BIOREMEDIATION AND LEACHING POTENTIAL OF PENTACHLOROPHENOL (PCP) IN BIODIESEL VERSUS DIESEL CARRIERS

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Abstract. Biodiesel is believed to be more environmentally friendly than petroleum-based diesel when used as a carrier for impregnating wood products with pentachlorophenol (PCP) for decay protection. A 6-mo study was conducted to evaluate bioremediation of PCP in biodiesel vs diesel in soil. Different percentages of biodiesel, diesel, and PCP were mixed with clean soil from a forested site and tested. Samples were taken bimonthly and analyzed for oil and grease, PCP concentration, and microbial enumeration. Soil moisture content was adjusted twice weekly if needed. In addition, toxicity and toxicity characteristic leaching potential were measured at Days 0 and 180. Results showed that with an increase in percentage of biodiesel, there was an increase in degradation of diesel and diesel-amended PCP. The greatest decrease of PCP concentration and toxicity occurred in biodiesel alone by Day 180. Results also showed a significant decrease with time in oil and grease concentration, PCP concentration, and toxicity among different treatments. Based on this study, it appears that the cometabolic effect of biodiesel on micro-organisms could accelerate degradation of PCP in treated wood after disposal.

Keywords: Biodiesel, bioremediation, diesel, pentachlorophenol, toxicity characteristic leaching potential.

INTRODUCTION

A survey in the western United States showed that 800,000 utility poles are disposed of each year and 50% of these poles are treated with pentachlorophenol (PCP) (Morrell 2003). All wood preservatives have a level of toxicity for different organisms and can present a challenge to environmental bioremediation (Chu and Kirsch 1972; Prewitt et al 2003; Kaoa et al 2005). PCP is one of the most recalcitrant
chemicals for microbial degradation among simple chlorinated phenols (McAllister et al 1996; Tuomela et al 1998). Methods approved by the US Environmental Protection Agency (EPA) for disposal of PCP-treated wood include landfill and incineration/thermal pyrolysis. It is predicted that these disposal methods will become more restricted because of the formation of hazardous byproducts, high costs (Kaoa et al 2005), and decreased land disposal space.

Bioremediation, an alternative to disposal of waste products, uses micro-organisms to degrade hazardous chemicals to less toxic or mineral compounds such as water, carbon dioxide, and inorganic elements (McGinnis et al 1991b; Langwaldt and Puhakka 2000). Bioremediation has been shown to be effective at degrading PCP (Duncan and Deverall 1964; Cserjesi 1967; McGinnis et al 1991a; Borazjani and Diehl 1998; Prewitt et al 2003). When PCP is impregnated into wood, it is usually dissolved in carrier petroleum-based hydrocarbons such as diesel. These carriers are also an environmental concern when PCP-treated wood is disposed of in landfills.

Biodiesel or mixtures of biodiesel with petroleum diesel are widely believed to be environmentally preferable to the neat petroleum product because of their rapid biodegradation in soil (Zhang et al 1998; Bonten et al 1999; Lapinskiene et al 2006; Khan et al 2007; Schleicher et al 2009) and a lower toxicity to aquatic and terrestrial organisms. Indeed, biodiesel has been suggested as an environmentally benign addition to help remediate coal tar polycyclic aromatic hydrocarbons (PAH) in soils (Taylor and Jones 2001), crude oil (Gloria Pereira and Mudge 2004), and heavy fuel oil spills on beaches (Fernández-Álvarez et al 2007). Another study demonstrated that the degradation rate of petroleum-based diesel increased in biodiesel/diesel mixtures (Zhang et al 1998).

Because they result in less environmental impact, these properties could be advantageous for diesel/biodiesel use in the wood preservation industry. Therefore, biodiesel/diesel mixture as a carrier for PCP in wood product treatment might facilitate the degradation of PCP and diesel. Also, a separate study is underway to evaluate the possible effect of biodiesel on efficacy of treated wood and biodegradability of PCP and its carriers in treated wood. The objective of this study was to compare biodegradation rates of PCP formulated with diesel/KB3 and diesel/biodiesel in soil.

MATERIALS AND METHODS

Sample Preparation

In this study, clean soil from a forested area was air-dried for 24 h and then sieved with a 6-mesh screen (3.35 mm) to remove clumps. Soil composition is provided in Table 1. One milliliter of selected solutions (Table 2) was mixed with 100 g clean dried soil. Fossil fuel diesel was obtained from Shell Company of Australia (Melbourne, Victoria) (Table 3) and mixed with 10% KB3, which is a ketone still-bottom petroleum byproduct that is used as a cosolvent to enhance solubility and effectiveness of PCP. Biodiesel was manufactured by the BioPreserve Company (Erie, PA) (Table 4). One milliliter containing 50 mg technical-grade PCP in acetone was added to the soil to provide a 500-mg/kg concentration for selected treatments. In this soil study, individual replications were hand-mixed for several minutes with a glass rod to ensure maximum homogenization. One set of samples contained only

<table>
<thead>
<tr>
<th>Table 1. Chemical composition of the soil.</th>
</tr>
</thead>
<tbody>
<tr>
<td>Extractable nutrient level (mg/kg)</td>
</tr>
<tr>
<td>Phosphorus</td>
</tr>
<tr>
<td>47.5</td>
</tr>
</tbody>
</table>
soil and was used as a control. Each treatment had three replicates. Mixed soil was put into 250-mL glass jars. The lids of the jars were pierced for free air exchange, and the jars were placed at room temperature for 6 mo. Moisture adjustment and aeration were provided twice weekly by adding deionized water and mixing to maintain soil moisture content of approximately 15% (wt/wt). Fifteen grams of soil were taken at bimonthly intervals for PCP analysis, oil and grease determination, and micro-organism enumerations. Toxicity characteristic leaching potential (TCLP) and microtox toxicity tests were performed only on Days 0 and 180.

Methylene Chloride Extraction Process

Ten grams of soil were placed into a cellulose extraction thimble and extracted using methylene chloride 200-mL soxhlet extraction according to EPA Method 3540 (Brilis and Marsden 1990). The extracts were condensed to 5 mL and used for determination of oil and grease and PCP concentration.

Oil and Grease Concentration

Oil and grease concentration was determined using a modified Standard Method 5520-F (Clesceri et al 1998). Two milliliters of condensed
methylene chloride extract from soil samples were put in preweighed 50-mL flasks, which were then placed in a fume hood to evaporate the methylene chloride. The difference between initial and final weight was calculated as the amount of oil and grease.

**Determination of Pentachlorophenol Concentration**

Five hundred microliters of condensed extract were placed in a 2-mL autosample vial followed by 100 µL of N, O-bis(trimethylsilyl) trifluoroacetamide and left at room temperature for 2 h. Four hundred microliters of hexane were then added to make the final volume. PCP concentration was determined according to EPA Method 8041 (EPA 2007) using an Agilent 6890 gas chromatograph equipped with a 63Ni electron capture detector and an Agilent Ultra II capillary column. Injector temperature was 250°C, column temperature was 175°C, and detector temperature was 315°C. Helium flow rate through the detector was 1.5 mL/min.

**Micro-organism Population**

To enumerate bacteria and fungi in samples, the serial dilution plate technique was used. One gram of soil sample was added to approximately 9 mL of sterile deionized water (room temperature) and mixed well for 1 min; then, immediately, serial dilutions were made accordingly for each sample. Two hundred fifty microliters from appropriate dilutions of the microbial suspension were spread on duplicate plates of nutrient agar and nutrient agar amended with 5 ppm PCP for bacteria and potato dextrose agar for fungi (dilution concentration was adjusted as needed). The plates were incubated 2 to 4 da at 28°C. After incubation, colonies on each plate were counted, averaged, and multiplied by the appropriate dilution factor.

**Toxicity**

Microtox was used to measure toxicity of soil samples. One gram of soil was added to 9 mL sterile water, centrifuged, pH-adjusted, and assayed by the abbreviated assay procedure found in the Beckman Instruments Instructions No. 015-555879 with a 15-min incubation period at 15°C.

**Toxicity Characteristic Leaching Procedure**

Leaching characteristics of the soil were determined using a modified version of the EPA TCLP procedure (EPA 1986). This test was performed at the beginning and the end of the experiment. EPA Method 8041 was used for evaluating PCP leached levels by gas chromatography.

**Statistical Analysis**

The microbiological and analytical results from this experiment were statistically analyzed using a completely randomized design with three replications for each treatment. Tukey’s multiple comparisons test was used to compare treatment mean differences at \( P = 0.05 \). Data were processed by SPSS statistics software.

**RESULTS AND DISCUSSION**

**Oil and Grease Concentration**

Results of oil and grease concentrations in soil are shown in Figs 1 and 2. In all treatments, there were considerable decreases (greater than 79%) in oil and grease concentrations (Fig 1) by the end of the study. However, actual concentrations of oil and grease in the soil showed significant differences with time within treatments (Fig 2). All treatments, except the control, showed significant decreases in oil and grease between Days 0 and 60. BIO and PBIO were the only treatments that did not show a significant decrease between Days 60 and 120, but they did significantly decrease by Day 180.

**Pentachlorophenol Concentration**

Changes in PCP concentration are shown in Figs 3 and 4. The highest rate of decrease was observed in PBIO by 31% and the lowest decrease was DKP by 10% (Fig 3). Statistical
analysis showed that there were significant differences in percentage decrease of PCP concentration between biodiesel alone and treatments containing biodiesel plus diesel (Fig 4).

Micro-organism Population

Results of micro-organism counts are shown in Tables 5, 6, and 7. Generally, the number of colonies decreased during the study. This could have been caused by natural adjustments to change in environment as well as toxicity of the chemicals that were added to the soil. Interestingly, the two treatments with biodiesel and no diesel, BIO and PBIO, supported higher fungal populations by Day 180.

Toxicity

Toxicity was also tested in this study. Results are depicted in Figs 5 and 6. The highest decrease in toxicity occurred in PBIO by 92%
Figure 4. Pentachlorophenol (PCP) concentrations in soil with time in different treatments. (Different letters indicate significant difference in time within a given treatment at $P = 0.05$.)

Table 5. PCP acclimated bacteria populations in treatments with time.

<table>
<thead>
<tr>
<th>Treatments</th>
<th>PCP resistant bacteria Day 0 (cfu/g)</th>
<th>PCP resistant bacteria Day 60 (cfu/g)</th>
<th>PCP resistant bacteria Day 120 (cfu/g)</th>
<th>PCP resistant bacteria Day 180 (cfu/g)</th>
</tr>
</thead>
<tbody>
<tr>
<td>DKP</td>
<td>4.32E+07</td>
<td>1.13E+05</td>
<td>5.20E+05</td>
<td>4.00E+05</td>
</tr>
<tr>
<td>BDP</td>
<td>4.33E+07</td>
<td>1.00E+05</td>
<td>3.50E+05</td>
<td>5.33E+05</td>
</tr>
<tr>
<td>PBIO</td>
<td>6.06E+07</td>
<td>2.04E+06</td>
<td>2.40E+05</td>
<td>1.08E+07</td>
</tr>
</tbody>
</table>

PCP, pentachlorophenol; cfu, colony-forming unit.

Table 6. Bacterial population in treatments with time.

<table>
<thead>
<tr>
<th>Treatments</th>
<th>Bacteria Day 0 (cfu/g)</th>
<th>Bacteria Day 60 (cfu/g)</th>
<th>Bacteria Day 120 (cfu/g)</th>
<th>Bacteria Day 180 (cfu/g)</th>
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<tr>
<td>C</td>
<td>1.23E+07</td>
<td>5.00E+08</td>
<td>1.76E+06</td>
<td>1.33E+05</td>
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<tr>
<td>DK</td>
<td>6.17E+07</td>
<td>3.00E+07</td>
<td>2.20E+06</td>
<td>5.33E+05</td>
</tr>
<tr>
<td>BD</td>
<td>3.67E+07</td>
<td>2.20E+07</td>
<td>1.40E+06</td>
<td>1.13E+06</td>
</tr>
<tr>
<td>DKP</td>
<td>3.93E+07</td>
<td>8.60E+06</td>
<td>2.06E+06</td>
<td>6.00E+05</td>
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<tr>
<td>BDP</td>
<td>4.68E+07</td>
<td>1.13E+08</td>
<td>2.33E+06</td>
<td>8.00E+05</td>
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<tr>
<td>BDD</td>
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<td>4.66E+07</td>
<td>9.33E+06</td>
<td>1.00E+06</td>
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<tr>
<td>BIO</td>
<td>3.17E+08</td>
<td>1.22E+07</td>
<td>8.60E+05</td>
<td>3.51E+04</td>
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<tr>
<td>PBIO</td>
<td>6.67E+07</td>
<td>1.85E+07</td>
<td>2.60E+06</td>
<td>1.50E+04</td>
</tr>
</tbody>
</table>

cfu, colony-forming unit.

Table 7. Fungal population in treatments with time.

<table>
<thead>
<tr>
<th>Treatments</th>
<th>Fungi Day 0 (cfu/g)</th>
<th>Fungi Day 60 (cfu/g)</th>
<th>Fungi Day 120 (cfu/g)</th>
<th>Fungi Day 180 (cfu/g)</th>
</tr>
</thead>
<tbody>
<tr>
<td>C</td>
<td>6.08E+05</td>
<td>2.00E+04</td>
<td>1.80E+04</td>
<td>2.73E+03</td>
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<tr>
<td>DK</td>
<td>2.97E+06</td>
<td>1.80E+04</td>
<td>2.80E+04</td>
<td>7.93E+03</td>
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<tr>
<td>BD</td>
<td>4.57E+06</td>
<td>2.20E+04</td>
<td>4.67E+03</td>
<td>7.40E+03</td>
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<tr>
<td>DKP</td>
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<td>2.70E+04</td>
<td>1.38E+04</td>
<td>1.07E+03</td>
</tr>
<tr>
<td>BDP</td>
<td>6.88E+07</td>
<td>7.30E+03</td>
<td>2.00E+02</td>
<td>1.53E+03</td>
</tr>
<tr>
<td>BDD</td>
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<td>4.60E+04</td>
<td>6.33E+03</td>
<td>6.53E+03</td>
</tr>
<tr>
<td>BIO</td>
<td>6.58E+05</td>
<td>2.40E+04</td>
<td>3.50E+04</td>
<td>5.84E+06</td>
</tr>
<tr>
<td>PBIO</td>
<td>9.33E+03</td>
<td>4.70E+04</td>
<td>2.80E+04</td>
<td>8.27E+06</td>
</tr>
</tbody>
</table>

cfu, colony-forming unit.
Figure 5. Toxicity differences in treatments during 180 da. (Different letters indicate significant difference among treatments at $P = 0.05$.)

Figure 6. Toxicity differences among treatments at Days 0 and 180. (Different letters indicate significant difference in time within a given treatment at $P = 0.05$.)

Figure 7. Percentage increase in pentachlorophenol (PCP) leaching during 180 da. (Different letters indicate significant difference among treatments at $P = 0.05$.)
Adding biodiesel to diesel also significantly decreased toxicity compared with diesel alone. Statistical analysis showed that there were significant differences with time among and within treatments in toxicity of samples (Fig 6).

### Toxicity Characteristic Leaching Potential

Results of TCLP are shown in Figs 7 and 8. The greatest rate increase in leaching was observed for PBIO by 73% and the lowest rate was in DKP by 16% after Day 180 (Fig 7). Results of statistical analysis showed that there were significant differences among treatments by the end of this study in samples containing biodiesel and diesel or biodiesel alone (Fig 7). However, there were significant differences in percentage increases for PCP leaching between Days 0 and 180 treatments (Fig 8).

### CONCLUSIONS

In general, our results indicated that increasing the percentage of biodiesel increased degradation of recalcitrant components in the soil, such as PCP. Toxicity (an indication of PCP or oil and grease degradation) was decreased by 54, 74, and 92% in diesel/PCP mixtures, biodiesel/diesel and PCP mixtures, and biodiesel/PCP mixtures, respectively. Increase of TCLP by Day 180 is an important economic consideration in disposing PCP-contaminated soil because it makes PCP bioavailable for micro-organisms, whereas the amount of PCP that leached from soil was lower than the permissible disposal level. The amount of hydrocarbon that remained in the soil was decreased up to 93% in samples that contained biodiesel compared with samples that contained diesel only. PCP degradation was 10, 17, and 31% in diesel/PCP mixtures, biodiesel/diesel and PCP mixtures, and biodiesel/PCP mixtures, respectively. An explanation for this behavior could be that cometabolic activities occur in the presence of biodiesel in this mixture. Biodiesel could initiate degradation of other chemicals such as diesel that are relatively recalcitrant. These results are in full agreement with findings of Zhang et al (1998), Bonten et al (1999), and Pasqualino et al (2006) who showed that biodiesel increased bioavailability of PAH, which increased degradation of PAH. This study showed that in soil contaminated with some percentage of biodiesel, the wood preservative PCP was degraded much faster than when the preservative contained a conventional carrier such as petroleum-based diesel. This could be a positive point in waste management and could offer an environmentally friendly alternative for disposal of PCP-treated wood waste. However, because adding biodiesel to wood preservative-treating solutions could also increase the rate of biodegradation of PCP in treated wood, the potential use of biodiesel may be limited to remedial treatments of contaminated soil and wastewater.
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REFERENCES


