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Degradation of *p*-nitrophenol and pentachlorophenol mixtures by *Sphingomonas* sp. UG30 in soil perfusion bioreactors

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The degradation of mixtures of pentachlorophenol (PCP) and *p*-nitrophenol (PNP) were evaluated in pure cultures of *Sphingomonas* sp. UG30, statically incubated soils (60% water-holding capacity) and soil perfusion bioreactors where encapsulated cells of UG30 were used as a soil inoculant. In pure-culture studies, conditions were optimized for mineralization of PCP and PNP mixtures at concentrations of 30 mg l^{-1} each. Optimum *in vitro* mineralization of PCP and PNP mixtures by UG30 was facilitated using ammonium phosphate as a nitrogen source, while inhibition was observed with ammonium nitrate. The bioreactor system used columns containing soil treated with mixtures of 100, 225 or 500 mg kg^{-1} of PCP and PNP. Rapid dissipation of both substrates was observed at the 100 mg kg^{-1} level. Inoculation with UG30 enhanced PCP degradation at the 100 mg kg^{-1} level in bioreactors but not in static soil microcosms. At higher PCP and PNP concentrations (225 mg kg^{-1}), occasional complete degradation of PNP was observed, and PCP degradation was about 80% compared to about 25% in statically incubated soils after 20 days at 22°C. There was no additional degradation of the PCP and PNP mixtures attributable to inoculation with encapsulated cells of UG30 in either soil system at concentrations of 225 or 500 mg kg^{-1}. *Journal of Industrial Microbiology & Biotechnology* (2000) **25,** 93–99.

Keywords: bioreactor; degradation; encapsulation; *p*-nitrophenol; pentachlorophenol; perfusion; remediation; soil; *Sphingomonas*

Introduction

Polluted soils are often contaminated with complex mixtures of xenobiotic chemicals. However, most bioremediation research has evaluated the degradation of a single chemical pollutant. Some studies have compared the biodegradation of related compounds with varying substitutions, e.g., chlorophenols, in straw compost [15,16] and in a fluidized-bed reactor [24]. Studies evaluating the simultaneous degradation of two different chemicals are not as common, or alternatively the research examines undefined mixtures such as petroleum hydrocarbons [8]. In one study, the fate of pentachlorophenol (PCP) and the insecticide lindane (hexachlorocyclohexane) in activated sludge reactors was assessed [13]. PCP and creosole contamination are often coupled and successful remediation of soils contaminated with mixtures of PCP and creosote has been reported [22].

Sphingomonas sp. strain UG30 was isolated from PCP-contaminated soil and demonstrated mineralization of up to 250 mg l^{-1} PCP [17]. Further studies revealed that UG30 was capable of degrading up to 100 mg l^{-1} *p*-nitrophenol (PNP) [18], 2,4-dinitrophenol and dinitrocresol [30]. In addition, PCP degradation in soil was enhanced when UG30 was introduced as cells encapsulated in κ-carrageenan [6]. To assess UG30 as a bioaugmentation agent for bioremediation of chemical mixtures, we investigated its potential for degradation of mixtures of PCP and PNP. Pure culture studies were initially used to assess the ability of UG30 to mineralize PCP and PNP mixtures, focusing on the influence of different nitrogen sources on PCP and PNP mineralization. Soil perfusion bioreactors were used to assess its ability to degrade these mixtures in soil. κ-Carrageenan-encapsulated UG30 cells were also introduced into the soil perfusion bioreactors to determine if cell encapsulation affected PCP and PNP degradation.

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Materials and methods

Bacterial strains and chemicals

Sphingomonas sp. UG30 was cultured and maintained in mineral salts (MS)+4% glutamate (w:v) medium (MMG) and on MMG agar. Stock cultures were maintained as frozen glycerol stocks at −20°C. Mineral salts solution (pH 7.0) contained (in mM) 2.39 K_2HPO_4, 0.97 KH_2PO_4, 0.41 $MgSO_4 \cdot 7H_2O$, and 0.02 $FeSO_4$, and 2.94 $NaNO_3$ [17]. Glucose and glutamate media contained 4.0 g l^{-1} of glucose and monosodium glutamate, respectively, *p*-nitrophenol (Sigma, St. Louis, MO, USA; anhydrous spectrophotometer grade) and uniformly labeled [^{14}C]PNP (Sigma; specific activity 6.6 mCi/mmol, purity 98%) were used. Sodium pentachlorophenate (Sigma; technical grade) and uniformly labeled [^{14}C]PCP (Sigma; specific activity 6.5 mCi/mmol, purity 95.5%) were used in this research.

Media optimization for mineralization of PCP and PNP mixtures in liquid culture

To determine a suitable carbon source for UG30 growth and mineralization in the PCP and PNP mixture, MS solution was

supplemented with 4.0 g l^{-1} glucose (MSG) and either 4.0 or 0.4 g l^{-1} glutamate. This was based on previous optimization of media for mineralization of 2,4-dinitrophenol by *Sphingomonas* sp. UG30 with glucose/glutamate mixtures (4.0 and 0.4 g l^{-1}, respectively) [30]. To determine a suitable nitrogen source for mineralization, glucose (4.0 g l^{-1}) was used as the sole carbon source and different supplemental nitrogenous compounds ($NaNO_3$; NH_4NO_3; $(NH_4)_2SO_4$; or $(NH_4)_2HPO_4$) were added at a concentration of 2.94 mM.

Mineralization studies were performed using 250-ml biometer flasks [23] containing 50 ml of media and an initial inoculum of 10^8 cells ml^{-1}. Mineralization was measured by trapping $[^{14}C]CO_2$ in 2 N NaOH and liquid scintillation counting. To assess mineralization of PCP and PNP in a mixture, two treatments were used. *Sphingomonas* sp. UG30 cells were initially grown in MSG medium amended with 50 μg ml^{-1} PNP to induce cells. After cells reached mid-exponential phase they were centrifuged, washed and transferred to MSG media amended with 30 mg ml^{-1} PCP and PNP each. In the first treatment, 0.003 mM (0.090 μCi l^{-1}) [^{14}C]PNP was added to determine PNP mineralization in the mixture. In the second treatment, 0.003 mM (0.090 μCi l^{-1}) [^{14}C]PCP was added to determine PCP mineralization in the mixture. As a positive control for PNP, one set of three flasks containing MSG media and UG30 cells had 30 mg ml^{-1} PNP with 0.003 mM [^{14}C]PNP added to the media to determine mineralization of PNP alone. As a positive control for PCP, one set of three flasks containing MSG media and UG30 cells had 30 mg ml^{-1} PCP with 0.003 mM [^{14}C]PCP added to the media to determine mineralization of PCP alone. Two replicates of two non-inoculated controls were added using the following media. One set contained glucose (4.0 g l^{-1}), glutamate (4.0 g l^{-1}) and $NaNO_3$ (2.94 mM). The second set contained glucose (4.0 g l^{-1}), glutamate (4.0 g l^{-1}) and $NaNO_3$ (2.94 mM). Flasks were incubated at room temperature (23°C) on a rotary shaker at 200 rpm for 18 days. Treatments to evaluate nitrogen sources were conducted in triplicate, while treatments to evaluate carbon sources were done in duplicate.

Preparation of soil

Soil used in this experiment was collected from an agricultural site in Cambridge, Ontario, Canada. Soil from this site has been used in our previous research [10]. The soil was sieved (2 mm) and amended with mixtures of PCP and PNP to attain concentrations of 100, 225, or 500 mg kg^{-1} PCP and PNP. The PCP and PNP mixture was added as a water/ethanol (50:50 vol:vol) solution (<60 ml kg^{-1} soil), added slowly and mixed with a sterile, stainless steel spatula. The soil with an initial moisture content of 8% to 13% was aged in 4-l glass containers for 2 weeks in the dark at 4°C to minimize microbial activity. For the first set of experiments with 100 mg kg^{-1} PCP and 100 mg kg^{-1} PNP, the mixed soil was aged for 4 weeks at 4°C before use.

Soil extraction

Soil samples were extracted at zero time (t=0 days) and at the end of the soil column experiment (20 days). Soil (10.0 g dry weight) was extracted with 30 ml of acidified acetone (acetone with 1.0% 1 N HCl) for 16 h on a rotary shaker at 200 rpm and centrifuged at 6,000×g for 10 min. The supernatant was removed and the soil re-extracted with 20 ml of acidified acetone (~2 h, 200 rpm). The extracts were combined and evaporated to dryness under N_2 gas. The residue was dissolved in 4 ml of methanol and filtered through a 0.22-μm pore-size nylon membrane prior to HPLC analysis.

Encapsulation of *Sphingomonas* sp. UG30 cells in κ-carrageenan

Sphingomonas sp. UG30 cells were harvested at late-exponential phase (OD_{600} of 1.0≈log 9.07 cfu ml^{-1}) by centrifugation at 16,300×g for 10 min. Beads were prepared as previously described [5,6] and dried in a laminar flow hood at 22°C for 12 h and stored at 4°C until used in soil columns. Culturable cell numbers in the κ-carrageenan beads were determined by enumerating colonies on MMG agar as described elsewhere [5,6].

Soil column bioreactors

A diagram of the bioreactors is shown in Figure 1. Soil columns consisted of Pyrex glass tubes (I.D. 25 mm, 400 mm length), containing 100 g of soil (dry weight) mixed with 25 g of washed gravel. Inoculated columns were also amended with beads containing the organism (1.0 g per column) mixed to achieve a good distribution. The length of the internal soil column was 20 cm. Soil bioreactors were established as a continuous soil perfusion system. A peristaltic pump (Ismatec, Cole-Parmer Instrument Company, Chicago, IL, USA) provided continuous percolation of

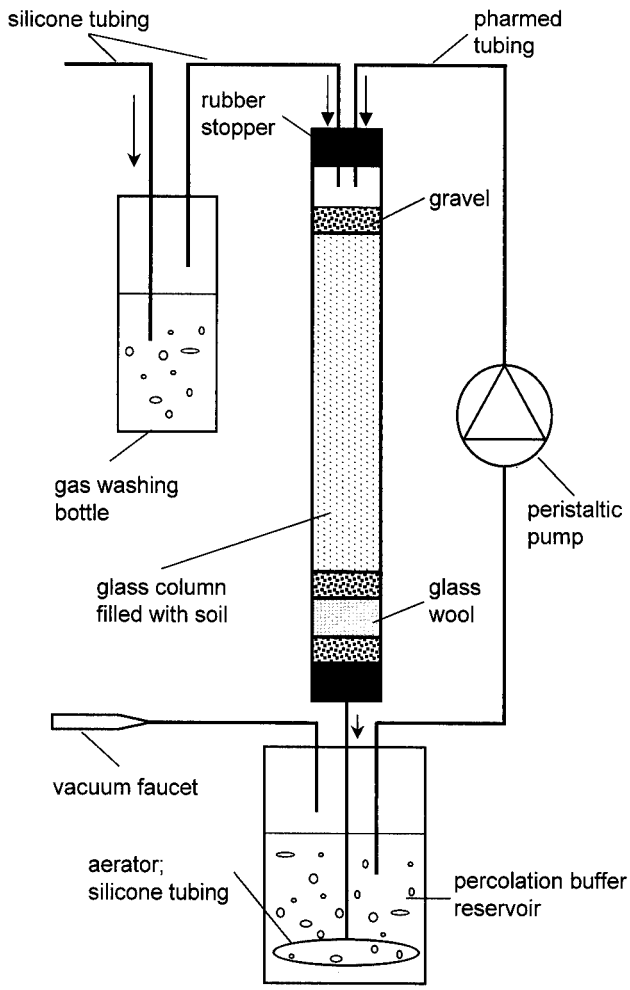

Figure 1 Schematic of soil perfusion column.

sterile potassium phosphate buffer (pH 7.0, 50 mM). The buffer reservoir contained 250 ml potassium phosphate buffer. The flow rate in the initial experiment, which used 100 mg kg^{-1} PCP and PNP was 1.2 l day^{-1}, while the flow rates for columns that used 225 and 500 mg kg^{-1} mixtures were 0.84 and 0.52 l day^{-1}, respectively. Aeration (perfusion buffer and bioreactor atmosphere) was facilitated by laboratory vacuum lines. The first perfusion buffer samples (15 ml each) were taken on day 1, and subsequent samples were taken every third day. About 5 to 10 h before sampling, buffer levels in the reservoirs were adjusted with deionized water to compensate for evaporation. Fresh buffer (15 ml) was always added after removal of a sample to maintain the perfusion buffer volume at 250 ml.

Static soil experiments

Degradation studies of the same PCP and PNP mixtures were also performed in 50-ml Corex tube (Fisher, Toronto, Ontario, Canada) microcosms to compare with results from soil bioreactor columns. Briefly, 20 g of soil (dry weight) were placed in each tube. Microcosms were maintained at constant moisture (18%) under static conditions. The 100, 250 and 500 mg kg^{-1} PCP and PNP mixtures were added to non-inoculated soil and soil inoculated with 0.2 g of dry beads containing *Sphingomonas* cells. Four replicates each of inoculated and of non-inoculated soil microcosms were used for each concentration of PCP and PNP. Tube caps were loosely placed to facilitate air exchange. Microcosms were incubated at room temperature (~22°C) for 20 days.

Analytical techniques

Nitrite concentrations in samples were estimated spectrophotometrically following diazonization [20]. Chloride release (an indicator of PCP dechlorination) was assessed using a chloride-specific electrode (Combination Chloride Electrode 94-17BN, Orion, Boston, MA, USA), and an Accumet Model 15 pH meter as described by Seech *et al.* [25]. Column effluent samples were concentrated for HPLC analysis using C18 solid phase extraction (SPE) columns (Bakerbond, 500 mg sorbent weight, J.T. Baker, Phillipsburg, NJ, USA). Samples (10 ml) were acidified with 1 ml of 0.1 N hydrochloric acid, diluted with 10 ml ultrapure water and passed through a conditioned SPE column under vacuum. Absorbed compounds were eluted with 4 ml of acetonitrile and filtered through a 0.22-μm pore-size nylon membrane.

HPLC analyses for the initial soil bioreactor experiment which used 100 mg kg^{-1} and for parts of the soil bioreactor experiment which used 500 mg kg^{-1} were performed on a Shimadzu HPLC (Shimadzu Instruments, Baltimore, MD, USA: SCL-6B system controller; 2 LC-6A pumps; SPD-M6A photodiode array UV–VIS Detector; and Class-VP version 4.1 software. A Phenomenex Spherex C18 reverse phase column (5 μm, 250×4.6 mm) (Phenomenex, Torrance, CA, USA) and gradient [(initial=60% acetonitrile: 40% acidified water (1% acetic acid)] attaining 100% acetonitrile at 14 min, maintained for 5 min and the composition returned to 60:40 within a minute and equilibrated. PCP was quantified at 250 nm while PNP was quantified at 320 nm. Samples were suitably diluted if necessary. The flow rate used with all methods was 0.6 ml min^{-1}. HPLC analyses for the other studies were performed on a Waters system (Waters, Medford, MA, USA: 2690 Alliance separation module, 996 photodiode array detector, and Millenium 32 software) using the same column and gradient conditions as described above. A 30-μl aliquot of the processed sample was injected for HPLC analysis and samples were suitably diluted if necessary.

Results and discussion

Optimization of carbon source for mineralization of PCP and PNP mixtures

Previous studies demonstrated that growth in, and mineralization of PCP alone by *Sphingomonas* sp. UG30 cells were effective in a minimal medium with 4 g l^{-1} glutamate (MMG) [5,17], but not with glucose as a carbon source [17]. Addition of glutamate to the medium may have prevented a loss of cell viability in the presence of PCP [11]. Mineralization studies using UG30 cells in MMG resulted in a maximum $[^{14}C]CO_2$ recovery of about 60% for PCP [5] and about 35% for PNP in minimal medium amended with glucose [18]. Inhibition of mineralization of PNP alone was observed using glutamate-amended minimal medium (MMG), while the best mineralization of PNP was observed using glucose with no additional nitrogen sources (MSG) [18]. Inhibition of PNP mineralization was observed at either glutamate concentration tested in the glucose medium. However, the two concentrations used did not affect PCP mineralization. We observed recovery of 54.1±7.2% and 62.5±6.4% as $[^{14}C]CO_2$ from the glucose medium with either 0.4 or 4.0 g l^{-1} glutamate, respectively. Topp *et al.* [29] reported that a combination of 1 g l^{-1} each of glutamate and glucose inhibited mineralization of 75 mg l^{-1} PCP alone by *Sphingomonas* sp. ATCC 39723. This was not observed in the study of mineralization of PCP alone by *Sphingomonas* sp. UG30 [26] and in this study. The minimal salts medium supplemented with 4.0 g l^{-1} glucose as a carbon source was therefore used for further investigations.

Effect of nitrogen source on mineralization of PCP, PNP, or PCP and PNP mixtures

The standard medium for growth and PCP-mineralization (MMG) included sodium nitrate ($NaNO_3$) as a nitrogen source [5,17]. However, Leung *et al.* [18] reported that mineralization of PNP by *Sphingomonas* sp. UG30 cells significantly decreased, to 22% from 35%, when $NaNO_3$ was included in the medium. They observed that a mineral salts medium with 4 g l^{-1} glucose (MSG) containing no other nitrogen source resulted in optimal PNP mineralization.

The use of $NaNO_3$ with either PCP or PNP alone resulted in cumulative $[^{14}C]CO_2$ evolution of about 65% for PCP but only 23% for PNP (Figure 2a). In the PCP and PNP mixture, PCP was mineralized despite a lag of 5 days. The large error bar at day 7 is due to a lack of activity in one of the replicates until after 7 days. PNP mineralization in the mixture was observed for only one replicate, but only at a slightly lower level (15%) than PNP alone (23%).

The same concentration (molarity) of each nitrogen source was added to the mineralization media. Since NH_4NO_3 contributes twice the inorganic N, levels of available N were twice the other treatments. Mineralization of PCP and PNP either alone or in combination was inhibited by these higher nitrogen levels (Figure 2b). The results for most treatments were variable. A lag-phase of 5 to 6 days was observed for both single-compound treatments. PCP in the mixture was mineralized to the same level as PCP alone,

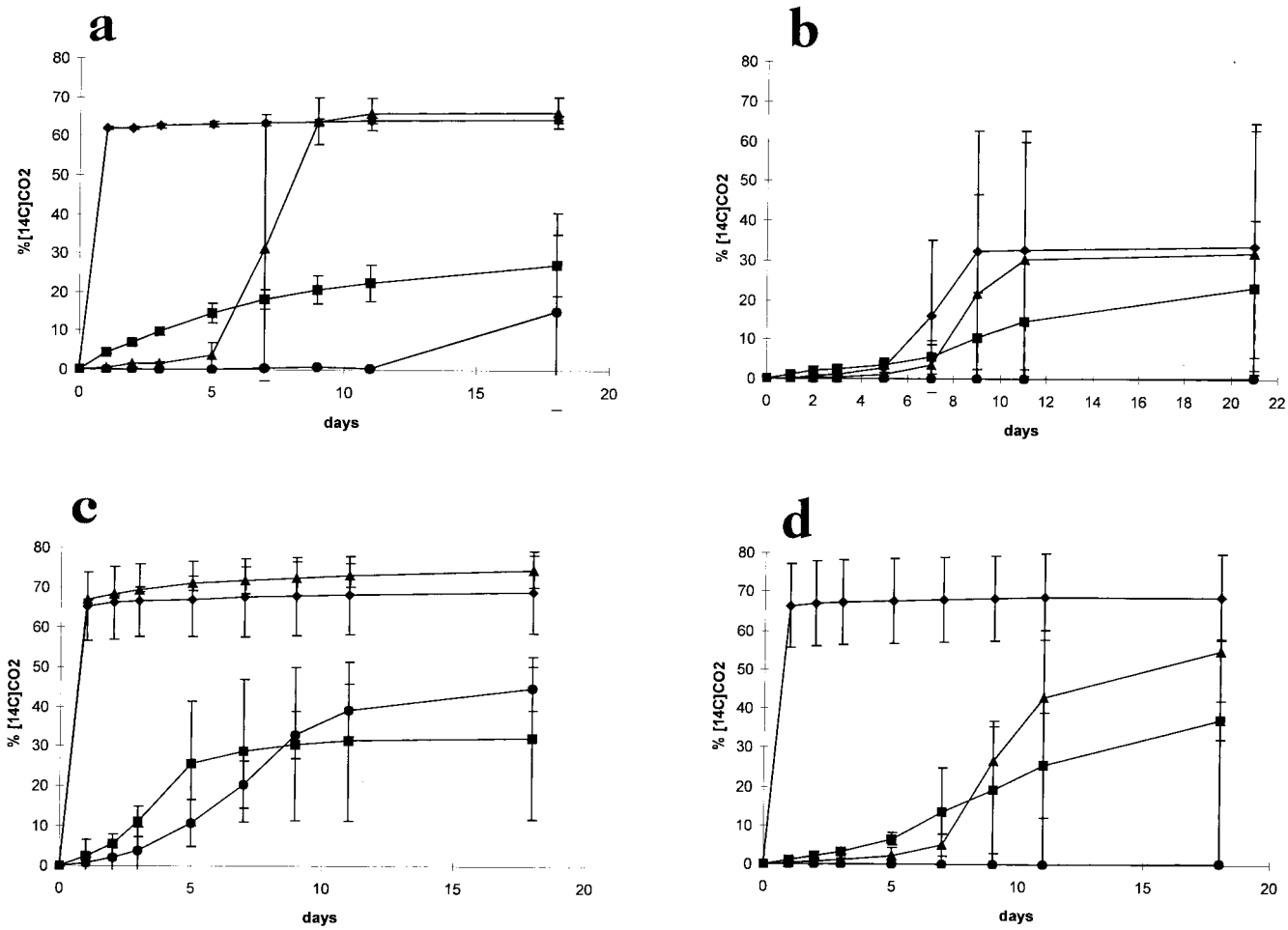

Figure 2 Mineralization of 30 mg l^{-1} PCP (✦), 30 mg l^{-1} PNP (■), or a mixture of 30 mg l^{-1} each of PCP and PNP (▲ = [^{14}C]PCP; = [^{14}C]PNP) by *Sphingomonas* sp. UG30 using (a) $NaNO_3$ as nitrogen source; (b) NH_4NO_3 as nitrogen source; (c) $(NH_4)_2HPO_4$ as nitrogen source; (d) $(NH_4)_2SO_4$ as nitrogen source. Data are means of three replicates ± one standard deviation.

whereas no mineralization was observed for PNP in the mixture over 18 days.

PCP and PNP were both mineralized (65% and 30–40%, respectively) either as single compounds, or in a mixture when $(NH_4)_2HPO_4$ was used as a nitrogen source (Figure 2c). There was no lag phase observed for PCP mineralization in the mixture. In previous studies of PCP mineralization Cassidy *et al.* [5,6] observed enhanced PCP mineralization with the addition of 1000 ppm phosphorus to medium or soil. The positive influence of phosphate seemed to overweigh the inhibitory affects of ammonium. The use of ammonium phosphate was determined to be useful for optimizing mineralization.

Ammonium sulphate ($(NH_4)_2SO_4$) was tested to ensure that any enhanced effect observed with ammonium phosphate was not due to the ammonium. Both PCP and PNP mineralization were complete, although a lag phase of 3 days was observed for PNP mineralization (Figure 2d). PCP mineralization in the mixture was slightly lower than for PCP alone, and a lag phase of 5 days was observed. No mineralization of PNP in the mixture was observed over 18 days.

Although determination of a suitable nitrogen source for degradation of PCP and PNP mixtures was the major interest for testing the various sources, the HPO_4^{2-} and SO_4^{2-} anions may have affected the degradative activity of *Sphingomonas* sp. UG30. Topp *et al.* [29] investigated effects of nutrient limitation using a PCP-degrading *Flavobacterium* sp. They reported that soil slurries with phosphate, glucose or glucose+PCP limitations were more sensitive to PCP toxicity compared to cells grown under ammonium-, sulphate- or PCP-limiting conditions. The authors concluded there is a relationship between phenotypic variation of *Flavobacterium* sp. and its biodegradation efficacy, apparently mediated through differences in sensitivity to PCP. Our data are similar to the results reported by Topp and Hanson [29]. Sulphate and ammonium inhibited PCP mineralization when available, and not inhibitory when they were limited. Enhanced mineralization was observed when phosphate was added, but not when phosphate was limited. Similar results were also observed with the mixtures of PCP and PNP. We did not explore mechanisms that could explain this observation further. However, results supported a conclusion that optimization of nutrient limitations for growth of inocula destined for use in degradation of polluted soils is necessary [29].

Soil perfusion bioreactors

Soil perfusion bioreactors containing mixtures of 100 mg kg^{-1} PCP and 100 mg kg^{-1} PNP allowed removal of PNP even when

Table 1 PCP recovery from soil treated with mixtures of 100 mg kg^{-1} PCP and PNP (10 mg total per column) after 7 days in a perfusion bioreactor compared to statically incubated soil. Effects of inoculation with *Sphingomonas* UG30. All PNP was degraded after 1 day (data not shown)

	Inoculated		Non-inoculated	
	Rep 1	Rep 2	Rep 1	Rep 2
mg PCP recovered per column				
PCP removed from buffer	0.07	0.08	0.2	0.2
PCP residual in buffer	0.0	0.0	3.3	4.9
Soil extractable	0.0	0.0	0.9	1.6
% Initial PCP recovered				
Total recovery (bioreactor)[a]	<1.0	<1.0	42.8	66.3
Avg. total recovery (static study)	65.2±0.2 (n=4)		70.2±2.6 (n=4)	

[a]Corrected for 15-ml sample removal.

Sphingomonas sp. UG30 was not inoculated into the bioreactor (data not shown). Within 24 h, no PNP was measured in the perfusion buffer. Initial concentrations of PCP in the perfusion buffer ranged between 12 and 15 mg l^{-1}. These concentrations decreased to non-detectable values when soil was inoculated with *Sphingomonas* sp. UG30 (Table 1). By contrast, about 15 mg l^{-1} PCP remained in the perfusion buffer in the non-inoculated soil. A mass balance of PCP recovery from the experiment which used the 100 mg kg^{-1} mixture is summarized in Table 1. About 99% removal of PCP occurred in inoculated columns, whereas 43 to 66% remained in columns containing non-inoculated soil. In experiments using 100 mg kg^{-1} mixture in static culture, a similar level of PCP was recovered from inoculated soil and non-inoculated soil (65% and 70%, respectively). These results suggest removal of PNP in the bioreactor system allowed degradation of 100 mg kg^{-1} PCP in soil when soil was inoculated with *Sphingomonas* sp. UG30. However, this was not observed in non-inoculated soils.

Since negligible degradation of PCP and PNP was observed at the 500 mg kg^{-1} level, data are presented only for the 225 mg kg^{-1} level. Rapid depletion of PNP in the perfusion buffer was observed in only one of four inoculated and one of four non-inoculated columns containing soil treated with 225 mg kg^{-1} of PCP and PNP (Table 2). No PNP remained in the soil from these two columns when the study was terminated. By contrast, complete degradation of PCP was not observed with the 225 mg kg^{-1} mixture (Table 3). The initial concentrations of PCP in the perfusion buffer solution ranged from 25 to 30 mg l^{-1}, and decreased to 10 to 15 mg l^{-1} following 20 days of incubation. Mass balances of PNP and PCP recovery from the study involving 25 mg kg^{-1} are summarized in Tables 2 and 3, respectively. In two columns (7 and 8) complete removal of PNP was observed, while about 65% remained in three inoculated and three non-inoculated bioreactors. This amount of PNP recovery was slightly lower than that determined for the static soil experiment. A lower recovery of PCP was observed in soils from the bioreactor compared to those in static soil, however no benefit of inoculation with *Sphingomonas* sp. UG30 was observed. Although complete degradation of PNP was observed in columns 7 and 8 (Table 2), PCP degradation in these columns was similar to columns 1 to 6 where PNP was not degraded (Table 3).

In soil perfusion bioreactors, biodegradation can be optimized due to control of moisture, aeration, dilution of substrate (to lower toxicity), facilitated desorption and enhanced bioavailability. Bruns-Nagel *et al.* [4] developed a system that optimized these parameters for remediating 2,4,6-trinitrotoluene (TNT)-contaminated soil. In their study the addition of nutrients (especially glucose) was necessary for optimal degradation. Our setup using the soil perfusion bioreactor, with phosphate buffer as the only added nutrient, was not satisfactory for degrading high concentrations of PNP and PCP. *In vitro* studies with ammonium phosphate suggest use of a phosphate buffer would be advantageous to degradation of PCP and PNP mixtures. Addition of glucose may also enhance co-metabolism of PCP and PNP. A ratio of 2.5:1 of perfusion buffer to soil was used. This resulted in initial equilibrium buffer concentrations of PNP of about 27, 51, and 135 mg l^{-1} in soil treated with 100, 225 or 500 mg kg^{-1} PNP and PCP, respectively. In liquid culture, *Sphingomonas* sp. UG30 mineralized 30 mg l^{-1} PNP but not 100 mg l^{-1} when amended with 100 mg l^{-1} PCP (data not shown). In a pure culture of the organism, maximum degradation of PNP occurred at about 42 mg l^{-1} PNP, and was limited at concentrations above 100 mg l^{-1} [18], while PCP-degradation in pure culture was limited at concentrations above 250 mg l^{-1} [17].

Other researchers have demonstrated a significant amount of chloride release accompanying PCP degradation [22,25]. Although PCP disappearance was observed from the perfusion buffer, and from the soil (in the amount of extractable PCP determined), we did not observe a corresponding chloride accumulation in the bioreactor study (data not shown). Dechlorination is necessary for mineralization of PCP and usually occurs as the first step in the degradation process [7]. If PCP was binding to soil, chloride release should have been detected since dehalogenation of chlorinated phenols has also been observed in polymeriza-

Table 2 PNP recovery from soil treated with mixtures of 225 mg kg^{-1} PCP and PNP (or 22.5 mg each per column) after 20 days in a perfusion bioreactor compared to statically incubated soil. Effects of inoculation with *Sphingomonas* UG30

	Inoculated columns				Non-inoculated columns			
	1	3	5	7	2	4	6	8
mg PNP recovered per column								
PNP removed from buffer	4.2	4.8	4.9	1.3	4.9	4.8	5.0	1.6
PNP residual in buffer	9.7	9.2	9.1	<0.1	9.5	9.5	8.8	<0.1
Soil extractable	2.1	2.3	2.4	<0.1	2.1	2.1	2.1	<0.1
% Initial PNP recovered								
Total recovery (bioreactor)	64.4	64.9	65.3	<1	65.9	65.5	62.2	1.6
Avg. recovery (bioreactor)	64.8±0.5			<0.1	64.5±2.0			<0.1
Total recovery (static)	79.1	90.2	82.3	89.8	81.8	78.7	79.1	75.6
Avg. recovery (static)	85.4±5.5 (n=4)				78.8±2.5 (n=4)			

Table 3 PCP recovery from soil treated with mixtures of 225 mg kg^{-1} PCP and PNP (or 22.5 mg each per column) after 20 days in a perfusion bioreactor compared to statically incubated soil. Effects of inoculation with *Sphingomonas* UG30

	Inoculated columns				Non-inoculated columns			
	1	3	5	7	2	4	6	8
mg PCP recovered per column								
PCP removed from buffer	1.5	1.3	1.8	1.6	1.9	1.8	2.0	1.7
PCP residual in buffer	2.3	2.5	2.6	2.1	1.9	1.8	2.0	1.7
Soil extractable	2.4	2.2	2.3	2.7	2.1	2.3	2.3	0.9
% Initial PCP recovered								
Total recovery (bioreactor)	27.1	21.9	23.6	22.9	19.4	19.8	21.0	12.5
Avg. recovery (bioreactor)	23.9±2.3				18.2±3.8			
Total recovery (static)	63.6	84.9	77.3	83.1	82.2	59.1	70.0	77.8
Avg. recovery (static)	77.2±9.6 (n=4)				72.3±10.1 (n=4)			

tion reactions with humic compounds [9]. From the results of our study, the fate of PCP could not be determined. However, during PNP degradation nitrite was released (data not shown) which indicated metabolism of the parent PNP compound.

At 100 mg kg^{-1}, concentrations of both PNP and PCP were low enough to permit degradation of PNP by indigenous soil microorganisms. At the 100 mg kg^{-1} level, enhanced PCP degradation was observed in soil columns inoculated with *Sphingomonas* sp. UG30. At higher concentrations of PCP and PNP infrequent but complete degradation of PNP was observed in columns resulting from indigenous microorganisms. The ability to stimulate intrinsic bioremediation of nitroaromatics was demonstrated [1,2], and of chlorophenols [14–16] using various bioremediation technologies. This is important since the successful introduction of specific degrading microorganisms at contaminated sites can be a problem [3]. Costs for bioremediation can be significantly reduced by strategies based on stimulation of intrinsic bioremediation instead of bioaugmentation with free or encapsulated cells.

Encapsulation of microbial cells has been researched to minimize adverse effects of PCP toxicity and enhance its mineralization [5,6,12,21,27]. However, in this study, encapsulation was not useful for overcoming effects of the toxicity of PCP and PNP mixtures for microbial degradation to occur. The use of adsorbents such as sawdust has been investigated to reduce bioavailability of PCP in the soil solution [19]. This approach may be suitable for the PCP and PNP mixtures. The importance of toxicity reduction affected by the methodology (e.g., type of bioreactor used or amendments applied) has been demonstrated [13,28]. These authors observed that optimization of adsorption, desorption and biodegradation processes are necessary to enable maximum degradation.

Conclusion

The mechanism for inhibition of degradation of PCP and PNP in mixtures is not understood. It appears the presence of both compounds increases toxicity interfering with growth and activity of the degrading microbial population. From the *in vitro* growth studies presented here and elsewhere there are conflicting nutritional requirements for PCP and PNP degradation. A perfusion reaction system offers the potential for enhanced degradation of PCP and PNP mixtures. However, by understanding the mechanisms of inhibition, more effective methods may be incorporated to optimize degradation of pollutant mixtures.

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