APPLICATION OF MICROBES AND EARTHWORMS FOR THE BIOLOGICAL REMEDIATION OF POLYCHLORINATED BIPHENYL CONTAMINATED SLUDGE

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ABSTRACT
Biological transformation of polychlorinated biphenyls (PCBs) is attractive since there is potential for complete biotransformation of contaminants without generation of toxic byproducts. In this paper, we report on the effectiveness two biological technologies: (1) the application of anaerobic and aerobic microbes in a cycling anaerobic-aerobic biological tilled-soil reactor (BTSR), and (2) the application of earthworms in vermicomposting bioreactors (VB). PCB contaminated sludge from the Ralston Street Lagoon, Gary, IN, USA, was the site media utilized. The first approach used the BTSR operated under cycling anaerobic-aerobic conditions with the sludge initially amended with anaerobic sediments followed by inoculation of aerobic microbes accompanied by switching to aerobic BTSR operation. The second approach utilized Eisenia fetida earthworms inoculated into VBs filled with varying mass fractions of contaminated sludge mixed with sterile potting soil. Natural PCB attenuation was investigated through the establishment of appropriate controls. Both BTSRs and VBs were monitored for the duration of the studies (ranging from six to twelve months) and samples were regularly removed, extracted and analyzed for PCB congener content by gas chromatography (GC) with electron capture detection (ECD). The results of these investigations suggest that both technologies have potential for application in PCB-contaminated site remediation and clean-up.

INTRODUCTION
Polychlorinated Biphenyl's (PCB's) are chlorinated aromatic organics which have been used extensively in diverse and widespread industrial applications including as key ingredients in adhesives, transformer dielectric fluids, and machine oils. PCB's were manufactured as Aroclors (USA), Kanecchlor (Japan) and Sovols (Soviet Union/Russia) (Tharakan et al., 1999). Their production was banned in 1976 by the United States Congress's Toxic Substances Control Act (TSCA) due to their adverse impact on health and the environment. Although this limited distribution and current usage of PCB products, past usage has resulted in global distribution of PCBs in various environmental media, as well as residual levels in fish and many marine and land mammals including humans (Chawla et al. 2000). Lax PCB disposal practices have contributed to an almost ubiquitous PCB environmental distribution and resultant environmental problem.

Biological Remediation: Effective and approved treatment technologies, such as incineration, are expensive and can generate harmful byproducts. Biologically mediated transformation of these PCB contaminants has potential as an alternative method for site cleanup. Pellizari et al. (1996) describes Rhodococcus erythropolis, strain NY05 as a microbes which demonstrated extensive aerobic PCB congener biodegradation, especially of lower chlorinated compounds.

Anaerobic-Aerobic Processes: These process configurations have demonstrated potential in the treatment of many chlorinated polyaromatics. Fatepure and Tiedje (1999) demonstrated that reductive and hydrolytic dehalogenation contribute to the degradation of chloroaromatics. Heterocyclic compounds may undergo anaerobic hydrolysis and haloaromatics usually undergo reductive dehalogenation under anaerobic conditions. Lower chlorinated PCB congeners have been cometabolically biodegraded (Hickey, 1999) using cosubstrates such as biphenyl, naphthalene and terpenes (Tharakan et al, 1999). The combination of anaerobic and aerobic conditions in sequence has potential for treatment of PCB contaminated media, has shown promise in lab-scale experiments, and is being investigated for possible field application.

Earthworms: Earthworms are key organisms in the breakdown of organic matter. Their widespread distribution and importance in soil systems makes them useful organisms for assessing contamination in soil environments. Several investigators have demonstrated that earthworms can take-up and bioaccumulate PCB congeners (Singer et al, 2001; Tharakan and Satterwaite, 2002). Additionally, earthworms can
be used to biomonitor and measure levels of contamination in solid residues of heavy metals and long-lived chlorinated organic compounds. It is also known that residues of various chlorinated organics can bioaccumulate and distribute to tissues of animals at higher trophic levels (Edwards, 1973). Recently, Tharakan et al (2004, 2005) have shown that PCB congeners in sludge from a TSCA contaminated hazardous site were biotransformed to some extent in vermicompost systems. The investigation and results presented here are continuing extensions of that study of the biologically mediated reduction of PCBs in PCB contaminated site sludge.

MATERIALS AND METHODS

Microbial Isolate: The microbial isolate was used in previous studies in our lab (Tharakan et al., 1999) and was obtained from Dr. J. Tiedje at Michigan State University (East Lansing, MI, USA). It is a gram positive aerobic cocci, isolated from Hudson River (NY) sediments and identified as Rhodococcus erythropolis (strain NY05), with a demonstrated (Pellizari et al.,1996) cometabolic biotransformative capability for several different PCBs. Microbial isolates were cultured in K1 minimal salt media (MSM) with biphenyl as a co-substrate; media composition and culture condition details have been provided elsewhere (Tharakan et al, 1999).

Anaerobic Sediments: Anaerobic sediments used to bioaugment the BTSRs were a gift from the Center for Microbial Ecology at Michigan State University, and were originally obtained from the Hudson River, NY. Anaerobic sediments were maintained under reduced anaerobic mineral media (RAMM).

PCB Contaminated Sludge: Sludge was obtained from the TSCA hazardous waste site at the Ralston Street Lagoon (RSL), Gary, IN. Three five-gallon buckets of sludge were received in our laboratory containing different levels of PCBs. These included samples from three locations: Northwest, Midwest, and Southwest, with approximate concentrations of 970 ppm, 780ppm, and 280ppm PCB, respectively, measured as Aroclor 1248. Midwest and Southwest sludge samples were used for the anaerobic-aerobic cycling bioreactor studies. The moisture content of these samples were 75 and 87%, respectively.

Reactor set-up: The BTSRs were constructed of ‘Nalgene’ polycarbonate desiccators (Model 5311-0250) modified for fluid circulation through the vessel (Figure 1). Total reaction volume was 4.5 L. The reaction bed was prepared by layering 1.5 L of gravel (for drainage purposes) followed by 3.0 L of the prepared contaminated sludge-soil mixture. Sludge was air dried in the hood, crushed and cleaned of debris (cigarette butts, band-aids, sticks, and rocks). The dried sludge was then crushed and 1.5 L was mixed with 1.5 L of sand, and then layered over gravel. Reactors were operated in an anaerobic glove box. At initiation, the sludge-sand mixture was amended with 200-ml anaerobic sediment under anaerobic conditions in a glove box. RAMM was circulated through the BTSR and the reaction bed was submerged under 1-inch of RAMM to ensure anaerobicity. Anaerobic conditions were maintained for four months. RAMM was then drained from the BTSR, which was then allowed to reach ambient aerobic condition over 1-month. BTSRs were then inoculated with NY05 and MSM was circulated. The BTSR was monitored for a month prior to NY05 inoculation to ascertain any indigenous biological activity. Figure 2 shows the schematic of the experimental protocol.
Earthworms: *E. foetida* earthworms reared in our laboratory was used in all vermicompost experiments. This species is commonly used for degrading organic wastes. *E. foetida* tends to be ubiquitous and many organic wastes are naturally colonized by this species (Edwards, 1988). It has a wide range of temperature and moisture level tolerance. In mixed cultures, this species usually dominates: field systems which begin with another species end up with large proportions of *E. foetida*.

**VB Experimental Setup:** The sludge from the Midwest sample was used in the VBs. Two sets of VB’s were established, each containing different mass fractions of contaminated sludge mixed with earthworm bedding, which enabled investigation of the effect of different initial PCB levels. Five starting PCB levels were used: 0%, 10%, 25%, 50%, 75% sludge mass fraction. VBs were designed with a bottom gravel drainage layer overlaid with a mesh screen. The sludge-earthworm bedding mixture was overlaid on this drainage layer. All VBs were covered with mesh so earthworms would not escape. One set of VBs was inoculated with 9 gms of live earthworms while the second set was maintained as a worm-free control. VBs were foil-wrapped to shield earthworms from light, permitting earthworms to work near the VB reactor wall.

**VB Experimental Protocol:** VB’s were periodically sprayed with water to maintain soil moisture content above 50%. Each VB was “fed” 3 gms of cornmeal every week. On a twice monthly basis, VBs were emptied and earthworms were separated from the sludge, counted and weighed to track biomass. The sludge-soil mixture was mixed and sampled for extraction and analysis by gas chromatography (GC).

**PCB Measurement:** All reactor samples (BTSR and VB) were placed in a laboratory fume hood to air dry. After drying, samples were extracted with acetonitrile. One gram of the dried sample was extracted in 15ml acetonitrile. The solvent was then filtered through a 0.4-um pore nylon filter. 1-ml of this filtered sample was injected into the GC and run using an ECD. The instrument used was a HP 5890 Series II GC utilizing a 0.32 mm internal diameter, 30 m fused silica column with a 0.5 µm film. Calibration curves with Aroclor 1248 were run prior to each set of samples.

**Vermicompost Experiments:** For the VBs, the experiments were terminated at varying points for the different sludge fraction levels. Total earthworm biomass in different various VB’s was measured. At the termination of the experiment, the complete sludge-bedding matrix for each VB was dried and extracted to provide information that would enable computation of the PCB mass balance to track fate and distribution. Total earthworm biomass was crushed, dried and Soxhlet extracted for 18 hours. Extracts were analyzed using GC with ECD.

**RESULTS AND DISCUSSION**

Figure 3 and 4 shows the average of the total PCB congener level in the two BTSRs through the course of the investigation, for the Midwest and Southwest sludge loaded reactors, respectively. The data covers both anaerobic and aerobic phases of the study.

![Figure 3: PCB levels as measured as total Aroclor 1248 in Midwest Sludge loaded BTSR.](image)

As seen in Figure 3, total PCB congener level is reduced by 70% within the first six weeks in the Midwest-sludge loaded BTSR. Subsequent to that, the overall reduction levels off so that there is only a small further decrease in total PCB level. At the lower PCB loading, in the Southwest-sludge loaded BTSR (Figure 4), initial PCB level reduction is 20% within first six weeks.

![Figure 4: PCB levels measured as total Aroclor 1248 in Southwest sludge loaded BTSR.](image)
The initial total PCB level in the Midwest sludge BTSR was high at close to 500ppm, while the initial PCB level in the Southwest sludge BTSR was only about 140ppm. The decreased reduction of total PCB level in the reactor with the lower initial PCB load could be reflective of decreases in PCB biotransformation kinetics at lower concentrations, suggesting first order kinetics for the biotransformation rates.

Samples were withdrawn from the BTSR on a monthly basis from four separate locations. There was a wide variation in PCB reductions demonstrated at the different sampling locations, underscoring the heterogeneity in the reaction bed within the BTSRs. The data in Figures 3 and 4 show the average total reduction across the four sampling locations and hence reflect potential total decreases that might be expected. The heterogeneities may also be reflective of local PCB migration and underscores the importance of sludge preparation prior to loading of the BTSRs.

**Reactor Controls:** Controls were maintained throughout the period of BTSR operation, including controls under biotic and abiotic conditions. PCB reductions in the controls demonstrated that there is substantial reduction under anaerobic conditions, especially when amendments of anaerobic sediments are incorporated into the control. The rates and extents of reduction did not always reflect expected outcomes. In one control, raw PCB contaminated sludge (neither dried nor crushed) was placed under RAMM with additional bioaugmentation of Hudson River anaerobic sediments, and the control vessel was placed in the anaerobic glove box. There was no perfusion of media into the sludge matrix and the control was maintained in a static condition. Samples were removed and analyzed by GC-ECD, demonstrating an initial reduction of PCB level from above 500 ppm to around 100ppm of PCB measured as Aroclor 1248. This is a reduction of approximately 80%, facilitated by the anaerobic sediments tilled into the control’s sludge surface, and suggests that anaerobic reduction of PCBs may be possible without additional RAMM circulation.

The results obtained in the controls also suggest that intrinsic anaerobic biotransformation may be a significant component of the overall reduction of PCB congeners. Potential advantages that might accrue from this are the possibility for elimination of bioaugmentation and mechanical manipulation of contaminated sludge with concomitant intrinsic biotransformation of PCB congeners. Field or site treatment may be possible by maintenance of anaerobic conditions.

**Vermicompost Bioreactors:** Figures 5 and 6 show the PCB level in the VBs loaded at a 10% and 50% sludge mass fraction, respectively, through the course of the vermicompost process. The reduction of total PCB levels demonstrates that these earthworms were able to remove the PCBs from the sludge-bedding matrix. This was also reflected in the increase in PCB concentration within the earthworm, indicating that bioaccumulation of PCBs was occurring. Bioaccumulation was measured by extraction and analysis of the final total earthworm biomass in each VB.
To monitor and discriminate between PCB reductions due to earthworm activity and that which might be occurring due to abiotic as well as other biotic mechanisms, reactors with identical sludge mass-fractions but without live earthworms were also established. Figures 7 and 8 show the total PCB level in these reactors through the course of the investigation. The data clearly demonstrates that there is PCB reduction taking place, even without worms, due to several other possible mechanisms, including volatilizations, irreversible adsorption, microbially mediated biological transformation, and photodegradation.

In addition to the reduction in PCB levels in the VBs and the controls without earthworms, the viability of the earthworms were monitored by tracking earthworm population and total earthworm biomass. The summary of this data is shown in Table 1. The data demonstrate that the higher the level of PCB, as would be the case with the VB’s with larger sludge mass fractions, the increase in earthworm biomass decreased. Apparently, earthworm reproduction and viability, as earthworms are exposed to increasing levels of PCBs, decreases. In the sludge free control with no PCBs in the VB, there was a 50% increase in earthworm biomass, while in the VB with a 75% mass fraction of sludge, the total earthworm biomass actually decreased by 54%. This suggests that PCBs provide some level of growth inhibition and contribute to reductions in viability and health of earthworms.

<table>
<thead>
<tr>
<th>Sludge Mass Fraction (%)</th>
<th>Initial worm mass (g)</th>
<th>Final worm mass (g)</th>
<th>Net gain or loss in biomass (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>0</td>
<td>9.0</td>
<td>13.2</td>
<td>47</td>
</tr>
<tr>
<td>10</td>
<td>9.0</td>
<td>12.2</td>
<td>35</td>
</tr>
<tr>
<td>25</td>
<td>9.1</td>
<td>5.6</td>
<td>-39</td>
</tr>
<tr>
<td>50</td>
<td>9.1</td>
<td>7.2</td>
<td>-21</td>
</tr>
<tr>
<td>75</td>
<td>8.9</td>
<td>4.1</td>
<td>-54</td>
</tr>
</tbody>
</table>

The level of PCB in the total earthworm biomass at the termination of the investigation was also measured. The entire earthworm biomass in each of the VBs was removed, dried and extracted, and analyzed using GC-ECD. The results of the GC-ECD analysis revealed the data shown in Table 2.

<table>
<thead>
<tr>
<th>Sludge Mass Fraction (%)</th>
<th>PCB Concentration in Earthworm Biomass (Ppm)</th>
</tr>
</thead>
<tbody>
<tr>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>10</td>
<td>148 ± 18</td>
</tr>
<tr>
<td>25</td>
<td>213 ± 17</td>
</tr>
<tr>
<td>50</td>
<td>189 ± 9</td>
</tr>
<tr>
<td>75</td>
<td>313 ± 9</td>
</tr>
</tbody>
</table>

The results showed that the PCB level in the earthworms in the different VBs ranged from 148ppm (in the 10% sludge mass fraction VB) to
313 ppm (75% sludge mass fraction VB). PCB bioaccumulation in the 25 and 50% sludge mass fraction VBs were similar, at around 200ppm.

The transport of the PCBs from the sludge to the earthworms occurs through two mechanisms: (1) direct absorption from the sludge-soil matrix through the earthworm body wall into the earthworm biomass, and (2) absorption of the PCBs as the sludge-soil matrix is processed through the earthworm’s intestine. In the earthworm gut, there is physical mastication of the sludge-soil matrix upon ingestion by the earthworm, and there is chemically and biochemically mediated transformations of the PCBs. This might include transformation from microbes that inhabit the earthworm gut (Edwards, 1988) or through enzymatic reactions or attack by gut secretions of the earthworm. In total, PCBs absorbed into the earthworm biomass are either simply bioaccumulated – as reflected in the data from Table 2 – or biotransformed.

In order to differentiate on biotransformation, bioaccumulation and other mechanisms for PCB transport in the VB systems, a rigorous mass balance is required. This would required complete GC-ECD analysis of all outgoing streams from the VB, and would have required gas phase sampling and analysis. The complete fate, transport and distribution mass balance analysis of PCBs through the course of the investigation requires attention to all possible routes of PCB transport as shown in Figure 9, which shows possible routes of transport in the VBs and identifies which compartments PCBs might eventually land up in.

![Figure 9: Possible routes of PCB transport and eventual fate.](image-url)

This compartmentalized model assumes PCBs may be subject to various mechanisms of transport out of the vermicompost reaction bed, including biotransformation by microbes or earthworms, bioaccumulation in earthworms, irreversible adsorption onto sludge-soil matrix and reactor walls, volatilization, photodegradation, and removal of PCB from the reaction bed through the monthly sampling protocol. As indicated, at the termination of the experiment, the earthworms were separated from the VB soil-sludge matrix and total earthworm biomass from each VB was dried, extracted, and analyzed (Table 2). The entire VB soil-sludge matrix was also extracted, along with extensive washing and extraction of VB reactor walls. All extracts and reactor wall washings were combined and concentrated prior to sample GC-ECD analysis. In this mass balance analysis, it is assumed that PCB volatilization is minimal (due to the extremely low vapor pressures of the various PCB congeners), photodegradation of PCBs is negligible (due to the shielding of VBs from all light), and irreversible adsorption of PCBs is negligible, because of the 18-hour, highly efficient Soxhlet extraction protocol used on the entire VB reaction bed. After obtaining all data on PCB levels in the various compartments, the mass balance analysis was conducted.

Thus for each VB and earthworm-free control, the overall reductions in PCBs was computed. All abiotic routes of PCB disappearance, as well as non-earthworm based biotic routes of PCB transport were accounted for. These calculations showed that the overall PCB removal in the VB with 10% sludge loading was 54%, whereas the total PCB reduction in an identical earthworm-free control was 48%. Thus, 48% of PCB congener mass was removed through abiotic mechanism, and the introduction of earthworms lead to an increase of only 6% in total PCB reduction. In the VB with a 50% sludge mass fraction loading, the overall PCB removal with the earthworms was 66%, while the total PCB removal from the earthworm-free control was 56%. This meant an increase of almost 10% in total PCB reduction with the introduction of earthworms. The data from the 25 and 50% sludge-fraction VBs did not demonstrate appreciable differences between the VB and the earthworm-free controls, suggesting that there may be an optimal level at which there is additional and enhanced reduction of PCB congeners in a vermicompost system.
The data from all our studies clearly demonstrates that earthworms are strong bioaccumulators of PCBs, transferring the bulk of the PCBs from the sludge-soil matrix to the earthworms themselves. The mass balance analysis suggests that, in addition to bioaccumulation, there is additional biotransformation taking place. This is reflected in the increases in overall PCB reduction measured in the earthworm inoculated VBs. Interestingly, this increased reduction occurs in the VB loaded with the higher sludge-mass fraction (50%), suggesting that the kinetics of biotransformation may be dependent on initial PCB level, indicative of a first order type kinetic biotransformation.

Rates of PCB removal were also calculated in an effort to quantify the kinetics of PCB bioaccumulation and biotransformation. The overall global rates of PCB reduction were calculated and fit to first order kinetic equations. This analysis demonstrated that the rates of PCB removal increased as the initial concentration of PCBs in the sludge-soil matrix increased, typical of first order kinetic behavior. The results are shown in Table 3. Table 4 shows the rates and extents of PCB reduction in the worm-free controls.

### Table 3: Rate of PCB reduction in VBs

<table>
<thead>
<tr>
<th>Sludge Mass Fract. (%)</th>
<th>Global PCB Reduction Rate (mg/d)</th>
<th>PCB Reduction (%)</th>
<th>Time (days)</th>
</tr>
</thead>
<tbody>
<tr>
<td>10</td>
<td>0.056</td>
<td>54.7</td>
<td>100</td>
</tr>
<tr>
<td>25</td>
<td>0.111</td>
<td>54.5</td>
<td>150</td>
</tr>
<tr>
<td>50</td>
<td>0.216</td>
<td>66.4</td>
<td>185</td>
</tr>
<tr>
<td>75</td>
<td>0.298</td>
<td>62.3</td>
<td>200</td>
</tr>
</tbody>
</table>

### Table 4: Rate of PCB reduction in Worm-Free Control Reactors

<table>
<thead>
<tr>
<th>Sludge Mass Fract. (%)</th>
<th>Global PCB Reduction Rate (mg/d)</th>
<th>PCB Reduction (%)</th>
<th>Time (days)</th>
</tr>
</thead>
<tbody>
<tr>
<td>10</td>
<td>0.048</td>
<td>48.5</td>
<td>100</td>
</tr>
<tr>
<td>25</td>
<td>0.109</td>
<td>55.4</td>
<td>150</td>
</tr>
<tr>
<td>50</td>
<td>0.181</td>
<td>55.9</td>
<td>185</td>
</tr>
<tr>
<td>75</td>
<td>0.323</td>
<td>68.9</td>
<td>200</td>
</tr>
</tbody>
</table>

These results demonstrated increases in rates of reduction with increasing initial PCB concentration. Interestingly, the rates and extents of PCB reduction in the BTSRs also demonstrated an increase with increases in the initial PCB levels in the BTSR. The BTSR with the Midwest sludge sample (~500ppm initial PCB level) supported a higher rate of PCB reduction than the BTSR with the Southwest sludge (150ppm PCB initially). In the BTSR, there is bioaugmentation with anaerobic and aerobic microbes. The results obtained here suggest biotransformation rates and extents will depend on initial PCB load as well as the nature and level of bioaugmentation strategies utilized.

Global rates of PCB reduction also appear higher in the reactors with the worms than in the worm-free controls. Total percentage reduction was higher in the case of the 10% and the 50% sludge mass fraction reactors, while the total PCB reduction percentage in the 50% sludge mass fraction reactor was essentially the same in both VB and worm-free reactor. In the case of the 75% sludge mass fraction reactor, the total PCB reduction percentage was 5% higher in the worm-free control, and this was also reflected in a higher global PCB reduction rate.

### CONCLUSION

In conclusion, the two separate biological treatment technology investigations have demonstrated the ability to both reduce and biotransform PCBs. The results demonstrate that the anaerobic-aerobic cycling BTSR has the potential to diminish the total level of PCB in a sludge sample, specifically the site samples from the Ralston Street Lagoon in Gary, IN. GC and MS analysis of the samples have demonstrated reduction of some higher chlorinated PCB congeners in the anaerobic phase. This has been followed by reduction of some of lower chlorinated PCB congeners in the aerobic phase. The total reduction of PCBs appears to be initially high, with higher percentage reductions seen with the sludge with a higher initial PCB loading.

In vermicompost bioreactors, bioaccumulation is the apparent primary means of PCB removal from the sludge matrix. This is accompanied by some level of biotransformation which demonstrated net increases in PCB reductions when earthworms were introduced into the sludge-soil matrix.
It is clear that much more research needs to be conducted to elucidate and differentiate bioaccumulation, biotransformation and overall PCB reduction, as well as measurable biological degradation through monitoring of by- and end-products of transformation. Research that focuses on evaluating and assessing these differences is critical to developing these technologies for potential application.

These studies do suggest that the use of biological technologies, whether encompassing anaerobic and aerobic microbiological agents or earthworms, can enhance PCB removal from hazardous contaminated sludge and hence contribute to the growing list of biological technologies that have potential for application in the remediation of PCB contaminated environmental media.

ACKNOWLEDGEMENTS
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REFERENCES


