

# Polymer Selection for Biphenyl Degradation in a Solid–Liquid Two-Phase Partitioning Bioreactor

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The commercially available thermoplastic polymer Hytrel was selected as the delivery phase for the hydrophobic model compound biphenyl in a solid–liquid two-phase partitioning bioreactor (TPPB), and 2.9 g biphenyl could successfully be degraded in 1-L TPPBs by a pure culture of the biphenyl-degrading bacterium *Burkholderia xenovorans* LB400 in 50 h and by a mixed microbial consortium isolated from contaminated soil in 45 h. TPPBs consist of an aqueous cell-containing phase and an immiscible second phase that partitions toxic and/or poorly soluble substrates (in this case biphenyl) on the basis of maintaining a thermodynamic equilibrium. This paper illustrates a rational strategy for selecting a suitable solid polymeric substance for the delivery of the poorly water-soluble model compound biphenyl. The partitioning of biphenyl between the selected polymers and water was analogous to partitioning of solutes between two immiscible liquid phases. The partitioning coefficients varied between 180 for Nylon 6.6 and 11,000 for Desmopan, where the later numerical value is comparable to biphenyl partitioning coefficients between water and organic solvents. Employing a solid delivery phase enabled the utilization of a surfactant-producing microbial mixed culture, which could not be cultivated in liquid–liquid TPPBs and thereby extended the range of biocatalysts that can be employed in TPPBs.

## 1. Introduction

Two-phase partitioning bioreactors (TPPBs) consist of a biocatalyst-containing aqueous phase and an immiscible second phase (1). The second phase functions typically as either a product sink removing potentially toxic products from the aqueous phase (2) or a substrate reservoir delivering substrates at low concentrations to the aqueous phase (3, 4). The second phase typically has a larger affinity for the target compounds, allowing in the case of substrate delivery the loading of large amounts of poorly water-soluble substrate into the second phase, thereby making TPPBs an excellent technology platform for the destruction of hydrophobic xenobiotics (4). The resulting equilibrium concentration in the aqueous phase is the only substrate available to the biocatalyst, and degradation of this available substrate will yield a disequilibrium, which in turn results in partitioning of additional substrate from the second phase into the aqueous phase, ideally allowing complete degradation of all substrate present in the reactor.

The second phase is typically an organic solvent (2, 5, 6), but examples of aqueous–aqueous two-phase systems (7), a cloud point system (8), and TPPBs employing ionic liquids (9) can also be found in the literature. More recently solid polymers have been used to replace the second liquid phase in TPPBs (10). It was found that small organic compounds can partition between an aqueous medium and a variety of thermoplastic polymers in the same way as between aqueous medium and immiscible organic solvents, and these polymers can therefore be used to replace organic solvents in TPPBs (10–12). The polymeric phase can theoretically be molded into any shape of

interest, but typically cylindrical or spherical beads with diameters of between 2 and 5 mm have been employed to date.

The various operational advantages over organic solvents are mainly due to the polymers' resistance to microbial degradation and their lack of cell toxicity. Furthermore, some organisms capable of degrading hydrophobic compounds tend to secrete biosurfactants to increase the availability of the hydrophobic substrate (13). In liquid–liquid TPPBs this can result in emulsification of the organic phase causing considerable operational difficulties (5), which would not occur in a solid–liquid TPPB. This enhanced compatibility of polymers extends the range of possible biocatalysts that can be used in TPPBs, in particular allowing the use of microbial consortia. This can be of importance for degrading persistent contaminants and mixtures of contaminant such as polycyclic aromatic hydrocarbons (PAHs) and polychlorinated biphenyls (PCBs). Examples of TPPBs employing organic solvents and microbial consortia are rare due to the difficulty of finding solvents that are non biodegradable by all members of the microbial consortium. The choice of the organic phases is therefore essentially limited to a few relatively microbial resistant compounds such as silicone oil (14) or the branched alkane 2,2,4,4,6,8,8-heptamethylnonane (HMN) (15).

To date various papers have studied the ability to use solid–liquid TPPBs to reduce aqueous-phase substrate and product concentrations below inhibitory levels (11, 16); however, the target compounds in all reported cases were moderately water-soluble substances. To be more generally applicable to the partitioning of a wider range of compounds, the performance of a solid–liquid TPPB would need to be confirmed for poorly water-soluble and crystalline substances. This paper describes the delivery of large quantities of biphenyl to degrading organisms in a solid–liquid TPPB. Biphenyl was chosen as a

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model compound that has successfully been degraded in liquid–liquid TPPBs (17), and which shares properties such as hydrophobicity with priority contaminants such as PAHs and PCBs.

It was the objective of this study to rationally select a polymer suitable for the degradation of the model compound biphenyl in a TPPB process and to demonstrate the enhanced spectrum of biocatalysts compatible with solid–liquid systems by employing a pure strain as well as a microbial consortium for the degradation of biphenyl.

## 2. Materials and Methods

All nutrients used in the fermentation media and solvents were obtained from either Sigma-Aldrich (Canada) or Fisher Scientific (Canada). Biphenyl (99%, assay) was obtained from Alfa Aesar (USA). The suppliers of the various polymers used in this study are listed in Table 3.

**2.1. Biocatalyst Selection.** *Pseudomonas* strain LB400 (strain NRRLB-18064), isolated by researchers at General Electric (Schenectady, NY), was obtained from the Northern Regional Research Laboratory (Peoria, IL). The strain has since been reclassified as *Burkholderia xenovorans* sp. nov (18). Cultivation conditions, medium formulation, and biomass analysis can be found elsewhere (17, 19).

A microbial consortium was isolated via selective enrichment from soil contaminated with a variety of petrochemicals including biphenyl. Mineral salt medium (50 mL) was spiked with 0.5 g L<sup>-1</sup> biphenyl, inoculated with 1 g of soil, and incubated on a rotary shaker (180 rpm) at 30 °C or 72 h. Samples of 0.1 mL were subsequently transferred to fresh medium (total of 10 transfers) to enhance the likelihood that only biphenyl-degrading organisms were left in the consortium.

**2.2. Delivery Phase Selection.** The biocompatibility of various polymers and organic solvents was tested by inoculating 125-mL shake flasks containing 50 mL of mineral salt medium (19) and 1 g of polymer or solvent with the degrading organisms in the absence of any additional carbon source. The polymer/solvent was considered bioavailable if the biomass concentration increased relative to a control after 5 days. Similarly shake flasks were incubated in the presence of 0.1 g of biphenyl. Lack of biomass formation after 5 days was attributed to toxicity of the delivery phase.

**2.2.1. Partitioning of Biphenyl between Water and Solid Phase.** The partitioning coefficient of biphenyl between biocompatible polymers and water was characterized to further identify the suitability of the selected polymers as a biphenyl delivery phase. Scintillation vials were filled with 10 mL of water and 0.2 g of the respective polymer. Biphenyl was dissolved in methanol at 66 g L<sup>-1</sup> and 2–250  $\mu$ L of this solution was added to the vials containing water and polymers. Biphenyl that is dissolved in methanol forms fine crystals once methanol dissolves in water. The initial amounts of biphenyl in the vials were above the solubility of biphenyl in water but were reduced below this level after equilibrium partitioning occurred; otherwise the vials were discarded and the polymer was tested with lower initial biphenyl concentrations. It was assumed that the small fractions of methanol did not affect the interaction of biphenyl with either polymer or water. Biphenyl concentrations in the aqueous phase were measured with an Ultraspec 3000 UV–vis spectrophotometer (Biochrom, UK) at  $\lambda = 250$  nm after agitating the vials for 5 days on a rotary shaker at 180 rpm at 30 °C to allow equilibrium to occur. The corresponding equilibrium concentration in the polymer was calculated via mass balance.

**2.2.2. Loading of Delivery Phase with Biphenyl.** In order to effectively transfer large amounts of biphenyl into polymers, methanol was used for its ability to dissolve substantial amounts of biphenyl. The partitioning behavior of biphenyl between methanol and the selected polymers was characterized to allow effective loading of the polymers with biphenyl and to be able to calculate biphenyl concentrations in polymers after methanol extraction during bioreactor operation. Scintillation vials were filled with 5 mL of methanol containing different initial concentrations of biphenyl and 2 g of polymers. The final equilibrium biphenyl concentration in methanol was measured after an incubation period of 5 days on a rotary shaker at 180 rpm and 30 °C, and the amount of biphenyl loaded into the polymer was calculated via mass balance. Control vials with no polymers were treated the same way.

**2.2.3. Release of Biphenyl into Aqueous Medium.** With the aim of determining the extent to which the selected polymer beads would actually release the loaded biphenyl into aqueous medium, polymers were loaded with different concentrations of biphenyl as described above (2.2.2), followed by equilibration with 10 mL of water on a rotary shaker for 5 days and photometric biphenyl determination in the aqueous phase. Methanol remaining on the polymer surface was removed by rinsing with water prior the equilibration. Removing methanol by water washing can be justified by the fact that the polymers had no affinity for methanol and methanol from the polymer surface could easily be removed with water while removal of poorly water-soluble biphenyl could be neglected.

**2.3. Biodegradation of Biphenyl in Solid–Liquid TPPBs: Proof of Concept.** As a final demonstration of the enhanced applicability of solid–liquid TPPBs, the biodegradation of a large amount of biphenyl as a poorly water-soluble model compound was shown by a pure microbial strain as well as by a surfactant-producing microbial consortium. Biodegradation of biphenyl in solid–liquid TPPBs was undertaken in parallel by *B. xenovorans* LB400 and a microbial consortium in two 1.5-L New Brunswick BioFlo I bioreactors, agitated each with two Rushton turbines at 600 rpm and aerated (sterile air) at 1 L min<sup>-1</sup>. The pH was maintained at 6.9. The aqueous phase volume was 1 L, and the delivery phase consisted of 50 g of Hytrel polymer beads at an initial biphenyl loading of 58 g kg<sup>-1</sup>. The beads were loaded with biphenyl from methanol in 1-L shake flasks, and the remaining methanol was removed with water prior the introduction of Hytrel to the bioreactors. Inocula for the two reactors were grown for 24 h on biphenyl crystals as the sole carbon source in mineral salt medium. Biphenyl crystals were removed via filtration through sterile glass wool, and the inocula sizes were adjusted to obtain initial biomass concentrations in the bioreactors of 10 mg L<sup>-1</sup>. Samples of the aqueous phase were taken periodically for biomass analysis, as were samples of the polymers. Biphenyl concentrations could not be measured directly from the polymers. The concentrations in the polymers were obtained via mass balance after equilibration of approximately 0.1 g of polymer with 10 mL of methanol and photometric biphenyl analysis in methanol. The polymer methanol partitioning coefficient of biphenyl was obtained before as described in section 2.2.2.

## 3. Results and Discussion

**3.1. Biocatalyst Selection.** Two different biocatalysts were chosen, one a pure strain and the other a microbial consortium, to demonstrate that biodegradation of poorly water-soluble substrates would be readily achieved with either type of cell system. The biphenyl and PCB degrader *B. xenovorans* LB400,

**Table 1. Properties of Solvents Typically Used in Liquid–liquid TPPBs and Their Biocompatibility with the Selected Biphenyl Degrading Consortium<sup>a</sup>**

solvent	log $K_{S/W}$	bioavailable	toxic	used in TPPBs (ref)
BES	4.39 <sup>b</sup>	+	–	(17)
octadecene	4.17 <sup>b</sup>	+	–	(17)
dodecane	4.26 <sup>b</sup>	+	–	(20)
decane	4.28 <sup>c</sup>	–	+	(20)
HMN		–	–	(14)
oleyl alcohol		+	–	(21)
silicone oil		–	–	(22)
hexadecane	4.22 <sup>c</sup>	+	–	(3)

<sup>a</sup> All solvents except dodecane and decane were compatible with *B. xenovorans* LB400. Log  $K_{S/W}$  represents the solvent water partitioning coefficient of biphenyl for the respective solvent. <sup>b</sup> Reference 17. <sup>c</sup> Reference 23.

with the ability to degrade biphenyl in liquid–liquid TPPBs and which has been characterized in previous work (17), and a biphenyl-degrading microbial consortium that was isolated from soil were the selected biocatalysts. The consortium was capable of degrading biphenyl as the sole carbon source if provided as crystals in mineral salt medium. Denaturing gradient gel electrophoresis (DGGE) revealed that the consortium was composed of at least five different species (data not shown).

**3.2. Delivery Phase Selection.** Liquid delivery phases commonly used in TPPBs were also evaluated to establish a benchmark for the polymers. However, silicone oil and HMN were the only delivery phases that were neither toxic nor bioavailable to the microbial consortium, as shown in Table 1. The pure strain *B. xenovorans* LB400 is compatible with a variety of organic solvents, as reported elsewhere (17). Biphenyl degradation by the microbial consortium in the presence of silicone oil or HMN was accompanied with strong biosurfactant production, which resulted in emulsification of the delivery phase. Similar problems have also been reported with surfactant-producing pure strains (5). A simple separation of the two phases of a TPPB is required if recycling of the delivery phase is desired and also for analytical purposes. Liquid–liquid systems were therefore not considered to be suitable for the employed microbial consortium.

As a guide for selecting a suitable delivery phase for a solid–liquid TPPB, a list of desirable characteristic of the delivery phase is proposed in Table 2 in analogy to previously described desirable solvent characteristics for liquid–liquid TPPBs (24).

A short list of seven polymers to be considered as delivery phases for biphenyl degradation in solid–liquid TPPBs was created on the basis of these characteristics and is shown in Table 3. All listed polymers fulfill requirements 1–5 and 7–9 (data not shown) and their affinity for the target molecule (requirement 6) will be described in the following sections.

**3.2.1. Partitioning of Biphenyl between Solid Phase and Water.** The seven different polymer listed in Table 3 were tested for their ability to sorb biphenyl from aqueous medium. Sorption equilibria for different biphenyl concentrations are shown in Figure 1. It can be seen that all polymers under consideration, with the exception of nylon, show a strong affinity for biphenyl. The equilibrium isotherms follow a linear trend for all polymers over the tested range of concentrations, as similarly observed for phenolic compounds (26). Linear isotherms indicate that the governing mechanism in these three-component systems is equilibrium partitioning analogous to partitioning of solutes between two immiscible liquid phases. The tested polymers show significant differences in their affinity for biphenyl; this can also be seen in their biphenyl partitioning coefficients shown

**Table 2. Desirable Polymer Characteristics for Delivery of Poorly Water-Soluble Substances to Degrading Organisms in Solid–Liquid TPPBs**

1. commercially available at a low cost
2. nonhazardous
3. nontoxic to the employed organisms
4. not available as carbon and energy source or otherwise biodegradable
5. not promoting biofilm formation under operational conditions
6. possessing desirable affinity for the target molecule(s)
7. thermally stable for sterilization purposes
8. stable in aqueous medium at the pH and electrolyte concentration of the employed culture medium
9. stable in medium employed to load polymer with target compounds

in Table 4, which were obtained from linear regression analysis of data presented in Figure 1.

Similar to the tested polymers, organic solvents also show differences in their affinity for biphenyl. For example, the biphenyl partitioning coefficient between *d*-limonene and water is  $K_{S/W} \approx 69,000$ , which is substantially higher than the biphenyl partitioning coefficient between octadecene and water ( $K_{S/W} \approx 15,000$ ) (17). The solubility of organic compounds in solvents and their partitioning between water and solvents depends on the molecular structure of both solute and solvent. Group contribution models such as UNIFAC and UNIQUAC have been developed on the basis of known properties of functional groups to predict vapor–liquid equilibria (27) and have since been extended to liquid–liquid equilibria (28), allowing such models to be applied to selected suitable solvents for liquid–liquid TPPBs (24). Initial attempts have been made to use the UNIFAC system for the estimation of adhesion enhancement between polymers and mineral surfaces treated with silane coupling agents (28). However, none of these models can readily be adopted to describe the interaction between polymers, solute, and water, although it is expected that the presence/absence of specific functional groups will also be important in the selection of effective polymers for different applications.

The partitioning coefficient of biphenyl between the tested polymers and water are very high, as shown in Table 4, and are comparable to biphenyl partitioning coefficients between organic solvents and water (Table 1). On the basis of these values, all tested polymers, with the exception of nylon, can be considered as potential delivery phases for TPPBs. The high partitioning coefficients, e.g.,  $K_{S/W} \approx 10,000$  for Desmopan, also suggest the possible use of polymers in environmental applications such as in scavenging for low concentrations of hydrophobic toxic contaminants from industrial wastewater streams. TPPBs have been discussed in a recent review as a possible technology to bioremediate trace organic compounds found in precious metals refineries' wastewaters (29). Extraction of these contaminants with an appropriate polymeric substance followed by biodegradation in a solid–liquid TPPB might also be a possible remediation technology.

**3.2.2. Loading of Delivery Phase with Biphenyl.** Biphenyl is crystalline at room temperature, and dissolving it in water prior to loading the delivery phase is not effective for generating high biphenyl concentrations in polymers due to the low solubility of biphenyl in water (7 mg kg<sup>-1</sup> at 30 °C (30)). In contrast, the solubility of biphenyl in methanol at 30 °C is 89 g kg<sup>-1</sup> (23), which facilitates dissolving large quantities of biphenyl in methanol to load the polymers.

Figure 2 shows linear equilibrium isotherms of biphenyl between methanol and the tested polymers, similar to what was observed when biphenyl partitioned between polymers and water. These results show again that equilibrium partitioning is

**Table 3. Properties of Polymers Considered as the Delivery Phase for Biphenyl in Solid–Liquid TPPB**

polymer	type	supplier	cost <sup>a</sup> (\$ kg <sup>-1</sup> )	structure
Kraton	G1657M	Kraton	n/a	styrene-ethylene/butadiene tribloc copolymer
Nucrel	925	DuPont	6.93	ethylene-methacrylic acid copolymer
nylon	6.6	DuPont	3.37–3.70	polycaprolactam
silicone rubber	GE-Mastercraft	Mastercraft	12.78–14.08	polydimethylsiloxane
Desmopan	DP 9370A	Bayer	4.07–5.61	polyurethane of poly(oxytetramethylene)glycol and methyldiisocyanate
Elvax	360	DuPont	1.02–1.14	poly(ethylene-co-vinyl acetate)
Hytrel	8206	DuPont	4.07–5.61	butylene terephthalate-butylene ether glycol terephthalate copolymer

<sup>a</sup> Average prices from various suppliers as of March 2007 in US\$ for same polymeric substances as listed assuming purchase of bulk quantities (25).

**Table 4. Polymer/Water Partitioning Coefficients ( $K_{S/W}$ ) of Biphenyl**

polymer	$K_{S/W}$	error of $K_{S/W}$	log $K_{S/W}$
Kraton	7072	56	3.85
Nucrel	2049	36	3.31
nylon	184	11	2.27
silicone rubber	2850	54	3.45
Desmopan	10987	56	4.04
Elvax	4781	50	3.68
Hytrel	3234	9	3.51

the governing process in the observed three-component systems. However, comparing Figures 1 and 2 shows that the maximum achievable concentration in the polymers is much higher in the biphenyl–methanol–polymer system in comparison with the corresponding biphenyl–water–polymer system. This effect is the most pronounced for Hytrel, which has the fourth highest biphenyl partitioning coefficient between polymer and water but the third highest for biphenyl partitioning between polymer and methanol. The partitioning coefficients of biphenyl between the three polymers Desmopan, Kraton, Hytrel, and water are  $>1$ , indicating that the relative affinity of these polymers for biphenyl is larger than the biphenyl affinity of methanol. Such high affinity would be of importance if hydrophobic compounds were to be extracted from media such as soil rather than water, followed by biodegradation in a TPPB. In a soil environment organic matter can have high affinity for hydrophobic compounds, and the ability of these three polymers to remove biphenyl from organic solvents such as methanol also indicates that polymers might be able to compete effectively for target molecules in soil with high organic content. Prpich et al. successfully demonstrated the effective extraction of phenol from soil by mixing dry soil with polymer beads (26), and the subsequent release of the phenol to a microbial consortium in a TPPB. The study was undertaken at laboratory scale and employed polymer beads with average diameters of 4–6 mm; for large-scale soil remediation other geometrical shapes and sizes of the polymer may be advantageous to simplify the soil polymer separation step.

Changes in the ability of polymers to sorb substances depending on the medium the polymers are immersed in are also known to occur when hydrogels, such as acrylic acid acrylamide copolymers, absorb water. The addition of 1.2% NaCl to distilled water was shown to reduce the water absorption capacity of hydrogels by 50% (31). Further, changes of the fluid compositions (e.g., the addition of ethanol and methanol to water) are known to affect the ability of hydrogels to absorb water substantially, as first shown by Tanaka (32). The opportunity to possibly influence the ability of polymers to sorb hydrophobic compounds by changing the medium conditions is very interesting, which may be a fruitful area of future work.

**3.2.3. Release of Biphenyl into Aqueous Medium.** The polymers loaded with biphenyl from methanol were transferred to aqueous medium after removal of residual methanol. The

resulting equilibrium aqueous phase concentrations were measured and are shown in Figure 3 as a function of the biphenyl concentration in the polymers. The data shown in Figure 3 are similar to the data shown in Figure 1 for low biphenyl concentrations. Under these conditions the same equilibria were reached, regardless of whether biphenyl was initially present in the aqueous phase (Figure 1) or in the polymer phase (Figure 3). At high biphenyl concentrations in the polymers the aqueous phase became saturated with biphenyl. The range of biphenyl concentrations in the polymer phases that results in a biphenyl saturated aqueous phases varied depending on the employed polymer. The upper section of Figure 3 shows this constant section for the different polymers.

The ability to load various polymers with large amounts of hydrophobic substances, which can then be released to their saturation concentration in aqueous media, provides an interesting delivery system for hydrophobic compounds to degrading organisms. This is of particular interest if bioavailability of the target compounds is the degradation rate-limiting step, which can be the case during biphenyl degradation (17). Under such conditions a polymer such as Hytrel that has a very wide range of concentrations resulting in a saturated aqueous phase should be chosen as the delivery phase. Hytrel loaded with 60 g kg<sup>-1</sup> would provide two-thirds of the initially available biphenyl at saturated aqueous phase concentrations to degrading organisms, until the concentration in Hytrel reaches 20 g kg<sup>-1</sup>, after which the equilibrium concentration in the aqueous phase would decrease linearly (assuming mass transfer rates are sufficiently large compared to microbial degradation rates). Desmopan with a similar initial loading would provide initial aqueous phase concentrations of only ~75% of the aqueous phase solubility followed by a linear decrease of the equilibrium concentration. Such partitioning behaviors would be of interest if elevated concentrations of the target compound were toxic to the degrading organisms as in the case of phenols (16).

The formation of free biphenyl crystals was not observed under the experimental conditions that resulted in biphenyl-saturated aqueous phases, showing that the release of biphenyl from the polymers did not continue once biphenyl saturation in the aqueous phase was reached. However, for the purpose of this study it is important that large quantities of biphenyl could be transferred into polymer beads and subsequently released to aqueous medium. Hytrel was chosen for bioreactor experiments as it can provide high aqueous-phase biphenyl concentrations over a large range of biphenyl concentrations in the polymer.

**3.3. Biodegradation of Biphenyl in Solid–Liquid TPPBs: Proof of Concept.** TPPB experiments were conducted to demonstrate the ability of solid–liquid TPPBs to deliver the hydrophobic model compound biphenyl to different degrading organisms that degrade biphenyl to completion. It is evident from the results shown in Figure 4 that both microbial systems could readily degrade the provided biphenyl within 50 h. The

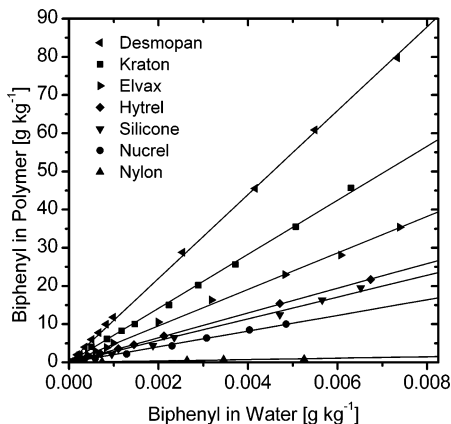


Figure 1. Biphenyl partitioning between water and polymer.

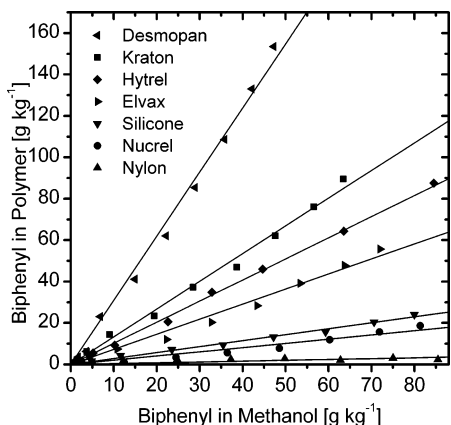


Figure 2. Biphenyl partitioning between methanol and the selected polymers.

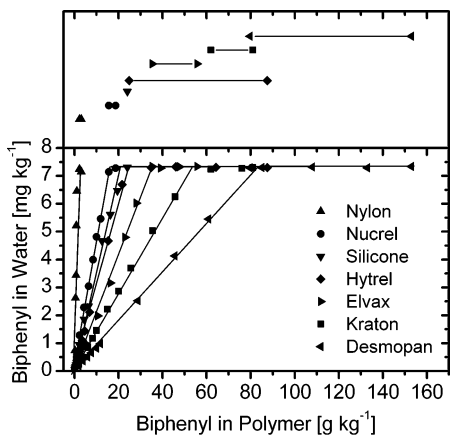


Figure 3. Release of biphenyl to water from polymers loaded in methanol. The upper part of the figure shows the concentration range of each polymer that results in a biphenyl-saturated aqueous phase. The upper part of the figure does not have a y-axis. The data points are presented on different vertical positions for clarification only.

microbial consortium exhibited a shorter acclimatization phase and also a higher overall biomass yield compared to the pure culture. Both microbial systems were able to degrade biphenyl to concentrations below the detection limit, showing the suitability of solid-liquid TPPBs for complete biphenyl degradation.

Similar amounts of biphenyl could be degraded by *B. xenovorans* LB400 within 25–30 h in liquid-liquid TPPBs (17). A possible reason for the slower degradation rates in solid-liquid TPPBs is that the mass transfer rate of biphenyl from the solid phase into the liquid phase might be rate-limiting. The

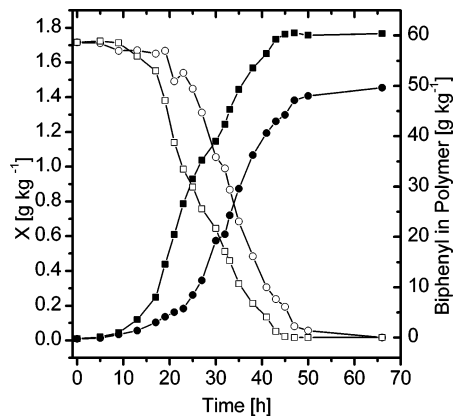


Figure 4. Biodegradation of biphenyl in solid-liquid TPPBs by *B. xenovorans* LB400 (circles) and a microbial consortium (squares). The closed symbols represent the biomass concentration in the aqueous phase (cell dry weight), and the open symbols represent the biphenyl concentration in the solid phase.

available surface area of the employed polymer beads (3–4 mm diameter) is significantly smaller than the surface area available for mass transfer in a liquid-liquid system (the estimated average droplet diameter is 30  $\mu\text{m}$  (33)). The biomass formation shown in Figure 4 also does not seem to follow the typical exponential trend of unrestricted microbial growth but rather a linear trend, a further indication of possible mass transfer limitation.

However, the biodegradation results clearly show that solid-liquid TPPBs can be used to deliver large amounts of poorly water-soluble substances to degrading organisms. The use of a solid delivery phase also allows the combination of TPPBs with large mixed microbial populations and surfactant-producing organisms, which expands the spectrum of utilizable biocatalysts and demonstrates that solid-liquid TPPBs can be used as a degradation platform for recalcitrant hydrophobic substances.

#### 4. Conclusions

Hydrophobic substances such as biphenyl partition between water and selected polymers similarly as between water and organic solvents. The observed partition coefficients are polymer-specific and can also reach values similar to those of organic solvents. The biphenyl capacity of the utilized polymers is higher when biphenyl is provided in methanol. Loading polymers such as Hytrel with large amounts of biphenyl from methanol and subsequently placing them in aqueous solution results in biphenyl saturation of the aqueous phase. The use of polymer delivery phases permits the use of microbial consortia and surfactant-producing bacteria in TPPBs, thereby extending the range of biocatalysts that can be employed relative to liquid-liquid TPPB systems

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