ARTICLE

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Enhancement of PCB Degradation by *Burkholderia xenovorans* LB400 in Biphasic Systems by Manipulating Culture Conditions

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ABSTRACT: Two-phase partitioning bioreactors (TPPBs) can be used to biodegrade environmental contaminants after their extraction from soil. TPPBs are typically stirred tank bioreactors containing an aqueous phase hosting the degrading microorganism and an immiscible, non-toxic and non-bioavailable organic phase functioning as a reservoir for hydrophobic compounds. Biodegradation of these compounds in the aqueous phase results in thermodynamic disequilibrium and partitioning of additional compounds from the organic phase into the aqueous phase. This self-regulated process can allow the delivery of large amounts of hydrophobic substances to degrading microorganisms. This paper explores the reactor conditions under which the polychlorinated biphenyl (PCB) degrader *Burkholderia xenovorans* LB400 can degrade significant amounts of the PCB mixture Aroclor® 1242. Aroclor® degradation was found to stall after approximately 40 h if no carbon source other than PCBs was available in the reactor. Sodium pyruvate was found to be a suitable carbon source to maintain microbial activity against PCBs and to function as a substrate for additional cell growth. Both biphenyl (while required during the inoculum preparation) and glucose had a negative effect during the Aroclor® degradation phase. Initial Aroclor® 1242 degradation rates in the presence of pyruvate were high (6.2 mg L^{-1} h^{-1}) and 85% of an equivalent concentration of 100 mg Aroclor® 1242 per L aqueous phase could be degraded in 48 h, which suggest that solvent extraction of PCBs from soil followed by their biodegradation in TPPBs might be a feasible remediation option.

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KEYWORDS: two-phase partitioning bioreactors; Aroclor®; polychlorinated biphenyls; ex situ; bioremediation

Introduction

Polychlorinated biphenyls (PCBs) are toxic xenobiotics, manufactured during the mid-twentieth century as congener mixtures. PCB mixtures were commercially available in North America under the trade name Aroclor® and found industrial applications in hydraulic and heat-transfer systems, inks, lubricants, paints, and adhesives. Prior to their ban in 1977, 570 million kg were sold in the Unites States, 75 million kg are estimated to have entered the environment, and 400 million kg are still in use and potential sources of future contamination (Cohen et al., 1993).

PCBs were designed to be non-flammable as well as chemically and thermally stable which results in their persistence in the environment. Magar (2003) concluded in a recent editorial that the challenge of PCBs in the environment today is virtually the same as it was 20 years ago (Magar 2003). Transport of PCBs and their fate in the environment are well understood (Dercova et al., 1999; Pier et al., 2003; Platonow et al., 1971; Thomann and St John, 1979; Weber et al., 1991) and precise analytical techniques have been developed for their enumeration (Cochran and Frame, 1999; Erickson, 1986; Frame et al., 1996; Rapaprot and Eisenreich, 1984; Rote and Murphy, 1971). A large number of PCB degrading organisms have been isolated (Bedard et al., 1987; Bopp 1986; Commandeur et al., 1995; Seto et al., 1995; Sierra et al., 2003) and, especially in the case of the aerobic PCB degrader *Burkholderia xenovorans* LB400, well characterized on a genomic and metabolic level (Chain et al., 2006; Denef et al., 2004, 2005). Despite these advances there is still a scarcity of treatment options for soils and sediments, with very few alternatives to in situ treatment (Magar, 2003).

This study examines factors affecting the second step of a proposed two stage process consisting of solvent extraction of PCBs from soil followed by microbial degradation in two-phase partitioning bioreactors (TPPBs). TPPBs are typically stirred tank bioreactors containing an aqueous phase in

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which microbial transformation can take place and an immiscible organic solvent phase acting as a reservoir for the target substrate(s) (Daugulis, 2001). Hydrophobic compounds such as polycyclic aromatic hydrocarbons (PAHs) or PCBs can be dissolved at high concentrations in the organic delivery phase and will, driven by thermodynamic phase equilibrium, partition at low concentrations into the aqueous phase where biodegradation occurs. Previously in an analogous strategy PAHs were extracted from soil using dodecane and ethanol and subsequently degraded by a *Sphingamonas* sp. in TPPBs (Janikowski et al., 2002), and high concentrations of biphenyl (equivalent to 5 $g\,L^{-1}$ aqueous phase) could also be degraded by *B. xenovornas* LB400 in TPPBs (Rehmann and Daugulis, 2006). PCBs are substantially more hydrophobic than biphenyl and low bioavailability in TPPBs might reduce degradation rates. Recent studies showed that sequestration of PCBs between water and oil in the environment have a rate reducing effect on PCB degradation (McNamara et al., 2005; Zwiernik et al., 1999).

Notwithstanding the success of degrading PAHs and biphenyl at high rates in TPPBs, preliminary experiments with resting cells (no carbon source other than PCBs present) could only achieve degradation of up to 90% of the initially available Aroclor® in a TPPB with low initial Aroclor® 1242 concentration equivalents of 10 $mg\,L^{-1}$. In these systems microbial degradation stopped, however, after approximately 40 h and resulted in significant PCB residues, depending on the operating conditions of the bioreactor. This limited time of degradative activity combined with low degradation rates therefore limits the amount of PCB that can be degraded per unit time and reactor volume resulting in inefficient PCB degradation. PCB degradation by growing cells of *B. xenovorans* LB400 in TPPBs containing large amounts of biphenyl (equivalent to 5 g per L in the aqueous phase) in the delivery phase (bis(2-ethylhexyl)sebacate) and Aroclor® 1242 concentration between 10 and 1,000 $mg\,L^{-1}$ could not be achieved, despite significant biomass formation (data not shown).

Arrested degradation with resting cells, and minimal degradation with growing cells (with biphenyl as the carbon source) are clear limitations to rapid and near complete degradation of PCBs in TPPBs. It was therefore the objective of this study to manipulate microbial cultivation conditions in TPPBs to enhance degradation rates and to prolong microbial activity to biodegrade the majority of PCB congeners in 100 mg Aroclor® 1242 per L aqueous phase.

Materials and Methods

Chemicals

All chemicals used in the fermentation media and the solvents were obtained from either Sigma–Aldrich (Oakville, ON, Canada) or Fisher Scientific (Ottawa, ON, 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, ONT).

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 *B. xenovorans* sp. nov. (Goris et al., 2004). Cultivation conditions, maintenance, and biomass determination were described previously (Rehmann and Daugulis, 2006).

Inoculum Preparation

B. xenovorans LB400 was cultivated in 5-L New Brunswick BioFlo® III bioreactors at 30°C, agitated with two Rushton turbines at 500 rpm and aerated (sterile air) at 3 $L\,min^{-1}$. Biphenyl was delivered from Hytrel™ polymer beads, which were pre-loaded from biphenyl saturated methanol, as the sole carbon source. Cultivating *B. xenovorans* in solid–liquid TPPBs allows the generation of high inoculum concentrations as well as subsequent complete biphenyl removal before use and is described in detail elsewhere (Rehmann and Daugulis, 2007b). Cells were harvested in mid exponential phase and the solid delivery phase was removed by filtration through sterile glass wool. Biomass was removed from culture medium via centrifugation at 3,000 rpm for 20 min at 4°C and re-suspended in fresh medium to the desired biomass concentration and distributed to three 1 L BioFlo® I bioreactors.

Cultivation Conditions

All PCB degradation experiments were undertaken in 1 L BioFlo® I bioreactors 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 were taken periodically for PCB and biomass analysis. The agitation rate was increase to 900 rpm prior to sampling to ensure that samples contained representative fractions of both liquid phases.

Enhancement Strategies

To examine the effect of varying a number of operating conditions to enhance PCB degradation in TPPBs, a variety of strategies were employed.

Strategy 1: Variation of Initial Biomass Concentration

Neat Aroclor® 1242 (100 mg) was dissolved in 10 mL silicone oil and added to three bioreactors. Biomass from a

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single inoclum preparation reactor was distributed unevenly over the three reactors to initial concentrations of 1, 1.2, and 3 $g\,L^{-1}$. The reactors were operated concurrently, under otherwise identical conditions.

Strategy 2: Addition of Supplementary Aroclor® After Fermentation Stalls

Neat Aroclor® 1242 (10 mg) was dissolved in 1 mL silicone oil and added to a bioreactor containing an initial biomass concentration of $X = 1\ g\,L^{-1}$. After 75 h of operation an additional 1 mL of silicone oil containing 10 mg neat Aroclor® 1242 was added to the reactor.

Strategy 3: Addition of Supplementary Biomass After Fermentation Stalls

Neat Aroclor® 1242 (100 mg) was dissolved in 10 mL silicone oil and added to a bioreactor containing an initial biomass concentration of $X = 0.5\ g\,L^{-1}$. After 75 h of operation an additional 10 mL of growth medium containing 0.5 g fresh biomass generated as described above were added to the reactor.

Strategy 4: Addition of Co-Substrates After Fermentation Stalls

Neat Aroclor® 1242 (100 mg) was dissolved in 10 mL silicone oil and added to three bioreactors. Biomass from the inoclum preparation reactor was distributed evenly to achieve initial concentrations of 0.3 $g\,L^{-1}$in all three reactors. The reactors were operated concurrently. After 40 and 65 h, 0.1 g of biphenyl, glucose or sodium pyruvate were added to the reactors.

Strategy 5: Addition of Co-Substrate at Outset

Neat Aroclor® 1242 (100 mg) was dissolved in 10 mL silicone oil and added to two bioreactors. The biomass from the inoclum preparation reactor was distributed unevenly to achieve initial concentrations of 0.01 and 0.3 $g\,L^{-1}$. The initial medium was supplemented with 10 and 7.5 g sodium pyruvate, respectively; providing growth substrate with the intention to investigate the PCB degradation ability of biomass growing on pyruvate from a small inoculum. The reactors were operated concurrently.

PCB Extraction and Analysis

Fermentation samples of 5 mL were extracted three times with 2 mL hexane (4 h of rotary shaker at 180 rpm and 30°C). At the time of extraction samples contained both aqueous phase and silicone oil. Biomass was not removed prior to extraction in order to extract PCBs that might physically be associated with the biomass. Control experiments with initial biomass concentration of 1 $g\,L^{-1}$ which had been inactivated via autoclaving showed no decrease of extractable PCBs over 80 h with a recovery of <95% of the initially added Aroclor® (data not shown). The hexane extract was analyzed with an Agilent 6890 gas chromatograph (Agilent Technologies Canada, Inc., Mississauga, ONT) 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, 180–240°C at 1.5°C min^{-1}, 240°C for 1 min, 240–300°C at 20°C min^{-1}, 300°C for 10 min. Aroclor® standards were run for every analysis and blank hexane was run after every four samples. Aroclor® was quantified by using the summed peak area according to EPA Method 304 h.

Results and Discussion

Strategy 1: Amount of Initial Biomass

Three TPPBs with different initial amount of the degrading organism, grown in the same seed fermentor, were run in parallel to observe the effect of initial active biomass concentration on the rate and extent of degradation. The conditions in all three reactors were identical except for the different amounts of biomass. The rate and extent of the degradation were both affected by the amount of biomass being present in the system, as shown in Figure 1. The

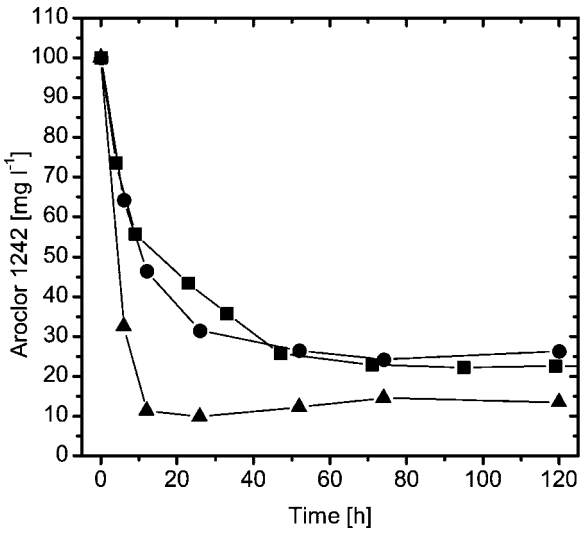

Figure 1. The effect of biomass concentration on the degradation of Aroclor® 1242 by *Burkholderia xenovorans* LB400. The initial biomass concentrations were 1 $g\,L^{-1}$ (squares), 1.2 $g\,L^{-1}$ (circles), and 3 $g\,L^{-1}$ (triangles). The initial amount of Aroclor® 1242 was 100 mg dissolved in 10 mL silicone oil. The PCB concentrations are shown as mg Aroclor® per L aqueous medium and represent an extraction of samples containing representative fractions of aqueous phase, biomass and silicone oil.

amount of Aroclor® 1242 in the reactors is reported as the total amount of PCBs extracted from samples containing representative fractions of aqueous phase, biomass and silicone oil, normalized by the total volume of aqueous phase for comparison purposes. This method of PCB extraction was chosen to ensure that the reported disappearance of PCB is due to biodegradation and not biosorption. The total amount of biomass did not change over the course of the fermentation (data not shown), indicating that no significant fraction of the degraded PCB congeners was used as carbon and energy source by the organism. Most aerobic PCB degraders utilize only monochlorinated congeners for cell growth (Ahmed and Focht, 1973), even though some have the ability to degrade congeners containing up to six chlorines, but presumably not to obtain energy for growth (Bopp, 1986). The volumetric rate at which Aroclor® 1242 was degraded, the slope in Figure 1, increased with an increase in initial biomass present. The effect of biomass concentration on the volumetric rate suggests that the mass transfer rate of PCBs from the delivery phase into the aqueous phase is not the rate-limiting step under the employed conditions. Mass transfer effects have been examined in a related study by varying the agitation rate under otherwise similar conditions (Aroclor® 1242 degradation in biphasic bioreactors) and it was found that the mass transfer rate was not limiting (Rehmann and Daugulis, 2007a). Mass transfer rates can become rate limiting in biphasic systems (Kose et al., 2003), however, the reactor system employed in this study is well mixed. The fact that the degradation rate does depend on the initial biomass concentration shows that the microbial degradation rate and not the mass transfer rate is the rate-limiting step under the employed conditions.

The degradation extent also varied depending on the initial biomass concentration as shown in Figure 1, with PCB degradation "stalling" after approximately 20–50 h. Kohler et al. (1988) also observed a decrease of biphenyl oxidation activity to less than 8% of its original value after 28 h when exposing resting cells of *Acinetobacter* sp. strain P6 and B1B to Aroclor® 1254. It can be concluded from the data in Figure 1 that the initial amount of active biomass dictates the rate and extent of degradation in the employed system. Each unit of biomass seems to have a limited ability to perform PCB transformation which is lost after a certain period of time. Possible reasons for the loss of activity toward PCBs include the depletion of degradable congeners, the formation of toxic by-products and the lack of carbon and energy source required to maintain microbial activity.

Strategy 2: Addition of Supplementary Aroclor® After Fermentation Stalled

The observed PCB residuals after 50 h (seen in Fig. 1) could have been due to the presence of a group of (highly chlorinated) congeners which cannot be degraded by the employed pure species. This however would suggest that

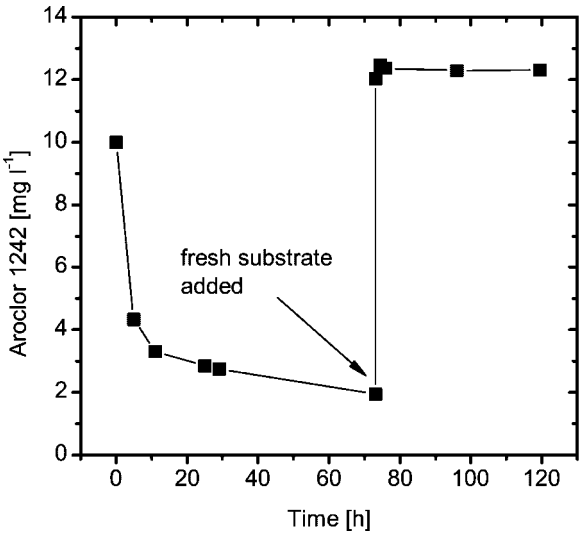

Figure 2. Effect of additional substrate after stall of degradation activity. The initial biomass concentration was 1 g L^{-1} and the initial Aroclor® 1242 concentration in the delivery phase (1 mL) was 10 g L^{-1}. After 75 h an additional 1 mL delivery phase containing 10 g L^{-1} Aroclor® 1242 was added. The PCB concentrations are shown as mg Aroclor® per L aqueous medium and represent an extraction of samples containing representative fractions of aqueous phase, biomass and silicone oil.

activity toward other congeners may still be present. Figure 2 shows the time course of the degradation of 10 mg L^{-1} Aroclor® 1242. Eighty percentage of the initially available PCBs were rapidly degraded within 20 h. The same amount of PCBs was added after 75 h, and no subsequent degradation occurred, despite the fact that a large number of low chlorinated congeners were reintroduced to the system. Degradation intermediates such as chlorobenzoic acids have been observed to accumulate with inhibitory effects on PCB degrading organisms (Seeger et al., 1999) and have therefore often been addressed by co-cultivating known benzoate degraders (Fava, 1996). Chlorobenzoate formations was not monitored, but it is unlikely that inhibitory levels were reached as significantly larger amount of Aroclor® 1242 were degraded under similar conditions as seen in Figure 1. To verify the suitability of the reactor environment for further PCB degradation after extensive incubation time fresh biomass was added to the reactor in Strategy 3.

Strategy 3: Addition of Supplementary Biomass After Fermentation Stalls

The suitability of the reactor environment for further PCB degradation after extensive incubation time was verified by the addition of fresh biomass after the degradation catalyzed by the initial biomass had stopped. A TPPB was inoculated with a relatively small initial amount of biomass (0.5 g L^{-1}). Based on the results shown in Figure 1 it was expected that high amounts of PCBs would remain in the reactor after the

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fermentation stalls, as expected and seen in Figure 3. No further degradation occurred after approximately 40 h when the remaining amount of Aroclor® 1242 was approximately 50 mg L^{-1}. The addition of fresh biomass after 75 h resulted in new degradation activity, which lasted for approximately 40 h (similarly to the activity of the initial biomass) and reduced the Aroclor® 1242 concentration to approximately 25 mg L^{-1}. The fact that fresh biomass showed activity toward the residual PCBs suggests that no inhibitory intermediates accumulated in the reactor medium and shows further that the remaining congeners can be degraded by active cells of *B. xenovorans* LB400, if present in the reactor. The cells present initially remained viable, which was confirmed by streaking out fermentation broth onto agar Petri dishes with biphenyl as the sole carbon source. No significant decrease in the number of colonies from samples during the entire fermentation was observed after 48 h of incubation (data not shown), confirming that viable cells were constantly present in the reactor. The observed inability of the cells to degrade PCBs for an extended period of time might therefore be due to the lack an available carbon and energy source. It can be speculated that in the absence of utilizable carbon source the cell metabolism slows down and PCB degrading enzymes or co-factors might not be re-generated.

Strategy 4: Addition of Co-Substrates After Fermentation Stalls

All reactors described above employed resting cell with no observed cell growth. Resting cell assays are typically used to study pathways and the formation of intermediates, however, for degradation applications growing cells are generally more suitable (Kohler et al., 1988). Actively growing cells might be able to maintain PCB degradation abilities over extended time periods; for example Lambo and Patel (2006) recently showed that the bacterium *Hydrogenophaga* sp. IA3-A maintained biphenyl dioxygenases activity when growing on tryptic soy broth or succinic acid (Lambo and Patel, 2006). The effect of additional carbon sources was investigated by adding 100 mg of three additional carbon source (biphenyl, glucose, and sodium pyruvate) to the reactors after 40 and 65 h of operation. The initial biomass concentrations were 0.3 g L^{-1} in each case which ensured that the degradation would stall while PCB concentrations were still reasonably high. Figure 4 shows that Aroclor® 1242 concentrations were reduced to approximately 75 mg L^{-1} in all three reactors before substrate addition. The addition of biphenyl and glucose could not reinitiate PCB degradation, even though an increase in biomass was observed, most dominantly in the case of added biphenyl. The immediate increase of biomass concentration after the addition of a suitable growth substrate is also an indication of the general viability of the organisms present.

The inability of biphenyl to reinvigorate PCB degradation is surprising as biphenyl naturally posses stronger inducing activity for the biphenyl degradation pathway (*bph*-pathway) than later intermediates (Denef et al., 2005). The fact that the cells are actively growing, as seen by the formation of new biomass, also shows that the *bph*-pathway is active. The lack of resulting PCB degradation might in this case be due

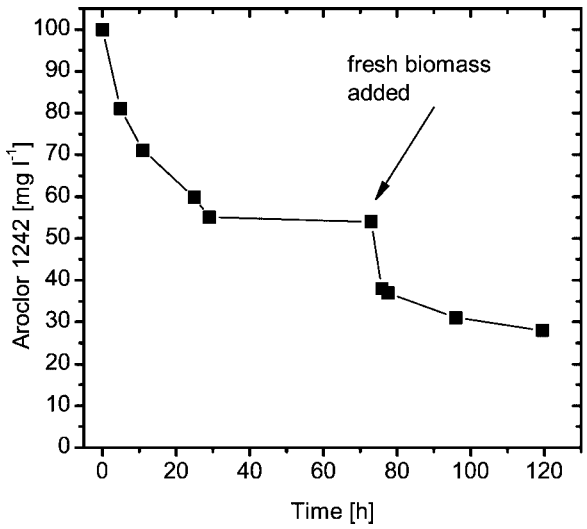

Figure 3. Effect of additional biomass after stall of degradation activity. The initial biomass concentration was 0.5 g L^{-1} and the initial Aroclor® 1242 concentration in the delivery phase was 10 g L^{-1}. After 75 h 0.5 g fresh biomass were added in 10 mL culture medium. The PCB concentrations are shown as mg Aroclor® per L aqueous medium and represent an extraction of samples containing representative fractions of aqueous phase, biomass and silicone oil.

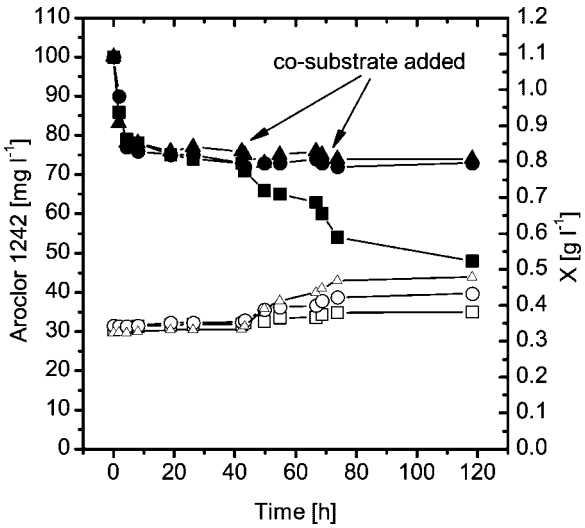

Figure 4. Effect of additional carbon sources after stall of degradation activity. The initial biomass concentration was 0.3 g L^{-1} and the initial Aroclor® 1242 concentration in the delivery phase was 10 g L^{-1}. After 40 and 65 h 0.1 g carbon source were added (triangles, biphenyl; circles, glucose; and squares, sodium pyruvate). The filled symbols represent the Aroclor® 1242 concentration (in mg Aroclor® per L aqueous medium, extracted from samples containing representative fractions of aqueous phase, biomass, and silicone oil) and the white symbols the biomass concentration.

to competition between PCB congeners and biphenyl for available enzymes. Biphenyl is approximately 30 times more soluble in water than Arcolor 1242 (Chou and Griffin, 1986; Paul, 1952), and the concentration difference between biphenyl and PCB congeners in the aqueous phase becomes further enhanced in a TPPB. The octanol/water partitioning coefficient of biphenyl ($\log K_{O/W} = 3.79$; Chou and Griffin, 1986) is substantially lower than the $\log K_{O/W}$ of Aroclor® 1242 congeners, which are reported to be in the range $\log K_{O/W} = 4.4$ and $\log K_{O/W} = 7$ depending on the level of chlorination of the specific congener (Hawker and Connell, 1988; Rapaport and Eisenreich, 1984). The biphenyl concentration in the aqueous phase of a biphasic octanol/water system would therefore be significantly larger than the aqueous phase Arolclor 1242 concentration in an octanol/water system and by analogy it is likely that the aqueous phase availability of biphenyl in the current system is orders of magnitude higher than PCB availability. With this higher concentration of biphenyl in the aqueous phase the *bph* enzymes might be likely to preferably degrade biphenyl over PCB congeners.

The addition of sodium pyruvate also resulted in a moderate increase of biomass, but additionally stimulated PCB degradation. The Aroclor® 1242 concentration was reduced to approximately 60 mg L^{-1} after the first sodium pyruvate addition and then further reduced to approximately 50 mg L^{-1} after the second addition. Pyruvate was chosen as a carbon source because it is a key downstream intermediate in the PCB degradation pathway of *B. xenovorans* LB400 (Seeger et al., 1995, 1997). *B. xenovorans* LB400 forms pyruvate from 4-hydroxy-2-oxovalerate by the *bph*I gene-product after six enzymatic conversions of biphenyl (Denef et al., 2004). Pyruvate does therefore not compete with PCBs for the early enzymes of the *bph*-pathway as biphenyl did. The biphenyl pathway in *B. xenovorans* LB400 is known to be regulated by at least two mechanisms, up-regulation by growth on biphenyl and down-regulation by growth on succinate (and probably other carbon sources; Beltrametti et al., 2001). The expression of the *bph*-genes was initiated during the cell growth on biphenyl, and might be continuously up-regulated, or not down regulated, by the presence of PCBs, while the decrease of degradative activity is most likely due to the fact that the cells have no available carbon or energy source, for example for the production of co-factors. Providing glucose might have had a down-regulating effect of the *bph*-genes as suggested by Beltrametti et al. (2001), resulting in cell growth, but no further PCB degradation as shown in Figure 4. Pyruvate does not seem to have this down-regulating effect and it can be speculated that this is due to its occurrence downstream in the *bph* pathway. It has previously been shown by Master and Mohn (2001) that *B. xenovorans* LB400 expresses the same levels of the *bph*A gene product in the presence of pyruvate as it expresses in the presence of biphenyl, which is consistent with the findings reported in this study. Another example of pyruvate having a similar effect is the biodegradation of naphthalene by *Pseudomonas putida* G7. Lee et al. (2003) showed in a chemostat study that the degradation rates of naphthalene by *P. putida* G7 were enhanced in the presence of pyruvate, while no diauxic growth was found. Pyruvate did not have the expected inhibitory effect on naphthalene degradation by *P. putida* G7 or on PCB degradation by *B. xenovorans* LB400, as shown here, which suggest that it might be of possible advantage in other cases of recalcitrant pollutants.

Strategy 5: Addition of Co-Substrate at Outset

The amount of initial biomass required to degrade 100 mg L^{-1} Aroclor® 1242 with resting cells of *B. xenovorans* LB400 to an appreciable extent is very high (Fig. 1). Growing cells of *Arthrobacter* sp. have been shown to be superior over resting cells in aqueous phase PCB degradation (Kohler et al., 1988). The fact that pyruvate was able to reinitiate PCB degradation by *B. xenovorans* LB400 indicates that it might function as a non-competitive growth substrate during PCB degradation of growing cells, which could allow using smaller inocula sizes. Figure 5a shows the time course of PCB degradation by a large initial biomass concentration

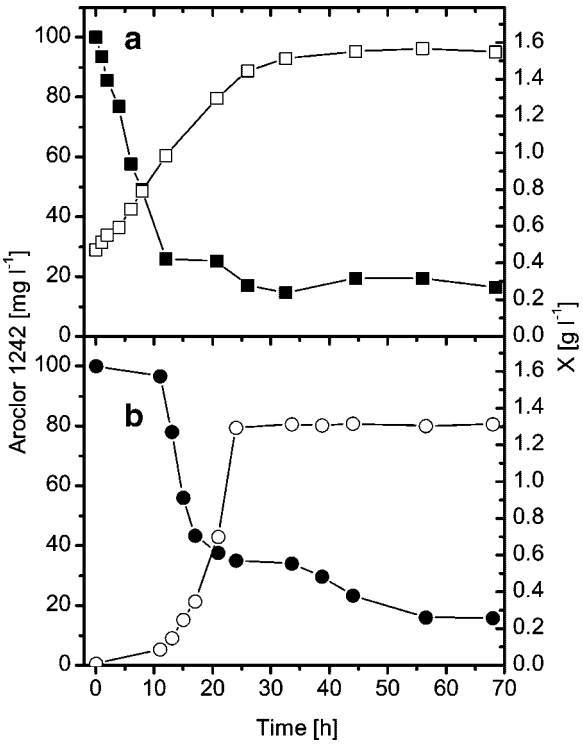

Figure 5. Degradation of Aroclor® 1242 in TPPB by growing cells of *B. xenovorans* LB400. The initial biomass concentration was 0.3 g L^{-1} (**a**) and 0.01 g L^{-1} (**b**). The initial Aroclor® 1242 concentration in the delivery phase was 10 g L^{-1}. The filled symbols represent the Aroclor® 1242 concentration (in mg Aroclor® per L aqueous medium, extracted from samples containing representative fractions of aqueous phase, biomass and silicone oil) and the white symbols the biomass concentration.

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(0.3 g L^{-1}) in the presence of 7.5 g L^{-1} pyruvate. Pyruvate is consumed at a much faster rate than PCBs are degraded and therefore this high amount of pyruvate was chosen to provide the cells with carbon and energy over an extended period of time. The concentration of Aroclor® 1242 was reduced to 25 mg L^{-1} after 10 h and to a final concentration of 15 mg L^{-1} after 70 h. Both rate and extent exceed the performance achieved with similar initial amount of resting cells (Fig. 3), and the residual of PCBs might be an accumulation of highly chlorinated congeners, or due to low bioavailability at these low PCB concentrations. However, the cells were growing during the time PCB degradation occurred, and the cell growth has to be attributed to pyruvate and not to PCBs, as cell concentrations remained constant when degrading similar amounts of PCBs in the absence of pyruvate (Fig. 3).

The addition of pyruvate to a reactor with a relatively high initial biomass concentration may have provided the cells initially present with sufficient energy to allow for extensive PCB degradation. However, it is not clear that the cells resulting from growth on pyruvate would possess *bph* activity. Accordingly, a modified experiment was conducted in which cells growing from an initial biomass concentration of 10 mg L^{-1} were still able to degrade the majority of 100 mg L^{-1} Aroclor® 1242 to a similar final concentration when growing on pyruvate as shown in Figure 5b. The measured PCB concentrations did not change significantly over the first 10 h due to the small amount of active biomass and decreased rapidly once sufficient microbial activity was present in the reactor. The observed activity toward PCBs under these conditions also shows the newly formed cells which grew on pyruvate as the carbon source still maintained their activity toward PCBs and still expressed the *bph*-genes. This metabolic state might either be inherited from the original inoculum or the upper *bph*-genes are constantly up-regulated by the PCBs present.

PCB degradation under both conditions shown in Figure 5 occurred at very high rates. The initial volumetric degradation rate under the conditions employed in Figure 5a can be estimated via linear regression analysis of the first seven data points to be 6.23 g L^{-1} h^{-1} [5.65, 6.81], the values in the brackets giving the upper and lower 95% confidence limits. A process with a similar scope applying soil washing with surfactant solutions followed by PCB biodegradation with *B. xenovorans* LB400 achieved up to 69% degradation after 24 h of the extracted PCBs, which was a mixture of Aroclor® 1242 and the highly chlorinated Aroclor® 1260 (Billingsley et al., 2002). The total amount of PCBs which can be introduced in a surfactant system is limited, however, with the initial PCB concentrations in surfactant solutions employed by Billingsley et al. being below 6 mg L^{-1}, while the biphasic system employed in this study could achieve initial aqueous phase equivalent concentrations of 100 mg L^{-1}. This value could easily be increased by employing a larger solvent fraction or high initial PCB-in-solvent loadings. The degradation rates achieved in this study are higher than the ones achieved by Billingsley et al., but the comparison is inadequate as this study did not employ any Aroclor® 1260. The high Aroclor® 1242 degradation rates achieved in this study suggest that solvent extraction of PCBs followed by biodegradation in TPPBs might be a viable option to remediate PCB contaminated soils under appropriate cultivating conditions.

Conclusions

Under appropriate conditions Aroclor® 1242 can be degraded by *B. xenovorans* LB400 in TPPBs by either resting or growing cells. Resting cells will lose their ability to degrade PCBs, and the addition of fresh PCB congeners cannot revert this, whereas the generation of active biomass can. A simple and effective method to generate active biomass is to provide a suitable carbon source during PCB degradation after initial inoclum growth on biphenyl. A growth substrate has to be chosen to avoid competition with PCBs for degrading enzymes and to avoid down-regulation of the *bph*-pathway; pyruvate was found to fulfill these requirements. High initial PCB concentrations (100 mg L^{-1}) were degraded to 15% of the initial concentration in less than 48 h at initial rates of 6.2 mg L^{-1} h^{-1}, which makes TPPBs following solvent soil extraction of PCBs an attractive option for PCB soil bioremediation.

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