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Using poly(vinyldodecylimidazolium bromide) for the *in-situ* product recovery of *n*-butanol

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The mitigation of end-product inhibition during the biosynthesis of *n*-butanol is demonstrated for an *in-situ* product recovery (ISPR) system employing a poly(ionic liquid) (PIL) absorbent. The thermodynamic affinity of poly(vinyldodecylimidazolium bromide) [P(VC_{12}ImBr)] for *n*-butanol, acetone and ethanol versus water was measured at conditions experienced in a typical acetone-ethanol-butanol (ABE) fermentation. In addition to providing a high *n*-butanol partition coefficient (PC=6.5) and selectivity ($\alpha_{BuOH/water}$=46), P(VC_{12}ImBr) is shown to be biocompatible with *Saccharomyces cerevisiae* and *Clostridium acetobutylicum*. Furthermore, the diffusivity of *n*-butanol in a hydrated PIL provides absorption rates that support ISPR applications. Using a 5 wt% PIL phase fraction relative to the aqueous phase mass, P(VC_{12}ImBr) improved the volumetric productivity of a batch ABE ISPR process by 31% relative to a control fermentation. The concentration of *n*-butanol in the P(VC_{12}ImBr) phase was sufficient to increase the alcohol concentration from 1.5 wt% in the fermentation medium to 25 wt% in the saturated PIL, thereby facilitating downstream *n*-butanol recovery.

Keywords: *n*-butanol, ABE fermentation, *Clostridium acetobutylicum*, *in-situ* product recovery, polyionic liquid

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Introduction

Continued interest in sustainable transportation fuel alternatives[1-3] has led to widespread consideration of biologically-produced butanol, whose advantages over ethanol include a higher energy content, reduced hygroscopicity, and greater miscibility with gasoline and diesel fuels.[1,3] Fermentations employing *Clostridium* microorganisms support well-established acetone-butanol-ethanol (ABE) processes, typically yielding these products in a 3:6:1 ratio.[3-5] Genetically-modified *Saccharomyces cerevisiae* has also been utilized to produce *n*-butanol and isobutanol in greater yields.[6-9]

Irrespective of the microorganism employed, the cytotoxicity of butanol establishes an upper limit on fermentation titres, thereby limiting bioreactor productivity[4] and making downstream product recovery more onerous. Note that conventional *n*-butanol / water separations are further complicated by a heterogeneous azeotrope, necessitating the use of two distillation columns to isolate the alcohol in high purity.[10-11] These issues can be mitigated by *in-situ* product removal (ISPR), wherein inhibitory products are removed during the fermentation process. [3,12-13] Two-phase partitioning bioreactor (TPPB) technology involving the use of a water-immiscible phase to extract cytotoxic products are of particular interest.[14-16]

An ideal extractant for a TPPB system is biocompatible with the microorganism and provides a combination of high target solute affinity, as measured by the partition coefficient (PC), along with a low affinity for water, as quantified by the solute selectivity ($\alpha = PC_{butanol}/PC_{water}$).[15] Although low molecular weight organic solvents are widely available, their suitability for TPPB applications is often limited by cytotoxicity and bioavailability concerns, as well as issues such as foaming and emulsification.[15,17-19] Crosslinked microporous resins are an alternative sequestering phase, in which *n*-butanol is adsorbed onto the polymer surface.[20-21] However, the high surface areas typical for these resins can result in cell adhesion, ultimately leading to biofilm formation.[20,22-23]

Adsorptive materials have also been used to sequester *n*-butanol for improvement in its bioproduction, however as their uptake mechanism is different from the absorptive one used in this study, they do not allow direct comparison.[24-28] In addition, many other materials and methods have

been used to improve *n*-butanol recovery processes,[29] including the use of membranes,[30-32] a technique which was not investigated in this work.

The low vapor pressure of ionic liquids has made them attractive materials for liquid-liquid extraction processes, and they have been examined in the context of *n*-butanol ISPR.[33] However, biocompatibility remains an ongoing concern, as many ionic liquids are toxic to microorganisms,[33-35] and extremely high viscosities make handling of ionic liquids problematic. Recent work has focused on polyionic liquids (PILs) that can provide favourable solute affinities along with desirable solid-state properties.[36] Unlike their low molecular weight analogues, PILs can be rendered non-bioavailable by tailoring their composition and chain architecture, yielding biocompatible TPPB absorbents with a unique combination of physical and chemical properties. Poly(vinyldodecylimidazolium bromide) [P(VC_{12}ImBr)] is a PIL of interest as it has been shown to have a high affinity for *n*-butanol and other fermentation products as compared to other imidazolium-based PILs tested.[36]

In this work, the utility of P(VC_{12}ImBr) for *n*-butanol production in a TPPB was examined from multiple perspectives. This includes careful determinations of PC and selectivity values over a range of compositions for the ternary PIL/BuOH/water system, analysis of the biocompatibility of P(VC_{12}ImBr) with *Clostridium acetobutylicum* and *Saccharomyces cerevisiae*, and assessments of *n*-butanol diffusivity within a PIL matrix. These studies culminate with ABE fermentation experiments that assess the performance of P(VC_{12}ImBr) as an ISPR extractant in terms of bioreactor volumetric productivity. The work concludes with a brief analysis of the recovery of volatile components from saturated P(VC_{12}ImBr), which demonstrate the absorbent's ability to concentrate *n*-butanol from dilute fermentation titres.

Materials and Methods

Chemicals

All chemical reagents were at the highest purity grade available and used as received from Fisher Scientific or Sigma-Aldrich. Type I ultrapure water (18.2 MΩ cm at 25°C) or HPLC grade water obtained from Fisher Scientific were used throughout all experiments and analytical procedures.

Synthesis of P(VC_{12}ImBr)

1-Vinyl-3-dodecylimidazolium bromide ([VC_{12}Im][Br]) was prepared, characterized and polymerized as previously described.[36] 1-Vinylimidazole (5 g) and 1-bromododecane (16.2 g, 1.1 molar equiv.) were dissolved in ethyl acetate (12.5 mL), then purged with nitrogen and refluxed for 24 h. The solution was then cooled to 0°C to promote crystallization of the IL monomer. The solids were recovered by filtration, washed with ethyl acetate and dried *in vacuo*.

Solution polymerization to give P(VC_{12}ImBr) was done by dissolving [VC_{12}Im][Br] (5 g) in a 9:1 toluene:ethanol solution (25 mL) before adding 2,2'-azobis(2-methylpropionitrile) (AIBN, 0.03 mol AIBN/mol monomer). The resulting mixture was heated to 90°C under nitrogen for 5 h under constant stirring. The reaction product was cooled to room temperature and the PIL recovered by precipitation from acetone (0.1 mL polymer/1 mL acetone). The solvent phase was decanted and the solid washed in fresh acetone for 24 h before drying under vacuum. The polymer, P(VC_{12}ImBr), was characterized by ^{1}H-NMR as described previously.[36]

Forming of dry P(VC_{12}ImBr) into consistent pieces was accomplished by grounding the material into a fine powder and immersing it in a 20% (v/v) ethanol /water solution using a 1 g PIL per 10 mL solvent ratio. The resulting plasticized material was consolidated using a hydraulic press into a 2 mm thick sheet, then cut into 9 mm by 6 mm pieces that were dried at 60°C to constant weight.

Microorganisms & medium formulations

Saccharomyces cerevisiae was obtained from Alltech (Nicholasville, Kentucky) and cultivated in a previously described medium,[37] that was modified to 10 g/L glucose. *Clostridium acetobutylicum* ATCC 824 was grown in medium prepared according to Barton & Daugulis,[17] that was sparged with nitrogen to ensure anaerobic conditions. All media were autoclaved separately prior to inoculation.

Partition coefficient (PC) and selectivity

The PC and selectivity values for P(VC_{12}ImBr) acting upon aqueous solutions were determined as previously described.[36] P(VC_{12}ImBr) samples were soaked in water for 24 hours prior to testing. Aqueous solutions (10 mL) were prepared for *n*-butanol (1 wt%), ethanol (1 wt%), or acetone (0.5 wt%),

and were added to scintillation vials with 5% (w/v) P(VC_{12}ImBr). The vials were sealed with a foil-lined cap and incubated at 30°C and 180 rpm for 24 hours. The resulting saturated samples of P(VC_{12}ImBr) were lightly patted dry with a paper towel and weighed before drying at 60°C to constant weight.

Mass balances were used to determine water and solute uptake by P(VC_{12}ImBr), using the mass of the water-soaked polymer, the mass of the polymer at equilibrium with the aqueous solution, the mass of the dry polymer, and the solute concentrations of the aqueous sample before and after equilibrium saturation. These calculations provided the aqueous and polymer weight fractions (w_i^{aq} and w_i^p), from which an average partition coefficient for each solute (PC_i) was determined.

$$PC_i = \frac{w_i^e}{w_i^{aq}} \quad \textbf{(1)}$$

The solute/water selectivity of P(VC_{12}ImBr) followed.

$$\alpha_{i/w} = \frac{PC_i}{PC_w} \quad \textbf{(2)}$$

Triplicate experimental trials provided the data needed to estimate the standard deviations for all PC and selectivity measurements.

Biocompatibility

The biocompatibility of P(VC_{12}ImBr) was determined as previously described.[33] For *S. cerevisiae*, growth medium (50 mL) was added to 125 mL Erlenmeyer flasks equipped with foam stoppers. P(VC_{12}ImBr) was sterilized separately from the medium and 5% (w/v) polymer was added aseptically to three flasks. Another three flasks contained no polymer. Each was inoculated with 2 mL of *S. cerevisiae* -80°C glycerol stock culture and incubated at 30°C and 180 rpm for 24 hours.

For *C. acetobutylicum* biocompatibility, P(VC_{12}ImBr) was added each to three 125 mL serum bottles, then sparged with N_2 to ensure anaerobic conditions and sterilized. *C. acetobutylicum* growth medium (50 mL) was prepared anaerobically and added to six 125 mL serum bottles, three of which contained the samples of P(VC_{12}ImBr) at 5% (w/v). Inoculation was by the addition of -80°C glycerol stock culture of *C. acetobutylicum*, followed by incubation at 37°C and 180 rpm for 24 hours.

Following 24 hours of incubation, biocompatibility was determined by comparing biomass growth and residual glucose levels of the samples with P(VC_{12}ImBr) to polymer-free controls.

Rate of Uptake and *n*-Butanol Diffusivity

The rate of *n*-butanol uptake by P(VC_{12}ImBr) was determined using a 1 L working volume reactor (Bioflo I, New Brunswick Scientific, USA), fitted with a six-blade Rushton impeller operating at 300 rpm and 22.5°C. At time zero, 5% (w/v) P(VC_{12}ImBr) was added to 1 L of an aqueous 2 wt% *n*-butanol solution. Aqueous samples were taken every 5 min until the 30-minute mark, after which the sampling frequency was decreased as the system approached equilibrium over 8 hours. GC analysis provided the loss of *n*-butanol from the aqueous phase to the PIL as a function of time.

These time course data, along with the average radius of the polymer pieces (0.27 cm), were used to estimate the solute diffusivity in the PIL using the Crank Equation.[38] A least squares regression analysis fit the model to the experimental data, as previously described.[39-40]

ABE fermentation

C. acetobutylicum was grown at 37°C and 180 rpm in airtight serum bottles, inoculated with of 2 mL of -80°C glycerol stock culture. After 24 hours, 10% (v/v) of the culture was propagated into six serum bottles containing fresh medium and grown for an additional 12 hours to reach the exponential growth phase for inoculation. The control ABE fermentation was conducted in a 5 L New Brunswick Scientific BioFlo III Bioreactor with a working volume of 3 L medium, which was autoclaved prior to inoculation. After sterilization of the bioreactor and medium, filtered N_2 gas was sparged through the reactor prior to inoculation to create an oxygen-free environment until anaerobic cell growth was observed.[21] The fermentation was initiated by the anaerobic addition of 10% (v/v) inoculum to the bioreactor, which was set at 37°C and agitated continuously at 150 rpm for 50 hours with pH monitored between 4 and 5.

The ISPR ABE fermentation was carried out under the same conditions as the control fermentation, up until the 18-hour mark, prior to anticipated inhibitory *n*-butanol concentrations, at which point the bioreactor contents were circulated through a column packed with P(VC_{12}ImBr) via a peristaltic pump. The flow of fermentation broth through the 500 mL glass column was at 50 mL/min, which contained approximately 150 g (dry weight) or 5% (w/v) of P(VC_{12}ImBr) polymer and circulated continuously back to the reactor upon conclusion of the fermentation.

For both the control and the ISPR fermentations, aqueous samples were taken in conjunction with expected ABE fermentation kinetics to determine cell growth, solvent and acid production, and glucose consumption. Immediate cell density measurements were followed by centrifuging the remaining sample in capped Eppendorf tubes at 10,000 rpm for 10 minutes at 10°C. The supernatant was then decanted, filtered through a sterile 0.2 μm syringe filter, and diluted accordingly for product and substrate concentration analysis.

Water + *n*-butanol + P(VC_{12}ImBr) system

To analyze the ternary water / *n*-butanol / P(VC_{12}ImBr) system over a range of *n*-butanol concentrations, P(VC_{12}ImBr) was first soaked in water for 24 hours. Once plasticized and patted dried with a paper towel, a 5% (w/v) fraction of P(VC_{12}ImBr) was added to multiple scintillation vials along with 10 mL of an aqueous *n*-butanol solution. The samples were then incubated for 24 hours at 37°C and 180 rpm to reach equilibrium, then a mass balance of each sample was performed using the mass of the equilibrated polymer pieces and the aqueous solution that was filtered for *n*-butanol detection.

Recovery of *n*-butanol from P(VC_{12}ImBr)

P(VC_{12}ImBr) was plasticized in water, then 30 g was added to each of 100 mL of an aqueous 2 wt% *n*-butanol solution in triplicate 250 mL Erlenmeyer flasks. The samples were then incubated for 48 hours at 37°C and 180 rpm to equilibrate. An aqueous sample was then taken from each flask to determine *n*-butanol content, and the polymer was removed from the flask and weighed, then placed in a container for thermal desorption. A mass balance was then performed to determine the amount of *n*-butanol and water taken up by the polymer.

To recover *n*-butanol from P(VC_{12}ImBr), the thermal desorption setup consisted of two 125 mL airtight straight-sided round jars that were connected by tubing through each lid. One vessel contained the equilibrated polymer from a single flask sample and was placed in a retrofitted microwave oven (Kenmore, USA), with the tubing connection leading out through the side of the microwave to the other container, which was submerged in ice chips. The microwave was run for 25 minutes on 50% power, to volatilize the liquid from P(VC_{12}ImBr), and the condensed aqueous *n*-butanol solution was collected in the chilled container, from which a sample was taken to determine the amount of *n*-butanol that was recovered from the polymer.

Analytical Methods

Aqueous samples were taken in triplicate for each experiment. *n*-Butanol, ethanol, and acetone concentrations were analyzed by gas chromatography using a Varian 450-GC gas chromatograph that was equipped with a CP-8410 AutoInjector, Restek RTX 502.2 capillary column, and an FID detector. Acetic acid and butyric acid concentrations were determined by HPLC with a Varian Prostar instrument equipped with a UV-Vis detector (PS 325, Varian Prostar) operating at 220 nm, using a Varian Hi-Plex H column (300 x 7.7 mm) at 60°C and a 9 mM H_2SO_4 mobile phase flowing at 0.4 mL/min. Glucose concentrations were determined through HPLC (Varian Prostar) with a refractive index detector (PS 356, Varian Prostar), using a Varian Hi-Plex H column (300 x 7.7 mm) at 60°C with a 9 mM H_2SO_4 mobile phase at 0.4 mL/min.

Cell growth was measured from triplicate samples by optical density measurements at 600 nm (OD_{600}) using the output of a Biochrom Ultrospec 3000 UV/Visible Spectrophotometer that was correlated with an established cell dry weight (g/L) calibration.

Results and Discussion

Thermodynamic affinity toward ABE fermentation products

The absorptive capacity of a TPPB material toward a given target molecule is quantified by its solute partition coefficient, $PC_i = w_i^p / w_i^{aq}$. The higher the PC provided by an absorbent, the less material is required to keep a solute below a specified cytotoxic concentration. The solute/water

selectivity, $\alpha_{i/w} = PC_i/PC_w$, quantifies the relative uptake of solute versus water, which impacts downstream product recovery significantly. The data listed in Table 1 for P(VC_{12}ImBr) absorption of *n*-butanol are consistent with those reported by Bacon et al.,[36] with PC = 6.5 and α = 46. This *n*-butanol partition coefficient is among the highest recorded for a biocompatible extractant, outperforming oleyl alcohol (PC = 3.6) by a significant margin.[17] Furthermore, PCs for P(VC_{12}ImBr) absorption of ethanol and acetone also surpass those reported for oleyl alcohol,[41] which are target by-products of an ABE fermentation.

The performance benefits associated with the higher absorptive capacities provided by P(VC_{12}ImBr) are offset somewhat by poorer selectivities. Although the PIL demonstrates a strong preference for *n*-butanol over water, the oleyl alcohol system provides selectivities that are several times higher than those of the polymer (Table 1). By taking up more water, the recovery of *n*-butanol from a saturated P(VC_{12}ImBr) phase may require more energy than a comparable oleyl alcohol-based process. Therefore, deciding between a PIL and conventional organic solvent will depend on the relative importance of absorbent capacity and selectivity, as well as the ease of absorbent handling in a TPPB environment.

Biocompatibility

If P(VC_{12}ImBr) is to be an effective ISPR absorbent, it cannot inhibit the growth of the biocatalyst, either through cytotoxicity or by the removal of essential nutrients from the fermentation medium.[22] Measurements of cell density[33,42] and residual glucose concentrations[43] for *S. cerevisiae* and *C. acetobutylicum* cultures that were incubated in the presence of P(VC_{12}ImBr) were compared to polymer-free controls. The data plotted in Figure 1 show that P(VC_{12}ImBr) did not inhibit microbial growth for either *S. cerevisiae* or *C. acetobutylicum*. As such, the material is biocompatible and appropriate for use in a biphasic system that involves absorbent contact with these organisms.

Solute Diffusivity and Uptake Rate

The diffusivity of *n*-butanol within P(VC_{12}ImBr) dictates whether the PIL can sequester *n*-butanol fast enough to support an ISPR-assisted ABE fermentation. Figure 2 provides a plot of *n*-butanol uptake by water-swollen P(VC_{12}ImBr) as a function of time, along with a fit of the Crank equation to the experimental data. After an initial 2-hour period of rapid *n*-butanol uptake, the system approached an equilibrium state by the 7-hour mark. Note that exponential growth in an ABE batch fermentation by *C. acetobutylicum* occurs over 10 hours to produce most of the *n*-butanol titer.[17] By comparison, nearly 90% of the *n*-butanol absorbed by P(VC_{12}ImBr) occurs within just 3 hours.

The diffusivity (D) of *n*-butanol in water-swollen P(VC_{12}ImBr) can be estimated by least square regression of the Crank Equation to the mass fraction uptake data plotted in Figure 2.[38] The P(VC_{12}ImBr) pieces used in the uptake experiment were modelled as equivalent spheres to facilitate fitting to the Crank equation in spherical coordinates,[38,40] yielding a diffusivity value of 6.5×10^{-8} cm^2/s. Note that this value is significantly below that of polydimethylsiloxane (PDMS), whose *n*-butanol diffusivity is on the order of 3.0×10^{-7} cm^2/s.[44] The transport properties of PDMS are exceptional, due in part to an exceedingly low glass transition temperature. However, the lack of polar / hydrogen-bonding functionality within PDMS results in a low solubility parameter and rather poor thermodynamic affinity for *n*-butanol, with a reported partition coefficient less than 1.0.[44]

Raising a polymer's solubility parameter toward that of *n*-butanol requires the introduction of associating functional groups that will inevitably raise the glass transition temperature. With imidazolium bromide functionality in every mer, P(VC_{12}ImBr) has a T_g in excess of 180 °C, and is absorptive only when plasticized by water and/or *n*-butanol, which confers enough molecular mobility to the polymer to support solute transport. The observed loss in *n*-butanol diffusivity is offset by a substantial gain in *n*-butanol partition coefficient. Using the methodology previously described,[40] in which the rates of sorption and biological reaction were compared as a function of sorbent size and diffusivity, it was estimated that for a particle size characteristic dimension of 0.27 cm and diffusivity of 6.5×10^{-8} cm^2/s, the rate of *n*-butanol uptake would be 30 mg/Lh. This rate compares favourably to the highest biological production rate without mass transfer limitation of 0.3 mg/Lh,[17] allowing the PIL to

maintain a pseudo-equilibrium concentration in a TPPB that is much lower than that encountered in a conventional bioreactor.

ABE fermentation

Although the ternary phase equilibrium data and biocompatibility studies presented to this point suggest that P(VC_{12}ImBr) can be an effective TPPB absorbent, the fermentation data plotted in Figure 3 demonstrate the performance gains that can be realized by using the material in an ISPR system. Figure 3a is a summary of a control experiment involving a conventional batch fermentation, while Figure 3b provides analogous data for an ISPR fermentation wherein product removal was initiated at the 18-hour mark by continuously passing the aqueous broth through a packed column of P(VC_{12}ImBr) and back into the bioreactor in a closed loop. Note that the fermentation broth pH was monitored at each sampling time to ensure that it was between 4 and 5, as this range is optimal for solvent production.[45]

Only acetic acid and butyric acid were produced during the first 10 hours of both fermentations (Figures 3a,b), at which point the system began converting these acids to acetone, *n*-butanol and ethanol. The progress of the control system slowed demonstrably after 34 hours, and after 38 hours, approximately 5 g/L glucose remained for the duration of the experiment. This residual glucose, along with stationary *n*-butanol, acetone, and ethanol concentrations, are consistent with end-product inhibition that limited the *n*-butanol yield to 37 g in the 3.7 L aqueous phase volume.

The enhancement provided by P(VC_{12}ImBr)-based ISPR is evidenced in multiple ways, including the consumption of glucose. In the ISPR fermentation, glucose was consumed at a rate similar to that of the control up until the 18-hour mark, at which point P(VC_{12}ImBr) was introduced. At the 22 hour mark, the ISPR glucose consumption rate reached its maximum of 4.6 g/L·h, which was 84% higher than that recorded for the control fermentation at this point, and 61% greater than the control's maximum glucose consumption rate observed at 26 hours. More importantly, the ISPR system reduced glucose concentration to near the analytical detection limit, thereby overcoming the end-product inhibition that plagued the control fermentation.

Evidence of improved process performance is also seen in the solvent production data. Whereas *n*-butanol, acetone, and ethanol production began to plateau after 26 hours in the control experiment (Figure 3a), aqueous phase concentrations in the ISPR system continued to increase up to the 36 hour mark (Figure 3b), with *n*-butanol reaching a peak of 10.7 g/L before declining to a stationary concentration of 10 g/L. The observed decreases in *n*-butanol and ethanol titers after 36 hours is attributed to continued absorption of ABE into the polymer at a rate that exceeded production by the biocatalyst.

After 50 hours, aqueous solvent concentrations in the ISPR fermentation medium were nearly identical to those in the control. However, this does not account for solute absorption by the polymer phase. At equilibrium, the fraction of the total solute residing in the polymer is given by $\mathrm{m}_{\mathrm{solute}}^{\mathrm{polymer}}/\mathrm{m}_{\mathrm{solute}}^{\mathrm{total}} = \mathrm{F}\cdot\mathrm{PC}_{\mathrm{solute}}/(1+\mathrm{F}\cdot\mathrm{PC}_{\mathrm{solute}})$, where F is the absorbent phase fraction and PC_{solute} is the solute partition coefficient. The ISPR fermentation used F = 0.05 g·polymer/g·aqueous phase. Based on the binary PC values for *n*-butanol/water (6.5), ethanol/water (1.1) and acetone/water (1.3) reported above, saturated P(VC_{12}ImBr) could contain 24% of the *n*-butanol, 5% of the ethanol, and 6% of the acetone residing in the ISPR system. However, this calculation assumes that binary PC values adequately describe phase partitioning of a quaternary system (*n*-butanol/acetone/ethanol/water). Independent testing of the equilibrium state of the saturated ISPR system revealed significant deviations from binary predictions, with the PIL containing 22% of the *n*-butanol, 11% of the ethanol and 2% of the acetone in the two-phase mixture. As such, the total amount of *n*-butanol produced from 3.2 L of ISPR fermentation medium reached 41 g, compared to just 32 g in the control experiment.

Further performance data for the control and ISPR fermentations are provided in Table 2. In addition to providing larger amounts of all three solvent products, the ISPR fermentation improved the volumetric productivity of the bioreactor by 31%. Note that these significant improvements were gained using a relatively small phase fraction of F=0.05, and further enhancements can be expected if more PIL absorbent is charged to the system, or the PIL bed is replaced periodically before reaching a saturated equilibrium condition.

n-Butanol Recovery

In addition to improving the volumetric productivity of a bioreactor by mitigating product inhibition, ISPR technology has the potential to enhance downstream recovery by concentrating the product within the absorbent phase. Conventional recovery of *n*-butanol from dilute fermentation media requires two distillation columns with an intermediate decanter to overcome complications derived from a heterogeneous azeotrope in the *n*-butanol-water system.[10,48] The partial liquid miscibility associated with this azeotrope presents an opportunity for an ISPR system to eliminate the first distillation column. If the *n*-butanol-water composition within a saturated polymer is greater than 7.7 wt% *n*-butanol, then a liquid mixture isolated from the absorbent will split into aqueous and organic phases with compositions of 7.7 wt% and 79.9 wt%, respectively, with the proportion of the two phases depending on the overall system composition.

As expected, an analysis of polymer saturated with the ISPR aqueous phase showed that it contained a substantial amount of water in addition to ABE. The volatiles within the PIL phase were comprised of 75 wt% water, 23 wt% *n*-butanol, 1 wt% acetone, and 1 wt% ethanol. This is well within the liquid-liquid equilibrium region of the *n*-butanol/water phase diagram, and if the trace organics are ignored, the aforementioned aqueous and organic phases would be present in a 21:79 wt:wt ratio, thereby obviating the need for two distillation columns to obtain high purity *n*-butanol. This selectivity for *n*-butanol versus water compares favourably with other reported ISPR systems, as demonstrated by the data listed in Table 3.

The recovery of *n*-butanol from P(VC_{12}ImBr) by thermal desorption was accomplished by heating saturated PIL and condensing the released volatile components. The PIL was saturated with an aqueous solution containing 1.5 wt% *n*-butanol before heating in a microwave oven to release water and alcohol. The isolated condensate separated into two distinct phases, indicating that the recovered composition was above the *n*-butanol + water solubility limit. Through measurement, the *n*-butanol-water mixture that was thermally removed from P(VC_{12}ImBr) consisted of 25.0 wt% *n*-butanol overall that partitioned between an organic phase (33 vol%) and an aqueous phase (66 vol%). Removal of *n*-butanol and water from P(VC_{12}ImBr) then allows for reuse of the absorptive material in subsequent ISPR processes.

Conclusion

Poly(vinyldodecylimidazolium bromide) [P(VC_{12}ImBr)] is a biocompatible absorbent that is well-suited for ISPR variations of ABE fermentation, providing favourable *n*-butanol affinity (PC=6.5) and selectivity ($\alpha_{BuOH/water}$ = 46). The diffusivity of *n*-butanol in water-saturated PIL was 6.51 x 10^{-8} cm^2/s, which is sufficient to ensure that alcohol absorption is competitive with bioproduction rates. The use of P(VC_{12}ImBr) in ABE ISPR fermentation at a 5 % phase fraction improved volumetric productivity by 31% relative to a control, while concentrating *n*-butanol in the PIL phase to beyond its aqueous solubility limit. This suggests that the ISPR process would require a single distillation column to recover high purity *n*-butanol, in contrast to the conventional distillation of a dilute fermentation broth.

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Table 1. Comparison of Absorption Performance Parameters for ABE Fermentation Products

Molecule	PC	α
P(VC_{12}ImBr)		
n-Butanol	6.5 ± 0.4[a]	46 ± 4[a]
Ethanol	1.1 ± 0.4[a]	7.2 ± 2[a]
Acetone	1.3 ± 0.3[a]	8.5 ± 2[a]
Oleyl Alcohol		
n-Butanol	3.6[17]	180[10]
Ethanol	0.22[41]	25[41]
Acetone	0.07[41]	-

Experimental error shows standard deviation from triplicate samples (n=3).
[a]This work

Table 2. Summary of Control and ISPR ABE Batch Fermentations by *C. acetobutylicum* ATCC 824

Parameter	ABE Fermentation	
	Control	ISPR
Aqueous phase volume (L)	3.2	3.2
PIL phase mass (g)	-	150
Glucose consumed (g)	172	234
Total ABE produced (g)	53.7	66.2
Acetone in aqueous phase (g)	18.9	20.6
Acetone in PIL (g)	-	0.5
BuOH in aqueous phase (g)	31.7	32.0
BuOH in PIL (g)	-	9.2
EtOH in aqueous phase (g)	3.1	3.5
EtOH in PIL (g)	-	0.4
Total ABE yield (g/g)	0.31	0.28
Total BuOH yield (g/g)	0.18	0.17
Volumetric ABE productivity (g/L·h)[a]	0.49	0.61
Volumetric BuOH productivity (g/L·h)[a]	0.29	0.38

a. Based on a total fermentation time of 34 hours

Table 3. Comparison of ISPR Methods for Selective *n*-Butanol Removal from ABE Fermentation Media

ISPR Extractant	*n*-Butanol in Fermentation Broth (wt%)	*n*-Butanol Content Extracted (wt%)	Reference
P(VC_{12}ImBr)	1.0	23	This work
Norit ROW0.8	1.87	25-31	46
Dowex® Optipore SD-2	0.34	15	20
Gas stripping	0.8-1.3	15	47
Vapor stripping- vapor permeation	1.0	21	30

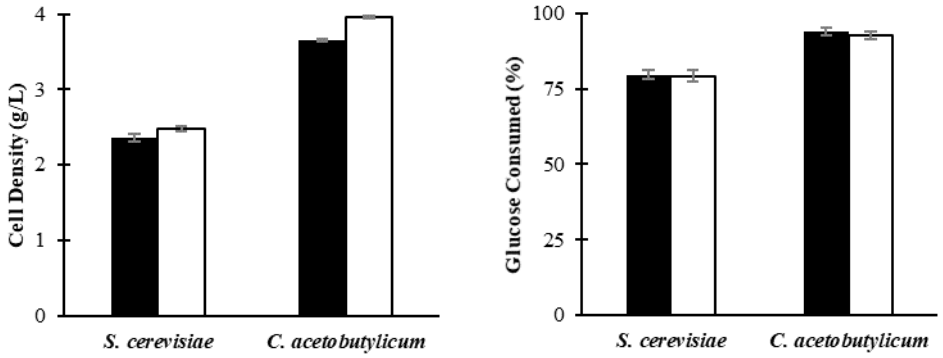

Figure 1. Cell density and glucose consumed after 24 hours of *S. cerevisiae* and *C. acetobutylicum* growth with (□) and without (■) the addition of 5 wt% P(VC_{12}ImBr). Error bars show standard deviation from triplicate samples (n=3).

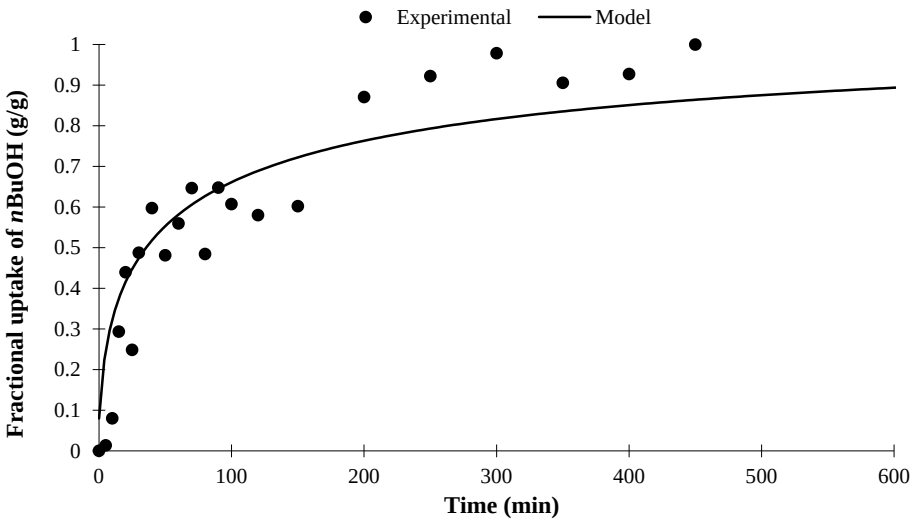

Figure 2. Experimental and model time course of the uptake of *n*-butanol by 5% (w/v) P(VC_{12}ImBr) in 1 L of a 2 wt% concentration *n*-butanol solution.

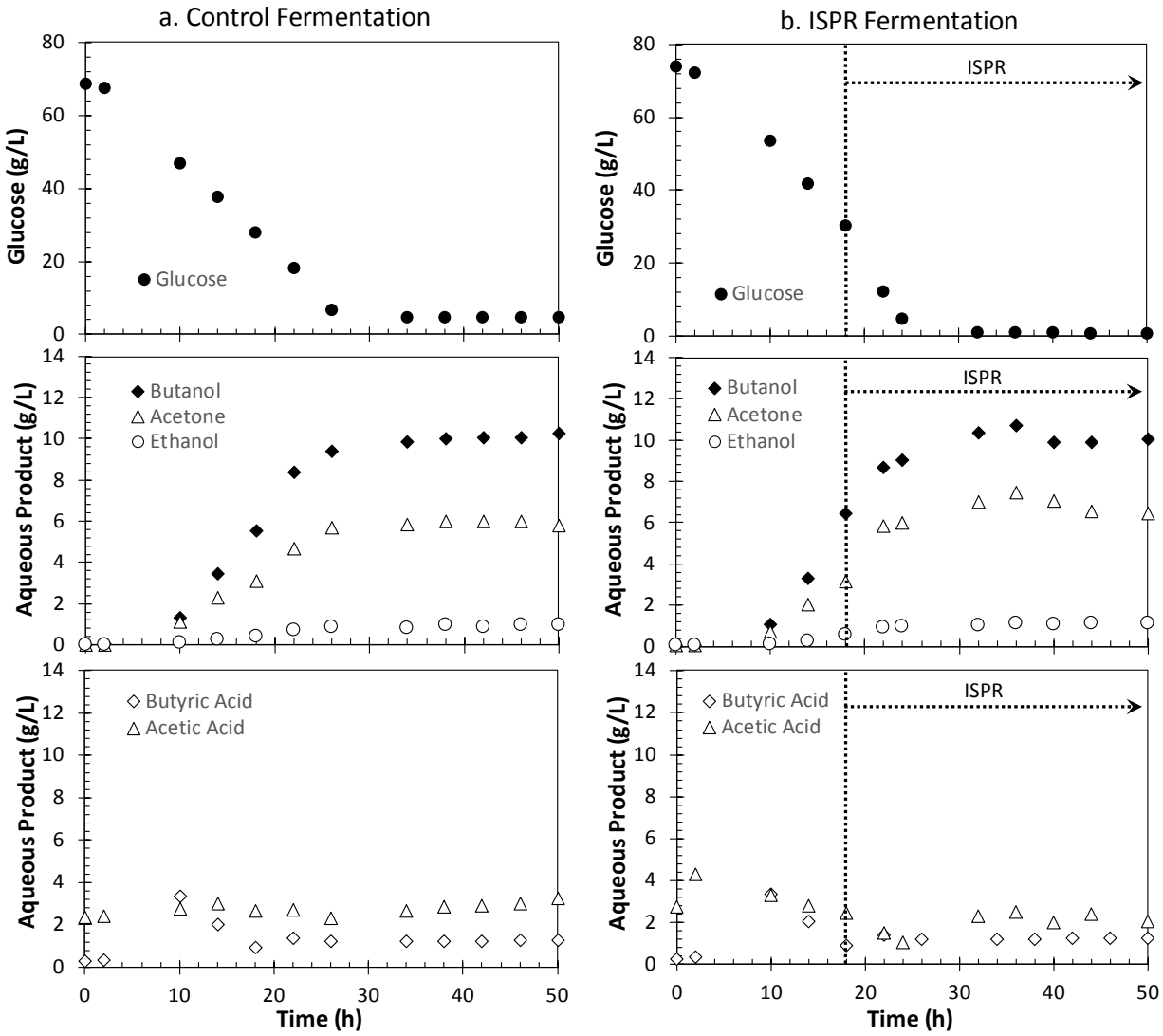

Figure 3. Aqueous phase concentration of batch fermentation components for *C. acetobutylicum* catalyzed ABE fermentation (a) control experiment; (b) ISPR experiment where contact of the fermentation broth with 5% (w/v) P(VC_{12}ImBr) was initiated at 18 hours.