Microbial barriers to the spread of pollution

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1. Microbial Barriers to the Spread of Pollution

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1. Introduction

Contamination of groundwater with toxic and carcinogenic compounds is a serious concern for public health and environmental quality. This problem is commonly manifested as a contaminant plume migrating in the direction of groundwater flow from a point source. Containment of the contaminant plume is important for preventing further migration and localizing the plume for *in situ* or *ex situ* remediation. Current containment methods include sheet pilings and grout curtains. These abiotic barriers require extensive physical manipulation of the site (e.g. excavation and back-filling) and are expensive to construct. An alternative approach, biobarrier technology, involves the use of microbial biomass produced *in situ* to manipulate groundwater flow (Figure 1). Biobarriers promise to be more cost effective and cause less surface disruption then conventional barrier technologies. Furthermore, containment using biobarriers can be combined with *in situ* biodegradation or biosequestration. This chapter will review published research that relates to biobarrier formation and present results from a mesocosm test of biobarrier longevity. These results demonstrate the effectiveness of microbial barriers for manipulation of hydraulics in mesoscale porous medium reactors.

2. Selective Plugging for MEOR and Biobarrier Formation

Containment of groundwater contaminant plumes using biobarriers is a nascent technology (Cunningham et al., 1997; Lappin-Scott and Costerton, 1992). However, the manipulation of subsurface fluid flow using microbial biomass has been studied for several decades in relation to secondary oil recovery. In 1958, Van Heiningen et al. patented a process to improve waterflood oil recovery by the *in situ* production of microbial polymers. However, oil industry research in the 1960 and 1970s focused on the *ex situ* production of bacterial polymers such as xanthan and scleroglucan for enhanced oil recovery (Jack, 1993). Nonetheless, laboratory evaluations demonstrated substantial reductions in the hydraulic conductivity of sand due to the *in situ* growth and polysaccharide polymer production by bacteria (Gupta and Swartzendruber, 1962; Mitchell and Nevo, 1964). Jenneman et

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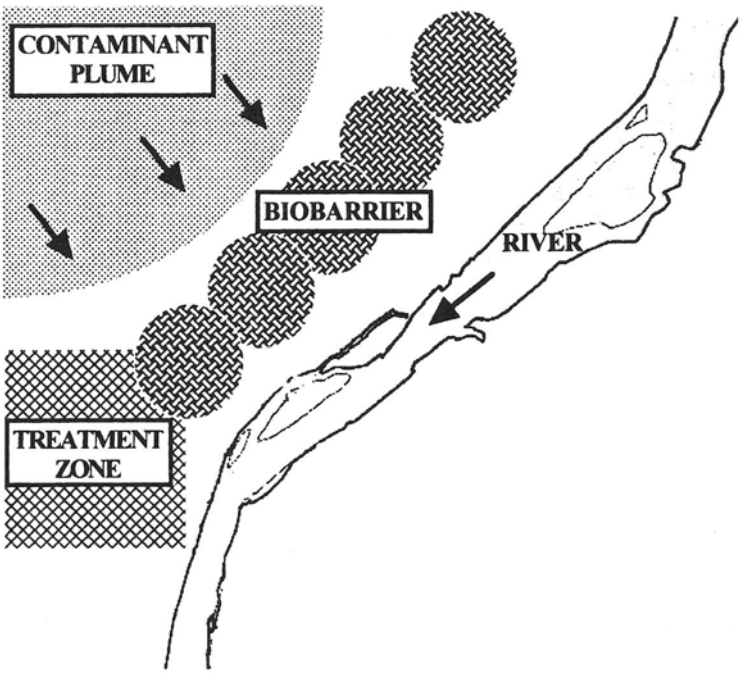

Figure 1. Diagrammatic representation of the use of biobarrier technology to prevent a groundwater contaminant plume from polluting an adjacent river. The permeability reduction caused by microbial biomass accumulation diverts groundwater flow to the treatment area. Furthermore, metabolism of the biobarrier microbial community aids in the degradation of the contaminant, contributing to remediation of the aquifer.

al. (1984) demonstrated significant permeability reductions in Berea sandstone cores by nutrient stimulation of microorganisms present within the cores. Further research by Raiders et al. (1986) revealed the preferential plugging of high permeability zones using dual core systems. Successful field tests of microbial profile modification were reported in 1993 by Coates et al. and more recently by Brown and Vadie (1997). In both of these cases, indigenous microbial communities within the waterflooded reservoirs were stimulated by the addition of molasses as a substrate and nitrate as an electron acceptor. Microbial growth and subsequent plugging of preferential flow zones resulted in a redirection of injected water and improved oil recovery. Much of the knowledge gained from the study of microbial profile modification can be directly applied to biobarrier formation for the containment of groundwater contaminants. However, groundwater reservoirs present a considerably different microbial habitat than oil reservoirs.

Environmental conditions experienced by microorganisms in groundwater habitats differ from those of oil reservoir habitats for a variety of factors including temperature, pressure, and salinity. The temperatures within oil reservoirs span the growth range of mesophilic (optimal growth temperature 25–40°C), thermophilic (40–60°C) and extremely thermophilic (> 60°C) bacteria. In contrast, growth conditions in groundwater aquifers are usually within the mesophilic to psychrophilic (5–15°C) bacterial growth range. In addition to the higher temperatures, bacteria within oil reservoirs may be exposed to relatively high pressures (200–300 atm). For a thermophilic microbial community enriched from a North Sea oil platform, growth was inhibited by pressures above 200 atm at 70°C (Sheenan and Vance, 1986). Nonetheless, under optimal growth conditions (pH, Eh, and temperature) bacteria were able to grow at pressures of over 1000 atm (Marquis, 1983). Although pressure tolerance is of concern in MEOR, it is unlikely to be a factor for microbial plugging in groundwater environments. The salinity of oil reservoir fluids is quite variable and can range from less than 1% to hypersaline environments with more than 10% salinity. A survey of thermophilic fermentative bacteria from 36 high temperature oil reservoirs, revealed that the salinity of the reservoir influenced the types of bacteria present (Grassia et al., 1996). In addition to influencing the composition of a bacterial community, salinity can affect the transport of bacteria through porous media (Cannon et al., 1991). Microbial attachment in porous media plugging is discussed in more detail below. Overall, the differences in microbial habitat between oil reservoirs and groundwater aquifers suggest differences in the types of bacteria to be stimulated for subsurface plugging in each environment. Whereas thermophilic and halophilic microorganisms are favored for growth under oil reservoir conditions, mesophilic or psychrophilic nonhalophiles are likely to predominate under groundwater conditions.

3. Biofilms in Porous Media

Imaging of microbial colonized porous media using scanning electron microscopy (SEM) and scanning confocal laser microscopy (SCLM) has revealed the presence of biofilms at the fluid interface with the substratum (Shaw et al., 1985; Stoodley et al., 1994). Biofilms on these surfaces caused a restriction of pore throat size, resulting in a reduction in hydraulic conductivity. Figure 2 shows a SCLM image of biofilm formed on glass beads by a *Pseudomonas fluorescens* strain isolated from a petroleum contaminated aquifer. These petroleum degrading bacteria, grown under denitrifying conditions, demonstrate the ability of biofilms to restrict pore throat size in porous media. SCLM imaging of biofilms has also revealed that these microbial communities are very open structures with as much as 73–98% extracellular

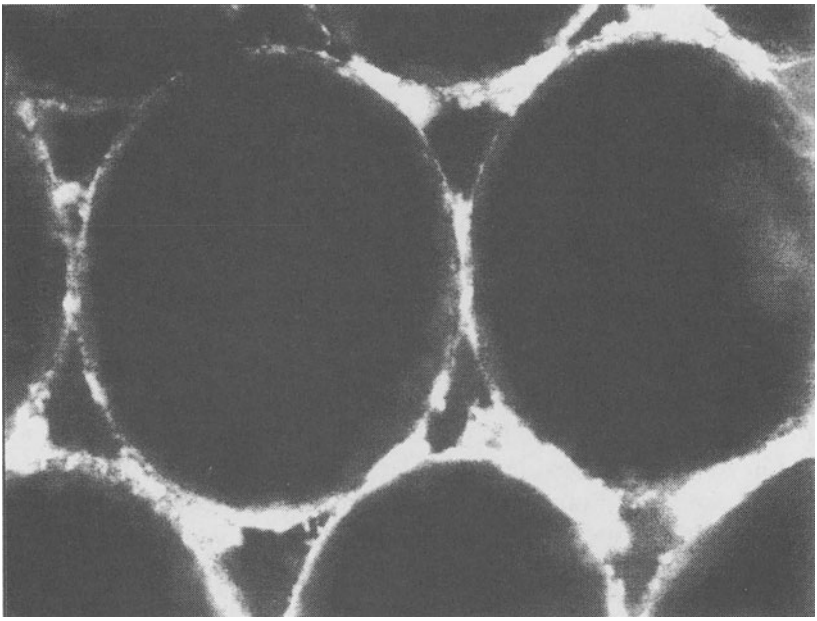

Figure 2. Scanning confocal laser micrograph image demonstrating the occlusion of pore throats caused by the growth of microorganisms in saturated porous media. This image was collected from a microscale reactor (50 × 3 × 2 mm, L × W × H) packed with 0.5 mm glass beads. The reactor was conditioned with simulated groundwater, inoculated with a *Pseudomonas fluorescens* strain isolated from petroleum contaminated aquifer, and then irrigated with a molasses and nitrate-based nutrient solution. Magnification = 1500×.

material and pore space (Lawrence et al., 1991). The free pore space has important implications for biofilm development, because it enables mass transfer rates to the biofilm organisms that could not be achieved with a homogeneous cell distribution (Costerton et al., 1994). The free pore space within biofilms suggests that porous media plugging by biofilms should be considered the induction of a secondary porosity (biofilm porosity) rather than simply a physical reduction of the original substratum porosity (Stoodley et al., 1994). Microscale and mesoscale evaluations of biofilm plugging of porous media suggested that a minimum hydraulic conductivity was obtained, regardless of the initial hydraulic conductivity (Cunningham et al., 1991; James et al., 1995). These results likely reflect that a certain amount of fluid flow was required for the maintenance of biofilm microorganisms. Although the restriction of pore throats within porous media by the growth of attached microorganisms has been demonstrated, it has also been suggested that the occlusion of pore throats by filtered microbial aggregates may be an important factor in the

plugging of porous media (Vandevivere, 1995). Nonetheless, it is likely that such microbial aggregates resulted from sloughing events within biofilm communities.

Overall, studies of microbial plugging of porous media indicate that: (1) microbial biomass accumulation significantly reduced hydraulic conductivity, (2) microbial plugging occurred selectively in higher permeability zones, and (3) entrained microbial aggregates may contribute to porous media plugging. Studies of the plugging of porous media by microorganisms have also revealed several factors influencing the uniformity and depth of plugging, these aspects will be considered below.

4. Subsurface Inoculation with Bacteria

Biostimulation refers to the addition of nutrients to stimulate the growth of indigenous microorganisms located within an environment, whereas bioaugmentation refers to nutrient stimulation combined with the addition of bacterial inoculants. Laboratory tests and field trials have demonstrated that biostimulation can be effective for selective plugging in enhanced oil recovery (Jenneman et al., 1984; Bhupathiraju et al., 1993; Coates et al., 1993; Brown and Vadie, 1997). Although similar strategies may be feasible for biobarrier formation, the use of specific inoculants (derived from the indigenous microbial community) may be more suitable for biobarrier applications because specific metabolic capabilities (i.e. degradation of the contaminant) are desired in addition to the ability to form a hydraulic barrier.

The use of microbial inoculants for biobarrier formation requires efficient subsurface transport and survival of the inoculated microorganisms. Microbial transport in porous media has received considerable study in relation to contamination of aquifers with pathogens, inoculation for *in situ* bioremediation, as well as microbial enhanced oil recovery (Harvey, 1997). The factors influencing microbial transport in the subsurface are complex and include characteristics of the porous medium, fluid phase, and bacterial cells. Porous medium factors influencing microbial transport included grain size and heterogeneity (Fontes et al., 1991; Harvey et al., 1993). Characteristics of the fluid phase influencing microbial transport included ionic strength (Fontes et al., 1991), salinity (Gannnon et al., 1991), and flow velocity (Camper et al., 1993). Enhanced microbial transport in low ionic strength solutions was likely due to less adsorption of bacteria to porous media surfaces. In addition to fluid phase ionic strength, a variety of bacterial characteristics influence adsorption to surfaces. These characteristics included motility (Korber et al., 1994), cell surface hydrophobicity (Rosenberg and Kjelleberg, 1986), and lipopolysaccharide composition (Williams and Fletcher, 1996). Several studies have shown that cell size was an important factor for transport of bacteria in porous media

(Fontes et al., 1991; Gannon et al., 1991; Camper et al., 1993). Thus, more efficient subsurface transport can be achieved through the use of small bacterial cells.

The starvation of bacteria resulted in a complex survival response that included a variety of changes in cell morphology and physiology (Amy and Morita, 1983; Kjelleberg, 1993). One of these changes was a reduction in cell size, often resulting in the formation of miniature cells or ultramicrobacteria (UMB). MacLeod et al. (1988) demonstrated the ability of UMB to more efficiently penetrate porous media than unstarved (vegetative) bacteria of the same species. The injection and resuscitation of UMB in porous media resulted in significant permeability reductions (Lappin-Scott et al., 1988; Cusack et al., 1992). Furthermore, the resuscitation of UMB resulted in more uniform plugging in sandstone cores (Lappin-Scott et al., 1988) and packed-sand columns (Cunningham et al., 1997) than observed for nutrient treatments alone. Overall, these results indicate that UMB are an effective delivery system for bacterial inoculation in porous media systems.

5. Stimulation of Subsurface Microbial Growth

An important factor in engineered microbial plugging of the subsurface is the ability to strategically place the barrier distal to the well bore. Plugging near the well bore can cause injection problems and require close spacing of injection wells for biobarrier formation. In sandstone core tests, a deeper plug was observed when UMB were resuscitated using a minimal medium than a rich medium (Lappin-Scott et al., 1988). More uniform plugging of sandstone cores was also observed under anaerobic than aerobic conditions (Raiders et al., 1986). These results suggest that slower microbial growth results in more uniform biomass distribution and resultant plugging of porous media. The slower growth of bacteria likely results in less nutrient consumption near the core inlet, allowing a more uniform distribution of nutrients and bacteria through the column.

Pulsed feeding of nutrients in sandstone columns also resulted in more uniform plugging than continuous feeding in sandstone cores (Raiders et al., 1986). Alternative pulses of limiting nutrients has been proposed as a strategy for eliminating well bore plugging in MEOR (Jenneman et al.,1993). In this method, required nutrients are injected in order of their predicted transport rates within the formation. Thus, a complete microbial growth medium is obtained distal to the well bore, where the nutrient pulses combine. Similar strategies, using pulses of electron donor and electron acceptor, have been evaluated for the prevention of well bore plugging during nutrient injection for *in situ* bioremediation (Peyton, 1996).

6. Mesocosm Evaluation of Biobarrier Longevity

To evaluate the longevity and maintenance (feeding) requirements of an engineered microbial barrier, a mesocosm study was performed. This study expanded on previous studies evaluating biobarrier formation in mesoscale systems and biobarrier resistance to heavy metals and solvents (Cunningham et al., 1997; James et al., 1995, 1996). These studies have focused on the use of UMB, formed from an isolate of *Klebsiella oxytoca* that copiously produces EPS during growth, and resuscitation with a citrate-based nutrient formulation. The biobarrier evaluated in this study was maintained for over one year with minimal nutrient maintenance requirements after initial formation of the barrier.

The mesocosm evaluation was conducted using a 1.2 × 0.9 × 0.3 m (L × W × H) stainless steel chamber. The design of this reactor has been previously described (Cunningham et al., 1997). Porous medium consisted of F-110 foundry sand to provide a porosity of approximately 35% and an initial hydraulic conductivity of 4.0 to 4.8 cm/min. The pore volume (PV) of the reactor was estimated to be 90 liters. Simulated groundwater flow was provided by a constant head tank to create a hydraulic gradient of approximately 3.8 cm across the length of the reactor. Bacteria and nutrients were injected perpendicular to simulated groundwater flow through an eleven port injection manifold located near the inlet section of the reactor. Bacteria and nutrients were injected at 10% of the total reactor flow rate using a multi-channel peristaltic pump.

Biobarrier formation was initiated by injecting 0.2 PV (total of 2 PVs, inoculum + hydraulic flow) of a starved cell suspension of *Klebsiella oxytoca*. The starved bacterial suspension was prepared as described by MacLeod et al. (1988) and contained 3.3×10^7 colony forming units (CFU) per ml. The starved ultramicrobacteria were diluted approximately 1:6 with nutrient solution ($Na_3C_6H_5O_7$-$2H_2O$ 7.36 g, $NH_4H_2PO_4$ 3.33 g , KH_2PO_4 3.36 g, K_2HPO_4 9.22 g, MgCl 0.21 g, $FeCl_3$ 0.0041 g, H_2O 1 L, pH 7.2) and injected into the reactor. Following UMB inoculation, nutrient solution alone was injected into the reactor until the reactor effluent flow rate equaled the injection flow rate (six days). Additional nutrient injection at 10% of the pre-inoculation effluent flow rate was initiated periodically, as described below.

The influence of biobarrier formation on the flow rate through the mesocosm reactor is shown in Figure 3. Initial inoculation with starved bacteria and injection of nutrients (days 1–6, injected volume 5.4 PV) resulted in a flow reduction of more than 90%. However, the flow rate began to increase approximately two days after cessation of the initial nutrient injection. Additional nutrient injections were performed on day 14 (0.25 PV), day 18 (0.06 PV), day 19 (0.04 PV), day 20 (0.03 PV), and day 21 (0.03 PV). These subsequent nutrient pulses resulted in a decrease in reactor flow rate of more than five orders of magnitude. The flow rate

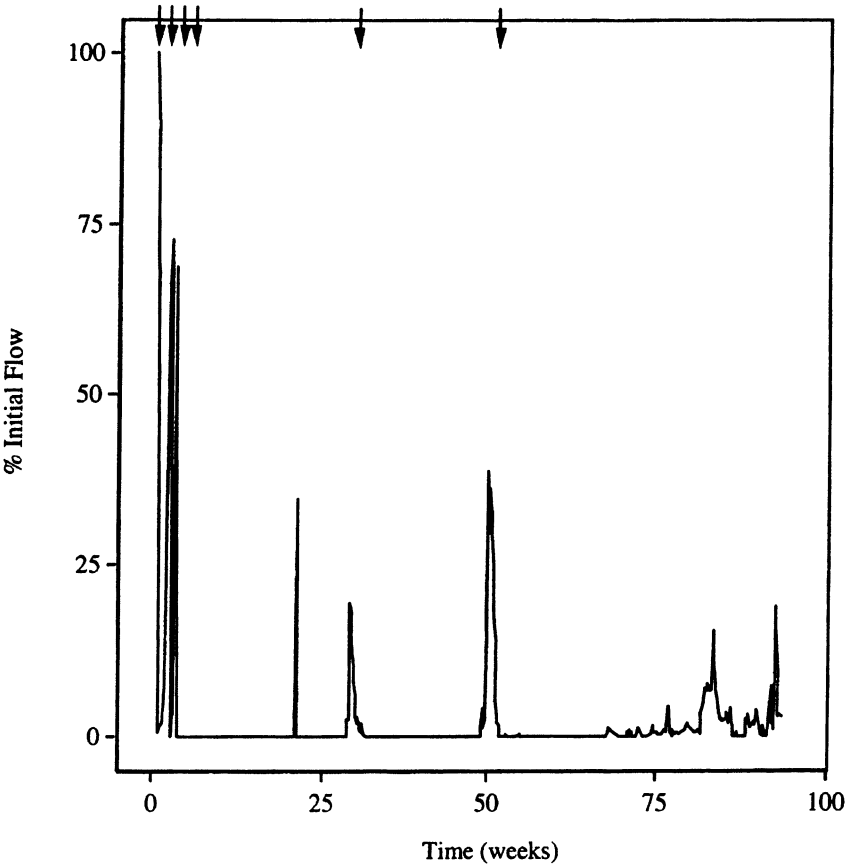

Figure 3. Flow rate (% of initial) vs. time in a groundwater mesocosm showing reduction in flow rate, due to biobarrier formation. The initial flow rate was approximately 300 ml min^{-1}. Biobarrier formation was initiated by inoculation with *Klebsiella oxytoca* UMB (first arrow). The UMB were suspended in a citrate-based nutrient medium immediately prior to injection Subsequent arrows indicate nutrient-only injections, as described in the text. Flow rate was reduced over five orders magnitude, but never completely stopped. The biobarrier was maintained for over 18 months with periodic nutrient additions (arrows).

remained at less than 0.004% of the initial flow rate for approximately 10 weeks then increased and stabilized at less than 0.04% of the initial flow rate for a further 10 weeks. Although flow of simulated groundwater through the porous medium was significantly reduced (as low as 0.0005 ml min^{-1}), flow was never completely blocked. Approximately 30 weeks after inoculation of the column, the flow rate began to increase and another nutrient injection (0.22 PV) was performed. This nutrient injection resulted in a similar reduction in flow rate as observed following

the initial inoculation and nutrient treatment and flow remained at less than 0.04% of the initial flow rate for a further 15 weeks. After approximately 50 weeks the flow rate again began to increase and another nutrient treatment (0.22 PV) was performed. The week 50 nutrient treatment did not result in as dramatic a decrease in flow rate as the previous treatments, although the flow rate was still reduced to less than 1% of the initial flow rate.

The results of the above study demonstrate the ability of microbial biobarriers to significantly reduce hydraulic flow through porous media in a two dimensional (bacteria and nutrients injected perpendicular to simulated groundwater flow) mesoscale test system. The effectiveness of pulse nutrient feeding for establishing an effective microbial barrier observed in this study agrees with previous studies using sandstone cores (Raiders et al., 1986). Loss of injectivity (plugging of the injection ports) due to the use of a complete growth medium was not observed in this study. Thus, sequential pulses of nutrients (Clark and Jenneman, 1992; Peyton, 1996) were not necessary. However, this evaluation was conducted using unconsolidated sand, which may be less prone to injection inlet plugging than sandstone cores. The reduction of flow without complete stoppage observed in this study supports the hypothesis that some flow is required through the biobarrier, resulting in a minimum hydraulic conductivity that can be achieved (Cunningham et al., 1991, 1997; James et al., 1995). In the closed test system used in this evaluation, the biobarrier required nutrient injection every 10–15 weeks to reestablish the reduction in flow rate. This nutrient maintenance requirement is likely due to wash-out of biomass from the porous media under low nutrient conditions. However, in a large-scale porous media system the wash-out rate may be much slower. The rapid washout observed in this experiment likely results from the selective plugging of more permeable zones within the porous media reactor.

7. Biobarrier Reactivity

One of the most encouraging aspects of biobarrier technology is the potential of combining contaminant biodegradation with containment. This dual capability could be accomplished by incorporating bacteria capable of degrading the contaminant within the microbial barrier. Metabolism of the contaminant by biobarrier bacteria could also reduce the maintenance (feeding) requirements for the barrier. A recent report has described a similar approach to remediate acid mine drainage using a semi-permeable reactive wall (Benner et al., 1997). However, this barrier was placed by excavation rather than produced *in situ* by the injection of bacteria and nutrients.

One of the important characteristics of bacteria to be used for biobarrier formation is the production of viscous extracellular polysaccharides (EPS). In a recent study by MSE Technology Applications (G. James, unpublished data), bacteria were isolated from a petroleum contaminated aquifer and screened for EPS production and the ability to metabolize benzene, toluene, ethyl-benzene, and xylene (BTEX) mixtures. Bacterial isolates that were capable of copious EPS production and BTEX metabolism included *Pseudomonas fluorescens* and *Pseudomonas aureofaciens*. Colonization of a porous medium by one of these isolates, *P. fluorescens*, is shown in Figure 2. Biobarrier formation by these isolates is currently being evaluated. Overall, the use of biobarriers to manipulate of groundwater flow and provide *in situ* contaminant degradation promises to provide effective remediation of contaminated groundwater.

8. Conclusion

The use of microbial barriers is a promising approach for the containment and remediation of contaminated groundwater. Research related to microbial enhanced oil recovery has previously demonstrated the feasibility of controlling subsurface fluid flow using biomass. However, this technology must be adapted for groundwater aquifer conditions. A mesoscale evaluation demonstrated the formation of a microbial barrier perpendicular to simulated groundwater flow. This barrier was maintained for over 18 months with periodic nutrient treatments. Overall, these results indicate that biobarriers are a feasible technology for the containment of groundwater contaminant plumes. Furthermore, the incorporation of contaminant-degrading microorganisms within the barrier may enable biodegradation as well as containment of the plume.

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References

Amy, P.S. and Morita, R.Y. (1983) Starvation-survival patterns of sixteen freshly isolated open-ocean bacteria, *Appl. Environ. Microbiol.* **45**, 1109–1115.

Benner, S.G., Blowes, D.W. and Ptacek, C.J. (1997) A full-scale porous reactive wall for prevention of acid mine drainage. *Ground Water Monitoring and Remediation* **17**, 99–108.

Bhupathiraju, V.K., McInerney, M.J. and Knapp, R.M. (1993) Pretest studies for a microbially enhanced oil recovery field pilot in a hypersaline oil reservoir. *Geomicrobiol. J.* **11**, 19–34.

Brown, L.R. and Vadie, A.A. (1997) The utilization of the microflora indigenous to and present in oil-bearing formations to selectively plug the more porous zones thereby increasing oil recovery during waterflooding. Annual Report, US Department of Energy, DOE/BC/14962-16.

Camper, A.K., Hayes, J.T., Sturman, P.J., Jones, W.L. and Cunningham, A.B. (1993) Effects of motility and adsorption rate coefficient on transport of bacteria through saturated porous media, *Appl. Environ. Microbiol.* **59**, 3455–3462.

Clark, J.B. and Jenneman, J.B. (1992) Nutrient injection method for subterranean processes. US Patent No. 5 083 611.

Coates, J.D., Chisholm, J.L., Knapp, R.M., McInerney, M.J., Menzie, D.E. and Bhupathiraju, V.K. (1993) Microbially enhanced oil recovery field pilot, Payne County, Oklahoma, in E. Premuzic and A. Woodhead (eds.), *Microbial Enhancement of Oil Recovery-Recent Advances*, Elsevier, New York, pp. 197–206.

Costerton, J.W., Lewandowski, Z., deBeer, D., Caldwell, D.E. and James, G.A. (1994) Biofilms, the customized microniche, *J. Bacteriol.* **176**, 2137–2142.

Cunningham, A.B., Characklis, W.G., Abedeen, F. and Crawford, D. (1991) Influence of biofilm accumulation on porous media hydrodynamics, *Environ. Sci. Technol.* **25**, 1305–1311.

Cunningham, A., Warwood, B., Sturman, P., Horrigan, K., James, G., Costerton, J.W. and Hiebert, R. (1997) Biofilm Process in porous media – Practical applications, in P.S. Amy and D.L. Haldeman (eds.), *The Microbiology of the Terrestrial Deep Subsurface*, Lewis Publishers, New York pp. 325–344.

Cusack, F., Surindar, S., McCarthy, C., Grieco, J., De Rocco, M., Nguyen, D., Lappin-Scott, H. and Costerton, J.W. (1992) Enhanced oil recovery-three dimensional sandpack simulation of ultramicrobacteria resuscitation in reservoir formation, *J. Gen. Microbiol.* **138**, 647–655.

Fontes, D.E., Mills, A.L., Hornberger, G.M. and Herman J.S. (1991) Physical and chemical factors influencing transport of microorganisms through porous media, *Appl. Environ. Microbiol.* **57**, 2473–2481.

Gannon, J., Tan, Y., Baveye, P. and Alexander, M. (1991) Effect of sodium chloride on transport of bacteria in a saturated aquifer material, *Appl. Environ. Microbiol.* **57**, 2497–2501.

Grassia, G.S., McLean, K.M., Glenat, P., Bauld., J. and Sheehy A.J. (1996) A systematic survey for thermophilic fermentative bacteria and archaea in high temperature petroleum reservoirs, *FEMS Microbiol. Ecol.* **21**, 47–58.

Gupta, R.P. and Swartzendruber, D. (1962) Flow-associated reduction in the hydraulic conductivity of quartz sand, *Soil Sci. Soc. Amer. Proc.* **26**, 6–10.

Harvey, R.W. (1997) In situ and laboratory methods to study subsurface microbial transport, in C.J. Hurst, G.R. Knudsen, M.J. McInerney, L.D. Stetzenbach, M.V. Walter (eds.), *Manual of Environmental Microbiology*, ASM Press, Washington, D.C., pp. 586–599.

Harvey, R.W., Kinner, N.E., MacDonald, D., Metge, D.W. and Bunn, A. (1993) Role of physical heterogeneity in the interpretation of small-scale laboratory and field observation of microorganism, microsphere, and bromide transport through aquifer sediments, *Water Resour. Res.* **29**, 2713–2721.

Jack, T.R. (1993) An overview of microbial enhanced oil recovery, in E. Premuzic and A. Woodhead (eds.), *Microbial Enhancement of Oil Recovery-Recent Advances*, Elsevier, New York, pp. 7–16.

James, G.A., Warwood, B.K., Cunningham, A.B., Sturman P.J., Hiebert, R. and Costerton J.W. (1995) Evaluation of subsurface biobarrier formation and persistence, in *Proceedings of the 10th Annual Conference on Hazardous Waste Research*, Great Plains – Rocky Mountain Hazardous Substance Research Center, Manhatten, KS, pp. 82–91.

James, G.A., Warwood, B.K., Horrigan, K., Hiebert, R., Cunningham, A.E. and Costerton, J.W. (1996) Containment of heavy metal and chlorinated organic solvent contamination using microbial barriers formed in packed-sand columns, in *96th General Meeting of the American Society for Microbiology*, New Orleans, LA, American Society for Microbiology, Washington, D.C.

Jenneman, G.E., Knapp R.M., McInerney, M.J., Menzie, D.E. and Revus, D.E. (1984) Experimental studies of *in situ* microbial enhanced oil recovery, *Soc. Pet. Engrg. J.* **24**, 33–37.

Jenneman, G.E., Clark, J.B. and Moffitt, P.D. (1993) A nutrient control process for microbially enhanced oil recovery applications, in E. Premuzic and A. Woodhead (eds.), *Microbial Enhancement of Oil Recovery-Recent Advances*, Elsevier, New York, pp. 319–333.

Kjelleberg, S. (1993) *Starvation in Bacteria*, Plenum Press, New York.

Korber, D.R., Lawrence, J.R. and Caldwell, D.E. (1994) Effect of motility on surface colonization and reproductive success of *Pseudomonas fluorescens* in dual-dilution continuous culture and batch culture systems, *Appl. Environ. Microbiol.* **60**, 1421–1429.

Lappin-Scott, H.M. and Costerton, J.W. (1992) Ultramicrobacteria and their biotechnological applications, *Curr. Opin. Biotechnol.* **3**, 283–285.

Lappin-Scott, H.M., Cusack, F. and Costerton, J.W. (1988) Nutrient resuscitation and growth of starved cells in sandstone cores: A novel approach to enhanced oil recovery, *Appl. Environ. Microbiol.* **54**, 1373–1382.

Lawrence, J.R., Korber, D.R., Hoyle, B.D., Costerton, J.W. and Caldwell, D.E. (1991) Optical sectioning of microbial biofilms, *J. Bacteriol.* **173**, 6558–6567.

MacLeod, F.A., Lappin-Scott, H.M. and Costerton, J.W. (1988) Plugging of a rock model system by using starved bacteria, *Appl. Environ. Microbiol.* **54**, 1365–1372.

Marquis, R.E. (1983) Barobiology of deep oil formations, in E.C. Donaldson and J.B. Clark (eds.), *Proceedings of 1982 International Conference on Microbial Enhanced Oil Recovery*, US Department of Energy, CONF-8205140, pp. 162–218.

Mitchell, R. and Nevo, Z. (1964) Effect of bacterial polysaccharide accumulation on infiltration of water through sand, *Appl. Microbiol.* **12**, 219–223.

Peyton, B.M. (1996) Improved biomass distribution using pulsed injections of electron donor and acceptor, *Water Res.* **30**, 756–758.

Raiders, R.A., McInerney, M.J., Revus, D.E., Torbati, H.M., Knapp, R.M. and Jenneman, G.E. (1986) Selectivity and depth of microbial plugging in Berea sandstone cores, *J. Ind. Microbiol.* **1**, 195–203.

Rosenberg, M. and Kjelleberg, S. (1986) Hydrophobic interactions: Role in bacterial adhesion, *Adv. Microb. Ecol.* **9**, 353–393.

Shaw, J.C., Bramhill, B., Wardlaw, N.C. and Costerton, J.W. (1985) Bacterial fouling in a model core system, *Appl. Environ. Microbiol.* **49**, 693–701.

Sheenan, J.L. and Vance, I. (1986) Microbial enhanced oil recovery techniques and offshore oil production, in E.C. Hill, J.L. Sheenan and R.J. Watkinson (eds.), *Microbial Problems in the Offshore Oil Industry*, John Wiley and Sons, New York, pp. 78–91.

Stoodley, P., deBeer, D. and Lewandowsky, Z. (1994) Liquid flow in biofilm systems, *Appl. Environ. Microbiol.* **60**, 2711–2716.

Vandevivere, P., Baveye, P., Sanchez de Lozada, D. and DeLeo, P. (1995) Microbial clogging of saturated soils and aquifer materials: Evaluation of mathematical models, *Water Resour. Res.* **31**, 2173–2180.

Van Heiningen, J., DeHaan, H.J. and Jansen, J.D. (1958) Process for the recovery of petroleum from rocks, Patent 89-580, The Netherlands.

Williams, V. and Fletcher, M. (1996) *Pseudomonas fluorescens* adhesion and transport through porous media are affected by lipopolysaccharide composition, *Appl. Environ. Microbiol.* **62**, 100–104.