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Simulation study of biobutanol production in a polymer-loaded two-phase partitioning bioreactor (PL-TPPB): Simulation and strategy for biobutanol production

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Abstract−A simulation study was performed for a two-phase partitioning bioreactor (TPPB) with polymer beads, Dowex Optipore L-493, as a second phase. When the initial glucose concentration is less than 30 g/L, a single-phase bioreactor is preferred, because it consumed all the glucose with 40% of biobutanol yield. Any glucose over the concentration remained in the single-phase bioreactor because cells were completely inhibited by products, mainly biobutanol, and thus glucose availability became less than 100%. The TPPB with 10% polymer beads completely consumed up to 120 g/L glucose and more polymer beads were required for the higher glucose concentration. Instead of increasing the proportion of polymer beads, 2 vvm of nitrogen gas was introduced continuously into the TPPB for the stripping of products, reducing product inhibitions. By applying gas stripping to the TPPB containing 10% polymer beads, 150 g/L of glucose was completely consumed and 99.7% acetone, 46.8% butanol and 82.5% ethanol was stripped out of the TPPB. Finally, on the basis of these estimations, a novel strategy based on the initial glucose concentration was suggested for high biobutanol production.

Key words: Biobutanol, Product Inhibition, Two-phase Partitioning Bioreactor, Second Phase, Polymer Beads, Gas Stripping

INTRODUCTION

Biobutanol has recently attracted great attention because of its distinct advantages over other biofuels [1-3]. It has a higher energy density (7,323 Kcal/L) than bioethanol (5,598 Kcal/L) comparable with fossil gasoline (7,656 Kcal/L), and it is more stable when mixed with conventional gasoline because of its lower oxygen content compared with bioethanol. In addition, since biobutanol does not have any corrosive properties, it can be delivered through a long-distance pipeline, which greatly reduces distribution costs.

Biobutanol has been mostly produced by the genus of *Clostridium* under anaerobic culture [4]. One of the critical problems for commercial production is product inhibition. The cells are completely inhibited at 10-15 g/L of biobutanol, and this reduces the productivity of biobutanol [5-7]. Novel processes with the aim of preventing product inhibition have been suggested, and they include perstraction, gas stripping, pervaporation, solid adsorption and extractive fermentation [5,6,8-10]. However, a novel process is still under exploration because each above-mentioned process has some drawbacks [11]. Two-phase partitioning bioreactors (TPPBs) containing conventionally an organic solvent as the second phase have long been applied to the bio-productions where substrate and/or product inhibitions are involved [12]. The organic solvent should be hydrophobic to ensure phase separation from the aqueous phase and needs to be biocompatible [13]. Therefore, the substrate and/or product should also be hydrophobic for the second phase to act as a reservoir of substrates and storage for products. Since biobutanol is a relatively hydrophilic compound, the conventional TPPB is not efficient for the production of biobutanol. As an alternative to the organic solvent, polymer beads are introduced to the TPPB as the second phase. This novel TPPB has many advantages over the conventional one [14]. In particular, for hydrophilic compounds such as biobutanol, hydrophilic polymer beads can be used in a TPPB because they would not be dissolved in the aqueous phase and readily absorb hydrophilic compounds [11,14].

In the previous manuscript [11], a mathematical model of a TPPB containing polymer beads as a second phase was developed for biobutanol production. Product inhibitions by butanol, acetic acid and butyric acid on the cells were considered in the model. The partitioning coefficients of butanol, acetone, ethanol, acetic acid and butyric acid for candidate polymers against an aqueous solution were determined, and Dowex Optipore L-493, a copolymer of styrene and divinyl benzene, showed the highest partitioning coefficient for butanol, acetone, ethanol and butyric acid compared with other candidate polymers. The mass transfer coefficients of the compounds from aqueous phase into polymer beads were experimentally determined, and those from the aqueous phase to nitrogen gas were also experimentally determined where gas stripping was introduced to the TPPB.

In this manuscript, a simulation for a polymer-loaded TPPB (PL-TPPB) was carried out. The performance of the PL-TPPB was compared with that of a single-phase bioreactor. In addition, the effect of gas stripping by nitrogen gas on enhancing biobutanol production was also investigated through a simulation study. Finally, a novel strategy based on initial glucose concentration was suggested for biobutanol production using the PL-TPPB.

MATERIALS AND METHODS

Clostridium acetobutylicum ATCC 824 was used for biobutanol production in this study. Details of the culture condition, PL-TPPB

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and analytical methods are described in the previous manuscript [11]. The PL-TPPB is composed of a 3 L aqueous phase and 0.3 Kg of polymer beads as the second phase. All the experimental procedures for the determination of mass transfer coefficients, and the mathematical model and relevant parameters are also suggested in the same manuscript. Following are some of the mathematical equations.

For product inhibition:

$$\mu = \frac{\mu_{max} C_G}{C_G + K_G} f(I) \quad (1)$$

$$f(I) = \left\{ 1 - \left(\frac{C_{BOH}}{C_{BOH-I}} \right)^{m_{BOH}} - \left(\frac{C_{AA}}{C_{AA-I}} \right)^{m_{AA}} - \left(\frac{C_{BA}}{C_{BA-I}} \right)^{m_{BA}} \right\} \quad (2)$$

Where μ, C_G and K_G represent specific cell growth rate (1/hr), glucose concentration (g/L) and half-saturation constant (g/L), respectively. f(I) is a function indicating the degree of inhibition by butanol (BOH), acetic acid (AA) and butyric acid (BA). C_{i-I} and m_i stand for the concentration of *i* compound completely inhibiting cell growth and the experimentally determined exponent reflecting the degree of inhibition.

For mass transfer of a compound from aqueous phase into polymer beads:

$$\frac{dC_{liq-i}}{dt} = -k_O a_i (C_{liq-i} - C^*_{p-i}) \quad (3)$$

Where $k_O a_i$, C_{liq-i} and C^*_{p-i} represent overall volumetric mass transfer coefficient of *i* compound (1/hr), the concentration of *i* compound in the aqueous phase (g/L), and that in the polymer beads equilibrated with the concentration in the aqueous phase (g/L).

For gas stripping:

$$\frac{dC_{liq-i}}{dt} = -k_L a_{N_2} (C_{liq-i} - C^*_{liq-i}) = -k_L a_{N_2} C_{liq-i} \quad (4)$$

Where $k_L a_{N_2}$ and C^*_{liq-i} represent mass transfer coefficient by gas stripping (1/hr) and the concentration of *i* compound in aqueous phase equilibrated with the concentration of *i* in nitrogen gas (g/L). Since nitrogen gas is continuously fed into and taken out of the PL-TPPB, C^*_{liq-i} can be assumed to be zero.

For polymer beads:

$$\frac{dC_{p-i}}{dt} = k_O a_i (C_{liq-i} - C^*_{p-i}) \frac{V_l}{V_p} \quad (5)$$

Where C_{p-i} represents the concentration of *i* compound in the polymer beads (g/L).

The concentration of the *i* compound in the aqueous phase was expressed based on the mass balance [11]. For example, the accumulation rate of butanol in the aqueous phase equals the production rates of butanol (by direct glucose metabolism and by the conversion of butyric acid to butanol) minus the elimination rates of butanol (by partitioning into polymer beads and by gas stripping).

RESULTS AND DISCUSSION

1. Biobutanol Production Using PL-TPPB

As predicted by simulation in the previous manuscript [11], the glucose availability, defined as the portion of glucose consumed to the initial glucose, in a single-phase bioreactor is only about 50% when the initial glucose concentration is 60 g/L. This value is very close to that of other research completed with the same initial glucose concentration [15]. This low availability is mainly due to product inhibitions and necessarily results in low biobutanol production. The simulation also shows that neither glucose consumption nor cell growth occurs when butanol concentration reaches the concentration limit (12 g/L) at 30 hours of operation, which results in no further butanol production. The simulation for a single-phase batch operation with 20 g/L of initial glucose concentration indirectly supports this explanation as shown in Fig. 1, where the final butanol concentration is 8.0 g/L below the limit of butanol concentration, and glucose availability approaches 100%. In this case, however, the concentrations of acetic and butyric acids remain around 1.0 g/L, which is due to the exhaustion of the glucose required to convert both organic acids to the corresponding compounds, as assumed previously [11]. Other research also shows that more than 90% of the initial 20 g/L glucose was used even without pH control [15]. Accordingly, when the initial glucose concentration is low, a single-phase bioreactor is sufficient for biobutanol production.

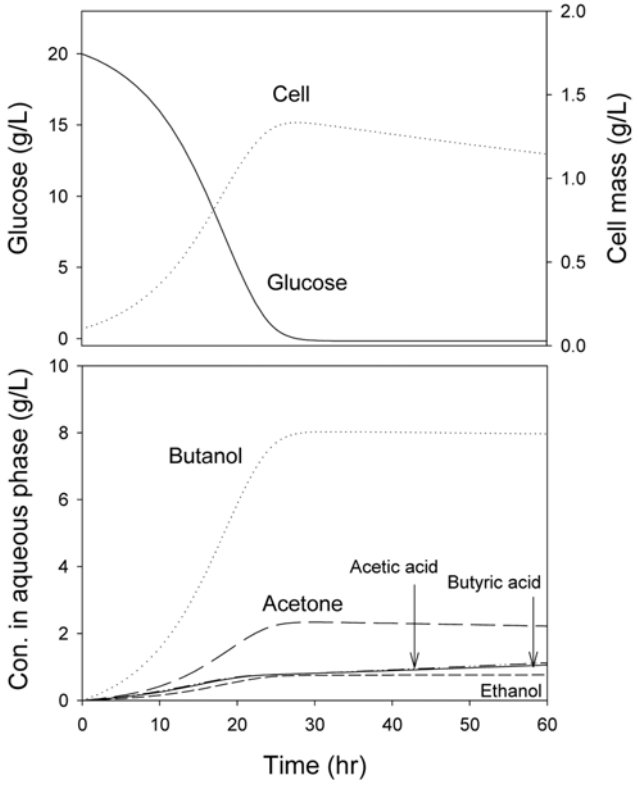

Fig. 1. Simulation of biobutanol production in a single-phase bioreactor for 20 g/L glucose.

Unlike butanol, the other inhibitory compounds, butyric and acetic acids, show negligible inhibitions on cells because the concentration of them produced in the PL-TPPB is very low compared with butanol, and their concentration limits completely inhibiting cells are as high as 11.0 g/L and 12.0 g/L, respectively [16]. Practically, therefore, butanol is the only compound causing inhibition on the cells, though inhibitions by butyric and acetic acids are considered in the mathematical model for biobutanol production. The

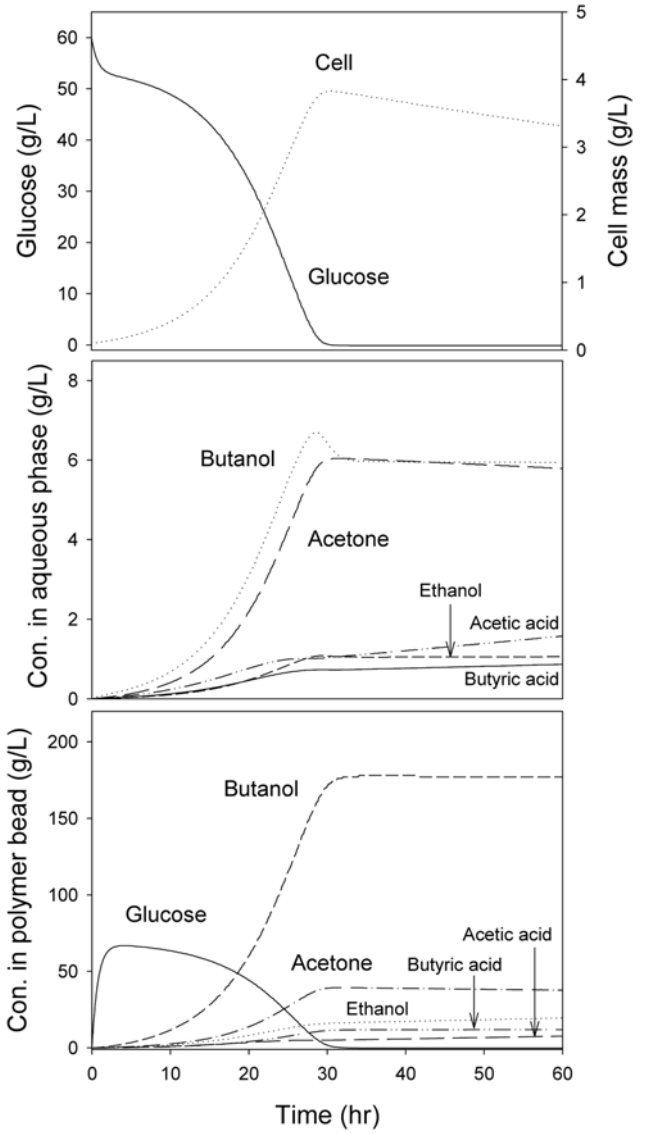

Fig. 2. Simulation of biobutanol production in the PL-TPPB with 10% polymer beads for 60 g/L glucose.

maximum initial glucose concentration without causing complete inhibition on cells was roughly estimated to be 30 g/L on the basis of 40% biobutanol yield.

To increase the glucose availability by reducing butanol concentration in the aqueous phase, by sequestering the butanol from the aqueous phase to the second phase, a PL-TPPB was employed. A simulation of the PL-TPPB with 3 L of aqueous phase along with 0.3 Kg polymer beads, 10% (w/w), was conducted for 60 g/L of initial glucose and the results are presented as Fig. 2. The figure shows that the glucose availability reaches 100% and the final cell concentration is 3.3 g/L, 83% higher than a single-phase bioreactor. The concentrations of butanol, acetone, ethanol, butyric acid and acetic acid in the aqueous phase were 5.94, 5.79, 1.06, 0.87 and 1.58 g/L, respectively. The final butanol concentration in the aqueous phase, 5.94 g/L, was much lower compared with the final butanol concentration in a single-phase batch operation, 12.2 g/L, which was due to the partitioning of the butanol between polymer beads

Table 1. Simulation results for biobutanol production from 60 g/L glucose in the PL-TPPB composed of 3 L working volume with 10% polymer beads

Parameter	Aqueous phase	Polymer beads	Total
Acetone (g)	17.4	11.3	28.7
Butanol (g)	17.8	53.1	70.9
Ethanol (g)	3.2	3.6	6.8
Total ABE (g)	38.4	68.0	106.4
ABE yield (%)		59.1	
Acetic acid (g)	4.7	2.3	7.0
Butyric acid (g)	2.6	5.9	8.5
Total organic acids (g)		15.5	
Initial glucose (g)	180	0	-
Final glucose (g)	0	0	0
Glucose availability (%)		100	
Final cell mass (g)	9.93	0	9.93
Cell yield (%)		5.5	

and the aqueous phase. The concentrations of the compounds in the polymer beads were estimated using partitioning coefficients, and butanol was found to be highest at 177 g/Kg-polymer, followed by 37.7 g/Kg for acetone, 19.5 g/Kg for butyric acid, 11.9 g/Kg for ethanol and 7.65 g/Kg for acetic acid. 117 g/Kg-polymer of butanol in the polymer beads is equivalent to 53.1 g of butanol, indicating that 74.9% of butanol produced in the PL-TPPB was absorbed into the polymer beads. The glucose in polymer beads was initially significantly increased but gradually decreased to zero because the polymer beads rapidly absorbed glucose and cells utilized glucose without being inhibited, releasing the glucose to the aqueous phase. To estimate the total amount of each compound produced in the PL-TPPB, the mass of each compound both in the aqueous phase and in polymer beads was combined, which is shown in Table 1. This table shows that the total amount of butanol produced is 70.9 g and this is equivalent to 23.6 g/L (on the basis of aqueous volume) or 21.5 g/L (on the basis of total volume). When compared with the single-phase operation obtaining 12.2 g/L biobutanol, the PL-TPPB shows 93.4% and 76% higher performance on the bases of aqueous and total volumes, respectively. Another important advantage of the PL-TPPB is a significantly reduced operating time. For example, it takes about 25 hours for 20 g/L of initial glucose concentration in a single-phase bioreactor and only 5 hours longer is required for 60 g/L of initial glucose concentration in the PL-TPPB. This is possible due to the virtuous circle, that is, products are absorbed into polymer beads, preventing product inhibition, and thus cells grow intensely and convert glucose to products at a high rate.

There may be some dispute about the concentration of each compound in the polymer beads. From a mathematical point of view, there is no concentration limit for each compound in the polymer beads. However, there should be a finite concentration limit for each compound because absorption is a physical phenomenon. The Langmuir isotherm was used to estimate the maximum capacity of the polymer beads for butanol, and it was found to be 646 g-butanol/Kg-polymer as previously reported [11]. This result implies that about 200 g of butanol can be absorbed into 300 mL of polymer

beads. Since butanol is present in the aqueous phase with many other compounds, the actual amount of butanol absorbed in the polymer beads may be smaller than estimated. The concentrations of other compounds including substrates, products, by-products, metabolic wastes *etc.* continuously change during PL-TPPB operation, and this in turn changes the influence of their presence on the absorption of compounds such as butanol into polymer beads [17]. Therefore, it is almost impossible to predict the exact amount of butanol absorbed into the polymer beads during PL-TPPB operation. For convenience, the partitioning coefficients are taken to be constant throughout, an assumption made by many other researchers in the PL-TPPB or conventional organic-solvent loaded TPPB (OL-TPPB) operations for the different compounds [17-20].

To reveal the relationship between the portion of polymer beads (Φ) and the glucose availability and the limit of PL-TPPB, the partitioning coefficient (P_C) is analyzed in different way. The following equation can be derived from the definition of the partitioning coefficient along with biobutanol yield of 0.4 on the initial glucose concentration (G) for the complete glucose consumption.

$$C_2 = \frac{C_1}{1+P_C\Phi} = \frac{0.4G}{1+\Phi P_C} \quad (6)$$

Where, C_1 and C_2 represent the butanol concentration before and after polymer beads addition, respectively. The dependency of butanol concentration in the aqueous phase on the initial glucose concentration and the partitioning coefficient is shown in Fig. 3(a). The figure shows that a higher portion of polymer beads results in lower butanol concentration in the aqueous phase. Since the partitioning coefficient of butanol between polymer beads and the aqueous phase, and the possible maximum butanol concentration in the aqueous phase are 29.8 and 12.0 g/L, respectively, the limit of initial glucose concentration ensuring complete consumption can be estimated by using Eq. (6) and shown in Fig. 3(b). When the portion of polymer beads increases from 0.1 to 0.3, the initial glucose also does from 119.4 to 298.2 g/L. For a PL-TPPB with Φ of 0.1, the relationship between initial glucose concentration and glucose availability is presented in Fig. 4. The figure shows that the glucose availability is maintained at 100% as long as initial glucose concentration is less than about 120 g/L. The glucose availability at 150 g/L of initial glucose concentration is approximately 80%, and that is reduced to 60% at 200 g/L of initial glucose concentration. The glucose availability can be enhanced by increasing the proportion of polymer beads in the PL-TPPB. The relationship between initial glucose concentration and the requirement of polymer beads to reduce the final butanol concentration in the aqueous phase to less than 12.0 g/L is presented in Fig. 5. As shown in this figure and explicitly anticipated from Eq. (6), the requirement of polymer beads increases linearly with the initial glucose concentration. For example, 200 and 300 g/L of initial glucose requires at least 19.0% and 30.2% polymer beads compared with the aqueous phase. Unlike with an organic solvent, polymer beads are completely mixed with the aqueous phase, and

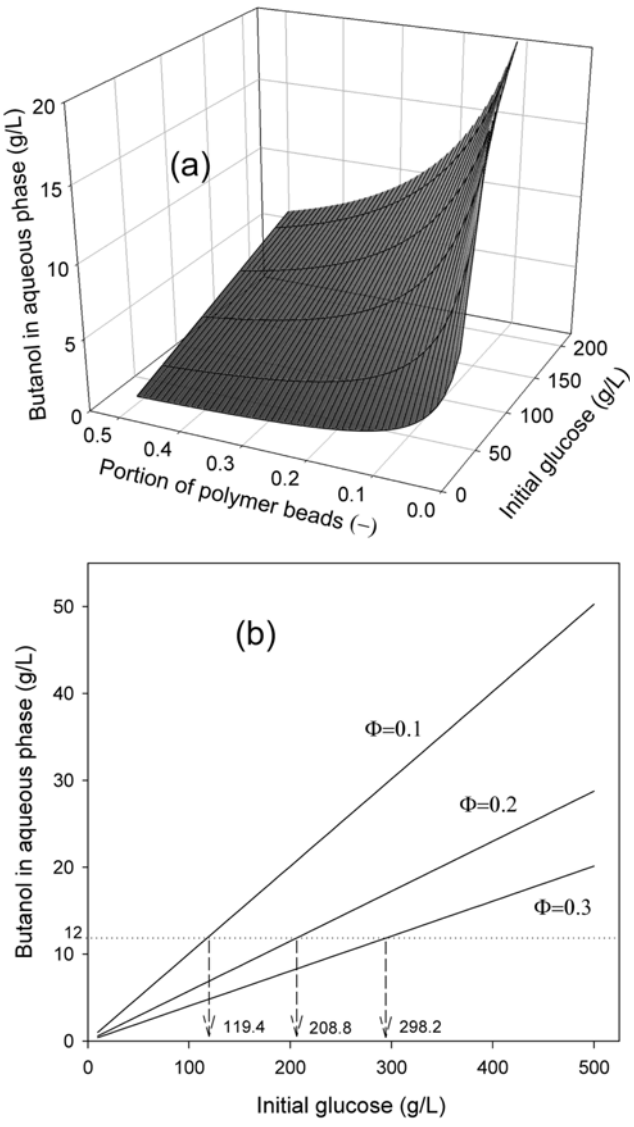

Fig. 3. (a) The dependency of butanol concentration in the aqueous phase on the initial glucose concentration and the portion of polymer beads in a PL-TPPB. (b) The relationship between initial glucose concentration and butanol concentration in the aqueous phase for various portions of polymer beads.

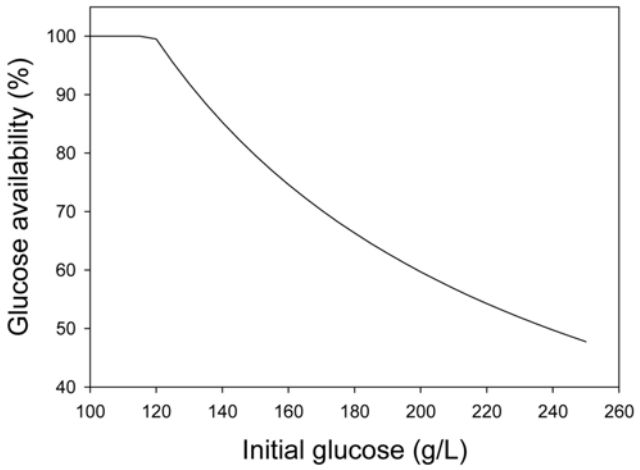

Fig. 4. The relationship between initial glucose concentration and glucose availability for the PL-TPPB with 10% polymer beads.

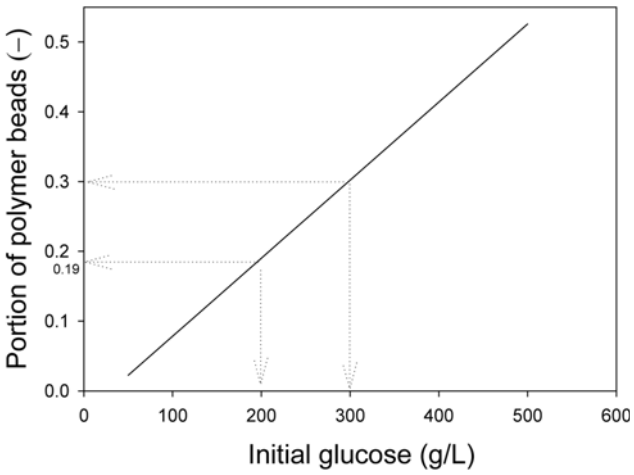

Fig. 5. The portion of polymer beads achieving butanol concentration below 12 g/L in the aqueous phase.

Table 2. Simulation results for biobutanol production from 150 g/L glucose in the PL-TPPB composed of 3 L working volume with 10% polymer beads

Parameter	Aqueous phase	Polymer beads	Total
Acetone (g)	38.1	24.8	62.9
Butanol (g)	36.7	109.5	146.2
Ethanol (g)	5.9	6.6	12.5
Total ABE (g)	80.7	140.9	221.6
ABE yield (%)		62.7	
Acetic acid (g)	0	0	0
Butyric acid (g)	0	0	0
Total organic acids (g)		0	
Initial glucose (g)	450	0	450
Final glucose (g)	85.9	11.0	96.9
Glucose availability (%)		78.5	
Final cell mass (g)	15.0	0	15.0
Cell yield (%)		4.2	

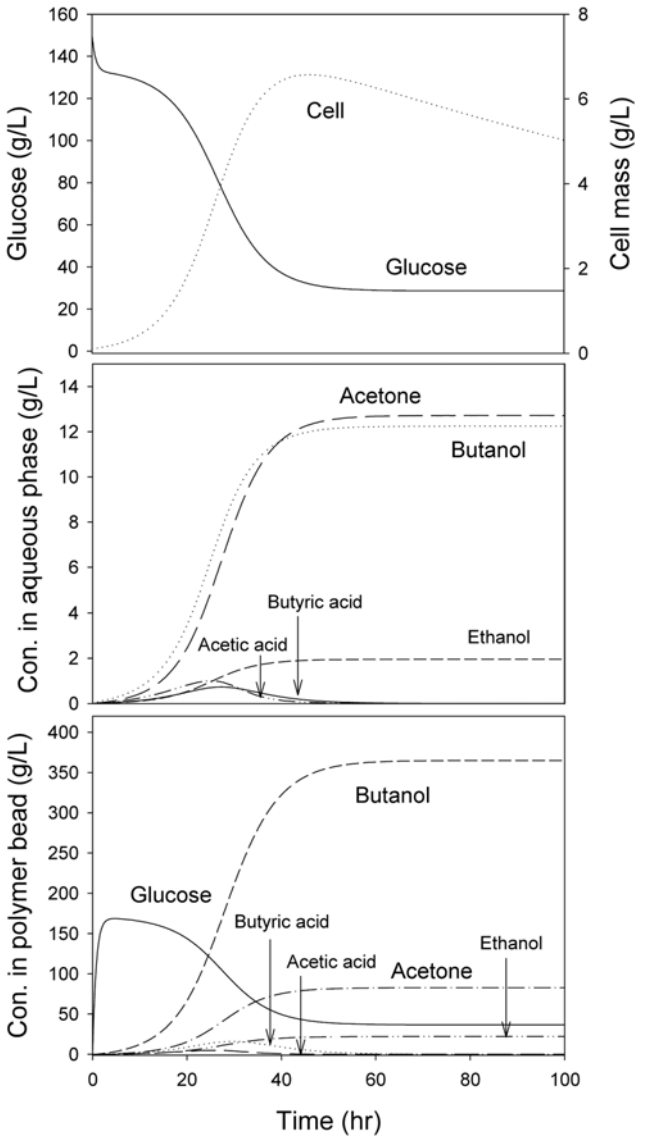

Fig. 6. Simulation of biobutanol production in the PL-TPPB with 10% polymer beads for 150 g/L glucose.

thus a high proportion of polymer beads may actually hinder PL-TPPB operation. According to our experience, less than 15% of polymer beads relative to the aqueous phase is desirable. Therefore, for an extremely high glucose concentration, a method other than adding more polymer beads should be explored. In this study, gas stripping was introduced to the PL-TPPB, and a simulation study for this new version of PL-TPPB was also performed.

2. Biobutanol Production Using a PL-TPPB with Gas Stripping

As shown in Fig. 4, glucose availability stands at 80% for a PL-TPPB with 10% of polymer beads and 150 g/L initial glucose concentration, which is also predicted in the simulation of PL-TPPB as shown in Fig. 6. That is, although a substantial amount of butanol is absorbed into the polymer beads, butanol concentration in the aqueous phase soon reaches 12.0 g/L and this inhibits the cells completely. With an increase in polymer beads, glucose availability and butanol production could be enhanced. However, since a high proportion of polymer beads could hinder PL-TPPB operation, gas stripping, widely used alone in biobutanol production [21-23], was introduced to the PL-TPPB. Nitrogen gas was used and it served to maintain the anaerobic condition as well as to strip volatile products such as butanol, acetone and ethanol out of the PL-TPPB. Gas flow rate was set at 2.0 vvm, and relevant mass transfer parameters were determined in the previous manuscript [11]. Fig. 7 shows a simulation for a PL-TPPB with gas stripping, and Table 3 shows the comparison of PL-TPPBs with and without gas stripping. Without gas stripping, glucose availability and biobutanol production for 150 g/L initial glucose stand at 78.5% and 146.2 g, respectively. The amount of biobutanol produced is equivalent to 41.4% based on consumed glucose and 32.5% based on initial glucose concentration. By attaching gas stripping to a PL-TPPB, glucose availability was enhanced to 100% and biobutanol production was estimated to be 177.3 g. This high production was due to the continuous stripping of butanol by nitrogen gas, which maintains the butanol concentration in the aqueous phase to less than 12 g/L. The portion of butanol stripped out

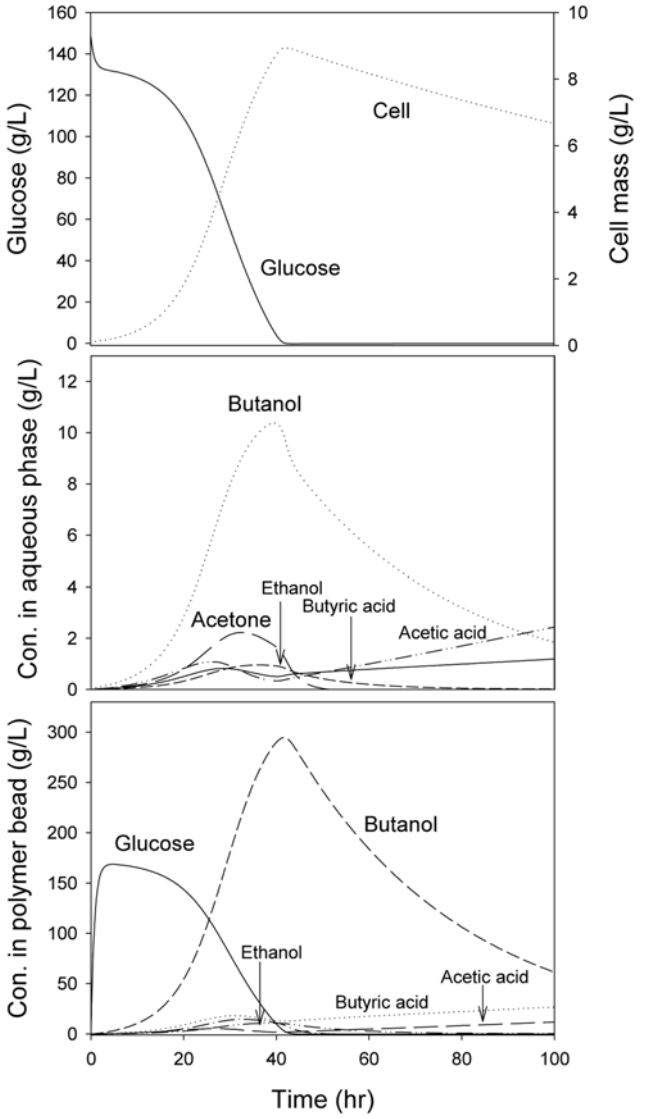

Fig. 7. Simulation of biobutanol production in the PL-TPPB with gas stripping for 150 g/L glucose.

Table 3. Simulation results for biobutanol production from 150 g/L glucose in the PL-TPPB with gas stripping

Parameter	Aqueous phase	Polymer beads	Stripping	Total
Acetone (g)	0.1	0.1	71.4	71.6
Butanol (g)	21.8	72.5	83.0	177.3
Ethanol (g)	1.3	1.7	14.1	17.1
Total ABE (g)	23.2	74.3	168.5	266
ABE yield (%)		59.1		
Acetic acid (g)	2.0	0.9	0	2.9
Butyric acid (g)	2.1	4.5	0	6.6
Total organic acids (g)		9.5		
Initial glucose (g)	450	-	-	450
Final glucose (g)	0	0	0	0
Glucose availability (%)		**100**		
Final cell mass (g)	25.8	0	0	25.8
Cell yield (%)		5.7		

of the PL-TPPB based on the total butanol production is 46.8%, 99.7% for acetone and 82.5% for ethanol at 50 hours when the glucose is completely consumed. That is, almost half of the butanol remains in the PL-TPPB while only trace levels of acetone are left. The dramatic decrease of acetone in the PL-TPPB is due to its high stripping mass transfer coefficient, 0.418 hr^{-1} compared with 0.117 hr^{-1} for butanol and 0.186 hr^{-1} for ethanol [11]. By extending the stripping period, the concentrations of butanol and ethanol would decrease continuously, and only organic acids would remain in the PL-TPPB. In a PL-TPPB using stripping, absorption of products into polymer beads cooperates with this stripping to prevent product inhibitions on cells, resulting in continuous biobutanol production and complete glucose consumption. Although the introduction of stripping prevents product inhibitions, it is not necessarily a good choice because it requires a great deal of energy, an inert gas such as nitrogen for stripping, and an additional unit, a condenser, in the actual bioprocess. Therefore, an economic analysis for PL-TPPB with stripping should be performed taking into account biobutanol productivity and the additional operating cost. Since the purpose of gas stripping is to maintain inhibitory products, mainly biobutanol, under the limiting concentrations, these compounds could be stripped for a limited period or intermittently to save operating costs. In that case, another important issue would be when to start and stop stripping. A detailed investigation of this issue is left for further research.

CONCLUSIONS

Two-phase partitioning bioreactors (TPPBs) have been used for the purpose of preventing substrates and/or product inhibitions. Dowex Optipore L-493, a copolymer of styrene and divinyl benzene, showed a partitioning coefficient of butanol against the aqueous phase of up to 29.8 and was chosen as the second phase for the production of biobutanol in the TPPB. We had previously set up a mathematical model of a TPPB with many parameters, some of which were measured experimentally or cited from other research. Through a simulation study, we suggest a novel strategy with respect to initial glucose concentration to enhance biobutanol production as follows. When initial glucose concentration is less than 30 g/L, a single-phase bioreactor is preferred and the final biobutanol concentration is estimated to be lower than 12.0 g/L. When the initial glucose concentration is over 30 g/L, a polymer-loaded TPPB (PL-TPPB) should be employed for high biobutanol production. The requirement for polymer beads is in proportion to the initial glucose concentration and the amount of polymer beads determines the maximum initial glucose concentration, achieving complete glucose consumption. When glucose is not completely used up in a PL-TPPB even in the presence of a high proportion of polymer beads, gas stripping can be introduced to the PL-TPPB. Butanol along with acetone and ethanol is continuously stripped out of the PL-TPPB, and absorption of products into polymer beads cooperates with this stripping to prevent product inhibitions on cells, resulting in continuous biobutanol production and complete glucose consumption. Another significant advantage of the PL-TPPB is the remarkably reduced operating time, which is possible due to the virtuous circle, that is, products are absorbed into polymer beads, preventing product inhibition, and thus cells grow intensely and convert glucose to products at a high rate.

This study suggests that the PL-TPPB has many advantages over

conventional organic-solvent loaded TPPBs (OL-TPPB) as well as single-phase bioreactors. Since this study was performed through simulation, actual experiments should be performed to modulate and elaborate the parameters of the model equations and to confirm the strategy suggested in this study, and these matters are left for further study. We believe that this study encourages researchers to apply a PL-TPPB to the production of many other hydrophilic compounds like biobutanol, causing substrate and/or product inhibitions, which are not efficiently carried out by conventional OL-TPPBs or single-phase bioreactors.

ACKNOWLEDGEMENT

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