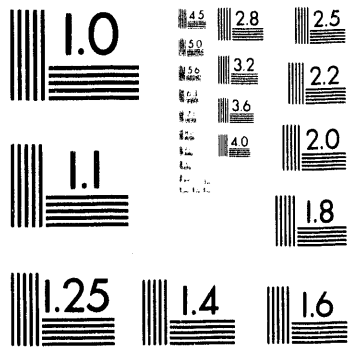
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PCB DECHLORINATION IN ANAEROBIC SOIL SLURRY REACTORS

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For presentation at the
Sixth International Institute of Gas Technology Symposium
on Gas, Oil and Environmental Biotechnology
Colorado Springs, Colorado

November 29, 1993

*Managed by Martin Marietta Energy Systems, Inc., for the U.S. Department of Energy under contract DE-AC05-84OR21400.

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ABSTRACT

Many industrial locations, including the U.S. Department of Energy's, have identified needs for treatment of polychlorinated biphenyl (PCB) wastes and remediation of PCB-contaminated sites. Biodegradation of PCBs is a potentially effective technology for the treatment of PCB-contaminated soils and sludges, including mixed wastes; however, a practical remediation technology has not yet been demonstrated.

In laboratory experiments, soil slurry bioreactors inoculated with microorganisms extracted from PCB-contaminated sediments from the Hudson River have been used to obtain anaerobic dechlorination of PCBs. The onset of dechlorination activity can be accelerated by addition of nutritional amendments and inducers. After 15 weeks of incubation with PCB-contaminated soil and nutrient solution, dechlorination has been observed under several working conditions. The best results show that the average chlorine content steadily dropped from 4.3 to 3.5 chlorines per biphenyl over a 15-week period.

PCB DECHLORINATION IN ANAEROBIC SOIL SLURRY REACTORS

INTRODUCTION

Polychlorinated biphenyls (PCBs) are a family of compounds that were used extensively in many industrial applications until the mid -1970s. They were produced as complex mixtures by direct chlorination of biphenyl and were marketed for use in transformers, capacitors, printing ink, paints, antidusting agents, pesticides, etc. The mixtures were marketed under various trade names (Aroclor, Clophen, Kanechlor, Phenoclor, and Pyralene) with associated numbers denoting the average chlorine content [e.g., Aroclor 1242, manufactured by Monsanto, contains 42% (by weight) chlorine].

The discovery of widespread environmental occurrence of these very stable compounds and the suspected carcinogenic effect in humans led to a ban on the use of PCBs in many countries. The public outcry in the United States culminated in the regulation of PCBs through the Toxic Substances Control Act (TSCA) in 1976. Even though a ban is in place, PCBs have persisted in the environment due to their low water solubility, strong adsorption characteristics, negligible volatilization, and low chemical reactivity. It is estimated that several million pounds have been released into the environment over time [1].

Although PCBs may seem to be inert, the aerobic microbial degradation of PCBs is well known and has been studied extensively. Recently, the anaerobic reductive dechlorination of PCBs by microorganisms has also been studied. A good review by Abramowicz [2] summarizes the research results of both the aerobic and anaerobic bioprocesses. The complete biodegradation of PCBs to form nonhazardous end products is complex and consists of three stages: (1) partial dechlorination of the biphenyl under anaerobic conditions; (2) aerobic attack on the biphenyl rings to cause ring cleavage; and (3) mineralization of the chlorinated benzoic acids to form H_2O, CO_2, Cl^-, and cell mass. Most of the current research is directed toward the first stage, and it is also believed that this first stage is rate limiting. Extensive efforts have been aimed at isolation of a pure culture capable of dechlorinating PCBs.

The anaerobic dechlorination 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 site or matrix (soil, sediment, etc.). The treatment period (or incubation period) is on the order of several months, but methods have been developed to enhance or induce dechlorination and thus shorten the incubation period. The most commonly used methods involve nutrient amendments and the addition of specific polyhalogenated biphenyls.

This paper presents the results of anaerobic dechlorination studies performed with historically PCB-contaminated soil and the effect of inoculation with endogenous organisms, carbon source addition, and dechlorination induction by single congener additions.

MATERIALS AND METHODS

Soil Collection

PCB-contaminated soil was obtained from a capacitor bank at a power substation located in Chattanooga, Tennessee. Over time, PCBs have been released through spills in and around the capacitors. The soil had the following characteristics: 26.1% clay, 24.7% sand, 49.2% silt, 0.86% organic matter, and 0.08% total nitrogen and had a pH of 8.1. The soil was combined with sterile water (equal volumes), and the slurry was ball milled antiseptically and anaerobically for 4 hours to ensure homogeneity. The PCB concentration was approximately 100 ppm in the soil, and the congener pattern resembled Aroclor 1248 or weathered Aroclor 1242.

Anaerobic Slurry Reactor Study

The slurry was combined with a mineral medium and a carbon source. Single chlorinated biphenyls were then added as a solution in acetone. Some reactors were inoculated with a medium containing organisms present in Hudson River sediments. Batch incubations were prepared under an anaerobic (nitrogen) environment in 150-mL nominal-volume serum bottles (Wheaton Scientific, Millville, NJ), and 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 bioreactors were (per liter): 200 g soil, 0.5 to 1.1 g carbon source [pyruvic acid (sodium salt) or yeast extract/nutrient broth mixture], 2.3 g acetone (as carrier of halogenated biphenyls), 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_4, 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 final pH of the slurry was 7 to 7.5, and the total slurry volume in each reactor was 35.1 mL. The reactors were incubated in the dark at room temperature.

Sixty-four slurry reactor experiments (each under different conditions) were conducted, and the variables studied are presented below.

Inoculum. Although it is generally believed that indigenous organisms in PCB-contaminated samples are capable of dechlorination, half the bioreactors were inoculated with a consortium of organisms eluted from Hudson River sediments. Naturally occurring organisms present in the Hudson River sediments have been responsible for extensive dechlorination [2]. The inoculum was prepared from equal volumes of dewatered Hudson River sediment and mineral medium. The

slurry was mixed for 1 hour followed by a 15-min settling time. The supernatant was removed and filtered through a cotton filter before use.

Carbon Source Addition. All reactors had acetone in the medium, but some of the reactors also received pyruvic acid or a complex nutrient mixture. The apparent benefits of carbon source amendments in dechlorination studies have been published elsewhere [3,4,5,6].

Single Congener Addition. The addition of single polychlorinated or polybrominated biphenyls has been used to induce dechlorination in river and pond sediments [7,8]. It appears that the addition of a "fresh" PCB congener jump starts the dechlorinating microorganisms and that the other PCB congeners present are dechlorinated simultaneously. Two congeners were used in this study: 2,3,6-trichlorobiphenyl (236-CB) and 2,4,6-trichlorobiphenyl (246-CB). The methodology for selecting these congeners was that one would induce dechlorination at the meta and the other would induce dechlorination at the para position. The stock solutions used in the study contained 10 mg of congener per milliliter acetone.

Sampling and PCB Analysis. One milliliter of slurry was removed at sampling time and was combined with 1 mL of acetone and 4 mL of hexane, shaken for 5 hours, and centrifuged at 1000 rpm for 5 min. One millititer of the organic phase was combined with 0.1 mL internal standard (octachloronaphtalene in hexane), and 2 μL of the mixture was injected into a gas chromatograph (Hewlett-Packard, Avondale, PA) equipped with an electron capture detector and a 30 m x 0.247 mm DB-1 capillary column (J&W Scientific, Folsom, CA). The splitless/split injector temperature was 270°C, and the detector temperature was 300°C. The 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 the oven temperature to 270°C. 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. The PCB congener composition of the Aroclors needed for calibration was supplied by Dr. Abramowicz (General Electric, Schenectady, NY), and similar data have been published elsewhere [9]. Data analysis using HP Chemstation software (Hewlett-Packard) was conducted on 68 congener-containing peaks. The peak identification has previously been published by Brown et al. [10]. The extracted soil was dried at 80°C for 20 hours to determine the dry soil weight of each sample.

PCB Chlorine Content. The average number of chlorine molecules per biphenyl molecule (based on the results from gas chromatogram analysis) was used as a measurement of overall dechlorination. The average initial chlorine content was 4.30 chlorines per biphenyl. A decrease in this number indicates a general dechlorination. In addition to the average chlorine content, the number of meta, ortho, and para chlorines was calculated. Neither of these methods is exact since complete congener separation was impossible in the analysis; however,

the methods are useful to show general trends and allow for comparisons between experiments.

RESULTS AND DISCUSSION

Sampling was done at startup and then after 3, 7, 11, and 15 weeks of incubation. Typical gas chromatograms obtained are shown in Figures 1 and 2. The PCB peak profile at startup is shown in Figure 1. As is noted, the large marked peak corresponds to the single congener (236-CB) added to this reactor; the remaining peaks are of course indicative of the original PCB contamination in the soil. In Figure 2, we see the PCB profile in the same reactor after 3 weeks of incubation. The size of the peak representing 236-CB has decreased, and the peak representing 26-CB has appeared. A closer comparison of the chromatograms in Figures 1 and 2 reveals that not only has the 236-CB been dechlorinated but the indigenous PCBs have undergone change. This was not the case after 3 weeks in experiments conducted without inducers (chromatogram data not shown).

Overall Dechlorination

In order to get a general indication about the dechlorination progress, Figure 3 was generated to show the average chlorine content in the indigenous PCBs. Each set of bars in Figure 3 corresponds to an experimental condition. The labels on the x-axis of the bottom graph also apply to the graph above. Several conclusions may be drawn based on the results presented in Figure 3.

Effect of Inoculum. Indigenous microorganisms (uninoculated reactors) did not show any dechlorinating activity over the 15-week study. The addition of endogenous organisms from Hudson River sediments caused PCB dechlorination in 7 of the 9 inoculated bioreactors.

Effect of Carbon Source. The addition of a carbon source beyond acetone resulted in improved dechlorination. Small improvements were noted using pyruvic acid as the second carbon source (acetone being the first); even better results were obtained from the bioreactors amended with the complex nutrient mixture.

Effect of Single Congener Addition. Dechlorination activity was generally induced faster in reactors amended with 236-CB, and activity was seen in as little as 3 weeks. However the extent of dechlorination was in most cases greater in "uninduced" reactors. It should be noted that direct comparisons between results from reactors amended with single congeners and results from nonamended reactors cannot be made since the addition of a single congener will cause the chlorine content to be overestimated in the calculations.

Dechlorination activity was observed in 7 of the 18 bioreactors, and the best results were obtained in inoculated reactors amended with acetone and pyruvate or the complex nutrient broth.

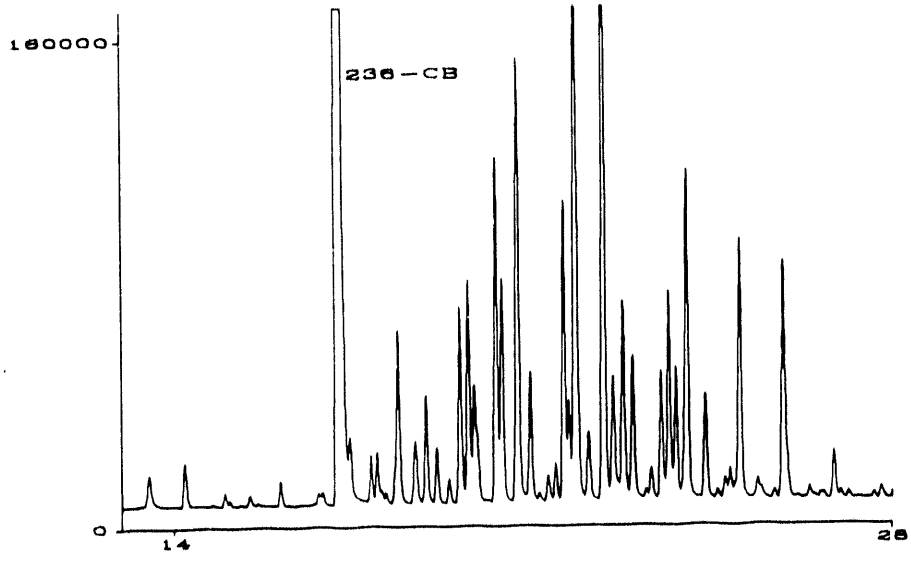

Figure 1. GAS CHROMATOGRAM OF SOIL PCB AT TIME ZERO. NOTE THE LARGE PEAK WHICH CORRESPONDS TO ADDED 236-CB

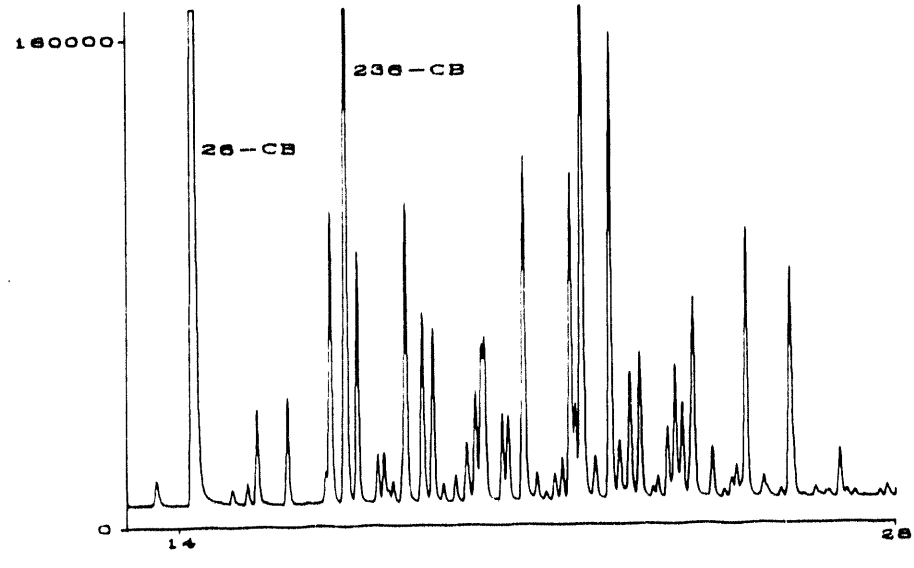

Figure 2. GAS CHROMATOGRAM AFTER 3 WEEKS OF INCUBATION IN REACTOR AMENDED WITH ACETONE/NUTRIENT BROTH AND 236-CB

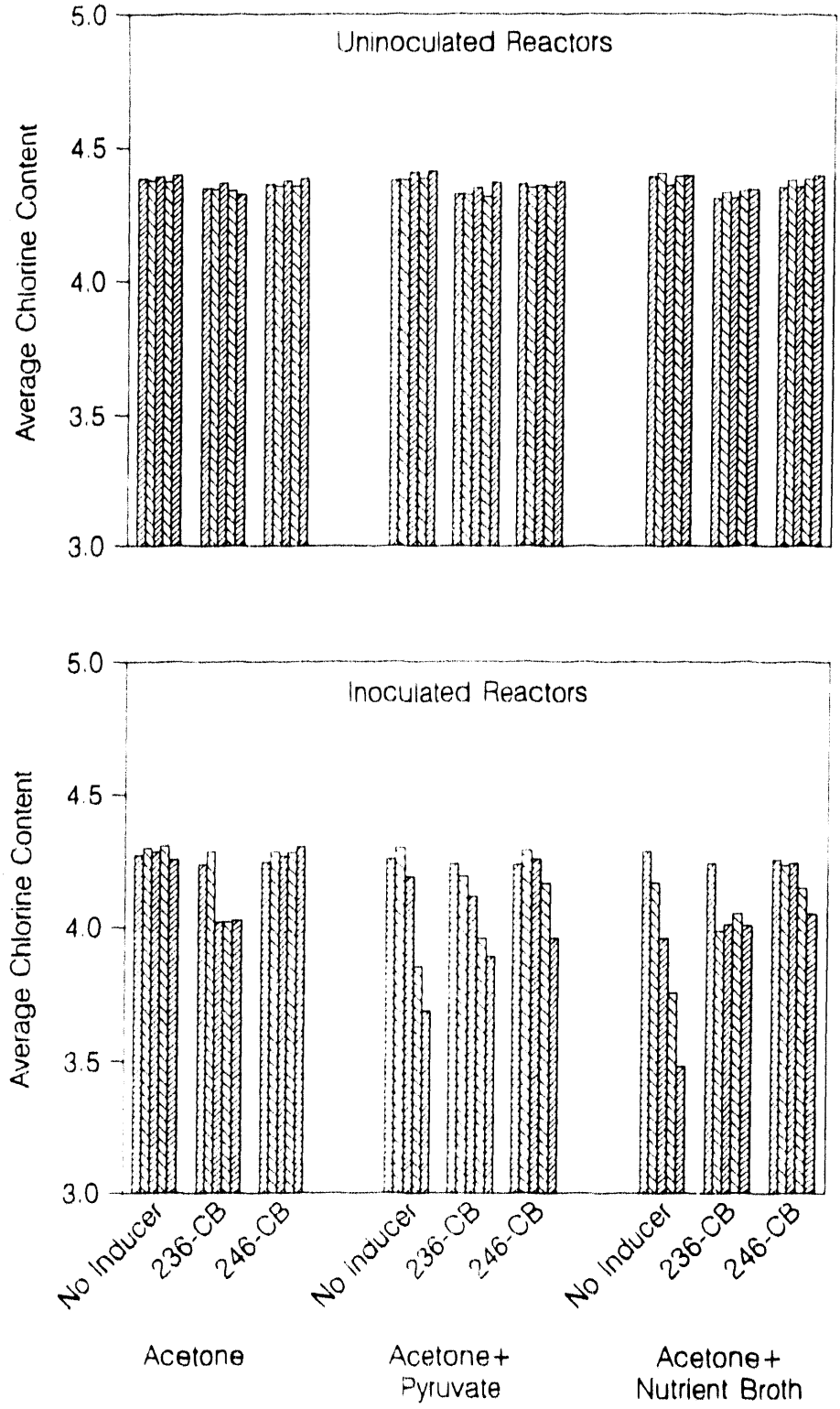

Figure 3. PCB CHLORINE CONTENT: EACH SET OF BARS CORRESPONDS TO SAMPLES TAKEN AT 0, 3, 7, 11, AND 15 WEEKS OF INCUBATION

Influence of the Chlorine Position on Dechlorination

As a complement to estimation of the PCB average chlorine content, estimations were also made of how many meta, para, and ortho chlorines were present in the PCBs. It was especially interesting to see how the various inducers affected the dechlorination pattern. In Figure 4, the number of meta, para, and ortho chlorines has been plotted as a function of the incubation time for inoculated reactors with acetone and pyruvate as the carbon sources. As is noted in the figure, regardless of the type of inducer that was added (236-CB; 246-CB; or none), only removal of meta chlorines was observed. Para dechlorination of the inducer 246-CB was not observed (data not shown). From this, we may conclude that the organisms present in the reactors are meta dechlorinators.

For the best condition studied (using acetone and complex nutrient broth as carbon sources), 50% of the meta chlorines were removed in 15 weeks from the indigenous PCBs in the soil.

Congener-Specific Dechlorination Results

Since the gas chromatograph analysis allowed for a near congener-specific analysis, it provided a good indication of which congeners were attacked. As an example, a histogram is displayed in Figure 5 showing the change in absolute mole percent of the different congeners in the inoculated reactor amended with acetone and the complex nutrient broth as carbon sources. Several peaks with lower peak numbers have increased in size, indicating an increase in the mole percent of the congener(s) present in the peak. In a similar fashion, peaks with higher peak numbers decreased in size. This is consistent with the dechlorination taking place. Peaks with a net change of more than 2 mole percent congener(s) have been listed in Table 1 together with the specific congeners represented by the peak. The congener and peak identification have been published by Brown et al. [10].

Knowing that meta chlorines (positions 3 and 5) are removed, it easy to see the link between congeners increasing in concentration and congeners decreasing in concentration; the increase in 2-2-CB corresponds to the decrease in 25-25-CB, 24-25-CB, and 23-25-CB. Similarly, the increase in 26-CB corresponds to the decrease in 236-3-CB, etc. In some cases it is more difficult to determine since a peak may contain two congeners, one of which is increasing and the other decreasing. As is noted in Table 1, some of the peaks increasing in size may contain congeners with meta chlorines, and it is possible that these chlorines will be removed with time (see Figure 4 for trend).

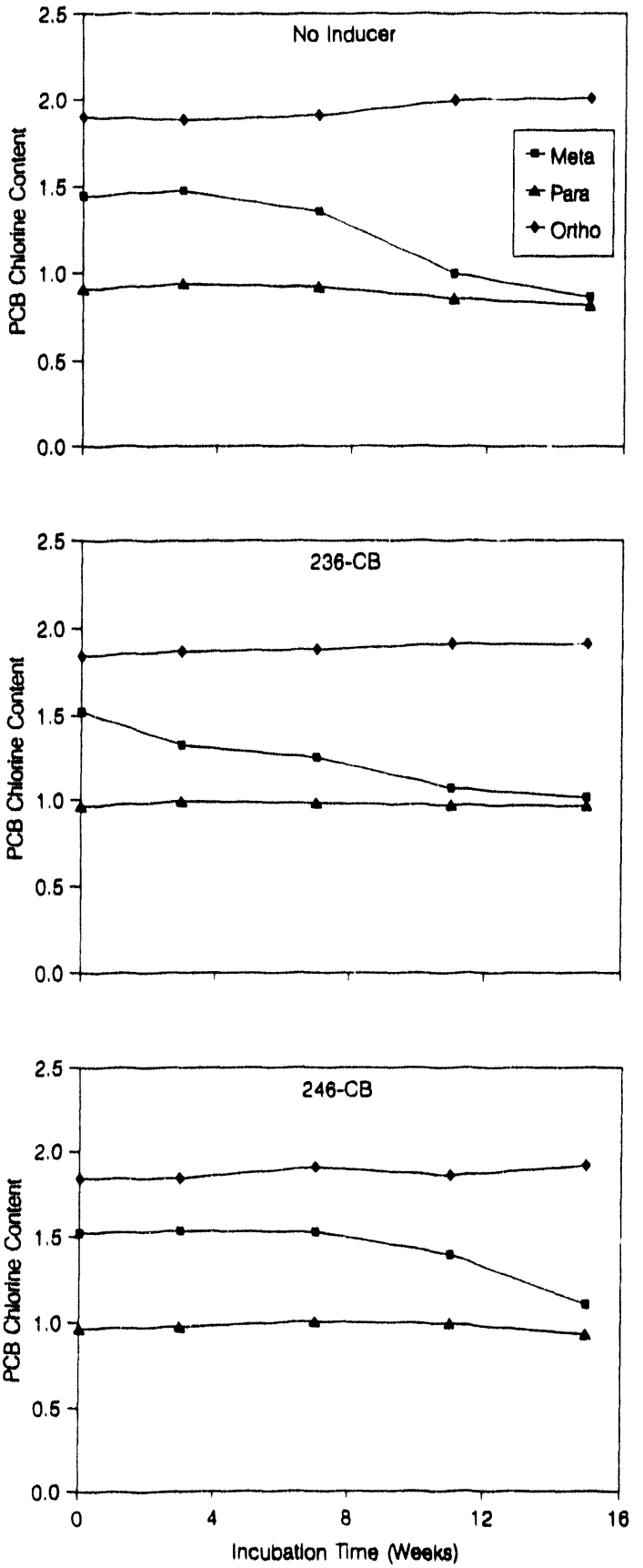

Figure 4. TIME VARIATION OF PCB CHLORINE POSITION IN REACTORS AMENDED WITH ACETONE/PYRUVATE

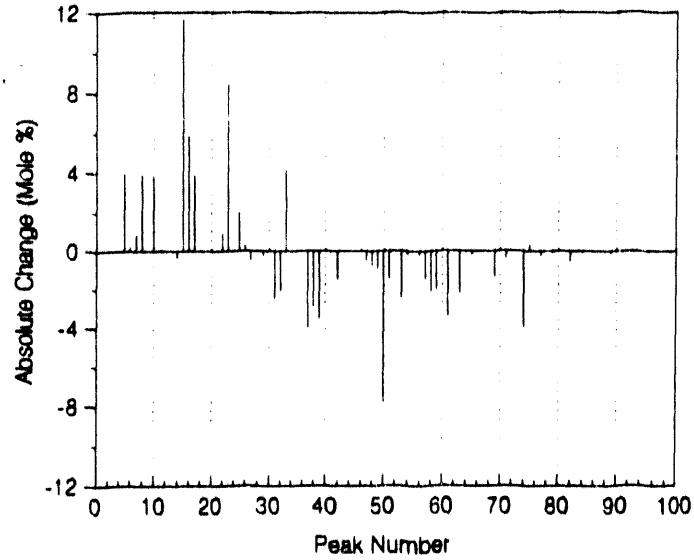

Figure 5. HISTOGRAM OF NET DECHLORINATION OBSERVED IN REACTOR AMENDED WITH ACETONE/NUTRIENT BROTH

Table 1. PCB CONGENERS CHANGING MORE THAN 2 MOLE PERCENT

Decreasing		Increasing	
Peak #	Congener	Peak #	Congener
31	25-25	5	2-2, 26
32	24-25	8	23, 2-4
37	23-25	10	26-2
38	34-4, 23-24, 236-3	15	24-2
39	236-4, 234-2	16	236, 26-3
50	23-34, 234-4	17	23-2, 26-4
53	245-25, 235-24	23	24-4, 25-4
58	234-25, 2346-4	33	24-24
61	34-34, 236-34		
63	234-23		
74	234-34, 234-236		

CONCLUSIONS

PCB-dechlorinating microorganisms were successfully transfered to a sandy soil contaminated with PCBs. The organisms expressed only meta-dechlorinating abilities in the 15-week study. The addition of 236-CB jump started meta dechlorination; however; the addition of 246-CB did not influence para dechlorination. The most extensive dechlorination of indigenous PCBs was observed in reactors without single congener amendments. The addition of carbon substrates (as a supplement to acetone) improved dechlorinating activity.

The data presented in this paper represent the initial results obtained from a long-term study, and experimentation is continuing.

ACKNOWLEDGEMENT

This work was supported by the U.S. Department of Energy's Office of Technology Development. The project is part of a Cooperative Research and Development Agreement between the Oak Ridge National Laboratory and the General Electric Company for collaborative development of bioremediation technologies for PCBs. Oak Ridge National Laboratory is managed by Martin Marietta Energy Systems, Inc., for the U.S. Department of Energy under contract DE-AC05-84OR21400.

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