Journal of Asian Scientific Research 2(11):705-710

Journal of Asian Scientific Research

journal homepage: http://aessweb.com/journal-detail.php?id=5003

BIOHYDROGEN PRODUCTION USING HYDROLYSATES OF PALM OIL MILL EFFLUENT (POME)

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ABSTRACT

Dark hydrogen fermentation using lignocellulosic biomass has been widely reported. In this study, raw and hydrolysed Palm Oil Mill Effluent (POME) were used as substrates to produce hydrogen by POME sludge in 30-mL serum bottle. A higher cumulative volume of hydrogen of 1439 mL $H_2.L^{-1}$ POME was obtained from hydrolysed POME as compared to raw POME. Fermentation process was then carried out in 2-L stirred tank bioreactor using hydrolysed POME as medium and it was found that a two-fold of hydrogen volumetric rate increase was achieved.

Key Words: Palm oil mill effluent (POME), Hydrolysis, Fermentation, Hydrogen.

INTRODUCTION

The crucial demands for energy, the critical condition of fossil fuel reserves as well as undesirable environmental pollution have driven the force to search for alternative fuels. Hydrogen is considered as viable alternative fuel and future energy carrier as its application for electricity generation in fuel cells proves to be environmentally friendly (Kapdan et al. 2006).

Among various hydrogen production processes, biological hydrogen production is less energy-intensive as it could be operated under mild operation condition (Wang et al. 2008). Biological hydrogen production through dark fermentation process is technically simple to be operated as well

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as high rate of hydrogen evolution has been achieved in comparison to photo-fermentation process (Das 2009 & Wang et al. 2008).

Various organic wastes could be used as substrate to produce hydrogen through dark fermentation (Hallenbeck et al. 2002), such as agro-industrial waste containing lignocellulosic materials. Nonetheless, degradation of lignocellulosic biomass is time-consuming since it is contained cellulose and hemicelluloses (Chong[a] et al. 2009). Hence, enzymatic hydrolysis could be employed to convert lignocellulosic biomass into monomeric sugars (Ibrahim et al. 2012). In Malaysia, Palm Oil Mill Effluent (POME) is an abundant agro-waste water that was produced about 15.2 million tons annually (Chong[a] et al. 2009). The organic substances composed in POME are opportunely applicable for the hydrogen fermentation process (Atif et al. 2005).

A pure or mixed culture could be employed to produce hydrogen during fermentation process (Wang et al. 2008). In comparison, mixed culture is more preferable to be used as its application does not involve sterilisation (Atif et al. 2005).

The aim of this study is to compare the hydrogen production from raw and hydrolysed POME conducted at mesophilic condition with initial pH of 6. Feasibility study was carried out in 2-L stirred tank bioreactor to determine the performance of hydrolysed POME to produce hydrogen in large scale. The results of hydrogen production, pH changes, cell densities, sugars degradation, and soluble metabolites production during fermentation were observed and documented.

EXPERIMENTAL PROCEDURE

POME sludge containing anaerobic microorganisms, used as a seed inoculum, were obtained from enriched acclimatised POME by our previous researcher (Ahmad Kamal et al. 2012). Fresh raw POME was collected from Sime Darby East Palm Oil Mill, Carey Island, Selangor and kept refrigerated at 4°C prior to usage. The cellulase and cellobiase used in this study was *Celluclast* 1.5L and *Novozyme* 188 supplied by Sigma-Aldrich (USA) with initial measured enzyme activities equal to 1651.80 and 2993.76 U – mL^{-1}, respectively. Determination of cellulase and cellobiase activities was based on the method described by Ghose (1987) and Yeoh et al. (1988), respectively. About 1% (v/v) enzyme cocktail was added to raw POME at pH 4.8 and hydrolysis process was done for 24 hours at 50°C with agitation speed of 150 rpm.

Two sets of experiment were conducted; in 30-mL serum bottles and using 2-L stirred tank bioreactor for hydrogen production by acclimatised POME sludge. The fermentation was initiated by adding 20% (v/v) POME sludge to 80% (v/v) POME medium. The anaerobic condition was established by sparging the nitrogen gas for 10 minutes during the start-up of fermentation process. The fermentation was then carried out at mesophilic condition (37°C) with initial pH of 6.0 and being agitated at the speed of 150 rpm.

The biogas evolved was collected in inverted graduated cylinder using water displacement method. Gas Chromatography (GC), model SRI 8610C equipped with TCD and HID detectors was used to determine the biogas composition. Helium gas was used as gas carrier at flow rate of 25 mL – min^{-1}. The temperature at the injection port, detector and oven were set at 41°C, 90°C and 100°C, respectively. DNS method was used to determine the reducing sugar consumption (Miller 1959), while soluble carbohydrate concentration was determined using phenol-sulfuric acid assay (Fournier 2001). Cell concentration was determined based on Volatile Suspended Solid (VSS) method (APHA 1998). COD was quantified using HACH reagent, whereas soluble metabolites was measured using High Performance Liquid Chromatography (HPLC), Agilent 1100 (Agilent Technologies, USA).

RESULT AND DISCUSSION

Enzymatic hydrolysis effects on hydrogen production

Acclimatised POME sludge was employed for hydrogen fermentation using fresh raw POME and enzyme treated POME (or hydrolysed POME) at mesophilic condition (37°C) in 30-mL serum bottles. As shown in Figure 1 (a) and (b), higher hydrogen production was attained when hydrolysed POME was used as the sole substrate, with hydrogen cumulative volume, productivity and yield equals to 1439 mL $H_2.L^{-1}$ POME, 87.19 mL $H_2.L^{-1}$ POME hr $^{-1}$ and 2.84 mol H_2.mol glucose consumed^{-1}, respectively. Hydrolysed POME basically contains monomeric sugars which are favourable for microbial growth. This statement is proven by the result shown in Table 1, where the cell concentration could only be detected when performing experiments using hydrolysed POME. Thus, it is concluded that efficient cell growth induced rapid hydrogen production.

Raw POME constituted high soluble metabolites as compared to hydrolysed POME (Table 1). Hydrogen was produced during acidogenesis process, along with soluble metabolites (Lee et al. 2002). As reported by Chong[a] et al. (2009), high concentration of acids will inhibit hydrogen production. Therefore, low hydrogen production from raw POME was contributed by high soluble metabolites accumulation.

Figure-1. Hydrogen production from raw and hydrolysed POME by acclimatised POME sludge after 24 hours fermentation.

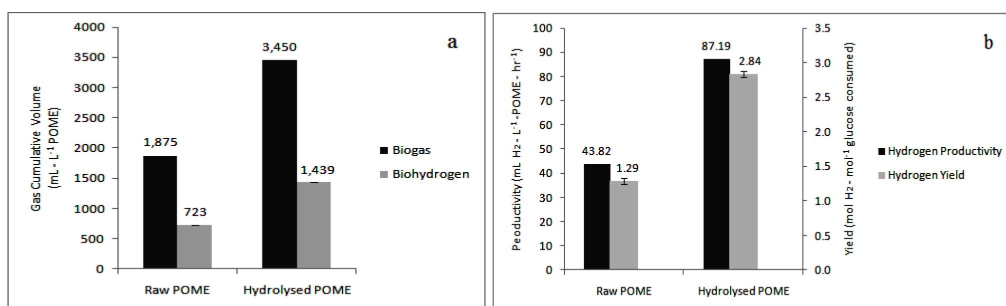


Table-1. Effects on substrates composition after 24 hours of fermentation in 30-ml serum bottle.

Type of substrates	Initial pH	Final pH	Cell density ($g.L^{-1}$)	COD removal (%)	Soluble metabolites ($g.L^{-1}$)		
					Acetate	Butyrate	Ethanol
Raw POME	6.00	4.96	nd	nd	2.36	1.65	2.12
Hydrolysed POME	6.00	5.89	1.60	10.76	0.69	1.09	1.72

[nd] not detected

Batch hydrogen production using hydrolysed POME as substrate

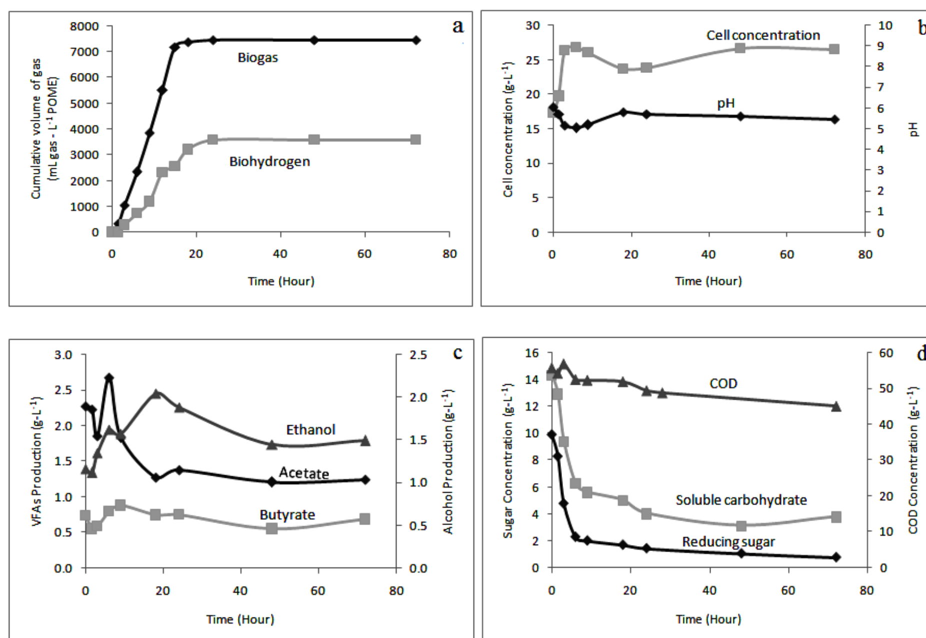
The hydrogen production from hydrolysed POME was further investigated in 2-L stirred tank bioreactor. The batch fermentation was conducted at initial pH of 6.0 using mesophilic condition (37°C). Based on the results, biogas production containing 40 – 50% hydrogen and carbon dioxide was obtained without methane.

As presented on Figure 2 (a) and (b), inoculation of acclimatised POME sludge had induced hydrogen production instantly. Adaptation of inoculum to hydrolysed POME took almost 1.5 hours of fermentation time, which represents lag phase. As cells entered exponential phase, hydrogen was vigorously produced and continued to evolve during stationary phase. According to Tanisho et al. (1987), hydrogen production was delayed when cells entered stationary phase as metabolic pathway was shifted to solventogenesis phase. Nevertheless, in this study, maximum hydrogen productivity was achieved during cell's stationary phase, which is equivalent to 155.65 mL $H_2.L^{-1}$ POME hr^{-1}.

Based on Figure 2 (b) and (c), pH values decreased from 6.0 to 5.2 as acidic metabolites started to accumulate during cell's exponential phase. However, bulk accumulation of acidic metabolites inhibits cell growth by penetrating the cell membrane, results in lower hydrogen production (Chong[a] et al. 2009). Hence, large energy is required to sustain cell's neutrality (Chong[b] et al. 2009). This statement is proved by the data presented on Figure 2 (d) in which almost 80% of sugar was consumed to supply the energy for maintaining the cell growth.

After 24 hours of fermentation, maximum hydrogen cumulative volume and yield was achieved, which are equivalent to 2859.72 mL $H_2.L^{-1}$ POME and 2.48 mol $H_2.mol^{-1}$ glucose consumed, respectively.

Figure-2. Batch hydrogen fermentation using hydrolysed POME as substrate by POME sludge. (a) Profile of cumulative hydrogen production (mL $H_2.L^{-1}$ POME), (b) Profile of cell growth and pH changes, (c) Profile of soluble metabolites production, (d) Profile of sugar consumption.



CONCLUSION

The enzymatic hydrolysis on POME improved hydrogen production by 50% as compared to raw POME. Hence, researches were carried out to determine the hydrogen fermentation profile using hydrolysed POME. The maximum hydrogen production, 2859.72 mL $H_2.L^{-1}$ POME was recorded after 24 hours fermentation.

ACKNOWLEDGEMENT

The authors gratefully acknowledge the financial support by the Universiti Kebangsaan Malaysia.

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