ASIA-PACIFIC JOURNAL OF CHEMICAL ENGINEERING
Asia-Pac. J. Chem. Eng. 2012; **7** (Suppl. 3): S324–S328
Published online in Wiley Online Library
(wileyonlinelibrary.com) DOI: 10.1002/apj.1651

Supplement article

Two-phase partitioning bioreactors: the use of polymers for the *in situ* removal of ethanol

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ABSTRACT: The use of polymers for *in situ* removal of ethanol, an inhibitory fermentation product, was investigated. Results from capacity experiments with six amorphous polymers showed that nylon 6,6 had the greatest capacity for ethanol, 0.123 g ethanol/g polymer. The Flory–Huggins solubility parameter for this polymer most closely matched that of ethanol and provided an initial indication that rational polymer selection based on the Flory–Huggins theory, which describes polymer–solute interactions, can be used to predict potential absorptive polymers for the uptake of target molecules. A hard, crystalline polymeric resin with surface-adsorptive properties, DOWEX Optipore L493, was found to have a higher ethanol capacity, 0.346 g ethanol/g polymer. Batch fermentations of a glucose medium using *Saccharomyces cerevisiae* were conducted in both the presence and absence of the polymeric adsorbent, DOWEX Optipore L493. Although the addition of the resin had a positive effect on reducing the aqueous ethanol concentration via *in situ* ethanol removal, its presence in the medium, perhaps due to abrasive effects on the cells, diminished this positive performance. © 2012 Curtin University of Technology and John Wiley & Sons, Ltd.

KEYWORDS: ethanol; fermentation; polymers; inhibition; partitioning bioreactor

INTRODUCTION

Two-phase partitioning bioreactors (TPPBs) consist of a cell-containing aqueous phase and a second immiscible phase that is used to partition toxic molecules either to the cells (in the case of toxic substrates) or away from the cells (in the case of inhibitory products), on the basis of equilibrium considerations.[1] To date, inhibitory products (and in particular, ethanol [2]) have been removed in TPPBs by immiscible organic solvents that traditionally needed to possess a variety of important properties including biocompatibility, nonbioavailability, low volatility, and low cost.[3] Replacement of the organic solvent phase by amorphous (absorptive) polymer beads to remove inhibitory fermentation end products has recently been demonstrated.[4–6]

Amorphous ('soft') plastics, which can be considered to be large molecular weight organic solvents, are capable of absorbing small molecular weight compounds by diffusion into the molecular structure of the polymers. Such polymers are inexpensive, can be formed into many shapes and sizes and, most importantly, are nonbiodegradable, and therefore can be readily used with virtually any type of cell in a bioreactor, including mixed culture systems. Because the polymers are solid, they provide several advantages relative to the use of immiscible organic solvents in TPPBs, including ease of handling, complete recovery from the bioreactor, and no potential for fouling of reactor materials such as rubber gaskets and seals.[7,8] Polymer beads can also be readily reused through multiple fermentation cycles with virtually no compromise in their performance. Limitations of solid polymer beads relative to organic solvents include lower diffusivity of the target molecule(s).

Absorptive polymer–solute behavior follows a strong negative deviation from Raoult's ideal solution law. To capture the nonideality of the polymer solutions, the Flory–Huggins theory was developed,[9] although this theory has yet to be confirmed for polar systems such as those involving ethanol. On the basis of the Flory–Huggins theory, a polymer with a solubility parameter close to that of ethanol should be effective in its absorption. Given that the hydrogen bonding interactions in ethanol contribute significantly to its overall solubility parameter, an effective polymer should not only have a similar solubility parameter, it should have a significant hydrogen bonding component. One limitation in applying the Flory–Huggins theory, however, is the lack of tabulated solubility parameters for most commercially available polymers and the scarcity of higher dimensional solubility parameters, which capture individual contributions for dispersive, polar, and hydrogen bonding interactions.

A contrasting mechanism for uptake of target molecules by polymers, adsorption, reduces the imbalance

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of attractive forces at the surface and hence reduces the surface free energy of heterogeneous systems.[10] Polymeric adsorbents (i.e. 'hard', crystalline polymers) are nonfunctionalized organic polymers, which are capable of removing organics from water. Synthetic, polymeric adsorbents are typically rigid, styrenic, plastic beads often possessing limited deformability, making them a suitable choice in a number of industrial applications.

Organic molecules are retained on the resin by weak van der Waals forces, and a reduction in the system's energy because of these forces causes the organic molecule to remain on the polymer surface. These adsorptive forces are heavily influenced by solution properties such as pH, temperature, and ionic strength, and the adsorbed organics can be desorbed from the resin, with a variety of possible methods including aqueous acids or bases, organic solvents or steam.

In this work, absorptive 'soft' polymers with a range of overall solubility parameters, along with different hydrogen bonding interactions, were investigated and compared based on their ability to remove ethanol from an aqueous solution. In addition, a crystalline adsorptive polymer was similarly tested. Ethanol fermentations were then conducted with one of the polymers to determine the possible impact of *in situ* ethanol removal on overall fermentation performance.

MATERIALS AND METHODS

Chemicals

Polymers under investigation were obtained from Sigma-Aldrich Canada Ltd., except Hytrel (DuPont Canada), and DOWEX Optipore L493 (Dow Chemical Company). High performance liquid chromatography (HPLC) grade ethanol used in polymer uptake experiments as well as all medium components was obtained from Sigma- Aldrich Canada Ltd.

Organism

An industrial strain of *Saccharomyces cerevisiae* (Alltech, Nicholsville, Kentucky) was used in fermentations and was grown on a glucose medium containing the following components: 150 g/L glucose, 10 g/L yeast extract, 11.6 g/L $(NH_4)_2SO_4$, 2.7 g/L KH_2PO_4, and 0.75 g/L $MgSO_4 \cdot 7H_2O$ (Daugulis *et al*., 1987). Inocula consisted of 50 mL growth medium in 125 mL erlenmeyer flasks, cultivated at 30 °C on a shaker for 24 h. As this industrial yeast is extremely robust, no special precautions were taken to provide obligate anaerobic conditions during inoculum preparation or fermentation.

Polymer capacity and release tests

Polymer beads (6 g) were placed in a sealed 125 mL erlenmeyer flask and shaken in the presence of 70 mL of an aqueous solution containing 110 g/L ethanol for 24 h at 30 °C, after which the aqueous concentration of ethanol was measured using HPLC and compared with the initial concentration. The capacity of the polymer was determined by mass balance (g of ethanol/g of polymer). The release of ethanol from Optipore polymer beads was investigated by placing 15 g of the polymer, previously loaded with ethanol from a capacity experiment, in 70 mL of water containing no ethanol. Aqueous samples were taken at various times and analyzed for ethanol concentration.

Single and two-phase batch fermentations

Parallel fermentations were conducted by adding 200 mL of medium (but with 250 g/L glucose) to each of 500 mL erlenmeyer flasks fitted with a foam stopper and inoculated with 20 mL of yeast inoculum. An 80 g/L of Optipore L493 beads was added to one of the flasks. Fermentations were conducted on an incubator shaker for 48 h at 30 °C, with aqueous samples removed periodically for analysis by optical density measurements, along with ethanol and glucose concentration in the aqueous phase.

Analytical procedures

Cell growth was determined by optical density measurements at 600 nm using a Biochrom Ultrospec 3000 UV/Visible Spectrophotometer. HPLC was used to determine the concentration of ethanol and glucose. The HPLC system consisted of a Waters 515 HPLC pump and a Waters 410 Differential Refractometer (Waters Millipore, Milford, MA, USA). A Waters SugarPak-I column, operating at 90 °C with water as the mobile phase, and a flowrate of 0.5 mL/min, was used. Prior to analysis, fermentation samples were centrifuged, and 1 mL of each sample was filtered three times using Waters C18 SepPak cartridges to remove proteins and cellular material prior to injection. A 0.25 mL of the filtered sample was injected into the column.

RESULTS AND DISCUSSION

The ability of various amorphous polymers to remove ethanol from an aqueous solution is displayed in Table 1, which shows ethanol uptake after 24 h of contact. Of the amorphous 'soft' polymers, nylon 6,6 was the most successful at removing the target solute, with a capacity of 0.123 g ethanol/g polymer.

On the basis of the results obtained from these capacity experiments, the validity of the Flory–Huggins theory to predict polymer–solute absorption for polar systems was assessed. Overall (δ) and higher dimensional solubility parameter values, which capture

DOI: 10.1002/apj

Table 1. Uptake of ethanol by polymers.

Polymer	Polymer capacity (g ethanol/ g polymer)	Partition coefficient (polymer phase concentration/ aqueous phase concentration)
Nylon 6,6	0.123	1.17
Poly (methylmethacrylate)	0.053	0.48
Hytrel 8206	0.011	0.10
Ethylene vinyl alcohol (32% VA)	0	0
Styrene butadiene (28% styrene)	0.049	0.44
Polypropylene	0	0
Optipore L493	0.346	4.44

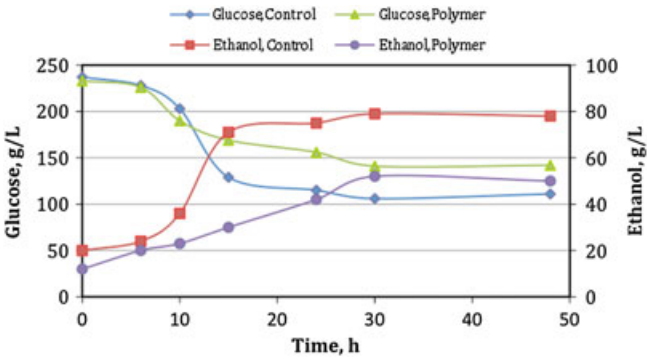

Figure 1. Time course of polymer and control ethanol fermentations. This figure is available in colour online at www.apjChemEng.com.

individual contributions for dispersive (δ_D), polar (δ_P), and hydrogen bonding (δ_H) interactions, were available for styrene butadiene, nylon 6,6, and poly(methylmethacrylate) and are summarized in Table 2, along with solubility parameters for ethanol. The Flory–Huggins theory predicts that the absorption of ethanol will be most successful with polymers whose overall and higher dimensional solubility parameters most closely match those of the target molecule. On the basis of the values in Table 1, the capacity of the polymers for ethanol should decrease in the following order: nylon 6,6, poly (methylmethacrylate), styrene butadiene, and this indeed was the case. This limited experimental evidence suggests that the Flory–Huggins theory may be extended in the future, with caution, to polar systems to predict which polymers will be most successful for solute absorption. In general, ethanol will be most readily absorbed by polymers that have similar dispersive, polar, and hydrogen bonding interactions, in this case nylon 6,6.

In light of this modest uptake of ethanol by soft, amorphous polymers, the ability of a hard, crystalline adsorbent to remove ethanol was investigated using Optipore L493, a macroporous styrenic polymer, and the results are also shown in Table 1. Given the significantly higher sorption by this polymer (0.346 g/g), it was used for *in situ* ethanol removal in a fermentation.

Figure 1 shows the results of the polymer and control fermentations. After approximately 24 h, the ethanol concentrations in batch fermentations in both systems reached a steady value. Because the residual glucose was present in excess of 100 g/L in both cases, it can be concluded that the production of ethanol did not stop because of substrate depletion. Over the course of the fermentation, 127 g/L and 91 g/L of glucose were consumed in the absence and presence of polymers, respectively, suggesting that the presence of polymer beads at a loading of 80 g/L had a negative impact on glucose uptake and ethanol production.

The inoculum for these fermentations used identically grown cells of *S. cerevisiae* which, when added to the fermentation flasks, would have provided very similar initial values of ethanol concentration. However, 20.38 g/L and 12.12 g/L of ethanol were present in the initial samples after polymer addition and mixing, in fermentations with 0 g/L and 80 g/L of polymer, suggesting that polymer uptake of ethanol was very rapid and had an immediate effect on reducing the initial ethanol concentration in the polymer fermentation. Figure 1 also shows that slightly less glucose was consumed during the polymer fermentation, but a disproportionately lower amount of ethanol was produced that can be accounted for by this reduced substrate consumption.

Over the course of the fermentation in the absence of polymer beads, 58 g/L of ethanol was produced, consuming 127 g/L of glucose. Therefore, the overall product yield with respect to substrate consumed is 0.46, a value very typical for *S. cerevisiae*. Using this yield coefficient along with the remaining measured concentrations of glucose, the theoretical amount of ethanol produced at each sampling time for the fermentation in the presence of adsorbent was estimated. This

Table 2. Overall (δ) and higher dimensional (δ_D, δ_P, δ_H) solubility parameters for ethanol and amorphous polymers investigated.[9]

Solubility parameter	Ethanol	Styrene butadiene	Nylon 6,6	Poly(methylmethacrylate)
δ_D ($MPa^{1/2}$)	15.80	17.55	18.62	18.64
δ_P ($MPa^{1/2}$)	8.80	3.36	5.11	10.52
δ_H ($MPa^{1/2}$)	19.40	2.70	12.28	7.51
δ ($MPa^{1/2}$)	26.60	18.07	22.87	22.69

DOI: 10.1002/apj

Table 3. Comparison of observed and theoretical ethanol concentrations throughout the batch fermentation in the presence of polymeric adsorbent Optipore L493.

Time (h)	Measured aqueous ethanol concentration (g/L)	Theoretical aqueous ethanol concentration increase based on glucose consumed and yield (g/L)	Actual aqueous ethanol concentration increase from previous period (g/L)	Difference (g/L)	Difference (g)
0	12.21	—	—	—	—
6	13.95	3.47	1.75	1.73	0.38
10	23.26	19.87	11.05	8.82	1.94
15	30.01	29.45	17.80	11.66	2.56
24	41.78	35.11	29.57	5.54	1.22
30	51.73	42.23	39.53	2.70	0.59
48	50.09	41.71	37.88	3.83	0.84

value was compared with the actual aqueous ethanol concentrations, as seen in Table 3.

Table 3 shows that when polymers were present, less ethanol was measured in the aqueous phase than predicted by the stoichiometric yield coefficient and the corresponding amount of glucose consumed. This is attributed to uptake of ethanol by the polymer. Thus, although the addition of polymers as a sequestering phase for ethanol uptake would appear to have achieved our objective, we have also observed that 'hard' adsorbents can be susceptible to abrasion and comminution of the polymers beads into very fine particles, as also noted by Galli,[11] and can cause shear and abrasion damage to the biomass.[12] This, along with possible leaching of residual additives from the Optipore beads, may be the cause of the reduced performance in the fermentation undertaken in this work. No such abrasion problems have been encountered with the use of 'soft' amorphous polymers in other systems that we have studied,[13,14] even with high degrees of mechanical agitation.

A final consideration in the use of polymers for uptake of target molecules is the rate and extent of release for final product recovery. The rate of release of ethanol into water from previously loaded Optipore is shown in Table 4. Because the uptake by this crystalline polymer is via adsorption (a surface phenomenon), it can be seen that the release of ethanol is extremely rapid, with almost complete desorption occurring within the first minute. This observation is consistent with the initially reduced amounts of ethanol found in the polymer fermentation system from the sample taken immediately after addition of the Optipore beads. However, a relatively small fraction (12%–14%) of the adsorbed ethanol was desorbed into water in one washing step, suggesting that, again, the use of hard, crystalline polymers in TPPB systems may not be an ideal choice.

Table 4. Desorption kinetics of polymeric adsorbent Optipore L493.

Time	Aqueous ethanol concentration (g/L)	Mass of ethanol in aqueous phase (g)	Percent of ethanol desorbed
0	0	0	0
1 min	5.36	0.375	12.69
5 min	5.48	0.384	12.97
30 min	5.25	0.368	12.43
1 h	5.26	0.368	12.45
24 h	6.00	0.420	14.19

CONCLUSION

In this work, a hard, crystalline polymer with high specific surface area has been shown to be superior to a number of amorphous, absorptive polymers for the uptake of ethanol. A similar result has also been found for the uptake of another biofuel, butanol by crystalline, styrenic polymers.[15] The use of the Flory–Huggins theory to predict effective absorptive polymers does, however, appear to be a promising first step in the search for superior 'soft' polymer absorbents. Nevertheless, the value of such pure-component parameters stems from their widespread availability, rather than their accuracy in describing polymer–solvent compatibility. Ultimately, a complete thermodynamic treatment of two-phase, three component systems is required for rigorous assessments of polymer performance. Activity coefficient and/or group contribution models such as Universal Functional Activity Coefficient (UNIFAC) may have value in this regard,[16] notwithstanding the relative paucity of thermodynamic information on solute–polymer mixtures.

Soft, amorphous materials, although potentially slower to take up target molecules because of the time required for molecules to diffuse into the polymer matrix, have a number of potentially superior features compared with hard polymeric adsorbents, including significantly lower cost, robustness during prolonged use because of their flexibility (retaining their structural

DOI: 10.1002/apj

integrity), and the ability to desorb the retained molecules essentially to completion[17]. We are currently more fully exploiting the Flory–Huggins theory to identify amorphous polymers for the absorption of a number of high value fermentation products (i.e. nutraceuticals), and the initial predictions involving a 'matching' of solubility parameters between solute and polymer appear to provide excellent results.

The use of inexpensive, nonvolatile, nonflammable, biocompatible and easily shaped polymers as the sequestering phase in a bioreactor is an enormous advance in designing high-efficiency and low-cost bioprocesses that eliminate cell toxicity and is key to the development of 'green', solvent-free processing strategies.

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DOI: 10.1002/apj