ABSTRACT: Simultaneous acetone butanol ethanol (ABE) fermentation by *Clostridium beijerinckii* P260 and in situ product recovery was investigated using a vacuum process operated in two modes: continuous and intermittent. Integrated batch fermentations and ABE recovery were conducted at 37°C using a 14-L bioreactor (7.0 L fermentation volume) containing initial substrate (glucose) concentration of 60 g/L. The bioreactor was connected in series with a condensation system and vacuum pump. Vacuum was applied continuously or intermittently with 1.5 h vacuum sessions separated by 4, 6, and 8 h intervals. A control ABE fermentation experiment was characterized by incomplete glucose utilization due to butanol toxicity to *C. beijerinckii* P260, while fermentation coupled with in situ recovery by both continuous and intermittent vacuum modes resulted in complete utilization of glucose, greater productivity, improved cell growth, and concentrated recovered ABE stream. These results demonstrate that vacuum technology can be applied to integrated ABE fermentation and recovery even though the boiling point of butanol is greater than that of water.

Introduction

Biobutanol is often proposed as a potential gasoline substitute due to its high energy content, low vapor pressure, low flammability and corrosiveness, and ability to be mixed with gasoline and diesel oil in high proportions (Qureshi and Ezeji, 2008). Butanol is highly toxic to microorganisms that catalyze its production, and for this reason less than 13 g/L of butanol is produced during batch fermentation. Therefore, typical acetone butanol ethanol (ABE) fermentation has been plagued by the use of dilute sugar solutions as substrates, large process volumes, high downstream process costs due to intensive energy requirements for recovery of low concentrations of ABE in the beer, and larger quantities of wastewater. Significant progress has been made in the past three decades on the development of alternative recovery technologies designed to simultaneously recover butanol from the fermentation broths so the concentration of butanol in the bioreactor is maintained below the threshold of toxicity. Integrated ABE fermentation processes such as adsorption, gas stripping, ionic liquids, liquid–liquid extraction, pervaporation, aqueous two-phase separation, supercritical extraction, and perstraction have been reported (Ezeji et al., 2007; Qureshi and Ezeji, 2008).
These techniques have advantages and disadvantages, and details on the mechanics of these processes have been reported previously (Ezeji et al., 2007, 2010; Groot et al., 1992; Oudshoorn et al., 2009; Vane, 2008).

The aim of the present research is to develop an integrated ABE fermentation and recovery technology that requires no membrane, sparger, or agitation; and is microorganism friendly. Integrated ABE fermentation featuring vacuum product recovery involves operating a bioreactor under vacuum so the fermentation broth boils at a low temperature to facilitate instant evaporation and recovery of ABE as it is formed. Vacuum fermentation has long been demonstrated as an effective method for continuous recovery of ethanol from fermentation beer (Cysewski and Wilke, 1977; Ghose et al., 1984; Lee et al., 1981; Nguyen et al., 2009). Despite these beneficial characteristics, an important technical difficulty of vacuum fermentation with regards to ethanol fermentation by *Saccharomyces cerevisiae* is that the small amount of oxygen required by yeast must be supplied in pure form due to low solubility of oxygen at reduced bioreactor pressure (Cysewski and Wilke, 1977). Because *Clostridium* species are strictly anaerobes, this problem will not exist in ABE fermentation.

Researchers have suggested that vacuum technology is suitable for recovering only products that are more volatile than water, limiting its use to production of ethanol and acetaldehyde (Roffler et al., 1984) while excluding production of butanol, which has a higher boiling point (118°C) than water. The application of vacuum technology to ABE fermentation may have been ignored due to this simplistic comparison of boiling points. Nevertheless, fundamental experimental evidence supports the application of vacuum technology to ABE fermentation. Butanol and water form a heteroazeotropic mixture, which, at concentrations below 77 g/L, boils at a lower temperature (or greater pressure in the case of an isothermal process) than the boiling point of either butanol or water (for vapor–liquid equilibrium see DECHEMA Chemistry Data Series; Gmehling and Onken, 1997). At this condition, the vapor will always be richer in butanol than the liquid mixture source. Interestingly, the concentration of butanol in the recovered stream will be greater than in the fermentation broth (Mariano et al., 2008). Driven by the favorable characteristics of the vapor–liquid equilibrium of the butanol–water mixture, the performance of vacuum fermentation technology on ABE fermentation was evaluated.

### Materials and Methods

#### Characterization of the Vacuum Recovery Process

To evaluate the vacuum process for ABE recovery from a dilute solution, the process was conducted at 37 ± 0.5°C using a model ABE solution in the presence and absence of fermentation intermediates, acetic, and butyric acid. Initial concentrations of butanol in the model solution were 5, 8, 10, and 15 g/L. Concentrations of acetone and ethanol used for formulation of the ABE model solution followed the ratio typically found in batch ABE fermentations, 6:3:1 (butanol/acetone/ethanol). Acid concentrations in the model solution were 3.5, 1.8, 1.4, and 0.90 g/L (acetic acid) and 1.0, 0.60, 0.50, and 0.30 g/L (butyric acid). A 2 L Erlenmeyer flask containing 1 L ABE model solution was connected in series to a condensation system and an oil-free Gast vacuum pump DAA-715A-EB (IDEX Corporation, Benton Harbor, MI). A water bath was used to control model solution temperature. At a vacuum range of 711–737 mm Hg, the solution boiled and the vapors were condensed in a 500 mL Erlenmeyer flask placed in a NESLAB RTE 17 refrigerated circulator (Thermo Electron Corporation, Newington, NH) set at 4°C. Vacuum was applied to the model solution mixture for various time durations (10, 30, 60, and 120 min; to fermentation reactor vacuum applied for 1.5 h), during which samples were taken from the Erlenmeyer flask and recovered stream (condensate) for ABE analysis.

#### Microorganism, Culture Maintenance, and Inoculum Preparation

The microorganism used in this study was *C. beijerinckii* P260. Laboratory stocks of the culture were routinely maintained as spore suspensions in sterile double distilled water at 4°C. *C. beijerinckii* P260 spores (600 μL) were heat shocked for 3 min at 60°C followed by cooling on ice. The heat shocked spores were inoculated into 40 mL anoxic pre-sterilized tryptone–glucose–yeast extract (TGY) medium (Ezeji et al., 2003, 2004) and incubated anaerobically for 12–14 h at 35 ± 1°C. This was followed by transferring 32 mL of actively growing culture (12–14 h old) to 368 mL anoxic pre-sterilized TGY medium. To create anaerobic conditions and to prepare anoxic medium, loosely capped bottles with sterilized TGY medium were kept in an anaerobic chamber (Coy Laboratory Products, Inc., Ann Arbor, MI) with a modified atmosphere of 82% N2, 15% CO2, and 3% H2 for 24 h to facilitate exchange of gases between the TGY medium and gases in the anaerobic chamber. Cells were grown anaerobically at 35°C for 4–5 h, during which the optical density of cells at 540 nm λ attained 0.9–1.1 (pre-culture).

#### Production of ABE in Batch Bioreactor

The bioreactor (New Brunswick Scientific Co, New Brunswick, NJ) containing 7 L of medium (glucose 60 g/L, yeast extract 1.5 g/L, and CaCO3 1.0 g/L) was autoclaved at 121°C for 20 min followed by cooling to 37°C under an O2-free N2 atmosphere. Filter sterilized P2 (70 mL) stock solutions (Ezeji et al., 2004; Qureshi and Blaschek, 1999) were added, followed by inoculation of the bioreactor with 350 mL of pre-culture. The bioreactor was maintained at 37 ± 1°C. To maintain anaerobic conditions in the
bioreactor during the early stages of cell growth, O₂-free N₂ gas was used to flush the headspace of the bioreactor for 18 h, during which the culture produced enough fermentation gases (CO₂ and H₂) to keep the bioreactor anaerobic. Culture samples (4 mL) were taken at intervals of 12 h to measure cells, glucose, and ABE concentrations. Samples were centrifuged at 10,000 rpm for 1 min in a refrigerated microcentrifuge. Clear supernatant was transferred into a fresh tube and stored at −20°C until analyzed for residual glucose and ABE concentrations. Results from this fermentation served as the control for comparison to the integrated vacuum ABE fermentation process.

Production of ABE by Vacuum Fermentation

The composition of the medium used for vacuum fermentation was the same as that used for batch fermentation experiments (control). A 14 L bioreactor (New Brunswick) containing 7 L fermentation medium (glucose 60 g/L, yeast extract 1.5 g/L, and CaCO₃ 1.0 g/L) was autoclaved at 121°C for 20 min. Following cooling to 37°C (under O₂-free N₂ headspace), 70 mL of each P2 stock solution was added. The bioreactor was inoculated with 350 mL of pre-culture, and fermentation was initiated as described above. The fermentation was allowed to proceed for 18 h, during which the ABE concentration approached 5–7 g/L, and was followed by ABE recovery by vacuum, where broth in the bioreactor boiled at the fermentation temperature (vacuum range 711–737 mm Hg) creating ABE and water vapors. The vacuum fermentation was operated in two different modes: (i) continuous and (ii) intermittent ABE recovery. In intermittent operation mode, 1.5 h vacuum sessions were separated by 4, 6, and 8 h intervals in three different experiments. Pressure inside the bioreactor was restored to atmospheric pressure by stopping vacuum and releasing N₂ into the bioreactor without which samples cannot be collected from the bioreactor. ABE and water vapors were condensed in a glass condenser (62 mm × 600 mm, cooling coil external surface area 1,292 cm²) at 4.0°C. The recovered ABE solution was pumped into the solvent collector using a peristaltic pump. To cool the condenser, a cooling machine (NESLAB RTE 17) with circulator was used. Cooling medium was 50% (v/v) ethylene glycol, and the circulation rate through the condenser was 600 mL/min. A schematic diagram of the process is shown in Figure 1. Oxygen-free distilled water was added to the reactor at intervals to maintain a constant amount of liquid (to compensate for water loss due to vaporization) inside the reactor. Foaming in the bioreactor was controlled by both addition of antifoam (added as needed) and pulse releases (1 s pulse) of N₂ into the bioreactor until foam collapsed. Samples were withdrawn at various intervals for analysis.

Analytical Procedures and Calculations

Cell growth was estimated by measuring optical density of C. beijerinckii 260 cells at 540 nm wavelength using a DU800 spectrophotometer (Beckman Coulter, Inc., Brea, CA). ABE and acid (acetic and butyric) concentrations were measured using a 7890A Agilent Technologies gas chromatograph.

Figure 1. Schematic diagram of batch acetone butanol ethanol (ABE) production by C. beijerinckii P260 and recovery by vacuum. Arrows show direction of vacuum, water, fermentation gases (CO₂ and H₂), and ABE flows during ABE recovery by vacuum.
Results and Discussion

Characterization of the Vacuum Recovery Process

In order to evaluate application of vacuum for butanol recovery from C. beijerinckii P260 fermentation broth, use of this technology was applied to a model ABE solution prior to recovery of butanol from an actual fermentation broth. The experimental conditions under which recovery was performed included: (i) time in which the solution was kept under vacuum (vacuum recovery time), (ii) initial organic compound (butanol/acetone/ethanol) concentration, and (iii) presence or absence of acetic and butyric acid that are present in ABE fermentation broth and are reaction intermediates of ABE. Butanol recovery effectiveness under vacuum was quantified in terms of the decrease in butanol concentration from the model solution, the rate of butanol removal, and the butanol concentration in the recovered condensate stream.

Decrease in butanol concentration of the model solution ranged from 14.4% to 77.0% of the initial butanol concentration, depending on vacuum recovery time, and initial butanol concentration. Vacuum recovery time and initial butanol concentration had a strong influence on the butanol concentration of the condensate. An increase in vacuum recovery time enhanced butanol recovery. As expected from the vapor–liquid equilibrium of the butanol–water binary system (Fig. 2), recovered condensate was 3–12 times richer in butanol (mass basis) than the feed solution. In the condensate, butanol concentration up to 161.5 g/L was obtained. The presence of acetic and butyric acid negatively affected the recovery of butanol from the solution and for that reason the above mentioned range (14.4–77.0%) decreased to 11.2–56.8% when acetic and butyric acid were present.

Greater initial butanol concentration in the ABE model solution yielded condensate streams with high concentrations of butanol. For example, when model solutions containing 5 and 15 g/L butanol (without acid) were subjected to vacuum recovery for 10 min, butanol concentrations in the condensate were 59.5 and 161.5 g/L, respectively. Rate of butanol removal varied from 1.4 to 16.6 g/L.h, depending on vacuum recovery time, initial butanol concentration, and presence of organic acids (Fig. 3). For a successful application of this technique to butanol/ABE recovery, the rate of removal should be greater than butanol/ABE productivity in the actual fermentation system. Because butanol removal rate (1.4–16.6 g/L.h) is greater than ABE productivity (<0.5 g/L.h), it could be applied for removal of microbial inhibitory products such as butanol/ABE. When gas stripping was applied to a model ABE solution containing 15.9 g/L butanol, it was necessary to operate the gas stripping for 20 h to reduce butanol concentration by 68.5% (Ezeji et al., 2003). Remarkably, it took vacuum recovery process only 2 h to accomplish the same percentage decrease in butanol concentration. This characteristic was effective in determining the duration of the vacuum sessions (1.5 h) used in the fermentation experiments of the present research.

The recovery of other fermentation products, such as acetone and ethanol was affected by vacuum time, their
initial concentration in the model solution, and presence of acetate or butyric acid. Decrease in acetate and ethanol concentration of the model solution ranged from 37.6% to 100% and 7.2% to 67.1%, respectively (data not shown). Concomitantly, an increase in the acid concentration (acetate: 0–43.6%; butyrate: 0.2–33.1%) of the feed solution was observed during vacuum recovery due to acetone and ethanol recovery. Previously, an increase in acid concentration during ethanol fermentation and in situ product recovery by vacuum was reported (Cysewski and Wilke, 1977), which resulted in loss of cell viability. Comparing this occurrence to butanol fermentation, there will be no accumulation of acetic and butyric acid in the bioreactor because of the continuous production and re-assimilation of this acid by solventogenic Clostridium species (Ezeji et al., 2010).

Production of ABE in Batch Bioreactor

Figure 4A shows the growth of C. beijerinckii P260 as measured by OD (λ = 540 nm) determination and decrease in glucose concentration over the course of 60 h of fermentation. Maximum cell growth was obtained at fermentation time of 12 h (Fig. 4A). ABE and acid production profiles of P. beijerinckii P260 over the course of 60 h are shown in Figure 4B. The culture produced 3.7 g/L acetone, 11.8 g/L butanol, and 0.6 g/L ethanol, resulting in a total ABE concentration of 16.1 g/L. At the end of fermentation, residual glucose concentration measured in the bioreactor was 13.9 g/L (Fig. 4A). The major reason for cessation of the fermentation process before exhaustion of substrate is butanol toxicity (Ezeji et al., 2003, 2004; Maddox
et al., 1995). The ABE productivity and yield were 0.26 g/L h and 0.35, respectively (Table I). This productivity is comparable to the productivity obtained by other butanol-producing cultures (Jesse et al., 2002; Lee et al., 2008).

Production of ABE by Vacuum Fermentation

To assess the performance of simultaneous butanol fermentation and recovery by vacuum, integrated batch fermentations and recovery were conducted. Experiments were designed to focus on the period of time *C. beijerinckii* P260 will be exposed to vacuum conditions. Consequently, vacuum was operated either continuously or intermittently. Growth of *C. beijerinckii* P260, glucose utilization, and concentrations of butanol, acetone, ethanol, and acetic acid and butyric acid produced at various times for each operational mode are given in Figure 5. In all cases, fermentation was allowed to proceed for 18 h before initiating the vacuum recovery. In the experiment in which vacuum was operated continuously or intermittently, the cell concentration of *C. beijerinckii* P260 as measured by OD (λ = 540 nm) attained 7.3 (Fig. 5A), which is approximately 1.7 times greater than the optical density of the control experiment (Fig. 4A). The total amount of ABE produced was 93.3 g, resulting in a productivity of 0.28 g/L h and a yield of 0.22. The total volume of condensate recovered was 5.6 L, which consisted of butanol and ABE average concentrations of 13.3 and 16.2 g/L, respectively. Butanol selectivity was 33.8 (Table I).

In comparison to the control experiment, the fermentation under continuous vacuum showed a 12 h decrease in fermentation time (from 60 to 48 h) accompanied by total consumption of substrate (as opposed to 13.9 g/L residual glucose concentration in control) due to increased concentration of cells and decreased butanol toxicity to the culture. However, total ABE yield was 37% less than that of the control (Table I). Two reasons were considered for the decreased yield: (i) losses of ABE through the connecting tubing and vacuum pump, and (ii) change in metabolic activity of the culture from predominantly butanol/ABE production to acid production due to exposure to vacuum. Based on the growth characteristics of *C. beijerinckii* 260 and concentration of acid in the bioreactor, the second reason was disregarded indicating that the culture was not negatively affected by exposure to vacuum. Previous reports on in situ recovery of ABE by pervaporation demonstrated 42–54% acetone and 10–18% butanol losses (Friedl et al., 1991; Qureshi et al., 1992) suggesting that losses to tubing and vacuum pump resulted in decreased ABE yield and productivity. Thus, values of ABE yield, productivity, and concentration in the condensate would be greater than the

<table>
<thead>
<tr>
<th>Parameters</th>
<th>Control experiment</th>
<th>Continuous vacuum</th>
<th>Intermittent vacuum 4 h interval</th>
<th>Intermittent vacuum 6 h interval</th>
<th>Intermittent vacuum 8 h interval</th>
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<tr>
<td>Total ABE (g)</td>
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<td>93.3</td>
<td>108.6</td>
<td>122.8</td>
<td>101.9</td>
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<td>Total acetone (g)</td>
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<td>Total butanol (g)</td>
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<td>76.4</td>
<td>85.1</td>
<td>103.0</td>
<td>84.6</td>
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<tr>
<td>Total ethanol (g)</td>
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<td>3.0</td>
<td>3.9</td>
<td>4.6</td>
<td>4.0</td>
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<td>Final cell concentration (OD⁴)</td>
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<td>7.3</td>
<td>8.5</td>
<td>8.2</td>
<td>8.7</td>
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<td>Initial glucose (g/L)</td>
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<td>58.1</td>
<td>62.2</td>
<td>59.6</td>
<td>61.7</td>
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<td>Residual glucose (g/L)</td>
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<td>0</td>
<td>0</td>
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<td>Glucose utilized (%)</td>
<td>76.4</td>
<td>100</td>
<td>100</td>
<td>100</td>
<td>100</td>
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<tr>
<td>ABE productivity (g/L/h)</td>
<td>0.26</td>
<td>0.28</td>
<td>0.34</td>
<td>0.28</td>
<td>0.23</td>
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<tr>
<td>ABE yield</td>
<td>0.35</td>
<td>0.22</td>
<td>0.26</td>
<td>0.29</td>
<td>0.24</td>
</tr>
<tr>
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<td>48</td>
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<tr>
<td>Vacuum time (h)</td>
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<td>30</td>
<td>7.5</td>
<td>9.0</td>
<td>7.5</td>
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<td>Condensate volume (L)</td>
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<td>1.9</td>
<td>1.9</td>
<td>2.1</td>
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<td>ABE conc. in cond. (g/L)</td>
<td>16.2</td>
<td>45.9</td>
<td>48.5</td>
<td>43.7</td>
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<td>Acetone conc. in cond. (g/L)</td>
<td>2.4</td>
<td>8.4</td>
<td>5.6</td>
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<td>Butanol conc. in cond. (g/L)</td>
<td>13.3</td>
<td>35.9</td>
<td>41.1</td>
<td>36.5</td>
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<td>Ethanol conc. in cond. (g/L)</td>
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<td>HAc conc. in cond. (g/L)</td>
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<td>0.2</td>
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<td>HBu conc. in cond. (g/L)</td>
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<tr>
<td>Butanol selectivity</td>
<td>33.8</td>
<td>15.5</td>
<td>16.1</td>
<td>17.3</td>
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*Optical density at 540 nm; cond., condensate; HAc, acetic acid; HBu, butyric acid. In intermittent mode, 1.5 h vacuum sessions were separated by 4, 6, and 8 h time periods.
obtained values if there were no losses. A mass balance over the model solution data indicated that acetone, butanol, and ethanol losses accounted for 12.5–61.8%, 0.0–10.4%, and 0.0–9.4% of the initial mass of each solvent, respectively (data not shown). Because losses of acetone were greater than that of butanol, the value of the obtained butanol/acetone mass ratio (5.5:1) was greater than normal (2:1), consequently, lower butanol selectivity could have been obtained. A butanol selectivity of 15–25 has been reported previously for a gas stripping system (Ezeji et al., 2004; Oudshoorn et al., 2009).

Operation of the vacuum in intermittent mode resulted in an oscillatory behavior of butanol, acetone, and ethanol concentrations in the fermentation broth. Concentrations of ABE in the bioreactor decreased during recovery by vacuum and increased in the intervals between vacuum sessions (Fig. 5C–E). In accordance with the model solution studies, operation of the vacuum for relatively short time (1.5 h) sufficed to significantly decrease butanol concentration in the bioreactor by 40–70% of the initial concentration of the butanol prior to vacuum sessions. Intermittent vacuum recovery were conducted at intervals of 4, 6, and 8 h, and butanol concentrations between these intervals ranged from 1.3 to 3.2 g/L, 1.3 to 5.0 g/L, 1.3 to 3.7 g/L, respectively. C. beijerinckii P260 cells were able to tolerate these levels of butanol concentration, achieving complete utilization of glucose and enhanced growth similar to values obtained in the experiment with continuous vacuum recovery (Fig. 5A and B). This demonstrates that the exposure of C. beijerinckii P260 to alternate pressure conditions (vacuum/atmospheric...
pressure) during ABE fermentation did not have detrimental effects on growth and ABE production. The rate of substrate consumption in the experiment with intervals of 4 h between vacuum sessions (with total consumption after 46 h) was similar to the value obtained in the ABE fermentation and recovery by continuous vacuum (with total consumption after 48 h). However, in experiments with extended intervals (6 and 8 h), the glucose consumption rate was slower and fermentations terminated after 63 and 65 h, respectively (Fig. 5B). Substrate consumption may have been delayed due to exposure of the cells to greater concentrations of butanol for longer periods of time (Fig. 5C). Consequently, ABE productivity decreased from 0.34 g/L h for 4 h interval to 0.28 and 0.23 g/L h for 6 and 8 h intervals, respectively, for a decrease of up to 32%.

Losses of ABE to the vacuum pump were lower under intermittent vacuum operation mode than under continuous vacuum as demonstrated by 9–32% improvement in ABE yield under intermittent vacuum recovery mode. For intermittent vacuum recovery, the bioreactor was under vacuum for a total of 9 h, during which ABE vapors and fermentation gases flowed through the connecting tubing, condensation system and vacuum pump compared to continuous vacuum recovery that lasted 30 h under similar conditions. Because ABE carriers are water vapors and fermentation gases, the shorter the time these gases/vapors are in motion, the lower the amount of ABE lost through the connecting tubing and vacuum pump. For this reason, ABE yield, productivity, and total ABE produced were greater in experiments with intermittent vacuum recovery than with continuous vacuum recovery (Table I). In addition to reduced vacuum recovery time and lesser ABE losses, average concentrations of butanol in the recovered stream (condensate) were 35.9, 41.1, and 36.5 g/L in experiments with vacuum recovery sessions conducted at intervals of 4, 6, and 8 h, respectively. For these cases, condensate volumes were 1.9, 2.1, and 1.9 L, respectively (Table I). As observed in the experiment with the ABE model solution, there was a significant reduction in the volume of recovered condensate (up to 66%) when vacuum time was reduced from 30 to 9 h.

Following integrated ABE fermentation and in situ product recovery by intermittent vacuum, residual ABE concentration in the bioreactor varied from 2.6 to 3.2 g/L, accounting for 17.1–18.5% of the total ABE produced in 7 L fermentation volume. The relatively high residual concentration of ABE in the bioreactor occurred because there was no ABE recovery 4 h preceding the end of fermentation (Fig. 5C–E). Because the residual ABE concentration in the bioreactor was an important portion of the total ABE produced and recovery of these products by down-stream distillation at this concentration is not economical, an additional vacuum session can be carried out to recover residual ABE in the bioreactor. In continuous vacuum recovery mode, residual ABE concentration in the bioreactor was 0.45 g/L and accounted for a lesser percentage (3.3%) of the total ABE produced. About 0.45 g/L residual ABE concentration was obtained in ABE fermentation and in situ product recovery by continuous vacuum, demonstrating the efficacy of the vacuum recovery process in reducing ABE concentration to an insignificant amount in the bioreactor.

Furthermore, butanol concentration in the fermentation broth reached its maximum value (5.2–6.5 g/L) after 18 h fermentation and prior to initiation of vacuum product recovery in both continuous and intermittent vacuum operational modes. The vacuum recovery process maintained ABE concentration in the bioreactor at low levels throughout the remaining fermentation time, especially in the experiment with continuous recovery in which butanol concentration did not exceed 1.8 g/L (Fig. 5C). Concurrently, butanol concentration in the recovered condensate decreased over time for most experiments as butanol concentration decreased in the broth (Fig. 5H). Thus, as observed in the model solution experiment (data not shown), the greater the butanol content in the fermentation broth, the greater the butanol concentration in the recovered condensate. This indicates that if the vacuum fermentation technology was combined with a mutant strain more tolerant to butanol, such as C. beijerinckii BA101 (Qureshi and Blaschek, 2000), butanol concentration in the bioreactor could be kept at higher levels to produce a more concentrated condensate stream. Besides the attainment of a more concentrated condensate during intermittent vacuum recovery, the energy requirements of vacuum fermentation are reduced due to reduction in vacuum time and subsequent reduction in energy demands of the vacuum pump and condensation system.

The amount of acetic and butyric acid recovered in the condensate during vacuum fermentation was low to insignificant, and their concentration in the condensate varied from 0 to 0.2 g/L (Table I). Despite the fact that acids were not significantly recovered, these compounds did not build up in the fermentation broth, as shown in Figure 5F and G. As fermentation intermediates, acetic and butyric acid were produced, re-assimilated, and converted to ABE, as expected. In this way, a bleed of fermentation broth was not necessary during the integrated butanol fermentation and recovery by vacuum. Moreover, this characteristic of Clostridium species to produce acetic and butyric acid during the acidogenic phase and the subsequent re- assimilation of the acid by C. beijerinckii 260 cells and conversion into ABE (solventogenic phase) have often been used to ascertain the integrity of solventogenic Clostridium cultures (Ezeji et al., 2010). The experiments performed in the present research provide sufficient evidence that C. beijerinckii P260 is not adversely affected during integrated ABE fermentation and recovery by vacuum operated in continuous or intermittent modes. As a result of high rates (up to 16.6 g/L h) of ABE removal, the present investigations demonstrated that ABE fermentation and in situ butanol recovery by vacuum is a novel technique and hence should be investigated further for scale up, economic and energetic evaluations.
Conclusions

The present research demonstrated that vacuum technology can be used to recover butanol from an aqueous solution and to promote enrichment of butanol in the recovered portion. Integrated ABE fermentation and recovery by vacuum enhanced performance of the ABE fermentation process because butanol was continuously removed from the bioreactor during fermentation and Clostridium beijerinckii P260 culture was not affected by the vacuum recovery process. Subsequently, the use of the vacuum fermentation technology resulted in a decrease in fermentation time, complete utilization of glucose, greater cell growth, and more concentrated product stream. The duration of vacuum recovery can be reduced by changing the operational mode of the vacuum from continuous to an intermittent regime without affecting the performance of the fermentation process if the interval between the 1.5 h vacuum sessions does not exceed 4 h. In operations with longer intervals, cells are exposed to greater concentrations of butanol for longer periods, therefore, delaying complete utilization of substrate. Operation in intermittent mode is desirable to reduce the energy requirements of the vacuum fermentation technology (distributed in the energy for the vacuum pump and condensation system) and to enhance ABE concentration in the recovered condensate because less water is vaporized under this operating condition. Operation of the vacuum in intermittent mode with vacuum sessions of 1.5 h at intervals of 4 h resulted in the best fermentation time and a product stream with butanol concentration 3.7 times greater than the value obtained in the conventional batch process. With this increase in butanol concentration in the product stream, a significant positive impact is expected on the economics of ABE production due to potentially reduced energy demands of the distillation unit.

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