

Bioavailability of PCBs in biphasic bioreactors

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Abstract

Polychlorinated biphenyls (PCBs) are xenobiotic and toxic contaminants of soil and sediment. A possible remediation scheme involves solvent extraction followed by microbial biodegradation in biphasic bioreactors. This study examined the effect of two water immiscible liquid phases on the extent and rate of aerobic Aroclor® 1242 biodegradation by *Burkholderia xenovorans* LB400 under controlled conditions. The immiscible phases were neither toxic nor biodegradable by the employed organism and it was found that reduced aqueous phase availability, caused by the immiscible phases, had a negative effect on the extent and rate of degradation. The initial PCB concentration in the immiscible phase and the nature of the immiscible phase was shown to influence biodegradation. It was further found that under the employed conditions, providing intensive mixing, the microbial degradation rate and not the mass transfer of PCBs from the immiscible phase into the aqueous phase was limiting the degradation process. Despite reduced specific microbial degradation rates in the presence of a water immiscible phase a maximum volumetric degradation rate of $0.44 \text{ mg l}^{-1} \text{ h}^{-1}$ in the presence of 0.1% silicone oil could be achieved. The findings in the study show that microbial degradation of PCBs in biphasic bioreactors might be a suitable technology for the treatment of solvent extracts of contaminated soils.

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1. Introduction

Polychlorinated biphenyls (PCBs) are toxic xenobiotics, manufactured until the late 1970s, and mixtures were commercially sold in North America under the trade name Aroclor®. PCBs had a wide industrial use prior to their ban in 1978 and are now widely distributed contaminants of soil and sediment [1,2]. PCBs are subject to microbial degradation under anaerobic conditions via reductive dehalogenation [3,4] and aerobic conditions via the biphenyl pathway (*bph*-pathway). One of the best studied aerobic PCB degraders is *Burkholderia xenovorans* LB400, which is known to be able to degrade up to hexachlorinated biphenyls [5–7] and was therefore used in this study.

Rates and extents of natural PCB degradation at different sites vary considerably [8] and the extent and rate of degradation depends on the congener composition of the contaminating PCBs (Aroclor® 1242 consists of mainly tri- and tetrachlorobiphenyl and is mainly subject to aerobic degradation, whereas Aroclor® 1260 consists of predominantly hexa- and heptachlorobiphenyl, making it mainly subject to reductive

dechlorination), on the physical parameters at the contaminated site, and on the presence of organisms capable of PCB degradation [2,9]. Another critical parameter is the presence of residual petroleum products which are, due to their widespread use in industrial applications, often found at PCB contaminated sites [8]. These hydrocarbons can form separate phases in soil or sediments and PCBs can dissolve in these non-aqueous phase liquids (NAPL), changing the soil- or sediment-water distribution coefficient of PCBs [10–12].

The negative effect of co-contaminates at PCB contaminated sites has often been attributed to physiological and physiochemical factors. Short chain aliphatic hydrocarbons are significantly more soluble in water than PCB congeners and can solvate the lipids in bacterial cell membranes and thereby alter the permeability of the membrane or destroy its structure [13]. Hydrocarbons also provide a major source of carbon and therefore a selective advantage for hydrocarbon utilizing bacteria, often resulting in an increase of less diverse microorganisms [14].

More recently it has been shown that the aqueous phase PCB concentration might be controlled by equilibrium partitioning of PCBs between oil and water [12,15]. It could further be shown that aqueous phase PCB concentration increased over time in sediments in which the degradation rate of NAPL was faster

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than the degradation rate of PCBs, thus resulting in an increase of PCB availability [15]. Increased PCB availability might be beneficial for biodegradation, but it also results in increased toxicity at the PCB contaminated site, increased bioaccumulation of PCBs in wildlife, as well as increased PCB mobility [16]. Some current studies have therefore focused on reducing PCB availability by adding various sorbents to sediments, reducing the overall toxicity [16–18].

A possible process to remediate PCB contaminated soils is solvent extraction followed by biodegradation in a biphasic reactor environment [9]. Such a reactor would operate according to the two-phase partitioning bioreactor (TPPB) concept, where large amounts of hydrophobic and/or toxic compounds are dissolved in a water immiscible phase before being introduced to a bioreactor, where microbial degradation of these compounds could take place in the aqueous phase [19]. Solvent extraction of soil contaminants followed by biodegradation in TPPBs has been demonstrated successfully for polyaromatic hydrocarbons (PAHs) [20]. Sequestration of PCBs between the aqueous phase and the immiscible phase might however reduce the availability to the degrading organism in a similar way as NAPL co-contamination do in soil environments. It is therefore the objective of this study to investigate the effect of hydrophobic water immiscible phases on the degradability of PCBs under controlled conditions.

2. Materials and methods

2.1. Chemicals

All chemicals used in the fermentation media and the solvents were obtained from either Sigma–Aldrich (Canada) or Fisher Scientific (Canada). Biphenyl, 99% (assay) was obtained from Alfa Aesar (USA) and Aroclor[®] 1242 (CAS Number: 53469-21-9) was obtained from Chromatographic Specialties Inc. (Brockville, Ontario).

2.2. Bacterial strain

Pseudomonas strain LB400 (strain NRRLB-18064), isolated by researchers at General Electric (Schenectady, NY) [5], was obtained from the Northern Regional Research Laboratory (Peoria, IL). The strain has since been re-classified as *B. xenovorans* sp. nov. [21]. Cultivation conditions and maintenance were described previously [22].

2.3. Scintillation vial experiments

A time course of PCB degradation in the absence of an immiscible phase was generated and experiments to investigate the effect of a wide range of immiscible to aqueous phase ratios were conducted in scintillation vials.

2.3.1. Time course of neat Aroclor[®] 1242 degradation

Sterile 20 ml scintillation vials were filled with 0.5 ml sterile medium (mineral salt medium (PAS) [5]). Neat Aroclor[®] 1242 was dissolved in acetone, filter-sterilized (0.22 μm nylon syringe

filter; Fisher Scientific, Canada) and added to the medium to a desired total concentration of 20 mg l^{-1} Aroclor[®] 1242. The vials were kept open in the fume hood for 48 h to allow the acetone to evaporate. *B. xenovorans* LB400 was grown for 24 h at 30 °C on an orbital shaker set at 180 rpm on biphenyl in PAS medium, filtered through sterile glass wool to remove excess biphenyl crystals, centrifuged at 3000 rpm for 20 min at 4 °C, washed twice with 0.05 M sodium phosphate buffer, pH 7.5, and re-suspended in PAS medium to a concentration of 0.6 g l^{-1} . 0.5 ml of these cell suspension was added to each of the vials containing medium and Aroclor[®] 1242, resulting in final biomass concentrations of 0.3 g l^{-1} and Aroclor[®] 1242 concentrations of 10 mg l^{-1} . The vials were closed with Teflon-lined caps and incubated for up to 5 days at 30 °C on an orbital shaker set at 180 rpm. Vials were periodically sacrificed and the cells were killed by addition of 10 μl 1N HCl and the remaining PCBs were extracted three times with 2 ml hexane. Controls vial with no biomass were treated identically.

2.3.2. Effect of presence of an immiscible phase on the extent of PCB degradation

Vials were prepared as described above. In addition to Aroclor[®] 1242 bis(2-ethylhexyl)sebacate (BES) and low viscosity silicone oil (5 cSt) dissolved and filter-sterilized in acetone were added to achieve final equivalent immiscible phase to aqueous phase concentrations between 1 mg l^{-1} and 10,000 mg l^{-1} . All vials were incubated on a rotary shaker at 180 rpm for 5 days, prior PCB extraction and analysis. Experiments were undertaken in duplicate and numbers are reported as the average values.

2.4. Bioreactor experiments

To ensure no physical limitations (e.g. mixing, pH, dissolved oxygen) bioreactors rather than scintillation vials were employed to investigate the effect of the presence of an immiscible phase on PCB degradation rates.

2.4.1. Inoculum preparation for bioreactors

The inoculum was prepared in a single fermentation vessel to generate enough biomass to operate three PCB degradation reactors concurrently with different amounts and types of immiscible phase but otherwise under similar conditions, including identical inocula. *B. xenovorans* LB400 was cultivated in a 5 l New Brunswick BioFlo[®] III bioreactor at 30 °C, agitated with two Rushton turbines at 500 rpm and aerated (sterile air) at 31 min^{-1} . Biphenyl was delivered from Hytre[™] 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; the rationale for, and details of, this procedure are described in detail elsewhere [23]. Cells were harvested in mid exponential phase and the solid immiscible phase was removed by filtration through sterile glass wool. Biomass was removed from culture medium via centrifugation at 3000 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.

2.4.2. Effect of presence of an immiscible phase on the rate of PCB degradation

Rate investigations were conducted in three parallel bioreactors. Aroclor[®] 1242 (10 mg) was dissolved in 1 ml silicone oil (Reactor 1), 1 ml BES (Reactor 2) and 10 ml silicone oil (Reactor 3). Bioreactor 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 aqueous phase volume was 1000 ml (PAS medium with 1.2 g l⁻¹ *B. xenovorans* LB400) and the initial amount of PCBs was 10 mg neat Aroclor[®] 1242 in each reactor. The pH was maintained at 6.9. Samples were taken periodically for PCB and biomass analysis. The agitation rate was increased to 900 rpm prior to sampling to ensure that samples contained representative fractions of both liquid phases. Samples were extracted three times with 2 ml hexane and analyzed via GC-ECD.

2.4.3. Effect of agitation rate in the presence of an immiscible phase on the rate and extent of PCB degradation

The effect of the agitation rate on microbial degradation was investigated in three parallel bioreactors. Aroclor[®] 1242 (10 mg) was dissolved in 10 ml silicone oil each and the agitation rate in the individual reactors were $n=200, 400$ and 600 rpm. The reactors were operated as described above.

2.5. PCB analysis

Samples were extracted three times with 2 ml hexane. The hexane extract was analyzed with an Agilent 6890 gas chromatograph (Agilent Technologies Canada Inc., Mississauga, Ontario) equipped with a fused silica capillary column (Supelco SPB-1, Sigma–Aldrich Corp., St. Louis, MO, USA), 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.

3. Results and discussion

3.1. Scintillation vial experiments

3.1.1. Time course of neat Aroclor[®] 1242 degradation

The time course for the degradation of neat Aroclor[®] 1242 in scintillation vials was determined to establish a benchmark degradation time to subsequently study the effect of the presence of an immiscible organic phase on the extent of Aroclor[®] 1242 degradation. The time course is shown in Fig. 1, and the achieved extent and rate are similar to degradation data reported elsewhere (~80% of Aroclor[®] 1242 at 10 mg l⁻¹ after 48 h) [24]. It can be seen that the initial degradation was rapid and no further degradation occurred after 48 h. The reduction of detectable Aroclor[®] is due to biodegradation and not due to reduced extractability or sorption to the biomass, as can be seen by the constant

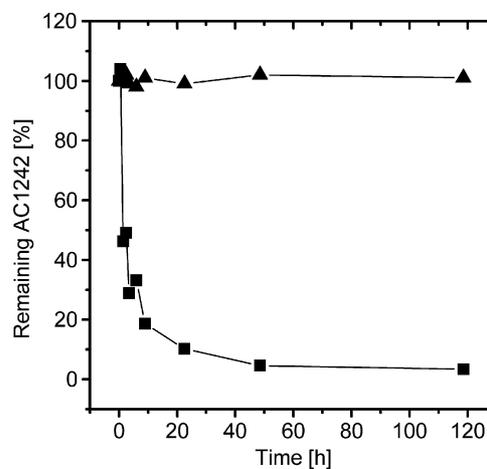


Fig. 1. Biodegradation of Aroclor[®] 1242 by *Burkholderia xenovorans* LB400 in scintillation vials at an initial biomass concentration of 0.3 g l⁻¹ in PAS medium with no biphenyl (squares). The triangles show the Aroclor[®] 1242 in a control experiment with killed biomass.

PCB levels in the presence of deactivated biomass (triangle in Fig. 1).

3.1.2. Effect of presence of an immiscible phase on the extent of PCB degradation

The applicability of the selected solvents for a possible soil extraction step of a PCB remediation process train was not a criterion during solvent selection for this experiment. Low viscosity, easy recovery and high affinity for the target compounds, among others, would be desired characteristics for such use. This experiment however investigates the effect of the affinity of the selected solvent towards the target molecules during the biodegradation step in a TPPB. Therefore two immiscible phases were chosen based on their different affinities for PCBs and on their compatibility with the employed organism; the organic solvent BES, a di-ester which is characterised by a very high octanol/water partitioning coefficient ($\log K_{O/W} = 10.08$) [22] and silicone oil, which is expected to have a significantly lower affinity for PCB congeners than most hydrophobic organic solvents [25]. Both immiscible phases are neither toxic to *B. xenovorans* LB400 nor can they be used as a carbon and energy source (data not shown). BES has previously been used as the delivery phase for rapid degradation of large amounts of biphenyl to *B. xenovorans* LB400 in two-phase partitioning bioreactors [22].

Different amounts of both immiscible phases were added to vials containing Aroclor[®] 1242 prior to the addition of the degrading organism. It was expected that Aroclor[®] 1242 would sequester between the two phases and that the equilibrium concentration in the aqueous phase would be affected by both the amount and nature of the immiscible phase. The aqueous phase PCB fraction is the only fraction available to the degrading organism and PCB degradation in the aqueous phase will result in partitioning of fresh PCBs from the immiscible phase into the aqueous phase to an extent which is dependent on the amount and type of the immiscible phase.

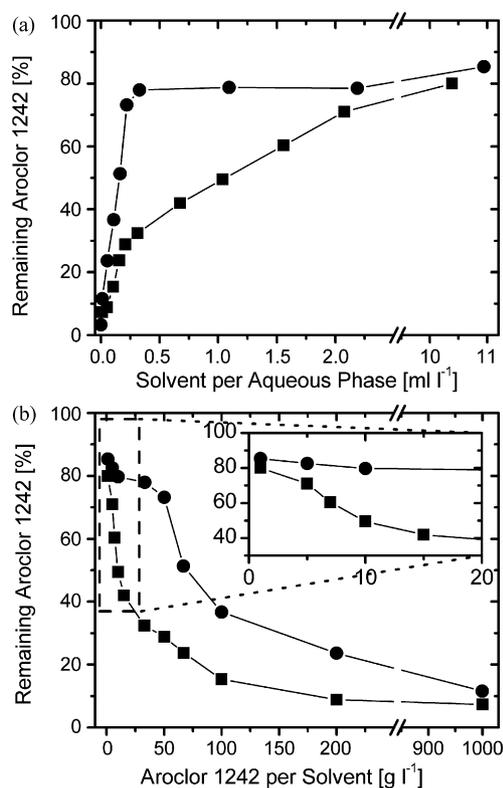


Fig. 2. Effect of different amount of immiscible phase (squares = silicone oil, circles = BES) on the achievable extent of Aroclor[®] 1242 (10 mg l^{-1}) degradation by *B. xenovorans* LB400. (a) and (b) are different plots of the same data. Plot (a) highlights the direct effect of increased immiscible phase fractions and plot (b) highlights the effect of initial PCB concentrations in the immiscible phases, important for PCB degradation process design.

Fig. 2 shows the effect of increased immiscible phase fraction on the extent of degradation after 5 days. While under the given conditions 10 mg l^{-1} PCBs were degraded almost to completion if provided directly in the aqueous phase (Fig. 1), increases in the fraction of the immiscible phases resulted in increased residual PCB concentrations, as shown in Fig. 2a. The addition of BES had a stronger effect than the addition of silicone oil due to the higher affinity of BES for PCBs and the corresponding lower PCB concentration in the aqueous phase, which has been shown to singularly govern the maximum dechlorination rate of Aroclor[®] 1242 under anaerobic conditions [10]. The aqueous phase biphenyl concentration and the possibility of influencing it with different volumes of an immiscible phase has also been shown to determine the degradation rate of biphenyl by *B. xenovorans* LB400 [22]. The aqueous phase PCB concentration has not been measured directly because of experimental difficulties as described by Chou and Griffin [26], however Luthy et al. [11] have shown that the aqueous phase Aroclor[®] 1242 concentration is reduced to approximately 20% of its original value if the hydraulic oil Fryquel 220 is present at an Aroclor[®] to Fryquel ratio of 50 g l^{-1} .

A linear relationship between the amount of BES present in the system and the achieved Aroclor[®] 1242 degradation can be seen in Fig. 2a until the BES fraction reaches 0.3 ml l^{-1} . In the presence of larger amounts of solvent, only 15–20% of

the initially available Aroclor[®] 1242 congeners were degraded. The effect of silicone oil on the extent of PCB degradation was substantially weaker than the effect of BES. At high silicone oil concentrations only 15–20% of the initially available Aroclor[®] 1242 congeners were degraded, similar to the results with BES. However, more than 20% degradation could be observed for silicone oil contents $< 2.1 \text{ ml l}^{-1}$ compared to similar degradation extents for BES contents $< 0.3 \text{ ml l}^{-1}$.

Plotting the same data in a different way (Fig. 2b) clearly shows that high initial PCB concentrations in the given immiscible phases are required for extensive biodegradation to occur. At low initial concentrations only 15–20% of the initially available Aroclor[®] 1242 congeners were degraded. Initial concentrations in BES have to be approximately 10 times higher than the initial concentrations in silicone oil to achieve similar degrees of degradation; for example the initial Aroclor[®] 1242 concentration of 7 g l^{-1} in silicone and 66 g l^{-1} in BES both result in approximately 50% degradation. This relationship can be seen only if the immiscible phase fraction is large enough to dissolve the available Aroclor[®]. At higher equivalent Aroclor[®] to immiscible phase ratios ($> 100 \text{ g l}^{-1}$) both aqueous phase and solvent are saturated with PCBs and a free Aroclor[®] phase is present. Under these conditions the type of immiscible phase does not have a strong effect.

The absolute numbers presented in Fig. 2 represent only the extent of degradation achieved under the given conditions (i.e. closed scintillation vials). The data presented in Fig. 2 show that under the given conditions even small fraction of the immiscible phase will severely limit the extent of PCB degradation. It was hypothesized that the limited extent of degradation in these closed systems was due to low degradation rates caused by low substrate levels, which was further investigated in bioreactor experiments.

3.2. Bioreactor experiments

3.2.1. Effect of presence of an immiscible phase on the rate of PCB degradation

The data presented in Fig. 2 do not give any information about degradation rates. To obtain degradation rates, bioreactors rather than scintillation vials were employed. As noted, bioreactors are expected to provide enhanced degradation conditions compared to the previously discussed scintillation vial experiments. The initial amount of Aroclor[®] 1242 per volume aqueous phase was maintained at 10 mg l^{-1} , while the initial biomass concentration was increased from 0.3 g l^{-1} to 1.2 g l^{-1} in comparison to the scintillation vial experiments. Fig. 3a shows the time course of PCB degradation in bioreactors in the presence of different amounts of immiscible phases (BES or silicone oil). Maintaining the total amount of PCBs at 10 mg l^{-1} and the initial PCB concentration in the solvents high, as suggested by the results shown in Fig. 2, resulted in solvent:aqueous phase ratio of 1:100 and 1:1000, while typical ratios employed in TPPBs are 1:10–1:4 [19]. Increasing the solvent:aqueous phase ratio while maintaining the PCB concentration in the immiscible phase constant would substantially increase the total amount of PCBs in the system, and exceed the scope of this study. The three reac-

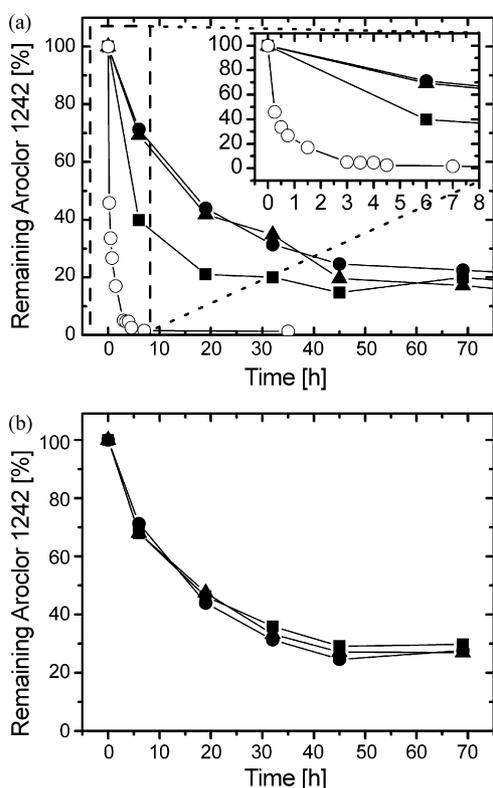


Fig. 3. Time course of Aroclor[®] 1242 (10 mg l^{-1}) degradation by *B. xenovorans* LB400 ($X_0 = 1.2 \text{ g l}^{-1}$) in BioFlo[®] I bioreactors. The effect of different amounts and types of immiscible phase (a): Reactor 0, no second phase (open circles); Reactor 1, 1 ml silicone oil (squares); Reactor 2, 1 ml BES (triangles); Reactor 3, 10 ml silicone oil (circles). Reactors 1 and 2 provided an effective initial Aroclor[®] concentration in the immiscible phase of 10 g l^{-1} and Reactor 3 of 1 g l^{-1} . The effect of agitation rate (b): 200 rpm (squares), 400 rpm (circles), 600 rpm (triangles). The immiscible phase in all three reactors was 10 ml silicone oil.

tors containing a second phase were operated concurrently and the initial biomass for the three reactors was grown in the same seed reactor, while the control reactor containing no solvent was inoculated separately. The biomass concentration did not change during the course of the experiment due to the small amount of available carbon source (data not shown).

The extent of degradation in all three reactors substantially surpassed the extent that was achieved at similar initial Aroclor[®]

1242 concentration in the immiscible phase in scintillation vials (Fig. 2). Reactors 1 and 2 provided an effective initial Aroclor[®] concentration in the immiscible phase of 10 g l^{-1} and Reactor 3 of 1 g l^{-1} . Similar initial PCB concentrations in the immiscible phase in scintillation vials as in Reactor 1 resulted in approximately 60% degradation, and initial PCB concentrations in the immiscible phase similar to those in Reactors 2 and 3 resulted in only 20% degradation. The rate and extent of degradation in the absence of an immiscible phase (Reactor 0) is substantially higher than in reactors containing any of the tested immiscible phase fractions. The rate is also higher than in the scintillation vial experiments (Fig. 1), mainly due to the above-mentioned increase of biomass.

To better compare reactor performances degradation rates were estimated and are presented in Table 1. Volumetric rates were calculated as the amount of PCB degraded per reactor volume and time required until no further degradation occurred ($\text{mg l}^{-1} \text{ h}^{-1}$). These volumetric rates were also normalized with respect to the initial biomass concentration to obtain specific rates ($\text{mg g}^{-1} \text{ h}^{-1}$). The volumetric rates are commonly used to assess the reactor performance, whereas the specific rates reflect on the performance of the catalyst/biomass. The volumetric degradation rates are very high, and in the case of Reactor 1 the volumetric rate of $0.44 \text{ mg l}^{-1} \text{ h}^{-1}$ is similar to the rate of $0.48 \text{ mg l}^{-1} \text{ h}^{-1}$ that was achieved in scintillation vials with pure Aroclor[®] 1242 (Fig. 1), however it is substantially lower than volumetric rate of $1.96 \text{ mg l}^{-1} \text{ h}^{-1}$ that was achieved under similar conditions in a bioreactor in the absence of a immiscible phase (Reactor 0). The specific rate in the reactor however is only 23% of the specific rate in the scintillation vials (Table 1), while the specific rates in scintillation vials and Reactor 0 are identical. The similarity of the volumetric rates in Reactor 1 and in the scintillation vials shows that high biomass concentration can compensate for the reduced substrate availability arising from a presence of the immiscible phase, which is the reason for the low specific rates. The fact that the specific substrate degradation rates (σ) of the bioreactor containing no immiscible phase (Reactor 0, $\sigma = 1.63 \text{ mg g}^{-1} \text{ h}^{-1}$) and the scintillation vials ($\sigma = 1.60 \text{ mg g}^{-1} \text{ h}^{-1}$) are within 3% of each other shows further that enhanced bioreactor aeration, agitation and pH control did not have a significant effect on the degradation of small amounts of PCBs. The fact that the biocatalyst performed identically in

Table 1
Performance parameters for different PCB degradation conditions

Reactor	X_0 (g l^{-1})	Volumetric degradation rate ($\text{mg l}^{-1} \text{ h}^{-1}$) ^a	Specific degradation rate ($\text{mg g}^{-1} \text{ h}^{-1}$) ^a	Relative specific rate (%) ^b	Extent (%)	Total time (h)
Vials	0.3	0.48	1.60	100.0	96	20
Reactor 0	1.2	1.96	1.63	102.1	98	5
Reactor 1	1.2	0.44	0.36	22.7	87	20
Reactor 2	1.2	0.17	0.14	8.8	81	48
Reactor 3	1.2	0.18	0.15	9.3	86	48

The initial amount of Aroclor[®] was 10 mg l^{-1} with respect to the aqueous phase volume under all conditions. The vials and Reactor 0 contained no immiscible phase. Reactor 1 contained 1 ml silicone oil with an initial Aroclor[®] concentration of 10 mg l^{-1} , Reactor 2 contained 1 ml BES with an initial Aroclor[®] concentration of 10 mg l^{-1} and Reactor 3 contained 10 ml silicone oil with an initial Aroclor[®] concentration of 1 mg l^{-1} .

^a Based on extent and time shown in this table.

^b Percentage of the specific rate in scintillation vials.

both system (vials and bioreactor) also allows direct comparison of results achieved in scintillation vials and bioreactors, under consideration of the different amounts of biomass. The observed lower extents of degradation in vials containing similar amounts of immiscible phase as Reactors 1–3 can therefore be contributed to the smaller initial amount of biomass.

The volumetric rates in Reactors 2 and 3 are lower than in Reactor 1, as expected. Reactor 2 was expected to result in a lower aqueous phase PCB concentration than Reactor 1 due to the higher PCB affinity of BES compared to silicone oil. Reactor 3 provides a lower aqueous phase PCB concentration due to the larger volume of the immiscible phase. The conditions provided in Reactors 2 and 3 still allow the degradation of >80% of the initially available Aroclor® 1242 congeners within 48 h; however the specific degradation rate drops to less than 10% of the rate achieved in the two single phase systems (scintillation vials and Reactor 0). The specific microbial degradation rate is a direct function of the aqueous phase substrate concentration, which is determined by the type and amount of immiscible phase that is present. The low specific rates however do not obviate the possibility of degrading PCBs in the presence of an immiscible phase as seen in the extents of degradation and in the volumetric rates at high biomass concentrations.

3.2.2. Effect of agitation rate in the presence of an immiscible phase on the rate and extent of PCB degradation

The observed decrease of volumetric and specific degradation rates when the amount of immiscible phase was increased shows that, under the given conditions, it is not the dissolution rates of Aroclor® 1242 but slow microbial kinetics caused by low aqueous phase substrate concentration that are rate limiting. This was also confirmed by varying the agitation rate in the bioreactors from 200 rpm to 600 rpm while keeping all other parameters identical (Fig. 3b). It can clearly be seen that moderate variations of the agitation rate have no effect on the rate and extent of microbial PCB degradation, showing that the microbial rate and not the mass transfer rate is limiting the degradation process under the employed reactor conditions. Similar results were found by Zwiernik et al. for achievable PCB dechlorination rates under anaerobic conditions [10]. In contrast Kose et al. [27] showed that the dissolution rate of PAHs from a NAPL and not the aqueous phase concentration can be the rate limiting step under conditions simulating oil spills on tidal flats. Those conditions did, due the objective of their study, not provide extensive mixing of the two phases. The intensive mixing in the bioreactors employed in this study results in the formation of a fine emulsion with large surface area for mass transfer to occur. It has been shown previously that under these circumstances instantaneous equilibrium conditions exist for a substance partitioning between an immiscible phase and aqueous media [22], which is substantially different from environmental condition as simulated by Kose et al. [27]. It can therefore be concluded that conditions created in biphasic bioreactors can be used to overcome mass transfer limitations during PCB biodegradation. Low aqueous phase PCB concentrations however can limit the microbial degradation rate and high initial PCB concentrations as well as high initial biomass concentrations can be used to overcome this.

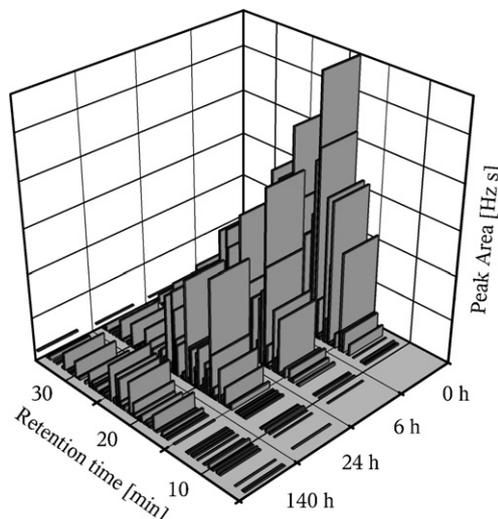


Fig. 4. Chromatograms (shown as peak area vs. retention time) of Aroclor® 1242 during biodegradation by *B. xenovorans* LB400 in the presences of BES (Reactor 2), showing the qualitative change in congener distribution.

The observed PCB disappearance is due to microbial activity, as control experiments in which the biomass was inactivated with hydrochloric acid prior to the incubation with Aroclor® in scintillation vials showed no significant degradation (Fig. 1). Fig. 4 shows chromatograms of samples at different incubation times from Reactor 2. It can clearly be seen that the congener distribution changes over the fermentation time, with faster degradation of lower chlorinated congeners (lower chlorinated congeners have typically lower retention times [28]), a sign of biodegradation [2]. Low chlorinated congeners are more susceptible to aerobic biodegradation than highly chlorinated congeners due to less steric hindrance by the chlorines. Also, low chlorinated congeners are less hydrophobic as shown in lower octanol water partitioning coefficients [29], which results in higher availability of those congeners in the aqueous phase.

The fact that Aroclor® 1242 could be degraded, if provided at initial concentrations of 1 g l^{-1} (Reactor 3) in silicone oil as the immiscible phase is important for possible engineered PCB biodegradation schemes, as concentrations of 1 g l^{-1} in the extract can be achieved via solvent extraction [9]. The results presented in this study show that the choice of this PCB-containing water immiscible liquid can have a profound impact on substrate availability to degrading organisms in the aqueous phase. For example a 10-fold lower initial PCB concentration in silicone oil was sufficient to achieve similar rates and extents of degradation compared to using BES (Table 1). In practice, this would mean that while choosing a solvent for PCB soil extraction or a solvent to reduce viscosity of a soil extract, the effect of this solvent on the aqueous phase concentration during biodegradation in a biphasic reactor environment should be taken into consideration. Solvents which result in high extraction efficiency during soil extraction may result in low aqueous phase concentrations during a possible subsequent biodegradation step. However, high initial PCB concentrations in the immiscible phase and high initial biomass concentrations can counteract the effect of low aqueous phase availability on vol-

umetric biodegradation rates to some extent, as shown in this study.

4. Conclusions

The aqueous phase PCB concentration is responsible for the rate and extent of degradation under laboratory conditions in the presence of an immiscible organic phase. The aqueous phase concentration is dependent on the nature and volume of the immiscible phase. The presence of an immiscible organic phase can be rate limiting during aerobic PCB degradation via the *bph*-pathway (this study), similar to what was found for PCBs in the presence of NAPL in a soil environment during anaerobic PCB dechlorination [10]. The rate of PCB release from the immiscible phase into the aqueous phase was, under the conditions in this study, not limiting the degradation rate. However, it could be limiting under conditions present in a soil NAPL environment. Low chlorinated PCB mixtures such as Aroclor® 1242 can be degraded in a biphasic reactor environment if provided at sufficiently high initial concentrations in the immiscible phase. This finding shows that TPPBs might be a suitable technology for the treatment of PCBs extracted from soil with organic solvents.

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