Bull. Environ. Contam. Toxicol. (1996) 57:737-742

Bulletin of Environmental Contamination and Toxicology

Influence of Chromium (VI) and Acidic Conditions on Removal of Pentachlorophenol from Soil by *Arthrobacter* Strain ATCC 33790

R. U. Edgehill

Department of Chemical Engineering, University of Queensland, St. Lucia, Queensland, 4072, Australia

Received: 8 February 1996/Accepted: 10 May 1996

The use of microbial inoculation for removal of chemical contamination from soil is a technology which has become increasingly important in recent years. There are now quite a few examples in the literature where inoculation has been shown to be beneficial in accelerating decontamination. Unfortunately, a number of papers has also documented failure of the approach, particularly when it has been used for remediation of soil contaminated with petroleum hydrocarbons. The majority of the investigations have examined variables such as inoculum size, temperature, soil moisture content, soil type, and concentration of contaminants on pollutant removal (Edgehill 1992).

A significant amount of information has been obtained on removal of pentachlorophenol (PCP), a constituent of wood preservative, from soil by the bacterial genera Arthrobacter, Flavobacterium, and Rhodococcus (Crawford and Mohn 1985; Edgehill and Finn 1983; Middledorp et al. 1990). In a recent study the effects of copper, chromium, and arsenate (CCA) on PCP utilization by Arthrobacter strain ATCC 33790 were examined (Edgehill 1996). CCA and PCP are often co-contaminants in soil near wood treating operations (Wall and Stratton 1994). In this investigation the effects of chromium (VI) and acidic conditions on PCP removal from soil by inoculated Arthrobacter strain ATCC 33790 were examined. No information currently exists in the literature on the effect of Cr (VI) as the sole CCA constituent and acidification without changing soil type on soil inoculation to remove PCP.

MATERIALS AND METHODS

The mineral salts medium (MSM), procedures for batch and continuous cultivation of Arthrobacter strain ATCC 33790, and clay soil used for the study have been previously described (Edgehill 1994; 1995). The procedure for analysis of PCP, which involved extraction of the soil with methanol and measurement of the UV absorbence at 320 nm (A_{320}) of the extract filtrate, have also been described (Edgehill 1995). A_{320} readings at various times during the experiment were compared with those at time zero to estimate the fraction of PCP remaining (C/Co).

To examine the effect of Cr (VI) on PCP removal by the inoculum, glass scintillation vials containing 5-g air dried soil, 2-ml of MS (20 mg/kg Cr (VI) study) or 2-ml of 1/2 strength MS (200 mg/kg Cr (VI) study) both without Ca, Fe, Mn, and EDTA, 0.1 mL 1% sodium pentachlorophenate (200 mg/kg PCP), and 0.1 mL Cr (VI) stock solution, were inoculated with 0.1mL inoculum grown in the chemostat or in batch culture. The stock solutions of Cr (VI) consisted of 0.2788 g/100 mL (1 mg/mL Cr (VI)) or 0.2895 g/10mL (10 mg/mL Cr (VI)) potassium dichromate dissolved in deionized water for the 20 or 200 mg/kg Cr (VI) studies, respectively. The inoculum size was 3.4-3.6 x 10^6/g. The moisture content, 0.46 (mass water/mass air-dried soil), was higher than used in previous work to prevent drying and to facilitate mixing of the soil. In that study, it had been shown that 0.54 moisture content did not inhibit utilization of 73 mg/kg PCP in 5 g soil by strain ATCC 33790 (Edgehill 1995). The vials containing inoculated or unioculated soil were incubated at room temperature.

Removal of PCP from acidified soil was examined. One half of the volumes of 10% H_2SO_4 (0.1 mL) and 1% sodium pentachlorophenate (0.2 mL) which lowered the pH of a 20-mL H_2O, 10-g soil slurry from 7.3 to 5.0 were added to 5 g soil. The soil was further moistened with 1.5 mL H_2O (containing 1 g/L $NaNO_3$ and 0.2 g/L $MgSO_4 7H_2O$) and 0.1 mL inoculum (4 x 10^6/g). The final moisture content of the inoculated soil was 0.35. Ammonium and phosphate were deliberated excluded to avoid alteration of the pH. Controls containing the same soil additives without the inoculum were also run. The vials were covered with Parafilm to limit water loss and incubated at 30°C. For both the Cr (VI) and pH 5 experiments duplicate or triplicate soils were sacrificed for analysis of PCP.

RESULTS AND DISCUSSION

In previous work the inhibition of hexavalent chromium on PCP removal from MSM and MSM containing 10% clay soil by Arthrobacter strain ATCC 33790 was examined (Edgehill 1996). The focus of this study was on the effect of Cr (VI) on utilization of PCP by strain ATCC 33790 in soil. The data in Figure 1 indicate that inoculation at 3.4-3.6 x 10^6/g resulted in removal of 70-80% of 200 mg/kg PCP in 5 days (120 h) from soil containing 20mg/kg Cr (VI). This is comparable to the 3-4 days required to remove 90% of 200 mg/kg PCP from soil containing 2000 mg/kg Cu (inoculum size, 4 x 10^6/g) but unavailable to the inoculated bacteria (Edgehill 1996). PCP was removed at a slightly faster rate in the inoculated soil not containing Cr (VI). Little or no disappearance of extractable PCP took place in uninoculated soil containing 20 mg/kg Cr (VI) in contrast to 20% disappearance in uninoculated soil not containing Cr (VI) which indicates that indigenous microbial activity on PCP was inhibited by 20mg/kg Cr.

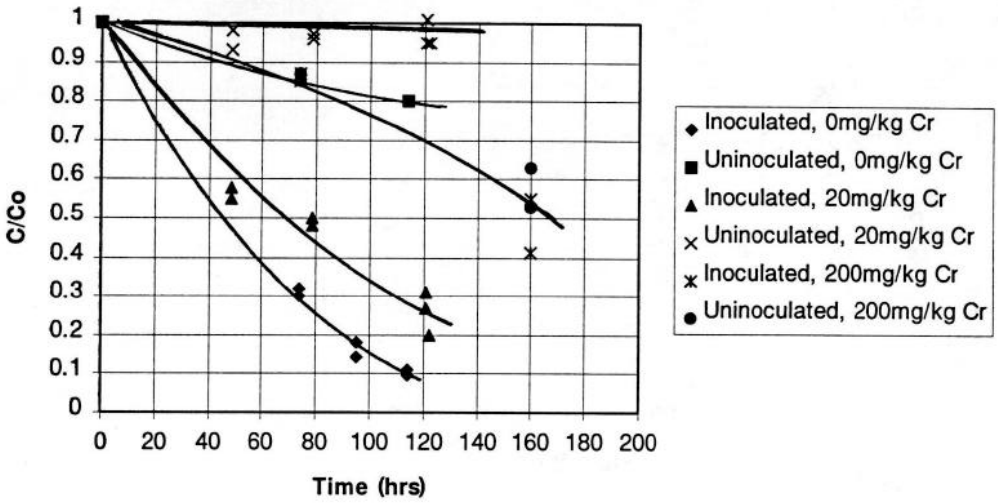

Figure 1. Effect of Cr (VI) on PCP Removal from Soil by Arthrobacter Strain ATCC 33790

At 200 mg/kg Cr (VI), removal of PCP by strain ATCC 33790 was severely inhibited. After 160 h, the A_{320} readings of the methanol extracts from uninoculated control and inoculated test soils were similar (Figure 1). The apparent decrease in PCP concentration in both was caused by disappearance of the yellow colour of methanol extractable chromate possible as a result of reduction of Cr (VI) to Cr (III) in the soil. Both aerobic microorganisms and soil organic matter are capable of reducing Cr (VI) to Cr (III) (Bartlett and Kimble 1976; Bopp and Ehrlich 1988). Chromium (III) is less toxic and precipitates under nonacidic conditions (Bopp and Ehrlich 1988). If slow reduction to Cr (III) did take place in the inoculated soil it was not sufficient to reduce the inhibitory effect of Cr (VI) to the added bacteria.

The resistance of the culture to 20 mg/kg Cr (VI) in soil was consistent with the earlier inoculation results with 10% soil-MSM. PCP was removed in 2 and 1 out of 2 experiments with 20 and 40 mg/L Cr (VI) in the medium, respectively (Edgehill 1996). At 0.46 soil moisture, 20 mg/kg Cr (VI) is equivalent to (with no sorption) 43.5 mg/L in the soil water.

Information in the literature indicates that most bacteria are sensitive to Cr (VI) (Baldi et al. 1990; Bopp and Ehrlich 1988; Luli et al. 1983; Mazierski 1995). Only 0.1% of the microorganisms in water samples from a sewage treatment plant receiving domestic and tannary wastes (100 mg/L Cr (VI)) and sediment near an industrial facility were capable of growth in medium containing 50 and 100 mg/L Cr (VI) respectively (Baldi et al. 1990; Luli et al. 1983). A hexavalent chromium tolerant Pseudomonas fluorescens, after loss of the plasmid conferring resistance to 1000 mg/L Cr (VI), was sensitive to concentrations of Cr (VI) greater than 3.5 mg/L (Bopp and Ehrlich 1988). The maximum specific growth rate of activated sludge organisms grown in

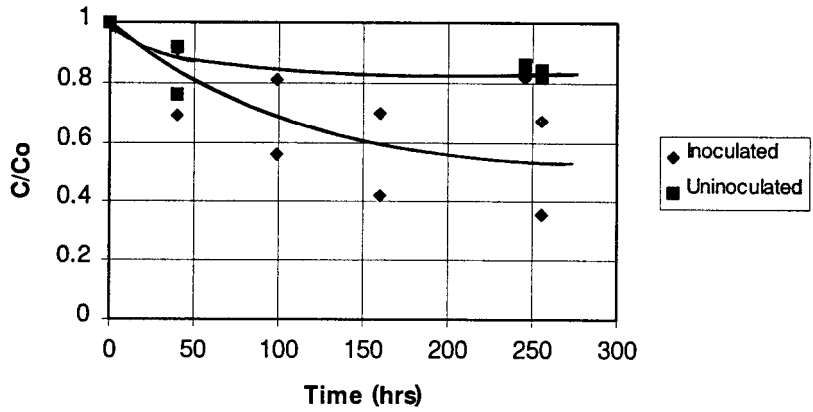

Figure 2. Effect of Acidic pH on PCP Removal from Soil by Arthrobacter Strain ATCC 33790

continuous culture on n-propyl alcohol was reduced by 40% with 11 mg/L Cr (VI) in the feed to a continuous culture (Mazierski 1995). Arthrobacter strain ATCC 33790 was resistant to 9.2 but not 23.2 mg/L Cr (VI) in MSM (Edgehill 1996).

Strain ATCC 33790 was rather ineffective in removing PCP from soil acidified to pH 5 during a 10-day incubation period. Methanol extract A_{320} readings of inoculated soils were much less reproducible than in previous neutral pH work and more than 1/2 of the readings were not appreciably less than those corresponding to uninoculated soil (Figure 2). The poor results were not surprising. The toxicity of PCP is enhanced under acidic conditions and is related to the increase in concentration of the undissociated form as pH approaches the pKa of 4.75 (Edgehill 1994; Lee et al. 1990). The low activity of the inoculum could have also been the result of the soil pH being near the lower physiological limit for growth of Arthrobacter strain ATCC 33790 in soil. Growth of strain ATCC 33790 on Trypticase Soy Broth in liquid culture is severely inhibited at pH less than 6.0 (Edgehill 1982). The results with Arthrobacter contrast the high tolerance which PCP - degrading fungi exhibit in low pH soil. Phanearochaete sordida was capable of removing 90% of 673 mg/kg PCP in 42 days from pH 3.8 soil (Glaser et al. 1993).

If inoculation is to be considered as a cleanup option for remediation of sites contaminated with PCP and CCA, soil conditions must be conducive to proliferation of the added microorganisms. The concentrations of CCA components (Cu, Cr, As) in the contaminated soil may have a significant influence on the kinetics of PCP removal by the inoculum. Previous results have shown that Arthrobacter strain ATCC 33790 was quite resistant to copper in the pH 7.3 clay soil used in this study. The copper was presumably

unavailable to the bacteria as a result of precipitation and/or adsorption (Edgehill 1996). Although soil inoculation tests with As have not been done, one might predict that As would not be strongly inhibitory if present at moderate concentrations. The culture was capable of rapidly removing nontoxic concentrations of PCP from MSM in the presence of 1604 mg/L As (Edgehill 1996).

Of the 3 CCA constituents, it appears that chromium would be most detrimental to the success of inoculation with Arthrobacter to remove PCP from soil also contaminated with CCA. Based upon the results of this study, one might consider a strategy of neutralization followed by dilution of the soil with uncontaminated soil or microbial inoculation with Cr (VI) reducing microorganisms to lower the concentration of Cr in the CCA to 20 mg/kg before inoculation. However, the degree of toxicity of Cr in soil will depend upon the soil type and whether Cr remains in the Cr (VI) form (Bartlett and Kimble 1976). With an unknown soil a laboratory test should be done first to determine what extent of removal (dilution factor) is needed to reduce Cr inhibition to an acceptable level.

REFERENCES

Bartlett RJ, Kimble JM (1976) Behavior of chromium in soils: II. Hexavalent forms. J. Environ Qual 5:383-386

Baldi F, Vaughan AM, Olson GJ (1990) Chromium (VI) - resistant yeast isolated from a sewage treatment plant receiving tannary wastes. Appl Environ Microbiol 56:913-918

Bopp LH, Ehrlich HL (1988) Chromate resistance and reduction in Pseudomonas fluorescens strain LB 300. Arch Microbiol 150:426-431

Crawford RL, Mohn WW (1985) Microbiological removal of pentachlorophenol from soil using a Flavobacterium. Enzyme Microb Technol 7:617-620

Edgehill RU (1982) Microbiol treatment of water and soil to remove pentachlorophenol (PCP). PhD Thesis Cornell University, Ithaca, NY

Edgehill RU (1992) Factors influencing the success of bioremediation. Aust Biotechnol 2:297-301

Edgehill RU (1994) Pentachlorophenol removal from slightly acidic mineral salts, commercial sand, and clay soil recovered Arthrobacter strain ATCC 33790. Appl Microbiol Biotechnol 41:142-148

Edgehill RU (1995) Removal of pentachlorophenol from soil by Arthrobacter strain ATCC 33790. In:Hinchee RE, Fredrickson J, Alleman BC (eds) Bioaugmentation for site remediation. Battelle Press, Columbus, Ohio, p 85

Edgehill RU (1996) Effects of copper-chrome-arsenate (CCA) components on PCP degradation by Arthrobacter strain ATCC 33790. Bull Environ Toxicol (in press, vol. 57, no. 2, August 1996).

Edgehill RU, Finn RK (1983) Microbial treatment of soil to remove pentachlorophenol. Appl Environ Microbiol 45: 1122-1125

Glaser JA Lamar RJ, Davis MW, Dietrich DM (1993) Field treatability trials for fungal treatment of soils contaminated with wood treating waste. Proceedings of the 19th Annual RREL Hazardous Waste Research Symposium Cincinnati, Ohio

Lee LS, Rao PSC, Nicedi-Kizza P, Delfino JJ (1990) Influence of solvent and sorbent characteristics on distribution of pentachlorophenol in octanol-water and soil-water systems. Environ Sci Technol 24:654-661

Luli GW, Talnagi JW, Strohl WR, Pfister RM (1983) Hexavalent chromium-resistant bacteria isolated from river sediments. Appl Environ Microbiol 45:846-854

Mazierski J (1995) Effect of chromium Cr (VI) on the growth rate of activated sludge bacteria. Wat Res 29: 1479-1482

Middledorp PJM, Briglia M, Salkinoja-Salonen MS (1990) Biodegradation of pentachlorophenol in natural soil by inoculated Rhodococcus chlorophenolicus Microb Ecol 20: 123-139

Wall AJ, Stratton GW (1994) Effects of chromated-copper-arsenate wood preservative on the bacterial degradation of pentachlorophenol. Can J Microbiol 40:388-392