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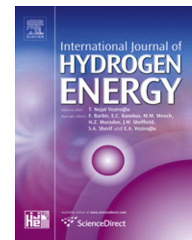

Enhanced bio-hydrogen production from sugarcane juice by immobilized *Clostridium butyricum* on sugarcane bagasse

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ABSTRACT

Immobilized *Clostridium butyricum* TISTR 1032 on sugarcane bagasse improved hydrogen production rate (HPR) approximately 1.2 times in comparison to free cells. The optimum conditions for hydrogen production by immobilized *C. butyricum* were initial pH 6.5 and initial sucrose concentration of 25 g COD/L. The maximum HPR and hydrogen yield (HY) of 3.11 L H_2/L substrate·d and 1.34 mol H_2/mol hexose consumed, respectively, were obtained. Results from repeated batch fermentation indicated that the highest HPR of 3.5 L H_2/L substrate·d and the highest HY of 1.52 mol H_2/mol hexose consumed were obtained at the medium replacement ratio of 75% and 50% respectively. The major soluble metabolites in both batch and repeated batch fermentation were butyric and acetic acids.
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1. Introduction

As a sustainable energy source, hydrogen is a promising alternative to fossil fuels. It is a clean and environmentally friendly fuel, which produces only water after combustion. Biologically, hydrogen can be produced by photosynthetic and fermentative routes [1]. Fermentative hydrogen can be generated by various types of microorganisms. *Clostridium* spp. is one of those organisms capable of converting sucrose to hydrogen and carbon dioxide [2,3].

Although the yield of hydrogen production by *Clostridium* spp. is considered high, it still requires further improvement for industrial application. Immobilization technique is a practical tool used to enhance the activity of microorganisms involved in the fermentation systems [4,5]. The advantages of immobilized cells over free cells include more tolerant to environment perturbation, reusable, process stability and higher biological activity since higher cell density can be applied [6]. Therefore, immobilization could lead to a high production rate and might be the best choice in terms of the feasibility of large scale and continuous processing [7]. Support materials for cell immobilization can be synthetic polymers, such as alginate and polyvinyl alcohol, or naturally available, such as lignocellulosic materials from agricultural residues. Synthetic support materials have the advantage of high stability. However, there are some disadvantages such as high cost, low substrate conversion efficiency and toxic to microorganisms [8]. Therefore, there is an interest toward the use of natural materials for cell immobilization in order to overcome these problems. In this study, sugarcane bagasse (SCB) was used to immobilize the seed inoculum for hydrogen fermentation in order to improve hydrogen production efficiency as

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well as to facilitate the reusability of the cells. SCB is an inexpensive local-available material possessing the advantages of being highly porous and having a good water retention capacity. The additional advantage of using cellulosic materials as support material for cell immobilization is that the spent materials can be sent back to the hydrolysis process for sugar production, hence minimizing waste generation [9,10].

Sugarcane is one of the most important industrial crops in Thailand. It can be cultivated in all parts of Thailand, except in the south, with a cultivation area of more than 960,000 ha. Approximately 48 million tons of sugarcane are produced per year [11]. Sugarcane juice is mainly used to produce sugar. However, based on a report of the Office of the Cane and Sugar Board (Thailand), sugar production from sugarcane is greater than sugar consumption [9]. Therefore, this research was designed to investigate an alternative way to add value to sugarcane by producing clean and renewable energy i.e., hydrogen. The main sugar found in sugarcane juice is sucrose at an approximate concentration of 200 g/L. Sucrose was reported as a substrate for producing hydrogen by various types of microorganisms such as *Clostridium butyricum* CGS5 [3] and *Thermotoga elfii* [12], with the yields ranging between 1.39 and 2.00 mol hydrogen/mol hexose. Therefore, sugarcane juice has the potential to be used as substrate for hydrogen production.

In the present study, the immobilization method was used to improve the hydrogen production efficiency in a batch system with an attempt to optimize the environmental conditions for hydrogen production. The optimum conditions obtained from the batch system were further used in repeated batch hydrogen fermentation with a variable medium replacement ratio in order to continuously producing hydrogen and to examine the reusability of the immobilized cells.

2. Materials and methods

2.1. Sugarcane juice

Sugarcane (*Saccharum officinarum* Linn.) was harvested from the field in Lopburi Province, Thailand. Sugarcane juice was prepared by crushing the sugarcane stalks in a squeezer and filtering through the sheet cloth, then kept at −20 °C. Frozen sugarcane juice was thawed at room temperature before use. The sugarcane juice consists of (all in g/L): sucrose, 199; glucose, 3.4; fructose, 3.2; with the total sugar concentration of 210 g/L.

2.2. Inocula

C. butyricum TISTR 1032 was pruchased from the Thailand Institute of Scientific and Technological Research (TISTR), Thailand. The cultivation protocol was conducted following the method of Pattra et al. [13].

2.3. Support material preparation and immobilization of *C. butyricum*

SCB was used as a support material for cell immobilization. It was cut using a knife into small pieces (approximately 0.5 × 0.5 × 0.5 cm) and passed through a 0.5–1 cm sieve. Delignification of SCB was conducted followed the previous report [14]. Immobilization was conducted by adding 7 g dry weight of delignified SCB to 63 mL of Tryptone Sucrose Yeast Extract (TSY) medium [15] in a serum bottle, capped with a rubber stopper and an aluminum cap and autoclaved at 121 °C for 15 min before inoculation with 10% (v/v) of *C. butyricum* (final cell density of 10^6 cell/mL). The bottle was flushed with argon to create anaerobic conditions and incubated at 37 °C for 10 h at 150 rpm on an orbital shaker. After incubation, the culture medium was drained and the immobilized cells were washed three times with sterile 0.85% NaCl. The final cell number in the support material was approximately 10^7 cells/g dry wt of the support material. The SCB and immobilized cells were visualized by scanning electron microscopy (SEM, JSM-5410LV, JEOL, Japan) [16].

2.4. Experimental procedure

The batch experiments for hydrogen production by free and immobilized *C. butyricum* were conducted by varying the initial pH and sucrose concentration in the substrate. Each 100 mL serum bottle with a working volume of 70 mL contained 60 mL of sugarcane juice, 7 mL of *C. butyricum* (10^7 cells/mL) as free cells or 7 g dry wt of immobilized cells (10^7 cells/g dry wt of support materials), 1.5 mL of 3.75% (w/v) L-cysteine as a reducing agent and 1.5 mL of nutrient stock solution [17]. After the replacement of the gas phase with argon to create anaerobic conditions, the serum bottle was incubated at 37 °C and 150 rpm for 24 h on the orbital shaker. All treatments were carried out in triplicates.

The effect of initial pH was determined at an initial sucrose concentration in the culture medium of 25 g COD/L by varying the initial pH in the range of 4.5–7.0 in order to obtain the optimum pH for hydrogen production. The effect of the initial concentration of sucrose as the substrate assessed in the range of 20–40 g COD/L was further investigated at the optimum pH.

2.5. Reusability of the immobilized cells

At the end of the batch experiment, culture medium in the serum bottle was replaced by 63 mL of fresh medium (60 mL of sugarcane juice, 1.5 mL of 3.75% (w/v) L-cysteine as a reducing agent and 1.5 mL of nutrient stock solution) with the optimum initial pH and sucrose concentration, then incubated at 37 °C and shaken 150 rpm for 24 h. The volume and content of biogas and VFAs and the sucrose concentration in the culture medium were determined during incubation. This process was repeated five times to determine the reusability of the immobilized *C. butyricum* for hydrogen production.

2.6. Repeated batch operation

The repeated batch experiment was conducted in a 2 L glass bioreactor (Biostat B, B. Braun Biotech International, Germany) with a working volume of 1.5 L at the optimum conditions obtained from the batch experiment. Immobilization was conducted by adding 150 g dry wt of delignified sugarcane bagasse into 1.5 L TSY medium in the reactor, which was then autoclaved at 121 °C for 15 min. After sterilization, the reactor was inoculated with 10% (v/v) of *C. butyricum* (10^6 cells/mL).

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