Biodegradation **8:** 15–19, 1997.

Indirect bioremediation: biodegradation of hydrocarbons on a commercial sorbent

William W. Mohn
Department of Microbiology & Immunology and Pulp & Paper Centre, University of British Columbia, Vancouver, BC, Canada V6T 1Z3

Accepted 14 January 1997

Key words: biodegradation, crude oil, hexadecane, phenanthrene, sorbent

Abstract

Urea-formaldehyde polymer is currently used as a sorbent for containment and clean up of hydrocarbons. The aerobic biodegradability of this polymer and hydrocarbons sorbed to the polymer were tested. Soil microorganisms readily grew on the polymer, and two organisms, a bacterium and a fungus, capable of growth on the polymer were isolated. However, biodegradation of the polymer was very slow and possibly incomplete. Biodegradation of the polymer was evident as a change in appearance of the polymer, but disappearance of the polymer was not detectable in liquid cultures incubated for six months or soil cultures incubated for one month. Destruction of the polymer by soil microorganisms at ambient temperature does not appear to be practical. Degradation of ^{14}C-labeled hexadecane and phenanthrene mixed with crude oil in liquid cultures inoculated with soil microorganisms was used as an estimate of general hydrocarbon degradation. When nitrogen was not limiting, the rates of hexadecane and phenanthrene degradation were the same, whether those hydrocarbons were sorbed to the polymer or not sorbed. When nitrogen was limiting, the polymer stimulated the rate of hexadecane degradation but not the rate of phenanthrene degradation. The polymer may stimulate hexadecane degradation by serving as a source of nitrogen. However, optimal degradation of sorbed hydrocarbons requires nitrogen addition. The results suggest that it may be feasible to decontaminate spent polymer by biodegradation of sorbed hydrocarbons.

Introduction

Hydrocarbon spills are a major international environmental concern. Sorbents are frequently useful for containing and cleaning up hydrocarbon spills (Mouche 1995). Rapid containment by a sorbent limits dispersal of hydrocarbon spills in aqueous environments and consequent environmental damage. Sorbents can also be used to stop movement of contaminants through soil. For clean up of hydrocarbons, sorbents are convenient and effective. After use, such sorbents are hazardous waste and require proper disposal. Biodegradation of the spent sorbent and associated hydrocarbons would be an ideal method of disposal as it could avoid the costs and risks of conventional hazardous waste disposal. If both sorbent and hydrocarbon contaminant could be mineralized, microbial biomass would be the only residue. Alternatively, if only the hydrocarbon contaminant could be mineralized, the residue would be sorbent and biomass which would be nonhazardous. This residue might even be reused, if its sorptive capacity was adequate. In this way, the sorbent might be biologically regenerated.

Sorption of hydrocarbons would likely affect their biodegradation via effects on their 'bioavailability.' Bioavailability is a general term describing numerous factors capable of limiting biodegradation which are poorly understood in complex environments. These factors have been well summarized by Alexander (Alexander 1994). These factors include dissolved concentration, surface area of a hydrocarbon and emulsification.

In this study, I examined the ability of soil microorganisms to aerobically degrade a commercial sorbent, a urea-formaldehyde polymer, as well as two hydrocarbons sorbed to the polymer. The hydrocarbons used

were hexadecane and phenanthrene which differ in chemical structure and relative recalcitrance. The ability of the polymer to serve as a nitrogen source to hydrocarbon-degrading organisms was also examined. The polymer was relatively recalcitrant, but the hydrocarbons were as readily degraded when sorbed or not sorbed. This demonstrates the potential for decontamination of spent hydrocarbon sorbents.

Materials and methods

Culture conditions

The mineral medium for all cultures contained (per liter) 2 g NaCl, 1.1 g K_2HPO_4, 0.53 g KH_2PO_4, 0.10 g Na_2SO_4, 30 mg $mgCl_2 \bullet 6H_2O$, 3.0 mg $CaCl_2 \bullet 2H_2O$ and 10 mg $FeSO_4$ as well as trace elements (Shelton and Tiedje 1984) and vitamins (Mohn 1995). The $MgCl_2$, $CaCl_2$and trace elements were combined and autoclaved separately. The $FeSO_4$ and vitamins were added to autoclaved medium from filter-sterilized stock solutions. A commercial hydrocarbon sorbent, a urea-formaldehyde polymer, was provided by Landmark Resources (Vancouver). Nonsterile polymer was added to autoclaved medium (1.0 g/liter). The polymer was not autoclaved, as it is not heat-stable. This material was in the form of very loosely associated white crystals which floated in medium. The polymer was a potential carbon and nitrogen source. Solid media were prepared by adding 1.5 g/liter agar with the polymer in a 5-ml agar overlay. Primary enrichment cultures were inoculated with 1 g/liter moist, organic-rich garden soil from the Vancouver area. Subsequent enrichment cultures and pure cultures received 0.1% inocula. All cultures were incubated at 30°C, and all liquid cultures were shaken. Microbial growth was monitored by microscopy, as the insoluble polymer interfered with protein quantification and other measures of biomass.

Analytical methods

Production of $^{14}CO_2$ was quantified in 125-ml Erlenmeyer flasks sealed with rubber stoppers. The stoppers were equipped with glass and rubber tubing to allow flushing the liquid medium, with inflow through the liquid medium and exhaust exiting from the headspace. Periodically the cultures were flushed with humidified air (approximately 200 ml/min. for 10 min.), and CO_2 in the exhaust was trapped by passage through a train of three scintillation vials each containing a mixture of 1 ml ethanolamine, 0.5 ml ethylene glycol monomethyl ether and 6 ml Beckman Ready-Solv scintillation cocktail. The samples were counted with a Beckman LS 6000IC scintillation counter. Total organic carbon was quantified with an automatic analyzer, Shimadzu TOC-500. Gravimetric determinations were made after drying polymer and soil to constant weight at 40°C, the low temperature in order to prevent thermal decomposition of the polymer.

Degradation of polymer

All treatments were in triplicate. Changes in dry weight were assayed by comparing 5.0-ml cultures dried after inoculation to ones dried after incubation for 14 days. Changes in total organic carbon were assayed by comparing 2.0-ml cultures, prepared in the analysis tubes, analyzed after inoculation to ones analyzed after incubation for 14 days. Soil cultures were prepared with 10.00 g moist soil in petri plates. For killed controls, the soil was autoclaved (20 min. at 121°C) on three consecutive days. To indicated treatments, 1.000 g of dry polymer was mixed with the soil. The petri plates were sealed with plastic film and incubated at 30°C. The weight of the plates was monitored and was maintained by addition of sterile water. After 28 d, the cultures were dried and weighed.

Degradation of hydrocarbons sorbed to polymer

Cultures contained 50 ml medium with or without 0.31 g/liter NH_4Cl as a nitrogen source. Crude oil was provided by Landmark Resources and was labeled by mixing with either ^{14}C-1-hexadecane (2.2 Ci/mol, >98% pure, Sigma) or ^{14}C-9-phenanthrene 13.3 Ci/mol, >98% pure, Sigma). The labeled compounds were dissolved in toluene prior to addition to crude oil. One gram of labeled crude oil was added per liter of medium, resulting in 50-ml cultures with either 2.6 x 10^6 dpm ^{14}C-1-hexadecane or 3.3 x 10^5 dpm ^{14}C-9-phenanthrene. The hydrocarbon sorbent was added to cultures (1 g/liter) after the crude oil, and the crude oil rapidly sorbed to this polymer, leaving no free oil visible. These cultures were inoculated with 1 g/liter moist soil.

Results and discussion

Recalcitrance of the polymer

Microbial growth occurred readily on medium with the hydrocarbon sorbent as the sole nitrogen and organic carbon source. Five different soil samples were used to inoculate separate enrichment cultures with the hydrocarbon sorbent as the sole organic substrate. An additional enrichment culture had no inoculum. After two weeks incubation, microbial growth occurred in all cultures, including the uninoculated control. Presumably, the nonsterile sorbent contained organisms capable of using it as a substrate. The enrichment cultures were serially transferred three times with growth always evident within one week. Microbial cell numbers typically increased at least 100-fold, from much less than one cell per microscope field-of-view to greater than 20. Two tertiary cultures were plated on homologous solid medium, yielding two isolates. The isolates did not cause clearing of polymer in the agar overlay. The first isolate was a small rod, presumably a bacterium and the second, was a large filament, presumably a fungus. The isolates were not characterized. Both isolates were maintained through several passages on homologous liquid medium, indicating use of the polymer as a growth substrate.

Despite the ability of microorganisms to grow on the sorbent polymer, only partial degradation of the polymer was visible in enrichment cultures and in cultures of the filamentous isolate, even after extensive incubation. During incubation, the polymer changed in color from white to yellow to brown, and it dispersed into increasingly fine particles. These changes did not occur in uninoculated control cultures incubated for the same period. However, most of the polymer was still visible after incubations of up to six months. Growth of either isolate in liquid medium for 14 days was not accompanied by significant decreases in dry weight or total organic carbon. Thus, any mineralization of the polymer by the isolates was below detectable levels. Growth might be supported by only a small fraction of the polymer, perhaps a fraction of relatively low molecular weight. This scenario is feasible, since one percent of the polymer would constitute 10 mg/liter, probably enough to support growth of a microbial population of the density observed.

The presence of sorbent polymer stimulated a decrease in dry weight of soil cultures (Table 1). The greater dry weight loss in cultures with polymer suggests degradation of the polymer but also may have been due to degradation of soil organic matter stimulated by the polymer (e.g., the polymer may have provided nitrogen which stimulated degradation of organic matter). Autoclaving the soil did not effect weight loss in the cultures. Thus, an abiological cause for the weight loss cannot be excluded. However, it is probable that the nonsterile polymer introduced microorganisms into the treatments with autoclaved soil, just as it appeared to do in the above uninoculated enrichment cultures. These results are consistent with slow or partial biodegradation of the polymer, but they do not allow estimation of rate or extent of polymer degradation.

Table 1. Loss of dry weight (g) in soil cultures with urea-formaldehyde polymer

Treatment	Dry weight (mean±SD)[a]	Weight loss[b]
Soil before incubation	6.22±0.01	-
Soil after incubation	6.10±0.03	0.12^{A}
Autoclaved soil + 1.000 g polymer after incubation	7.02±0.04	0.20^{B}
Soil + 1.000 g polymer after incubation	7.03±0.05	0.19^{B}

[a] n = 3
[b] Means followed by different uppercase letters are significantly different at the 0.1 probability level

Degradation of hydrocarbons sorbed to the polymer

Hexadecane

In the absence of added ammonium, hexadecane degradation in soil-inoculated cultures was very limited, with only 5% conversion of labeled hexadecane to CO_2 (Figure 1). Addition of the sorbent polymer initially reduced the rate of hexadecane degradation but allowed relatively slow, sustained degradation to occur over a period of 200 days, resulting in 13% conversion. The initial inhibitory effect of polymer could be due to reduced bioavailability of hexadecane caused by its sorption to the polymer. The overall stimulatory effect of the polymer could be due to use of the polymer as a nitrogen source. Addition of ammonium greatly increased the rate of hexadecane degradation and also increased hexadecane conversion to 17%. This effect indicates that nitrogen was a limiting factor in the above cultures and supports use of the polymer as a nitrogen source as the explanation for the polymer's stimulatory effect. Addition of polymer to cultures with ammonium caused no measurable change in hexadecane degra-

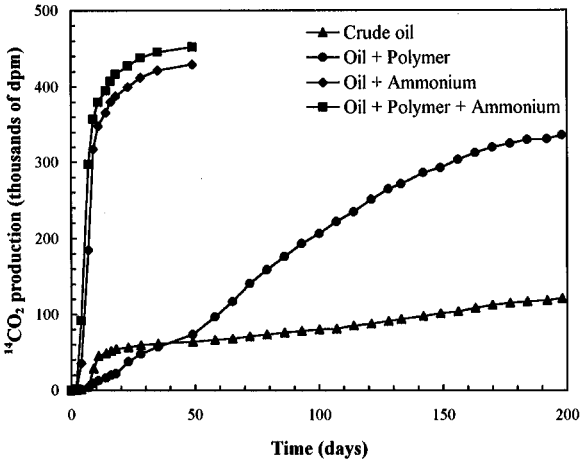

Figure 1. Effects of sorbent polymer and added nitrogen on degradation of ^{14}C-hexadecane mixed with crude oil in liquid cultures with soil inocula (n = 3).

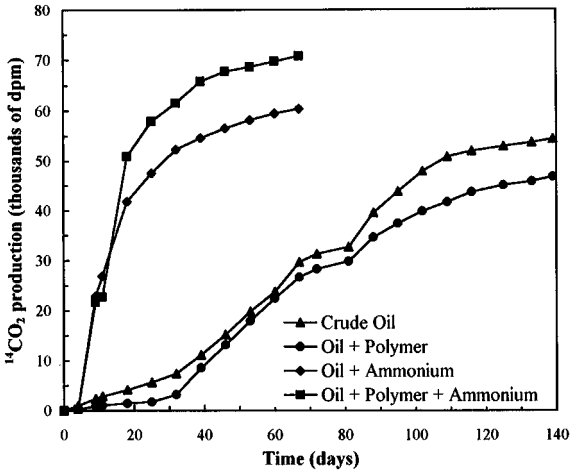

Figure 2. Effects of sorbent polymer and added nitrogen on degradation of ^{14}C-phenanthrene mixed with crude oil in liquid cultures with soil inocula (n = 3).

dation. The means for total hexadecane conversion in cultures with ammonium and with or without polymer were within one standard deviation. Since the polymer had no inhibitory effect on hexadecane degradation in the presence of added ammonium, it did not noticeably reduce bioavailability of hexadecane under that condition. A possible explanation for the different effects in cultures with and without ammonium is that abundant nitrogen allowed growth of a population better able to aquire sorbed hexadecane via bioemulsification or some other process.

Phenanthrene

Like the case of hexadecane, phenanthrene degradation in the absence of added ammonium was limited to a relatively low rate and low total conversion of labeled phenanthrene to CO_2 (Figure 2). Unlike the case of hexadecane, addition of the sorbent polymer did not measurably reduce the initial rate of hexadecane degradation nor stimulate the final amount of conversion of phenanthrene. Total phenanthrene conversions in cultures without ammonium and with or without polymer were approximately 15%, and those means were within one standard deviation. The experiments with hexadecane and those with phenanthrene were not run simultaneously, so the inocula may account for the different effects of added polymer. The inoculum for the latter experiment may have included significantly more nitrogen or more nitrogen-fixing organisms. Hexadecane degraders in the former experiment may have been less able to compete for limited nitrogen than phenanthrene degraders in the latter experiment. Like the case of hexadecane, addition of ammonium greatly increased the rate of phenanthrene biodegradation and also increased phenanthrene conversion to 18%. Thus, despite substantial phenanthrene degradation in the absence of added ammonium, nitrogen was a limiting factor in those cultures, as it was for the hexadecane cultures. Addition of polymer to cultures with ammonium caused no measurable change in phenanthrene biodegradation. The means for total phenanthrene conversion in cultures with ammonium and with or without polymer were within one standard deviation.

Because of the instability of the polymer, sterile control cultures were not used in the above experiments. However, the observed results (e.g., degradation kinetics and nitrogen limitation) strongly suggest that the primary mechanisms of hexadecane and phenanthrene degradation were biological. The total conversions of hydrocarbons to CO_2 were less than would be expected for complete mineralization of the hydrocarbons. However, since the compounds were not uniformly labeled, definite conclusions about their complete mineralization cannot be made. Complete mineralization of these hydrocarbons may not be possible in this system.

The neutral effect of the polymer on hydrocarbon biodegradation was not necessarily expected, as there are numerous ways which sorption might affect hydrocarbon biodegradation. Biodegradation of some hydrocarbons, particularly lighter ones, appears to be

limited by dissolution (Wodzinski & Bertolini 1972; Wodzinski & Coyle 1974; Thomas et al. 1986; Stucki & Alexander 1987) which could be affected by a sorbent. Biodegradation of other hydrocarbons, particularly heavier ones, appears to be limited by available surface area (Wang & Ochoa 1972; Nakahara et al. 1977; Fogel et al. 1985) which could be affected by a sorbent. Emulsification of hydrocarbons by biosurfactants from certain organisms is thought to facilitate their biodegradation (Singer & Finnerty 1984), and this process could be affected by a sorbent. Sorption of hydrocarbons to soil was found to have variable effects on their biodegradation (Subba-Rao & Alexander 1982; Mihelcic & Luthy 1988; Guerin & Boyd 1992; Tsomides et al. 1995).

Conclusions

The urea-formaldehyde polymer tested is relatively recalcitrant and cannot be easily destroyed by aerobic biodegradation. Hydrocarbons sorbed to the polymer appear to be as biodegradable as ones which are not sorbed. Thus, it may be possible to clean up a hydrocarbon spill with the polymer and then to destroy the hydrocarbons by biodegradation. This is essentially an indirect form of bioremediation. This process may sufficiently decontaminate the polymer for it to be treated as nonhazardous waste. It remains to demonstrate the degree of decontamination which is possible with different hydrocarbon mixtures.

Acknowledgment

I thank Joanna Zheng and Jacqueline Chung for technical assistance. This work was supported by Landmark Resources, Ltd.

References

Alexander M (1994) Biodegradation and Bioremediation. Academic Press, San Diego

Fogel S, Lancione R, Sewall A & Boethling RS (1985) Application of biodegradability screening tests to insoluble chemicals: hexadecane. Chemosphere 14: 375–382

Guerin WF & Boyd SA (1992) Differential bioavailability of soil-sorbed naphthalene to two bacterial species. Appl. Environ. Microbiol. 58: 1142–1152

Mihelcic JR & Luthy RG (1988) Degradation of polycyclic aromatic hydrocarbon compounds under various redox conditions in soil-water systems. Appl. Environ. Microbiol. 54: 1182–1187

Mohn WW (1995) Bacteria obtained from a sequencing batch reactor that are capable of growth on dehydroabietic acid. Appl. Environ. Microbiol. 61: 2145–2150

Mouche C (1995) Managing used oil. Pollution Engineering 27: 40–43

Nakahara T, Erickson LE & Gutierrez JR (1977) Characteristics of hydrocarbon uptake in cultures with two liquid phases. Biotechnol. Bioeng. 19: 9–25

Shelton DR & Tiedje JM (1984) General method for determining anaerobic biodegradation potential. Appl. Environ. Microbiol. 47: 850–857

Singer ME & Finnerty WR (1984) Microbial metabolism of straight-chain and branched alkanes. In: Atlas RM (Ed) Petroleum Microbiology (pp 1–60). Macmillan Publishing Co., New York

Stucki G & Alexander M (1987) Role of dissolution rate and solubility in biodegradation of aromatic compounds. Appl. Environ. Microbiol. 53: 292–297

Subba-Rao RV & Alexander M (1982) Effect of sorption on mineralization of low concentrations of aromatic compounds in lake water samples. Appl. Environ. Microbiol. 44: 659–668

Thomas JM, Yordy JR, Amador JA & Alexander M (1986) Rates of dissolution and biodegradation of water-insoluble organic compounds. Appl. Environ. Microbiol. 52: 290–296

Tsomides HJ, Hughes JB, Thomas JM & Ward CH (1995) Effect of surfactant addition on phenanthrene biodegradation in sediments. Environ. Toxicol. Chem. 14: 953–959

Wang DIC & Ochoa A (1972) Measurements on the interfacial areas of hydrocarbon in yeast fermentations and relationships of specific growth rates. Biotechnol. Bioeng. 14: 345–360

Wodzinski RS & Bertolini D (1972) Physical state in which naphthalene and biphenyl are utilized by bacteria. Appl. Microbiol. 23: 1077–1081

Wodzinski RS & Coyle JE (1974) Physical state of phenanthrene for utilization by bacteria. Appl. Microbiol. 27: 1081–1084