ENHANCED BIODEGRADATION OF ORGANIC WOOD-PRESERVATIVE CONTAMINATED WASTEWATER BY COMMERCIAL SURFACTANTS

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INTRODUCTION

At creosote and pentachlorophenol (PCP) wood treatment sites, a large volume of contaminated process wastewater is produced from several different sources, including steaming of wood, vapor drying or oil seasoning, vacuum condensate, steam and oil leaks around the system, and rainwater runoff. This process water contains high concentrations of PCP, polycyclic aromatic hydrocarbons (PAHs) from the creosote, including sixteen which have been listed by the EPA as priority pollutants, carrier oil, and soluble high molecular weight aromatics extracted from the wood.

Bioremediation, where microorganisms detoxify or degrade wastes, is well established as a less expensive technology that can meet clean-up standards for many contaminants (Thayer 1991) including PCP and creosote (Stroo et al. 1989; Cerniglia 1992). This technology, however, can be inefficient for oily wastes containing high concentrations of PCP or high molecular weight PAHs. These recalcitrant compounds often remain in the oil phase, not the aqueous phase, thus are not accessible to microorganisms for breakdown (Mueller et al. 1989). In addition, many of these compounds are co-metabolized and not completely mineralized due to their low aqueous solubility and strong sorption properties. Toxicity may not be reduced because of the accumulation of by-products resulting from this co-metabolic process (Mueller et al. 1989; Cerniglia 1992).

The use of surfactants in biodegradation processes represents a possible means of stimulating the microbial breakdown of environmental pollutants such as slightly soluble high molecular weight chemicals. Surfactants are amphipathic molecules consisting of a hydrophilic/polar head and a hydrophobic/non-polar tail (Rouse et al. 1994). In bioremediation of soil-water systems, surfactants have been found to either enhance or inhibit biodegradation, depending upon the surfactant concentration. When surfactants were below their critical micelle concentration (CMC), the water surface tension was reduced and the solubility of the weakly soluble organic contaminants was enhanced. When the surfactant concentrations were above their CMCs, enhanced solubility of such contaminants was due to partitioning into the surfactant micelles. In this last situation, the organic chemicals may not be available to the microorganisms for degradation to occur (Jahan and Maier 1992).

The broad objective of this study was to evaluate different commercial surfactants for enhancing the biodegradation of wood-preserving process wastewater containing a high concentration of PCP, PAHs and oil and grease. Specific objectives were: (1) screening selected anionic and non-ionic surfactants for interference on PCP and PAHs, analyses by gas chromatography (GC); (2) testing for the toxicity of the surfactant itself to degrading microorganisms; and (3) testing different concentrations of selected surfactants to determine the best concentration for degradation as well as the least inhibition toward microbial growth.

METHODS AND MATERIALS

Screening Surfactants for Interference of Contaminant Analyses by Gas Chromatography

Nonionic surfactants (Span 20, Span 80, Brij 35, Tergitol, Triton X-100, Tween 20, and Tween 80) and anionic surfactants (caprylic acid and lauryl sulfate) were screened for analytical interference at a concentration ten-fold above their CMC. Samples consisted of only surfactant in water (no PAHs or PCP were present). Samples were extracted by liquid-liquid extraction with methylene chloride (EPA Method 3520; U.S. EPA 1992), concentrated to 10 ml by Kuderna-Danish apparatus, eluted (3 ml) through a silica gel clean-up column with pentane: methylene chloride (40:60), and analyzed by GC (EPA Method 8040 and 8100; U.S. EPA 1992) for interference or overlap at peaks of selected PAHs and PCP. PAHs analyzed throughout this study included Naphthalene, Acenaphthene, Fluorene, Phenanthrene, Anthracene, Fluoranthene, Pyrene, Chrysene, and Benzo(a)pyrene.

Testing for Surfactant Toxicity to Microorganisms

Three concentrations of each surfactant (ten-fold above, ten-fold below, and at each CMC) in distilled water were assayed by the Microtox® Toxicity test procedure (Azur Corporation, Carlsbad, California) which determines the toxicity of a substance by measuring the light levels of viable luminescent bacteria upon their exposure to test substrates. Procedures followed the manufacturer's directions for the

Basic Acute Toxicity Protocol. The toxicity results were expressed as EC_{50}, which is the effective concentration of the toxicant causing a 50% decrease in the light output by the bacteria under exposure time (five minutes) and test temperature (15 °C). Since these values are inversely related (the higher the EC_{50}, the lower the toxicity), results are presented in Toxicity Units (TU). Toxicity Units are defined as $1/EC_{50} \times 100$ which results in a lower value indicating a lower toxicity. TU values for clean water will be <1.

Evaluating Different Concentrations of Selected Surfactants for Enhanced Degradation

Three concentrations of four selected surfactants (ten-fold above, ten-fold below, and at each CMC) were tested in four-liter batch tank reactors containing a blend of process water from three different wood-treating sites contaminated with high levels of PCP, PAHs, and oil and grease. Nutrients were added as a 0.5% solution of Miracle Grow (15:30:15). Oxygen was added as pumped air. Each test was run as a completely random design with three replications per treatment and three replications of the control consisting of no surfactant addition. pH levels were maintained around 7. Samples were analyzed at Days 0, 30, and 60 for PCP and PAH concentrations by methods given above, oil and grease concentrations according to Standard Methods for the Examination of Water and Wastewater (Taras et al. 1989), microbial enumerations (bacteria, fungi, and actinomycetes) by the spread plate method on selective media, and toxicity by the Microtox® toxicity assay. It is not easy to correlate a Microtox TU value to absolute toxicity levels. In this study, Microtox is used as a measure of relative toxicity. When contaminant levels are high, TU levels should also be high. As degradation proceeds, toxicity levels should go down as contaminants are degraded to non-toxic or less toxic forms.

RESULTS

Screening Surfactants for Interference of Contaminant Analyses by Gas Chromatography

None of the surfactants screened for interference of contaminant extraction and analyses by gas chromatography exhibited peak overlap above the detection limit of 0.55 mg/L for any of the PAHs (data not shown). Only one surfactant, Span 20, exhibited slight peak interference for PCP above the detection limit of 0.13 mg/L. A PCP concentration of 1.72 mg/L was exhibited for Span 20, although no PCP was actually present. One anionic surfactant, lauryl sulfate, interfered with the extraction process. This surfactant foamed excessively, causing the extractors to clog such that the methylene chloride could not adequately reflux.

Testing for Surfactant Toxicity to Microorganisms

The non-ionic surfactants Tween 20 and Tergitol exhibited no toxicity (TU<1) at any of the three concentrations tested (Table 1). Tween 80 and Span 80 were slightly toxic (TU<1.5) only at the highest concentration tested. The anionic surfactant, caprylic acid, exhibited high toxicity (25.3 TU) at the highest surfactant concentration, while the other anionic surfactant, lauryl sulfate, was extremely toxic (TU>100) at the two highest surfactant concentrations. Toxicity of the surfactants to the degrading microbial populations were ranked from least toxic to most toxic as follows: Tween 20 and Tergitol < Tween 80 and Span 80 < Brij 35 < Triton X-100 < Span 20 < Caprylic acid < Lauryl sulfate. Based on these results and the results from Objective 1, lauryl sulfate was not evaluated further and caprylic acid and Span 20 where also dropped.

Evaluating Different Concentrations of Selected Surfactants for Enhanced Degradation

The first 60-day bioreactor study evaluated the non-ionic surfactant Brij 35. At Day 60, PCP concentrations decreased by 57.7% in the treatment with ten-fold above the CMC (Figure 1). No statistical changes in PCP concentrations were observed in the other two treatments or controls. The PAH concentrations dropped to below detection in all treatments and controls (Figure 1). Initial PAH concentrations were much lower than desired. The subsequent bioreactor studies contained contaminated water from another heavily contaminated site in order to increase these concentrations. Oil and grease concentrations decreased the most in the control samples, followed by the ten-fold below, CMC, and finally the ten-fold above treatments (data not shown). Addition of Brij 35 appeared to inhibit oil and grease degradation. Bacterial populations did not differ in any of the treatments or controls for any sampling date. Levels remained at 10^5 cfu/ml and the populations did not significantly decrease over time (data not shown). No fungi or actinomycetes were recovered. Toxicity decreased in all of the treatments from Day 0 to Day 60 (data not shown). At Day 60, the treatment containing the highest concentration of Brij 35 was the least toxic, followed by the other two treatments containing surfactant, and then the control.

The second 60-day bioreactor study tested the non-ionic surfactant Tergitol. The process water in this study contained greater concentrations of PAHs than were present in the first study. No significant degradation of PCP occurred in any treatment or control (Figure 2). Significant degradation of total PAHs (greater than 90%) was observed in all treatments and controls by Day 60 (Figure 2). No significant change in oil and grease concentrations occurred in any treatment or control (data not shown). No differences

in the bacterial population were seen in any of the treatments or control for any sampling date. Levels remained at 10^5 cfu/ml and the populations did not significantly decrease over time (data not shown). No fungi or actinomycetes were recovered. Toxicity decreased by Day 60 in all treatments and control (data not shown), but never reached the level as observed in the previous study with the surfactant Brij 35.

The third 60-day bioreactor study tested the non-ionic surfactant Tween 20. The process water in this study also contained greater concentrations of PAHs than were present in the first study. No significant degradation of PCP occurred in any treatment or control (Figure 3). Significant degradation of total PAHs was observed in all treatments and controls by Day 60 (90% or greater for treatments and controls except for below CMC treatment which was 80%) (Figure 3). Oil and grease concentrations significantly decreased in two of the treatments and control (data not shown). Only the above CMC treatment did not decrease in oil and grease concentrations. No differences in the bacterial population were seen in any of the treatments or control for any sampling date. Levels remained at 10^5 cfu/ml and the populations did not significantly decrease over time (data not shown). No fungi or actinomycetes were recovered. Toxicity decreased by Day 60 in all treatments and the control (data not shown). Treatments containing Tween 20 were less toxic at Day 60 compared to the control.

A fourth bioreactor study evaluating the non-ionic surfactant Span 80 is in progress. Unfortunately, this series also contains water low in PAHs. Process water that has been recently collected has been diluted with excessive amounts of stormwater run-off due to the high levels of rainfall. This is one of the drawbacks of working with genuine process water versus water that has been spiked with contaminants in the laboratory. Since degradation of PAHs has consistently been greater than 90% in all of the above studies, even those containing high PAH concentrations, the focus of further studies will be on enhanced degradation of PCP. PAHs will continue to be included in the analyses, but we will not be concerned if levels are low.

CONCLUSIONS

In all bioreactor studies, degradation of total PAHs in controls and with all concentrations of surfactants were significant (>90%), even at the higher PAH concentrations. Microtox toxicity levels decreased as PAH-contaminant levels decreased. Addition of surfactants may not be needed for PAH degradation. PCP degradation, however, only occurred thus far in the Brij 35 study at the highest surfactant concentration. Most Day 60 samples (except Brij 35 above CMC treatment) maintained toxicity in relation to PCP levels. Oil and grease concentrations were variable. This is an on-going project and other surfactants will continue to be tested in bioreactor studies over the coming year. Surfactant-enhanced degradation should result in faster degradation (reduced treatment times), more complete degradation (no intermediate by-products), improved water quality, and reduced clean-up costs.

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Table 1. Results from the Microtox® toxicity screening of the surfactants at three concentrations. Values are expressed as Toxicity Units ($1/EC_{50}$ x100). Water and phenol values are presented for comparison.

	Surfactant Concentration[1]		
Surfactant	Above	CMC	Below
Brij 35	2.9	1.9	1.9
Tween 20	<1	<1	<1
Tween 80	1.4	<1	<1
Triton X-100	13.1	1.0	<1
Span 20	10.2	2.6	1.9
Span 80	1.1	<1	<1
Tergitol	<1	<1	<1
Caprylic acid	25.3	3.9	1.2
Lauryl Sulfate	>100	>100	9.9
Water	Toxicity units = <1		
Phenol[2]	Toxicity units = 5.5		

[1]Surfactant concentrations (mg/L) are ten-fold above, ten-fold below and at Critical Micelle Concentration (CMC).
[2]Phenol was tested at 100 mg/L.

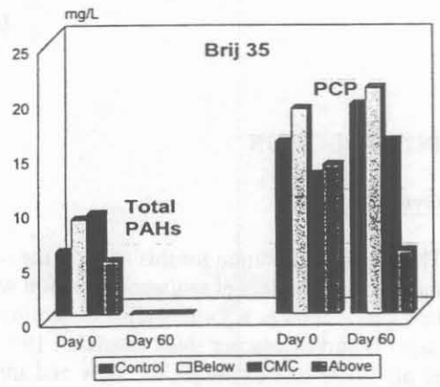

Figure 1. Degradation of PCP and PAHs in bioreactor study containing the surfactant Brij 35. Each bar represents an average of three replicates.

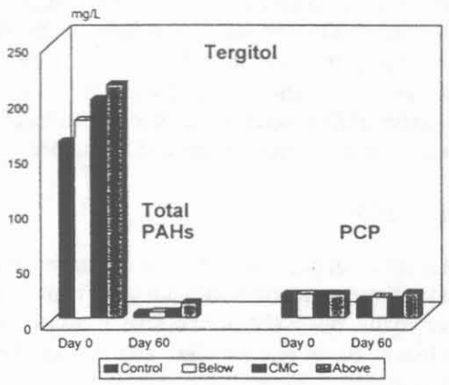

Figure 2. Degradation of PCP and PAHs in bioreactor study containing the surfactant Tergitol. Each bar represents an average of three replicates.

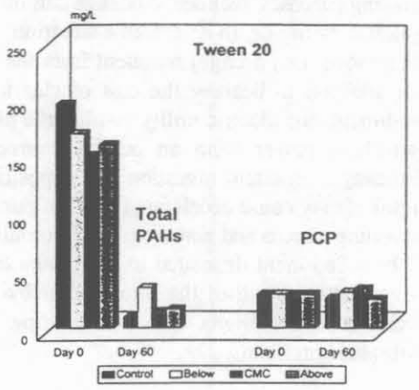

Figure 3. Degradation of PCP and PAHs in bioreactor study containing the surfactant Tween 20. Each bar represents an average of three replicates.