Butanol production from the effluent of hydrogen fermentation


ABSTRACT

The purpose of the study was to recover butanol from the effluent of the hydrogen-producing bioreactor containing acetate, butyrate, and carbohydrate. The butanol production by *Clostridium beijerinckii* NRRL B592 was evaluated under both unsterilized and sterilized conditions for examining the potential of butanol production for the practical application. Sucrose of 10 g/L and butyrate of 2 g/L coupled with acetate buffer were used to mimic the effluent. Sucrose was completely consumed in the both unsterilized and sterilized conditions during acetone-butanol-ethanol (ABE) fermentation. However, the results illustrate that the carbohydrate consumption rate in the unsterilized condition was higher than that in the sterilized condition. The maximum butanol concentrations of 3,500 and 3,750 mg/L were achieved in the sterilized and unsterilized conditions, respectively. Meanwhile, it was found that the acetate and the butyrate concentrations of 600 and 1,500 mg/L, and 300 and 1,000 mg/L were ingested to yield butanol in the sterilized condition and in the unsterilized condition, respectively. The results concluded that high levels of acetate and butyrate could eliminate the interference of other microbial populations, resulting in the enrichment of *C. beijerinckii* NRRL B592 in the fermentor. The butanol production by *C. beijerinckii* NRRL B592 could be, therefore, produced from the effluent of the hydrogen-producing bioreactor. It promised that the microbial butanol production is one of attractive bioprocesses to recover energy from wastes.

Key words | ABE fermentation, biofuel, butanol, butyrate, *Clostridium*, hydrogen

INTRODUCTION

There have been growing research interests on biorenewable energy production in the worldwide. In particular, acetone-butanol-ethanol (ABE) fermentation has been regained lots of attentions lately due to energy shortage and environmental concerns. In ABE fermentation, the ratio of acetone to butanol to ethanol typically is 3:6:1 where butanol is the major species. One of the superior properties of butanol is that it contains 30% more energy than ethanol and only 5% less than gasoline. Butanol is one of the most attractive alternatives to replace fossil fuel. As been reported, solvent-producing *Clostridia* produce hydrogen, acetate and butyrate in an exponential growth phase, whereas the metabolism shift to acetone, butanol, and ethanol productions during the stationary phase in a batch culture (*Afshar et al. 1986; Brosseau et al. 1986*). On the other hand, the metabolic pathway can toward to either acidogenesis for organic acid productions or solventogenesis for solvent productions.

During acidogenesis, the accumulation of the organic acids results in a decrease pH of the fermentation broth, while solvents are produced at lower pH (*Lee et al. 2008*). Previous investigations found that *Clostridium acetobutylicum* is favored to evolve solvents at a pH below 5 (*Monot et al. 1984; Gottwald & Gottschalk 1985*). In addition, it has been observed that the organic acids can be reutilized to form acetone, butanol and ethanol (*Afshar et al. 1986; Brosseau et al. 1986*). Hartmanis et al. (*1984*) confirmed the
uptake of butyrate to form butanol by *Clostridium acetobutylicum* ATCC 824. Tashiro *et al.* (2004) reported the butanol production from a mixture of butyrate and glucose by *Clostridium saccharoperbutylacetonicum* N1-4 in a pH-stat fed-batch reactor. In addition to butyrate, a study found butanol production by *C. beijerinckii* BA101 would be increased by the addition of acetate (Chen & Blaschek 1999).

In the last decade, biohydrogen production from organic wastes has been intensively explored. As been well known, biohydrogen production through dark fermentation is always accompanied with acetate and butyrate productions. The effluent also contained some residual carbohydrate contents while the hydrogen-producing reactor was being operated at a very short hydraulic retention time (Lin & Chang 1999; Kim *et al.* 2006; Chen *et al.* 2009). From the perspective of environmental protection, the effluent has been considered as a waste stream. There is a need to dispose off this effluent in an environmentally friendly manner. Since this effluent is rich with high levels of organic acids and carbohydrates, the process of microbial butanol production achieves the needs of waste reduction/treatment and renewable bioenergy recovery simultaneously. In the study, a bio-process in terms of ABE fermentation was proposed to yield butanol from the effluent of hydrogen-producing bioreactors containing acetate, butyrate, and carbohydrates. The butanol production by *C. beijerinckii* NRRL B592 was evaluated under both unsterilized and sterilized conditions. The investigation performed under the unsterilized condition would examine the potential of butanol production for the practical application.

**MATERIALS AND METHODS**

**Culture development**

*C. beijerinckii* NRRL B592 obtained from Bioresource Collection and Research Center (BCRC), Hsinchu, Taiwan was developed in peptone-yeast extract-glucose (PYG) medium under anaerobic condition for 24 h at 35 ± 1°C. The medium composed of glucose (10 g/L); yeast extract (10 g/L); peptone (5 g/L); triptone (5 g/L); cysteine-HCl (0.5 g/L); glutathione (0.25 g/L); K2HPO4 (2.04 g/L); KH2PO4 (0.04 g/L); FeSO4 · 7H2O (1.1 mg/L); CaCl2 (0.008 g/L); MgSO4 · 7H2O (0.0192 g/L); NaCl (0.08 g/L); and NaHCO3 (0.4 g/L). The medium was sterilized at 121°C for 15 min before culture development. The cell culture was then centrifuged to remove the liquid portion before inoculation.

**Batch assays**

For comparison, the batch assays were performed in the sterilized condition and in the unsterilized condition, respectively. The experiment carried out under the sterilized condition was used as a control test. In the batch tests, the butanol production experiments were conducted in 500-mL batch reactors with 3.33 mL of nutrient solution and 100 mL of acetate buffer solution. Each liter of the acetate buffer solution consisted of 357 mL of 0.1 M acetic acid and 643 mL of 0.1 M sodium acetate. The composition of the nutrient solution was described elsewhere (Chen *et al.* 2009). Sucrose of 10 g/L and butyrate of 2 g/L coupled with the acetate buffer solution (0.9 g/L) were used to mimic the effluent from the hydrogen-producing bioreactors. This was equivalent to a total chemical oxygen demand (COD) of 15.8 g/L. The centrifuged cells with the corresponding mixed liquor volatile suspended solids (MLVSS) concentration of 210 mg/L developed from PYG medium were inoculated into the batch reactor. The initial pH in the batch reactor was adjusted to 5.2 ± 0.1 using 0.1 N potassium hydroxide or hydrochloric acid. The batch reactor was purged with nitrogen gas, was sealed with butyl rubber stoppers, and then was incubated in a shaker at 30 ± 1°C and 100 rpm. The samples from the batch reactor were drawn during the tests and were analyzed for the solvents (butanol, acetone, and ethanol), the organic acids (acetate, and butyrate), carbohydrate contents, and pH.

**Analytical methods**

MLVSS and pH were measured following the procedures described in *Standard Methods* (1998). Carbohydrate in the mixed liquor was determined based on the method described in Frolund *et al.* (1996). Solvents and individual volatile fatty acid (VFA) were analyzed using gas chromatograph (SRI 8610C) equipped with a flame ionization detector (FID). The capillary column was a 30 m by 0.53 mm packed nitrotetraphilic acid modified polyethylene glycol. The operational temperatures of the injection port and the detector were both maintained at 240°C, respectively. The temperatures of the oven were set from 45 to 240°C for determining each species of solvents and VFAs. Nitrogen was used as the carrier gas at a flow rate of 5 mL/min.

**RESULTS AND DISCUSSION**

Figure 1 illustrates the variation of pH during the experimental period under the individually sterilized and
unsterilized conditions. As revealed in the figure, the pH values in the control test (sterilized one) slowly dropped from 5.2 to 5.0 between day 0 to day 4, and then drastically increased to 5.8 on day 8 followed by a decrease to pH 5.4 in the end of the experiment. However, the result under the unsterilized condition shows that the pH remained consistent around 5.2 from day 0 to day 4, and it stayed constantly about 5.0 between day 5 and day 8. The pH values then increased to around 5.2 and remained unchanged. The increase of the pH values was not as sharp as that in the sterilized condition. In addition, the pH increase in the sterilized condition was occurred earlier than that in the unsterilized condition.

Figure 2 indicates the solvent concentrations during the experimental period in the sterilized and unsterilized conditions. As illustrated from Figure 2a, the butanol concentration in the sterilized condition began to increase on day 4 and reached plateau by 10 days. The maximum butanol concentration was achieved nearly 3,500 mg/L. A similar pattern was observed from the acetone concentration where it was achieved to the maximum of approximately 1,800 mg/L on day 10, and then reduced to 1,300 mg/L. The ethanol concentration, however, increased to the peak of 1,000 mg/L, and then gradually declined with time. In Figure 2b, the butanol concentration in the unsterilized condition increased since day 6, and then remained consistent since day 10. The maximum butanol concentration was obtained at a concentration of 3,750 mg/L. Meanwhile, the maximum acetone concentration of 1,200 mg/L was found on day 10. The peak of the ethanol concentration of approximately 600 mg/L also appeared on day 10, and then decreased with time. Based on the results in Figure 2, the production of solvents resulted in the increase of the pH values of the fermentation broth. The result was in agreement with previous studies (Soni & Jain 1997a; Soni & Jain 1997b; Tashiro et al. 2004).

The variations of acid concentrations with time under the sterilized and unsterilized conditions are shown in Figure 3. As revealed in Figure 3a, the butyrate concentration in the sterilized condition dramatically declined from 2,000 mg/L on day 4 to 500 mg/L on day 8. In this period, the butanol concentration as shown in Figure 2a was sharply increased. It illustrates that C. beijerinckii NRRL B592 assimilated butyrate to form butanol during ABE fermentation. The metabolism in acidogenesis was more dominated than that in solventogenesis in this stage. In comparison with the results in Figure 1, the butyrate consumption coupled with the butanol production increased the pH values of the
fermentation broth. However, the butyrate concentration began increased to 1,000 mg/L in the end of fermentation. It reflects that this increase of pH values could derive the shift of the metabolism from solventogenesis to acidogenesis for the butyrate production. In addition to butyrate, the acetate concentration also gradually decreased from 900 mg/L to 300 mg/L. It was apparent that acetate was capable to be ingested by \textit{C. beijerinckii} NRRL B592 during ABE fermentation. This finding was in consistent with previous reports (Formanek, \textit{et al}. 1997; Chen & Blaschek 1999; Ezeji \textit{et al}. 2003). However, it was found that the acetate concentration increased in the end of the fermentation because of the prevailing of acidogenesis. In Figure 3b, the butyrate concentration under the unsterilized condition gradually reduced from 2,000 mg/L on day 5 to 1,000 mg/L on day 9. In opposite to the sterilized condition, the increase of butyrate concentration was not observed in the end of ABE fermentation. The acetate concentration decreased from 900 mg/L to 600 mg/L in the unsterilized condition. The utilization of acetate by \textit{C. beijerinckii} NRRL B592 was not as apparent as that in the sterilized condition.

Figure 4 depicts the carbohydrate concentrations during the experimental period in the sterilized and unsterilized conditions. As evident from the figure, the consumption of carbohydrate in the sterilized condition occurred two days earlier than that in the unsterilized condition. The carbohydrate was completely utilized on day 12 for both conditions. Sucrose was fully consumed during ABE fermentation. The carbohydrate consumption rate in the unsterilized condition was higher than that in the sterilized condition.

In the study, sucrose was completely consumed to produce acetate, butyrate, acetone, butanol, and ethanol. However, it should be noted that acetate and butyrate were ingested simultaneously by \textit{C. beijerinckii} NRRL B592. A COD balance should not be set up without including acetate and butyrate. In the COD balance, the total COD of 15.8 g/L

Table 1 | A summary of the COD percentages of the final products

<table>
<thead>
<tr>
<th>Condition</th>
<th>Organic acids</th>
<th>Solvents</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Acetate (%)</td>
<td>Butyrate (%)</td>
</tr>
<tr>
<td>Sterilized condition</td>
<td>4</td>
<td>11</td>
</tr>
<tr>
<td>Unsterilized condition</td>
<td>4</td>
<td>11</td>
</tr>
</tbody>
</table>
in the feed was balanced against the COD of the products as shown in Table 1. As revealed in the table, 91% of COD under the sterilized condition and 87% of COD under the unsterilized condition were converted into organic acids and solvents. Moreover, 56% of COD was utilized to form butanol in the sterilized condition while 58% of COD was for butanol production in the unsterilized condition. Butanol was the main product of ABE fermentation.

CONCLUSIONS

The butanol productions from the synthetic medium simulating the effluent from the hydrogen-producing bioreactor were determined in both sterilized and unsterilized conditions. The uptake of butyrate and acetate to form butanol by \textit{C. beijerinckii} NRRL B592 had been confirmed in the sterilized condition. The production of solvents coupled with the ingestion of acetate and butyrate could increase the pH values of the fermentation broth. Thereafter, this increase could derive the shift of the metabolism from solventogenesis to acidogenesis during ABE fermentation. In the unsterilized condition, butyrate was also assimilated by \textit{C. beijerinckii} NRRL B592 to yield butanol as well. However, the uptake of acetate was not as apparent as that of butyrate. The butanol production was successfully achieved under the unsterilized condition. This was mainly due to the presence of high concentrations of acetate and butyrate, inhibiting the interference of other microbial populations, and thereby enriching \textit{C. beijerinckii} NRRL B592 in the fermentor. It promised the microbial butanol production from the effluent of the hydrogen-producing bioreactor. The microbial butanol production through ABE fermentation is one of attractive bio-processes to recover energy from wastes.

ACKNOWLEDGEMENTS

This study was supported by a research grant (NSC 97-2221-E-197-028) from National Science Council, Taipei, Taiwan.

REFERENCES


