INTRODUCTION

Processes influencing the fate of contaminants in the subsurface include biodegradation, sorption, and transport. Our overall goal was to use an integrated biological and chemical strategy to affect these fate-controlling processes and thereby enhance the remediation of mixed wastes. This meant enhancing the biodegradation of the less recalcitrant organic waste components, and enhancing the desorption and transport of the nonbiodegradable components to facilitate their removal by "pump and treat".

Our approach to biodegradation enhancement was unique in that we considered bioavailability, rather than genetic potential, to be the critical limiting factor controlling the biodegradation rates of many organic contaminants. Any hazardous waste or spill site may contain soil organisms with the appropriate gene sequences that code for degradation. However, degradation of contaminants may be affected by factors other than gene sequence limitations. One major constraint is the aqueous solubility of organic chemicals, which is critical for uptake of the potential substrate by microorganisms. Enhancing the uptake of slightly soluble organics requires an alteration of the environment surrounding the organic compound to allow its accommodation in an aqueous environment. Surfactants are examples of molecules which have the ability to surround nonpolar solutes and disperse or solubilize them in aqueous solution. Of particular interest, because they are natural products, are surfactants produced by microorganisms (biosurfactants). In this research project, we hypothesized that use of biosurfactants would increase the solubility, and hence, the cellular uptake of many contaminants with limited water solubility. In addition, we hypothesized that biosurfactants could be used to enhance the remediation of highly sorbed recalcitrant contaminants, including metals, by modifying their solution-phase activity so that desorption and, thus, transport was enhanced. This would allow their removal by flushing via induced hydraulic gradients (i.e., pump and treat).
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PROPOSAL OBJECTIVE

To determine the influence of biosurfactants on contaminant sorption\desorption and the resultant impact on biodegradation and transport of mixed wastes.

RESULTS ACHIEVED

Summary

The overall results of this project suggest that in situ treatment with biosurfactants has the potential to be an effective, economical, and nontoxic remediation technology. Specifically, we have demonstrated that a rhamnolipid biosurfactant may be used to increase the apparent solubility and the biodegradation rate of organic compounds (see Appendix 1 and 4). We have demonstrated a structure-function relationship between various rhamnolipid biosurfactants and have shown that enhanced biodegradation occurs in the presence of concentrations as low as 10 mg/L biosurfactant (Appendix 9). We have examined rhamnolipid aggregate types and sizes formed in the presence of organics and metals (Appendix 6). We have demonstrated that biosurfactants may also be used to enhance the desorption and transport of highly sorptive contaminants (e.g., recalcitrant organics; metals) and, thus, to facilitate the flushing of contaminated subsurface environments by pumping. For example, rhamnolipid is a very effective surfactant for use in removal of residual hydrocarbon from sand (Appendix 11). We have demonstrated that a second biosurfactant, cyclodextrin can increase in the solubility of various organic compounds and result in enhanced desorption and transport (Appendix 2, 3) of organics and of organic-metal mixtures (Appendix 7). The effect of a cosolvent, cyclopentanol, on solubilization of cyclodextrin was further examined (Appendix 8). In addition, two scholarly reviews have been written concerning the use of biosurfactants in remediation (Appendix 5, 10). Thus, the results from this project add information to the literature on the effects of biosurfactants on i) biodegradation enhancement, ii) adsorption\desorption behavior of organic and inorganic compounds, and iii) transport of organic compounds.

Specific Results

The specific results of this grant are 11 peer-reviewed publications which are summarized below and are attached as appendices. In addition there have been 3 proceedings published and 18 abstracts presented at national meetings resulting from this work.

One unique aspect of this project is that the collaboration between environmental microbiology and chemistry led to a wide audience for this work. Publications have or will appear in: Applied and Environmental Microbiology, Environmental Science and Technology, Journal of Contaminant Hydrology, Journal of the American Chemical Society, Journal of Colloid and Interface Science, and Soil Science Society of America. Meetings which have been attended include: American Chemical Society, American Society for Microbiology, Soil Science Society of America, Third International Symposium on In Situ and On-Site Bioreclamation, and National Ground Water Association.
Peer-reviewed publications to date


**Abstract**
The use of a microbial surfactant (biosurfactant) was investigated for its potential to enhance bioavailability, and hence, the biodegradation of octadecane. The rhamnolipid biosurfactant used in this study was extracted from culture supernatants after growth of *Pseudomonas aeruginosa* ATCC 9027 in phosphate-limited proteose peptone/glucose/ammonium salts medium (PPGAS). Dispersion of octadecane in aqueous solutions was dramatically enhanced by rhamnolipid, increasing by a factor of more than 4 orders of magnitude, from 0.006 mg/l to over 250 mg/l, in the presence of 300 mg/l biosurfactant. The relative enhancement of octadecane dispersion was much greater at low rhamnolipid concentrations than at high concentrations. Rhamnolipid-enhanced octadecane dispersion was found to be dependent on pH and shaking speed. Biodegradation experiments using an initial octadecane concentration of 1500 mg/l, showed that 20% of the octadecane was mineralized in 84 hours in the presence of 300 mg/l rhamnolipid, compared to only 5% of the octadecane with no surfactant present. These results indicate that rhamnolipids may have potential for facilitating bioremediation of sites contaminated with hydrocarbons of limited water solubility.

SEE APPENDIX I FOR FULL REPRINT


**Abstract**
Hydroxypropyl-β-cyclodextrin (HPCD), a microbially produced compound, was investigated for its potential to increase the apparent aqueous solubilities of low-polarity organic compounds. The results show that the apparent solubilities of trichloroethene, chlorobenzene, naphthalene, anthracene, and P,P'-DDT were significantly increased in HPCD solutions. The relative aqueous-phase concentrations ($S/S_o$) of the compounds increased linearly with increasing HPCD concentration. The solubilization power of HPCD is dependent on the size and relative polarity of its cavity and, unlike surfactants, HPCD has no critical micelle concentration. The partition mechanism was shown to be a valid approach for interpreting the solubilization activity of HPCD. The potential application of HPCD in the remediation of contaminated groundwater is briefly discussed.

SEE APPENDIX II FOR FULL REPRINT

Abstract
The removal of low-polarity organic compounds from soils and aquifers by water flushing is often constrained by sorption. There is great interest in developing systems that can enhance the transport of organic compounds through porous media, thus facilitating remediation. We investigated the potential of hydroxypropyl-β-cyclodextrin (HPCD), a microbially produced compound, to reduce the sorption and enhance the transport of several low-polarity organic compounds. The results show that cyclodextrin does not interact with the three soils tested. As a result, there is no retardation of cyclodextrin during transport. The retardation of compounds such as trichlorobiphenyl, phenanthrene, and anthracene was significantly (orders of magnitude) reduced in the presence of cyclodextrin.

SEE APPENDIX III FOR FULL REPRINT


Abstract
Previous work has shown that a rhamnolipid biosurfactant produced by Pseudomonas aeruginosa ATCC 9027 increased the aqueous dispersion and biodegradation of octadecane (C₁₈) in a concentration dependent manner. This study further investigated the effect of rhamnolipid concentrations on the uptake and biodegradation of octadecane. It was found that at rhamnolipid concentrations above the critical micelle concentration (CMC), biodegradation was enhanced relative to controls, but below the CMC, biodegradation was initially inhibited relative to controls. To explore this inhibition at low rhamnolipid concentrations, changes in cell hydrophobicity were monitored during cell growth using the BATH (bacterial adherence to hexadecane) method. Results showed that cells grown on octadecane alone had a relative BATH = 12% in comparison to a BATH = 80% in the presence of rhamnolipid after 4 days of growth. The rate at which the cells became hydrophobic was found to depend on rhamnolipid concentration and was directly related to the rate of octadecane biodegradation. These results suggest that the bioavailability of octadecane in the presence of rhamnolipid is controlled by both aqueous dispersion of octadecane and cell hydrophobicity.

SEE APPENDIX IV FOR FULL REPRINT

Abstract
Rising remediation costs have increased interest in the development and application of innovative remediation technologies. Specifically, development of new bioremediation technologies is attractive because of the potential for both successful and economical in situ treatment of contaminated sites. A common constraint to efficient bioremediation of contaminated sites is the low aqueous solubility of many organic contaminants. These include a variety of fuel-related compounds (e.g., aliphatics, aromatics and heterocyclics), and higher molecular weight chlorinated organic compounds. Limited contaminant solubility has several consequences for remediation. For slightly soluble biodegradable contaminants both degradation rates and levels are reduced. For slightly soluble recalcitrant contaminants, removal by flushing is retarded because of extensive sorption by soil. Development of efficient bioremediation technologies will require that contaminant availability issues be addressed. To accomplish this, the mechanisms underlying microbial solubilization and biodegradation of slightly soluble organic contaminants must first be understood.

SEE APPENDIX V FOR FULL REPRINT


Abstract
A rhamnolipid biosurfactant produced by *Pseudomonas aeruginosa* ATCC 9027 is reported to increase the aqueous dispersion and biodegradation of petroleum hydrocarbons and to complex heavy metals. These reports indicate the potential for application of rhamnolipids in remediation of contaminated sites. Effective use of rhamnolipids will require understanding of rhamnolipid morphology and the effects of pH and organic and inorganic contaminants on that morphology. We used cryo-transmission electron microscopy to investigate the morphology of vitrified, frozen hydrated suspensions of rhamnolipid over a pH range of 5.5 to 8.0, and to determine the effect of a model alkane, octadecane, and a model heavy metal, cadmium, on rhamnolipid morphology. Micrographs clearly showed that rhamnolipid morphology was a function of pH, changing from lamellar, to vesicular, to micellar as pH increased. The effect of cadmium and octadecane on rhamnolipid morphology was determined at pH 6.8 and 7.0, where maximum cadmium complexation and maximum octadecane dispersion occurs. Cadmium seemed to stabilize rhamnolipid vesicle structures as shown by an increase in vesicle number and a decrease in vesicle diameter. In contrast, octadecane favored the micellar structure as shown by the complete absence of vesicles.

SEE APPENDIX VI FOR FULL REPRINT
Abstract

The cleanup of contaminated soil and groundwater at hazardous waste sites has become a major focus of research and policy debate. A major factor complicating the cleanup of many sites is the co-occurrence of organic compounds and heavy metals the so-called mixed wastes. We investigated the ability of a modified cyclodextrin to simultaneously complex low-polarity organic compounds and heavy metals. The results of the experiments showed that carboxymethyl-beta-cyclodextrin could simultaneously increase the apparent aqueous solubilities of the selected organic compounds (anthracene, trichlorobenzene, biphenyl, and DDT) and complex with Cd^{2+}. This complexation was not significantly affected by changes in pH or by the presence of relatively high concentrations of Ca^{2+}. It is possible that this reagent can be used successfully to remediate hazardous waste sites contaminated by mixed wastes.

SEE APPENDIX VII FOR FULL REPRINT

Abstract

The effect of cyclopentanol on the solubilization of six polycyclic aromatic hydrocarbons (PAHs) by β-cyclodextrin (β-CD) and γ-cyclodextrin (γ-CD) is reported in this paper. The addition of 0.1% v/v cyclopentanol significantly enhances the solubilization power of β-CD for pyrene, acenaphthene, phenanthrene and fluoranthene which form a 1:2 complexes with β-CD. However, the solubilization of acenaphthene and phenanthrene by β-CD in the presence of cyclopentanol decreases at higher β-CD concentrations following an initial increase. This phenomenon is attributed to the cyclopentanol-induced formation of insoluble β-CD aggregates. In contrast, cyclopentanol produces no pronounced effect and a marked decrease in the solubilization power of β-CD for naphthalene and anthracene, respectively. These two compounds form 1:1 complexes with β-CD. The solubilization of all six PAHs by γ-CD is significantly enhanced by 1% v/v cyclopentanol. This result indicates that the addition of cyclopentanol increases the "hydrophobicity" of the γ-CD cavity and increases the solubilization power of γ-CD without inducing structure-dependent selectivity. On the basis of linearity and nonlinearity observed in the solubilization curves of the six PAHs in the presence of cyclopentanol, it is suggested that naphthalene, anthracene, acenaphthene and phenanthrene form 1:1 complexes with γ-CD, while fluoranthene and pyrene form both 1:1 and 2:2 complexes.

SEE APPENDIX VIII FOR FULL REPRINT

Abstract
A study was conducted to quantify the effect of rhamnolipid biosurfactant structure on degradation of alkanes by a variety of Pseudomonas isolates. Two dirhamnolipids were studied, a methyl ester (dR-Me) and an acid form (dR-A). These rhamnolipids have different properties with respect to interfacial tension, solubility, and charge. For example, the interfacial tension between hexadecane and water was decreased to < 0.1 dyn/cm by the dR-Me but was only decreased to 5 dyn/cm by the dR-A. Solubilization and biodegradation of two alkanes in different physical states, liquid and solid, was determined at dirhamnolipid concentrations ranging from 0.01 to 0.1 mM (7 to 70 mg l⁻¹). The dR-Me markedly enhanced hexadecane (liquid) and octadecane (solid) degradation by eight different Pseudomonas strains except for one strain which exhibited extremely high cell surface hydrophobicity, the growth of which was inhibited on octadecane. The dR-A also enhanced hexadecane degradation by all degraders but more modestly than the dR-Me. For octadecane, the dR-A only enhanced degradation by strains with low cell surface hydrophobicity.

SEE APPENDIX IX FOR FULL REPRINT


Abstract
The use of water flushing (pump and treat, in situ soil washing) is one of the predominant methods currently in use for remediation of contaminated subsurface environments. While this method has been successful in some cases, its effectiveness is often constrained by one or more factors related to contaminant transport and fate. Recent research has focused on chemical additives that might be useful for enhancing contaminant removal during flushing. Examples include the addition of surfactants, cosolvents, and complexing agents. We are involved in the study of cosolvents and microbially produced biodegradation, and removal of residual phases from the subsurface. In this paper, we summarize our recent results and provide a comparison of the advantages and disadvantages in the use of biosurfactants and cosolvents in remediation of contaminated subsurface environments.

SEE APPENDIX X FOR FULL REPRINT

Abstract

An anionic monorhamnolipid biosurfactant produced by Pseudomonas aeruginosa was investigated for its potential to remove residual hexadecane from sand columns. In a series of column experiments, residual hexadecane saturation was established by pumping 14C-hexadecane into water-saturated sand columns and then flushing with water at a velocity of 25 cm hr⁻¹. Monorhamnolipid solutions of varying concentration were then applied to the columns at a velocity of 15 cm hr⁻¹ to remove the residual hexadecane. Of the rhamnolipid concentrations tested, which ranged from 40 to 1500 mg L⁻¹, the optimal concentration for residual removal was 500 mg L⁻¹, approximately 10 x critical micelle concentration. Approximately 84% of the residual was removed from the column packed with 20/30 mesh sand and 22% was removed from the 40/50 mesh column. The primary mechanism for residual removal was mobilization (displacement and dispersion), while solubilization was found to be insignificant. The performance of monorhamnolipid was compared with that of two synthetic surfactant solutions on a mass basis (500 mg L⁻¹) for the 40/50 mesh sand. Sodium dodecyl sulfate (0.2 x cmc), and polyoxyethylene (20) sorbitan monooleate (38 x cmc), removed 0% and 6.1% of the residual saturation, respectively.

SEE APPENDIX XI FOR FULL REPRINT

Proceedings


Abstracts presented


1995 Miller, R.M. Potential in situ applications of biosurfactants in the remediation of contaminated sites. 209th National Meeting of the American Chemical Society, Anaheim, CA, April 2-7.


