EFFECT OF INITIAL SUBSTRATE CONCENTRATION AND PH ON BIO HYDROGEN FERMENTATION FROM OIL PALM MESOCARP FIBER (OPMF) HYDROLYSATE IN BATCH FERMENTATION

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ABSTRACT: *In the recent years, the interest using lignocellulosic biomass for biological hydrogen fermentation has gain significant trantions. In this study, biohydrogen gas was produced from Oil Palm Mesocarp Fiber (OPMF) hydrolysate by Clostridium butyricum via dark fermentation. OPMF hydrolysate was prepared by pretreating OPMF with alkaline sodium hydroxide (NaOH) and followed by enzymatic hydrolysis. The resulting OPMF hydrolysate was used as the substrate medium for biohydrogen production in batch fermentation. The effect of NaOH concentration on OPMF pretreatment was observed by varying the concentration from 2 – 10 % (w/v). The optimal condition for the pretreatment was utilized when OPMF was pretreated by 6% of NaOH at 70°C which yielded around 20 g/L of reducing sugar. Anaerobic biohydrogen fermentation was performed at various initial pH (5, 6, 7, 8 and 9) and various initial substrate concentrations (5, 10, 15 and 20 g/L) in 30mL serum bottles at mesophilic (37°C), anaerobic condition. The best hydrogen yield was obtained at initial pH of 7 and initial soluble sugar concentration of 5 g/L respectively with acetate, butyrate and ethanol as major metabolites.*

1. INTRODUCTION

As part of modern living, rapid growth in the consumption of energy has been witnessed in recent years. However, the world's energy consumption is still heavily dependent on fossil fuel, especially in the transportation sector [1]. This over dependency on fossil fuel has led to serious environmental problems and energy security issue. One of the major associated with fossil fuel is the emission of greenhouse gases (GHG) and pollutant such as CH_4, SO_2, CO_2, NOx when fossil fuel was combusted hence leading to environmental catastrosphic such as acid rain and global warming [2].

Being an efficient, sustainable, renewable and clean energy carrier, hydrogen gas is poised as promising and potential alternative to polluting and limited fossil fuel [3].Many routes of hydrogen production process are available through various technologies and sources. Currently, almost all hydrogen gas is manufactured from fossil fuels by partial oxidation or steam or methane reforming [4] [5]. Both of these processes require extensive industrial inputs and consume a significant amount of fossil resources. Other than that, hydrogen gas can be obtained through splitting of water via electrolysis but the operating cost is relatively very high [6]. Biological hydrogen production in dark fermentation process has attracted a lot of attention in recent decades due to the operation simplicity, renewable resources and improving the rate of hydrogen production [7] [8].

The lignocellulosic biomass have been reported as viables substrates for biohydrogen production through dark fermentation process due to their abundance. As a product of photosynthesis, biomass is the most valuable non-petroleum renewable source that can be utilized for the sustainability of biohydrogen production [3]. Generally, lignocellulosic biomass is a heterogeneous material of three types of biopolymer, which are cellulose, hemicelluloses and lignin [9]. Several researches and development activities has been previously reported in order to extract biopolymer to biomass and convert it into higher valued compounds [10]. Pretreatment process is required to disrupt the lignin seal and improve the accessibility of cellulolytic enzyme during the enzymatic saccharification process [11]. Pretreatment technology is sometimes applied to eliminate lignin in order to produce the best substrate or carbohydrate sugar to be used for the fermentation process.

The palm oil industry is reported to generate great volumes of lignocellulosic biomass during plantation and palm oil extraction process. Oil Palm Mesocarp Fiber (OPMF) is an attractive feedstock due its abundance, renewability and rich in various fermentable sugars and other valuable biochemical monomer. Annually, more than 10 million tons of OPMF was produced as palm oil, hence, adds to the increase of lignocellulosic material found in Malaysia [12]. Previously, OPMF was either applied as mulching or fed into boilers for power generation, however, both methods are proven to be damaging to the environment, hence a more sustainable process is mandatory.

Thus, the aim of this research was to extract fermentable sugars from oil palm mesocarp fibers using alkaline pretreatment and enzymatic hydrolysis process and then to find the optimum parameter for biohydrogen production via light independent fermentation process.

2. MATERIALS AND METHODS

Oil Palm Mesocarp Fiber (OPMF)

The oven dried OPMF with 6% overall moisture content was collected from Carey Island, Sime Darby in Selangor. The fibers were grounded into 2-mm average size using a grinder (model FRITSCH UCM 19) to increase the fiber surface area for enzymatic hydrolysis reaction. The ground OPMF were sealed in plastic bags and stored at 4°C until further use.

Inoculum Preparation

Clostridium butyricum used for hydrogen production in this study was a local strain isolated by our previous researcher [13]. The pure culture was inoculated in Reinforced Clostridia Media (RCM) for 15 to 20 hours at 37°C with the agitation speed of 150rpm until the optical density (OD) value reached 0.9 to 1.1. The inoculum was then prepared in a 100 mL serum bottle by adding 10% (v/v) seed culture and 90% (v/v) of RCM.

Pretreatment and Hydrolysis of OPMF

The pretreatment of OPMF was carried out at an ambient pressure (1atm) using different concentrations of NaOH varying from 2% to 10% (w/v) and heated at temperatures of 50°C and 70°C. The ratio of biomass to liquid used in this experiment was 1:10. After 2 hours of pretreatment, the resulting fibers were washed, neutralized and air dried. The dried pretreated fibers were kept in a sealed plastic bag at -8°C until it was used for enzymatic hydrolysis. The enzymatic hydrolysis of pretreated fibers was carried out in 250 mL Erlenmeyer flasks using an incubator shaker (ECOTRON EC 25, Switzerland) which was set at 50°C and 150rpm. About 5 g (dry basis) of the pretreated fibers was immersed in 0.05M sodium citrate buffer to maintain a desired pH of 4.8. The enzymes, Cellulase (Celluclast 1.5L, Novozymes A/S, Denmark) and cellobiase (Novozym 188, Novozymes A/S, Denmark) were added at 60 FPU per g glucan loading (FPU = filter paper unit) and 64 pNPGU per g glucan (pNPG = p-Nitrophenyl β-D-glucoside), respectively and incubated for 72 hours. At the end of the 72nd hour, the enzymes were denatured by boiling the mixture for 10 minutes. The hydrolysate were then filtered and the supernatant was stored at -8°C for sugar determination. The filter paper activity of Celluclast 1.5L (85FPU/ml) was determined according to a method by Ghose [14] while β-glucosidase activity of Novozyme 188 (1946 pNPGU/ml) was determined according to [15], respectively.

Fermentation in Serum Bottle

Batch fermentation was conducted in 30-mL serum bottle with 20mL working volume. Nitrogen gas was purged in order to remove the oxygen creating anaerobic condition in the serum bottle. Fermentation was carried out by adding 90% (v/v) OPMF hydrolysate as substrate and 10% (v/v) inoculum. The effect of initial pH (5, 6, 7, 8 and 9) was carried out at constant initial substrate of 5g/L, whereas the study for the effect initial substrate concentrations (5, 10, 15 and 20 g/L) were carried out by fixing the pH at 7.

Analytical Procedure

The compositional analysis of oil palm mesocarp fiber such as structural carbohydrate, lignin, extractives and ash was analyzed according to the laboratory analytical procedure (LAP) [16].Total reducing sugars and total carbohydrates in each sample were determined by dinitrosalicylic acid (DNS) reagent and phenol sulphuric acid assay, respectively. The hydrogen content in the biogas was characterized by using gas chromatography (GC) (model SRI 8600C, USA) with thermal conductivity detector (TCD) and helium ionization detector (HID). The system was equipped with two GC columns, 6' silica gel column and 3' Mol Sieve A column (Restek, USA). Helium gas was used as the carrier gas (MOX 99.99% at 25mL/min). The temperatures of the oven, injector port and detector were 100°C, 41°C and 90°C respectively. The amount of volatile fatty acids (VFAs) and alcohol analysis were measured using High Performance Liquid Chromatography (HPLC), (Agilent 1100 series, Agilent Technologies, USA).

3. RESULTS AND DISCUSSION

Composition of OPMF

The composition of monomeric sugar and other components of raw OPMF were characterized as shown in Table 1.

Table 1: Composition of the raw OPMF

Composition	Content (%)
Glucan	28.8 ± 0.48
Xylan	25.3 ± 0.65
Arabinan	1.91 ± 0.12
Acid Insoluble Lignin (AIL)	28.97 ± 2.07
Ash	2.6 ± 0.34
Ethanol Extractive	6.32 ± 0.51

Glucan was the major component in the fiber followed by xylan, arabinan and acid insoluble lignin. Hence xylan was the main component of hemicelluloses in the oil palm mesocarp fiber.

From the result obtained, most of the component produced was lesser compared to the previous study except for acid insoluble lignin composition [17]. The composition indicated that indeed OPMF is rich in carbohydrates and a potential source of biohydrogen production.

Pretreatment and Hydrolysis Process

The experimental results of enzymatic hydrolysis of oil palm mesocarp fiber using different pretreatment econditions are shown in Fig. 1. The effect of the amount of sodium hydroxide with different temperature were evaluated by comparing the total reducing sugar produced in the hydrolysis process.

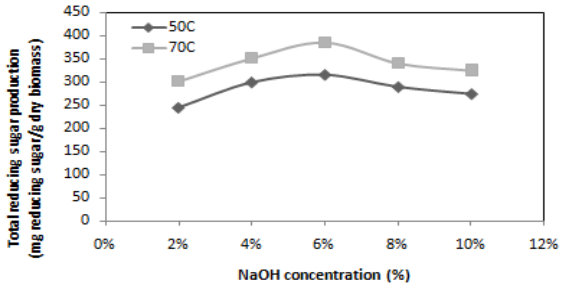

Fig. 1: Total reducing sugars production in hydrolysis process from pretreated oil palm mesocarp fibers with NaOH concentration of 2%, 4%, 6%, 8% and 10% (w/v) at 50°C and 70°C for 2 hours.

It was observed that the pretreatment with 6% NaOH and higher temperature of 70°C lead to the highest total reducing sugar after enzymatic hydrolysis. However, further increment of NaOH concentration led to the decrease amount of total reducing sugar production in the hydrolysate. The results also showed that some of the sugar component (glucan, xylan and arabinan) of pretreated fiber dissolved into the black liquor during the pretreatment process. The solubilisation rate of OPMF structural sugars was found to be higher at high

sodium hydroxide (NaOH) concentration and in high temperature condition.

This finding was corroborated to [18] who also pointed out that temperature, concentration and treatment period are the most important parameters that must be manipulated during the pretreatment process in order to maintain or retain the carbohydrate sugars in the treated fiber.

Effect of Initial pH and Biohydrogen Production

Wang [19] observed that manipulating the culture pH is one of the critical factors affecting the efficiency of fermentation. In this study, the initial pH varied from 5 to 9 with the increment of 1. The effect of an initial pH of OPMF hydrolysate on the hydrogen production was investigated at a fixed initial total sugar concentration of 5 g/L.

Fig. 2 and Table 2 showed that the maximum cumulative hydrogen of 1243.1 mL/L-hydrolysate and the highest hydrogen yield of 2.51 mol H_2/mol sugar consumed were produced at an initial pH of 7. The initial pH value of OPMF hydrolysate dropped to a final pH of around 4.7 to 5.2 during the cultivation. This result is parallel to the findings by Syafawati [20] and Fan [21] who reported that the initial pH 7 represented the optimum pH for biohydrogen production.

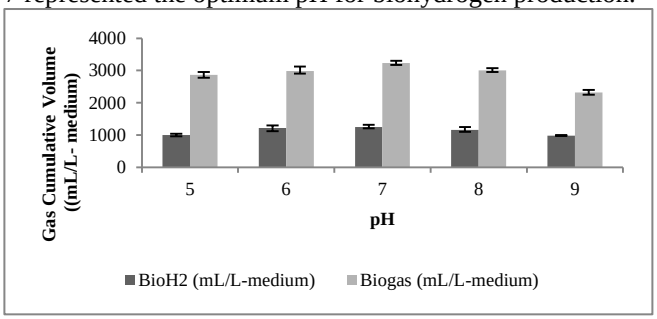

Fig. 2: Hydrogen Production from OPMF hydrolysate by *Clostridium butyricum* at various initial pH after 48 hours fermentation

Table 2: Effect of Initial pH on biohydrogen production after 48 hours fermentation

Parameter		Sugar Consumed (g/L)	Hydrogen Yield (mol H_2/mol sugar consumed)	Soluble Metabolites (g/L)		
Initial pH	Final pH			Ethanol	Acetate	Butyrate
5	4.70	4.15	2.05	2.49	3.01	1.10
6	4.90	4.57	2.43	1.09	1.42	0.22
7	5.03	4.80	2.51	1.03	2.84	0.46
8	5.05	4.31	2.37	1.03	2.25	0.02
9	5.22	4.77	1.97	0.86	1.40	0.14

The biohydrogen production was accompanied by the production of volatile fatty acid, VFAs and alcohol [22]In this study, acetate, butyrate and ethanol were the main metabolites produced during the fermentation process while no lactate and propionate were detected (Table 2). The amount of VFAs and alcohol produced in 48 hours fermentation was acetate; (2.84 g/L), butyrate; (0.46 g/L) and ethanol; (1.03 g/L). As shown in Table 2, biohydrogen yield at the initial pH of 5 was the lowest which can be which can be correlated with higher accumulation of soluble metabolites as reported by Chong [23] where it was found that high concentrations of acids such as VFAs inhibited the biohydrogen production.

It was reported that the lowe initial pH values caused longer lag periods in the hydrogen production [24] whereas the high initial pH values decreased the lag time and cause the lower yield of hydrogen production [25].

Effect of Initial Total Sugar Concentration on Biohydrogen Production

In this study, the effect of the initial sugar concentration from 5 g/L to 20 g/L with the increment of 5 on the hydrogen production was investigated. The initial pH of the hydrolysate was fixed at the optimum pH of 7.

Fig. 3 and Table 3 showed that the maximum cumulative hydrogen of 1153.7 mL/L-hydrolysate and the highest hydrogen yield 2.09 mol H_2/mol sugar consumed were produced at the initial total sugar concentration of 5 g/L. The results showed that the cumulative hydrogen production increased while biohydrogen production yield decreased when the concentration of the initial total sugar concentration was increased (Fig. 3).

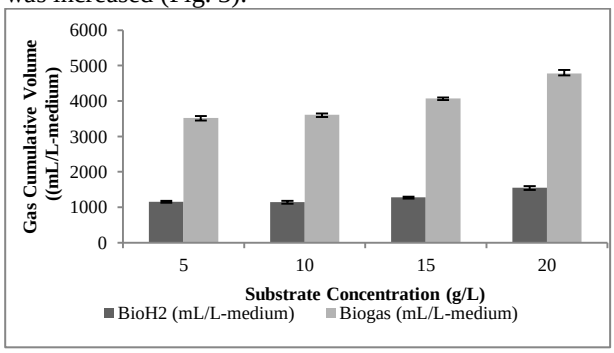

Fig. 3: Hydrogen production from OPMF hydrolysate by *Clostridium butyricum* at different substrate concentration

Based on the result shown in Table 3, acetate, butyrate and ethanol were the main metabolites produced during the fermentation process with no lactate and propionate were detected (Table 3). The amount of VFAs and alcohol produced after 48 hours of fermentation were acetate; (2.53 g/L), butyrate; (0.9 g/L) and ethanol; (1.39 g/L). This study showed that changes of the initial total sugar concentration affected the cumulative hydrogen production volume, hydrogen production yield, VFAs production and alcohol production.

Ginkel [26] and Fan [27] reported that, the increase of initial total sugar concentration would increase the partial pressure in the headspace of serum bottle and caused the switching of hydrogen production pathway to solvent production pathway, thus inhibiting the hydrogen production. Other than that, the increase of initial total sugar concentration could enhance the accumulation of VFAs and other solvent which can inhibit the growth of the microorganism [21].

Table 3: Effect of initial substrate concentration on biohydrogen production after 48 hours fermentation

Substrate Concentration (g/L)	Sugar Consumed (g/L)	Hydrogen Yield (mol H_2/mol sugar consumed)	Soluble metabolites (g/L)		
			Ethanol	Acetate	Butyrate
5	4.61	2.09	1.39	2.53	0.90
10	9.04	1.12	2.35	2.85	1.56
15	13.54	0.74	3.46	3.12	2.26
20	18.00	0.68	3.52	4.41	2.19

4. CONCLUSION

The optimal condition for the pretreatment was obtained when OPMF was pretreated with 6% of NaOH at 70°C which yielded around 20g/L sugar. The optimal condition of the biohydrogen production in dark fermentation process were obtained at the initial pH 7.0 and initial sugar concentration 5g/L respectively. The best hydrogen yield obtained at this condition were 2.09 molH_2/mol sugar consumed.

5. ACKNOWLEDGEMENTS

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