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The microbial degradation of chlorophenolic preservatives in spent, pressure-treated timber

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Abstract

The reduction of pentachlorophenol in treated timber, after inoculation with pentachlorophenol-degrading bacterial species, *Rhodococcus chlorophenolicus* and *Flavobacterium* sp., and the white-rot fungus *Phanerochaete chrysosporium*, was monitored in solid substrate systems and in liquid culture suspensions. In solid substrate systems there was no significant pentachlorophenol degradation by the bacterial species under a variety of conditions. Under similar conditions, *Phanerochaete chrysosporium* transformed over 80% of the starting concentration of 500 ppm to pentachloroanisole. In liquid culture suspensions however, mid-exponential phase *Flavobacterium* sp. cells were able to degrade over 99% of the pentachlorophenol in sawdust and wood chips due to the extraction of PCP from the timber as a water soluble salt. There were however no significant changes in the chlorinated dioxin components during this treatment.

Abbreviations: ATTC – American type culture collection, AWPA – American Wood Preservers' Association, DSM – Deutsche Sammlung für Mikroorganismen, GC/MS – gas chromatograph/mass spectrometer, HpCDD – heptachlorodibenzo-*p*-dioxin, HpCDF – heptachlorodibenzofuran, HxCDD – hexachlorodibenzo-*p*-dioxin, HxCDF – hexachlorodibenzofuran, ^{13}C-OCDD – carbon 13-labelled octachlorodibenzo-*p*-dioxin, OCDD – octachlorodibenzo-*p*-dioxin, OCDF – octachlorodibenzofuran, PCDDs – polychlorinated dibenzo-*p*-dioxins, PCDFs – polychlorinated dibenzofurans, PCP – pentachlorophenol, PnCDD – pentachlorodibenzo-*p*-dioxin, PnCDF – pentachlorodibenzofuran, TCDD – tetrachlorodibenzo-*p*-dioxin, TCDF – terachlorodibenzofuran, TeCP – tetrachlorophenol, WHC – water holding capacity, w/v – weight for volume ratio

Introduction

Although the use of oil-borne pentachlorophenol (PCP) as a timber preservative is diminishing throughout North America and Europe, there remains a large reservoir of treated timber in service. Despite a practical life of greater than 50 years, treated utility poles can still contain PCP concentrations close to the original treatment retentions (12,000–27,000 ppm) when removed from service (Cui & Ruddick 1994). In Canada alone, over 100,000 PCP-treated utility poles are expected to be removed from service this year, and, although a portion of these poles can be reused, the disposal options for the remainder are extremely limited. The environmental restraints on the release of chlorinated organics in pulp mill effluent exclude pulping for the disposal of PCP-treated poles, and the alternative routes of landfilling and high-temperature incineration also impart significant problems, including the limited capacity of landfill sites, with the attendant requirements for leachate monitoring and control, or the public resistance to the construction of high-temperature incineration facilities. Today, utility companies are often unable to dispose of removed poles and must rely on temporary storage. Over the past decade, much research into the biological reme-

diation of chlorophenol-contaminated soils has been carried out (Compeau et al. 1990; Lamaer & Dietrich 1990; Salkinoja-Salonen et al. 1989; Valo & Salkinoja-Salonen 1986; Crawford & Mohn 1985), and the application of such technologies to remove and/or destroy the preservative in spent timber offers an extremely promising disposal method. The choice of bioremediation as a disposal solution is enhanced by both the availability of timber processing techniques, such as chipping and milling, and the contained nature of the waste-wood. Conversely, however, PCP in pressure treated timber may not be readily accessible to microbial mineralisation; a problem compounded by the organic structure of wood, and the carrier oils and chlorinated impurities in the preserving solution. This paper examines the suitability of known chlorophenol-degrading microorganisms for timber bioremediation, and describes the results from the use of composting and liquid culture remediation methods.

Materials and methods

Organisms and media

The following three aerobic, PCP-degrading organisms were chosen for this study.

i) *Rhodococcus chlorophenolicus* DSM 43826, a Gram positive actinomycete, isolated by Apajalahti and Salkinoja-Salonen (1986) from a column packed with soil and bark chips and exposed to PCP for two years.

ii) *Flavobacterium* sp. ATCC 53874, a Gram negative species, isolated by Saber and Crawford (1985) from PCP-contaminated soil. This organism can tolerate higher concentrations of PCP than *R. chlorophenolicus* in liquid culture. Both organisms however exhibit a high degree of PCP mineralisation (Apajalahti & Salkinoja-Salonen 1986; Saber & Crawford 1985) and have been used in soil decontamination studies (Salkinoja-Salonen et al. 1989; Crawford & Mohn 1985).

iii) *Phanerochaete chrysosporium* BKM-F-1767, a ligninolytic fungus capable of PCP metabolism (Mileski et al. 1988). Although this organism exhibits a low degree of PCP mineralisation (2%; Lamar et al. 1990), its ability to penetrate into wood cell lumens, thus access PCP in wood, made it an appropriate choice. *Ph. chrysosporium* has been used in both soil and wood decontamination studies, but biotransformation, as well as degradation, was also a removal mechanism (Lamar & Dietrich 1990, 1992; Lamar et al. 1990).

Flavobacterium sp. was grown in the glutamate-containing medium of Steiert et al. (1987), and induced for PCP degradation at mid-exponential phase by the addition of 100 μM PCP. *R. chlorophenolicus* was cultured in DSM-65 medium containing (gl^{-1}): glucose (4.0); yeast extract (4.0); malt extract (10.0); pH = 7.0, and induced with 10 μM PCP at late exponential phase. *Ph. chrysosporium* was grown on 1% malt extract agar plates containing 10 μM PCP. A mineral salts buffer, containing (gl^{-1}): K_2HPO_4 (0.64); KH_2PO_4 (0.31); NH_4Cl (0.5); $MgSO_4 \cdot 7H_2O$ (0.2); $CaCl_2$ (0.02); $FeSO_4 \cdot 7H_2O$ (0.005); pH = 7, was used for cell washes.

Sources of PCP treated timber

Material treated with PCP in carrier oil was obtained from the groundline of a 1967 jack pine pole removed from service in 1989. The outer 20 mm contained approximately 15,000 ppm PCP and was either ground to 20 mesh sawdust, or chipped (particle size approx. $2 \times 1 \times 0.5$ cm). For experiments at lower PCP retentions, strips of *Pinus ponderosa* sapwood ($5 \times 2.5 \times 0.3$ cm) were treated in the laboratory to 500 ppm PCP according to the standard method of the American Wood Preservers' Association (AWPA 1989).

Extraction and analysis of chlorinated phenols

Pentachlorophenol concentration in culture samples was determined by modification of the method of Apajalahti and Salkinoja-Salonen (1986). Acetyl esters were prepared from culture samples by dilution with 2 volumes of 0.06 M sodium bicarbonate buffer (pH = 9.9), containing 2,4,6-tribromophenol as an internal reference compound, and addition of excess acetic anhydride. The samples were shaken and left to stand for 30 minutes. Acetylated chlorophenols were then extracted into n-hexane. Aliquots of the hexane layer were analysed on an HP5890 series 2 gas chromatograph equipped with a VG Trio 1000 mass selective detector (VG Instruments, Pointe Claire, Québec). Splitless injections were made onto a 25 m, 0.2 mm, 0.33 μm film HP-5 column. The chromatography conditions were: injector 200° C; interface temperature 250° C; initial oven temperature 50° C, held for 2 minutes, then at 25° C min^{-1} to 100° C and at 10° C min^{-1} to 250° C, with the final temperature held for 1 minute. The carrier gas was helium at a flow rate

of 1 ml min^{-1}. The detection limit, with an ionisation potential of 70 eV, was 10 ppb. For extraction of PCP from pole material, all samples were first ground to 20 mesh sawdust. Samples (0.5 g) were then sonicated for 15 minutes with 10 ml of 1% (v/v) glacial acetic acid in methanol. After centrifugation (5000 × g, 10 minutes), the supernatant was transferred to a 25 ml volumetric flask. The sawdust residue was then further extracted as above, and extracted for a third time with 5 ml of methanol. All extracts were then combined. This extract was purified to remove carrier oils on a florisil column as follows. A small volume of the extract (accurate to 0.01 ml, containing 10–20 μg PCP) was mixed with the internal standard (2,4,6-tribromophenol, 20 μg) in a 5 ml vial and evaporated to dryness with a stream of nitrogen. The residue was transferred with the help of small amount of 1:1 hexane/methylene chloride (1–2 ml) to a microcolumn (30 × 0.1 cm) containing 1 gram florisil. The column was eluted with 10 ml 1:1 hexane/methylene chloride. This fraction, which contains mainly oil, was discarded. The column was then eluted with 10 ml 1:9:0.1 diethyl ether/hexane/acetic acid (volume ratio) and the eluent, which contained mainly PCP, TeCP, and the internal standard, collected. A portion of this eluent (1 ml) was evaporated to dryness with nitrogen. The residue was dissolved in 1 ml of pH 9.9 sodium bicarbonate buffer, and the sample derivatised with acetic anhydride as described above. The acetates were then extracted with 1 ml isooctane and 1 μl of the extract injected into GC as above. The extraction efficiency was greater than 95%.

*Extraction and analysis of polychlorinated dibenzo-*p*-dioxins and dibenzofurans*

All solvents used for extraction of these chemicals were omnisolv® (spectral grade) solvents from BDH, and the Brockman Activity I basic alumina (Fisher Scientific) was activated at 130° C over night before use. Prior to use, the acid purified sea sand was extracted with acetone and dried. The ^{13}C-OCDD was purchased from Cambridge Isotope Laboratories, California, USA and the chlorinated dibenzo-*p*-dioxin and dibenzofuran standard mixtures were obtained from Chromatographic Specialties Inc., California, USA. All other chemical were of analytical grade. Sawdust samples (1 gram, 20 mesh) were Soxhlet extracted with 100 ml toluene for 24 hours and liquid samples (200 ml) were extracted four times with 50 ml toluene. The toluene extract from all samples was evaporated on a Rotavapor at 40° C and cleaned up as follows. The residue was dissolved in a minimal amount of methylene chloride and mixed with purified sea sand. After evaporation of the methylene chloride with a stream of nitrogen, the sea sand was added to the top of a column (30 × 0.7 cm) containing 5 g of activated basic alumina with the help of small amount of hexane. The column was then washed with 30 ml of 3:97 methylene chloride/hexane (volume ratio), 15 ml of 20:80 methylene chloride/hexane, and 10 ml of methylene chloride. The second fraction, containing the PCDDs and PCDFs, was collected, evaporated and the residue dissolved in 1 ml toluene. The toluene solution (0.1 ml) was mixed with 0.5 μg ^{13}C-OCDD (internal standard) in 0.1 ml toluene and diluted to 0.5 ml. The resulting solution was analysed by GC/MS, as described for chlorophenols, with the following exceptions. The column was a 30 meter J & W DB-5 column with an internal diameter of 0.25 mm and a film thickness of 0.25 μm. The operating conditions were: injector temperature 300° C, interface temperature 270° C, initial oven temperature 100° C held for 2 minutes, then at 15° C min^{-1} to 240H° C, and at 4° C min^{-1} to a final temperature of 280° C held for 15 min. The quantification of PCDDs and PCDFs was carried out using selected ion monitoring with ^{13}C-OCDD as the internal standard. The responses of different congeners of PCDDs and PCDFs relative to ^{13}C-OCDD were determined by injecting a mixture containing 0.5 ng each of ^{13}C-OCDD, OCDD, 1,2,3,4,6,7,8-HpCDD, 1,2,3,4,7,8-HxCDD, 1,2,3,7,8-PnCDD, 2,3,7,8-TCDD, OCDF, 1,2,3,4,6,7,8-HpCDF, 1,2,3,4,7,8-HxCDF, 1,2,3,7,8-PnCDF, and 2,3,7,8-TCDF. Because of the large concentration difference between OCDD and lower chlorinated dibenzo-*p*-dioxins (five chlorine and less) in PCP-treated timber, dibenzo-*p*-dioxins and dibenzofurans substituted by five chlorines or less could not be quantified by this method.

Experimental

Both solid substrate fermentation and liquid culture methods were investigated for the decontamination of treated wood. Initial studies were made with low concentrations of PCP (500 ppm), but pole material containing 15,000 ppm PCP, and its associated impurities, was used to further test the more promising liquid culture methods. For the inoculation of sawdust in solid substrate systems, bacterial species, *R. chlorophenolicus* and *Flavobacterium* sp., were grown in DSM-65 and glutamate medium respectively at 25° C and

induced for PCP degradation. After PCP degradation by both species had been confirmed, stationary phase cells were centrifuged (5,400 × g, 10 minutes), washed in mineral salts buffer, and resuspended in fresh buffer. The fungal strain, *Ph. chrysosporium* was grown for 7 days at 39° C in autoclaved ponderosa pine sapwood sawdust (20 mesh), with periodic additions of sterile distilled water. Aliquots (0.5 g dry weight) of sawdust, treated with PCP to 500 ppm, were set at 60% water holding capacity with sterile mineral salts solution. The effects of sterilisation of the sawdust, and the addition of labile carbon sources (1% w/w; sodium glutamate for the *Flavobacterium* sp., sucrose for *R. chlorophenolicus*, and cellobiose for *Ph. chrysosporium*), prior to inoculation were examined. The sawdust was inoculated with 10% w/w untreated sawdust colonised with *Ph. chrysosporium* mycelium, and to a level of 7.7×10^9 cells g^{-1} for *R. chlorophenolicus* and 9.4×10^8 cells g^{-1} for *Flavobacterium* sp. (viable counts). All samples were incubated at 25° C and pentachlorophenol concentration determined after 2 and 8 weeks incubation. The results given are compared with uninoculated controls and are the average of duplicate treatments. Liquid culture suspensions consisted of sawdust or wood chips suspended in cultures of mid-exponential phase, PCP-induced *Flavobacterium* sp., or *R. chlorophenolicus*. These cultures were incubated at 25° C and shaken at 120 rpm. Chlorophenol and chlorinated dioxin concentrations were determined in both the liquid and solid phases, the wood being separated by coarse filtration through Whatman No. 1 cellulose filters. Live cultures were compared to controls heat-sterilised at mid-exponential phase, due to possible culture environment changes caused by cell growth.

Results and discussion

Solid substrate systems

The degradation of PCP in timber by both bacterial species was extremely limited in solid substrate systems. *R. chlorophenolicus* degraded approximately 20% of the PCP in non-sterile and pre-sterilised sawdust after 8 weeks, and 14% in the presence of 1% w/v sucrose. *Flavobacterium* sp. degraded 36% under non-sterile conditions, 31% with pre-sterilised sawdust, and 24% in the presence of 1% w/v sodium glutamate after 2 weeks, with no further degradation after 8 weeks (Table 1). In similar experiments with PCP-contaminated soil, *R. chlorophenolicus* in particular has performed better (Briglia et al. 1990; Middeldorp et al. 1990), although in these cases the addition of other carbon sources also reduced PCP degradation. Conversely, the addition of sodium glutamate has been shown to significantly aid PCP degradation in liquid culture and in contaminated soils by the *Flavobacterium* sp. (Topp et al. 1988; Topp & Hanson 1990).

The limited bacterial degradation under these conditions was not due to inhibition of the inoculum by PCP. *R. chlorophenolicus* has been shown to survive and degrade PCP well in soils with initial concentrations up to 750 ppm PCP (Briglia et al. 1990); although *Flavobacterium* has been reported to be inhibited at PCP concentrations greater than 500 ppm in soil (Crawford & Mohn 1985). There are however other factors in timber which may have been inhibitory to these species, such as the presence of antimicrobial resin acids and terpenes, and although the toxicity of the extractives present in pine heartwood was not examined in this study, many naturally occurring resin acids and terpenes do have biocidal properties (McCarthy et al. 1990). The most likely explanation for the lack of PCP degradation in wood by bacteria however was the limited accessibility of the PCP to bacterial enzymes, and this was confirmed by liquid culture studies described later. During pressure treatment of softwood species, the majority of PCP is deposited on the primary cell wall of the longitudinal tracheids, although there will be some penetration of PCP into the S2 layer (Zicherman 1975). Due to their hyphal growth, fungi are able to enter the tracheid lumen via pits, but without the aid of a wood swelling solvent, such as water, the ability of most bacteria to enter wood cells is restricted. Fungi are also able to produce large quantities of extracellular enzymes which aid penetration. Pentachlorophenol degradation by bacteria is thought to be an intracellular process however (Xun & Orser 1991; Apajalahti & Salkinoja-Salonen 1987), and therefore dependent on the rate of transportation or diffusion of PCP into the cells. There was a high degree of PCP metabolism in sawdust by the fungus *Ph. chrysosporium* in solid substrate systems. Over 97% of PCP disappeared in 2 weeks (Table 1), and similar results have been reported for contaminated wood and soil (Lamar & Dietrich 1992; Lamar et al. 1990). The disappearance of PCP however was not mineralisation, and, after 8 weeks, 70% to 80% of the PCP was recovered as pentachloroanisole under all treatment conditions (Table 1). Dechlorination by *Ph. chrysosporium* was minimal, although

Table 1. Pentachlorophenol and pentachloroanisole concentration (ppm) in sawdust after inoculation and incubation as a solid substrate at 60% WHC.

	Time (weeks)	Non-sterile	Prior sterilisation of sawdust	Addition of carbon source
Uninoculated control	2	499	n.d.[1]	n.d.
	8	493	n.d.	n.d.
R. chlorophenolicus	2	508	429	401
7.7×10^9 cells gram^{-1}	8	395	450	387
Flavobacterium sp.	2	310	348	375
9.4×10^8 cells gram^{-1}	8	328	336	373
Ph. chrysosporium	2	15	13	16
10% w/w sawdust with	8	7	7	7
mycelium	8	(387)[2]	(350)	(372)

[1] n.d. = not determined.

[2] Numbers in brackets indicate concentration of pentachloroanisole (ppm) detected after 8 weeks.

some (less than 1 ppm) 2,3,4,6-tetrachloroanisole was detected. The extent and mechanism of dechlorination by lignin peroxidases from white-rot fungi remains unclear (Roy-Arcand & Archibald 1991; Morgan et al. 1991). As with the bacterial species, sterilisation or the addition of labile carbon made no significant difference to the amount of PCP metabolism. Other factors may influence methylation however; Whitfield et al. (1991) reported that chloroanisole production by *Paecilomyces variotii* was significantly affected by relative humidity. However, the effect was only noticeable for 2,4,6-tri- and 2,3,4,6-tetrachlorophenol as virtually no PCP was methylated by this organism. Chloroanisoles still represent an environmental hazard; pentachloroanisole is more volatile, and considerably more lipophilic than PCP and thus is more liable to bioaccumulation. A process in which this compound was generated in such significant amounts would not be suitable for treatment of preserved timber.

Liquid culture suspensions

Since the lack of PCP availability for bacterial degradation in treated timber appeared to be the major cause of the failure of solid substrate systems, we examined liquid culture suspensions for their ability to allow the extraction of PCP, and other lesser chlorinated phenols, as a water-soluble salts into the liquid phase, with simultaneous/subsequent bacterial degradation in this phase. Figure 1 shows the distribution of PCP from sawdust, with an initial PCP concentration of 500 ppm and a weight to volume ratio of 1%, between the solid and liquid phases. The initial pH of these *Flavobacterium* sp. cultures was 7.2; metabolism of sodium glutamate as the sole carbon and energy source, however, overcame the weak phosphate buffering of the growth medium and resulted in an increase of pH to 9.1 by stationary phase. The extraction of PCP from the wood into the liquid phase, without any further degradation (sterilized controls) was rapid (< 15 minutes) as expected at alkaline pH, reaching an equilibrium value of 80% of the starting concentration. In the presence of live PCP-degrading *Flavobacterium* sp., the rate of extraction in the first 15 minutes matched that of the sterile controls. After this however, the PCP extracted from the wood was degraded by the cells in the aqueous phase, thus the aqueous phase acted as a sink for PCP, resulting in continued extraction from the wood to well below the 20% equilibrium value observed in the sterile controls (Fig. 1). The coupling of degradation in the aqueous phase with extraction therefore enabled complete removal of PCP from the wood (residual concentrations less than 1 ppm) and complete loss in the aqueous phase (below detection) within 6 hours. The results from a similar experiment with actual pole material removed from service (initial PCP concentration 15,000 ppm; initial 2,3,4,6-TeCP concentration 800 ppm), rather than laboratory treated timber, are shown in Fig. 2. After onset of PCP degradation, the rate of degradation was not significantly affected by an increase of weight to volume ratio from 1% to 8%. At 8% w/v, both the PCP and the 2,3,4,6-TeCP in the aqueous phase dropped below detection after 2 days, and after 7 days, the concentration of PCP in the saw-

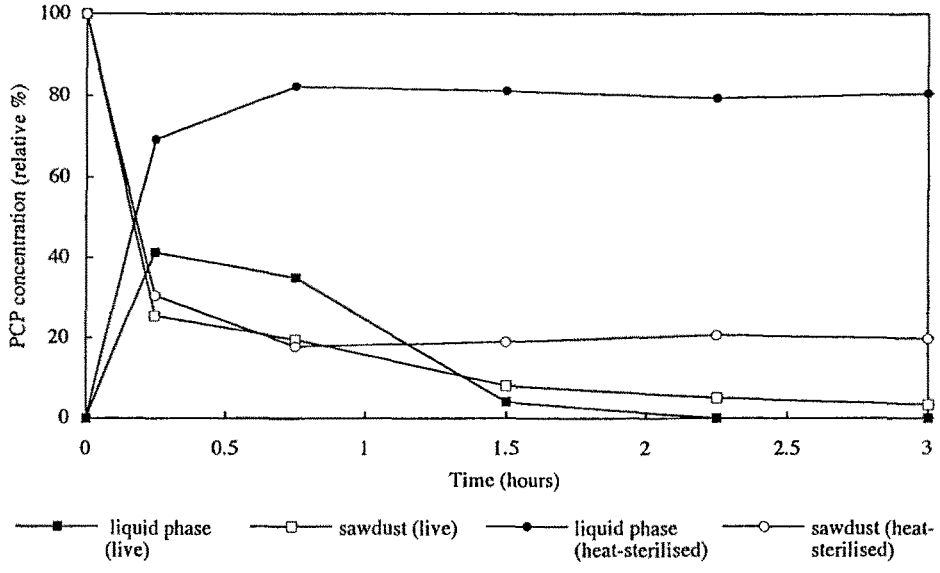

Fig. 1. Distribution and degradation of PCP from sawdust in live and heat-sterilised *Flavobacterium* sp. cultures.

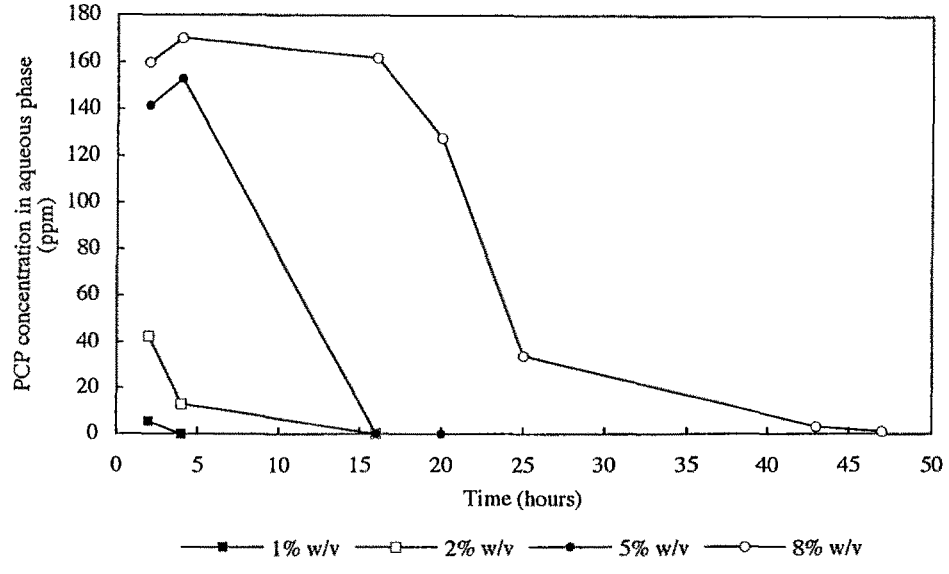

Fig. 2. Degradation of PCP from pole material by *Flavobacterium* sp. in liquid culture suspensions.

dust was reduced from 15,000 ppm to 7 ppm, and the TeCP was below detection. At the higher weight to volume ratios, the concentration of PCP extracted from the timber in the first 2 hours approaches its maximum equilibrium value; thus the increase in weight to volume ratio from 5% to 8% does not proportionally increase the concentration in the aqueous phase (Fig. 2). The *Flavobacterium* sp. can tolerate PCP concentrations between 100 and 200 ppm in the aqueous phase (Saber & Crawford 1985), and the degradation of initial concentrations as high as 300 ppm, in the presence of polyurethane support have been reported (O'Reilly & Crawford 1989). The oil components, which are present, at the time of treatment, at greater than 10 times the PCP concentration, did not prevent PCP degradation by the *Flavobacterium* sp., but, as expected, there was no significant removal of the chlorinated dioxin components by this method. The major-

Table 2. Distribution of PCDDs and PCDFs between the aqueous and solid phases of *Flavobacterium* sp. cultures after 2 days.

	Flavobacterium sp.		Sterilised control	
	sawdust (ppm)	liquid phase (ppt)	sawdust (ppm)	liquid phase (ppt)
OCDD	89	880	90	1180
OCDF	1.3	96	1.6	124
HpCDD[1]	19.1	48	33.4	118
HpCDF[1]	1.0	38	1.2	120
HxCDD[1]	0.6	b.d.[2]	1.0	b.d.
HxCDF[1]	0.1	b.d.	0.1	15

[1] Concentrations reported as the sum of detected isomers.

[2] b.d. = below detection limit.

ity of the chlorinated dioxin components remained in the wood material, with no more than 1.2 parts per billion in the liquid phase (Table 2). Jackson and Bisson (1990) reported a similar association of PCDDs and PCDFs in the oil phase of soils contaminated with wood-preserving solutions.

The extremely high extraction and degradation achieved in these systems can in part be explained by the reduction of the pole material to sawdust, a process which is clearly not economical for large scale waste treatment. However, with chipped material (particle size approx. 2 × 1 × 0.5 cm), a more realistic choice, comparable results were achieved (Fig. 3). As with sawdust, the PCP concentration in the aqueous phase rose rapidly after the addition of the pole material (5% w/v, initial PCP concentration 12,000 ppm) to a maximum concentration of 130 ppm. This was degraded below detection in the first 6 hours of the process – whilst the cells were still in exponential phase. However, the decrease in surface area to volume ratio, caused by the increase in particle size, slowed the extraction of PCP from the wood, and after 21 hours, the residual concentration of PCP in the wood was 750 ppm (94% removal). The addition of wood material to the batch cultures extended the stationary phase of the cells, and, after 14 days, the concentration in the wood chips was reduced to 49 ppm (> 99% removal). The *Flavobacterium* sp. was recovered on agar plates containing sodium glutamate after this period, although as the wood chips were not sterilised, other bacterial species were detected. These species were inhibited by 50 μM PCP on agar plates, and their ability to grow in the liquid culture suspensions was probably due to complete degradation of PCP by the *Flavobacterium* sp. Under identical experimental conditions, *R. chlorophenolicus* could not be recovered in a viable state after 48 hours, nor was there degradation of PCP in the aqueous phase, reflecting the lesser tolerance of this species to PCP in the aqueous phase (Apajalahti & Salkinoja-Salonen 1986). With respect to scale-up of this process, the time taken for the degradation of chlorophenolics from the wood, together with the residual level of PCP in the wood, will be influenced by factors such as surface area to volume of the wood material. The residual level could perhaps be reduced by further treatments, and this is currently under investigation. The maximum equilibrium PCP concentration in the aqueous phase was not toxic to the *Flavobacterium* sp., and the determining factor for the ideal weight to volume ratio will be a function of final bioreactor configuration, as a high ratio will require significant energy input to keep the particles in suspension, whilst providing adequate aeration. The ability to degrade PCP from the timber at the maximum equilibrium concentration in the aqueous phase should also allow for the natural variation in input PCP concentration in timber which results from wood species, treatment method, and depth of penetration. This biological treatment process is in effect a leaching of the pole material with the microbial medium, combined with the degradation of all the acutely toxic chlorophenolic preservative components. The process therefore generates waste water containing bacterial cells and some treating oils in an emulsion, and pole material containing a low residual of PCP, the majority of the treating oil, and chlorinated dioxin components. The waste water should not present a problem as such material could be treated in a conventional activated sludge system, as the acutely toxic chlorophenolics have been removed. The cellular catalyst can also be recovered to initiate new batch processes, although re-use maybe limited by the growth of contaminating species. The question of disposal of wood material containing chlorinated dioxin components remains, and further physicochemical treatment would be required to dispose of this material; however the difficulty, cost and scale of this should be considerably reduced by prior biological destruction of the chlorinated phenolics.

Conclusions

Solid substrate fermentation of PCP-treated wood would not appear to be suitable for bacterial inocu-

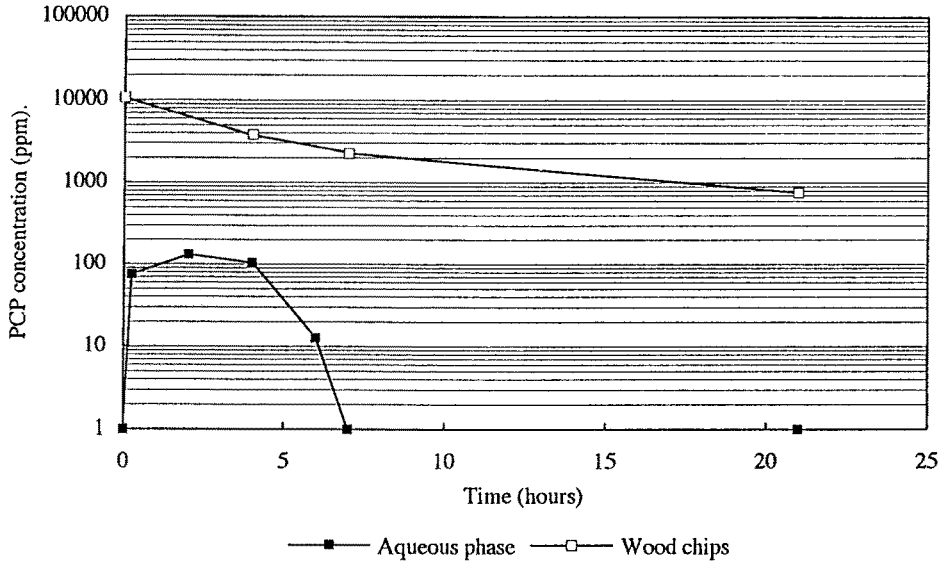

Fig. 3. Distribution and degradation of PCP from chipped utility poles (5% w/v) by *Flavobacterium* sp. in liquid cultures.

lants. Certain fungal species can access the PCP in such systems at low PCP retentions, but the generation of pentachloroanisole may be a problem. Suspended solids systems seem a much more appropriate choice since extremely rapid removal of PCP can be achieved in model systems. The degradation of chlorophenolic preservatives in chipped utility poles can be readily accomplished by the use of exponentially growing, PCP degrading *Flavobacterium* sp. A reduction of greater than 99% can be achieved in 14 days, however the chlorinated dioxin components are not significantly reduced in this process, and remain in the wood material. Although scale-up is required, the procedure itself is promising for the removal of PCP, due to the large degree of process control points it offers. These include primary factors affecting PCP concentration in the aqueous phase, such as weight to volume ratio of suspended solids below 5%, which is in turn influenced by factors affecting the rate of extraction, such as pH of the aqueous phase, particle size and the rate of degradation.

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