

# Production of Acetone Butanol Ethanol (ABE) by a Hyper-Producing Mutant Strain of *Clostridium beijerinckii* BA101 and Recovery by Pervaporation

Nasibuddin Qureshi and Hans P. Blaschek\*

University of Illinois at Urbana-Champaign, Biotechnology and Bioengineering Group,  
Department of Food Science and Human Nutrition, 1207 W Gregory Drive, Urbana, Illinois 61801

A silicone membrane was used to study butanol separation from model butanol solutions and fermentation broth. Depending upon the butanol feed concentration in the model solution and pervaporation conditions, butanol selectivities of 20.88–68.32 and flux values of 158.7–215.4 g m<sup>-2</sup> h<sup>-1</sup> were achieved. Higher flux values (400 g m<sup>-2</sup> h<sup>-1</sup>) were obtained at higher butanol concentrations using air as sweep gas. In an integrated process of butanol fermentation–recovery, solvent productivities were improved to 200% of the control batch fermentation productivities. In a batch reactor the hyper-butanol-producing mutant strain *C. beijerinckii* BA101 utilized 57.3 g/L glucose and produced 24.2 g/L total solvents, while in the integrated process it produced 51.5 g/L (culture volume) total solvents. Concentrated glucose medium was also fermented. The *C. beijerinckii* BA101 mutant strain was not negatively affected by the pervaporative conditions. In the integrated experiment, acids were not produced. With the active fermentation broth, butanol selectivity was reduced by a factor of 2–3. However, the membrane flux was not affected by the active fermentation broth. The butanol permeate concentration ranged from 26.4 to 95.4 g/L, depending upon butanol concentration in the fermentation broth. Since the permeate of most membranes contains acetone, butanol, and ethanol (and small concentrations of acids), it is suggested that distillation be used for further purification.

## Introduction

Butanol is an important chemical and can be used as a feedstock for plastic industries and fuel and as an extraction solvent in the food industry. As a fuel, butanol has several advantages over other fermentatively produced fuels and these include high energy content, miscibility with diesel fuel, and a low vapor pressure. Butanol is produced by fermentation using *Clostridium acetobutylicum* or *C. beijerinckii*. The other solvents produced by these cultures include acetone or 2-propanol and ethanol. *C. acetobutylicum* fermentation was carried out from World War I and until after World War II. In 1945 over 60% of the total butanol produced was obtained by fermentation (1). By the 1960s, efficient production of acetone and butanol by the petrochemical industry in combination with higher costs of carbohydrate substrate sources saw the virtual elimination of industrial ABE fermentation. The last butanol fermentation plant (National Chemical Products, South Africa) was closed in 1982 (2) due to the increased cost of butanol recovery. The uncertainty of petroleum supplies and the finite nature of fossil fuels has revived research efforts aimed at obtaining liquid fuels from sources other than oil. In 1989, 4.5 billion pounds of butanol was produced from petrochemical processes.

During the early eighties, efficient reactor systems were developed to achieve high reactor productivities (3–4). However, these high reactor productivities were at the expense of low butanol concentration in the effluent streams and low sugar utilization. To solve these problems, product removal techniques were investigated for

the in-situ/online removal of butanol. These recovery techniques include adsorption (5–8), gas stripping (5, 9–12), liquid–liquid extraction (13–21), perstraction (22–26), pervaporation (27–32), and reverse osmosis (33). The details of these methods and their application to the ABE fermentation have been detailed elsewhere (34). These developments have played an important role in achieving high reactor productivities and high sugar utilization (up to 100%) (12, 35), and hence in improving the economics of butanol production by fermentation (36).

Recovery of butanol by pervaporation has received more attention than any other technique. Pervaporation results in an increased rate of solvent production, a higher rate of sugar utilization, and recovery and partial concentration of butanol and acetone. Pervaporation does not harm the culture (28, 29, 37). Although several membrane types have been studied (silicone [poly(dimethylsiloxane), PDMS], polypropylene, liquid (oleyl alcohol and polypropylene), poly(tetrafluoroethylene), and silicone membranes filled with silicalite; ref 37) for the removal of butanol from fermentation broth, silicone-based membranes are still considered to be the best available membranes.

Interestingly, a superior butanol-producing *C. beijerinckii* strain was developed in 1997 (38). This culture produces 23–27 g/L total solvents from 60 g/L glucose, with a yield of 0.39–0.45, compared to a yield of 0.29–0.33 for the parental strain. Further improvements to 33 g/L total solvents have been successful under optimized conditions (39). These improvements make the ABE fermentation process more attractive for several envi-

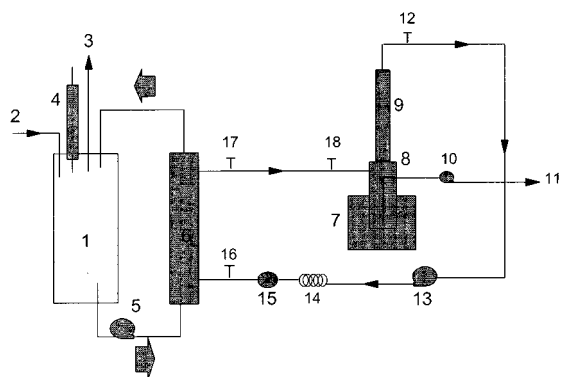


**Figure 1.** A schematic diagram of butanol production by *C. beijerinckii* BA101 and recovery by pervaporation: 1, reactor; 2, nitrogen gas in; 3, nitrogen gas out; 4, condenser; 5, fermentation broth recycle pump; 6, pervaporation membrane; 7, cooling bath; 8, condensate collector; 9, condenser; 10, product removal pump; 11, product (ABE); 12, thermometer; 13, gas recycle pump; 14, heat exchanger; 15, flow-meter; 16–18, thermometers.

ronmental and economic reasons (40). Prominent among the economic factors is the current surplus of agricultural wastes or byproducts that can be utilized as inexpensive fermentation substrates. One particular example is mycotoxin contaminated corn, which is unsuitable for use as animal feed and is difficult to dispose of. *C. beijerinckii* BA101, a hyperamylolytic strain, was developed by using *N*-methyl-*N'*-nitro-*N*-nitrosoguanidine (NTG) together with selective enrichment on the nonmetabolizable glucose analogue 2-deoxyglucose (41). *C. beijerinckii* BA101 is able to utilize starch and has an elevated level of amylolytic enzymes.

Although a superior culture has been developed, process engineering can still be applied to the ABE fermentation. In-situ/online recovery of butanol by pervaporation is expected to improve the rate of sugar utilization and will result in removal and partial concentration of acetone and butanol. The objective of this study was to utilize pervaporation in combination with the *C. beijerinckii* BA101 hyper-butanol-producing strain to further increase the efficiency of butanol production.

## Materials and Methods

**Organism.** *C. beijerinckii* BA101 was used in these studies (38, 41). The spores of the culture were stored in distilled water at 4 °C. The spores (0.1 mL) were heat shocked at 80 °C for 10 min in Trypticase–glucose–yeast extract (TGY) medium followed by incubation at 35 °C for 14–18 h. Five to ten milliliters of the actively growing cells were inoculated into 100 mL of P2 medium (38). TGY and P2 media were kept in an anaerobic chamber for 24 h prior to inoculation. P2 medium contained 60–80 g/L glucose, 1 g/L yeast extract, and stock solutions (mineral, vitamin, and buffer solutions). Glucose and yeast extract were sterilized at 121 °C for 15 min while stock solutions were filter-sterilized and added to the glucose–yeast extract solution when cooled to room temperature.

**Bioreactor and Cultivation.** A 1 L glass bioreactor (New Brunswick Scientific Co., New Brunswick, NJ) was used to cultivate the microorganism. The reactor containing glucose–yeast extract solution was sterilized at 121 °C for 15 min. After autoclaving, the medium was cooled to 35 °C under an oxygen-free nitrogen gas atmosphere. The reactor containing 700 mL of medium was inoculated with 35 mL of actively growing cells of *C. beijerinckii* BA101. Nitrogen gas was swept across the surface of the

medium until the culture initiated gas (CO<sub>2</sub> and H<sub>2</sub>) production on its own. The fermentation was run at 34 ± 1 °C. Samples were withdrawn regularly for glucose and solvent analysis. Soon after the removal of the samples, they were refrigerated at 4 °C followed by centrifugation at 13 000 rpm for 2 min. The clear supernatant was stored at –18 °C until analyzed for glucose and solvent production. Cells were suspended in saline water to measure optical density. When the total solvent concentration reached 4.5–13.5 g/L, pervaporation was applied to remove ABE. The pervaporation membrane was sterilized by circulating sterilized distilled water at 80–85 °C for 2 h. Following sterilization the membrane was cooled to 25–35 °C by passing oxygen-free nitrogen gas through the membrane. Sterilized distilled water was added to the fermentor to replace the lost volume through the pervaporation membrane. Chemically resistant tubing was used to connect the pervaporation membrane with the bioreactor. These tubes were sterilized using 50% (v/v) ethanol solution overnight followed by washing with presterilized (cooled to room temperature under oxygen-free nitrogen gas) distilled water. The condenser, which was used to condense the vapors from the reactor, was sterilized using 50% (v/v) ethanol overnight, followed by washing with sterile distilled water. Batch fermentation was allowed to proceed for 17–39 h after which continuous product removal commenced. The integrated fermentations were run with 80–150 g/L initial glucose concentrations. During the recovery process the fermentors were not fed with glucose or nutrient solution.

**Pervaporation Membrane.** The membrane area was 0.08 m<sup>2</sup> based on internal diameter (3.4 mm) of the tube. The details of the membrane module and operating conditions have been published elsewhere (42). For pervaporation optimization studies, the membrane module was operated at two different temperatures (43 and 80 °C). Air was used as a sweep gas to remove diffused butanol from the surface of the membrane and was circulated using a gas recycle pump (Regenair; Gast Manufacturing Corporation, Benton Harbor, MI). The flow rate of sweep gas was controlled at 2–18 L/min using a flow measuring device (Cole-Parmer Instrument Co., Vernon Hills, IL). The air was cooled to 0 to –15 °C in a condenser. The gas was used in a closed cycle and was circulated through a heat exchanger (copper tube 5 mm internal diameter and 4 m length, wound as a coil), flow device, pervaporation module, and condenser and back to the pump. A coolant (50% [v/v], ethylene glycol) was circulated through the condenser jacket at a flow rate of 600 mL/min. The condensate was collected in a round-bottom conical flask and was removed intermittently using a mini peristaltic pump (Fisher Scientific, Fair Lawn, NJ) and weighed in order to determine flux. Thermometers and pressure gauges were used to measure the temperatures and pressures of various streams.

The butanol model solution was circulated through the pervaporation membrane at a flow rate of 500 mL/min, while active fermentation broth was circulated at a flow rate of 100–150 mL/min. This was done using a Masterflex pump (Cole-Parmer Instrument Co., Vernon Hills, IL). The circulation rate of the fermentation broth was kept low because the *C. beijerinckii* culture is affected by high circulation rate, which causes agitation. The temperature difference between the module inlet and outlet was 1–2 °C. Figure 1 shows a schematic diagram of the apparatus used for butanol removal from the fermentation broth of *C. beijerinckii* BA101. For the

**Table 1. Removal of Butanol from Model Solution by Pervaporation<sup>a</sup>**

| run no. | butanol concentration (g/L) |       | feed temp. (°C) | condensation temp. (°C) | sweep gas flow rate (L/h) | selectivity (—) | flux (g m <sup>-2</sup> h <sup>-1</sup> ) |
|---------|-----------------------------|-------|-----------------|-------------------------|---------------------------|-----------------|-------------------------------------------|
|         | initial                     | final |                 |                         |                           |                 |                                           |
| 1.      | 34.8                        | 4.9   | 43              | -14                     | 120                       | 25.37–37.18     | 27.8–33.6                                 |
| 2.      | 36.4                        | 5.4   | 43              | 0                       | 120                       | 23.39–30.12     | 30.6–38.6                                 |
| 3.      | 30.7                        | 0.3   | 80              | -14                     | 120                       | 29.30–75.51     | 98.0–146.0                                |
| 4.      | 33.0                        | 1.3   | 43              | -14                     | 1080                      | 25.19–58.19     | 69.0–105.0                                |
| 5.      | 37.6                        | 1.9   | 80              | -14                     | 1080                      | 20.88–68.32     | 158.7–215.4                               |

<sup>a</sup> For the effect of condensation temperature, compare runs 1 and 2. For feed temperature effect, compare runs 1 and 3 and runs 4 and 5. For the effect of sweep gas flow rates, compare runs 1 and 4 and runs 3 and 5.

recovery of butanol from the fermentation broth, oxygen-free nitrogen gas was used as a sweep gas.

**Analyses.** Acetone, butanol, ethanol, and acids (acetic and butyric) were determined by gas chromatography (Hewlett-Packard Gas Chromatograph 6890) using a flame ionization detector and a capillary column (cross-linked FFAP; dimension 30 m × 0.53 mm and film thickness 1 μm). Glucose was measured enzymatically using a Sigma Diagnostics Kit, Glucose HK (Sigma Chemicals, St. Louis, MO). For these determinations absorbances were measured at 340 nm using a Beckman DU-40 spectrophotometer. The reactors were monitored and controlled by the simultaneous injection of samples for ABE and glucose analysis. If needed, gas flow rates were varied to control the system. Cell concentration was determined by measuring the optical density at 540 nm. For the batch fermentor (without recovery) the solvent productivity was calculated as the total solvent produced in the culture (g/L) divided by the total fermentation time. For this system, yield was calculated as total solvents produced divided by total glucose utilized.

**Material Balance Equations.** For calculations of the different fermentation and pervaporation parameters, the following mass balance equations were used.

The productivity/rate of solvent production, yield and glucose utilization were calculated using the following equations:

$$R_p = [V_e C_s + V_t C_{st} + \Sigma(V_1 C_{s1} + V_2 C_{s2} + \dots + V_n C_{sn})]/V_e h \quad (1)$$

$$Y_{p/s} = [V_e C_s + V_t C_{st} + \Sigma(V_1 C_{s1} + V_2 C_{s2} + \dots + V_n C_{sn})]/[V_e (C_{go} - C_{ge})] \quad (2)$$

$$R_g = (C_{go} - C_{ge})/h \quad (3)$$

The selectivity and flux were calculated using eqs 4 and 5.

$$\alpha = [y/(1 - y)]/[x/(1 - x)] \quad (4)$$

$$F_t = W/Ah_t \quad (5)$$

## Results

**Membrane Characterization.** The silicone membrane was characterized using 30.7–37.6 g/L butanol model solution in distilled water. The final butanol concentrations in the experiments were 0.3–5.4 g/L (Table 1). Feed temperature, rate of sweep gas, and condensation temperatures were altered, and their effects on selectivity and flux were evaluated. Feed temperature (43 °C) and sweep gas flow rate (120 L/h) were kept constant and condensation temperatures were changed to evaluate the effect of condensation temperature. For the first experiment, condensation temperature was kept at -14 °C. In the butanol concentration range of 4.9–34.8 g/L, the selectivities and flux were 25.37–37.18 and

27.8–33.6 g m<sup>-2</sup> h<sup>-1</sup>, respectively (run 1). At 0 °C condensation the selectivity and flux were 23.39–30.12 and 30.6–38.6 g m<sup>-2</sup> h<sup>-1</sup>, respectively (run 2). The results suggested that there was not a significant effect of condensation temperature on the selectivity. However, the flux values were similar.

Next, condensation temperature and the flow rate of sweep gas were kept constant at -14 °C and 120 L/h, respectively, and feed temperature was raised to 80 °C. This resulted in selectivity and flux of 29.30–75.51 and 98.0–146.0 g m<sup>-2</sup> h<sup>-1</sup>, respectively (run 3). An additional experiment was run at a high gas flow rate (run 4). Compared to run 1, both selectivity and flux improved. After this experiment, the effects of high feed temperature and high rate of sweep gas were evaluated (run 3 and 5). At a flow rate of 1080 L/h and 80 °C feed temperature, the flux and selectivity were 158.7–215.4 g m<sup>-2</sup> h<sup>-1</sup> and 20.88–68.32, respectively.

An experiment was run at various butanol concentrations (0.8–58.8 g/L), high feed temperature (80 °C), and high sweep gas rate (1080 L/h). The results of this experiment are shown in Figure 2. At 55 g/L butanol, total flux was over 400 g m<sup>-2</sup> h<sup>-1</sup> and selectivity was 19.0. At 0.8 g/L butanol, selectivity was between 49.68 and 75.89 and flux was 137.5 g m<sup>-2</sup> h<sup>-1</sup>. Between butanol concentrations of 2.5 and 15 g/L, flux was almost constant at 137.5 g m<sup>-2</sup> h<sup>-1</sup>. As butanol concentration increased to 55 g/L, flux sharply increased. With the increase in butanol concentration, selectivity decreased. For a system where sweep gas is used, these flux and selectivity values are high.

**Batch Fermentation and Recovery.** A batch fermentation experiment was run with 59.8 g/L initial glucose in P2 medium using *C. beijerinckii* BA101. Over the course of 72 h, the culture produced 19.6 g/L butanol, 4.3 g/L acetone, and 0.3 g/L ethanol, resulting in a total solvent concentration of 24.2 g/L and a yield of 0.42 (Figure 3). At the end of fermentation, the unused sugar was 2.6 g/L. This demonstrated that the culture could not utilize the remaining sugar because of toxic effect of butanol. The maximum cell concentration was 1.70 g/L at 41 h. The average rate of cell growth was 0.04 g L<sup>-1</sup> h<sup>-1</sup>. The solvent productivity was 0.34 g L<sup>-1</sup> h<sup>-1</sup>. At the end of fermentation, acids were at 0.4 g/L. In this experiment the cell growth lag phase was 6 h. The values of various kinetic parameters are given in Table 2.

To produce and remove butanol from a batch reactor of *C. beijerinckii* BA101, we started a batch fermentation with an initial glucose concentration of 78.2 g/L and yeast extract added at 1 g/L in P2 medium. After 17 h of cell growth and fermentation, the culture accumulated 3.2 g/L butanol and 1.4 g/L acetone (Figure 4b). At this time glucose concentration in the reactor was 60.2 g/L (Figure 4a). At this stage butanol and acetone removal by pervaporation was started and continued until there was no glucose left in the reactor. At zero time, acid concentration was 1.6 g/L, which was reduced to 0.7 g/L at the

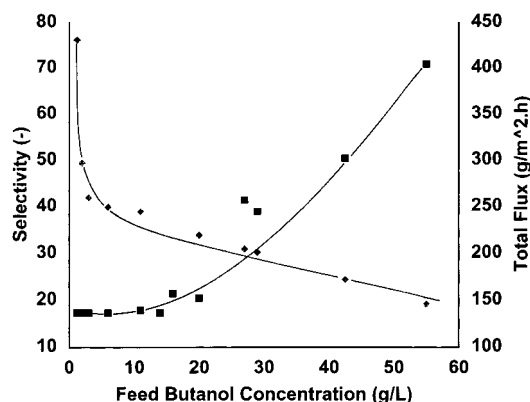


**Figure 2.** Butanol flux and selectivities at various butanol concentrations in model butanol solution:  $\diamond$ , butanol selectivity;  $\blacksquare$ , total flux.

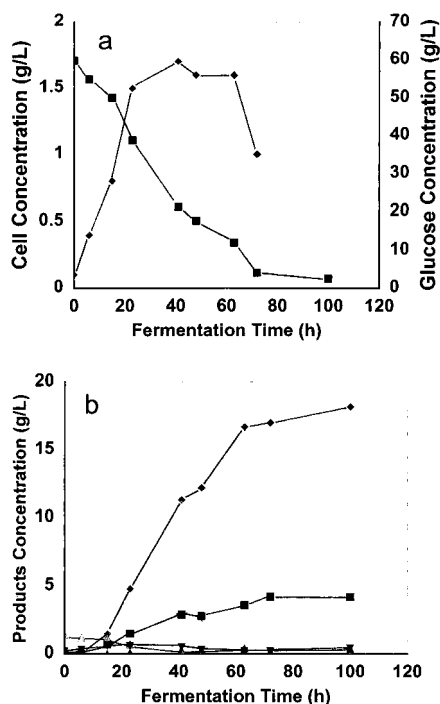


**Figure 3.** Production of acetone butanol ethanol in a batch reactor employing *C. beijerinckii* BA101 grown in P2 medium plus 60 g/L glucose: (a)  $\blacksquare$ , glucose concentration;  $\diamond$ , cell concentration; (b)  $\diamond$ , butanol;  $\blacksquare$ , acetone;  $\blacktriangle$ , ethanol;  $\triangle$ , acetic acid;  $\nabla$ , butyric acid.

end of 65 h fermentation and 0 g/L at the end of 89 h. The culture took about 65 h to utilize all of the sugar, thus producing 32.8 g/L total solvents (Table 3). The cell concentration in the reactor was 4.4 g/L at 41 h. The rates of sugar utilization and solvent production at various times are shown in Figure 4c. During the initial fermentation period (without product removal), the rate of sugar utilization was  $1.06 \text{ g L}^{-1} \text{ h}^{-1}$ , which increased to  $1.48 \text{ g L}^{-1} \text{ h}^{-1}$  after the recovery was started. During the recovery process the average rate of glucose utilization and the average rate of solvent production were 1.20 and  $0.50 \text{ g L}^{-1} \text{ h}^{-1}$ , respectively. For these calculations, 65 h has been taken as the fermentation time. The average values of the various fermentation parameters are given in Table 3.

During ABE removal from the fermentation broth, condensate samples were taken to measure total flux and selectivities of various components at different time intervals. Butanol selectivities ranged from 7.34 to 17.45, and the total flux ranged from  $23.2$  to  $27.1 \text{ g m}^{-2} \text{ h}^{-1}$ .

Acetone, ethanol, and butyric acid were also removed during the separation process. The selectivities of acetone, ethanol, and butyric acid ranged from 1.46 to 7.44, 1.31 to 3.26, and 1.66 to 3.28, respectively. Acetic acid was identified only in the first pervaporate sample, and its selectivity was 1.61. Butanol concentration in the pervaporate varied from 21.5 to 49.2 g/L.

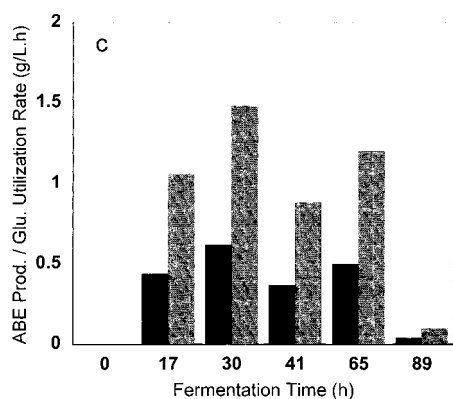
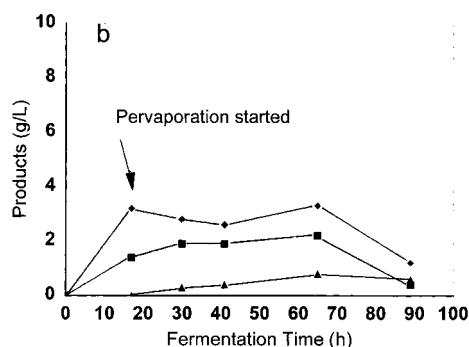
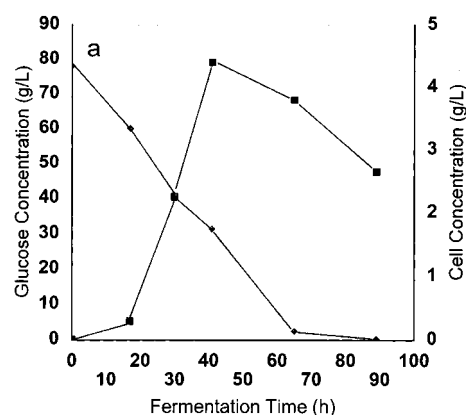
Further, to economize the batch fermentation, we produced butanol in 102.3, 158.6, and 200 g/L glucose P2 medium containing 1 g/L yeast extract (Table 2). The results of butanol production from 60 g/L glucose using *C. beijerinckii* BA101 are given in Table 2. At 102.3 g/L initial glucose the culture produced 23.0 g/L solvents and utilized 53.5 g/L glucose. At the end of fermentation 48.8 g/L glucose was left in the reactor. Similarly, at 158.6 g/L initial glucose the culture utilized 48.6 g/L glucose and produced 21.9 g/L total solvents. In all the three experiments solvent yields were 0.42–0.45. At 200 g/L glucose only 1.7 g/L total solvents were produced and the maximum cell concentration was 0.35 g/L. To compare the effect of high glucose concentration on butanol production, we plotted butanol concentrations for these glucose concentrations in Figure 5. The highest concentration of butanol was achieved at 60 g/L initial glucose concentration followed by 102.3 and 158.6 g/L, respectively. Figure 5 shows that fermentation was slow when using 158.6 g/L glucose. At 250 g/L initial glucose there was no growth. From the raw data, the maximum rates of butanol production were calculated and these are plotted in Figure 6. Up to a glucose concentration of 100 g/L, a constant butanol production rate of  $0.45 \text{ g L}^{-1} \text{ h}^{-1}$  was observed. At glucose concentrations higher than 100 g/L, lower rates of butanol production were observed.

Product recovery can be applied to produce butanol from concentrated substrates, where product is removed before it reaches a toxic level. To examine this, we initiated fermentation with 151.2 g/L initial glucose in P2 medium. To overcome growth inhibition, we increased the inoculum level to 15% (v/v). For 39 h the fermentation was allowed to proceed without product removal. At that time butanol concentration in the reactor was 9.7 g/L and total solvent concentration was 13.5 g/L. The cell concentration in the reactor was 0.6 g/L. The reactor productivity during this 39 h period was  $0.35 \text{ g L}^{-1} \text{ h}^{-1}$ , which is close to the productivity of a batch reactor operated at an initial glucose level of 60 g/L. At that time product removal was initiated by pervaporation using a membrane with  $0.16 \text{ m}^2$  surface area. The glucose concentration in the reactor at 39 h was 120 g/L. The concentrations of ABE, cells, and glucose are shown in Figure 7a,b. Fermentation product removal was continued for 75 h. At that time the fermentation stopped as shown by cell growth and glucose utilization. In the reactor, 30.4 g/L glucose remained unused. The rates of glucose utilization and solvent production are shown in Figure 7c. As soon as the product removal was started, the rates of glucose utilization and of solvent production increased to  $3.73$  and  $1.57 \text{ g L}^{-1} \text{ h}^{-1}$ , respectively. At about 75 h fermentation stopped, while pervaporation continued until 90 h.

During the recovery, acetone selectivity ranged from 8.41 to 10.23 (selectivity of 1.74 ignored due to dilution), while that for ethanol ranged from 3.11 to 10.20. The selectivity of butanol ranged from 9.27 to 18.80 (Table 4). Acetic and butyric acids were identified only in the first samples, and their selectivities were 5.9 and 7.34, respectively. Flux ranged from  $25.1$  to  $31.0 \text{ g m}^{-2} \text{ h}^{-1}$ . The first reading of the flux was high because some water

**Table 2. Various Kinetic Parameters for ABE Production from Concentrated Substrate in Batch Reactors Containing *C. beijerinckii* BA101**

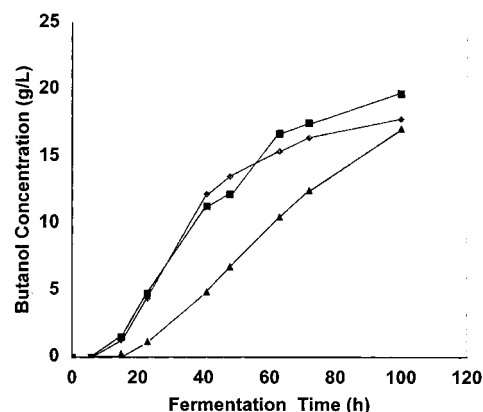
| parameter                                                        | glucose <sup>a</sup><br>(60 g/L) | glucose<br>(102 g/L) | glucose<br>(158.6 g/L) | glucose<br>(200 g/L) |
|------------------------------------------------------------------|----------------------------------|----------------------|------------------------|----------------------|
| total ABE produced (g/L)                                         | 24.2                             | 23.0                 | 21.9                   | 1.7                  |
| solvent yield (g/g)                                              | 0.42                             | 0.43                 | 0.45                   |                      |
| solvent productivity (g L <sup>-1</sup> h <sup>-1</sup> )        | 0.34                             | 0.23                 | 0.16                   |                      |
| acids produced (g/L)                                             | 0.4                              | 0.8                  | 0.2                    | 1.8                  |
| initial glucose (g/L)                                            | 59.8                             | 102.3                | 158.6                  | 200                  |
| final glucose (g/L)                                              | 2.6                              | 48.8                 | 110.0                  |                      |
| utilized glucose (g/L)                                           | 57.3                             | 53.5                 | 48.6                   |                      |
| glucose utilization (%)                                          | 95.71                            | 52.3                 | 30.65                  |                      |
| glucose utilization rate (g L <sup>-1</sup> h <sup>-1</sup> )    | 0.80                             | 0.54                 | 0.49                   |                      |
| max. cell concentration (g/L) at (h)                             | 1.70 (41)                        | 1.70 (41)            | 1.60 (72)              | 0.35 (100)           |
| average rate of cell growth (g L <sup>-1</sup> h <sup>-1</sup> ) | 0.04                             | 0.04                 | 0.02                   |                      |
| lag time for cell growth (h)                                     | 6                                | 15                   | 23                     | 63                   |
| total fermentation time (h)                                      | 72                               | 100                  | 100                    | 140                  |

<sup>a</sup> Control experiment.**Figure 4.** Production of acetone butanol ethanol in an integrated batch reactor using *C. beijerinckii* BA101 and recovery by pervaporation: (a) ◇, glucose concentration; ■, cell concentration; (b) ◇, butanol; ■, acetone; ▲, ethanol; (c) ■, solvent production; □, glucose utilization rates.

condensate was left in the condenser during the sterilization process.

**Table 3. Values of Various Fermentation Parameters for an Integrated ABE Fermentation Product Recovery Process<sup>a</sup>**

|                                                               |                    |
|---------------------------------------------------------------|--------------------|
| total ABE produced (g/L)                                      | 32.8               |
| solvent yield (g/g)                                           | 0.42               |
| ABE production rate (g L <sup>-1</sup> h <sup>-1</sup> )      | 0.50               |
| initial glucose concentration (g/L)                           | 78.2               |
| final glucose concentration (g/L)                             | 0.3                |
| glucose utilization rate (g L <sup>-1</sup> h <sup>-1</sup> ) | 1.20               |
| glucose utilized (%)                                          | 100                |
| acids produced (g/L)                                          | 0.0                |
| maximum cell concentration (g/L)                              | 4.4                |
| cell yield (g/g)                                              | <i>b</i>           |
| total fermentation time (h)                                   | 65–89 <sup>c</sup> |

<sup>a</sup> ABE production in a batch reactor using *C. beijerinckii* BA101.<sup>b</sup> Not calculated. <sup>c</sup> Glucose utilized by 65th h and residual acids utilized by 89th h.**Figure 5.** Production of butanol by *C. beijerinckii* BA101 in a batch reactor containing P2 medium plus glucose added at various concentrations: ■, 60 g/L glucose; ◇, 102 g/L glucose; ▲, 158 g/L glucose.

In the fermentation product recovery integrated system, 51.5 g/L total solvents (Table 5) were produced compared to 24.2 g/L in a batch fermentation without recovery (Table 2). The solvent yield was 0.42–0.44. The initial glucose level in the reactor was 151.2 g/L, and the final glucose level was 30.0 g/L. Interestingly, no acids accumulated at the end of fermentation. The cell concentration in the reactor was 2.80 g/L at 90 h.

In the above fermentation, all of the sugar was not utilized and the fermentation stopped when the sugar level was 30 g/L. To examine why the fermentation stopped, we centrifuged the fermentation broth at 8000 rpm for 10 min and removed the supernatant. Glucose was added to the supernatant to make the final glucose concentration of 75–80 g/L. Then the supernatant was

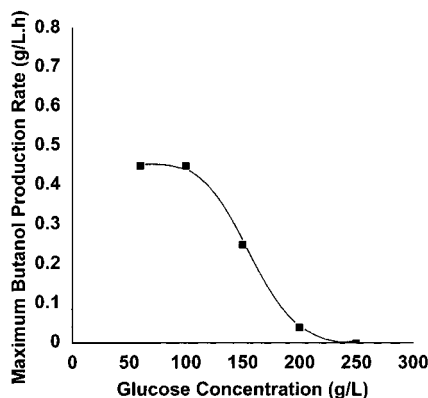


**Figure 6.** Maximum butanol production rates at various glucose concentrations in batch reactors of *C. beijerinckii* BA101.

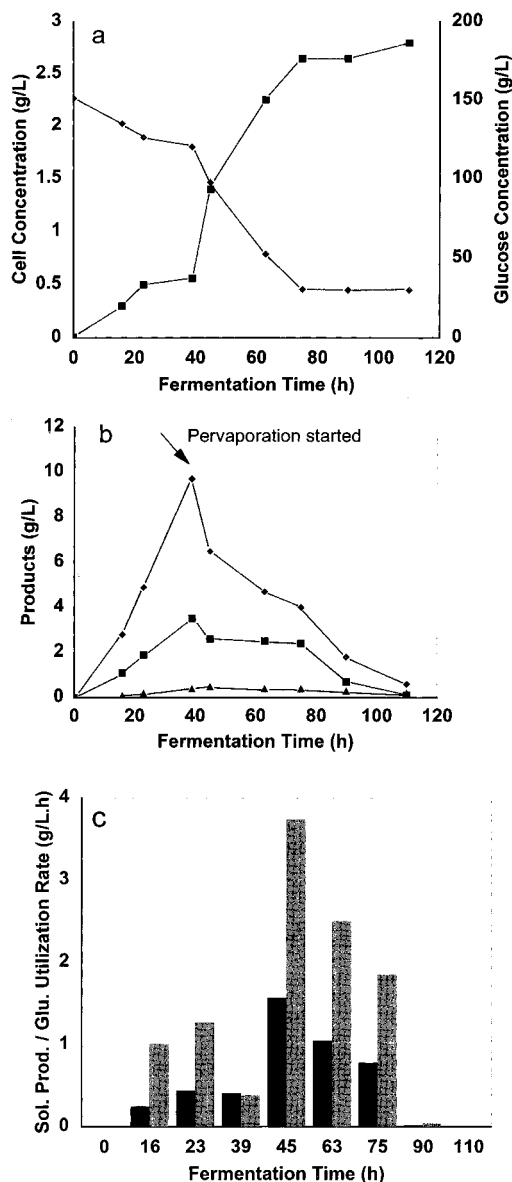


**Figure 7.** Production of acetone butanol ethanol by *C. beijerinckii* BA 101 in an integrated batch reactor and recovery by pervaporation: (a) ◇, glucose concentration; ■, cell concentration; (b) ◇ butanol; ■, acetone; ▲, ethanol; (c) ■, solvent production; □ glucose utilization rates.

divided into two equal volumes. To one volume was added 1 g/L yeast extract and P2 medium stock solutions, while no nutrients were added to the other volume. Both of the

bottles (containing 115 mL of supernatant in each bottle) were sterilized as described in Materials and Methods. On cooling to room temperature (in an anaerobic chamber), both of the bottles were inoculated with 10 mL of freshly grown cells and the fermentation was initiated. The cell growth was vigorous in the bottle containing nutrients. Figure 8 shows products and glucose utilization in the bottle containing nutrients at various times. Between 55 and 65 h, the fermentation stopped and at that time the total solvent concentration was 25.1 g/L of which 19.6 g/L was butanol. Total solvent yield was 0.42. During the fermentation the culture produced 0 g/L total acids. In the bottle without nutrients, the maximum cell concentration was 0.4 g/L and 1.12 g/L solvents were produced. This culture became acidogenic and produced 3.2 g/L acids. The initial and final glucose concentrations in the bottle not containing nutrients were 78.7 and 76.0 g/L, respectively.

## Discussion

The results on membrane characterization suggested that there was a significant effect of feed temperature and sweep gas on butanol flux and selectivity. The flux increased with an increase in temperature because of an increase in vapor pressure of butanol at high temperature. Such an increase in flux and selectivity (with temperature) has been investigated for silicone and silicalite membranes (silicone membrane filled with silicalite) (37). A higher rate of sweep gas also improved selectivity and flux. This was due to efficient removal of diffused butanol from the membrane surface. A combination of high temperature and high rate of sweep gas increased the flux to  $215.4 \text{ g m}^{-2} \text{ h}^{-1}$ . Application of a vacuum would give a much higher flux than the sweep gas. On the basis of our experience, it appears that this membrane has the potential to be an industrial membrane for butanol separation. With spent fermentation broth the membrane did not foul (42). In this article we compared butanol selectivity and total flux values for spent fermentation broth and active fermentation broth. Selectivity decreased by a factor of 2–3 with the active fermentation broth. Such a decrease in selectivity (180 to 66) has been reported for liquid membranes (31). At this stage there is no explanation for this decrease in selectivity. It is interesting to note that the flux remained unchanged as compared to model butanol solution in water or spent fermentation broth. Larrayoz and Puigjaner (29) studied butanol pervaporation in a batch fermentation. However, they did not report membrane fouling with fermentation broth.

A control batch fermentation of acetone butanol ethanol resulted in 2.6 g/L unused sugar at the end of fermentation due to butanol toxicity. To avoid solvent toxicity, we initiated a fermentation product removal system with 78.2 g/L initial glucose. As can be seen in Figure 4a, there was no glucose left at the end of 65 h, suggesting that butanol toxicity was reduced. The average solvent productivity in the integrated system was  $0.50 \text{ g L}^{-1} \text{ h}^{-1}$ , compared to the control experiment average solvent productivity of  $0.34 \text{ g L}^{-1} \text{ h}^{-1}$ . This increase in productivity occurred for two reasons: First, there was decreased butanol inhibition due to low butanol concentration (less than 4 g/L) in the fermentation broth, and second, there was a high cell concentration (4.4 g/L) in the reactor. Furthermore, there was no adverse effect of pervaporation on the *C. beijerinckii* BA101 mutant culture.

In the integrated experiment, both acetone and ethanol were removed from the culture broth efficiently. The

**Table 4. Selectivities and Flux in an Integrated Process of ABE Production Using *C. beijerinckii* BA101 and Pervaporation<sup>a</sup>**

| time of<br>PV sample<br>collection (h) | AC <sub>2</sub> O<br>(g/L) | AC <sub>2</sub> O<br>(α) | BuOH<br>(g/L) | BuOH<br>(α) | EtOH<br>(g/L) | EtOH<br>(α) | HAc<br>(g/L) | HAc<br>(α) | HBu<br>(g/L) | HBu<br>(α) | flux<br>(g m <sup>-2</sup> h <sup>-1</sup> ) |
|----------------------------------------|----------------------------|--------------------------|---------------|-------------|---------------|-------------|--------------|------------|--------------|------------|----------------------------------------------|
| 45                                     | 5.3                        | 1.74                     | 67.0          | 8.83        | 2.1           | 4.80        | 1.7          | 5.90       | 0.7          | 7.34       | 34.8                                         |
| 63                                     | 21.2                       | 8.41                     | 95.4          | 18.80       | 3.0           | 7.86        | 0.0          | 0.00       | 0.0          | 0.00       | 29.3                                         |
| 75                                     | 24.8                       | 10.23                    | 45.7          | 10.95       | 3.5           | 10.20       | 0.0          | 0.00       | 0.0          | 0.00       | 31.0                                         |
| 90                                     | 0.9                        |                          | 26.4          | 9.27        | 0.8           | 3.11        | 0.0          | 0.00       | 0.0          | 0.00       | 25.1                                         |

<sup>a</sup> AC<sub>2</sub>O, acetone; BuOH, butanol; EtOH, ethanol; HAc, acetic acid; HBu, butyric acid; PV, pervaporation.

**Table 5. Values of Kinetic Parameters in an Integrated ABE Production Product Recovery System<sup>a</sup>**

|                                                               |           |
|---------------------------------------------------------------|-----------|
| total ABE produced (g/L)                                      | 51.5      |
| solvent yield (g/g)                                           | 0.42–0.44 |
| ABE production rate (g L <sup>-1</sup> h <sup>-1</sup> )      | 0.69      |
| acids produced (g/L)                                          | 0.0       |
| initial glucose (g/L)                                         | 151.2     |
| final glucose (g/L)                                           | 30.0      |
| glucose utilization rate (g L <sup>-1</sup> h <sup>-1</sup> ) | 1.61      |
| glucose utilized (%)                                          | 80.15     |
| maximum cell concentration (g/L) at (h)                       | 2.80 (90) |
| rate of cell growth (g L <sup>-1</sup> h <sup>-1</sup> )      | 0.04      |
| cell yield (g/g)                                              |           |

<sup>a</sup> ABE production in batch reactor containing *C. beijerinckii* BA101 and recovery by pervaporation.

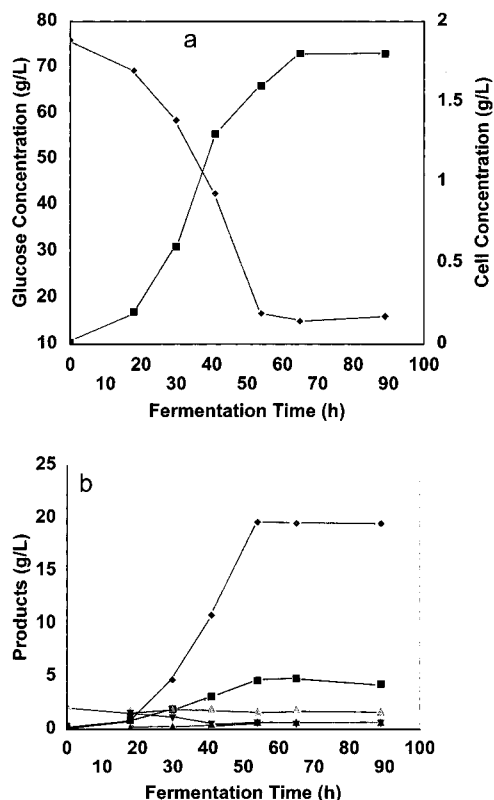


**Figure 8.** Production of acetone butanol ethanol by *C. beijerinckii* BA101 in batch reactor. Fermentation of supernatant of high glucose concentration batch reactor effluent supplemented with nutrients: (a) ◇, glucose; ■, cell; (b) ◇, butanol; ■, acetone; ▲, ethanol; △, acetic acid; ▽, Butyric acid.

selectivity of butanol decreased with the application of active fermentation broth as compared to model butanol solution (Table 1) and spent fermentation broth (42). With the model butanol solution and spent fermentation broth, butanol selectivities were similar. We do not know the reason why butanol selectivity decreased when using the active fermentation broth. While using active fermentation broth, butyric acid diffused through the membrane and its selectivities ranged from 1.47 to 6.20. Acetic

acid was identified only in the first pervaporate sample, and its selectivity was 1.61. Acid removal during the pervaporation process using polypropylene membrane (25), silicalite-filled silicone membrane (butyric acid) (37), and silicone membrane (butyric acid) has been reported (43).

Culture growth was inhibited above 100 g/L initial glucose and strongly inhibited above 160 g/L initial glucose level. The maximum butanol production rates decreased with increased glucose concentrations in the fermentation broth (Figure 6). Substrate inhibition is another commonly known problem in solvent fermentations. *C. beijerinckii* BA101 hyper-butanol-producing mutant strain differs in osmo-tolerance when compared to the *Clostridium acetobutylicum* P262 industrial strain (35), which was able to tolerate 300–350 g/L lactose in whey permeate. Further attempts are continuing to make the *C. beijerinckii* BA101 culture osmo-tolerant.

In the integrated fermentation containing concentrated glucose medium the average rates of solvent production and glucose utilization were 0.69 and 1.61 g L<sup>-1</sup> h<sup>-1</sup> (Table 5), respectively, which are much higher (200%) than the rates achieved in the control experiment (Table 2). Furthermore, application of concentrated substrates would reduce the size of the fermentation equipment and the volume of waste disposal streams. The selectivities of acetone butanol and ethanol were similar as in the previous fermentation product recovery experiment. Although there was 30 g/L glucose at 75 h, the fermentation stopped. Several reasons may be the cause for the cessation of fermentation, including diffusion of oxygen through the connecting tube, lack of nutrients, and the effect of culture recycle through the pump. However, further experiments showed that the culture became deficient in nutrients (Figure 8). In this experiment (supplemented with nutrients), the final concentration of total solvents was similar to the total solvents in the control experiment.

It is interesting to note that the ABE yield of 0.41–0.45 was obtained with the *C. beijerinckii* BA101 culture. These yields are much higher than the *C. beijerinckii* 8052 parental strain yields of 0.29–0.33 in batch culture. This would economize the process of making butanol from renewable resources. The values of yield and productivity in integrated experiments were calculated using material balance equations.

## Conclusions

At a feed temperature of 43 °C and a sweep gas flow rate of 120 L/h, membrane selectivities of 25.37–37.18 and flux values of 27.8–33.6 g m<sup>-2</sup> h<sup>-1</sup> were achieved using model butanol solution in water. High selectivities (20.88–68.32) and high flux values (158.7–215.4 g m<sup>-2</sup> h<sup>-1</sup>) were obtained at 80 °C feed temperature and 1080 L/h sweep gas flow rate. The flux value of 400 g m<sup>-2</sup> h<sup>-1</sup> has been obtained at high butanol concentration (55 g/L).

The *C. beijerinckii* BA101 strain produced 24.2 g/L total solvents from 57.3 g/L glucose and had a productiv-

ity of  $0.34 \text{ g L}^{-1} \text{ h}^{-1}$  and a yield of 0.42. Glucose concentrations higher than 100 g/L inhibited cell growth. Above 200 g/L glucose level, the culture did not grow. Butanol fermentation recovery experiments demonstrated high productivities (200% of control batch fermentation), and higher glucose utilization was achieved. In the integrated experiments, acids did not accumulate, as all the acids were converted into acetone and butanol. The culture produced 51.5 g/L total solvents in the integrated experiment.

Butanol selectivities of 8.83–18.80 were obtained with the active culture. These selectivities are about one-half to one-third that of the model solution in water. This resulted in a decreased butanol concentration in the permeate; however, flux remained constant. The use of vacuum will enhance flux manyfold. The permeate concentration of butanol ranged from 26.4 to 95.4 g/L, depending upon the butanol concentration in feed.

### Acknowledgment

This work was supported, in part, by the Illinois Corn Marketing Board grant ICMB Blaschek and Hatch 50-304FSHN.

### Notation

|           |                                                                                                                  |
|-----------|------------------------------------------------------------------------------------------------------------------|
| $A$       | pervaporation membrane area ( $\text{m}^2$ )                                                                     |
| $F_t$     | total flux ( $\text{g m}^{-2} \text{ h}^{-1}$ )                                                                  |
| $C_{ge}$  | glucose concentration in the reactor at the end of the fermentation (g/L)                                        |
| $C_{go}$  | glucose concentration in the reactor at time 0 (g/L)                                                             |
| $C_s$     | concentration of solvent (g/L)                                                                                   |
| $C_{st}$  | concentration of solvent in the condensate recovered from the top of the reactor (g/L)                           |
| $C_{sn}$  | concentration of solvent in $n$ th condensate ( $n = 1, 2, 3, \dots, n$ ) (g/L)                                  |
| $h$       | fermentation time (h)                                                                                            |
| $h_r$     | time period during which permeate $W$ was collected (h)                                                          |
| $R_g$     | glucose utilization rate ( $\text{g L}^{-1} \text{ h}^{-1}$ )                                                    |
| $R_p$     | solvent production rate ( $\text{g L}^{-1} \text{ h}^{-1}$ )                                                     |
| $V_e$     | liquid volume in the reactor at the end of fermentation (L)                                                      |
| $V_t$     | condensate volume recovered from the effluent fermentation gases (L)                                             |
| $V_n$     | volume of $n$ th condensate recovered from the condensate receiver (pervaporate) ( $n = 1, 2, 3, \dots, n$ ) (L) |
| $x$       | weight fraction of butanol/solvent in reactor (—)                                                                |
| $y$       | weight fraction of butanol/solvent in pervaporate (—)                                                            |
| $Y_{p/s}$ | butanol or solvent yield (g/g)                                                                                   |
| $W$       | weight of permeate condensate collected in time $h_r$ (g)                                                        |
| $\alpha$  | butanol or solvent selectivity (—)                                                                               |

### References and Notes

- Rose, A. H. *Industrial Microbiology*; Butterworths: London, 1961; pp 160–166.
- Jones, D. T.; Woods, D. R. Acetone-butanol fermentation revisited. *Microbiol. Rev.* **1986**, *50*, 484–524.
- Qureshi, N.; Maddox, I. S. Reactor design for the ABE fermentation using cells of *Clostridium acetobutylicum* immobilized by adsorption onto bonechar. *Bioprocess Eng.* **1988**, *3*, 69–72.
- Afschar, A. S.; Biebl, H.; Schaller, K.; Schugerl, K. Production of acetone and butanol by *Clostridium acetobutylicum* in continuous culture with cell recycle. *Appl. Microbiol. Biotechnol.* **1985**, *22*, 394–398.
- Ennis, B. M.; Qureshi, N.; Maddox, I. S. In-line toxic product removal during solvent production by continuous fermentation using immobilized *Clostridium acetobutylicum*. *Enzyme Microb. Technol.* **1987**, *9*, 672–675.
- Groot, W. J.; Luyben, K. Ch. A. M. In-situ product recovery by adsorption in the butanol/2-propanol batch fermentation. *Appl. Microbiol. Biotechnol.* **1986**, *25*, 29–31.
- Maddox, I. S. Use of silicalite for the adsorption of  $n$ -butanol from fermentation liquors. *Biotechnol. Lett.* **1982**, *4*, 759–760.
- Nielson, L.; Larsson, M.; Holst, O.; Mattiasson, B. Adsorbents for extractive bioconversion applied to the acetone-butanol fermentation. *Appl. Microbiol. Biotechnol.* **1988**, *28*, 335–339.
- Ennis, B. M.; Marshall, C. T.; Maddox, I. S.; Paterson, A. H. J. Continuous product recovery by in-situ gas stripping/condensation during solvent production from whey permeate. *Biotechnol. Lett.* **1986**, *8*, 725–730.
- Groot, W. J.; van der Lans R. G. J. M.; Luyben K. Ch. A. M. Batch and continuous butanol fermentation with free cells: integration with product recovery by gas stripping. *Appl. Microbiol. Biotechnol.* **1989**, *32*, 305–308.
- Park, C.-H.; Okos, M. R.; Wankat, P. C. Acetone-butanol-ethanol (ABE) fermentation and simultaneous separation in a trickle bed reactor. *Biotechnol. Prog.* **1991**, *7*, 185–194.
- Qureshi, N.; Maddox, I. S. Integration of continuous production and recovery of solvents from whey permeate: Use of immobilized cells of *Clostridium acetobutylicum* in a fluidized bed reactor coupled with gas stripping. *Bioprocess Eng.* **1991**, *6*, 63–69.
- Evans, P. J.; Wang H. W. Enhancement of butanol formation by *Clostridium acetobutylicum* in the presence of decanol-oleyl alcohol mixed extractants. *Appl. Environ. Microbiol.* **1988**, *54*, 1662–1667.
- Ishii, S.; Taya, M.; Kobayashi, T. Production of butanol by *Clostridium acetobutylicum* in extractive fermentation system. *J. Chem. Eng. Jpn.* **1985**, *18*, 125–130.
- Qureshi, N.; Maddox, I. S. Continuous production of acetone butanol ethanol using immobilized cells of *Clostridium acetobutylicum* and integration with product removal by liquid liquid extraction. *J. Ferment. Bioeng.* **1995**, *80*, 185–189.
- Roffler, S. R.; Blanch, H. W.; Wilke, C. R. In-situ recovery of butanol during fermentation. I. Batch extractive fermentation. *Bioprocess Eng.* **1987**, *2*, 1–12.
- Roffler, S. R.; Blanch, H. W.; Wilke, C. R. In-situ recovery of butanol during fermentation II. Fed-batch extractive fermentation. *Bioprocess Eng.* **1987**, *2*, 83–92.
- Roffler, S. R.; Blanch, H. W.; Wilke, C. R. In-situ extractive fermentation of acetone and butanol. *Biotechnol. Bioeng.* **1988**, *31*, 135–143.
- Shukla, R.; Kang, W.; Sirkar, K. K. Toxicity of organic solvents to *Clostridium acetobutylicum* for extractive ABE fermentation. *Appl. Biochem. Biotechnol.* **1988**, *18*, 315–324.
- Taya, M.; Ishii, S.; Kobayashi, T. Monitoring and control for extractive fermentation of *Clostridium acetobutylicum*. *J. Ferment. Technol.* **1985**, *63*, 181–187.
- Traxler, R. W.; Wood, E. M.; Mayer, J.; Wilson, M. P., Jr. Extractive fermentation for the production of butanol. *Dev. Ind. Microbiol.* **1985**, *26*, 519–525.
- Eckert, G.; Schugerl, K. Continuous acetone-butanol production with direct product removal. *Appl. Microbiol. Biotechnol.* **1987**, *27*, 221–228.
- Grobbe, N. G.; Eggink, G.; Cuperus, F. P.; Huizing, H. J. Production of acetone, butanol and ethanol (ABE) from potato wastes: fermentation with integrated membrane extraction. *Appl. Microbiol. Biotechnol.* **1993**, *39*, 494–498.
- Jeon, Y. J.; Lee, Y. Y. In situ product separation in butanol fermentation by membrane-assisted extraction. *Enzyme Microb. Technol.* **1989**, *11*, 575–582.
- Qureshi, N.; Maddox, I. S.; Friedl, A. Application of continuous substrate feeding to the ABE fermentation: Relief of product inhibition using extraction, perstraction, stripping, and pervaporation. *Biotechnol. Prog.* **1992**, *8*, 382–390.

- (26) Shukla, R.; Kang, W.; Sirkar, K. K. Acetone-butanol-ethanol (ABE) production in a novel hollow fiber fermentor-extractor. *Biotechnol. Bioeng.* **1989**, *34*, 1158–1166.
- (27) Friedl, A.; Qureshi, N.; Maddox, I. S. Continuous acetone-butanol-ethanol (ABE) fermentation using immobilized cells of *Clostridium acetobutylicum* in a packed bed reactor and integration with product removal by pervaporation. *Biotechnol. Bioeng.* **1991**, *38*, 518–527.
- (28) Groot, W. J.; Den Reyer, M. C. H.; Baart de la Faille, T.; van der Lans, R. G. J. M.; Luyben K. Ch. A. M. Integration of pervaporation and continuous butanol fermentation with immobilized cells. I: Experimental results. *Chem. Eng. J.* **1991**, *46*, B1–B10.
- (29) Larrayoz, M. A.; Puigjaner, L. Study of butanol extraction through pervaporation in acetobutylic fermentation. *Biotechnol. Bioeng.* **1987**, *30*, 692–696.
- (30) Matsumura, M.; Kataoka, H. Separation of dilute aqueous butanol and acetone solutions by pervaporation through liquid membranes. *Biotechnol. Bioeng.* **1987**, *30*, 887–895.
- (31) Matsumura, M.; Takehara, S.; Kataoka, H. Continuous butanol/2-propanol fermentation in down-flow column reactor coupled with pervaporation using supported liquid membrane. *Biotechnol. Bioeng.* **1992**, *39*, 148–156.
- (32) Qureshi, N.; Meagher, M. M.; Hutkins, R. W. Silicalite membrane to recover and concentrate acetone butanol from model solutions and fermentation broth. *J. Membr. Sci.* **1999**, *158*, 115–125.
- (33) Garcia, A.; Ianotti, E. L.; Fischer, J. L. Butanol fermentation liquor production and separation by reverse osmosis. *Biotechnol. Bioeng.* **1986**, *28*, 785–791.
- (34) Maddox, I. S. The acetone-butanol-ethanol fermentation: Recent progress in technology. *Biotechnol. Genet. Eng. Rev.* **1989**, *7*, 189–220.
- (35) Maddox, I. S.; Qureshi, N.; Roberts-Thomson, K. Production of acetone-butanol-ethanol from concentrated substrates using *Clostridium acetobutylicum* in an integrated fermentation-product removal process. *Process Biochem.* **1995**, *30*, 209–215.
- (36) Qureshi, N.; Maddox, I. S. Application of novel technology to the ABE fermentation process: An economic analysis. *Appl. Biochem. Biotechnol.* **1992**, *34/35*, 441–448.
- (37) Qureshi, N. Recovery of alcohol fuels using selective membranes by pervaporation. Ph.D. Dissertation. University of Nebraska, Lincoln, NE, 1997.
- (38) Formanek, J.; Mackie, R.; Blaschek, H. P. Enhanced butanol production by *Clostridium beijerinckii* BA101 grown in semidefined P2 medium containing 6% maltodextrin or glucose. *Appl. Environ. Microbiol.* **1997**, *63*, 2306–2310.
- (39) Chen, C.-K.; Blaschek, H. P. Acetate enhances solvent production and prevents degeneration in *Clostridium beijerinckii* BA101. *Appl. Microbiol. Biotechnol.* **1999**, In press.
- (40) Blaschek, H. P. Approaches to making the food processing industry more environmentally friendly. *Trends Food Sci. Technol.* **1992**, *3*, 107–110.
- (41) Annous, B. A.; Blaschek, H. P. Isolation and characterization of *Clostridium acetobutylicum* mutants with enhanced amylolytic activity. *Appl. Environ. Microbiol.* **1991**, *57*, 2544–2548.
- (42) Qureshi, N.; Blaschek, H. P. Fouling studies of a pervaporation membrane with commercial fermentation media and fermentation broth of hyper-butanol producing *Clostridium beijerinckii* BA101. *Sep. Sci. Technol.* **1999**, In press.
- (43) Geng, Q.; Park, C.-H. Pervaporative butanol fermentation by *Clostridium acetobutylicum* B18. *Biotechnol. Bioeng.* **1994**, *43*, 978–986.

Accepted May 28, 1999.

BP990080E