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Performance and population analysis of hydrogen production from sugarcane juice by non-sterile continuous stirred tank reactor augmented with *Clostridium butyricum*

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ABSTRACT

Non-sterile operation of continuous stirred tank reactor (CSTR) augmented with *Clostridium butyricum* and fed with sugarcane juice was studied at various hydraulic retention time (HRT). The maximum hydrogen production rate and yield of 3.38 mmol H_2/L/h and 1.0 mol H_2/mol hexose consumed, respectively, were achieved at HRT 4 h. The relationship of the augmented microorganism and normal flora in the fermentation system under non-sterile condition were analyzed by polymerase chain reaction-denaturing gradient gel electrophoresis (PCR-DGGE). Initially, at HRT 36 h, other species related to *Lactobacillus harbinensis* and *Klebseilla pneumoniae* were present as a major group in the reactor. When HRT was decreased to 12, 6 and 4 h, *C. butyricum* was present with a competition between *L. harbinensis* and *K. pneumoniae*. Results indicated that augmented *C. butyricum* could compete with contaminated microorganisms during non-sterile operation at low HRT (12–4 h) with the support of normal flora (*K. pneumoniae*).

1. Introduction

Being a sustainable energy source, hydrogen is a promising alternative to fossil fuels, as it is a clean and environmental friendly fuel, which produces water instead of greenhouse gases after combustion. Hydrogen has a high energy yield of 122 KJ/g which is 2.75 times greater than that of hydrocarbon fuel. Hydrogen can be generated mainly from fossil fuels, biomass and water by chemical or biological process [1,2]. Biologically, hydrogen can be produced by the photosynthetic and fermentative routes which are more environmentally friendly and less energy intensive compared to thermo-chemical and electro-chemical processes [3]. In comparison to photosynthetic microorganisms, fermentative hydrogen-producing microorganisms are advantageous in that hydrogen can be evolved in a reactor continuously without light [4].

Among the fermentative hydrogen producers, the organisms of genus *Clostridium* such as *Clostridium butyricum* [5,6], *Clostridium acetobutylicum* [7] *Clostridium saccharoperbutylacetonicum* [8], *Clostridium pasteurianum* [9,10] are often used to produce hydrogen [11]. *Clostridium* sp. the spore forming anaerobic

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bacteria, is one of those organisms capable of converting sucrose to hydrogen with the yield ranged between 2.0 and 4.8 mol of hydrogen/mol sucrose [5,6,10] which is higher than that of the other fermentative bacteria such as *Enterobacter* sp. (1 mol hydrogen/mol hexose) [3]. When pure culture is used for hydrogen fermentation, the reactors are mostly started up and operated under sterile condition which requires high cost for hydrogen production in industrial scale. To overcome this problem, the addition of pure culture to the reactor under non-sterile condition has drawn our attention.

During hydrogen production under non-sterile condition, the relationship of competitive and cooperative of microbial populations could be found. For the competitive relationship, some isolates augmented into the reactors cannot become dominant due to their inability to grow in the communities depending on the environmental condition [12]. In contrast, for the cooperative relationship, the mixed indigenous microorganisms in the substrate could consume volatile fatty acids (VFAs) produced during hydrogen production, which in turn facilitate hydrogen production of bioaugmented producer by reducing the product inhibitory effect [12]. According to these previous reports, the microbial community in the hydrogen production system under non-sterile condition should be investigated for the dominate species responsible for hydrogen production. Therefore, in this study the microbial community in the hydrogen fermentation system after adding *C. butyricum* under non-sterile condition was explored in order to verify the relationship between the augmented microorganism and normal flora in the substrate i.e. sugarcane juice.

Sugarcane is one of the important industrial crops in Thailand. It can be cultivated in all parts of Thailand, except in the Southern of Thailand, with the cultivation area of more than 960,000 ha. Approximately 48 million tons of sugarcane is produced per year [13]. Sugarcane juice is mainly used to produce sugar. However, from the report of Office of the Cane and Sugar Board (Thailand), sugar production from sugarcane every year is greater than sugar consumption [13]. Therefore, this research was designed to investigate an alternative way to value-added sugarcane by producing a clean and renewable energy i.e. hydrogen. The main sugar found in sugarcane juice is sucrose, which can be used as substrate for hydrogen production by various microorganisms such as *C. pasteurianum* [10] and *C. butyricum* CGS5 [6] with yields of 4.80 and 2.78 mol-H_2/mol-sucrose, respectively. Therefore, sugarcane juice has a potential to be used as substrate for hydrogen production.

In the present study, sugarcane juice was used as a substrate to produce hydrogen continuously by *C. butyricum* in the continuous stirred tank reactor (CSTR). The relationship of the augmented microorganism i.e. *C. butyricum* and normal flora in the fermentation system under non-sterile condition were analyzed by the polymerase chain reaction-denaturing gradient gel electrophoresis (PCR-DGGE) method.

2. Materials and methods

2.1. Fermentative medium preparation

Sugarcane (*Saccharum officinarum* Linn.) used in this study was harvested from sugarcane field, Lopburi Province, Thailand. Sugarcane juice was prepared by crushing the sugarcane stalk using squeezer and filtrating through a thin layer cloth and then boiled to concentrate in order to obtain the sugarcane syrup. The sugarcane syrup has a final concentration of 2000 g/L (2245 g-COD sucrose/L) of total sugar and was kept at −20 °C until the usage. Frozen sugarcane syrup was thawed by placing at room temperature prior to use as substrate for hydrogen production. For medium preparation, the sugarcane syrup was diluted with distilled water to a concentration of 25 g-COD sucrose/L and supplemented with sufficient inorganic nutrients for bacterial growth including (mg/L); NH_4HCO_3 5240, K_2HPO_4 125, $MgCl_2.6H_2O$ 15, $FeSO_4.7H_2O$ 25, $CuSO_4.5H_2O$ 5, $CoCl_2.5H_2O$ 0.125, $NaHCO_3$ 6720 [14].

2.2. Seed inoculum preparation

C. butyricum TISTR 1032 was obtained from Thailand Institute of Scientific and Technological Research (TISTR), Thailand. This microorganism is able to produce hydrogen as previously been reported by Pattra et al. [15]. It was grown in cooked meat medium (CMM) (Himedia, India) at 37 °C under the anaerobic condition for 10 h and kept at 4 °C as a stock culture. Prior to cultivation, *C. butyricum* was activated by transferring 1 mL of the stock culture at a cell concentration of 10^7 cells/mL into 10 mL of fresh Tryptone Sucrose Yeast Extract (TSY) medium, incubated at 37 °C for 10 h at 150 rpm using an orbital shaker under the anaerobic condition. The culture was further enriched by inoculating 10% v/v, cell concentration of 10^6 cells/mL, of the culture into 60 mL fresh TSY medium and incubated at the given conditions before using as inoculum [16]. Each liter of TSY containing 5.0 g of tryptone; 3.0 g of sucrose; 5.0 g of yeast extract; 1.0 g of K_2HPO_4 [17].

2.3. CSTR operation and monitoring

A 1-L CSTR with a working volume of 900 mL was used to produce hydrogen from sugarcane juice. The schematic diagram of the bioreactor was depicted in Fig. 1. The reactor was fed with 810 mL of fermentative medium and 90 mL of *C. butyricum* (10% (v/v), final cell density of 10^6 cell/mL) as seed inoculum under anaerobic condition and operated at a controlled temperature of 37 °C with a constant stirring at 150 rpm using impeller. A pH of fermented broth was maintained at 6 using a pH controller and 1N NaOH. The oxidation–reduction potential (ORP) was measured using ORP meter (MV7615, B&C Electronic, Italy) to ensure the anaerobic condition. After 12 h of reactor operation, the fermentative medium was continuously fed from medium tank to the feed-in port at the bottom of the reactor using peristaltic pump. The effluent overflowed from the reactor at the feed-out port. Samples were taken at the sampling port. The starting hydraulic retention time (HRT) was 36 h and the HRT was further shortened to 24, 12, 6 and 4 after a steady state was reached. Steady-state conditions reached when the product concentration including hydrogen gas content, biogas volume and metabolite concentration (i.e. VFAs and ethanol) were stable (less than 10% variation) for 5–7 days.