Appl Microbiol Biotechnol (1994) 40:926–932

Applied and Microbiology Biotechnology

Activation of an indigenous microbial consortium for bioaugmentation of pentachlorophenol/creosote contaminated soils*

Marie-Paule Otte[1,3], Josée Gagnon[2,3], Yves Comeau[2], Nathalie Matte[3], Charles W. Greer[3], Réjean Samson[3]

[1] Environmental Sciences Institute, University of Quebec in Montreal, P.O. Box 8888, Station A, Montreal, Quebec, Canada H3C 3P8
[2] Environmental Engineering Section, Department of Civil Engineering, Ecole Polytechnique of Montreal, P.O. Box 6079, Station A, Montreal, Quebec, Canada H3C 3A7
[3] Environmental Engineering Group, Biotechnology Research Institute, National Research Council Canada, 6100 Royalmount Avenue, Montreal, Quebec, Canada H4P 2R2

Received: 18 June 1993/Received revision: 1 September 1993/Accepted: 9 September 1993

Abstract. Soil activation, a concept based on the cultivation of biomass from a fraction of a contaminated soil for subsequent use as an inoculum for bioaugmentation of the same soil, was studied as a method for the aerobic biodegradation of pentachlorophenol (PCP) and polycyclic aromatic hydrocarbons (PAH) in contaminated soils. A microbial consortium able to degrade PCP and PAH in contaminated soil from wood-preserving facilities was isolated and characterized for PCP degradation and resistance. To obtain an active consortium from the contaminated soil in a fed-batch bioreactor, the presence of soil as a support or source of nutrients was found to be essential. During the 35 days of bioreactor operation, residual PCP in solution remained near zero up to a loading rate of 700 mg/l per day. The PCP mineralization rate increased from 70 mg/l per day when no PCP was added to the bioreactor to 700 mg/l per day at the maximum loading rate. The consortium tolerated a PCP concentration of 400 mg/l in batch experiments. Production of a PCP-degrading consortium in a fed-batch slurry bioreactor enhanced the activity of PCP biodegradation by a factor of ten. PAH biodegradation increased, during the same time period, by a factor of 30 and 81 for phenanthrene and pyrene, respectively. Preliminary laboratory-scale results indicated that a significant reduction in the time required for degradation of PCP and PAH in contaminated soil could be achieved using activated soil as an inoculum.

Introduction

The extensive use of toxic compounds such as PCP, polycyclic aromatic hydrocarbon (PAH)-laden creosote, and copper, chromium, arsenate solutions (CCA) in the wood-preserving industry has led to serious soil contamination problems. Bioremediation of these soils is a considerable challenge due to the complex mixture of organic and inorganic pollutants. Aerobic and anaerobic biodegradation of PAH and PCP has been demonstrated for pure cultures both in the laboratory and in the field (Topp and Hanson 1990; Lamar and Dietrich 1990; Weissenfels 1992). Nevertheless, the activity of pure cultures is often restricted to a limited number of contaminants and is less applicable to the complex mixture of pollutants encountered in the wood-preserving industry. Mixed indigenous strains were also shown to degrade PCP and PAH and to have the advantage of being more resistant to extreme environmental changes and predation (Fewson 1988).

The bioremediation of wood-treatment-facility soils contaminated with PCP and creosote, can be performed using several technologies. Aerobic biopile treatment has been successfully used for the biodegradation of PCP (Briglia et al. 1990; Crawford and Mohn 1985) and chlorophenols (Valo and Salkinoja-Salonen 1986) and some PAHs (Grosser et al. 1991). Although the technology is simple and cost-effective, inhibition of the growth and activity of the biomass can occur due to high concentrations of contaminants, low bioavailability of substrates, or lack of some essential nutrient(s) or O_2 (Kearney and Kellogg 1985). The observed decline in the survival and activity of pure laboratory cultures introduced into soil is caused by extreme changes in environmental conditions (Acea et al. 1988; Goldstein et al. 1985). Soil/slurry bioreactors provide a more effective method than biopile treatment for controlling environmental conditions (Mueller et al. 1991), although this is not always practical. Some of the benefits of a soil/slurry bioreactor can be applied by culturing the indigenous micro-organisms that possess pollutant biodegradation potential initially in a separate bioreactor and then using the inoculum to enhance biopile treatment.

The objective of the present research was to evaluate a process of activating the indigenous pollutant-degrading micro-organisms to enhance biopile treatment by bioaugmentation. A portion of the contaminated soil was used as inoculum and cultured in a soil/slurry

* Issued as NRC 33861

Correspondence to: R. Samson

bioreactor to enhance the activity of this indigenous biomass. The validity of the process was examined by determining the resistance of the acclimated consortium to PCP and some PAHs and maximizing activity by increasing the PCP loading rates in a fed-batch soil/slurry bioreactor.

Materials and methods

Soils

The contaminated soil was obtained from two wood-preserving industrial sites, at Tracy (soil 1) and at Delson (soil 2) Quebec (Canada). These silty sand soils had been contaminated for several years with PCP, PAH and CCA.

Soil samples were taken from three different zones at each site: near the condenser tanks (1A and 2A), from the pretreated pole storage area (1B and 2B) and from an adjacent non-contaminated forest (1C and 2C). Samples were collected from a depth of 30 cm. Each sample consisted of a composite of five subsamples from an area 3 m × 3 m. Soil was sieved (2 mm) and kept at 4° C in amber-coloured 1-l glass jars until used. Soil was characterized by Sylvestre et al. (1991) using the following tests: granulometry, moisture, pH, cation exchange capacity (CEC), mineral oil and grease, PCP, PAH and heavy metals (Table 1).

Growth medium

A mineral salts medium (MSM) was used in all experiments (Greer et al. 1990). Media were sterilized by autoclaving at 121° C for 20 min.

Chemicals

Reagent-grade PCP (99.5% pure) was obtained from Sigma (St. Louis, Mo., USA). For mineralization studies, [U-^{14}C]PCP (specific activity 440.3 MBq/mmol), [9-^{14}C]phenanthrene (484.7 MBq/mmol) and [4,5,9,10-^{14}C]pyrene (2035 MBq/mmol) were purchased from Sigma.

Microcosm experiments

Batch experiments to determine the PCP-degrading activity, PCP tolerance and the effect of soil on consortium activity were conducted in 125-ml culture flasks (microcosms) containing 20 ml of a soil slurry (5% w/v) in MSM. Flasks were sealed with rubber septa, incubated at 23° C and agitated at 200 rpm.

Characterization of the indigenous PCP-degrading consortium. To determine PCP-degrading activity of the biomass in each soil, an equivalent amount of each of the soils 1A, 1B, 2A and 2B was taken to prepare the 5% slurry. PCP (100 mg/l) spiked with [^{14}C]PCP (150000 dpm) was added to each soil slurry to follow PCP mineralization.

Isolation and identification of PCP degraders. Pure cultures were isolated from a composite mix of soils 1A, 1B, 2A and 2B. Samples were plated on a selective medium containing PCP (100 mg/l), diluted MSM (1/50 of phosphates), Oxoid agar (10 g/l, Difco), and 20 mg/l of bromothymol blue as pH indicator (Saber and Crawford 1985). Colonies that had yellow zones were isolated and purified by transferring three times on agar plates (PCP, 100 ppm; MSM; NB, 0.2 g/l, Oxoid agar, 10 g/l). Identification of bacteria in the consortium was performed using the API Rapid NFT method (Analytical Profile Index, API Analytab Products, Plainview, N.Y., USA).

PCP tolerance. The consortium tolerance to PCP was assessed by exposure to increasing concentrations of PCP (100, 200, 400, 800 and 1600 mg/l). In each microcosm 150000 dpm of [^{14}C]PCP was added to monitor mineralization during 30 days. In this experiment, tolerance was defined as the ability of the consortium to degrade PCP in 30 days.

Soil effect. The effect of the slurry soil concentration on consortium viability and activity was tested by exposing different concentrations of soil/slurries to a spiked (150000 dpm) PCP (100 mg/l) solution. After incubation the initial 5% slurry (2 days, 25° C, 200 rpm) was diluted to a 0.05% soil slurry and incubated under the same conditions for another 2 days and then a second dilution was made (0.0005% soil slurry). To evaluate the effect of soil addition on the activity of the consortium, a gamma-irradiated (50 kGray at a dosage rate of 28.3 kGray/h by ^{60}Co) (Wolf et al. 1989) silty clay agricultural soil was added to the most diluted slurry.

Fed-batch bioreactor

For the experiments on biomass production, tolerance, and activity, a 15-l fed-batch soil slurry Bioengineering bioreactor was used. The bioreactor was continuously fed without being discharged. O_2 (98% saturation), pH (7.0), temperature (25° C) and agitation (400 rpm) were controlled. Gas emissions were trapped

Table 1. Characterization of soils from wood preserving industries

Sites	pH	CEC (meq/100 g)	Water content (%)	Oil and grease (mg/kg)	Granulometry	PAH (mg/kg)	PCP (mg/kg)	Cr (mg/kg)	Cu (mg/kg)	As (mg/kg)	Zn (mg/kg)
1A	7.1	17.0	20.4	23500*	Clayed sand	919	1362	49	50	12	160
1B	7.5	7.0	8.6	2400	Loamy sand	4	16	15	25	3	89
1C	7.1	12.8	23.1	900	Uniform sand	6	<0.1	20	75	4	76
2A	6.4	2.9	10.6	51500	Uniform sand	4686	684	304	38	32	446
2B	7.1	6.5	6.5	2900	Silty sand	15	120	40	66	5	61
2C	4.0	3.3	17.4	1100	Uniform sand	<0.1	<0.1	16	8	1	21
Quebec criteria B				1000		20	0.5	250	100	30	500

CEC, Cation exchange capacity; PAH, polycyclic aromatic hydrocarbon; PCP, pentachlorophenol
* Underlined when exceeding Quebec criteria B, the level that must be attained for restoration of industrial sites

by a NaOH (0.1 M) trap followed by an activated carbon trap. The bioreactor contained a 5% (w/v) soil slurry (500 g of contaminated soil in 10.0 l of MSM).

Consortium tolerance and activity. To determine the PCP-tolerance level of the consortium in fed-batch culture, the bioreactor was fed for 35 days at increasing PCP-loading rates (0, 50, 100, 300, 500, 700 and 900 mg/l per day) with flow rates ranging from 144 to 316 ml/day. The feed solution was PCP in water adjusted to pH 10.0 with NaOH. Slurry samples (15 ml) were taken from the bioreactor every day to quantify soluble PCP and chloride by HPLC. The concentration of other ions such as HPO_4^-, NO_3^-, NH_4^+, Na^+, K^+ was also determined by HPLC.

Consortium degradation rate. PCP degradation rates were evaluated both in the bioreactor and in microcosms. In the bioreactor, continuous loading was temporarily stopped and three successive 100 mg PCP/l spikes were added. The residual PCP concentration in the bioreactor was then monitored.

PCP-degrading activity was determined in microcosms by mineralization studies with slurry samples taken from the bioreactor just before each increase in the PCP loading rate. At the 0 and 100 mg/l per day loading rates, five microcosms were prepared, receiving 0.1, 1, 10, 100 and 500 mg/l of PCP spiked with 60000 dpm of [^{14}C]PCP. At each of the 300 and 500 mg/l per day loading rates, five other microcosms were prepared, receiving 10, 100, 300 and 500 mg/l of PCP spiked with 60000 dpm of [^{14}C]PCP. PCP mineralization was then monitored in each microcosm as described above.

Mass balance

A mass balance for PCP and PAH was performed on the contents of the bioreactor. The solid-phase, the liquid-phase and the gas traps were extracted as described below, at the beginning and at the end of the experiment, and abiotic losses, due to volatilization or adsorption, were determined.

PAH degradation

The PAH-degrading activity of the consortium was evaluated in 125-ml microcosms. Soil/slurry samples (20 ml) taken from the bioreactor at a 100 mg/l per day loading rate were spiked with radioactive phenanthrene (25000 dpm corresponding to 7.8 μg/l) and pyrene (10000 dpm corresponding to 10 μg/l), and mineralization was monitored as described above.

Efficiency of soil activation

To test the efficiency of the acclimated, resistant and active consortium produced by activating the soil, preliminary qualitative studies were conducted in which contaminated soils were inoculated with the biomass produced in the bioreactor. PCP- and PAH-contaminated soil (mix of an equivalent amount of soils 1A, 1B, 2A and 2B) was amended with 50 mg/kg of PCP spiked with 90000 dpm of [^{14}C]PCP and bioaugmented with centrifuged slurry (10% w/w) taken from the bioreactor at a loading rate of 600 mg/l per day. Mineralization was monitored for 15 days.

Analytical methods

PCP mineralization in 125 ml microcosms was followed by trapping $^{14}CO_2$ in a 5 ml tube containing 1.0 ml of 0.5 M KOH. After recovery and rinsing, a scintillation cocktail (ACS, Amersham, UK) (18 ml) was added to KOH samples and the solution was analysed using a liquid scintillation counter (Packard Instrument, Tri-Carb model 4530).

Contaminant extraction. PCP and PAH in soil samples were Soxhlet extracted for 16 h with CH_2Cl_2 following drying of the samples with sodium sulphate and spiking with *p*-terphenyl (recovery standard). In soil slurries, the samples were centrifuged at 5000 rpm for 15 min and the water phase and solid phase (pellet) were extracted separately. The water phase (100 ml) was extracted three times with CH_2Cl_2 (15 ml) and the pellet was Soxhlet-extracted as described above. The extracts were concentrated to 5 ml using a rotary evaporator (Büchi model RE 120) and purified by column chromatography. Two millilitres of the extract were added to the top of the column (300 mm × 10.5 mm) containing 8 g silica gel (100–200 mesh) previously activated at 105° C for 16 h. The extract was first eluted with 35 ml hexane to extract oil and grease and then with 75 ml hexane containing 20% CH_2Cl_2 to extract alkylated and non-alkylated PAH. The column was finally eluted with 35 ml of methanol to obtain PCP.

Contaminant analysis. Soluble PCP was analysed on filtered samples (1 ml; Millex-HV 0.45-μm filters, Millipore) by HPLC (Spectra-Physics SP 8800) on an ODS-1 type chromatography column (250 mm × 4.6 mm) with an injection volume of 10 μl. The mobile phase was an isocratic mix of 80% methanol and 20% water acidified to pH 3.5 with concentrated H_3PO_4 (0.7 ml/min, 55° C). Detection was at 308 nm using a Spectra-Physics detector (model SP8440XR). HPLC grade PCP (Sigma, St. Louis, Mo., USA) was used as standard.

PAH were analysed by gas chromatography/mass spectrometry (GC/MS) in the single-ion monitoring mode. Mass-spectral analysis of the fractions was performed using a HP 5890 GC coupled with an HP 5970 MS detector. The GC was equipped with a 30 m × 0.25 mm DB5 capillary column with a 0.25-μm stationary phase. One microlitre of the sample was injected, the carrier gas being helium. The injector was at 250° C and the detector at 280° C. The oven was maintained at an initial temperature of 55° C for 3 min, then increased by 4° C/min to a final temperature of 280° C, which was maintained for 10 min, for a total elution time of 70 min.

Soluble ion analysis. Soluble anions were analysed on filtered samples (1 ml; Millex-HV 0.45-μm filters, Millipore) with a conductivity detector (Waters model 431) by HPLC (Spectra-Physics SP 8800) on a Hamilton PRP-X100 chromatography column (250 mm × 4.1 mm) with an injection volume of 50 μl. The mobile phase was an isocratic mix of 10% methanol and 7 mM *p*-hydroxybenzoic acid adjusted to pH 8.5 with 5 M NaOH (1.75 ml/min, 40° C).

Soluble cations were analysed on filtered samples (1 ml; Millex-HV 0.45-μm filters, Millipore) with a conductivity detector (Waters model 430) by HPLC (Spectra-Physics SP 8100XR) on a Hamilton PRP-X200 chromatography column (250 mm × 4.1 mm) with an injection volume of 10 μl. The mobile phase was a mix of 35% methanol and 6 mM HNO_3 (0.75 ml/min, 40° C).

Results and discussion

Microcosm experiments

Characterization of the consortium. Biodegradation tests showed that three of the four contaminated soil samples (1A, 1B and 2A) contained PCP-degrading organisms. The time required to obtain the maximum mineralization rate ranged from less than 4.5 to 9.0 days. These maximum rates corresponded to the inflection

point of the growth curves, at which point, according to previous studies with 2,4-dichlorophenoxyacetic acid, the substrate was completely removed from solution (Comeau et al. 1993). Bacterial and fungal colonies were obtained by growth of the consortium on non-selective nutrient medium. Only bacteria grew on selective media employing PCP as the sole carbon source and many of the colony types could not be successfully maintained in culture. Two PCP-degrading strains were isolated from the consortium and one was identified as *Pseudomonas luteola.* In uncontaminated forest soils, sampled adjacent to the wood-treatment facility, no biodegradation of PCP was detected after 30 days of incubation.

PCP tolerance. In batch culture, PCP degradation by the consortium was inhibited at concentrations greater than 400 mg/l, which compares favourably with tolerance levels of 160 to 200 mg/l reported for *Pseudomonas* sp. (Radehaus and Schmidt 1992), *Flavobacterium* sp. (Gonzales and Hu 1991) and *Arthrobacter* strain NC (Stanlake and Finn 1982).

Soil effect. The consortium activity decreased significantly with a reduced soil concentration in the slurry (Fig. 1). The biodegradation rate decreased from 33 mg/l per day for the undiluted slurry to 16 mg/l per day for the 0.05% slurry and to 1 mg/l per day for the most diluted slurry. The addition of sterile soil (1%, w/v) to the most diluted slurry resulted in a 25 mg/l per day activity corresponding to a 92% recovery of the consortium activity. Soil addition clearly enhanced the PCP biodegradation rate of the consortium.

These results suggested that the positive effect of soil addition can be attributed to the provision of more surface area for the attachment of bacteria and substrate, or the provision of nutrients available in the sterile soil. Soil particles can provide support for the biomass, increasing the global biomass activity and they can also adsorb contaminants on their surface, decreasing the toxicity to the micro-organisms. Other studies indicated that fine silt and clay particles gave similar results for benzene, toluene, ethylbenzene, xylene (BTEX) and naphthalene biodegradation in aquifer sediments (Holm et al. 1992; Oldenhuis et al. 1989). The use of bark chips as a solid support for a PCP-degrading consortium also significantly decreased PCP toxicity for the biomass by adsorbing the pollutant and allowing degradation to proceed at higher PCP concentrations (Apajalahti and Salkinoja-Salonen 1984). Immobilization of bacteria on calcium alginate enhanced their toxicity threshold and increased the biodegradation rate of PCP and *p*-cresol (O'Reilly et al. 1988).

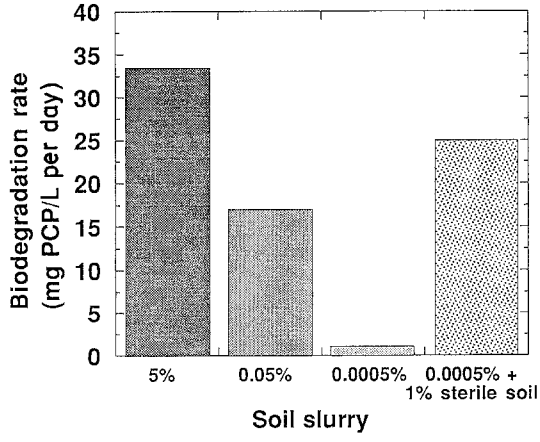

Fig. 1. Effect of soil concentration on the degradation of 100 mg/l of pentachlorophenol (PCP)

Fed-batch bioreactor studies

Consortium PCP tolerance and activity. Results obtained using the fed-batch soil/slurry bioreactor showed that during the first 2 days of operation, when no PCP was added to the system, a low level of PCP was detected in the solution, originating from PCP desorption from the soil particles to the water phase (Fig. 2A, B). After this time, residual PCP disappeared gradually until day 4, demonstrating degradation activity by the indigenous biomass. Preliminary studies had shown that a lag phase of 2.5 days was necessary to initiate PCP-degradation activity, which was in agreement with the lag phase observed in the bioreactor study. After adding 50 mg/l per day of PCP to the system, a shorter acclimation period (1 day) was needed to enable the biomass to degrade this PCP concentration. During the subsequent increases in the PCP loading rate from 50 mg/l per day to 700 mg/l per day of PCP, soluble PCP was undetectable in the bioreactor. These results showed an increase in the activity of the consortium with increasing PCP loading rates to the bioreactor and a tolerance level of up to 700 mg/l per day in fed-batch culture. Studies on PCP tolerance using epilithic consortia (Brown et al. 1986) showed similar tolerance levels, whereas PCP-degradation activity by pure cultures (*Flavobacterium* sp.) reached 40 to 90 mg/l per day (Saber and Crawford 1985).

After 35 days of bioreactor operation the soluble PCP concentration increased dramatically within 3 days. At the 700 mg/l per day PCP loading rate, the NaCl concentration was 6077 mg/l (104 mM) and soluble PCP was undetectable, whereas at the 900 mg/l per day PCP loading rate, the NaCl concentration increased to 7785 mg/l (133 mM) and soluble PCP concentration increased from 0 to 1560 mg/l. This indicated an inhibition of consortium activity that could be attributed to a combination of the high salt concentration and the inhibitory level of PCP in solution. A concentration of 86 mM sodium chloride was shown to be responsible for inhibition of PCP degradation by *Flavobacterium* sp. (Gonzalez and Hu 1991).

Consortium degradation rate. When the bioreactor was spiked with three successive 100 mg/l additions of PCP, the time for complete degradation, as measured by

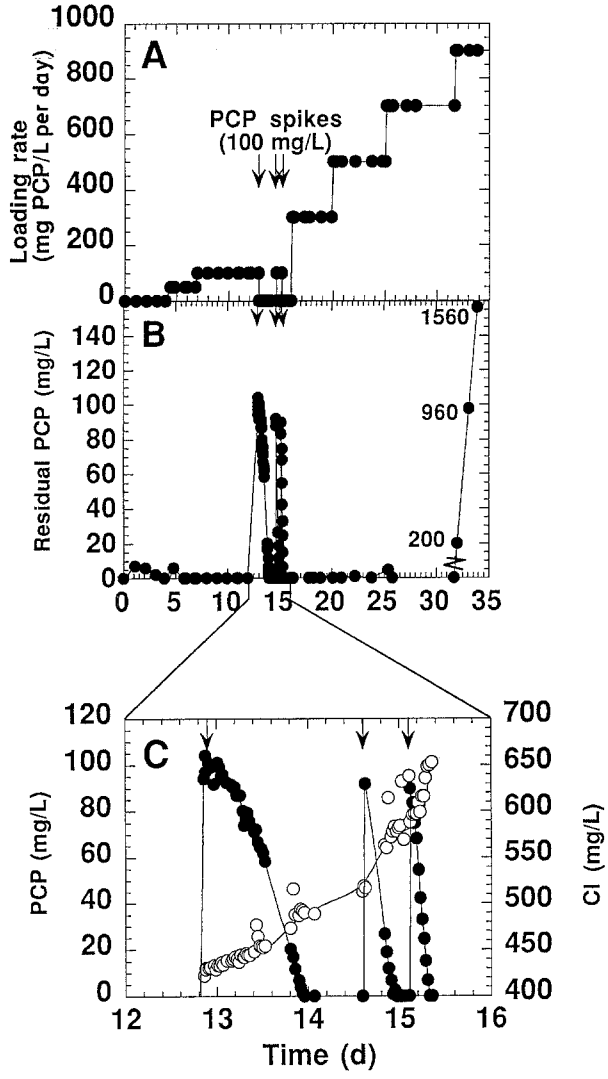

Fig. 2A–C. PCP biodegradation in fed-batch slurry bioreactor. **A** PCP loading rate. **B** Residual PCP. **C** Spikes of PCP (zoom from 12 to 16 h): ●, PCP; ○, Cl^- measured; —, Cl^- calculated

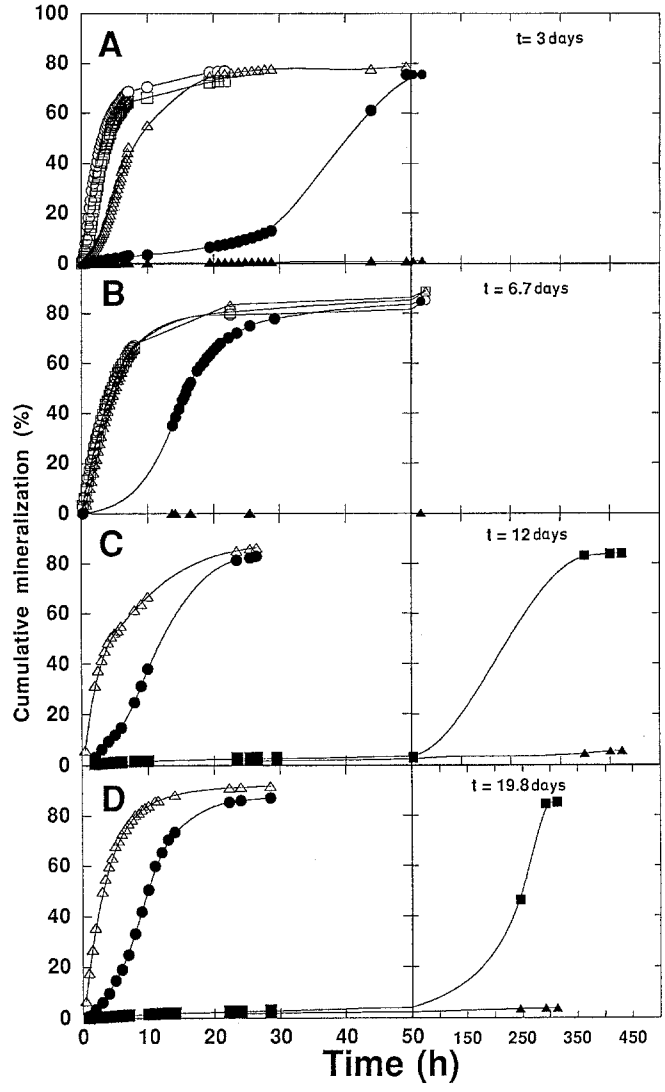

Fig. 3A–D. Effect of loading rate on the mineralization of PCP. Samples were taken from the soil/slurry reactor for determination of mineralization rates when the PCP loading rates were: **A** 0 mg/l per day, **B** 100 mg/l per day, **C** 300 mg/l per day, or **D** 500 mg/l per day. Concentrations of the ^{14}C[PCP] spikes were: ○, 0.1 mg/l; □, 1 mg/l; △, 10 mg/l; ●, 100 mg/l; ■, 300 mg/l; ▲, 500 mg/l. Data are shown for 0–50 h (*left frame*) and for 50–500 h (*right frame*)

HPLC, decreased from 25 h to 10 h to 5 h for each successive spike (Fig. 2C). This corresponded to an increase in the degradation rates from 140 to 275 to 450 mg/l per day, calculated from the slope of the curve at the maximal degradation rate. Chloride ion analysis demonstrated a stoichiometric production of Cl^- from the added PCP. The PCP-degrading activity was increased by 300% by spiking three times with 100 mg/l of PCP over a 3-day period. This suggested that an efficient way to increase the activity and possibly the tolerance of the consortium would be to add consecutive spikes of PCP instead of loading PCP continuously at increasing levels.

A step-wise increase in the continuous PCP loading rate from 0 to 500 mg/l per day in the bioreactor increased the maximum biodegradation rate of the consortium, as demonstrated by parallel microcosm studies in which samples were removed from the bioreactor and spiked with PCP (0.1, 1, 10, 100, 300 and 500 mg/l) (Fig. 3).

Before the bioreactor was loaded with PCP, a lag phase in the mineralization activity was observed. This occurred at concentrations equal to or greater than 10 mg/l (Fig. 3A). The lag phase increased as the PCP spike concentration increased. Similar results have been observed with pure cultures (Gonzalez and Hu 1991; Stanlake and Finn 1982). Increasing the PCP loading rate to the bioreactor resulted in a progressive decrease in the lag phase at the different PCP spike concentrations. For example, when the bioreactor loading rate was 100 mg/l per day (Fig. 3B), concentrations of PCP from 0.1 to 10 mg/l were degraded at the same rate with no lag phase. With increasing loading rate, the lag phase for the 100-mg/l spike decreased from 20 h and reached a minimum at a loading rate of 500 mg/l per day. The lag phase for the 300-mg/l spike

was more than 50 h but PCP mineralization occurred after 250 h, whereas no mineralization occurred at the 500 mg/l-spike after more than 400 h incubation (Fig. 3C, D). The marked reduction of the lag phase demonstrated that increasing the PCP loading rate of the bioreactor resulted in a good acclimation of the consortium and the PCP tolerance limit for the consortium was between 300 and 500 mg/l. This tolerance was identical to that initially found in batch cultures.

Mass balance

A mass balance for PCP and PAH was performed on the contents of the bioreactor (Table 2). In the liquid fraction, PCP was always below the detection limit (<0.5 mg/l), which confirmed that PCP biodegradation occurred continuously. In addition, PCP in the solid fraction of the bioreactor remained almost constant in spite of a continuous addition of PCP for a total amount of 120000 mg added in 30 days.

The concentration of several PAH with two to four rings decreased substantially in 500 h in the liquid and solid fractions. For example, acenaphthene was reduced from a total of 99 to 10 mg, phenanthrene from 40 to 13 mg, fluoranthene from 112 to 29 mg, pyrene from 73 to 26 mg and benzo(*a*)anthracene from 21 to 9 mg. Since the traps did not show any significant accumulation of PAH, volatilization and adsorption could not explain this reduction in concentration (Table 2). It suggested that the biomass maintained a PAH-degrading activity while PCP was added at increasing loading rates. Culturing the biomass on PCP in the slurry bioreactor stimulated both PCP- and PAH-degrading activity.

Interestingly, several of the high-molecular mass PAHs increased in concentration during the operation of the bioreactor (Table 2). In the absence of biodegradation, this may be attributed to breaking up of the soil particles during bioreactor operation resulting in a more efficient extraction of non-bioavailable substrate, as has been seen in other investigations (Middleton et al. 1991).

PAH degraders

To confirm that the activated biomass loaded with PCP could improve PAH degradation, liquid microcosms were prepared in which [^{14}C]phenanthrene and [^{14}C]pyrene were added to the consortium. A 20% mineralization level was obtained in 2 h for phenanthrene and in 4 h for pyrene, whereas in the native soil, the same level was obtained in 60 h for phenanthrene and in 324 h for pyrene. These results showed that acclimation of the consortium in the bioreactor increased the mineralization rate of phenanthrene and pyrene 30-fold and 81-fold, respectively, over the rates observed in the native soil.

Efficiency of soil activation

The efficiency of the produced consortium was qualitatively tested using contaminated soil (mix of an equivalent amount of soils 1A, 1B, 2A and 2B). Inocula from the soil/slurry were introduced into static soil microcosms and PCP mineralization was monitored. Within 36 h, 50% of the PCP was mineralized in the inoculated soil, while no mineralization was observed

Table 2. Mass balance of PCP and creosote in the 10-l bioreactor

Compounds	Time = 0 days			Time = 35 days				
	Total (mg)[a]	Liquid (mg)	Solid (mg)	Total (mg)	Liquid (mg)	Solid (mg)	Traps (mg)	Solid residues (mg)
Naphthalene	6	0	6	21	0	19	0.0	2
Acenaphthylene	3	1	2	1	0	0	0.0	0
Acenaphthene	99	31	68	10	0	7	0.3	4
Fluorene	35	8	27	2	0	0	0.0	2
Phenanthrene	40	5	35	13	0	9	0.0	3
Anthracene	30	3	27	13	0	10	0.0	3
Fluoranthene	112	6	106	29	0	17	0.0	12
Pyrene	73	4	69	26	0	16	0.0	10
Benzo(*a*)anthracene	21	1	20	9	0	6	0.0	3
Chrysene	22	1	21	14	0	10	0.0	4
Benzo(*b*)fluoranthene	10	0	10	63	3	50	0.0	10
Benzo(*k*)fluoranthene	9	0	9	0	0	0	0.0	0
Benzo(*a*)pyrene	9	0	9	37	2	29	0.0	6
Indeno(1,2,3-*cd*)pyrene	5	0	5	18	0	15	0.0	3
Dibenzo(*ah*)anthracene	1	0	1	13	0	12	0.0	1
Benzo(*ghi*)perylene	0	0	0	3	0	1	0.0	2
Total PAH	475			273				
Pentachlorophenol	136	60	76	208	11	103	0.0	94

[a] Milligrams per reactor

in the uninoculated soil during the same time period. These preliminary data suggested that activating the soil significantly increased PCP mineralization activity of the consortium in contaminated soil.

Activation of an indigenous microbial consortium to give acclimated, resistant and active biomass can be performed in a bioreactor using contaminated soil as an initial inoculum. The presence of soil enhanced consortium-degrading activity. Loading the bioreactor with PCP as sole source of carbon stimulated the biodegradation of both PCP and some PAHs. These results indicate that the activated soil process is a promising approach for the restoration of sites contaminated by the wood-preserving industry. Extrapolation to full scale will require the determination of the most favourable (environmental) conditions for the production and use of activated soil.

Acknowledgements. We thank Dr. Jalal Hawari for valuable discussion, Chantale Beaulieu, Danielle Ouelette, Alain Corriveau and Stéphane Deschamps for technical assistance, the Natural Sciences and Engineering Research Council and the National Research Council for financial support to M-P.O.

References

Acea MJ, Moore CR, Alexander M (1988) Survival and growth of bacteria introduced into soil. Soil Biol Biochem 20:509–515

Apajalahti JHA, Salkinoja-Salonen M (1984) Absorption of pentachlorophenol (PCP) by bark chips and its role in microbial PCP degradation. Microbial Ecol 10:359–367

Briglia M, Nurmiaho-Lassila E-L, Vallini G, Salkinoja-Salonen M (1990) The survival of the pentachlorophenol-degrading *Rhodococcus chlorophenolicus* PCP-1 and *Flavobacterium* sp. in natural soil. Biodegradation 1:273–281

Brown EJ, Pignatello JJ, Martinson MM, Crawford RL (1986) Pentachlorophenol degradation: a pure bacterial culture and a epilithic microbial consortium. Appl Environ Microbiol 52:92–97

Comeau Y, Greer CW, Samson R (1993) Role of inoculum preparation and density on the bioremediation of 2,4-D contaminated soil by bioaugmentation. Appl Microbiol Biotechnol 38:681–687

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

Fewson CA (1988) Biodegradation of xenobiotics and other persistent compounds: the causes of recalcitrance. TIBTECH 6:148–153

Goldstein RM, Mallory LM, Alexander M (1985) Reasons for possible failure of inoculation to enhance biodegradation. Appl Environ Microbiol 50:977–983

Gonzalez JF, Hu W-S (1991) Effect of glutamate on the degradation of pentachlorophenol by *Flavobacterium* sp. Appl Microbiol Biotechnol 35:100–104

Greer CW, Hawari J, Samson R (1990) Influence of environmental factors on 2,4-dichlorophenoxyacetic acid degradation by *Pseudomonas cepacia* isolated from peat. Arch Microbiol 154:317–322

Grosser RJ, Warshawsky D, Vestal JR (1991) Indigenous and enhanced mineralization of pyrene, benzo[*a*]pyrene, and carbazole in soils. Appl Environ Microbiol 57:2462–3469

Holm PE, Nielsen PH, Albrechtsen H-J, Christensen TH (1992) Importance of unattached bacteria and bacteria attached to sediment in determining potentials for degradation of xenobiotic organic contaminants in an aerobic aquifer. Environ Microbiol 58:3020–3026

Kearney PC, Kellogg ST (1985) Microbial adaptation to pesticides. Pure Appl Chem 57:389–403

Lamar RT, Dietrich DM (1990) In situ depletion of pentachlorophenol from contaminated soil by *Phanerochaete* spp. Appl Environ Microbiol 56:3093–3100

Middleton AC, Nakles DV, Linz DG (1991) The influence of soil on bioremediation of PAH-contaminated soils. Remediation (Autumn):391–406

Mueller JG, Lantz SE, Blattmann BO, Chapman PJ (1991) Bench-Scale evaluation of alternative biological treatment processes for the remediation of pentachlorophenol and creosote contaminated materials: slurry-phase bioremediation. Environ Sci Technol 25:1055–1061

O'Reilly KT, Kadakia R, Korus RA, Crawford RL (1988) Utilization of immobilized-bacteria to degrade aromatic compounds common to wood-treatment wastewaters. Water Sci Technol 20:95–100

Oldenhuis R, Kujik L, Lammers A, Janssen DB, Withold B (1989) Degradation of chlorinated and non-chlorinated aromatic solvents in soil suspension by pure bacterial cultures. Appl Microbiol Biotechnol 30:211–217

Radehaus PM, Schmidt SK (1992) Characterization of a novel *Pseudomonas* sp. that mineralizes high concentrations of pentachlorophenol. Appl Environ Microbiol 58:2879–2885

Saber DL, Crawford RL (1985) Isolation and characterization of *Flavobacterium* strains that degrade pentachlorophenol. Appl Environ Microbiol 50:1512–1518

Stanlake GJ, Finn RK (1982) Isolation and characterization of a pentachlorophenol-degrading bacterium. Appl Environ Microbiol 44:1421–1427

Sylvestre P, Hawari J, Mayer R (1991) Sols contaminés par l'industrie de préservation du bois – extraction et photodéchloration du PCP. In: Proceedings of the 14th International Symposium on Wastewater Treatment, Montreal, Quebec, Environment Canada, Ottawa, Canada, pp 1–24

Topp E, Hanson RS (1990) Degradation of pentachlorophenol by a *Flavobacterium* species grown in continuous culture under various nutrient limitations. Appl Environ Microbiol 56:541–544

Valo R, Salkinoja-Salonen M (1986) Bioreclamation of chlorophenol-contaminated soil by composting. Appl Microbiol Biotechnol 25:68–75

Weissenfels WD (1992) Adsorption of polycyclic aromatic hydrocarbons (PAH) by soil particles: influence on biodegradability and biotoxicity. Appl Microbiol Biotechnol 36:689–696

Wolf DC, Dao TH, Scott HD, Lavy TL (1989) Influence of sterilization methods on selected soil: microbiological, physical, and chemical properties. J Environ Qual 18:39–44