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Continuous Direct Solvent Extraction of Butanol in a Fermenting Fluidized-Bed Bioreactor with Immobilized *Clostridium acetobutylicum*†

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ABSTRACT

Immobilized *Clostridium acetobutylicum* was used to ferment glucose into acetone and butanol in a fluidized-bed bioreactor. A nontoxic immiscible solvent, oleyl alcohol, was added to, and removed directly from, the fermenting columnar reactor and extracted the majority of the inhibitory butanol from the aqueous broth. The extracting solvent had a distribution coefficient of near 3 for butanol. Nonfermenting system tests indicated that equilibrium between the phases could be reached in one pass through the column. Steady-state results are presented for the fermentation with and without the extractive solvent addition. One run, with a continuous aqueous feedstream containing 40 g/L glucose, was operated for 23 d. A steady state was established with just the aqueous feedstream. Approximately half of the glucose was consumed, and the pH fell to 4.5 from 6.5. Then, during multiple intervals, the flow of organic extractive solvent (oleyl alcohol) was begun into the fermenting columnar reactor. A new apparent steady state was reached in about 4 h. The final aqueous butanol concentration was lowered by more than half. The total butanol production rate increased by 50–90% during the solvent extraction, as the organic-to-aqueous ratio increased from 1 to 4,

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respectively. There was an observed maximum volumetric productivity of 1.8 g butanol $h^{-1}L^{-1}$ in this nonoptimized system. The butanol yield apparently improved because of the removal of the inhibition. More substrate is going to the desired product, butanol, and less to maintenance or acid production, resulting in 10–20% increases in the ratio of butanol relative to all products.

Index Entries: Solvent extraction; fluidized-bed reactor; butanol; separation and fermentation; immobilized cell.

INTRODUCTION

Butanol is a commodity chemical feedstock and solvent that, early in this century, was primarily made by industrial fermentation (*see* reviews [*1,2*]). The fermentation process declined after World War II owing to both the rise of the petroleum industry and the increase of raw material costs (molasses). Continued research in bioconversion of renewable resources, and the actual and potential increases in petroleum costs have encouraged interest in the butanol fermentation. Butanol may also have an additional market as an oxygenated fuel additive (*3*). The economics of extraction in a free-cell fed-batch butanol fermentation have also been examined (*4*).

Butanol is the primary product of the fermentation of sugars by various bacteria, in particular *Clostridium acetobutylicum*. This is a complex fermentation, with, first, an acidogenic phase producing butyric and acetic acids and, then, a solventogenic phase producing butanol, acetone, and ethanol. Both the products and the lowered pH can be inhibitory to the continued fermentation. This has limited final butanol concentrations to a maximum of 15 g/L in batch culture, with generally much lower yields. The removal of the inhibitory product from the ongoing fermentation has been suggested by many researchers as a method to alleviate the product inhibition and improve the process. Possibilities for product removal include pervaporation (*5*), the use of hollow-fiber reactors (*6,7*), and the use of solid adsorbents (*8–11*).

The approach here for product removal is to use an immiscible extractive solvent. Roffler et al. (*12*) and Groot et al. (*13*) review several possibilities of extractive bioconversions, but not an extractive fluidized-bed bioreactor. Key issues are the extractant toxicity and capacity, as well as the actual contacting scheme devised and its operability (*14*). Many solvents have been tested for the acetone-butanol fermentation (*6,15,16*). Oleyl alcohol was chosen as the primary extractant from these results based on its nontoxicity, and its having both a reasonable distribution coefficient and selectivity for butanol. Dibutyl phthalate (*17*) has also been used as an extractant.

Most studies of extractive acetone-butanol fermentation have been performed in a batch reactor (*15,18*) with free cells. Wayman and Parekh (*17*) performed a sequential batch extractive fermentation with cell recycle. Another approach has been to extract the butanol continuously in a side stream of the fermentor. Roffler et al. (*19*) performed a fed-batch fermentation with a concentrated glucose feed using a extraction column on a recycled side stream and achieved a butanol productivity of 1 g $L^{-1}h^{-1}$. Eckert and Schugerl (*20*) used a continuous-stirred fermentor with a membrane filter to recycle the cells and provide a cell-free broth to an extraction cascade. The lack of direct contact of the cells with the organic extractant allowed them to use the more toxic extractant, decanol.

A class of high-productivity bioreactors is the fluidized-bed bioreactor (FBR) with immobilized cells (*21*) with demonstrated 10-fold increases in productivity over free-cell fed-batch reactors for ethanol fermentation (*22*). FBRs can contain a solid biocatalyst, an aqueous stream of substrates and products, and a gas phase. It is possible to increase the number of phases present further by adding an extractive phase, such as an immiscible liquid. Allen and coworkers have proposed this type of liquid extraction in a fluidized bed (*23*) for the acetone-butanol fermentation, but did not report experimental results of the system. Oleyl alcohol is lighter than water and must be operated in the cocurrent mode in the FBR. Dibutyl phthalate is denser than water and may be operated in a countercurrent mode. As suggested by the free cell extractive fermentation experiments above, the *in situ* extraction should increase the butanol productivity and accomplish some product separation. This work tests the toxicity and distribution coefficient of oleyl alcohol, confirms the hydrodynamic operability of the extractive FBR, and reports on the first continuous fermentation experiments in the extractive FBR with improved butanol production.

MATERIALS AND METHODS

Stocks of *Clostridium acetobutylicum* NRRL-B643 were maintained on potato media. The inocula were checked by the colony morphology technique (*24*) to ensure that they had retained high-solvent capability. The inocula were then grown in a 3-L airlift fermentor with a continuous nitrogen sparge. The cells were centrifuged and immobilized in gel beads, typically 4% κ-carrageenan as described previously (*25*). The beads were measured under a lower-power microscope to be 1.0–1.4 mm in diameter. The cell concentration in the gel beads was measured with a hemocytometer. Dry weights were made of the cells added.

The media consisted of (per liter): $MgSO_4 \cdot 7H_2O$ (0.2 g), $MnSO_4 \cdot H_2O$ (0.01 g), $FeSO_4 \cdot 7H_2O$ (0.01 g), NaCl (0.01 g), K_2HPO_4 (0.25 g), CH_3COONH_4 (0.55 g), yeast extract (5 g), and glucose (40 g). KCl (0.05 *M*) was added to the media for the reactor to stabilize the bead and Dow Silicone antifoam

(0.2 g/L) to control foaming. The media were sparged with nitrogen before any use in the fermentor, as was the oleyl alcohol (Aldrich Chemical Co., Milwaukee, WI). Reagent-grade oleyl alcohol and dibutyl phthalate (Sigma Chemical Co., St. Louis, MO) were tested for toxicity on growth of *Clostridia* by batch cultivation and microscopic examination.

The distribution coefficients of five components (butanol, acetone, acetic acid, ethanol, and butyric acid) were measured between the extractants and an aqueous solution of five components. Known volumes and concentrations of each solution were mixed and allowed to equilibrate overnight, and samples of each phase were analyzed. Extraction experiments were also performed in the columnar contactor without microorganisms. A known aqueous solution of butanol and the other products was contacted with a stream of oleyl alcohol extractant. Effluent samples were monitored until a steady state was reached. The glucose was measured with a YSI glucose analyzer (Yellow Springs Instr. Co., Yellow Springs, CO). The organic acids and solvents were measured by gas chromatography (GC) at 160°C on Chromosorb 101 for both aqueous and extractant phases. The samples were first acidified to below pH 3 with HCl.

A known volume of immobilized-cell beads was placed into a column (122-cm length), with a taper from 1.3 to 3.8 cm id, schematically shown in Fig. 1. The reactor volume was 0.75 L, and the volume including the head space for separation of the two liquid phases was 2.9 L. The column was water-jacketed and maintained at 30°C. The aqueous feed was continuously provided to the base of the column using a calibrated peristaltic pump (Masterflex, Cole-Parmer, Niles, IL). The extractant was added as described below through a needle at the base of the column. Viton and special organic-resistant Tygon® tubing (Cole-Parmer) were used to decrease both organic and oxygen permeability. Samples of both phases could be collected through ports along the column. Most samples were taken at the top of the tapered section or at the effluent. The total amounts of aqueous and organic effluent phases were monitored to confirm flow rates. Any beads that left the reactor were collected and measured. The samples were filtered (0.4 μm) within a minute, and frozen until analysis for glucose and organics. The effluent samples were removed and measured for pH. A YSI dissolved-oxygen probe (Model 5739, Yellow Springs Instr. Co.) was placed in the top of the reactor and periodically removed to be recalibrated. A straight fluidized-bed contactor (61-cm length, 2.5 cm id) was used for the extraction tests and the initial fermentation tests.

RESULTS AND DISCUSSION

Toxicity Tests

Initial toxicity tests were made with two candidate extraction solvents in which toxicity was reported to be minimal. These solvents were oleyl alcohol (*26*) and dibutyl phthalate (*17*). The *Clostridium* was cultured in 5

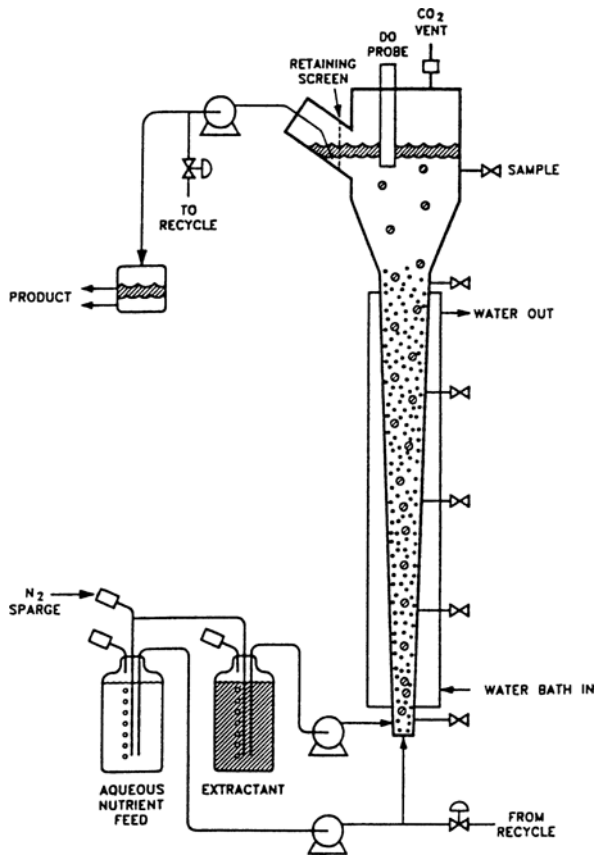

Fig. 1. Schematic of experiment extractive fermentation fluidized-bed bioreactor.

mL of anoxic media in the presence of 1 mL of the solvent. The optical density and glucose consumption were monitored in triplicate runs, and compared to a control (no solvent) over several days. The bacteria remained viable (motile and intact) by microscopic examination in all cases. The presence of the organic phase interfered with the optical density measurements making only qualitative comparisons possible. Table 1 lists the measured glucose concentrations after incubation for 72 h. The glucose consumption was slightly less in the presence of oleyl alcohol when compared to the control tests, but was significantly less in the presence of dibutyl phthalate. These results are in agreement with the qualitative growth observations, and they indicate that oleyl alcohol is the least toxic solvent of the two tested.

Distribution Coefficient

The acetone-butanol fermentation produces several products in addition to acetone and butanol. Aqueous mixtures of the five product compounds were equilibrated with oleyl alcohol. The equilibrated samples

Table 1
Glucose Consumption in the Presence of Solvents
(Initial Glucose Concentration 5 g/L)

Solvent	Glucose after 72 h, g/L
Control (no solvent)	0.0
Oleyl alcohol	0.9
Dibutyl phthalate	4.0

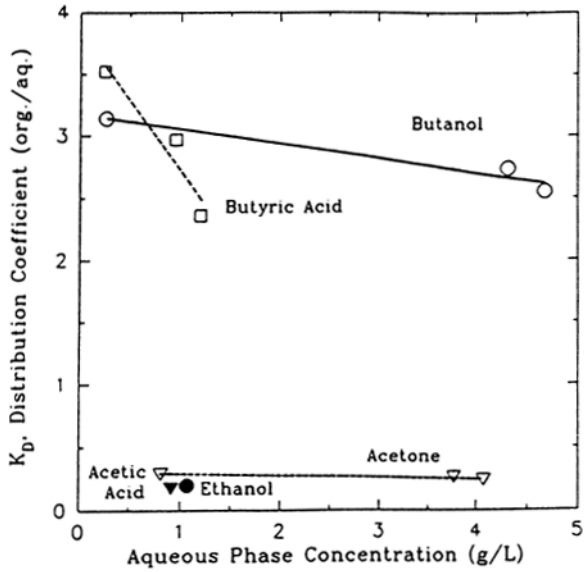

Fig. 2. Distribution coefficients of fermentation products in oleyl alcohol. ○ Butanol; □ butyric acid; ▽ acetone; ▼ acetic acid; ● ethanol.

were then analyzed by GC. Figure 2 shows some of the results from these tests. It can be seen that butanol and butyric acid have distribution coefficients >1. At a concentration near 5 g/L (aqueous), butanol has a distribution coefficient of 2.6, which increases to over 3 as the concentration decreases. These values are in approximate agreement with other reports (3.2 in Evans and Wang [*16*]; 4 in Ishii et al. [*15*]). Since butanol is the desired product and is present at the highest concentrations, it will be preferentially removed. The lesser components will also be removed during an extractive fermentation, but to a lesser degree. Glucose was not removed by the oleyl alcohol. The removal of the butyric acid may present a problem if the fermentation is not in the solventogenic phase.

Contactor Tests

A preliminary test of the contactor was performed. An aqueous mixture of butanol, acetone, ethanol, acetic acid, and butyric acid was

prepared and pumped through the 1-in id columnar reactor in one pass. Oleyl alcohol was added to the base of the reactor through a mixer. Both phases separated and were collected at the top exit of column at 30-min intervals. The aqueous residence time of the system was about 15 min. The total continuous system reached steady state within 60 min with an initial peak in the amount of butanol removed in the organic phase (because of the larger amount of uncontacted aqueous phase initially present when the organic flow began). The distribution coefficients remained relatively constant throughout the experiment, indicating a fast approach to equilibrium between the phases. The extraction was also tested in contact with suspended gel beads. The column was filled with κ-carrageenan gel beads and fluidized with an aqueous solution of 9.6 g/L butanol. A 10:1 organic:water dispersion was made and pumped into aqueous flow at the bottom of the column. The organic coalesced into ~3-mm droplets and quickly rose through the bed. The contact time was < 1 min. Samples of each phase were collected at the top of the column and analyzed by GC. After 1 min, the effluent butanol concentrations were 5.4 g/L aqueous and 13.7 g/L organic. After several residence times, the concentrations were 5.5 g/L aqueous and 14.3 organic for a distribution coefficient of 2.6 at an overall phase ratio of one part organic to three parts aqueous in the FBR. In another test, 5 mL of solvent were injected as a pulse directly into the FBR. The distribution coefficient of that extraction was also 2.6. This indicates that equilibrium can be reached between the phases in the fluidized bed during a short contact time.

Extractive Fermentations

Several long runs were performed using immobilized *C. acetobutylicum* in both columnar reactors. One 20-d run in the tapered column will be described in more detail. Preliminary experiments were performed in the straight 2.5-cm column. Preliminary experiments showed that a rapid extraction of butanol occurred with an apparent distribution coefficient of only 2.3. The lower apparent K_D indicates that equilibrium had not been completely reached in this experiment or that other materials in the media affect the K_D. Slugging due to gas production was also a problem necessitating the change to the tapered bed. Beads of alginate were found to be less stable in this fermentation.

The immobilized biocatalysts were placed in the column, and fed an anaerobic medium of glucose and other nutrients and salts at pH 6. There was no pH control or buffering. This run was operated with a continuous aqueous feedstream containing 40 g/L glucose for 23 d. The initial quantity of beads in the columnar reactor was 220 mL with a cell loading of 12 g dry wt/L. The beads were fluidized by the aqueous flow of about 3 mL/min; this flow rate was necessary to fluidize the bed.

The system was operated for a week with just the aqueous feedstream to establish a steady-state condition (Table 2). Approximately half of the glucose was consumed, and the pH fell to 4.5 at the effluent. The slight

Table 2
Butanol Production and Simultaneous Extraction in an FBR[a]

Flow rate, L/h		Aqueous concentration, g/L						Organic concentration, g/L					Butanol production rates	Ratio
Aqueous	Organic	Glucose	EtOH	Acetone	AcCOOH	BuOH	BuCOOH	EtOH	Acetone	AcCOOH	BuOH	BuCOOH	g/h	Butanol/total products g/g
0.14	0.00	19	0.7	1.5	0.7	3.7	0.5	-	-	-	-	-	0.52	0.52
0.14	0.15	19	0.4	1.3	0.6	1.8	0.3	0.1	0.3	0.07	3.3	0.2	0.76	0.61
0.18	0.00	20	0.6	1.7	0.6	3.5	0.4	-	-	-	-	-	0.63	0.52
0.18	0.24	20	0.5	1.7	0.6	1.6	0.3	0.09	0.4	0.05	3.0	0.3	1.02	0.57
0.11	0.00	21	0.5	1.7	0.7	4.1	0.4	-	-	-	-	-	0.45	0.56
0.11	0.48	20.5	0.3	1.3	0.7	0.8	0.2	0.04	0.2	0.0	1.5	0.0	0.81	0.67
0.17	0.00	17	0.4	1.6	0.9	4.1	0.3	-	-	-	-	-	0.68	0.54
0.17	0.23	15	0.4	1.8	0.9	2.1	0.3	0.09	0.5	0.0	4.5	0.2	1.36	0.65

[a]Organic extractant was oleyl alcohol. Aqueous feed was 40 g glucose/L. Each datum represents steady-state values with and without extraction.

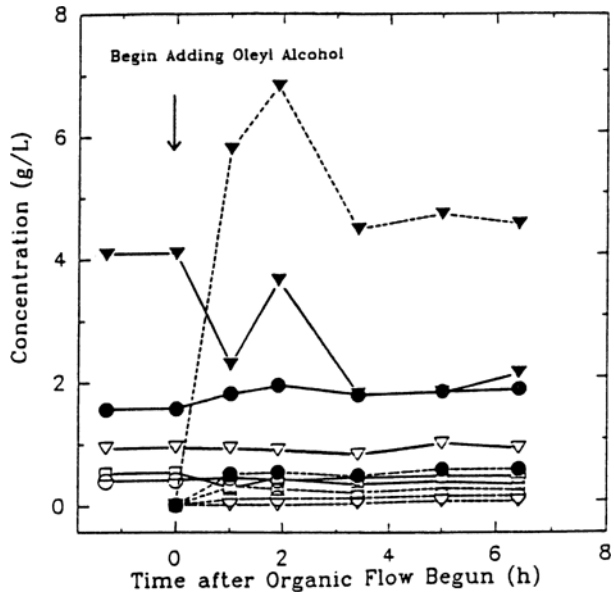

Fig. 3. Acetone-butanol fermentation with simultaneous extraction by oleyl alcohol (ratio organic:aqueous = 1.4) approach to steady state in continuous FBR. ▼ Butanol; ● acetone; ○ ethanol; ▽ acetic acid; □ butyric acid. Dashed lines ----- are organic concentrations; solid lines ——— are aqueous.

changes in the glucose concentration over the entire run are the result of the slow loss of beads in the effluent and the increase of the cell loading in the beads resulting from growth. The final amount of beads was 180 mL. After steady state was established, during four intervals over the next week, the flow of organic extractive solvent (oleyl alcohol) was begun into the fermenting columnar reactor. The oleyl alcohol was also sparged with nitrogen. The oleyl alcohol rapidly left the bed with an estimated residence time of <5 min. Thus, the aqueous residence time was not greatly changed. The extractive solvent was fed to the reactor for 6–8 h until a new apparent steady state was reached. Figure 3 shows a representative time-course of the transients for one of these experiments. This figure shows that the fermentation reached a new steady state in about 4 h after an initial overshoot in the butanol extraction. The final aqueous butanol concentration was lowered by more than half. Table 2 lists several of the steady-state operating conditions for this reactor. The distribution ratio was 1.9 during the extraction cycles. This indicates that the extraction is not reaching equilibrium during the extractive fermentation. This may be partially because of interference from the cells in solutions, since cells are known to be attracted to interfaces and can slow the mass transfer by physical shielding. Lower distribution ratios have been noted by others in cell-free fermentation broth and the cell-containing broth (*15*).

The volumetric productivities based on the reactor volume ranged from 0.6 to 0.9 g $L^{-1}h^{-1}$ for the continuous aqueous system and from 1.0 to 1.8 g $L^{-1}h^{-1}$ for the continuous extraction for this nonoptimized system. These values are significantly higher than traditional batch fermentation of <0.5 g $L^{-1}h^{-1}$ (*1*) and comparable to other optimized extraction schemes (i.e., 2.5 g $L^{-1}h^{-1}$ [*27*] and 1.0 g/L/h [*19*]).

As the organic-to-aqueous ratio increased, the aqueous butanol concentration was reduced from half its initial value to less than a fourth. The ethanol, acetone, and acetic acid were not greatly affected by the extraction because their distribution coefficients are lower than those of butanol. The total butanol production rate (measured in g/butanol produced/h) increased by 50–90% during the solvent extraction. The greatest increase was at the highest organic:aqueous ratio of 4.1:1, where the aqueous butanol concentrations were the lowest. Because the overall glucose conversion was not greatly changed by the solvent extraction, the increases in butanol production must be because of improvements in yield owing to the removal of the inhibition. The butyric acid is also extracted by the oleyl alcohol, and its total production also increased in the extractive reactor. More substrate is going to the desired product, butanol, and less to maintenance or other products. This can be seen in the 10–20% increases in butanol relative to all products in Table 2. In batch studies with oleyl alcohol, Evans and Wang (*16*) also observed increases in both butanol and butyric acid production, and in the overall yield in batch extraction.

The immobilized cell FBR was also operated in full aqueous recycle mode in order to test the limits of conversion. The changing glucose concentration was monitored as it was consumed. When the glucose was constant for more than 10 h, the fermentation was presumed to have halted, and oleyl alcohol was added and removed from the recirculating FBR. This was repeated until all the glucose was consumed (*see* Fig. 4). The other concentrations did not change significantly. It appears that butanol concentrations >6 g/L significantly reduce the fermentation and production of butanol. Under these conditions, very little organic acids, acetic or butyric, were produced and butanol was by far the major product. This further supports the benefit of removing the butanol from the immobilized *C. acetobutylicum* fermentation and indicates that (with a sufficiently long column) complete glucose conversion could be achieved with improved butanol yields.

CONCLUSIONS

An FBR with immobilized *C. acetobutylicum*, and direct simultaneous extraction and fermentation was tested for the first time. The butanol was removed in the continuous system by the nontoxic extractant, oleyl alcohol. The extractant preferentially removed the butanol over the other

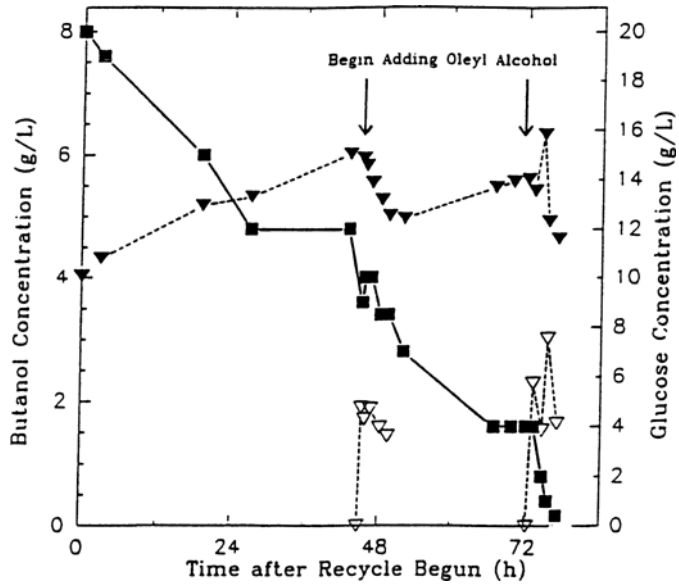

Fig. 4. Acetone-butanol fermentation with sequential extraction (FBR in complete recycle—batch). ■ Glucose (aqueous); ▼ butanol (aqueous); ▽ butanol (organic).

components, and butanol production was enhanced. The extractant removes the inhibitory product, butanol, from the system allowing more production by the bacteria. The system has shown 50–90% increase in product yield and butanol productivity over the nonextractive case. The overall productivities were as much as 1.8 g butanol $L^{-1}h^{-1}$, which are comparable to those of other advanced bioreactor schemes. Further optimization is necessary in the continuous system to increase the glucose conversion, which should be easily accomplished in a longer column.

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