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Simulation study of biobutanol production in a polymer-loaded two-phase partitioning bioreactor (PL-TPPB): Model development

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Abstract−The conventional two-phase partitioning bioreactor (TPPB) containing an organic solvent as a second phase was found to be hardly efficient for biobutanol production because of the relatively low partitioning coefficient of butanol between the organic solvent and aqueous solution. Polymer bead was alternatively employed as the second phase in the TPPB, and Dowex Optipore L-493, a copolymer of styrene and divinyl benzene, was chosen as the optimum polymer because it shows the highest partitioning coefficients of butanol, acetone, ethanol and butyric acid against the aqueous phase among candidate polymers. The mass transfer coefficients of compounds from the aqueous phase into polymer beads were experimentally determined with respect to agitation speed. The mass transfer coefficient related to the stripping of volatile compounds by nitrogen gas was also determined, and the influence of gas flow rate turned out to be greater than that of the agitation speed, though both influences were remarkable. A mathematical model for the TPPB containing the polymer beads was suggested and as many as 40 parameters were cited from other publications or determined in this study. This mathematical model will be subsequently used for the detailed simulation study.

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

INTRODUCTION

Global warming and energy shortages have been imminent worldwide problems [1]. Fuels account for about 30% of total energy usage, and this urgently requires sustainable alternative fuels that emit less toxic exhaust and greenhouse gases than fossil fuels [2,3]. Biofuels such as biodiesel and bioethanol, capable of using an existing fossil fuel supply infrastructure, have proved very attractive due to their renewable, environmentally-friendly and carbon-neutral properties, and have been commercialized in many European countries, the USA and Brazil [4].

Recently biobutanol has attracted attention because it has several distinct advantages over other biofuels [4-6]. First, since biobutanol has lower oxygen content compared with bioethanol, it is more stable when mixed with conventional gasoline. For example, gasoline mixed with over 10% of bioethanol degrades an existing gasoline engine whereas the property of gasoline mixed with 10% of biobutanol was reported to be similar to that of unleaded gasoline, and approximately 16% of biobutanol can be mixed with gasoline without changing the gasoline engine [4]. Second, biobutanol has a higher energy density than bioethanol because it has a longer carbon chain. That is, biobutanol contains 7,323 Kcal/L, 30% higher than ethanol (5,593 Kcal/L), and this is comparable with fossil gasoline (7,656 Kcal/L). Third, since biobutanol does not have any corrosive properties, it can be delivered through a long-distance pipeline, which greatly reduces distribution costs. Finally, it can be handled easily due to its relatively lower vapor pressure and higher hydrophobicity compared with bioethanol.

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Despite these advantages, several critical problems, mostly related to the fact that biobutanol is largely produced by anaerobic microorganisms, need to be solved for commercial production. The most widely used genus, *Clostridium*, produces biobutanol along with acetone and ethanol in the ratio of 6 : 3 : 1, which inevitably requires a separation process. Another problem is product inhibition. The cells are completely inhibited at 10-15 g/L of biobutanol and this reduces the productivity of biobutanol [4,7-14]. Novel processes with the aim of preventing product inhibition during biobutanol production have been suggested since the 1980s and they include perstraction [6,7], gas stripping [8,9], pervaporation [10,11], solid adsorption [12] and extractive fermentation [13,14]. After these processes were compared, a fermentation associated with gas stripping or perstraction was concluded to be the most promising [4]. However, gas stripping demanding the separation of biobutanol from water and other compounds requires large amounts of energy, and perstraction, requiring a membrane with quite a broad surface, does not guarantee high efficiency [4].

Two-phase partitioning bioreactors (TPPBs) have long been applied to bio-production where substrate and/or product inhibition is involved [15]. Organic solvents have been conventionally used as the second phase for TPPBs and a systematic selection procedure for organic solvents was suggested previously [16,17]. TPPBs with organic solvent as a second phase appeared to be successful in many cases, but some problems have been addressed as follows [18]. The organic solvent should be hydrophobic to ensure phase separation from the aqueous phase and needs to be biocompatible [16]. Accordingly, the substrate and/or product should also be hydrophobic for the second phase to act as a reservoir of substrates and storage for products. Therefore, the researchers who developed strategic approaches for the development of TPPBs suggested that a single-

phase bioreactor was preferred to a TPPB for the treatment of relatively hydrophilic compounds [19]. In addition, if plural substrates and/or products in which physical properties such as hydrophobicity are different from each other are involved in bio-production, it is very difficult to select the proper organic solvents. Emulsions are often observed during the operation of a TPPB and this hinders the separation of the two phases. The loss and gradually reduced purity of the organic solvent are also problems. As an alternative to organic solvents, polymer beads were used as the second phase and they have showed many successful applications [18,20]. Polymer beads are usually much cheaper than organic solvents and show excellent durability. For hydrophilic compounds such as biobutanol, hydrophilic polymer beads, unlike organic solvents, can be used in a TPPB because they are not dissolved in the aqueous phase and readily absorb hydrophilic compounds. Emulsions are not produced in a TPPB containing polymer beads, which can be reused without loss. In addition, the polymer beads can be applied to a mixed culture of different species. Various polymer beads with different properties can be placed into a TPPB at the same time or sequentially to facilitate production and separation of target compounds without substrates and/or products inhibitions. For example, polymer beads with a high partitioning coefficient for substrates are introduced first, and those for products are introduced later to prevent both substrate and product inhibition. In addition, polymer beads impregnated with magnetic particles can be devised, which would make their separation from the TPPB quite easy [18].

In this study, optimum polymer beads were selected for a polymer-loaded two-phase partitioning bioreactor (PL-TPPB) for biobutanol production. The mathematical model for the PL-TPPB was developed, and the parameters of the model were determined.

MATERIALS AND METHODS

Clostridium acetobutylicum ATCC 824 was used for biobutanol production in this study. Subculture was carried out in an anaerobic jar (A-100, MGC, Japan). Fifty (50) mL of medium was poured into a 150 mL bottle and purged with nitrogen gas for 10 minutes to remove oxygen. Once the cells were inoculated into the bottle, the bottle was sealed with a rubber septa and aluminum cap and placed in a shaking incubator (IS-971RF, Jeiotech, Korea) operating at 30 °C and 150 rpm. For the subculture, RCM medium (3.0 g/L yeast extract, 5.0 g/L glucose, 10.0 g/L beef extract, 5.0 g/L tryptone, 5.0 g/L peptone, 1.0 g/L soluble starch, 0.5 g/L cystein hydrochloride, 5.0 g/L NaCl and 3.0 g/L sodium acetate) was used while YEM medium (8.0 g/L yeast extract, 0.2 g/L $MgSO_4 \cdot 7H_2O$, 0.01 g/L $MnSO_4 \cdot 4H_2O$, 0.01 g/L $FeSO_4 \cdot 7H_2O$, 1.0 g/L Asparagine, 0.5 g/L cystein, 2.2 g/L casein hydrolysate, 1.0 mg/L Thiamine HCl, 1.0 mg/L p-amino benzoic acid and 0.02 mg/L biotin) supplemented with glucose was used for other cultures. Batch culture was carried out in a 5 L fermentor (KoBioTec, Korea) with a 3 L working volume at 35 °C and a pH of 5.5. Agitation was at 200 rpm and nitrogen was supplied into the fermentor intermittently to ensure an anaerobic condition. Butanol, acetone, ethanol, acetic acid and butyric acid were measured with a GC (HP 5890 II, USA) equipped with an FID detector and ZB-WAX 7HG-G007-11 column (Phenomenox, USA). Helium was used as the carrier gas. The temperatures of the injector and detector were 210 and 230 °C, respectively, and that of the column was elevated from 40 to 210 °C at 10 °C/min after the oven temperature was initially maintained for 4 min. Glucose was measured using the DNS method [21]. Simulation was performed with Matlab R009b®. Polymer beads were kindly donated by Dr. Daugulis at Queen's university in Canada or purchased from a manufacturer (Sigma, USA). The biocompatibility of polymer beads was carried out by measuring cell mass change in a 250 mL flask containing a 100 mL culture medium with 1 g/L of cell and 3 g of polymer beads. To measure the partitioning coefficient and the maximum absorption capacity of polymer beads for a compound, each 5 g of polymer beads was added to a 250 mL flask containing 100 mL of the compound ranging from 5-100 g/L and placed in the shaking incubator at 30 °C for 24 hours to ensure equilibrium [18]. After incubation, an aqueous sample was taken from each flask to determine the concentration of the compound, and the amount of the compound absorbed into the polymer beads was estimated from the concentration. To measure the mass transfer coefficient of a compound from the aqueous phase into the polymer beads, various concentrations of the compound were prepared in the fermentor with 1 L of working volume of YEM medium and 20 g of the polymer beads was placed in the fermentor. Samples were taken every 3 minutes to measure the concentration of the compound in the aqueous phase, which enabled an estimation to be made of the amount of the compound absorbed into the polymer beads. The stripping mass transfer coefficient of the compound was measured in the same fermentor by introducing nitrogen gas and measuring the concentration of the compound in the aqueous phase every 3 minutes to estimate the amount of the compound stripped by the nitrogen gas. The partitioning coefficient of butanol between the organic solvent and aqueous phase was determined according to the protocol previously reported [16,18].

RESULTS AND DISCUSSION

1. Selection of an Optimum Polymer

Partitioning coefficients of five compounds involved in the biobutanol production, butanol, acetone, ethanol, acetic acid and butyric acid, were determined for the five different candidate polymers. Some of these candidates were recommended by Dr. Daugulis and other research was also referred to [22]. As shown in Table 1, two types of Dowex polymer beads, a copolymer of styrene and divinyl benzene, exhibit higher partitioning coefficients for those five compounds against an aqueous solution compared with other polymer beads, in particular, five and ten times higher for butanol and butyric acid, respectively. Due to the high partitioning coefficients for compounds known to cause severe inhibition on cells such as butanol, acetic and butyric acids [23], product inhibitions are expected to be reduced significantly in biobutanol production using the PL-TPPB. In the case of SD-2, the partitioning coefficients of butanol and ethanol are much higher than that for acetone. In the case of L-493, the partitioning coefficient of butanol is much higher than those of ethanol and acetone. Since butanol is produced in a dominant quantity as well as the major compound causing product inhibition on the cells, L-493 seems to be the most promising polymer beads for the second phase in the PL-TPPB. Before finalizing polymer selection, a biocompatibility test with *Clostridium acetobutylicum* ATCC 824 for SD-2 and L-493 was carried out [22]. Minor cell

Table 1. Partitioning coefficients of compounds between polymer beads and aqueous solution

Polymer	Butanol	Acetone	Ethanol	Acetic acid	Butyric acid
Hytrel 8206 (Polyether-ester) (DuPont, USA)	4.8	2.7	0.8	0.3	1.0
Desmopan 453 (Polyurethane) (Bayer, GER)	5.4	3.8	2.8	0.6	2.2
Elvax 770 (Ethylene/vinylacetate) (DuPont, USA)	3.3	2.5	3.0	0.9	2.3
Dowex Optipore SD-2 (Poly(styrene-co-DVB)) (Dow, USA)	20.1	5.6	5.2	8.0	21.6
Dowex Optipore L-493 (Poly(styrene-co-DVB)) (Dow, USA)	29.8	6.5	11.3	4.9	22.9

decrease was observed for both L-493 and SD-2 (data now shown), which differs from previous results by other researchers [22], who reported noticeable *E.coli* decrease in the presence of L-493. Therefore, L-493 was finally selected as the optimum polymer beads in the PL-TPPB.

2. Requirement of Polymer Beads

The partitioning coefficient is defined in Eq. (1).

$$\frac{\frac{m_1 - m_2}{M}}{\frac{m_2}{V}} = P_c \quad (1)$$

where m_1, m_2, M, V and P_c represent the masses of butanol (g) before and after polymer beads addition, the mass of polymer beads (g), volume of aqueous phase (mL) and partitioning coefficient (mL/g). The mass of polymer beads can be converted to a corresponding volume (mL) using specific gravity but the unit of mass is used in this study for convenience sake. Eq. (1) can be rearranged to Eq. (2) which estimates the mass of polymer beads required to reduce high butanol concentration to a target concentration. The resultant butanol concentration after polymer beads have been introduced to a PL-TPPB can be also estimated using Eq. (3).

$$M = \frac{V(C_1 - C_2)}{P_c C_2} \quad (2)$$

$$C_2 = \frac{VC_1}{V + P_c M} \quad (3)$$

Where, C_1 and C_2 represent the butanol concentration before and after polymer beads addition, respectively. Eqs. (2) and (3) can be rearranged to Eqs. (4) and (5), respectively.

$$C_2 = \frac{C_1}{1 + P_c \Phi} \quad (4)$$

$$\Phi = \frac{C_1 - C_2}{P_c C_2} \quad (5)$$

where Φ represents the ratio of the mass of second phase to the volume of the aqueous phase (in Kg/L). According to our experience, the value of Φ should be less than 0.2 in order not to disturb the PL-TPPB operation. Eqs. (2) and (5) indicate that the amount of polymer beads required for reducing 1 L of 30 g/L butanol to 10 g/L or 5 g/L is estimated to be 67 g (Φ=0.067) or 168 g (Φ=0.168), respectively.

PL-TPPB was briefly compared with conventional organic solvent loaded-TPPB (OL-TPPB) for biobutanol production on the basis of partitioning coefficients. The partitioning coefficients of butanol between an aqueous solution and three promising organic solvents which other researchers had used for biobutanol production [14,24] were experimentally determined in this study, and they were 3.70 for PPG 1200, 3.21 for oleyl alcohol and 4.86 for olive oil. Eq. (5) indicates that the Φ (here, dimensionless) values of olive oil for reducing 1 L of 30 g/L of butanol to 10 g/L or 5 g/L are estimated to be as high as 0.412 or 1.03, respectively. A high Φ requires large amounts of organic solvent and a Φ value over 1.0 may mean that the second phase is no longer the second phase because the volume of organic solvent exceeds that of the aqueous solution. Another comparison concerns total butanol concentration reducible to a certain concentration in a TPPB. When Φ is fixed at 0.2, the total butanol concentration reducible to 5 g/L is 34.8 g/L for a PL-TPPB containing L-493, while it is only 9.86 g/L for an OL-TPPB containing olive oil. These all estimations imply that the PL-TPPB shows much higher performance than an OL-TPPB from the point of view of preventing product inhibition and biobutanol production.

However, the estimates from these equations were not able to reflect physical phenomena exactly. In particular, when the butanol concentration is extremely high, the equations might predict a butanol concentration in the polymer beads exceeding the maximum capacity of the polymer beads for butanol. To provide a limit for butanol concentration in the polymer beads, the maximum absorption capacity of the polymer beads for butanol was estimated using the Langmuir isotherm, conventionally used for adsorption but often used for absorption [18], and it was found to be as high as 646 g-butanol/Kg-polymer.

3. Overall Mass Transfer Coefficient from an Aqueous Solution into Polymer Beads

The overall mass transfer coefficient is used to describe the mass transfer of a compound from an aqueous phase into polymer beads with a single coefficient, and must consider both aqueous and polymer resistances to mass transfer [20]. The overall mass transfer coefficient can be expressed as the sum of two reciprocal mass transfer coefficients as shown in Eq. (6): one is for the aqueous phase and the other is for polymer beads [20].

$$\frac{1}{k_O} = \frac{1}{k_L} + \frac{1}{a k_P} \quad (6)$$

where k_O, k_L and k_P represent the overall mass transfer coefficient (m/hr), the mass transfer coefficient for the aqueous phase (m/hr) and the mass transfer coefficient for polymer beads, respectively. The symbol a represents a specific surface area per volume of polymer beads. In practice, the mass transfer rate of a compound from the bulk aqueous phase to polymer beads can be expressed using the overall volumetric mass transfer coefficient ($k_O a$, 1/hr) as follows, and this coefficient can be determined experimentally using

Table 2. Mass transfer coefficients of compounds from aqueous solution to the polymer beads, Dowex Optipore L-493

Agitation (rpm)	Overall mass transfer coefficient (k_La hr^{-1})				
	Butanol	Acetone	Ethanol	Acetic acid	Butyric acid
100	0.12	0.28	0.06	0.13	0.27
200	0.54	0.82	0.21	0.43	0.64
400	0.79	1.09	0.48	0.63	0.85

Eq. (7).

$$\frac{dC_{liq-i}}{dt} = -k_O a_i (C_{liq-i} - C^*_{p-i}) \quad (7)$$

Where C_{liq-i} and C^*_{p-i} represent the concentration of compound i in the aqueous phase, and that in the polymer beads equilibrated with the concentration in the aqueous phase. These overall mass transfer coefficients were determined experimentally and are summarized in Table 2. As expected, the overall mass transfer coefficients increase with agitation speed. The overall mass transfer coefficients increased by 2.9-4.5 times when the agitation speed doubled from 100 to 200 rpm, but they only increased by 1.3-2.3 times when the speed was changed from 200 to 400 rpm. From the point of view of the compounds, the overall mass transfer coefficient of acetone was highest among the five, followed by butanol and ethanol.

4. Stripping Effect by Nitrogen Gas

When biobutanol is produced anaerobically by *Clostridium acetobutylicum* ATCC 824, carbon dioxide and hydrogen are produced as well, and they may serve to strip volatile compounds from the fermentation broth, reducing product inhibition. Accordingly, bioreactor systems utilizing the evolving gases to strip out the volatile compounds from the culture broth have been developed [7-9,25]. However, the evolving carbon dioxide and hydrogen in the fermentation are simply 2 mol-CO_2/mol-glucose and 2 mol-H_2/mol-glucose, respectively, which means that, on the assumption that carbon dioxide and hydrogen are ideal gases, 22.4 L of each gas is produced when 30 g/L glucose in a 3 L-working volume bioreactor is completely consumed. Therefore, the total volume of the two gases from the fermentation corresponds to 1 vvm of gas flow rate for 15 minutes or 2 vvm for 7.5 minutes. Since it usually takes about 30 hours for batch fermentation for biobutanol production, the circulation of the evolving gases or an additional gas supply is required to strip volatile compounds and reduce product inhibitions [9,25]. In this study, nitrogen gas was supplied to the PL-TPPB for the stripping of the compounds from the bioreactor and this inert gas also plays a role in maintaining the anaerobic condition of the fermentation. The parameters affecting the stripping rate are the mass transfer coefficient ($k_La_{N_2}$), generally proportional to the superficial gas flow rate [19], and the concentration gradient of a compound between aqueous solution and nitrogen gas, serving as a driving force. The mass transfer of compound i from aqueous solution to gaseous nitrogen can be expressed as follows [17].

$$\frac{dC_{liq-i}}{dt} = -k_L a_{N_2} (C_{liq-i} - C^*_{liq-i}) = -k_L a_{N_2} C_{liq-i} \quad (8)$$

Where C^*_{liq-i} represents the concentration of i compound in aqueous phase equilibrated with the concentration of i in nitrogen gas. Since nitrogen gas is continuously fed into and taken out of the PL-TPPB, C^*_{liq-i} can be assumed to be zero. This equation implies that the stripping of *i* compound by nitrogen gas can be expressed as a first-order reaction rate, and this is in good agreement with observations by other researchers [9,27]. In a stirred-tank type bioreactor, it is the superficial flow rate and agitation speed that affect mass transfer coefficient. In this study, the mass transfer coefficients with respect to these two parameters were experimentally determined and are shown in Table 3. Mass transfer coefficients increase with gas flow rate and agitation speed, irrespective of compounds. The increase in agitation speed makes the gas bubbles smaller, which leads to the increase of the total specific surface area of nitrogen gas bubbles (a) and decreases the resistance to mass transfer through the air-liquid interface (k_L) [19]. The increase in the gas flow rate definitely increases the number of gas bubbles or gas hold-up in the bioreactor, which increases the mass transfer coefficient. Although the increase in both gas flow rate and agitation speed enhances the mass transfer coefficient, they cannot be increased indefinitely, and thus a proper operating condition should be determined. The first three columns in Table 3 show that the increase in agitation speed from 200 to 400 rpm enhances the mass transfer coefficients by 1.5 (acetone)-8.7 (butanol) times while an increase from 400 to 600 rpm does the same by 1.0 (ethanol)-acetic acid (3.6) but the mass transfer coefficient of acetic acid is as low as 4.3×10^{-2} hr^{-1}, 8.6% compared with acetone. When it comes to compounds, acetone shows the highest mass transfer coefficient, followed by ethanol and butanol. For gas flow rate, when it was increased from 2.0 to 4.0 vvm at a constant 200 rpm, the mass transfer coefficient was enhanced by 1.7 (acetone)-11.6 (butanol) times while the mass transfer coefficient was increased by approximately 20% when the gas flow rate was increased from 4.0 to 6.0 vvm.

Table 3. Mass transfer coefficients of compounds via nitrogen gas stripping

Operating condition (vvm; rpm)	Mass transfer coefficient (k_La, hr^{-1})$\times10^2$				
	Butanol	Acetone	Ethanol	Acetic acid	Butyric acid
2.0; 200	1.35	28.5	4.16	0.6	1.1
2.0; 400	11.7	41.8	18.6	1.2	2.7
2.0; 600	18.4	49.9	18.2	7.3	5.5
4.0; 200	15.6	48.2	16.2	3.3	2.3
6.0; 200	19.9	59.5	18.5	1.2	6.1

Looking at the mass transfer coefficients of absorption and gas stripping from an economic viewpoint, an operating condition of 2.0 vvm and 400 rpm was determined as optimal. The mass transfer coefficients of acetic and butyric acids are much lower than those of the other the compounds, and the stripping of these two fatty acids is ignored in the following modeling and simulation study.

5. Model Development for PL-TPPB

A mathematical model for batch PL-TPPB operation was developed, and this can be readily modified for continuous operation. For the development of model equations for biobutanol production in a PL-TPPB, several assumptions are suggested as follows [23, 26]. First, glucose is the only limiting substrate, that is, other nutri-

ents including the nitrogen source are not limited during operation. Second, cells are inhibited by butanol, acetic acid and butyric acid. Third, acetic and butyric acids are metabolic intermediates and they are reduced to acetone and butanol, respectively. Fourth, both acetone and butanol can also be directly produced from a carbon source, glucose. Fifth, ethanol is produced only from a carbon source. Sixth, fermentation is operated under anaerobic conditions at optimal temperature and pH. Seventh, all cells are active and no cell decay is assumed. Eighth, the partitioning coefficients of each compound between polymer beads and the aqueous phase are constant throughout the operation, and the partitioning coefficient of a compound is not influenced by the presence of other compounds, nutrient medium or cells [18]. Ninth, acetone, butanol and ethanol are stripped by nitrogen gas, but acetic acid, butyric acid and glucose are not.

5-1. Models for Microorganisms in the Aqueous Phase

Cells are not absorbed into the polymer beads due to their large size. In addition, with the assumptions of batch operation and no cell decay, the change of cell concentration in the aqueous phase can be expressed as Eq. (9).

$$\frac{dX}{dt} = \mu X \tag{9}$$

where X, t and μ represent cell concentration (g/L), time (hr) and specific cell growth rate (1/hr), respectively. According to the second assumption, butanol and two organic acids cause inhibition on cells and this is expressed by the Eq.s. (10) and (11) [23].

$$\mu = \frac{\mu_{max} C_G}{C_G + K_G} f(I) \tag{10}$$

$$f(I) = \left\{ 1 - \left(\frac{C_{BOH}}{C_{BOH-I}}\right)^{m_{BOH}} - \left(\frac{C_{AA}}{C_{AA-I}}\right)^{m_{AA}} - \left(\frac{C_{BA}}{C_{BA-I}}\right)^{m_{BA}} \right\} \tag{11}$$

where C_G and K_G are glucose concentration (g/L) and half-saturation constant (g/L), respectively. f(I) is a function indicating the degree of inhibition by the three inhibitory compounds. 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. In this study, most parameters were cited from reference [23,26], but μ_{max}, K_G and C_{BOH-I} were experimentally determined within this study through batch fermentations. The parameters cited from other publications may not be the exact values for this study because culture conditions, medium composition, configuration of bioreactor, *etc.* are different from each other, though the same species or microorganism is used. Therefore, this study will provide approximate results, which would be valuable data for the bioreactor development.

5-2. Model for Glucose in the Aqueous Phase

Glucose is used for cell growth, cell maintenance and the production of products and by-products. It is also consumed for the conversions of butyric and acetic acids to butanol and acetone, respectively. Glucose is partitioned between the aqueous phase and polymer beads and no stripping of glucose is assumed. The resulting equation for glucose in the aqueous phase is suggested as Eq. (12).

$$\frac{dC_G}{dt} = -k_L a_G (C_G - C^*_{G-p}) - k_1 \mu X - mX - k_2 \frac{C_G}{C_G + K_G} \frac{C_{BA}}{C_{BA} + K_{BA}} X - k_3 \frac{C_G}{C_G + K_G} \frac{C_{AA}}{C_{AA} + K_{AA}} X \tag{12}$$

where $k_L a_G$ and C^*_{C-P} represent the mass transfer coefficient of glucose from the aqueous phase into polymer beads (m/hr) and the glucose concentration in polymer beads (g/L) equilibrated with that in the aqueous phase, which can be expressed using the partitioning coefficient (P_G), and the glucose concentration in polymer beads (C_{G-P}) as follows.

$$C^*_{G-P} = \frac{C_{G-P}}{P_G} \tag{13}$$

The parameters k_1, k_2, k_3 and m represent glucose consumption by cell growth, the conversion of butyric acid to butanol, the conversion from acetic acid to acetone and the glucose consumption for cell maintenance, and they were cited from other research [23,26].

5-3. Model for Butyric Acid in the Aqueous Phase

According to mass balance, the conversion rate of butyric acid to butanol subtracted from the production rate of butyric acid becomes the accumulation rate of butyric acid in the aqueous phase (C_{BA}). As stated previously, the stripping of butyric acid by nitrogen gas is ignored.

$$\frac{dC_{BA}}{dt} = k_4 \left[k_1 \mu_{max} \frac{C_G}{C_G + K_G} f(I) X + mX \right] - k_5 \frac{C_{BA}}{C_{BA} + K_{BA}} \frac{C_G}{C_G + K_G} X - k_L a_{BA} (C_{BA} - C^*_{BA-P}) \tag{14}$$

5-4. Model for Butanol in the Aqueous Phase

The accumulation rate of butanol in the aqueous phase (C_{BOH}) equals the butanol stripping rate by nitrogen gas subtracted from the sum of the butanol production rate by direct glucose metabolism and the conversion rate of butyric acid to butanol.

$$\frac{dC_{BOH}}{dt} = k_6 \left[k_1 \mu_{max} \frac{C_G}{C_G + K_G} f(I) X + mX \right] + k_7 \frac{C_{BA}}{C_{BA} + K_{BA}} \frac{C_G}{C_G + K_G} X - k_L a_{BOH} (C_{BOH} - C^*_{BOH-P}) - k_L a_{BOH} C_{BOH} \tag{15}$$

Where $k_L a_{BOH}$ represents the mass transfer coefficient of butanol stripped by nitrogen gas.

5-5. Model for Ethanol in the Aqueous Phase

The stripping rate of ethanol subtracted from the ethanol production rate from glucose equals the accumulation rate of ethanol in the aqueous phase (C_{EOH}).

$$\frac{dC_{EOH}}{dt} = k_8 \left[k_1 \mu_{max} \frac{C_G}{C_G + K_G} f(I) X + mX \right] - k_L a_{EOH} (C_{EOH} - C^*_{EOH-P}) - k_L a_{EOH} C_{EOH} \tag{16}$$

5-6. Model for Acetic Acid in the Aqueous Phase

The conversion rate of acetic acid to acetone subtracted from the acetic acid production rate becomes the accumulation rate of acetic acid in the aqueous phase (C_{AA}). As stated previously, no stripping of acetic acid was assumed.

$$\frac{dC_{AA}}{dt} = k_9 \left[k_1 \mu_{max} \frac{C_G}{C_G + K_G} f(I) X + mX \right] - k_{10} \frac{C_{AA}}{C_{AA} + K_{BA}} \frac{C_G}{C_G + K_G} X - k_L a_{AA} (C_{AA} - C^*_{AA-P}) \tag{17}$$

5-7. Model for Acetone in the Aqueous Phase

The stripping rate of acetone by nitrogen gas subtracted from the sum of the direct production rate of acetone from glucose and the conversion rate of acetic acid to acetone equals the accumulation of acetone (C_{AT}).

$$\frac{dC_{AT}}{dt} = k_{11}\left[k_1\mu_{max}\frac{C_G}{C_G+K_G}f(I)X + mX\right] + k_{12}\frac{C_{AA}}{C_{AA}+K_{AA}}\frac{C_G}{C_G+K_G}X - k_La_{AT}(C_{AT}-C^*_{AT-P}) - k_La_{AT}C_{AT} \quad (18)$$

5-8. Model for Polymer Beads

The six compounds including five products and glucose are absorbed into the polymer beads, and their concentration in the polymer beads can be expressed as follows:

$$\frac{dC_{P-i}}{dt} = k_O a_i (C_{liq-i} - C^*_{p-i})\frac{V_l}{V_p} \quad (19)$$

Where i represents glucose, acetone, butanol, ethanol, acetic acid or butyric acid. The parameters were previously explained in Eq. (7). V_l and V_P stand for the volumes of the aqueous phase and polymer beads, respectively.

6. Parameters and Validation of Model

The parameters required for simulation are listed in Table 4. These parameters can be classified into three categories: those for cells, polymer beads and gas stripping. The parameters associated with cells include those for cell growth, product inhibitions on cells, product formations from glucose and the conversions of acetic and butyric acids to acetone and butanol, respectively. The parameters associated with polymer beads are the partitioning coefficients and mass transfer coefficients of the six compounds from the aqueous phase to polymer beads. The mass transfer coefficients of these compounds for stripping by nitrogen gas are also involved in the model. Most of the parameters associated with cells were cited from another publication [23,26], but some were modified through experiments for better prediction. All the parameters for mass transfer were determined experimentally in this study.

In this study, batch fermentation without polymer beads and gas stripping was performed and compared with the simulation result. Since the lag phase, of approximately 15 hours, was not considered in the model, it was omitted in both experimental and simulation results. As shown in Fig. 1, the butanol concentration from simulation was slightly higher than that from the actual experiment, and a similar trend was observed for ethanol. These differences may be due to the stripping of butanol and ethanol by the evolution of carbon dioxide and hydrogen gas. However, on the whole, the model predicted the experimental results well. The characteristics of simulated and experimental batch fermentation for biobutanol production are summarized in Table 5. The availabilities of glucose from experiment and simulation were 53.8% and 49.3%, respectively, for 60 g/L of initial glucose concentration. That is, approximately 50% of glucose remained unused in the culture broth. As results, although the yield of solvents (ABE) and biobutanol on the basis of glucose consumed is 48% and 35%, respectively, those on the basis of initial glucose concentration are only 26% and 18.5%, respectively. These low glucose availabilities may be due to the fact that the cells could not metabolize the glucose further, because of severe cell inhibition when the butanol reached the maximum concentration, approximately 12 g/L in this study. Therefore, the purpose of PL-TPPB development can be said to be to maximize glucose availability by reducing product inhibitions, resulting in high productivity of biobutanol.

CONCLUSIONS

To enhance the productivity of biobutanol by reducing product

Table 4. Parameters for the simulation of biobutanol production in a PL-TPPB

Parameter	Value	Ref.	Parameter	Value	Ref.
μ_{max} (hr^{-1})	0.15	26*	P_{AA} (-)	4.9	This study
K_G (g/L)	2.862	26*	P_{BOH} (-)	29.8	This study
m (hr^{-1})	0.081	23	P_{AT} (-)	6.5	This study
C_{BOH-I} (g/L)	12.0	23	P_{EOH} (-)	11.3	This study
C_{BA-I} (g/L)	11.0	23	$k_La_{N_2-BOH}$ (hr^{-1})	0.117	This study
C_{AA-I} (g/L)	12.0	23	$k_La_{N_2-AT}$ (hr^{-1})	0.418	This study
m_{BOH} (-)	2.0	23	$k_La_{N_2-EOH}$ (hr^{-1})	0.186	This study
m_{BA} (-)	2.5	23	k_1 (-)	14.3	26*
m_{AA} (-)	1.5	23	k_2 (hr^{-1})	0.193	26*
K_{AA} (g/L)	1.818	23	k_3 (hr^{-1})	0.2862	26*
K_{BA} (g/L)	2.862	23	k_4 (-)	0.071	26*
k_La_G (m/hr)	0.15	This study	k_5 (hr^{-1})	0.39	26*
k_La_{BA} (m/hr)	0.85	This study	k_6 (-)	0.39	26
k_La_{BOH} (m/hr)	0.79	This study	k_7 (hr^{-1})	0.36	26
k_La_{EOH} (m/hr)	0.48	This study	k_8 (-)	0.039	26*
k_La_{AA} (m/hr)	0.63	This study	k_9 (-)	0.081	26*
k_La_{AT} (m/hr)	1.09	This study	k_{10} (hr^{-1})	0.356	26*
P_G (-)	1.28	This study	k_{11} (-)	0.1	26*
P_{BA} (-)	22.9	This study	k_{12} (hr^{-1})	0.196	26*

*: Modified from original values

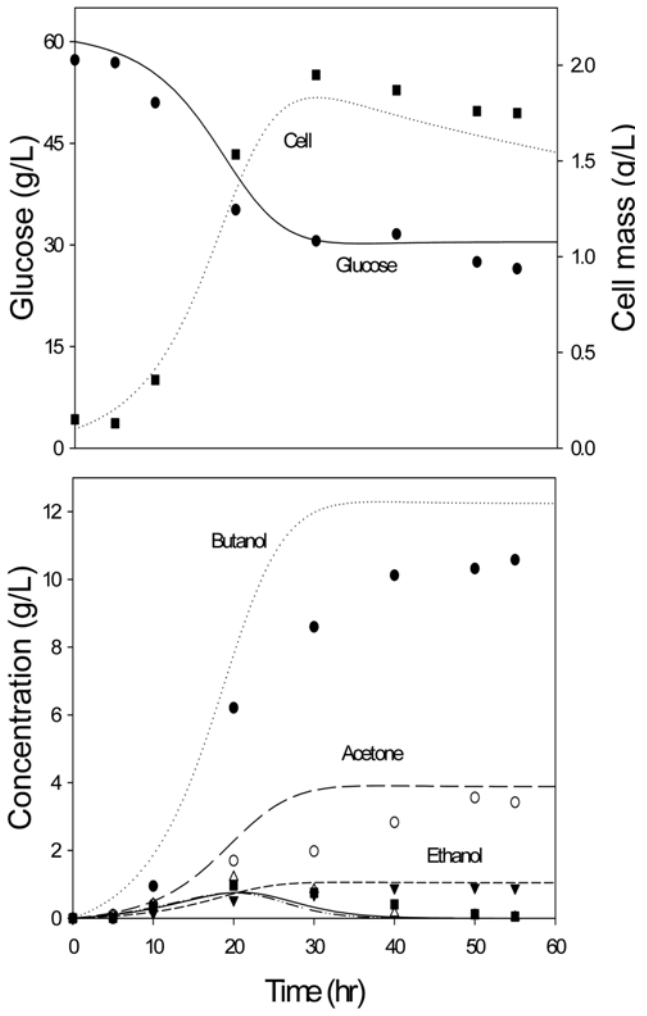

Fig. 1. Comparison of simulation and experimental results for a batch operation for biobutanol production.

Table 5. Characteristics of batch fermentation for biobutanol production

Parameter	Experiment	Simulation
Acetone (g/L)	3.42	3.89
Butanol (g/L)	10.58	12.2
Ethanol (g/L)	0.89	1.05
Total ABE (g/L)	14.9	17.14
ABE yield (%)	48.3	57.9
Acetic acid (g/L)	0.032	0
Butyric acid (g/L)	0.05	0
Total organic acids (g/L)	0.082	0
Initial glucose (g/L)	57.3	60
Final glucose (g/L)	26.5	30.4
Glucose availability (%)	53.8	49.3
Final cell concentration (g/L)	1.75	1.54
Cell yield (%)	0.06	0.05

inhibitions, a novel two-phase partitioning bioreactor (TPPB) was employed, and its performance was evaluated through a simulation study. For part I, a mathematical model was developed and many relevant parameters were determined. We originally considered a conventional TPPB containing an organic solvent as a second phase, but this system proved to be inadequate for biobutanol production because of the low partitioning coefficient of butanol between candidate organic solvents and an aqueous solution. Instead of organic solvents, polymer beads, which have many advantages over organic solvents, were employed as the second phase for the TPPB. Since polymer beads are rigid, non-bio available and not dissolved in the aqueous phase irrespective of their hydrophilicity, they can be used in any TPPB without limitation. Since Dowex Optipore L-493 shows a partitioning coefficient as high as 29.8 for butanol against an aqueous solution, it was chosen as the second phase. We developed a mathematical model with many parameters, some of which were determined experimentally or cited from other research. This model will be used as a simulation study to investigate the performance of a TPPB containing polymer beads as the second phase.

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