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Effect of Different Temperature, Initial pH and Substrate Composition on Biohydrogen Production from Food Waste in Batch Fermentation

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Abstract: The aim of this study was to establish the optimum operating parameters for biohydrogen production from food waste. Batch fermentation was conducted using a 150 mL serum vial incubated in anaerobic condition. Heat-treated Palm Oil Mill Effluent (POME) sludge was used as the seed culture for biohydrogen production. Biohydrogen production was performed at different temperatures (35, 40, 50, 55 and 60°C), initial pH (5, 6, 7 and 8) and various compositions of sludge to substrate (10:90, 20:80, 30:70 and 40:60% (v/v)). The highest biohydrogen yield was 593 mL H_2 g^{-1} carbohydrate for the experiment conducted at a temperature of 55°C, initial pH 7 and composition of sludge to substrate at 30:70% (v/v). The biohydrogen production from the waste was accompanied by the production of organic acids and the ratio of Hac/HBu was 0.87. Treatment efficiency as shown by Total Carbohydrate (TC), Total Suspended Solids (TSS) and Total Volatile Solids (TVS) reduction were 38, 25 and 18%, respectively.

Key words: Biohydrogen, food waste, anaerobic digestion, palm oil mill effluent sludge

INTRODUCTION

Hydrogen is a clean energy that could be produced from renewable materials, such as organic waste, crop straw and wastewater (Li and Chen, 2007). Biohydrogen produced from various renewable resources is less energy intensive than that from chemical or electrochemical processes since its production is carried out at ambient temperature and pressure (Jo *et al*., 2007) and it is considered as an alternative to fossil fuel.

Biological hydrogen production has so far been produced from wastewater (Van Ginkel *et al*., 2005; Mohan *et al*., 2007), sugars (Ren *et al*., 2006; Ustak *et al*., 2007), food waste (Jo *et al*., 2007), starch (Wang *et al*., 2007), palm oil mill effluent (O'Thong *et al*., 2007) and other sources of biomass. The majority of the research focusing on biohydrogen production from organic waste and carbohydrate rich substances employ mixed microflora to get higher yields of hydrogen. Food waste and Palm Oil Mill Effluent (POME) sludge have been considered as suitable substrates for biohydrogen production due to their abundance in Malaysia. Jo *et al*. (2007) stated that the major recycling methods such as composting and feed stuffing are not suitable to treat food wastes due to their high salinities. POME sludge from palm oil mills is costly to dispose of and treat. Therefore, biological fermentation of food waste and POME sludge is attractive to produce hydrogen as well as to treat these problematic wastes because they are carbohydrate-rich and easily hydrolysable. In addition, biohydrogen production would be accompanied by the formation of acidic metabolites (e.g., acetic acid, butyric acid, etc.). A proper control of the culture pH is a critical factor affecting the efficiency of the fermentation (Wang *et al*., 2007; Cheong and Hansen, 2007).

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Much research was conducted to produce biohydrogen from carbohydrate-rich substrate in the mesophilic and thermophilic temperature ranges. The thermophilic fermentation process gave high production rates and thus could be interest for industrial applications especially for plant discharges at high temperatures (Cheong and Hansen, 2007). It is of great interest to understand the correlation between pH and hydrogen production since pH could influence the activity of the hydrogen producer (Wang *et al*., 2007). Although many studies have been performed on biohydrogen production from organic wastes, but none on biohydrogen from food waste seeded with POME sludge.

The objective of this study was to determine the suitable parameters (temperature, initial pH and substrate composition) for biohydrogen production from anaerobic degradation of food wastes under non-sterile conditions using natural mixed microflora.

MATERIALS AND METHODS

Seed Sludge

The seed microflora for hydrogen production was taken from an anaerobic digester of Palm Oil Mill Effluent (POME) treatment plant at Felda Serting Hilir, Negeri Sembilan, Malaysia. The pH and the Volatile Suspended Solid (VSS) concentrations of the sludge were 7.12 and 55 g L^{-1}, respectively. The sludge was heat-treated at 80°C for 30 min to inactivate the hydrogen consumer and to harvest spore-forming anaerobic bacteria (Zhang *et al*., 2007). *Clostridium* sp. which is the main hydrogen producers is abundant in natural environments form spores in unsuitable circumstance (Kim *et al*., 2008).

Substrate

Food waste was collected from several restaurants in Seri Serdang, Selangor, Malaysia. Food wastes containing the ratio of 2:1:1 (carbohydrate: protein: fibre) was then ground using a Waring blender. The ratio of water added was 2 times greater than the weight of the food waste. Food waste with a Chemical Oxygen Demand (COD) of 100 g L^{-1} was used. Sodium bicarbonate (NaH_2CO_3) was added at 0.84 g/100 mL substrate to adjust the total carbohydrate/alkalinity ratio to be 1.0±0.1. Table 1 indicates the characteristics of the food waste used in this study.

Batch Fermentation

Batch fermentation was conducted in a 150 mL serum vial with 100 mL working volume. Nitrogen was purged for 10 min to eliminate the oxygen present in the system. Acclimatization was carried out by adding 50 mL heat-treated POME sludge in 50 mL Reinforced Clostridial Media (RCM) in water bath at different temperatures depending on the temperatures tested during fermentation for 24 h. The RCM medium contained (g L^{-1}): meat extract 10, peptone 5, yeast extract 3, D+ glucose 5, starch 1, sodium chloride 5, socium acetate 3, L-cycteine 0.5, agar 0.5. A 50 mL of this broth was added to 50 mL food waste for 24 h. Fermentation was carried out by adding the inocula and substrate at certain amounts (10:90, 20:80, 30:70 and 40:60% (v/v)) with adjusted initial pH values (5, 6, 7 and 8). The fermentation was conducted at different temperatures; 35, 40, 50, 55 and 60°C.

Table 1: Characteristics of the food waste used

Parameters	Values
pH	6.1-6.4
Total solid (g L^{-1})	285.8-376.6
Ammonia (g L^{-1})	0.145-0.266
Total sugar (g L^{-1})	49.26-62.10
Moisture (%)	71-73
Protein (%)	24.49-31.20
Fat (%)	22.98-28.58
Fiber (%)	1.07-1.34
Ash (%)	3.99-6.28

Analytical Methods

The biohydrogen generated from the batch fermentation was stored in a Hungate tube and estimated by using a gas chromatograph (Shimadzu Co. Ltd., GC-8A) equipped with a thermal conductivity detector with nitrogen as a carrier gas. The temperature of the column was 50°C while that of the injection and detector ports was 100°C. A pure hydrogen gas was used as the calibration standard. The total Chemical Oxygen Demand (COD), total carbohydrate, alkalinity, Total Suspended Solids (TSS) and Volatile Suspended Solids (VSS) were measured according to the Standard Methods (APHA, 1998). Organic acids were measured by HPLC (Shimadzu LC-10AS with UV-VIS detector SPD-10A) with 4 mM sulphuric acid as the mobile phase at a flow rate of 0.6 mL min^{-1}. Cumulative biogas production curves and total specific hydrogen production were obtained over time by using STATISTICA 6.0 for batch experiment. The modified Gompertz equation was used to analyze biogas production in batch mode (Zhang *et al*., 2007):

$$H = P.\exp\left\{-\exp\left[\frac{Rm.e}{P}(\lambda - t) + 1\right]\right\}$$

where, H is the cumulative hydrogen production (mL), P is the hydrogen production potential (mL), Rm is the maximum hydrogen production rate (mL h^{-1}), λ is lag-phase time (h) and e is 2.718.

RESULTS AND DISCUSSION

Food waste fermentation for biohydrogen production was carried out under strictly anaerobic fermentation. This study was conducted to reveal the influence of temperature, initial fermentation pH, and composition of substrate and sludge on biohydrogen generation. In all of the batch experiments, the biogas produced contained hydrogen (41-62%) and carbon dioxide (35-50%). There was no methane detected in the evolved gas.

Effects of Temperature

Hydrogen production was relatively low at mesophilic range (30-40°C) but higher at thermophilic range (50-55°C). The thermophilic condition reduces the solubility of hydrogen and thereby alleviates inhibition from hydrogen partial pressure (O'Thong *et al*., 2007). Extreme thermophilic temperature (60°C) resulted in slow hydrogen production rate. Figure 1 shows the effects of temperature on biohydrogen production from anaerobic digestion of food waste. Table 2 shows the values of hydrogen production potential (P), maximum hydrogen production rate (Rm), lag duration time and yield for all the experiments conducted in this study. A temperature of 55°C gave high yield (526 mL H_2 g^{-1} carbohydrate) and hydrogen production potential of 209 mL day^{-1}. The yield and hydrogen production potential were relatively low at 35°C. Cheong and Hansen (2007) reported that hydrogen production potential for thermophilic condition at 55°C was higher (134 mL day^{-1}) compared to mesophilic condition (67 mL day^{-1}). Therefore, 55°C was considered as the suitable temperature for biohydrogen production in this study.

Effect of Initial pH

It is known that the pH value plays a crucial role in influencing the biohydrogen production efficiency for anaerobic degradation of food waste. In this study, different initial pH values (5.0, 6.0, 7.0 and 8.0) were tested for biohydrogen production. Figure 2 shows the accumulated biohydrogen profile for different initial pH values in 24 h of anaerobic fermentation. The result of the biohydrogen profile for initial pH 5 cannot be plotted because the production of the accumulated biohydrogen was very low (0.12±0.02 N mL H_2 day^{-1}). Mohan *et al*. (2007) found that the initial pH 5 was not suitable

Table 2: Kinetics parameters and yield for biohydrogen production at different temperatures, initial pH values and compositions of substrate for 24 h of incubation

Conditions						
Temperature (°C)	Initial pH	Mixed sludge (v): substrate(v)	R_m (mL h^{-1})	λ (h)	P (mL)	Hydrogen yield (mL g^{-1} carbohydrate)
35	6	20:80	39	3	16	44±12.8
40	6	20:80	38	3	73	179±9.6
50	6	20:80	26	2	187	485±29.5
55	6	20:80	56	2	209	526±15.9
60	6	20:80	11	7	210	220±14.6
55	5	20:80	0.2	3	0.1	0.42±0.1
55	7	20:80	24	3	212	563±2.6
55	8	20:80	22	4	195	467±29.3
55	7	10:90	15	3	144	391±14.6
55	7	30:70	25	2	233	593±10.3
55	7	40:60	24	4	196	282±2.1

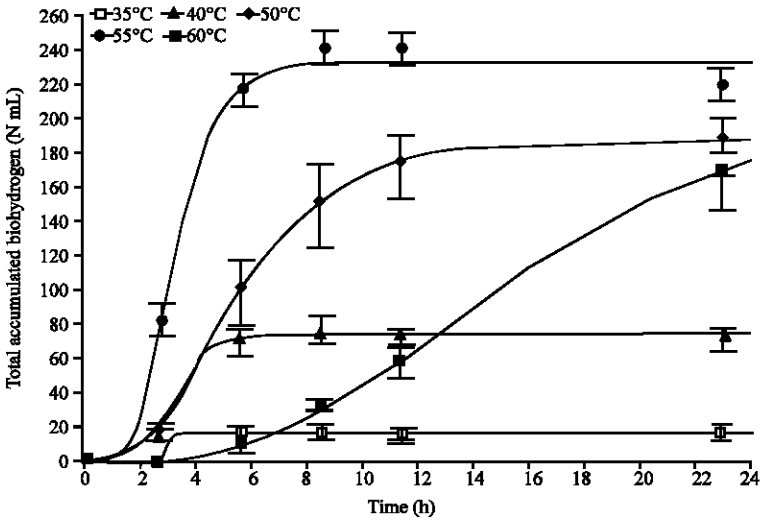

Fig. 1: Time course of biohydrogen production from food waste at different temperatures

for biohydrogen production. One of the possible reasons for the lower hydrogen yield at initial pH 5 was due to acids production in the system (Fig. 3). The acidogenic metabolisms shift the metabolic path to solventogenesis which might result in the suppression of hydrogen production (Mohan *et al*., 2007). Generally, acid accumulation in the system causes a sharp drop of the pH, thus inhibiting biohydrogen production. The bacteria involved could not sustain its metabolic activity at pH values less than 5.0 and complete inhibition was reported in the pH range of 4.0-5.0 (Li and Chen, 2007; Mohan *et al*., 2007).

Li and Chen (2007) and Zhang *et al*. (2007) stated that the highest yield of hydrogen was found at initial pH 7.0. The optimal initial pH for biohydrogen production from food waste in this study was pH 7.0, followed by initial pH 8.0 and 6.0. Mohan *et al*. (2007) reported that initial pH 6.0 gave higher hydrogen yield compared to initial pH 5.0 and 7.0. Figure 3 shows that the final pH values dropped to 4.3-5.4 due to the volatile fatty acids being produced. Similar, findings were reported by Li and Chen (2007). Wang *et al*. (2007) reported that extreme pH values (pH 4.0 and 9.0) appeared to inhibit hydrogen production. When the pH value in the system dropped below 4.0, the hydrogen production stopped. The control of pH could significantly affect hydrogen production and stimulate microorganisms to produce hydrogen (Zhang *et al*., 2007). When the initial pH was higher than 8.0 the culture required more adaptation time for hydrogen production (Zhang *et al*., 2007).

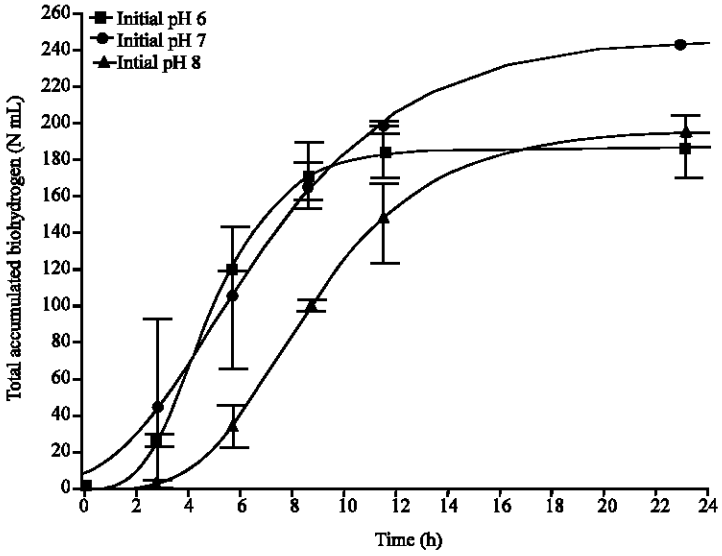

Fig. 2: Time course of biohydrogen production from food waste at different initial pH values

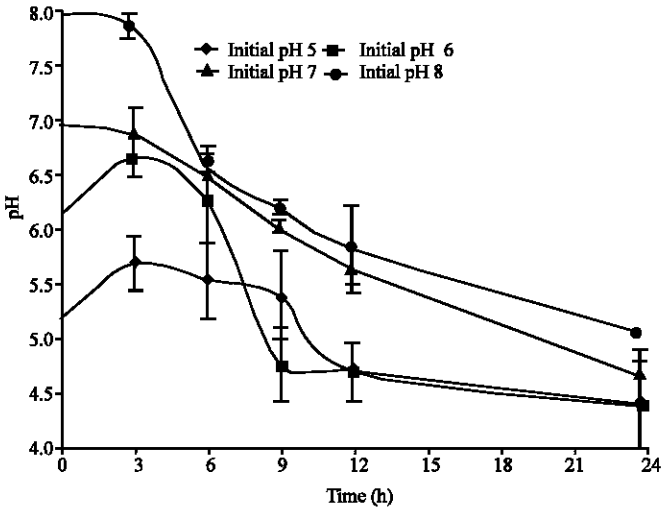

Fig. 3: pH profile during anaerobic digestion of food waste for biohydrogen production

Effect of Different Composition of Mixed Substrate and Sludge

The composition of the substrate had a significant influence on the overall hydrogen production (Mohan *et al*., 2007). The fermentation was done at 55°C and initial pH 7 were found to be suitable in the earlier experiments for biohydrogen production from anaerobic degradation of food waste. The main substrate used in this study was food waste and the addition of POME sludge as the hydrogen producer. Figure 4 shows the accumulated biohydrogen profile for different compositions of POME sludge and food waste (10:90, 20:80, 30:70 and 40:60% (v/v)). The results showed that 30% (v/v) inocula was suitable for optimal biohydrogen production which gave 214 mL day^{-1} of accumulated hydrogen. Kim *et al*. (2004) found the maximum specific hydrogen production potential was

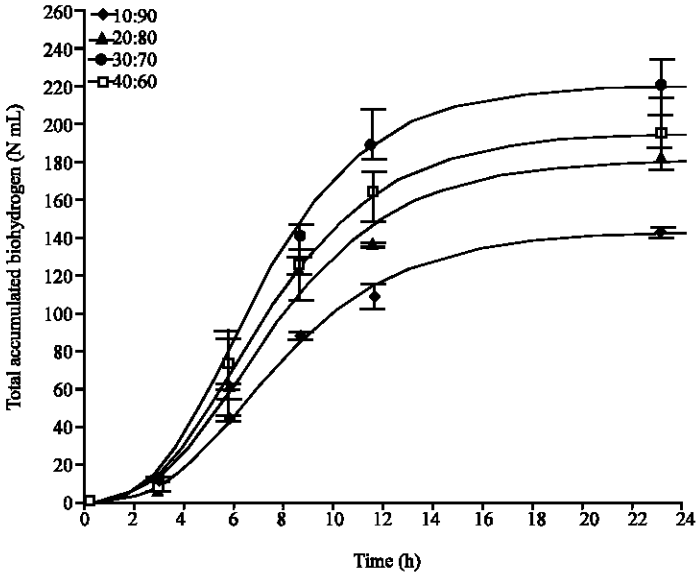

Fig. 4: Time-course of biohydrogen production from food waste at different compositions of substrate and sludge

113.2 mL day^{-1} for the waste composition of 87:13 (food waste:sludge). In this study, the lowest biohydrogen production obtained was at 10% (v/v) inocula (144 mL day^{-1}). This might be due to the high carbohydrate concentrations in the food waste causing product inhibition. Decreased in the carbon loading rate showed enhancement in hydrogen yield (Van Ginkel *et al*., 2005). Mohan *et al*. (2007) reported that the addition of sludge in the batch fermentation as co-substrate enhanced the performance of biohydrogen production.

In order to quantitatively describe the cumulative hydrogen production, a modified Gompertz equation was used to fit the experimental data (Zhang *et al*., 2007). Table 2 shows the summary of the kinetic parameters using the modified Gompertz equation and the yield of biohydrogen from fermentation of food waste. The highest biohydrogen yield (593 mL H_2 g^{-1} carbohydrate) was found at temperature 55°C, initial pH 7 and composition of sludge to substrate of 30:70% (v/v). Table 3 shows the comparison of hydrogen yield (per carbohydrate basis) obtained in this study with other findings. It can be noted that the result of this study showed higher yield compared to most other reports. This was due to the type of substrate used and the suitability of the natural mixed microflora (POME sludge) to convert the waste into biohydrogen. Moreover, the thermophilic condition would eliminate methane-producing bacteria.

Production of Soluble Metabolites

Biohydrogen production from food waste was also accompanied by the production of organic acids coupled with solvent production due to the acidogenic metabolism. The generation of these acidic intermediates reflects changes in the metabolic pathway of the microorganisms (Mohan *et al*., 2007). Table 4 shows the organic acids produced during fermentation of food waste in this study. During incubation, the pH in the broth dropped due to the acidogenesis in anaerobic fermentation and all the organic acids concentrations increased along the fermentation time. The main by-products obtained were lactate, acetate and butyrate. Acetate was the main product at start-up, while butyrate increased

Table 3: Comparison of biohydrogen yield in batch mode

Feedstock	Temperature (°C)	Initial pH	Biohydrogen yield (mL H_2 g^{-1} carbohydrate)	References
Rice	37	4.5*	346	Fang *et al.* (2005)
Food waste and sewage sludge	35	5.0-6.0*	122.9	Kim *et al.* (2004)
Starch	55	6.0	92	Zhang *et al.* (2003)
Cellulose	60	7.0	193	Ueno *et al.* (1995)
Food waste and POME sludge	55	7.0	593	This study

* pH controlled during the treatment

Table 4: Production of organic acids and acetate/butyrate ratios at 24 h fermentation of food waste

Temperature (°C)	Initial pH	Mixed sludge (v): food waste(v)	Concentration (g L^{-1}) Acetate	Butyrate	Propionate	Lactate	(Hac/HBu) ratio
35	6	20:80	2.09	3.25	0.96	8.62	0.64
40	6	20:80	1.35	1.68	1.24	6.83	0.80
50	6	20:80	6.23	6.84	-	3.06	0.91
55	6	20:80	0.75	1.31	0.13	5.31	0.57
60	6	20:80	2.71	2.78	0.60	4.57	0.97
55	5	20:80	0.35	1.01	-	1.53	0.35
55	7	20:80	1.20	1.94	0.54	2.81	0.62
55	8	20:80	0.36	2.36	0.15	1.44	0.15
55	7	10:90	0.65	1.15	-	1.95	0.57
55	7	30:70	4.45	5.13	0.32	4.62	0.87
55	7	40:60	1.46	1.78	-	0.97	0.82

Table 5: TSS, TVS and carbohydrate reduction after 24 h of anaerobic digestion of food waste

Temperature (°C)	Initial pH	Mixed inocula (v): substrate (v)	Reduction (%) TSS	TVS	Carbohydrate
35	6.0	20:80	12.4±1.10	12.9±0.7	33.9±1.70
40	6.0	20:80	22.5±0.60	11.3±0.6	38.0±0.70
50	6.0	20:80	26.5±0.50	13.5±0.8	37.5±1.70
55	6.0	20:80	28.4±2.30	12.4±2.3	36.7±1.70
60	6.0	20:80	25.3±0.30	14.8±0.7	70.6±1.80
55	5.0	20:80	31.7±1.70	11.6±0.4	27.1±3.00
55	7.0	20:80	34.0±9.30	17.4±2.7	36.2±8.00
55	8.0	20:80	43.0±7.20	25.5±2.5	39.9±22.8
55	7.0	10:90	24.9±1.00	10.2±3.9	48.0±10.2
55	7.0	30:70	25.5±1.80	17.9±1.5	37.9±7.10
55	7.0	40:60	30.5±14.1	31.4±1.4	66.4±6.90

significantly and become the dominant metabolite after reaching the steady-state for the case of POME as the substrate for biohydrogen production (O'Thong *et al*., 2007). Theoretically, the production of lactate causes the environment acidic thus inhibiting the production of biohydrogen (Jo *et al*., 2007). In this study, the concentration of lactic acid was much higher in mesophilic condition compared to thermophilic condition. Hence, the amount of biohydrogen produced in mesophilic condition (35 and 40°C) was relatively low. The metabolism of hydrogen-producing bacteria might be shifted to the formation of acids rather than hydrogen (Morimoto, 2004). A low concentration of propionate was observed in this study. Propionate was found to be insignificant in batch operation and propionic acid bacteria were inhibited by heat-treatment of anaerobic sludge (Kim *et al*., 2008). The ratio of Hac/HBu for the optimized conditions (55°C, initial pH 7, 30:70 (sludge:food waste) was 0.87. This finding is in agreement to the report by O'Thong *et al*. (2007).

Treatment Efficiency

Organic matter in food waste was converted to hydrogen, soluble by-product (organic acids and alcohols) and biomass through biohydrogen fermentation. The conversion of food waste to organic acids and alcohols would enhance the reduction of waste volume (Kim *et al*., 2004). The degradation

of substrate in the present study was analyzed by measuring Total Suspended Solid (TSS), Total Volatile Solid (TVS) and total carbohydrate (Table 5). The highest TSS removal achieved in this study was 43% for the experiment conducted at initial pH 8, 55°C and sludge: food waste at 20:80% (v/v). Kim *et al.* (2004) reported that suspended solids removal could be achieved up to 72-76% from food waste. For the case of biohydrogen production using mixed anaerobic cultures, an increase of total biomass is not always accompanied by an increase of hydrogen production due to microbial population changes (Hawkes *et al.*, 2002). Carbohydrate reduction shows the efficiency of the anaerobic system for degrading organic substances by the fermentation of food waste for the production of hydrogen. The highest carbohydrate reduction obtained in this study was 70.6% at temperature 60°C, initial pH 6.0 and 20:80 (sludge:food waste). The best conditions for biohydrogen production in this study (55°C, initial pH 7, 30:70 (sludge:food waste)) gave 38% of carbohydrate reduction and 25% reduction of TSS.

CONCLUSION

In this study, the biogas produced contained hydrogen (41–62%) and carbon dioxide (35-50%) with no detectable methane gas. Enhanced biohydrogen production could be achieved under proper controlled conditions with enrichment of biohydrogen producing bacteria from POME sludge in thermophilic conditions. The experimental results showed that biohydrogen production from food waste was optimal at a temperature of 55°C, initial pH 7, 30:70% (v/v) (sludge:food waste) which gave 593 mL H_2 g^{-1} carbohydrate and biohydrogen production rate of 25 mL h^{-1}. Biohydrogen production from anaerobic fermentation of food waste was accompanied with the production of organic acids and reduction of TSS, TVS and total carbohydrate. The HAc/HBu ratio obtained was 0.87 at the end of the experiment. Hence, food waste can be considered as a good substrate for biohydrogen production under suitable operating conditions.

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