Full-Scale Removal of PCP from Granite Timber Post and Pole Site Soil

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ABSTRACT: The Granite Timber Post and Pole site is located near Philipsburg, Montana, and is the site of a former wood treating facility. Site soil was found to be extensively contaminated with pentachlorophenol (PCP). In July 2005, a site visit was conducted to collect soil samples for bench-scale activity screening and solid-phase studies. Activity screening studies resulted in the isolation of a highly active PCP-degrading *Sphingomonas sp.* which was later used as a bioaugmenting agent in bench and field studies. Results from bench-scale solid-phase studies showed that starting PCP levels were reduced by 87% in 28 days. Full-scale cleanup of contaminated soil was initiated in July 2006 with construction and bioaugmentation of a land treatment unit (LTU). The initial soil treatment cycle (Lift 1) was completed in early September 2006 and the final soil treatment cycle (Lift 2) initiated in late September 2006. Results showed that average Day 0 soil PCP levels in Lift 2 decreased from 75 mg/kg to 21 mg/kg by June 2007 with a decrease of 72% in 257 days. The PCP content in all samples analyzed was below the action level of 48 mg/kg.

INTRODUCTION

Pentachlorophenol (PCP) is a biocide that has been extensively used as a preservation agent in the lumber and timber industries. While primarily being used as a wood preservative, PCP has also been used in other industries including applications as a fungicide, bactericide, herbicide, insecticide, algaecide, and molluscicide (Crosby 1981). PCP was listed as a priority pollutant by the U.S. EPA (U.S. EPA 1989), and designated as a persistent organic pollutant (POP) because of its toxicity and environmental stability.

Site Background. The Granite Timber Post and Pole site is located in Granite County, Montana, near the town of Philipsburg. The site covers approximately 25 acres (10.1 hectares) and has been used for the commercial treatment of wood products since 1963. The wood treatment solution consisted of 5 percent (%) PCP dissolved in diesel fuel and was prepared in a mixing tank. Wood products were transferred to a 6,700-gal (25,362-L) pressure tank and treated with the PCP solution under pressure. After treatment, the wood products were placed on racks to dry.

The site has been under investigation by the State of Montana since 1987 and has been under a series of administrative and judicial orders to clean up PCP-contaminated soil in several areas. Investigations showed that site soil was extensively contaminated with PCP and creosote components.

Soil samples were collected in 4-oz (118-mL) wide-mouth jars during a site visit in July 2005. The samples were evaluated in activity screening studies for PCP-degrading activity. Bulk samples of PCP-contaminated and “clean” soil were also collected and

initially sieved prior to preparation of soil blends having a desired PCP concentration. The soil blends were used in bench-scale solid-phase studies to evaluate the performance of bioremediation technology as a remedy for site cleanup.

In late July 2006, a land treatment unit (LTU) was constructed on-site to treat contaminated soil using solid-phase bioremediation technology. The unit, having a capacity of 1,900 yd^3 (1,453 m^3), was amended with ingredients from a standard recipe, developed by Response Engineering and Analytical Contract (REAC) personnel, and augmented with an indigenous PCP-degrading microorganism identified in the activity screening study. The purified culture, designated as strain GPP-5, was sent to a commercial laboratory for characterization and identified as a *Sphingomonas sp.* Bench-scale solid-phase studies later showed that this culture rapidly degraded PCP in site soil over short incubation periods with concomitant production of chloride (Cl).

MATERIALS AND METHODS

Activity Screening Studies. Studies were initiated to identify soil samples containing PCP-degrading microorganisms. Twelve soil samples were screened for activity in flasks containing 100-mL mineral salts (MS)-PCP medium with an initial PCP concentration of 100 µg/mL. The flasks were incubated on a gyratory shaker set at a temperature of 30°C and an agitation rate of 200 rpm. The study was conducted over a 28-day period with samples collected weekly. Samples were clarified by centrifugation, syringe filtered and the filtrate analyzed for soluble Cl content and the culture pH measured. An increase in soluble Cl was an indication of PCP-degrading activity in soil enrichment cultures.

B**ench-Scale Solid-Phase Studies.** Bench-scale solid-phase studies were conducted to assess the performance of the Environmental Response Team (ERT)/REAC recipe in promoting the removal of PCP from site soil by microbial degradation. Test soil blends were prepared from PCP-contaminated and "clean" soil samples to achieve a target PCP concentration of approximately 180 mg/kg.

Strain GPP-5 was grown in shake flask culture in minerals salts-glutamate medium at 30°C and 200 rpm for 48 hours. The culture growth was monitored daily by measuring the absorbency at 560 nm. After 48 hours, an aliquot of the culture was diluted to an estimated population density of 1.25×10^7 Colony Forming Units (CFU)/mL in deionized water and the diluted culture placed in an ice bath until used.

Duplicate 4.5-qt (4.26-L) stainless steel trays fitted with stainless steel lids were used as bench-scale reactors. Soil blends were prepared in each reactor at a rate of 1,175 g/reactor and supplemented with sawdust and calcium carbonate at rates of 50 g/kg and 10 g/kg, respectively, based on a soil bed weight of 1,250 g. The soil blend was quite acidic and required a higher rate of calcium carbonate to raise the pH to neutrality. The final rate of calcium carbonate addition was 30 g/kg.

Due to the presence of elevated levels of diesel fuel (analyzed as diesel range organics [DRO]) in soil blends, inorganic nitrogen (ammonium nitrate) and phosphorus (dibasic sodium phosphate) sources were added to the soil beds to stimulate the growth of DRO-degrading microorganisms. The nitrogen and phosphorus sources were added at rates based on the estimated total organic carbon (TOC) content in the soil beds. The

carbon to nitrogen (C:N) and carbon to phosphorus (C:P) ratios were 30:1 and 400:1, respectively.

A 1-mL aliquot of the diluted culture was added to sterile deionized water used to hydrate the soil beds. Upon soil hydration, the theoretical population density of the bioaugmenting agent was 1 x 10^4 CFU/g with a target moisture level of 20%.

Soil PCP, DRO, Cl, pH, and moisture content (MC) were monitored in reactors over a test period of 41 days at incubation temperatures ranging from 25°C to 30°C. PCP, DRO and pH were measured biweekly while Cl and MC were measured weekly.

Scale-up of the Bioaugmenting Agent. Scale-up studies were conducted to produce sufficient biomass to inoculate the LTU. The culture was grown in a nutrient medium that promoted high cell densities over short incubation periods. The medium recipe was identified during medium development studies reported in an earlier communication (Allen et al. 2007). Due to the small size of the LTU, strain GPP-5 was produced in bulk in shake flask culture. The cell suspensions were concentrated by centrifugation, the cell concentrates suspended in 50% v/v aqueous glycerol, and then frozen at –20ºC until shipped to the site. Approximately 97 g of biomass (wet wt.) were produced during the study.

Field-Scale Studies

LTU Construction. The LTU was placed in the north central portion of the site and constructed in July 2006. Wood chips were placed in the graded area to prevent punctures in the liner from rocks and sticks. The treatment cell was then lined with a high density polyethylene (HDPE) liner (30 mil [0.076 cm] thickness). A 6-in (15.2-cm) layer of wood chips was applied on top of the liner and the wood chip layer then overlaid with a 2-in (5.1-cm) layer of small rocks. The wood chips were again added to prevent punctures to the liner when the rocks were added.

Soil Treatment Cycle (Lift 1). Bulk excavated soil was amended with sawdust (approximately 10% v/v), mixed and pushed into one large pile. Crushed limestone was then added to the amended soil (approximately 1% wt/wt) and then sieved to remove large rocks using a vibrating screen having a 4-in (10.2-cm) grid. The sieved, amended soil was collected on a conveyer belt and transported into the treatment cell. The final depth of the soil bed was approximately 18 in (45.7 cm). The remainder of the amended soil was stockpiled at the edge of the LTU.

The LTU was then bioaugmented with strain GPP-5, the highly active PCP-degrading strain isolated during activity screening studies. The microbial cells were dispensed into the soil bed using a cell dispensing apparatus fastened to the frame of a spring tooth harrow. The harrow was pulled by a tractor to apply the microbial cells. The apparatus consisted of a 300-gal (1,136 L) plastic feed tank, a diaphragm pump, and a custom-made manifold-tubing assembly to dispense the microbial suspension. A PVC pipe exiting the feed tank was fitted with a gate valve and attached to the diaphragm pump. The diaphragm pump was connected to the manifold-tubing assembly with Teflon® tubing attached to the back of the teeth of the harrow. As the spring teeth created furrows in the soil, the cell suspension was trickled into the furrows and covered as the soil collapsed.

The flow rate of the dispensing apparatus was initially calibrated with tap water. The cell mass was added to the feed tank containing 250 gal (946 L) and mixed. After opening the valves on the dispensing apparatus, the tractor was driven at a defined speed in order to uniformly apply the inoculum to LTU soil. The use of the dispensing apparatus has been described in a previous communication (Allen et al. 2007).

After inoculation, the LTU was then hydrated to 20% MC using a sprinkling system. The LTU was watered twice weekly and tilled immediately after sample collection. Soil moisture was monitored twice weekly and adjusted accordingly with the sprinkler system. The initial treatment cycle (Lift 1) was monitored for 6 weeks with samples collected weekly. PCP analysis of all of the Lift 1 samples showed that the PCP concentration was below the action level of 48 mg/kg apparently due to insufficient mixing of the soil stockpile.

Soil Treatment Cycle (Lift) 2. The remainder of the stockpiled soil was added to the unit in September 2006 to a final depth of 18 in (45.7 cm). Day 0 control samples were collected in late September 2006 and test samples collected on Days 222 and 257 in May and June 2007, respectively. Samples were submitted for PCP analysis and the extent of PCP degradation determined.

Field Sampling. The LTU was divided into 10-ft (3.05 m) by 10-ft grids (3.05 m) and sampling grids were selected by a random number generating program. At Day 0, 10 grids were randomly selected for sampling. From each grid, three aliquots were collected at respective depths of 0-6 in (0-15.2 cm), 6-12 in (15.2-30.5 cm) and 12-18 in (30.5-45.7 cm). The aliquots collected at each depth were composited into one sample. Three sets of 10 grids were sampled with three composite samples generated at each soil depth. The total grids sampled represented approximately 12% of the total LTU grids. The three composite samples were submitted for PCP analysis.

For test samples, four grids were randomly selected and four aliquots from each grid were collected at the respective depths described previously. The three composite samples were submitted for PCP analysis.

Similar sampling protocols were utilized when collecting test and control samples from Lift 2. Due to the setup of Lift 2 at the end of the growing season in 2006, the frequency of sample collection was considerably different as test samples were not collected until the Spring of 2007.

RESULTS AND DISCUSSION

Activity Screening Studies. Results showed that all of the soil enrichment cultures exhibited PCP-degrading activity. Enrichment cultures developed from 11 of 12 soil samples exhibited weak PCP-degrading activity while the remaining culture exhibited strong activity. Strong activity was defined as the complete degradation of 100 µg/mL PCP with stoichiometric production of Cl in 7 days. Soil enrichment cultures requiring longer time periods to completely degrade PCP were defined as weak degraders. Further studies were conducted with the enrichment culture exhibiting strong activity and resulted in the isolation and purification of the PCP-degrading microorganism. The

purified culture was later identified as a *Sphingomonas sp*. and was used as a bioaugmenting agent in bench- and field-scale solid-phase studies.

Bench-Scale Solid-Phase Studies. Results of the bench-scale solid-phase studies are summarized in Figure 1. The data represent the average of PCP, DRO and Cl analyses from both reactors.

Soil PCP was rapidly degraded over a 41-day test period with PCP levels reaching a minimal concentration by Day 28. The rapid degradation of PCP was confirmed by the rapid increase in soil Cl content with Cl levels reaching a maximum concentration over the same time period that PCP levels were reduced to a minimal concentration. Further incubation showed no significant decreases in PCP or increases in Cl concentration.

The solid-phase study was conducted to demonstrate that the ERT/REAC recipe could promote the rapid degradation of PCP after bioaugmentation with strain GPP-5. This recipe has been used successfully in previous studies (Allen et al. 2007, Allen et al. 2005, Allen et al. 2000). From an average initial concentration of 119 mg/kg, the PCP concentration was reduced by 80% (24 mg/kg) in 14 days, 87% (16 mg/kg) by Day 28 and 85% (18 mg/kg) by Day 41. The Cl production curve showed that Cl reached a maximum concentration at Day 28 with relatively little change in concentration over the remaining 13 days of the study. The final PCP concentration of 16 to 18 mg/kg was well below the action level of 48 mg/kg established for this site.

Soil DRO was also analyzed over the 41-day study in order to determine its susceptibility to microbial degradation. The DRO degradation profile was considerably different from the profile observed for PCP degradation. In contrast to rapid degradation observed for PCP, DRO were degraded at a much lower rate and extent.

From an initial concentration of 1,045 mg/kg, DRO levels were reduced by 15% (893 mg/kg) by Day 14, 26% (779 mg/kg) by Day 28 and 29% (743 mg/kg) by Day 41. The rate and extent of DRO removal was clearly reduced after Day 28. Whether DRO degradation continued after Day 41 was not determined.

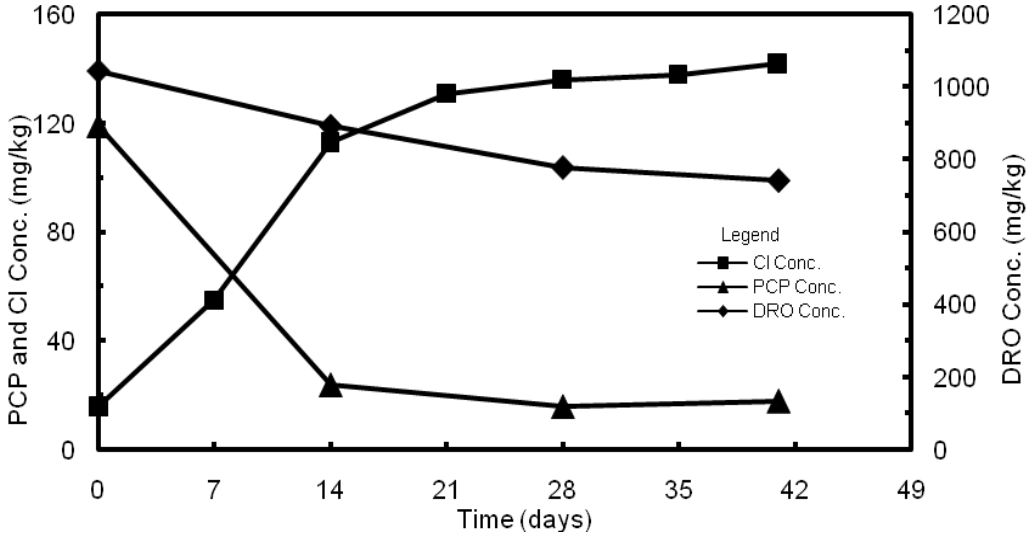

FIGURE 1. Biodegradation of PCP and DRO in contaminated soil.

Additional solid-phase studies were conducted with stockpiled site soil during the development of a most probable number (MPN) assay to estimate the population density of PCP-degrading microorganisms in soil. However, activity screening studies indicated that the stockpiled soil exhibited strong PCP-degrading activity. Solid-phase studies showed that most if not all of the soil PCP already had been degraded as evidenced by high initial soil Cl levels, small increases in soil Cl detected over time of incubation and a decreasing population of indigenous PCP-degraders. These data indicate that the strong PCP-degrading activity exhibited by the stockpiled soil should result in rapid degradation of PCP in Lift 2.

Field-Scale Studies

Soil Treatment Cycle (Lift) 2. The performance of the field unit during Lift 2 is summarized in Table 1. The final 18-in (45.7-cm) layer of soil was added to the unit during late September 2006 and Day 0 samples collected. Results showed that Day 0 PCP levels were 90.6, 98.3 and 37.4 mg/kg at soil depths of 0-6 in (0-15.2 cm), 6-12 in (15.2-30.5 cm) and 12-18 in (30.5-45.7 cm), respectively, with an average PCP level of 75 mg/kg. After collection of Day 0 samples, the unit was not sampled until the start of the 2007 field season (May 2007). At Day 222, results showed that PCP levels were dramatically reduced to an average PCP concentration of 10 mg/kg with the extent of removal at 87%. Samples were collected again in June 2007 (Day 257) to confirm that PCP levels had been reduced to below the action level of 48 mg/kg. PCP levels were found to be somewhat higher at an average PCP concentration of 31 mg/kg with a 59% reduction. The overall average PCP concentration determined from the May and June sampling events was 21 mg/kg with a 72% reduction. The PCP concentration in all composite soil samples collected was below the action level of 48 mg/kg and site soil was deemed to be remediated.

TABLE 1. Biodegradation of PCP in lift 2.

Time (Days)	PCP Conc. (mg/kg)			Avg. PCP Conc. (mg/kg)	Degradation (%)
	0-6 in	6-12 in	12-18 in		
0	90.6	98.3	37.4	75	0.0
222	8.1	7.0	15.0	10	86.7
257	14.4	43.2	35.9	31	58.7
222 & 257	11.3	25.1	25.5	21	72.0

CONCLUSIONS

Activity screening studies resulted in the isolation and identification of a highly active PCP-degrading *Sphingomonas sp*. The culture was used as a bioaugmenting agent to accelerate the removal of PCP from soil in bench- and field-scale studies.

The use of the ERT/REAC recipe in combination with the use of a bioaugmenting culture resulted in rapid removal of PCP with 80% of available PCP degraded by Day 14 and 87% by Day 28 in bench-scale solid-phase studies. Residual PCP levels were quite

low at 24 mg/kg and 16 mg/kg, respectively. Thus, even as early as 14 days, the cleanup goal of 48 mg/kg had been achieved.

DRO residues were also susceptible to degradation by indigenous microorganisms. However, the degradation profile was considerably different from PCP with a slow rate of removal and a much lower extent of removal. Only 15% of DRO residues were degraded by Day 14, 26% by Day 28 and 29% by Day 41. These data suggest that DRO removal from site soil to levels below 700 mg/kg will require lengthy incubation times.

Solid-phase bioremediation technology performed well in removing PCP from LTU soil in the 2006 and 2007 field seasons. Degradation results suggest that PCP-degrading microbial populations were able to survive the severe Montana winter. Site cleanup goals were already met by the May 2007 sampling even though there was no watering or tilling conducted after the application of the second lift.

While variability in soil PCP concentrations was observed when comparing May and June 2007 results, all of the soil composites had PCP concentrations below the action level of 48 mg/kg. The overall reduction in PCP concentration was 72% with an average final PCP concentration of 21 mg/kg.

REFERENCES

Allen, H.L., J. McKeown, A. Lange, R. Laramie, and T.F. Miller. 2007. "Full-Scale Removal of PCP from Pringle Post and Pole Site Soil." Paper D-03. *In* A.R. Gavaskar and C.F. Silver (Symposium Chairs), In Situ and On-Site Bioremediation – 2007, *Proceedings of* the *Ninth International In Situ and On-Site Bioremediation Symposium*, Battelle Press, Columbus, Ohio.

Allen, H.L., K. Lawrence, D. Zuroski, S. Journell, W. Wellendorf, K. Harris, and T.F. Miller. 2005. "Performance and Process Economics of the Aerobic Bioremediation of Pentachlorophenol." Paper F-05. *In* B.C. Alleman and M.E. Kelley (ed.), In Situ and On-Site Bioremediation – 2005, *Proceedings of* the *Eighth International In Situ and On-Site Bioremediation Symposium*, Battelle Press, Columbus, Ohio.

Allen, H.L., K. Lawrence, S. Journell, W. Wellendorf, K. A. Harris, and T.F. Miller. 2000. "Field-Scale Studies on Removal of Pentachlorophenol from Contaminated Soil." In G. B. Wickramanayake, A. R. Gavaskar, J. T. Gibbs, and J. L. Means, (Eds.), *The Second International Conference on Remediation of Chlorinated and Recalcitrant Compounds*, pp. 161-168, Vol. C2-7, Battelle Press, Columbus, Ohio.

Crosby, D.G. 1981. "Environmental Chemistry of Pentachlorophenol." *Pure Appl. Chem.* 53: 1051-1080.

United States Environmental Protection Agency. 1989. "Hazardous Wastes from Non-Specific Sources." 40 CFR Subpart 261.31. U.S. Environmental Protection Agency, Washington, D.C.