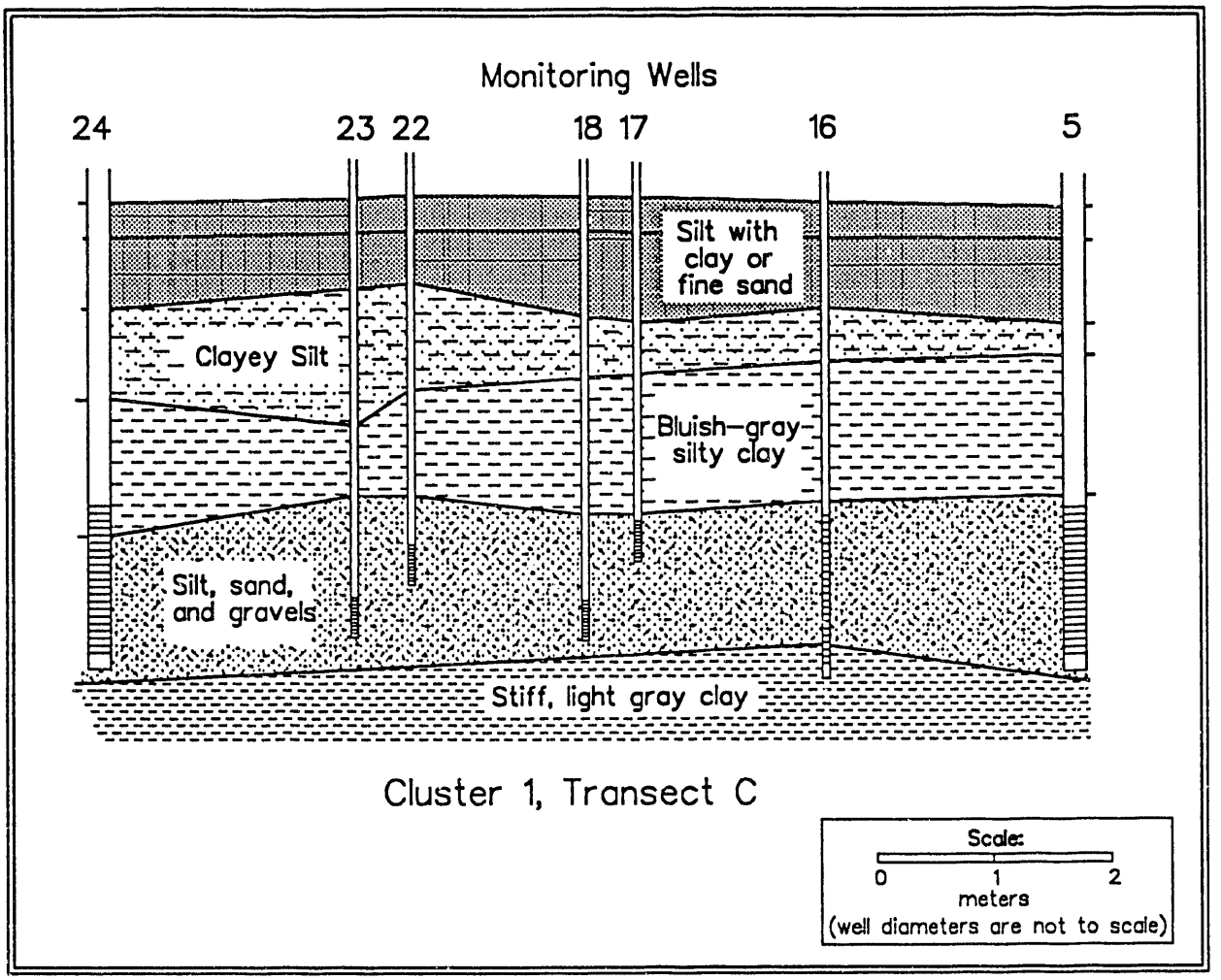

Figure 3. Cross Section along Transect C in Well Cluster 1

Literature Cited

1. Crawford, R. L., and K. T. O'Reilly. 1990. Bacterial degradation of chlorophenols, p. 113-143. In *Reviews in Environmental Toxicology*, Vol. 4. E. Hodgson (ed.). Toxicology Communications, Raleigh, North Carolina.
2. Stormo, K. E., and R. L. Crawford. 1992. Preparation of encapsulated microbial cells for environmental applications. Appl. Environ. Microbiol. 58:727-730.
3. Stormo, K. E., and R. L. Crawford. 1993. Pentachlorophenol degradation by microencapsulated *Flavobacterium* and their enhanced survival for *in situ* aquifer bioremediation. *In* In- situ and on-site bioreclamation. Proceedings, Second International Symposium, San Diego, April 5-8, 1993. Battelle Environmental Systems and Technology Division, Columbus, Ohio.
4. Levinson, W. E., K. E. Stormo, H. L. Tao, and R. L. Crawford. 1993. Hazardous waste treatment using encapsulated or entrapped microorganisms. *In* G. R. Chaudhry (ed.), Biological degradation and bioremediation of toxic chemicals. Timber Press, Portland, Oregon. In press.
5. Pignatello, J., M. Martinson, J. Steiert, R. Carlson, and R. L. Crawford. 1983. Biodegradation and photolysis of pentachlorophenol in outdoor channels. Appl. Environ. Microbiol. 46:1024-1031.
6. Garabedian, S. P., D. R. LeBlanc, K. M. Hess, and R. D. Quadri. 1987. Natural-gradient tracer test in sand and gravel: Results of spatial moments analysis. USGS Open File Report 87 109:87-109.
7. LeBlanc, D. R. 1987. Description of the hazardous waste research site. USGS Open File Report 87-475.
8. LeBlanc, D. R., S. P. Garabedian, S. M. Hess, and R. D. Quadri. 1987. Natural gradient tracer test in sand and gravel: Objective approach, and overview of tracer movement. USGS., Open File Report 87-109.
9. Mackey, D. M., D. L. Freyburg, P. V. Roberts, and J. A. Cherry. 1986. A natural gradient experiment on solute transport in a sand aquifer 1. Approach and overview of plume movement. Water Resources Research 22:2017-2029.

Publications Resulting from this Research

Crawford, R. L. 1991. Bioremediation of groundwater pollution. Curr. Opin. Biotechnol. 2:436-9.

Stormo, K. E., and R. L. Crawford. 1992. Preparation of encapsulated microbial cells for environmental applications. Appl. Environ. Microbiol. 58:727-730.

Levinson, W. E., K. E. Stormo, H. L. Tao, and R. L. Crawford. 1993. Hazardous waste treatment using encapsulated or entrapped microorganisms. *In* G. R. Chaudhry (ed.), Biological degradation and bioremediation of toxic chemicals. Timber Press, Portland, Oregon. In press.

Stormo, K. E., and R. L. Crawford. 1993. Pentachlorophenol degradation by microencapsulated *Flavobacterium* and their enhanced survival for *in situ* aquifer bioremediation. *In* In- situ and on-site bioreclamation. Proceedings, Second International Symposium, San Diego, April 5-8, 1993. Battelle Environmental Systems and Technology Division, Columbus, Ohio.

Stormo, K. E., D. B. Knaebel, and R. L. Crawford. 1993. Detection and quantification of PCP-degrading *Flavobacterium* sp. in environmental samples using PCR and internal standard plasmids. Abstract, 93rd General Meeting of the American Society for Microbiology, Atlanta, Georgia. May 16-20, 1993.

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