Bull. Environ. Contam. Toxicol. (1996) 57:258-263


Bulletin of Environmental Contamination and Toxicology

# Effects of Copper-Chrome-Arsenate (CCA) Components on PCP Degradation by *Arthrobacter* Strain ATCC 33790

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Received: 25 September 1995/Accepted: 15 March 1996

Bioremediation with microbial inoculation (bioaugmentation) is a relatively new technology for contaminated site cleanup. The feasibility of the technology is dependent upon properties of the target pollutants, inoculated microorganisms, and contaminated environment. A significant amount of attention has been given to evaluating its feasibility for removal of pentachlorophenol (PCP), a chemical often found at wood preservative-contaminated sites (Mueller et al. 1989). Results of studies have shown that PCP-contaminated soil may be treated successfully using the bacterial genera Arthrobacter, Flavobacterium, and Rhodococcus (Crawford and Mohn 1985; Edgehill and Finn 1983; Middeldorp et al. 1990). Concentrations of PCP at least as high as 600mg/kg were reported to be removed from soil inoculated with 4.1 x $10^7$/g Arthrobacter strain ATCC 33790 (Edgehill 1995). Information has been provided on the effects of inoculum size, temperature, PCP concentration, soil type, and soil moisture content on removal of PCP from soil (Crawford and Mohn 1985; Edgehill and Finn 1983; Middeldorp et al. 1990). Although the results have increased the current level of understanding of the applicability and limitations of bioaugmentation, there is still little known about the role of inhibitory substances on kinetics of PCP removal and the effects of short-or long-term exposure of the inoculum to extremely high levels of PCP (hot spots). For decontamination of real sites, as opposed to synthetic ones produced in the laboratory, where contamination is neither uniform nor homogeneous, both of these factors become quite important.

Copper chrome arsenate (CCA) is a wood preservative in common use (Wall and Stratton 1994). In some areas it supplements or has replaced the creosote-PCP formulation also used for wood treatment. At facilities where both are or have been used, contamination may be a mixture of metals and organics such as PAHs and PCP. PCP is of particular concern because it may migrate to surrounding areas with water runoff and is acutely toxic to fish (Rao 1977).

For bioaugmentation to be effective at sites having mixed contamination, PCP must be degraded in the presence of the organics and metals. Data exist on the influence of naphthalene, phenanthrene, and cresols (creosote components) on PCP degradation (Edgehill 1994). Naphthalene at its solubility limit (30

mg/L) and cresols at 1000mg/L prevented removal of nontoxic levels of PCP by Arthrobacter strain ATCC 33790 (Edgehill 1994). However, there have been no reports in the literature on the influence of arsenic and metals such as copper and chromium, found in CCA on PCP removal by strain ATCC 33790. The response of the culture to extremely high levels of PCP has also not been explored. With inoculation of environments containing highly variable amounts of PCP, temporary exposure of the cells to extremely toxic concentrations of PCP may occur before concentrations are lowered with diffusion or by intentional dilution with uncontaminated soil or water.

The aim of this work was to examine the effect of CCA components on utilization of PCP by Arthrobacter strain ATCC 33790. The effect of the CCA components on PCP removal from aqueous phase, soil slurry, and soil was examined in laboratory studies. The aqueous phase results supplement those recently obtained with PCP-degrading Flavobacterium ATCC 53874 (Wall and Stratton 1994). However, the results described here provide insights into which components of CCA are most detrimental to PCP degradation. An appropriate remedial technology may be one which alleviates toxicity by selectively removing only those component (s). It was also of interest to investigate whether strain ATCC 33790 could regain its ability to degrade PCP following exposure to extremely high concentrations.

## MATERIALS AND METHODS

For all experiments examining the influence of metals and arsenic on PCP removal, Arthrobacter strain ATCC 33790 was used. Inocula were taken from batch or chemostat (where indicated) cultures grown on mineral salts medium (MS) containing PCP as the sole carbon source as previously described (Edgehill 1994). For some of the experiments, inocula were grown on MS containing 1mg/L rather than 5mg/L $FeSO_4 7H_2O$. Calcium, Fe, Mn, and EDTA were excluded from MS containing Cu and/or containing soil. Inoculum size was estimated using the relationship that $A_{600} = 0.25$ corresponds to $2 \times 10^8$/ml (Edgehill 1982). Lawes soil was used for soil slurry and soil inoculation studies. The soil contains approximately 60% clay and has a pH of 7.3 (Edgehill 1994). All inoculated media were shaken at room temperature (20-25°C).

The ability of Arthrobacter strain ATCC 33790 to survive high and moderate concentrations of PCP was examined. High concentration flasks containing 21mL of mineral salts medium and PCP concentrations of 997, 1039, 1910 and 2078 mg/L were inoculated with 0.5-1mg (23.8-47.6 mg/L) cells and incubated at 30°C for 7.5hr. Moderate concentration flasks containing 24.3 mL MS, 547 and 536 mg/L PCP, 0.6 mg (24.7 mg/L) cells were incubated for 13.5 hr at 30°C. The pH of the medium before autoclaving ranged from 7.3-7.7. The high and moderate concentration media were diluted with neutralized sterile MS to 200-217 and 125-127 mg/L PCP, respectively, and incubated at 30°C for 5 d.

Copper was added to MS using 1/10, 1/100, and 1/1000 dilutions of a stock solution containing 0.2010 g $CuSO_4 5H_2O$ dissolved in 50mL of deionized water (1 g/L Cu). The Cu concentrations in 50mL ranged from 0-8.0 mg/L. PCP was added to the solutions at 89-124 mg/L. The flasks were inoculated with 2.4mg/L chemostat-grown cells.

PCP-MS solutions containing arsenate were made up using a stock solution of 4.37 g/L $Na_2HAsO_4 7H_2O$ (1.05 g/L As) and its 1/100 dilution in deionized water. The solutions were added to MS to give As concentrations of 0-708 mg/L. Higher concentrations (1033, 1604, and 2120 mg/L) were obtained by adding $Na_2HAsO_4 7H_2O$ directly to the flasks before inoculation. Solution volumes of 21-55 mL contained an initial PCP concentration of 75-102mg/L PCP and 5.0-5.2 mg/L inoculum.

Chromium was added to MS as 984 mg/L (2.782 g/L $K_2Cr_20_7$) or 9.84 mg/L stock solutions in deionized water to give concentrations of 0-46.4 mg/L. Solution volumes of 21.2-21.5 mL containing 81-116 mg/L PCP were inoculated with 4.8 mg/L of the culture.

For soil inoculation studies examining the effect of Cu, 5g air-dried Lawes soil (Edgehill 1994) blended with 1 mL MS (pH-7), 0.1 mL 1% sodium pentachlorophenate (200 mg/kg PCP), and 0.1 mL of a 1000 mg/L Cu solution (20 mg/kg), 0.1 mL or 0.5 mL of a 100 mg/mL Cu solution (2000 mg/kg or 10,000 mg/kg, respectively) were inoculated with 0.1 mL culture ($4x10^6$/g). The 100mg/mL Cu stock solution was a slightly cloudy suspension. Duplicate soils were analyzed at various times for PCP.

The influence of chromium on PCP removal in soil slurry was examined. MS (30 mL) containing 10% Lawes soil (3 g), 50-70 mg/L PCP, and Cr concentrations of 0-203 mg/L was inoculated with 1 mL inoculum ($4.4-7.7x10^6$/mL).

For analysis of PCP in aqueous solutions, low turbidity cultures ($A_{600}$<0.05) were diluted using deionized water. Turbid cultures and 1/6 dilutions of soil slurry were filtered using a 0.22-um filter and Swinnex cartridge (Millipore Corp.). Soil PCP was analyzed by methanol extraction as previously described (Edgehill 1995). Culture, soil slurry, and methanol filtrates were read at 320 nm ($A_{320}$) using a UV-visible spectrophotometer. The $A_{320}$ values were compared with those at time zero to estimate the fraction of PCP remaining.

## RESULTS AND DISCUSSION

Microbial inoculation (bioaugmentation) with PCP-degrading microorganisms represents one option for removal of PCP. However, for the technology to be successful at sites where CCA is also present, the inoculated organisms must be capable of degrading the PCP in the presence of the component metals and arsenic. This study examined the PCP degradation behavior of Arthrobacter strain ATCC 33790 with various concentrations of CCA components in

solution, soil slurry, and soil.

Copper was quite inhibitory to utilization of 89-124 mg/L PCP in solution by Arthrobacter strain ATCC 33790. PCP was removed immediately from flasks containing copper concentrations up to 0.1 mg/L. At 0.5 and 1 mg/L Cu, PCP utilization was delayed. The times corresponding to 90% disappearance from the mineral salts medium at Cu concentrations of 0, 0.02, 0.04, 0.08, 0.1, 0.5, and 1.0 mg/L were 96, 120, 120, 167, 120, 191, and 456 hr, respectively. PCP was not removed from the medium containing 2 and 8 mg/L Cu in 1.5 mon.

The culture was able to remove at least 90% of 200mg/kg PCP in less than 4 d from Lawes soil containing concentrations of copper as high as 2000 mg/kg. At a soil concentration of 10,000 mg/kg Cu, approximately 50% of the initial extractable PCP was removed with inoculation in 4 d. The amount of PCP remaining in uninoculated soil not containing Cu after 46 hr, 77% of the initial amount, is consistent with results from previous work with this soil (Edgehill 1995). Much of the copper in the soil is apparently unavailable to the inoculated bacteria as a result of precipitation or adsorption. In additional experiments not described here, 90.3 mg/L PCP was removed from MS containing 5% Lawes soil and 100mg/L Cu. However, a blue precipitate was observed in the MS-soil mixture (Edgehill unpublished).

The inhibitory effect of hexavalent chromium in solution was less strong than that of copper. The behavior of the culture was similar to that of the control not containing Cr at concentrations of Cr less than or equal to 9.2 mg/L. The PCP was depleted in these flasks in 1-1.5 d. At concentrations tested above 9.2 mg/L, 23.2 and 46.4 mg/L, the inhibition was apparently irreversible. No utilization of PCP occurred in the presence of an initial concentration of 23.2 mg/L Cr in 2 Mon.

The organism appeared to be more tolerant to chromium if soil was also present in the MS. After one week the PCP was substantially depleted, as indicated by removal of more than 50% of the $A_{320}$, at chromium concentrations of 20 mg/L and 40 mg/L in 2 and 1 out of 2 experiments, respectively. Little or no removal of $A_{320}$ from inoculated MS containing 60.3 mg/L Cr and 10% Lawes soil occurred after one week.

The Arthrobacter culture showed high tolerance to arsenate in solution. PCP (75-102 mg/L) was rapidly removed from mineral salts medium containing arsenic concentrations as high as 1604 mg/L. The disappearance kinetics were similar below 1000 mg/L where removal of the PCP occurred in a day or less. Inhibition of substrate utilization was apparent at 1604 and 2120 mg/L. At the former concentration, the half life of PCP was approximately 1 d. With 2120 mg/L in the growth medium, only 25% of the initial absorbance of the solution was removed in 7.5 d.

Recent results have been reported in the literature on the ability of Flavobacterium sp. to remove PCP from medium containing various amounts of CCA preservative (Wall and Stratton 1994). The synthetic (lab) and actual (technical) preservative formulations used for the work consisted of 135.2 g/L Cr, 127.5 g/L As, and 82.4 g/L Cu and 124.6 g/L Cr, 119.6 g/L As, and 81.5 g/L Cu, respectively. Lag periods before removal of 10, 50, and 100 mg/L PCP were increased by the presence of CCA. Concentrations of the preservative of 11.64 x $10^{-4}$% w/v (synthetic) and 6.19 x $10^{-4}$% w/v (technical) were completely inhibitory at concentrations of PCP ≥50 mg/L. The individual metal ion concentrations in these mixtures were 0.77 mg/L Cr, 0.5 mg/L Cu, and 0.74 mg/L As (technical) and 1.57 mg/L Cr, 0.96 mg/L Cr, and 1.48 mg/L As (laboratory). Assuming a composition of CCA equal to that described in the literature and no toxicity enhancement with synergistic interaction of the metals, the concentration of CCA capable of being tolerated (corresponding to 1 mg/L Cu) by strain ATCC 33790 is 1.2 x $10^{-3}$% (w/v) for both lab and technical formulations.

An additional characteristic of importance in evaluating the effectiveness of bioaugmentation for removal of PCP is the ability to withstand high pollutant concentrations. Concentrations of PCP exceeding 135 mg/L are toxic to strain ATCC 33790 but growth will occur immediately at 300 mg/L (Edgehill and Finn 1982). There is an interest in knowing whether extremely high concentrations inactivate the organism.

The culture was unable to degrade 200 mg/L PCP after exposure to 1000 or 2000 mg/L PCP for 7.5 hr. However, 24.7 mg/L cells did survive 540 mg/L for 13.5 hr. After dilution of the medium to 125 mg/L, the culture removed the PCP.

An additional experiment examined the ability of chemostat-grown cells of strain ATCC 33790 to remain viable after long-term incubation in MS with 500 mg/L PCP. With an inoculum size of 2.5 mg/L, no PCP disappearance was observed in 800 hr. After dilution of the culture (on day 33) to 250 mg/L, no removal of PCP was evident when analysed 9 d later

The results of this study indicate that dispersed cells of Arthrobacter strain ATCC 33790 are quickly inactivated by high concentrations of PCP. A biofilm culture of strain ATCC 33790 was found to be more tolerant to PCP, surviving a concentration of 1000 mg/L for 1.5 d (Edgehill 1996). The organism has shown extremely high tolerance in clay soil where 600 mg/kg, corresponding to a soil water concentration (assuming no sorption) of 3000 mg/L, was removed with inoculation (Edgehill 1995).

Acknowledgments: The author thanks the Process Environmental Analysis Centre (PEAC) for loan of equipment and supplies and the Australian Research Council for funding the project.

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