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Sequential Anaerobic–Aerobic Biodegradation of PCBs in Soil Slurry Microcosms

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ABSTRACT

Many industrial locations have identified the need for treatment of polychlorinated biphenyl (PCB) wastes and remediation of PCB-contaminated sites. Biodegradation of PCBs is a potentially effective technology for treatment of PCB-contaminated soils and sludges; however, a practicable remediation technology has not yet been demonstrated. In laboratory experiments, soil slurry microcosms inoculated with microorganisms extracted from PCB-contaminated Hudson River sediments have been used for anaerobic dechlorination of weathered Aroclor 1248 in contaminated soil with a low organic carbon content. Anaerobic incubation was then followed by exposure to air, addition of biphenyl, and inoculation with *Pseudomonas* sp. LB400, an aerobic PCB degrader. The sequential anaerobic–aerobic treatment constituted an improvement compared to anaerobic or aerobic treatment alone by reducing the total amount of PCBs remaining and decreasing the tendency for end products to accumulate in humans. A 70% reduction of PCBs was observed during sequential treatment with products containing fewer chlorines and having a shorter half-life in humans than the original PCBs. The aerobic treatment alone was also quite effective as a stand-alone treatment reducing the PCBs by 67%. The results represent a case in which anaerobic river sediment organisms have been successfully transferred to a matrix free of river or lake sediments.

Index Entries: PCB; anaerobic–aerobic biodegradation; soil slurry microcosms; bioremediation.

INTRODUCTION

Polychlorinated biphenyls (PCBs) are a family of compounds produced as complex mixtures by direct chlorination of biphenyl and were employed extensively in many industrial applications until the mid-1970s. The mixtures were used in transformers, capacitors, printing ink, paints, antidusting agents, pesticides, and so forth, and sold under various trade names (Aroclor, Clophen, Kanechlor,

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Phenoclor, and Pyralene). Associated numbers with each name denoted average chlorine content (e.g., Aroclor 1242, manufactured by Monsanto, contains 42% [by weight] chlorine) *(1)*.

The discovery of widespread environmental occurrence of these very stable compounds and their suspected carcinogenic effect in humans led to the regulation of PCBs through the Toxic Substances Control Act in 1976. Even if not in production, PCBs have persisted in the environment, and it is estimated that several million pounds have been released *(1)*. The health effects as PCBs relate to humans have recently been reviewed *(2,3)*.

Although PCBs may seem inert, aerobic microbial degradation of lower-chlorinated PCBs is well known and studied. Anaerobic reductive dechlorination of PCBs by microorganisms in river sediments has also been investigated, and a review by Abramowicz *(4)* summarizes research results on both aerobic and anaerobic bioprocesses. An engineered process for complete biodegradation of PCBs to form nonhazardous end products is complicated and may consist of three stages:

1. Partial dechlorination yielding 3 or 4 chlorines per biphenyl under anaerobic conditions;
2. Aerobic attack on a biphenyl ring to cause ring cleavage; and
3. Mineralization of resulting chlorinated benzoic acids to form H_2O, CO_2, Cl^-, and cell mass *(4)*.

In rare cases, anaerobic degradation of the biphenyl ring has been reported *(5–7)*. As the individual processes become well known, it is possible that an engineered approach may be taken for PCB treatment under environmentally controlled conditions to maximize efficiency.

Anaerobic dechlorination of PCBs has been observed in river and pond sediments, and has been proven and repeated in laboratory settings. Isolation of dechlorinating organisms is difficult, and mixed cultures capable of PCB dechlorination found at a specific site tend to lose their activities when transferred to another matrix void of humic acids *(8)*. In contrast, isolation of bacteria capable of dechlorinating 3-chlorobenzoate or 2-chlorophenol has been successful *(9,10)*. Anaerobic dechlorination of halogenated compounds has recently been reviewed by Mohn and Tiedje *(11)*.

The anaerobic treatment period (or incubation period) is on the order of several months, but methods have been developed to enhance or induce dechlorination of halogenated aromatics and thus shorten the incubation period. The most commonly used methods involve nutrient amendments *(12–15)* and addition of specific polyhalogenated biphenyls *(15–17)*. The result of sequential anaerobic–aerobic treatment of PCB-contaminated soils has been published by Shannon et al., but few details about the procedure were presented *(18)*. An anaerobic study with PCB-contaminated soil has been presented by Tiedje et al., who combined noncontaminated sediments (as a source of nutrients and organic carbon), Hudson River organisms, and contaminated soil to form sediment/soil slurries *(19)*. Anid et al. have presented results of sequential anaerobic–aerobic degradation of PCB-spiked Hudson River sediments in both microcosms and a river model with 800 kg sediment *(20)*.

The objective of this work was to investigate the feasibility of employing engineered sequential anaerobic–aerobic biodegradation schemes to effect reduction of PCBs in contaminated soils with low organic carbon *(21)*. The second objective was to determine the possibility of using exogenous organisms without the need for

addition of actual river sediments. Results are presented from anaerobic dechlorination studies performed with historically PCB-contaminated soil, as well as results from aerobic biodegradation of PCBs subsequent to anaerobic treatment or as a stand-alone treatment. Anaerobic studies were performed in (river) sediment-free soil slurry microcosms and represent a case in which a consortium has been successfully transferred from native river sediments to soil with very low organic carbon content.

Methods

Soil Collection

PCB-contaminated soil was collected from a capacitor bank at a power substation located in Chattanooga (TN). Over time, PCBs were released through spills in and around these capacitors. The soil was analyzed and found to have the following characteristics: 26% clay, 25% sand, 49% silt, 0.6% organic carbon, and 0.04% total nitrogen; pH of 8.3. Collected soil was combined with sterile deionized water (equal volumes), and the slurry was ball-milled antiseptically and anaerobically for 4 h to ensure homogeneity. The PCB concentration was approx 100 mg/kg in dry soil, and the congener pattern resembled weathered Aroclor 1248 with decreased levels of trichlorobiphenyls (mainly 23-2, 26-4, 24-4, and 25-4 trichlorobiphenyl). The congener pattern of Aroclor 1248 has been published elsewhere *(18,22)*.

Soil Slurry Microcosm Study

Anaerobic batch incubations were prepared under a nitrogen environment in 150-mL nominal-volume serum bottles (Wheaton Scientific, Millville, NJ) by combining steam-sterilized medium with the soil slurry. The bottles were capped with Teflon-lined caps (The West Co., Phoenixville, PA) and aluminum crimp-seals (Wheaton).

The final concentrations of nutrients in the anaerobic microcosms were (per liter): 200 g soil, 1.1 g sodium pyruvate, 2.3 g acetone, 1.2 g $NaHCO_3$, 525 mg NH_4Cl, 100 mg $MgCl_2 \cdot 6H_2O$, 75 mg $CaCl_2$, 35 mg K_2HPO_4, 27 mg KH_2PO_4, 21.5 mg $FeCl_2 \cdot 4H_2O$, 300 μg H_3BO_3, 200 μg $CoCl_2 \cdot 6H_2O$, 100 μg $ZnSO_4 \cdot 7H_2O$, 30 μg $MnCl_2 \cdot 4H_2O$, 30 μg $Na_2MoO_4 \cdot 2H_2O$, 20 μg $NiCl_2 \cdot 6H_2O$, 10 μg $CuCl_2 \cdot H_2O$, 10 μg Na_2SeO_3, and 1 mg resazurin. The medium was boiled under a nitrogen atmosphere for 2 min and cooled to room temperature before the $NaHCO_3$ was added. The final pH of the slurry was 7–7.5, and the total slurry volume in each microcosm was 35 mL. No chemical reducing agent was added in this study, and anaerobic conditions (as indicated by resazurin color change) were achieved after 1 wk in the bottle inoculated with organisms eluted from Hudson River sediments. Anaerobic conditions in the uninoculated bottle did not develop, indicating the lack of general aerobic organisms in the soil capable of growth under the conditions employed. The microcosms were incubated without agitation in the dark at room temperature.

During the aerobic phase, previously anaerobic microcosms were exposed to air and supplemented with additional nutrients (1.5 g biphenyl, 2.1 g NH_4Cl, 4.4 g K_2HPO_4, and 1.7 g KH_2PO_4/L slurry). The microcosms were placed in an upright position on an orbital shaker (200 rpm) at room temperature and exposed to air by the introduction of sterile cotton-filled syringe needles through the Teflon-lined caps. Adequate oxygenation of the microcosms was verified by the resazurin color change in the medium. Steam-sterilized microcosms were exposed to the atmosphere to account for any PCB evaporation.

Inocula

Naturally occurring organisms present in Hudson River (NY) sediments have been shown to be responsible for extensive dechlorination of PCBs in that environment *(23–25)*. The anaerobic inoculum was prepared from equal volumes of dewatered Hudson River sediments (site H7 *[23]*) and mineral medium. The slurry was mixed for 1 h followed by a 15-min settling time. The supernatant was removed and filtered through sterile cotton before use. One microcosm was inoculated with the filtrate (5 mL into 30 mL) and the other microcosm was left uninoculated, but 5 mL sterile medium were added. The aerobic culture (*Pseudomonas* sp. LB400 [NRRL-18064]) was supplied by General Electric and maintained on the medium used in the aerobic studies. Prior to use, the seed culture was grown for 2 wk in serum bottles exposed to air as described above. Indigenous aerobic biphenyl degraders were absent in the contaminated soil (data not shown).

Sampling and PCB Analysis

One milliliter of slurry was removed using sterile techniques at each sampling time and was combined with 1 mL of acetone and 4 mL of hexane, shaken for 4 h, and centrifuged at 1000 rpm (112*g*) for 5 min. One milliliter of the organic phase was combined with 0.1 mL internal standard (8 μg/L octachloronaphtalene in hexane), and 1 μL of the mixture was injected into a gas chromatograph (GC) (Hewlett-Packard, Avondale, PA) equipped with an electron capture detector and a 30 m × 0.247 mm DB-1 (0.25-μm film thickness) capillary column (J&W Scientific, Folsom, CA). The splitless/split injector temperature was 270°C, and the detector temperature was 300°C. Initial oven temperature was kept at 40°C for 2 min, after which two temperature ramps (20°C/min to 160°C and 5°C/min thereafter) were used to increase oven temperature to 270°C. Multilevel calibration was performed using an Aroclor (Ultra Scientific, North Kingstown, RI) mixture of 70% (by weight) Aroclor 1242, 20% Aroclor 1254, and 10% Aroclor 1260 in hexane diluted to levels appropriate to ensure compatibility with samples. The PCB congener composition of the Aroclors and peak identification needed for calibration were supplied by Abramowicz (General Electric, Schenectady, NY), and similar data have been published elsewhere *(24,26)*. Data analysis using Hewlett-Packard Chemstation software was conducted on 68 congener-containing peaks. The extracted soil was dried at 80°C for 20 h to determine the dry soil weight of each sample and allow PCB concentrations to be calculated.

The number of mono-, di-, tri-, and so forth, chlorobiphenyls were calculated based on the known PCB congener composition in the standards and peak identification mentioned above. In addition to homolog calculations, the expected half-life in humans of the PCB congeners was calculated based on data compiled by Brown *(3)*.

RESULTS AND DISCUSSION

Sampling was conducted at start-up and then continued about every 4th week. A typical PCB GC peak profile for microcosms at start-up is shown in Fig. 1A. Clear evidence of dechlorination was first noted after 7 wk; dechlorination then slowly progressed for another 12 wk (data not shown). The PCB GC peak profile after 19 wk of incubation with organisms from the Hudson River may be seen in

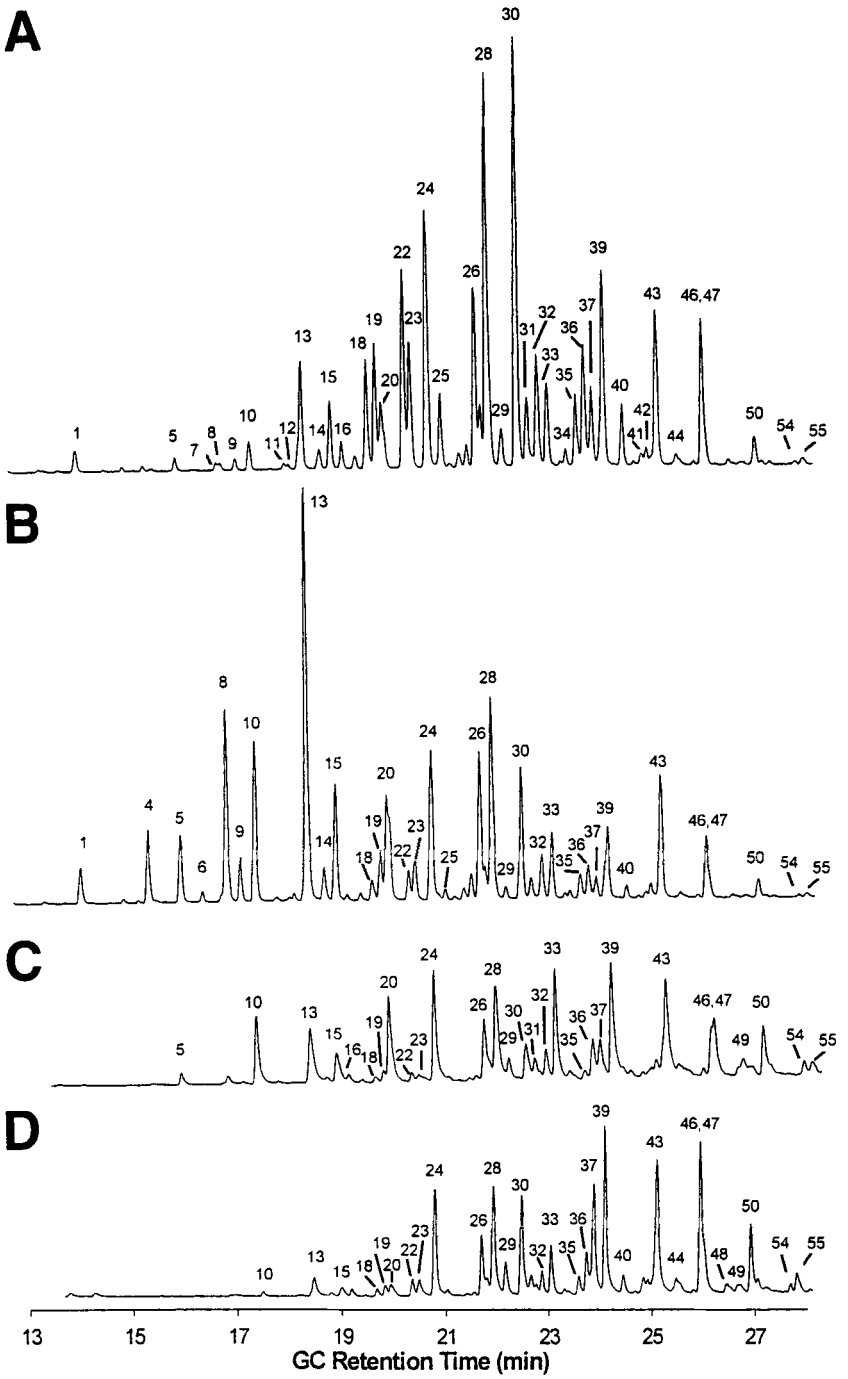

Fig. 1. PCB GC peak profiles of samples taken from contaminated-soil reactors at start-up and after the various incubation conditions. Numbers above peaks correspond to peak numbers. **(A)** Initial GC peak profile (weathered Aroclor 1248). **(B)** GC peak profile after anaerobic incubation. **(C)** peak profile after anaerobic and aerobic incubation. **(D)** GC peak profile after aerobic incubation.

Fig. 1B. Additional incubation for over 60 wk did not result in additional dechlorination under the conditions described (data not shown). It is clear from inspecting the chromatograms in Figs. 1A,B that there is an increase in peak sizes around 14–20 min GC retention time and a decrease in sizes for peaks occurring after 20 min. This shift in peaks is indicative of dechlorination. The PCB peak profile of samples taken from the uninoculated control microcosm did not change over the same time period; additional incubation time of 60 wk did not result in dechlorination in this type of microcosm (data not shown).

The shift in PCB GC peak profile during active dechlorination (*see* Fig. 1B) was very similar to that presented by Shannon et al., indicating similar microbial activation, however, information about the anaerobic consortium or techniques was not described in Shannon et al.'s work *(18)*. Detailed inspection of chromatograms and congener patterns revealed that 50% of the *meta*-substituted chlorines was removed during the anaerobic incubation, resulting in a decrease from 1.5 to 0.75 *meta*-chlorines/biphenyl. The number of *para*- and *ortho*-substituted chlorines remained the same (0.9 and 1.9 chlorines/biphenyl, respectively). As previously mentioned, dechlorination was not observed in the uninoculated bottle.

The major congeners present in relevant GC peaks are shown in Fig. 2 where the calculated mole percentage and mole percentage change have been plotted for GC peaks 1–55. The major products of the dechlorination were congeners 24-24, 24-26, 24-4, 26-4, 24-2, 2-4, and 2-2. This dechlorination pattern is consistent with pattern M described by Quensen et al. *(27)*, which is generally associated with *meta*-dechlorination of Aroclor 1242, yielding mostly *para*- and *ortho*-substituted PCBs. The M pattern is similar to pattern N, but has a lower tendency for dechlorination of 245-trichlorobiphenyl groups. Dechlorination of virgin Aroclor 1248 by Hudson River organisms in river sediment spiked with the Aroclor followed pattern C (*para*- and *meta*-removal) in Quensen et al.'s study *(27)*.

The anaerobic incubation period of inoculated and uninoculated microcosms was followed by inoculation with *Pseudomonas* sp. LB400 and aerobic incubation for an additional 19 wk. Results from analyses indicate a substantial decrease in peak sizes below 24 min GC retention time and sizes of peaks occurring after 24 min remaining the same (*see* chromatograms in Figs. 1C,D). No evaporation was noted in sterile microcosms conducted in parallel and vented to the atmosphere in a similar fashion (data not shown). The decrease in individual peak sizes during this aerobic phase (*see* Fig. 1C) is similar to that reported by Shannon et al., by a proprietary organism *(18)*. The decrease in peak sizes in Fig. 1D is similar to that reported by Bedard et al. for NRRL 15940 *Alcaligenes eutrophus* H850 *(22)*. The superior abilities of *Pseudomonas* sp. LB400 to degrade aerobically PCBs in mixtures in the presence of biphenyl has been documented by Bedard et al. *(28)*.

The change in homolog composition during fermentations is shown in Fig. 3. A decrease in tetra-, penta-, and hexachlorobiphenyls was accompanied by an increase in di- and trichlorobiphenyls during anaerobic incubation of the microcosm inoculated with organisms eluted from Hudson River sediments. During subsequent aerobic incubation, all dichlorobiphenyls and a large fraction of the tri-, tetra-, and pentachlorobiphenyls were degraded. Overall, a 70% decrease in total concentration of PCBs was observed for the sequential anaerobic–aerobic fermentation. The aerobic treatment alone proved quite effective in reducing the total PCB concentration by 67%, leaving mainly tetra- and pentachlorobiphenyls (*see* Fig. 4).

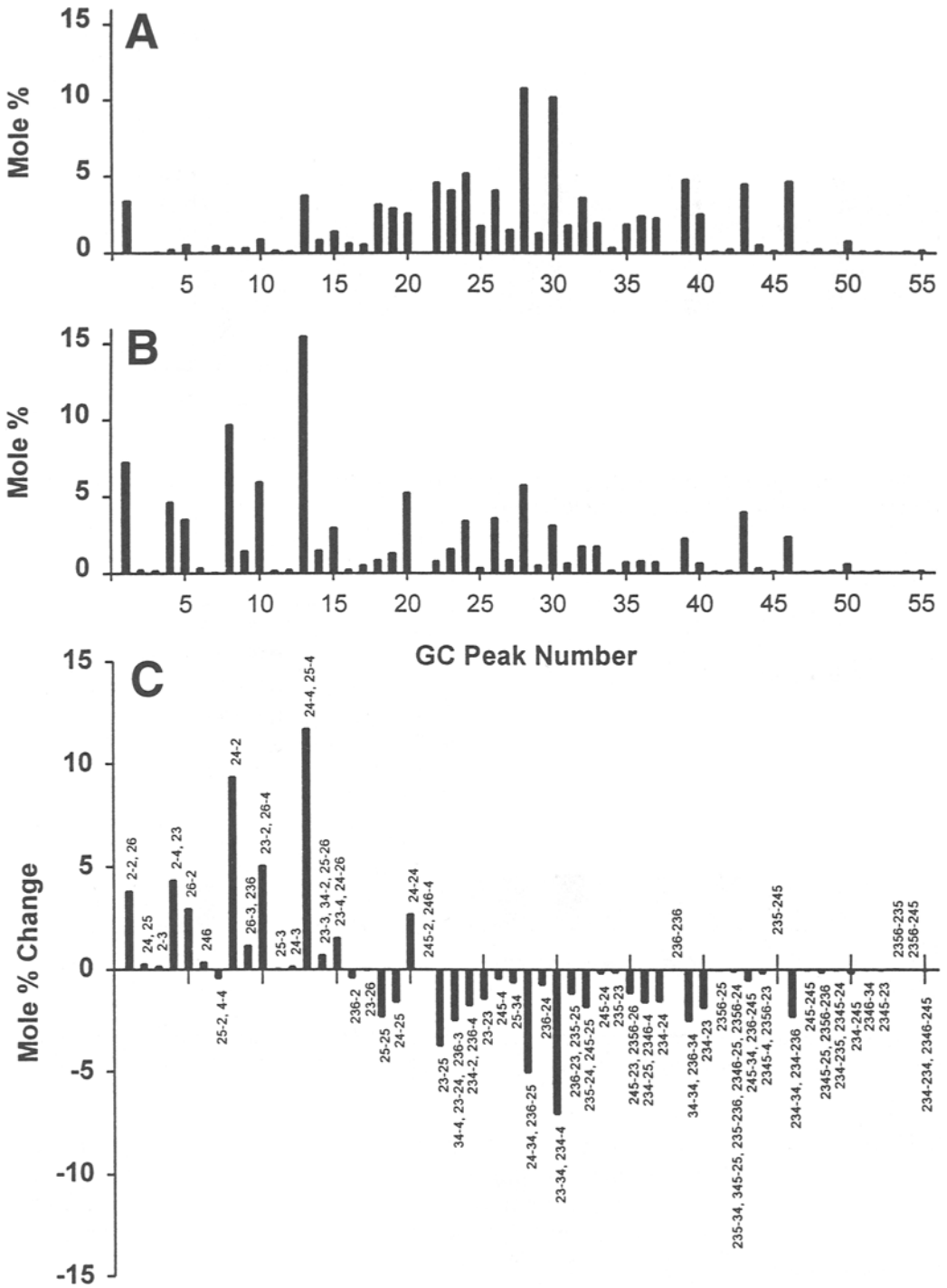

Fig. 2. PCB concentration profiles of samples taken from contaminated-soil reactors at start-up and after anaerobic incubation for 19 wk. Peak labels indicate the congeners present in the peak. **(A)** Initial PCB profile (weathered Aroclor 1248). **(B)** PCB profile after anaerobic incubation. **(C)** Change in PCB profile after anaerobic incubation.

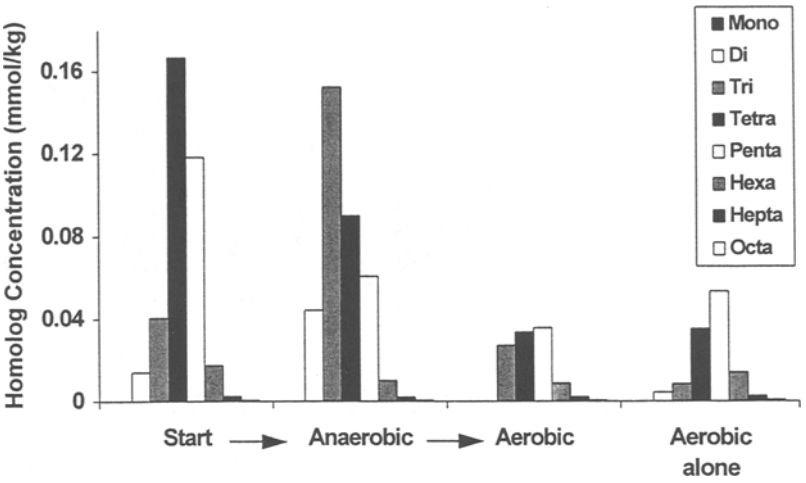

Fig. 3. Calculated PCB homolog concentrations of samples taken from contaminated-soil reactors during the various incubations.

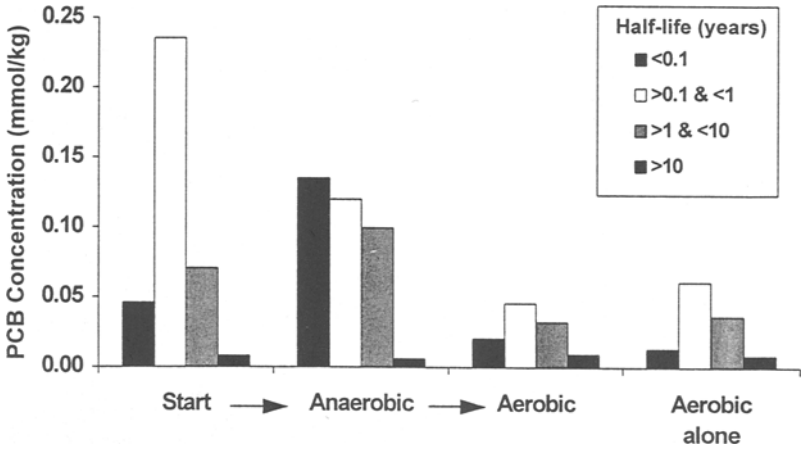

Fig. 4. Calculated PCB concentrations of samples taken from contaminated-soil reactors during the various incubations grouped by half-life expectancy in humans.

The achieved overall reduction of PCBs in the sequential anaerobic–aerobic treatment compares well with Shannon et al., who noted an 81% reduction of PCBs in a contaminated soil (1240 mg/kg Aroclor 1248) *(18)*. The greater degradation noted by Shannon et al. may be contributed to the lack of easily degraded trichlorobiphenyls *(22,28)* not originally present in weathered soil used in our studies. The results in the current study represent an improvement over the sequential treatment results presented by Anid et al., who observed an approx 40% reduction of 300 mg/kg (spiked) Aroclor 1242 in Hudson River sediments *(20)*. The betterment noted in the current study is attributed to limited capability of the aerobic isolate (S3) used in the Anid et al. study to degrade trichlorobiphenyls.

The near-congener-specific analysis also allowed for calculation of PCB half-life in humans during the incubations. Initially, over 50% of the PCB congeners had a half-life between 0.1 and 1 yr in humans (*see* Fig. 4). After anaerobic incubation, redistribution of congeners resulted in an increase in PCB congeners with a half-life shorter than 0.1 yr. This was accompanied by a decrease in PCB congeners with a slightly longer half-life. Interestingly, the fraction of PCB congeners with a half-life longer than 1 yr increased, indicating that some of the generated congeners during anaerobic conditions have a longer half-life than their predecessors.

As noted in Fig. 4, the aerobic fermentation proved very effective both in conjunction with anaerobic fermentation and by itself. Overall, sequential anaerobic–aerobic fermentation represented a substantial improvement over anaerobic fermentation alone and offered a slight improvement over aerobic fermentation alone when evaluating results based on expected half-life in humans.

The data presented in this article are part of a larger study during which 110 duplicate samples were collected from microcosms under various environmental conditions and PCB concentrations, but having the same soil type. These samples were subjected to the same sample preparation and analytical procedures as the original samples. The pooled standard deviations *(29)* of PCB concentrations and chlorine content were 11 mg/kg and 0.02 chlorines/biphenyl, respectively. In addition, an instrument reproducibility test was also performed by duplicate injection of the same sample into the GC of 110 samples. The pooled standard deviations in this case were 4 mg/kg and 0.02 for the PCB concentration and the calculated average number of chlorines per biphenyl respectively. The PCB concentration in the samples subjected to data quality assurance ranged from 14 to 1300 mg/kg (based on dry solids), and the calculated average number of chlorines per biphenyl ranged from 3 to 4.6.

CONCLUSIONS

PCB-dechlorinating microorganisms were successfully transferred from Hudson River sediments to a sandy soil contaminated with PCBs. This represents one of a few cases in which anaerobic river sediment organisms have been successfully transferred to a nonnative matrix (free of river or lake sediments *[15]*). Higher-chlorinated biphenyls were dechlorinated to less-chlorinated products more susceptible to aerobic degradation. The total concentration of PCBs decreased by 70% for sequential anaerobic–aerobic treatment compared with a 67% decrease for aerobic treatment alone. The sequential treatment resulted in PCB products with fewer chlorines and shorter half-lives in humans compared with either anaerobic or aerobic treatment alone.

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