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Utilization of palm kernel cake as a renewable feedstock for fermentative hydrogen production

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ABSTRACT

Fermentative hydrogen generation was studied using palm kernel cake (PKC) as sustainable cellulosic biomass. PKC was subjected to an acid hydrolysis approach using dilute H_2SO_4 (7% v/v). PKC hydrolysate obtained was then diluted (70%) and used as a substrate for hydrogen generation. Chemical analysis showed that the main fermentable sugars in diluted PKC hydrolysate were glucose, xylose and mannose with the concentrations of 2.75 g/L, 2.60 g/L and 27.75 g/L, respectively. Hydrogen production was carried out by the cultivation of *Clostridium acetobutylicum* YM1 on PKC hydrolysate. The effect of incubation temperature, the initial pH of culture medium and microbial inoculum size on hydrogen production was studied using a statistical model. The analysis of the model generated showed that the initial pH value of the culture medium and inoculum size had significant effects on the hydrogen production. The study showed that the optimum conditions for the biohydrogen production were 30.57 °C temperature, pH 5.5 and 20% inoculum size. A verification experiment was performed in the optimum conditions determined. Experimental results of the verification test showed that a cumulative hydrogen volume of 1575 ml/L was generated with consuming 2.75 g/L glucose, 2.20 g/L xylose and 16.31 g/L mannose.

1. Introduction

Nowadays, the conventional production of energy from fossil fuel resources has been found detrimental to the environment due to the increasing greenhouse gas emission such as carbon dioxide (CO_2) and nitrous oxide (N_2O) which has led to a rise in the air pollution and global warming. Furthermore, the increasing depletion of the fossil fuel sources and the high fluctuation in oil and petroleum-derived fuel prices have brought about a rising trend in the consumption of the renewable and green energy [1–3]. Hydrogen is known as a clean energy source with a high energy yield, accounting for 112–122 kJ/g (about 2.75-fold higher than fossil fuels) which produces only H_2O when it burns [4–7].

At present, hydrogen is mainly generated by the transformation of the fossil fuels to hydrogen which leads to the release of the high quantity of the greenhouse gasses. The continual generation of hydrogen entails renewable energy resources. In this view, the different types of renewable feedstocks have already been utilized for the hydrogen generation such as agro-industrial residues, energy crops, municipal solid wastes, industrial wastewater and forest residues [8–10].

Palm kernel cake (PKC) is known as an agro-industrial residue which is obtained after the extraction of oil from palm fruit in palm oil industry [11]. It has been reported that Malaysia is one of the main countries for the production of palm oil in the world so that Malaysia produced a total of 19,666,953 tons of crude palm oil and

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2,518,947 tons of PKC in 2014 [12].

The polysaccharide content of the agro-industrial residues are mostly composed of cellulose and hemicellulose which are linked with lignin by the covalent bonds [13]. In this regard, PKC has been found as a renewable feedstock which is mostly comprised of cellulose and hemicellulose content. The analysis of the chemical composition of PKC has revealed that cellulose and hemicellulose content constitute 11.6% and 61.5% of the PKC fibre, respectively. Similar studies have shown that the hemicellulose content of the PKC polysaccharide is composed of mannan to a large extent (57.8%), which is followed by xylan with forming 3.7% of the hemicellulose content [14,15].

Fermentative hydrogen production (dark fermentation) is an environmentally clean method in which microorganisms basically grow on carbohydrate-based substances to evolve hydrogen gas in anaerobic conditions [16]. In this regard, bacterial genus *Clostridium* has been shown as the main microorganisms used in the fermentative hydrogen generation [17]. A number of *Clostridium* spp. have been applied in biohydrogen production such as *Clostridium beijerinckii*, *Clostridium butyricum*, *Clostridium tyrobutyricum* [18], *Clostridium acetobutylicum* [19] and *Clostridium saccharoperbutylacetonicum* [20].

It has been found that the hydrogen generation in the dark fermentation is affected by the environmental variables such as incubation temperature, pH value of the culture medium and microbial inoculums. Hence, it is important to optimize the production conditions to achieve the elevated hydrogen evolution [18,21,22].

Although many attempts have been made to produce hydrogen in the fermentative process using different hydrogen-generating microorganisms, further studies for introducing new strains of hydrogen-producing bacteria are necessary in line with the sustainable production of energy. In this regard, the mesophilic strain *C. acetobutylicum* YM1 has newly been isolated from the local areas by Al-Shorgani et al. [23] which has shown its high potential for the hydrogen generation [24]. On the other hand, a variety of lignocellulosic biomass have been already used for the biohydrogen generation such as oat straw, corn stalk, rice straw, banyan leaves, wheat straw and maize leaves [25–30], however, to the best of authors' knowledge no research work has so far been performed to use PKC in the biohydrogen production. In this view, PKC is largely produced in Malaysia, offering an inexpensive raw material for the sustainable production of hydrogen energy.

The current research work aimed to evaluate the feasibility of the hydrogen production by the new isolate *C. acetobutylicum* YM1 using PKC as the low-cost renewable feedstock in Malaysia. The effect of the three pivotal process parameters, namely incubation temperature, the initial pH value of the culture medium, and the inoculum size of microorganism on the hydrogen production was also studied by fitting a regression model on the experimental results using the response surface methodology (RSM) on the basis of a central composite design (CCD). The interaction effects of the environmental factors tested were analysed to determine optimum conditions for the hydrogen generation. Since this strain has previously not been used for the hydrogen evolution from PKC, this research work presents novel findings in the context of hydrogen energy production.

2. Materials and methods

2.1. Microorganism

C. acetobutylicum YM1 was obtained from Biotechnology Pilot Plant Laboratory, Department of Chemical and Process Engineering, Faculty of Engineering and Built Environment, University Kebangsaan Malaysia [23]. A synthetic medium, namely tryptone-yeast extract-acetate (TYA) medium was used in this study. The composition of TYA medium was as follows (g/L): tryptone, 6; yeast extract, 2; ammonium acetate, 3; KH2PO4, 0.5; $MgSO_4 \cdot 7H_2O$, 0.3, and $FeSO_4 \cdot 7H_2O$, 0.01 [31]. *C. acetobutylicum* YM-1 was grown anaerobically in TYA medium to use it as the inoculum source in fermentative hydrogen production.

2.2. Preparation and hydrolysis of PKC

PKC was obtained from Sime Darby Jomalina Sdn Bhd, NURI Edible Oil Complex, Port Klang, Malaysia and stored in 4–8 °C prior to use. The amount of 350 g of PKC was mixed in H_2SO_4 (7%v/v) in 1 L Schott-Duran bottle and shaken properly to produce a PKC solution with a concentration of 35% (w/v). Mixture was then treated by heating for 1 h at 121 °C using an autoclave (HVE50, Hirayama, Japan). Subsequently, the hydrolysate solution was cooled to the room temperature. The hydrolysate solution was then filtered using the filter paper Whatmann No. 1 to obtain a clear filtrate. The filtrate of the PKC hydrolysate was diluted up to 70% to make PKC hydrolysate with a concentration of 30% in comparison to its undiluted form [31]. The dilution of the PKC hydrolysate was carried out by mixing 690 ml of distilled water and 10 ml of a concentrated TYA medium (100 times concentrated). The solution obtained was then added to 300 ml of the undiluted PKC hydrolysate and mixed well to make 1 L of the diluted PKC hydrolysate for sugar analysis and hydrogen fermentation.

2.3. Hydrogen production

A determined volume of the diluted PKC hydrolysate was transferred into a 250 ml Schott-Duran bottle. The initial pH of the diluted PKC hydrolysate was adjusted according to the values determined by CCD using 6 M NaOH and 1 M HCl. The bottles were then sterilized at 121 °C for 15 min and left to cool at the room temperature. Subsequently, anaerobic conditions inside the bottles were provided by sparging oxygen-free nitrogen to the void space of the bottles for 10 min using a membrane air filter (0.22 μm pore size). All air filter and air tube set were sterilized by an autoclave prior to utilization. The diluted PKC hydrolysate was then inoculated with the inoculums sizes determined by CCD. For inoculation, the defined volume of inoculum source (grown *C. acetobutylicum* YM1 in TYA medium) was transferred into the prepared PKC hydrolysate to make a total volume of 200 ml of the culture medium. Microbial cultures were then incubated at different temperatures according to CCD. When hydrogen generation decreased to a great extent, hydrogen fermentation was stopped and culture samples were taken. The fermentation time of all experimental runs based on CCD are shown in Table 1. Hydrogen gas produced was then collected to measure cumulative hydrogen volume.

2.4. Central composite design and regression model

Hydrogen production experiments were performed on the basis of CCD in which the simultaneous effects of the three independent variables, namely incubation temperature (°C), the initial pH of the culture medium, and the inoculum size of the microorganism (%) on hydrogen generation were evaluated. The total number of the experiments of the fermentative hydrogen production was $2^k + 2k + n_o$, where k denotes the number of the independent variables and n_o is the number of centre points in the experimental design. The design matrix of the fermentation experiments is shown in Table 1. Experimental variables were coded at the three levels including +1, 0 and −1, representing the high level, middle level (centre) and low level, respectively [32]. The coded setting and