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Anaerobic biodegradation of pentachlorophenol in mixtures containing cadmium by two physiologically distinct microbial enrichment cultures

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Anaerobic biodegradation of pentachlorophenol (PCP), in mixtures containing cadmium (Cd), by sulfidogenic (SRB) and methanogenic (MET) enrichment cultures, was studied. Removal of 91–93% of PCP occurred in both SRB- and MET-enriched cultures, in the absence of Cd, within 82 days. The presence of soluble Cd initially decreased the rate of PCP removal by the enrichment cultures, but PCP removal rates improved as the Cd precipitated. GC-MS, ^{14}C-PCP, and ^{13}C-PCP studies confirmed mineralization of PCP by both enrichment cultures, as well as the incorporation of PCP carbon into specific phospholipid fatty acids (PLFAs) of the cell membranes of PCP-degrading anaerobes. This is the first report on anaerobic biodegradation of PCP by SRB- and MET-enriched cultures in the presence, with simultaneous precipitation, of the toxic heavy metal Cd, and of the incorporation of PCP carbons into specific PLFAs of the anaerobic bacterial cells. *Journal of Industrial Microbiology & Biotechnology* (2001) **27,** 11–17.

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Introduction

Pentachlorophenol (PCP) has been produced and used widely in the past as a herbicide, fungicide, and wood preservative [18]. Inadequate handling, accidental spills, and leaching from dumpsites have resulted in its contamination into groundwater and soil [17]. PCP is an inhibitor of oxidative phosphorylation and in addition to its toxicity, it is carcinogenic [1]. The U.S. Environmental Protection Agency (EPA) has listed PCP as a priority pollutant [11]. Many studies on the biodegradation of halogenated compounds including PCP have been done using aerobic culture techniques. Aerobic biodegradation of chlorinated aromatic compounds was considered more efficient and applicable for bioremediation. It is now thought that biodegradation by reductive, anaerobic dechlorination is an equally important natural process [10,15].

Degradation of PCP has been studied under anaerobic mixed culture conditions [5,11,12,15,18,22]. The degradation of PCP is dependent upon several factors, including the type of microbial community, its interaction with other co-contaminants, and the influences of other environmental factors. Anaerobic PCP biodegradation occurs by reductive dechlorination, in which the chlorine atoms on the ring are replaced by hydrogen [5]. This process is of great importance for bioremediation because of the ability of anaerobes to dechlorinate even highly chlorinated compounds [15,22]. In fact, some anaerobes can couple reductive dechlorination to energy production via an electron transport system [8,23,24,32]. Stuart and Woods [33] presented evidence that a reductively dechlorinating bacterial population can use PCP as a terminal electron acceptor.

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If PCP is used as a carbon and energy source, the PCP carbons will be utilized in the formation of biomass, including various fatty acids, by the cells. Phospholipids are an important component of all cell membranes [6,16,19], including anaerobic bacteria [20,21,34,36]. Phospholipids, as reflected in the composition of the associated long-chain fatty acids in a given sample, can be used as a fingerprint to identify members of microbial communities [26,31]. Using ^{13}C-enriched substrates, it is possible to follow the assimilation of carbon from a given substrate into the phospholipid fatty acids (PLFAs) of organisms capable of assimilating that substrate [6,13].

There is still much to be learned about how different anaerobes carry out the reductive dechlorination of PCP, particularly in the presence of co-contaminants. In the natural environment, chlorinated compounds are usually present along with other contaminants. If effective bioremediation processes are to be developed for these environments, we must better understand how microbial populations respond and adapt to the presence of both toxic organic compounds and heavy metals. We studied the anaerobic biodegradation of PCP in the presence of a co-contaminant heavy metal, cadmium (Cd). We chose Cd as the co-contaminant because it is one of the most mobile heavy metals in soils, and it can leach into the groundwater and in turn produce long-term toxic effects on humans and other organisms [28]. Other than a report of significant zinc toxicity to PCP-degrading anaerobes [14], little is known about the physiological responses of PCP-degrading anaerobic microbes growing in the presence of a toxic heavy metal such as Cd.

In this work, we studied the anaerobic degradation of PCP under two different physiological conditions, sulfidogenic (SRB) and methanogenic (MET). We monitored the degradation and transformation of PCP and the co-precipitation of Cd from its initial soluble phase by the two distinct enrichments. In addition, we monitored the ability of these anaerobic bacteria to mineralize PCP

and incorporate the carbon from PCP into biomass, by supplementing cultures with either ^{14}C-PCP or ^{13}C-enriched PCP and following the appearance of $^{14}CO_2$ and/or $^{14}CH_4$, and incorporation of ^{13}C into the PLFAs of the microorganisms.

Materials and methods

Culture media

Modified Starkey's Medium C (ATCC Medium 207) was used to culture the sulfate-reducing bacteria with lactate/sulfate acting as the electron donor/acceptor, respectively [3]. The medium contained 0.5 g KH_2PO_4, 1.0 g NH_4Cl, 1.0 g Na_2SO_4, 0.1 g $CaCl_2{\cdot}2H_2O$, 2.0 g $MgSO_4{\cdot}7H_2O$, 5.8 g 60% sodium lactate, and 1.0 g yeast extract per 1.0 l of water. The solution was boiled under nitrogen flow and then immediately placed on an ice bath to cool rapidly. Once cooled, the medium was dispensed into serum bottles, which were kept under nitrogen flow for 1 min, sealed, and then autoclaved. Separately, 7.5% cysteine–HCl and 1.0% ferrous ammonium sulfate were prepared, filter-sterilized, and autoclaved. Just prior to use, 0.5 ml of both solutions were aseptically added to the serum bottles.

Methanogens were grown using the basic Medium 1, described by Balch et al [4], with acetate/CO_2 acting as their electron donor/acceptor, respectively. The solution was prepared under 80% N_2 and 20% CO_2 flow, and later shifted to 80% H_2 and 20% CO_2 flow after addition of the reductants. The medium was dispensed into serum bottles, which were kept under a mixture of 80% H_2 and 20% CO_2 flow for 1 min, sealed, and then autoclaved.

Culture conditions

PCP degradation in the presence and absence of soluble Cd was studied in 100-ml serum bottles, which were sealed with rubber stoppers and crimped aluminum caps after introduction of 50 ml of specific defined medium. Sewage sludge obtained from the anaerobic digester of the Moscow, ID municipal wastewater treatment plant was used as the primary inoculum. Sludge (100 ml) was centrifuged at 7000 rpm. The supernatant was discarded and the solid pellet was resuspended in water (100 ml). Then, 5 ml of the resuspended sewage sludge (approx. 500 mg wet sludge) was than added to the 100-ml serum bottles using a 5-ml syringe and 18G needle. PCP from an ethanolic stock solution (1200 or 1300 mg/l) and Cd from an aqueous stock solution (1300 mg/l) of $Cd(NO_3)_2$ (98%) were introduced into the 100-ml serum bottles using a 5-ml syringe and 22G needle, before addition of the sludge. Triplicates were run for each condition, and PCP was introduced both in the presence and absence of Cd. Simultaneously, controls were inoculated with autoclaved sewage sludge. The headspaces of the serum bottles were pressurized with N_2 or a mixture of 80% H_2 and 20% CO_2 in the SRB and MET enrichment cultures, respectively [3,4]. The cultures were incubated without shaking at 37°C, the approximate temperature of the sludge when taken from the treatment plant.

As cells began to grow, the SRB enrichment culture produced gas, which was periodically removed using a syringe. In contrast, the MET enrichment culture consumed the gas mixture in the headspace creating a vacuum. MET headspaces were repressurized aseptically with a mixture of 80% H_2 and 20% CO_2 whenever necessary. To establish microbial communities more resistant to PCP and Cd, at regular time intervals, subcultures (10% v/v) were removed and inoculated to fresh culture media containing PCP and Cd.

Confirmation of desired physiological activity

During growth, the two enrichment cultures were tested for their production of sulfide and methane to establish that each had the desired physiological activity. An enrichment culture was confirmed to be sulfidogenic by measuring the production of sulfide from sulfate over time, using the methylene blue assay [9]. Controls were inoculated with autoclaved sewage sludge and incubated under similar conditions. An enrichment culture was confirmed to be methanogenic by introducing radioactive ^{14}C-bicarbonate into the medium and then measuring $^{14}CH_4$ production. $^{14}CH_4$ analyses were performed as previously described, by sacrificing the entire culture [25]. Briefly, 100,000 dpm of ^{14}C-bicarbonate was introduced into the medium. The culture was allowed to grow for 1 week at 37°C. Then, the bottle was removed and the gas flushed through a series of vials containing 10 ml Carbosorb (Packard Instruments, Meriden, CT) to trap any $^{14}CO_2$ present. The exit gas from this trap was then passed through a tube furnace (800°C) to combust its $^{14}CH_4$ to $^{14}CO_2$. The gas exiting the furnace was flushed through a series of bottles containing Carbosorb to trap the $^{14}CO_2$. Then, an equal volume of cocktail (Ecolite, ICN, Costa Mesa, CA) was added to each vial, and the radioactivity present was counted in a liquid scintillation counter (model Tricarb 2100TR; Packard Instruments). To study the distribution of radioactivity between the cell mass and the liquid media, the media were centrifuged at 7000 rpm for 30 min. Then, 1 ml of the supernatant was mixed with 5 ml of cocktail and its radioactivity counted. The solid pellet was extracted with 5 ml of tissue solubilizing agent (Protosol, Dupont NEN, Boston), and then 1 ml of the extract was dissolved in 5 ml of cocktail and its radioactivity counted. Mass balances were calculated for each culture to determine the amount of the initial ^{14}C-bicarbonate present as $^{14}CH_4$, $^{14}CO_2$, soluble ^{14}C compounds, and insoluble (biomass) ^{14}C compounds. The controls were inoculated with autoclaved sewage sludge and incubated under similar conditions.

Sampling procedure

PCP analysis

Samples were periodically removed from each culture bottle during the course of the experiment using a syringe and 18G needle. Serum bottles were shaken well before sampling to insure that the sample would contain a representative sample of all solids. The samples were centrifuged at 14,000 rpm for 5 min, and the supernatant was collected and passed through a 0.45-μm pore size filter (Acrodisk GMP, Gelman Laboratories, Ann Arbor, MI, USA) into HPLC vials. The solid pellet obtained after centrifugation was extracted by vortexing it with ethanol (95%) and then centrifuged at 14,000 rpm for 5 min. The supernatant was collected and passed through 0.45-μm pore size filter into HPLC vials. To determine the amount of PCP present, each filtered sample was chromatographed using a Hewlett-Packard 1090 HPLC equipped with a diode array detector set at 217 nm. The column was a reverse phase Envirosep PP column (125×3.2 mm) (Phenomenex, Torrance, CA). The column temperature was 41°C and the flow rate was 0.8 ml per minute. The injection volume was 10 μl and the mobile phase was an isocratic mixture

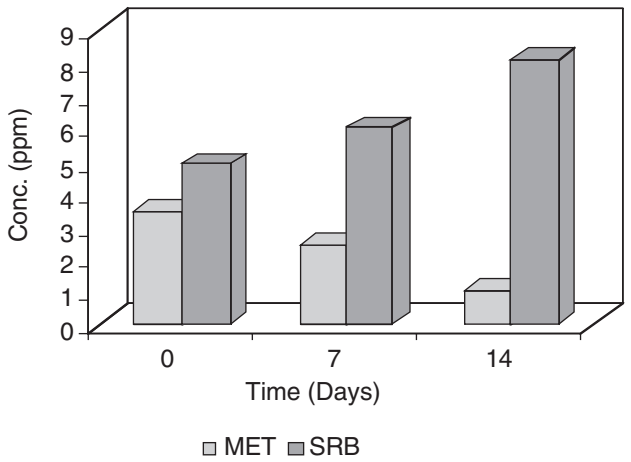

Figure 1 Sulfide production in SRB and MET enrichment cultures grown in the presence of cadmium.

of 40% phosphoric acid (100 mM) and 60% acetonitrile. The values obtained for the respective aqueous and solid phases were combined to represent the total PCP left in the medium.

Cd precipitation

The sampling procedure for estimating soluble versus insoluble Cd was similar to PCP extraction, but instead of 1 ml, a 5-ml sample was removed, and HNO_3 was used instead of ethanol in the extraction procedure. Each filtered sample was assayed for Cd using inductively coupled plasma-atomic emission spectroscopy (ICP-AES). Assays were run by the EPA-certified analytical laboratory of the College of Agriculture, University of Idaho, Moscow, ID.

PCP dechlorination

To determine qualitatively the presence of intermediary PCP degradation products, 2-ml samples from each culture bottle were removed periodically and mixed with 1 ml of 50% aqueous acetonitrile, and then acidified with 0.5 ml 1 N HCl. This mixture was extracted three times with 8 ml of ethyl acetate. The combined ethyl acetate layers were concentrated to 50 μl using a rotary evaporator. The concentrated ethyl acetate sample was subjected to GC-MS analysis [29]. GC-MS analysis was performed with a gas chromatograph (5890 series II; Hewlett-Packard, Wilmington, DE), which was connected to a mass spectrometer (model 5989; Hewlett-Packard). The injector temperature was 250°C, the oven temperature was 105°C, and the detector temperature was 300°C. The carrier gas used was H_2 with a flow rate of 2 ml/min.

PCP mineralization

To determine if PCP was being mineralized into CO_2 and CH_4, radioactive ^{14}C-PCP (Sigma Chemical Company, St. Louis, MO) was used as a substrate. The enrichment cultures were grown with radioactive ^{14}C-PCP and later, cultures were checked for the presence of $^{14}CO_2$ and $^{14}CH_4$ by sacrificing the whole bottle. Initially, 100,000 dpm of ^{14}C-PCP was introduced into the previously described media. Cultures were allowed to grow for a month in an incubator at 37°C. Then, $^{14}CO_2$ and $^{14}CH_4$ were recovered and measured by the same method as used earlier for determining the $^{14}CH_4$ from ^{14}C-bicarbonate.

PLFA analysis

^{13}C-PCP (C/D/N Isotopes, Quebec, Ont, Canada) from an ethanolic stock solution (10 mg/10 ml) was introduced into both the SRB and MET enrichment cultures, and the cultures were incubated for 2 weeks. Similar enrichment cultures supplemented only with ^{12}C-PCP containing a natural abundance of ^{13}C were simultaneously grown under the same conditions. Controls consisted of sterilized cultures and PCP-free enrichment cultures. After 2 weeks of incubation at 37°C, 2-ml samples were removed from the ^{13}C-PCP, PCP, and control bottles and centrifuged at 14,000 rpm for 2 min. The solid pellets were then saponified, methylated, and extracted for PLFAs by the procedure of Microbial Identification, Newark, DE [21]. Extracted samples were analyzed using a gas chromatograph (5890 series II; Hewlett-Packard), connected to a mass spectrometer (model 5989; Hewlett-Packard).

Nomenclature of fatty acids

The fatty acids are designated by the number of carbon atoms:number of double bonds, followed by the position of the first double bond from the ω (methyl) end of the molecule. Branched-chain fatty acids are indicated by prefixes *i* or *a* for *iso*- and *anteiso*-branchings, respectively [26], while *cis* and *trans* isomers are indicated by the suffixes *c* or *t*.

Results and discussion

The two enrichment cultures were tested for their production of sulfide during the first 2 weeks of incubation. The SRB enrichment culture showed a steady increase in the concentration of sulfide during this period. Since sulfate was the only source for the sulfide in the medium, the increase in the concentration of sulfide was attributed to the reduction of sulfate, indicating the growth of sulfate-reducing bacteria. Approximately 8.25% of the initial sulfate present in the medium was reduced to sulfide during the first 2 weeks of incubation. In contrast, the MET enrichment culture showed an initial low level of sulfide (3–4 mg/l) that decreased as methanogenic conditions became dominant (Figure 1). The radioactive ^{14}C-bicarbonate study showed the production of $^{14}CH_4$ in the MET enrichment culture after 1 week of incubation. MET enrichment cultures first consumed the gas mixture in the headspace, creating a vacuum, as previously observed [4], and then produced methane, confirming they were methanogenic. About 16% of the initial radioactive ^{14}C-bicarbonate added to the MET enrichment culture was converted into $^{14}CH_4$ after 1 week of incubation (Table 1). No significant $^{14}CH_4$ was produced by the

Table 1 Percentage distribution of ^{14}C from ^{14}C-bicarbonate in SRB and MET enrichment cultures after 1 week of incubation

Added bicarbonate recovered as	Percentage recovered±SD (n=3)	
	SRB	MET
CO_2	45.14±2.8	56.6±3.40
CH_4	0.46±0.28	16.4±0.87
Aqueous	4.45±0.93	8.7±4.57
Particulate	39.43±3.2	17.3±1.80
Unaccounted	4.98±1.2	1.0±0.83

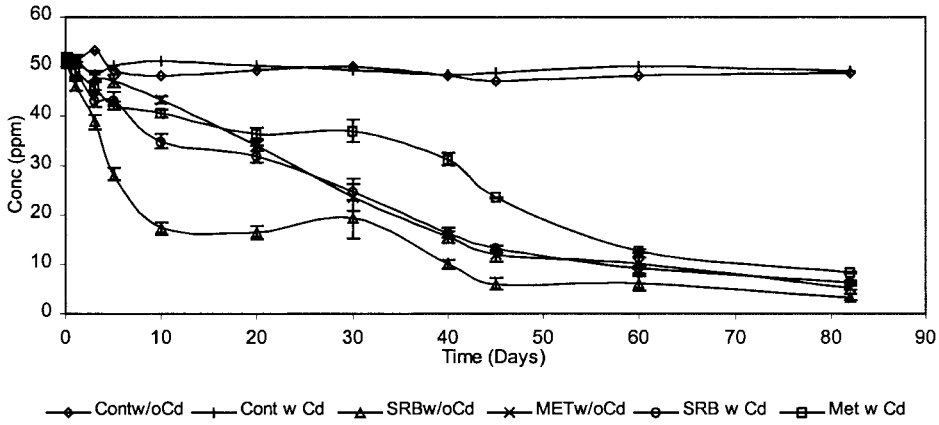

Figure 2 Removal of PCP over time by SRB and MET enrichment cultures in the absence and presence of cadmium.

SRB enrichment culture over this time period. The controls inoculated with autoclaved sewage sludge and incubated under the same conditions did not produce CH_4 or sulfide over the same time (data not shown).

After confirming that the two enrichment cultures were sulfate-reducing and methanogenic, respectively, the disappearance of PCP in the presence and absence of initial soluble Cd in the enrichment cultures was studied over 82 days. Initial concentrations of soluble PCP and Cd (50 mg/l each) were present in both media. Periodic assays showed 91–93% disappearance of PCP from the medium of both enrichment cultures, in the absence of Cd, within this period (Figure 2). The pH of the culture medium decreased slowly over time, and was periodically adjusted to neutrality. Other experiments showed that regardless of the initial concentration of PCP in the medium, up to 100 mg/l, the removal of PCP by both cultures reached 85–95% in the absence of Cd within 82 days (data not shown). In the presence of 50 mg/l initially soluble Cd, the percentage of PCP removal decreased somewhat, to 83–87%, in both enrichment cultures (Figure 2). Within 20 days from inoculation, 90–95% of the initially soluble Cd had been removed from the soluble phase of both cultures. Figure 3 shows the increase in the concentration of Cd in the insoluble phase as it was precipitated by the enrichment cultures over a 20-day period. The presence of Cd in the soluble phase initially inhibited the rate of PCP removal by both enrichment cultures, though PCP removal rate improved as Cd was precipitated. During the same time, the controls showed no significant decrease in the concentration of soluble PCP or Cd.

The appearance of PCP dechlorination products in both enrichment cultures, grown in the presence of Cd, was determined qualitatively at specific time intervals. Table 2 shows the various dechlorination products detected in the SRB and MET enrichment cultures over the first 30 days of inoculation. By day 10, TeCP isomers appeared in both cultures. By day 30, various TriCP and DiCP isomers were also detected. TeCP and TriCP isomers, including 2,3,4,5-TeCP, 2,3,4,6-TeCP, 2,3,5,6-TeCP, 2,3,6-TriCP, and 2,4,5-TriCP, were observed in both enrichments. We did not detect any monochlorophenols. This may be because any monochlorophenols produced were short-lived. Another metabolite, 2,5-dichloro-1,4-benzenediol (Figure 4), with a relative abundance of 8.5% in relation to PCP was detected in the MET enrichment culture. It may have been produced by a side reaction, or it could be an intermediate in an alternate PCP biodegradation pathway [30]. This was an interesting observation as hydroquinone formation was detected after growth under strictly anaerobic conditions, and after conducting all sampling in anaerobic hoods. In addition, to avoid any chemical transformations not the result of microbial activity, all samples were frozen immediately after sampling until the GC-MS analysis was done. Recently, Shink *et al* [30] discovered a pathway with hydroxyhydroquinone as a central intermediate, and through which nitrate-reducing bacteria degrade phenolic compounds under anaerobic conditions. They demonstrated that the breakdown of these compounds was determined by the overall reaction energetics, and by the redox potentials of the electron acceptors

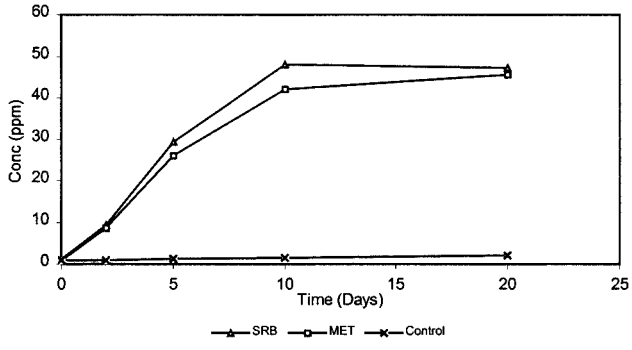

Figure 3 Increase in cadmium concentration over time in the insoluble phase of SRB and MET enrichments.

Table 2 Intermediary reductive dechlorination products detected in SRB and MET enrichment cultures

Enrichment culture	Intermediate products detected[a]
SRB	2,3,4,5-TeCP, 2,3,5,6-TeCP, 2,3,4,6-TeCP, 2,4,5-TriCP, 2,3,6-TriCP, 2,3,5-TriCP, 2,4-DiCP
MET	2,3,4,6-TeCP, 2,3,4,5-TeCP, 2,3,5,6-TeCP, 2,3,6-TriCP, 2,4,6-TriCP, 2,4,5-TriCP, 2,6-DiCP, 2,3-DiCP, 2,5-dichloro, 1,4-benzenediol

[a]TeCP: tetrachlorophenol; TriCP: trichlorophenol; DiCP: dichlorophenol.

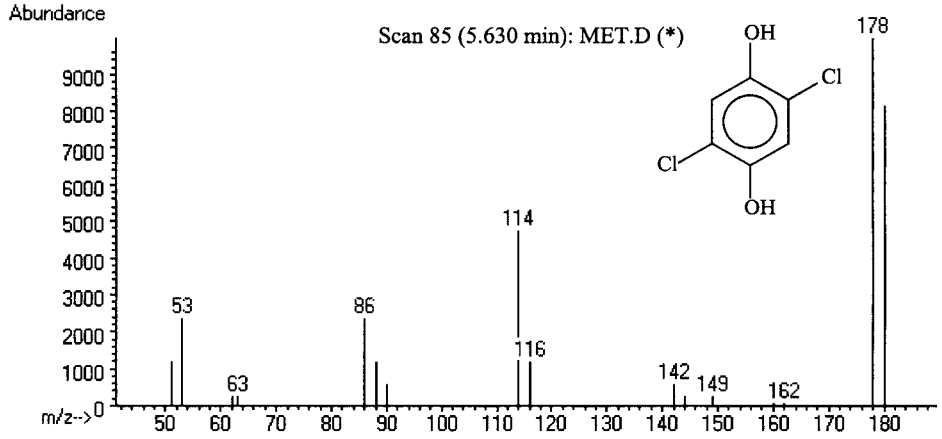

Figure 4 Mass spectra of 2,5-dichloro-1,4-benzenediol. The cultures were extracted by ethyl acetate and analyzed by GC-MS.

used. In addition to reductively dechlorinating PCP, the appearance of significant amounts of the hydroquinone in MET enrichment cultures showed that they may also utilize this pathway. Alternatively, in the enrichments, nitrate reducers may have been growing along with the methanogenic bacteria resulting in the production of hydroquinone.

The appearance of these less-chlorinated phenols over time demonstrated that both enrichment cultures were degrading PCP by reductive dechlorination. Earlier studies showed that sulfate-reducing conditions are a good environment for the growth of anaerobic bacteria capable of reductively dechlorinating and degrading PCP [7,27]. Controls inoculated with autoclaved sewage sludge produced no dechlorination products during the same period.

Radioactive ^{14}C-PCP was used as a substrate to confirm that in the presence of initially soluble Cd, PCP was being mineralized into CO_2 and/or CH_4 by the SRB and MET enrichment cultures. After 30 days, 48% of initially added ^{14}C-PCP was converted to $^{14}CO_2$ and 14% to $^{14}CH_4$ under the sulfate-reducing conditions, whereas 17% and 35% of the initial radioactive ^{14}PCP was converted into $^{14}CO_2$ and $^{14}CH_4$, respectively, under methanogenic conditions (Table 3). Controls showed neither PCP degradation nor precipitation of Cd. Production of $^{14}CH_4$ in the SRB enrichment culture after a longer incubation period of 30 days (Table 3) indicated that methanogens eventually became active at a low level in this enrichment culture. This was in contrast to the earlier experiment carried out for 1 week in order to confirm the establishment of the SRB and MET enrichment cultures (Table 1). CH_4 production was not observed in that SRB enrichment culture. These data showed that methanogens appeared late in a culture medium that favored the growth of SRBs.

Concentrations of 50 mg/l PCP were used in the experiment, since groundwater below wood treatment plants have been reported to have PCP concentrations typically ranging from 25 to 100 mg/l or more [35]. Cd at an initial soluble concentration of 50 mg/l did not prove highly toxic to either enrichment culture, although it transiently slowed the rate of PCP degradation. Both enrichment cultures were able to precipitate most of the Cd within 20 days, which probably reduced its toxic effect on the growing cultures. PCP, in the presence of initially soluble Cd, was still degraded by reductive dechlorination, as observed by the appearance of lesser-chlorinated phenols over time. This was in accordance with the fact that reductive dechlorination of chlorinated xenobiotics forms the central basis for their anaerobic detoxification and degradation [2]. This is, however, the first report of anaerobic biodegradation of PCP by SRB and MET enrichment cultures in the presence and with simultaneous precipitation of the toxic heavy metal Cd. The SRB enrichment culture did show a better rate of PCP removal than the MET enrichment culture, in both the presence and absence of Cd. The degradation of PCP in the presence of Cd in both enrichment cultures was not due to abiotic mechanisms since the controls did not degrade PCP or precipitate Cd.

Microbial communities that were more resistant to PCP and Cd mixtures could be obtained by subculturing regularly into fresh media. These subcultures were able to remove PCP at a much faster rate compared to the first enrichment from sewage sludge. Subcultured inocula removed 90% of the PCP (50 mg/l) in the presence of an equal concentration of initially soluble Cd within 30 days, compared to 82 days initially (Figure 5).

The PLFAs of the bacteria in the enrichment culture, supplemented with ^{13}C-PCP, PCP, and the PCP-free sterile controls, were characterized using the GC-MS. Fatty acids from

Table 3 Percentage mineralization of ^{14}PCP into carbon dioxide and methane by SRB and MET enrichment cultures after 30 days of incubation at 37°C

Enrichment culture	Percentage recovery±SD (n=3) of ^{14}C as				
	CO_2	CH_4	Aqueous	Particulate	Unaccounted
SRB	48.49±3.2	13.91±1.51	0.235±0.005	18.72±0.88	18.65±3.39
MET	16.74±4.37	35.19±2.1	0.69±0.1	28.36±2.8	12.35±1.71

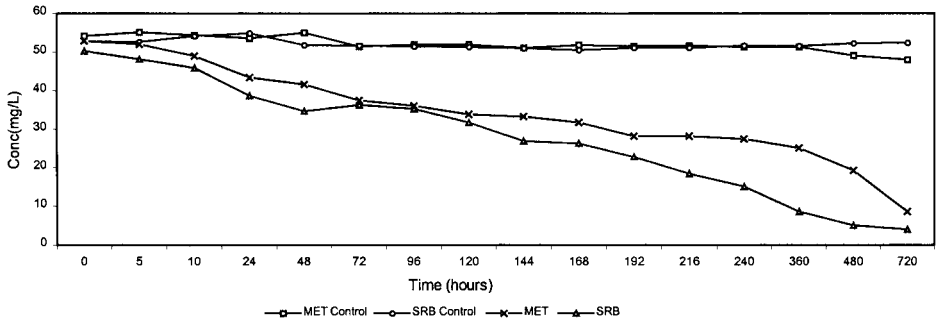

Figure 5 Degradation of PCP by subcultured enrichment cultures over a 30-day period.

C_{12} to C_{20} were found in both enrichment cultures grown with ^{13}C-PCP and PCP. Fatty acids from C_{12} to C_{19} are commonly found in bacteria [16,31]. Similar individual peaks obtained for the PLFAs in the enrichment cultures grown with PCP and ^{13}C-PCP were then analyzed for their relative differences in the percentage of ^{13}C in the peaks and are listed in Table 4. All the PLFA peaks obtained, whether the cultures were supplemented with PCP or ^{13}C-PCP, showed a ^{13}C isomer peak. This is because all compounds contain a small percentage of ^{13}C isomer [13]. An increase in the relative percentage of ^{13}C isomer peak for any PLFA obtained from ^{13}C-PCP–supplemented culture, in comparison to the same peak obtained from PCP-supplemented culture, would suggest that ^{13}C from ^{13}C-PCP was being incorporated into the PLFAs. Eight of the 15 PLFAs in the SRB enrichment culture and 7 of the 14 PLFAs in the MET enrichment culture showed an increase in their relative content of ^{13}C. Sterile (autoclaved) uninoculated control, as expected, did not show any PLFA peaks. The PLFAs from MET enrichment cultures showed enrichment in specific ^{13}C, ranging from 0.6% to 1.94%. The SRB enrichment cultures showed a better incorporation of the ^{13}C into PLFAs, with ^{13}C enrichments ranging from 0.85% to 9.28% depending upon the fatty acid. We obtained various PLFAs, which were common to both enrichment cultures, and some which were unique for each enrichment culture. The fatty acids detected in the SRB and MET enrichment culture have been observed in earlier studies of these microbial groups confirming that both the enrichment cultures were enriching specified microbial types [20,34,36]. There was a greater percentage enrichment of ^{13}C in PLFAs of the SRB enrichment culture (1.86% as in *14:0* to 9.28% as in *20:0*), compared with the MET enrichment culture (0.6% and 1.65% for the same fatty acids). The incorporation of ^{13}C into certain *iso*-branched fatty acids, for example *i16:0* and *i17:0*, were observed only in the MET enrichment culture. The SRB enrichment culture, in contrast, showed ^{13}C incorporation into specific straight-chained fatty acids like *12:0*, *15:0*, *17:0*, and *18:17c* and into an *anteiso*-branched fatty acid, *a15:0*. This shows that as PCP was reductively dechlorinated and mineralized to CO_2 and/or CH_4 by the enrichment cultures, PCP carbon was also incorporated biosynthetically into the fatty acids of the cell membranes of the bacteria. Further studies to isolate and characterize individual microorganisms growing and utilizing PCP will be necessary before firm conclusions can be drawn as to the specific microorganisms that were assimilating PCP.

Table 4 Percentage increase of selected ^{13}C fatty acid peaks in ^{13}C-PCP–supplemented SRB and MET cultures after 2 weeks of incubation

Fatty acid detected	Percentage increase of ^{13}C fatty acid peak in ^{13}C-PCP supplemented bottles	
	SRB	MET
12:0	1.6	ND[a]
14:0	1.86	0.6
15:0	0.85	–
16:0	–	1.05
17:0	2.08	–
18:0	–	–
20:0	9.28	1.65
*i*15:0	–	–
*a*15:0	5.09	–
*i*16:0	ND[a]	1.94
*i*17:0	–	0.97
*a*17:0	ND[a]	–
*i*18:0	–	ND[a]
16:1ω7*c*	–	–
16:1ω9*c*	2.73	0.86
18:1ω7*c*	3.66	ND[a]
18:1ω9*c*	–	0.71

[a]Not detected.

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