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Evaluation of biodegradation potential of foam embedded *Burkholderia cepacia* G4

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Foam embedded *Burkholderia cepacia* G4 removed up to 80 % and 60 % of a 3 mg/l solution of trichloroethylene (TCE) and a 2 mg/l solution of benzene, respectively. Removal of TCE and benzene decreased more than 50% when readily metabolizable carbon sources were present. TCE degradative activity was observed with G4 cells induced with phenol or benzene prior or after immobilization of cells.

Introduction

Burkholderia cepacia G4 has recently received much attention due to its demonstrated ability to degrade pollutants like aromatic hydrocarbons and chlorinated solvents (Folsom and Chapman, 1991; Shields and Reagin, 1992; Luu *et al*., 1995). The use of this organism in bioreactors has been limited in part by its lack of competitive ability when compared to other toluene degradative bacteria (Massol-Deya *et al*., 1997), seemingly related to its poor adhesion capabilities. One alternative to biofilm bioreactors is to use immobilized bacteria to treat wastewater effluents. The optimization of techniques for embedding *B. cepacia* in hydrophilic polyurethane foam in such a manner that cells are effectively entrapped and retain metabolic activity has been previously reported (Santo Domingo *et al*., 1997a, b). In this study, we examined the ability of immobilized *B. cepacia* to degrade TCE and benzene. The objective of this study was to evaluate the potential of polyurethane embedded bacteria for the remediation of contaminated groundwaters.

Materials and methods

Bacterial growth conditions and foam preparation

Axenic batch cultures of *Burkholderia cepacia* G4 were grown in a yeast-glucose medium (YGM), which consisted of basal salts medium (BSM; Shields *et al*., 1989) plus 1 g glucose/l and 0.5 g yeast extract/l. Batch cultures were grown in 250 ml shaker flasks (200 rpm, 30°C) or in 4 l polycarbonate bottles as previously described (Santo Domingo *et al*, 1997a). Cultures were harvested for foam embedding by centrifugation (11,000 x *g*, 10 min, 15°C). Bacterial slurries were prepared by resuspending pellets in BSM medium to a density of 2.2 – 5.5% dry wt. Bacteria were routinely embedded in hydrophilic polyurethane foam within 2 h after slurry preparation.

In experiments involving the induction of toluene monooxygenase subsequent to slurry preparation, slurries were stored overnight at 4°C prior to induction and embedding. Ingredients of the foam were: bacterial slurry, 20 g; polyurethane prepolymer, 13.33 g; and lecithin-based surfactant HS-3, 0.54 g. A 5% (dry wt) slurry yielded approximately 3 g dry wt bacteria per 100 g wet wt foam. A 1% solution of the lecithin-based compound HS-3 (Amisol) was used as the surfactant, and Bipol 6B (Matrix, Inc., Dover, NH, USA) was used as the polyurethane prepolymer (Santo Domingo *et al*., 1997a). Controls consisted of cell-free foams which were generated by substituting 20 g BSM medium for bacterial slurry. Foam samples were reduced to a particulate state by means of a Waring blender, stored at 4°C, and used for experiments within 2-4 h unless otherwise stated.

Induction of enzyme activity

Toluene monooxygenase activity in G4 was induced by adding phenol (2 mM) or benzene (2 mM) to cultures 2 h before the commencement of harvesting. In some experiments, induction was performed prior to cell immobilization by adding phenol to the bacterial slurry for 2 h, after which the cells were centrifuged, resuspended in BSM, and embedded in foam. In attempts to induce enzyme activity in pre-embedded G4 cells, 0.1 g quantities of G4/foam aggregate were placed in 22 ml borosilicate glass vials to which 10 ml BSM and phenol (2 mM) or benzene (2 mM) were added. Vials were sealed and shaken (200 rpm) for 2, 4, or 21 h. After induction, excess liquid was removed and replaced with 10 ml fresh BSM. For comparison purposes,

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Table 1 Removal of TCE and benzene by embedded and unembedded G4[a]

Inoculum type	*Media used*	*% TCE removed*	*% benzene removed*
Foam embedded G4	BSM	98.5 (1.3)[b]	91.3 (5.6)
Foam embedded G4	YGM	37.6 (41.1)	52.3 (37.5)
G4 bacterial slurry	BSM	95.1 (4.8)	98.1 (0.5)
G4 bacterial slurry	YGM	39.2 (38.9)	45.2 (18.8)

[a]Bacterial slurries and foams were explosed to TCE (3.1 mg/l) or benzene (2 mg/l) in BSM or YGM medium. Induction was performed prior to slurry preparation.
[b]Numbers in parenthesis represent standard deviation of the mean.

G4 slurry equivalent to the embedded biomass was placed in flasks containing BSM medium and phenol or benzene. Bottles were sealed and shaken in the same manner as foam samples, after which cells were pelleted (9300 x *g*, 15 min) and the supernatant decanted. Pellets were resuspended in 50 ml of BSM and 10 ml of each suspension was placed in quadruplicate vials for use in TCE degradation assays. Controls consisted of uninduced embedded G4 and uninduced free cells. Statistical analyses were performed using Excel (Microsoft Corp., Redmond, WA, USA) or JMP (SAS Institute, Cary, NC, USA).

Degradation assays

Assays for trichloroethylene (TCE) biodegradative capability were typically conducted using quadruplicate glass vials containing 10 ml BSM and 0.1 g foam/bacterial aggregate or the equivalent biomass (0.059 ml) of bacterial slurry. TCE (3 mg/l) was added as a 0.01% methanol solution (v/v), after which vials were immediately sealed with crimp caps and Teflon-coated septa, incubated 3 – 5 days at 30°C, and subjected to headspace analysis by gas chromatography. Results were interpreted with the aid of a standard curve generated from TCE-inoculated BSM samples, incubated under the same conditions as the experimental samples. Benzene (2 mg/l) was also added using methanol as the carrier solution. TCE and benzene were measured by gas chromatography as previously described (Santo Domingo *et al*., 1997a). Detection limits for TCE and benzene were 1.0 and 20.0 µg/l, respectively. TCE degradation was also studied using TCE contaminated groundwater samples collected from Savannah River Site monitoring wells after purging a minimum of 3 well volumes, and turbidity, pH, and conductivity readings had stabilized (Westrick *et al*., 1984). Samples were collected in 22 ml glass vials which were immediately sealed with Teflon-coated septa and crimp caps. Only vials containing no air bubbles or bubbles of less than approximately 2 mm diameter were used to provide water for experiments (U.S. EPA 1986). Samples were used within 6 h after collection.

Results and discussion

BSM-suspended cells removed up to 97.5% of TCE, with approximately 16% removal due adsorption to the foam material (Table 1). Embedded and unembedded G4 removed statistically similar amounts of TCE ($P < 0.05$). In addition, immobilized cells degraded 90% of the benzene (2 mg/l) in BSM media (Table 1), although 29% of benzene loss was attributed to adsorption to the foam. In contrast, the equivalent amount of unembedded cells achieved 97% degradation of benzene. The addition of carbon sources (1 g glucose/l, 0.5 g yeast extract /l) significantly decreased TCE and benzene removal ($P < 0.01$). Similar results were seen in the presence of 0.1 g glucose/l and 0.05% yeast extract (data not shown). Thus, it appears that organic compounds in YGM were used as competing carbon sources, effectively inhibiting TCE cometabolism as well as benzene utilization.

The requirement for induction of toluene monooxygenase activity in G4 means that its utility for groundwater treatment will depend in part on the duration of enzyme activity, once induced. To explore the useful lifetime of induced and embedded cells, we tested foam and slurry samples 3 h and 72 h after preparation (Table 2). Lower TCE degradation activity was observed with these foam

Table 2 Duration of TCE degradative activity following enzyme induction.

	% TCE (3 mg/l) removed[a]	
Inoculum type[b]	*3 h after induction*	*72 h after induction*
Foam embedded G4 (5.1%)	68.3 (7.5)[c]	32.7 (12.9)
Foam embedded G4 (2.5%)	31.5 (6.7)	8.2 (3.9)
G4 bacterial slurry (5.1%)	75.3 (1.9)	34.3 (18.7)
G4 bacterial slurry (2.5%)	68.2 (5.9)	14.4 (2.1)

[a]Quadruplicate samples were explosed to 3 (mg/l) TCE in BSM shortly (3 h) after embedding and after 3 days' storage at 4°C.
[b]Foams were prepared using 2.5% and 5.1% (dry wt) slurries; induction was performed prior to slurry preparation.
[c]Numbers in parentesis represent standard deviation of the mean.

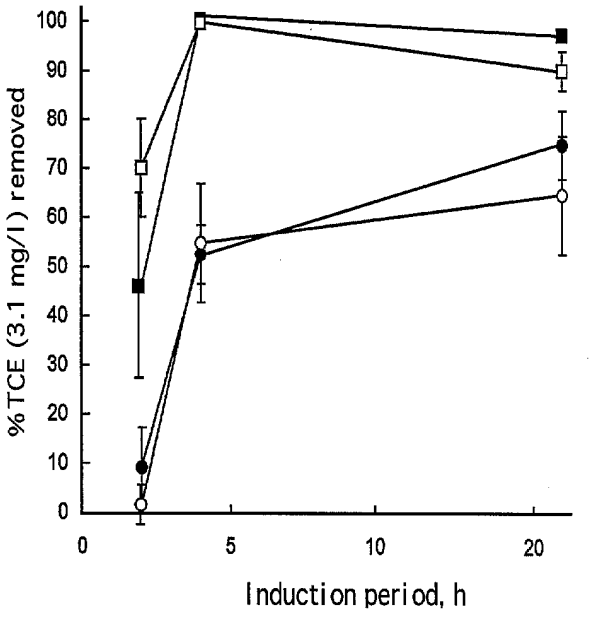

Figure 1 Induction of previously embedded G4. Embedded G4 cells and slurry samples (5.2% dry wt) were induced with phenol or benzene (2 mM) for 2, 4, or 21 h prior to TCE exposure. Legend: closed circle, phenol-induced foam-G4 aggregate; opened circle, benzene-induced foam-G4 aggregate; closed square, phenol-induced slurry; opened square, benzene-induced slurry.

preparations, perhaps due to variations in foam composition in the batch treatments. More importantly, TCE degradation dropped significantly ($P < 0.01$) in both 72 h old foams and slurries compared to 3 h old foams. Enzyme activity in previously embedded cells, however, could be induced in foam/G4 aggregates by exposing the cells to 2 mM phenol or 2 mM benzene (Figure 1). A 2 h induction (such as was used in previous experiments) had some effect on slurry preparations, but had no noticeable effect on foam embedded G4. In contrast, most TCE degradation was achieved after a 4 h induction, although activity of phenol-induced and benzene-induced foam preparations was significantly less than bacterial slurries even after a 21 h induction. It is not known whether longer induction periods would have further increased TCE removal.

The ability of embedded G4 to degrade TCE in contaminated groundwaters was evaluated using samples from aquifers containing different concentrations of TCE (i.e., 1.09 to 2.71 mg/l) of TCE (Figure 2). Both slurry and foam achieved significant removal of TCE ($P < 0.05$). Slurries removed 88 to 100% of TCE from all groundwater samples. However, foam embedded G4 yielded variable results, ranging from 12% to nearly 80% removal. These

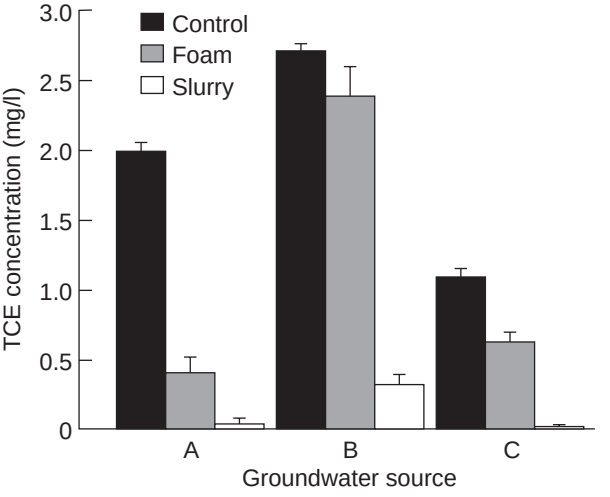

Figure 2 TCE removal from groundwater. Slurry samples (5.2% dry wt) and embedded G4 cells were exposed groundwaters containing 1.99 mg/l (A) , 2.71 mg/l (B), and 1.09 mg/l (C) of TCE respectively. Induction was performed prior to slurry preparation. Control consisted on unammended groundwater.

differences were not correlated with the inital TCE concentration in the samples.

The present study demonstrated that polyurethane-embedded G4 cells can degrade TCE and benzene. These results are relevant since previous studies have indicated that polyurethane immobilization causes bacteria to become nonculturable (Santo Domingo *et al*., 1997a). In addition, we showed that TCE cometabolism could be induced by phenol amendments even after G4 cells were embedded in polyurethane. Although G4 was responsible for most of the observed removal, TCE and benzene were found to bind to the foam material. It is not at present known whether compounds absorbed in this manner are still available for bacterial degradation.

Additional considerations will be of importance in designing a groundwater treatment process using embedded G4 cells. For example, the relatively short duration of toluene monooxygenase activity, once induced, presents an obstacle to the practical use of immobilized G4 in bioreactors. An alternative approach to this shortcoming would be to use microbial strains that constitutively express oxygenase activity (Shields and Reagin, 1992). Nevertheless, the option of inducing the enzyme in previously embedded cells raises the possibility that foam/bacterial aggregates could be prepared and stored for later use, with induction being performed at the bioremediation site (perhaps during several consecutive induction/TCE degradation cycles).

Process design will also be affected by the fact that TCE degradation is inhibited by the presence of readily metabolizable carbon sources. This is particularly a problem if such carbon sources do not induce oxygenases that can cometabolically transform TCE. In theory, this might not be a significant problem in the ex situ treatment of TCE-contaminated deep subsurface waters due to their usually oligotrophic nature. However, variable results obtained with groundwater samples suggest that groundwater composition strongly affects degradative activity of embedded G4 in an as yet unclear manner. Although the present study demonstrates that the potential exists for the development of G4/hydrophilic foam-based processes, future work must address the nature of this variablity for such processes to be commercially viable.

Acknowledgments

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