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Utilization of Anaerobic Bioreactor and Sampling Systems for Long-Term Bioremediation Research

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Abstract

An anaerobic bioreactor system was designed, constructed and operated in a batch recycle mode to establish the microbial biodegradation of Aroclor 1248 spiked sediment. Long-term experiments conducted with the bioreactor systems (SF 4-6) confirm that significant dechlorination of Aroclor 1248 spiked sediments occurred. After 124 weeks of operation, the average total chlorine/biphenyl of the original Aroclor was reduced by 25 % for SF 4 and 26 % for SF 5, with the majority of dechlorination occurring within 7 weeks. A series of sanitary landfill leachate and volatile fatty acid amendments during the course of the experiment produced no additional dechlorination activity. Chromatograms from Florisil volatile (gaseous) traps collected from SF 4-6 after 50 weeks of bioreactor operation indicate the production of biphenyl and other currently unidentified metabolic products. The long-term operation of kilogram-scale anaerobic bioreactor and monitoring systems demonstrates the effectiveness of these systems to: provide consistent and representative sampling over extended periods; the ability to easily manipulate operational parameters; and to facilitate the introduction of co-factors and amendments.

Introduction

The utilization of anaerobic processes for the bioremediation of PCB-contaminated sediments is currently confounded by an apparent "dechlorination plateau effect" reported by several investigators [1-10]. A time course characterization of reductive dechlorination of PCB-contaminated sediments would include: an initial lag period; followed by an brief interval of rapid dechlorination; and an extended period of reduced or no additional dechlorination activity (plateau). Although significant progress has been made investigating the complex mechanisms of this limitation, extensive basic research is still required to further identify, define and optimize the variables required for understanding the complex microbial dynamics associated with enhancing the reductive dechlorination of PCB-contaminated sediments.

Various configurations of anaerobic (methanogenic) bioreactor systems have been widely used as an effective treatment for many industrial and sanitary waste streams. The recycling-upflow fixed bed (R-UFB) reactor is a second generation system or hybrid of the original upflow anaerobic sludge bed (UASB) reactor developed and extensively studied by Lettinga [11]. 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 [12]. The experimental bioreactor and sampling systems designed and utilized for this research are based on the design and operational principles of a R-UFB bioreactor.

The purpose of this on-going investigation is to utilize the innovative design and sampling systems of the experimental bioreactor systems for long-term bioremediation research; to identify, define and optimize the reductive dechlorination of PCB-contaminated sediment.

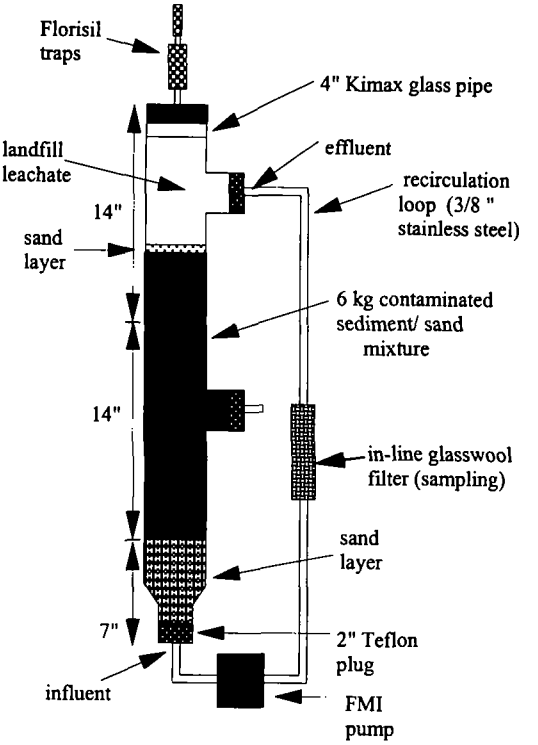

Figure 1. R-UFB bioreactor currently operating at the SUNY Oswego Environmental Research Center.

Experimental Methods

The 6 L bioreactor systems (designated Superfund (SF) - SF 4-6) were constructed by the SUNY Oswego Center for Innovative Technology Transfer (CITT) and are shown in Figure 1 and are described fully by Pagano et al.[9]. Sediment utilized in the R-UFB reactors was collected from Contaminant Cove near the Massena Inactive Hazardous Waste Site (MIHWS), Massena, NY. Introduction of approximately 6 kg of the sediment/sand mixture into the bioreactor systems was accomplished by combining MIHWS sediment with Ottawa sand 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 through the sediment. The combined sediment/sand mixture was spiked with technical grade Aroclor 1248 to bring the concentration of the mixture 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 collected at the Nanticoke Landfill 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 of the experiment. After week 3, the bioreactors were placed in a recycle mode and recirculated at 7.2 mL/minutes resulting in a hydraulic retention time (HRT) of approximately 0.5 days. Analytical and sample preparation methods are fully described by Pagano et al.[9]

Results and Discussion

The results after 124 weeks of bioreactor operation indicate that significant microbial induced dechlorination (expressed as reduction of Cl per biphenyl) occurred in SF 4 and 5 (Figure 2). In general, the extent of dechlorination as measured by the average total Cl/biphenyl (Cl/BP), was nearly the same in SF 5 compared to SF 4 (2.88 vs. 2.91). The reduction in chlorine content per biphenyl after 124 weeks of operation was 25% for SF 4 and 26 % for SF 5, compared with Aroclor 1248. The majority of dechlorination apparently occurred between weeks 3-7 of the experiment, since methanogenic conditions were not established in the bioreactor systems until week 3. Notable shifts were observed in mole % homologue chlorination levels between SF 4, SF 4* (reactor

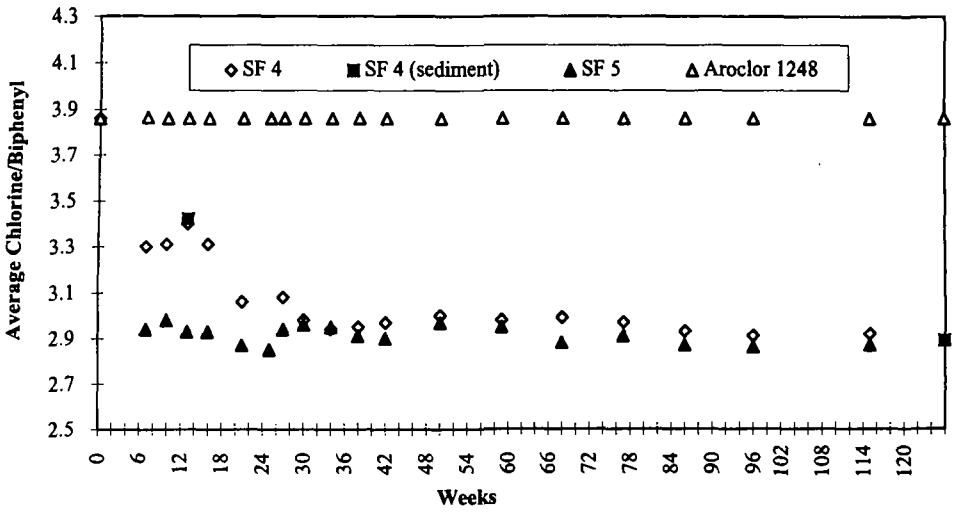

Figure 2. Time course comparison of total dechlorination over 124 week experiment.

sediment) and SF 5 as compared to Aroclor 1248. As compared to Aroclor 1248, the majority of microbial dechlorination in both SF 4 and SF 5 was associated with *meta*- Cl/biphenyl removal, with 55 % removal in SF 4 (0.60 vs. 1.33) and 54 % in SF 5 (0.61 vs.1.33). Approximately 12 % dechlorination occurred at the *para*- Cl position for both reactors, as well as a limited (10 %) ability for *ortho*- Cl/BP removal as compared with the original Aroclor in both SF 4 and 5.

A comparison of chromatograms from Florisil volatile (gaseous) traps collected from SF 4-6 after 50 weeks of bioreactor operation demonstrates the effectiveness and value of collecting the more volatile dechlorination products of the bioreactor system (Figure 3). The chromatograms are a direct reflection of the current PCB equilibrium (degree of dechlorination) within each bioreactor. As expected, the chromatographic patterns reflect the increase in lower chlorinated (most volatile) congeners (2,2'- + 2,6'- ; 2,4'- + 2,3- ; and 4,4'- + 2,4,2'-) found in SF 4 and 5 compared to SF 6 (control reactor). Additionally, several early eluting (non-PCB) peaks are evident in the Florisil trap chromatography from SF 4 and 5, which are not present in SF 6. Utilizing gas chromatography-mass spectroscopy (GC-MS) we have identified biphenyl, and tentatively identified other potential metabolic products. These results require further laboratory validation and development/refinement of sample preparation methods to enhance metabolic product detection limits.

The vast majority of current research being conducted on the anaerobic dechlorination of PCB is micro-scale, utilizing serum bottles as reaction chambers where the entire chamber is sampled for analysis. Subsampling of larger laboratory scale anaerobic bioreactors for PCB offers unique challenges and advantages. One of the most critical problems to be addressed in the development and long-term utilization of the R-UFB sediment reactors was the validation of sampling and analytical methodologies which would adequately represent the "current internal status" of a laboratory scale bioreactor; without introducing sampling bias (ie. small or unrepresentative samples); without biasing the congener-specific integrity of the sample matrix; and without disturbing the established anaerobic and microbial environments. Long-term validation of the in-line glasswool filter system has permitted the collection of an integrated (monthly) and representative samples, with minimal disturbance to the bioreactor environments. The basic concept of the in-line

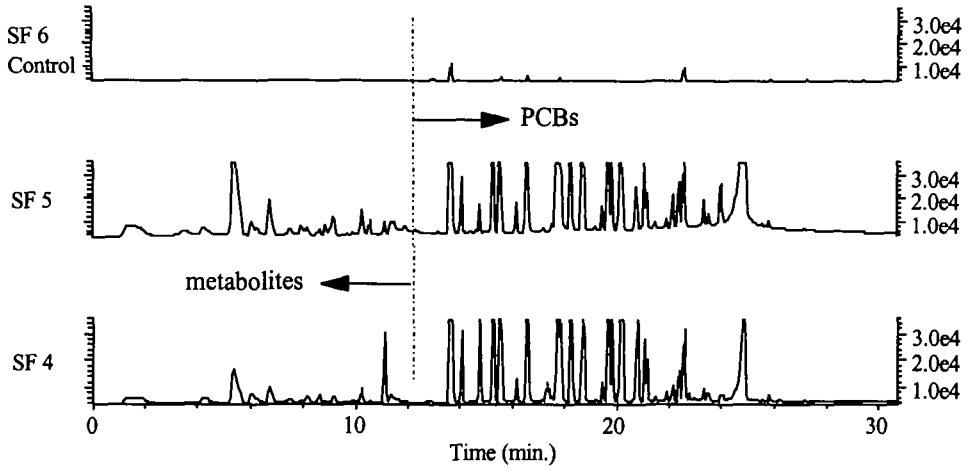

Figure 3. Comparison of chromatograms from Florisil volatile traps collected from SF 4-6 after 50 weeks of bioreactor operation.

filter system is to collect the sediment fines and biological granules (particles) over time on the glasswool surface. The overall sub-sampling scheme is based on a general PCB equilibrium set up in the bioreactor system due to continuous recycle, effectively measuring the entire bioreactor environment. This system insures that a representative sample of the entire bioreactor system is collected. The results from the 124 week experiment conclusively demonstrate that no significant homologue or Cl substitutional bias exists in the utilization of the in-line filter system as a sub-sampling device.

Several bioreactor operational and design features utilized for this research could be incorporated into the design of containment disposal facilities to establish 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 the containment disposal facility to optimize in-place bioremediation of PCB contaminated sediments and soils. The effective design for an in-place containment facility treatment system would incorporate the basic infrastructure to promote moisture, nutrient and microbial movement within the contaminated sediments and soils.

Long-term bioremediation experiments continue utilizing the innovative design concepts and sampling methodologies of the R-UFB bioreactor systems (SF 4 and 5). It is a significant advance for PCB bioremediation that the proper microbial and environmental conditions for dechlorination activity could be established and maintained over a 124 week period within kilogram-scale reactors. The operational and sampling systems of the bioreactors have permitted accurate and reproducible monitoring over a period of 124 weeks; conclusively demonstrating that no significant homologue or Cl substitutional bias exists in the utilization of the in-line filter system as a sub-sampling device. Additionally, data collected from the volatile Florisil traps suggest the production of metabolic products associated with the reductive dechlorination of PCB contaminated sediment. Additional manipulations and amendments to the bioreactor systems are planned to further study the dechlorination plateau effect. Innovative bioreactor design concepts and sampling methodologies utilized in this research have direct application to the design, operation, maintenance and monitoring of in-place bioremediation at hazardous waste containment facilities.

Acknowledgements

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