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Manipulating the composition of absorbent polymers affects product and by-product concentration profiles in the biphasic biotransformation of indene to *cis*-1,2-indandiol

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ABSTRACT

The biotransformation of indene to the pharmaceutical intermediate, 1,2-indandiol, by *Pseudomonas putida* 421-5 (ATCC 55687) produces large amounts of the inhibitory by-product, 1-indenol, as well as several different minor degradation products. Three segmented block copolymers, Hytrel® 8206, Hytrel® 3078, and Pebax® 2533, containing varying types and amounts of soft segment components, exhibited different affinity toward the product and by-product. The polymers were each used as the sequestering phase for metabolite removal from this transformation in a two-phase partitioning bioreactor (TPPB). The polymer with the highest affinity for each metabolite enhanced its production via more extensive partitioning into the polymer phase. This work reports the first use of polymers whose differing compositions are attributed to target molecule affinity and the different production profiles seen during two-phase biocatalysis. The uniquely high water content of 30 wt% in Hytrel® 8206 emphasized the importance of accounting for water uptake in partition calculations, and potentially conferred enhanced affinity and diffusivity by providing an expanded polymer network, significantly improving biotransformation completion time compared to the low-water-absorbing polymers.

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1. Introduction

Biotransformations are subject to limitations arising from concentrations of substrate, product, or by-product(s) which have an inhibitory or toxic effect toward the microbial biocatalyst [1]. The addition of a dispersed absorbent polymer phase, having affinity toward target molecules, has been shown to improve productivity by providing a reservoir, separate from the cell-containing aqueous phase, into which compounds can partition [2–5]. The ratio of a target molecule's concentration in the polymer phase relative to the aqueous phase is the partition coefficient (PC), a measure of polymer–solute affinity, which determines the extent of target molecule uptake by the auxiliary phase at a given aqueous concentration.

The biphasic biotransformation of indene to the pharmaceutical intermediate 1,2-indandiol by *Pseudomonas putida* 421-5 (ATCC 55687) was first investigated at Merck & Co. using silicone oil as a reservoir for delivering the hydrophobic substrate, indene [6]. The metabolic pathways of indene biotransformation are complex, with two primary products and five total possible products [6]. The initial selection of a hydrophobic liquid was rational considering the substrate's hydrophobicity; however, silicone oil has almost no affinity toward 1-indenol, the major by-product, and the dominant inhibitor of this process [7]. That is, the work by Merck was able to gradually deliver the hydrophobic substrate to the cells, but silicone oil did not alleviate the inhibition caused by the by-product, 1-indenol. Our initial investigation into this system replaced the liquid solvent with a polymer, Hytrel® 8206, having affinity toward both the major inhibitory by-product, 1-indenol, and the desired product, 1,2-indandiol, which is not inhibitory to this biotransformation (Table 1). The use of the polymer was found to out-perform the silicone oil partitioning phase and provide operational advantages due to its phase stability [7].

Polymers which have been used as absorbent auxiliary phases in biotransformations all feature a soft, amorphous polymer chain network above its glass transition temperature (T_g), permitting chain mobility for permeation of target molecules [8]. This feature is distinct from adsorbent resins, where the material is held in its porous architecture by rigid polymer chains which are below their T_g, such that target molecule interactions occur at the material's surface, and diffusion occurs only within the bulk fluid filling the pore spaces rather than within the material itself [9,10].

Our motivation for investigating soft, absorbent polymers, in contrast to glassy, macroporous adsorbent resins, applied as in situ auxiliary phases in two-phase partitioning bioreactors (TPPBs) is

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Table 1
Polymer properties.

Polymer name	% Hard segment	% Soft segment	T_g (°C)	Water absorption at equilibrium (%)	Density (kg/m^3)
Hytrel® 3078	~50% Poly(butylene terephthalate) [24]	~50% N/A[a]	−60 [34]	0.8 [31]	1060 [31]
Hytrel® 8206	~50% Poly(butylene terephthalate) [24]	~50% N/A[a]	−59 [29]	30 [32]	1170 [24]
Pebax® 2533	20% Poly(amide)-12 [28]	80% Poly(butylene oxide) [28]	−65 [34]	1.2 [33]	1000 [33]

[a] Composition information not available.

several-fold. Adsorbent resins are already well-studied and characterized for many biotransformation applications [11], and suffer from mechanical resilience and fouling problems [12]. In contrast to adsorbents, absorbent polymers act similarly to immiscible liquid solvents, operating passively by diffusion governed thermodynamically by concentration gradients, resulting in partition coefficients based on linear sorption isotherms in the concentration ranges of interest rather than competitive site occupation as seen in porous adsorbent isotherms [2]. Finally, the attractive cost of commodity polymers relative to specialized adsorbent resins provides an economic incentive in process development [12].

The majority of polymers used in TPPBs are in the family of poly(ether)-containing block copolymers [4,13–16], where the soft poly(ether) segments available for solute permeation are structurally supported by domains of a hard, glassy segments such as a poly(ester) (e.g. Hytrel®), or poly(amide) (e.g. Pebax®). The soft segment's poly(ether) composition determines affinity toward a target molecule, while the hard segment is thought to have a negligible contribution toward target molecule absorption due to glassy polymers' lack of chain mobility. Furthermore, segmented block copolymers containing poly(ether) segments which are highly polar will absorb significant amounts of water, while less-polar poly(ethers) will absorb almost no water. Until recently, this difference in water uptake among different poly(ether)-based block copolymers has been neglected in the literature comparing polymers for use in TPPBs, but this property affects measurements of target molecule affinity, which are based on mass balance calculations assuming constant phase volumes.

By adjusting the polarity of the soft segment of block copolymers via changing the poly(ether) monomer length, it was hypothesized that segmented block copolymers having different soft segment compositions would produce unique distributions of metabolites under identical conditions, and could have multiple, compound effects on this model complex biotransformation. Our aim was to manipulate the outcome of this biotransformation using three different commercial poly(ether)-based segmented block copolymers, having different soft segment compositions and amounts, described in Table 1, which impart differential affinity toward the toxic, hydrophobic major by-product and the relatively non-toxic, hydrophilic minor desired product, described in Table 2. This is the first report to attribute block copolymer composition to differences in relative target molecule affinity, which produced different outcomes in this model biotransformation.

2. Materials and methods

2.1. Chemicals

All chemicals were purchased from either Sigma–Aldrich (Canada) or Fisher Scientific (Canada) except 1,2-indandiol, which was purchased from Wako Chemicals (USA). Hytrel® was provided by DuPont (Canada), and Pebax® was purchased from Foster Corporation (Putnam, CT).

2.2. Microorganism

P. putida 421-5 (ATCC 55687) was purchased from ATCC, grown for 24 h at 30 °C in TSB at 180 RPM and stored at −80 °C as 1 mL aliquots in 10% glycerol. The same growth conditions were used to generate inoculum flasks from frozen stocks.

2.3. Partition coefficient experiments

To measure polymer affinity toward the biotransformation metabolites, high-purity water, or spent medium from a previous experiment, was used to provide a measurement under both "ideal" and realistic conditions, respectively. The medium contained realistic ion concentrations, which may affect partition coefficients via water activity (A_W) modification. The medium was harvested and centrifuged for 1 h at 3600 RPM, and 10 mL of the supernatant was placed in sealed vials with polymer bead samples of 2, 3, and 4 g (wet mass, surface dried by blotting on absorbent paper) for 24 h shaking at 180 RPM to reach equilibrium, providing triplicate measurements of the PC. Concentrations in the polymer-containing vials were measured by HPLC and compared to a control vial without polymer, and the change in concentration was used to calculate the partition coefficient via mass balance, assuming negligible losses. 1,2-indandiol and 1-indanone PCs were measured in both fluids, while 1-indenol was only measured in spent medium because it is not commercially available as an analytical standard.

2.4. Biotransformations

2.4.1. Polymer loading with substrate

Seven hundred grams (wet mass, surface dried) of the appropriate polymer beads (Hytrel® 8206, Hytrel® 3078, or Pebax® 2533) were split evenly into two 1 L Erlenmeyer flasks and 500 mL water was added to each. The polymer was sterilized in the flasks by autoclaving (Hytrel®) or by boiling for 15 min with agitation to avoid gelation due to a lower melting point (Pebax®). Indene was loaded into the polymers by filtering 14 mL into each flask through a 0.2 μm nylon syringe filter, sealing with a sterile rubber stopper, and equilibration on a shaker for 24 h at approximately 250 RPM. The water was then decanted and the polymer beads were added directly from the flasks to the bioreactor 30 min prior to inoculation. This method effectively loaded indene into the polymer at 40 g/kg for each experiment because its low aqueous solubility (ca. 100 mg/L) avoided a significant remainder in the aqueous phase upon reaching equilibrium. A high loading was chosen to ensure a relatively constant driving force for substrate delivery regardless of consumption differences between experiments.

2.4.2. Bioreactor operation

The bioreactor (5 L BioFloIII, New Brunswick Scientific, Edison, NJ) was filled with 2.64 L of medium, containing (g/L): K_2HPO_4 (2.0); $(NH_4)_2SO_4$ (2.0); $MgSO_4 \cdot 7H_2O$ (0.4); $FeSO_4 \cdot 7H_2O$ (0.02); yeast extract (3.0); tryptone–peptone (3.0); glycerol (20.0); and 3 mL trace element solution, containing (mg/L): H_3BO_3 (300); $ZnCl_2$

Table 2
Major metabolite properties.

Name	Role	Toxic concentration (g/L) [30]	Structure	$K_{O/W}$[a]
1,2-Indandiol	Desired product	15		5
1-Indenol	Major inhibitory by-product	3		16
1-Indanone	Minor isomerization product of 1-indenol	1.5		25
Indene	Biotransformation substrate	5		1000

[a] Logarithmic values from [35] converted into linear values for PC comparison.

(50); $MnCl_2{\bullet}4H_2O$ (30); $CoCl_2$ (200); $CuCl_2{\bullet}2H_2O$ (10); $NiCl_2{\bullet}6H_2O$ (20); and $Na_2MoO_4{\bullet}2H_2O$ (30) and autoclaved.

Glucose was autoclaved separately as a 50% (w/v) solution and 120 mL was added to obtain 20 g/L. pH was automatically maintained at 7.0 using 6 M KOH. Dissolved oxygen was monitored and maintained above 30% saturation by adjusting agitation between 500 and 700 RPM. Anti-foam was added dropwise as required.

Each biotransformation was initiated by adding six 125 mL shake flasks containing 40 mL of a 24-h late-exponential phase culture of *P. putida* 421-5 (ATCC 55687) in tryptic soy broth to 2.76 L of medium containing 700 g (wet mass) polymer beads in the bioreactor. At 3.5 h after inoculation, a 50 mL bolus containing 6 g $(NH_4)_2SO_4$ and 6 g yeast extract was added aseptically, and a 50% (w/v) glucose feed was initiated at 60 mL/h for 12 h using an Imed® Gemini intravenous pump. The aqueous volume of the reactor was maintained near 3 L by removing a volume equivalent to the aqueous substrate additions at each time point.

Aqueous samples were periodically removed, centrifuged at 16,000 × g for 5 min, and passed through a 0.2 μm syringe filter for HPLC analysis. Polymer samples (40 beads, ca. 1 g) were also periodically removed using a small sieve, their surface rinsed for 3 s with sterile water, then immersed in 10 mL isopropanol (IPA) for 12 h at 180 RPM to establish equilibrium. The polymer samples were equilibrated in fresh IPA twice more to ensure full extraction of metabolites. The IPA fractions were then analyzed by the same HPLC method to determine metabolite concentrations in the polymer. No metabolites were detectable in the third extraction except for indene, which was present in a large amount. Overall (aqueous + polymer) concentrations of 1,2-indandiol and 1-indenol were calculated by adding the mass present in both phases and dividing by the total reactor volume. 1-Indanone was not considered to be a reliable indicator of biocatalyst performance because it is an isomerization product of 1-indenol rather than a biocatalytic product, and is present in exceedingly small concentrations <100 mg/L.

2.5. Analytics

Biomass was measured by relating optical density at 600 nm to a calibration line giving cell dry weight (g/L). Major metabolites, 1,2-indandiol, 1-indenol, 1-indanone, indene, with retention times of 6.8, 9.6, 10.5, and 16 min, respectively, were separated on a Varian Polaris C-18A 4.6 × 150 mm HPLC column at 1 mL/min using gradient elution from 15:85 to 85:15 acetonitrile:water over 15 min, and detection was at 220 nm on a Varian ProStar 325 UV/vis detector. Concentrations were determined using standard calibrations with the exception of 1-indenol which is commercially unavailable. This compound, being the major product, produced the largest peak, and was identified based on previous reports of relative retention time and M–S confirmation in similar biocatalytic systems [6,17]; 1-indenol was quantified using peak area only assuming a linear detector response. Minor other metabolites appeared as small peaks on the chromatogram emerging at later times in the biotransformation, however, being the desired product, maximum aqueous 1,2-indandiol concentration dictated the completion of each biotransformation.

3. Results and discussion

3.1. Partition coefficient experiments

3.1.1. Effect of water absorption by polymers on partition coefficient calculations

The use of wet polymer mass (with the surface dried) in both the partition coefficient and bioreactor experiments was intended to avoid the inevitable transfer of water into the hydrophilic polymer as much as practically possible, which would lower the aqueous volume in the two-phase system as the polymer phase became swollen with water. Although the introduction of water within the polymer has the potential to slightly dilute the medium to which it is added, by introducing the polymer as a pre-swollen water–polymer complex, volumes are held relatively constant and mass balance calculations reflect the system composition at equilibrium.

The significant uptake of water by hydrophilic polymers has not been previously reported in the literature during TPPB polymer selection, despite moderately water-absorbing polymers being investigated and applied in over a dozen publications to date, for example: [2,14,18,19]. This effect most strongly affects PC values

for compounds having modest partition coefficients in polymers with high water uptake by skewing phase volumes if water uptake is unaccounted for. An alternative point of view would be that absorbed water remains separate from the polymer and continues to be part of the bulk aqueous phase; however, the entire swollen polymer volume and its contents are indeed separated from the cells' perspective in the aqueous phase. In reality, there is likely both "bound" water associating directly with the polymer chains and "bulk" water existing as an aqueous phase somewhat entrapped in the polymer chain network [20]. The amount of each type of water would depend on many factors including hard and soft segment composition and polymer microphase morphology. This property would be an exceedingly important parameter to determine if thermodynamic predictions for TPPB polymer selection are to be attempted, as the presence of water in the polymer alters the chemical environment encountered by absorbed solutes. Unfortunately, predictive thermodynamic tools are currently unable to account for both types of water present in the polymer phase [21], and this aspect is a focus of ongoing research in our group. Accounting for water uptake permits the comparison of polymers that exhibit different extents of water absorption on an equal basis, such that partition coefficient values can be realistically compared.

3.1.2. Effect of medium salts on partition coefficients

It was not possible to compare 1-indenol partitioning in high-purity water to bioreactor medium because this compound is not commercially available. Instead, 1-indanone, a minor degradation product of 1-indenol, was used as a second target molecule, representative of a hydrophobic metabolite. The PC values measured for the various polymers in biotransformation medium were significantly higher, by approximately two-fold, for each compound than those measured in high-purity water, shown in Fig. 1. This is likely a result of medium components such as dissolved salt ions competing for hydration, thereby lowering the water activity (A_W) and increasing solute activity in the aqueous phase, driving more into the polymer at equilibrium.

Salt effects have been noted as being of potential importance in TPPB systems based on abiotic experiments containing high salt concentrations exceeding 100 g/L, corresponding to relatively low A_W values of approximately 0.95 [22], at which point PC values toward relatively hydrophobic target molecules begin to deviate [23]; however, the salt concentrations at these levels are unrealistic for biocatalytic systems due to osmotic stress on the cells. The influence on PC values seen here in actual bioreactor medium, due to much lower salt concentrations of approximately 20 g/L by the endpoint, is a particularly noteworthy phenomenon. Note that the relative differences in PC values shown in Fig. 1 among the metabolites across all polymers correspond to their relative lack of affinity toward the aqueous phase, exemplified by their $K_{O/W}$ values (Table 2), which is directly related to their relative affinity for the polymer (Fig. 1). That is, the more hydrophobic a solute is, indicated by a higher $K_{O/W}$ value, the more affinity it exhibits toward the polymer phase. In each case, the additional osmotic pressure from the aqueous phase salts is constant, and the differences in affinity among the polymers toward the target molecules are due to the different compositions of the polymers.

3.1.3. Effect of soft segment proportion on partition coefficients

As discussed previously, the polymers employed in this investigation share segmented block architecture, containing soft, amorphous segments which allow solute absorption and permeation within the bulk polymer mass. This mechanism is distinct from the well-established use of glassy, styrene–divinylbenzene-based adsorbent resins upon which functionality may be grafted, and which engage solutes by surface interaction rather than dissolution and permeation [11]. This distinction is important in understanding the performance of these materials, and the following sections discuss the effects of this feature.

We assume that both Hytrel® grades are composed of approximately 50 wt% hard poly(ester) and 50 wt% soft poly(ether) [24], because hard segment proportion has been shown to have a dominant influence on physical and mechanical properties [25], and both grades share very similar glass transition temperatures (Table 1). Based on their similar soft segment proportion, differences in target molecule affinity between the two Hytrel® grades were therefore due to the type of soft segments rather than the amount available. In contrast to both Hytrel® grades, Pebax® 2533 contains a much greater proportion (80 wt%) of relatively non-polar, hydrophobic soft segments, poly(butylene oxide), PBO (Table 2), and also had higher affinity toward the more hydrophobic metabolite, 1-indenol, than either Hytrel® grade, shown in Fig. 1b, suggesting that the greater proportion of soft segment provided additional absorptive material for solute uptake. The greater proportion of absorptive material in Pebax® 2533 did not provide the same advantage toward the hydrophilic metabolite 1,2-indandiol. The non-polar, hydrophobic PBO soft segment in Pebax® 2533 lacked the polarity to absorb 1,2-indandiol as extensively as the more hydrophilic poly(ether) in Hytrel® 8206, which was present at only 50 wt%, signifying the importance of soft segment composition in addition to its proportion within the copolymer.

3.1.4. Effect of soft segment composition on partition coefficients

The relative affinity toward each metabolite among the two grades of Hytrel®, illustrated in Fig. 1, showed differences due to the polymers' different soft segment compositions because both grades share an approximately equal soft segment proportion, and differ in polarity. Hytrel® 8206 absorbs significantly more water which indicates higher polarity, and had the highest affinity toward the more polar, hydrophilic, non-inhibitory product 1,2-indandiol. Hytrel® 3078 exhibits relatively low water absorption and thus has low polarity (Table 1), and exhibited higher affinity toward the less-polar, hydrophobic inhibitory by-product, 1-indenol. The polarity difference between the two grades of Hytrel®, while having soft segments present in similar amounts, explains the dissimilar water uptake among the polymers, and also explains the different affinity toward the two target molecules of varying polarity, shown in Fig. 1.

Additionally, a more polar, hydrophilic polymer may provide higher affinity toward relatively hydrophilic target molecules such as 1,2-indandiol due to the presence of water within the polymer. This effect has previously been systematically demonstrated for a polymer absorbing solutes under varying controlled moisture conditions, where absorption of hydrophilic solutes was preferentially enhanced at higher polymer water contents [26]. However, in a bioreactor, polymer composition is the only way to control water uptake.

There are two aspects of block copolymers to evaluate in order to achieve favorable partitioning of a particular target molecule: the soft segment present must be a relatively large proportion of the material, and it should be composed of a poly(ether) which has affinity for the target molecule.

3.2. Bioreactor experiments

3.2.1. Biomass

The 1,2-indandiol concentrations in these experiments were similar to our previous experiment comparing Hytrel® 8206 to a liquid extractant [7], yet the maximum biomass concentration was approximately 50% higher here at over 4 g/L compared to 2.7 g/L previously in the same growth period. The previous experiment's polymer loading method using indene dissolved in IPA, a good solvent for indene, resulted in the exposure of the culture to released

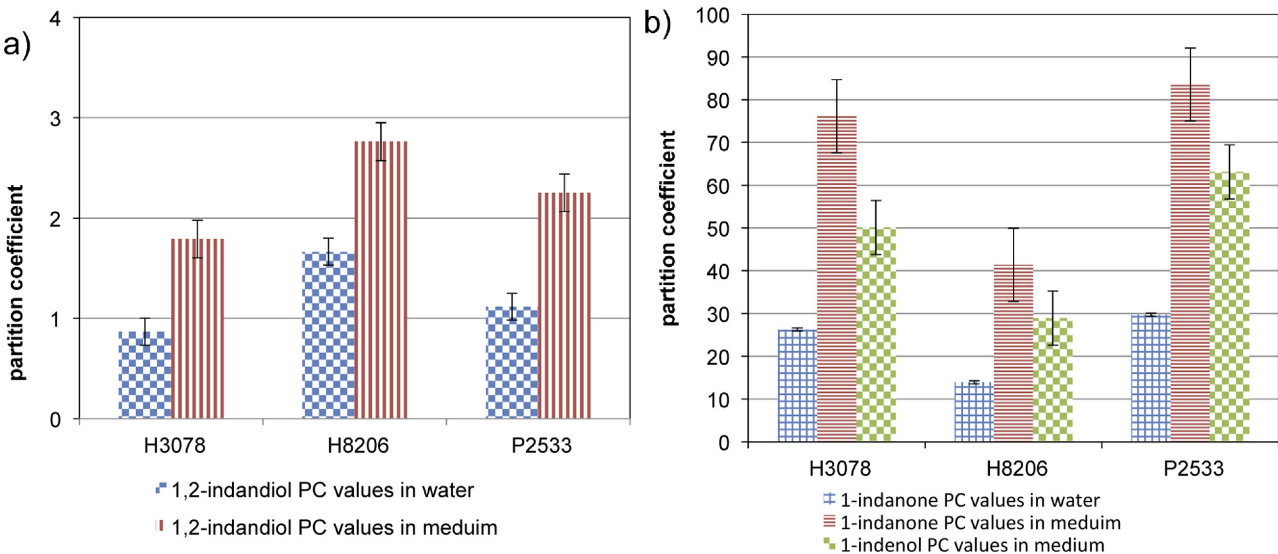

Fig. 1. (a) Partition coefficient values of 1,2-indandiol measured in high-purity water or medium in the three polymers. Error bars represent the standard deviation within the triplicate samples for each metabolite. (b) Partition coefficient values of 1-indanone measured in high-purity water or medium, and 1-indenol PC values measured in medium in the three polymers. Error bars represent the standard deviation within the triplicate samples for each metabolite.

IPA, which is water-miscible, upon addition of the polymer to the bioreactor. To avoid biocatalyst exposure to the toxic solvent, the present method using a dispersed indene phase in water with the polymer was developed, resulting in higher biomass levels due to the lack of IPA exposure. This procedure mimicked the direct loading of indene into hydrophobic silicone oil in the original Merck & Co. process while allowing good mixing of the polymer. The similar 1,2-indandiol concentrations despite significantly more biocatalyst suggest that the system was limited in production by the concentrations of inhibitory metabolites in the aqueous phase, which was addressed in this work using polymers of different composition.

3.2.2. *Relative polymer affinity*

The aqueous concentrations of the non-inhibitory product 1,2-indandiol, and the inhibitory by-product 1-indenol, are shown in Figs. 2 and 5, respectively. The bioreactor experiments produced similar aqueous concentrations in each run at early time points; while at later time points, the differences in aqueous product/by-product concentrations due to the polymers' differing compositions became evident: the 1-indenol aqueous concentration profile diverged at approximately 6 h among the different polymers used, while 1,2-indandiol reached fairly similar endpoint concentrations at different times.

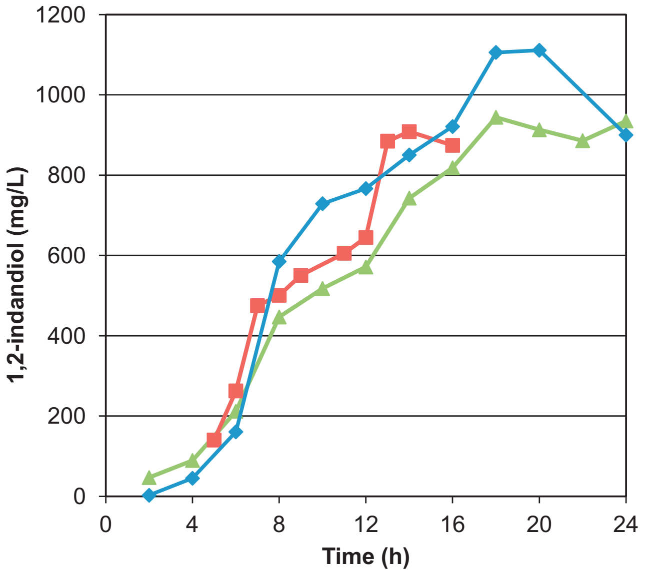

Fig. 2. Aqueous 1,2-indandiol concentration with the three polymer auxiliary phases. Symbols: ■, Hytrel® 8206; ♦, Hytrel® 3078; ▲, Pebax® 2533.

The corresponding concentrations of 1,2-indandiol and 1-indenol in the polymer are shown in Figs. 3 and 6, respectively, and the overall (aqueous + polymer) concentrations are shown in Figs. 4 and 7, respectively. The polymer having the highest affinity for the product 1,2-indandiol, Hytrel® 8206, sequestered the largest amount (Fig. 3), resulting in the highest overall 1,2-indandiol concentration (Fig. 4). Similarly, Pebax® 2533 sequestered the most by-product, 1-indenol (Fig. 6), and that experiment produced significantly more of that metabolite overall (Fig. 7). The bioreactor experiments have shown that the differences in affinity toward the target molecules, due to the polymers' distinct proportion and composition of soft segments, are responsible for manipulating the metabolite concentrations seen in the aqueous phase and encountered by the biocatalyst. This affected the biocatalyst's production and is responsible for the deviations in the time course plots and the outcomes of the biotransformations.

3.2.3. *Metabolic flux vs. polymer flux*

The overall (aqueous + polymer) concentration differences between bioreactor runs using different polymers would be expected as a result of the polymers' differential affinity toward the two major metabolites seen in the partition coefficient experiments (Fig. 1). However, the extent of partitioning out of the aqueous phase in this complex system at equilibrium accounts for only part of the observed effects occurring in situ, because the metabolic rate of *production* also affects aqueous titer, and presumably depends on both biomass concentration and extent of inhibition. In other words, there are at least two dynamic processes occurring (absorptive removal and metabolic production) that affect the rates of changing concentrations, measured in each phase at any point in time. Furthermore, the relative rate of each process influences the other, resulting in a system with compounded effects. That is, the removal of 1-indenol may allow its further production by the biocatalyst, but the rate of production would depend on the level of biocatalyst inhibition imposed by

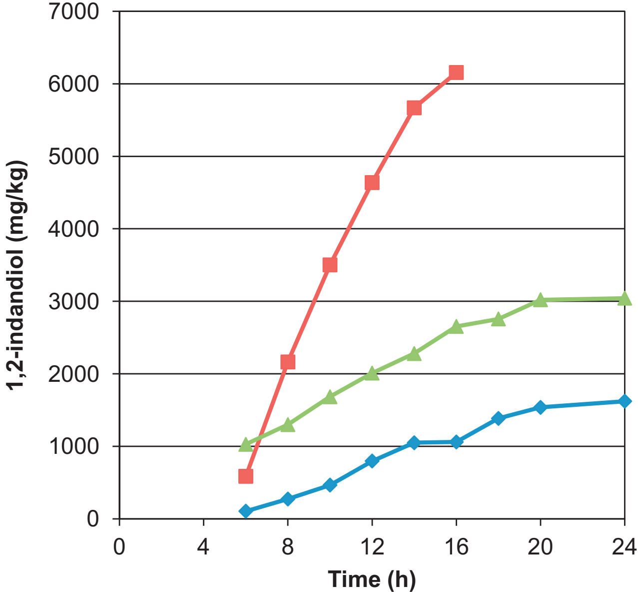

Fig. 3. 1,2-Indandiol concentration in the polymers. Symbols: ■, Hytrel® 8206; ♦, Hytrel® 3078; ▲, Pebax® 2533.

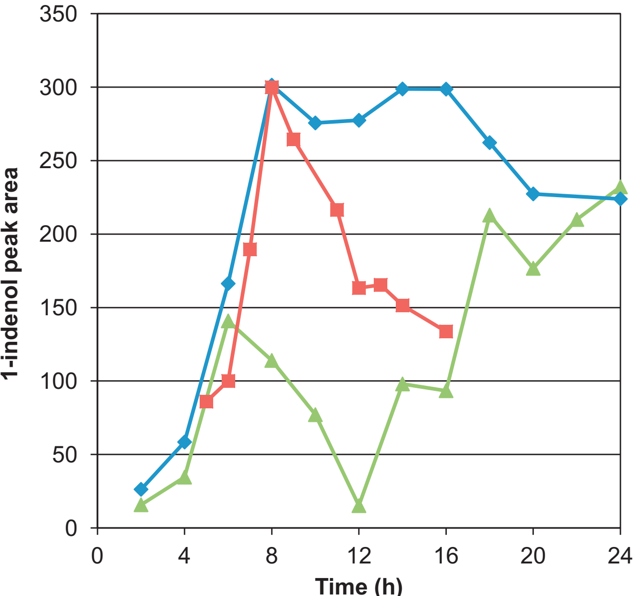

Fig. 5. Aqueous 1-indenol concentration with the three polymer auxiliary phases. Symbols: ■, Hytrel® 8206; ♦, Hytrel® 3078; ▲, Pebax® 2533.

the presence of aqueous 1-indenol. Because the rates of biocatalyst production and polymer absorption are not equal, the system is in a state of constant feedback, causing multiple, compounded effects.

Because this system features several possible routes for substrate bioconversion with additional minor degradation products, establishing a closed mass balance on the substrate is exceedingly difficult due to different fluxes possible through the various pathways, further affected by the polymers' differential action on aqueous phase concentrations. However, by monitoring the two major, primary products, a reasonable grasp of the relative flux of the substrate can be proposed, as described below. A detailed metabolic flux balance analysis would be required for a complete understanding; however, the influence of a polymer would still skew the expected outcome unless the entire system, including the polymer, could be accurately modeled as a dynamic system.

3.2.4. 1,2-Indandiol

Being the desired product, a high overall concentration of 1,2-indandiol was the goal of this investigation; however, due to the toxicity of the by-product, 1-indenol, relative to 1,2-indandiol (Table 1), it was speculated whether a higher overall 1,2-indandiol concentration would be better achieved by sequestration of 1,2-indandiol itself; or, whether removing 1-indenol, the major inhibitory by-product, would allow a less-inhibited biocatalyst to produce more overall desired product. The accumulation of aqueous 1,2-indandiol in all three experiments, near 1000 mg/L, was similar to the titer obtained by Merck researchers employing a controlled indene feeding strategy in single phase

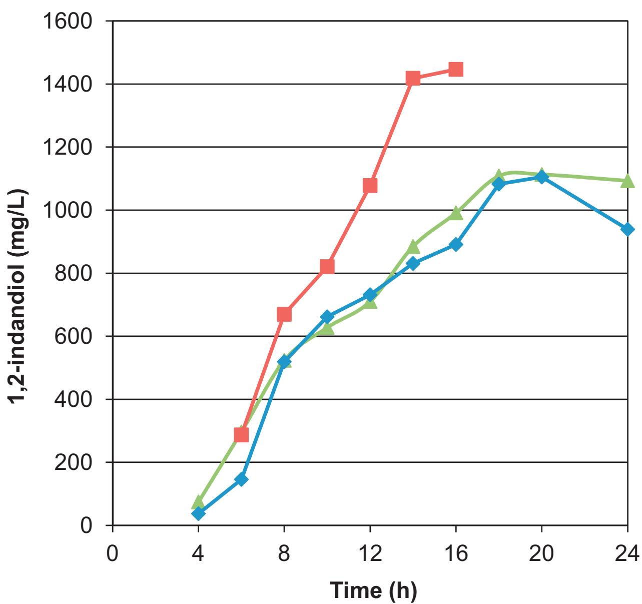

Fig. 4. Overall 1,2-indandiol concentration in the reactor (aqueous and polymer phases combined). Symbols: ■, Hytrel® 8206; ♦, Hytrel® 3078; ▲, Pebax® 2533.

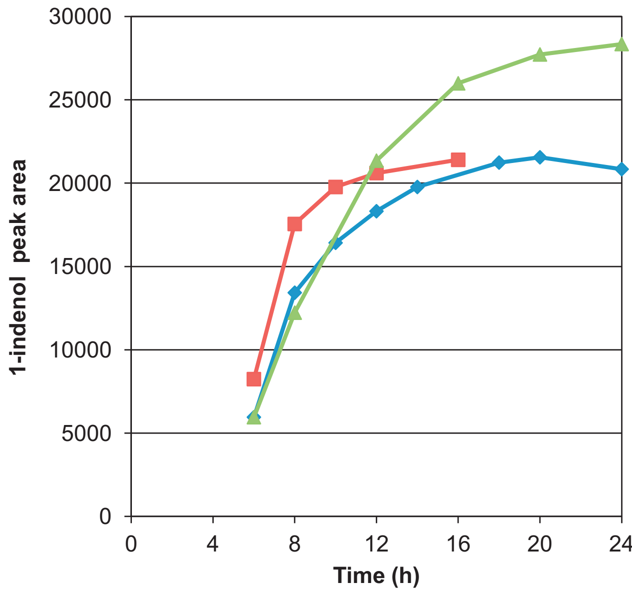

Fig. 6. 1-Indenol concentration in the polymers. Symbols: (■) Hytrel® 8206; (♦) Hytrel® 3078; (▲) Pebax® 2533.

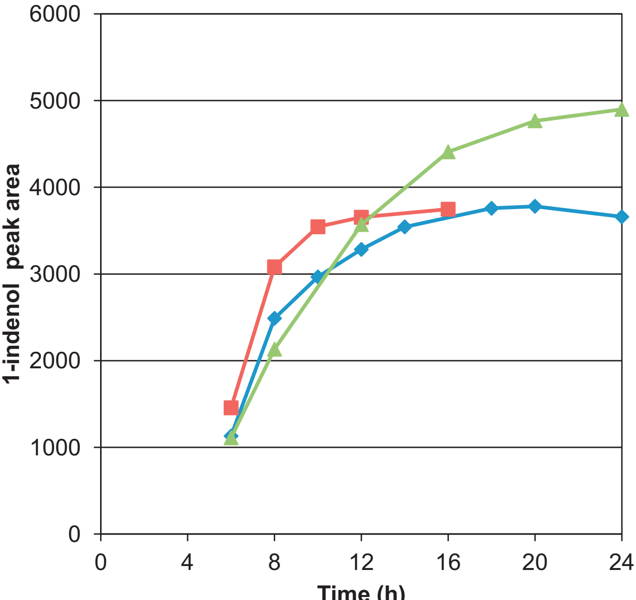

Fig. 7. Overall 1-indenol concentration in the reactor (aqueous and polymer phases combined). (■) Hytrel® 8206; (♦) Hytrel® 3078 (▲); Pebax® 2533.

fermentation using an optimized biocatalyst [17], and higher than titers reported for this specific strain [6,27]. However, these lacked the benefit of additional products being sequestered in the auxiliary phase.

The reason for the continuous accumulation of 1,2-indandiol in both phases until the endpoint of the biotransformation, unlike 1-indenol, is likely due to the fact that it is not a strong inhibitor of the biotransformation, while 1-indenol is a strong inhibitor (Table 1). The cells would presumably be less prone to altering their production in response to aqueous concentrations, such that the overall differences in 1,2-indandiol production (Fig. 4) are more obvious in its accumulation within the polymers (Fig. 3) due to their differential affinity, despite similar aqueous concentrations (Fig. 2). The apparently large difference between concentrations of 1,2-indandiol in Hytrel® 8206 and the aqueous phase suggest a greater PC value than was measured in medium. However, it is stressed that PC experiments reach thermodynamic equilibrium while the TPPB system is dynamic, constantly driving toward an ever-changing equilibrium endpoint. The discrepancy could be due to a steep drop in 1,2-indandiol aqueous concentration at the endpoint of the experiment, seen in Fig. 2, which is impossible to attribute entirely to either a change in the biocatalyst's metabolism or the polymer's action.

3.2.5. 1-Indenol

The accumulation of 1-indenol relative to 1,2-indandiol was similar to that reported in a previous study with this biocatalyst [6]. However, there is a distinct difference in the pattern of aqueous 1-indenol accumulation beyond relative differences in concentration; the concentration plateaued using Hytrel® 3078; peaked near the same concentration then declined using Hytrel® 8206; and peaked at a lower concentration then increased again using Pebax® 2533 (Fig. 5). These dynamic differences could be caused by more extensive removal of 1-indenol by Pebax® 2533 compared to either grade of Hytrel®, causing a response by the cells to the lower concentration of toxic 1-indenol. While the aqueous 1-indenol concentration dropped dramatically using Pebax® 2533, Fig. 6 shows that the concentration in the polymer steadily increased throughout the experiment, indicating that the biocatalyst was less inhibited by 1-indenol due to its sequestration by the polymer, and responded to its removal by producing additional amounts. The rate of production between 8 and 12 h was obscured by the faster rate of removal by the polymer, which was later overcome by biocatalyst production when 1-indenol began to accumulate in the aqueous phase again (Fig. 5).

The 1-indenol profiles with both Hytrel® grades reached a similar, higher aqueous concentration at 6 h than with Pebax® 2533 (Fig. 6), approximately corresponding to the polymers' relative partition coefficients toward 1-indenol (Fig. 1). The deviation in aqueous 1-indenol concentrations between the Hytrel® grades, shown in Fig. 5, was apparent in its accumulation in the polymer, shown in Fig. 6, where Hytrel® 8206 absorbed 1-indenol faster than Hytrel® 3078, and again appears to have out-paced the rate of production by the biocatalyst, evident by 1-indenol's decline in the aqueous phase (Fig. 5). Note that the overall 1-indenol plot (Fig. 7) closely resembles the polymer 1-indenol plot. The major changes in 1-indenol concentration were occurring in the polymer phase due to the large partition coefficient toward this compound, which overshadows the deviations seen in the aqueous 1-indenol plot.

3.2.6. Rate of production

Among the different polymers, Hytrel® 8206 absorbed both 1,2-indandiol and 1-indenol at a faster rate than the hydrophobic polymers, shown in Figs. 3 and 6, respectively. Because this polymer absorbs a significant amount (30 wt%) of water within its chain network, it is reasonable to assume that water within the swollen network could provide an additional diffusive pathway to a dense polymer containing no water; not only would intermolecular chain interactions be loosened by bound water through plasticization, but the presence of bulk water between the chains could provide a continuous liquid path for solute diffusion which would be more rapid than diffusion through the amorphous solid network. This enhancement may have been equally effective in supplying the substrate to the aqueous phase, improving the biocatalytic rate, and was responsible for the experiment using Hytrel® 8206 reaching completion approximately 8 h earlier than the experiments using the other polymers.

4. Conclusion

Three different commercial segmented block copolymers, varying in soft segment composition and proportion, exhibited different affinity toward the target molecules, extracting the hydrophilic, non-inhibitory product, and hydrophobic, inhibitory by-product to different extents owing to their different compositions. Water uptake by hydrophilic polymers was found to skew partition coefficient calculations and should be accounted for, and may also provide an expanded network with greater chain mobility, enhancing target molecule affinity and mass transfer to and from the aqueous phase. This study has shown that the addition of a polymer auxiliary phase having affinity toward a target metabolite can selectively enhance a system's productivity, where the polymer's relative affinity toward each metabolite is attributed to its soft segment composition and proportion. By using a polymer having affinity toward a certain metabolite, it was extracted from the aqueous phase, and more of that metabolite was produced by the cells in response to its removal. This metabolic response suggests that simply targeting an inhibitory by-product may not necessarily detoxify a system due to consequent responses in metabolic flux, and this would depend on the biocatalytic pathways' plasticity and ability to divert substrate. However, this also points toward targeting multiple, individual molecules by using multiple, different polymers in amounts chosen based on the biotransformation's specific metabolic profile.

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