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Review paper

Microbial degradation of pentachlorophenol

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Abstract

Pentachlorophenol (PCP) was the most prevalent wood preservative for many years worldwide. Its widespread use had led to contamination of various environments. Traditional methods of PCP clean-up include storage in land-fill sites, incineration and abiotic degradation processes such as photodecomposition. Some aerobic and anaerobic microorganisms can degrade PCP under a variety of conditions. Axenic bacterial cultures, *Flavobacterium* sp., *Rhodococcus* sp., *Arthrobacter* sp., *Pseudomonas* sp., *Sphingomonas* sp., and *Mycobacterium* sp., and fungal cultures, *Phanerochaete* sp. and *Trametes* sp. exhibit varying rates and extent of PCP degradation. This paper provides some general information on properties of PCP and reviews the influence of nutrient amendment, temperature and pH on PCP degradation by various aerobic and anaerobic microorganisms. Where information is available, proposed degradation pathways, intermediates and enzymes are reviewed.

Introduction

Pentachlorophenol (PCP) is listed as one of the priority pollutants by the United States Environmental Protection Agency and European Community (Wild et al. 1993). In 1985, world-wide production of PCP was 100,000 tons (Wild et al. 1993). As of 1978, PCP consumption ranged between two and three million kg per year in Canada. Eighty percent of this was used for wood preservation. The United States produced 23.6 million kg of PCP in 1974 (Rao 1978). In Japan, PCP was used as a herbicide for paddy and upland rice with production levels of 13.3 million kg per year. In 1971, use of PCP as a herbicide was banned in Japan due to its lethality (fish kills), and production decreased to 3 million kg per year (Crosby 1981). In Finland, the estimated total use since the 1930's was 25,000 tons. In 1984, chlorophenol production ceased and in 1988 became illegal (Salkinoja-Salonen 1990).

PCP can be manufactured by two methods. One method involves chlorination of phenol in the presence of catalytic amounts of aluminum chloride and organic-chlorination promoters or stabilizers. The second method is hydrolysis of hexachlorobenzene in the presence of either sodium hydroxide, sodium carbonate, or by the Raschig-Hooker method (hexachlorobenzene is exposed to catalysts, calcium phosphate or silicate) (Crosby 1981). PCP has a pKa of 4.7 in water (Crosby 1981). The acidity is due to the electron withdrawing properties of the chlorines on the ring. It is a white crystalline solid that melts at 190° C, boils at 300.6° C and is relatively volatile (Crosby 1981). It is soluble in organic solvents such as methanol, acetone and benzene but only slightly soluble in water. It has a pH-dependent absorbance maximum of 303 nm. The sodium salt of PCP (NaPCP) is often used industrially. In contrast to pure PCP, this form has a high melting temperature, is soluble in water, methanol, and acetone and is non-volatile (Crosby 1981).

Technical grade PCP contains 85–90% PCP and other phenolic compounds, such as, tri- and tetrachlorophenols, and predioxins and iso-predioxins. Predioxins and iso-predioxins are formed when two molecules of PCP, trichlorophenol or tetrachlorophenol undergo condensation. Neutral fractions of technical grade PCP can contain vary-

ing amounts of tetrachlorodibenzo-*p*-dioxin (TCDD), pentachlorodibenzo-*p*-dioxin (PCDD), hexachlorodibenzo-*p*-dioxin (HCDD), heptachlorodibenzo-*p*-dioxin (HeCDD), octachlorodibenzo-*p*-dioxin (OCDD), tetrachlorodibenzo-*p*-furan (TCDF), pentachlorodibenzo-*p*-furan (PCDF), hexachlorodibenzo-*p*-furan (HCDF), heptachlorodibenzo-*p*-furan (HeCDF), octachlorodibenzo-*p*-furan (OCDF), and hexachlorobenzene (HCB). The dioxin present in the highest concentration is usually OCDD and is comparatively non-toxic (Crosby 1981).

Toxicity of PCP

PCP has been used as a bactericide, fungicide, herbicide, algicide, insecticide, and molluscide. It is capable of performing these biocidal functions due to its toxic nature. PCP uncouples oxidative phosphorylation by making cell membranes permeable to protons, resulting in dissipation of transmembrane pH gradients and electrical potentials (Steiert et al. 1988). Smejtek et al. (1989) observed that PCP caused an isothermal phase transition from gel to fluid state in model lecithin membranes. In addition, PCP may adsorb preferentially to disordered regions in the biological membrane, leading to alterations in membrane function (Smejtek et al. 1989). Interference by PCP at pituitary or hypothalamic levels was observed in rats (Jekat et al. 1994). In barnacle muscle fibres, PCP stimulated the ouabain-insensitive sodium efflux by increasing free internal calcium ions (Nwoga & Bittar 1991).

PCP absorbs into mammalian skin from soil. A Rhesus monkey absorbed 24.4% of 17 mg/L applied PCP from soil (Wester et al. 1993). This is significant since PCP is corrosive to skin and can cause burns and blisters. In mammals, acute exposure leads to elevated body temperature, increased respiratory rate, elevated blood pressure, hyperglycemia, and cardiovascular stress (Crosby 1981). Despite this, there is little evidence for adverse health effects in people with chronic exposure to PCP (Gilbert et al. 1990). PCP is a suspected carcinogen, teratogen and is highly embryotoxic (Crosby 1981).

Environmental contamination by PCP

The highest reported usage of PCP is in the wood preserving and treatment industry, particularly for utility poles, fences and railway ties. PCP offers the advantages of oil and partial water solubility, biocide effectiveness and limited environmental persistence. PCP is used as a dip treatment in freshly sawn lumber to prevent sapstaining and blue-staining by fungi. This process usually occurs in open-air basins and can cause pollution of soil and water in the area (Salkinoja-Salonen 1990). Kitunen et al. (1987) investigated persistence of technical grade PCP in soils of four Finnish wood-preserving facilities. The sawmills were in operation for 3–40 years and all soils were contaminated with chlorophenols. PCP and other more acidic chlorophenols migrated into soil less than neutral chlorophenols. Polychlorinated phenoxyphenols and polychlorinated dibenzofurans were found at high concentrations, 1–50 mg/kg dry weight soil and 0.2–5 mg/kg dry weight respectively, in the top layer of soil. All compounds were recalcitrant and there was little reduction by abiotic or biotic degradative processes (Kitunen et al. 1987). Salkinoja-Salonen (1990) reported chlorophenol (CP) concentrations in soil of a saw mill to be 10,000 mg CP/kg soil. Surface waters, ground waters and lake waters in the area of saw mills were also contaminated with the same chlorophenols used in wood preservatives (Valo et al. 1984). Leaching of chlorophenols through soil permits PCP to enter water systems, allowing spread to other environments.

In Canada, PCP treatment of utility poles accounted for a significant portion of PCP usage from 1941–1970. There are concerns about contamination of soil and water during pressure treatment, the loss of preservative while treated poles are in service, and disposal of PCP-contaminated poles. The latter is important because many utility poles, in use for thirty years or more, are ready to be replaced, and options for disposal are limited. Waste containing above 5 g PCP/200 kg wood requires special disposal. Disposal options include burial in an approved and secure area, chemical landfill or incineration. Concrete encapsulation and bioremediation are future possibilities for PCP management (Ruddick 1991). Depletion of PCP from utility poles can occur by movement with the carrier oil, evaporation, water leaching, photochemical decomposition, and biological degradation.

Wegman & Hofstee (1979) sampled some surface waters in the Netherlands for chlorophenols. PCP was the most prevalent chlorophenol and maximum concentrations ranged from 1–10 g/L. Other dominant chlorophenols were trichlorophenols and tetrachlorophenols. Wegman & van der Broek (1983) investigated chlorophenols in sediments of rivers in the Netherlands. Thirty-five sites were sampled and in sediment samples with high overall chlorophenol contamination, PCP was present. In sediment samples with

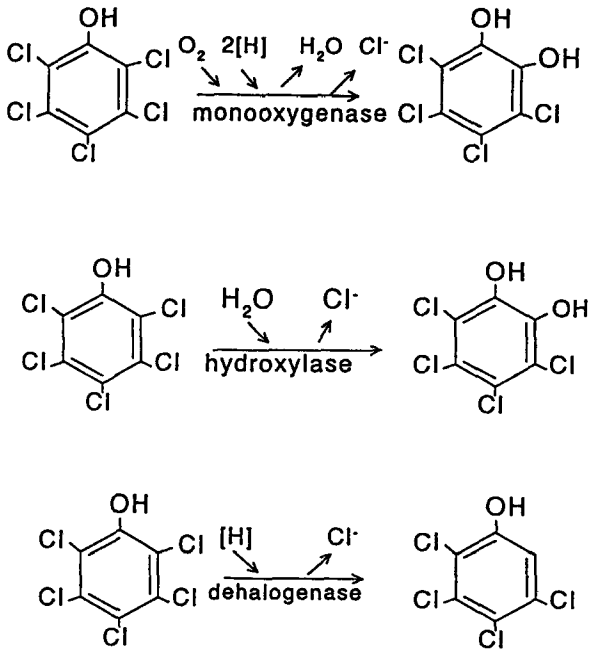

Fig. 1. Pathways of PCP degradation.

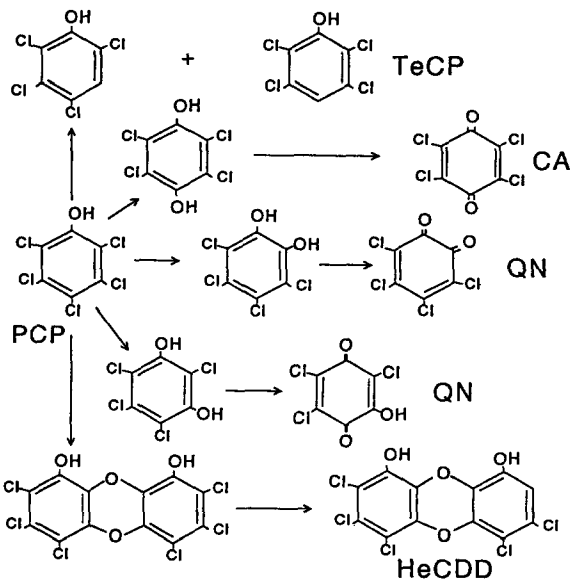

Fig. 2. Photodecomposition of PCP. TeCP: Tetrachlorophenol, CA: Chloroanil, QN: Quinone, HeCDD: Heptachlorodibenzodioxin.

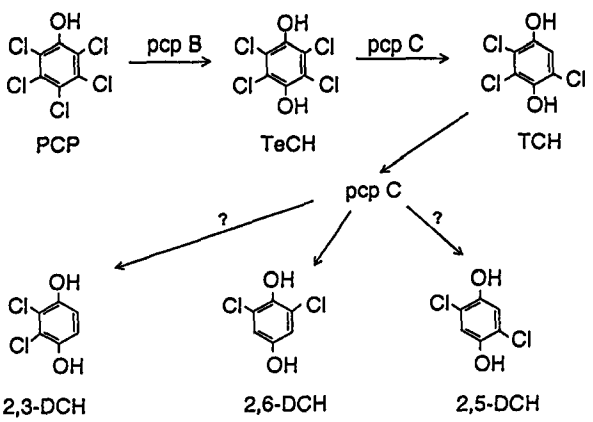

Fig. 3. Pathway of PCP metabolism by *Flavobacterium* sp. ATCC 39723. TeCH: Tetrachlorohydroquinone, TCH: Trichlorohydroquinone, DCH: Dichlorohydroquinone.

low chlorophenol content, PCP was detected between 0.2–4.6 g/kg dry weight sediment. Chlorophenols were also found in sewage sludge from the U.K. (Wild et al. 1993). The dominant chemical present in 12 sewage sludges was 2,4-dichlorophenol with concentrations of 19.6–86.3 g/L and 7.2 to 52.6 mg/kg dry sludge. PCP was present in all sludges but at concentrations of 0.21 to 3.76 g/L and 0.2 to 2.04 mg/kg dry weight (Wild et al. 1993). This suggests the presence of chlorophenols in a variety of environments, many without primary exposure to PCP. The incidence of chlorophenol pollution stresses the necessity of finding methods to remove low-level chlorophenols from the environment.

PCP degradation

Biodegradation of PCP can occur by three processes: hydroxylation, oxygenolysis, and reductive dechlorination (Fig. 1). PCP removal can occur by abiotic processes such as volatilization, photodecomposition, and absorption. Biotic degradation occurs by plant or animal uptake and transformation, and microbial degradation.

Sodium PCP is the form often used in technical formulation of PCP. As the sodium salt of PCP is not volatile, the contribution of volatilization to abiotic degradation is minimal (Crosby 1981). Photodecomposition, in contrast, has been implicated in up to 25% of PCP degradation in different environments under a variety of conditions (Pignatello et al. 1983; Seigle-Murandi et al. 1991). Mills & Hoffman (1993) found 47 μM PCP (11.36 mg/L) in the presence of titanium oxide decreased rapidly when irradiated with a UV light source. After 3 h of illumination no PCP was detected. Tetrachlorohydroquinone (TeCH) and the quinone, *p*-chloroanil, appeared as

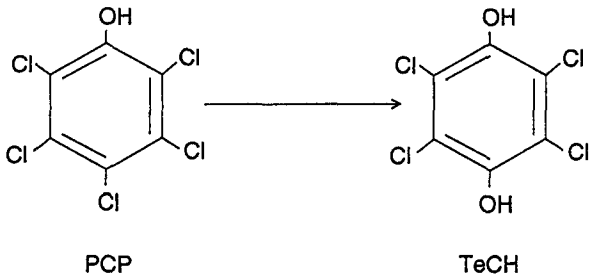

Fig. 4. Pathway of PCP degradation by *Rhodococcus chlorophenolicus* PCP-1. TeCH: Tetrachlorohydroquinone.

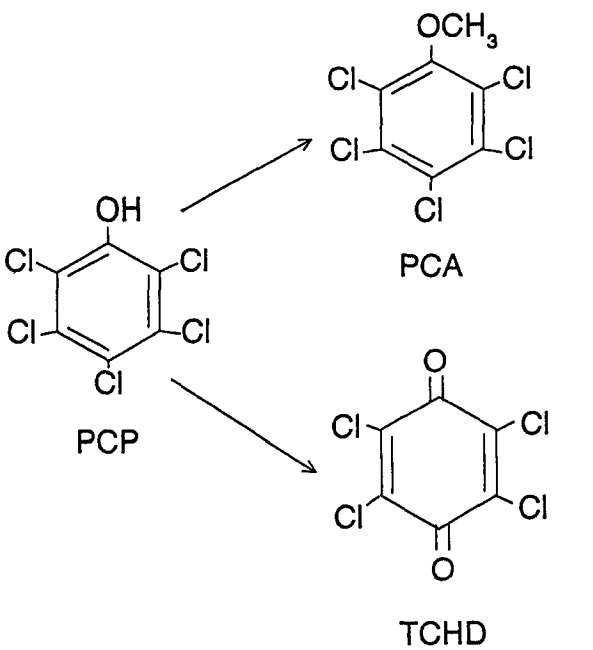

Fig. 5. Pathway of PCP metabolism by *Phanerochaete chrysosporium*. PCA: Pentachloroanisole, TCHD: 2,3,5,6-Tetrachloro-2,5-Cyclohexadiene-1,4-Dione.

intermediates. Formation of chloride and hydrogen ions increased linearly when a suspension of titanium oxide and PCP was illuminated (Mills & Hoffman 1993). Octachlorodibenzodioxin was also an intermediate of photodecomposition of PCP (Crosby 1981) (Fig. 2).

PCP absorbs to organic matter causing removal of PCP from water into sediment. This process can affect the availability of PCP to other degradation processes. It can also influence attempts to establish levels of pollution in water systems and methods for removal of the pollutant. Jacobsen et al. (1993) found 50% of

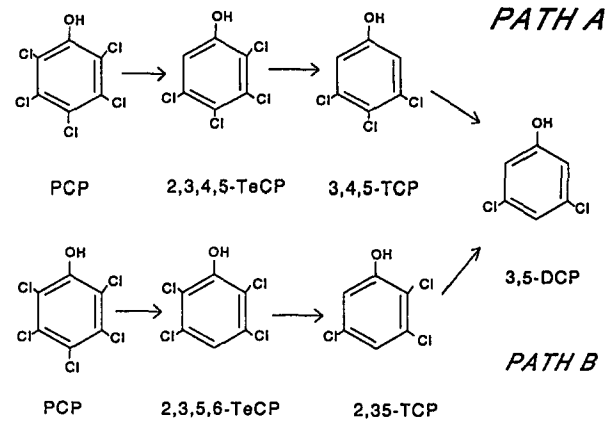

Fig. 6. PCP degradation pathways for anaerobic microbes. TeCP: Tetrachlorophenol, TCP Trichlorophenol, DCP: Dichlorophenol.

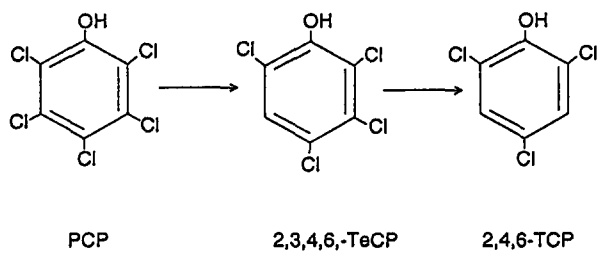

Fig. 7. PCP degradation by *Desulfomonile tiedjei* DCB-1. TeCP: Tetrachlorophenol, TCP: Trichlorophenol.

the influent PCP was absorbed to activated sludge at low solids retention times (high-flow through rates). Bellin et al. (1990) and Shimizu et al. (1992) found absorption of PCP to organic matter was dependent on pH i.e. sorption decreases as pH increases. At pH 3.0, 80% of the PCP was sorbed to a natural solids mix (consisting of organic matter, clay minerals and metal oxides) while at pH 12, 30% of the PCP was absorbed (Shimizu et al. 1992).

Uptake of PCP by plants and animals can contribute to PCP removal from the environment. Most animals can metabolize and eliminate PCP, producing intermediates such as TeCH and the glucuronide conjugate of PCP (Crosby 1981). Aquatic animals can metabolize PCP to PCP acetate, tetrachlorophenol, TeCH and its conjugates (Crosby 1981). Frank et al. (1990) reported that 35% of farm animals tested in Ontario, Canada between 1986 and 1988, contained detectable levels of PCP in their fat. Reports on the ability of plants to take up and transform PCP are inconsistent

and inconclusive. Bellin & O'Connor (1990) reported little uptake of PCP by fescue, lettuce, carrots and chili peppers. It was observed that degradation of PCP by soil microorganisms was high, thereby, limiting availability of PCP for plant uptake. Scheunert et al. (1986) found 6.56% total uptake of 1 mg/L PCP by carrots and 3.07% uptake of 1 mg/L PCP by barley. These uptake values are not significant. They found a large portion of the added PCP was bound to soil. Casterline et al. (1985) demonstrated the ability of spinach and soybean plants to take up PCP and identified the presence of PCP, 2,3,4,6-tetrachlorophenol, methoxytetrachlorophenol, 2,3,4,6-tetrachloroanisole and pentachloroanisole in the non-polar and polar fractions of extracts from both plants.

One area that has potential for removal of PCP is microbial degradation. This paper focuses on degradation of PCP by pure and mixed cultures of aerobic and anaerobic microorganisms. Conditions that inhibit and enhance degradation, and pathways, intermediates and enzyme systems implicated in PCP degradation by bacteria and fungi are reviewed.

Aerobic biodegradation of PCP: mixed cultures

Aerobic PCP degradation by mixed microbial cultures is important since most PCP-contaminated sites are surface soil or sediments which may support growth and activity of aerobic microbial consortia. PCP degradation can occur by the combined efforts of microorganisms in these consortia. In addition, some pure cultures of bacteria isolated from PCP-contaminated sites are capable of mineralizing a high concentration of PCP (200 mg/L).

To degrade PCP, the initial barrier that must be overcome by an aerobic microbial consortium is PCP toxicity. Watanabe (1978) reported the most probable numbers (MPN) of PCP-decomposers in field soil was affected by the presence of 40 mg/L PCP. After 4 weeks, no PCP was degraded, indicating that microbial activity may have been influenced. PCP concentrations of 10 mg/L also had an inhibitory effect (Watanabe, 1978). In experiments to determine the kinetics of PCP degradation in liquid batch cultures inoculated with industrial raw sewage, concentrations of 800–1600 μg/L PCP were inhibitory to growth of microorganisms. The specific growth rate decreased as PCP increased to 300 μg/L. Low cell yields of 0.01–0.04 g dry cell weight/g substrate consumed were reported. The substrate used was PCP in mineral salts medium. Large numbers of protozoa were also found in the culture and it is believed the low cell yields were due to predation of bacteria by protozoans (Klecka & Maier 1985). Liu (1989) studied degradation by a mixed bacterial culture, of pure PCP and a commercial PCP formulation in cyclone fermenters. In the presence of 25 mg/ml PCP, microorganisms increased from 2×10^5 to 9.7×10^5 CFU/ml over 4 days. Upon exposure to 25 mg/L commercial PCP, CFUs increased from 2×10^5 to 7×10^6/ml by day 14. While pure PCP was initially less inhibitory to growth, growth in the presence of commercial PCP was enhanced over time.

Acclimation of inocula to be used for PCP degradation may increase the tolerance of microorganisms to PCP. Pignatello et al. (1983) reported that artificial freshwater streams pulsed with 432 μg/L PCP inhibited microbial growth and killed two species of fish. Once acclimated, the high PCP-dose streams were no longer lethal to aquatic organisms and degradation by microorganisms proceeded. Rutgers et al. (1993), using a continuous culture, found the soil inoculum was, initially, sensitive to pulses of 10 μM PCP. Upon acclimation, growth and degradation at 77 μM PCP occurred. A report by Watanabe (1978) indicated PCP-treated (acclimated) soils supported more microbial growth than untreated soils in the presence of PCP. Acclimation of microbial communities to PCP appears to increase tolerance to PCP and/or select for PCP-tolerant microorganisms.

The presence of PCP in an environment may enhance a selected population of microorganisms. Soils amended with PCP concentrations of 200 mg/L demonstrated an initial increase in total viable bacteria to 10^8 bacteria/g dry soil, followed by an increase to 10^9 bacteria/g dry soil. The higher the PCP concentrations the longer the maximum number of viable cells was maintained (Sato 1983, 1987). The population of Gram-negative bacteria in the culture increased at a similar rate to numbers of viable bacteria, suggesting the viability increase was dependent on the increase in Gram-negative bacteria (Sato 1983, 1987; Watanabe 1978). Sato (1985) studied the predominance of different groups of bacteria in soil treated with PCP and untreated soil. PCP was found to modify the bacterial community in soil, allowing only selected groups of bacteria to grow. The prevalent morphology seen in the presence of PCP was a Gram-negative, coccoidal shaped, polarly flagellated bacterium. The same soil sample without PCP contained a diversity of microorganisms. Rutgers et al. (1993) used light microscope observations of PCP-treated soil to determine

two major groups of Gram-negative bacteria. These bacteria remained the dominant groups throughout the experiment. Upon addition of PCP, PCP-tolerant microorganisms were selected for. There is a strong correlation between the presence of PCP-tolerant and Gram-negative bacteria. This result is not unexpected, since studies by Ruckdeschel et al. (1987) and by Izaki et al. (1981) indicate, in general, Gram-negative bacteria, are less sensitive to PCP than Gram-positive bacteria. Of the bacterial strains studied most are Gram-negative. An exception is *Rhodococcus* sp. which is Gram-positive or Gram-variable but exhibits sensitivity to PCP in liquid culture (Briglia et al. 1990).

PCP is known to affect different cellular processes. Sato (1983, 1987) studied the effect of PCP on glycine transfer to nitrate via ammonification and nitrification. In the presence of PCP, glycine disappeared rapidly with the concomitant accumulation of ammonium. Ammonification was not affected by PCP. Nitrification began after a lag period indicating the inhibitory effect of PCP on this process. Sato (1987) reported an increase in pH associated with disappearance of glycine, in the presence of PCP. An increase in pH indicates ammonification, a drop in the pH following ammonification indicates nitrification. At PCP concentrations of 50 and 200 mg/L the pH remained high (between pH 6.5 and 8.0 over 25 days), indicating inhibition of nitrification. At 10 mg/L PCP, the pH dropped, therefore no inhibition of either process occurred at lower PCP concentrations (Sato 1987). Nevalainen et al. (1993) studied the effect of 2,4,6-trichlorophenol (TCP) on an established nitrifying biofilm. Initially, upon addition of TCP, nitrification was inhibited. After acclimation with TCP, nitrification proceeded without a lag period and the biofilm demonstrated the ability to dechlorinate PCP. The evidence presented attests to the potential of PCP to inhibit nitrification. Fortunately, the acclimation of a culture to PCP appears to at least partially reverse this inhibition or reduce the sensitivity of the microorganism to a second exposure of the toxicant (Trevors et al. 1982).

PCP biodegradation

Aerobic microorganisms capable of PCP biodegradation have been identified in a variety of environments: industrial sewage (Klecka & Maier 1985), activated sewage sludge (Jacobsen et al. 1991; Moos et al. 1983), soils (Bellin et al. 1990; Rutger et al. 1993; Watanabe 1977, 1978), and freshwater sediments (Larsson & Lemkemeier 1989; Pignatello et al. 1983). PCP degradation, mineralization and dechlorination by microbial communities from different environments are summarized in Table 1. It is noted that a number of sites pre-exposed to PCP did not demonstrate the presence of PCP-degrading microorganisms under conditions tested. Seech et al. (1991) were unable to find PCP-degraders in PCP-contaminated soil from a wood treatment plant, as indicated in Table 1. Degradation was not observed unless cells of a PCP-degrading *Flavobacterium* sp. were added to soil samples. Likewise, Mueller et al. (1991, 1993) could not establish PCP degradation in creosote and PCP contaminated groundwater using an inoculum of soil from a creosote-contaminated site.

There is strong evidence to support the role of acclimation in increasing the number of PCP-tolerant microorganisms in a given environment. There can also be enhancement of PCP degradation by microbial communities acclimated to PCP. In general, studies which used acclimated inocula, demonstrated enhanced degradation in shorter times and minimized lag phases. Pignatello et al. (1983) observed, at PCP concentrations of 144 μg/L, unacclimated sediments mineralized 15% PCP while acclimated sediments mineralized 60% PCP. Unacclimated clear-water and humic lake sediments demonstrated mineralization of 14.3% and 18%, respectively (Larsson & Lemkemeier 1989). Two studies, using acclimated bark chip biofilters, reported 35–63% mineralization at 160 mg PCP/kg solids and 80% mineralization at 200 μM PCP (52.6 mg/L) (Apajalahti & Salkinoja-Salonen 1984; Valo et al. 1985). Moos et al. (1983) reported 67% mineralization of 2 mg/L PCP in 6 h using acclimated activated sludge as the inoculum. Watanabe (1978) reported the effect of acclimation of PCP degradation was negligible in the soil tested. At 40 μg PCP/g fresh soil, PCP remaining after 14 days was 4.7 μg/g and 4.5 μg/g in unacclimated and acclimated soil, respectively. A similar result was found using 80 μg PCP/g fresh soil. In most cases, acclimation of inoculum appears to enhance degradation. This likely occurs because acclimation allows selection of microorganisms tolerant to PCP, thereby increasing viability and activity.

Adsorption of PCP to organic matter may have an impact on biodegradation of PCP by aerobic microorganisms. It is important to monitor binding of PCP to organic matter as it can reduce bioavailability of PCP to degrading microorganisms. This can lead to a false conclusion about the microbial contribution to PCP degradation if it is not accounted for (Apajalahti &

Table 1. PCP degradation by mixed culture aerobic microorganisms.

Source of inoculum	Conditions	Design	Acclimation	[PCP]	Degradation	Mineralization	Dechlorination	Adsorption	References
Bark chips from PCP-degrading bio-filter	Liquid	Shake flasks	+	200 μM (52.6 mg/L)	+	80%	NR	+ 17–48 μM/10 ml	Apajalahti & Salkinoja-Salonen 1984
Industrial sewage	Liquid	Continuous culture	+	8.0 mg/L 80.7 mg/L	< 10 μg/L 37.8 μg/L	NR	NR	NR	Klecka & Maier 1985
Wood chip biofilm	Liquid		+	160 mgPCP/kg biosolids	+	35–63%	81–85%	+	Valo et al. 1985
Rock-colonizing from freshwater	Liquid	Continuous culture	+	9–250 mg/L	60–75 μg/L remaining	60–80%	5 mol Cl^-/mol PCP	NR	Brown et al. 1986
Mixed culture	Liquid	Cyclone fermenters	+	25 mg/L Pure PCP	98% 100 h	NR	16 mg/L 100 h	NR	Liu 1989
				25 mg/L Technical grade PCP	100%	NR	23 mg/L 14 d	NR	
Mixed culture	Liquid	Cyclone fermenters	+	25 mg/L	+ 6 d	NR	NR	NR	Liu et al. 1991
Soil	Soil	Flooded	NR	100 μg PCP/g soil dry weight	10–70 d half-life	NR	NR	+	Kuwatsuka & Igarashi 1975
		Upland			20–120 d half-life	NR	NR	+	
Soil	Volcanic ash soil	Plots	NR	20 kg/ha	2 wk half-life	NR	NR	NR	Watanabe 1977
Soil	Low moisture content	NR	-	40 μg/g fresh soil	4.7 μg/g 14 d	NR	NR	NR	Watanabe 1978
			+		4.5 μg/g 14 d	NR	NR	NR	
	Low moisture content	NR	-	80 μg/g fresh soil	6 μg/g 28 d	NR	NR	NR	
			+		5 μg/g 28 d	NR	NR	NR	
				PCP Celite 40 μg/g fresh soil	2 μg/g 4	NR WK	NR	NR	

Table 1. Continued.

Source of inoculum	Conditions	Design	Acclimation	[PCP]	Degradation	Mineralization	Dechlorination	Adsorption	References
Activated sludge	NR	Flasks	+	2 mg/L	+	67% 6 h	NR	0.8–0.31%	Moos et al. 1983
		Continuous-stirred tank reactor	+	20 mg/L	600 μg/L effluent (97%)	NR	NR	NR	
Garden soil	Soil + glycine	Percolation flask and column	NR	10, 50, 200 mg/ml	-	NR	NR	NR	Sato 1987
Wood preservation site soil		Fed-batch culture	+	50 μM (13.15 mg/L)	2 μM/h (0.526 mg/L/h)	+	+	NR	Rutgers et al. 1993
		Nutristat	+	171 μM (45 mg/L)	64 nmol/min/mg protein	NR	100%	NR	
Norfolk soil low OC, acid	Soils + Sewage sludge	Air-flow system	NR	0.75 mg/kg soil	38 d Half-life	75%	NR	+	Bellin et al. 1990
Bluepoint soil high OC, basic					8% remaining 33 d	100%	NR	NR	
	Sludge	Fill and draw reactors	NR	50–250 μg/L	70–90% Removal at high SRT	NR	NR	40–55% Low SRT	Jacobsen et al. 1991
	Artificial freshwater streams	NR	+	32.6 μg/L	75% 120 h	NR	NR	15%	Pignatello et al. 1983
			+	76 μg/L	100% 70 h	NR	NR	NR	
			+	199 μg/L	100% 40 h	NR	NR	NR	
			-	144 μg/L	+	15%	NR	NR	
			+	144 μg/L	+	60%	NR	NR	
	Humic sediments clear-water sediments	NR	-	48 μg/L	NR	14.3%	NR	NR	Larsson & Lemkemeier 1989
			-	48 μg/L		18%	NR	NR	

Table 1. Continued.

Source of inoculum	Conditions	Design	Acclimation	[PCP]	Degradation	Mineralization	Dechlorination	Adsorption	References
Soil microbes from creosote site	Groundwater contaminated with PCP and creosote	Shake flasks	NR	52 mg/L	-	NR	NR	NR	Mueller et al. 1991
Unacclimated activated sludge	Simulated chlorophenol-contaminated groundwater	Continuous-flow, fluidized-bed reactor	+ Mono- and dichlorophenols	3.8 mg/L	92.5%	NR	+	NR	Puhakka & Järvinen 1992
Activated sludge	Chlorophenol-contaminated groundwater	Continuous-flow fluidized-bed reactor	-	2.5 mg/L	85–99% 30 d	NR	+	NR	Järvinen et al. 1994
Aerobic biomass	Chlorophenol-contaminated groundwater	Continous-flow fluidized-bed reactor	+ Dichlorophenols	2.5 mg/L	98% 98 d	NR	+	NR	Järvinen & Puhakka 1994
Mixed culture microbes	Groundwater from American Creosote Works	Creosote microbes	NR	16.47 mg/L	-	NR	NR	NR	Mueller et al. 1993
		Creosote microbes + PCP-degrader	NR	541 mg	71.9%	NR	NR	25.5%	

NR: not reported, +: denotes positive observation, -: denotes no response.

Salkinoja-Salonen 1984; Bellin et al. 1990; Jacobsen et al. 1991).

Organic matter such as wood chips or sludge are often added to reactors to study their effect on PCP degradation. Apajalahti & Salkinoja-Salonen (1984) used wood chips to attempt to enhance degradation of PCP by a mixed culture of bacteria. Without chips the culture was inhibited at 10 μM (2.6 mg/L) PCP. On addition of 0.5 g and 5.0 g of chips, PCP degradation proceeded at 20 μM (5.2 mg/L) and 200 μM (52.6 mg/L), respectively. In the presence of 200 μM PCP, it was determined that upon amendment with of 5, 3, or 1 g of wood chips/10 ml of media, 17, 29 and 48 μM PCP remained adsorbed to the chips, respectively. The highest adsorption observed was at 5.0 g of chips with 25% adsorption. Bellin et al. (1990) found the effect of sludge additions on PCP degradation was minimal and reversible in the two soils tested. Jacobsen et al. (1991) demonstrated PCP sorption by sludge occurred as the solids retention time (SRT) increased in fill and draw reactors. At an SRT of 2 to 3 days, 40–60% of PCP influent was sorbed to the sludge. Jacobsen et al. (1993) also found 50% of PCP was sorbed to sludge at low SRTs. Removal of PCP by degradation occurs best at high SRTs, therefore by manipulating the SRT, sorption and degradation of PCP can be controlled under these conditions. It is possible to use the sorption properties of PCP to the benefit of degradative microorganisms. High influent concentrations can be decreased to less toxic levels by adsorption to allow maximum degradation to proceed. If sorption is reversible, conditions in the reactor may be adjusted to allow desorption of the bound PCP, thereby liberating it for further degradation.

Amendments

Soils and sediments can be carbon-limited environments for microorganisms. By amending degradation sites with carbon sources it may be possible to enhance degradation of PCP. Few studies have addressed the effect of different carbon sources on the degradative capacity of microorganisms in aerobic communities. Valo et al. (1985) attempted to enhance PCP degradation by an active biomass by supplementing the culture with glutamic acid, *p*-OH-benzoic acid, *m*-OH-benzoic acid, phenol, benzoic acid, peptone or yeast extract. None of these carbon sources enhanced degradation of PCP. Liu et al. (1991) explored the effect of cometabolism on degradation of chlorophenols. Cometabolism involves partial transformation of a chemical, not accompanied by carbon incorporation or energy utilization by degrading microorganisms. PCP was found to function as a cometabolite for degradation of persistent dichlorophenols, trichlorophenols and tetrachlorophenols (Liu et al. 1991).

The organic carbon content to which the microbial consortium is exposed can influence degradation. Kuwatsuka & Igarashi (1975) investigated PCP degradation in eleven different soils. They reported that PCP degradation was related to the organic matter content in the soils. Factors such as soil texture, clay content, degree of base saturation, soil pH and phosphorus content showed minimal effect on PCP degradation. Larsson & Lemkemeier (1989) tested the efficiency of PCP degradation in a clear-water and humic lake. The humic lake mineralized PCP at almost twice the rate of the clear-water lake. On amendment with sediment, however, rates in both lakes increased and were comparable. The authors suggested the higher bacterial load in sediment compared to water was the reason for the increased PCP mineralization rate.

Valo et al. (1985) studied the effect of nutrient supplementation on PCP biodegradation by an active biomass. With 0.4 mM NH_4Cl, 50% of PCP was mineralized in 250 h, but with 40 mM NH_4Cl, 150 h was necessary to degrade 50% PCP. Without amendment, degradation of 50% PCP took 500 h. PCP degradation was investigated in the presence of glycine (Sato 1983, 1987) and 2,4,6-TCP degradation by a nitrifying biofilm was studied (Nevalainen et al. 1993). These studies focused on the effect of PCP on conversion of the nitrogen source, with no information provided on the effect of the nitrogen source on PCP degradation.

Temperature and pH effects on PCP biodegradation

Evaluation of the effects of temperature and pH on an active microbial biomass is important for potential bioremediation applications. Conditions at a PCP-contaminated site are rarely ideal. Valo et al. (1985) found PCP mineralization did not proceed after 700 h at 8° C or at 50° C. Optimum degradation occurred at 28° C with 50% mineralization occurring in 150 h. At 37° C, there was a 400 h lag time after which degradation rapidly increased. In contrast, Järvinen & Puhakka (1994) and Järvinen et al. (1994) found 99% of PCP present in chlorophenol-contaminated ground water was degraded at temperatures of 5–10° C (ambient ground water temperature) in continuous-

flow fluidized-bed reactors. Valo et al. (1985) also evaluated the effect of pH on PCP degradation. Degradation occurred between pH 5.6–8.0. A neutral or slightly acidic pH was optimum. At pH 8.0, a lag time of 200 h was observed prior to PCP degradation.

PCP metabolism

Kuwatsuka & Igarashi (1975) reported the intermediates of PCP degradation in soil were 2,3,4,6-, 2,3,4,5-, 2,3,5,6-tetrachlorophenols, 2,3,5-, 2,3,4-, 2,4,6-, and 2,3,4- or 2,4,5-trichlorophenol. Of these, 2,3,4,5-tetrachlorophenol, 2,3,6-trichlorophenol, and 2,4,6-trichlorophenol were the major products. The dominant pathway in this microbial biomass was initiation of degradation by *ortho*-dechlorination. After incubating 2,4,6-trichlorophenol with a nitrifying biofilter, the product 2,6-dichlorohydroquinone was detected. It was suggested this reaction was catalyzed by ammonium monooxygenase (Nevalainen et al. 1993). These reports suggest different mechanisms of PCP degradation by different microbial consortium. Kuwatsuka & Igarashi (1975) used a culture which degraded PCP primarily by reductive dehalogenation. In contrast, the intermediate found in the study by Nevalainen et al. (1993) suggested an oxygenolytically dehalogenating activity in the nitrifying biofilter.

PCP degradation by bacteria

A number of bacterial isolates with the ability to degrade PCP and other chlorinated compounds have been isolated from a variety of environments, usually with a history of PCP exposure. Bacteria known to degrade PCP include: *Flavobacterium* sp., *Rhodococcus chlorophenolicus*, other *Rhodococcus* sp., *Arthrobacter* sp., *Mycobacterium* sp., *Sphingomonas* sp. and *Pseudomonas* sp.

Flavobacterium strains were isolated from sediments of a man-made channel dosed with PCP (Pignatello et al. 1983), and from three sites in Minnesota, U.S.A. with a history of PCP exposure (Saber & Crawford 1985). The *Flavobacterium* strain isolated by Pignatello et al. (1983), designated ATCC 39723, has been widely studied.

A bacterial strain found to degrade PCP was isolated from lake sediment (Apajalahti & Salkinoja-Salonen 1986) and designated *Rhodococcus chlorophenolicus* PCP-1 based on API tests, the presence of cell wall diamino acids and sugars, and lipids (Apajalahti et al. 1986). This strain was reclassified as *Mycobacterium chlorophenolicus* PCP-1 based on 16S ribosomal RNA analysis (Briglia et al. 1995). Häggblom et al. (1988c) isolated two *Rhodococcus* spp. with the ability to mineralize PCP. One was isolated from the sludge of an aerated lagoon treating pulp and paper mill effluents, while the other was isolated from soil collected at a sawmill timber-treating facility that had undergone bioremediation. These strains were nocardioform actinomycetes and exhibited changes in cell morphology from cocci to rods and then back to cocci. The colonies were yellowish-orange and mucoid, containing menaquinones with nine isoprenoid units and one hydrogenated double bond, mycolic acids with 32–36 carbon atoms and tuberculostearic acid.

Chu & Kirsch (1972) isolated a bacterial strain, KC-3, from industrial waste-water capable of mineralizing PCP. This strain was later characterized as a member of the genus *Arthrobacter*. Stanlake & Finn (1982) isolated four strains of *Arthrobacter* sp. from a variety of PCP-contaminated environments. One strain, NC, demonstrated effective mineralization of PCP. It was characterized as *Arthrobacter* sp. by Gram variable staining, rod to coccus morphological transitions, lack of motility, yellow pigmentation, positive catalase and cytochrome oxidase tests, and most significantly, presence of cell-wall constituent diaminopimelic acid (Stanlake & Finn 1982). Edgehill & Finn (1983) also isolated a strain of *Arthrobacter*, designated ATCC 33790, which was similar in characteristics to strains isolated by Chu & Kirsch (1972) and Stanlake & Finn (1982).

A *Mycobacterium* sp. strain that degraded PCP was isolated from soil (Suzuki 1983). Characterization by morphological and physiological tests yielded a Gram-positive non-motile rod with no mycelium, that was catalase and oxidase positive, urease-negative, that reduced nitrate to nitrite, produced acid but no gas from glucose and grew between 22–37° C (Suzuki 1983). Häggblom et al. (1988c) isolated a PCP-degrading *Mycobacterium* strain from soil at a sawmill timber-treatment facility. The characteristics of the isolate were as follows: it exhibited cyclic changes in morphology from coccus to rod; colonies were white and wrinkled; the cells contained menaquinones with nine isoprene units and one hydrogenated double bond, had mycolic acids with more than 60 carbons, and tuberculostearic acid.

Trevors (1982) isolated three bacterial strains able to degrade PCP from agricultural soil and from a freshwater stream. They were identified as *Pseudomonas*

sp. by morphological and biochemical criteria (they were Gram- negative, nonspore-forming, motile rods, oxidase- and catalase-positive, gelatin-liquefied, used glucose as a carbon source, and produced fluorescent pigment). Other researchers have reported a variety of *Pseudomonas* sp. that can dehalogenate substituted phenols (Slater et al. 1979). Radehaus & Schmidt (1992) recently isolated a bacterial strain from a heavily-contaminated wood products site. The strain was classified as *Pseudomonas* sp. RA2 based on motility, presence of poly-β-hydroxybutyrate granules, ability to use different carbon and energy sources, cellular fatty acid composition, and the adsorption maxima of acetone-extracted yellow pigment. It has since been reclassified as *Sphingomonas* sp. RA2 (SK Schmidt, University of Colorado, Boulder, Colorado, U.S.A., personal communication).

Bacterial growth on PCP

Pentachlorophenol is an uncoupler of oxidative phosphorylation. Uncouplers render the cell membrane permeable to protons, resulting in dissipation of transmembrane pH gradients and electrical potential (Steiert et al. 1988). Izaki et al. (1981) surveyed various Gram-negative and positive bacterial strains, and reported many *Pseudomonas* strains demonstrated the highest tolerance to PCP, showing the ability to tolerate PCP concentrations of 500 mg/L and higher. Strains from genera such as *Salmonella, Enterobacter* and *Escherichia* exhibited moderate tolerance to PCP concentrations between 200–300 mg/L and genera such as *Bacillus, Flavobacterium, Streptomyces, Staphylococcus,* and *Corynebacterium* were sensitive at concentrations of 10–20 mg/L PCP. Ruckdeschel et al. (1987) demonstrated *Pseudomonas aeruginosa, Streptomyces* sp. and *Salmonella typhimurium* were the most PCP-tolerant of a range of Gram-negative and -positive bacteria tested. Gram-positive bacteria tend to be more sensitive to PCP than Gram-negative bacteria. It was hypothesized this may be due to a protective effect of lipopolysaccharide (LPS) in Gram-negative cells (Sato 1987). Izaki et al. (1981) noted that many of the more sensitive strains such as *Bacillus* were PCP-degraders while more resistant strains displayed negligible degradation.

Steiert et al. (1988) reported that PCP did not cause dissipation of the pH gradient or a reduction of nucleotide pools due to stimulation of ATPase activity, when added to cultures of uninduced or induced *Flavobacterium* cells. While some *Flavobacterium* sp. are sensitive to low PCP concentrations, the strain isolated from a PCP-contaminated environment by Pignatello et al. (1983) was resistant to 100 mg/L PCP. Reports differ on the maximum concentration of PCP that allows growth of *Flavobacterium* sp. Saber & Crawford (1985) found growth to occur on 200 mg/L PCP, while Hu et al. (1994) found that at 200 mg/L PCP, cell death rate was higher than growth rate. Both Topp et al. (1988) and Topp & Hanson (1990a) reported a transient loss in viability at 50 mg/L PCP. Brown et al. (1986) found *Flavobacterium* sp. ATCC 39723 utilized 600 mg/L PCP in the presence of cellobiose and had a maximum carbon utilization rate of 0.15 g (dry weight) of PCP carbon per h. At 808 mg/L PCP, growth of *Flavobacterium* sp. was inhibited. Saber & Crawford (1985) isolated viable *Flavobacterium* sp. strains from soil containing up to 873 mg PCP/kg dry weight soil. Crawford & Mohn (1985) and Seech et al. (1991) demonstrated *Flavobacterium* sp. ATCC 39723 degraded PCP in soil at concentrations of 100 mg/L and 175 mg PCP/kg, respectively. Crawford & Mohn (1985) reported *Flavobacterium* sp. to degrade 298 and 321 mg/L PCP in soils from wood treatment landfill sites, but this required four inoculations to accomplish. In contrast, Briglia et al. (1990) could not show PCP degradation by *Flavobacterium* sp. in peaty soil at 15 μM (7.8 mg/L) PCP. A seven order of magnitude decrease in cell number was also observed. Peaty soil contained a large population of indigenous microorganisms and it was believed the decrease in viability of *Flavobacterium* sp. cells may be due to predation by protozoans (Briglia et al. 1990).

Rhodococcus (Mycobacterium) chlorophenolicus PCP-1 appears to exhibit lower tolerance to PCP in liquid culture. Apajalahti & Salkinoja-Salonen (1986) observed a 50% inhibition of growth at less than 20 μM PCP (about 5 mg/L) in liquid culture. When 10^4 cells/g soil were immobilized in polyurethane foam at 15 μM (7.8 mg/L) PCP, the cells remained viable for at least 200 days (Briglia et al. 1990). In soil, Middeldorp et al. (1990) found cell activity to be uninhibited by 630 mg PCP/kg soil. After 154 days, Briglia et al. (1994) found a three-orders-of-magnitude increase in cell numbers in soil, containing 30 mg PCP/kg, inoculated with 500 cells/g soil. The *Rhodococcus* sp. isolated by Häggblom et al. (1988c) showed 50% reduction of growth at 5–10 μM PCP in liquid culture. *Rhodococcus* sp. strains grew poorly in liquid cultures but showed an impressive ability to grow and degrade high levels of PCP in soils. Increased viability was

reported in liquid culture when an immobilizing agent such as polyurethane foam was added.

Chu & Kirsch (1972) found that the KC-3 isolate (renamed *Arthrobacter* sp.) grew well in liquid PCP culture. At 26 mg/L PCP, after 84 h, there was a 50-fold increase in viable cell count in liquid culture. The growth rate of *Arthrobacter* sp. increased with PCP concentrations up to 130 mg/L and growth inhibition was due to increase in pH and production of chloride ions (Stanlake & Finn 1982). PCP, in the undissociated form, inhibited growth at 2 mg/L and higher (Stanlake & Finn 1982). There is no information on the viability of *Arthrobacter* sp. in PCP-contaminated soils.

PCP toxicity has been studied in *Mycobacterium* and *Pseudomonas* sp. Suzuki (1983) found the *Mycobacterium* sp. could not grow in the presence of 50 mg/L PCP. Häggblom et al. (1988c) found their *Mycobacterium* strain showed a 50% reduction in growth yield at 5–10 μM PCP. *Mycobacterium* sp. demonstrated limited growth and viability in the presence of PCP in liquid culture. Few studies exhibited the ability, in comparison to these strains, to remain viable in soil. Some *Mycobacterium* sp. are recognized pathogens to various animals and this fact may impede further studies on their use in potential field applications. A *Pseudomonas* strain isolated by Trevors (1983) grew rapidly at 10 mg/L but was completely inhibited at 75 mg/L. He concluded the LC_{50} for resting cells treated with PCP at 1 h and 12 h was 29 and 19 mg PCP/L, respectively. Radehaus & Schmidt (1992) found cells of *Pseudomonas (Sphingomonas)* sp. RA2 to exhibit decreased viability at 200 mg PCP/L. Growth rate was 0.09/h at 40 mg/L but dropped to 0.05/h at 150 mg/L. *Pseudomonas* sp., in contrast to *Mycobacterium* sp., may have good potential in bioremediation studies. *Pseudomonas* sp. are ubiquitous in nature and strains with PCP-degrading ability have been isolated from highly PCP-contaminated soil sites.

Flavobacterium sp. ATCC 39723 and *Sphingomonas* sp. RA2 are probably the most PCP-tolerant bacteria of those studied. *Arthrobacter* sp. also shows a high level of tolerance. While *Rhodococcus* isolates do not seem to demonstrate high tolerance in the laboratory, two studies reported their ability to survive in high PCP concentrations in soil or upon addition of an immobilization agent in liquid culture. Both the soil environment and the immobilizing agent may encourage adsorption of PCP, thereby reducing the free concentration of PCP available to exert toxicity to cells.

PCP degradation

While tolerance to PCP may be an essential feature of bacterial strains selected for study of PCP degradation, more important is the ability of the microorganism to mineralize PCP to CO_2, Cl^- and H_2O. Table 2 summarizes the available information on degradation, mineralization and dechlorination of PCP by different bacteria. In most studies, mineralization refers to the release of CO_2 from [^{14}C]-PCP.

As shown in Table 2, *Flavobacterium* sp. mineralizes PCP in liquid culture (Gonzalez & Hu 1991; Hu et al. 1994; Saber & Crawford 1985; Steiert et al. 1987). In some cases a lag phase was observed before the onset of PCP degradation by this strain. Topp et al. (1988) found 50 mg/L PCP was degraded as a sole source of carbon but only after a lag phase of 90 h. Gonzalez & Hu (1991) observed that lag phases of 10 h occurred at 10 mg/L PCP, 30 h at 20 mg/L, 55 h at 44 mg/L, 80 h at 80 mg/L and 200 h at 200 mg/L. Few investigators have tested the effectiveness of PCP degradation by *Flavobacterium* sp. ATCC 39723 in soil. Crawford & Mohn (1985) found mineralization occurred in soil at 100 mg/L. They also found that *Flavobacterium* sp., inoculated into soil sites contaminated with 298–321 mg/L PCP, degraded about 80% of the PCP, although no evidence of mineralization was provided. Multiple (four) inoculations were required to attain this level of PCP degradation. *Flavobacterium* sp. ATCC 39723 exhibits a broad substrate range. It can dechlorinate various chlorinated phenols such as three tetrachlorophenols, four trichlorophenols and five dichlorophenols, to varying degrees (Steiert et al. 1987). Xun & Orser (1991a) showed this strain can degrade 40 mg/L tribromophenol and triiodophenol. *Flavobacterium* sp. can also degrade the herbicide bromoxynil. In the presence of 5 mg/L bromoxynil, after 24 h a strong odor of cyanide was evident, indicating its degradation by *Flavobacterium* sp. Degradation of bromoxynil was slower than PCP by whole cells (Topp et al. 1992).

Rhodococcus (Mycobacterium) chlorophenolicus PCP-1 maintains higher viability in soil than in liquid culture and consequently is more effective at mineralizing PCP in soil (Table 2) (Briglia et al. 1994; Middeldorp et al. 1990). Immobilization in polyurethane foam further enhanced PCP degradation in liquid culture by this strain (Briglia et al. 1990). *R. chlorophenolicus* PCP-1 can also degrade PCP anaerobically in the presence of iodosobenzene at a rate similar to aerobic degradation of PCP (Uoti-

Table 2. Degradation of PCP by pure cultures of bacteria.

Bacteria	Culture conditions	[PCP]	Degradation	Mineralization	Dechlorination	Incorporation	References
Flavobacterium sp. ATCC 39723	Liquid	100 mg/L	+	73–83% 3.7–7.2 h	NR	17–27%	Crawford & Mohn 1985
	Liquid	0.2 mM (53.4 mg/L)	+	NR	100%	NR	Steiert et al. 1987
	Liquid	50 mg/L	+	NR	NR	NR	Topp et al. 1988
	Liquid	100–150 mg/L	1.51×10^{-13} g PCP/cell/h	NR	NR	NR	Topp et al. 1988
	Liquid	200 mg/L	+ 420 h	NR	NR	NR	Hu & Gonzalez 1991
	Liquid batch + PUF	10–300 mg/L 10–50 mg/L	+ 5 d	70–80%	+	NR	O'Reilly et al. 1989
	Semi-continuous feed + PUF		+ 150 d	86%	NR	NR	
	Continuous feed + PUF	55 mg/L 3 ml/min	6.65 g (93%)	NR	+	NR	
	Liquid + PUF	150 mg/L	+, 1 wk	NR	NR	NR	Briglia et al. 1991
	Liquid	150 mg/L	+	+	+	NR	Hu et al. 1994
	Liquid + calcium alginate	250 mg/L	+	+	+	NR	
	Liquid + PUF	700 mg/L	+	+	+	NR	
	Soil	100 mg/L	+	100% 1 wk	NR	NR	Crawford & Mohn
	In situ	298 mg/L	58 mg/L remaining 41 mg/L remaining	NR	NR	NR	1985
		321 mg/L		NR	NR	NR	
	Soil	175 mg/kg soil	+	55% 84 d	35 mg/kg	NR	Seech et al. 1991
	Increased soil moisture		+	65% 84 d	49 mg/kg		
	Increased pH		+	63% 168 d	22 mg/kg	NR	
	Increased soil organic matter		+	53% 168 d	102 mg/kg		
	Increased chloride		+	20% 196 d	310 mg/kg	NR	
	Anoxic conditions		+	1% 196 d	0		

	Timber compost	500 mg/L	24–36%	NR	NR	NR	McBain et al. 1995
			2 wk +	+			
		500 mg/L	7 mg/L remaining		NR	NR	
	Timber extract	15,000 mg/L	7 d				
Rhodococcus chlorophenolicus PCP-1	Liquid	10 μM (2.6 mg/L)	+, 250 h	70%	NR	NR	Apajalahti & Salkinoja-Salonen 1986
	Timber solids	15 mg/mL	38%	NR	NR	NR	McBain et al. 1995
			3.7 mg PCP/d/kg soil				
	Soil + PUF	15 mg/ml	38%	NR	NR	NR	Briglia et al. 1990
			3.7 mg PCP/d/kg soil				
	Soil	630 mg/kg soil	+	150–250 mg/kg soil	NR	NR	Middeldorp et al. 1990
		30 mg/kg soil	+	13–18 mg/kg soil			
	Soil	350–600 mg/kg soil	+	600–2000 pg/cell/month	NR	NR	Briglia et al. 1994
		30 mg/kg soil		100 pg/cell/month			
			+		NR	NR	
Rhodococcus sp.	Liquid						Häggblom et al. 1988
CP-1		10 μM (2.6 mg/L)	+, 1 d	70%	NR	NR	
CG-1		10 μM	+ 1 d	40%	NR	NR	
Arthrobacter KC-3	Liquid	2.6 mg/L	80%	NR	NR	NR	Chu & Kirsch 1972
		72 μg	+	73%	NR	NR	
Arthrobacter sp.	Continuous-feed	525 mg/L	97%	NR	95%	NR	Stanlake & Finn 1982
		0.045 h					
Arthrobacter sp.	Soil	120–150 mg/L	1 d half-life NR	NR	NR	Edgehill & Finn 1983	
ATCC 33790		150–200 mg/L	50% 5 d				
	Outside soil-unmixed	150–200 mg/L		NR	NR	NR	
	Outside soil-mixed		85% 5 d				
				NR	NR	NR	
	Liquid	117 μM (40.7 mg/L)	+	50%	NR	NR	Siahpush et al. 1992
	co-immobilized		45 h				
	Liquid	100–110 mg/L	2–2.2 g 57 h	NR	NR	NR	Edgehill 1994
		1800 mg/L	+				
	Continuous-feed	340–500 mg/L	4 mg/L residual	NR	NR	NR	
Mycobacterium sp.	Liquid	55.1 nmol	+ 4 h	NR	NR	NR	Suzuki 1983
	Liquid	10 μM	60% 1 d	NR	NR	NR	Häggblom et al. 1988
Pseudomonas sp.	Liquid	50 mg/L	50%	NR	NR	NR	Trevors 1982
Pseudomonas resinovorans	On-site of industrial soils Carbon amended	680 mg/kg	6 mg/kg left 2.7 d	12% 48 h			Seech et al. 1994
Sphingomonas RA2	Liquid	40 mg/L	+	14%	26 mg/L Cl^-	14%	Radehaus & Schmnidt 1992

NR: not reported, +: denotes positive observation, -: denotes no response, PUF: polyurethane foam.

la et al. 1992). Both *R. chlorophenolicus* PCP-1 and other *Rhodococcus* sp. strains exhibited the ability to degrade various chlorinated phenol, chlorinated guaiacols, chlorinated syringols and 0-methylate chlorinated *p*-hydroquinones (Häggblom et al. 1988a, 1988b, 1988c). While *R. chlorophenolicus* PCP-1 did not exhibit much PCP metabolizing activity in liquid culture, it was effective at degrading high concentrations of PCP in soils.

Arthrobacter sp. ATCC 33790 mineralized an influent of 525 mg/L PCP in a chemostat reactor (Edgehill 1994; Stanlake & Finn 1982) (see Table 2 for values). This strain also removed 85% of PCP at concentrations between 150–200 mg PCP/kg from soil (Edgehill & Finn 1983). *Arthrobacter* sp. ATCC 33790 is a possible candidate for bioremediation studies. However, more research must be undertaken to determine its degradative potential under different soil conditions.

Immobilization and encapsulation

Immobilized cells are defined as cells that are entrapped within or associated with an insoluble matrix (Cassidy et al. 1995; Trevors et al. 1992). Alginate and polyurethane foam have been used to immobilize PCP-degrading bacteria. Alginate immobilization of *Flavobacterium* sp. ATCC 39723 permitted degradation of PCP at concentrations as high as 150 mg/L in batch reactors. Concerns about the use of alginate included its low mechanical strength, incompatibility with anions such as phosphate and susceptibility to biodegradation (O'Reilly & Crawford 1989). Polyurethane foam enhanced the ability of *R. chlorophenolicus* PCP-1 cells to maintain viability and degrade PCP in liquid culture at high concentrations (Briglia et al. 1990). O'Reilly & Crawford (1989) demonstrated the ability of polyurethane foam-immobilized *Flavobacterium* sp. ATCC 39723 cells to degrade 300 mg/L PCP, with 70–80% mineralization. In the presence of polyurethane foam, lag phases were also decreased. Polyurethane-immobilized *Flavobacterium* sp. ATCC 39723 cells degraded up to 700 mg/L PCP in liquid culture (Hu et al. 1994). Siahpush et al. (1992) investigated the effect of co-immobilization of *Arthrobacter* sp. ATCC 33790, using powdered activated carbon in calcium alginate capsules, on PCP degradation. Co-immobilization is defined as the use of microorganisms, with known degradative capabilities immobilized together with an absorbent within a spherical permeable gel matrix or membrane (Siahpush et al. 1992). Co-immobilization offers some advantages over cell-immobilization: the presence of adsorbents quickly remove the contaminant, allowing the use of high flow rates in reactor systems; adsorbents dampen variations in contaminant concentrations; they help lower concentrations when the contaminant is toxic; and they help retain any extracellular enzymes produced by bacterial cells. Co-immobilized *Arthrobacter* cells were exposed to 117 μM PCP (30.7 mg/L). Complete PCP removal was seen in 30 h and 50% of the PCP was mineralized. Degradation of PCP by co-immobilized cells was dependent on the type and concentration of adsorbent used. It was reported that the larger the quantity of adsorbent used, the lower the PCP concentration, and the lower the mineralization rate by bacteria.

Conditions affecting PCP degradation

Effect of inoculum on PCP degradation

Some researchers have noted that *Flavobacterium* requires multiple inoculations for effective degradation of PCP, particularly in heavily contaminated (298–321 mg/L PCP) soils (Briglia et al. 1990; Crawford & Mohn 1985). Middeldorp et al. (1990) incubated *R. chlorophenolicus* in peaty soil and found 500 cells/g soil gave no significant degradation, 8×10^4 cells/g soil at 630 mg PCP/kg soil mineralized 15%, and 10^8 cells/g soil at 30 mg PCP/kg soil mineralized 10%. In sand, 500 cells/g soil induced mineralization of 30 and 630 mg PCP/kg soil, 8×10^4 cells/g were effective at degrading 30 mg PCP/kg soil, and 10^8 cells/g doubled the mineralization rate (Middeldorp et al. 1990). Edgehill & Finn (1983) found the incubation times necessary for *Arthrobacter* sp. to reduce PCP by 90% for 10^6, 10^5 and 10^4 cells/g soil was 24 h, 40 h and 100 h, respectively. Edgehill (1994) reported the time for *Arthrobacter* sp. to reduce PCP by half was dependent on the inoculum size. In commercial sandy soil supplemented with 77 g/ml PCP, an inoculum of 6.6×10^6 cells/g soil required 3 h to reduce PCP by half, 6.6×10^4 cells/g required 19 h, and 656 cells/g required 42 h.

Nutrient amendment

Topp et al. (1988) found that addition of 3 g/L glutamate stimulated PCP degradation by *Flavobacterium* sp. ATCC 39723 in liquid culture. Pentachlorophenol removal decreased when cells were shifted from a glutamate-supplemented medium to a medium with PCP as the sole carbon source. Full activity was re-

established 1.5 h later upon addition of 4 g/L glutamate. Gonzalez and Hu (1991) also reported the ability of glutamate and other carbon sources to reduce lag phase for growth. Topp & Hanson (1990b) found the generation time for *Flavobacterium* sp. decreased in the presence of glutamate to 4.4 h and after 45 h, there was a 13-fold increase in degradation. This stimulatory effect was not seen at 150–225 mg/L PCP or at 10^5, 10^6 or 10^7 cells/ml. Gonzalez & Hu (1991) found the specific degradative activity of *Flavobacterium* sp. cells in a fermenter increased when glutamate was present but decreased as it became depleted, suggesting glutamate may play a role in maintaining PCP degradative activity. Glucose, at 20 mg/L, enhanced degradation of 50 mg/L PCP by *Flavobacterium* sp. (Topp et al. 1988). These researchers also found the decline in viable *Flavobacterium* sp. cells was rapid under glucose and PCP limitation, with a rate of loss of 3.5×10^5 CFU/ml/h. Glucose stimulated PCP degradation by *Flavobacterium* sp. under nitrogen, sulfate and glucose limitations (Topp & Hanson 1990b). Generation time of *Flavobacterium* in the presence of 4 g/L glucose was 14.1 h, and after 45 h the cell population had increased 3-fold (Topp & Hanson 1990b). Some studies found that adding glutamate and glucose together to reaction mixtures repressed PCP degradation (Hu et al. 1994; Topp et al. 1988; Topp & Hanson 1990b) whereas Hu et al. (1994) suggested the enhanced degradation seen with glutamate and glucose may be due to an increase in cell growth rate, an increase in biomass, an adaptation to the environment and improved maintenance ability.

Conversely, Radehaus & Schmidt (1992) found with *Pseudomonas (Sphingomonas)* sp. RA2, glucose supplementation had no effect on the kinetics of PCP mineralization and the growth rate was not affected. They concluded that addition of PCP increased rate of glucose utilization but not the reverse. This strain has a preference for PCP over glucose. This appears to be unique to this isolate and could be a valuable characteristic for in situ bioremediation.

Some researchers have investigated the effect of organic carbon content in soil on PCP degradation. Middeldorp et al. (1990) tested the effect of peaty soil, which is high in organic matter, and sandy soil, which is low in organic carbon, on PCP degradation by *R. chlorophenolicus* PCP-1. In peaty soil, 20% of 630 mg PCP/kg soil was degraded, and 50% of 30 mg PCP/kg soil was degraded, before inoculation with PCP-1. Upon inoculation, 45% of 630 mg/kg dry soil and 45% of 30 mg PCP/kg soil was mineralized. In contrast, degradation was not found in sandy soil before inoculation. After inoculation, mineralization (25% of 630 mg PCP/kg soil and 60% of 30 mg PCP/kg soil) occurred. This indicates the peaty soil contained a natural PCP-degrading population (Middeldorp et al. 1990). When considerations are made for the level of degradation in control samples, the activity of PCP-1 was not enhanced in peaty soil containing higher organic carbon. Adding an extra carbon source, in the form of distillery waste enhanced degradation by *R. chlorophenolicus*. In the presence of distillery waste, 30 mg PCP/kg soil was degraded, releasing 2.3 ng Cl^-/kg in peaty soil and 0.4 ng Cl^-/kg in sandy soil after 130 days. A portion of the chloride ions liberated may be due to the activity of the natural PCP-degrading population in the peaty soil (Middeldorp et al. 1990). Briglia et al. (1990) reported the activity of *R. chlorophenolicus* increased in peaty soil amended with distillery waste and wood chips. Without carbon source addition, 38% of PCP was degraded, with distillery waste added 63% PCP was degraded, and with addition of distillery waste and wood chips 80% PCP was degraded. Briglia et al. (1994) found with sandy soil, 500 cells/g soil led to significant mineralization while with peaty soil, an inoculum of 8×10^4 cells/g soil was required.

A limitation in phosphate in a *Flavobacterium* sp. culture caused a 100-fold decline in degradation (Topp & Hanson 1990b). Other nutrients such as nitrogen, sulfur (Topp & Hanson 1990a, 1990b) and magnesium (Topp & Hanson 1990a) had no effect on PCP degradation by this strain. Copper, chromate and arsenic (CCA) inhibited PCP removal at 2, 2, 10 ng/L, respectively (Wall & Stratton 1994). They observed that exposure of *Flavobacterium* sp. cells to 11.64×10^{-4} and 6.19×10^{-4}% of lab and technical grade CCA, respectively, caused complete inhibition of PCP degradation used at concentrations greater than 50 mg/L PCP. While 5–50 mg/L creosote did not affect removal of 35 mg/L PCP, it was toxic to bacteria and caused a reduction in viability (Topp & Hanson 1990a). From the studies reviewed, addition of a supplemental carbon in most situations greatly enhances degradation. Glutamate is effective at enhancing PCP degradation by *Flavobacterium* sp. Its effect on the degradation by other bacteria should be explored. It was not evident in studies with *R. chlorophenolicus* if soil with a high organic carbon content was better for PCP degradation.

Temperature

Temperature can affect PCP degradation. *Flavobacterium* sp. ATCC 39723 showed significant removal of PCP between 24 and 35° C, but is ineffective below 12° C or above 40° C (Crawford & Mohn 1985). Apajalahti & Salkinoja-Salonen (1987) reported the PCP degradation rate by *R. chlorophenolicus* increased as temperature increased up to 41° C. No PCP degradation was observed at 44° C. Häggblom et al. (1988c) reported the optimum temperatures for two *Rhodococcus* sp. isolates concurred with the information for *R. chlorophenolicus*. Trevors (1982) tested the effectiveness of PCP degradation by 3 *Pseudomonas* strains at 0, 4 and 20° C. At 0° C, no degradation was seen after 100 days; at 4° C strains 1, 2, and 3 degraded 50%, 23% and 11%, respectively, after 100 days; and at 20° C all three strains degraded about 50% of the PCP.

pH

Stanlake & Finn (1982) found degradation of PCP by *Arthrobacter* sp. ceased, as pH dropped to 6.15, and resumed when pH was adjusted to 7.1. They also found that the lag phase increased as pH decreased. A decrease in pH is a natural consequence of cleavage of chloride ions from the PCP ring. Suzuki (1983) investigated the effect of pH on degradation of PCP by a *Mycobacterium* strain and found maximum methylation occurred between pH 6.5–7.0 and hydroxylation was dominant at pH 6.0. Edgehill (1994) reported that the growth rate for *Arthrobacter* sp. at pH 7.4 was higher than at pH 6.5 in the presence of PCP.

Moisture content

In soil, water content can affect PCP degradation by bacteria. Crawford & Mohn (1985) found that with *Flavobacterium* sp., under the conditions tested, a 15–20% soil water content was most effective. At 50% water content, no mineralization was evident for 10 days, however, activity then increased and equalled that of drier soils. Briglia et al. (1994) investigated the effect of moisture content on PCP degradation in peaty and sandy soils by *R. chlorophenolicus*. At 30 mg PCP/kg soil, moisture contents of 66% and 58% enhanced degradation in sandy soils. At the same PCP concentration, 79, 58 and 66% water content greatly increased mineralization (Briglia et al. 1994). Seech et al. (1991) established PCP degradation by *Flavobacterium* sp. ATCC 39723 proceeded most rapidly in treatments of increased soil water content. A PCP concentration of 175 mg PCP/kg soil was mineralized by 66% with 60% soil water content, as compared to 55% mineralization at 30% soil water content.

PCP degradation pathways, intermediates, and associated enzymes

Xun & Orser (1991c) showed that the first step of PCP degradation by *Flavobacterium* sp. ATCC 39723 was by oxygenolytic dechlorination. They found that PCP was converted to tetrachlorohydroquinone (TeCH) in 20 min in the presence of O_2 and NADPH. The following reaction was proposed:

$$2NADPH+2H^{+}+O_2+Ar-Cl \rightarrow 2NADP^{+}+H_2O+Ar-OH+HCl$$

The oxygenolytic nature of this reaction was confirmed by using radiolabelled H_2O and O_2 (Xun et al. 1992c). Degradation of the oxygenolytic product, TeCH, proceeds by reductive dechlorination, i.e. direct replacement of chlorine by hydrogen. The end-products were tri- and di-chloro-*p*-hydroquinones (Steiert & Crawford 1986). The reductive dehalogenase requires reduced glutathione as a cofactor (Xun et al. 1992a). Glutathione stimulated dehalogenation 75-fold more than in controls and a conversion of 100 μmol TeCH to 108 μmol glutathione was reported.

The enzyme responsible for the initial dechlorination and hydroxylation of PCP by *Flavobacterium* sp., PCP-4-monooxygenase, requires induction by PCP. A number of researchers demonstrated this by using chloramphenicol to block protein synthesis. If uninduced cells are exposed to chloramphenicol, PCP degradation does not occur, however, if cells are first induced using PCP, the presence of chloramphenicol does not block PCP degradation (Steiert et al. 1987; Topp et al. 1988). Xun & Orser (1991b) isolated a periplasmic protein, designated PcpA, which became detectable 40 min after exposure to PCP, reached a maximum level at 1 h exposure but was not present after 6 h. The gene encoding this protein was cloned and sequenced. It encodes a translation product of 271 amino acids with a predicted MW of 30 kDa. This product shows 35% homology with mammalian cytochrome P-450 monooxygenase and *P. putida* catechol dioxygenase genes (Xun & Orser 1991b).

The *Flavobacterium* PCP-4-monooxygenase (with a specific activity of 11.51 U/ml protein) was purified 160-fold from crude cell extract via a 7-step protocol. Information on the conversion of PCP to TeCH, some properties of the enzyme and optimal

conditions for enzymatic activity are summarized in Table 2. PCP monooxygenase exhibits a broad substrate range. It is active towards various halogenated phenols including 2,3,5,6-tetrachlorophenol, 2,4,6-, 2,3,6-, 2,3,4-trichlorophenols, 2,6- and 2,3-dichlorophenol, pentafluorophenol, triiodophenol, tribromophenol and dibromophenol. It also catalyzes the release of an amino group as hydroxylamine, a nitro group as nitrite, a cyano group as cyanide, and iodine as iodide from the *para* position of substituted phenols (Xun et al. 1992b). The purified enzyme exhibits the ability to degrade the herbicide bromoxynil (3,5-dibromo-4-hydroxybenzonitrile). Bromoxynil (100 μM or 52 mg/L) was completely degraded in 20 min with accumulation of the end-product, dibromohydroquinone. Bromoxynil, oxygen and NADPH were consumed in a 1:1:2 ratio and 1 mol of cyanide was produced per mol of bromoxynil consumed (Topp et al. 1992).

Xun et al. (1992d) isolated the enzyme (TeCH reductive dehalogenase) responsible for the reductive dehalogenation of TeCH to form TCH (trichlorohydroquinone), DCH (dichlorohydroquinone) and MCH (monochlorohydroquinone) from *Flavobacterium* sp. ATCC 39723. Expression of this enzyme was constitutive, as it does not require induction by PCP and is unaffected by chloramphenicol (Xun et al. 1992d). Its physical characteristics and some properties are summarized in Table 3.

The genes encoding PCP-4-monooxygenase (*pcpB*) and for TeCH reductive dehalogenase (*pcpC*) from *Flavobacterium* sp. ATCC 39723 were cloned, sequenced and expressed in *Escherichia coli* (Orser et al. 1993a, 1993b). Analysis of *pcpB* showed this gene to be encoded by an open reading frame of 1,614 bp that translates to a product of 538 amino acids with a predicted MW of 59,932 Da. In Southern analysis, a *pcpB* gene probe from *Flavobacterium* sp. hybridized to DNA sequences in *Pseudomonas* sp. SR3 and *Arthrobacter* sp. ATCC 33790. These bacteria exhibit similar PCP-degradation profiles as *Flavobacterium* sp. and this information suggests the initial step of PCP degradation in these strains is similar. The *pcpB* gene probe did not hybridize to DNA from *R. chlorophenolicus, Arthrobacter* sp. DSM 20407, a 100-kb plasmid from *Flavobacterium* sp. ATCC 39723 or the *E. coli* control. The *pcpB* gene exhibits 56% nucleotide similarity to the *tfdB* gene encoding 2,4-dichlorophenol hydroxylase and *vanB* gene encoding vanillate demethylase. Northern analysis revealed two possible transcripts, of 2400 and 1400 nucleotides in length, suggesting *pcpB* may be a dicistronic message. The *pcpB* gene was expressed in *E. coli* and one clone, CCL3, was inducible for the production of PCP-4-monooxygenase as shown by Western blot analysis. The CCL3 clone also produced TeCH from PCP.

The *Flavobacterium pcpC* gene coding for TeCH reductive dehalogenase is encoded in an open reading frame of 747 nucleotides, corresponding to 248 amino acids and a MW of 28,263 Da (Orser et al. 1993b). Northern analysis revealed one transcript which was not influenced by a range of PCP concentrations, indicating it is a constitutive, 800-nucleotide monocistronic message. The gene was cloned into *E. coli* and a positive clone, JD01, was identified. Cell extracts from the clone and the vector were prepared and only the clone was capable of converting TeCH to TCH, DCH and MCH. Sequence comparison analysis revealed some similarity to glutathione-S-transferases from carnations and maize. Degradative steps subsequent to MCH are not yet characterized. It is likely that ring cleavage occurs without prior removal of the final chlorine by either an intradiol or extradiol pathway.

R. chlorophenolicus PCP-1 transformed PCP to two major metabolites by *p*-hydroxylation, TeCH and TCH (Apajalahti & Salkinoja-Salonen 1987). The authors used radiolabelled 0_2 and H_2O to trace the origin of the oxygen atom in the hydroxyl group. It was determined the oxygen atom came from water, not molecular oxygen, based on a shift in the mass spectrum of TCH in the presence of water. Hydroxylation of trichlorophenol (TCP) to TCH occurred via donation of a hydroxyl group from molecular oxygen (Apajalahti & Salkinoja-Salonen 1987). The hydroxylation activity was induced by PCP as indicated by exposure of induced and uninduced cells to chloramphenicol (Apajalahti & Salkinoja-Salonen 1987). *R. chlorophenolicus* also transformed chlorinated guaiacols and syringols to trichloromethoxydihydroxybenzene and trichlorodimethoxydihydroxybenzene, respectively (Häggblom et al. 1988a). O-Methylation activity is also seen with *R. chlorophenolicus*. TeCH was O-methylated to tetrachloromethoxyphenol as were lower chlorinated hydroquinone intermediates (Häggblom et al. 1988b). O-Methylation was found to be a constitutive process and did not require induction by PCP. Hydroxyl groups flanked by two chlorine atoms seemed to be selectively O-methylated (Häggblom et al. 1988b). Uotila et al. (1991, 1992) demonstrated the ability of *R. chlorophenolicus* PCP-1 cells and membrane associated enzymes, to dehalogenate compounds under anaerobic condi-

Table 3. Enzymes, pathways and intermediates implicated in PCP degradation by bacteria.

Bacteria	Enzyme	Location & activity	Substrate	End-products	Character istics	Substrate conversion	Optimum conditions	References
Flavobacterium sp. ATCC 39723	PCP-4-monooxygenase	Purified cell-free extract 11.51U/ mg protein	PCP NADPH O_2	Tetrachloro-*p*-hydroquinone (TeCH)	63 kDa (132 kDa dimer) 1 Flavin mol/enzyme Inducible gene pcpB	37 μM PCP → TeCH 20 min	- pH 7.5–9.0 - Temp. 35–40° C	Xun & Orser 1990
			Triiodophenol (TIP) Trichlorophenol (TCP) Tribromophenol (TBP)			40 mg/L 40 min		Xun & Orser 1991
			Bromoxynil NADPH O_2	Dibromo hydroquinone		100 μM (52.6 mg/L) 20 min		Topp et al. 1992b
	TeCH Reductive dehalogenase	Purified cell-free extract 122.8 U/ mg protein	TeCH Glutathione (GSH)	Trichlorohydro-quinone (TCH) Dichlorohydro-quinone (DCH)	60 kDa dimer Oxygen sensitive constitutive gene pcpC	100 μmol → DCH	pH 6.0–7.0 Temp 40° C 100 μm Salts	Xun et al. 1992d
Rhodococcus chlorophenolicus PCP-1		Crude membrane extracts *p*-hydroxylase	PCP NADPH FAD	TeCH	Cytochrome P-450 involved inducible	1.75 nmol PCP/h/ mg protein		Uotila et al. 1991
		Crude cell-free extract	TeCH	- TeCMP from. uninduced -1,2,4-Trihydroxybenzene induced		13.3 nmol TeCH/h/ mg protein		Apajalahti & Salkinoja-Salonen 1987

Table 3. Continued.

Bacteria	Enzyme	Location & activity	Substrate	End-products	Character istics	Substrate conversion	Optimum conditions	References
Rhodococcus sp. CP-1		Crude cell-free extract	TeCH	Chlorinated trihydroxy benzene		50 μM TeCH → 8 μM monochlorotrihydroxy-benzene + 20 μM trihydroxybenzene		Häggblom et al. 1989
Arthrobacter sp. ATCC 33790		Membrane associated 5.5–12 mU/mg protein	PCP NADPH O_2	TeCH	Inducible	PCP decreased 1.6 mM– 0.75 mM		Schenk et al. 1989
Mycobacterium sp.		Crude cell-free extract	PCP + methyl-donor	PCA	Constitutive, heat stable			Suzuki 1983

tions in the presence of iodosobenzene and Na_2SO_3. Iodosobenzene-supplemented cells were as effective at PCP degradation under anaerobic conditions as cells were under aerobic conditions. Rates of consumption were 1.31 and 1.28 nmol PCP/h under aerobic and anaerobic conditions, respectively. Iodosobenzene is believed to activate the transfer of oxygen molecules from water (Uotila et al. 1992). *R. chlorophenolicus* enzyme extracts had rates of consumption of 2.26 nmol PCP/h/mg protein.

A *Rhodococcus* sp. isolated by Häggblom et al. (1988) also exhibits an inducible PCP degradation pathway, as shown by use of chloramphenicol. Chlorinated hydroquinones were detected, indicating this reaction is a *p*-hydroxylation. O-Methylation was also seen and, as in the case for *R. chlorophenolicus* PCP-1, was constitutive. Intermediates from O-methylation were chloromethoxyphenols and chlorodimethoxyphenols (Häggblom et al. 1988). Using a crude extract prepared from cells of a *Rhodococcus* strain, Häggblom et al. (1989) demonstrated disappearance of TeCH with formation of dichlorotrimethoxybenzene, monochlorotrimethoxybenzene and trimethoxybenzene, suggesting that hydroxylation and dechlorination of TeCH to dichlorotrimethoxybenzene occurred by three reductive dechlorination reactions. This pathway is similar to that in *R. chlorophenolicus* PCP-1.

Little information is available on the pathway and metabolites PCP metabolism by *Arthrobacter* sp ATCC 33790. Schenk et al. (1989) isolated a membrane fraction capable of *p*-hydroxylating PCP to TeCH. Schenk et al. (1990) used this membrane fraction to investigate the source of the hydroxyl group by radiolabel studies. They found both hydroxyl groups on TeCH, one associated with the phenol ring and one donated, were labelled in the presence of labelled water. This occurred in controls with and without the addition of enzyme. It was not possible to determine where the hydroxyl group originated from. If dioxygen had donated the hydroxyl group, it may have been replaced by a hydroxyl group from water, as the original phenol hydroxyl group evidently was (Schenk et al. 1990). The origin of the hydroxyl group remains to be determined to obtain a more complete picture of the pathway of PCP degradation in *Arthrobacter* sp. ATCC 33790.

Suzuki (1978) reported the transformation of PCP to pentachloroanisole (PCA) by cell-free extracts of *Mycobacterium* sp. *S*-Adenosylmethionine was added as a methyl donor. The degradation intermediates by this strain was first found to be tetrachloro-2-methoxyphenol (after 1 h), and subsequently PCA, trichlorodimethoxybenzene, and tetrachlorodimethoxybenzene (after 4 h) (Suzuki 1983). Trichlorocatechol and TeCH were also detected. The *Mycobacterium* strain isolated by Häggblom et al. (1988c) degraded several chlorinated phenols, guaiacols and syringols without accumulation of end-products except small amounts of anisoles. The intermediates were identified as TeCH and TCH by mass spectroscopy. Contrary to *Flavobacterium, Rhodococcus* and *Arthrobacter* spp., the initial step of PCP transformation by *Mycobacterium* sp. proceeded in uninduced or PCP-induced cells in the presence of chloramphenicol, indicating activity was constitutive rather than inducible (Häggblom et al. 1988c).

Johri et al. (1991) reported that *Pseudomonas ovalis* exhibited a plasmid-mediated dechlorinase activity. When the plasmid was cured, dehalogenase activity was lost. Orser et al. (1993a) were unable to detect any dehalogenase or degradative activity associated with a plasmid in *Flavobacterium* sp.

PCP degradation by fungi

Degradation of xenobiotics by fungi offers some advantages over bacteria. Many fungi implicated in PCP degradation are members of the white-rot Basidiomycetes and are capable of degrading lignin, a structural polymer in woody plants. Lignin is insoluble and extracellular, therefore the fungus excretes extracellular enzymes. The excreted enzymes are instrumental in catalyzing the initial oxidative depolymerization of lignin molecules (Mileski et al. 1988). The degradation of lignin releases nutrients and plays a role in recycling of photosynthetically-fixed carbon (Lamar & Dietrich 1992).

Fungi, in particular white-rot Basidiomycetes, have been implicated in the non-specific degradation of a number of xenobiotic compounds including DDT, chlorinated phenoxyacetates, chlorinated anilines, PCP, chlorinated alicyclic insecticides, biphenyl, polychlorinated biphenyls, polycyclic aromatic hydrocarbons, chlorinated dioxins, 2,6,6-trinitrotoluene, azo dyes, and triphenylmethane dyes (Higson 1991). With this broad range of degradative activity, fungi may be suitable organisms for inoculation into PCP-contaminated sites with all the associated toxic compounds. Almost all studies on PCP degradation were conducted with members of the white-rot Basid-

iomycetes. Among these, *Phanerochaete chrysosporium* and *Phanerochaete sordida* have received particular attention. Members of the *Trametes* genus such as *Trametes versicolor* and *Trametes hirsuta* have also shown potential as PCP degraders. A number of other families of fungi were tested for PCP degradation including the yeasts, Zygomycetes, Deuteromycetes (Melanconiale, Mucedinaceae, Dematiaceae, Tuberculariales), Basidiomycetes and Ascomycetes (Seigle-Murandi et al. 1991).

Different families of fungi exhibit different tolerances to PCP, with a large amount of heterogeneity existing in the PCP tolerances of individual genera in the families. Seigle-Murandi et al. (1991) conducted a study of PCP toxicity to, and degradation by, fungi. At 0.5 g/L, all families and species were inhibited except *Fusarium moniliforme*. Lowering the pH in the medium caused precipitation of PCP and highlighted strains with halo regions around them, indicating degradation and possibly growth. Of fifty strains selected, one half had spores sensitive to PCP and required 48 h mycelial growth before PCP toxicity was overcome. Fungi tolerant of 1 g/L PCP and able to degrade PCP included 1 yeast genus; 4 Zygomycete genera; 1 Deuteromycetes (Melanconiale); 14 Deuteromycetes (Mucedinaceae); 1 Basidiomycete; 22 Deuteromycetes (Dematiaceae); 3 Deuteromycetes (Tuberculariales) and 3 Ascomycetes.

Most studies on fungal degradation of PCP have focused on white-rot Basidiomycetes. Alleman et al. (1992) investigated the toxicity of PCP to six species of white-rot fungi. They found growth of *P. chrysosporium* seized at 17 μg PCP/mg mycelium. The most tolerant species was *T. versicolor*. All six species failed to grow at PCP concentrations higher than 5 mg/L when added as a young culture (less than 1 day old). This indicates growth of the fungus, resulting in greater biomass, may favourably affect tolerance to PCP (Alleman et al. 1992).

Lamar et al. (1990b) tested the sensitivity of *Phanerochaete* sp. to PCP. *P. chrysosporium* and *P. sordida* strains were the most resistant, still showing mycelial extension at 25 mg/L PCP. *P. laevis, P. chrysorhiza, P. sanguinea*, and *P. filamentosa* were sensitive to 5 mg/L PCP. Mileski et al. (1988) found *P. chrysosporium* spores were sensitive, with loss of viability to PCP at 4 mg/L. The establishment of a mycelial mat seemed to counteract PCP toxicity. They also reported that a decrease in fungal respiration, in the presence of 500 mg/L PCP, was only detected in nitrogen-limited cultures. Alleman et al. (1992) found that with *P. chrysosporium*, growth became inhibited at 17 mg/L PCP and suggested the high value reported by Mileski et al. (1988) was due to the high biomass used. Lamar & Dietrich (1990) studied the ability to re-isolate *P. chrysosporium* and *P. sordida* from wood chips in soil plots exposed to PCP. *P. chrysosporium* was isolated on days 1, 8, 15, 46 but not on days 20 and 29. *P. sordida* was re-isolated on all days tested.

Most studies assessing PCP toxicity to fungal growth involved measuring hyphal length of the mycelial mat (Mileski et al. 1988; Lamar et al. 1990b; Alleman et al. 1992). Mileski et al. (1988) measured the effect of PCP on fungal respiration by following metabolism of ^{14}C-glucose to $^{14}CO_2$. Alleman et al. (1993) developed a rapid screening method for detection of PCP-resistant fungi. This method involved adding a PCP-soaked cellulose disc to a fungal culture (4 cm mycelial diameter) and measuring the zones of inhibition formed around the disc. At 24 h, all strains tested were inhibited by 10 mg/L PCP. By day 14, 17 of 18 strains had overgrown the discs. The latter observation was believed to reflect a weakness in the assay. By day 5, the PCP in the disc had leached into the agar such that the PCP in the disc was no longer as concentrated as initially applied and thus was no longer as toxic. This method could prove useful for short-term screening.

Most fungi involved in degradation of PCP exhibited the ability to non-specifically degrade a range toxic and persistent chemicals. Table 4 summarizes information on PCP degradation by different genera and species of fungi. It appears that fungi, in general, are not efficient at mineralizing PCP. Mileski et al. (1988) reported the highest percentage of PCP mineralized in liquid culture by *Phanerochaete chrysosporium* as 23% of 1.1 mg/L PCP. The only other fungal strain to show the potential to mineralize PCP in liquid culture was *P. sordida*. Lamar et al. (1990b) reported this strain mineralized 11.64% of 25 mg/L PCP compared to 1.9% mineralization by *P. chrysosporium* under the same conditions. *T. hirsuta* demonstrated the ability to mineralize 27.23% of 382 μg PCP/g soil in softwood chips. This was the only reported case of mineralization of PCP in soil by a fungal strain.

A large proportion of PCP removed in liquid culture by fungi was converted to non-transformable products such as pentachloroanisole. Many fungal species exhibited the ability to degrade PCP almost completely. However, very little of the degradation was accounted for by mineralization, none by dechlorination, and only one report indicates incorporation of ^{14}C-label

Table 4. PCP degradation by pure cultures of fungi.

Fungi	Conditions	[PCP]	Degradation	Mineralization	Dechlorination	Incorporation	Adsorption	References
Phenerochaete chrysosporium	Liquid N-limited	1.1 mg/L	97%	23%	NR	NR	NR	Mileski et al. 1988
	Liquid N-limited	13.6 nmol	78%	50.5%	NR	2.8%	NR	
	Liquid N-sufficient	13.6 nmol	45%	10.2%	NR	7.6%	NR	
	Liquid	1.9 mM	1.349 mM	NR	NR	NR	NR	
	Liquid	25 mg/L	16%	1.9%	NR	NR	NR	Lamar et al. 1990b
	Liquid	19 μM	70%	NR	NR	NR	NR	Roy-Arcand & Archibald 1991
	Liquid	40 mg/L	95%	NR	NR	NR	NR	Alleman et al. 1992
	Soil	50 μg/g	< 1 μg/g remaining	2%	NR	NR	NR	Lamar et al. 1990a
		80 μg/g	96% 64 d	NR	NR	NR	NR	Lamar et al. 1990b
		250–400 μg/g	51% 22 d	NR	NR	NR	NR	Lamar & Dietrich 1990
			86% 46 d	NR	NR	NR	NR	
		250 μg/g	63–72% 6 wk	NR	NR	NR	NR	Lamar & Dietrich 1992
		672 μg/g	72% 56 d	NR	NR	NR	NR	Lamar et al. 1993
Phanerochaete sordida	Liquid	25 mg/L	+	11.64%	NR	NR	NR	Lamar et al. 1990b
	Soil	80 μg/g	82% 64 d	NR	NR	NR	NR	Lamar et al. 1990b
		250–400 μg/g	77% 22 d	NR	NR	NR	NR	Lamar & Dietrich 1990
			82% 46 d	NR	NR	NR	NR	
			45–60% 42 d	NR	NR	NR	NR	Lamar & Dietrich 1992
		672 μg/g	89%	NR	NR	NR	NR	Lamar et al. 1993
Tramates versicolor	Liquid	24 μM	82.1% 3 h	NR	NR	NR	NR	Roy-Arcand & Archibald 1991
		40 mg/L	0.2 mg/L	NR	NR	NR	NR	Alleman et al. 1992
Tramates hirsuta	Soil	382 μg/g	62%	27.23%	NR	24%	43%	Lamar & Dietrich 1992
		672 μg/g	55% 56 d	NR	NR	NR	1%	Lamar et al. 1993
Phoma glomerata	Liquid	1 g/L	15%	NR	NR	NR	NR	Seigle-Murandi et al. 1991

Table 4. Continued.

Fungi	Conditions	[PCP]	Degradation	Mineralization	Dechlorination	Incorporation	Adsorption	References
	Dark	100 mg/L	14% 24 h 31% 12 d	NR	NR	NR	NR	
	Light		22% 24 h 50% 12 d	NR	NR	NR	NR	
Ceriporiopsis subvermispora	Soil	448 mg/L	37%	NR	NR	NR	NR	Lamar & Dietrich 1992
Zygomycetes	Liquid 3/4 strains tested	1 g/L	25%	NR	NR	NR	NR	Seigle-Murandi et al. 1991

NR: not reported, +: denotes positive response.

from radiolabelled PCP into fungal biomass (Mileski et al. 1988).

The situation is similar in soil, however, adsorption of PCP to soil components was evident. Lamar & Dietrich (1992) reported 43% of the PCP added (250–448 μg/g soil) adsorbed to the wood chips. In addition, substantial amounts of PCP degradation often occur in uninoculated controls. Lamar et al. (1990a) reported 43% of 50 μg PCP/g soil was removed in uninoculated soils, and Lamar et al. (1990b) found 20% of PCP (80 μg/g soil) removal in controls. In these studies, degradation in controls was attributed to abiotic degradation since there was no growth of any microorganisms from the soil on selective media. This does not rule out indigenous PCP degraders since microorganisms may be non-culturable on the media or under the growth conditions used. Seigle-Murandi et al. (1991) investigated PCP degradation by *Phoma glomerata*. Abiotic degradation of PCP in uninoculated controls reached 25% and 8% after 12 days in light and dark, respectively.

The use of wood chips in PCP degradation experiments in soil is common for fungi. Often wood chips are used as vehicles of fungal inoculation into soil (Lamar et al. 1990a, 1990b; Lamar & Dietrich 1990). In some cases the wood chips serve as a soil amendment, providing an additional carbon source for fungi (Lamar & Evans 1993). Since PCP is used as a wood preservative, PCP-contaminated wood is processed into chips to facilitate fungal degradation. *T. hirsuta* caused a 25% weight loss after 4 weeks in PCP-contaminated softwood chips from ammunition boxes. In addition, 27% mineralization of 382 μg PCP/g soil was observed (98% total degradation) (Lamar & Dietrich 1992). In sawdust-treated with 500 mg PCP/L, *P. chrysosporium* degraded over 97% PCP in two weeks. Most (70–80%) of the PCP was recovered as pentachloroanisole (McBain et al. 1995).

Nutrient amendments

Mileski et al. (1988) reported that PCP mineralization by *P. chrysosporium* was enhanced at PCP concentrations of 1.1 mg/L under nitrogen-limited conditions, 50.5% as compared to 10.2% when nitrogen was not limiting. In contrast, Lamar et al. (1990a) found the rate of degradation by *P. chrysosporium* was most rapid in the soil with the highest amounts of carbon and nitrogen. Although the rate was faster, the eventual amount of PCP degraded was similar in all soils, regardless of nutrient components. At 5 μg PCP/mg mycelium, cultures of *P. chrysosporium*, under nitrogen deficiency, were completely inactivated and could not be revived (Alleman et al. 1992). This concentration of PCP was not lethal to nitrogen-sufficient cultures. Unlike *P. chrysosporium*, *Inonotus dryophilus* and *T. versicolor* remained viable and had comparable biomass production in both nitrogen-limited and nitrogen-sufficient conditions (Alleman et al. 1992). Siegle-Murandi et al. (1991) also found that glucose at concentrations above 5 g/L, repressed PCP degradation by several fungal strains. While degradation may be

Table 5. Degradation of PCP by anaerobic microbial cultures.

Source of inoculum	Conditions	Design	Acclimation	[PCP]	Degradation	Mineralization	Dechlorination	References
Desulfomonile tiedjei DCB-1	Liquid + formate		+ 3-chlorobenzoate	10 μM	less than 50 nM remaining	NR	54 μmol Cl^-/h/g protein	Mohn & Kennedy 1992
Anaerobic digestor sludge	Liquid	Anaerobic serum bottles	-	40 μM (11 mg/L)	+	22.4% (38.3% CH_4)	+	Mikesell & Boyd 1986
			+ (monochloro phenols)			22.9% (32.0% CH_4)	+	
Anaerobic sewage sludge	Liquid	Anaerobic upflow bioreactor	+ monochlorophenols phenol	1.61 mg/L/d	35.2% 18 d	NR	NR	Krumme & Boyd 1988
Municipal sludge digestor	Liquid	Continuous-flow upflow anaerobic reactor	NR	0.384 μmol/L (100 μg/L)	95%	NR	+	Woods et al. 1989
				0.32 μmol/L	94.9%	NR	+	
				1.04 μmol/L	96.5%	NR	+	
				1.92 μmol/L	94.9%	NR	+	
Municipal digestor sludge	Liquid	Anaerobic serum bottles	+ PCP	10 μM	+ (brief lag period)	NR	+	Madsen & Aamand 1991
Granular sludge	Liquid	UASB reactor	+	2.0–4.5 mg/L	95% 1.5 months	NR	20%	Hendriksen & Ahring 1993
Anaerobic digested sewage sludge	Liquid + glucose	Fixed-film reactor	+	2 mg/L	98% 9 months (60% controls)	NR	20–40% (1% in controls)	
				3 mg/L	98% (21% controls)	NR		
Anaerobic sludge	Wastewaters	Sealed anaerobic reactor	+ PCP	0.5–10 mg/L 46 d	+	+	NR	Kudo 1989
Sediments		Screw-cap glass vessels	-	0.82 μg/g sediment	+ (5 d lag period)	NR	+	Abrahamsson & Klick 1991
			+		+ (no lag)	NR	+	
			+	5 μg/g sediment	+	NR	+	

Table 5. Continued.

Source of inoculum	Conditions	Design	Acclimation	[PCP]	Degradation	Mineralization	Dechlorination	References
Sediment	Sediment slurries	Anaerobic chamber	-	10, 20 mg/L	- (40 d)	NR	-	Bryant et al. 1991
				65 mg/L	29 mg/L remaining (144 d)	NR	+	
			+ (2,4-dichlorophenol) + (3,4-dichlorophenol)	20 mg/L	3.1 mg/L remaining (5 d)	NR	+	
					1.0 mg/L remaining (7 d)	NR	+	
Sewage sludge wastewater treatment plant	Soil (loamy, fine sand)	Flasks soil + sludge	NR	70 μmol/kg soil	0.5 μmol/kg soil 28 d (left)	NR	NR	Mikesell & Boyd 1988
		Soil			45% (degraded) 46 d			
		Soil + sludge (aerobic)			20% 28 d	NR	63%	
		Soil + sludge (anaerobic)			100% 32 d	NR	64%	
Anaerobic sewage sludge	NR	Anaerobic serum vials	+ PCP, phenol	7.5–37.5 μM 8 months	7–17%	NR	+	Larsen et al. 1991
Thermophilic sewage sludge		50° C			3%	NR	+	
Freshwater sediments					80–90% 1.61 μmol/L/d at 7.5 μmol 7.5 μmol/L/d at 37.5 μmol	NR	+	
Cattle manure digestor					70%	NR	+	
Granular sludge from sugar-containing waste-water		UASB reactor and glucose	+ PCP, phenol	3.0 mg/L	75% 4 d 100% 10 d	NR	NR	Hendriksen et al. 1992
				4.5 mg/L	90% 2.2 mg PCP/l/reactor/d	NR	NR	
Anaerobic digestor sludge		Dual-reactor system: continuous flow & batch reactor	-	1.35 μM	97% 10 d	NR	+	Nicholson et al. 1992
			+	4.38 μM	99.7% 1.2 d	NR		

Table 5. Continued.

Source of inoculum	Conditions	Design	Acclimation	[PCP]	Degradation	Mineralization	Dechlorination	References
Volatile fatty acid granules + PCP-degrading granules		UASB reactor	NR	60 mg/L 21.8–36.5 mg/day/reactor 171–205 d	98–99% removal	18% (52% CH_4)	NR	Wu et al. 1993
				40 mg/L 3 d	22.5 mg/d removal	19.4 mg/g VSS/d	NR	

NR: not reported, +: denotes positive observation, -: denotes no response.

Table 6. Pathways and intermediates of PCP degradation by anaerobic cultures.

Source of inoculum	Conditions	Acclimation	Substrate	Intermediates	Dechlorination pathway	References
Desulfomonile tiedjei		+ 3-chlorobenzoate	10 μM PCP	2,3,4,5-TeCP 2,4,6-TCP	*meta* *meta*	Mohn & Kennedy 1992
Sediments	Glass vessels	-	0.82 μg/g PCP	2,3,4,5-TeCP 3,4,5-TCP 3,5-DCP 3 MCP	*o* *o* *o* *o*	Abrahamsson & Klick 1991
		+		2,3,4,5-TeCP 3,4,5-TCP 3,5-DCP 2,3,5,6-TeCP	*o o > p > m* *o* *o* *p*	
			5 μg/g PCP	2,4,5-TCP 3,4-DCP	*m*	
Sediments	Anaerobic chamber	-	65 μg/ml PCP	2,3,5,6-TeCP 2,3,5-TCP	*p* *o*	Bryant et al. 1991
		2,4-DCP		2,3,4,5-TeCP	*o*	
		3,4-DCP		2,3,5,6-TeCP	*p*	
		1:1 1,4-DCP:3,4-DCP		2,3,5,6-TeCP 2,3,5-TCP 3,5-DCP 3-CP Phenol	*p* *o* *o* *m*	
Thermophilic manure	Serum vials 50° C	-	7.5–37.5 μM PCP	2,3,4,5-TeCP 3,4,5-TCP 3,5-DCP	*o* *o A* *p*	Larsen et al. 1991
Freshwater lake sediment				2,3,5,6-TeCP 2,3,5-TCP 3,5-DCP	*p* *o B* *o*	
Alder swamp sediment					*A + B*	
Freshwater stream sediment					*A + B*	
Municipal sewage sludge		2-MCP	10 μg/ml PCP	3,4,5-TCP 3,5-DCP	*o*	Mikesell & Boyd 1986
		3-MCP		2,3,4,6-TeCP 2,4,6-TCP	*m*	
		4-MCP		2,3,5,6-TeCP 2,3,5-TCP	*p*	
		2-,3-,4-MCP		3,4,5-TCP 3,5-DCP 3-MCP	*o*	
Granular sludge	UASB reactor		PCP 95%	2,3,5,6-TeCP 2,3,5-TCP 3,5-DCP	*p*	Hendriksen et al. 1992
			5%	2,3,4,5-TeCP 3,4,5-TCP 3,5-DCP	*o*	

Table 6. Continued.

Source of inoculum	Conditions	Acclimation	Substrate	Intermediates	Dechlorination pathway	References
Sewage sludge	Fixed-film reactor		PCP	2,3,4,5-TeCP	*o*	Hendriksen & Ahring 1993
				3,4,5-TCP	*o*	
Granular sludge	UASB reactor		PCP	2,3,4,5-TeCP	*o*	
				3,4,5-TCP	*o*	
				2,3,5,6-TeCP	*p*	
				2,3,5-TCP	*o*	
Anaerobic digestor sludge	Batch reactor	-	1,35 μM PCP	2,3,4,5-TeCP	*o*	Nicholson et al. 1992
				3,4,5-TCP	*o*	
		+	4.38 μM PCP	2,3,4,5-TeCP	*o*	
		PCP		2,3,5,6-TeCP	*p*	
				2,3,4,5-TeCP	*m*	
				3,4,5-TCP		
				2,4,5-TCP		
				2,3,5-TCP		

MCP: monochlorophenol, DCP: dichlorophenol, TCP: trichlorophenol, TeCP: tetrachlorophenol, *o*: ortho, *p*: para, *m*: meta, +: denotes acclimation, -: denotes absence of acclimation, A: pathway A, B: pathway B.

enhanced under nitrogen-limitation by *P. chrysosporium*, cell viability at higher PCP concentrations is increased when nitrogen is present. It would be advantageous to find a fungal strain with as few nutrient or growth constraints as possible since conditions in situ are usually less than ideal.

Fungal enzymes involved in PCP degradation

Unlike bacteria, fungi generally do not utilize PCP as a source of carbon and energy. Degradation of PCP, and of many other xenobiotic compounds, is not the consequence of enzyme systems targeted to this function. Fungal enzyme systems generally exist to serve other purposes such as degradation of wood components like ligninocellulose. Enzymes isolated and identified as having PCP degradative potential are the phenol oxidases; lignin peroxidase, manganese (Mn) peroxidase and laccase.

Lignin peroxidase (ligninase) catalyzes the initial oxidative depolymerization of lignin and has been implicated in oxidation of a number of xenobiotic compounds (Mileski et al. 1988). Manganese peroxidase oxidizes Mn(II) to Mn(III) which, in turn, oxidizes phenolic substrates to phenoxy radicals (Michel et al. 1991; Périé & Gold 1991). Mileski et al. (1988) purified a ligninase from the extracellular fluid of *P. chrysosporium* cultures. A mixture of ligninase, hydrogen peroxide, dimethylformamide and PCP resulted in the loss of 9.3 μM PCP and the appearance of 7.3 μM of a metabolite (2,3,5,6-tetrachloro-2,5-cyclohexadiene-1,4-dione or TCHD). Lin et al. (1990) examined the effect of extracellular and cell-associated enzymes on PCP degradation. They found the cell mass component was able to mineralize PCP but when cell biomass and extracellular enzyme components were used in conjunction, 70% of 10.45 μmol/L PCP added was mineralized in 22 days. Lin et al. (1990) suggested the enzyme preparation included extracellular oxidases, Mn peroxidases in the oxidizing state, and peroxidases. The cell mass was believed to contain glucose oxidases. The hydrogen peroxide required by extracellular enzymes may be produced by the glucose oxidase or other extracellular oxidases (Lin et al. 1990).

Few studies have demonstrated the ability of fungi to dechlorinate PCP. The extracellular enzyme, laccase, isolated from *T. versicolor*, was shown to be responsible for a significant portion of chlorophenol degradation. Laccase is a phenol oxidase that was originally named because of its ability to clot the sap of the lacquer tree when exposed to oxygen (Roy-Arcand & Archibald 1991). These researchers isolated four laccases from *T. versicolor*. After a 30 min incubation with a crude laccase preparation, 100% of 2,4-dichlorophenol; 4,5- and 4,5,6-chloroguaiacol; 86% of 2,3,4,6-tetrachlorophenol; 23–40% of PCP, 2,4,6-trichlorophenol, and tetrachloroguaiacol were degraded. Dechlorination was found to proceed rapidly and

in conjunction with oxygen consumption and substrate disappearance. When 800 μM of a mixture of chlorophenols and chloroguaiacols were exposed to the crude laccase, 540 μM Cl^- were released within 30 min while 100 μM O_2 was consumed and 544 μM of substrate was used. By 63 min the chloride ion level reached 740 μM. Over 30 min, in the presence of PCP, the crude laccase preparation consumed no oxygen and released no chloride ions. After 17 h, 24% of PCP was consumed and 6.6% chloride ions liberated. Laccase, in the presence of ABTS, resulted in 69% disappearance of PCP compared to 24% without, after 17 h.

Seigle-Murandi et al. (1993) investigated the PCP degrading potential of 999 fungal strains from different fungal families. Also investigated was the relationship between production of phenol oxidase activities and PCP degradation. In Zygomycetes, the highest rate of degradation was found in species that did not produce any phenol oxidase activity. In the Basidiomycetes, the highest producers of phenol oxidase activity did not degrade PCP. In all the families tested, no clear correlation between production of phenol oxidase activity and PCP degradation was observed.

In general, a negligible amount of PCP is mineralized by most fungi studied. Most of the PCP was transformed, often by O-methylation, to intermediates such as pentachloroanisole. Several researchers report pentachloroanisole as an intermediate of PCP degradation by *P. chrysosporium*. Lamar et al. (1990b) found 64–71% of 80 μg PCP/g soil was transformed to pentachloroanisole and as the amount of PCP in the soil decreases, pentachloroanisole increases. Lamar & Dietrich (1990) reported *P. chrysosporium* transformed 13% of 250–400 μg PCP/g soil to pentachloroanisole in PCP-contaminated soil over 15 days. *P. chrysosporium* transformed 65% of 250 μg PCP/g soil in hardwood chips to pentachloroanisole and 72% of PCP in softwood chips to pentachloroanisole. McBain et al. (1995) observed 70–80% of 500 mg/L PCP degraded by *P. chrysosporium* was recovered as pentachloroanisole, indicating dominance of methylation in PCP degradation. Lamar et al. (1990b) determined most of the volatilization of PCP by *P. chrysosporium* was due to accumulation of pentachloroanisole. Researchers have found that *P. sordida* accumulated less pentachloroanisole than *P. chrysosporium*. Lamar & Dietrich (1990) reported transformation by *P. sordida* to be 8% of 250–400 μg PCP/g soil to pentachloroanisole over 22 days, and Lamar & Dietrich (1992) reported transformation of 7% PCP to pentachloroanisole in softwood chips and 17% in hardwood chips. Lamar et al. (1990b) also found *P. sordida* had the ability to mineralize 13.07% of pentachloroanisole. Purified lignin-ase from *P. chrysosporium* produced an intermediate other than pentachloroanisole, 2,3,5,6-tetrachloro-2,5-cyclohexadiene-1,4-dione (TCHD) (Mileski et al. 1988). No accumulation of pentachloroanisole was demonstrated by *T. hirsuta* and *C. subvermispora* (Lamar & Evans 1993). *T. versicolor* laccases demonstrated effective dechlorination of PCP, generating a brown colour in treated samples which suggested the presence of quininoid derivatives (Roy-Arcand & Archibald 1991). Seigle-Murandi et al. (1991) showed intermediates from PCP degradation by *Phoma glomerata* with mass spectrum retention times identical to tetrachlorodiphenols and tetrachloromuconic acid. Trace amounts of pentachloroanisole were also detected. O-Methylation is a dominant degradative process in fungi. There are no reports of isolation and characterization of the enzymes responsible for O-methylation of PCP.

Lin et al. (1991) proposed two models for degradation of PCP by *P. chrysosporium*. The first involves initial degradation by extracellular enzymes, followed by conversion of the degradative intermediate (TCHD) to CO_2 by cell-bound enzymes. The second model suggests the cell-bound enzymes directly mineralize the PCP without using extracellular enzymes. Both models may work together. It is known extracellular fungal enzymes convert PCP to TCHD. Adsorption of PCP to mycelium of fungi also occurs, especially at high PCP concentrations, thereby limiting availability of PCP to extracellular enzymes. It is believed that direct mineralization of PCP by cell-associated enzymes also occurs.

More studies are needed to elucidate pathways of PCP degradation and the influence of enzyme systems on degradation by fungi. Because strains of *Trametes* sp. do not accumulate the intermediate pentachloroanisole as the *Phanerochaete* strains do, possibly due to different enzyme systems, they should be studied more closely for use as degradative organisms. Pentachloroanisole is known to be less toxic to microorganisms than PCP but it is also more lipophilic and may bioaccumulate. The purified lignin peroxidase from *P. chrysosporium* appears to show promise for PCP degradation, especially in conjunction with cell-bound enzymes. Efforts to purify and characterize cell-bound factors that aid in PCP mineralization should be made. *T. versicolor* and *T. hirsuta* may be

better candidates for direct inoculation into sites than members of *Phanerochaete*. They are more resistant to PCP than other fungal genera and do not accumulate known toxic intermediates. *T. versicolor* produces laccase, which is effective in dechlorination of chlorophenols. Unlike lignin peroxidase from *P. chrysosporium*, laccases do not require addition of hydrogen peroxide.

Anaerobic degradation of PCP

Research on anaerobic degradation of PCP is of critical importance. Sites contaminated with PCP include anaerobic environments as such as soils, water, sediments and industrial sludge. Anaerobic environments contain microbial consortia involved in methanogenesis and sulfate reduction. It is therefore important to evaluate the effect of PCP on these anaerobic processes.

Few researchers have successfully isolated and characterized PCP-degrading anaerobes. To date, only one anaerobic bacterium capable of dehalogenating PCP has been isolated. *Desulfomonile tiedjei* DCB-1 is a pure-culture anaerobe capable of aromatic reductive dehalogenation. This organism was originally isolated from sewage sludge enriched for mineralization of 3-chlorobenzoate (Deweerd et al. 1990). The bacterium is a Gram-negative, non-sporulating, obligately anaerobic rod with an invagination of the cell wall. It was designated as a new genus in the class *Proteobacteria* based on 16S rRNA sequence analysis. It was granted the genus name *Desulfomonile*, a 'collared' sulfate-reducer, and species *tiedjei*, in deference to J.M. Tiedje, who was instrumental in its isolation (Deweerd et al. 1990).

Dietrich & Winter (1990) enriched for a 2-chlorophenol-degrading consortium in sewage sludge. They distinguished 3 morphologically distinct bacteria by phase contrast microscopy and scanning electron microscopy. The most dominant organism was a spirochaete-like bacterium, while the other two were coccoid and rod-shaped. Unlike *D. tiedjei* DCB-1, the bacteria in this mixed culture did not use sulfate or nitrate as electron acceptors. The presence of *n*-butyrate or other fatty acids was required as the electron acceptors (Dietrich & Winter 1990). No attempt was made to isolate or characterize these bacteria and no possible role for them was suggested by the researchers.

One other report attempted to determine the dominant bacteria present on PCP-degrading granules in an UASB reactor (Wu et al. 1993). *Methanothrix*-like rods growing as long filaments or as chains of three to five cells long were the main species evident. *Methanosarcina* sp. and *Methanobacterium* sp. were also prevalent in granules. No attempt was made to isolate these bacteria or to determine their contributions to PCP degradation.

Microorganisms capable of PCP degradation exist in a number of anaerobic environments, many without previous exposure of PCP. In anaerobic conditions, PCP has been implicated in inhibition of methanogenesis and volatile fatty acid degradation. Guthrie et al. (1984) found methane production was inhibited in unacclimated digested sewage at 0.2–0.4 mg PCP/L. Wu et al. (1993) also reported inhibition of activity of acetogens and methanogens by 1.0–2.5 mg PCP/L in volatile fatty-acid granules. At 2.5 mg and 20 mg PCP/L, methane production in volatile fatty-acid granules was reduced to 43% and 1.6%, respectively. In PCP-degrading granules (pre-exposed to PCP), inhibition of methane production was reduced. The presence of 5 mg PCP/L was required to initiate inhibition of methanogenesis with PCP-degrading granules, compared to 2.5 mg PCP/L in volatile fatty-acid granules. Wu et al. (1993) also commented on the inhibition of propionate-degraders by 1.0 mg/L PCP, and complete inhibition of the acetate-degraders and partial inhibition of the butyrate-degraders at 2.5 mg/L PCP. There is some evidence to suggest acclimation to PCP may decrease the inhibition of methanogenesis. Similar evidence was found for PCP inhibition of nitrification under aerobic conditions.

Mohn & Kennedy (1992a) found *D. tiedjei* DCB-1 growth was inhibited at PCP concentrations of 10 μM (2.63 mg/L) if cells were uninduced and PCP transformation did not occur. If 3-chlorobenzoate was present in the medium, dehalogenation occurred (Mohn & Kennedy 1992a). From the available information it is evident PCP can affect methanogenic processes in the environment. Anaerobic environments rely on all the indigenous organisms to work together to catalyze the degradation of substrates to provide nutrients and energy for cellular processes. Any factor which threatens this delicate balance can adversely affect the viability of the whole community. Very little information is available on how PCP affects the spectrum of reactions occurring in anaerobic environments. Acclimation to PCP or other chlorophenols can enhance tolerance of microorganisms and microbial processes to PCP. Care

must be taken with the concept of acclimation, however, because it may not be creating tolerance so much as selecting for specific microorganisms in the complex consortia.

Under anaerobic conditions, PCP degradation proceeds by reductive dechlorination. This means intermediates are lower chlorinated phenol, such as tetrachlorophenols (TeCP), trichlorophenols (TCP), dichlorophenols (DCP) and monochlorophenols (MCP). Table 5 summarizes available information on PCP degradation, mineralization and dechlorination by anaerobic cultures. There are few reports which demonstrate mineralization of [^{14}C]PCP by anaerobic consortia (Mikesell & Boyd 1986, 1988; Wu et al. 1993). Anaerobic consortia from sewage sludge, water sediments and granular sludge were used as sources of inoculum. Of the inoculum sources used, some showed limited ability to degrade PCP. Krumme & Boyd (1988) found anaerobic sewage sludge degraded little PCP at 1.61 mg/L in an anaerobic upflow reactor even when acclimated with monochlorophenols. Larsen et al. (1991) tested three anaerobic sludges and one thermophilic sewage sludge, acclimated with PCP and phenol, and observed degradation of PCP at 50° C was minimal.

Acclimation of inocula with chlorophenols may enhance biodegradation of PCP. Sediment, unacclimated, was unable to degrade PCP. However, when pre-exposed to 2,4-DCP and 3,4-DCP, dechlorination occurred (Bryant et al. 1991). Several studies found that PCP-acclimated inocula exhibit enhanced PCP degradation and reduced lag periods (Abrahamsson & Klick 1991; Madsen & Aamand 1991; Nicholson et al. 1992). *D. tiedjei* requires acclimation with 3-chlorobenzoate before PCP dechlorination will occur. Attempts to acclimate *D. tiedjei* with chlorophenols were unsuccessful (Mohn & Kennedy 1992a). Acclimation with monochlorophenols often does not lead to enhanced PCP degradation (Krumme & Boyd 1988). Mikesell & Boyd (1986) demonstrated the amount of PCP mineralized in an anaerobic digester was lower by a monochlorophenol-acclimated culture (54.9%) than by an unacclimated culture (65.7%).

Different reactor types can affect the efficiency of PCP degradation. The highest reported concentration of PCP degraded was achieved using an upflow anaerobic sludge blanket reactor (UASB) with PCP-degrading granules. Degradation of 60 mg/L PCP was observed over 171–205 days. At day 144, 52% of [^{14}C]PCP was liberated as CO_2 and 18% was recovered as CH_4 (Wu et al. 1993). Hendriksen & Ahring (1993) compared PCP degradation in a fixed-film reactor inoculated with anaerobic digested sewage sludge and an upflow anaerobic sludge blanket (UASB) reactor inoculated with granular sludge, over an extended time period. The fixed-film reactor was less stable than the UASB reactor even when supplemented with glucose. The fixed-film reactor failed to function after 15.5 months and only addition of yeast extract could renew activity, while the UASB reactor functioned without fail for the duration of the 18-month long experiment. This provides some evidence for the superior ability of one reactor type over another. Reactors capable of handling large influents containing PCP over extended time can be more useful in the bioremediation of PCP-contaminated substrates. The study by Hendriksen & Ahring (1993) provides insight on the performance of aerobic microbial communities over long time periods.

Nutritional amendments

Thorough investigations of the effect of carbon sources, nitrogen sources and other nutrients on PCP degradation by anaerobic microorganisms have not been done. Degradation of 2-chlorophenol stopped completely when yeast extract and peptone were omitted from the media. Dehalogenation of 2-chlorophenol required the presence of *n*-butyrate or another fatty acid as an electron acceptor (Dietrich & Winter 1990). The effect of 0.9 g/L glucose on PCP-degrading anaerobic granules was evaluated by Hendriksen et al. (1992). In 10 days, at 3 mg/ml PCP, no PCP could be detected in the effluent in the glucose-amended reactor, while 35% remained in the control glucose-unamended reactor. On day 61, at 4.5 mg/L PCP, there were 500 μg/L PCP in the effluent of the glucose amended reactor and at days 277–353, 99.8% PCP was removed with a removal rate of 2.2 mg PCP/L/reactor/day. The control reactor had effluent PCP concentrations ranging from 200–5000 μg/L at day 61 and 50% PCP remained in the reactor at day 353. Glucose amendment resulted in greater degradative capacity and stability. It also led to 2–3 times more biomass than the reactor without glucose amendment (Hendriksen et al. 1992). Therefore, amendment of reactors with a carbon source such as glucose can increase PCP degradation, decrease lag phases, increase stability of the degradative process and cause concomitant increase in biomass.

D. tiedjei DCB-1 has some specific growth requirements, such as thiamine, 1,4-naphthoquinone, nicotinamide, hemin and lipoic acid. Rumen fluid will also

satisfy its growth requirements (Deweerd et al. 1990). *D. tiedjei* requires pyruvate and formate for growth, and requires acclimation by 3-chlorobenzoate to allow PCP dehalogenation to proceed (Mohn & Kennedy 1992a).

As important as it is to determine the conditions that will enhance degradation, it is also necessary to be aware of inhibitors of PCP degradation. Madsen & Aamand (1991) demonstrated the inhibitory effect of sulfate, sulfite and thiosulfite on PCP degradation. In the presence of 10 mM sulfate initial PCP transformation rate was slightly lower than in methanogenic cultures. By 30 h, the decline in PCP dechlorination in sulfate-amended cultures was significant. Sulfite and thiosulfite at 10 μM were very inhibitory to PCP degradation. The addition of molybdate, an inhibitor of sulfate-reduction, reversed the effect of the sulfate on PCP degradation but not the effect of sulfite or thiosulfite. It is believed that competition for H_2 and other electron donors was responsible for the inhibition of dechlorination by sulfate. Sulfate reduction is a more thermodynamically favourable reaction and could more effectively use low concentrations of H_2 (Madsen & Aamand 1991). Kohring et al. (1989) also found 2,4-DCP degradation proceeded more slowly in the presence of sulfate. Although this inhibition of chlorophenol degradation by sulfate was seen in at least two reports, Häggblom & Young (1990) found a very different situation. They studied the degradation of 3 monochlorophenols and 2,4-dichlorophenol in sulfidogenic sediments. Upon initial incubation in the sulfidogenic sediments, a substantial lag phase (120–220 days) was observed before degradation of the chlorophenols occurred. Upon re-feeding of sediments with the chlorophenols, the lag phase decreased to less than 10 days. Molybdate could inhibit sulfate-reduction, thereby, enhancing PCP degradation (Madsen & Aamand 1991). In the study by Häggblom & Young (1990) molybdate inhibited sulfate reduction and completely inhibited degradation of the chlorophenols, suggesting sulfate-reducing bacteria were responsible for chlorophenol degradation. The differences in these reports could be due to a number of factors. The source of anaerobic microorganisms was different for all three reports, therefore, the sources may contain different microbial consortia. The report by Häggblom & Young (1990) was continued for a longer time than that by Kohring et al. (1989). Perhaps if their experiment was extended, similar results would have been seen. Research by Häggblom & Young (1990) and by Kohring et al. (1989) was conducted on chlorophenols other than PCP. PCP may be degraded by a different initial pathway than lower chlorophenols, suggesting the possibility that the necessary factors may not have been present in those environments.

Temperature effects

Most studies on PCP dechlorination by anaerobes are conducted at temperatures ranging from 30–37° C. One group of researchers investigated the ability of different inoculum sources to degrade PCP at 50° C (Larsen et al. 1991). Degradation of PCP at higher temperatures may be useful because of the higher metabolic rate and lower sludge produced by the microorganisms. Eight different inocula sources were used: three anaerobic sludges operating at 35° C; one thermophilic sludge (55° C); three fresh water sediments (10–15° C) and one manure digester (55° C). Interestingly, the four sludge samples showed very little capacity for PCP degradation at 50° C. In contrast, fresh water sediments, unlikely to have prior exposure to PCP, degraded PCP very effectively at 50° C. Abrahamsson & Klick (1991) found 60 days was required to remove half of the added PCP at 6° C. An increase in degradation of chlorophenols was seen as temperature increased from 6–30° C and then decreased as temperature was raised to 50° C. In contrast, Mohn & Kennedy (1992b) reported the sludge granules used performed optimally at 50° C for removal of chlorophenols. Kohring et al. (1989) also found the freshwater sediments degraded chlorophenols optimally at the more moderate temperatures between 25–35° C.

Pathways and intermediates

It is important to elucidate the pathways of PCP degradation in different anaerobic environments under different conditions. Information on pathways can determine whether consortia from similar environments have the same mechanisms of PCP degradation. Table 6 summarizes the degradative pathways and intermediates found in anaerobic microbial communities. In many cases, mineralization is not evident in anaerobic environments, therefore, by following the transformation products it is possible to determine limiting steps. Using this knowledge, alterations in conditions can be made to permit mineralization to occur. Accumulation of end-products needs to be monitored since the intermediates of PCP degradation are often more toxic than the parent compound.

There is some evidence that similar environments support similar anaerobic consortia. Anaerobes from sediment samples tested seem to show a preference for dechlorination at the *para* or both *para* and *ortho* positions (Bryant et al. 1991; Larsen et al. 1991). This is only based on limited information and manipulation of conditions may easily change the findings. For example, acclimation can affect pathways, usually by decreasing preference for a particular dechlorinating position. Abrahamsson & Klick (1991) found unacclimated sediment preferred to dechlorinate *ortho*-substituted chlorines while acclimated sediment showed *para* and *meta* dechlorination. Similar results are evident in anaerobes from sludges (Mileski & Boyd 1986; Nicholson et al. 1992). More studies with a large number of similar sites under identical conditions would be needed to confirm this observation. It is noted, however, that *D. tiedjei* is capable of only *meta*-dechlorination, the least evident pathway seen in the environment.

It is necessary to be aware of the effect of degradation intermediates on microorganisms and other organisms. Intermediates from *ortho*-dechlorination tend to be persistent and have a substantial toxicity associated with them. Ruckdeschel et al. (1987) confirmed the toxicity of *ortho*-dechlorinated intermediates to Gram-negative and Gram-positive bacteria. The toxicity of these intermediates may partly explain why so few anaerobes can completely mineralize PCP. It may be beneficial to avoid using conditions which show a strong preference for the *ortho*-dechlorination pathway in favour of the *para*-pathway which produces significantly less toxic intermediates (Ruckdeschel et al. 1987).

Conclusions

Different microorganisms from a variety of environments demonstrate the ability to degrade PCP. How completely and how efficiently this occurs depends on the microorganisms and the environmental conditions. In general, aerobic microorganisms have a greater ability to mineralize higher PCP concentrations than do anaerobic microorganisms. In addition, more efficient mineralization of PCP occurs by axenic cultures of bacteria than by fungi. Almost all microorganisms, pure and mixed culture, performed better upon amendment with a carbon source. Immobilizing also appears to enhance PCP degradation.

While microorganisms in anaerobic environments have not demonstrated the potential for degradation of PCP at high concentrations (e.g. 200 mg/L) they remain important for study. Many PCP-contaminated sites are oxygen-limited or have regions of oxygen limitation. To allow degradation by aerobic microorganisms, sites must be aerated. The inoculation of oxygen-limited environments with microorganisms which thrive in such environments could be a favourable option. To aid in the practical use of PCP-degrading anaerobes, further characterization of the microbial ecology within the anaerobic consortium is necessary. Isolation and characterization of anaerobic PCP-degrading microorganisms should provide information on the diversity of PCP-degrading anaerobes, the prevalent degradative pathways involved and conditions which may enhance viability, leading to greater degradative potential.

Fungi also have an important role in PCP degradation. They tend not to be desirable as PCP degraders due to the low incidence of mineralization and the accumulation of potentially toxic end-products. Much of the PCP wastes are wood products heavily impregnated with PCP. Bacteria do not have the ability to access PCP from these wastes. Fungi, however, with their unique ability to degrade lignin can break down the wood, making PCP more bioavailable to degrading microorganisms. Unfortunately, there are no reports of fungi being isolated from PCP-contaminated sites. Fungal spores tend to be sensitive to PCP so survival in heavily PCP-contaminated sites may be unlikely. It is unclear whether the lack of fungal isolates is due to a lack of isolation attempts or due to a failure to isolate them. The most effective PCP-degrading bacteria were isolated from PCP-containing sites so the possibility exists a fungal strain isolated from such a site may also possess a superior ability to degrade PCP.

The most widely studied PCP-degrading microorganisms are the pure-culture bacterial strains, *Flavobacterium* ATCC 39723 and *Rhodococcus chlorophenolicus* PCP-1. Information is also available on *Arthrobacter* sp. ATCC 33790, *Sphingomonas* sp. RA2, some *Pseudomonas* isolates, and *Mycobacterium* strains. The enzymes responsible for initial steps of PCP metabolism by *Flavobacterium* sp. ATCC 39723 have been isolated and characterized. In addition, the genes encoding these enzymes have been characterized and cloned into *E. coli*, which then demonstrated the ability to degrade PCP. This technological advance could lead to a variety of research areas. *E. coli* may not be viable as an inoculum into PCP-contaminated sites

because it may not have the necessary mechanisms for survival in low nutrient environments with high predation, or it may out-compete indigenous organisms for nutrients and release toxins which could permanently disrupt the local ecology. Because *E. coli* has a significantly higher generation time than *Flavobacterium* sp. ATCC 39723, it could be used to manufacture large amounts of the degradative enzymes for use either directly or in immobilized form in bioremediation efforts, thus bypassing the inherent problems associated with using live organisms. Cloning the genes for the enzymes into an indigenous strain such as *Pseudomonas* sp. that has a high tolerance to PCP but does not have PCP-degrading properties could overcome some of the problems of introducing new organisms into contaminated environments. Furthermore, the properties of PCP-degradative enzymes can be improved by protein engineering methods to enhance their industrial potential. Erickson & Mondello (1993) were able to improve the substrate range of a biphenyl dioxygenase from *Pseudomonas* sp. LB400 by site-directed mutagenesis. The mutagenesis resulted in an enzyme variant which combined the broad substrate specificity of the enzyme from *P. pseudoalcaligenes* strain with increased activity of the enzyme from LB400. This approach offers a good potential for use to improve properties of catabolic enzymes involved in PCP degradation.

Little information exists on the pathogenic possibility of PCP-degrading bacteria. Both *Mycobacterium* and *Flavobacterium* are known to contain species which are pathogenic to other organisms. This fact may limit their usefulness in bioremediation. In addition, while PCP toxicity is well-documented, the toxicity of degradation intermediates is not well-known but may affect the viability of certain microorganisms. Most fungal strains studied degrade PCP by O-methylation resulting in pentachloroanisole, which is more lipophilic and can accumulate in tissues of higher organisms. Bryant & Schultz (1994) tested the toxicity of PCP and 25 intermediates on growth of *Tetrahymena* sp., and found PCP, 2,3,4,5-tetrachlorophenol, 2,3,5-trichlorophenol and tetrachlorohydroquinone to be inhibitory.

Little is known about how PCP affects microbial cells. Whether it binds to surface structures and enters by an established transport route or enters by some other mechanism is unknown. More studies are needed in the area of microbial PCP degradation. With sufficient information, the potential for bioremediation of PCP-contaminated sites using microorganisms is possible.

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