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Bioremediation of pentachlorophenol-contaminated soil by bioaugmentation using activated soil

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Abstract The use of an indigenous microbial consortium, pollutant-acclimated and attached to soil particles (activated soil), was studied as a bioaugmentation method for the aerobic biodegradation of pentachlorophenol (PCP) in a contaminated soil. A 125-l completely mixed soil slurry (10% soil) bioreactor was used to produce the activated soil biomass. Results showed that the bioreactor was very effective in producing a PCP-acclimated biomass. Within 30 days, PCP-degrading bacteria increased from 10^5 cfu/g to 10^8 cfu/g soil. Mineralization of the PCP added to the reactor was demonstrated by chloride accumulation in solution. The soil-attached consortium produced in the reactor was inhibited by PCP concentrations exceeding 250 mg/l. This high level of tolerance was attributed to the beneficial effect of the soil particles. Once produced, the activated soil biomass remained active for 5 weeks at 20 °C and for up to 3 months when kept at 4 °C. The activated attached soil biomass produced in the completely mixed soil slurry bioreactor, as well as a PCP-acclimated flocculent biomass obtained from an air-lift immobilized-soil bioreactor, were used to stimulate the bioremediation of a PCP-impacted sandy soil, which had no indigenous PCP-degrading microorganisms. Bioaugmentation of this soil by the acclimated biomass resulted in a 99% reduction (from 400 mg/kg to 5 mg/kg in 130 days) in PCP concentration. The PCP degradation rates obtained with the activated soil biomass, produced either as a biomass attached to soil particles or as a flocculent biomass, were similar.

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Introduction

Pentachlorophenol (PCP) is one of the most prevalent wood preservatives worldwide. Its widespread use had led to the contamination of considerable volumes of soil. Traditional methods dealing with PCP-contaminated soil include disposal in landfill sites, incineration, soil washing and chemical extraction. Many bacterial strains are known to degrade PCP but they are not often found in natural soil even after years of contamination with this xenobiotic chlorinated compound. Bioaugmentation of a contaminated soil with an acclimated microbial consortium is a promising approach for the restoration of sites contaminated by PCP.

Aerobic and anaerobic biodegradation of PCP by bacterial pure cultures has been demonstrated in laboratory and field studies (Stanlake and Finn 1982; Topp and Hanson 1990; Lamar and Dietrich 1990). Nevertheless, once inoculated in contaminated soils, the activity of these pure cultures is often limited by extreme changes in environmental conditions (Goldstein et al. 1985). Furthermore, the use of pure cultures can be associated with the accumulation of partial biodegradation products (Apajalahti and Salkinoja-Salonen 1986). Mixed indigenous consortia have also been shown to degrade PCP and to have the advantage of being more resistant to natural environmental conditions and predation (Fewson 1988). These consortia can be produced as a flocculent biomass or as an immobilized biomass on organic or inorganic supports, which provide a large surface area for bacterial attachment and substrate adsorption (Morsen and Rehm 1987). Soil activation is a method of biomass production that produces a PCP-acclimated consortium (Otte et al. 1994). With this method, soil particles act not only as a support for biomass growth but also as a source of microorganisms

and nutrients. Furthermore, by adsorbing the contaminant to their surface, soil particles assist in reducing the contaminant toxicity in the liquid phase. The concept of soil activation was also used for the development of an air-lift immobilized-soil bioreactor for the treatment of PCP-contaminated waters (Karamanev et al. 1996). Owing to the positive effect of soil particles on the global biomass activity and of the air-lift reactor hydrodynamics, the volumetric PCP degradation rate reached as much as 900 mg l^{-1} h^{-1}, a biodegradation rate 7–4000 times higher than those reported in the literature (Otte et al. 1994; Frick et al. 1988; Salkinoja-Salonen 1983). For full-scale bioremediation applications, soil activation can be used to produce an activated soil biomass to enhance pollutant biodegradation in biopile treatment. However, the mobility and distribution of the activated soil biomass after inoculation in the contaminated soil can limit the efficiency of the biodegradation.

The main objective of this research was to study the effect of the bioaugmentation of a PCP contaminated soil with activated soil biomass at a bench-scale level. Two different forms of activated soil inoculums, an attached soil biomass and a flocculent biomass, were used to compare the effect of the biomass attachment state on treatment efficiency. The attached soil biomass was produced in an aerated-slurry-phase bioreactor while the flocculent biomass was produced in an air-lift immobilized-soil bioreactor (Karamanev et al. 1996).

Materials and methods

Soil origin and characterization

PCP-contaminated soil 1 was obtained from a wood-mill site located in southwest Quebec (Canada). This sandy soil had been contaminated with PCP (500 mg/kg) for 10 years (1960–1970) by condenser tank flushing to the ground every autumn. PCP-contaminated soil 2 (80 mg/kg) was collected from a pole storage area located at Pointe-aux-Trembles (Quebec, Canada). This silty loam soil had been contaminated for 30 years by the leaching of PCP technical-grade solutions from treated wood poles. All soil samples were collected at a 30-cm depth and from an area of 2 m × 2 m. The soils were sieved on-site (8 mm) and kept at 4 °C in the dark until use. Soil 2 was used to produce the PCP-acclimated consortium in the bioreactor while soil 1 was treated by bioaugmentation.

Samples of soils 1 and 2 were characterized for the following physical and chemical parameters: granulometry, water content, organic matter content and pH. Granulometry was determined using ASTM standard method D 422 (ASTM 1990) based on particle-size distribution. The granulometry of particles larger than 75 μm was determined by sieving, while the distribution of particles smaller than 75 μm was determined by soil particle sedimentation using the hydrometer method. The water content of soil samples was performed using the ASTM standard method D 2216 (ASTM 1992) based on gravimetric principles. The organic matter content in soil samples was determined according to the ASTM standard method D 2974 (ASTM 1988) based on mass loss following dry sample ignition at 500 °C. Soil pH analysis was performed using potentiometry (Accumet electrode model 25) according to ASTM standard method D 4972 (ASTM 1989).

Soil samples were also characterized for their chloride ion and PCP concentrations, and for the PCP-degrading activity of the indigenous biomass (techniques described later).

Chemicals

Reagent-grade PCP (purity of 99.5%), [U-^{14}C]PCP (specific activity 440.3 MBq/mmol, purity >98%) and 2,4,6-tribromophenol (purity >99%) were obtained from Sigma (St. Louis, Mo.). The composition of the mineral salts medium (MSM) was as described by Greer et al. (1990). All the mineral salts composing the MSM solution were ACS grade and were purchased from Anachemia Science (Lachine, Quebec, Canada). Methylene chloride (pesticide grade) was purchased from Sigma and methanol (pesticide grade) was obtained from Anachemia Science.

Biomass production

Activated soil

The production of the activated attached soil biomass was performed in a 125-l fed-batch completely mixed soil slurry bioreactor using an aeration system (0.12 vvm) and a mechanical agitator system (40 rpm), which kept soil particles in suspension. The bioreactor had an aluminum cover to minimize compound volatilization. The growth medium was composed of 10% (w/v) soil (10 kg soil 2 in 100 l MSM). The bioreactor was fed with PCP previously dissolved in 0.25 M NaOH at increasing PCP concentrations (50, 100, 150, 200, 250, 300 mg/l) added each time the PCP concentration in the aqueous phase fell below the detection limit (0.5 mg/l). Every day, slurry samples were taken from the bioreactor to determine the filtered PCP and chloride ion concentrations. The pH of the slurry was monitored on-line with a pH-meter. The bioreactor was operated for a period of 31 days. A mass balance for PCP, based on its distribution between the solid phase and the liquid phase, was performed at the end of the activated soil biomass production.

Flocculent biomass

The PCP-acclimated flocculent biomass was produced in an immobilized-soil bioreactor. This air-lift reactor contained fixed immobilized soil particles (soil 2) on a semi-permeable support (nonwoven polyethylene geotextile) that divided the cylinder bioreactor (volume 500 ml, diameter 50 mm) into two semi-cylindrical vertical sections, as described by Karamanev et al. (1996). The flocculent biomass used for the bioaugmentation experiment contained 10^8 cfu PCP-degrading bacteria/g suspended solids.

PCP-degrading activity

Mineralization experiments using radiolabelled PCP were conducted to determine the PCP-degrading activity of the two studied soils and to verify whether the activated soil biomass, once produced, remained active when kept at room temperature and at 4 °C. PCP mineralization tests were performed in 125-ml glass serum bottles equipped with a CO_2 trap (5-ml glass tube filled with 1 ml 0.5 M KOH). Samples of 10.0 ± 0.5 g moist contaminated soil and 25 ml MSM solutions spiked with [U-^{14}C]PCP (100 000 dpm/microcosm) were added to the microcosms. These were immediately sealed with rubber septa, incubated at room temperature and agitated at 150 rpm. To assess any abiotic loss of the radiolabelled compound, abiotic controls containing 0.04% (w/w) sodium azide were prepared. Periodically the KOH containing the trapped $^{14}CO_2$ was removed for analysis and replaced by fresh alkali. KOH samples (2 ml) were mixed with 9 ml liquid scintillation cocktail (Optiphase Hisafe-3, Wallac, Montreal, Quebec) and analyzed by a liquid scintillation counter (Wallac, model 1409).

Bioaugmentation

The bioaugmentation treatment was performed in 4-l glass jars containing 2 kg soil 2. The inoculation of contaminated soil with

the acclimated biomass was carried out whatever the physical form of the inoculum, to give a final PCP-degrading bacterial concentration of 10^5 cfu/g dry soil. Three physical forms of inoculum were used for the bioaugmentation experiment: (1) slurry activated soil, (2) centrifuged slurry activated soil (4000 rpm for 10 min) from which excess water and chloride ions had been removed and (3) PCP-acclimated flocculent biomass produced by the air-lift immobilized-soil bioreactor. Five different experimental conditions, tested in duplicate, were studied: (1) soil amended with 13 ml slurry activated soil (121 g activated soil/l MSM) and 87 ml MSM, (2) soil amended with 7 g centrifuged activated soil, previously mixed with 95 ml MSM, (3) soil amended with 42 ml mixed liquor (22.9 g acclimated flocculent biomass/l MSM) and 58 ml MSM, (4) soil amended with 100 ml MSM (biotic noninoculated soil) and (5) soil amended with 100 ml MSM and 10 g sodium azide (sterilized noninoculated soil). After vigorous mixing with a stainless-steel spatula, all macrocosms were covered with aluminum caps and incubated at 20 °C in the dark. To ensure good oxygen transfer and soil homogenization, soil in all macrocosms were manually mixed every 3 days. Soil pH adjustment during treatment was performed with the addition of 10.00 g $CaCO_3$/macrocosm to the soils under treatment (0.5% $CaCO_3$ w/w). Periodically, each macrocosm was sampled for soil pH analysis, PCP concentration and total heterotrophic counts.

Statistical analysis

Statistically significant differences between PCP biodegradation curves obtained from the five different treatments were evaluated at a 95% confidence level by analysis of variance.

Analytical methods

Chloride ion analysis

Chloride ion concentration in soil slurry samples was measured with an Orion electrode 9417B (Fisher, Ottawa, Ont., Canada). For soil samples, 10.0 ± 0.5 g soil was mixed with 100 ml distilled water for 30 min prior to electrode immersion. The detection limit was 5.0 ± 0.1 mg Cl^-/l. At chloride ion concentrations higher than 350 mg/l, the standard error was 5 mg/l.

Soil bacterial counts

For both total heterotrophic and PCP-degrading bacterial counts, the soil biomass extraction was performed by mixing, for 15 min on a wrist-action shaker, 10.0 ± 0.01 g soil with 100 ml sterilized (autoclaved 20 min, 121 °C, 103.4 kPa) saline solution (0.85% w/v NaCl) containing 20 glass spheres (3 mm).

For PCP-degrading bacterial counts, slurry samples (1 ml) were then successively diluted in glass culture tubes containing 9 ml sterilized saline solution (0.85% NaCl). A volume of 0.1 ml of each dilution was plated on selective medium containing PCP (100 mg/l), MSM solution and Oxoid agar (20 g/l, Unipath, Montreal, Quebec). In parallel, controls were made using plates without added PCP. After incubation during 14 days in the dark at 20 °C, colonies were counted. For total heterotrophic counts, slurry samples (1 ml) were successively diluted in glass culture tubes (a series of five tubes per dilution) containing 9 ml nutrient broth (8 g/l, BBL Becton Dickinson, Md.) according to the multiple-tube fermentation technique. After 7 days of incubation at 30 °C, the number of positive tubes (turbid culture media) was recorded and the most probable number (MPN) was calculated according to MPN statistical table (95% confidence limits).

PCP extraction

Residual soil PCP was extracted with a Soxtec extractor unit HT 1043 (Tecator, Sweden). A 10-g sample of soil (± 0.001 g) was air-dried and ground with a mortar to obtain a fine dry powder. Soil samples were then deposited in an extraction cartridge (model 1522-0018, Fisher Scientific, Canada) and soaked in methylene chloride at 110 °C for 20 min. Samples were then washed with the same solvent for 30 min. The solvent was then evaporated and the PCP was dissolved in methanol. Before each PCP extraction, soil samples were previously spiked with 2,4,6-tribromophenol (100 µl 15 g 2,4,6-tribromophenol/l methanol) as a recovery standard. For each soil sample, the water content was determined so that results could be expressed on a dry-weight basis.

PCP analysis

PCP in the liquid phases (PCP in water or in methanol) was analyzed in filtered samples (0.45-µm Teflon filters; Millipore Co., Neppean, Ont., Canada) by HPLC Spectra-Physics SP AS3000 (Thermo Separation Products, Fremont, Calif.) on an ODS-1 type chromatography column (250 mm × 4.6 mm) with an injection volume of 10 µl. The HPLC UV detector was set at 210 nm. The mobile phase was constituted of 10% acetonitrile and a gradient mix of 20%–70% (v/v) methanol and acidified water (pH 3.5 with concentrated H_3PO_4). The pumping rate was 0.7 ml/min at 55 °C. The analysis time for the PCP was about 55 min. The PCP detection limit was 0.5 mg/l and the standard error was 0.05 mg/l.

Results

Soil characterization

Physical and chemical characterization

The physical and chemical characteristics of soils 1 and 2 are presented in Table 1. Soil 1 is a sandy lightly acidic soil (pH = 6.2) and soil 2 is a silty loam lightly alkaline soil (pH = 7.5). The water content in the sandy soil (14.9%) is lower than that of the silt loam soil (21.9%).

Table 1 Physical and chemical characterization of soils 1 and 2. *PCP* pentachlorophenol

Parameters	Soil 1: wood-mill site	Soil 2: pretreated pole storage site
Physical		
Water content (%)	14.9 ± 0.1	21.9 ± 0.2
Soil texture	Sand	Silt loam
Chemical		
PCP (mg/kg)	500	80
Choride ions (mg/kg)	450 ± 10	2115 ± 50
pH	6.2 ± 0.5	7.5 ± 0.5
Organic matter content (%)	1.86 ± 0.03	11.46 ± 0.11

Both soil samples exceed the admissible provincial governmental criteria for industrial sites of 5 mg PCP/kg (MENVIQ 1988) indicating that these soils should be remediated. The very high PCP concentration in soil 1 (500 mg/kg) was unexpected since a sandy soil is generally associated with a high hydraulic conductivity and a low adsorption capacity. PCP was probably strongly adsorbed onto soil organic matter, as suggested by many studies discussing hydrophobic organic pollutant adsorption (Hassett and Anderson 1979; Means 1980; Fabre et al. 1990). On the other hand, the high chloride concentration found in soil 2 (2115 mg/kg) could have originated from intrinsic PCP degradation.

PCP-degrading activity

Results from the PCP mineralization experiments showed that only soil 2 (pretreated pole storage site) contained a PCP-degrading microflora. As much as 80% of the [^{14}C]PCP was recovered in CO_2 from soil 2 within 8 days of incubation compared to only 2% for both the soil from the wood-mill site (soil 1) and the sterilized soil (soil background [^{14}C]CO_2 release level) (results not shown). This high $^{14}CO_2$ recovery level (80%) indicates the presence of a very active indigenous biomass. In comparison, only 24% of $^{14}CO_2$ was recovered in 30 days in a similar study conducted by Laine and Jorgensen (1996) on the PCP-degrading activity of a PCP-contaminated soil. The time required to obtain the maximum PCP biodegradation rate with the soil 2 sample ranged from less than 5 to 10 days. On the basis of these results, soil 2 was chosen for the production in bioreactors of a PCP-acclimated biomass (activated soil and flocculent biomass) and soil 1 to be treated by bioaugmentation with the PCP-acclimated biomass produced in the bioreactor.

Soil activation

Consortium PCP-degradation rate

Activated soil biomass was produced with increasing PCP concentrations in the slurry-phase bioreactor. Along with increasing PCP concentration, the rate of PCP degradation by the consortium increased by a factor of 24 (from 7 mg l^{-1} day^{-1} to 167 mg l^{-1} day^{-1}) within 21 days of operation (Fig. 1). The maximum degradation rate (200 mg PCP l^{-1} day^{-1}) was observed on day 30, after a temporary inhibition of the consortium. This maximum degradation rate indicated that chloride ion accumulation in the culture medium did not inhibit the consortium activity even at a level of 800 mg Cl/l.

PCP tolerance

During the production of the activated soil biomass in the completely mixed soil slurry bioreactor, PCP degradation by the consortium was inhibited by PCP concentrations greater than 300 mg/l (Fig. 1). As shown in Fig. 1, approximately 50% of the PCP added to the liquid phase of the CMB seemed to disappear in the first 5 min. This was probably due to the sorption of a fraction of the PCP added onto soil particles since no volatilization was detected.

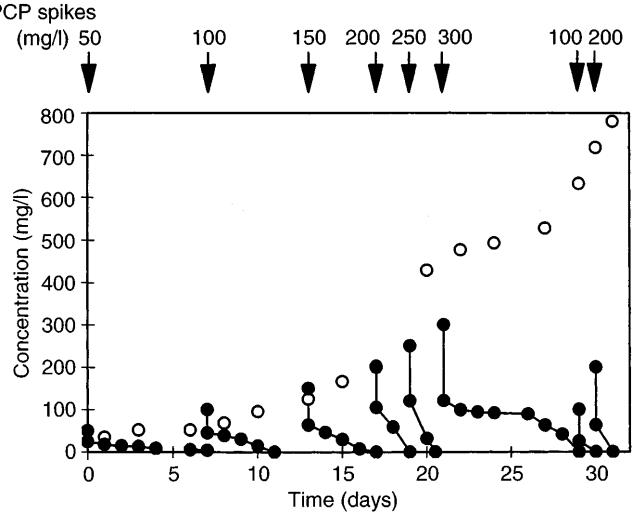

Fig. 1 Levels of pentachlorophenol (PCP; ●) and chloride ion (○) concentrations in the liquid phase during the production of the activated soil biomass in the bioreactor

Mass balance

A mass balance was performed on the PCP and chloride ion content in the bioreactor. The PCP vapor fraction was not considered because of the very low vapor pressure of PCP (1.1×10^{-4} mm Hg; 14.7 mPa). A previous study had confirmed that no PCP was detectable in an activated-carbon gas trap after 35 days of soil activation in a similar slurry bioreactor (Otte et al. 1994). As presented in Table 2, PCP in the liquid fraction was below the detection limit (< 0.5 mg/l) after 31 days of bioreactor operation. Only 0.3% (400 mg) of the total added PCP (125 000 mg) was found adsorbed onto soil particles, implying that 99.7% of the total PCP added to the bioreactor was degraded by the consortium. The accumulation of chloride ions in solution indicated that 91% of the PCP added was degraded. Furthermore, the complete biodegradation of PCP was proved by there being no sign of intermediate metabolite accumulation (tetra- or trichlorohydroquinones or pentachloroanisole, for example) on HPLC chromatograms.

The adsorption of PCP onto soil particles increased by 50% during the 31-day activation period (Table 2) from 80 mg/kg soil to 120 mg/kg. Since activated soil is to be used as an inoculum for bioaugmentation, the process of soil activation can produce a PCP-contaminated inoculum. After a 10-day maturation period, however, the PCP concentration in the activated soil

Table 2 Mass balance of PCP and chloride ions in the 125-1 completely mixed bioreactor. *t* day of operation

Fraction	PCP content of the reactor						PCP degraded (%)
	$t = 0$ (mg)	Added (mg)	$t = 31$ (mg)	Accumulated in soil (mg)	Predicted (mol)	Observed (mol)	
Liquid	0	–	0	0	–	–	–
Solid	800	–	1 200	400	–	–	–
Total	800	125 000	1 200	400	0.47	0.47	99.7%[b]
Chloride ions	3 000	82 230[a]	78 000	75 000	2.35	2.14	91%[c]

[a] If PCP mineralized
[b] By PCP mass balance
[c] By Cl^- mass balance

decreased to its original level (80 mg/kg soil). For a 0.5% (w/w) inoculum fraction for bioaugmentation of a contaminated soil to be treated, the resulting PCP concentration originating from the inoculation would be much lower (0.4 mg PCP/kg soil) than the bioremediation goal (5 mg PCP/kg soil).

Characterization of the consortium (activated soil biomass)

After 31 days of incubation in the completely mixed soil bioreactor, the total heterotrophic bacteria had increased from 10^7 cfu/g soil to 10^{10} cfu/g while the PCP-degrading bacteria had increased from 10^5 cfu/g soil to 10^8 cfu/g. Controls made with plates without added PCP did not show any growth of colonies.

Stability of the PCP-degrading activity in the soil-attached consortium

The stability of the PCP-degrading activity of the activated soil biomass after production in the bioreactor was monitored over time. Using this type of inoculum for a soil bioremediation application at an industrial scale may require the activated inoculum produced to be stored. As shown in Fig. 2, the activated soil biomass kept its PCP-degrading activity up to 35 days when stored at room temperature. During this period, the lag phase lasted between 2 h and 20 h but the maximal cumulative mineralization level always reached between 77% and 87%. When kept at 4 °C, the PCP-degrading activity of the activated soil biomass remained constant over 3 months of storage (results not shown). These results indicate that the simple storage of the produced biomass at 4 °C would be sufficient to maintain the PCP-degrading activity of the consortium.

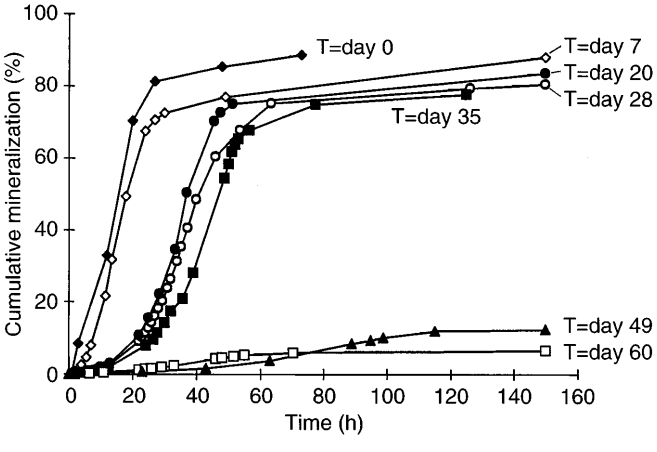

Fig 2 Effect of storage time (at 20 °C) on the PCP-degrading activity of the activated soil biomass

Bioaugmentation

PCP biodegradation

The efficiency of the activated soil consortium was tested for the treatment of a PCP-contaminated soil (soil 1), which had no or few indigenous microorganisms capable of degrading PCP. Three forms of inoculum (slurry activated soil, centrifuged activated soil and flocculent biomass) were introduced into static soil macrocosms to evaluate the effect of the biomass immobilization and the effect of chloride concentration in the slurry activated soil on the overall treatment efficiency. As shown in Fig. 3A, the PCP concentration in inoculated soils decreased from 400 mg/kg to 5 mg/kg in 130 days, independently of the type of inoculum used, while the PCP concentration remained relatively constant in the non-inoculated soil. Thus, 98% of the PCP found initially in the contaminated soil was degraded when the acclimated consortium (activated soil or flocculent biomass) was added to the soil.

Statistical analysis at a 95% confidence level did not show any significant difference between the PCP concentration levels associated with the different types of inoculum (slurry activated soil, centrifuged slurry activated soil or flocculent biomass). Consequently, neither the chloride concentration in the slurry activated soil inoculum nor the immobilized state of the activated soil biomass seemed to affect the efficiency of bioaugmentation under the conditions studied. The maximum degradation rates were similar in all inoculated soils (25 mg PCP kg^{-1} day^{-1}).

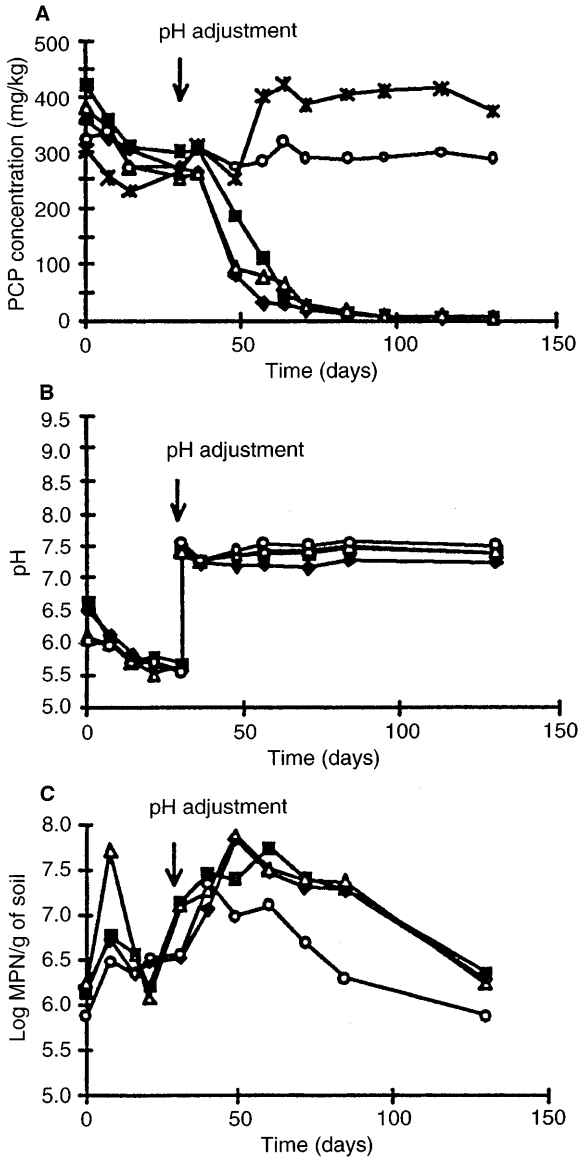

Fig. 3A–C Changes of PCP concentration (**A**), pH (**B**) and the biomass density (total heterotroph) (**C**) in soil inoculated with centrifuged activated soil (◆), with slurry activated soil (■), with flocculent biomass (Δ), in the noninoculated soil (○) and in the sterilized noninoculated soil (*)

pH effect

After 30 days of treatment, the pH of the inoculated soils decreased greatly as a result of PCP degradation (Fig. 3B). Initially at a value of 6.6, the pH in the inoculated soils decreased to 5.7 in 30 days. This acidification apparently reduced the biomass activity since the total heterotrophic bacterial population density decreased by more than a factor of 10 when the pH fell below 6.0 (Fig. 3C). Moreover, the PCP concentration in the inoculated soils reached a stationary level between 250 mg and 300 mg PCP/kg during the same period of time (Fig. 3B). After pH adjustment on day 30 with $CaCO_3$ addition, the PCP biodegradation resumed and the PCP concentration reached 5 mg/kg as the biomass density increased to $10^{7.5}$ MPN/g. The subsequent decrease in heterotrophic bacterial counts was associated with the reduction in available carbon substrate for growth.

Discussion

Activation of the indigenous microbial consortium, with PCP as the unique carbon source, has produced an adapted, resistant and active biomass. Results showed that, within 30 days, PCP-degrading bacteria increased to 10^8 cfu/g soil and an increasing PCP degradation rate (from 7 to 167 mg l^{-1} day^{-1} within 21 days) was obtained. Moreover, the mass balance performed on PCP and chloride ion content in the reactor indicated that more than 90% of the added PCP was biodegraded by the consortium. Studies on PCP degradation rate using epilithic consortia (Brown et al. 1986) have shown a similar degradation rate, whereas PCP degradation by a pure culture (*Flavobacterium*) reached 40–90 mg l^{-1} day^{-1} (Saber and Crawford 1985). In this study, the observed maximum PCP-degrading rate was 200 mg l^{-1} day^{-1}.

During the production of the activated soil biomass in the completely mixed soil slurry bioreactor, PCP degradation by the consortium was inhibited by PCP concentrations greater than 300 mg/l, which compared favorably with tolerance levels of 160–200 mg PCP/l reported in the literature for *Pseudomonas* sp. (Radehaus and Schimdt 1992), *Flavobacterium* sp. (Gonzales and Hu 1991) and *Arthrobacter* strain NC (Stanlake and Finn 1982). Furthermore, this tolerance level is similar to the one reported by Otte et al. (1994), who showed that a PCP-acclimated consortium attached to soil particles was inhibited by PCP concentrations exceeding 300 mg/l. The pollutant adsorption by soil particles probably decreased the toxicity level associated with high PCP concentrations and allowed the biomass cultivation to proceed at higher PCP concentrations. This phenomenon was also reported by O'Reilly and Crawford (1989), who showed that polyurethane afforded protection to *Flavobacterium* exposed to very high concentrations of PCP (300 mg/l), and by Ehrhardt and Rehm (1985) in a study on the degradation in bioreactor of phenol by *Pseudomonas* sp. immobilized on activated carbon.

The bioaugmentation of the PCP-impacted sandy soil, which proved to have no indigenous microorganisms able to use PCP, showed that bioaugmentation of this soil by an adapted biomass was necessary to reduce PCP concentration by 98%. Indeed, while PCP concentration in inoculated soils decreased greatly from 500 mg/kg to 5 mg/kg in 130 days, the PCP concentration in noninoculated soil remained constant. This result can be compared favorably with results reported by Mueller et al. (1991) on the bioremediation of a PCP-contaminated sandy soil, which showed that biostimulation alone (adjustment and control of pH, nutrients and water content) resulted in a 28% PCP decrease (from 265 mg/kg to 74 mg/kg) in 90 days of

treatment. Edgehill and Finn (1983) reported an 85% reduction in PCP concentration (from 2 mg/kg to 0.3 mg/kg) in a soil inoculated with a pure culture of *Arthrobacter* sp. previously acclimated to PCP, whereas no reduction in PCP concentration was observed in the noninoculated soil control.

No significant difference was observed between the PCP concentration evolution associated with the slurry activated soil and that obtained with the soil inoculated by the nonimmobilized biomass (flocculating biomass). The immobilized state of the activated soil biomass produced in the completely mixed bioreactor had thus no effect on the PCP degradation rate. The presence of the soil is important for the soil activation technique in which the contaminant sorption allowed the cultivation of the biomass at higher PCP concentrations while the presence of the soil did not interfere with the PCP biodegradation in the bioaugmentation technique. In fact, the main difference between these two experiments was in the mode of biomass activation (while the same type of soil was used). In the slurry reactor, the biomass was activated on the surface of the soil particles which were in continuous motion. In the immobilized-soil bioreactor (Karamanev et al. 1996), however, the same type of soil particles, containing the same microbial consortium, were immobilized in space and were also activated. The present results show that activity of the consortia obtained in a slurry reactor is the same as that in an immobilized-soil bioreactor since they are used in equal quantities (10^5 cfu/g dry soil). Therefore, the mode of soil activation affects the rate of consortium production, but not its specific activity per unit quantity of biomass. During the bioaugmentation experiment, an acidification of the soil associated with PCP degradation was observed. Consequently, inhibition of PCP biodegradation had occurred. Stanlake and Finn (1982) also observed the inhibition of PCP degradation by *Flavobacterium* sp. when the soil pH fell below 6.15. In that study, the PCP-degrading activity recovered once the pH was adjusted to 7.1. Therefore, pH control during soil biorestoration, especially with soil contaminated with halogenated compounds, is crucial to the success of a biotreatment.

The results of this study indicate that the soil activation process is a very effective method for the production of an acclimated biomass. The use of soil particles for indigenous biomass production significantly decreased PCP toxicity to the biomass by adsorbing the pollutant and allowing cultivation to proceed at higher PCP concentrations. Finally, the activated soil biomass represents an excellent solid inoculum for bioaugmentation of contaminated soils from the wood-preserving industry.

Practical applications

Bioremediation of PCP-contaminated soil by bioaugmentation using an activated soil consortium is a promising method to maintain the activity of the PCP-degrading biomass. This concept is currently used in a static above-ground pile for PCP-contaminated soil. It could also be used to accelerate the degradation of other contaminants such as polycyclic aromatic hydrocarbons or other recalcitrant compounds.

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