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Original Research

Evaluation of Hydrogen and Methane Production from Co-digestion of Chicken Manure and Food Waste

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Abstract

Recently, the rapid expansions of agricultural waste, including chicken manure and food waste, has increased the amount of organic waste produced. Therefore, the main objective of this study is to evaluate the possibility of using the co-digestion of food waste and chicken manure for the production of biogas, hydrogen and methane. An anaerobic co-digestion of chicken manure (CM) and food waste (FW) was carried out using a 150 mL serum vial at different ratios: 0:1,1:9, 2:8, 3:7, 4:6, 5:5 and 1:0 of CM to FW, and incubated at 35ºC. The highest hydrogen and methane yields were 239.2 and 60.8 mL/gVS, respectively, for the experiment conducted at a selected ratio of 3:7 of CM:FW by using a 500 mL reactor. Tagged 16S rRNA gene pyrosequencing analysis for selected ratio 3:7 of CM:FW showed that the seed culture was comprised largely of uncultured bacteria from phyla *Proteobacteria*, *Bacteroidetes* and *Firmicutes*. During mesophilic hydrogen fermentation, phylum of Firmicutes (40%) was dominant at day 1, while phylum of Firmicutes (15%) dominated at day 13. *Clostridium sp.* was the main species detected in the acidogenic phase, while *Methanosaeta consilii* and *Methanosaeta hungatei* were detected during the methanogenic phase.

Keywords: co-digestion, food waste, chicken manure, Mesophilic, pyrosequencing

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Introduction

Biogas is known as a clean energy source due to its high specific energy content. The process of biological gas production in dark fermentation is less energy intensive than non-biological processes. Dark fermentation is a preferred method for resource recovery and energy conversion from food waste. Various types of feedstock such as municipal waste, livestock manure, food waste, and wastewater have been utilized as substrates in dark fermentation [1, 2]. Food waste, consisting primarily of carbohydrates, proteins and fats, also represents a source of bioenergy. Food waste (FW) is a suitable residual substrate mainly due to its high carbohydrate content and abundant availability [3].

The demand for chicken meat and eggs has increased in Malaysia. Chicken meat is one of the most consumed as a protein source in Malaysia among urban and rural residents [4]. The upward trend of chicken meat consumption is seen in Malaysia from 36 to 39 kg per capita consumption from 2000 to 2011, and 3200 broiler grower farms producing 523 million birds was reported in 2010, in which 43 million live birds were exported to Singapore [5]. The amount of the manure, for instance, has been estimated at 0.08-0.1 kg/day for chicken [6].

The biochemical pathway of dark fermentation is well established. It is generated as a product of acidogenesis and acetogenesis in the anaerobic digestion (AD) process, but is rapidly consumed by methanogenic bacteria in a single-phase digestion process. In general, the production of CH4 and H2 is a two-stage process involving separation of the acidogenic and the methanogenic stages. To produce hydrogen from a dark fermentation metabolism, the blocking of the methanogenesis in the anaerobic pathway is one of the key considerations due to the conversion of hydrogen to methane in this step. Efficient production of biogas depends on many factors, including operating conditions, substrate compositions, and microbial community. The use of complex microbial seed cultures as starting inocula is advantageous for biogas production from complex organic substrates. These advantages include higher operating stability and tolerance to indigenous microorganisms' presence in the feedstock, as well as capability for producing a wide range of hydrolytic enzymes [7].

The community structures of microorganisms and their metabolic capability play important roles in fermentation processes. Various culture-independent molecular methods have been used to explore dynamics in dark fermentation niches based on diversity of these phylogenetic markers, e.g., denaturing gradient gel electrophoresis [8, 9], clone library [10], and pyrosequencing of biodiversity marker genes [11, 12]. The tagged pyrosequencing approach is the current method of choice as it allows for high-throughput quantitative parallel analysis of microbial community structures and functions from different environmental and engineered systems [13].

The objectives of this study were (a) to evaluate the fermentative hydrogen and methane production from co-digestion of chicken manure and food waste at different ratios, (b) to determine the effect of pH adjustment of the substrate for hydrogen and methane production, and (c) to assess the microbial community in both hydrogenesis and methanogenesis stages by using 16S rRNA gene pyrosequencing.

Materials and Methods

Substrate

Food waste was collected from the cafeteria of the Engineering Faculty, Universiti Putra Malaysia (UPM). Food waste containing carbohydrates (rice), protein (fish or meat) and fiber (vegetables) at a ratio of 3:1:1 based on weight was ground using a Waring blender. The amount of water added was 2 times greater than the weight of the food waste. The chicken manure was collected at a UPM Chicken Farm and prepared at a ratio of 1:1 (chicken manure:tap water) based on weight. This feedstock was made once a week and then stored

Table 1. Characteristics of food waste and chicken manure used in this study.

Parameter	Unit	Food waste	Chicken manure
pH	--	5.67±0.1	8.3±0.3
Total Solids (TS)	g/L	93.33±1.7	105±1.7
Total Suspended solids (TSS)	g/L	82.00±2.1	98.0±2.5
Total Volatile Solids (TVS)	g/L	73.50±0.2	76.83±0.6
Volatile Suspended Solids (VSS)	g/L	72.30±0.8	69.50±0.7
Chemical Oxygen Demand (COD)	g/L	85.4±5.3	101.4±12.7
Carbon	%	41.5	28.9
Nitrogen	%	1.75	3.8
C:N	-	23.71	7.61

at 5ºC in a chiller. The characteristics of food waste and chicken manure used in this study are shown in Table 1.

Batch Fermentation

Batch fermentation was carried out using 150 mL serum vial with a working volume of 100 mL. The sample was prepared at different ratios 0:1, 1:9, 2:8, 3:7, 4:6, 5:5 and 1:0 of chicken manure to food waste. Chicken manure was used as an inoculum at the different ratio according to weight ratio. A bioreactor, 500 mL schott glass bottle with a working volume 400 mL was also set up for the selected ratio of substrate to determine the effect of pH adjustment at 7.0 on biogas production.

The fermentation using a single substrate, either food waste or chicken manure, was used as a control. The initial pH was adjusted at 7.0 using 2M NaOH and 2M HCl and then flushed with nitrogen gas for 10 min to eliminate the oxygen presence in the system. During fermentation, the temperature of the culture was maintained at 35ºC by incubation of the vial and Schott glass bottle in a water bath (Memmert). All experiments were carried out in duplicate. During fermentation, total gas volume was measured using a syringe (Terumo 50 mL), and gas composition was periodically monitored using gas chromatography (Carboxen-1010 PLOT l).

16S Metagenomics Analysis

The sample obtained from chicken manure to food waste fermentation at a ratio of 3:7 was submitted to the First BASE laboratories Sdn Bhd for metagenomics analysis. The 16S metagenomics analysis was performed based on MEGAN5 processing using a BlastN 2.2.30 tool. MEGAN5 shorten alignment files were filtered first based on significant threshold, and then the sequence was placed in the correct taxonomical branch.

Analytical Methods

Total solids (TS), total volatile solids (VS), chemical oxygen demand (COD) and ammonium-nitrogen (NH_4-N) were analyzed according to Standard Methods [14]. The amount of biogas generated was measured using a gas bag and syringe (Terumo 50 mL). Biogas content (H_2, CH_4, and CO_2) was measured using a gas chromatograph (Sigma-Aldrich Co. LLC) equipped with a thermal conductivity detector (TCD) and a column Carboxen-1010 PLOT, 30 m x 0.53 mm I.D. helium was used as a carrier gas. The temperatures of the injection port and the detector were 200 and 230ºC, respectively. VFA were analyzed by using high-performance liquid chromatography (HPLC) with a cation resin column (Aminex HPX-87H column, 300 mm x 7.8 mm). H_2SO_4 (4 mM) was used as a mobile phase at a flow rate of 0.6 mL/min. The wavelength and pressure was set at 210 nm and 150 psi, respectively. Cumulative biogas production curves were obtained over time by using STATISTICA 13.0 for batch experiment. The modified Gompertz equation was used to analyse biogas production in batch fermentation [15].

$$H = P \exp\left(- \exp\left(\frac{R_m\, e}{p}(\lambda - t) + 1\right)\right) \quad (1)$$

…where H is cumulative hydrogen or methane produced (mL), P is hydrogen or methane production potential (mL), Rm is rate of hydrogen or methane production (mL/h), λ is the lag phase (h), t is fermentation time (h) and e is 2.718281828.

Results and Discussion

Characteristics of Food Waste and Chicken Manure

The characteristics of TS, TSS and TVS were higher for chicken manure than food due to the different compositions of organic fraction of municipal solid wastes (Table 1). The COD of food waste was 85.4 g/L and the COD of chicken manure was shown to be much higher than food waste at 101.4 g/L. The pH of chicken manure was in alkaline condition at 8.3 while food waste showed acidic pH at 5.67.

Performance of Biogas Production from Food Waste and Chicken Manure

The characteristics of sample with different CM:FW ratios are listed in Table 2. According to the previous study, the pH value plays a crucial role in influencing the biogas production efficiency for anaerobic degradation of waste.

In this study, the initial pH of fermentation was adjusted at pH 7 to produce biogas. After 14 days fermentation, the result shows that the final pH decreased for the ratio CM:FW (0:1, 1:9, 2:8 and 3:7) and the final pH increased for the ratio CM:FW (4:6, 5:5 and 1:0). From the results of this study, we found that the pH increased with decreasing ratios of food waste. Increases in the addition of food waste caused the pH to be reduced dramatically. This might be due to the accumulation of volatile fatty acids (VFA) of food waste, resulting in the decrease of pH and even the failure of anaerobic digestion [16, 17]. Therefore, the addition of CM is required to act as a buffer to neutralize the acid produced and accumulated in the culture. Changes in pH are thus reflected by variations in substrate and energy utilization, synthesis of proteins and various storage products, and metabolite production [18]. The C:N ratio varied from 23.71 to 7.61 with increasing percentages of CM. Among the seven different ratios tested in this study, the C:N ratio for fermentation using CM:FW at a ratio of 3:7 was found to be optimal, which gave the highest biogas production (972 mL). The C:N ratio

Table 2. Performance of anaerobic digestion at different ratios of CM and FW at 14 days fermentation.

Parameters		CM:FW						
		0:100	10:90	20:80	30:70	40:60	50:50	100:0
Cumulative gas volume (mL)		214	421	574	972	710	626	480
pH	Initial	7	7	7	7	7	7	7
	Final	3.29	3.9	4.9	5.8	7.3	7.45	8.45
C:N		23.71	21.52	19.41	18.35	15.22	10.58	7.61
Reduction efficiency (%)	TSS	47.32	36.51	41.39	45.1	47.1	45.9	50.1
	VSS	22.61	25.43	33.97	45.4	36.1	39.32	34.39
NH_4^+-N (mg/L)		159.5	214.3	298.4	443.2	657.0	895.9	2342.9

was an important parameter, and the optimal C:N ratio has a significant effect on the efficiency of anaerobic digestion (AD). A substrate with low C:N ratio resulted in the production of high amounts of total ammonia nitrogen (TAN) and volatile fatty acids (VFAs), and these substances are important intermediate products produced during anaerobic digestion [19]. The C:N ratio of single CM was 7.61, indicating that CM contain high nitrogen composition. The NH_4-N concentration of CM was high (over 2000 mg L^{-1}). The high NH_4-N concentration led to a high NH_3 concentration, which may result in an unstable AD process due to loss of methanogenic activity [20]. The inhibition value for NH_4-N inhibition was started at 1700 mg L^{-1} [21]. One of the methods used to avoid excessive production of ammonia during AD is to increase the C:N ratio of feedstock. This can be done by co-digesting with other waste that high in biodegradable carbon to improve the performance of AD. Co-digestion of chicken manure is a suitable substrate for AD than the single substrate, and higher biogas yield can be obtained from this mixture. Fig. 1 shows cumulative biogas production at different ratios of chicken manure and food waste (0:1, 1:9, 2:8, 3:7, 4:6, 5:5 and 1:0).

In batch fermentation using serum vials, the highest biogas production (972 mL) was obtained when a mixture of CM and FW at a ratio 3:7 was used as substrate. The

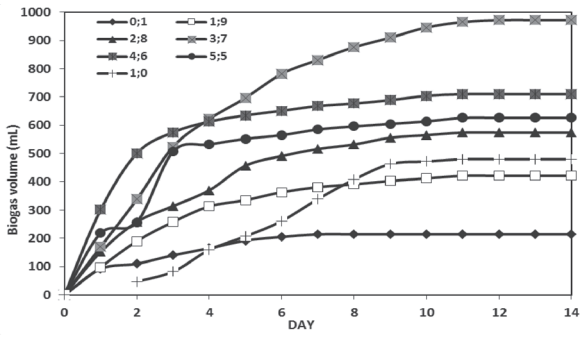

Fig. 1. Cumulative biogas production at various proportions of chicken manure and food waste (CM:FW).

production of biogas was increased drastically during the early stages of the fermentation (day 1 to 5). After day 7, reduced production of biogas was observed, but the production at a low rate was continued constantly until day 14. Very low biogas production (214 mL) was observed in a single waste digestion of food waste (0:1). In general, a single substrate fermentation process gave very low biogas production as compared to co-digestion fermentation. According to Serrano et al. (2014), the co-digestion process provides more balanced nutrients for efficient digestion with high biogas production [22]. Thus, co-digestion could be used to achieve higher digestion efficiency. The short biogas accumulation time and low biogas accumulation might also be due to the inhibition caused by the fast accumulation of VFA [23].

Theoretically, the early stages of fermentation process will release hydrogen gases, while methane gases will be released in the later stages of the process. Results from this study suggested that a mixture of CM and FW at a ratio of 3:7 was suitable for optimal biogas production, which produced 972 mL of accumulated biogas for 14 days of fermentation.

Fig. 2. shows the cumulative of hydrogen and methane gas produced from different ratios of CM:FW. The data of hydrogen production at various CM proportions, corresponding to Eq. (1) using the best-fitted kinetic parameters, are summarized in Table 3, also showing the data of hydrogen and methane yields, maximum specific hydrogen and methane production rates at various CM proportions. The H_2 yield (97.2 ml/gVS) obtained in digestion with CM at a proportion of 30% was higher as compared to the H_2 yield (55.2 mL/g VS) obtained from the digestion of food waste to microbial seed (F/M) at a ratio of 7.5, as reported by Chananchida et al. [24]. The yield and production rate of H_2 reported in the literature were varied due to the use of different proportions of carbohydrate in the feedstocks, the nature of feedstocks, fermentation pH and temperature [25]. The single digestion of food waste very low H_2 yield (possibly due to food overloading) and acidogenic microorganisms converted the food waste to volatile fatty acids,

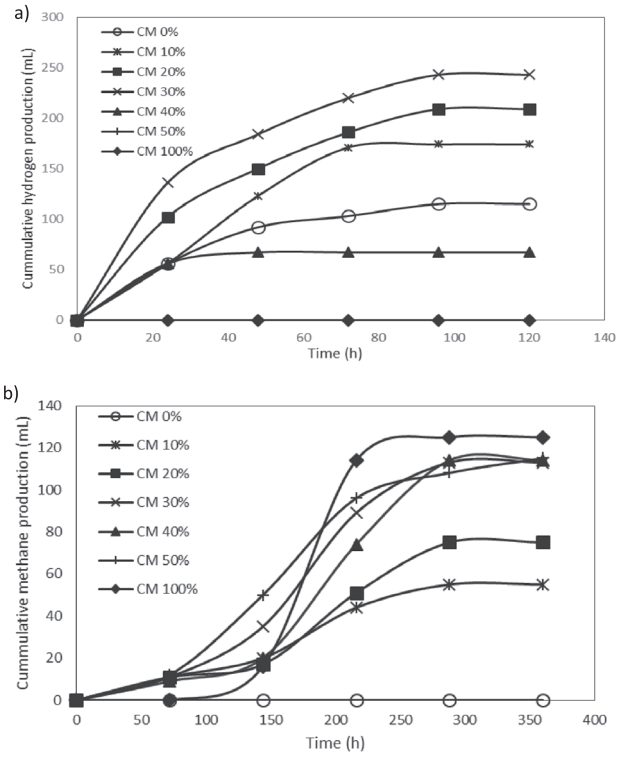

Fig. 2. Cumulative a) hydrogen and b) methane gas production at various proportions of CM.

rapidly resulting in an acidic condition (pH dropped to 3.29) in the reactor content. This phenomenon causes the inhibition effect on the hydrogen-producing microorganisms.

Fig. 2 shows that only hydrogen gas was produced in a single digestion of CM at concentration ranging from 0% to 40%. On the other hand, only gas methane was produced in a single digestion of CM at concentration ranging from 10% to 100%. The highest hydrogen gas production (243 mL) was obtained at 30% CM, while the highest methane gas production (125 mL) was obtained at 100% CM. The highest hydrogen yield (97.2 mL/gVS) and methane yield (45.2 mL/gVS) was obtained at 30% CM mixed with FW. The mixture of CM and FW at a ratio of 3:7 brought the highest yield of hydrogen and methane gas. In order to quantitatively describe the cumulative biogas production, a modified Gompertz equation was used to fit the experimental data.

Table 3 shows the kinetic parameters for H_2 and CH_4 production, the corresponding hydrogen and methane yields, and maximum specific hydrogen and methane production rates at various chicken manure proportions. Table 2 indicates that hydrogen and methane yield for co-digestion of food waste with chicken manure at 30% CM were higher than those obtained in a single digestion.

The highest hydrogen yield (97.2 mL/gVS) obtained in this study for digestion using 30% CM was higher compared to that reported by Kim et al. [26] for digestion using a single food waste. Results of this study have indicated that the co-digestion process produced a higher yield of hydrogen as compared with a single digestion process. According to Serrano et al. (2014), the co-digestion process provides more balanced nutrients for efficient digestion with higher biogas production, suggesting that it could be used to achieve higher digestion efficiency [22]. The yield of hydrogen and methane was significantly increased with increasing proportion of CM from 0% to 30%.

Table 3. Kinetic parameters for H_2 and CH_4 production, the corresponding hydrogen and methane yields, maximum specific hydrogen and methane production rates at various chicken manure proportions.

CM Propotion (%)	Hydrogen					Methane			
	ʎ (h)	Rm (mL/h)	P (mL)	HY[a] (mL/gVS)	MSHPR[b] (mL/h/gVS)	MMPR[c] (mL/h)	CMP[d] (mL)	MY[e] (mL/gVS)	MSMPR[f] (mL/h/gVS)
0	3.6	16.2	115	38.3	5.4	0	0	0	0
10	4.4	15.4	174	62.1	5.5	0.02	55	19.6	0.01
20	5.4	9.8	209	83.6	3.9	0.09	75	30.0	0.04
30	6.9	7.6	243	97.2	3	1.2	113	45.2	0.5
40	3.2	19.4	67	25.8	7.5	0.7	114	43.8	0.3
50	0.1	0	0	0	0	0.8	115	42.6	0.3
100	0.2	0	0	0	0	0.5	125	41.6	0.2

[a] Hydrogen yield
[b] Maximum specific hydrogen production rate
[c] Maximum methane production rate
[d] Cumulative methane production
[e] Methane yield
[f] Maximum specific methane production rate

Table 4. Concentrations and compositions of VFA.

CM Proportion (%)	VFA (mg/l)	VFA Composition (%)			
		Acetate	Propionate	Butyrate	Lactate
0	2135.6	12.4	0.5	34.2	0
10	1876.4	14.5	0.5	29.4	2.1
20	1324.2	17.3	0.6	31.9	3.5
30	1143.1	12.9	0.4	32.4	7.6
40	897.5	16.9	0.7	32.1	1.2
50	556.3	20.9	1.2	31.6	3.2
100	125.6	56.2	4.2	2.3	1.6

Volatile Fatty Acids (VFA) Accumulation

It is well known that three steps – (i) hydrolysis, (ii) acidogenesis and (iii) methanogenesis – are involved in anaerobic digestion. The production of a large amount of volatile fatty acids (VFAs) through hydrolysis and acidogenesis can lead to a decrease in pH when alkalinity in the anaerobic digester is insufficient. Non-methanogenic microorganisms responsible for hydrolysis and acidogenesis can be adapted to low pH while the activity of methanogens may be lost at low pH, suggesting that methanogenesis can be inhibited at low culture pH. The total VFA concentrations for various proportions of chicken manure are shown in Table 4. The total VFA concentration decreased with increasing proportion of CM.

For hydrogen fermentation from a single food waste, acetate and butyrate were the main VFA produced. Table 4 shows that butyrate was the main composition of VFA (31.6-34.2%), followed by acetate (12.4-56.2%) for the co-digestion of chicken manure and food waste. According to the metabolic pathway during hydrogen fermentation, the production of acetate and butyrate would be accompanied with hydrogen production [27]. The biogas produced was low when a large amount of acetic acid was produced in a single fermentation of food waste. However, hydrogen was not produced and reduced hydrogen content was observed when propionate was accumulated during hydrogen fermentation [28]. Reduced butyric acid accumulation for all fermentations might be due to the sharp drop of pH, which triggered the excessive accumulation of acetic acid [29].

The main methods for reducing the VFA inhibition on methanogenesis activities were focused on the adjustment of C:N ratio [30]. High concentrations of VFA lead to the pH drop and high accumulation of undissociated acids, both of which inhibit methanogenic activity and biogas production [31, 32]. The highest sum of VFA is 64.3% from the 100% of CM (Table 4). However, 100% of CM produced one of the lowest biogas production rates, maybe due to the effect of the concentration of VFA.

Based on Table 2, the initial pH was 7.0 for different CM proportions. However, the final pH varied from 3.29 to 5.8 of hydrogen production for a single and co-digestion fermentation and decreased with the CM proportions (0, 10, 20 and 30%), which were likely due to the accumulation of VFA. At the end of methane fermentation, the culture pH of various CM proportions CM proportions was 7.3, 7.45 and 8.45, respectively. This range of culture pH could maintain methanogen activity. For fermentation with CM proportion of 0% and 10%, the VFA concentration for propionate was 1068 mg/L and 938 mg/L, respectively. Propionate at a concentration of above 900 mg/L would inhibit methane production and methanogen activity [33].

Effect of pH Adjustment on Hydrogen and Methane Production in Bioreactor

Fig. 3 shows the time course of the biogas production in 500 mL reactor using a mixture of CM and FW at the selected ratio of 30:70. The total volume of biogas produced was 1600 mL for 14 days fermentation.

The culture pH was initially set at 7.0 and after 3 days of fermentation the pH was adjusted to pH 6.9, in which stable production of biogas was observed. The optimal pH value was 5.5 for batch fermentation of

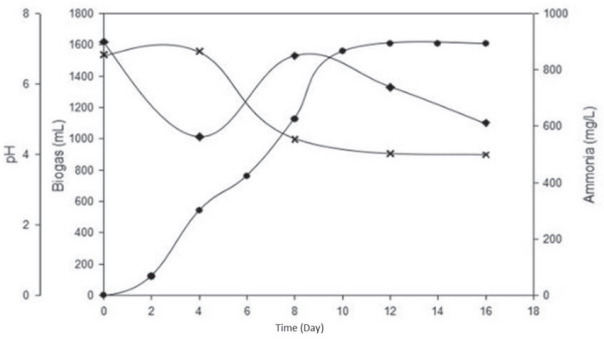

Fig. 3. Profile of biogas (●), ammonia (x) and pH (♦) for fermentation using a mixture of chicken manure and food waste at a ratio of 3:7.

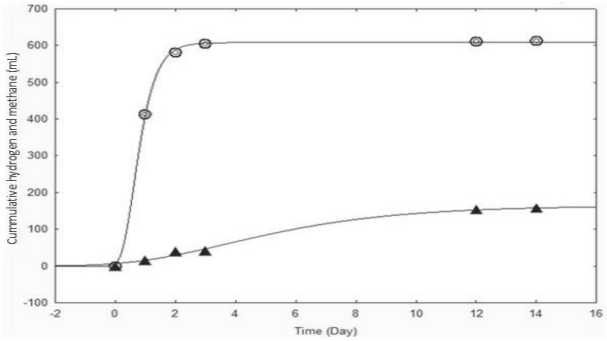

Fig. 4. Cumulative H_2 (⊛) and CH_4 (▲) produced during fermentation using a mixture of fresh CM and FW at a ratio of 3:7.

food waste [26]. High acidic culture pH gave negative effect to the activity of hydrogen-producing bacteria, since ATP is used to ensure cell neutrality rather than to produce hydrogen [4]. Low culture pH may also inhibits the activity of hydrogenase [4, 34]. Generally, pH is a key parameter in fermentation that affects the degree of substrate hydrolysis, the activity of hydrogenase, as well as metabolic pathways [35]. In the early stage of fermentation, the ammonia concentration was stable and started to be reduced at day 5, and reduction of ammonia was continued until day 14. The total ammonia accumulated in the culture was decreased from 854 mg/L to 576 mg/L in 2 weeks of fermentation (Fig. 3). The decreasing ammonia concentration after fermentation was due to stable pH and the concentration of ammonia being below 2000 mg/L along fermentation, which has no effect on biogas production. The activity of methanogenic bacteria was inhibited at ammonia concentrations of above 2000 mg/L [36]. However, results of this study revealed that biogas production was increased during fermentation due to low accumulation of ammonia in the culture.

The cumulative production of hydrogen and methane in fermentation using a mixture of CM at FW at a selected ratio 3:7 is presented in Fig. 4.

The volume of biogas produced during the fermentation of a mixture of CM and FW at a ratio of 3:7 was increased steadily from day 1 until day 14 with a total volume of 1600 mL. Hydrogen gas was produced from the early stage of fermentation until day 4, and after that the methane gas started to produce up to day 14. The hydrogen yield was 239.2 mL H_2/gVS, and no methane gas was detected at the early stage of fermentation. The methane gas was detected at day 5 and onwards with a yield of 60.8 mL CH_4/g VS. Anaerobic co-digestion of a mixture of CM and FW at a ratio of 3:7 was beneficial for the production of hydrogen and methane.

Table 5 shows the comparison of hydrogen and methane yield in batch fermentation using different types of feedstock, including a mixture of food waste and agricultural product at various ratios. All fermentations were carried out at mesophilic temperature, but the culture volume was not the same.

Bacterial Community Diversity during co Digestion of a Mixture of CM and FW

The bacterial community profile was investigated by 16S rRNA gene pyrosequencing, which was conducted for sample collected from co-digestion of CM and FW at a ratio of 3:7. The fermentation culture producing H_2 (day 1) and CH_4 (day 13) gas were selected for pyrosequencing analysis because these were the times of the highest H_2 and CH_4 gas production, respectively. The pyrosequencing data are summarized in Table 6. The dataset comprises a total of 111638 reads with an average read length of 349.13 bases.

Table 7 shows the statistical analysis of alpha diversity of the pyrosequencing dataset of the sample. The sample at day 1 and day 13 had the highest Shannon-Weaver and the lowest Simpson index, indicating high bacterial species diversity in the seed inoculum [41].

Advances in molecular biology have improved our understanding in the bacterial community in methane and hydrogen fermentation. Understanding the bacterial

Table 5. Comparison of hydrogen and methane yields in batch mode operation.

Feedstock	Ratio	Culture Volume (mL)	Temperature (°C)	Initial pH	Hydrogen yields (mL/gVS)	Methane yield (mL/gVS)	References
Food waste and waste activated sludge	ND	150	37	5.5	106.4	353.5	[37]
Food waste	ND	ND	35	5.3	80.9	ND	[26]
Food waste and sludge	ND	70	30	6.8	98.14	ND	[38]
Food waste and pig manure	1:01	500	35	ND	ND	409.5	[39]
Food waste and straw	1:04	600	35	7.14	ND	171	[40]
Food waste and chicken manure	70:30	350	35	7	239.2	60.8	This study

*ND= Not determine

Table 6. Summary of pyrosequencing dataset.

Sample	Description	Number of read	Avg. read length
Day 1	H_2 produced	57,934	333.086
Day 13	CH_4 produced	53,704	365.181

Table 7. Statistical analysis diversity of the pyrosequencing dataset.

Sample	Shannon	Simpson	Chao1
Day 1	3.903	0.885	106
Day 13	3.667	0.870	101

mechanism of methane and hydrogen fermentation will contribute to the development of improved processes through better identification of the good bacterial for high yield of biogas. The relative abundance of bacterial diversity community in samples was characterized from the clone libraries (Fig. 5).

Based on Fig. 5a), the sample for day 1 was represented mainly by *Firmicutes* (40%), followed by *Proteobacteria* (22%) and *Bacteroidetes* (20%). These dominant phyla accounted for approximately 82% of total sequences. The minority groups of phyla were *Actinobacteria* (15%), *Tenericutes* (1.3%), *Deinococcus-Thermus* (0.7%) and *Fusobacteria* (0.2%). Overall, *Firmicutes, Proteobacteria* and *Bacteroidetes* were the most abundant phyla, corresponding to nearly 80% of the sequences. Clostridium sp. clusters represented 76% of total Firmicutes during the acidogenic phase. The high level of butyrate produced by fermentation (Fig. 3) is thus consistent with Clostridium as the dominant microbial group during fermentation. Kim et al. [42] suggested that the butyrate-related pathways are the main routes for H_2 production that yielded 2 mol H_2/mol hexose or 69% of the theoretical H_2 yield.

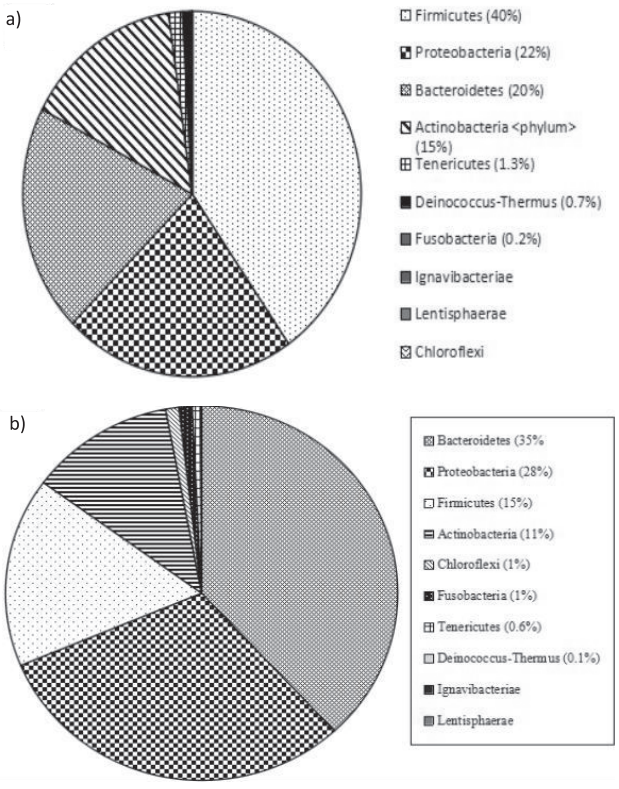

Fig. 5. Relative abundance of bacterial community phyla in a) Day 1 and b) Day 13 inoculum based on 16S rRNA gene clone library sequences.

Meanwhile for day 13 (Fig. 5b) the culture sample was dominated by members of the phylum *Bacteroidetes* (35%), followed by *Proteobacteria* (28%), and *Firmicutes* (15%). The dominant phyla covered approximately 80% of total sequences. Other minor groups were *Actinobacteria* (11%), *Tenericutes* (0.6%), *Fusobacteria* (0.2%) and *Deinococcus-Thermus* (0.1%).

The shift of the microcosm suggested that the dominant type of bacteria are able to consume the nutrients present in the sludge for growth [41]. Figure 7 shows the classification of microbial of phylum for the samples collected on days 1 and 13 from the co-digestion of FW and CM at a ratio of 70:30. Based on the results, during methane gas production (day 13), Clostridium clusters populations were reduced due to lack of substrate and the domination of other genera of Firmicutes.

Table 8 shows the abundant bacterial species in H_2 production (day 1) and CH_4 production (day 13). The abundant bacteria presence in the sample collected on day 1 was closely related to the genus Clostridium. The presence of a high number of *Clostridium* sp. clusters is due to the fact that these microorganisms are the predominant strains involved in hydrogen production [43].

The yields of hydrogen and methane depend on the composition of food waste and chicken manure. It is well known that carbohydrates produced the most hydrogen through biological hydrogen fermentation, compared with protein or lipids [44]. The high carbohydrate composition of food waste is conducive to hydrogen production. Therefore, the microbial community during H_2 production was also investigated. It was found that *Clostridium sp.* Strain Z6 and ASF356 represent similarity at 91 and 96%, respectively. These results indicate that *Clostridium sp.* as H_2-producing bacteria played an important role in improving the yield of hydrogen. According to the previous study, the clostridium species that are involved in the H_2 fermentation process are categorized into mesophilic and thermophilic. The mesophilic clostridium, including *Clostridium pasteurianum* [45], *Clostridium butylicum* [46], and *Clostridium acetobutylicum* [47], is in agreement in this study for the sample of day 1 (Table 8)

Bacillus spp, which are facultative anaerobes, spore-forming, low G + C content gram-positive bacteria that produced H_2, was detected [48]. *Bacillus sp.* IDA4740 (day 1) showed similarity at 87%. Shin et al. [49]

Table 8. Abundant bacterial species.

Sample	Species	Similarity (%)	Abundance (%)
DAY 1	*Clostridium* sp. Strain Z6	91	70
	Clostridium sp. ASF356	96	3
	Clostidium pasteurianum	89	12
	Clostridium butylicum	95	4
	Bacillus sp. IDA4740	87	2
	Alkalibacterium sp. 8B	93	2
DAY 13	*Methanosaeta consilii*	95	72
	Methanosaeta hungatei	93	23

reported that *Bacillus species* were detected using PCR-DGGE analysis for semi-continuous fermentation fed with food waste operated at 35ºC, 5 days HRT and pH 5.6 at steady state. These results indicated that *Bacillus* spp. exist during the H_2 fermentation stage. However, *Bacillus* spp. and *Lactobacillus* spp. have been shown to utilize parts of H_2 for lactic acid production and thus to decrease H_2 yield [50].

In this study the abundant species for CH_4 production at day 13 consists of *Methanosaeta consilii* and *Methanosaeta hungatei* with similarity of 95 and 93%, respectively. *M. hungatei* is hydrogenotropic methanogens, which produce CH_4 from H_2/CO_2. *M. hungatei* also uses formate as alternative substrate for CH_4 production. However, they do not use acetate for CH_4 production and assimilate only small amounts of it into biomass [51]. In general, acetic acid decomposition is responsible for approximately 70% of the CH_4 generation, and the remainder is considered $H_2 + CO_2$ derived. *M. concilii* and *M. hungatei* were considered to be the acetate-utilizing and H_2-utilizing methanogens in the methanogenic reactor, respectively [52].

Conclusion

Results from this study have demonstrated that a suitable concentration and combination of waste is one of the key factors in enhancing biogas production in the digestion process. The optimal ratio of a mixture of chicken manure with food waste was 30:70, which gave the highest total biogas production (1600 mL) for 14 days of fermentation in a 500 mL bioreactor. In this co-digestion, the percentage of H_2 and CH_4 in biogas produced was 64% and 19%, which corresponds to the yield of H_2 and CH_4 of 239.2/gVS and 60.8 mL/gVS, respectively. Very low biogas production (214 mL) was obtained from a single FW digestion. The microbial community obtained from the co-digestion of food waste and chicken manure have the capability to generate hydrogen and methane at mesophilic temperature. 16S rRNA gene pyrosequencing revealed rapid enrichment of key bacterial strains in bio-diversified microbial communities in the sample, leading to microcosms highly enriched for H_2- (*Clostridia*) and CH_4-producing microorganisms (*Methanosaeta*). The information generated from his study provides an important basis for process optimization and a platform for further development of high-rate H_2 and CH_4 fermentation.

Conflict of Interest

The authors declare no conflict of interest.

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