Technical Note

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Medium composition effects on solute partitioning in solid–liquid two-phase bioreactors

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Abstract

Biphasic systems such as two-phase partitioning bioreactors (TPPBs) have been used to alleviate biological inhibition by sequestering inhibitory compounds within an immiscible phase. The use of solid polymer beads as this auxiliary phase provides a fully biocompatible alternative to potentially toxic organic solvents. While guidelines exist for the rational selection of the polymer phase, the effect of the aqueous phase composition on molecular sequestration has not been explored in the literature. This work aims to identify aspects of medium composition that influence the partitioning of target molecules into the sequestering phase. Using benzaldehyde as the target molecule and Hytrel G3548L (DuPont) as the polymer phase, pH, temperature, salt and glucose concentrations, as well as ethanol concentrations, were examined for their effects on the partition coefficient. pH and temperature were observed to have no significant effect on benzaldehyde partitioning. Salt and glucose additions increased the partition coefficient by 173% and 30%, respectively, compared with pure reverse osmosis (RO) water, while increasing ethanol concentration was found to decrease the partition coefficient from 44 (±1.6) to 1 (±0.3). Strategic changes to the aqueous phase can be made to improve affinity of the sequestering phase for target molecules. This provides a simple and cost-effective method to potentially improve TPPB system performance.

Keywords: polymer beads; two-phase partitioning bioreactor; partition coefficient; biotransformation

INTRODUCTION

The bioproduction of high-value compounds, such as pharmaceuticals or food additives, has great potential for application in industry due to the high purity and stereoselectivity achieved in the final product.[1] The major limitation of biological synthesis routes compared with chemical synthesis is low final product concentrations, often caused by toxicity of substrate, product, or by-product to the micro-organism performing the transformation. Biphasic systems, such as two-phase partitioning bioreactors (TPPBs), are often employed to alleviate inhibition by sequestering the inhibitory compounds into an auxiliary immiscible phase.[2]

The selection of an appropriate immiscible phase is an essential component of TPPB design. This phase must demonstrate high affinity for the target compound(s), which is characterized by the partition coefficient(s). The partition coefficient is the ratio of the concentration of the target compound in the sequestering phase relative to its concentration in the aqueous phase at equilibrium. Organic solvents have often been employed as the immiscible phase, but can have limitations such as cytotoxicity, bioavailability, high viscosity, flammability and high cost.[3] However, recent advances in the field of TPPBs have demonstrated that solid polymer beads can provide an effective alternative sequestering phase.[4]

Solid–liquid TPPBs have been applied to the production of several high value compounds, such as 3-methylcatechol[5], carvone[6], 2-phenylethanol[7], L-phenylacetylcarbinol[8], and benzaldehyde[9]. These systems have all shown greater than 100% improvement in performance over single phase systems, demonstrating the potential of solid–liquid TPPBs to approach concentration requirements for industrial applications. The TPPB literature has focused on providing strategies for sequestering phase selection, with little to no attention being given to the composition of the aqueous phase.[3,10,11]

A difference in partitioning between abiotic and biologically determined partition coefficients has been observed in the solid–liquid TPPB literature, but the cause for this discrepancy is not currently understood. As polymer properties remain largely constant, the most reasonable source of this variation comes from the aqueous phase, which will change from its starting composition over the course of the biotransformation. While many studies observe between 5 and 30% decrease in the partition coefficient for biological studies compared with abiotic studies,[5,6,7,8] increases in partitioning have also been reported.[12] Partition coefficients are often used in mass balances to account for the target compound in the polymer phase, emphasizing the need to account for variation in partition coefficients over the course of a biotransformation.[7,13] By independently analyzing factors that may affect partitioning, the discrepancies observed in the literature can be better understood and applied to future systems to allow greater operational control over TPPB partitioning behavior.

The objective of this study was to investigate some common elements of medium composition that may change during

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biotransformation and their individual effects on the partition coefficient. Benzaldehyde was used as the target molecule with the polymer Hytrel G3548L (DuPont), as there have recently been reported studies in the field of solid–liquid TPPBs with Hytrel G3548L demonstrating affinity for benzaldehyde.[8,9] By identifying how medium composition may alter the equilibrium distribution of compounds, some simple strategies to improve the performance of a solid–liquid TPPB have also been suggested.

EXPERIMENTAL

Chemicals and polymers

For experiments testing growth medium as the aqueous phase, a typical minimal medium with all necessary macronutrients for growth[8] was used with all components (glucose, $(NH_4)_2SO_4$, KH_2PO_4, $MgSO_4$, $CaCl_2$, $FeSO_4$, $ZnSO_4$, $MnSO_4$, $CuSO_4$) purchased from Fisher Scientific Canada (Oakville, ON, Canada). Additional materials for modifying the aqueous phase (KCl, ethanol, H_2SO_4 and KOH) were also purchased from Fisher Scientific Canada. Cylindrical Hytrel G3548L polymer beads (4 × 3 × 2 mm) were graciously donated by DuPont Canada. Hytrel G3548L is a co-polymer of poly(butylene terephthalate) and polyether with a specific gravity of 1.16, glass transition temperature of −45 °C, melting point of 156 °C and shore hardness rating of 35D.[8]

Concentration measurements

HPLC-UV detection (Varian, Prostar, Model # PS325, Polaris 5u C18-A 150 × 4.6 mm column) was used to quantify benzaldehyde at 283 nm. The mobile phase of 30% v/v acetonitrile was maintained at 1 mL min^{-1}.

Partition coefficients

Partition coefficients were determined using a previously described method.[14] 10 mL of stock solution were incubated (24 h, 30 °C, 180 rpm) with a set mass of polymer (1–5 g) for each test. Error bars in all figures show the 95% confidence interval obtained from the linear regression to determine the partition coefficient. Medium composition effects were tested using independent stock solutions of RO water with 2 g L^{-1} of benzaldehyde. pH was tested at 5, 7 and 9. KCl was tested from 0 to 360 g L^{-1}, glucose from 0 to 500 g L^{-1} and ethanol from 0 to 100%v/v. To test the effect of temperature, samples were sealed to avoid losses due to volatilization and incubated at room temperature (23 °C), 30 °C, and 45 °C for 24 h at 180 rpm.

RESULTS AND DISCUSSION

The effect of reverse osmosis (RO) water, tap water, growth medium

The first objective was to analyze RO water, tap water, and a simple growth medium to see what, if any, effect these had on partitioning, and to select a standard aqueous phase for subsequent testing (Fig. 1(a)). Figure 1 (a) shows that within the 95% confident intervals, there is no statistical difference in partition coefficient for RO water, tap water, or the minimal growth medium tested. RO water was selected as the aqueous phase for subsequent tests to avoid any unforeseen interactions between compounds in the aqueous phase and the variable being tested.

When designing the medium composition for biocatalysis, some important aspects that must be considered are pH, salt concentrations, and glucose (or another carbon source) concentration. This study examined these elements of medium composition, and how they may be altered to effect partitioning. Additionally, the effect of ethanol on the partition coefficient was examined, as ethanol is an example of an inhibitory fermentation product produced in large quantities, often at high concentrations, and is also a common anaerobic fermentation by-product.

Effect of pH

The effect of pH on partitioning of benzaldehyde was examined over a range of pH values typical of biological operation.[8,9] Fig. 1(b) shows that there is no statistical difference in partitioning of benzaldehyde, which was expected because the functional group of benzaldehyde is an aldehyde, and therefore was unlikely to ionize over this range. It is expected that some molecular change, such as ionization, would be required in order for pH to affect partitioning. However, this effect may be the least broadly applicable of all the variables tested in this study, as it likely depends on the chemical nature of the target molecule and is an area under investigation in our group with a variety of molecules possessing different molecular features.

Effect of temperature

The effect of temperature on partitioning was examined from room temperature (23 °C) to 45 °C, shown in Fig. 1(c). This range was selected to avoid experimental complications due to volatilization of benzaldehyde at high temperatures as well as to avoid adding additional power requirements with bioreactor operation to cool the medium to lower temperatures. There is no significant effect of temperature on partitioning shown in Fig. 1(c). However, temperature may affect the rate of partitioning due to the temperature dependence of diffusivity, which may be useful to evaluate for a given system but is beyond the scope of this investigation.

Effect of salt concentration

To determine the effect of salt concentration, potassium chloride (KCl) was selected as it is fully ionizable, has been used in similar studies on liquid–liquid partitioning, and may be representative of other dissociable salts present in growth medium.[15] The full range of KCl solubility at 30 °C was tested, and the effect is shown in Fig. 1(d).

Salt concentration provided the most significant change in partition coefficient of all variables explored in this study, with a 173% increase over pure RO water with KCl at its solubility limit (precipitated KCl was observed at 360 g L^{-1}, 4.8 mol L^{-1}). It appears as though low concentrations of KCl have a minimal effect on partitioning, with overlapping 95% confidence intervals seen for up to 75 g L^{-1}. However, above this point, the partition coefficient increases, until a plateau is reached when approaching the solubility limit, with negligible differences between 300 g L^{-1} (below the solubility limit) and 360 g L^{-1} (above the solubility limit). An increase in partitioning with salt addition in liquid–liquid TPPBs has been previously observed[15] and was explained by hydration theory, in which the electrolyte prefers to associate with water molecules, resulting in the effective removal of the water molecules from their role as a solvent. This causes an increase in the activity of nonelectrolyte (in this study, benzaldehyde) in solution corresponding to the increase in partitioning. There exists a limiting water activity below which further improvements in partitioning are not observed,[15] which may explain the plateau observed above 300 g L^{-1} KCl.

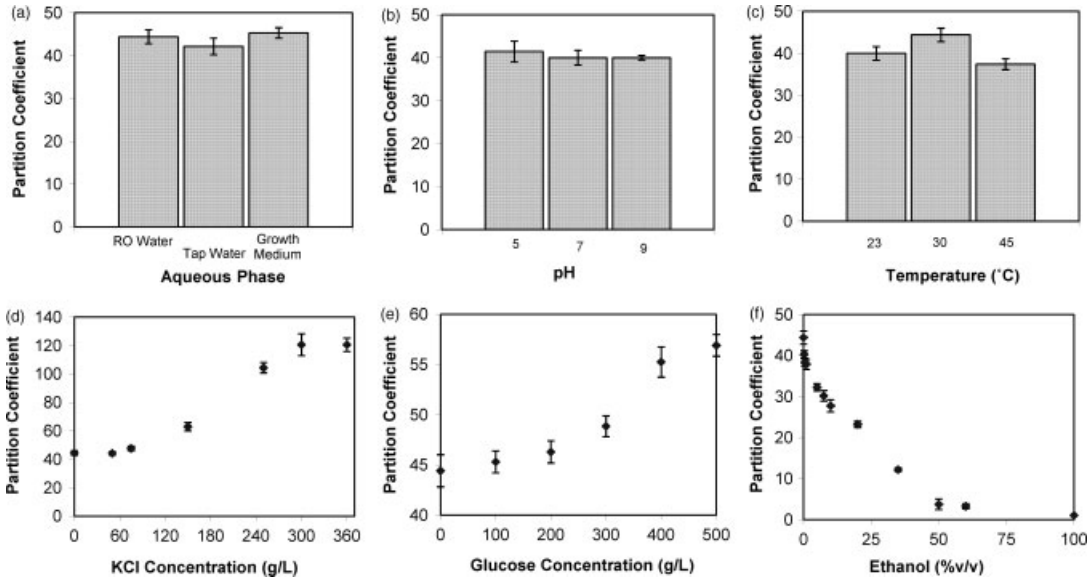

Figure 1. Partition coefficient of benzaldehyde toward Hytrel G3548L varying (a) RO water, tap water, growth medium, (b) pH, (c) temperature, (d) KCl concentration, (e) glucose, and (f) ethanol concentration.

The salt effect has great potential to improve TPPB performance in a broad range of applications. While the addition of electrolytes at high concentrations may damage active microbes (by osmotic pressure effects), the addition of salt at the end of a biotransformation may provide a way to improve extraction in batch processes.

Effect of glucose concentration

Glucose, when present in a growth medium, can be present at high concentrations, such as those used in high gravity ethanol fermentation (up to 500 g L^{-1}).[16] This presents a potentially significant change to medium composition. Therefore, the effect of glucose concentration on partitioning was selected as a variable of interest for this study.

Figure 1(e) shows that the effect of changing glucose levels and displays the same general shape as the salt effect shown in Fig. 1(d). However, the change in partitioning is much less significant, with only a 30% increase over pure RO water at 500 g L^{-1} glucose. Based on hydration theory suggested for the salt effect, an analogous trend would be expected for glucose, since non-electrolytes still cause a reduction in water activity, which, as previously described, would increase partitioning.[15] It should be noted that a statistically significant change in partitioning was not observed until a glucose concentration of 300 g L^{-1} was reached. Therefore, partitioning of target molecules into the polymer beads may be improved over the course of the biotransformation by maintaining high concentrations of glucose, which is less likely to disrupt microbial activity than high levels of electrolytes such as KCl.

Effect of ethanol concentration

Fermentation for ethanol production yields ethanol concentrations in excess of 70 g L^{-1}.[16] Traditional acetone–butanol–ethanol fermentation has cumulatively lower yields due to end-product inhibition of approximately 20 g L^{-1}.[17] This may represent a significant change to the medium composition over the course of a biotransformation, and has been presented in the literature as a potential source of discrepancy between abiotic partition coefficients relative to those determined using medium obtained from a biotransformation containing ethanol.[8]

Figure 1(f) shows a decrease in partition coefficient with increasing ethanol concentration, reducing from 44.4 (±1.6) for pure RO water to 1 (±0.3) for 100% ethanol. The trend displayed in Fig. 1(f) shows sharp decreases in partition coefficient for up to 10% ethanol by volume, with the slope declining after this point. This may be due to ethanol increasing the solubility of benzaldehyde in the aqueous phase, reducing the thermodynamic preference of benzaldehyde for the polymer phase, as benzaldehyde is moderately hydrophobic (water solubility of 6.5 g L^{-1}). Therefore, during an anaerobic biotransformation, the gradual accumulation of ethanol may result in a reduction of the partition coefficient. In systems in which the product is being sequestered by the polymer beads, this reduced affinity may decrease system productivity. However, in systems where substrate is being delivered from polymer beads, this reduction may facilitate the release of the compounds from the polymers. Previous work in the field of solid–liquid TPPBs has demonstrated that some systems with hydrophobic target compounds display such a high affinity for the sequestering phase that their release from polymer beads is hindered.[14] With a statistically significant decrease in partitioning at 0.5% ethanol by volume, even low levels of ethanol may reduce the partition coefficient enough to help improve their delivery. This concept has been demonstrated to improve substrate release in a solid–liquid TPPB for the biodegradation of phenols, with 0.5% methanol added as a co-solvent.[18]

CONCLUSIONS AND SUGGESTIONS FOR ENHANCED TPPB PERFORMANCE

This work demonstrated a high dependency of the partition coefficient of benzaldehyde on medium composition. Therefore, it is recommended that studies reporting partition coefficients not only describe the medium used for testing, but also use a composition that is as similar as possible to the aqueous phase of any biotransformation involved. Additionally, solid–liquid TPPB

studies using partition coefficients in mass balances must account for variation over the course of the biotransformation.

The effects determined in this study provide a broad range of strategies to improve TPPB performance. In a batch biotransformation, the addition of a strong electrolyte, such as KCl, to approach the solubility limit may provide significant improvements in product recovery. For batch or continuous processes, the maintenance of high levels of glucose may provide a moderate increase in partitioning without damage to microbes that could result at high levels of electrolytes. When a biotransformation involves the delivery of a substrate from the polymer phase, the presence of ethanol as a co-solvent may improve performance by facilitating the release of substrate from the polymer.

With the application of these simple changes to medium composition, the performance of a solid–liquid TPPB may show significant improvement as an alternative to designing specifically tailored polymers. Therefore, while rational polymer selection or even customization of polymers are important aspects of solid–liquid TPPB design, the aqueous phase should not be ignored, as simple alterations may be able to provide relatively high improvements in performance. Current work is investigating other target molecules and polymers to develop heuristics for determination of the aqueous phase composition, as has been done previously for organic solvent and polymer selection, and implement these strategies in fermentation studies.[3,10]

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