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Reductive Dechlorination of Chlorophenols by a Pentachlorophenol-Acclimated Methanogenic Consortium

DAVID K. NICHOLSON,† SANDRA L. WOODS,* JONATHAN D. ISTOK, AND DANIEL C. PEEK‡

Environmental and Water Resources Engineering Program, Department of Civil Engineering, Apperson 202, Oregon State University, Corvallis, Oregon 97331-2302

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Anaerobic digester sludge fed 5,300 mg of acetate per liter, 3.4 μM pentachlorophenol, and nutrients for 10 days biotransformed pentachlorophenol by sequential *ortho* dechlorinations to produce 2,3,4,5-tetrachlorophenol and 3,4,5-trichlorophenol. Upon acclimation to 3.4 μM pentachlorophenol for 6 months, the methanogenic consortium removed chlorines from the *ortho*, *meta*, and *para* positions of pentachlorophenol and its reductive dechlorination products. Pentachlorophenol was degraded to produce 2,3,4,5-tetrachlorophenol, 2,3,4,6-tetrachlorophenol, and 2,3,5,6-tetrachlorophenol. Dechlorination of 2,3,4,5-tetrachlorophenol produced 3,4,5-trichlorophenol, which was subsequently degraded to produce 3,4-dichlorophenol and 3,5-dichlorophenol. 2,3,4,6-Tetrachlorophenol was dechlorinated at the *ortho* and *meta* positions to produce 2,4,6-trichlorophenol and 2,4,5-trichlorophenol. 2,3,5,6-Tetrachlorophenol yielded 2,3,5-trichlorophenol, followed by production of 3,5-dichlorophenol. 2,4,6-Trichlorophenol was degraded to form 2,4-dichlorophenol, and 2,4,5-trichlorophenol was dechlorinated at two positions to form 2,4-dichlorophenol and 3,4-dichlorophenol. Of the three dichlorophenols produced (2,4-dichlorophenol, 3,4-dichlorophenol, and 3,5-dichlorophenol), only 2,4-dichlorophenol was degraded significantly within 3 weeks, to produce 4-chlorophenol.

A knowledge of biotransformation pathways is essential to assess risks at contaminated sites, implement biological treatment processes, or design effective soil and groundwater bioremediation strategies. However, pathways observed in the laboratory with unacclimated organisms often differ from those observed in the field or in treatment processes with consortia exposed to the contaminant for a long period of time.

Pentachlorophenol (PCP) was selected for study because it is toxic to a wide variety of organisms (5), it is widely distributed in the environment, and the anaerobic biotransformation pathways for chlorophenols (CPs) have been well studied (Fig. 1). PCP has been used extensively as a wood preservative and pesticide. Hundreds of sites in the United States are contaminated with PCP as a result of wood-treating activities. Many of these sites are on the National Priority List for cleanup under the Superfund program. Additionally, CPs are produced in the anaerobic biotransformation of 2,4-dichlorophenoxyacetic acid or 2,4,5-trichlorophenoxyacetic acid (14). Thus, understanding the biodegradation of CPs will also allow better prediction of the environmental fate of these compounds.

Anaerobic biodegradation of CPs occurs by reductive dechlorination, a process by which chlorines are replaced with hydrogen. Degradation is dependent upon characteristics of the parent compound, the microbial consortium, and environmental factors. Reductive dechlorination of CPs has been observed for unacclimated and acclimated anaerobic sewage sludges, sediments, soils amended with sewage sludge, and aquifers (Fig. 1). The reductive dechlorination process is of environmental importance because the less-chlorinated metabolic products are generally less toxic and degraded more easily by aerobic bacteria than the more highly chlorinated parent compounds. However, less-chlorinated phenols may also be more mobile in contaminated soils because of their increased water solubility.

Studies of the distribution and fate of PCP in Japanese rice paddy fields provide some of the earliest information concerning anaerobic CP biotransformations (10, 13, 20, 25, 26). Ide et al. (10) were first to identify tetrachlorophenols (TeCPs) and trichlorophenols (TCPs) as degradation products of PCP, and they attributed degradation to a microbially mediated reductive dechlorination process. Chlorines in positions *ortho* to the hydroxyl group were removed more readily than those in the *meta* or *para* positions, a phenomenon that has been verified in later studies (3, 14, 15, 28).

Biotransformation pathways vary with the characteristics of the microbial consortium. Unacclimated consortia preferentially remove chlorines from the position *ortho* to the hydroxyl group. For example, unacclimated consortia transform PCP to produce 2,3,4,5-TeCP and 3,4,5-TCP (2, 3, 14, 28). Similarly, degradation of 2,4,6-TCP results in the formation of 2,4-dichlorophenol (2,4-DCP) and 4-CP (3, 7, 14, 23, 28).

Acclimated microbial consortia may yield biotransformation pathways different from those of unacclimated consortia. Boyd and Shelton (3) observed that sludges acclimated to 2-CP degrade 4-CP and 2,4-DCP but not 3-CP and that those acclimated to 3-CP are incapable of degrading 2-CP. Additionally, sludges acclimated to individual monochlorophenols produce different initial PCP degradation products (15). Anaerobic sludge acclimated to 2-CP produces 2,3,4,5-TeCP from PCP by reductive dechlorination of the *ortho* chlorine. Sludge acclimated to 3-CP dechlorinates PCP at the *meta* position to yield 2,3,4,6-TeCP, and sludge acclimated to 4-CP yields 2,3,5,6-TeCP by *para* dechlorination. In a similar study (4), CP reductive dechlorination pathways were determined for organisms acclimated to either 2,4-DCP or 3,4-DCP. The 2,4-DCP-acclimated consortium produced

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* Corresponding author.

† Present address: Woodward-Clyde Consultants, Denver, CO 80237.

‡ Present address: PTI Environmental Services, Lake Oswego, OR 97035.

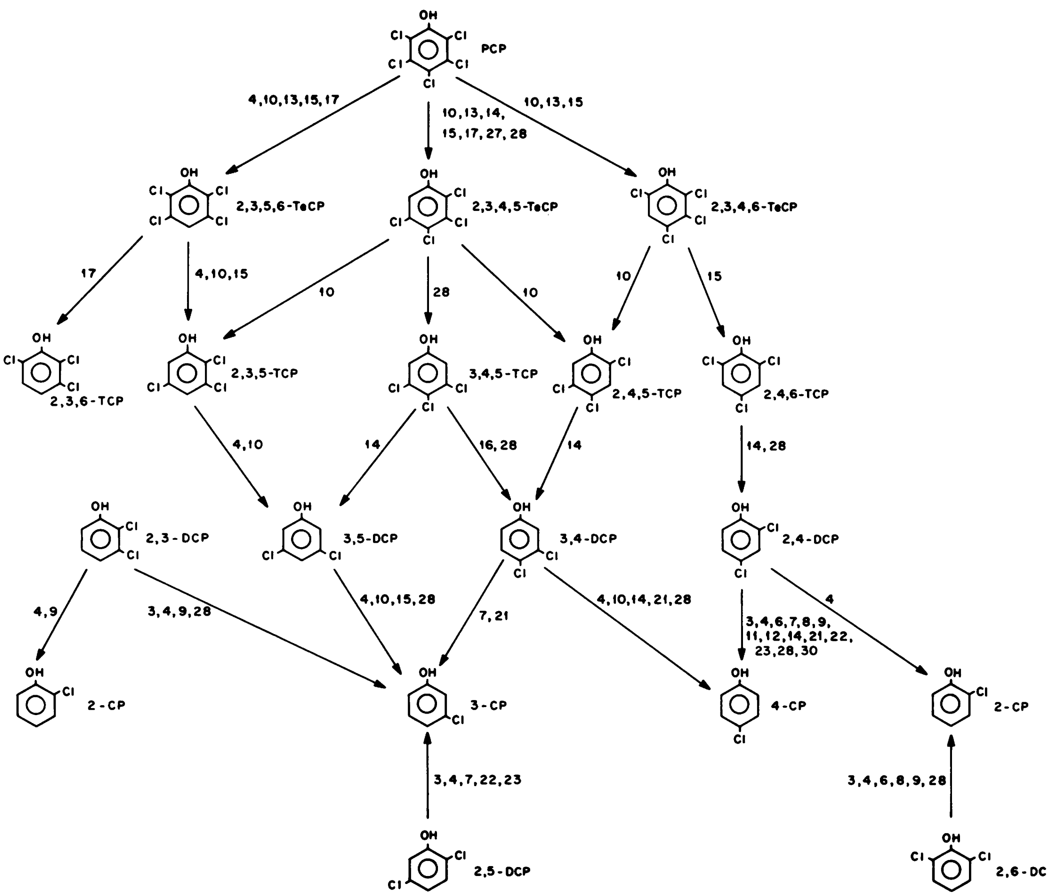

FIG. 1. Summary of previously observed CP reductive dechlorination pathways by acclimated and unacclimated anaerobic consortia. Numbers next to arrows refer to references.

ortho-dechlorination products, while the 3,4-DCP-acclimated consortium produced *para*-dechlorination products.

The degradation products for CPs in Fig. 1 include those observed for acclimated and unacclimated soils, sediments, and sludges. These data suggest that environments exposed to PCP over a long period may yield a large number of metabolites and a complex PCP biotransformation pathway. This paper describes the observed pathway for the reductive dechlorination of PCP. The kinetics of these biotransformations will be discussed elsewhere (29).

Metabolites were identified for PCP and each of its metabolic products in separate batch biodegradation experiments. These individual experiments yielded a more complex PCP degradation pathway than previously observed, but one that is consistent with results presented in Fig. 1.

MATERIALS AND METHODS

PCP biotransformation pathways were determined for methanogenic consortia fed 5,300 mg of acetate per liter, 3.4 μM PCP (0.9 mg/liter), and nutrients for periods between 10 days and 9 months. Anaerobic digester sludge continuously fed the acetate-PCP-nutrient solution for 6 to 9 months at a 10-day hydraulic residence time represented the acclimated consortium. During this period, the reactor was brought to steady state with respect to retention time, substrate removal, gas production, E_h, and pH to provide similar initial conditions for each batch experiment.

Reductive dechlorination pathways were determined by using a dual-reactor system. A 9.5-liter continuous-flow "mother" reactor served as a source of inocula for experiments conducted in smaller batch reactors. The original source of organisms was anaerobic digester sludge from the Corvallis, Oreg., municipal wastewater treatment plant. The digester was operated at 35°C with a solid-retention time of 30 days. Treatment plant influent is largely domestic sewage.

The mother reactor was fed a solution formulated by using a modification of the anaerobic media described by Owen et al. (19). Concentrations of inorganic nutrients and vitamins (in milligrams per liter) in the feed solution were as follows: $CaCl_2 \cdot 2H_2O$, 139.2; NH_4Cl, 221.6; $MgCl_2 \cdot 6H_2O$, 1,000.7; KCl, 722.6; $MnCl_2 \cdot 4H_2O$, 11.2; $CoCl_2 \cdot 6H_2O$, 16.7; H_3BO_3, 3.23; $CuCl_2 \cdot 2H_2O$, 1.51; $Na_2MoO_4 \cdot 2H_2O$, 1.44; $ZnCl_2$, 1.18; $NiCl_2 \cdot 6H_2O$, 8.42; $(NH_4)_2HPO_4$, 73.4; biotin, 0.015; folic acid, 0.015; pyridoxine hydrochloride, 0.075; riboflavin, 0.038; thiamine, 0.038; nicotinic acid, 0.038; pantothenic acid, 0.038; vitamin B_{12}, 0.0009; *p*-aminobenzoic acid, 0.038; thioctic acid, 0.038. Each day, 135 mg of $Na_2S \cdot 9H_2O$ and 100 mg $FeCl_2 \cdot 4H_2O$ were injected directly into the reactor. Acetic acid, sodium acetate, and sodium bicarbonate were added to the feed solution to provide alkalinity and 5,300 mg of acetate per liter. PCP was added at 0.9 mg/liter (3.4 μM).

Reactor system. The reactors were constructed of Kimax beaded-process pipe with stainless steel and Teflon-lined flange fittings (Ace Glass Company, Vineland, N.J.). Brass valves on the top plate provided a sampling port, influent port, and gas collection port. Microorganisms were kept in suspension by continuous stirring with a Teflon paddle driven by an electric motor. The reactor was housed in an environmental chamber maintained at 31°C. Feed solution was kept under nitrogen in a glass container at 4°C to minimize microbial growth. Feed solution was continuously delivered to the reactor through Teflon tubing by a FMI RP-G6 laboratory pump (Fluid Metering Inc., Oyster Bay, N.Y.) to provide a hydraulic retention time of 10 days.

Batch experiments were conducted in a 2.5-liter batch reactor of similar construction. The batch reactor had a gas relief valve in addition to sampling and influent valves and was stirred continuously by a magnetic stir bar. The batch reactor was fitted with E_h and pH electrodes, and gas production was measured by a wet test gas meter connected to the gas relief valve.

Transfer of the sludge from the mother reactor to the batch reactor was conducted under anaerobic conditions. The headspace of the batch reactor was purged with nitrogen until oxygen was undetectable by gas chromatography with thermal conductivity detection. Nitrogen was also flushed through the headspace of the mother reactor while the sludge was transferred to the batch reactor via the bottom port of the mother reactor. Prior to each experiment, the batch reactor was filled with sludge and allowed to equilibrate until the E_h fell below −330 mV to ensure conditions necessary for methanogenesis. An aqueous solution containing the CP of interest and sodium acetate was adjusted to pH 7.5 with concentrated HCl. The solution was then injected through the influent valve by using a 50-ml glass syringe. The initial acetate concentration was 1,500 mg/liter for all batch experiments.

Additional batch experiments for the degradation of DCPs were conducted with 60-ml amber serum bottles. CP solution was delivered by pipette, and sludge was added to fill the bottle. After several weeks of incubation, the contents of the bottle were filtered and extracted in the same manner as samples from the batch reactor.

Sampling procedure. Liquid samples (20 ml) were taken immediately after injection of the CP-acetate solution and at 2- to 6-h intervals during the course of the experiments by using a 30-ml glass syringe. Samples were filtered by using a Millipore filtration apparatus and Gelman type A/E glass fiber filters with a 1-μm pore size. The first 3 ml of filtrate was discarded, and CP concentrations were measured for the remaining sample.

Chemical sources. 2,3,4,5-TeCP (98% purity) was obtained from Pfaltz and Bauer Co., Waterbury, Conn. 2,3,5,6-TeCP (99%) and 3,4,5-TCP (98%) were obtained from Ultra Scientific Co., Hope, R.I. All other chlorophenols, 2,6-dibromophenol, and 2,4,6-dibromophenol were obtained from Aldrich Chemical Company, Inc., Milwaukee, Wis., in purities of at least 98%. 2,3,4,6-TeCP was obtained through the Aldrich Chemical Co. rare chemical library. All chemicals were used without further purification.

Analytical procedures. Aliquots (10 ml) were used for CP analyses. Samples were acetylated and extracted into hexane by a modification of the method described by Voss et al. (24) and the National Council of the Paper Industry for Air and Stream Improvement (18). The internal standard (2,6-dibromophenol or 2,4,6-tribromophenol) was added by using a 50-μl syringe. One milliliter of a 0.7-g/ml solution of K_2CO_3 and 1 ml of acetic anhydride were added, and the funnel was shaken for exactly 2 min. After 20 min, 5 ml of hexane was added and the funnel was again agitated for 2 min. After the water was drawn off, the hexane layer was collected with a new Pasteur pipette and stored at 4°C in a 2-ml amber glass vial with a Teflon-lined cap until analysis by capillary gas chromatography.

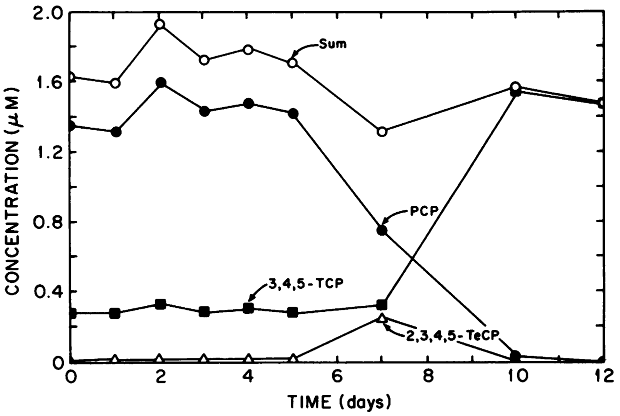

FIG. 2. Progress curve for PCP reductive dechlorination by a methanogenic consortium fed PCP for 10 days.

Injections of hexane extracts (1 μl) were made by autosampler into a Hewlett-Packard model 5890A gas chromatograph equipped with a ^{63}Ni electron capture detector and a DB-5 fused-silica capillary column (inner diameter, 30 m by 0.323 mm; J & W Scientific, Orangeville, Calif.). Helium (351 g/cm^2, 5 lb/in^2) was used as the carrier gas, and 95% argon–5% methane was used as the electron capture detector auxiliary gas. The detector was held at 320°C, and the injector temperature was 250°C. The temperature program was as follows. An initial oven temperature of 45°C was held for 2 min, increased by 15°C/min to 105°C, and then increased by 5°C/min to a final temperature of 215°C that was held for 5 min. To separate 2,3,4,6-TeCP from 2,3,5,6-TeCP and 2,3,5-TCP from 2,4,5-TCP, the oven was held at an initial temperature of 45°C for 2 min, followed by temperature increases of 15°C/min to 105°C, 1°C/min to 150°C, and 5°C/min to a final temperature of 215°C that was held for 5 min. Peak areas were determined by a Hewlett-Packard Model 3392A Integrator.

Total and volatile suspended solids were determined by using standard methods 2540D and 2540E (1).

RESULTS AND DISCUSSION

PCP biotransformation by a methanogenic consortium fed PCP for 10 days. PCP was added to the batch reactor to yield an initial concentration of 1.35 μM (Fig. 2). Moderate amounts (0.28 μM) of 3,4,5-TCP also were present at the beginning of the experiment because it was present in the mother reactor as a result of the reductive dechlorination of PCP.

PCP degradation began after a lag period of 5 days. Nearly complete conversion (97%) of PCP to metabolites was observed within 10 days. 2,3,4,5-TeCP appeared transiently; its concentration increased with the removal of PCP and then decreased with a concurrent increase in the concentration of 3,4,5-TCP. No other CPs were observed during the experiment. Therefore, the consortium sequentially reductively dechlorinated PCP at the *ortho* positions to form 2,3,4,5-TeCP and 3,4,5-TCP. 3,4,5-TCP was not further degraded over 10 days.

TABLE 1. Summary of experimental conditions for CP reductive dechlorination experiments by acclimated organisms

Parent compound	Mass balance (%)[a]	Initial concn (μM)	Parent compound removal (%)[b]	Time (days)
PCP	107 ± 8.9	4.38	99.7	1.2
2,3,4,5-TeCP	—[c]	9.35	61.2	1.5
2,3,4,6-TeCP	106 ± 4.6	4.21	99.9	1.0
2,3,5,6-TeCP	99 ± 4.2	3.80	62.1	1.5
3,4,5-TCP	103 ± 2.4	7.31	89.8	1.5
2,4,5-TCP	110 ± 5.5	4.75	24.3	1.5
2,4,6-TCP	102 ± 8.2	5.84	74.3	1.5
2,3,5-TCP	101 ± 3.0	3.95	87.0	1.1

[a] Mass balances include all measured CP concentrations. Values given are the mean of the sum of the CP concentrations divided by the initial conditions followed by the coefficient of variation (standard deviation/mean).

[b] Based on initial and final measured concentrations.

[c] A mass balance was not observed. Only 47.4% of the mass of 2,3,4,5-TeCP that was biotransformed appeared as 3,4,5-TCP. The systematic loss of mass (29) suggests that one or more metabolic products were undetected.

PCP biotransformation by an acclimated methanogenic consortium. Acclimation of the sludge to PCP over a period of 6 months resulted in the elimination of the lag period, more-rapid biotransformation rates, and the development of a split biodegradation pathway. PCP (4.38 μM) was biotransformed at 99.7% efficiency within 1.2 days (Table 1 and Fig. 3). Degradation of PCP was accompanied by the production of all three TeCPs, as well as 3,4,5-, 2,4,5-, and 2,3,5-TCP. The "sum" identified in Fig. 3 represents the sum of the concentrations of PCP and all TeCPs and TCPs identified in the reactor. After 0.6 days, the mass balance appears to fall off as a result of the production of DCPs which were not included in the sum.

2,3,4,5-TeCP reached a maximum concentration of 0.37 μM after 0.5 days and was not detected in the reactor after 1 day. 2,3,5,6-TeCP accumulated rapidly in the reactor to a maximum concentration of 3.07 μM or approximately 70% of the initial PCP concentration. The accumulation of 2,3,5,6-TeCP ceased after 0.88 days when PCP was removed from the reactor. 2,3,4,6-TeCP, the *meta* dechlorination product, was observed at very low levels (less than 0.07 μM) during the experiment.

The experiment demonstrates that the consortium acquired the ability to dechlorinate PCP at all three chlorine positions after exposure to PCP for 6 months. Although dechlorination at the *ortho* position is preferred by unacclimated consortia (3, 14, 15, 28), removal of *para* and *meta* chlorines from PCP to produce 2,3,5,6-TeCP and 2,3,4,6-TeCP is observed following acclimation. The development of a split degradation pathway has generally not been observed in previous laboratory experiments, although it has been documented for rice paddy soils exposed to PCP (10, 13). Additionally, each of the TeCPs observed in this study were also reported in studies performed examining PCP biodegradation by sludges individually acclimated to either 2-CP, 3-CP, or 4-CP (15).

TeCPs. 2,3,4,5-TeCP was rapidly degraded, accompanied by the production of 3,4,5-TCP (Fig. 4), consistent with the

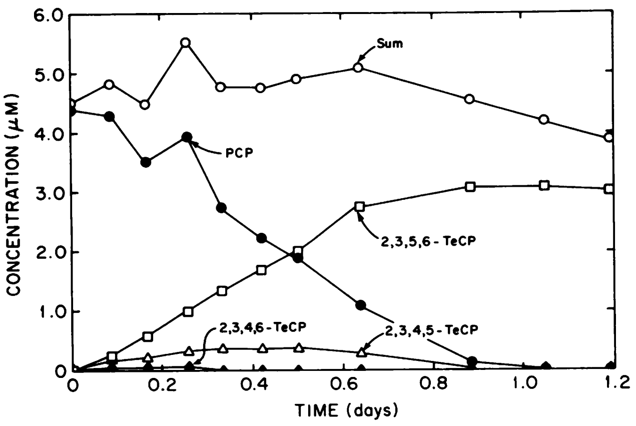

FIG. 3. Progress curve for PCP reductive dechlorination by a PCP-acclimated methanogenic consortium.

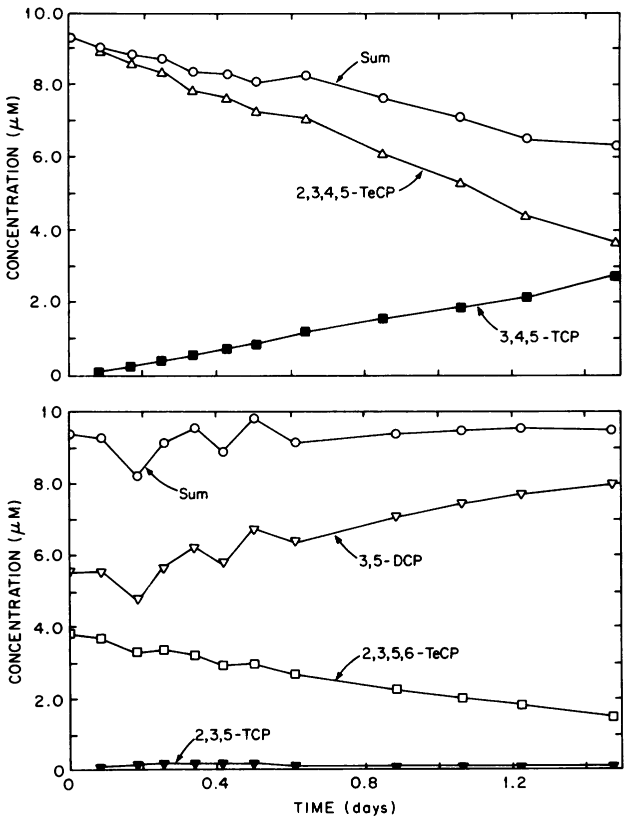

FIG. 4. Progress curves for 2,3,4,5-TeCP and 2,3,5,6-TeCP reductive dechlorination by a PCP-acclimated methanogenic consortium.

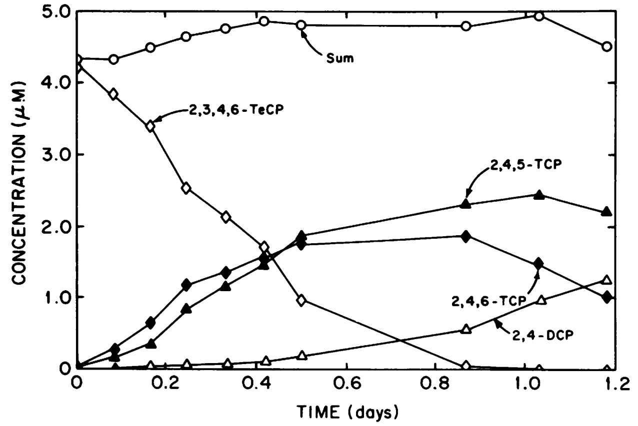

FIG. 5. Progress curve for 2,3,4,6-TeCP reductive dechlorination by a PCP-acclimated methanogenic consortium.

reductive dechlorination pathway observed for 2,3,4,5-TeCP in experiments with PCP as the parent compound. After 1.5 days, the production of 3,4,5-TCP accounted for only 47.4% of the mass of 2,3,4,5-TeCP that was removed. Although *meta-* and *para*-dechlorination products of 2,3,4,5-TeCP (2,3,4-TCP, 2,4,5-TCP, and 2,3,5-TCP) were not detected during this or repeated experiments with 2,3,4,5-TeCP, the systematic loss of mass suggests that either 3,4,5-TCP was being rapidly converted to an undetected metabolite or another TCP was being produced and rapidly biotransformed.

2,3,5,6-TeCP removal was accompanied by production of 2,3,5-TCP and accumulation of 3,5-DCP (Fig. 4). The appearance of these metabolites suggests sequential dechlorination of 2,3,5,6-TeCP's *ortho* chlorines. 2,3,6-TCP, the only other TCP that could be produced by dechlorination of 2,3,5,6-TeCP's *meta* chlorine, was not detected. The average sum of the concentrations of measured chlorophenols was 99.0% of the initial concentration, indicating a satisfactory mass balance.

2,3,4,6-TeCP was removed from the reactor at 99.9% efficiency within 1 day, accompanied by the accumulation of nearly equal amounts of 2,4,6- and 2,4,5-TCP and moderate amounts of 2,4-DCP (Fig. 5). The average sum of the concentrations of 2,3,4,6-TeCP, 2,4,6-TCP, 2,4,5-TCP, and 2,4-DCP during the experiment was 4.63 μM, 7.4% higher than the initial conditions. Because the mass balance accounted for the mass of 2,3,4,6-TeCP added to the reactor, 2,4,6- and 2,4,5-TCP were identified, and 2,3,4- and 2,3,6-TCP were not observed, the data suggest that *meta* dechlorination and removal of the *ortho* chlorine from the 2 position were the predominant pathways for 2,3,4,6-TeCP degradation by the acclimated anaerobic consortium.

TCPs. In the experiments with PCP or the three TeCPs, four TCPs were identified as metabolic products (3,4,5-, 2,4,6-, 2,4,5-, and 2,3,5-TCP); biotransformations of these TCPs were evaluated in separate batch experiments (Table 1).

3,4,5-TCP was removed at 89.8% efficiency after 1.5 days and was undetectable after 2.0 days (Fig. 6). The removal of 3,4,5-TCP was accompanied by a stoichiometric increase in the concentration of two DCPs. 3,5-DCP accounted for 96.2% of the mass of DCPs formed, and 3,4-DCP accounted for the remaining 3.8%. Thus, 3,4,5-TCP was predominantly dechlorinated at the *para* position to produce 3,5-DCP, but it

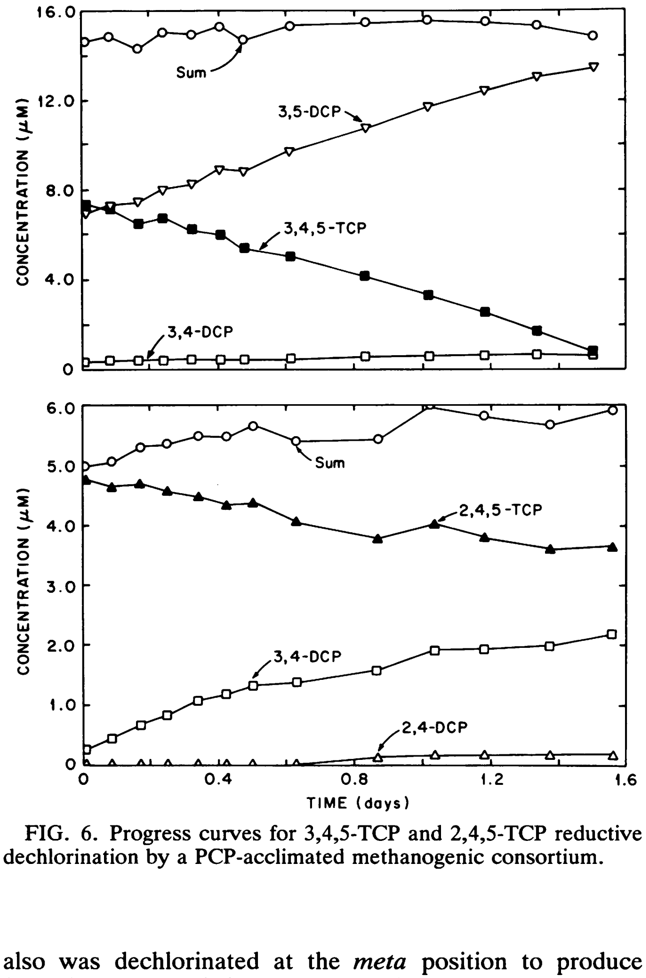

FIG. 6. Progress curves for 3,4,5-TCP and 2,4,5-TCP reductive dechlorination by a PCP-acclimated methanogenic consortium.

also was dechlorinated at the *meta* position to produce 3,4-DCP at a much lower rate. Both pathways have been observed in previous studies, *para* dechlorination was demonstrated in experiments with sewage sludges by Mikesell and Boyd (14, 15), and *meta* dechlorination was observed in reactor studies by Woods et al. (28) and in soils amended with sewage sludge (16).

2,4,5-TCP was slowly removed from the reactor, accompanied by the production of 3,4- and lesser amounts of 2,4-DCP (Fig. 6), reflecting dechlorination at the *ortho* and *meta* positions, respectively. The *para*-dechlorination product, 2,5-DCP, was not detected. After 1.5 days, the *ortho*-dechlorination product, 3,4-DCP, represented 93.6% of the mass of the two DCPs.

2,4,6-TCP was biotransformed by the production of 2,4-DCP (Fig. 7). No other CPs were observed. Thus, 2,4,6-TCP was dechlorinated at the *ortho* position, as has been previously reported (14, 28). An average of 99.5% of the initial mass of 2,4,6-TCP was recovered as 2,4,6-TCP and 2,4-DCP during the 1.5-day experiment.

2,3,5-TCP was dechlorinated at the *ortho* position to produce 3,5-DCP (Fig. 7). The two *meta*-dechlorination products, 2,5-DCP and 2,3-DCP, were not detected.

DCPs. Batch reactor experiments were performed to evaluate the degradation of the three DCPs observed in the pathways. Progress curves for 2,4-, 3,4-, and 3,5-DCP suggested extremely slow degradation kinetics for these compounds. Only 2,4-DCP was degraded within 2 days. No metabolites were detected. Extended batch experiments were then performed by using 60-ml serum bottles to test the

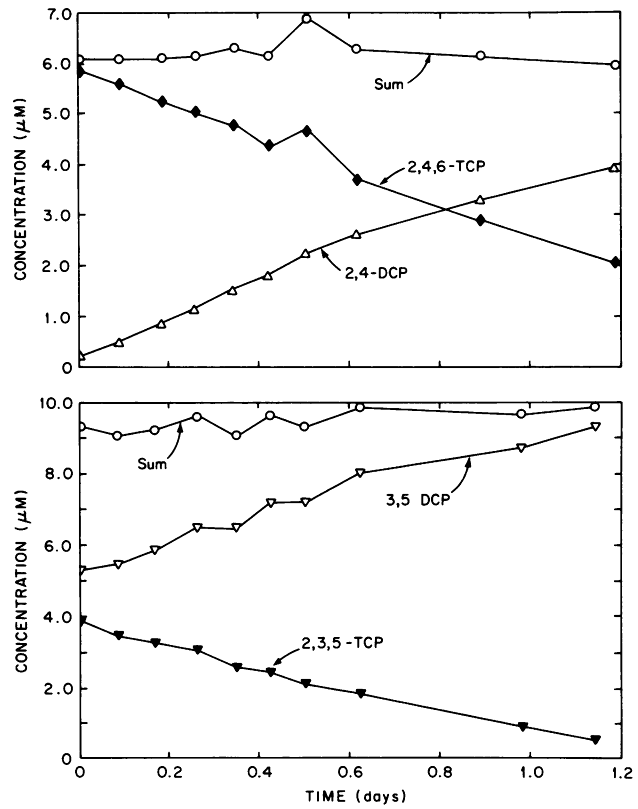

FIG. 7. Progress curves for 2,4,6-TCP and 2,3,5-TCP reductive dechlorination by a PCP-acclimated methanogenic consortium.

degradation of 2,4-DCP, 3,4-DCP, and 3,5-DCP over a period of 3 weeks. 2,4-DCP was partially removed during this period, accompanied by the accumulation of 4-CP, indicating removal of the *ortho* chlorine. 3,4-DCP and 3,5-DCP were not significantly removed, and metabolites were not detected.

Conclusions. The methanogenic consortium exposed to PCP for 10 days dechlorinated PCP principally at the *ortho* position. This preference for *ortho* dechlorination is consistent with earlier studies with unacclimated consortia (2, 3, 14, 28). However, acclimation of the organisms to PCP over a period of 6 months produced a microbial consortium with the ability to remove chlorines from the *ortho*, *meta*, and *para* positions of PCP.

On the basis of the results of the experiments, the complete biotransformation pathway observed for the reductive dechlorination of PCP by this acclimated consortium is shown in Fig. 8. PCP was dechlorinated at three positions to produce 2,3,4,5-TeCP, 2,3,4,6-TeCP, and 2,3,5,6-TeCP. 2,3,4,5-TeCP was dechlorinated at the *ortho* position to form 3,4,5-TCP, which then gave 3,5-DCP and lesser concentrations of 3,4-DCP as persistent products. 2,3,4,6-TeCP produced both 2,4,6-TCP and 2,4,5-TCP. 2,4,6-TCP was dechlorinated sequentially at the *ortho* positions to produce 2,4-DCP and 4-CP, and 2,4,5-TCP was dechlorinated to produce 3,4-DCP and 2,4-DCP. Sequential *ortho* dechlorination of 2,3,5,6-TeCP yielded 2,3,5-TCP and 3,5-DCP.

ortho dechlorination was observed most frequently. Every possible metabolite due to *ortho* dechlorination was observed except for the production of 2,3,4-TCP from 2,3,4,6-TeCP. Therefore, eight of the nine possible *ortho*-dechlorination products were observed. In contrast, only two *para*-dechlorination products (of seven possible prod-

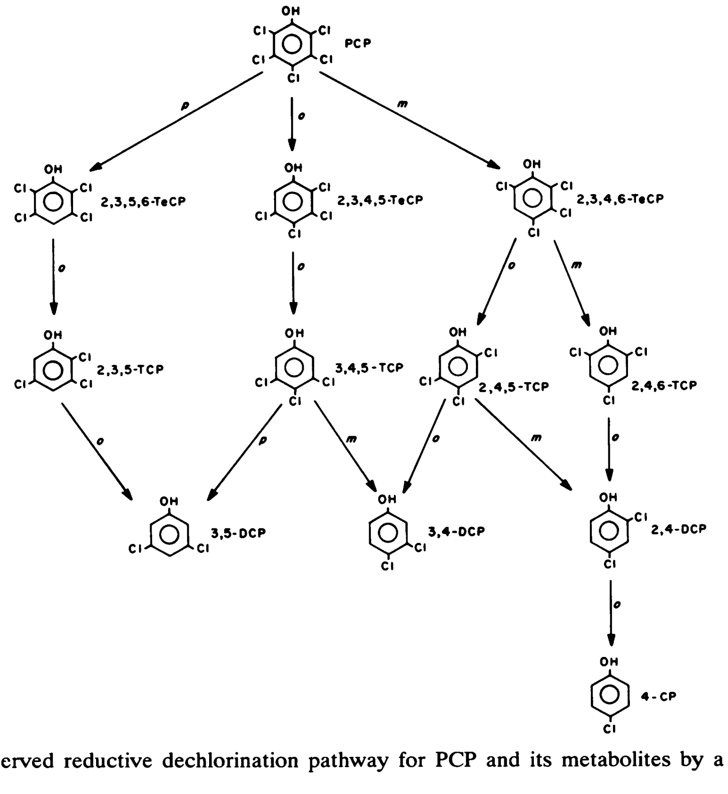

FIG. 8. Summary of the observed reductive dechlorination pathway for PCP and its metabolites by a PCP-acclimated methanogenic consortium.

ucts) and four *meta*-dechlorination products (of nine possible products) were observed. Of the possible polychlorinated phenolic congeners, only five are not included in the pathway: 2,3-DCP, 2,5-DCP, 2,6-DCP, 2,3,4-TCP, and 2,3,6-TCP. It is important that these five undetected DCP and TCP congeners possess at least one *ortho* chlorine and would not be expected to be observed because of the consortium's preferential removal of *ortho* chlorines.

The observed PCP dechlorination pathway for the acclimated consortium based on these experiments with PCP and its metabolites (Fig. 8) closely resembles the composite chlorophenol dechlorination pathway based on the literature for acclimated and unacclimated consortia shown in Fig. 1. Only one previously observed PCP metabolite was not observed in this series of experiments (2,3,6-TCP observed by Murthy et al. [17]). However, the likelihood of dechlorinating 2,3,5,6-TeCP at the *meta* position to produce 2,3,6-TCP instead of *ortho* dechlorination to produce 2,3,5-TCP is questionable. Similarly, this study has resulted in the identification of a new reductive dechlorination pathway. 2,4,5-TCP was dechlorinated at the meta position to produce 2,4-DCP, although the rates were very slow.

In soils and groundwaters contaminated with PCP, it is unlikely that certain CPs such as 2,6-DCP or 2,3,6-TCP would be observed. Production of compounds which do not appear in Fig. 8 may suggest contamination by other chlorinated phenols or the development of alternative pathways due to acclimation or environmental conditions.

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