Biohydrogen production from palm oil mill effluent by dark fermentation using clostridium butyricum

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Abstract. The process of producing oil from fresh fruit bunches produces palm oil effluent (POME). Good processing of POME is needed to prevent emissions that can damage the environment. POME can be used as a substrate to produce biohydrogen gas. This study aims to treat palm oil liquid waste-producing biohydrogen by dark fermentation using Clostridium butyricum. The initial stage is the addition of NaOH to POME which functions to degrade lignin and stop methanogenic bacteria. Then the hydrolysis method was carried out during dark fermentation using Clostridium butyricum with varying concentrations of NaOH (0.3; 0.5; 1% w/v POME) and hydraulic retention time. The results showed the highest biohydrogen production was obtained at a concentration of NaOH 0.3% w/v with a hydraulic retention time of 32 hours with an amount of 2824.2 mg H\textsubscript{2}/L POME.

1. Introduction

Palm oil is one of the important industrial plants. The largest amount of vegetable oil is produced from crude palm oil (CPO). CPO is an industrial raw material that is widely used for various industrial sectors, including the food industry, cosmetics industry, pharmaceutical industry, steel industry and others. The increase in the palm oil industry in addition to having an impact on meeting the needs of the community for palm oil consumption, also impacts the environment. From an environmental perspective, the palm oil processing industry produces waste in the form of solid waste and liquid waste. One type of waste produced by the CPO industry is palm oil mill effluent (POME). Processing every 1 ton of oil palm produces more than 50% of POME \cite{1}. The large amount of POME produced is an environmental problem for the CPO industry \cite{2}. This is due to the high organic content of POME which can reduce dissolved oxygen levels which are known from the COD parameter values of 50 gr / L and BOD 25 gr / L \cite{3,4} One type of processing carried out is the conversion of POME into a more valuable product using the help of microorganisms. One product that can be produced from POME processing is biohydrogen.

Biohydrogen is a renewable energy that is environmentally friendly. Therefore biohydrogen has the potential as alternative energy that can be used to overcome the depletion of fossil oil reserves and reduce the impact of emissions produced on the environment \cite{5}. The great demand for hydrogen in the future prompted a number of researchers to optimize biohydrogen production. Parameters that
affect biohydrogen production include residence time and substrate concentration. This research aims to produce biohydrogen from POME processing by dark fermentation using Clostridium butyricum. The variables used in this study are variations in residence time and variations in NaOH concentration in the pretreatment of POME substrates.

2. Experimental Methods

2.1. Pretreatment
POME storage was carried out in a 4°C freezer. POME was centrifuged for 7 minutes with 21,000 rpm before adding NaOH. Solids obtained were collected and taken as much as 40 gr to the flask. There are 3 flask with NaOH concentrations variations 0.3% (w/v), 0.5% (w/v) and 1% (w/v) which was added as much as 400 ml. The result of mixing was heated at a temperature of 30°C for 4 hours. The POME solid was then filtered and washed with distilled water to remove the base. POME was centrifuged again for 7 minutes with a speed of 21,000 rpm to remove the water content.

2.2. Bacteria cultivation
Cultivation of Clostridium butyricum was carried out using RCM as much as 10 mL in a 50 mL ampoule bottle for one week. Then the results of cultivation were transferred in 40 mL RCM media to obtain new 50 mL inoculums.

2.3. Dark fermentation
1 L POME of the enzymatic hydrolysis was put into the 2 L flask. Then nitrogen gas has flowed for 5 minutes in the flask. Furthermore, bacterial culture in RCM media as much as 100 mL was inserted into the flask. Fermentation was carried out for 4 days by taking and analyzing samples and products in 32 hours, 64 hours and 96 hours.

2.4. Analysis of biohydrogen products in gas chromatography
Biohydrogen products were analyzed after fermentation had completed in 32 hours, 64 hours and 96 hours sample variations in each NaOH pretreatment concentration substrate 0.3: 0.5 and 1% w/v POME. Biohydrogen products were analyzed using gas chromatography.

2.5. Glucose analysis in the fermentation process at HPLC
HPLC was used to analyze glucose content in palm oil liquid waste substrate. Theoretically, the glucose content in POME hydrolysis is enzymatically around ± 1-5%. The concentration of glucose standard solution used is 1%, 2%, 5% and 8% with a ratio of acetonitrile: water (80 : 20% v/v). The samples which to be analyzed are filtered to remove noise particles.

3. Results and Discussion
The sugar content obtained from the hydrolysis of POME is used by Clostridium butyricum as an energy source to increase the rate of cell growth. Initial glucose concentration has an important role in the yield and rate of hydrogen production during fermentation. The relatively low initial glucose concentration causes the fermentation rate to be also low [6].

(Figure 1) shows that the highest concentration of hydrogen gas occurs at a retention time of 32 hours, while at 64 and 96 hours, the concentration of hydrogen decreases. This is inversely proportional to the graph of glucose concentration. Longer retention time causes greater hydrolyzed glucose content.

(Figure 2) with 0.5% NaOH and (figure 3) 1% NaOH concentration show that at a retention time 32 and 64 hours increase glucose concentration result but decreases at retention time 96 hours and 0.5% NaOH concentration. Biohydrogen production has an increased concentration at a retention time of 32 hours to 64 hours, but the decrease that occurs is much lower than in the condition of 0.5% NaOH concentration.
Figure 1. Effect of glucose concentration to hydrogen gas production in the addition of 0.3% NaOH

Figure 2. Effect of glucose concentration to hydrogen gas production in the addition of 0.5% NaOH

Figure 3. Effect of glucose concentration to hydrogen gas production in the addition of 1% NaOH
Glucose as an inhibitor occurs when glucose inhibits the action of the hydrogenase enzyme in which the active center of this enzyme in the form of ionized ion groups cannot maintain the conformation of the active site to bind the substrate and catalyze the reaction [6]. This results in a decrease in the acquisition of biohydrogen. Decreased glucose concentration is influenced by the initial pH value. This happens because of an increase in the concentration of VFA (Volatile Fatty Acid) which causes a decrease in pH thereby inhibiting hydrogen production [7].

Decreasing glucose substrate concentration causes increasing partial pressure in the fermentation process. This will divert acidogenesis in the process to solventogenesis, thereby inhibiting hydrogen production. The process of hydrogen production by fermentation bacteria also accompanies the formation of volatile fatty acids (propionic acid) and alcohol as metabolic byproducts. This product formation reduces equivalents like NADH. This reduced NADH equivalent supports the metabolism of glucose into ethanol and the process of propionogenesis. As a result, the production of ethanol and propionic acid will cause a decrease in the acquisition of hydrogen products so that the ratio of NADH/NAD⁺ in cells must be maintained.

Several research reports show that biohydrogen production which initially obtains high production yields, will tend to decrease to low levels further processing due to the formation of propionic acid and the emergence of partial hydrogen pressure in the reactor [8]. If the concentration of propionic acid is more, it will reduce the production of biohydrogen. Conversely, if the ratio of acetic acid and butyric acid formation increases, the concentration of biohydrogen produced increases as well. Failure to maintain the level of carbon source (glucose) in the form of a liquid phase in the reactor also optimally affects the growth of microorganisms, the level of substrate utilization, enzyme activity and the overall results of the process itself. It is concluded that the hydraulic retention time affects the amount of glucose hydrolysis produced.

4. Conclusion
The conclusions obtained from this research are:
1. Biohydrogen can be produced from palm oil liquid waste using the bacterium Clostridium butyricum
2. The highest level of biohydrogen production is the concentration of NaOH 0.3%, which is 2824.2 mg H₂/L POME.
3. The best fermentation retention time in producing the highest levels of biohydrogen is at 32 hours at a 0.3% NaOH concentration.

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