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Reductive Dechlorination of PCB-Contaminated Sediments in an Anaerobic Bioreactor System

JAMES J. PAGANO,*
RONALD J. SCRUDATO,
RICHARD N. ROBERTS, AND
JEFFREY C. BEMIS

*Environmental Research Center, 319 Piez Hall, State University
of New York at Oswego, Oswego, New York 13126*

An anaerobic bioreactor system was operated in a batch recycle mode to establish the microbial biodegradation of Aroclor 1248-spiked sediment, utilizing sanitary landfill leachate as a novel carbon, nutrient, and/or microbial source. Experiments conducted on two bioreactors confirmed that significant dechlorination of Aroclor 1248-spiked sediments occurred. After 13 weeks of operation, the average total chlorine/biphenyl of the original Aroclor was reduced by 11% and 23%, with the majority of dechlorination occurring within 7 weeks. No dechlorination was observed in the sterilized control reactor. The overall significance is the first reported occurrence of anaerobic dechlorination of a PCB-contaminated sediment in a low-cost laboratory-scale bioreactor system. The environmental significance is the reduction in chlorine content of the original Aroclor, an important component in any environmental bioremediation program. Innovative approaches to laboratory-scale bioreactor monitoring and bioreactor design principles applicable to hazardous waste containment areas are also discussed.

Introduction

Various configurations of anaerobic (methanogenic) bioreactor systems have been widely used for many industrial and sanitary waste streams. The upflow anaerobic sludge bed (UASB) reactor and hybrid offshoots have been used to effectively treat sanitary landfill leachate (1, 2) and several chlorinated compounds (3, 4). The recycling-upflow fixed bed (R-UFB) reactor is a second generation system or hybrid of the original UASB reactor developed and extensively studied by Lettinga (5). The R-UFB bioreactor technology has been employed as an effective treatment of various high-solid wastes (6-8) and chlorinated aromatic compounds (9, 10).

The advantages of the R-UFB bioreactor design include a stable microbial biomass and uniform distribution of nutrients, pH, and temperature due to continuous recycle

(11). Although these systems are not normally considered for the treatment of recalcitrant or hydrophobic compounds, the operational and design principles associated with the reactor systems are consistent with the development of a stable reactor configuration (microbial biomass, nutrient and carbon transfer, and metabolic product removal) when operated in a batch recycle mode.

The properly operated municipal sanitary landfill is a highly active microbial (methanogenic) environment producing large volumes of methane over the course of decades. It has been reported by Rhee (12) that methanogenic conditions, not methanogenic bacteria, are required for PCB dechlorination in sediments. Since most landfills generally contain low levels of halogenated compounds (13, 14), it was hypothesized that the indigenous microflora may be able to degrade xenobiotic compounds, such as PCB. Sanitary landfill leachate is a complex mixture of readily available organic carbon (mainly volatile fatty acids), proteins, amino acids, trace metals, nutrients, and microbes (15). The environmental conditions typical in sanitary landfills and optimized in anaerobic bioreactor systems should be conducive to the development and/or optimization of native microbial populations that degrade PCB.

Since the original report of in-situ dechlorination by Brown et al. (16) and subsequent laboratory confirmation by Quensen et al. (17), the evidence for the anaerobic microbial dechlorination of PCB-contaminated sediments continues to accumulate (18). Numerous studies have demonstrated that the microbial consortia that dechlorinate PCB are readily transferred from anaerobic lake or riverine environments to the laboratory (17, 19-22). Although extensive basic research is still required to further identify, define, and optimize the operative variables, the necessary components for the development and optimization of laboratory-scale or larger bioreactors are presently available. The purpose of this study was to utilize anaerobic bioreactor systems for the dechlorination of PCB-contaminated sediments, employing sanitary landfill leachate as a novel nutrient, carbon, and/or microbial source.

Materials and Methods

Design: Sediment Bioreactors. The anaerobic systems utilized for these experiments are based on the design and operational principles of a R-UFB bioreactor. The 6-L bioreactor systems are designated Superfund (SF) SF 4-6 and are illustrated in Figure 1. Reactors were constructed of glass, Teflon, and stainless steel to minimize adsorption of PCBs. Stock materials were used to minimize cost per reactor. Specially fabricated Teflon parts (reactor end fittings) required for the bioreactors were manufactured. Recirculation pumps were Fluid Metering, Inc. (FMI) pumps (QG-50) constructed of stainless steel, Teflon, and hardened ceramic piston and liner. An in-line filter sampler was developed utilizing a 10 cm (length) × 2.0 cm (diameter) glass chromatography column (Ace Glass Co.) packed with 1.5 g of silanized glass wool. This in-line filter system collects a suspended solids sample that can be easily removed for periodic analysis and also maintains the mechanical integrity of the pumping system by protecting the ceramic piston/liner of the FMI pump from particulates. Several sample ports were also available for direct leachate

* Corresponding author telephone: 315-341-3639; fax: 315-341-5346; e-mail address: Pagano@oswego.oswego.edu.

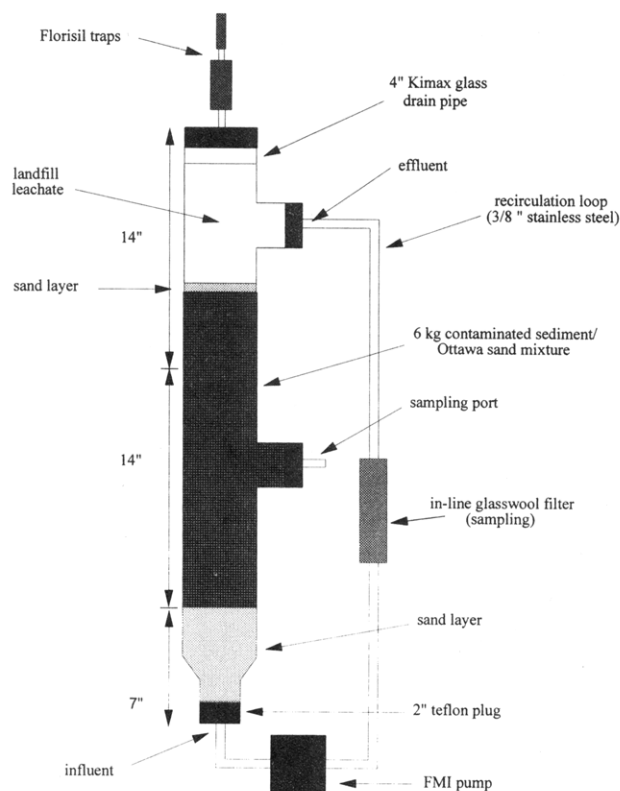


FIGURE 1. Recycling upflow fixed bed bioreactor currently operating at the Environmental Research Center to treat approximately 6 kg of contaminated sediment/Ottawa sand mixture.

sampling. In order to characterize volatilized PCBs and possible daughter products from the reactors, a dual trap system consisting of fully activated Florisil-filled volumetric pipettes (25 + 10 mL) was developed. The second Florisil trap acts as a reserve, if the first trap becomes overloaded with water vapor, and/or as a volatile PCB method blank. Gases produced were collected in Tedlar bags for CH₄/CO₂ determination. The reactors were maintained at 25 ± 1 °C in insulated cabinets equipped with automatic temperature monitoring and control devices (Omega 6100).

Leachate for the experiments was collected from the Nanticoke Sanitary Landfill located near Binghamton, NY. The leachate was moderately acidic, high in total ionic strength, rich in phosphorous and nitrogen, and contained large quantities of volatile fatty acids (8). Leachate sufficient for the experiment was pumped from an underground sump at the Nanticoke Landfill into 19-L dark Nalgene containers continually sparged with zero-grade nitrogen during collection and maintained at 4 °C until used. A baseline analysis of the leachate feedstock material was conducted, including chemical oxygen demand (11 552 mg/L), volatile fatty acids (5099 mg/L), total organic carbon (3686 mg/L), total phosphorus (1.1 mg of P/L), total Kjeldahl nitrogen (310 mg/L) and pH (6.4 units). Sediment utilized in the R-UFB reactors was collected from an embayment of the St. Lawrence River located near the Massena Inactive Hazardous Waste Sites (MIHWS), Massena, NY. Grab sediment samples were collected in precleaned 1 gal paint containers and maintained at 4 °C until used. The sediment consisted of approximately 75% sand and 25% silt/clay with a PCB concentration of 35 mg/kg (dry weight).

Experimental Design. Approximately 6 kg of the sediment/sand mixture was introduced into the bioreactor

systems (SF 4, SF 5, and SF 6) by combining St. Lawrence River sediment with Ottawa sand (Fisher Scientific) necessary to produce a 1:3 weight/weight ratio. This ratio was experimentally determined by percolation testing to provide the required permeability to allow fluid migration. The combined sediment/sand mixture was spiked with technical-grade Aroclor 1248 (Chem Services, Inc.) to 400 mg/kg (dry weight). The sediment and sand mixture acted as the fixed bed media in the bioreactor. Approximately 3 L of sanitary landfill leachate was pumped into the bioreactor system continually sparged with zero-grade nitrogen to maintain anoxic conditions. Stabilization of the microbial populations within the reactors, as indicated by the production of methane, occurred during week 3. Once a stable reactor configuration was achieved, the reactors were placed in a recycle mode and recirculated at 7.2 mL/minute. All control reactor (SF 6) components were sterilized by autoclave, including the sediment/sand mixture and landfill leachate. Routine operational monitoring of the bioreactor systems were conducted monthly and included congener-specific PCB analysis of liquid (leachate), in-line glass wool filters, and gaseous Florisil traps. Direct sediment sampling from SF4 was conducted after 13 weeks to validate in-line filter monitoring results.

Analytical Methods. Congener-specific PCB analyses were conducted using capillary column procedures and standards developed at the Wadsworth Center for Laboratories and Research, New York State Department of Health (23, 24). The calibration standard was a 1:1:1:1 mixture of Aroclors 1221, 1016, 1254, and 1260 from the EPA Pesticide Repository, each at 200 ng/mL, and HCB (5 ng/mL), DDE, and Mirex at 10 ng/mL. Analytical instruments were calibrated every six samples. Congener identification, IUPAC No., and assigned congener number are reported in Table 1. The capillary column utilized was a Hewlett-Packard (HP) Ultra II 25-m DB-5 with 0.22 mm i.d. and 0.33 μm film thickness. The gas chromatographic system used helium as the carrier gas and argon/methane (P5) as the makeup gas. The system was temperature programmed after 2 min at 100 to 160 °C at 10 °C/min and then increased by 3 °C/min to 270 °C and held for 16 min. The injection port and detector were maintained at 330 and 270 °C, respectively. A HP Model 5890 II gas chromatograph with an electron capture detector (Ni⁶³) was used for data acquisition. Quality assurance/quality control was based on a program of replicate analyses, surrogate recoveries (decachlorobiphenyl), matrix spikes/matrix spike duplicates, method, and reagent blanks.

Chromatographic data were collected and processed by use of the HP 3365 Series II ChemStation software and Microsoft Excel 5.0 spreadsheet procedures. The HP software system generated the identity and amount of each PCB congener. Data were further processed such that the mole percent (congener specific), mole percent (homologue), mole percent Cl substitution (homologue), and average Cl/biphenyl (total, homologue, and Cl substitution) were generated. Coeluting congeners were assumed to be in equal proportions for all spreadsheet calculations.

Organic acid analyses were conducted by high-performance liquid chromatography using an ion exclusion chromatographic method developed by McDowell et al. (25). Methane and carbon dioxide analyses were conducted with an isothermal gas chromatograph (Gow Mac 550) with a thermal conductivity detector and Porapak Q column. Total organic carbon (TOC) was measured utilizing a modified ampule-persulfate method (26).

TABLE 1

PCB Congener Identification, IUPAC Numbers, and Assigned Congener Number Utilized for PCB Congener Identification, Quantitation, and Figure Captions

congener	IUPAC No.	assigned no.	congener	IUPAC No.	assigned no.
2 Cl	1	1	34/34 + 236/34	77 + 110	37
4 Cl	3	2	2356/25 + 34/23	151 + 82	38
2/2 + 2/6	4 + 10	3	235/236	135	39
24 + 25	7 + 9	4	2356/24	147	40
2/3	6	5	236/245	149	41
2/4 + 23	8 + 5	6	245/34	118	42
HCB		7	2356/23	134	43
26/2	19	8	235/245	146	44
25/2	18	9	245/245 + 234/236	153 + 132	45
4/4 + 24/2	15 + 17	10	234/34	105	46
236 + 26/3	24 + 27	11	2345/25	141	47
23/2 + 26/4	16 + 32	12	2356/236	179	48
25/3	26	13	234/235	130	49
24/3	25	14	2346/236 + 2356/34	176 + 163	50
25/4	31	15	234/245	138	51
24/4	28	16	2346/34	158	52
34/2	33	17	2345/23	129	53
23/4	22	18	2356/245	187 + 181	54
236/2	45	19	2346/245	183	55
25/25	52	20	234/234 + 245/345	128 + 167	56
24/25	49	21	23456/25	185	57
24/24 + 245/2	47 + 48	22	2345/236	174	58
23/25	44	23	2356/234	177	59
236/2 + 23/24 + 34/4	59 + 42 + 37	24	2346/234 + 2345/34	171 + 156	60
236/4	64	25	2346/2356	201	61
23/23	40	26	2345/235	172	62
235/26 + 245/4	94 + 74	27	2345/245	180	63
25/34	70	28	23456/236	200	64
24/34	66	29	MIREX		65
234/4 + 34/23	60 + 55	30	2345/234 + 23456/34	170 + 190	66
245/25	101	31	2345/2356	199	67
245/24	99	32	23456/245 + 2345/2346	203 + 196	68
245/23	97	33	23456/234	208	69
234/25	87	34	2345/2345	194	70
DDE		35	23456/2345	206	71
236/236	136	36	DCB	209	72

Sample Preparation. In-line filter (glass wool) samples were soaked overnight in 75 mL of concentrated HNO₃ and 25 mL of deionized (DI) water to remove oxidizable organics. After acid treatment, the liquid portion of the in-line filter sample was extracted three times with 20 mL of hexane in a separatory funnel and the hexane fractions stored. The glass wool was extracted sequentially with 20 mL each of acetone, acetone/hexane, and hexane with each aliquot added to a separatory funnel. DI water (100 mL) was added to the separatory funnel, and the hexane fraction was removed and stored. The acetone/water fraction was back-extracted three times with 20 mL of hexane, with the hexane fractions stored. The combined hexane fractions were dried over anhydrous sodium sulfate and concentrated to about 2 mL in a Kuderna–Danish (K–D) apparatus. Elemental sulfur was removed from the condensed extract with tetrabutyl ammonium hydrogen sulfate (27). The extract was further cleaned utilizing a 10-g chromatography column of 4% deactivated Florisil (Sigma, PR grade, 60–100 mesh) (23, 24). Sediment samples of 0.25 g were extracted sequentially by ultrasonication at high power (Fisher Scientific, Model 550) with 50 mL each of acetone, acetone/hexane, and hexane and placed in a separatory funnel. The sample extract was back-extracted and dried, sulfur was removed, and it was cleaned with Florisil as described above. Florisil volatile trap samples were transferred directly into a 10 × 350 mm chromatography column and eluted with 60 mL of hexane. The eluate is concentrated in a K–D

apparatus to 1 mL for gas chromatographic analysis.

Results

Sampling and Bioreactor Control Validation. A series of experiments were conducted to validate the in-line glass wool filter sampling system developed for this research. A PCB congener and homologue mole percent comparison between an in-line filter sample (SF 4) and sediment collected directly from SF 4* (both collected after approximately 13 weeks of operation) are depicted in Figures 2 and 4. SF 4* denotes a sediment directly sampled from SF 4 as a validation of the SF 4 in-line filter sample. A mole percent comparison (congener and homologue) between SF 6, the sterilized control, and the original Aroclor 1248 spike are illustrated in Figures 3 and 4. A significant positive correlation was noted between the PCB congeners (expressed as mole percent) of the in-line filter sample (SF 4) and sediment sampled directly from SF 4* ($r = 0.9968$). A similar correlation was noted in the congener-specific mole percent comparison of the Aroclor 1248 spike and control reactor SF 6 ($r = 0.9798$). The efficacy of the in-line filter system was further confirmed by a very close fit of data between validation samples in homologue mole percent (Figure 4) and average *o*-, *m*-, *p*-, and total Cl/biphenyl (Figures 5 and 6). These results conclusively demonstrate that no significant homologue or Cl substitutional bias resulted from the use of the in-line filter system as a subsampling device. These results also established that SF

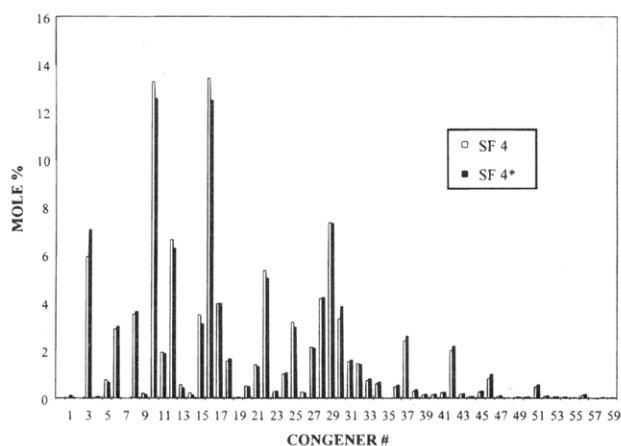


FIGURE 2. Mole percent comparison between SF 4 in-line glass wool filter and sediment directly sampled from SF 4* after approximately 13 weeks of operation.

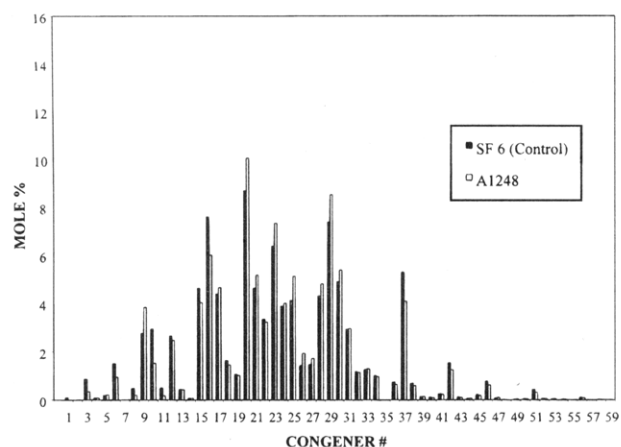


FIGURE 3. Mole percent comparison between SF 6 (control) and the original Aroclor 1248 spike after approximately 13 weeks of recirculation in the R-UFB reactor.

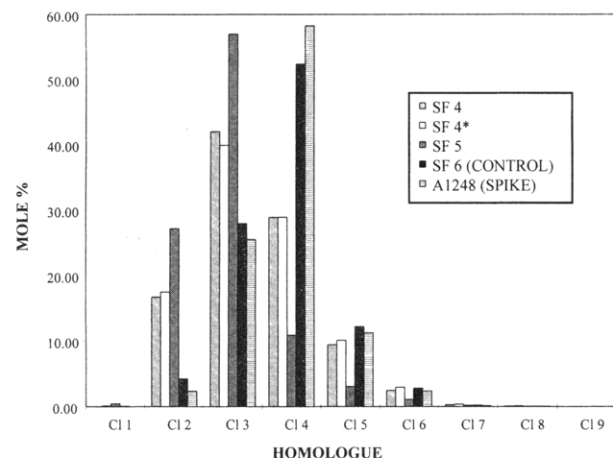


FIGURE 4. Mole percent homolog comparison of SF 4, SF 4*, SF 5, SF 6, and Aroclor 1248. SF 4* indicates sediment directly sampled from bioreactor.

6 adequately represented a "control bioreactor" and further confirmed the effectiveness of the in-line sampling system.

Bioreactor Results. The results after 13 weeks of bioreactor operation indicate that significant microbially induced dechlorination (expressed as the reduction of Cl per biphenyl) occurred in SF 4 and 5, whereas no dechlorination was observed in the control reactor (Figure 5). In general, the extent of dechlorination as measured by the

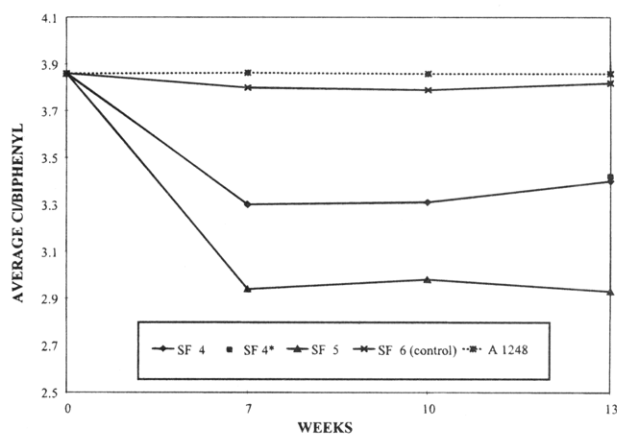


FIGURE 5. Time course comparison of total dechlorination in SF 4, SF 4*, SF 5, SF 6, and Aroclor 1248. SF 4* indicates sediment directly sampled from bioreactor at week 13.

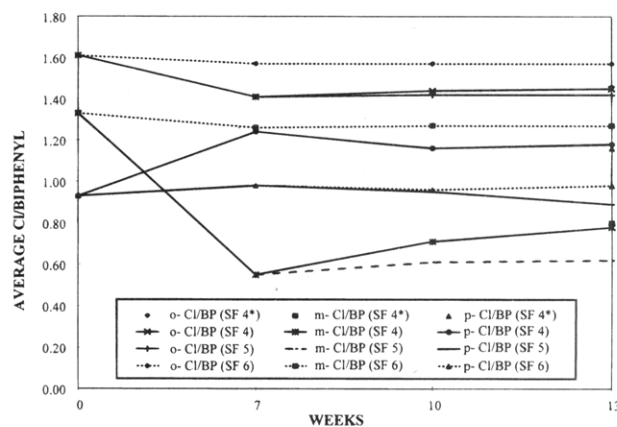


FIGURE 6. Time course comparison of average *o*-, *m*-, and *p*-chlorine per biphenyl in SF4, SF 4* (week 13), SF 5, and SF 6. Values for the original Aroclor 1248 spike are given at week 0.

average total Cl/biphenyl (Cl/BP) was greatest in SF 5 compared to SF 4 (2.93 vs 3.40). The reduction in chlorine content per biphenyl after 13 weeks of operation was 11% for SF 4 and 23% for SF 5 compared with the control reactor (SF 6). The majority of dechlorination apparently occurred between weeks 3 and 7 of the experiment since methanogenic conditions were not established in the bioreactor systems until week 3. Notable shifts were observed in the mole percent homologue chlorination levels between SF 4, SF 4*, and SF 5 as compared to SF 6 and Aroclor 1248 (Figure 4). The data also indicate that SF 5 compared to SF 4 exhibited enhanced dechlorination competence for the higher chlorinated homologues, especially the tetra-, penta-, and hexa-PCB homologues (Figure 4).

No apparent difference was observed in average *o*- and *m*-Cl/BP removal comparing SF 4 and SF 5, although a notable difference was observed in the complete lack of competence (or inhibition) for *p*-Cl/BP removal in SF 4 (Figure 6). As compared to the control reactor, the majority of microbial dechlorination in both SF 4 and SF 5 was associated with *m*-Cl/biphenyl removal, with 39% removal in SF 4 (0.78 vs 1.27) and 51% in SF 5 (0.62 vs 1.27). No or little dechlorination occurred at the *p*-Cl position in SF 4 and SF 5. Both bioreactors demonstrated a limited (10%) ability for *o*-Cl/BP removal as compared to the control reactor. Although ortho-dechlorination was repeatedly observed in both SF 4, SF 4*, and SF 5 (Figure 6) over the course of the experiment (weeks 7, 10, and 13), these data require further laboratory validation. A congener-specific

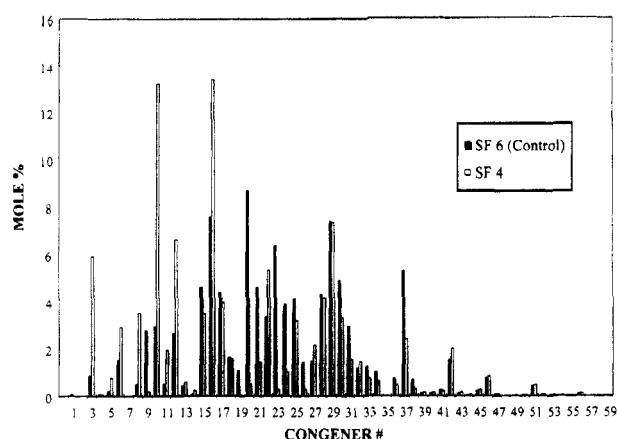


FIGURE 7. Mole percent comparison between SF 4 and the control reactor (SF 6) spike after approximately 13 weeks of recirculation in the R-UFB reactor.

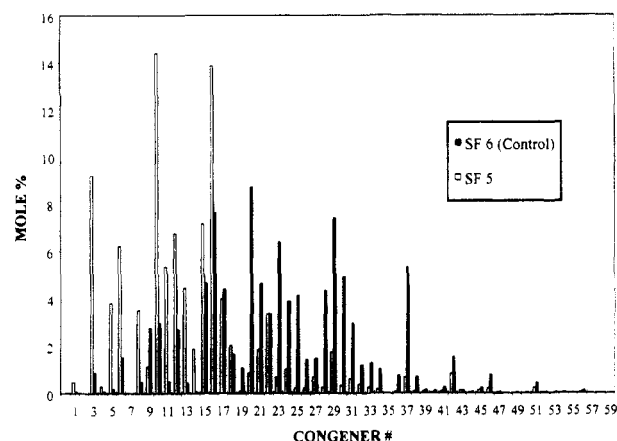


FIGURE 8. Mole percent comparison of PCB congeners between SF 5 and the control reactor (SF 6) after approximately 13 weeks of recirculation in the R-UFB reactor.

comparison of the in-line filter system samples from SF 4 and SF 5 further confirmed the anaerobic dechlorination of the Aroclor 1248-spiked sediment/sand mixture. Comparisons between the control and the experimental reactors after 13 weeks of operation are illustrated in Figures 7 and 8. SF 4 showed substantial mole percent increases (≥ 4.00) in five PCB congeners (2,2'- + 2,6'-; 4,4'- + 2,4,2'-; 2,3,2'- + 2,6,3'-; and 2,4,4'-) with decreases (≥ 4.00) in 2 congeners (2,5,2',5'- and 2,3,2',5'-). Whereas SF 5 showed substantial mole percent increases (≥ 4.00) in seven PCB congeners (2,2'- + 2,6'-; 2,4'- + 2,3'-; 4,4'- + 2,4,2'-; 2,3,6'- + 2,6,3'-; 2,3,2'- + 2,6,3'-; 2,5,3'-; and 2,4,4'-) with decreases (≥ 4.00) in six congeners (2,5,2',5'-; 2,3,2',5'-; 2,3,6,4'-; 2,5,3',4'-; 2,4,3',4'-; and 2,3,4,4'- + 3,4,2',3').

A comparison of Florisil volatile (gaseous) traps collected after 13 weeks of bioreactor operation reflect the current PCB equilibrium within the bioreactor systems. As expected, an increase in lower chlorinated (most volatile) congeners was observed, with the most significant increases reported in congeners 2,2'- + 2,6'-; 2,4'- + 2,3'-; and 4,4'- + 2,4,2'-, corresponding with congeners that increased most significantly in the in-line filter samples for SF 4 and SF 5.

Discussion

Bioreactor Design and Operation. Several technical, material, design, sampling, and analytical problems were encountered and mitigated during the development, con-

struction, and operation of the 6-kg R-UFB sediment bioreactors. The majority of current research being conducted on the anaerobic dechlorination of PCB is micro-scale, utilizing serum bottles as static reaction chambers where the entire chamber is sampled for analysis. Effective subsampling of larger laboratory-scale bioreactors offers unique challenges. One of the most critical problems addressed in this study was the validation of sampling and analytical methodologies that would adequately represent the "current internal status" of a laboratory scale bioreactor; without introducing sampling bias (i.e., small or unrepresentative samples), without biasing the congener-specific integrity of the sample matrix, and without disturbing the established anaerobic and microbial environments. Development and validation of the in-line glass wool filter system allowed an integrated (monthly) and representative sample to be readily collected with minimal disturbance to the bioreactor environments. The basic concept of the in-line filter system is to collect the sediment fines and biological granules (particles) over time. The overall subsampling scheme is based on a general PCB equilibrium setup in the bioreactor system due to continuous recycle, effectively characterizing the entire bioreactor environment.

The bioreactor system is based on design principles that maximize the retention of microbial biomass within the system. The upflow velocity in the R-UFB bioreactor system was chosen to maintain the fixed bed permeability (prevent bed settling) and to avoid microbial washout. A layer of Ottawa sand was utilized on top of the contaminated sediment/sand mixture to maintain a stable reactor environment by preventing washout of microbial biomass. Ottawa sand was also utilized to increase the permeability of the St. Lawrence River sediment in order to ensure optimal moisture, nutrient, and microbial movement and with metabolic waste product removal. The Ottawa sand also served as the fixed bed media in the bioreactor system, providing additional sites (surface area) for microbial colonization.

Design Features: Applications to Containment Areas. Microbial degradation of PCB-contaminated sediment is inhibited or does not occur in hazardous waste disposal sites due to regulated engineering designs, which create dry, sterile environments (18). In related research conducted at the Seneca Meadows Landfill near Waterloo, NY, tightly capped sanitary landfill cells were essentially devoid of biological activity due to the lack of adequate moisture (28). It is becoming increasingly clear that in order to promote active microbial degradation of contaminated sediments, adequate moisture along with the required nutrients must be able to migrate through the contaminated material while maintaining anaerobic conditions.

Several bioreactor operational and design features utilized for this research could be incorporated into the design of containment disposal facilities to promote in-place bioremediation of PCB-contaminated sediments and soils. Additional basic research is required to design and operate a pilot-scale containment system to assess design features such as moisture, nutrient, and microbial movement within containment disposal facilities to optimize in-place bioremediation. The effective design for an in-place containment facility treatment system would incorporate the design and operational features utilized in this study to promote moisture, nutrient, and microbial movement.

Bioreactor Performance: Microbial Considerations. Additional studies are needed to determine whether the microbial consortia responsible for the dechlorination activity in the R-UFB bioreactor systems were due to the native sediment microbial population, the sanitary landfill leachate, or a combination of both. A congener-specific analysis of the St. Lawrence River sediment used for this study revealed a chromatographic pattern resembling in-situ dechlorination (increase in lower chlorinated homologues and congeners) relative to the major contaminant (Aroclor 1248) of the MIHWS area. A recent study by Sokol et al. (22) confirmed the in-situ dechlorination of St. Lawrence River sediment collected near the sediment utilized for this study. Additionally, several congeners found to increase substantially in our bioreactors were similar to dechlorination patterns found with inocula from the St. Lawrence River (22). This suggests that the sediment is the most probable source of the microbial flora responsible for PCB dechlorination in the bioreactor systems.

Although comparisons between bioreactor and micro-scale research are problematic, the overall PCB dechlorination reported in this study is analogous to the 24-week investigation of Aroclor 1248 dechlorination utilizing inocula from the St. Lawrence River (22). As depicted in Figure 5, the majority of dechlorination occurred in the bioreactor systems during the first 7 weeks, followed by a period of reduced activity. Similar results from several micro-scale dechlorination studies have been reported, and theories have been proposed to address this effect (17, 20–22, 30). One major advantage in the use of kilogram-scale bioreactor systems is the ability to manipulate the system or change operational parameters when the dechlorination activity has slowed. Additional manipulations to the bioreactor systems are planned to enhance or reinvigorate the microbial dechlorination. Although many basic questions remain, it is a significant advance for PCB bioremediation research that the proper microbial and environmental conditions for dechlorination activity were established in a kilogram-scale R-UFB bioreactor system.

Conclusions

Reductive dechlorination of PCB-contaminated sediment was demonstrated in the R-UFB bioreactor systems. Sanitary landfill leachate was successfully utilized as a novel source of organic carbon, nutrients, and/or microorganisms. An innovative in-line sampler was developed and validated to measure the current internal status of the bioreactor systems. The environmental significance is an overall reduction in chlorine content of the original Aroclor, an important component in any bioremediation program. The bioreactor design concepts utilized in this research have direct application to the design, operation, maintenance, and monitoring of in-place bioremediation at hazardous waste containment facilities.

Acknowledgments

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