Examination of Pentachlorophenol Biodegradation in Contaminated Soil

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Abstract

Biodegradation behavior is very site specific, therefore, the use of actual site contaminated soil is useful in determining the biological fate of the hydrophobic pollutants such as pentachlorophenol (PCP) present at a site. Although the site described in this study was originally contaminated with both pentachlorophenol and 2,3,4,6-tetrachlorophenol, the presence of other chlorophenols indicates that intrinsic degradation of PCP may be occurring at the site. In order to determine the potential for, and extent of, biodegradation of the chlorophenol contamination at the site, pentachlorophenol contaminated soil from different depths at a former wood treatment site were placed under both aerobic and anaerobic conditions in the laboratory and monitored by HPLC. The desorption behavior of PCP from the soil was also monitored by HPLC in other experiments. Biodegradation of pentachlorophenol contamination occurred anaerobically and aerobically. Reductive dechlorination of pentachlorophenol in anaerobic samples resulted in predominantly metachlorinated products including 3,5-dichlorophenol and 3-monochlorophenol, which tend to be more toxic than corresponding ortho- or para-chlorinated phenols. The 3-chlorophenol was further degraded with the appearance of phenol, but not in quantifiable amounts. However, PCP desorption from the contaminated soil was significant during the first few weeks and the extent dependent on the contaminated soil mass to solution volume ratio. In addition, a sorbed fraction of the initial pentachlorophenol was not available for biodegradation in the anaerobic studies and higher concentrations remained compared to the aerobic degradation experiments. A recent bioremediation trend has focused on sequential anaerobic/aerobic treatment for complete degradation of highly chlorinated organics. To test this possibility, aerobic surface soils were spiked with monochlorophenols, which are the degradation products of anaerobic PCP degradation. Both 2- and 4-monochlorophenol were degraded aerobically in the spiked surface soil samples, but 3-monochlorophenol was not significantly degraded, effectively eliminating a sequential treatment approach. The aerobic microorganisms appear to be more competent PCP degraders at the site and may provide the most effective remediation approach.

Introduction

Pentachlorophenol (PCP) has been widely used as a wood preservative. The site in this study was originally contaminated with PCP and 2,3,4,6-tetrachlorophenol (2,3,4,6-TeCP) from a dip operation in the 1950s. The fate of PCP contamination at the site is controlled by the presence or absence of indigenous PCP degrading microorganisms and the bioavailability of the compound. Microbial degradation is the key removal mechanism for PCP in contaminated soil and has recently been reviewed [1,2]. Aerobic microorganisms have been shown able to degrade and use PCP as a source of carbon and energy [1]. In addition, anaerobic microorganisms are capable of biodegrading PCP to lesser chlorinated phenols, and even degrading PCP to complete mineralization. The degradation products tend to be less hydrophobic and more mobile in soil systems, but sometimes more toxic than PCP [3].

The biological availability of PCP is controlled by physical and chemical properties of both the soil and PCP. Soil characteristics which influence the environmental fate of PCP include pH and organic carbon content. Compound characteristics include partitioning behavior, solubility, and, if it is an ionizable compound, the acid dissociation coefficient. Being a relatively hydrophobic organic compound (log K_{ow} = 5.24 [4]), sorption of PCP to soils significantly affects biodegradation. PCP has been reported with a solubility of 14-20 mg/L, which actually only accounts for the neutral species. With a dissociation constant (pKa) of 4.75, PCP exists in the environment in both the neutral form and more soluble, ionic form, pentachlorophenolate. At a typical environmental pH (7-8), more than 99% of the PCP present will exist as pentachlorophenolate, dramatically increasing the aqueous solubility of PCP. Therefore, above a pH of 7, sorption of the phenolate ion and metal complexes between the phenolate anions and calcium cations become important [5].

Studies have indicated that a residual amount of PCP, up to 30 mg/kg, was not degradable in soils that have been contaminated for a long period of time [6,7]. It has also been noted that chlorophenols in soil with long-term contamination were not as accessible to biodegradation as short-term, spiked chlorophenol [8].

Potential PCP bioremediation strategies have included using inoculated or indigenous microorganisms under strictly aerobic or anaerobic conditions. In addition, a recent trend for degradation of highly chlorinated organics has been sequential anaerobic/aerobic treatment.

The focus of this study was to evaluate the presence and biodegradation capability of indigenous microorganisms and determine the extent of desorption and biodegradation, using contaminated soil from the site for the study. PCP contaminated soil samples from the site were placed in the laboratory under varying mass to volume ratios to monitor desorption of PCP and under anaerobic and aerobic conditions to monitor the disappearance of PCP and the appearance of chlorophenol intermediates. Monochlorophenols have not been detected in contaminated soil from the site, but anaerobic degradation of PCP can lead to the formation of monochlorophenols which are difficult to further degrade anaerobically. Thermodynamic calculations suggest that anaerobic degradation would be more effective with higher chlorinated compounds and aerobic degradation would be more effective with lesser chlorinated compounds; therefore, remediation of the site with a sequential anaerobic/aerobic scheme could potentially be a more effective

method for treatment chlorinated compound contamination. The potential use of a sequential anaerobic/aerobic treatment was examined by spiking aerobic surface soil samples with monochlorophenols and monitoring their concentrations by HPLC.

Experimental Methods

PCP was extracted from soil samples with 1:1 mixture of acetonitrile and water and from soil slurries with a 1:1 mixture of acetonitrile and soil slurry. Analysis for PCP and all other chlorophenols was done by HPLC. Detection limits were less than 0.5 mg/L for all chlorophenols. Soil cores from a contaminated area at the site were taken using a manual core sampler. The soil was a fine grained silty clay.

The soil for aerobic degradation experiments was taken from near the surface where no PCP was present and conditions were expected to be aerobic due to close proximity to the surface. A sample of soil was placed in a serum bottle and partially filled with mineral media to completely saturate and cover the soil. A live sample and an autoclaved sample were spiked to approximately 20 mg/L, and then incubated with each monochlorophenol.

Soil for aerobic PCP degradation experiments was taken from a shallow depth where conditions were also assumed aerobic due to the close proximity to the surface. Soil was added to serum bottles and mineral media was added to wet the soil, but not over saturate the soil. Adequate headspace was available to maintain aerobic conditions and all serum bottles were flushed with air weekly. PCP concentrations were less than 10 mg/kg when sampled.

Soil used in the anaerobic experiments was taken from the deepest point sampled was added to serum bottles saturated with mineral media. Soil PCP concentrations varied at this depth from 40 to 60 mg/kg. The headspace was flushed with nitrogen, sealed, and acetone, methanol, and dextrose were added to the bottles to induce methanogenic conditions. An anaerobic enrichment culture was transferred to uncontaminated soil which were spiked with PCP and methanol and dextrose.

For desorption studies, contaminated soil was placed in unbuffered, nanopure water at varying solid mass to liquid volume ratios and the concentrations monitored. Aqueous samples were also taken from samples with a fixed mass to volume ratio for several months to monitor the time required to reach equilibrium.

Results and Discussion

Pentachlorophenol was anaerobically and aerobically degraded in contaminated soils and spiked samples by microorganisms from the PCP site. Aerobically, PCP biodegraded to less than 0.5 mg/kg with a relatively short lag-period, all in less than 20 days. The addition of moisture and nutrients to the soil resulted in aerobic PCP biodegradation. It is not known currently if both are required to induce degradation. Subsequent addition of PCP was also biodegraded at a rate similar to the original, long-term PCP contamination.

Anaerobically, the PCP was dechlorinated in contaminated soils and subsequent inoculums into PCP-spiked soils with preferential removal of ortho > para > meta chlorines. This preferential removal resulted in mainly metachlorinated products and the accumulation of

3-monochlorophenol (3-MCP) as the only monochlorophenol metabolite. Phenol was detected, but not in quantifiable amounts, so it is not certain if dechlorination to phenol is the only degradation pathway for 3-MCP. Futher identification of the degradation products of 3-MCP is needed.

Similar to the anaerobic results of preferential removal of ortho- and para-chlorines and difficulty with meta-chlorines, aerobic degradation occurred in samples spiked with ortho and para-monochlorophenol (2- and 4-MCP), but not with meta-monochlorophenol (3-MCP). Bearing in mind that the primary product of anaerobic degradation is 3-MCP, which is recalcitrant to aerobic degradation, a sequential anaerobic/aerobic treatment scheme is not likely to be effective at this site.

Slow desorption occurred in desorption studies and the extent of desorption was dependent on the soil mass to solution volume ratio, with the fraction of PCP desorbing to the aqueous phase varying from 20% to 80% of the initial contamination as mass to volume varied from 10 to 0.01 kg/L. The effect the slow desorption had on anaerobic PCP degradation is seen in Figure 1. Significant desorption occurred in the anaerobic samples during the first two weeks and only after the aqueous concentration peaked, did degradation begin and products, primarily 3-MCP, appear (Figure 2). In addition, a residual amount of PCP existed at the conclusion of the anaerobic experiments, up to 20 mg/kg (Figure 1). Anaerobic samples inoculated with microbes from the contaminated soil degraded PCP in newly spiked soils to much lower levels, approximately to five-fold lower concentrations, further indicating that desorption was limiting the degradation in the contaminated soil.

In this study, the initial PCP concentration was approximately an order of magnitude greater in the anaerobic experiments with deeper soils than the aerobic experiments with shallow soils. Desorption of PCP does not appear limiting degradation under aerobic conditions. Whether the lack of a non-biodegradable fraction in this aerobic experiment is related to the relatively low PCP concentration, approximately 7 mg/kg, or some biological factor is not known. An initial desorption model was developed using contaminated soil and varying soil mass to solution volumes, monitoring the concentrations, and modeling the aqueous and sorbed concentrations with a Freundlich isotherm (Figure 3). This model allows for prediction of aqueous concentrations that can be expected from the degradation studies. Based on this initial model, the aerobic microbes appear to be degrading to significantly lower aqueous concentrations even when compared to the spike anaerobic samples, indicating a lower biological threshold in the aerobic microbes than with the anaerobic microbes.

There is some indirect evidence of natural, intrinsic biodegradation occurring at the site including the relatively short acclimation period for the almost complete aerobic degradation of PCP and the presence of anaerobic metabolites in the contaminated soils. Further investigation of the potential differences in threshold concentrations between aerobic and anaerobic microbes is needed prior to choosing a remediation scheme at the site.

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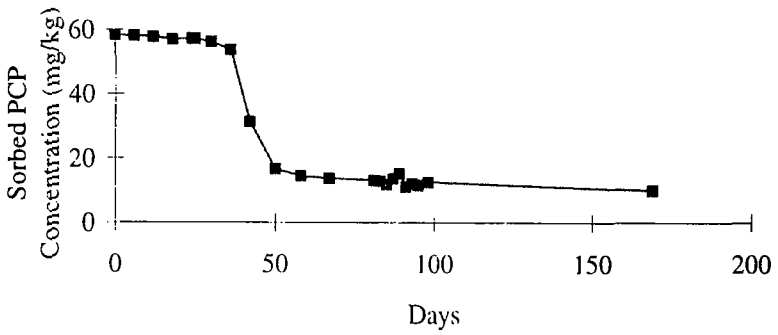

Figure 1. Sorbed pentachlorophenol soil concentration in anaerobic degradation experiment.

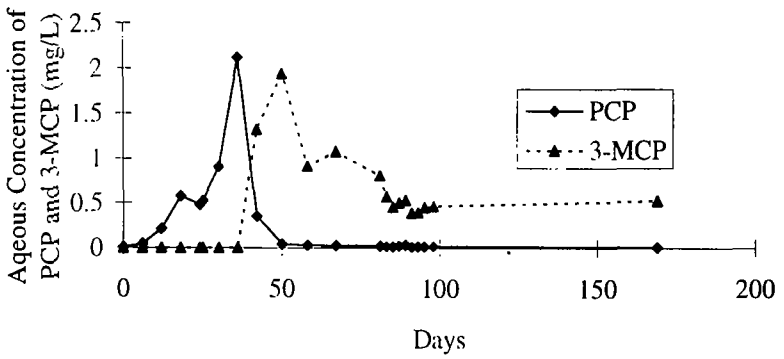

Figure 2. Aqueous PCP and 3-MCP concentration in anaerobic degradation experiment.

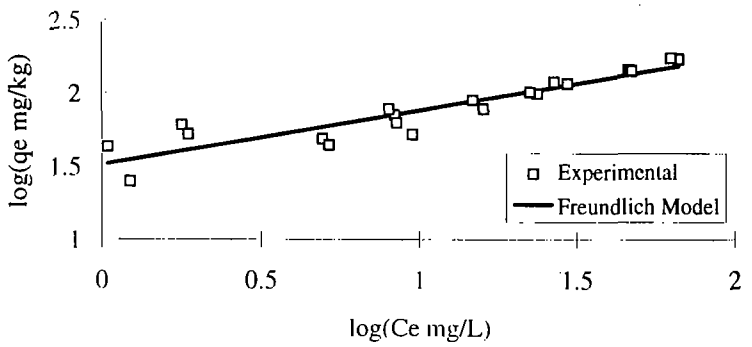

Figure 3. Log-log Desorption isotherm for PCP from soil contaminated over 30-40 years.