Biotechnol Lett (2006) 28:2027–2031
DOI 10.1007/s10529-006-9189-1

ORIGINAL RESEARCH PAPER

# Ex situ bioremediation of phenol contaminated soil using polymer beads

**George P. Prpich · Rachel L. Adams · Andrew J. Daugulis**

Received: 16 June 2006 / Revised: 3 August 2006 / Accepted: 18 August 2006 / Published online: 29 September 2006


**Abstract** Polymer beads have been used to absorb high concentrations of phenol from soil decreasing the initial concentration of 2.3 g $kg^{-1}$ soil to 100 mg $kg^{-1}$ soil and achieving a phenol loading within the polymer beads of 27.5 mg phenol $g^{-1}$beads. The phenol-loaded polymer beads were removed from the soil and placed in a bioreactor, which was then inoculated with a phenol-degrading microbial consortium. All of the phenol contained within the polymer beads was shown to desorb from the polymer matrix and was degraded by the microbial consortium. The beads were used again (twice) in a similar manner with no loss in performance.

**Keywords** Phenol contamination · Polymer absorption · Soil bioremediation · Two-phase bioreactor

## Introduction

Ex situ technologies for the bioremediation of soil contaminated with toxic organic compounds include land farming, composting and bioreactors. Operation of these bioremediation strategies involves the excavation and or agitation of soil, which often leads to enhanced abiotic loss of contaminants. For a technology such as land farming, in which soil is tilled on a regular basis, volatilization has been shown to be responsible for the bulk of removal of an oily sludge with a 76% loss of total hydrocarbon contaminants (Hejazi and Husain 2004a). Although volatilization of contaminants, and their escape into the atmosphere, is often viewed as a feasible manner for soil remediation, the contaminants still persist in the environment. Research has shown that volatilization of hydrocarbons from land farms poses a risk to both humans and the environment (Hejazi et al. 2003), therefore, it is necessary to improve on current soil remediation technologies in order to reduce the possible risks.

Physical factors such as temperature, moisture content, organic fraction as well as molecular weight of contaminants affect abiotic losses (Hejazi and Husain 2004b, Trably and Patureau 2006). Of these factors the organic fraction of soil tends to be the most important causing the sorption of contaminants and a reduction in volatilization losses. Research has shown that volatilization may reduce the effectiveness of a bioremediation process, therefore, it was hypothesized that by retaining the contaminants (or minimizing volatilization) by way of increasing organic content, a greater percentage of the target

G. P. Prpich · R. L. Adams · A. J. Daugulis (✉)
Department of Chemical Engineering, Queen's University, Kingston, Ontario K7L 3N6 Canada
e-mail: daugulis@chee.queensu.ca

contaminants would be destroyed (Hickey and Paek 1996).

A variety of sorbent materials have been provided to soils in order to enhance the ability of the soil to retain volatile toxic compounds as well as to dilute possibly inhibitory levels of contaminants. Sewage sludge or compost has been shown to decrease the volatilization of low molecular weight alkanes from soils (Namkoong et al. 2002), as have wood chips, peat and activated carbon (Rhykerd et al. 1998). Synthetic materials, such as XAD-2 ion exchange resins, readily adsorb organic contaminants from soil (Guerin 1999, Lei et al. 2004) although, in a manner similar to activated carbons, the presence of these materials inhibits the biodegradation process by reducing the bioavailability of the target contaminant (Guerin 1999). Polymers are another sorbent material that have been shown to be effective for the removal of pesticides, PAHs, volatile organic compounds and phenols from an aqueous phase (Amsden et al. 2003, Patterson et al. 2002, Streat and Sweetland 1998, Prpich and Daugulis 2006), but to date, there has been little research pertaining to their ability to remove contaminants from soil. In this paper we propose the use of solid polymer beads for the removal of a semi-volatile model contaminant (phenol) from soil and its subsequent destruction in a bioreactor via a microbial consortium isolated for its ability to degrade phenol.

## Materials and methods

### Chemicals and polymers

All chemicals were reagent grade and were purchased from Fisher Scientific (Canada). Hytrel polymer beads were received as a gift from DuPont Canada. Hytrel 8206 is a polyether-ester block copolymer thermoplastic elastomer consisting of a 50:50 blend of poly(butylene terephthalate):poly ether glycol. It has a melting temperature of 189°C, a glass transition temperature of –59°C, and the beads are rice-shaped, 5 mm length × 1.5 mm diam., with a density of 1.17 g $cm^{-3}$.

### Organisms, media and cultivation

A microbial consortium was utilized to degrade phenol in the bioreactor studies. It has been described previously and was used based on its demonstrated ability for enhanced rates of phenol biodegradation (Prpich and Daugulis 2005). The consortium was isolated by selective enrichment (on phenol as sole carbon source) from an activated sludge sample taken from a pulp and paper waste water treatment facility and consisted of four major isolates identified (using API) as *Pseudomonas putida, Pseudomonas alcaligenes, Acinetobacter baumannii* and *Acinetobacter johnsonii.* Culture media, growth conditions and inoculum preparation have also been described previously (Prpich and Daugulis 2005).

### Soil

Uncontaminated soil was collected onsite from Queen's University, Kingston, Ontario. The soil was air dried for 48 h and then passed through a 2 mm sieve. The soil was classified as sandy-loam (99.09% sand and 0.91% clay) with a pH of 7.9 in distilled, deionized water and moisture content of 35%. Loss of ignition test was used to calculate the organic content of the soil, which was found to be 0.91%.

### Analysis of phenol in soil

Soil samples were analyzed for the presence of phenol by contacting 1 g of soil with 1 ml of deionized water in a 15 ml test tube. The contents of the test tube were vortexed for 1 min and then centrifuged for 15 min at 2370 g, a procedure which has previously been confirmed by us to provide equilibrium conditions. One ml of supernatant was removed and analyzed for the presence of phenol using the 4-aminoantipyrine colorimetric test (Yang and Humphrey 1975). Samples were analyzed at 510 nm and the results were correlated to a previously developed calibration curve. Soil samples were analyzed three times and an average value was calculated for each data point.

### Contaminating soil with phenol

From a stock solution of phenol at 40 g $l^{-1}$, 50 ml lots were used to contaminate 0.8 kg soil to give an initial loading of 2.5 g phenol $kg^{-1}$ soil. The contamination procedure, conducted in a fume hood, involved spreading the soil out in a large tray, spraying the phenol solution evenly over the soil using a spray bottle, mixing the soil to achieve homogeneity and then transferring the soil to a 2 l Erlenmeyer flask. The flask was sealed with a rubber stopper, mixed once more and then soil samples were taken to determine initial phenol concentration.

### Absorption of phenol from soil by polymer beads

To 0.8 kg of phenol-contaminated soil in a 2 l Erlenmeyer flask, 80 g as-received polymer beads were added. The flasks were sealed with rubber stoppers, mixed thoroughly and maintained at 30°C. The soil was mixed manually twice a day and samples were periodically taken to determined phenol concentrations. Abiotic and biotic controls were set up to determine the fate of phenol within the system. The abiotic control involved sterilization of 0.8 kg soil for 2 h at 121°C and 1.4 bar. The sterilization process was repeated three times over 48 h (Scott et al. 1982). The sterilized soil was then contaminated with phenol as described above. The biotic control consisted of 0.8 kg of non-sterilized soil contaminated with phenol. The abiotic control was used to determine any physical/chemical loss of phenol while the biotic control was used to determine possible phenol loss due to the presence of naturally occurring microorganisms. Mixing and sampling of phenol were undertaken in both cases as described above.

### Assessment of phenol loading in polymer beads

Desorption tests were performed on phenol-loaded beads taken from the soil as well as from the bioreactor upon completion of phenol biodegradation experiments. A known mass of beads was transferred from the soil or bioreactor to a sealed 125 ml Erlenmeyer shake-flask containing 50 ml of distilled, deionized water and agitated at 180 rpm for 24 h at 30°C. The aqueous phase was then tested to determine phenol content and the mass of phenol remaining in the beads was calculated based on a previously constructed partition coefficient for phenol between polymer and aqueous phase (Prpich and Daugulis 2004).

### Biodegradation of phenol in 5 l bioreactor

After 24 h of contact, the polymer beads were separated from the soil using a 2.36 mm sieve and then added to a 5 l bioreactor (New Brunswick Scientific, USA) along with the phenol-degrading microbial consortium. The aqueous working volume of 3 l was maintained at a pH 6.7, 30°C, agitation of 400 rpm and aeration of 1 l $min^{-1}$. Aqueous samples were periodically drawn to determine the concentration of phenol and biomass. After completion of biodegradation the culture medium was strained (through a 2.36 mm sieve) in order to separate the polymer beads. The beads, which were recovered quantitatively from the soil and the fermentation fluid by the sieve size used, were prepared for re-use (twice) by air-drying, and the culture medium was returned to the bioreactor for the next biodegradation cycle.

## Results and discussion

### Absorption of phenol from soil by polymer beads

The results of the phenol absorption experiment, as well as the abiotic and biotic controls are shown in Fig. 1. It can be seen that in the presence of the polymer beads the phenol concentration in the soil decreased rapidly, within 24 h, from an initial concentration of 2.3 g phenol $kg^{-1}$ soil to below 100 mg phenol $kg^{-1}$ soil. The reduction in phenol concentration was attributed to phenol absorption into the polymer beads as the two control experiments suggest that physical, chemical and biological degradation were not responsible for the dramatic decrease in phenol concentration. Absorption of phenol into the

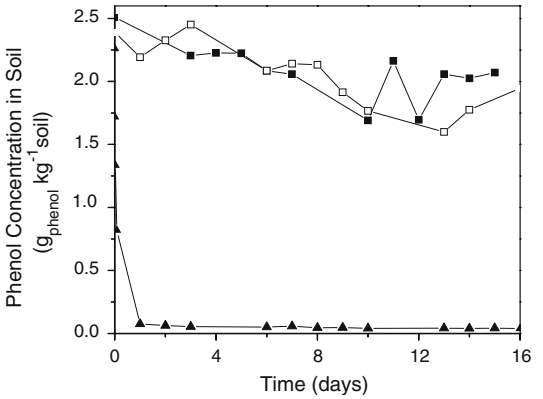


**Fig. 1** Absorption of phenol by polymer beads from soil. The solid squares represent the abiotic control, hollow squares represent the biotic control and the triangles represent the phenol remaining in soil after addition of polymer beads

polymer beads was confirmed by a desorption test of phenol-loaded beads in fresh water. Based on a partition coefficient for phenol between aqueous and polymeric phases an overall mass balance of the system accounted for all of the phenol.

The concentration of phenol in the polymer beads was calculated to be 27.5 mg phenol $g^{-1}$ polymer beads, which is similar to the phenol loading obtained previously in absorbing phenol from an aqueous solution (Prpich and Daugulis 2004).

## Biodegradation of phenol in a 5 l bioreactor

The removal of phenol from soil and absorption into the polymer beads for the three trials is shown in Table 1. For each trial the polymer beads effectively reduced phenol levels to below 100 mg $kg^{-1}$ soil.

When the polymer beads from the initial loading were placed in a bioreactor and inoculated with the mixed population of phenol degrading microbes the cells experienced a lag phase as phenol was desorbed from the beads into the aqueous phase (Fig. 2). The phenol concentration in the aqueous phase reached a level of 365 mg $l^{-1}$ ($t$ = 6 h) at which time the cells entered into exponential growth. The biodegradation of phenol was rapid and by $t$ = 10 the phenol concentrations in the aqueous phase were below detectable limits. To determine whether any phenol remained within the polymer beads a known mass of beads was taken from the bioreactor and contacted with fresh water for 24 h. No phenol was detected in the aqueous phase and coupled with reports on the release of phenols from Hytrel (Prpich and Daugulis 2004, 2006) it was determined that all the phenol had been released.

The two remaining trials were performed in a similar fashion to the initial trial with the used polymer beads absorbing high levels of phenol from the soil (Table 1) and then desorbing the phenol in the aqueous environment of the bioreactor prior to biodegradation (Fig. 2). The slight difference in phenol degradation profiles between the three fermentations is likely due to cell viability. The second fermentation occurred 24 h after the completion of the first and it is apparent that the cells maintained high viability over this period of time, as no lag phase was observed. The biodegradation of phenol commenced immediately upon addition of the polymer beads, resulting in a lower maximum phenol concentration in the bioreactor. The third trial experienced a comparatively lengthy lag phase of 5 h arising from an extended downtime between trial 2 and 3. During the 72 h of inactivity the cells were not provided with a carbon source and therefore, upon introduction of phenol loaded polymer beads, the cells required a period of adjustment before resuming biodegradation. This resumption of phenol degradation after 3 days without substrate demonstrates the robust nature of the microbial consortium in withstanding periods of starvation.

**Table 1** Results of the three phenol biodegradation cycles

| Trial | Initial concentration of phenol in soil $mg_{phenol}$ $kg^{-1}$ $_{soil}$ | Final concentration of phenol in soil $mg_{phenol}$ $kg^{-1}$ $_{soil}$ | Mass of phenol in beads $mg_{phenol}$ | Biomass Yield $g_{biomass}$ $g^{-1}$ $_{phenol}$ |
|---|---|---|---|---|
| 1 | 2720 | 70 | 2286 | 0.98 |
| 2 | 2457 | 74 | 2484 | 1.27 |
| 3 | 2848 | 78 | 2797 | 1.21 |

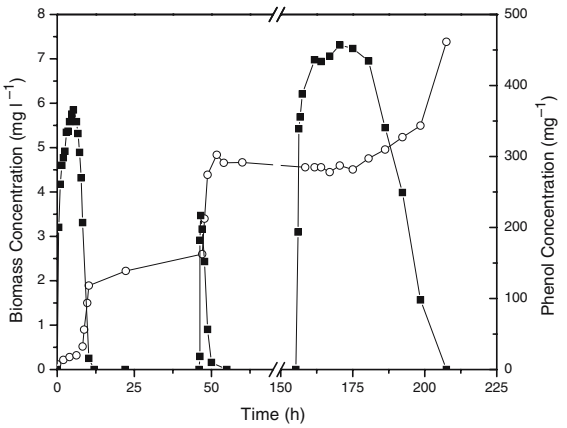


**Fig. 2** Biodegradation of phenol desorbed from polymer beads. Polymer beads were used to reduce phenol concentrations in the soil. The loaded beads were then introduced into a bioreactor, which was inoculated, and phenol was subsequently biodegraded via a microbial consortium. Solid squares represent phenol concentration and hollow shapes represent biomass concentration

The metabolic performance of the three trials, described in terms of biomass yield, are comparable and calculated values are given in Table 1. These cell yield values are similar to those obtained previously for this consortium growing on phenol (Prpich and Daugulis 2005).

## Conclusion

Polymer beads have been demonstrated to readily absorb high levels of phenol from soil reducing the concentration to levels that may result in activity of indigenous organisms. The loaded polymer beads, used in conjunction with a solid-liquid partitioning bioreactor, were shown to be an effective method for the ex situ bioremediation of phenol contaminated soil.

**Acknowledgements** Financial support from the Natural Sciences and Engineering Research Council of Canada is gratefully acknowledged.

## References

Amsden BG, Bochanysz J, Daugulis AJ (2003) Degradation of xenobiotics in a partitioning bioreactor in which the partitioning phase is a polymer. Biotechnol Bioeng 84:399–405

Guerin TF (1999) Bioremediation of phenols and polycyclic aromatic hydrocarbons in creosote contaminated soil using ex-situ land treatment. J Hazard Mater 65:305–315

Hejazi RF, Husain T (2004a) Landfarm performance under arid conditions. 1. Conceptual framework. Environ Sci Technol 38:2449–2456

Hejazi RF, Husain T (2004b) Landfarm performance under arid conditions. 2. Evaluation of parameters. Environ Sci Technol 38:2457–2469

Hejazi RF, Husain T, Khan FI (2003) Landfarming operation of oily sludge in and region–human health risk assessment. J Hazard Mater 99:287–302

Hickey WJ, Paek J (1996) Ventilation effects on mineralization and volatilization of naphthalene in a gasoline-contaminated subsurface soil. Chemosphere 32:1655–1667

Lei L, Suidan MT, Khodadoust AP, Tabak HH (2004) Assessing the bioavailability of PAHs in field-contaminated sediment using XAD-2 assisted desorption. Environ Sci Technol 38:1786–1793

Namkoong W, Hwang EY, Park JS, Choi JY (2002) Bioremediation of diesel-contaminated soil with composting. Environ Pollut 119:23–31

Patterson BM, Davis GB, Mckinley AJ (2002) Laboratory column experiments using polymer mats to remove selected VOCs, PAHs, and pesticides from ground water. Ground Water Monit R 22:99–106

Prpich G, Daugulis AJ (2006) Biodegradation of a phenolic mixture in a solid-liquid two-phase partitioning bioreactor. Appl Microbiol Biot DOI: 10.1007/s00253-006-0311-z

Prpich G, Daugulis AJ (2005) Enhanced biodegradation of phenol by a microbial consortium in a solid liquid two phase partitioning bioreactor. Biodegradation 16:329–339

Prpich GP, Daugulis AJ (2004) Polymer development for enhanced delivery of phenol in a solid-liquid Two-Phase Partitioning Bioreactor. Biotech Prog 20:1725–1732

Rhykerd RL, Sen D, McInnes KJ, Weaver RW (1998) Volatilization of crude oil from soil amended with bulking agents. Soil Sci 163:87–92

Scott HD, Wolf DC, Lavy TL (1982) Apparent adsorption and microbial degradation of phenol by soil. J Environ Qual 11:107–112

Streat M, Sweetland LA (1998) Removal of pesticides from water using hypercrosslinked polymer phases: Part 2 - Sorption studies. Process Saf Environ 76:127–134

Trably E, Patureau D (2006) Successful treatment of low PAH-contaminated sewage sludge in aerobic bioreactors. Environ Sci Pollut R 13:170–176

Yang R, Humphrey A (1975) Dynamics and steady state studies of phenol degradation in pure and mixed cultures. Biotechnol Bioeng 17:1211–1235