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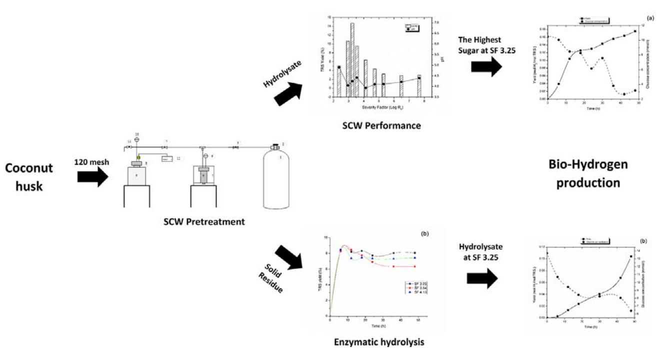
An integrated green process: Subcritical water, enzymatic hydrolysis, and fermentation, for biohydrogen production from coconut husk

Maktum Muharja, Fitri Junianti, Dian Ranggina, Tantular Nurtono, Arief Widjaja*

Department of Chemical Engineering – Institut Teknologi Sepuluh Nopember, Surabaya 60111, Indonesia

GRAPHICAL ABSTRACT



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ABSTRACT

The objective of this work is to develop an integrated green process of subcritical water (SCW), enzymatic hydrolysis and fermentation of coconut husk (CCH) to biohydrogen. The maximum sugar yield was obtained at mild severity factor. This was confirmed by the degradation of hemicellulose, cellulose and lignin. The tendency of the changing of sugar yield as a result of increasing severity factor was opposite to the tendency of pH change. It was found that CO_2 gave a different tendency of severity factor compared to N_2 as the pressurizing gas. The result of SEM analysis confirmed the structural changes during SCW pretreatment. This study integrated three steps all of which are green processes which ensured an environmentally friendly process to produce a clean biohydrogen.

1. Introduction

The world faces the progressive depletion of its energetic resources that mainly based on non-renewable fuels. One renewable solution is the use of biohydrogen form of biomass (Sánchez and Cardona, 2008). In agricultural dominating countries like Indonesia, the crop residue and waste which is renewable, sustainable, biodegradable, and environmentally friendly have great potential for biohydrogen production.

Coconut husk (*Cocos nucifera*) (CCH) which is produced about 5.1 million tons per year is the one of abundant crop residue in Indonesia (Mahmud and Ferry, 2005). CCH which has the main contains 26.72% cellulose, 17.73% hemicellulose can be a potential feedstock candidate for biohydrogen production (Sangian et al., 2015a). However, because of the highness of lignin content (about 41.19%), it is not easy work to directly convert CCH into biohydrogen.

Production of biohydrogen was well performed from lignocellulosic material using acid pretreatment (Gonzales et al., 2016b; Kumar et al.,

* Corresponding author.
E-mail address: arief_w@chem-eng.its.ac.id (A. Widjaja).

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2017a). An acid or alkaline solution is often used in the pretreatment process due to its low price, simplicity of the process, resulting in a high yield. Although acid and alkaline hydrolysis are relatively fast methods that produce high sugars concentrations, the reaction medium must be neutralized after the process, generating solid waste (Prado et al., 2016). The subcritical water (SCW) technique is one of the most promising technologies, which is essentially break down the recalcitrant structure of the lignocellulosic material in order to increase the accessibility of cellulose and hemicellulose polymers to cellulolytic enzymes (Koppram et al., 2014; Sangian et al., 2015b). One of the best strategies to convert coconut husk into fermented sugars is by using enzymatic hydrolysis – due to its low energy requirement and less pollution caused – however, the major problem of enzymatic hydrolysis is the low accessibility of cellulose, due to the rigid association on cellulose with lignin. It is also required to alter the biomass macroscopic and microscopic size and structure as well as its sub-microscopic chemical composition and structure so that hydrolysis of the carbohydrate fraction to monomeric sugars can be achieved more rapidly and with greater yields (Mosier et al., 2005). In other words, SCW is crucially and costly unit process in converting coconut husk into hydrogen.

Due to the severe process conditions, industrial application of this processes suffers from various challenges (Toor et al., 2011). Depend on the operational conditions, the degradation products are also formed, both from sugars (furan and its derivates and weak acids) and, to a less extent, from lignin (phenolics). These compounds may also inhibit the later fermentation processes, leading to lower hydrogen yields and productivities and, therefore, might be required to carry out a prior detoxification treatment (Gírio et al., 2010). Despite these limitations, the SCW technique has been proved to be an environmentally benign medium for a number of chemical and related processes in the last few decades (Wen et al., 2009). The challenge of this pretreatment method is the adequate fractionation of hemicelluloses, cellulose and lignin, together with a minor degradation, in order to get maximal fermentation yields and rates.

Although subcritical water process is able to degrade hemicellulose into dissolved sugars in the liquid fraction of SCW, there is still a residue of cellulose in the solid fraction. Therefore combining subcritical water and enzymatic hydrolysis was investigated to degrade the remaining cellulose in the SCW solid fraction into glucose which can be fermented into hydrogen (Ren et al., 2009). The combination of supercritical CO_2 and enzymatic hydrolysis has been studied by Alinia et al. (2010) and Gao et al. (2010) by using a mixture of commercial *cellulase* and β-*glucosidase* enzymes to enhance sugar yield from rice and wheat straw. Kumar et al. (2017b) analyses biohydrogen production from lignocellulosic biomass using dark fermentation and studied the bottlenecks associated with the pretreatment. However, as long as the literature studied, there has been no publication that reported studies of applying integration of three subsequent green processes, subcritical water-enzymatic hydrolysis-fermentation to produce hydrogen from coconut husk.

The objective of this work is to develop methods for efficient SCW and enzymatic hydrolysis of coconut husk into fermentable sugars and fermentation of hydrolysate to hydrogen. The effect of the severity factor on sugar yield was also discussed to determine the best condition of SCW.

2. Materials and methods

2.1. Materials

Coconut husk was obtained in Manado, North Sulawesi, Indonesia, it was firstly dried under the sunlight for 2 d, then in an oven at 105 °C. The material was milled and screened by using screener (*Retsch GmBh Rheinische Strade 36 4278, Haan, Germany*) to obtain a particle size of 120 mesh. After that it was washed with distilled water, dried at 105 °C until constant weight and stored in a desiccator.

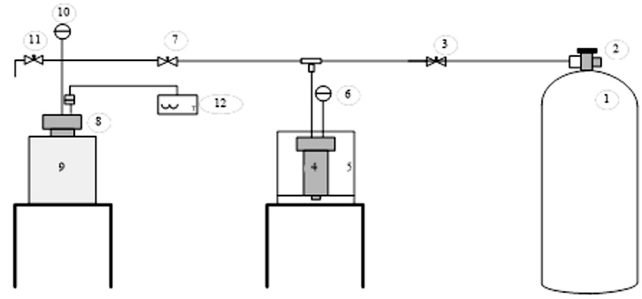

Fig. 1. The design of subcritical water apparatus.

2.2. Methods

2.2.1. Subcritical water pretreatment

Fig. 1 shows apparatus design of SCW that is adapted from work by Ju et al. (2011). It consists of three main parts, a subcritical reactor, heater, and control devices. They are a stainless-steel reactor with inner volume about 100 ml in total, a tube of carbon dioxide (CO_2), pressure regulator valve, output ball valve, jacket heater, a temperature controller (PID), and a pressure gauge. PID and pressure indicator were connected to the reactor. The process was run under batch mode.

Briefly, 6 g of coconut husk and 120 ml of deionized water were added to the reactor. CO_2 was supplied to the reactor until reached 80 bar and set the temperature at 150, 160, 180, 200 °C with a reaction time of 60 min when the temperature inside the reactor reached the desired temperature of the reaction. In the end of the process, the reactor was immediately cooled down by cooling water until 30 °C and the pressure was released instantaneously using the ball valve. The extracted sample then filtered using filter paper, and the residue was washed with deionized water. It was then dried in the oven at 60 °C for 2 d until constant weight and stored for analysis.

In order to make comparison, nitrogen gas was also employed in the present study.

2.2.2. Enzymatic hydrolysis

Enzymatic hydrolysis was conducted at the optimal condition based on previous work (Sangian et al., 2015a). The results of the solid from SCW-treated were hydrolyzed using commercial *cellulase* from *Aspergillus niger* (Sigma Aldrich, Japan) and Commercial *endo-1,4-*β*-xylanase* from *Trichoderma longibrachiatum* (Sigma Aldrich, Mexico). Enzymatic hydrolysis was conducted at following reaction conditions: pH of 3, the temperature of 60 °C, with incubation in a shake flask at 125 rpm for 48 h.

2.2.3. Fermentation

Enterobacter aerogenes NBRC 13534 was used in this fermentation studies. *E. aerogenes* was cultured on potato dextrose agar (glucose 20 g L^{-1}, peptone 10 g L^{-1}, yeast extract 5 g L^{-1}, and agar 16 g L^{-1}) at 30 °C. The hydrolysate was obtained from SCW-treated coconut husk that has the highest total reducing sugar (TRS) concentration. Inoculums were prepared by acclimation process, inoculating 10 oses of *E. aerogenes* to 8 mL pre-seed medium which added 0.35 g L^{-1} $FeSO_4 \cdot 7H_2O$ and incubating for 14–16 h. After incubation, the cell concentration was confirmed about 10 million cells/mL. The fermentation process was conducted on the 50 ml of working volume of fermentor which connected to hydrogen bag (CEL Scientific Tedlar Gas Sampling Bags, USA), incubated at 37 °C, adjusted to pH 7 using 4 M NaOH, agitated at 125 rpm for 48 h, and insured no leak for the anaerobic process.

2.2.4. Analytical methods

The composition of hemicellulose, cellulose and lignin at the untreated and SCW-treated condition were analyzed gravimetrically

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