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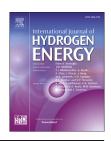
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Pretreatment conditions of palm oil mill effluent (POME) for thermophilic biohydrogen production by mixed culture

Safa Senan Mahmod a, Jamaliah Md Jahim a,b,*, Peer Mohamed Abdul a,b

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ABSTRACT

The availability of fermentable sugars in POME is one of the critical factors that determine the fermentative biohydrogen production yield. This study was carried out to determine the pretreatment conditions viz., temperature, hydrolysis time and acid concentration that can yield the highest monomeric sugars, to be utilized for production of biohydrogen from POME by mixed culture dark fermentation. Two different acids were used, nitric acid and phosphoric acid in the pretreatment of POME. Batch fermentation was performed to determine the potential of pretreated POME as a substrate for the production of biohydrogen under the tested pretreatment conditions. Higher hydrogen yield was successfully achieved using pretreated POME as compared to raw POME by mixed culture. Maximum hydrogen production was 0.181 (mmol/L/h), which corresponded to the yield of 1.24 mol H₂/mol glucose achieved at 0.8% (w/v) phosphoric acid with initial total reducing sugar concentration of 18.47 g/L. Hence, the results implied that POME pretreated with 0.8% (w/v) phosphoric acid is a potential substrate for efficient biohydrogen fermentation yield that is 97% higher than untreated POME. While POME pretreated with 1% (w/v) of nitric acid showed 65% improvement in biohydrogen yield as compared to untreated POME. Therefore, the results implied that both pretreatment methods of POME showed significant increase in the biohydrogen production.

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Introduction

Malaysia has been known for its high production of palm oil with an average crude palm oil production of more than 13 million tons per year. Hence, the more crude palm oil

produced the larger the amount of palm oil mill effluent (POME) is generated. However, POME is a highly polluting wastewater with high COD and BOD which can lead to serious environment pollution, particularly contamination of the water resources [1]. The conventional method used by most of the palm oil mills is the open ponds for POME treatment

E-mail address: jamal@ukm.edu.my (J.Md. Jahim).

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^a Department of Chemical and Process Engineering, Faculty of Engineering and Built Environment, Universiti Kebangsaan Malaysia, 43600 Bangi, Selangor, Malaysia

^b Research Centre for Sustainable Process Technology, Faculty of Engineering and Built Environment, Universiti Kebangsaan Malaysia (UKM), 43600 Bangi, Selangor, Malaysia

^{*} Corresponding author. Department of Chemical and Process Engineering, Faculty of Engineering and Built Environment, Universiti Kebangsaan Malaysia, 43600 Bangi, Selangor, Malaysia.

mainly for the low capital and operational cost of the open lagoons. The main disadvantages of this method are the huge land area required, the long retention time that is 20–200 days and the emission of greenhouse gases that are hard to be captured [2].

In the past few years, several methods have been considered in utilizing lignocellulosic biomass for the production of the different types of biofuel; including biogas, biodiesel, pyrolytic bio-oil, and bioethanol, whereby biogases can play significant role in term of developing energy security [3]. Meanwhile, POME and its derivatives have been considered as high potential substrates for the production of biohydrogen [4], it is comparatively resistant to biodegradation, however it is considered as a potential substrate for biohydrogen production [5], due to the high content of carbohydrates, protein, nitrogenous compounds, lipids and minerals that are present in POME [6,7]. It is worth to mention the advantage of microbial hydrogen fermentation from organic waste for being an environmental friendly process with high energy production yield that is 2.75 times higher than hydrocarbon fuels [8]. Furthermore, the process is relatively inexpensive, requires low energy and generates low pollution [4].

Many researches have been carried out on the pretreatment of POME, in order to increase its solubilization and release the fermentable monomeric sugars that could be converted to hydrogen in the anaerobic fermentation process. For instance, POME has been treated by heating [9,10], ozonation [9,11], drug fluvastatin and Na₂SO₄ dosing [9], acid or alkaline pretreatment [10,12], autoclaving [13], ultrasonication [14]. In pretreating other lignocellulosic materials, Ozkan and her co-workers [15] showed that H₂ production from pretreated sugar beet-pulp was highest in alkaline, thermal, microwave and thermal-alkaline treatments, respectively. Moreover, dilute acid pretreatment had significant impact on increasing the fermentable sugars yield of rice straw [16].

Dilute acids such as sulfuric [10], hydrochloric [16], phosphoric [17] or nitric [18] acids are the most common catalysts used in pretreatment of lignocellulosic materials. The product of the acid hydrolysis solution mainly contains monomeric sugars such as xylose, glucose and arabinose depending on the initial carbohydrate composition of the lignocellulosic material. Other by-products could also be released like oligosaccharides, furfural, hydroxylmethyl furfural (HMF), phenolic compounds and acetic acid [18]. It has been reported that nitric acid had shown a short reaction time and higher efficiency for saccharification. Also, residual nitric acids can be used as a nitrogen source for the fermentation process, by forming nitrate when neutralized, to replace other common nitrogen sources such as yeast extract ammonium, amino acid, and urea [19]. On the other hand, phosphoric acid has been known to fractionate the lignocellulosic biomass and enhance cellulose digestibility [3]. Phosphoric acid is environmentally friendly and the solid residues can be used as fertilizers [20]. Moreover, the absence of oxidizability in phosphoric acid makes it more advantageous in the treatment of lignocellulosic biomass over sulfuric acid, although the latter is one of the most commonly used acids in the industry [21].

Raw POME is discharged from the mill at high temperatures around 80-90 °C, hence it is more expected to be treated at thermophilic anaerobic condition with less difficulties. Furthermore, a higher substrate degradation and biogas production rate have been commonly reported as advantages of the thermophilic anaerobic POME digestion upon mesophilic digestion [22]. Khemkhao and co-authors [23], found that thermophilic anaerobic digestion can be operated at high OLR with satisfactory COD removal and biogas production, while little or almost no microbial washout was recorded as compared to mesophilic reaction. In addition, a higher concentration of volatile fatty acids (VFAs) was accumulated under mesophilic condition, which is related to the lower productivity of hydrogen [8]. Nevertheless, the mesophilic condition still can be preferable due to greater process stability [22].

The use of pure cultures for the fermentative H_2 production requires strict sterile conditions [24]. Mixed cultures have been suggested as an alternative to pure cultures, which eliminate the need for sterilization. In addition, mixed culture has the ability to adapt to the microbial diversity and handle different substrates [25].

In dilute acid concentration, it has been reported that reaction temperature, and reaction time, variation of acid concentration showed the highest sensitivity on sugar recovery and fermentation performance [26,27]. In the present study, we aimed to enhance the sugar solubility of POME by pretreating it with phosphoric or nitric acid in order to improve the hydrogen production using mixed culture enriched from anaerobic sludge. Herein, the variations in pretreatment time, reaction temperature and acid concentration were evaluated by one-factor-at-a-time design to examine the pretreatment conditions of POME.

Materials and methods

Pretreatment of POME

Palm oil mill effluent was collected from a local palm oil mill located in Selangor, Malaysia. The collected POME was stored in cold room at 4 °C for further use. Phosphoric acid (85%, analaR, BDH) and nitric acid (69–70%, J.T.Baker) were separately used to hydrolyse raw POME. The range of hydrolysis reaction time, hydrolysis reaction temperature and concentrations of both acids was 5–30 min, 25–75 °C and 0.1–3% (w/ v), respectively. The hydrolysis was carried out in 250 mL flask, and all tests were conducted in triplicates.

Inoculum preparation

The seed sludge used in this study was collected from a digested sludge originated from a thermophilic sequential batch reactor treating POME. The sludge was pre-heated at 80 °C for 60 min in a shaking water bath (Model SW22, Julabo, Germany) to inactivate the methane-producing bacteria and other non-hydrogen producing microorganisms, and then it was centrifuged and resuspended in POME and used as inoculum for biohydrogen fermentation. The typical pH, total COD, volatile suspended solid (VSS) and total suspended solid

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