APPLIED AND ENVIRONMENTAL MICROBIOLOGY, Dec. 1996, p. 4556–4562 Vol. 62, No. 12
0099-2240/96/$04.00+0

Microbial Dechlorination of Historically Present and Freshly Spiked Chlorinated Dioxins and Diversity of Dioxin-Dechlorinating Populations

ANDREI L. BARKOVSKII* AND PETER ADRIAENS

Department of Civil and Environmental Engineering, University of Michigan, Ann Arbor, Michigan 48109-2125

Received 10 June 1996/Accepted 23 September 1996

The ability of a microbial consortium eluted from dioxin-contaminated Passaic River sediments to dechlorinate polychlorinated dibenzo-*p*-dioxins (PCDDs) was investigated under methanogenic conditions. Aged 2,3,7,8-tetraCDD, which had partitioned into the microbial consortium from sediments, was stoichiometrically converted to tri- and monoCDD congeners. During dechlorination, dominant microbial activity within the consortium shifted from methanogenic to nonmethanogenic activity. Freshly spiked octaCDD was converted to hepta-, hexa-, penta-, tetra-, tri-, di-, and monochlorinated isomers, but the reaction stoichiometry was not determined. No methanogenic activity was observed, and the maximum yield of protein coincided with the production of less-chlorinated DD congeners. Two distinct pathways of dechlorination were observed: the *peri*-dechlorination pathway of 2,3,7,8-substituted hepta- to pentaCDDs, resulting in the production of 2,3,7,8-tetraCDD, and the *peri*- lateral dechlorination pathway of non-2,3,7,8-substituted congeners. Direct evidence of further lateral dechlorination of 2,3,7,8-tetraCDD was obtained from the historically contaminated incubations; no isomer-specific identification of triCDDs in spiked incubations was determined. Pasteurized cells exhibited no *peri*-dechlorination pathway, and triCDDs were the least-chlorinated congeners produced in these treatments. These results demonstrate that (i) both freshly spiked and aged PCDDs are available to microbial reductive dechlorination, (ii) the *peri* and triCDD dechlorinations are attributed to activities of nonmethanogenic, non-spore-forming microbial subpopulations, and (iii) the 2,3,7,8-residue patterns in historically contaminated sediments are likely affected by microbial activity.

Polychlorinated dibenzo-*p*-dioxins (PCDDs), in particular the 2,3,7,8-substituted (lateral) isomers, are among the most hazardous environmental pollutants because of their molecular planarity and ability to bind to biological receptors (21, 33). Because of their high toxicity and suspected genotoxic potential, their determination and fate in the environment are of great interest. The lack of a clear source identification, based on characteristic congener profiles for the processes which generate them, has been speculated to be due to natural attenuation (weathering) of the PCDDs by biological or abiotic processes. Indeed, it was shown previously that spiked PCDD could be dechlorinated when incubated with soils and sediments under reducing conditions (2, 3, 7). The contribution of abiotic reactions to reductive dechlorination of PCDDs by organic and inorganic electron transfer molecules in reduced environments has been recently demonstrated (1).

The contribution of anaerobic microorganisms as the agents effecting PCDD dechlorination in sediments has received limited attention (2, 3, 11). Evidence for initial reductive dechlorination of PCDDs by sediment-derived microorganisms has been reported for 1,2,3,4-tetraCDD (11) and 1,2,3,4,6,7,8-heptaCDD (3). The profiles of PCDD residues in sediments indicate a predominance of the highly chlorinated congeners (hepta- and octaCDDs) (40, 41), with 2,3,7,8-substituted isomers being the dominant congeners (14), thus rendering 1,2,3,4-tetraCDD an inadequate model to investigate natural attenuation processes of PCDDs in sediments.

* Corresponding author. Mailing address: Department of Civil and Environmental Engineering, University of Michigan, 1351 Beal Ave., 219 EWRE Bldg., Ann Arbor, MI 48109-2125. Phone: (313) 764-6350. Fax: (313) 763-2275. Electronic mail address: abark@engin.umich.edu.

The predominant impediment to microbial catalysis during transformation of hydrophobic contaminants in general, and PCDDs in particular, is the issue of bioavailability (4, 29). Adriaens et al. (2) recently investigated the effect of long-term (2 to 3 year) incubations on the availability and transformation of freshly spiked hepta- to pentaCDDs and dibenzofurans in sediments and calculated the half-lives of these compounds to be on the order of years. Alternatively, bioconcentration of historically present hydrophobic pollutants in microbiota may play a role in enhancing their natural microbial attenuation (9, 42), according to the following rationale. Whereas bioconcentration of PCDDs and related compounds in animal and plant tissues has been extensively described (24, 25, 34, 36), the high affinity of structurally similar polychlorinated biphenyls (PCBs) for microbial membrane lipids (phosphatidyl cholines) was only recently demonstrated (18). Moreover, on the basis of in vivo experiments with *Shewanella putrefaciens*, membrane-associated electron transfer components, such as the menaquinone redox system, were recently found to catalyze dechlorination reactions (31, 32). Thus, interactions between the electron flux in bacterial membrane systems and polymer (42)- or lipid (18)-solubilized PCB or PCDD molecules may increase the susceptibility of aged hydrophobic contaminants to microbial reductive dechlorination.

Information on the involvement of specific physiological groups of microorganisms during reductive dechlorination processes in general, and of PCDDs in particular, in anaerobic sediments is scarce. Previous experiments with sediments (2, 3) and sediment-derived microorganisms (11) were performed under methanogenic conditions analogous to those of experiments in which the anaerobic dechlorination of PCBs was examined (30, 39, 44). Whereas methanogens have been dem-

onstrated to dechlorinate PCBs (44), methanogenic activity is not always required (13, 43) as PCBs have been shown to be dechlorinated by nonmethanogenic anaerobic sporeformers (43). The activities could be differentiated on the basis of differences in dechlorination patterns (43, 44). This communication (i) addresses the extent of dechlorination of aged and freshly spiked dioxins by a sediment-derived community and (ii) attempts to relate specific microbial activities to dechlorination patterns observed.

MATERIALS AND METHODS

Media and inoculum. Historically dioxin-contaminated estuarine lower Passaic River (N.J.) sediment samples were collected near a former Agent Orange production plant (12). The prereduced anaerobic (methanogenic) nutrient medium (pH 7.2) and primary substrate cocktail (acetate, 75 mg/liter; butyrate, 15 mg/liter; benzoate, 10 mg/liter) used in all incubation studies have been described previously (3). All stock solutions were autoclaved separately at 120°C for 20 min, excluding vitamin stock and resazurin indicator solutions which were sterilized by filtration (0.2-μm pore size; Millipore). Sediments were transferred anaerobically (N_2-CO_2-H_2, 85:10:5) into a stirred 2-liter sterile Fernbach flask, mixed with 2 volumes of sterile medium, spiked with substrate cocktail (100 mg/liter, total), and incubated in the dark at room temperature. After a 2-month preincubation period, the slurry was allowed to settle for 1 h and the supernatant containing the eluted microorganisms was gently transferred into a 2-liter sterile vessel and supplied again with the substrate mixture. The supernatant containing the eluted consortia was analyzed for the presence of PCDD congeners, and three subsamples (5 ml each) were transferred into sterile 20-ml vials sealed with gas-tight Teflon-coated butyl rubber stoppers. The head space in the vials was analyzed for the presence of methane on a weekly basis. When the concentration of methane in subsamples (measured under isothermal conditions at 40°C with a model 5890A Hewlett-Packard gas chronometer equipped with flame ionization detection and a model DB-624 column of 60 m by 0.53 mm [3.00-μm film thickness]) reached 200 nM per flask, the medium containing the microbial consortia enriched from anaerobic sediments was used as the inoculum for all experiments. The experiment with freshly spiked PCDDs was conducted in an aqueous-organic biphasic system (5, 6, 26). Decane (4%, vol/vol) was chosen from three of the compounds tested (decane, toluene, and xylene) as the organic phase carrying PCDDs on the basis of its minimal effect on methanogenic activity (8). No decane was present in historically contaminated treatments.

Pasteurization. The inoculum (5 ml each, 28 ± 4 μg of protein per ml) was anaerobically transferred into sterile N_2-CO_2 (80:20, vol/vol)-flushed 20-ml serum bottles and sealed with gas-tight Teflon-coated butyl rubber stoppers. The concentration of protein was measured in three independent replicates by a modified Lowry assay for whole cells (15). Bottles were treated in a water bath at 75°C for 30 min on four consecutive days, with incubation at 30°C between treatments. Such treatment is generally regarded as sufficient for elimination of nonsporeformers, including most of those that are thermophilic (20), and does not change the chemical and physical characteristics of environmental samples (22). It was previously demonstrated that heating at 80°C for 15 min eliminated methanogenic activity in sediment-derived consortia (43).

Experimental setup. Five different treatments were established in triplicate for four time points: (i) sediment-derived microbial cells with historically present PCDD congeners, (ii) the same microbial cells pasteurized as described above with historically present CDD congeners, (iii) sediment-derived microbial cells spiked with a mixture of PCDDs (AccuStandard, Inc.) dissolved in decane, (iv) pasteurized microbial cells spiked with the mixture of PCDDs, and (v) anaerobic media spiked with the PCDD mixture as a chemical control. The mean final spiked concentrations of PCDD congeners (in nanomoles per liter ± standard errors) were 11,413 ± 543, octaCDD; 42.8 ± 2.0, heptaCDDs (1,2,3,4,6,7,8- and 1,2,3,4,6,7,9-heptaCDDs); and 1.8 ± 0.8, hexaCDDs (1,2,4,6,7,9- and 1,2,3,4,6,8-hexaCDDs) (medium-decane, 96:4, vol/vol). The values represent measurements from three independently spiked replicates. All manipulations were performed in an anaerobic chamber under an N_2-CO_2-H_2 atmosphere. The serum bottles were sealed with Teflon-coated butyl rubber stoppers and incubated at 30°C under methanogenic conditions in the dark with agitation (270 rpm). Every two months the cell suspensions were supplied with 100 mg of the substrate cocktail per liter. After 0, 1, 3, and 7 months, triplicates of each treatment were sacrificed for PCDD analysis.

Sample analysis. After the concentrations of methane and protein were measured, freshly contaminated cell suspensions and chemical controls were spiked with 1,2,3,4-tetraCDD (10 μl of a 50-mg/liter stock solution in toluene to a final concentration of 0.1 μg/ml) as an internal standard to correct for PCDD recovery efficiencies. Manual triplicate extractions with equal volumes of toluene were performed at each time point. Historically contaminated suspensions were extracted similarly but without internal standard to avoid chromatographic interference of 1,2,3,4-tetraCDD with trace concentrations of historical tetraCDD (35). The pooled extracts of each treatment were concentrated to 100 μl and analyzed with a model 5890-II Hewlett-Packard gas chromatograph-mass spectrometer equipped with a model 5972A mass selective detector. For analysis of octa- through tetraCDD congeners, the following gas chromatograph-mass spectrometer operating conditions were used: column, Ultra-2 of 50 m by 0.2 mm (0.33-μm film thickness); pressure, 30 lb/in^2; flow rate, 0.8 ml/min (He); linear flow velocity, 25 cm s^{-1}; split-splitless injection; injector temperature, 280°C; detector temperature, 300°C; initial temperature, 90°C; hold, 1 min; rate 1, 25°C min^{-1}; final temperature 1, 230°C; final time, 15 min; rate 2, 2°C min^{-1}; final temperature 2, 300°C; final time, 20 min; and total run time, 75.67 min. Samples were scanned for M^+, $(M+1)^+$, $(M+2)^+$, $(M+3)^+$, $(M+4)$ ions at a dwelling time of 100 ms per ion. Recovery efficiencies for PCDD congeners were 90 to 95% on the basis of the internal standard used. Quantitation was based on a six-level calibration curve established for a custom-made mixture of dioxin congeners, representing the five PCDD homolog groups monitored (tetra- through octaCDDs; AccuStandard, Inc.). The threshold level of reliable quantitation was 0.2 nmol/liter. Identification was based on the comparison of the retention times of isomers and relative ion abundances of key ion clusters in the mass spectra with calibrated and published values (19).

Mono- through tetraCDD congeners were analyzed under the same operating conditions of the gas chromatograph-mass spectrometer, except for the temperature program (injector temperature, 280°C; detector temperature, 300°C; initial temperature, 90°C, hold, 5 min; rate 1, 5°C min^{-1}; final temperature 1, 240°C; final time, 40 min; rate 2, 60°C min^{-1}; final temperature 2, 290°C; final time, 35 min; total run time, 110.83 min). Samples were scanned for M^+, $(M+1)^+$, $(M+2)^+$, $(M+3)^+$, $(M+4)^+$, and M^+-COCl ions at a dwelling time of 100 ms per ion. Identification of tetraCDD congeners was based on the absolute and relative (to 1,2,3,4-tetraCDD) retention times and relative ion abundances of key ion clusters in the mass spectra for tetraCDD congeners from specific window-defining mixtures (Cambridge Isotope Laboratories). Identification of mono- through triCDD congeners was based on the comparison of the retention times of isomers (AccuStandard, Inc.) and relative abundances of key ion clusters in the mass spectrum with calibrated and published values (National Bureau of Standards, Spectra Library). Quantitation was based on detector response values for each homolog group, as extrapolated from a correlation between detector responses for tetra- to octaCDD.

RESULTS

PCDD dechlorinating activity. Dechlorination of historically present and freshly spiked PCDDs was evaluated during a 7-month experiment using enriched microbial consortia derived from historically contaminated Passaic River sediments. Analysis of the cell suspension eluted from Passaic River sediments for historically present PCDD congeners revealed the presence of one tetraCDD isomer, identified as 2,3,7,8-tetraCDD on the basis of its absolute retention time (coelution with a standard) and its retention time relative to the retention time of 1,2,3,4-tetraCDD (35). The total concentration of 2,3,7,8-tetraCDD was 62.3 ± 12.5 nmol/liter or 0.6 ± 0.1 μg of total cell protein per mg. The more highly chlorinated DDs were present in trace concentrations and could not be quantified. No less-chlorinated congeners were observed.

The microbial reductive dechlorination of 2,3,7,8-tetraCDD commenced soon after the beginning of the experiment: after 1 month of incubation, two triCDD congeners, (one of which did not exceed 0.7 nmol/liter), a 2-monoCDD congener, and traces of a 1-monoCDD congener were observed (Fig. 1). The diCDD congeners were found in trace concentrations only. Between 1 and 3 months of incubation, the triCDD congeners decreased to nonquantifiable levels, and after 3 months of incubation, only 2-monoCDD remained. None of these congeners were produced in pasteurized cell suspensions during that time. After 7 months of incubation, both triCDD isomers (one of which was in trace concentration) and 2-monoCDD were observed again in the native cell suspensions (Fig. 1). The concentration of aged tetraCDD decreased by 30% to 43.6 ± 6.2 nmol/liter or to 0.24 ± 0.04 μg/mg of total cell protein, resulting in the total production of 9.8 ± 2.8 nmol of triCDD per liter and 9.7 ± 1.4 nmol of 2-monoCDD per liter. The dechlorination reaction was stoichiometric, as 19 nmol of tetraCDD per liter disappeared, resulting in the production of 19.5 nmol of triCDD and 2-monoCDD per liter. One of the triCDD congeners was found in pasteurized cell incubations as well, but in trace concentration (below 0.2 nmol) only. No

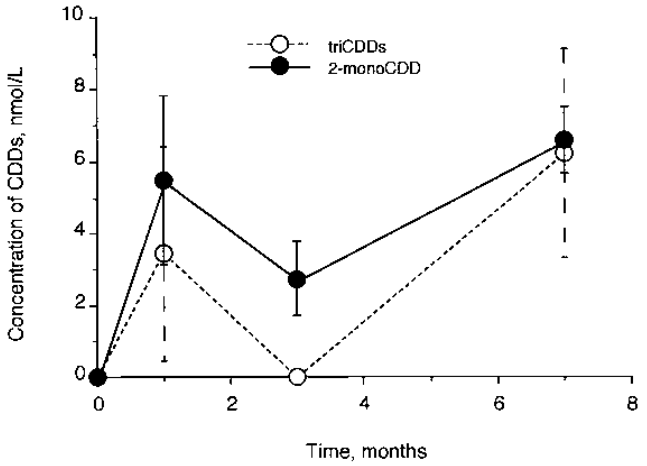

FIG. 1. Dynamics of the production of mono- and triCDD congeners during dechlorination of aged 2,3,7,8-tetraCDD by native cells.

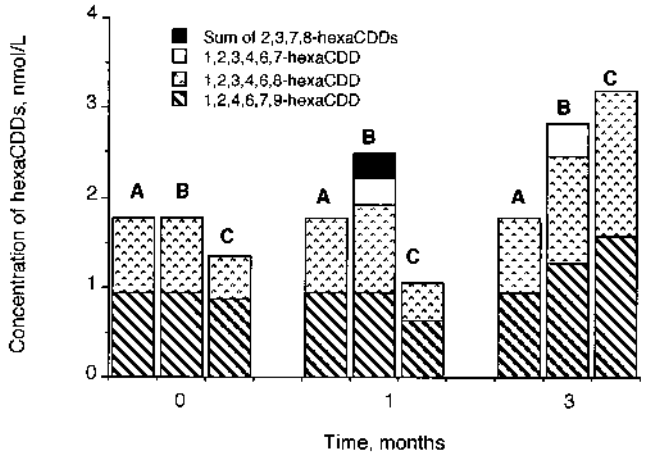

FIG. 3. Dynamics of hexaCDD congeners in chemical controls (A), native cell suspensions (B), and pasteurized cell suspensions (C) during dechlorination of freshly spiked PCDDs. Standard errors of the means were <10%.

decrease of 2,3,7,8-tetraCDD was observed, and no di- and monoCDD congeners were produced by pasteurized cells.

The patterns of bacterial dechlorination of a freshly spiked mixture of highly chlorinated (octa- through hexaCDD) dioxins were more complicated. The concentration of both 1,2,3,4,6,7,8- and 1,2,3,4,6,7,9-heptaCDD congeners doubled in the native and pasteurized microcosms during the first 3 months of incubation to 82 to 94 nmol/liter (Fig. 2). This increase was followed by a decrease in the heptaCDD concentration to 19 to 24 nmol/liter between 3 and 7 months of incubation. The net decrease of heptaCDD was correlated to the appearance of stoichiometric (native cell suspensions) or nonstoichiometric (pasteurized cells) amounts of hexa-, tetra-, tri-, and (in the case of native suspensions) monoCDDs. No increase nor decrease of heptaCDD congener concentrations was observed in chemical controls.

The hexaCDD congeners appeared after 1 month of incubation. Five hexaCDD isomers were produced by native cell suspensions and identified as two coeluting 2,3,7,8-substituted (1,2,3,6,7,8- and 1,2,3,4,7,8-) hexaCDD isomers and three non-2,3,7,8-substituted (1,2,3,4,6,7-; 1,2,4,6,7,9-; and 1,2,3,4,6,8-) hexaCDD isomers (Fig. 3). Two of the non-2,3,7,8-substituted hexaCDD isomers (1,2,4,6,7,9- and 1,2,3,4,6,8-hexaCDD) were produced by pasteurized cells. The total concentration of hexaCDD congeners produced was relatively low (3.6 nmol/liter, twice the initial spiked concentration). Whereas both of the isomers produced and identified in pasteurized cell incubations were identical to the spiked hexaCDD congeners, all three hexaCDD isomers which distinguished the native from the pasteurized incubations were produced de novo (Fig. 3). After 7 months, none of the hexaCDD congeners was found in quantifiable concentration. No changes in the hexaCDD congeners' profiles and concentrations were observed in chemical controls.

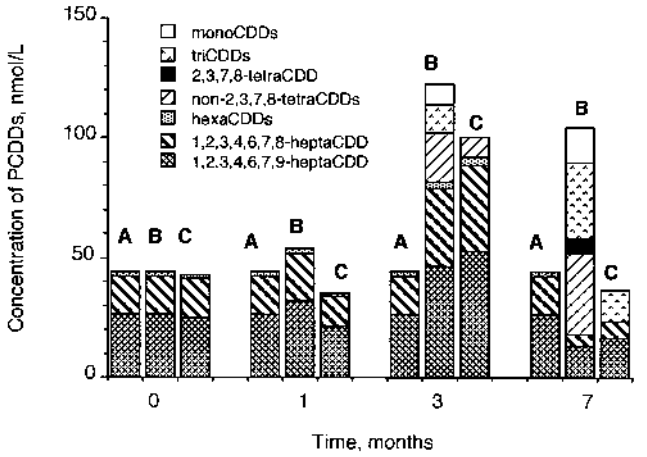

FIG. 2. Dynamics of hepta- through monoCDD congeners in chemical controls (A), native cell suspensions (B), and pasteurized cell suspensions (C) during dechlorination of freshly spiked PCDDs. Standard errors of the means were <10%.

The reductive dechlorination of hepta- and hexaCDD congeners resulted in the production of tetraCDD congeners and subsequent dechlorination to triCDD (in both native and pasteurized cell suspensions) and monoCDD (native suspensions only) by the end of the experiment (Fig. 2). PentaCDDs were found in both treatments in trace concentrations only. Four tetraCDD isomers were produced in the native suspensions, with 2,3,7,8-tetraCDD representing approximately 14% of the total amount (Fig. 2). These tetraCDD isomers subsequently dechlorinated to five triCDDs whose isomers could not be specifically identified, several diCDD congeners (in trace concentrations only), and both possible monoCDD congeners. Overall, the total production of tetra- to monoCDDs (approximately 87 nmol/liter) exceeded the disappearance of hepta- and hexaCDDs (approximately 64 nmol/liter) 1.4-fold (Fig. 2).

Three tetraCDD congeners, including 2,3,7,8-tetraCDD (trace concentration) were produced by pasteurized cells after 3 months of incubation. After 7 months, they were nearly stoichiometrically converted to two uncharacterized triCDD congeners, which were the end products of reductive dechlorination of PCDD by pasteurized cells. No di- or monoCDD congeners were discovered in these treatments (Fig. 2). The triCDD congeners produced made up approximately 18% of the total concentration of hepta- to tetraCDD congeners, which disappeared during the experiment. The final concentrations of tetra- to monoCDD congeners in the native and pasteurized cell suspensions were approximately 87 and 13 nmol/liter, respectively. No mono- through tetraCDD congeners appeared in chemical controls.

Throughout the experiment, the losses of octaCDD were 8%

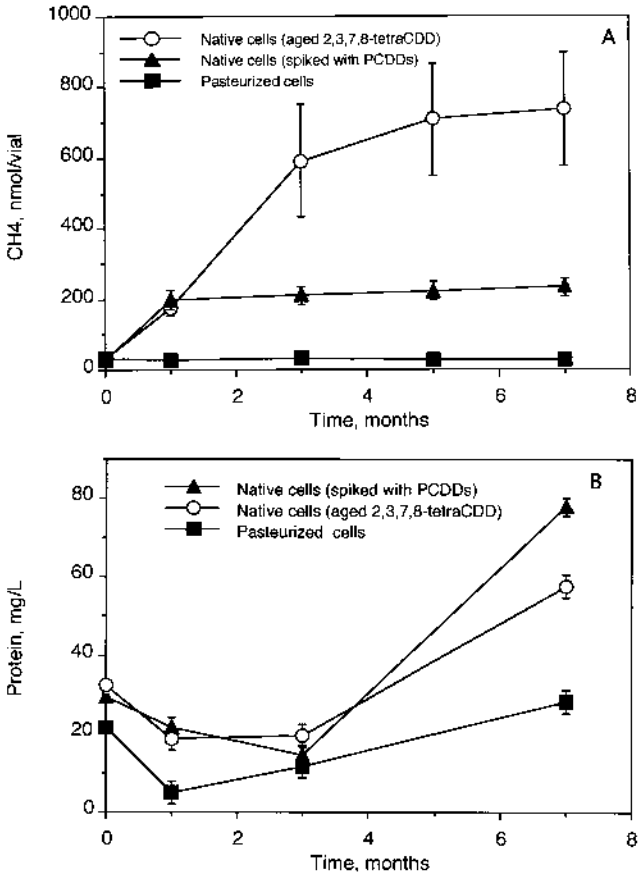

FIG. 4. Production of methane (A) and protein (B) in native and pasteurized cell incubations. Dynamics of methane and protein production by historically contaminated and freshly spiked pasteurized cells were indistinguishable from each other.

for pasteurized cell suspensions and 26% for native cell suspensions (data not shown). Since the net loss of octaCDD caused by the presence of native cell suspension was approximately 1,630 nmol/liter, no more than 10% of that loss could be accounted for by the appearance of less-chlorinated isomers. No loss of octaCDD was observed in chemical controls.

Microbial respiratory activity and growth. The rate of methane production in historically contaminated native cell suspensions decreased after 3 months of incubation, and methane production ceased after 5 months (Fig. 4A). The freshly spiked cell suspensions produced methane during the first month of incubation only, after which methanogenesis stopped completely. No methane was produced by both historically contaminated and freshly spiked pasteurized cells.

The time-dependent protein profiles show a trend opposite to that of methanogenesis. In historically contaminated cells, the decreasing protein concentration during the first month was followed by an increase, with maximum yield at the end of the incubation, i.e., when methanogenesis ceased (Fig. 4B). In freshly spiked cell suspensions, the decrease of protein content continued until the third month of incubation and was followed by a steep increase up to 80 μg/ml. This concentration was the highest yield reached in any treatment. This increase in protein content was not correlated to methanogenesis. Pasteurization initially decreased protein concentrations, which reached the initial value at the end of experiment (Fig. 4B).

DISCUSSION

Both aged and freshly spiked PCDD congeners were reductively dechlorinated by a microbial consortium eluted from Passaic River sediments. One congener, identified as 2,3,7,8-tetraCDD, was found in the eluted cell suspension consisting of sediment pore water and microorganisms. The partitioning of 2,3,7,8-tetraCDD to the aqueous phase is unlikely because of its low water solubility (0.6 nmol/liter at 25°C) (28). More likely, partitioning of 2,3,7,8-tetraCDD into the aquatic cell suspension resulted in bioconcentration by microbial cells, presumably in membrane lipids. Sediment concentrations of 2,3,7,8-tetraCDD in tidal estuaries of the Passaic River are on the order of 0.11 to 6.9 μg/kg, or 0.3 to 21.5 nmol/kg (12). Analyses of sediment, laboratory-exposed fish, and tissues of resident and migratory biota show that 2,3,7,8-substituted congeners are selectively enriched, with concentrations reported from 38 to 500 pg/g of lipid, depending on the species analyzed (10).

Whereas macrobiota accumulate PCDD via sediment ingestion, direct partitioning of PCDDs from sediment organics to cell lipids is presumably responsible for accumulation in microbial cells. The partitioning of 2,3,7,8-tetraCDD into the lipid tripalmitin was observed, and the structure of the lipid–2,3,7,8-tetraCDD complex was hypothesized (27). Recently, Dulfeer and Govers (18) demonstrated the affinity of (planar) PCBs to microbial cell lipids. Alternatively, accumulation of hydrophobic contaminants in bacterial cells may proceed via solubilization by extracellular polymers. For example, the herbicide diclofop, a chlorinated biaryl compound, and its aromatic breakdown products were shown to bioaccumulate in cell capsules of a biofilm microbial community (42).

During reductive dechlorination of aged 2,3,7,8-tetraCDD isomer, the activity of the natural microbial consortium shifted from predominantly methanogenic to predominantly nonmethanogenic, as differentiated by the yield of methane and protein in the natural microbial consortia. The first phase of tetraCDD dechlorination (Fig. 1) took place during active methanogenesis (Fig. 4A) and concomitant decreasing protein concentrations (Fig. 4B). Two triCDD isomers (one of which did not exceed 0.7 nmol/liter) and both monoCDD isomers (1-monoCDD was in trace concentration) were observed. 1-MonoCDD cannot be a product of 2,3,7,8-tetraCDD dechlorination, unless molecular rearrangement occurred. This isomer and the minor triCDD isomer are hypothesized to result from dechlorination of highly chlorinated congeners which were found in the eluted microbial cell suspension in trace concentrations.

Despite decreasing methane production, dechlorination of tetraCDD continued until the end of the experiment (Fig. 1). During this second phase, the native consortium exhibited directly opposite trends with respect to methane and protein production. The increase in protein concentration indicated that the cessation of methanogenesis was not due to substrate limitation (Fig. 4). The reason for the shift of microbial consortium from methanogenic to predominantly nonmethanogenic during tetraCDD dechlorination remains unknown. Whereas chlorinated aromatic compounds have been demonstrated to be used as terminal electron acceptors (17, 23), the dioxin concentrations used in this study were too low to effect significant changes in electron fluxes. 2-MonoCDD was the end product of tetraCDD dechlorination during this phase. In contrast to the native cell suspensions, pasteurized cells produced no methane, resulted in low cell yields, and produced trace amounts of a triCDD congener as the end product of dechlorination. The data obtained suggest that (i) the reduc-

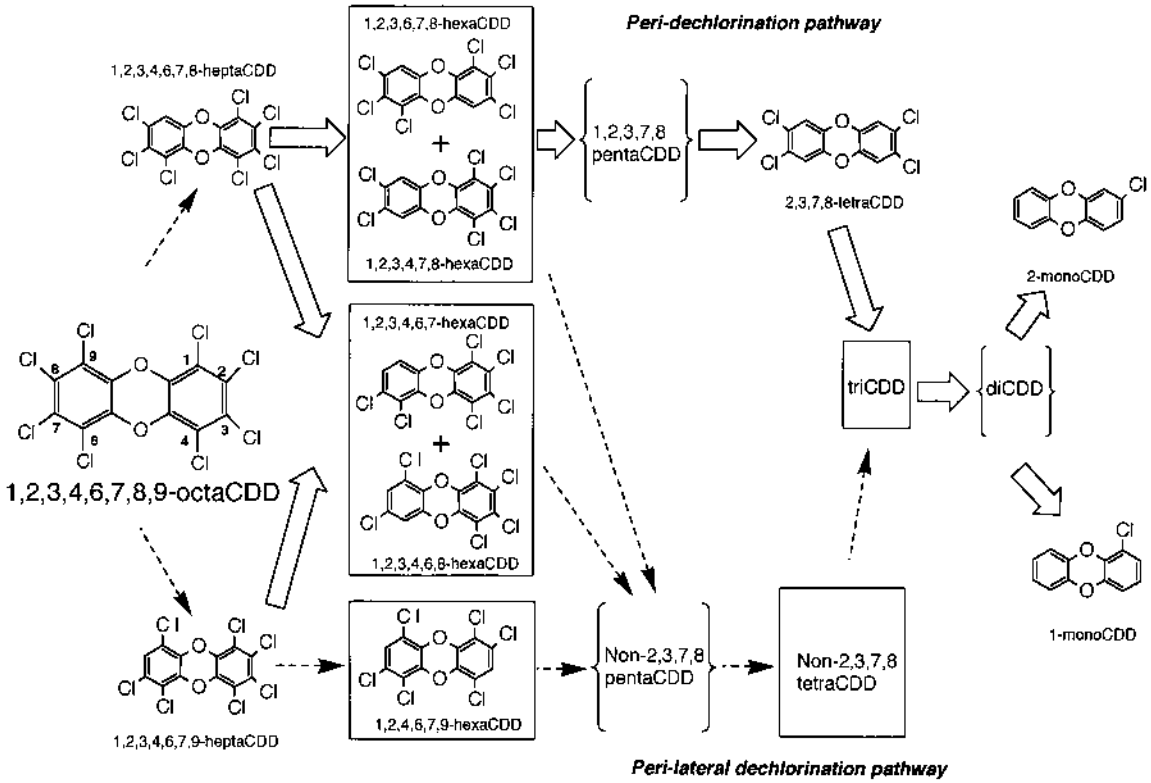

FIG. 5. Branched pathway of PCDD microbial dechlorination. Broad, open arrows designate dechlorination caused by activity of nonmethanogenic, non-spore-forming microorganisms. Intermediates in braces were found in trace concentrations.

tive dechlorination of triCDD congeners was mediated by subpopulations of nonthermoresistant, non-spore-forming microorganisms and (ii) this subpopulation was not necessarily methanogenic.

The comparison of patterns of freshly spiked PCDD dechlorination by the native and pasteurized cells revealed distinctive differences based on both highly (hepta- and hexa-) and less (tri)-chlorinated dioxins. Whereas the non-2,3,7,8-substituted hexaCDD congeners (except for 1,2,3,4,6,7-hexaCDD) found as intermediates of heptaCDD dechlorination were shared by both treatments, the 2,3,7,8-substituted hexaCDD congeners were discovered in the native cell suspensions only (Fig. 3). 2,3,7,8-TetraCDD was also found in quantifiable concentration as an intermediate in the native but not pasteurized cell suspensions (Fig. 2). This observation is evidence for a dichotomous dechlorination pathway for highly chlorinated PCDD congeners in this microbial consortium: (i) a mixed *peri*-lateral dechlorination pathway for non-2,3,7,8-substituted congeners and (ii) a *peri*-dechlorination pathway for 2,3,7,8-substituted hepta- through pentaCDD isomers (Fig. 5). The first proposed pathway is hypothesized to represent combined microbial (due to subpopulation of thermoresistant anaerobic sporeformers) and chemical (due to the coelution of unknown redox-active compounds) activities. The reductive dechlorination of PCDD congeners caused by metals, polyphenolic compounds, or vitamins was reported earlier (1).

The second pathway represents the result of microbial activity, because its intermediates were not present in pasteurized cell incubations. Pasteurization as applied is generally regarded as sufficient for elimination of nonsporeformers, including most thermophilic ones (20). Thus, a subpopulation of anaerobic non-spore-forming sediment microorganisms is postulated to be responsible for *peri* dechlorination of 2,3,7,8-substituted PCDD congeners. A similar observation was made by Ye et al. (43) during an investigation of PCB dechlorination, who found that microorganisms which survived heat and ethanol treatments preferentially removed *meta* chlorines, while non-thermo- and non-ethanol-resistant subpopulations mainly contributed to the *para*-dechlorination activity.

In our experiment, the total amount of the hexaCDD produced was relatively low regardless of the treatment and the pentaCDDs were found in trace concentrations only. It is of interest that the analysis of environmental samples for the presence of total (14) and 2,3,7,8-substituted PCDDs (40, 41) indicated low hexa- and pentaCDD concentrations except in samples directly affected by incineration outfall (37). Whether this phenomenon is caused by high susceptibility of these congeners to microbial reductive dechlorination or their inherent chemical reactivities remains unclear.

Up to 10% of the total loss of octaCDD can be accounted for by the appearance of less-chlorinated isomers. As no loss of octaCDD was observed in chemical controls, sorption losses to glassware can be excluded. Even though octaCDD loss can be partially explained by (i) different adsorption capacities of the native and pasteurized microbial cells (38) or (ii) reactions other than reductive dechlorination of octaCDD and its dechlorination products, this experimental phenomenon is not really understood. The excess of tetraCDD-to-monoCDD production (approximately 23 nmol/liter) relative to hepta- and hexaCDD disappearance (Fig. 2) indicates that the real amounts of hepta- and hexaCDDs exceeded those observed during the experimental sampling points. To date, the only stoichiometric conversion of octaCDD to less-chlorinated

products was observed in the presence of zerovalent zinc in organic or aqueous solution (1).

During reductive dechlorination of freshly spiked dioxins by the native consortium, directly opposite trends of methanogenic activity and protein production were observed (Fig. 4). As less-chlorinated (tetra-, tri-, and monoCDD) dioxins appeared after the third month of incubation (Fig. 2), their production coincided with an increase in protein content and the cessation of methanogenic activity. The pattern of decreasing methane production with increasing dechlorination has been observed during biologically mediated reductive dechlorination of other chlorinated compounds, such as 2,3,6-trichlorobiphenyl (13), tetrachloroethene (16), and 3-chlorobenzoate (17). We observed no production of methane (Fig. 4A), low amounts of protein (Fig. 4B), and the triCDD congeners as dead-end products of the chlorinated dioxins' reductive dechlorination (Fig. 2) in the pasteurized microcosms. These data lend support to the hypothesis that subpopulation(s) of anaerobic, nonmethanogenic, non-spore-forming microorganisms may be responsible for both *peri* dechlorination of 2,3,7,8-substituted hepta- to pentaCDD isomers and further dechlorination of the triCDD congener group in lower Passaic River sediments.

On the basis of evidence for the accumulation of 2,3,7,8-substituted hepta- and tetraCDD congeners in sediments (14, 40, 41), the microbially mediated *peri* dechlorination of 2,3,7,8-substituted PCDD isomers seems to be an important pathway of natural attenuation of chlorinated dioxins. This evidence for a microbially mediated pathway suggests the need to include the contribution of microorganisms in the source-sink analysis of patterns of distribution of PCDD congeners observed in contaminated sediments.

ACKNOWLEDGMENTS

This research was supported in part by NIEHS Superfund Grant ES-91-02 and by EPA Grant CR 821918-01-0 awarded to P.A.

We thank Tom Yavaraski for his help with method development for PCDD analysis.

REFERENCES

1. **Adriaens, P., P. R. Chang, and A. L. Barkovskii.** 1996. Dechlorination of PCDD/F by organic and inorganic electron transfer molecules in reduced environments. Chemosphere **32:**433–441.
2. **Adriaens, P., Q. Fu, and D. Grbic'-Galic'.** 1995. Bioavailability and transformation of highly chlorinated dibenzo-*p*-dioxins and dibenzofurans in anaerobic soils and sediments. Environ. Sci. Technol. **29:**2252–2260.
3. **Adriaens, P., and D. Grbic'-Galic'.** 1994. Reductive dechlorination of PCDD/F by anaerobic cultures and sediments. Chemosphere **29:**2253–2259.
4. **Alexander, M.** 1991. Research needs in bioremediation. Environ. Sci. Technol. **25:**1972–1973.
5. **Ascon-Cabrera, M., and J.-M. Lebeault.** 1993. Selection of xenobiotic-degrading microorganisms in a biphasic aqueous-organic system. Appl. Environ. Microbiol. **59:**1717–1724.
6. **Barkovskii, A., and P. Adriaens.** 1995. Biological and abiotic dechlorination of highly chlorinated dioxins in biphasic microcosms, p. 73–82. *In* R. E. Hinchee, D. B. Anderson, and R. E. Hoeppel (ed.), Bioremediation of recalcitrant organics. Batelle Press, Columbus, Ohio.
7. **Barkovskii, A., Q. Fu, and P. Adriaens.** 1994. Biological and abiotic dechlorination of highly chlorinated PCDD/PCDF: issues of bioavailability and pathways. Organohalogen Compounds **21:**469–473.
8. **Barkovskii, A. L., and P. Adriaens.** Unpublished data.
9. **Baughman, G. L., and D. F. Paris.** 1981. Microbial bioconcentration of pollutants from aquatic system—a critical review. Crit. Rev. Microbiol. **8:** 205–228.
10. **Belton, T. J., R. Hazen, B. E. Ruppel, K. Lockwood, R. Mueller, E. Stevenson, and J. J. Post.** 1985. A study of dioxin (2,3,7,8-tetrachlorodibenzo-*p*-dioxin) contamination in select finfish, crustaceans and sediments of New Jersey waterways. New Jersey Department of Environmental Protection, Trenton.
11. **Beurskens, J. E. M., M. Toussaint, J. de Wolf, J. M. D. van der Steen, P. C. Slot, L. C. M. Commandeur, and J. R. Parsons.** 1995. Dehalogenation of chlorinated dioxins by an anaerobic microbial consortium from sediment. Environ. Toxicol. Chem. **14:**939–943.
12. **Bopp, R. F., M. L. Gross, H. Tong, H. J. Simpson, S. J. Monson, B. L. Deck, and F. C. Moser.** 1991. A major incident of dioxin contamination: sediments of New Jersey estuaries. Environ. Sci. Technol. **25:**951–956.
13. **Boyle, A. W., C. K. Blake, W. A. Price II, and H. D. May.** 1993. Effects of polychlorinated biphenyl congener concentration and sediment supplementation on rates of methanogenesis and 2,3,6-trichlorobiphenyl dechlorination in an anaerobic enrichment. Appl. Environ. Microbiol. **59:**3027–3031.
14. **Charles, M. J., W. G. Green, and G. D. Marbury.** 1995. Appraisal of the mass spectrometry/mass spectrometry of polychlorinated dibenzo-*p*-dioxins and dibenzofurans using new data. Environ. Sci. Technol. **29:**1741–1747.
15. **Daniels, L., R. S. Hanson, and J. A. Phillips.** 1994. Chemical analysis, p. 512–555. *In* P. Gerhardt, R. G. E. Murray, W. A. Wood, and N. R. Krieg (ed.), Methods for general and molecular bacteriology. American Society for Microbiology, Washington, D.C.
16. **DiStefano, T. D., J. M. Gosset, and S. H. Zinder.** 1991. Reductive dechlorination of high concentration of tetrachloroethene by an anaerobic enrichment culture in the absence of methanogenesis. Appl. Environ. Microbiol. **57:**2287–2292.
17. **Dolfing, J., and J. M. Tiedje.** 1986. Hydrogen cycling in a three-tiered food web growing on the methanogenic conversion of 3-chlorobenzoate. FEMS Microbiol. Ecol. **38:**293–298.
18. **Dulfeer, W. J., and H. A. J. Govers.** 1995. Membrane-water partitioning of polychlorinated biphenyls in small unilamellar vesicles of four saturated phosphatidylcholines. Environ. Sci. Technol. **29:**2548–2554.
19. **Environmental Protection Agency.** 1986. The analysis of polychlorinated dibenzo-p-dioxins and polychlorinated dibenzofurans (EPA method 8280), p. 8280/1–8280/33. *In* Test methods for evaluating solid waste, vol. IB. Laboratory manual physical/chemical methods. Environmental Protection Agency, Washington, D.C.
20. **Gottschalk, G., J. R. Andersen, and H. Hippe.** 1981. The genus Clostridium (nonmedical aspects), p. 1767–1803. *In* M. P. Starr, H. Stolp, H. G. Trüper, A. Bakkows, and H. G. Schlegel (ed.), The prokaryotes, vol. II. Springer-Verlag, New York.
21. **Hansen, D. J.** 1991. Dioxin toxicity: new studies prompt debate, regulatory action. Chem. Eng. News **69:**7–14.
22. **Heijman, C. G., C. Holliger, M. A. Glaus, R. P. Schwarzenbach, and J. Zeyer.** 1993. Abiotic reduction of 4-chloronitrobenzene to 4-chloroaniline in a dissimilatory iron-reducing enrichment culture. Appl. Environ. Microbiol. **59:** 4350–4353.
23. **Holliger, C., and G. Schraa.** 1994. Physiological meaning and potential for application of reductive dechlorination by anaerobic bacteria. FEMS Microbiol. Rev. **15:**297–305.
24. **Hülster, A., J. F. Müller, and H. Marscher.** 1994. Soil-plant transfer of polychlorinated dibenzo-*p*-dioxins and dibenzofurans to vegetables of the cucumber family (*Cucurbitaceae*). Environ. Sci. Technol. **28:**1110–1115.
25. **Kim, J.-G., T. Sakurai, N. Suzuki, and J. Nakanishi.** 1995. Levels of PCDDs and PCDFs in fish and shrimp from lake Kasumigaura. Organohalogen Compounds **24:**313–315.
26. **Köhler, A., M. Schüttoff, D. Bryniok, and H.-J. Knackmuss.** 1994. Enhanced biodegradation of phenanthrene in a biphasic culture system. Biodegradation **5:**93–103.
27. **Leenheer, J. A.** 1991. Organic substance structures that facilitate contaminant transport and transformation in aquatic sediments, p. 3–23. *In* R. A. Baker (ed.), Organic substances and sediments in water, vol. I. Lewis Publishers, Chelsea, Mich.
28. **Mackay, D., W. Y. Shiu, and K. C. Ma.** 1992. Illustrated handbook of physical-chemical properties and environmental fate for organic chemicals, vol. 2. Lewis Publishers, Chelsea, Mich.
29. **Mihelcic, J. R., D. R. Lueking, R. J. Mitzell, and J. M. Stapleton.** 1993. Bioavailability of sorbed- and separate-phase chemicals. Biodegradation **4:**141–153.
30. **Øfjord, G. D., J. A. Puhakka, and J. F. Ferguson.** 1994. Reductive dechlorination of Aroclor 1254 by marine sediment cultures. Environ. Sci. Technol. **28:**2286–2294.
31. **Petrovskis, E. A.** 1995. Transformation of chlorinated solvents by metal-reducing bacteria. Ph.D. thesis. The University of Michigan, Ann Arbor.
32. **Petrovskis, E. A., T. M. Vogel, D. A. Saffarini, K. H. Nealson, and P. Adriens.** 1995. Transformation of tetrachloromethane by *Shewanella putrefaciens* MR-1, p. 61–67. *In* R. E. Hinchee, D. B. Anderson, and R. E. Hoeppel (ed.), Bioremediation of chlorinated solvents. Batelle Press, Columbus, Ohio.
33. **Poland, A., and J. C. Knutson.** 1982. 2,3,7,8-Tetrachlorodibenzo-*p*-dioxin and related halogenated aromatic hydrocarbons: examination of the mechanism of toxicity. Annu. Rev. Pharmacol. Toxicol. **22:**517–554.
34. **Reiner, E. J., K. A. MacPherson, T. M. Kolic, A. Hayton, and R. E. Clement.** 1995. Characteristic levels of chlorinated dibenzo-*p*-dioxins and dibenzofurans in fish from Ontario Great Lakes. Organohalogen Compounds **24:** 379–384.
35. **Ryan, J. J., H. B. S. Conacher, L. G. Panopio, B. P.-Y. Lau, and J. A. Hardy.**

1991. Gas chromatographic separations of all 136 tetra- to octa- polychlorinated dibenzo-*p*-dioxins on nine different stationary phases. J. Chromatogr. **541:**131–183.

36. **Schrock, M. E., E. S. Barrows, and L. B. Rosman.** 1995. TCDD/TCDF levels in bioaccumulation test tissues and their corresponding sediments. Organohalogen Compounds **24:**363–367.
37. **Thomas, V. M., and T. G. Spiro.** 1996. The U.S. dioxin inventory: are there missing sources? Environ. Sci. Technol. **30:**82A–85A.
38. **Tsezos, M., and J. P. Bell.** 1989. Comparison of the biosorption and desorption of hazardous organic pollutants by live and dead biomass. Water Res. **23:**561–568.
39. **Van Dort, H. M., and D. L. Bedard.** 1991. Reductive *ortho* and *meta* dechlorination of a polychlorinated biphenyl congener by anaerobic microorganisms. Appl. Environ. Microbiol. **57:**1576–1578.
40. **Wenning, R. J., M. A. Harris, B. Finley, D. J. Paustenbach, and H. Bedbury.** 1993. Application of pattern recognition techniques to evaluate polychlorinated dibenzo-*p*-dioxin and dibenzofuran distributions in surficial sediments from the lower Passaic River and Newark Bay. Ecotoxicol. Environ. Saf. **25:**103–125.
41. **Wenning, R. J., M. A. Harris, M. J. Ungs, D. J. Paustenbach, and H. Bedbury.** 1992. Chemometric comparison of polychlorinated dibenzo-*p*-dioxin and dibenzofuran residues in surficial sediments from Newark Bay, New Jersey and other industrialized waterways. Arch. Environ. Contam. Toxicol. **22:**397–413.
42. **Wolfaardt, G. M., J. R. Lawrence, R. D. Robarts, and D. E. Caldwell.** 1995. Bioaccumulation of the herbicide diclofop in extracellular polymers and its utilization by a biofilm community during starvation. Appl. Environ. Microbiol. **61:**152–158.
43. **Ye, D., J. F. Quensen III, J. M. Tiedje, and S. A. Boyd.** 1992. Anaerobic dechlorination of polychlorobiphenyls (Aroclor 1242) by pasteurized and ethanol-treated microorganisms from sediments. Appl. Environ. Microbiol. **58:**1110–1114.
44. **Ye, D., J. F. Quensen III, J. M. Tiedje, and S. A. Boyd.** 1995. Evidence for *para* dechlorination of polychlorobiphenyls by methanogenic bacteria. Appl. Environ. Microbiol. **61:**2166–2171.