BIOTRANSFORMATION OF PCB-CONTAMINATED SLUDGE USING CYCLING ANAEROBIC-AEROBIC BIOREACTORS

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ABSTRACT: In this study, we investigated the biotransformation of PCB’s in sludge and sediment from the Ralston Street Lagoon SUPERFUND site in Gary, IN. A biological tilled soil reactor (BTSR), designed and developed for cycling anaerobic-aerobic biotreatment of PCB contaminated solid-phase environmental media, was used to investigate the biotransformation of various congeners and components in the sludge. The bioprocess is a sequential anaerobic-aerobic cycling of micro-environmental conditions in the contaminated sludge loaded into the BTSR, and which has been amended, initially with PCB-dechlorinating anaerobic sediment, and then with aerobic PCB biodegrading aerobic microbes. Anaerobic sediments with demonstrated PCB-dechlorinating activity were tilled into the sludge, and then anaerobic conditions maintained by re-circulation of reduced anaerobic mineral media (RAMM). Aerobic conditions were subsequently introduced and aerobic minimal salt media (MSM) supplemented with cosubstrates and PCB biodegrading microorganisms were re-circulated through the BSTR. Appropriate positive (biotic) and negative (abiotic) controls were maintained to monitor, quantify, discriminate and measure the biological transformation of the PCB’s. All samples were analyzed for PCB congener concentrations using gas chromatography with electron capture detection (ECD). The results obtained demonstrate that, with suitable amendments, biotransformation of hazardous sludge may be a potentially viable option for on-site remediation of the Ralston Street Lagoon.

INTRODUCTION
Polychlorinated Biphenyl’s (PCB’s) are chlorinated aromatic organics extensively used in industry until being banned in 1976 by the United States Congress’s Toxic Substances Control Act (TSCA) thereby limiting the distribution and future usage of PCB products, (Chawla et al. 2000), due to their adverse impact on health and environment. PCB’s used to be manufactured as Aroclors in the USA, Kanechior in Japan, and Sovols in the former Soviet Union, (Tharakan et al., 1999). The Aroclors vast range of utility as key ingredient in various adhesives, transformer dielectric fluid, and machine oils due to its capacity for heat resistance led to its global distribution and subsequent contamination of multiple environmental matrices such as sediments, soils, and inland water-bodies, (Tharakan et al., 1999). Lax PCB disposal practices have lead to almost ubiquitous PCB environmental distribution. Approved treatment technologies, such as incineration, are expensive and can generate harmful byproducts. Biotransformation of contaminants is a potential alternative method.
to develop viable processes for site cleanup. Pellizari et al. (1996) describes *Rhodococcus erythropolis* (NY05) as “one of the microbes tested that exhibits the most extensive PCB degradation”.

**MATERIALS AND METHODS**

**Microbial Isolate.** The microbial isolate, was leftover from the previous Howard University experiment (Tharakan et al., 1999) obtained from Dr. J. Tiedje at Michigan State University. This organism is a gram positive, aerobic cocci isolated from the Hudson River (NY) sediments and is identified as *Rhodococcus erythropolis* (strain NY05). Pellizari et al. (1996) declares that, “it can cometabolically biotransform several different PCBs upon supplementing with biphenyl.”

**Soil Matrix.** PCB Contaminated Sludge was obtained from the SUPERFUND site at the Ralston Street Lagoon in Gary, Indiana. The Gary Sanitary District (GSD) was responsible for the packaging and delivery of the sludge to the University premises. Three five-gallon buckets of sludge were delivered to the Howard University Biochemical Engineering Lab (HUBEL) containing 3 different levels of PCB contamination. The samples were from different locations within the lagoon, Northwest, Midwest, and Southwest with approximate concentrations of 1000ppm, 780ppm, and 220ppm respectively, as reported to us by GSD. The Midwest and Southwest samples were chosen for our experiments.

**Reactor set-up.** The reactors are made from ‘Nalgene’ polycarbonate desiccators (Model 5311-0250) and modified so that the fluid contents can be pumped in from the top and out from the bottom of the vessel. The tube in the bottom of the reactor is covered with fiberglass to prevent clogging when the drainage pump is in operation (Figure 1). A total volume of 4.5 L is used as the reaction volume. The reaction bed is prepared by layering the bottom with 1.5 L of gravel for drainage purposes; directly on top of the gravel 3.0 L of ‘soil’ is loaded and packed in. Prior to loading, the sludge is completely air dried in the laboratory hood and ground up so that undesired debris can be removed such as cigarette butts, band-aids, sticks, and rocks. After the debris was removed, 1.5 L of dried and powdered sludge is mixed with 1.5 L of sand to enable fluid flow and percolation through the reaction bed. The reactors are then placed in a glove box and amended with 200 ml of anaerobic sediments while under the positive pressure of nitrogen gas; afterwards RAMM was pumped into the reactor saturating the bed and completely covering it creating an inch of fluid layer above the bed. The glove box and reactors remained in the presence of nitrogen for four months allowing the possibilities of anaerobic metabolic processes. At the completion of the anaerobic phase the RAMM was drained from the reactors and they were allowed to sit for a month to see if there would be any change naturally prior to inoculation with NY05 and nutritional support with MSM. MSM was then provided to the reactor for a month prior to inoculation with NY05 to observe whether or not any indigenous aerobes may work, after which, the inoculation was conducted and fresh MSM re-circulated (Figure 2).
**FIGURE 1. Biological Tilled Soil Reactor (BTSR) Design.**

**FIGURE 2. Experimental Protocol for Biological Tilled Soil Reactor**

**Growth Media.** *Anaerobic:* During the anaerobic phase Reduced Anaerobic Mineral Medium (RAMM) was circulated through the reactors in a semi-batch manner. The medium preparation is described in Quensen et al (1996) which includes the dye Resazurin serving as an oxygen indicator.

*Aerobic:* During the aerobic phase the Minimum Salt Medium (MSM) ‘K1 medium’ described in Reis (1997) was pumped and re-circulated through the
reactor bed to provide the inoculated NY05 with the required nutrition. The K1 was amended with 300ppm biphenyl to serve as a cosubstrate for PCB biotransformation by this particular the microbe.

**Anaerobic Maintenance.** On a daily basis the glove box was purged with nitrogen gas and monitored with FYRITE® Gas Analyzer CO₂ and O₂ Indicators; the reactor headspaces were also monitored and purged with nitrogen gas if traces of oxygen were present.

**Aerobic Maintenance.** While operating in the aerobic phase the MSM is changed once a week for Biochemical Oxygen Demand (BOD) and microbial waste considerations.

**PCB Measurement.** The reactor samples and their respective controls were placed in a laboratory fume hood to air dry. After drying, samples were extracted with methylene chloride in a soxhlet extraction apparatus for 18 hrs with a reflux rate of 3-4 cycles per hour. The extract is then concentrated down to approximately 2 ml and the solvent is exchanged to hexane ([6], GSD QAPP) and brought to a final volume of 10 ml. The 10 ml sample is washed with the equivalent volume of concentrated sulfuric acid until the sample becomes a perfectly transparent liquid. The washed sample is now ready for injection into the Gas Chromatograph-Electron Capture Detector (ECD). The column and program used is a HP 5890 Series II GC utilizing a 0.32 mm i.d., 30 m fused silica column with a 0.5 \( \mu \)m film ECD. Tetrachloro-m-xylene was the internal standard spiked into the samples prior to extractions. This standard was able to also prove that the soxhlet extraction process had an efficiency of almost 100%.

**RESULTS AND DISCUSSION**
Figure 3 shows the chromatogram of the Midwest Sludge Reactor from the initial through the end of a four month period. As time goes during the anaerobic phase the higher chlorinated congeners decrease in quantity while the lower chlorinated compounds such as monochlorinated, dichlorinated, and trichlorinated biphenyls increase in quantity. This is the result of chlorine atom liberation by anaerobic microbial action. However, in the initial stages of dechlorination the reactor concentration itself does not decrease since the change that occurs is merely movement of chlorine atoms. At times the concentration increases but with a less toxic form of PCB. Figure 4 shows the occurrence of increased concentration after one month due to the generation of many lower chlorinated congeners and subsequent reduction in concentration from that point onward. The negative control took two months to achieve similar biotransformation, as illustrated in Figure 5. The negative control is prepared and kept in the same environment as the reactor but RAMM recirculation does not occur within the vessel. One may be able to conclude that the lack of availability of fresh nutrients stymied the progress of the anaerobes although they were exposed to an ample supply of organics.
The experiment is currently in the aerobic phase with the inoculated NY05. It remains to be seen how well this microbe will survive and work in this type of environment since it is not indigenous to the Ralston Street Lagoon.

FIGURE 3. Chromatograms showing congener transformations and reduction in the Midwest Reactor.
FIGURE 4. Mid-West Sludge Reactor

FIGURE 5. Negative Control for Mid-West Sludge Reactor

Conclusion
As expected the anaerobic phase of the cyclic anaerobic/aerobic experiment shows evidence of biotransformation credited to Hudson sediment anaerobes.
The results obtained demonstrate that, with suitable amendments, anaerobic
dechlorination biotransformation of hazardous sludge may be a potentially viable
option for on-site remediation of the Ralston Street Lagoon. This is only the
beginning, since the aerobic phase of the experiment has yet to be analyzed. Prior
results suggest that the NY05 microbe will biotransform the lower chlorinated
PCBs seen in the Figure 3 chromatograms.

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