

# Biodegradation of biphenyl in a solid–liquid two-phase partitioning bioreactor

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## Abstract

Biphenyl was successfully degraded by *Burkholderia xenovorans* LB400, initially described as *Pseudomonas* sp. LB400, in a solid–liquid two-phase partitioning bioreactor (TPPB). Solid–liquid TPPBs are comprised of an aqueous, cell containing phase, and a solid polymeric phase that partitions toxic and/or poorly soluble substrates (in this case biphenyl) based on maintaining a thermodynamic equilibrium. The employed polymer was Hytrel™, a thermoplastic polyester elastomer. The surface area available for mass transfer of biphenyl was limiting and resulted in mass transfer limited growth, as demonstrated experimentally by employing two different geometric shapes (cylinders with different aspect ratios) of the polymer phase with different specific surface area, while keeping all other parameters constant. The linear microbial growth rates were substantially higher when more polymer surface area was provided.

The mass transfer coefficient of biphenyl from Hytrel™ to water was measured under experimental conditions, which allowed predicting the release rate based on the biphenyl concentration gradient. The partitioning behaviour of biphenyl between Hytrel™ and culture medium was measured as well, which allowed the development of a simple mechanistic model describing microbial growth based on known microbial properties in combination with substrate delivery from the solid polymer phase. The model was capable of describing the experimental data well and can be used to predict degradation rates for other geometric shapes of a solid delivery phase such as sheets or rods, which might be of operational advantage in various applications of TPPBs to the controlled uptake and release of other recalcitrant molecules.

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## 1. Introduction

Biphenyl is an aromatic hydrocarbon, comprised of two, six-sided aromatic rings connected at one carbon on each ring. It was mainly used as a precursor for polychlorinated biphenyls (PCBs), but is also industrially used as a dyestuff carrier for textiles and copying paper, as a solvent in pharmaceutical production and as a fungistat in transportation containers of oranges and other citrus fruits [1,2]. Biphenyl can be degraded aerobically by a variety of soil bacteria which are often also capable of degrading low chlorinated PCBs [3,4]. Biphenyl has low solubility in water and high solubility in organic solvents suggesting the use of two-phase partitioning bioreactors (TPPBs) for microbial biphenyl degradation. Kinetic parameters for the strain *Burkholderia xenovorans* LB400 and the Monod model were previously estimated in liquid–liquid TPPBs [5]. TPPBs

are typically stirred tank bioreactors containing two immiscible phases, an aqueous phase containing the biocatalyst (bacteria, yeasts, other fungi or mammalian cells) and a second phase functioning either as a substrate reservoir or a product sink [6]. Depending on the process, the second phase has to be carefully chosen to show high affinity for the desired product or the substrate, without interfering with the microbial system. TPPBs can be used to deliver large amounts of hydrophobic substrates to degrading organisms. Large amounts of substrates can be dissolved in the second phase, resulting in substrate partitioning to a reduced equilibrium concentration in the aqueous phase. Only substrate in the aqueous phase is available to the biocatalysts and degradation will result in a disequilibrium, which in turn will result in partitioning of additional substrate from the second phase into the aqueous phase. The substrate delivery into the aqueous phase is controlled by the microbial degradation rate if the mass transfer rate is significantly larger than the microbial consumption rate. Substances for the second phases in TPPBs have traditionally been hydrophobic organic solvents such as octyl-alcohol or octadecene [7]. Aqueous–aqueous

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two-phase systems have been employed [8] and more recently solid polymers have been used [9]. It was found that some thermoplastics have strong affinities for hydrophobic organic molecules and show partitioning behaviour similar to organic solvents.

Solid–liquid TPPBs have several advantages over liquid–liquid TPPBs in terms of biological compatibility of the second phase. Solid polymeric substances which are typically used in TPPBs are biologically inert, being neither toxic to the organism of choice nor can they substitute as an alternative carbon source and divert biological activity away from the target substrate [10].

The organic phase in liquid–liquid TPPBs has to be selected carefully to also fulfill these requirements, which often limits the choice of the delivery phase, and generally limits liquid–liquid TPPBs to pure strains or small well-defined microbial consortia [11]. The advantages of liquid–liquid TPPBs are the physical properties of the delivery phases. The fundamental principles of solvent extraction and the involved chemical and physical interactions are well understood, allowing systematic screening for organic solvents with high capacities for most target molecules [12]. A further advantage is the large interfacial area available for mass-transfer between aqueous and delivery phase under conditions of high mixing intensity. The two phases form a fine emulsion under operational conditions in a stirred tank bioreactor ( $n = 600$  rpm,  $Re > 10,000$ ). The average droplet diameter can be estimated to  $30 \mu\text{m}$ , resulting in a total specific interfacial area of  $8.7 \text{ m}^2 \text{ dm}^{-3}$  ( $\text{m}^2$  interfacial area per  $\text{dm}^3$  fermentation broth at 50 mL organic phase per L aqueous phase) [13]. Such conditions can allow the assumption of instantaneous equilibrium formation of a given substance partitioning between the two liquid phases [5,14]. The available surface area for mass-transfer in solid–liquid TPPBs is determined by the size and shape of the employed solid phase. Typically employed delivery phases are spherical or cylindrical beads with diameter of 2–4 mm resulting in a total specific interfacial area of  $0.07 \text{ m}^2 \text{ dm}^{-3}$  ( $\text{m}^2$  interfacial area per  $\text{dm}^3$  fermentation broth at 50 g solid phase per L aqueous phase). Other geometrical shapes with even smaller surface to mass ratios such as sheets or rods might hold operational advantages, but mass transfer limitations are likely to occur under such conditions.

The objective of this study was to determine whether solid–liquid TPPBs are suitable for biphenyl degradation by *B. xenovorans* LB400, which occurs at high microbial rates and to gain an understanding of the mass transfer processes involved in this system. Mass transfer limited cell growth has been shown to occur when insoluble substrates are delivered as solid crystal [15] and liquid–liquid TPPBs have been shown to overcome these limitations in some instances [16], whereas mass transfer rates are found limiting in others [13]. This is the first study to investigate the effects of mass transfer rates on microbial growth in solid–liquid TPPBs.

## 2. Theory

A schematic diagram of a solid–liquid TPPB is shown in Fig. 1. A simple model for microbial growth on a single substrate

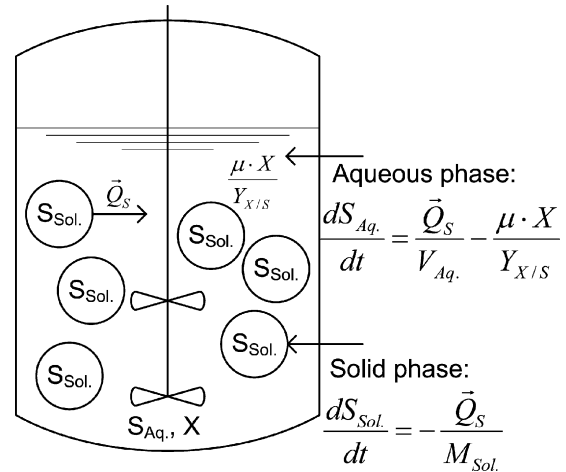


Fig. 1. Schematic of solid liquid TPPB; the size of individual polymer beads is not to scale.

delivered from a solid phase can be described as follows

$$\frac{dX}{dt} = \mu X \quad (1)$$

where  $X$  is the biomass ( $\text{g m}^{-3}$ ),  $\mu$  the specific growth rate ( $\text{h}^{-1}$ ) and  $t$  is the time (h). The change of substrate concentration in the aqueous phase consists of two terms, microbial degradation, which is linked to biomass formation via a yield coefficient [17] and the substrate flux from the solid phase  $\bar{Q}_S$ :

$$\frac{dS_{aq}}{dt} = \frac{1}{V_{aq}} \cdot \bar{Q}_S - \mu X \frac{1}{Y_{X/S}} \quad (2)$$

where  $S_{aq}$  is the aqueous phase substrate concentration ( $\text{g m}^{-3}$ ),  $\bar{Q}_S$  the substrate flux from the solid phase ( $\text{g h}^{-1}$ ) and  $V_{aq}$  is the volume of the aqueous phase ( $\text{m}^3$ ). The biomass yield coefficient has previously been estimated to be  $Y_{X/S} = 0.48 \text{ g g}^{-1}$  [5]. The substrate concentration in the solid phase changes according to

$$\frac{dS_{sol}}{dt} = -\frac{1}{M_{sol}} \cdot \bar{Q}_S \quad (3)$$

where  $S_{sol}$  is the concentration in the solid phase ( $\text{g kg}^{-1}$ ) and  $M_{sol}$  is the mass of the solid phase (kg). The specific growth  $\mu$  ( $\text{h}^{-1}$ ) is dependent on the substrate concentration in the aqueous phase and can be modelled via the Monod model:

$$\mu = \frac{\mu_{max} S_{aq}}{K_S + S_{aq}} \quad (4)$$

where  $\mu_{max}$  is the maximum specific growth rate, previously estimated to  $\mu_{max} = 0.25 \text{ h}^{-1}$  and  $K_S$  is the half saturation constant  $K_S = 0.1 \text{ g m}^{-3}$  [5]. The flux from the solid phase to the liquid phase can be adopted from models describing flux from solid substrate crystal into solution [18]:

$$\bar{Q}_S = K_t A (S_{aq}^{eq} - S_{aq}^t) \quad (5)$$

where  $K_t$  is a constant ( $\text{m h}^{-1}$ ),  $A$  the interfacial surface area ( $\text{m}^2$ ),  $S_{aq}^{eq}$  the substrate concentration in the aqueous phase in equilibrium to the substrate concentration in the solid phase

( $\text{g m}^{-3}$ ) and  $S_{\text{aq}}^t$  is the substrate concentration in the aqueous phase at the time  $t$  ( $\text{g m}^{-3}$ ).

A correlation between  $S_{\text{aq}}^t$  and  $S_{\text{so1}}$  as well as the mass-transfer coefficient  $K_f$  have to be determined experimentally.

### 3. Materials and methods

#### 3.1. Chemicals

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). Hytrel™ (Hytrel™ is a registered trademark of E.I. du Pont de Nemours and Company) is a thermoplastic polyester elastomer with a density of  $1.17 \text{ g cm}^{-3}$ . The polymer chain contains approximately 50% poly-butylene terephthalate (PBT) and 50% butylene ether glycol terephthalate. It was found not to be available as a carbon source to the employed microorganism and no biofilm formation on the polymer surface has been observed (data not shown). Hytrel™ polymer beads were obtained from DuPont Canada, in cylindrical shapes with a specific surface area of  $1.49 \text{ m}^2 \text{ kg}^{-1}$  ( $\text{m}^2$  polymer surface per kg polymer). A fraction of these polymer beads was reduced in size to smaller cylinders with a higher specific surface area of  $2.38 \text{ m}^2 \text{ kg}^{-1}$ .

#### 3.2. Bacterial strain

*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 re-classified as *B. xenovorans* sp. nov. [19]. Cultivation conditions, medium formulation and biomass analysis can be found elsewhere [5,20].

#### 3.3. Biphenyl uptake

Hytrel™ beads (1 g) were added to scintillation vials containing 10 mL methanol and varying amounts of biphenyl. The system was allowed to equilibrate by placing it on a rotary shaker at  $30^\circ\text{C}$  for 48 h. A control vial containing methanol and biphenyl but no Hytrel™ was added for every vial containing Hytrel™. Biphenyl concentrations were measured with an Ultraspec 3000 UV–vis Spectrophotometer (Biochrom, UK) at  $\lambda = 250 \text{ nm}$ . The biphenyl concentration in methanol was measured after equilibration and the difference in concentration between the vials containing beads and the control vials was attributed to partitioning into the Hytrel™ beads. The concentration in beads was calculated via mass balance and was considered to be in equilibrium with the final concentration remaining in methanol.

#### 3.4. Biphenyl release

Hytrel™ polymer beads (1 g) were loaded with different amounts of biphenyl from methanol as described above.

Methanol was removed by washing of the beads with water for 3 min. This step removed methanol from the bead surface and did not significantly reduce the amount of biphenyl present in the beads (confirmed by re-equilibrating the washed beads with methanol and measuring biphenyl concentration in methanol, data not shown). The methanol free beads were added to 50 mL cell-free culture medium and agitated at 600 rpm. Aqueous phase samples were periodically analyzed spectroscopically for their biphenyl concentration.

#### 3.5. Biphenyl biodegradation

Experiments were undertaken in parallel in two 5-L New Brunswick BioFlo® III bioreactors, agitated each with two Rushton turbines at 600 rpm and aerated (sterile air) at  $3 \text{ L min}^{-1}$ . The aqueous-phase volume was 2000 mL and the mass of Hytrel™ beads was 105 g. Conditions were automatically maintained at  $30^\circ\text{C}$ , and at pH 6.9 by adding 3 M KOH. A 50 mL inoculum was grown in a 100 mL shake flask on biphenyl for 24 h, split into two equal volumes and inoculated into the two reactors. The first reactor contained Hytrel™ beads with a specific surface area of  $0.079 \text{ m}^2 \text{ dm}^{-3}$  and the second reactor the same amount of beads with a specific surface area of  $0.126 \text{ m}^2 \text{ dm}^{-3}$ . The beads were previously equilibrated with 200 mL methanol and 20 g biphenyl on a rotary shaker for 48 h at  $30^\circ\text{C}$  and washed with sterile water, resulting in beads containing 78 g biphenyl per kg beads. Biomass concentration in the reactors was periodically measured spectroscopically as described elsewhere [5].

## 4. Results and discussion

The hydrophobic substrate biphenyl is a solid crystal at ambient conditions. It has to be transferred into the designated solid phase of a TPPB in order to be delivered to the degrading organisms. This substrate uptake was mediated by dissolving biphenyl initially in methanol. Different amounts of biphenyl were dissolved in methanol, and Hytrel™ was added as a solid phase. Hytrel™ has no affinity for methanol (data not shown) and methanol physically attached to the polymer surface can easily be removed with water, prior to the release experiments. The amounts of biphenyl removed during this washing are insignificant due to the low solubility of biphenyl in water. The calculated biphenyl concentration in the solid phase (based on mass balance) can be plotted against the equilibrium concentration in methanol to determine the partitioning behaviour. Biphenyl partitions between Hytrel™ and methanol with a constant partitioning coefficient in the measured range, as shown in Fig. 2. Similar linear behaviour has been found for the partitioning of phenols between aqueous medium and Hytrel™ [21]. The partitioning coefficient for biphenyl concentrations below  $45 \text{ g kg}^{-1}$  in methanol was found to be  $K_{\text{S/L}}^{\text{m}} = 1.019 \pm 0.021 \text{ g g}^{-1}$ , showing that the relative affinities of Hytrel™ and methanol for biphenyl are almost identical. The constant partitioning coefficient allows calculation of the

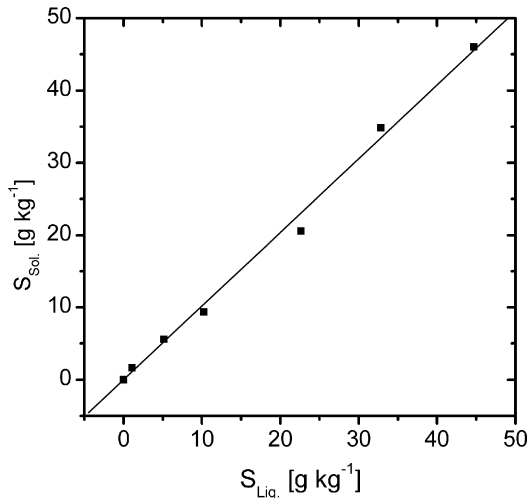


Fig. 2. Partitioning of biphenyl between Hytrel™ and methanol at 30 °C.

equilibrium biphenyl concentration in Hytrel™ according to Eq. (6):

$$S_{\text{sol}} = \frac{M_{\text{BP}}}{M_{\text{sol}} + M_{\text{liq}}/K_{\text{S/L}}^m} \quad (6)$$

where  $M_{\text{BP}}$  is the mass of biphenyl in the system (g) and  $M_{\text{liq}}$  is the mass of methanol (kg).

The dynamic release of biphenyl from Hytrel™ polymers into cell-free microbial culture medium was investigated under reactor conditions. Fig. 3 shows the biphenyl concentration in the aqueous phase as a function of time for different initial biphenyl concentrations in Hytrel™. It can be seen that both the rate and extent of the release vary substantially with the initial biphenyl concentration in Hytrel™. The extent of the release is expected to be the concentration in the aqueous phase in equilibrium with the biphenyl content in the polymer. However, initial biphenyl concentration in the solid phase  $>35 \text{ g kg}^{-1}$  released biphenyl at the same rate and to the same final concentration in the aqueous phase. The highest achieved aqueous phase concentration was  $0.00692 \text{ g dm}^{-3}$ , which is the solubility of biphenyl

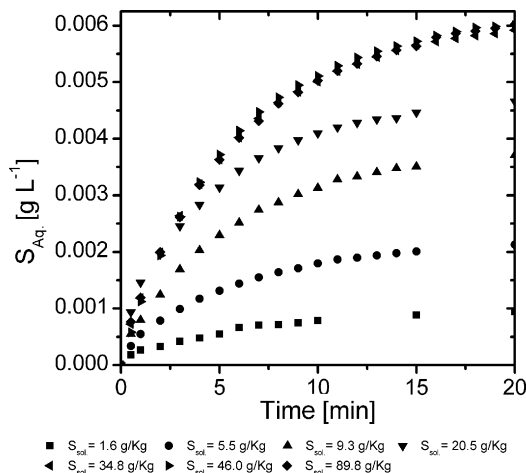


Fig. 3. Biphenyl release from 1 g Hytrel™ into 50 mL water at different initial biphenyl concentrations in Hytrel™.

in the employed medium at 30 °C. The solubility of biphenyl has been reported to be  $0.00608 \text{ g dm}^{-3}$  at 25 °C for aqueous phase solutions with similar salt contents [22], which is very similar to the value found here. The observed release rates are very rapid if compared to solubilization rates of other hydrophobic compounds such as PAHs. Viamajala et al. [23] achieved constant concentrations of phenanthrene, fluorene and fluoranthene in aqueous solution after 20–40 h during solubilization experiments. Delivery of biphenyl from Hytrel™ beads achieved saturation/equilibrium concentration within 20–40 min. The differences in the rates might be due to differences in aqueous phase solubility of the PAHs compared to biphenyl (the solubility of the above mentioned PAHs is approximately one order of magnitude lower than the solubility of biphenyl) and the lower agitation rates employed by Viamajala et al. [23].

The biphenyl concentration in the solid phase remains essentially constant over the time of the release experiments due to the high initial concentrations in the polymer and the low solubility of biphenyl in the aqueous phase. The total change in concentration in the polymer phase under experimental conditions is between 0.3% and 1.7%, depending on the initial concentration of biphenyl in Hytrel™.

Neglecting the change of biphenyl concentration in Hytrel™ and assuming no biphenyl consumption or loss in the aqueous phase allows simplifying Eq. (5) to

$$\bar{Q}_S = \frac{dS_{\text{aq}}}{dt} V_{\text{aq}} = K_t A (S_{\text{aq}}^{\text{eq}} - S_{\text{aq}}^t) \quad (7)$$

where the equilibrium biphenyl concentration in the aqueous phase  $S_{\text{aq}}^{\text{eq}}$  equals the final biphenyl concentrations  $S_{\text{aq}}^{\infty}$  in the release experiments, which allows the estimation of  $K_t$  by plotting  $\bar{Q}_S/A$  versus  $S_{\text{aq}}^{\infty} - S_{\text{aq}}^t$ , as shown in Fig. 4.

The same linear relationship between release rate and the driving force ( $S_{\text{aq}}^{\infty} - S_{\text{aq}}^t$ ) can be seen for all experimental conditions.  $K_t$  was estimated by least square regression to be  $K_t = 0.33397 \pm 0.00533 \text{ m h}^{-1}$ . The rate at which biphenyl is released from Hytrel™ at any given time therefore depends

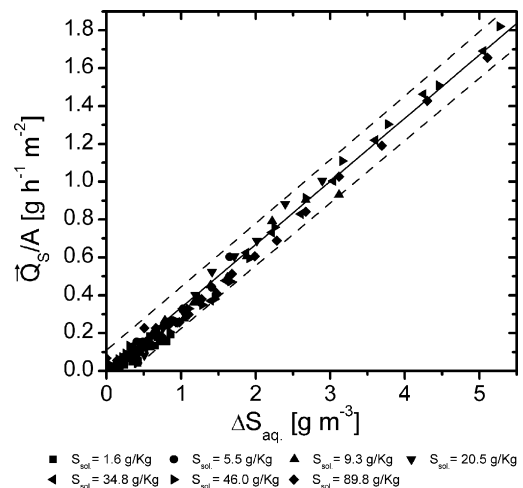


Fig. 4. Normalized biphenyl release rate vs. driving force during biphenyl release from 1 g Hytrel™ into 50 mL culture medium at different initial biphenyl concentrations in Hytrel™. The solid line shows a linear regression of the data and the dashed lines the 95% confidence limit.



on the concentration gradient, the mass transfer coefficient, and the available surface area, with the latter two expected to remain constant during the course of a fermentation.

Predicting release rates during bioreactor operation requires a relationship between the concentrations in the solid and aqueous phases. From the initial Hytrel™ loadings and the equilibrium aqueous concentrations presented in Fig. 3 it can be deduced that there is no linear relationship between the equilibrium concentration in the aqueous phase and the initial (and final) concentration in the solid phase. Fig. 5 shows the relationship between the equilibrium biphenyl concentration in the aqueous phase and in the solid phase. The data clearly show that the partitioning coefficient is not constant over the employed range of concentrations. A constant partitioning coefficient of approximately  $\log K_{S/W}^m = 3.5$  can be found for  $S_{\text{sol}} < 25 \text{ g kg}^{-1}$ . This value is similar in magnitude to the octanol water partitioning coefficient of biphenyl,  $\log K_{O/W}^m = 3.89$  [24]. The partitioning coefficient increases for biphenyl concentration in the solid phase  $> 25 \text{ g kg}^{-1}$ . An empirical asymptotic equation was used to predict the aqueous phase equilibrium concentration as a function of the biphenyl concentration in the solid phase over the entire concentration range, as shown by the solid line in Fig. 5. The data could be described by

$$S_{\text{aq}}^{\infty} = 7.05417 \times 10^{-3} (1 - 0.92902^{S_{\text{sol}}}) \quad (8)$$

Various methods to describe solid–liquid equilibria thermodynamically can be found in the literature [25–27]. Different methods have been applied, ranging from predicting activity coefficients of the target compound in both phases via the UNI-FAC model [28], to treating the solid phase containing the target molecule as a new compound [25], or to describing the interaction between the solid phase and target compound as surface adsorption [27]. Some of these methodologies might be applicable to the situation in this study, however, a fundamental thermodynamic description of the solute polymer interaction

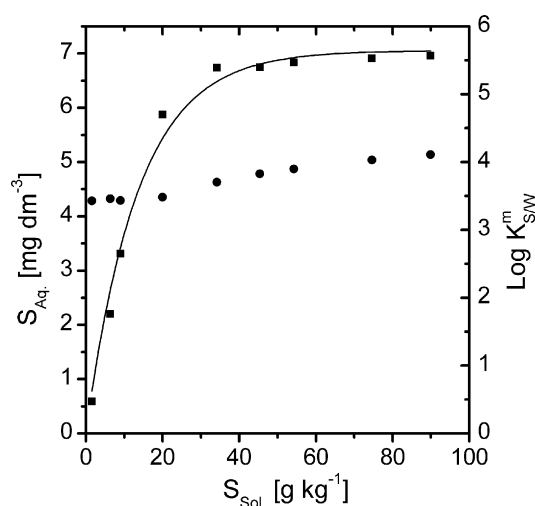


Fig. 5. The log solid–liquid partitioning coefficient of biphenyl  $\log K_{S/W}^m$  as a function of biphenyl concentration in the Hytrel™ (circles) and the equilibrium aqueous phase biphenyl concentration  $S_{\text{aq}}^{\infty}$  as a function of the initial biphenyl concentration in Hytrel™  $S_{\text{sol}}$  (squares). The solid line shows an empirical non-linear regression of the data.

is beyond the scope of this study and also is not required to describe the effects of mass transfer limitations on microbial performance in TPPBs.

Using the estimated parameters for  $K_t$ ,  $\mu_{\text{max}}$  and  $K_S$  and the empirical equation (8) allows simulating microbial biphenyl degradation in a solid–liquid TPPB by solving Eqs. (1)–(5). These equations were solved numerically using the ordinary differential equation solver of Matlab (ODE23s). Fig. 6 shows the biomass formation during biphenyl degradation by *B. xenovorans* LB400. The circles show the biomass formation and the corresponding line the simulated values. It is evident that the model describes the experimental data very well. Initially, biomass formation follows the typical exponential growth curve. After approximately 20 h biomass seems to increase linearly rather than exponentially, a clear indication of a mass transfer limited system, as captured by the model. The dashed line shows the expected course of the fermentation assuming instantaneous equilibrium formation (e.g. no mass transfer limitation), as observed in liquid–liquid TPPBs [5]. The effect of the mass transfer limitation is vast; the total fermentation time increases from 25 to 44 h. The squares in Fig. 6 represent the same fermentation with Hytrel™ beads of a smaller diameter. The total amount of Hytrel™ and the initial biphenyl concentration in the solid phase were identical, but the specific surface area was increased by approximately a factor of 1.6. It can also be seen that the overall rate increases significantly over the rate observed with the larger beads and the total fermentation time decreases to approximately 35 h, whereas the time course also contains the characteristic linear growth kinetics. The corresponding solid line shows the solution of Eqs. (1)–(5) and (8) with the same parameters as before and the increased surface area  $A$ .

The simulated and the experimental data fit very well, showing that the model describes the system within the range of

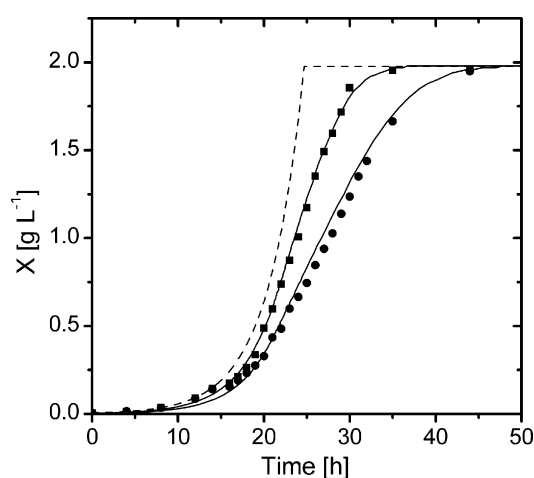


Fig. 6. Biodegradation of biphenyl by *Burkholderia xenovorans* LB400 delivered from Hytrel™ polymer beads. The circles represent the biomass when biphenyl was delivered from Hytrel™ beads with a specific surface area of  $0.079 \text{ m}^2 \text{ dm}^{-3}$  and the squares when biphenyl was delivered from beads with a specific surface area of  $0.126 \text{ m}^2 \text{ dm}^{-3}$ . The solid lines represent the simulated biomass formation and the dashed line the biomass formation with no mass transfer limitation.

operating conditions and can also potentially describe delivery phases with different geometries. Using geometries with larger specific surface areas (e.g. smaller beads) would result in curves more similar to the ones observed in liquid–liquid systems, whereas geometries with smaller specific surface areas (sheets or rods) would yield curves with linear segments with a smaller slope. Such geometries are of interest for bioremediation applications. Prpich et al. [21] recently applied polymer beads to extract phenol contaminations from soil followed by ex situ biodegradation of phenol in a solid liquid TPPB. Sheets or rods would provide operational advantages during the soil extraction step, and the results of this study can help predict the effects of these geometries on substrate release to the degrading organisms.

The effect of mass transfer limitation is shown more dramatically in simulations plotted in Fig. 7. Taking into account the rate of microbial uptake of biphenyl, as well as its rate of release from the polymer, allows to predict the non-equilibrium aqueous phase biphenyl concentration by solving Eqs. (1)–(5) and (8) for  $S_{aq}$ . The non-equilibrium biphenyl concentration in the aqueous phase declines at almost similar rates for both delivery phase geometries and falls below  $0.1 \text{ mg dm}^{-3}$  after approximately 25 h. At this time the biomass concentration is  $X = 0.84 \text{ g dm}^{-3}$  for the larger beads and  $X = 1.23 \text{ g dm}^{-3}$  for the smaller beads as shown in Fig. 6, indicating that 50% more biphenyl has been consumed in the case of the smaller beads. This can also be seen through the larger predicted equilibrium concentration displayed as the dashed lines in Fig. 7. This aqueous biphenyl concentration would be achieved if phase equilibrium was established due to partitioning without simultaneous microbial uptake, but is never reached in the observed system as the microbial biphenyl consumption is faster than the mass transfer rate of biphenyl from the Hytrel™ beads into the aqueous phase. The difference between the actual biphenyl concentration and the equilibrium concentration is the mass

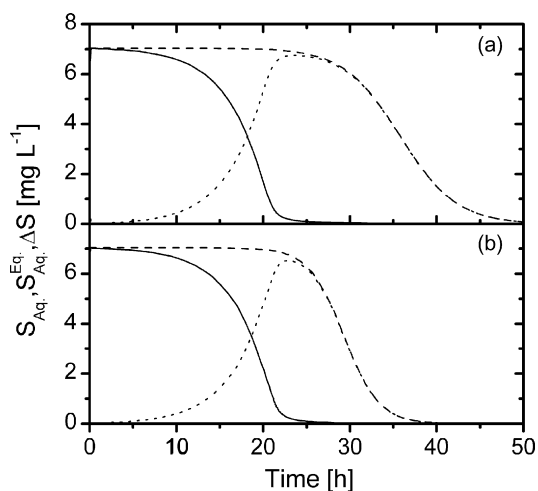


Fig. 7. Simulated non-equilibrium aqueous phase substrate concentration (solid lines), hypothetical equilibrium substrate concentration (dashed lines) and the mass transfer driving force  $\Delta S$  (dotted lines) as a function of fermentation time. The simulated values for Hytrel™ beads with a specific surface area of  $0.079 \text{ m}^2 \text{ dm}^{-3}$  are shown in plot (a) and the simulated values for Hytrel™ beads with a specific surface area of  $0.126 \text{ m}^2 \text{ dm}^{-3}$  are shown in plot (b).

transfer driving force  $\Delta S = S_{aq}^{\infty} - S_{aq}^t$ , represented by the dotted lines in Fig. 7. The driving force is low during the initial period of both fermentations, indicating that the mass transfer rate is sufficiently large compared to the microbial substrate consumption rate, allowing the biphenyl concentration to be almost at equilibrium in both phases (solid and aqueous phase). The driving force reaches a maximum between 25 and 30 h and decreases towards the end of the fermentation. The reactor providing more specific surface area (Fig. 7b) operates at aqueous phase biphenyl concentrations far below the equilibrium concentration only for the last 15 h of the fermentation (seen by the maximum in the driving force), whereas the reactor providing less surface area (Fig. 7a) operates under these conditions for the last 30 h of the fermentation. The reactor using the larger bead size operates under mass transfer limited conditions for a longer period of time than the reactor providing more surface area, which limits the bioavailable amount of substrate and results in lower microbial degradation rates and longer fermentation times.

## 5. Conclusions

Hydrophobic compounds such as biphenyl can be delivered to degrading organisms from Hytrel™ polymers is solid–liquid TPPBs. The mass transfer rate of the substrate into the aqueous phase is lower than in liquid–liquid two-phase systems [5], but higher than in aqueous systems containing pure substrate crystals [23]. The mass transfer rate can be lower than the microbial degradation rate resulting is substrate limited growth, which could be described successfully with a simple mechanistic model. The framework of this model can potentially be extended to other solid phase geometries which might be advantageous as easily removable contaminant absorbents in bioremediation applications [29].

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