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Characterization of pH dependence in organic acid absorption with non-reactive and reactive polymers for application in two-phase partitioning bioreactors

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HIGHLIGHTS

- Species distribution clearly described during acid partitioning in an absorptive polymer.
- Effect of acid absorption on equilibrium pH well characterized.
- High pressure CO_2 (60 bar) can reversibly lower pH to facilitate acid absorption.
- Amine-functionalized hydrogels achieve high acid recovery with low polymer fractions.

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ABSTRACT

Strategies for extracting organic acids from aqueous solutions using polymeric absorbents are demonstrated and discussed in the context of two-phase partitioning bioreactor (TPPB) design. Experimental data and material balances for the uptake of butyric acid and benzoic acid by a poly(ether-block-amide) copolymer (Pebax 2533) establish the inherent limitations of unreactive absorbents for organic acid bioprocesses that operate at near-neutral pH. Improvements to TPPB performance are achieved by lowering pH temporarily with CO_2 to enhance acid absorption, and removing the solute-rich polymer before restoring pH to fermentative values by releasing the CO_2 pressure. Butyric acid removal by Pebax 2533 improved from 3% to 40% upon acidifying a pH 6 solution with 60 bar of CO_2, while benzoic acid absorption increased from 1% to 80% using this pressure manipulation technique. A reactive extraction approach involving a newly-synthesized amine functionalized hydrogel is also described wherein acid/base reaction equilibrium governs the extent of solute uptake. Copolymerization of 2-(dimethylamino)ethyl acrylate (DMAEA) and trimethylolpropane triacrylate (TMPTA) yielded a thermoset material with sufficient basicity to remove 80% of both butyric and benzoic acid from aqueous solution using just 1 wt% polymer relative to the aqueous phase mass.

1. Introduction

Two phase partitioning bioreactor (TPPB) technology can improve the productivity of biological processes by sequestering inhibitory fermentation products and/or compounds intended for biodegradation [1]. Polymeric absorbents have attracted recent attention, with considerable effort expended on developing methods for selecting polymers with high thermodynamic affinity for target solutes [2] . This affinity is generally expressed in terms of the partition coefficient (PC), defined as the ratio of the equilibrium concentration of solute in the polymer ($[s]^{pol}$, mole/g) to that in the aqueous phase ($[s]^{aq}$, mole/g) (Eq. (1)),

$$PC = \frac{[S]^{pol}}{[S]^{aq}} = \frac{n_S^{pol}/m_{pol}}{n_S^{aq}/m_{aq}} \quad (1)$$

Abbreviations: TPPB, two-phase partitioning bioreactor; ISPR, in situ product recovery; PC, partition coefficient; D, distribution coefficient; n^{tot}, total moles of solute; n_S^{aq}, moles of solute in the aqueous phase; n_S^{pol}, moles of solute in the polymer phase; n_{HA}^{aq}, moles acid in aqueous phase; n_{HA}^{pol}, moles acid in polymer phase; $n_{A^-}^{aq}$, moles conjugate base in aqueous; m_{aq}, aqueous mass (g); m_{pol}, polymer mass (g).

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where n_S^{pol} and n_S^{aq} are the moles of solute in the polymer and aqueous phases, respectively, and m_{pol} and m_{aq} are the phase masses.

TPPB systems involving hydrophobic target solutes are relatively simple to design, with PC values often exceeding 3000 for polyaromatic hydrocarbons [3], and values in the range of 50–100 for slightly more hydrophilic substrates such as 2-phenylethanol [4]. In these favorable cases, the amount of polymer required to bring solute concentrations below their inhibitory levels, as expressed in terms of the phase ratio (F, Eq. (2)) is typically below 0.1.

$$F = \frac{m_{pol}}{m_{aq}} \tag{2}$$

Sequestering hydrophilic molecules such as has been seen for cis-indandiol is considerably more challenging, since they generally possess PC values less than 5 in organic solvents and polymers [5]. As a result, higher phase ratios are required to regulate aqueous solute concentrations below inhibitory levels. Since this comes at the expense of TPPB volumetric productivity, strategies for improving the uptake capacity of the polymer phase are of considerable interest.

Longstanding challenges encountered when extracting ionizable solutes from aqueous solution, in particular the pH sensitivity of solute partitioning, are well documented [6,7], and strategies for recovering organic acids from fermentation media have been the subject of several recent reviews [8,9]. At the near-neutral pH levels needed to support the growth of many microorganisms, solutes such as butyric acid (pK_a 4.8) and benzoic acid (pK_a 4.2) exist predominately in their conjugate base form. Given that alkali metal carboxylate salts are virtually insoluble in organic solvents and polymers [10,11], solute extraction potential is limited to the small amount of conjugate acid present in typical fermentation media. This fact, combined with the low PC values generally observed for organic acids, results in exceptionally poor performance in conventional TPPB systems. In this work, butyric acid has been selected as several studies have focused on *in situ* product recovery (ISPR) of this molecule [12–14], as it has been shown that this acid exerts a general inhibitory effect on fementative bacteria [15], and reductions in acid concentrations during fermentation would improve microbial performance. Additionally, benzoic acid has been selected due to its strong partitioning behavior, which provides comparison to the relatively low partitioning achieved by butyric acid, while also permitting model validation for a second acid.

One strategy for improving TPPB efficiency involves acidifying the system intermittently to promote organic acid absorption, then restoring the fermentation medium to a pH that supports the biotransformation. Repeated pH shifts can be accomplished without increasing inorganic salt concentrations by acidifying reversibly with carbon dioxide. Some success has been achieved by sparging CO_2 at ambient pressure [13,16], while higher partial pressures (pCO_2) have been shown to drop pH values further by virtue of increased CO_2 concentration [17]. Therefore, a sequence of CO_2 pressurization, absorption, and depressurization has the potential to enhance organic acid extraction without raising the ionic strength of the aqueous phase.

Another strategy for removing organic acids from fermentation media uses water-insoluble, organic bases such as tertiary amines to extract acids in the form of alkylammonium carboxylate salts [18]. However, such reactive extractants have been reported to exhibit strong toxic effects despite their extremely low solubility in water [12], limiting their use for ISPR. However, these amine-based extractants possess attractive qualities, encouraging research to overcome these toxicity issues. Specifically, reactive extractants avoids phase equilibrium limitations by exploiting reaction equilibrium between a weak acid and a weak base, potentially allowing for greatly improved recovery of acids demonstrated low PC values. Kertes and King demonstrated high extraction of carboxylic acids with mixtures of organic bases and various solvents [19], and recently the reactive extraction of biologically-produced solutes such as succinic acid [20] and butyric acid [12] has stimulated research in developing novel reactive extractants. Interestingly, recent studies have developed acrylamide-based polymeric hydrogels that demonstrate reactive extraction of organic acids due to their amine functionality [21], and this approach may be able to overcome toxicity issues, as the extractants are covalently bonded to the polymeric matrix.

This report begins with the development of a material balance framework that describes the effects of polymer phase fraction (F), polymer/solute affinity (PC), and aqueous solution pH on solute uptake by an unreactive (i.e. absorptive) polymer. Knowledge of the influence of pH on organic acid absorption is then used to demonstrate the benefits of applying high CO_2 pressure to acidify the solution reversibly. We conclude with a demonstration of organic acid extraction by reaction with a thermoset acrylate hydrogel bearing a high concentration of pendant trialkyl amine functionality.

2. Materials and methods

2.1. Polymers and materials

A polyether-co-amide block copolymer (Pebax® 2533, Arkema, Inc) was selected on the basis of previous polymer selections strategies for butyric acid [13], and this polymer is comprised of 80% poly(tetramethylene glycol) and 20% 12-poly(amide) by weight [22], and was soaked in water and dried prior to use. An amine functionalized hydrogel was prepared by solvent-fee copolymerization of 2-(dimethylamino)ethyl acrylate (DMAEA, 4.25 g) with trimethylolpropane triacrylate (TMPTA, 0.75 g) at 70 ± 1 °C for 24 h using 2,2′-azobis(2-methyl-propionitrile) (AIBN, 0.2 g) as a radical initiator. Soluble material within the resulting thermoset was removed by swelling the polymer with THF and recovering from excess acetone. The purified product was dried under vacuum at 23 °C. It was assumed that any acid recovery achieved by these polymers was based on absorption, as previous work has demonstrated that uptake of solutes into amorphous polymers is based on absorption rather than adsorption [23].

2.2. Analytical methods

Aqueous samples were analyzed for butyric and benzoic acid concentration using an HPLC instrument (Varian Prostar) equipped with a UV–Vis detector (Varian Prostar, PS325) operating at 220 nm. Butyric acid separations employed a Varian Hi-Plex H column (300 × 7.7 mm) operating at 60 °C with a 10 mM H_2SO_4 mobile phase at 0.7 mL/min, while benzoic acid separations used a Varian Pursuit C8 5 μm column (250 × 4.6 mm), with a mobile phase of 20 mM H_3PO_4 in 50:50 water/acetonitrile at 1 mL/min. Butanol concentrations were determined with a Varian 450-GC gas chromatograph equipped with a CP-8410 AutoInjector, VF-5 ms 30 m column and a FID detector.

2.3. Partition coefficient determinations

Partition coefficients for butanol, butyric acid, and benzoic acid absorption by Pebax 2533 were determined in triplicate by contacting polymer (1 g) with the requisite aqueous solution of solute (10 mL). Butanol and butyric acid experiments were conducted

using 20 g/L solutions. The low solubility limit of benzoic acid restricted its aqueous concentration to 2.5 g/L, necessitating the addition of 5% v/v 1 M H_2SO_4 to acidify the solution to the point where the conjugate base concentration was rendered insignificant. After 24 h equilibration at 30 °C, the aqueous phase was decanted and analyzed for solute concentration as described above. The solute concentration in the polymer was calculated by material balance. As water absorption by Pebax 2533 is 1.2% [24], any possible effects on partitioning were considered negligible. Polymer-free control samples were subjected to the same testing regimen to ensure the constancy of initial solution concentrations throughout the equilibration period. PC values for solute absorption by Pebax 2533 at 30 °C were calculated from Eq. (1) to be: 2.0 ± 0.2 for butanol, 4.2 ± 0.3 for butyric acid, and 70 ± 1for benzoic acid with error reported as standard deviation (n = 3).

2.4. Influence of polymer phase fraction, acid concentration and initial pH on acid partitioning

Solute partitioning tests were performed in triplicate by adding varying amounts of Pebax 2533 (0.5, 1.0, 1.5, 2.0, 2.5, 3.5 and 5.0 g) to 10 mL of 2.5 g/L aqueous solutions of butanol, butyric acid, or benzoic acid, which respectively represented polymer fractions of 0.05, 0.10, 0.15, 0.20, 0.25, 0.35, 0.50, reflecting the range of polymer fractions employed in TPPBs. After 24 h equilibration, the pH of the aqueous phase was measured before determining the aqueous phase and polymer phase solute concentrations by the methods described above. Solute speciation between its conjugate acid and conjugate base forms was calculated from the measured solution pH (see Supplemental Information), from which the species fractions in the two phases (i.e. n_{HA}^{pol}/n^{tot}, n_{HA}^{aq}/n^{tot}, and $n_{A^-}^{aq}/n^{tot}$) were calculated. To determine the effect of initial acid concentration on changes to pH arising from absorption, triplicate samples of Pebax 2533 (5 g, F = 0.5) were shaken in butyric acid solutions (10 mL) of varying concentrations (2.5, 5, 10, 15, 20, 25, 35, and 50 g/L) for 24 h, after which the equilibrium pH and solute distribution were determined as described above. A polymer fraction of F = 0.5 was selected as this is the highest practical polymer fraction, and thus represents the maximum values for changes in equilibrium pH. Since typical fermentations for organic acid production tolerate pH near neutral values, knowledge of species distribution as a function of pH is an important consideration. Butyric acid and benzoic acid solutions (10 mL, 2.5 g/L) were pH-adjusted with 3 M KOH or H_2SO_4 to yield values ranging from 2 to 7 at equilibrium, after which partitioning and solute distribution were determined as described above.

2.5. Absorption assisted by CO_2 pressurization

Aqueous solutions (500 mL) of 2.5 g/L butyric acid or benzoic acid were adjusted to pH 6.0 using 3 M KOH, since this is the optimal pH for butyric acid fermentation by *Clostridium tyrobutyricum*. Pebax 2533 (100 g) was added to the solution and the mixture was transferred to a 1-L stainless steel pressure vessel equipped with a liquid sampling tube (Parr Instrument Company, Moline, IL, USA). The vessel was sealed and pressurized with CO_2 (15, 30, 45, or 60 bar) and stirred at 500 rpm for one hour. The aqueous phase was then sampled under pressure and the acid concentration determined as described above. Since solution pH could not be measured within the pressure vessel, conjugate acid and conjugate base mole fractions were not determined, but rather reported as the fraction remaining in aqueous solution (n_{tot}^{aq}/n^{tot}) and absorbed by the polymer (n_{HA}^{pol}/n^{tot}).

2.6. Reactive extraction with amine-functionalized hydrogel

Samples of amine functionalized hydrogel (20–100 mg) were added to 10 mL aliquots of 2.5 g/L butyric and benzoic acid. These were allowed to equilibrate with pH monitoring to ensure completion, an equilibrium was reached at 2 h. Considering the extremely low polymer fractions employed (0.002–0.01), the small degree of water absorption observed was considered negligible. Samples of aqueous solution were then analyzed following the HPLC method described above.

3. Results and discussion

3.1. Absorption of unreactive solutes

Designing a TPPB process requires knowledge of the solute distribution between the organic/polymer phase and the aqueous phase. For systems involving unreactive target molecules, a partition coefficient (PC) is all that is needed to calculate the phase ratio (F) that will maintain aqueous solute concentrations below cytotoxic levels. Eqs. (3) and (4) are material balances expressed in terms of the fraction of the total solute loading in the polymer (n_S^{pol}/n^{tot}) and aqueous (n_S^{aq}/n^{tot}) phases.

$$\frac{n_S^{pol}}{n^{tot}} = \frac{F \cdot PC}{F \cdot PC + 1} \tag{3}$$

$$\frac{n_S^{aq}}{n^{tot}} = \frac{1}{F \cdot PC + 1} \tag{4}$$

In a batch bioreactor operating at a given phase ratio, the total amount of solute in the system (n^{tot}) is time dependent, but the fraction of solute partitioning between the phases is fixed, providing that PC remains constant. Note that the PC for a polymer/solute combination is equal to the solute activity coefficient in the polymer phase divided by that in the aqueous phase [2]. Since these activity coefficients are different functions of phase composition, PC values are, strictly speaking, dependent on n^{tot}. However, variations in PC are often sufficiently small to be ignored without introducing significant error to the analysis.

Consider the data plotted in Fig. 1 for butanol absorption, a biomolecule that has been studied previously in the context of TPPB development [4,25]. Independent measurements of butanol uptake by Pebax 2533 from an aqueous solution containing 2.5 g/L of solute (not shown) provided a PC estimate of 2.0 ± 0.2. Using this single value in Eqs. (3) and (4) gave predictions of solute partition-

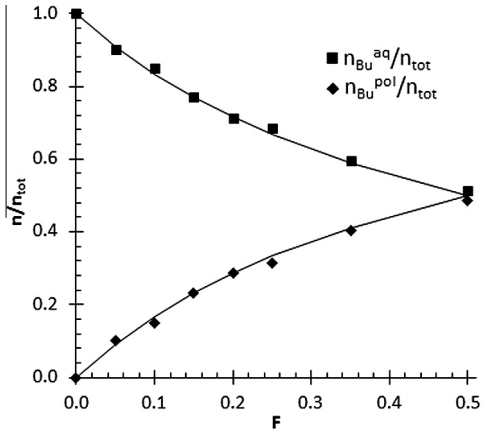

Fig. 1. Partitioning of butanol between Pebax 2533 and the aqueous phase as a function of polymer fraction (30 °C; $[Butanol]_{initial}$ = 2.5 g/L; Lines represent Eqs. (3) and (4) with PC = 2.0).

ing with respect to phase ratio that agree with experimental values. This confirms that a constant PC, when measured at a solute loading that is relevant to bioprocess design, can be used with confidence to predict solute partitioning.

3.2. Absorption of organic acids

As described above, organic acid extraction is complicated by dissociation of the acid within the aqueous phase. Predicting the extent of ionization requires knowledge of the acid dissociation constant in water (pK_a) and the solution pH, which when combined with material balances, gives Eqs. (5)–(7). Note that three process variables must be quantified; conjugate acid in the polymer (n_{HA}^{pol}/n^{tot}), conjugate acid in water (n_{HA}^{aq}/n^{tot}), and conjugate base in water (n_{A-}^{aq}/n^{tot}), assuming that carboxylate anion is not absorbed by the polymer ($n_{A-}^{pol}/n^{tot} = 0$).

$$\frac{n_{HA}^{pol}}{n^{tot}} = \frac{F \cdot PC}{F \cdot PC + \left[1 + 10^{(pH - pK_a)}\right]} \tag{5}$$

$$\frac{n_{HA}^{aq}}{n^{tot}} = \frac{1}{F \cdot PC + \left[1 + 10^{(pH - pK_a)}\right]} \tag{6}$$

$$\frac{n_{A-}^{aq}}{n^{tot}} \cdot = \frac{10^{(pH - pK_a)}}{F \cdot PC + \left[1 + 10^{(pH - pK_a)}\right]} \tag{7}$$

These expressions were validated by comparing calculated values to experimental data acquired for butyric acid (PC = 4.2 ± 0.3, pK_a 4.8) and benzoic acid (PC = 70 ± 1, pK_a = 4.2) absorption by Pebax 2533. Note that these are *intrinsic* PC values for conjugate acid absorption [26], measured at a low enough pH to supress acid dissociation, in contrast to *observed* PC values calculated at an arbitrary pH, which can be affected by solute ionization. Fig. 2 illustrates the effect of phase fraction on polymer and aqueous phase compositions, as well as the equilibrium pH. Agreement between calculated and measured solute partitioning values is good, providing confidence that our material balance and reaction equilibrium framework is useful for TPPB design. Moreover, as observed for butanol absorption, a PC value derived from measurements that are appropriate to TPPB operation gave good material balance predictions.

The data in Fig. 2 demonstrate two other principles of TPPB development: the sensitivity of solute absorption to polymer/solute affinity (PC), and the pH buffering capacity of a polymeric absorbent. Although Pebax 2533 absorbed both solutes, the removal efficiency of benzoic acid was far greater than that of butyric acid, owing to significant differences in PC (70 ± 1 versus 4.2 ± 0.3, respectively). Unreactive absorbents for bioreactions yielding multiple organic acids can, therefore, be designed to remove one solute preferentially, if a large difference in PC values exists. This opportunity for selective removal on the basis of thermodynamic partitioning is not afforded by basic absorbents such as amine-functionalized hydrogels, which we demonstrate in the final section of this paper.

The sensitivity of aqueous phase pH to the polymer phase fraction is particularly important. In the case of butyric acid, pH increased from 3.4 in the absence of polymer to 4.1 at $F = 0.5$, while benzoic acid showed a rise from 3.0 to 4.8 over the same range. Since these changes result from selective removal of acid from the aqueous solution, the large pH shift observed for benzoic acid absorption is consistent with the greater affinity of Pebax 2533 for this solute. The ability of an absorptive phase to raise pH by sequestering organic acid is widely reported [27], and is not unique to the system of present interest. However, our data suggest that pH buffering can impose an upper limit on extraction efficiency. The data plotted in Fig. 2b show that experimental measurements of n_{HA}^{pol}/n^{tot} for benzoic acid approached an asymptotic value of 0.87. Consider that the limit of Eq. (5) as $F \to \infty$ is 1.0 when pH is con-

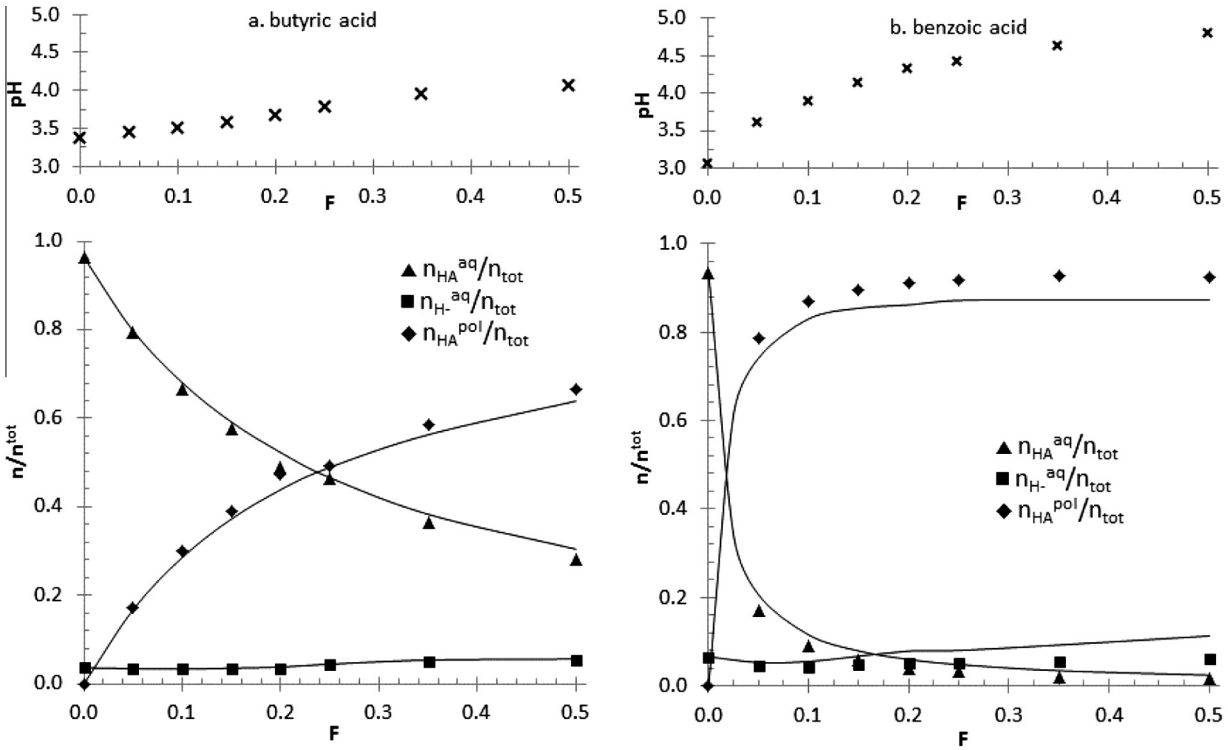

Fig. 2. Equilibrium pH and acid-carboxylate partitioning as a function of Pebax® 2533 fraction (a. butyric acid; b. benzoic acid; 30 °C; $[acid]_{initial}$ = 2.5 g/L; lines calculated from Eqs. (5)–(7)).

stant, in which case all organic acid can be extracted given a sufficiently large amount of polymer. On the other hand, since pH is an *increasing* function of F, n_{HA}^{pol}/n^{tot} can approach a plateau that is dictated by the magnitude of PC versus $(1 + 10^{(pH-pKa)})$. We conclude that, unlike unreactive solutes such as butanol, the fractional uptake of an acidic solute may not be quantitative, and limitations should be considered when setting absorption efficiency targets in TPPB process design.

Further insight into the relationship between solution pH and organic acid uptake was gained from experiments in which the butyric acid concentration was varied while holding the polymer phase fraction at $F = 0.5$. Fig. 3 presents plots of initial pH (prior to polymer addition) and equilibrium pH (24 h in the presence of polymer). Differences in pH ranging from 0.5–0.7 units were observed at all acid concentrations, confirming that acid absorption by the polymer can lessen the need for chemical pH adjustment and ease the osmotic stress arising from base addition [28]. While similar observations have been made for adsorptive ion-exchange resins [29] and reactive extraction systems [12], pH regulation with unreactive polymers has not been explored previously.

3.3. Effect of pH on species distribution

Up to this point in our study, pH was not manipulated using additional reagents, but left to reach an equilibrium position produced by water, polymer and organic acid alone. However, the observed pH fell below the 5–7 range required by many bioconversions, and buffering and/or the addition of strong base would, therefore, be required. Fig. 4 illustrates partitioning experiments wherein aqueous phase pH was adjusted deliberately using strong mineral acid or base. The experimental data, along with calculations based on Eqs. (5)–(7), confirm that, as pH is increased, conjugate acid in the polymer and aqueous phases are lost to the aqueous conjugate base fraction. At pH 6.0, which represents the optimal pH value for butyric acid produced by *C. tyrobutyricum* [28], 91% of total butyric acid and 89% of total benzoic acid remained in the aqueous phase in the form of a carboxylate salt.

Partitioning data for organic acids is often expressed in terms of the distribution coefficient, D, defined as the concentration of conjugate acid in the organic phase divided by the aqueous phase concentrations of conjugate acid and conjugate base. This quantity is readily derived from Eqs. (5)–(7) to give the distribution coefficient at any pH, as follows.

$$D = \frac{[HA]^{pol}}{[HA]^{aq} + [A-]^{aq}} = \frac{n_{HA}^{pol}/m_{pol}}{(n_{HA}^{aq} + n_{A-}^{aq})/m_{aq}}$$

$$D = \frac{PC}{1 + 10^{(pH-pK_a)}} \quad (8)$$

Unlike PC, which is relatively constant, D is a dynamic value that changes as a function of PC and pH. Thus D represents a value that approaches PC as pH decreases, providing a measure of achievable partitioning at a given pH value. The distribution coefficient data provided in Fig. 4 highlight the sensitivity of solute partitioning to PC and aqueous solution pH. Clearly, TPPB systems that are buffered or otherwise maintained near neutral pH cannot provide high organic acid absorption levels, irrespective of the polymer/acid affinity. This realization has motivated studies of alternative approaches, as described below.

3.4. Effect of CO_2 acidification on acid absorption

One strategy to improve TPPB performance involves acidifying the system temporarily to promote absorption, then removing the solute-rich polymer before restoring pH to fermentative values. Whereas shifting the pH using mineral acids and bases increases the ionic strength of the solution, CO_2 can acidify a solution reversibly by introducing carbonic acid and its various conjugate bases. Small improvements in organic acid uptake have been demonstrated by sparging CO_2 through acidic solutions at atmospheric pressure [10,11], However, higher CO_2 pressures have a greater potential to affect solution pH [30,31] and, by extension, to improve butyric and benzoic acid absorption. Fig. 5 demonstrates the effect of CO_2 acidification on the performance of Pebax 2533. Test solutions contained 2.5 g/L of organic acid that were brought to an initial pH of 6.0 using KOH. After loading the polymer at $F = 2.0$ to these solutions, they were pressurized with CO_2 and allowed to equilibrate at room temperature for 1 h.

Fig. 5a shows that the distribution coefficient for butyric acid increased from $D = 0.1$ at $pCO_2 = 1$ bar to $D = 3.0$ at 60 bar pCO_2, which represents an increase in recovery from 3% to 40%. The improvement in benzoic acid absorption was even greater, as D rose from 0.7 to 24.0 using CO_2 addition, corresponding with recovery increase from 1% to 80% (Fig. 5b). The heightened response of the benzoic acid system is a by-product of its polymer/solute affinity, which is reflected by its high partition coefficient. These results demonstrate that CO_2 pressurization has a strong positive effect on solute partitioning, owing to medium acidification, thereby providing a means of changing pH without increasing the osmotic pressure on a microorganism. Additionally, previous work has studied the effect of high pressure CO_2 on microbial activity, explicitly demonstrating that fermentative cells could withstand a one hour exposure to high pressure with no observable effects, while also outlining many important practical considerations for the application of high pressure techniques to achieve ISPR in acid fermentations [14]. In particular, is important to note that fermentation media containing pH buffers are more difficult to acidify, and TPPB development involving CO_2 pressurization techniques require careful analysis of overall pH control strategies.

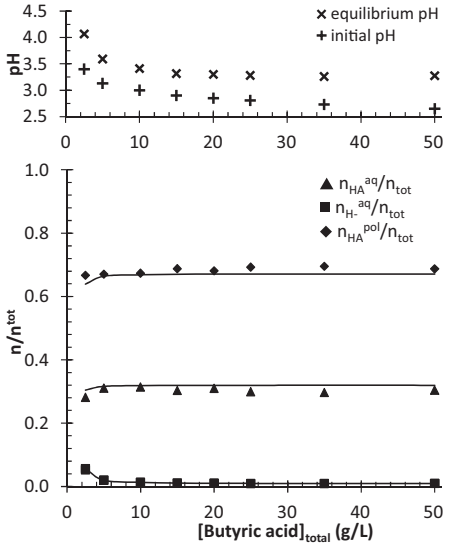

Fig. 3. pH and species distribution as a function of initial butyric acid concentration ($F = 0.5$; 30 °C; lines represent Eqs. (5)–(7)).

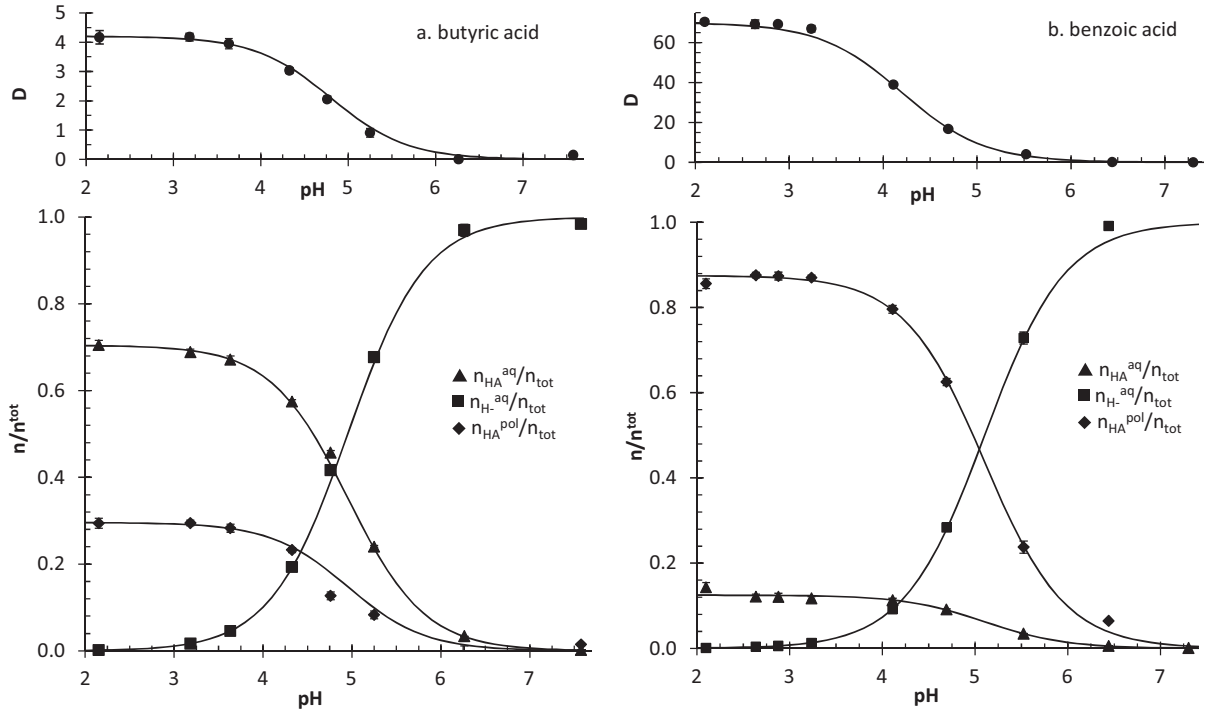

Fig. 4. Distribution coefficient (D) and conjugate acid-conjugate base fractions as a function of equilibrium pH ($F = 0.1$, $[acid]_{initial} = 2.5$ g/L, lines represent Eqs. (5)–(8)).

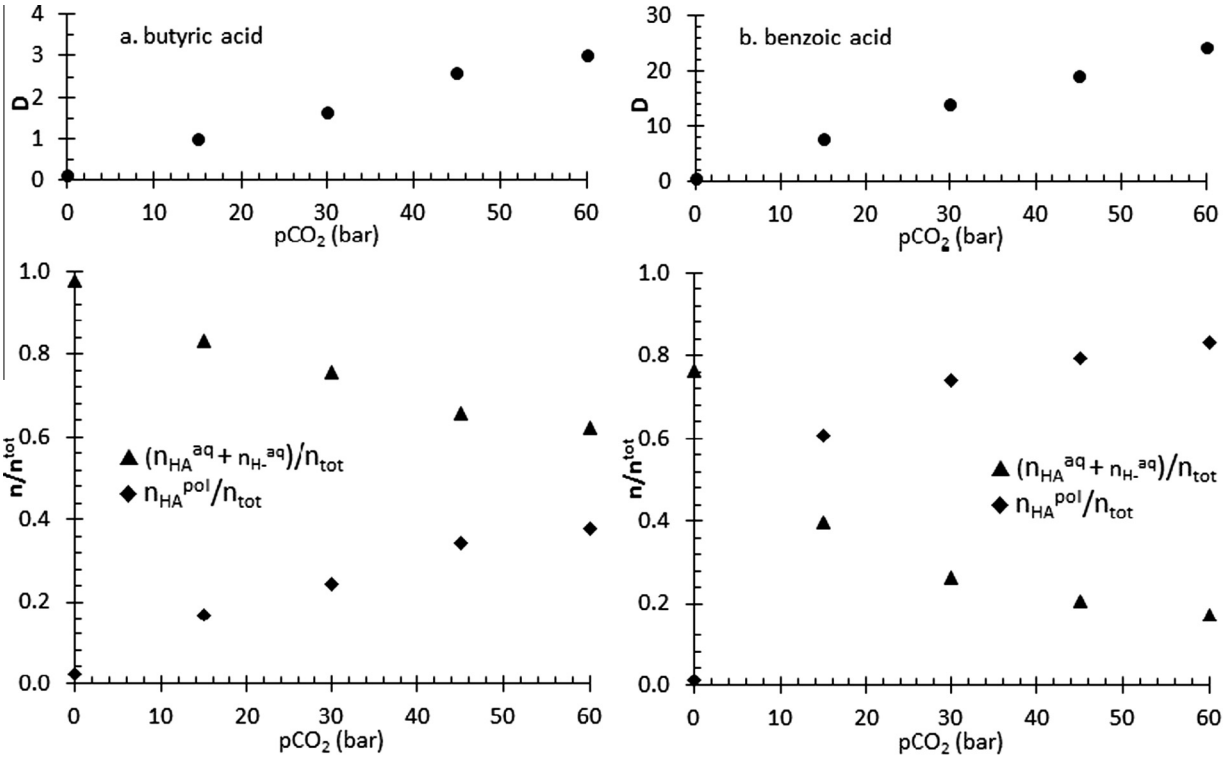

Fig. 5. Distribution coefficients (D) and solute partitioning as a function of CO_2 partial pressure ($F = 0.2$; $[acid]_{initial} = 2.5$ g/L; initial pH = 6.0).

3.5. Reactive extraction by base-functionalized hydrogel

Our studies have confirmed that, irrespective of the affinity of an unreactive polymer for an organic acid, the influence of pH on the conjugate acid/conjugate base distribution is such that TPPBs operated at near-neutral pH are inherently inefficient. An alternate extraction strategy involves exploiting the solute's acidity using a base-functionalized hydrogel to sequester the target molecule as a carboxylate salt [32]. Covalent bonding of the reactive functionality to the polymer matrix renders it insoluble in the aqueous phase, potentially avoiding cytotoxicity reported for small molecule extractants [12,33]. Gonzalez-Saiz et al. applied this hydrogel

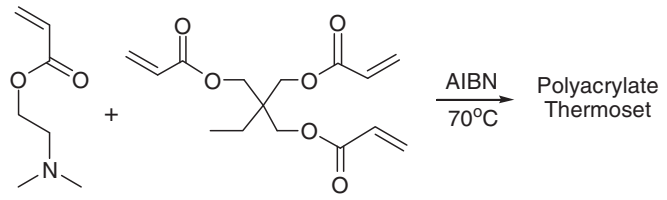

Fig. 6. Synthesis of a tertiary amine functionalized thermoset.

approach to sequester citric acid with a thermoset comprised of acrylamide, N,N′-methylenebisacrylamide, and small amounts of N,N-dimethylaminoethyl methacrylate (7–20%) [34]. Using a phase fraction of $F = 0.02$, they removed 31% of citric acid from a 10 g/L solution. Similarly, Asci and Hasdemer used a poly(acrylamide-co-N,N′-methylenebisacrylamide) hydrogel to extract acetic, lactic, citric, and tartaric acid. Due to the low basicity of primary amides, they required extremely high phase fractions on the order of $F = 2.5$ to achieve removal efficiencies of 70–75% [21].

Our attempts to prepare a base-functionalized hydrogel containing high amine concentrations began with peroxide-Initiated crosslinking of a commercially available homopolymer, poly(2-(N,N-dimethylamino)ethyl acrylate). Unfortunately, the extent of crosslinking introduced by the cure was insufficient, giving a thermoset that generated space-filling gel when immersed in dilute aqueous solutions of benzoic acid. Hydrogels with the requisite crosslink density were prepared by radical copolymerization of 2-(N,N-dimethylamino)ethyl acrylate (DMAEA) with trimethylolpropane triacrylate (TMPTA) using azobisisobutyronitrile (AIBN) as a radical initiator (Fig. 6). A purified thermoset comprised of 85 mol% DMAEA and 15 mol% TMPTA swelled to three times its original mass when submerged in a 2.5 g/L solution of benzoic acid in water, giving particles that resisted mechanical breakup over prolonged periods of agitation in suspension.

The data presented in Fig. 7 demonstrate the efficiency of this thermoset to absorb carboxylic acids. Applying a phase ratio of $F = 0.01$ to a 2.5 g/L solution of butyric acid removed 81% of the solute, giving an equilibrium pH of 5.4 (Fig. 7a). Note that each gram of thermoset contained 5.9 mmoles of tertiary amine functionality. Loading the material at $F = 0.01$ to a 2.5 g/L solution of butyric acid corresponds to an amine:acid molar ratio of 2.1:1, meaning that there was enough amine to bind all the butyric acid in solution. That only 81% of the acid was removed is a product of weak acid/weak base interactions, as well as the affinity of butyric acid for the hydrogel phase [35,36]. In the case of benzoic acid, treating a 2.5 g/L solution with $F = 0.01$ gave an amine/acid ratio of 2.9, resulting in a removal efficiency of 74% and a final pH of 6.0 (Fig. 7b).

This is in sharp contrast to Pebax 2533 (Fig. 2), which required nearly an order of magnitude more polymer to approach these removal efficiencies. To gain a better perspective of this performance gap, we used Eq. (5) to calculate n_{HA}^{pol}/n^{tot} for Pebax 2533 operating at $F = 0.01$ and pH 5.8, which predicted a value of just 0.8% for butyric acid, and 1.1% for benzoic acid. The amine hydrogel not only provided better removal efficiencies, it did so at moderate pH without appreciable sensitivity to organic acid structure. Consistent absorption values were observed for butyric and benzoic acid, implying that selectivity for organic acid separation is not provided by basic extractants, unlike those recorded for Pebax 2533, whose absorption capacity is dictated by water/polymer phase equilibrium as opposed to acid/base reaction equilibrium. However, some degree of separation could be afforded in reactive absorbent by careful consideration of extraction pH, if sufficient difference in dissociation constants between two given acids exists [27].

Hydrolytic instability is an important deficiency of an acrylate-based hydrogel, owing to the sensitivity of esters to water under both acidic and basic conditions. Indeed, the self-catalyzed hydrolysis of poly(N,N-dimethylaminoethyl acrylate) is well documented

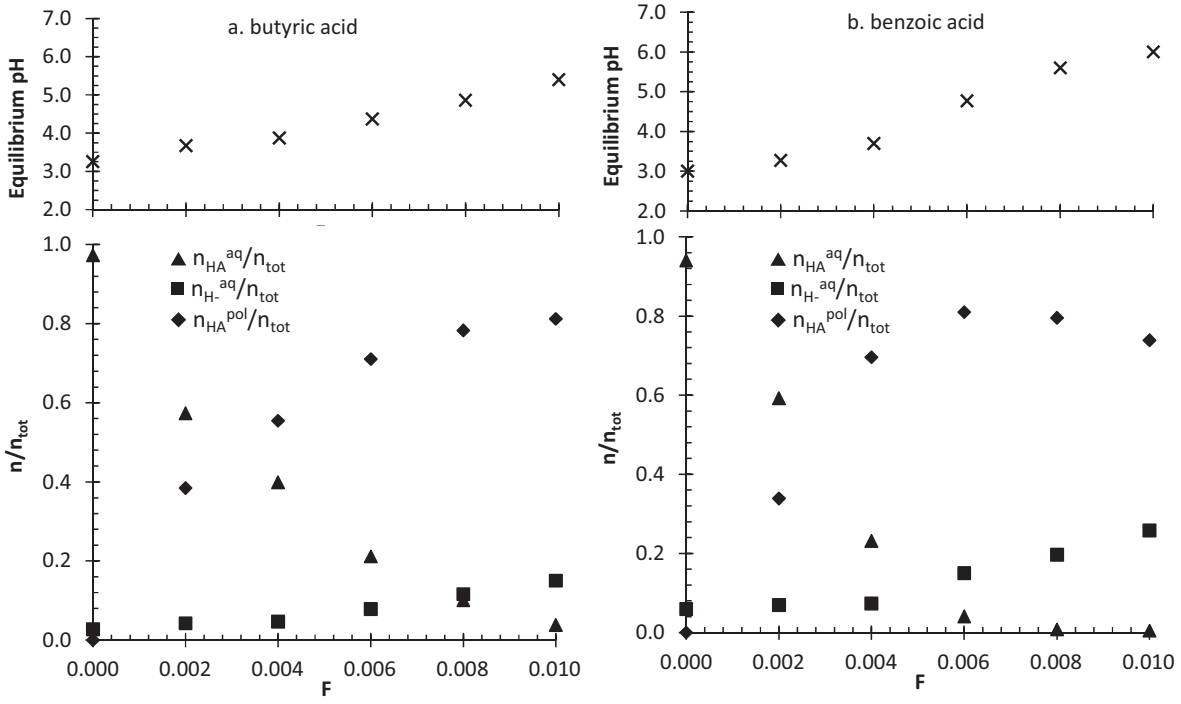

Fig. 7. Equilibrium pH and acid-carboxylate partitioning as a function of amine hydrogel fraction (a. butyric acid; b. benzoic acid; 30 °C; $[acid]_{initial} = 2.5$ g/L).

[37], cleaving 2-(N,N-dimethylamino)ethanol from the polymer backbone. This releases organic base into aqueous solution where it neutralizes organic acid without sequestering it within the hydrogel. The timescale of this hydrolysis is on the order of hours under the conditions of use [33], making it unsuitable for TPPB applications. As described above, Asci and Hasdemer prepared a polyacrylamide hydrogel that is more robust to hydrolysis than that used in this work [21], but the low basicity of acrylamides resulted in far inferior performance to the tertiary amine functionality of present interest. We are preparing a second generation of thermoset hydrogels to overcome this instability, while retaining the basicity that makes this class of materials so promising for organic acid bioprocesses. Furthermore, this future work will also focus on describing the reaction kinetics and mechanisms for these reactive hydrogels.

4. Conclusion

Experimental data and material balances describing organic acid partitioning have confirmed that TPPBs operating at near neutral pH suffer performance limitations due to acid dissociation. Indeed, the pH buffering effect provided by acid absorption into the polymer phase can establish an upper limit to solute removal, such that further increases in phase fraction have little effect. These limitations can be overcome to some degree by reversible acidification using high pressure CO_2, which facilitates conjugate acid uptake without increasing the ionic strength of the fermentation medium. However, reactive extraction by thermoset materials containing organic base functionality is more effective, since high acid recoveries can be achieved at moderate pH using relatively small amounts of polymer, although more selective extraction may be achievable with non-reactive absorbents.

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Appendix A. Supplementary data

Supplementary data associated with this article can be found, in the online version, at http://dx.doi.org/10.1016/j.cej.2015.11.068.

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