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Short Communication

Continuous hydrogen and butyric acid fermentation by immobilized *Clostridium tyrobutyricum* ATCC 25755: Effects of the glucose concentration and hydraulic retention time

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ABSTRACT

The effects of the hydraulic retention time (HRT = 8, 10, 12 or 16.7 h) and glucose concentration (30, 40 or 50 g/L) on the production of hydrogen and butyrate by an immobilized *Clostridium tyrobutyricum* culture, grown under continuous culturing conditions, were evaluated. With 30 g/L glucose, the higher HRTs tested led to greater butyrate concentrations in the culture, i.e., 9.3 g/L versus 12.9 g/L with HRTs of 8 h and 16.7 h, respectively. In contrast, higher biogas and hydrogen production rates were generally seen when the HRT was lower. Experiments with different glucose concentrations saw a significant amount of glucose washed out when 50 g/L was used, the highest being 22.7 g/L when the HRT was 16.7 h. This study found the best conditions for the continuous production of hydrogen and butyric acid by *C. tyrobutyricum* to be with an HRT of 12 h and a glucose concentration of 50 g/L, respectively.

1. Introduction

With the current changes in the global climate and environment, it is necessary for humankind to evaluate alternative fuel sources, with a strong preference towards renewable, sustainable and green energy areas. Two potential areas that meet these requirements are the use of hydrogen and biofuels. Hydrogen is an attractive energy source since it has a higher energy yield than hydrocarbon-based fuels and, yet, only generates water as a by-product during combustion. Biological methods offer a less energy intensive means for hydrogen production than other technologies since they can be performed under ambient pressure and temperatures (Cheong and Hansen, 2007; Elam et al., 2003). Anaerobic bacteria are one of the means of biological hydrogen production and are known to utilize a wide assortment of energy sources for this purpose (Kapdan and Kargi, 2006; Nishio and Nakashimada, 2007).

To optimize the amount of hydrogen generated, it is best to utilize cultures that produce acetate and butyrate (Hawkes et al., 2002), such as *Clostridium tyrobutyricum*, since organisms producing other by-products, such as acetate, ethanol and lactic acid, produce less hydrogen due to the redox balance within the cell (Vavilin et al., 1995; Nath and Das, 2004; Kapdan and Kargi, 2006). Another benefit of *Clostridia* is their ability to produce acids and alcohols, such as butyric acid and butanol, which can be used in a variety of applications, including the use of butanol as a biofuel.

Both of these biofuels, i.e., hydrogen and butanol, offer a variety of benefits, including cleaner fuel sources and the potential for renewable energy. Aside from the obvious clean-fuel applications that exist with burning bio-hydrogen, it is also possible to use the hydrogen produced to generate electricity using proton exchange membrane fuel cells (PEMFC) (Levin et al., 2004). Using a PEMFC developed in our lab (Jeon et al., 2008), current work includes the coupling of these two technologies and the optimization of this coupled system. However, the conditions leading to the highest hydrogen and butyric acid production levels from *C. tyrobutyricum* have not been elucidated.

Therefore, in this study, the continuous production of hydrogen and butyric acid by an immobilized culture of *C. tyrobutyricum* ATCC 25755 was evaluated. In particular, the effects of different hydraulic retention times (HRTs) and the glucose concentration were studied to determine the optimal conditions for the continuous production of butyric acid and hydrogen by this strain.

2. Methods

2.1. Bacterial strain, media and growth

C. tyrobutyricum was purchased from the Korean Collection for Type Cultures (KCTC). The culture was grown in argon-reduced Reinforced Clostridial Medium (RCM), using the recipe from DIFCO

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(USA), but without the addition of agar. The spores were activated at 70 °C for 10 min in a water bath before being inoculated into 20 ml RCM. After growth overnight, the cells were sub-cultured (10%) into RCM, grown for 12 h and then sub-cultured (10%) into RCM and grown for 12 h more. The culture of 50 ml was used to inoculate the initial batch reactor, which contained 500 ml of reduced RCM and 30 g/L glucose. The optical density of the culture was measured using a UV-1700 (SHIMAZU) set at 600 nm.

2.2. Batch and fed-batch fermentation

After inoculating the reactor, the batch culture was grown for 19 h, at which time the glucose content in the media was nearly depleted (Fig. 1a). At this time, the media contents of the two reactors were mixed continuously (100 ml/min). As needed, concentrated RCM (4× nutrients along with 1× salts and concentrated glucose), was fed into the reactor to bring the glucose concentration up to 30 g/L and to provide nutrients for the culture. The immobilization matrix used was porous polyurethane-activated carbon media (Lee et al., 2008). Both reactors were operated at 37 ± 0.5 °C but the pH was controlled solely within the batch reactor to a value of 5.7 through the addition of 8 M NH_4OH. The batch reactor was stirred at 120 rpm to ensure complete mixing. After the butyrate concentration reached a maximum, the reactor was switched to a continuous mode with fresh RCM added continuously. The total culture volume within the reactors was 2 L.

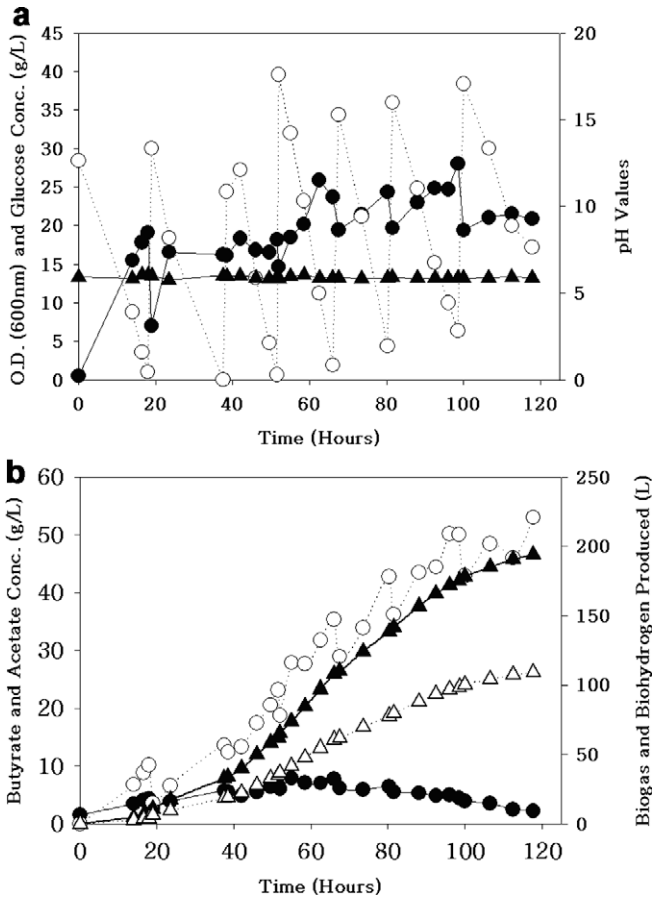

Fig. 1. Results from the fed batch operation with *C. tyrobutyricum*. (a) Values for the optical density (●), pH (▲) and glucose concentrations (○). (b) Results showing the total biogas (▲) and bio-hydrogen (Δ) produced and the volatile fatty acid concentrations (acetate (●) and butyrate (○)). The immobilization and fed batch operation was begun after 19 h, as seen by the sudden decrease in the optical density when the media in the two reactors were mixed.

2.3. Analytical methods and data analysis

The samples were analyzed as described previously (Lee et al., 2008). The effects of the HRT and glucose concentration were conducted twice. Error bars represent the standard deviation calculated using the sample points taken for each condition.

3. Results and discussion

3.1. Fed batch operation

C. tyrobutyricum was initially immobilized within a polyurethane matrix during a fed-batch operation. For the first 19 h, the culture was grown solely in the batch half of the reactor system. When the glucose was nearly depleted, the contents of the two reactors (batch and immobilized) were mixed, thus initiating the fed-batch culture. The fed batch operation was continued until the butyrate concentration reached a maximum value, which in this study was about 53 g/L (Fig. 1b), a value that is slightly higher than that obtained in other studies that also employed immobilized wild-type *C. tyrobutyricum* cultures (Zhu and Yang, 2003; Zhu et al., 2005) and was comparable with the *ack* and *pta* mutants described by Liu et al. (2006) and Zhu et al. (2005), respectively. Although this enhanced production is very likely due in part to the richer nature of the media used in this study, i.e., RCM media has more yeast extract and tryptone than the clostridial growth media (CGM) used by Dr. Yang's group (Huang et al., 1998), the yield seen here (0.45 g/g glucose) is just below the theoretical max-

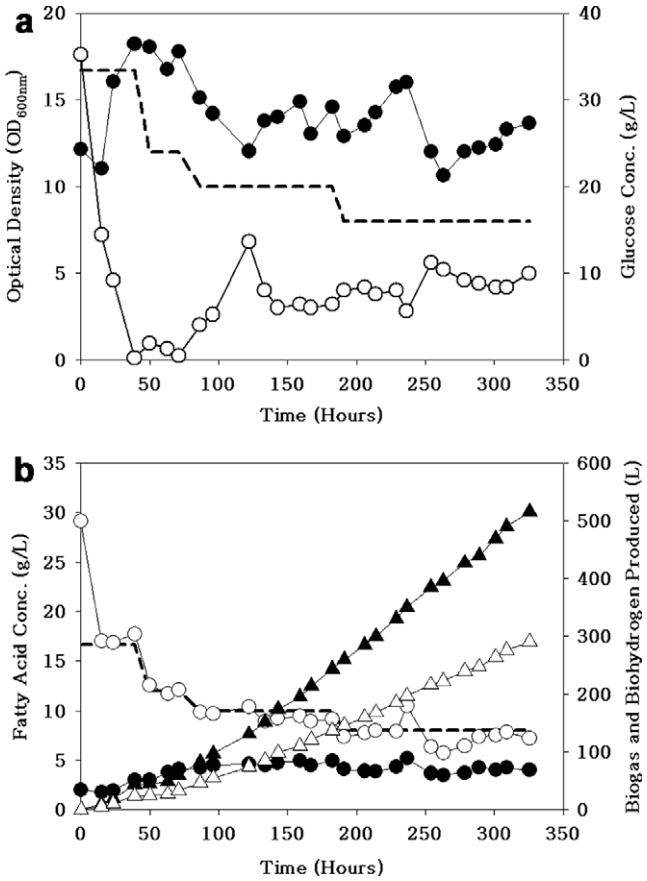

Fig. 2. Effect of the hydraulic retention time (HRT) on the characteristics of the immobilized *C. tyrobutyricum* culture. (a) Results for the optical density (●) and residual glucose concentrations (○). The dashed line (- - -) denotes the HRT used. (b) Results showing the total biogas (▲) and bio-hydrogen (Δ) produced and the acetate (●) and butyrate (○) concentrations within the reactors during these tests.