Polymer Development for Enhanced Delivery of Phenol in a Solid−Liquid Two-Phase Partitioning Bioreactor

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Two-Phase Partitioning Bioreactors (TPPBs) have traditionally been used to partition toxic concentrations of xenobiotics from a cell-containing aqueous phase by means of an immiscible organic solvent and to deliver these substrates back to the cells on the basis of metabolic demand and the maintenance of thermodynamic equilibrium between the phases. A limitation of TPPBs, which use organic liquid solvents, is the possibility that the solvent can be bioavailable, and this has therefore limited organic liquid TPPBs to the use of pure strains of microbes. Solid polymer beads have recently been introduced as a replacement for liquid organic solvents, offering similar absorption properties but with the capability to be used with mixed microbial populations. The present work was aimed at identifying a polymer with a greater capacity for and more rapid uptake and release of phenol for use as the second phase in a mixed culture TPPB. Polarity and hydrogen bonding capabilities between polymer and phenol were considered in the screening and selection process of candidate polymers. Hytrel (a copolymer of poly(butylene terephthalate) and butylene ether glycol terephthalate) polymer beads, offered improved capacity (19 mg phenol/g polymer at a fixed initial phenol concentration of 2000 mg/L) and a greater diffusivity (1.54×10^{-7} cm^2/s) when compared to the capacity and diffusivity of previously used EVA (ethylene vinyl acetate) beads (12.4 mg phenol/g polymer and 3.73×10^{-9} cm^2/s, respectively). Hytrel polymer beads were then used in a TPPB for the investigation of various substrate feeding strategies (fed-batch, bead replacement, and concentrated spikes of phenol), with rapid and complete phenol degradation shown in all cases.

Introduction

Controlled bioremediation of man-made xenobiotics has received a great deal of attention due its appeal as a "green" cleanup strategy. Challenges that impede the biodegradation of xenobiotics include substrate toxicity, limited availability to microbes of relatively water-insoluble substrates, and maintenance of a threshold substrate concentration. In an effort to overcome these limitations the Two-Phase Partitioning Bioreactor (TPPB) concept has been proposed (*1*, *2*).

Traditionally, a TPPB uses an immiscible organic liquid that acts as a reservoir and delivery agent to effectively partition toxic substrates to/from the aqueous phase. The transport of substrates between the organic and aqueous phase is governed by thermodynamic equilibrium. Microbial consumption of toxic molecules in the aqueous phase disrupts this equilibrium, resulting in the transfer of substrate from the organic phase to the aqueous phase to maintain equilibrium. In this manner, the rate at which substrates are provided to the system is dependent on the metabolic activity of the microorganisms rather than mass transfer, as seen by exponential cell growth, and therefore the system can be regarded as being self-regulating. TPPBs have proven to be effective at reducing toxic substrate concentrations to subinhibitory levels and offering improved loading and delivery of relatively water-insoluble compounds (*3*−*5*) but have also been limited by the potential bioavailable nature (i.e., biodegradability) of the delivery solvent. Because of the possible consumption of the organic solvent, single species of microorganisms and aseptic techniques generally must be used, and therefore in order to expand the biodegradation possibilities of TPPBs there must be improvement of the second phase.

The restriction of using pure cultures in TPPBs is compensated by the fact that, through rational solvent selection (*6*), an organic delivery phase can be tailored to a substrate/organism pairing, usually with excellent results. Several studies (*4*, *5*, *7*) have employed silicone oil as a delivery phase, solely because of its nonbiodegradability, along with mixed cultures; however, the properties of silicone oil are fixed. Thus, the use of silicone oil provides no opportunity for enhancing important solvent properties (e.g., substrate solubility, substrate distribution coefficient, viscosity, surface tension) to accommodate different substrates. This fundamental limitation of the use of silicone oil has been amply demonstrated in TPPB systems degrading polyaromatic hydrocarbons (PAHs), in which rational solvent selection (*8*) provided solubilities for PAHs substantially higher than are possible for silicone oil (*7*).

The use of solid polymer beads has recently been shown to be an effective replacement for immiscible organic liquids in TPPBs (*9*). The ability of polymers to provide controlled absorption and release of small molecular weight molecules has been well documented in the area of drug delivery (*10*) and, to a lesser extent, xenobiotics (*9*, *11*, *12*). The nonbiodegradable nature of the solid polymer beads allows the use of a microbial consortium

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in a TPPB, which has been shown to offer improved rates of degradation of phenol over systems that contain a single pure microbial species (*13*). Moreover, as is the case for organic liquid TPPBs, polymers can be tailored and modified, for example, through monomer selection, functionalization, copolymerization, cross-linking, and polymer processing, to adapt their uptake/release properties to different target molecules.

The first objective of this work was to rationally select polymers that would offer improved capacity for and delivery of a target molecule (phenol) for degradation in a TPPB containing a microbial consortium. Previous work with EVA beads for the absorption and release of phenol (*9*, *13*) were the standard for which improvement was to be compared. Screening and selection of candidate polymers requires an understanding of the molecular structure of the polymer, possible functional groups contained within the polymer, and their affinities for the target molecule. Previous work with polyurethane foam (*11*) has shown that hydrogen bonding has been a possible mechanism for the absorption of phenol from an aqueous solution.

The second purpose of this work was to investigate the performance of a TPPB and selected polymer when presented with a variety of substrate feeding scenarios. Three such strategies were investigated: fed-batch operation, the addition of concentrated phenol spikes, and the replacement of spent polymer beads with beads loaded with phenol. Because polymer beads do not have as large an affinity for phenol as do many specifically selected liquid solvents, it was important to determine if the beads are capable of managing concentration fluctuations arising from aggressive substrate feeding, and as well, it was important to determine how the microbial consortium reacted to the stress of the feeding scenarios.

Materials and Methods

Chemicals/Polymers. Samples of polymer beads were obtained from Dupont (Hytrel, Nucrel, EVA, ELVAX) and Scientific Polymer Products Inc. (nylon 6, nylon 6/6, nylon 4/6, nylon 6/9, styrene-copolymer-butadiene). Dr. S. Parent of Queen's University supplied polypropylene beads. The polymer beads were roughly spherical with diameters of about 2−5 mm. Phenol (with an assay of 99% minimum) and all other chemicals (see below) were purchased from Sigma-Aldrich Chemical Co. (Oakville, ON).

Organisms and Culture Conditions. A microbial consortium was used for the degradation of phenol for all fermentation experiments. The consortium was isolated, by means of selective enrichment, for its superior performance in phenol degradation relative to a widely used pure strain of *Pseudomonas putida* (ATCC 11172) and was used in previous work (*13*). The culture contains four separate entities: *Acinetobacter baumannii*, *Acinetobacter johnsonii*, *Pseudomonas alcaligenes*, and *Pseudomonas putida*, all of which aid in the degradation of phenol (*13*). Microscopic examination of samples was used throughout the experiments to confirm the presence of the four microorganisms. The consortium was maintained by serial transfers in liquid culture which consisted of (g/L): K_2HPO_4, 2.56; KH_2PO_4, 2.08; NH_4Cl, 1; $MgSO_4 \cdot 7H_2O$, 0.5; ferric ammonium citrate, 5×10^{-5}; $CaCl_2$, 5×10^{-6}; and carbon source 250 mg/L phenol and 590 μg/L of succinic acid. The cultures were grown overnight and stored at 4 °C. Maintenance and inoculum preparation were undertaken aseptically; however, all fermentation experiments were operated without consideration of sterile techniques.

Inocula. Inocula for use in the TPPB system were grown in 125-mL Erlenmeyer flasks containing 50 mL of mineral salts solution and 500 mg/L of phenol. Phenol was used as the sole available carbon source in order to fully acclimatize the organisms prior to inoculation. The cultures were incubated at 30 °C and 180 rpm for a 24-h period, spun down at 3400 rpm for 10 min, and resuspended in 50 mL of fresh carbon-free medium prior to inoculation. A consistent mass of 0.07 g cell dry weight (CDW) was used to inoculate each of the fermentations.

Analytical Methods. Cell turbidity was measured using an Ultraspec 3000 spectrophotometer at 600 nm, and concentrations were determined from previously constructed calibration curves.

Phenol concentration was determined using the 4-aminoantipyrine method (*12*), which is effective at phenol detection to concentrations of 5 μg/L. Absorbances were measured at 510 nm using an Ultraspec 3000 spectrophotometer, and subsequent concentrations were determined with a calibration curve.

Phenol Absorption Capacity of Hytrel. A 6-g portion of each tested polymer was added to 125-mL Erlenmeyer flasks containing 70 mL of a 2000 mg/L phenol/salts medium solution. The flasks were sealed with a rubber stopper and agitated at 180 rpm and 30 °C for a 24-h period, after which a sample was withdrawn to determine the remaining phenol concentration in solution. A mass balance on total phenol present was performed to determine the absorption capacity of the polymer.

Partitioning Coefficient of Hytrel. To determine the partitioning coefficient of Hytrel for phenol, 6 g of Hytrel polymer beads were added to each of six 125-mL Erlenmeyer flasks containing 70 mL of phenol/salts medium solution at phenol concentrations of 500, 1000, 2000, 3000, 4000, and 5000 mg/L. The flasks were sealed with rubber stoppers, agitated at 180 rpm, and maintained at 30 °C for a period of 24 h. The remaining phenol in solution was then determined, and a mass balance was performed to calculate the mass of phenol absorbed by the polymer beads.

Diffusivity Coefficient of Hytrel. To determine the diffusivity coefficient of phenol in Hytrel, nine 125-mL Erlenmeyer flasks were prepared with each flask containing an equal volume (70 mL) of salts solution and an equal concentration of phenol (1940 mg/L). Next, 6 g of fresh Hytrel polymer beads was placed in each of the flasks, and the flasks were sealed with rubber stoppers and agitated at 180 rpm and 30 °C. Samples were analyzed for phenol concentration at various times over a 25-h period. Once a sample had been drawn from a flask, the flask was discarded, and this process was continued until the last flask was sampled at 25 h. From the phenol concentrations in solution a mass balance was performed, and the mass of phenol absorbed by the beads at various times was determined. To determine the diffusivity coefficient of Hytrel for phenol, a least squares regression was performed on the uptake data as described in ref *9*.

Biodegradability of Hytrel. To determine if the selected polymer beads were biodegradable, 6 g of beads was placed in a 125-mL Erlenmeyer flask containing 50 mL of mineral salts solution. The flask was inoculated with 5 mL of TPPB inoculum and agitated at 180 rpm and 30 °C for a period of 2 weeks. Samples were periodically taken to determine cell concentrations.

Batch Fermentation in a TPPB using Hytrel. Fermentation in a TPPB was undertaken in a New Brunswick Scientific Co., Bioflo III fermentor. The bioreactor had a working volume of 3 L consisting of mineral

salts solution and a phenol concentration of approximately 1850 mg/L. The bioreactor was agitated at 400 rpm and aerated at 3 L/min with the temperature and pH being automatically controlled at 30 °C and 6.8, respectively. A predetermined mass (200 g), based on capacity experiments, of Hytrel polymer beads was added to the reactor, and the system was allowed to equilibrate for a period of 15 h. Upon reaching phenol equilibrium the system was inoculated with 0.07 g CDW of the microbial consortium. A dissolved oxygen (DO) probe measured DO levels, and liquid samples were periodically removed to measure phenol and cell concentrations.

TPPB Feeding Strategies using Hytrel. ***Fed-Batch Feeding Strategy.*** This experiment was initiated with a batch fermentation in which 3 L of mineral salts medium at a phenol concentration of 5000 mg/L was prepared, and 380 g of fresh Hytrel polymer beads were added to the system and allowed to equilibrate, with respect to phenol concentration, for a period of 15 h. The fermentor was inoculated with a mass of 0.07 g CDW of microbial consortium, and the system was agitated at 600 rpm and aerated at 4 L/min, with the temperature and pH automatically controlled at 30 °C and 6.8, respectively. Fed-batch mode occurred upon completion of the initial batch growth. Then, 1.5 L of medium was removed and replaced with a peristaltic pump at a rate of 6 L/h (i.e., within 15 min). The removed volume was replaced with 1.5 L of fresh mineral salts medium loaded with 9 g of phenol (6000 mg/L), which would result in a total phenol concentration of 3000 mg/L in the bioreactor. Three medium removals and additions were undertaken in fed-batch mode.

Bead Replacement, Phenol Spike, and Process Validation Feeding Strategies. Upon completion of a batch fermentation, as discussed above, the bioreactor was stopped, and the beads contained within the system were removed and replaced with phenol-loaded beads and 150 mL of concentrated salt solution. Loading of the beads occurred in 1 L of mineral salts solution containing 15 g of phenol; 380 g Hytrel polymer beads were agitated in this solution with a magnetic stirrer and left overnight. Sampling of the solution before the beads were added and after the beads were removed was used to determine the mass of phenol absorbed by the polymer beads. Two bead replacement feeding strategies were performed to show reproducibility.

Upon completion of the bead replacement feeding strategy, 150 mL of concentrated salt solution containing 60 g/L phenol was added to the bioreactor. Samples were taken 15 min after addition and then periodically over the duration of the fermentation. Two phenol spike fermentations were performed to show reproducibility.

After the above feeding strategies had been completed, a final test was performed to confirm the essential role that the polymer beads play in reducing phenol concentrations in a TPPB. All of the polymer beads were removed from the bioreactor, and the system was loaded with a significantly larger concentration of phenol (5800 mg/L). The system was agitated and aerated for 22 h during which time samples were taken to determine if cell concentration and phenol concentration were changing as a result of degradation. At 22 h the beads were returned to the bioreactor, and the system was sampled for a further 28 h period.

Results and Discussion

Polymer Selection. Phenol is an aromatic alcohol that exhibits weak acid properties and is the simplest member of the hydroxyl benzene class. As a result of the hydroxyl functional group attached to the benzene ring, phenol is a relatively polar compound when compared to other aromatic hydrocarbons such as benzene and is miscible in water to a concentration of 1 g phenol/15 mL at 20 °C (*14*). The hydroxyl functional group readily lends itself to forming a hydrogen bond with electronegatively charged molecules, which is a convenient characteristic when selecting a polymer with an affinity for phenol.

Table 1. Initial Capacity of Phenol for Candidate Polymers for an Initial Phenol Concentration of 2000 mg/L

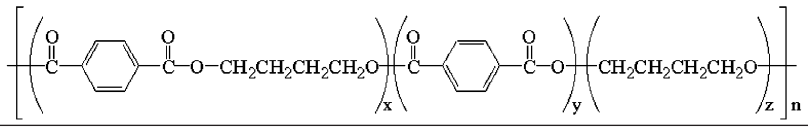
candidate polymers	type	polymer loading mg phenol/g polymer
Hytrel	8206	19
EVA	32% vinyl acetate	11.5
	23% vinyl acetate	7.7
	9.5%vinyl acetate	3.7
nylon	6	9.9
	6/6	7.8
	4/6	4.2
	6/9	1.7
styrene/butadiene, ABA block copolymer	28% styrene	0
polypropylene		0
Nucrel	925	0
ELVANOL	3175	0

Screening and selection of a polymer can be approached in a manner similar to that of selecting an organic solvent TPPB delivery agent. The initial objective is to generate a short list of candidate polymers based on defining characteristics (in this case functional groups exhibiting polarity and possible hydrogen bonding) and then follow up with experimental investigations. The polymer chosen should also be amorphous, which would allow for greater diffusion of target molecules into and out of the polymer bead, rather than a more crystalline polymer that is rigid and therefore permits little flow in and out. Finally, the polymers chosen for experimentation must be inexpensive and commercially mass-produced to ensure availability and consistent quality.

Initial efforts at identifying superior polymers for phenol uptake involved the use of the Extractive Screening Program database (ESP) (*6*). The ESP program is based on the UNIFAC (UNIversal quasichemical Functional group Activity Coefficient) group contribution method for predicting thermodynamic equilibrium, along with a database of 1500 components. The program predicts likely candidate extractants for removing target molecules (in this case phenol) on the basis of functional group interactions. The database produced a lengthy list of possible extractants for the uptake of phenol, and an assessment of the functional groups of the most likely solvent candidates produced a few common chemical families, namely, esters, amines, and carboxylic acids. These chemical families are polar and exhibit the ability to accept hydrogen bonding, both sought-after characteristics when searching for a polymer with an affinity for phenol. Once common traits are found, the search is directed toward locating polymers that may contain these functional groups in their molecular structure.

Table 1 shows a resulting list of polymers that were tested for their ability to absorb phenol in solution. Hytrel (ester), EVA (ester of acetic acid), and nylon (amine) were chosen on the basis of their functional groups, polarity, and anticipated affinity for phenol. Additional polymers, Nucrel (ethylene-methacrylic acid copolymer), ELVANOL (poly(vinyl alcohol)), polypropylene, and styrene-copolymer-butadiene, were chosen and tested to demonstrate the validity of selecting a polymer on the basis of the criteria of polarity and hydrogen bonding abilities. These polymers did not comply with the polymer selection criteria

Table 2. Properties of Hytrel (50% Poly(butylene terephthalate))[a]

molecular structure	
glass transition temperature	−59 °C
melting point	189 °C
biological stability	no degradation observed over 2-week control experiment
density	1.17 g/cm^3
bead dimensions	oval shaped beads; 5 mm length, 1.5 mm diameter

[a] Taken from ref 14.

and, upon experimentation, failed to absorb phenol, as anticipated. This rational polymer selection, like rational organic solvent selection for liquid-phase TPPBs, is an important methodology in obtaining improved system performance. As noted, this is in contrast to the use of a generic delivery phase, such as silicone oil, which does not permit the tailoring of the delivery phase to the target molecules.

Hytrel 8206 is an experimental grade of polymer, and information as to the exact composition of the polymer is unavailable except for the knowledge that Hytrel 8206 contains approximately 50% poly(butylene terephthalate) (PBT) and 50% butylene ether glycol terephthalate. From this information, experimental data for Hytrel containing 50% PBT (*16*) were found that showed phenol, at a molten state or high aqueous concentrations, to be an excellent solvent for Hytrel, whereas other solvents showed no affinity to Hytrel. Hytrel is an amorphous polymer, and Table 2 contains additional details concerning the polymer as well as an illustration of the molecular structure, showing the occurrence of the ester functional group, which is believed to be the site of hydrogen bonding with the phenol molecule.

Partitioning Coefficient. Initial work with polymer beads as a second phase in a TPPB (*9*) characterized the polymers in terms of their absorption capacities (mg phenol absorbed/g of polymer) based on an initial aqueous phenol concentration of 2000 mg/L, which was chosen because it is at a concentration that is inhibitory to most phenol degrading microbes (*17*, *18*) and specifically to the microbial consortium being used (*13*). Here we determined if capacity was in fact a function of concentration; if so, polymers would behave in a manner similar to immiscible organic solvents in TPPB systems, and consideration of a polymer's distribution coefficient could be used to predict required polymer loading for solutions of different phenol concentrations.

Figure 1 shows the results of the partitioning coefficient experiment. The data show a linear relationship (describing phenol uptake as mg phenol/g polymer vs aqueous phenol concentration) that represents the partitioning coefficient between the two phases. On the basis of the data it is apparent that by increasing the driving force (concentration of phenol in the aqueous phase) there exists a linear relationship corresponding to an increase in loading capacity of the polymer beads.

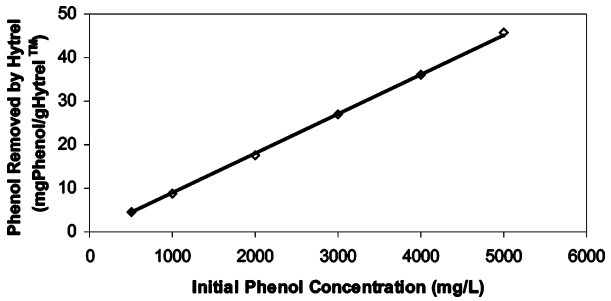


Figure 1. Relationship between phenol absorption by Hytrel and aqueous phenol concentration.

Diffusivity Coefficient of Hytrel. The objective of using a polymer as a second phase is 2-fold: the first is to ensure that toxic substrates at high concentrations are absorbed quickly in order to minimize exposure times thus minimizing subsequent damage to microbes; the second is to ensure that toxic substrates are released at a rate that will not create a mass transfer rate-limited environment.

Diffusivity is a physical property, dependent on temperature and the properties of the target molecule, that quantifies the rate at which a molecule can diffuse into and out of a polymer matrix. Diffusivity remains constant at a fixed temperature, and therefore the rate at which molecules diffuse into the polymer is mirrored by the rate at which they will diffuse out. Figure 2 illustrates the absorption of phenol expressed as normalized phenol concentration absorbed versus time. The uptake of phenol is very rapid, and after a 3-h period 99% of the total amount of phenol absorbed had been taken up. In comparison, identical experiments (*9*) showed that 20 h are required for the absorption of 99% of total absorbed phenol by EVA. The diffusivity of Hytrel was found to be 1.54×10^{-7} cm^2/s, which is 2 orders of magnitude higher than that found for EVA with a vinyl acetate concentration of 40% (3.73×10^{-9} cm^2/s). Coupling this with the increased capacity of Hytrel over EVA at 2000 mg/L (19 mg/g of Hytrel and 12.4 mg/g of EVA), the selection of Hytrel polymer beads would appear to provide a potential improvement for a TPPB system using a polymer as the second phase. In addition to the diffusivity of the target molecule in the polymer, other factors that would affect the overall rate of delivery in a TPPB include film resistance around the beads, which could be reduced (as we have done) by rapid mixing, and the diffusional length, which would be determined by the bead dimensions.

Comparatively, Hytrel polymer beads offer improved uptake of phenol compared to adsorption techniques. For example, bentonite (*19*) was used as an adsorption surface for phenol and required 8 h of contact time between phenol and bentonite in order to achieve equilibrium. With decreased loading capacities in the bentonite (9.9 mg phenol/g bentonite) and a longer contact time required for equilibrium, the polymer beads offer improved performance. Also, because of the absorption phenomena, release of phenol out of the polymer requires only a concentration gradient, whereas many adsorption processes requires thermal or chemical treatment to regenerate the target surface.

Biodegradability of Hytrel. With Hytrel beads as the only source of carbon, no bacterial growth was detected over a two-week incubation period, suggesting that this polymer is not biodegradable.

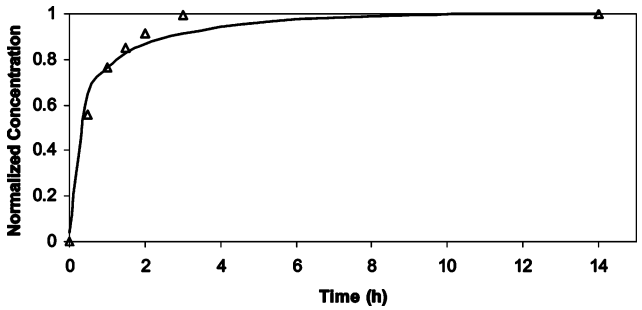


Figure 2. Absorption of phenol by Hytrel polymer beads comparing normalized concentration of phenol absorbed vs time. The experimental data are shown by the symbols, and the line represents the best fit, using the method of least squares, corresponding to a diffusivity coefficient of 1.54×10^{-7} cm^2/s.

Batch Fermentation in a TPPB using Hytrel. A batch fermentation was performed using Hytrel beads in a manner identical to previous work with EVA polymer beads (*9*, *13*) in order to offer a direct comparison. The system was loaded with 1840 mg/L of phenol and 200 g of fresh Hytrel polymer beads. Figure 3 shows that although a 14-h period of equilibration was provided, the phenol concentration was reduced to 580 mg/L in about 5 h, resulting in a capacity of 19 mg phenol/g Hytrel. The system was inoculated (at 14 h) and experienced a lag phase of approximately 6 h, after which time rapid degradation of phenol and prolific growth of cells were observed as characterized by a decrease in dissolved oxygen (not shown). Note that the DO never dropped to below 40% of saturation, suggesting that the system was not oxygen-limited. In addition, the exponential increase in CDW seen here and previously (*13*) suggest that the system was not mass transfer (substrate) limited. Phenol concentrations in solution were fully depleted at the 26-h mark, representing a total fermentation time of 12 h. The volumetric phenol consumption rate (based on volume of aqueous phase) was determined to be 150 mg phenol/L·h and is similar to the volumetric rate observed in a liquid−liquid two-phase system (*20*) (135 mg phenol/L·h), even though the bead volume fraction in the present work was much smaller than the organic volume fraction in ref 20.

Upon completion of the fermentation 20 g of polymer beads was removed from the system and placed in 500 mL of water. The flask was agitated overnight, and the aqueous phase was tested for phenol concentration the next day. No phenol was detected, confirming the ability of the polymer beads to completely release all absorbed phenol. Furthermore, the polymer beads were inspected for microbial growth, such as a biofilm, but the beads appeared clean and undamaged.

The fermentation was repeated with used polymer beads to show reproducibility, and from Figure 3 it is evident that the system performed in an identical manner and that the Hytrel polymer beads are capable of repeated use.

Comparison of the fermentations between Hytrel polymer beads and previous work with EVA (*9*, *13*) demonstrates that Hytrel has a greater capacity for phenol (19 mg/g of Hytrel and 12.4 mg/g of EVA), a more rapid uptake of phenol (diffusivity of 1.54×10^{-7} cm^2/s for Hytrel and 3.73×10^{-9} cm^2/s for EVA), and therefore a quicker release. The first objective of this work, the search for an improved polymer, was therefore successfully completed, and work focused on the ability of the improved polymer to handle various substrate-feeding strategies.

TPPB Feeding Strategies Using Hytrel. Several feeding strategies were employed to investigate the performance of the solid−liquid TPPB system to handle phenol loadings that may arise in a variety of phenol-contamination situations. In the first, representing the treatment of a highly contaminated water source, a 6000 mg/L phenol solution was added to the TPPB system in a fed-batch mode (repeated three times). In the second, representing the use of polymer beads to "decontaminate" a very highly contaminated (15,000 mg/L) phenol source, Hytrel beads were used to absorb phenol, and the loaded beads were then used in a batch TPPB fermentation (repeated twice) to degrade the phenol. This was followed immediately by the third feeding strategy, mimicking the biotreatment of highly concentrated aqueous phenol "spikes" (essentially, a saturated phenol solution of 60 g/L), repeated once. Finally, to test that the beads are necessary for the degradation of high phenol concentrations, the beads were removed, a spike of phenol (giving 5800 mg/L) was added, the system was maintained under aerated and agitated conditions, the beads were replaced, and the degradation allowed to go to completion.

The batch phase of the fermentation was undertaken with an initial phenol concentration (4570 mg/L) well above the initial phenol concentration from the previous fermentations (approximately 2000 mg/L) before the polymer beads were added. The reason for the elevated initial phenol concentration was to highlight the phenomena of increased capacity by the beads when presented with an increase in aqueous phenol concentration

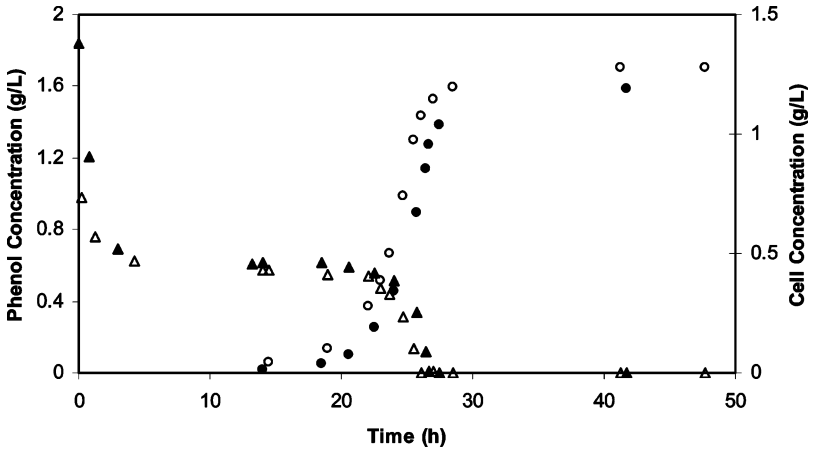


Figure 3. Batch fermentation with Hytrel polymer beads at an initial phenol concentration of 1840 mg/L. The open triangles and circles represent phenol and cell concentrations for fresh beads, respectively, and the closed symbols are for reused Hytrel beads.

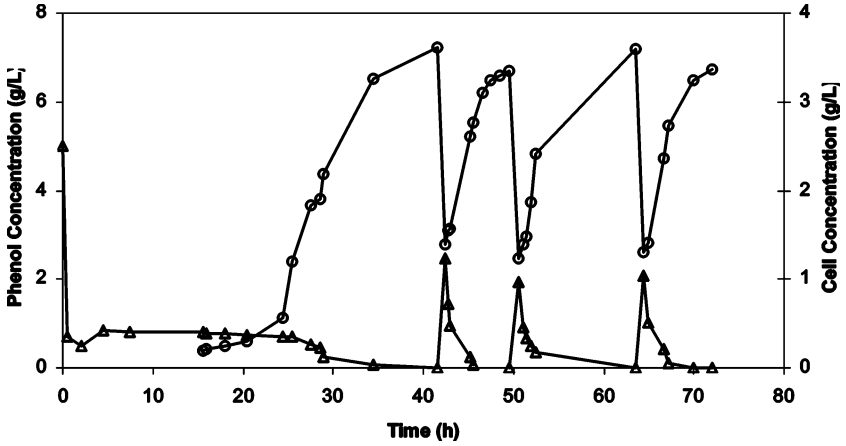


Figure 4. Batch fermentation of phenol followed by three fed-batch fermentations. The triangles represent phenol concentration, and the circles represent cell concentration.

(i.e., the polymers can also be characterized by a partitioning coefficient). From Figure 1 the quantity of polymer beads required to decrease the phenol concentration to a subinhibitory level was calculated to be 380 g. After bead addition and an equilibration period of 15 h an overall mass balance for phenol was performed on the system, and the phenol capacity of the polymer beads was determined to be 36 mg phenol/g polymer bead, similar to what was expected from Figure 1.

After inoculation with the microbial consortium the entire mass of phenol in the system was consumed in 26 h (Figure 4); however, the cell concentration continued to increase even after phenol depletion, leveling off about 8 h after phenol was no longer detected in the medium. It has been observed (*21*, *22*) that even though the molecules targeted for degradation may no longer be detected in solution, metabolites resulting from the initial destruction of the molecules may still be present. The presence of metabolites is suggested by the additional growth of cells after consumption of the targeted molecules. To gain an accurate calculation of the yield coefficient, the fermentations were left until cell concentrations remained unchanged.

Upon complete degradation of phenol in the batch fermentation and leveling off of the cell concentration, three fed-batch fermentations were undertaken. Fermentation medium (1.5 L) was pumped from the fermentor and replaced with 1.5 L of mineral salts medium containing 6000 mg/L of phenol. From Figure 4 it is apparent that during the addition of phenol-loaded medium (15 min) the beads were capable of reducing phenol levels from the diluted medium concentration of 3000 to 2400, 1900, and 2100 mg/L for each separate feeding. Within 1.5 h of entering the system the phenol concentration had been reduced to subinhibitory levels of 950, 930, and 1010 mg/L, respectively. During this time cell concentration did not increase, which would have signified the consumption of phenol, and therefore it has been shown that the disappearance of phenol in solution is due to its uptake by the polymer beads.

The reduction of phenol in solution to subinhibitory concentrations resulted in the subsequent degradation of all available phenol by the microbial consortium. The rapid absorption of phenol by the polymer beads ensured that the cells were not exposed to high concentrations of phenol for prolonged periods of time and, on the basis of their performance, suffered no adverse affects. Volumetric consumption of phenol for the three feeding strategies (based on the volume of the aqueous phase) was determined to be 320 mg phenol/L·h which is similar to the rate reported for liquid−liquid systems (175 mg phenol/L·h (*20*), 510 mg phenol/L·h (*17*)) even though the bead volume fraction in this work was less than the organic fractions in the two-liquid TPPB systems.

The next feeding strategy investigated was initiated with a batch fermentation. In this instance (Figure 5), inoculation occurred 3.5 h after introduction of polymer beads rather than 15 h in order to illustrate the increased diffusivity of Hytrel polymer beads relative to EVA (*6*, *10*). Upon completion of the batch fermentation, replace-

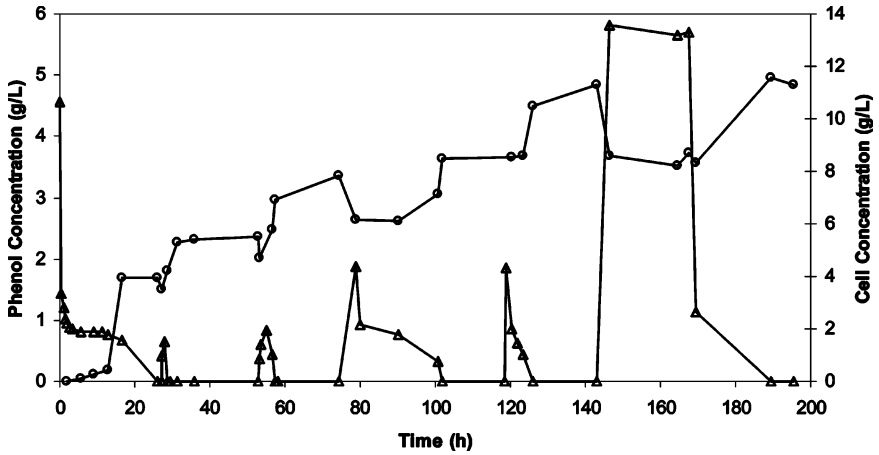


Figure 5. Batch fermentation of phenol followed by two bead replacements, two concentrated phenol spikes, and a final validation of the need for polymers in a TPPB. The triangles represent phenol concentration, and the circles represent cell concentration.

ment of the polymer beads was undertaken twice (Figure 5, 27 h, 74.5 h). Phenol-loaded beads (380 g) were introduced to the system containing a mass of 41 and 39 mg phenol/g Hytrel for each respective replacement. Upon introduction of the beads into the bioreactor the concentration of phenol in solution increased from 0 to 660 and 780 mg/L for each respective addition. The beads introduced the phenol to the system at a concentration that was below the inhibitory concentration of the cells and, coupled with the acclimatized nature of the cells and elevated cell concentration, phenol degradation occurred rapidly, with complete disappearance of phenol in 4 h in each case.

The removal of beads and addition of phenol-loaded beads demonstrates a potential practical application of the system. Polymer beads could be introduced into a highly contaminated environment, allowed to absorb high concentrations of targeted contaminants, removed, and then added to a bioreactor for remediation. The use of the polymer beads to transport the targeted contaminants would suggest that a treatment facility might possibly be located offsite, a smaller (TPPB) system could be used to treat a large mass of toxic material, and the decrease of concentrations in the original environment might speed up and aid in the process of natural attenuation.

Previous work (*23*) demonstrated the feasibility of adding concentrated feed spikes of phenol to a TPPB containing an immiscible organic liquid delivery solvent phase. The liquid solvent, with its high affinity for phenol and aided by vigorous mixing, was capable of absorbing the phenol spike quickly, limiting any potential damage to the microbial population. Although polymer beads are effective at absorbing phenol, it was important to determine if the uptake would be rapid enough to prevent the adverse effects that elevated concentrations of phenol would have on the microbial population. Concentrated phenol spikes that would lead to a bulk aqueous phenol concentration of 3000 mg/L were introduced (Figure 5) to the system at hours 79 and 119. Very rapidly (15 min after addition) phenol concentrations were decreased to 1870 and 1850 mg/L for each feeding. After a short delay of approximately 4 h, presumably due to phenol shock, the system began to degrade phenol and performed similarly to previous fermentations. Absorption of phenol by the polymer beads, although not as rapid as absorption by an immiscible organic solvent, demonstrated the ability of the beads to act as a buffer to mitigate toxic shocks arising from addition of concentrated substrate solutions.

A final question to be answered was whether polymer beads are necessary in a TPPB for the degradation of high concentrations of phenol. After the successive feedings employed in this work it is possible the microbial consortium had become acclimatized to elevated levels of phenol and therefore were capable of degrading phenol at concentrations much higher than the inhibitory concentrations determined from previous work (*13*). To establish if the microbial consortium had in fact become acclimatized to elevated phenol concentrations, the polymer beads were removed after complete degradation of phenol from the phenol spike feeding strategy, and the system was loaded with phenol to a concentration of 5800 mg/L. The system was allowed to mix, with aeration, for a period of 22 h during which time phenol concentrations remained constant and cell concentrations did not change (Figure 5). At the 22-h mark it was determined that the microbial consortium was unable to degrade phenol as a result of its high concentration, and the polymer beads were placed back into the bioreactor. Absorption began immediately with phenol concentrations decreasing to 1130 mg/L in 2 h, and after a 4-h lag phase, vigorous degradation of phenol took place, verified by the decrease in dissolved oxygen (data not shown). The fermentation demonstrated the consortium's ability to withstand temporarily elevated concentrations of phenol until levels were decreased by bead addition to a value where degradation could occur. The necessity of the polymers to decrease elevated phenol concentrations confirms their requirement within a TPPB.

Conclusion

This work was performed to find a polymer exhibiting superior capacity and delivery of phenol in a TPPB system compared to a previously studied polymer (EVA). The search for a polymer was based on the assumption that absorption of phenol into a polymer is largely due to hydrogen bonding interactions. Hytrel proved to be the best candidate polymer, exhibiting increased capacity and a larger diffusivity over previously used EVA. Using a variety of phenol feeding strategies we have confirmed that Hytrel is effective at quickly reducing elevated phenol concentrations, thus providing a favorable environment for biodegradation. Furthermore, it was shown that the inclusion of polymer beads in a TPPB to reduce initial phenol concentrations to subinhibitory levels is necessary before biodegradation can occur, even with a highly acclimatized microbial consortium.

The emergence of inexpensive polymer beads as another workable option for a second phase in a TPPB suggests roles the polymer beads may possibly play in future bioremediation strategies. As a transport vehicle, the beads may be capable of reducing toxic concentrations of targeted contaminants in water sources followed by the transport and remediation of the toxic contaminants in a TPPB located offsite. The ability to rationally select polymers and to modify polymer properties suggests that they can be tailored to specific contaminants (*24*). Furthermore, the polymer beads may be considered for the in situ reduction of toxic concentrations of contaminants in soil or other environments. Once concentrations are reduced to subinhibitory levels, natural attenuation could take place with a demand-based delivery of the toxic contaminants. In either case, it is import to note that retrieval and reuse of the beads are certainly possible. Future work will attempt to degrade multiple toxic substrates with a microbial consortium in a TPPB where polymers comprise the second phase.

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