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Optimization of biohydrogen production from sugarcane bagasse by mixed cultures using a statistical method

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ABSTRACT

The aim of this study is to determine the optimum condition for biohydrogen production from sugarcane bagasse (SCB) hydrolysate using a central composite design and response surface methodology (RSM). SCB was hydrolyzed with 0.5% (v/v) sulfuric acid at 121 °C, 0.15 MPa for 60 min in an autoclave at a solid to liquid ratio of 1:15 (g:mL). Heat-treated bacterium obtained from a hydrogen producing fermentor was used as the inoculum. The interaction of three factors, i.e., substrate concentration, substrate:buffer ratio and inoculum:substrate ratio on hydrogen production potential (*P*) were investigated. The results indicated that the substrate concentration, substrate:buffer ratio and inoculum:substrate ratio had a significant influence effect on *P*. An optimal condition was found at substrate concentration of 22.77 g-total sugar L^{-1}, 4.31 substrate:buffer ratio, and 0.31 inoculum:substrate ratio resulted in a maximum *P* of 6980 mL H_2 L^{-1}. The confirmation experiment results indicated that optimum *P* was statistically significant, from the predicted value obtained by RSM which suggests that RSM could be efficiently used to optimize a biohydrogen production from SCB hydrolysate using mixed cultures. These results indicates that the SCB hemicellulose hydrolysate is suitable as a fermentation media for producing biohydrogen. This approach will add value to SCB by converting agricultural waste into a safe and clean form of energy.

1. Introduction

Our energy supply comes mainly from fossil fuels for transportation and industrialization resulting in not only environmental pollution, but also economic and political problems owing to their limited reserves and uneven distribution. Nowadays, alternative energy can come from natural resources (wind, sunlight, geothermal power and biomass) which are inexhaustible. Using these resources to supply our energy needs supports sustainable development, while also lowering greenhouse gas emissions. The different characteristics of an energy resource can be evaluated in terms of sustainability and their ability to replace traditional fuels at different levels including power generation, heating, as a transport fuel and for rural energy. In developing countries, biomass represents the major source of renewable energy because of its commonly used as the local energy supply. Bioenergy is a fuel derived from a biological source (biomass) and is also referred to as biofuel. Biomass is defined as any organic material coming from any form of life or its derived metabolic production. Biodiesel and bioethanol are biofuels that currently are the only alternative energy source able to replace transportation fuel in vehicle engines without involving major modifications. Previous researches study biofuel from biomass such as agricultural waste [1], food waste [2] and microalgae [3–5].

On the other hand, some scientists predict hydrogen as the "fuel of the future", and the worldwide trend is the shift away from non-renewable fuels towards the establishment of renewable hydrogen

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energy. Many companies are working to develop technologies that might efficiently exploit the potential of hydrogen energy for use in motor vehicles. As of November 2013 there are demonstration fleets of hydrogen fuel cell vehicles undergoing field testing including; the Chevrolet Equinox Fuel Cell, Honda FCX Clarity, Hyundai ix35 FCEV and Mercedes-Benz B-Class F-Cell [6]. Hydrogen is considered to be an ideal energy source for the future due to its cleanliness and high energy yield of 142 kJ g^{-1}, which is 2.75 times greater than that of any hydrocarbon fuel. Hydrogen production technologies can be classified as physical–chemical and biological methods. Physical–chemical methods are very energy intensive and result in the emission of harmful greenhouse gases (CO, CO_2 and CH_4) that have an impact on global warming. In contrast, biological methods for biohydrogen production are more environmentally friendly, lower in energy consumption and have a cheaper substrate cost because hydrogen can be produced from raw materials such as organic waste, agricultural waste [7,8], industrial wastewater [9] and municipal waste [10]. These substrates are readily available and have been widely used in anaerobic fermentation to produce hydrogen gas in environmentally friendly ways [11].

Biohydrogen from cellulose is a high value-added product, since cellulosic biomass is the most abundant renewable resource on earth. In addition, biohydrogen can be produced from non food crops of inedible waste products and does not divert food away from the animal or human food chain. However, pretreatment is an important tool for the cellulosic bioconversion process because it has great potential for improving the efficiency of the anaerobic process. Thailand is an agricultural country and sugarcane is one of the important industrial crops. 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 Mt of sugarcane are produced each year [12]. Sugarcane bagasses (SCB) are a waste left after the sugarcane extraction process. Since the bagasse accounts for approximately 25% of sugarcane mass, about 12 Mt of SCB are produced annually. The most common use for SCB is for energy production by combustion [13] with can cause environmental problems because of the emissions of CO_2. SCB consists of three main fractions, i.e., cellulose, hemicelluloses and lignin of which 30–35% is hemicelluloses [14]. The generation of hydrogen from SCB using anaerobic fermentation usually requires substrate pretreatment procedures. Diluted acid treatment of hemicelluloses fraction in SCB yields a solution containing mainly glucose and xylose with a small amount of arabinose [14,15]. Since bonds in cellulose are stronger than in hemicelluloses, a solid waste of cellulose and lignin is obtained in the diluted acid hydrolysis of SCB [15]. Due to its composition, hydrolysate of SCB is a very attractive raw material for the production of hydrogen. An important consideration in hydrogen production from SCB hydrolysate though, is that during hydrolysis several inhibitory compounds are formed [16].

Several factors affect biohydrogen production in addition to nature of the microbial flora [17] such as temperature, pH, mineral medium formulation, the type of inoculums, the profile of organic acids produced and the type and concentration of substrate. Particularly, pH is a key parameter in biological processes as it affects enzyme activities, metabolite transporters, and the microbial community. Therefore the production media formulation must include buffering compounds such as sodium or ammonium bicarbonate to reduce pH variations during cultivations. Teli et al. [18] reported the buffering capacity, strongly affects the biohydrogen yield. However, high concentrations of a buffer may have an inhibitory effect on anaerobic fermentation. An optimal substrate:buffer ratio of 2–2.5 was found which maintained the pH around 5–5.5. Therefore, the optimization of fermentation conditions are importance for biohydrogen production.

In conventional multifactor experiments, optimization is usually carried out by varying a single factor while keeping all other factors fixed at a specific set of conditions. This is not only time-consuming, but it is usually impossible to reach the true optimum because the process ignores the interactions among the other variables. As a result, response surface methodology (RSM) has been proposed to determine the influences of individual factors and their interactive influences. RSM is a statistical technique for designing experiments, building models, evaluating the effects of several factors, and searching for optimum conditions [19]. Recent studies have looked at the optimization on hydrogen production [1,20]. However, the statistical optimization on biohydrogen production from cellulosic hydrolysate is still lacking in the literature.

The present study investigates the effects of substrate concentration, substrate:buffer ratio and inoculum:substrate ratio on hydrogen production from SCB hydrolysate. A central composite experimental design was employed in planning the experiment in order to find out which experiment variables affect hydrogen production potential by using RSM and a predictive polynomial quadratic equation.

2. Material and methods

2.1. Sugarcane bagasse hydrolysate

SCB used in this study was obtained from a local sugar processing plant (Thai Roong Reuang sugar industry, Phitsanulok, Thailand). The SCB was air dried, milled and sieved through a 0.5 mm screen before being stored at room temperature prior to usage. The composition (w/v) of the SCB was 38.1% cellulose, 21.2% hemicelluloses, 8.3% lignin, 2.5% ash and 40.1% of other components.

Acid hydrolysis of the SCB hemicellulose fraction was conducted at 121 °C, 0.15 MPa for 60 min with 0.5% (v/v) sulfuric acid in an autoclave at a solid to liquid ratio of 1:15 (g:mL). After hydrolysis, a solid residue was separated by filtration through a thin layer cloth. The pH of the hydrolysate was adjusted to 10 with $Ca(OH)_2$. The resulting precipitate was removed by centrifugation (1500 rpm, 15 min) and then re-acidified to pH 7, followed by further centrifugation. The detailed procedures are delineated in a previous study [1]. The supernatant was concentrated until a total-sugar content of 200 mg L^{-1} was achieved and kept at 4 °C prior to use. Total sugar was determined by the phenol sulfuric acid method [21].

2.2. Seed microorganisms

The seed microorganisms in this study were taken from a 200 L hydrogen pilot plant, with a volatile suspended solid (VSS) of 3.23 g L^{-1}. This reactor was continuously fed with 20 mg-total sugar L^{-1} and maintained at pH 6. Prior to use, the seed sludge was first washed with 0.75% w/v of $NaCl_2$ and heated at 94 °C for 60 min to inhibit the bioactivity of the hydrogen consumers and to harvest spore-forming anaerobic bacteria.

2.3. Experimental design

In order to assess the optimization of factors affecting hydrogen production from SCB hydrolysate, a central composite experimental design (CCD) and RSM were conducted to evaluate the key variables influencing hydrogen production potential from three factors; substrate concentration (X_1), substrate:buffer ratio (X_2) and inoculum:substrate ratio (X_3). RSM is an empirical statistical technique employed for multiple regression analysis using quantitative data obtained from properly designed experiments to simultaneously