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# Mass transfer considerations in solid–liquid two-phase partitioning bioreactors: a polymer selection guide

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## ABSTRACT

**BACKGROUND: Selection of polymers for two-phase partitioning bioreactors (TPPBs) has been focused primarily on predicting a polymer's affinity for the target molecule. Although the *extent* to which a polymer absorbs a solute is important, the *rate* of uptake/release must be sufficiently rapid such that a TPPB is not mass transfer limited. This work focused on developing a guide to identify combinations of polymer diffusivities and diffusional path lengths that will ensure a TPPB is not limited by substrate delivery.**

**RESULTS: TPPB systems limited by substrate delivery yielded linear growth, while biologically limited systems exhibited exponential growth. Release rates of phenol from various polymer phases increased as polymer diffusivity increased, or as diffusional path length (polymer bead size) decreased. A polymer selection guide was developed identifying combinations of polymer diffusivity and bead size that will ensure a TPPB is not mass transfer limited, for a desired maximum substrate consumption rate.**

**CONCLUSION: In selecting polymers for TPPB applications, solute affinity (extent of uptake) has been relatively well characterized using first principles methods, and the present work has now 'completed the picture' by providing a description of polymer transport properties (diffusivity and diffusional path length) to be able to generate a guide for selecting polymers.**
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**Keywords:** Bioreactors; Diffusion; Kinetics; Mass Transfer

## INTRODUCTION

Conventional bioremediation strategies for the degradation of toxic contaminants can be limited by substrate toxicity.[1] Two-phase partitioning bioreactors (TPPBs) overcome this limitation by utilizing a second immiscible phase that selectively partitions compounds to/from the cell containing aqueous phase. Substrates with low water solubility will partition into the sequestering phase at much higher concentrations than the cell-containing aqueous phase, thus maintaining the aqueous concentrations below inhibitory levels. The partitioning phase then acts as a reservoir which continually delivers the substrate to the aqueous phase at a rate that maintains a balance between thermodynamic equilibrium of the system and the metabolic demand of the cells.[2]

Immiscible organic solvents have proven to be effective partitioning phases for the degradation of phenol,[3] benzene,[4] toluene,[5] and polycyclic aromatic hydrocarbons.[6,7] However organic solvents must meet several stringent requirements including biocompatibility, nonbioavailability, low volatility, and low cost.[8] Furthermore, the nonbioavailability requirement often limits immiscible solvent TPPB applications to pure cultures, as mixed cultures are often capable of degrading a wide spectrum of organic molecules, including the selected solvent itself.[9]

Polymers have shown to be a promising alternative as the partitioning phase in TPPBs that can overcome these limitations. Commercial polymers are readily available in a wide range of homo-and-copolymer chemistries, can be formed into a variety of shapes with varying sizes, are non-biodegradable, non-toxic, non-flammable, can be easily handled, recovered, and reused,[10] and are generally much less expensive than organic solvents. Although polymers have been successfully used to sequester a wide range of toxic organic substrates, further success in identifying effective polymers requires that a rational approach be taken in selecting them, presumably on the basis of sound scientific principles. One absolute property that an effective sequestering phase must possess is a high affinity for the target molecule, usually characterized by the partition coefficient of the solute in the polymer. Using rigorous first principles thermodynamics, we have generated frameworks for selecting high affinity polymers by consideration of Hildebrand and Hansen Solubility Parameters, Flory–Huggins solution theory, and UNIFAC activity coefficient based models.[11–13] The TPPB community can now use these tools in selecting polymers on the basis of predicted solute affinity.

Although thermodynamic affinity considers the *extent* to which a polymer will sorb a solute, implementation of the TPPB

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**Table 1.** Summary of relevant properties of the different polymers used, and abiotic mass transfer results

| Polymer | $\rho$ (g cm$^{-3}$) | $M_P$ (°C) | $T_g$ (°C) | $D_{avg}$ (cm) | Diffusivity (cm$^2$ s$^{-1}$) | *Ka* (min$^{-1}$) | Partition coefficient |
|---|---|---|---|---|---|---|---|
| Hytrel 8206S | 1.17 | 180 | −60 | 0.19 | $1.567 \times 10^{-7}$ | $0.0247 \pm 0.00148$ | 42 |
| Hytrel 8206 M | 1.17 | 180 | −60 | 0.26 | $1.577 \times 10^{-7}$ | $0.0208 \pm 0.00081$ | 41 |
| Hytrel 8206 L | 1.17 | 180 | −60 | 0.32 | $1.582 \times 10^{-7}$ | $0.0124 \pm 0.00076$ | 41 |
| Hytrel 3548 | 1.16 | 156 | −45 | 0.38 | $1.352 \times 10^{-7}$ | $0.0093 \pm 0.00032$ | 45 |
| Hytrel 5544 | 1.22 | 215 | −35 | 0.33 | $4.297 \times 10^{-8}$ | $0.0061 \pm 0.00035$ | 25 |

$M_P$ – Melting point
$T_g$ – Glass transition temperature
Ka – Overall mass transfer coefficient

technology platform also requires that the *rate* of uptake/release be sufficiently fast such that the overall process is reaction, rather than mass transfer rate, limited. This was seen in some of our earlier work,[14] and was confirmed recently when we showed that although liquid–liquid TPPB systems may generally not be mass transfer limited because of the small droplet size of the dispersed organic phase, polymer TPPBs, operating above a few hundred rpms where external mass transfer limitations become negligible,[15] may have significant mass transfer resistance due to the rate of solute diffusion within the polymer structure. Another recent article identified that the substrate mass transfer between the polymer phase and the aqueous phase may be too slow in a high rate biological system, and that a thorough investigation of mass transfer is urgently needed.[16]

With external mass transfer limitations being negligible above a few hundred rpms, there are two main factors that affect the overall substrate diffusion rate into/from a polymer: polymer diffusivity, and diffusional path length. Diffusivity is a physical property that characterizes the rate of molecular transport of the target molecule within the polymer structure, and polymers with higher diffusivities would be expected to provide higher overall mass transfer rates. Diffusional path length also affects the overall rate of solute transport, as smaller polymer bead sizes would be expected to have a faster overall release/uptake rate of the target molecule.

As noted, overall degradation rates in TPPBs should be determined by the maximum volumetric microbial substrate demand, and not limited by the substrate mass transfer rate between the two phases. This study focused on characterizing the biological volumetric rate of a mixed population system degrading phenol as a substrate by evaluating the population's inherent kinetics. We then examined the effect of diffusivity and diffusional path length (polymer bead size) on abiotic phenol release by several grades/sizes of Hytrel beads by estimating their impact on the mass transfer coefficient, and the overall mass transfer rate. Experiments were then conducted to determine which combinations of diffusivity/bead size caused the biological system to become mass transfer rather than reaction rate limited. Finally a guide was created based on the critical polymer properties of polymer bead size and diffusivity to identify combinations of these two, controllable aspects, to allow a user to select polymers for TPPBs that would result in the system being reaction rate rather than mass transfer rate limited, for a given maximum microbial substrate demand. This new mass transfer guide, along with our earlier thermodynamic framework for selecting polymers on the basis of affinity, now provides the user with the tools needed to rationally select effective polymers in TPPB applications

## EXPERIMENTAL

### Polymers, organisms and medium formulation

Table 1 summarizes some relevant properties of the different Hytrel polymers used; Hytrel 8206 has been effectively used in the past as the sequestering phase for phenol degradation in TPPBs.[17] The as-received 0.32 cm Hytrel 8206 beads were also reduced in size using a rotary mill, to achieve two distinct smaller bead fractions by passing through standard mesh sieves, and the average bead diameter ($D_{avg}$) was estimated assuming the beads were spherical. The resulting three sizes of Hytrel 8206 polymer beads were labeled with an L (large), M (medium), or S (small) depending on the average bead diameter, as defined in Table 1.

A phenol-degrading microbial consortium consisting primarily of *Acinetobacter baumannii, Acinetobacter johnsonnii, Pseudomonas alcaligenes* and *Pseudomonas putida* was used as previously described.[17] The culture medium consisted of 2.08 g L$^{-1}$ $KH_2PO_4$, 2.56 g L$^{-1}$ $K_2HPO_4$, 1.0 g L$^{-1}$ $NH_4Cl$, 0.5 g L$^{-1}$ $MgSO_4{\bullet}7H_2O$, 0.1 g L$^{-1}$ yeast extract, and 0.5 g L$^{-1}$ phenol. All chemicals were purchased from either Sigma-Aldrich (Canada) or Fisher Scientific (Canada). Inoculum for the biological experiments was prepared 16 h in advance, with 50 mL samples incubated at 30 °C, and 180 rpm in 125 mL Erlenmeyer flasks. Each flask was inoculated initially with 100 µL frozen stock culture of the microbial consortium.

### Phenol uptake experiments

Diffusivity and overall mass transfer coefficients of different grades/sizes of Hytrel polymers were determined using a 1 L working volume New Brunswick Scientific reactor, fitted with two six-blade Rushton impellers and operated at 21 °C and 300 rpm. The initial aqueous phenol concentration was 2400 mg L$^{-1}$ with a polymer phase ratio of 3% added at time zero. Diffusivities were determined by performing a least squares regression analysis to fit the Crank Equation to the fractional uptake vs time data as previously described.[10]

Overall mass transfer coefficients between the aqueous and polymer phases were determined by performing a phenol mass balance on the system, wherein a decrease in the phenol aqueous concentration was attributed to an increase of phenol in the polymer phase. The slope of a $\ln(C_L - C^*)$ vs time plot gave the overall mass transfer coefficient, *Ka*, where $C_L$ is the measured bulk aqueous phase substrate concentration, and $C^*$ (theoretically the substrate concentration at the liquid surface adjacent to the polymer[15]) was determined by multiplying the mass-balance-estimated polymer concentration by the partitioning coefficient (PC). PCs were determined at equilibrium (after



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24 h contact it was assumed the system had reached equilibrium) as the ratio of the phenol concentration in the polymer phase divided by the phenol concentration in the aqueous phase.

### Biological control experiments

Biological control experiments (no polymer) were conducted to determine the kinetic parameters of the microbial consortium during phenol biodegradation, and these parameters were used to model microbial rates. These experiments used a 3 L working volume New Brunswick Bioflo III bioreactor, with a two six-blade Rushton impellor, and four baffles. The bioreactor was operated at 30 °C and 300 rpm, with aeration supplied at 0.3 vvm and an initial phenol concentration of 500 mg $L^{-1}$.

Microbial growth utilizing a single substrate can be modeled by definition of the specific growth rate, μ, as described in Equation (1),

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

The microbial specific growth rate μ ($h^{-1}$) is further assumed to follow Monod kinetics:

$$\mu = \frac{\mu_{max} S}{S + K_s} \quad (2)$$

where $\mu_{max}$ is the maximum specific growth rate under no substrate limitation, $S$ is the growth limiting substrate concentration, and $K_s$ is the Monod half-saturation constant for the cell – substrate combination. The biological volumetric rate of substrate consumption can then be related to the volumetric rate of biomass production by Equation (3) if a constant biomass-to-substrate yield coefficient is assumed:

$$\frac{dS}{dt} = \frac{1}{Y_{x/s}} * \frac{dX}{dt} \quad (3)$$

By manipulating Equations (1) – (3) it is possible to generate a differential equation in a single variable, which can be solved analytically as shown in Equation (4), with initial conditions that at $t = 0$, $S = S_0$ and $X = X_0$,

$$\left(X_o + Y_{\frac{x}{s}} \left(S_o + K_s\right)\right) * \ln\left(\frac{X_o + Y_{\frac{x}{s}}\left(S_o - S\right)}{X_o}\right) - K_s Y_{\frac{x}{s}} * \ln\left(\frac{S}{S_o}\right) = \mu_{max} t \left(X_o + Y_{\frac{x}{s}} S_o\right) \quad (4)$$

Values of the maximum specific growth rate ($\mu_{max}$), half saturation constant ($K_s$) and the yield coefficient ($Y_{x/s}$) were determined for the culture growing on phenol by finding values that best fit Equation (4) from the batch biological control experiments.

### Biodegradation experiments in the presence of polymers

Biodegradation experiments using a TPPB were conducted to determine under what conditions of polymer bead size and diffusivity, a TPPB system would be limited by substrate mass transfer from the polymer partitioning phase. Qualitatively it was anticipated that three situations could arise: (1) the system was never mass transfer limited, and the cells would experience exponential growth; (2) the system would be entirely mass transfer limited resulting in linear microbial growth; and (3) the system would transition from unlimited growth to mass transfer limited growth which would appear as an exponential increase in cells, switching to linear growth as mass transport became limiting. These experiments were conducted in a similar manner to the biological control experiments, with the exception that phenol was not added directly to the culture medium, rather, it was provided to the cells by preloading 100 g of polymer bead with a desired amount of phenol. There were two different loading strategies used in the biodegradation experiments. The first strategy involved loading the polymers with enough phenol, such that, should the system reach equilibrium quickly, the aqueous phenol concentration would be 500 mg $L^{-1}$ (example: 3300 mg in 100 g of Hytrel 8206 polymers, in 3 L of fermenter aqueous volume). The second strategy involved loading the polymers with the same total mass of phenol used in the biological control experiments (1500 mg in total).

In addition to observing such reaction/mass transport limitations through experimental results, mathematically describing the biological and mass transfer rates was also undertaken to confirm whether/when mass transport became limiting. Finally, such modeling was also used to formulate a polymer selection guide that would allow a user to answer the question: if the highest anticipated/desired biological volumetric rate is known, what polymer size and polymer diffusivity must be used in order for the system not to be mass transfer limited?

The volumetric mass transfer rate of substrate delivery can be described by Equation (5)

$$\overline{Q}_s = Ka\left(S^* - S_{aq}\right) \quad (5)$$

where $Ka$ is the overall mass transfer coefficient ($h^{-1}$), $S^*$ is the aqueous substrate concentration in equilibrium with the polymer substrate concentration, and $S_{aq}$ is the actual substrate concentration in the cell containing aqueous phase.[14] $Ka$ can be determined experimentally (as described above), and the aqueous and polymer phenol concentrations, respectively, can be found from experimental measurements of aqueous phase phenol concentrations, and mass balance, knowing the partition coefficient for phenol of the polymer.

The instantaneous biological volumetric rate of substrate consumption can be calculated from Equation (3) with measured values of cell and substrate concentration determined experimentally, and previously-determined kinetic coefficients. By comparing the calculated instantaneous values of the volumetric mass transfer rate and the volumetric biological rate, it was possible to see which rate was higher during the course of the experiments, potentially as also reflected in the cell biomass trajectories (exponential/linear).

### Analytical methods

Aqueous phenol concentrations were determined using either the 4-aminoantipyrene method (triplicates were tested) at 505 nm as previously described,[18] or by the use of a Varian Pro Star HPLC with UV/VIS detection (duplicates were tested) as previously described,[19] depending on availability of equipment. Separate experiments confirmed the interchangeability of these two methods. Cell concentrations were measured using optical density (OD) values at 600 nm and converted to cell dry weight using a predetermined calibration curve.



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## RESULTS AND DISCUSSION

### Characterizing phenol uptake by polymers: diffusivity, partition coefficients, and mass transfer coefficients

As was previously demonstrated,[15] as long as mixing in small scale bioreactors exceeds 200 rpm, the overall resistance to mass transfer of a solute between an aqueous phase and a polymer bead resides exclusively within the polymer itself, and depends on the diffusional path length and the solute diffusivity. Both aspects were independently examined via abiotic dynamic uptake experiments at 300 rpm for different polymer bead sizes of a single grade of Hytrel (8206) and three grades of Hytrel possessing different phenol diffusivities (8206, 3548 and 5544), in conjunction with data fits to the Crank Equation as previously described.[10] In addition, end point phenol concentration measurements and the use of mass balances were used to determine the partition coefficients of phenol for each of the polymers studied.

Figure 1 shows the phenol uptake data, along with one example of the Crank Equation fit, and a replicate run for Hytrel 8206. The data clearly show that smaller bead sizes of a specific grade of Hytrel (8206) result in more rapid uptake of phenol, and that different Hytrel grades, possessing different chemical structures, have both different uptake rates for similar bead sizes (reflecting differences in phenol diffusivity) as well as different affinities (partition coefficients). By way of specific example, after 40 min the Hytrel 8206S beads reached 80% of their final equilibrium concentration, while the medium and large beads reached 67% and 53% of equilibrium, respectively. Furthermore, after 40 min the Hytrel 5544 beads reached only 23% of their equilibrium concentration, less than half of that which the 8206 L polymers had achieved. As seen in Table 1, the polymer diffusivities vary across the various grades of Hytrel, and remain essentially constant for the varying sizes of Hytrel 8206 polymer at $1.567 \times 10^{-7}$ cm$^2$ s$^{-1}$, which is close to previously reported values.[8] The PC values for the various polymers are also shown in Table 1, and reflect the slightly different chemical compositions of the various grades of Hytrel. Partition coefficients determined at equilibrium for the Hytrel 8206 polymer sizes were all approximately 41, which is also close to values reported previously.[11,15,20] The overall mass transfer coefficients (Table 1) decrease as the polymer size increases (within Hytrel 8206), or as the diffusivity of the polymer decreases (across the various Hytrel grades), also as expected.

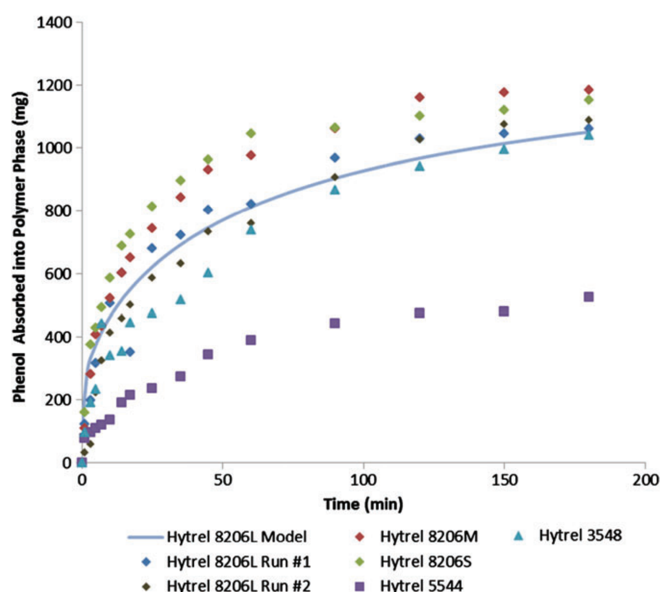


**Figure 1.** Effect of polymer bead size and diffusivity on the mass of phenol absorbed into the polymer phase.

**Table 2.** Comparison of kinetic parameters of microbial consortium used to literature values

| | $S_o$ (mg L$^{-1}$) | $\mu_{max}$ (h$^{-1}$) | $Y_{x/s}$ | $K_s$ (mg L$^{-1}$) | Ref |
|---|---|---|---|---|---|
| This work | 500 | 0.3 | 0.69 | 45 | |
| *Pseudomonas putida* ATCC 11172 | 600 | 0.28 | 0.53 | N/A | 17 |
| *Pseudomonas putida MTCC 1194* | 1000 | 0.216 | 0.65 | 20.59 | 22 |
| Mixed culture | 600 | 0.3 | 0.7 | N/A | 17 |
| Mixed culture | 0-800 | 0.37 | N/A | 144.68 | 23 |

### Kinetic parameters

Table 2 shows the kinetic parameters for the phenol degrading consortium utilized in this study, as determined from biological control experiments; the values obtained are similar to literature values for other organisms degrading phenol at a similar initial sub-inhibitory substrate concentration. Dissolved oxygen limitations were assumed negligible as aeration was supplied at 1 L min$^{-1}$; significantly higher than previous literature values which found no oxygen limitations.[21]

### Effect of polymer diameter/diffusivity on TPPB performance

As noted, biological systems that are not mass transfer limited should exhibit exponential cell growth, whereas mass transfer limitations can cause exponential growth to become linear or, in the worst case, to be linear throughout a bioconversion process. The effect of diffusional path length (bead diameter) and polymer diffusivity on substrate delivery was therefore investigated by employing different sizes and grades of Hytrel polymer. Figure 2(a) shows the biomass and substrate concentration time course for TPPBs employing different Hytrel 8206 bead sizes, using the first loading strategy as described in Materials and methods. For the same initial biomass concentration of 0.065 g L$^{-1}$ for both systems, higher aqueous substrate concentrations were achieved, initially and throughout the fermentation, for the 8206S beads compared with the Hytrel 8206 L beads, signifying a greater substrate delivery rate. It appears as though, for this loading strategy, neither system was mass transfer limited, as the instantaneous substrate concentrations were all well above $K_S$, resulting in only exponential growth. Figure 2(b) and (c) confirm that neither system was mass transfer limited, as the calculated substrate mass transfer rate was greater than the biological rate for both polymers, for the duration of the experiment. It appears that at the initial biomass concentration employed, and the substrate levels and rates of release arising from loading strategy 1, mass transfer limitations do not occur, which led to devising a new (reduced) loading strategy that might result in mass transfer limitations.

The second (reduced) substrate loading strategy was therefore employed, along with an increased initial cell concentration (0.08 g L$^{-1}$) in order to reduce the substrate delivery rate and increase the biological demand such that a transition from exponential to linear growth might occur. Figure 3(a) shows the aqueous substrate and biomass concentration time course for TPPBs employing the Hytrel 8206 L and 8206S polymers. In

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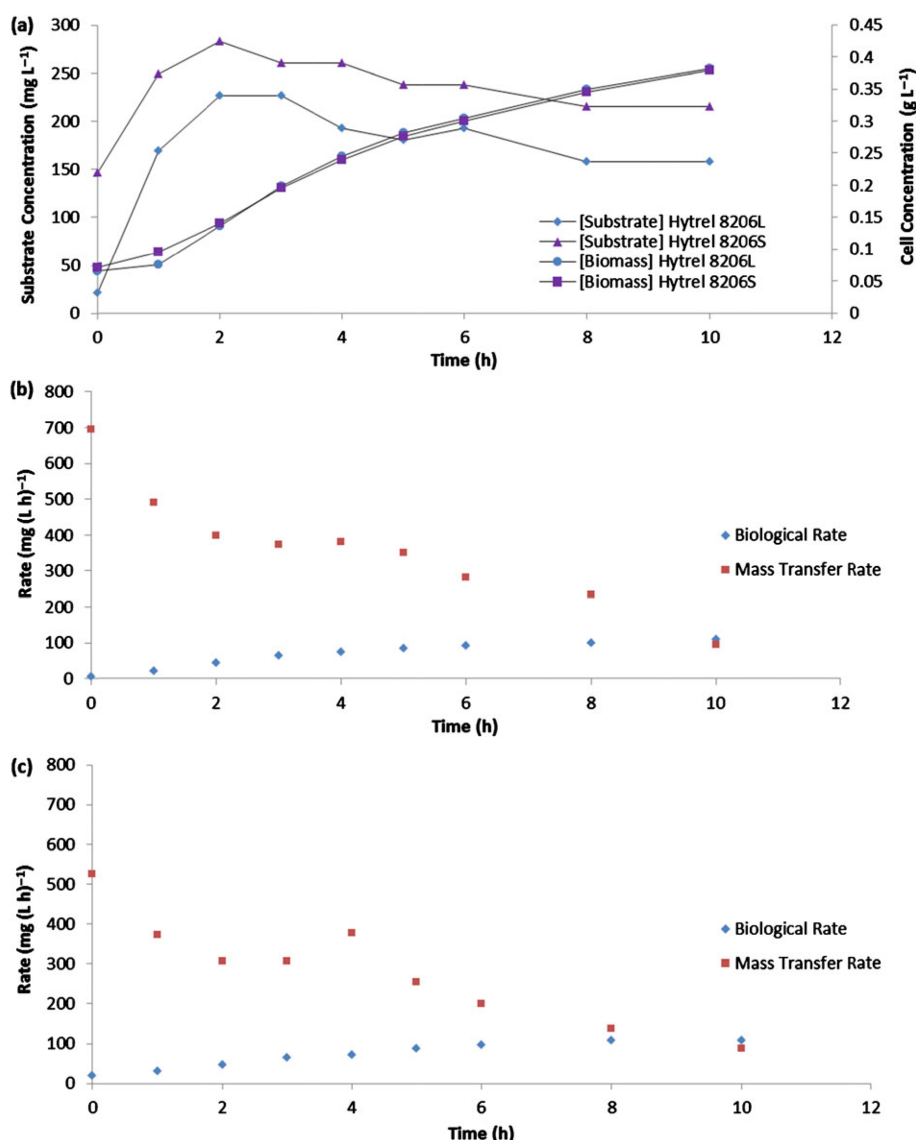


**Figure 2.** (a) Substrate and biomass concentration time course for the bioreactors employing Hytrel 8206 L and 8206S beads (1st loading strategy). Parts (b) and (c) compare the calculated biological and mass transfer rates for the bioreactors employing Hytrel 8206 L and 8206S beads, respectively.

this case the aqueous substrate concentrations for both TPPBs, decrease sharply after 2 h operation, and approach levels that might lead to substrate limiting conditions. The biomass data show that after about 6 h operation the 8206 L beads appear to exhibit linear growth suggesting that a mass transfer limitation may be occurring. Figure 3(b) mathematically confirms this observation, as the mass transfer rate decreases to below the biological rate, after 6 h operation, and remains the dominant (lowest) rate for the rest of the experiment. In contrast the 8206S beads appear to result in only exponential cell growth, until the final data point where the aqueous phenol concentration is 44.9 mg $L^{-1}$ similar to the value of the half saturation constant (45 mg). Figure 3(c) shows that the calculated mass transfer rate for Hytrel 8206S beads decreases from 245 mg $(L\,h)^{-1}$ as phenol is released to the aqueous phase, and does not drop below the biological rate until the final data point. Overall these data show that smaller polymer beads sizes can significantly improve the performance of a TPPB by reducing/eliminating mass transfer limitations, and that polymer bead size can now be used as a selection criterion to counteract the suggestion that mass transfer limitations are inevitable in polymer-based TPPB systems.

The second polymer property, diffusivity, was also investigated by employing two different grades of Hytrel polymer, 8206 L and 5544, possessing similar bead diameters but different diffusivities (Table 1). The second (reduced substrate) loading strategy was again used in this experiment, and the initial biomass concentration in both cases was 0.04 g $L^{-1}$. Higher aqueous substrate concentrations were generated by the Hytrel 8206 L polymers, as seen in Fig. 4(a), indicating a faster release rate, which is consistent with the phenol uptake data and calculated diffusivities, seen in Fig. 1 and Table 1, respectively. After 6 h operation, however, both systems appear to transition from exponential to linear cell growth, suggesting that mass transfer limitations may be occurring as biological rates exceed mass transfer rates. Figure 4(b) and (c) mathematically substantiate this, as the biological and mass transfer rates intersect after 6 h operation for both systems, and the mass transfer rate becomes the limiting overall aspect of the process. Although both biomass curves appear to be linear after

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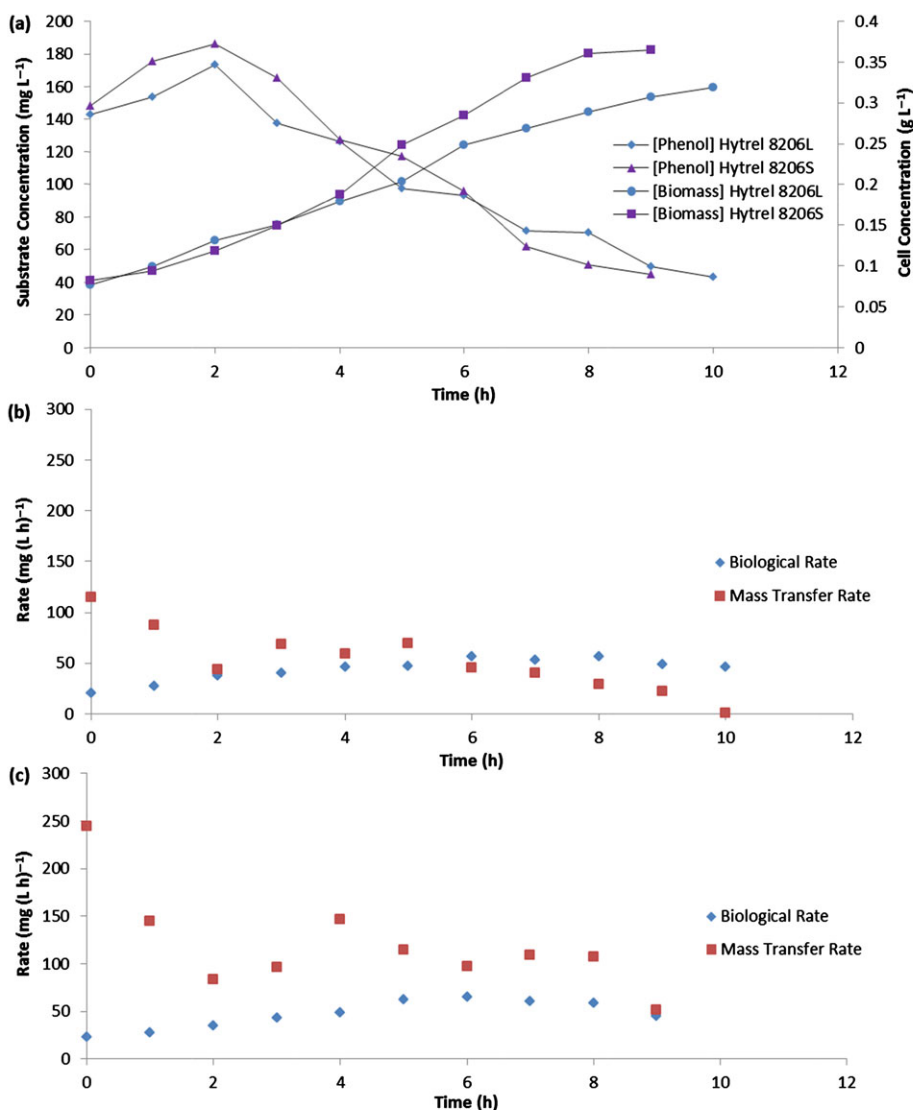


**Figure 3.** (a) Substrate and biomass concentration time course for the bioreactors employing Hytrel 8206 L and 8206S polymers (2nd loading strategy). Parts (b) and (c) compare the calculated biological and mass transfer rates for the bioreactor employing Hytrel 8206 L and 8206S beads, respectively.

6 h (Fig. 4(a)), the higher diffusivity of the 8206 L polymer led to a higher linear rate of biomass change. These results have shown that a second polymer property, diffusivity, in addition to polymer bead size, can also be used to select polymers that will provide increased mass transfer rates in polymer-based TPPBs.

## Polymer selection guide

A polymer selection guide, based on polymer diffusivity and bead size, was then developed to allow a user to select polymers for TPPBs that will ensure that a system is not limited by the rate of substrate delivery, for a target maximum microbial volumetric consumption rate. In many instances the performance of a bioreactor (characterized by the volumetric rate of substrate consumption) will be known in order to ensure that a process meets its performance objectives, and this guide can identify which size and type of polymer will be needed to ensure that a TPPB achieves this rate. The guide is based on the use of Hytrel 8206, overwhelmingly the most commonly used polymer in TPPB research, although as long as the partition coefficient for the target substrate is known, similar guides can be prepared for different polymer/substrate combinations.

The constant diffusivity and diameter curves in Figs 5 and 6 were generated for different assumed combinations of polymer diffusivities (ranging from $1.0 \times 10^{-6}$ to $1.0 \times 10^{-9}$ cm$^2$ s$^{-1}$) and bead diameters (ranging from 0.1 to 0.5 cm) by initially using the Crank Equation in 'reverse'. That is, for an assumed pair of diffusivity and bead size values, the Crank Equation was used to generate a mass transfer uptake curve (similar to that shown in Fig. 1). The concentration time course was then linearized as described in Materials and methods, and the corresponding overall mass transfer coefficients were calculated from the slope of the $\ln(C_L - C^*)$ vs time plot.

In order to then calculate the overall mass transfer rate (Equation (5)), a concentration driving force ($S^* - S_{aq}$) had to be selected. The concentration driving force selected would need to ensure that a system is not mass transfer limited, until lower substrate concentrations are reached, ones that would cause reduced rates based on Monod kinetics, at which point mass transfer rates become insignificant. That is, as substrate depletion is neared, the Monod

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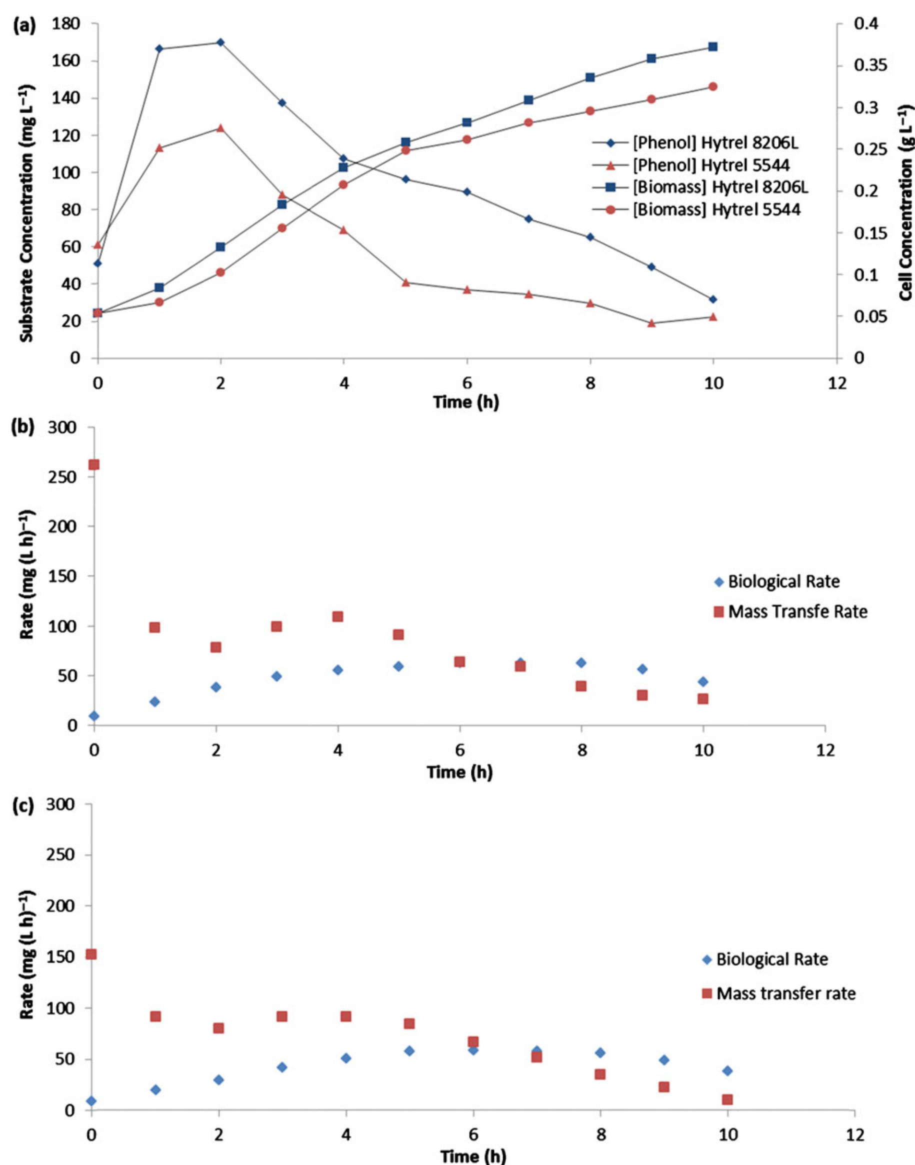


**Figure 4.** (a) Substrate and biomass concentration time course for the bioreactors employing Hytrel 8206 L and 5544 beads (2nd loading strategy). Parts (b) and (c) compare the calculated biological and mass transfer rates for the bioreactor employing Hytrel 8206 L and 5544 beads, respectively.

model predicts ever-diminishing rates of biological substrate consumption, regardless of the substrate mass transfer rate. With this criterion in mind, an aqueous substrate concentration ($S_{aq}$) of 100 mg L$^{-1}$ was selected, as this value reduces the specific growth rate to 70% of $\mu_{max}$ (based on our value of $K_S$) as the system's kinetics become substrate limited and mass transfer rates are inconsequential. Then $S^*$ was calculated as 260 mg L$^{-1}$ using a PC of 41, and conducting a phenol mass balance on the system. Using this driving force ($S^* - S_{aq}$) and the previously calculated overall mass transfer coefficients, the maximum mass transfer rate was determined for each combination of diffusivity and diffusional path length. This rate determines the maximum biological rate possible, allowing for the generation of the curves displayed in Figs 5 and 6.

As an example of using the guide, if the target maximum substrate consumption rate is selected to be 600 mg L$^{-1}$ h$^{-1}$ then, using Fig. 5, polymers with a bead diameter of 0.17 cm and a diffusivity of $5.0 \times 10^{-7}$ cm$^2$ s$^{-1}$ or a bead diameter of 0.24 cm and a diffusivity of $1.0 \times 10^{-6}$ cm$^2$ s$^{-1}$ would need to be selected to ensure that the system can achieve this rate, without mass transfer limitations occurring. Figure 5 is useful if polymers are available in fixed sizes (y-axis), but different polymer types with associated different diffusivities. Figure 6 represents the same data based on knowing a polymer's diffusivity, and determining what bead size of that polymer would be required to achieve the same performance.

## CONCLUSIONS

TPPBs have shown to be effective in the biodegradation of toxic contaminants, as the partitioning phase acts as a reservoir that regulates their concentration in the cell containing aqueous phase to below inhibitory levels. Solid polymer partitioning phases possess numerous advantages over traditional immiscible organic solvents,[8] however, solid polymers possess internal mass transfer limitations, based on the critical properties of diffusivity and diffusional path length, which may limit their potential benefits. This

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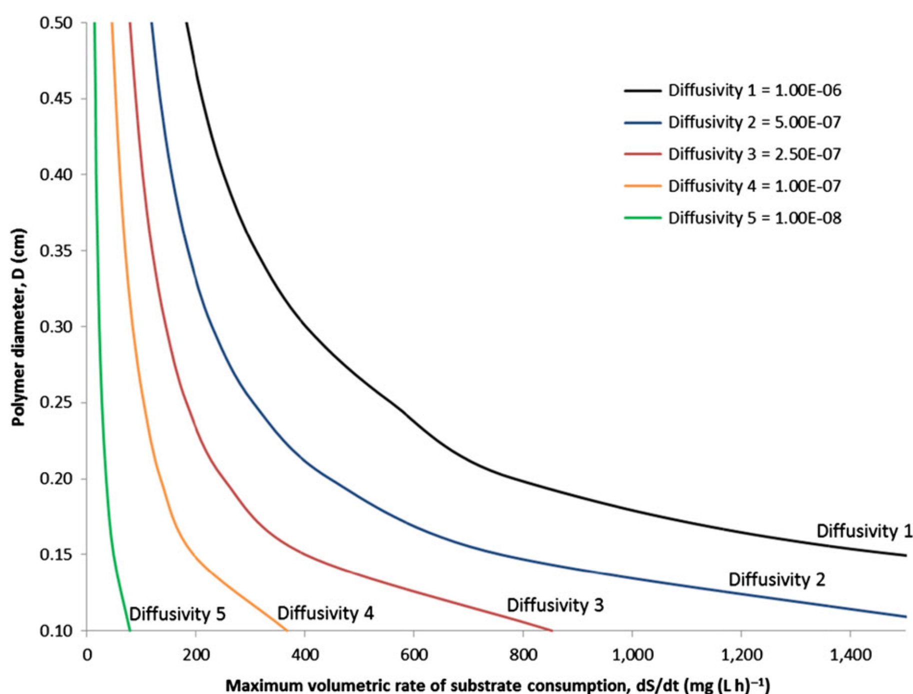


**Figure 5.** Polymer selection guide with constant diffusivity curves (cm$^2$ s$^{-1}$). Polymers with higher diffusivity allow for higher maximum microbial volumetric consumption rates to be achieved for a given bead diameter.

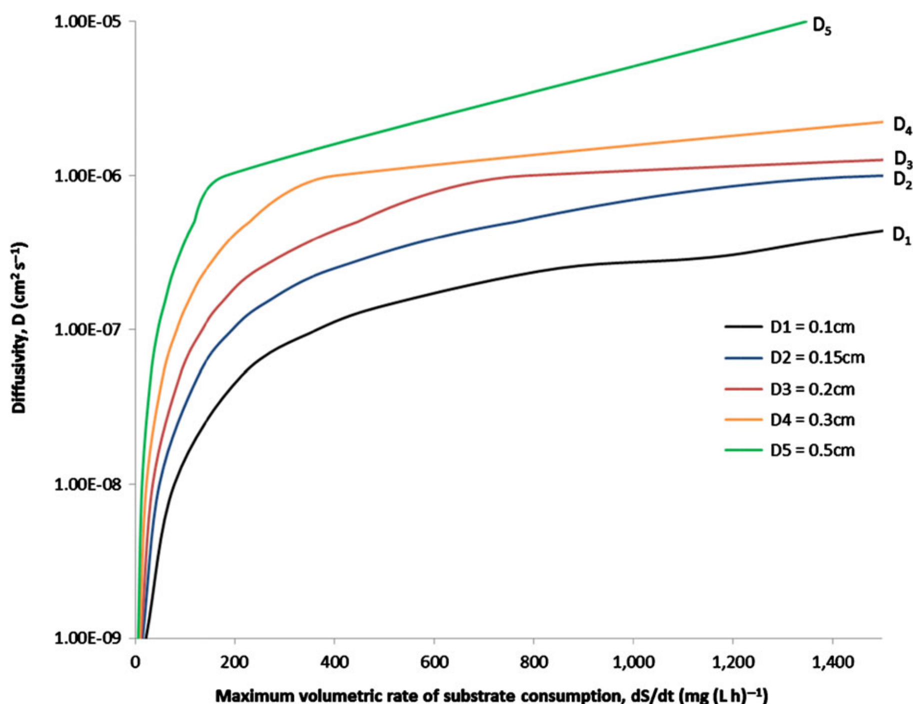


**Figure 6.** Polymer selection guide with constant diffusional path lengths (cm) curves. A decrease in diameter allows higher maximum microbial volumetric consumption rates to be achieved, for a given polymer diffusivity.

work has shown that the rate of uptake of a target molecule into the polymer phase increases as the diffusivity increases, or when the diffusional path length (polymer bead size) decreases, and can result in higher biological consumption rates being achieved. A polymer selection guide based on microbial kinetic relationships and convective mass transfer equations has been created to allow a user to select a polymer, based on diffusivity and polymer bead size, to ensure that a TPPB is reaction rate rather than mass transfer limited, for a target maximum volumetric substrate consumption rate. Future work will confirm that TPPB systems that deal with *in situ* product removal of inhibitory target molecules can also be characterized using the approach described in this work, and that the guide presented here is equally applicable to such reverse situations.

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