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Biodegradation of PCBs in Two-Phase Partitioning Bioreactors Following Solid Extraction From Soil

Lars Rehmann, Andrew J. Daugulis

Department of Chemical Engineering, Queen's University, Kingston, Ontario, Canada, K7L 3N6; telephone: 613-533-2784; fax: 613-533-6637; e-mail: andrew.daugulis@chee.queensu.ca

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ABSTRACT: This article demonstrates the feasibility of a novel process concept for the remediation of PCB contaminated soil. The proposed process consists of PCB extraction from soil using solid polymer beads, followed by biodegradation of the extracted PCBs in a solid–liquid two-phase partitioning bioreactor (TPPB), where PCBs are delivered from the polymer beads to the degrading organisms. The commercially available thermoplastic polymer Hytrel™ was used to extract Aroclor® 1242 from contaminated artificial soil in bench scale experiments. Initial PCB contamination levels of 100 and 1,000 mg kg^{-1} could be reduced to 32% ± 1 to 41% ± 7 of the initial value after 48 h mixing in the presence of a mobilizing agent at polymer-to-soil ratios of 1% (w/w) and 10% (w/w). The decrease of detectable PCBs in the soil was consistent with an increase of PCBs in the polymer beads. It was further shown that Aroclor® 1242 could be delivered to the PCB degrading organism *Burkholderia xenovorans* LB400 in a solid–liquid TPPB via Hytrel™ beads. A total of 70 mg Aroclor® 1242 could be degraded in a 1 L solid–liquid TPPB within 80 h of operation.

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Introduction

Polychlorinated biphenyls (PCBs) are toxic xenobiotics, manufactured in North America until the late 1970s as congener mixtures under the trade name Aroclor®. PCBs had broad industrial use prior to their ban in 1978 and are now widely distributed contaminants of soil and sediment (Abramowicz, 1990; Robinson and Lenn, 1994). PCBs are subject to limited microbial degradation under anaerobic conditions via reductive dehalogenation (Mohn and Tiedje, 1992; Tiedje et al., 1993) and under aerobic conditions via the biphenyl pathway (*bph*-pathway). The bacterium *Burkholderia xenovorans* LB400 is one of the best studied aerobic PCB degraders, known to be able to degrade up to hexachlorinated biphenyls (Bopp, 1986; Fain and Haddock, 2001; Hofer et al., 1994), and was therefore used in this study.

Natural biodegradation of PCBs in contaminated soils and sediments occurs at low rates (Young and Cerniglia, 1995), and various attempts to accelerate biodegradation have been undertaken (Fava et al., 2003; Manzano et al., 2003; Singer et al., 2003). In situ and ex situ processes have been applied. Phytoremediation is a promising technique for in situ soil remediation (Whitfield Aslund et al., 2007), and recently the *bph*-genes of *B. xenovorans* LB400 have been cloned into tobacco plants to increase PCB degradation (Mohammadi et al., 2007). Other in situ strategies focus on reducing PCB availability and toxicity by adding sorption material such as activated carbon to sediments (Millward et al., 2005; Werner et al., 2005, 2006; Zimmerman et al., 2004). This method however does not degrade PCBs. Recently iron nano-particles have been shown to dechlorinate PCBs when added to soil (Varanasi et al., 2007).

Ex situ methods include excavation of soil followed by incineration (Magar, 2003), the application of soil-slurry bioreactors (Fava et al., 2000), and various extraction techniques, such as microwave assisted steam extraction (Di et al., 2002), solvent extraction (EPA, 1994; Jakher et al., 2007), supercritical fluid extraction (Anitescu and Tavlarides, 2002; Wu and Marshall, 2001), and surfactant soil washing (Berselli et al., 2006; Billingsley et al., 1999, 2002). Prpich et al. (2006) recently demonstrated that polymers can be used to extract phenols from contaminated soil by mixing solid polymer beads with dry soil followed by phenol degradation in a solid–liquid two-phase partitioning bioreactor (TPPB).

TPPBs are typically comprised of an aqueous phase containing a biocatalyst, and a water-immiscible phase containing large amounts of hydrophobic and/or toxic substrate (Daugulis, 2001). The substrate partitions, at low concentrations, from the water-immiscible phase into the aqueous phase, where degradation occurs. TPPBs employing organic solvents (Rehmann and Daugulis, 2006) or solid polymer beads (Rehmann and Daugulis, 2007b) as water-immiscible phases have been shown to be effective systems for the degradation of biphenyl by *B. xenovorans* LB400.

Correspondence to: A.J. Daugulis

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TPPBs can be combined with PCB extraction from soil to form a soil remediation process. However solvents which are used during soil extraction typically have low viscosity and low boiling points, such as methanol, acetone or iso-propyl alcohol (EPA, 1994; Jakher et al., 2007) and do not posses the desired characteristics of solvents to be used in TPPBs, including immiscibility (Bruce and Daugulis, 1991). This limitation does not occur with solid polymers and Figure 1 illustrates a possible process of polymer bead PCB extraction followed by PCB degradation in a solid–liquid TPPB.

This is to our best knowledge the first study to demonstrate the feasibility of the process shown in Figure 1 in bench scale studies. The soil extraction step was demonstrated by contaminating model soils with Aroclor® 1242 followed by its extraction with polymer beads, while the feasibility of the TPPB step was shown by degrading an equivalent of 100 mg Aroclor® 1242 per l aqueous phase in a solid–liquid TPPB.

Materials and Methods

Chemicals

All chemicals used in the fermentation media and the solvents were obtained from either Sigma–Aldrich (St. Louis, MO) or Fisher Scientific (Ottawa, Canada). Biphenyl, 99% (assay) was obtained from Alfa Aesar (Ward Hill, MA) and Aroclor® 1242 (CAS Number: 53469-21-9) was obtained from Chromatographic Specialties, Inc. (Brockville, Ontario). Hytrel™ (Hytrel™ is a registered trademark of E. I. du Pont de Nemours and Company, Kingston, Canada) is a thermoplastic polyester elastomer with a density of 1.17 $g\,cm^{-3}$. The polymer chain contains approximately 50% poly-butylene terephthalate (PBT) and 50% butylene ether glycol terephthalate. It was found not to be available as a carbon source to *B. xenovorans* LB400 and no biofilm formation on the polymer surface has been observed (Rehmann and Daugulis, 2007b). Hytrel™ polymer beads were obtained from DuPont Canada, in cylindrical shapes with a specific surface are of 1.49 $m^2\,kg^{-1}$ (m^2 polymer surface per kg polymer).

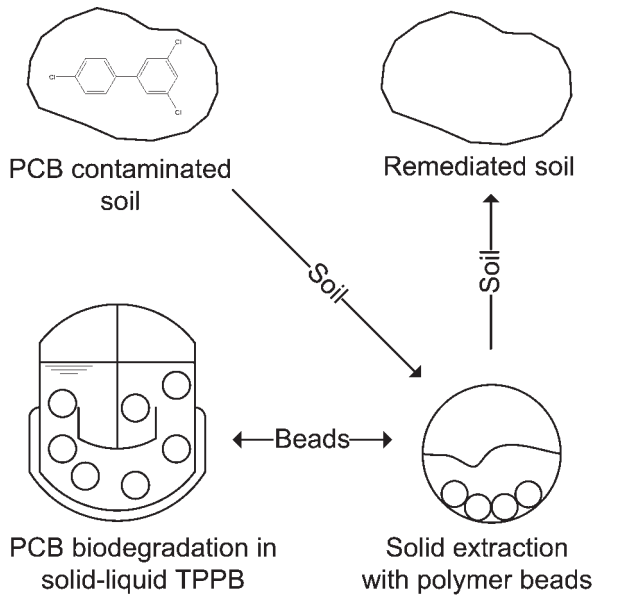

Figure 1. Schematic of soil remediation process. PCB contaminated soil is mixed with a solid extraction phase (polymeric material) in the presence of iso-propyl alcohol. PCBs are transferred from the soil to the polymers, the remediated soil is returned to the environment and the polymers are treated in a solid-liquid TPPB for microbial PCB destruction.

Bacterial Strain

Pseudomonas strain LB400 (strain NRRLB-18064), isolated by researchers at General Electric (Schenectady, NY) (Bopp, 1986), was obtained from the Northern Regional Research Laboratory (Peoria, IL). The strain has since been reclassified as *Burkholderia xenovorans* sp. nov. (Goris et al., 2004). Cultivation conditions, maintenance and biomass determination were described previously (Rehmann and Daugulis, 2006).

Uptake of Aroclor® 1242 by Hytrel™

The affinity of Hytrel™ for Aroclor® 1242 was demonstrated by measuring the partitioning coefficients of Aroclor® 1242 between Hytrel™ and water and between Hytrel™ and methanol. Methanol solutions containing 3–9 $g\,kg^{-1}$ Aroclor® 1242 were prepared and 1 g of each solution was equilibrated with 0.1 g Hytrel™ in 10 mL glass scintillation vials sealed with an aluminum capped lid and agitated for 48 h on a rotary shaker at 180 rpm and 30°C. The remaining Aroclor® 1242 was measured in methanol and compared to control vials containing no Hytrel™. The concentration in the polymer was calculated via mass balance and was assumed to be in equilibrium with the final concentration in methanol. The Hytrel™ water partitioning coefficient was estimated by equilibrating the Hytrel™ previously loaded with PCBs from methanol with 10 L water (48 h). The remaining PCB concentration in the beads was calculated via mass balance after re-equilibrating the Hytrel™ with methanol, which then allowed estimating the amount released to the water.

Solid Extraction of Aroclor® 1242 From Soil

All experiments were undertaken with artificial soil composed of 10% organics (peat), 20% clay and 70% industrial sand at pH 6 as outlined in OECD method 207. Soil was contaminated in open 10 mL glass scintillation vials

with Aroclor® 1242 dissolved in Isopropyl alcohol (IPA). The initial PCB concentration in the IPA was varied in order to add the same relative amount of IPA to all soil samples (10% w/w) resulting in final PCB in soil concentrations of either 100 or 1,000 mg kg^{-1}, followed by 48 h mixing (rotary shaker at 240 rpm and 22°C) to ensure distribution of the PCBs throughout the soil and evaporation of IPA.

Hytrel™ polymer beads and 15% IPA (mobilizing agent) were subsequently added to the contaminated soil in the scintillation vials and closed with aluminum sealed lid to form four extraction series with different initial conditions: 0.1 g beads were each added to 12 vials containing 1 g of soil contaminated with 100 mg kg^{-1} Aroclor® 1242 (extraction series E1) and to 12 vials containing 1 g soil contaminated with 1,000 mg kg^{-1} Aroclor® 1242 (extraction series E2), 0.03 g beads were added to vials containing 3 g of soil contaminated with 100 mg kg^{-1} Aroclor® 1242 (extraction series E3) and to 12 vials containing 3 g soil contaminated with 1,000 mg kg^{-1} Aroclor® 1242 (extraction series E4). Extraction series E1 and E2 employed a polymer to soil ratio of 10% (w/w) and an initial PCB level of 100 and 1,000 mg kg^{-1} respectively, while E3 and E4 employed a polymer to soil ratio of 1% (w/w) at similar initial contamination levels. All vials were incubated on a rotary shaker at 240 rpm and 22°C. Three vials of each extraction series and one control vial per extraction series containing no beads were removed from the shaker after 6, 12, 24, and 48 h. Polymers and soil were separated manually and PCBs were extracted from soil and polymer as described below.

Biodegradation of Aroclor® 1242 in a Solid–Liquid TPPB

Hytrel™ polymer beads (2.5 g) were loaded with Aroclor® 1242 by equilibrating them with 3.5 g methanol containing 160 mg Aroclor® 1242. The final Aroclor® 1242 concentration in the polymer was 48 mg kg^{-1}. The polymers were added to a 1 L BioFlo® I bioreactor at 30°C, agitated with two Rushton turbines at 500 rpm and aerated (sterile air) at 1 L min^{-1}. The pH was maintained at 6.9. Samples of the polymer beads were taken periodically for PCB analysis as described below and of the aqueous phase for biomass analysis as described elsewhere (Rehmann and Daugulis, 2006). The reactor was inoculated with PAS medium (Bopp, 1986) containing *B. xenovorans* LB400 at initial concentrations of 2 g L^{-1}. The biomass for the inoculum was grown in a solid–liquid TPPB with biphenyl as the sole carbon source as described elsewhere (Rehmann and Daugulis, 2007b). Sodium pyruvate was added at the outset of the fermentation (6 g) and after 48 h (3 g) to provide the degrading organism with a carbon and energy source that promotes cell activity and PCB degradation (Rehmann and Daugulis, 2007c). A control experiment with heat-inactivated biomass was undertaken under similar conditions.

PCB Extraction and Analysis

PCBs were extracted from soil samples of 1 or 3 g with 10 mL hexane and from Hytrel™ (0.03 or 0.1 g) with 5 mL methanol (24 h on a rotary shaker at 180 rpm and 30°C). The extract was analyzed with an Agilent 6890 gas chromatograph (Agilent Technologies Canada, Inc., Mississauga, Ontario, Canada) equipped with a fused silica cap capillary column (Supelco SPB-1, Sigma–Aldrich Corp. St. Louis, MO), an electron capture detector (ECD) (280°C) and split injector (250°C). The temperature program was as follows: 100°C for 4 min, 100–180°C at 10°C min^{-1}, 180°C for 1 min, 180°C to 240°C at 1.5°C min^{-1}, 240°C for 1 min, 240°C to 300°C at 20°C min^{-1}, 300°C for 10 min. Aroclor® standards in hexane and methanol were run for every analysis and blank hexane/methanol was run after every four samples. Aroclor® was quantified by using the summed peak area according to EPA Method 304 h.

Results and Discussion

Uptake of Aroclor 1242 by Hytrel™

Hytrel™ had been selected from a variety of polymeric substances as a suitable delivery phase for biphenyl in solid–liquid TPPBs in a previous study (Rehmann et al., 2007). It was found that Hytrel™ had a suitable polymer/water partitioning coefficient for biphenyl of $\log K_{S/W} = 3.51$, and it was also shown that Hytrel™ had the ability to extract biphenyl from methanol or water and subsequently release it into aqueous medium where biodegradation by *B. xenovorans* LB400 took place (Rehmann and Daugulis, 2007b). The ability of Hytrel™ to sorb large amounts of PCBs, which are environmentally more significant than biphenyl, from methanol was evaluated, and the equilibrium data are shown in Figure 2. The equilibrium isotherm follows a linear trend over the observed range of concentrations, and it can be seen that Aroclor® 1242 partitions preferentially into Hytrel™. Similar partitioning behavior had also been found for biphenyl, where the Hytrel™ methanol partitioning coefficient was $K_{S/M} \sim 1.3$ (Rehmann and Daugulis, 2007b). Aroclor® 1242 partitions between Hytrel™ and methanol with a partitioning coefficient of $K_{S/M} \sim 4.2$.

The fact that Hytrel™ has an approximately four times higher affinity for PCBs than organic solvents such as methanol indicates the possibility of using a material such as Hytrel™ in PCB remediation schemes. However, if the remediation scheme requires subsequent biodegradation of PCBs in aqueous medium, then reversible release of PCBs from the polymeric carrier material is required. The PCB-loaded Hytrel™ was therefore equilibrated with water and the equilibrium isotherm is also shown in Figure 2. Linear partitioning of Aroclor® 1242 could be found over the observed range of concentrations, with a high Hytrel™/water partitioning coefficient of $\log K_{S/W} = 5.52$. The

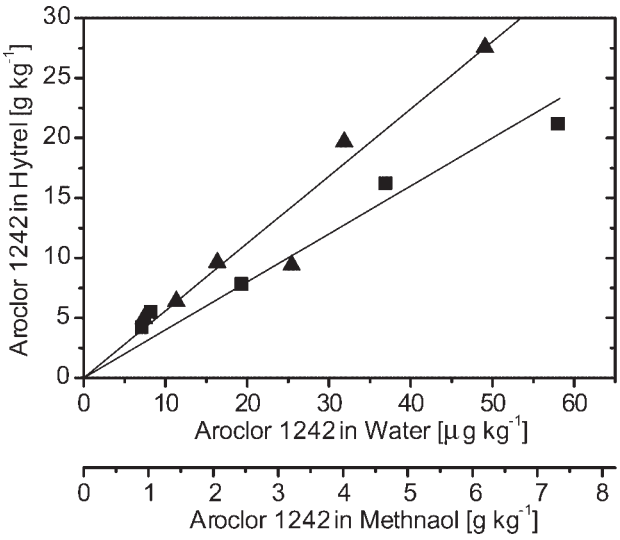

Figure 2. Partitioning of Aroclor® 1242 between methanol and Hytrel™ (triangles) and between water and Hytrel™ (squares).

observed partitioning coefficient is significantly larger than the octanol/water partitioning coefficient of Aroclor® 1242 of log $K_{O/W} = 4.2$ (Cohen et al., 1993), again showing the high affinity of the polymeric substance for PCBs.

Solid Extraction of Aroclor® 1242 From Soil

Extraction of Aroclor® 1242 from soil was evaluated in four different extraction series employing different initial contamination levels and initial Hytrel™ to soil ratios. IPA was added as a mobilizing agent to facilitate mass transfer of PCBs from soil particles into the polymer beads. Mobilizing agents have been shown to significantly enhance PCB extractability in soil (Berselli et al., 2006). IPA was chosen as it has been approved by the US environmental protection agency in a commercial soil extraction process (EPA, 1994), however solvent free extraction with polymer beads would be highly beneficial and is currently under investigation. Table I shows the initial contamination levels, Hytrel™ to soil ratios and PCB distribution after 48 h for the four different extraction series. The PCB concentration in the soil was reduced to 30–40% of its original value after mixing for 48 h, while between 50% and 70% could be recovered in the polymer. Comparing the results of extraction series E1 and E3 shows that the amount of Aroclor® 1242 in soil contaminated at initial concentrations of approximately 100 mg kg^{-1} could be reduced to 37 and 35 mg kg^{-1} using either 10% (w/w) or 1% (w/w) of Hytrel™. The 10-fold smaller amount of Hytrel™ in E3 in comparison to E1 did not result in the removal of a smaller PCB fraction, showing the efficiency of the extraction process. The lower bead-to-soil ration resulted in a substantially increased PCB loading in the polymer. The final PCB concentration in the polymer was >4,000 mg kg^{-1} in the case of E3, which is lower than the range of concentrations shown in Figure 2, indicating that a further reduction of the Hytrel™ to soil ratio might be possible without losing performance.

Even though the final PCB removal in E1 and E3 was similar, as shown in Table I, the rate at which the extraction occurred was significantly higher in E1, as can be seen in Figure 3a and c. In the case of E1 the majority of PCBs was extracted within the initial 12 h, whereas it took 48 h to reach similar levels in E3. The difference can readily be explained by the tenfold higher available surface area in E1, as a result of the higher polymer to soil ratio. The mass transfer rate of biphenyl from Hytrel™ to water has been shown to be directly proportional to the available surface area (Rehmann and Daugulis, 2007b). The surface to volume ratio of the Hytrel™ beads employed in this study was high, as the average bead diameter was 4–6 mm. Such geometries are suitable for fundamental lab-scale studies, however for field applications it would not be suitable to separate rice grain sized polymer beads from soil. The performance of different geometries such as polymer sheets or rods will have to be evaluated for large scale applications, which however is beyond the scope of this study. In a related study Hytrel™ sheets have been used for the in situ removal of 3-methylcatechol from a fermentation vessel, and the performance of the sheets was comparable to the performance of beads of similar mass as far as the extend of removal was concerned, however the removal rate was lower due to the decrease in surface area (Prpich and Daugulis, 2007).

Due to the high affinity of the selected polymer for PCBs a ratio of only 1% (w/w) was enough to reduce the concentration of Aroclor® 1242 in soil to below 50 mg kg^{-1} (E3), which is the concentration in soil set by the US code of federal regulation (40 CFR 761.65) above which PCB spills

Table I. Extraction of Aroclor® 1242 from soil using Hytrel™ polymer beads under different initial conditions.

Test series	Initial concentration in soil (mg kg^{-1})	Bead/soil ratio (g g^{-1})	Final concentration in soil (mg kg^{-1})	Final concentration in polymer (mg kg^{-1})	Residual in soil (%)	Amount extracted in polymer (%)
E1	100	0.1	37 ± 3	621 ± 124	34 ± 3	57 ± 11
E2	1,000	0.1	271 ± 2	5,653 ± 641	32 ± 1	67 ± 8
E3	100	0.01	35 ± 6	4,039 ± 337	42 ± 7	48 ± 4
E4	1,000	0.01	334 ± 27	68,036 ± 7,746	33 ± 3	68 ± 8

The presented values represent the averages of triplicates after 48 h ± 95% confidence limits.

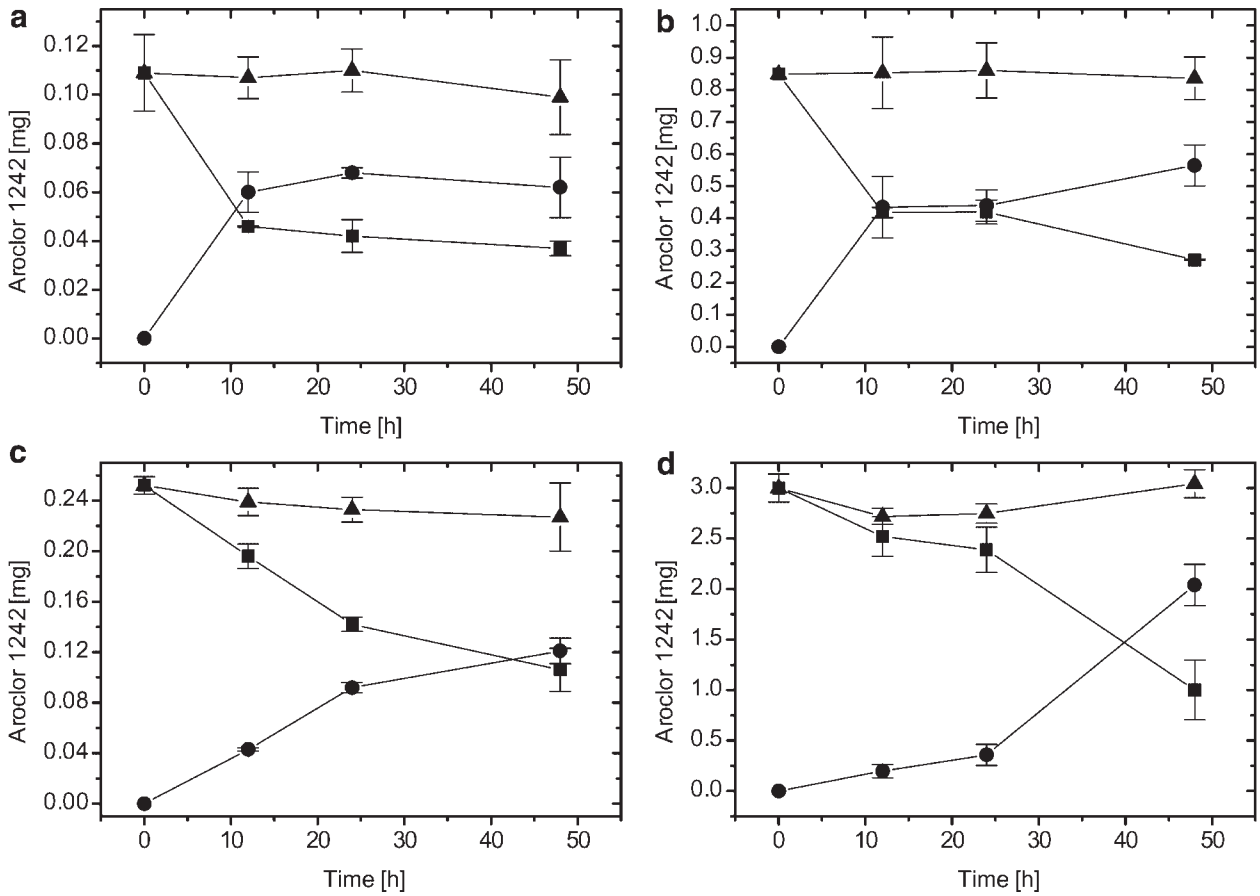

Figure 3. Time course of PCB extraction from soil with Hytrel™ polymer beads. The triangles represent the total amount of Aroclor® 1242 recovered, the squares represent the amount in remaining in soil and the circles the amount extracted to the polymer beads. Test series E1 is shown in (**a**), E2 in (**b**), E3 in (**c**) and E4 in (**d**).

have to be reported to the authorities for further actions. This key value was not achieved if the initial PCB concentration in the soil was 1,000 mg kg^{-1} (E2 and E4), however the PCB concentration in soil was reduced significantly in both extraction series and the possibility of two or more sequential extraction will be investigated in future research.

The conditions in E1 and E2 were similar except that the total amount of PCBs present in the soil was increased from 100 to 1,000 mg kg^{-1}. The residual amount of PCBs after 48 h increased from 37 to 271 mg kg^{-1}, however, there is no significant difference in the final% distribution of PCBs between soil and polymer (Table I). The time course of E2 resembles the time course of E1 more than the time course of E3 (Fig. 3). E2 and E1 share the same polymer to soil ratio, while E2 and E3 share the same polymer to PCB ratio. It can be seen in Figure 3 that the bead to soil ratio seems to govern the rate at which PCBs are removed from soil. The final concentration of Aroclor® 1242 in Hytrel™ is higher in E2 ($5{,}653 \pm 641$ mg kg^{-1}) than in E3 ($4{,}039 \pm 337$ mg kg^{-1}), suggesting that more PCBs could have been extracted in E3 given additional extraction time. Figure 3c seems to confirm this as it appears that the PCB concentration in both phases did not reach a constant level after 48 h. This is most likely due to the hydrophobic nature of PCBs, which can associate with soil particles (Ehlers and Luthy, 2003) resulting in slow transfer of PCBs from soil to the polymer despite the addition of IPA as a mobilizing agent. More hydrophilic substances have been shown to transfer from soil into polymers at a much faster rate. Pripch et al. could reduce the amount of phenol in soil from 2,300 mg kg^{-1} to approximately 100 mg kg^{-1} in 24 h under otherwise similar conditions to the ones employed in this study (Prpich et al., 2006).

In series E4 the initial PCB concentration was 1,000 mg kg^{-1}, as in series E2, and the bead to soil ratio was 1% (w/w) as in series E3. The final concentration remaining in the soil is only slightly higher than in E2 (334 mg kg^{-1} compared to 271 mg kg^{-1}), despite the tenfold decrease of the amount of extractant. The time course of the extraction generally follows the same trend as E3 (Fig. 3), confirming that the extraction rate is strongly affected by the polymer to soil ratio. The total amount of PCBs extracted in series E4 is substantially higher than in the other extraction

series. The final Aroclor® 1242 concentration in Hytrel™ was 68,000 mg kg^{-1}, showing the strong PCB sorption capacity of Hytrel™. These finding are significant for possible PCB remediation strategies even if no biodegradation of the extracted PCBs follows, as the amount of contaminated material can be reduced by orders of magnitude if PCBs are extracted from soil using 1% (w/w) of extractant. Even though soil extraction was shown only for Aroclor® 1242 and not for higher chlorinated PCB mixtures such as Aroclor® 1260, it is expected that that the methodology can be extended towards other Aroclors. Figure 4 shows chromatograms of PCB extracts from control soil, and from the soil and Hytrel™ fraction of extraction series E2. Each peak represents one or more congeners, and it can clearly be seen that the congener distribution in the treated soil (Fig. 4b) is not significantly altered from non treated soil (Fig. 4a). The extracted PCB fraction consequently follows the same distribution (Fig. 4c). The fact that highly chlorinated congener (high retention times) are extracted to the same extent as low chlorinated (short retention times) suggests that other Aroclors can be extracted the same way as Aroclor® 1242.

Biodegradation of Aroclor® 1242 in a Solid–Liquid TPPB

In order to recycle the polymer beads in a possible soil remediation scheme it would be necessary to remove PCBs from the polymer followed by PCB degradation, as suggested in Figure 1. This can be achieved in solid–liquid TPPBs, which have previously been demonstrated to be an effective technology platform for biodegradation of phenols (Prpich and Daugulis, 2006) and biphenyl (Rehmann and Daugulis, 2007b). Introducing polymer beads containing large amounts of PCBs into a bioreactor will result in equilibrium partitioning of PCBs into the aqueous medium (as shown in Fig. 2), where they can be degraded by appropriate microorganisms. Hydrophobic substances such as PCBs will result in low aqueous phase PCB concentrations and it can be inferred from Figure 2 that the PCB concentrations in the aqueous phase delivered from Hytrel™ beads will be below 100 μg kg^{-1}. This low PCB level is the only bioavailable fraction in TPPBs and studies in liquid-liquid TPPBs have shown that substrate availability of PCBs can be the rate limiting factor in biphasic systems during aerobic PCB degradation by *B. xenovorans* LB400 (Rehmann and Daugulis, 2007a).

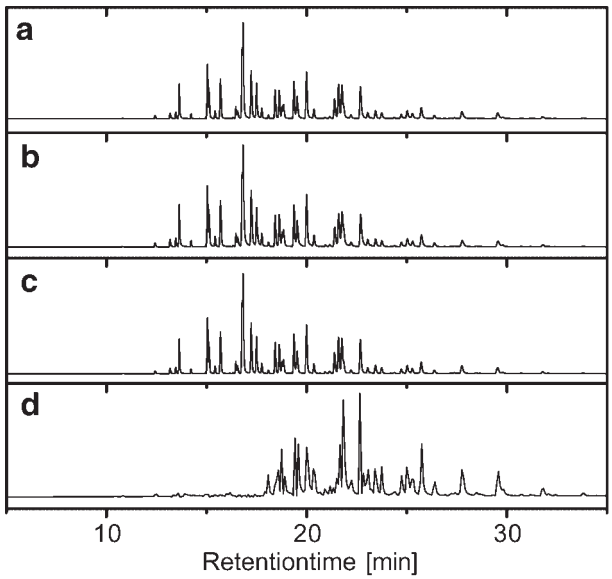

Figure 4. Chromatogram of Aroclor® 1242 extracted from: (**a**) control soil, containing no polymer beads and an initial Aroclor® 1242 concentration of 1,000 mg kg^{-1} after 48 h, (**b**) soil containing 10% (w/w) Hytrel™ and an initial Aroclor® 1242 concentration of 1,000 mg kg^{-1} after 48 h, (**c**) Hytrel™ beads, which were in contact with soil from (b) after 48 h, and (**d**) Hytrel™ beads after treatment in bioreactor. No scale is shown on the *y*-axis to emphasis the similarities in the congener distribution in (a–c) and the differences in (d).

It has previously been shown that the specific microbial PCB degradation rate in the presence of an immiscible liquid phase was reduced to less than 10% of its value in a single phase system, and that the resulting low degradation rates can be partly circumvented by employing large amounts of biomass (Rehmann and Daugulis, 2007a). It was further shown that cells of *B. xenovorans* LB400 lost their *bph*-activity after approximately 40 h when degrading PCBs in the absence of a metabolizable carbon source, and that the addition of sodium pyruvate could provide carbon and energy to the organism while sustaining its activity towards PCBs (Rehmann and Daugulis, 2007c). In anticipation of similar limitations in solid-liquid TPPBs, the initial amount of biomass was therefore chosen to be 2 g kg^{-1} and sodium pyruvate was added at the outset of the fermentation and after 48 h. The initial PCB loading in the polymer beads was chosen to be 50 g kg^{-1} based the concentrations achieved during soil extraction (Table I, E4). PCBs were loaded into the polymers from methanol and not extracted from soil in order generate a large amount of equally loaded beads without artificially contaminating soil.

Figure 5 shows the time course of Aroclor® 1242 degradation in a solid–liquid TPPB. The initial Aroclor® 1242 concentration of 50 g kg^{-1} in the solid phase was reduced to 16 g kg^{-1} over a total fermentation time of 80 h. Biomass formation due to growth on pyruvate was observed during the first 10 h after inoculation and after the addition of supplementary pyruvate after 48 h. The fact that new biomass was formed after the second addition of pyruvate shows that the cells were viable. The minimal effect on PCB degradation at this point might be due to low PCB availability. However, the general performance achieved in this solid-liquid TPPB is comparable to the performance of liquid–liquid TPPBs employing silicone oil as a water-immiscible phase (Rehmann and Daugulis, 2007c). Approximately 70% of the initially present PCBs, an equivalent of 70 mg PCB per l aqueous phase, was degraded within 80 h. The reduction in Aroclor® is clearly due to biological activity as the control experiment with inactivated

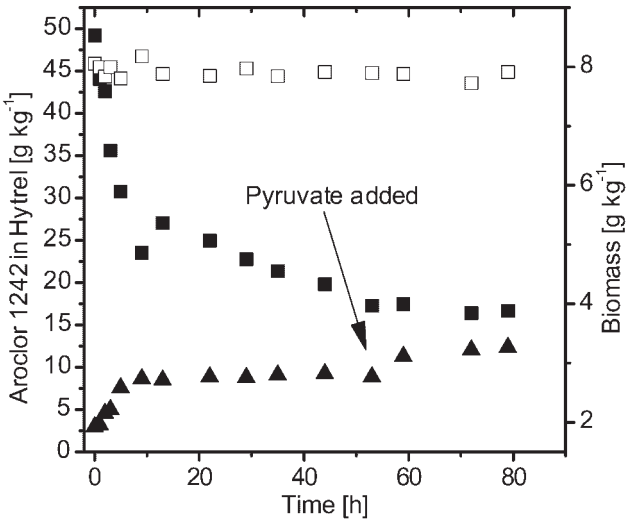

Figure 5. Biodegradation of Aroclor® 1242 by *B. xenovorans* LB400 in a solid–liquid TPPB. The filled squares show the PCB concentration in Hytrel™ beads during biodegradation and the empty squares show the PCB concentration during a control experiment with deactivated biomass. The triangles represent the biomass concentration during the biodegradation experiment.

biomass did not yield in any significant PCB reduction (Fig. 5). Further, a clear change in the congener distribution of the extracted PCBs can be seen in Figure 4d, with a preferential reduction of the lower chlorinated congeners (small retention times), which is typical for aerobic PCB degradation (Abramowicz, 1990).

Conclusions

The individual steps constituting a PCB soil remediation process comprised of soil extraction with polymers followed by PCB degradation in a solid–liquid TPPB were demonstrated in this paper. It can be concluded that the volume of PCB contaminated material can be reduced significantly if PCBs are extracted from soil into polymers, and that low chlorinated PCB mixtures can subsequently be degraded aerobically in a solid–liquid TPPB. Future work will investigate the use of weathered soil and the possibility of treating higher chlorinated Aroclors.

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References

Abramowicz DA. 1990. Aerobic and anaerobic biodegradation of PCBs: A review. Crit Rev Biotechnol 10:241–300.

Anitescu G, Tavlarides LL. 2002. Methanol as a cosolvent and rate-enhancer for the oxidation kinetics of 3,3′,4,4′-tetrachlorobiphenyl decomposition in supercritical water. Ind Eng Chem Res 41:9–21.

Berselli S, Benitez E, Fedi S, Zannoni D, Medici A, Marchetti L, Fava F. 2006. Development and assessment of an innovative soil-washing process based on the use of cholic acid-derivatives as pollutant-mobilizing agents. Biotechnol Bioeng 93:761–770.

Billingsley KA, Backus SM, Ward OP. 1999. Effect of surfactant solubilization on biodegradation of polychlorinated biphenyl congeners by *Pseudomonas* LB400. Appl Microbiol Biotechnol 52:255–260.

Billingsley KA, Backus SM, Wilson A, Singh A, Ward OP. 2002. Remediation of PCBs in soil by surfactant washing and biodegradation in the wash by *Pseudomonas* sp. LB400. Biotechnol Lett 24:1827–1832.

Bopp LH. 1986. Degradation of highly chlorinated PCBs by *Pseudomonas* strain LB400. J Ind Microbiol 1:23–29.

Bruce LJ, Daugulis AJ. 1991. Solvent selection strategies for extractive biocatalysis. Biotechnol Prog 7:116–124.

Cohen RM, Mercer JW, Matthews J. 1993. DNAPL site evaluation. Boca Raton, FL: C.K. Smoley.

Daugulis AJ. 2001. Two-phase partitioning bioreactors: A new technology platform for destroying xenobiotics. Trends Biotechnol 19:457–462.

Di P, Chang DP, Dwyer HA. 2002. Modeling of polychlorinated biphenyl removal from contaminated soil using steam. Environ Sci Technol 36:1845–1850.

Ehlers LJ, Luthy RG. 2003. Contaminant bioavailability in soil and sediment. Environ Sci Technol 37:295A–302A.

EPA. 1994. Innovative technology evaluation report: Terra-Kleen Response Group, Inc. Solvent Extr Technol 540R94521:1–68.

Fain MG, Haddock JD. 2001. Phenotypic and phylogenetic characterization of *Burkholderia (Pseudomonas)* sp. strain LB400. Curr Microbiol 42:269–275.

Fava F, Di Gioia D, Marchetti L. 2000. Role of the reactor configuration in the biological detoxification of a dump site-polychlorobiphenyl-contaminated soil in lab-scale slurry phase conditions. Appl Microbiol Biotechnol 53:243–248.

Fava F, Bertin L, Fedi S, Zannoni D. 2003. Methyl-beta-cyclodextrin-enhanced solubilization and aerobic biodegradation of polychlorinated biphenyls in two aged-contaminated soils. Biotechnol Bioeng 81:381–390.

Goris J, De Vos P, Caballero-Mellado J, Park J, Falsen E, Quensen JF III, Tiedje JM, Vandamme P. 2004. Classification of the biphenyl- and polychlorinated biphenyl-degrading strain LB400T and relatives as *Burkholderia Xenovorans* sp. nov. Int J Syst Evol Microbiol 54:1677–1681.

Hofer B, Backhaus S, Timmis KN. 1994. The biphenyl/polychlorinated biphenyl-degradation locus (bph) of *Pseudomonas* sp. LB400 encodes four additional metabolic enzymes. Gene 144:9–16.

Jakher A, Achari G, Langford CH. 2007. An investigation into solvent extraction of pcbs from weathered soils. Environ Technol 28:49–57.

Magar VS. 2003. PCB treatment alternatives and research directions. J Envir Eng 129:961–965.

Manzano MA, Perales JA, Sales D, Quiroga JM. 2003. Enhancement of aerobic microbial degradation of polychlorinated biphenyl in soil microcosms. Environ Toxicol Chem 22:699–705.

Millward RN, Bridges TS, Ghosh U, Zimmerman JR, Luthy RG. 2005. Addition of activated carbon to sediments to reduce PCB bioaccumulation by a polychaete (*Neanthes arenaceodentata*) and an amphipod (*Leptocheirus plumulosus*). Environ Sci Technol 39:2880–2887.

Mohammadi M, Chalavi V, Novakova-Sura M, Laliberté J, Sylvestre M. 2007. Expression of bacterial biphenyl-chlorobiphenyl dioxygenase genes in tobacco plants. Biotechnol Bioeng 97:496–505.

Mohn WW, Tiedje JM. 1992. Microbial reductive dehalogenation. Microbiol Rev 56:482–507.

Prpich GP, Daugulis AJ. 2006. Biodegradation of a phenolic mixture in a solid–liquid two-phase partitioning bioreactor. Appl Microbiol Biotechnol 72:607–615.

Prpich GP, Daugulis AJ. 2007. A novel solid-liquid two-phase partitioning bioreactor for the enhanced bioproduction of 3-methylcatechol. Biotechnol Bioeng 98:1008–1016.

Prpich GP, Adams RL, Daugulis AJ. 2006. Ex situ bioremediation of phenol contaminated soil using polymer beads. Biotechnol Lett 28:2027–2031.

Rehmann L, Daugulis AJ. 2006. Biphenyl degradation kinetics by *Burkholderia Xenovorans* LB400 in two-phase partitioning bioreactors. Chemosphere 63:972–979.

Rehmann L, Daugulis AJ. 2007a. Bioavailability of PCBs in Biphasic Bioreactors. Biochem Eng J 10.1016/j.bej.2007.07.004 (in press).

Rehmann L, Daugulis AJ. 2007b. Biodegradation of biphenyl in a solid-liquid two-phase partitioning bioreactor. Biochem Eng J 36:195–201.

Rehmann L, Daugulis AJ. 2007c. Enhancement of PCB degradation by *Burkholderia xenovorans* LB400 in biphasic systems by manipulating culture conditions. Biotechnol Bioeng 99:521–528.

Rehmann L, Sun B, Daugulis AJ. 2007. Polymer selection for biphenyl degradation in a solid-liquid two-phase partitioning bioreactor. Biotechnol Prog 23:814–819.

Robinson GK, Lenn MJ. 1994. The bioremediation of polychlorinated biphenyls (PCBs): Problems and perspectives. Biotechnol Genet Eng Rev 12:139–188.

Singer AC, Smith D, Jury WA, Hathuc K, Crowley DE. 2003. Impact of the plant rhizosphere and augmentation on remediation of polychlorinated biphenyl contaminated soil. Environ Toxicol Chem 22:1998–2004.

Tiedje JM, Quensen JF III, Chee-Sanford J, Schimel JP, Boyd SA. 1993. Microbial reductive dechlorination of PCBs. Biodegradation 4:231–240.

Varanasi P, Fullana A, Sidhu S. 2007. Remediation of PCB contaminated soils using iron nano-particles. Chemosphere 66:1031–1038.

Werner D, Higgins CP, Luthy RG. 2005. The sequestration of PCBs in Lake Hartwell sediment with activated carbon. Water Res 39:2105–2113.

Werner D, Ghosh U, Luthy RG. 2006. Modeling polychlorinated biphenyl mass transfer after amendment of contaminated sediment with activated carbon. Environ Sci Technol 40:4211–4218.

Whitfield Aslund ML, Zeeb BA, Rutter A, Reimer KJ. 2007. In situ phytoextraction of polychlorinated biphenyl—(PCB)contaminated soil. Sci Total Environ 374:1–12.

Wu Q, Marshall WD. 2001. Approaches to the remediation of a polychlorinated biphenyl (PCB) contaminated soil—A laboratory study. J Environ Monit 3:281–287.

Young LY, Cerniglia C. 1995. Microbial transformation and degradation of toxic organic chemicals. New York: Wiley-Liss. 654 p.

Zimmerman JR, Ghosh U, Millward RN, Bridges TS, Luthy RG. 2004. Addition of carbon sorbents to reduce PCB and PAH bioavailability in marine sediments: Physicochemical tests. Environ Sci Technol 38:5458–5464.