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# Sequential fermentation of hydrogen and methane from steam-exploded sugarcane bagasse hydrolysate

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## ABSTRACT

The goal of this study was to sequential fermentation of hydrogen and methane from sugarcane bagasse (SCB). Steam explosion conditions for pretreating SCB were optimum at 195 °C and 1.5 min, which yielded 36.35 g/L of total sugar and 2.35 g/L of total inhibitors. Under these conditions (all in g/L): glucose, 11.33; xylose, 24.41; arabinose, 0.61; acetic acid, 2.33; and furfural, 0.02 were obtained. The resulting hydrolysate was used to produce hydrogen by anaerobic mixed cultures. A maximum hydrogen production rate of 396.50 mL H<sub>2</sub>/L day was achieved at an initial pH of 6 and an initial total sugar concentration of 10 g/L. The effluent from the hydrogen fermentation process was further used to produce methane. Response surface methodology with central composite design was used to obtain the suitable conditions for maximizing methane production rate (MPR). An MPR of 185.73 mL/L day was achieved at initial pH, Ni and Fe concentrations of 7.59, 3.61 mg/L and 8.44 mg/L, respectively. Total energy of 304.11 kJ/L-substrate was obtained from a sequential fermentation of hydrogen and methane. This approach will not only add value to SCB, in the form of safe and clean energy, but also provide a solution for making use of this abundant waste.

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## Introduction

Environmental problems due to the use of rapidly diminishing supplies of fossil fuel have led to ever increasing need for an alternative energy that is both environmentally friendly and renewable [1,2]. Hydrogen is one of the solutions to these problems since it is considered as an efficient energy carrier with high energy content. The two main categories of

biological hydrogen production are the photo production process and the dark fermentation process [3]. The dark fermentation process has been found to be more advantageous of the two in terms of all day long continuous production, and higher hydrogen production rates [3]. Lignocellulosic materials have recently received a lot of attention as one of the best feedstocks for the production of hydrogen due to its underlying monosugars (such as glucose, arabinose, and xylose) [4,5]. Sugarcane bagasse (SCB), a waste product from

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the sugar industry, is one of the lignocellulosic materials that has a great potential to be used for hydrogen production [6,7]. More than 400 million tons of SCB are generated annually from sugar manufacturers around the world [8]. It consists of hemicelluloses (25–27%), cellulose (38–42%) and lignin (20–25%) [8]. Prior to use, the pretreatment of the lignocellulosic material is required in order to liberate its underlying monosugars such as glucose, xylose, and arabinose. Pretreatment methods that have been used include physical, chemical and enzymatic techniques [6,9]. Two phase end products, solid and hydrolysate phases, are obtained after the pretreatment. The hydrolysate is mainly comprised of glucose and xylose with a small amount of arabinose. These sugars are solubilized from the hemicellulosic fraction [10,11]. The solid phase is cellulosic fraction which can be subsequently hydrolyzed into glucose that can be further used to produce energy products such as hydrogen and ethanol.

Several pretreatment methods are applied to hydrolyze the lignocellulosic materials such as acid hydrolysis, alkaline hydrolysis, and enzymatic hydrolysis [10,11]. Among the available pretreatment methods, the enzymatic hydrolysis method is considerable effective and generates less toxic chemicals [11]. However, the cost of enzyme is much higher than the other chemicals used for the pre-treatment. Therefore, the interests have been paid to acid or alkaline hydrolysis methods due to its simplicity (easy to conduct), cost effectiveness and efficiency. The drawbacks of acid or alkaline hydrolysis include hazardous chemical usage, strong reactions, corrosion and the production of microbial inhibitors (acetic acid, furfural, hydroxymethyl-furfural and soluble lignin) [10,12,13]. To overcome these problems, the steam explosion process was chosen to pretreat the SCB. Steam explosion is a thermomechanicochemical process consisting of two stages which are, vapor cracking and explosive decompression [14]. This process is considered to be an environmentally friendly method by virtue of the usage of only water steam to operate the process. In addition, this method is conducted under a high temperature with a short reaction time resulting in more energy efficiency and less generated hazardous chemicals, especially in comparison to acid and base hydrolysis [14].

Effluent generated after the fermentation of bio-hydrogen process contained volatile fatty acids (VFAs) such as butyric acid (HBu), acetic acid (HAc) and residual sugars. These compositions have been reported as valuable substrates for ethanol and methane production by various types of mixed and pure cultures of fermentative bacteria. Therefore, in order to completely and efficiently utilize the SCB, the effluent from the hydrogen fermentation process was further used to produce methane.

The goal of this study was to produce hydrogen and methane from SCB. Steam explosion conditions for pretreating SCB were optimized in order to obtain the SCB hemicellulose hydrolysate suitable as a fermentation medium for producing hydrogen by anaerobic mixed cultures. Factors affecting hydrogen production from the hydrolysate i.e. initial pH and initial total sugar concentration were investigated. The effect of Endo-nutrient addition was also studied. The effluent from the hydrogen production process was subsequently used as the substrate for methane fermentation by

anaerobic mixed cultures. Key factors affecting the methane production process were optimized using response surface methodology (RSM) with central composite design (CCD). This approach will not only add value to SCB, in the form of safe and clean energy, but also provide a solution for making use of this abundant waste.

## Materials and methods

### SCB

SCB was obtained from a local shop selling sugarcane juice in Khon Kaen, Thailand. The SCB was air dried, cut with scissors and blended in a blender to a size of approximately  $0.5 \times 0.5$  mm. SCB consists of (all in % (w/w)): cellulose, 33.63; hemicellulose, 23.88; lignin, 4.31; ash, 1.29; and other components, 31.96.

### Seed inoculum

The seed inoculum for hydrogen production was upflow anaerobic sludge blanket (UASB) granules from a brewery wastewater treatment plant in the northeastern part of Thailand. Prior to being used to produce hydrogen, the UASB granules were spread on an aluminum tray to a thickness of about 0.5 cm and then heat shocked in a hot air oven at  $90^\circ\text{C}$  for 5 h in order to inactivate methanogens and to select the hydrogen producers (spore-forming microbes). The heat-treated UASB granules were directly used as the inoculums for hydrogen production without enrichment.

### Steam explosion pretreatment of SCB

SCB was soaked in a 0.5% (v/v)  $\text{H}_2\text{SO}_4$  solution for 2 h. After draining and pressing out the remaining hydrolyzing solution, the moisture content of the soaked SCB was 77.51%. Approximately 540 g (120 g dry weight) of soaked SCB were then added to a 2 L steam explosion reactor (CHINO, Japan) and treated with high pressure steam. The combination of process temperature in the steam explosion reactor (195 and  $215^\circ\text{C}$  which is equivalent to the pressure of 14.04 and 21.12 bar, respectively) and the reaction time (1.5 and 4.5 min) were set as the desired process conditions. Following the steam explosion pretreatment, each sample was collected, washed and adjusted to a final volume of 2 L using tap water. The solid residue was then separated from the liquid phase (hydrolysate) by filtration through a thin layer cloth. The resulting filtrate was centrifuged at  $20^\circ\text{C}$ , 8000 rpm for 10 min. The pH of the SCB hydrolysate obtained from each pretreatment condition was in the ranges of 1.54–1.64. Prior to being used as the substrate for hydrogen production, the hydrolysate was heated on a hot plate stirrer until the temperature of the hydrolysate reached  $80^\circ\text{C}$ . This step was carried out to reduce the concentration of volatile components (furfural and phenolic compounds) [15]. The pH of the hydrolysate was then adjusted to 10 by  $\text{Ca}(\text{OH})_2$  to partially eliminate phenolic compounds and other acid components e.g. acetic acid and tannic acid [15]. The precipitate was removed by centrifugation at 6000 rpm for 10 min. The resulting hydrolysate was

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