CHAPTER IV
RESULTS AND DISCUSSION

4.1 Characteristics of food waste and seed sludge

In the experiment, food waste was as a substrate and seed sludge was as microorganisms that were produced hydrogen production. Characteristics of food waste and seed sludge were summarized in Table 4.1. In order to calculate F/M ratio, COD was calculated for the initial food waste, while VS was calculated for the initial seed sludge.

Table 4.1 Characteristics of food waste and seed sludge

Parameter	Value	
	Food waste	Seed sludge
Total Solid (g/l)	334.55±0.65	89.29±0.25
Volatile Solid (g/l)	318.81±0.02	59.64±0.05
Total COD (g/l)	186.67±12.22	1.60±0.06
Total Kjeldal nitrogen (%)	134.49±37.13	773.69±0.85
Protein (%)	962.5±36.13	-
Carbohydrate (mg/l)	12.68±1.34	-
FOG (g/l)	26.57±2.64	-
pH	4.11±0.05	7.47±0.06

4.2 Phase I: Investigation of the optimal environmental condition on bio-hydrogen production

4.2.1 Step I: Investigation of initial pH (4–12)

In step I, F/M ratio was fixed at 10 and added the nutrient element for microorganism. Batch experiments were operated for 7 days and 10 days afterward they were out of biogas under mesophilic and thermophilic conditions, respectively.

Cumulative hydrogen production, biogas component, COD removal, pH and VFAs were daily monitored, the results was shown in below.

1) Biogas composition

Table 4.2 and Table 4.3 show the cumulative biogas production in all experiments under mesophilic and thermophilic conditions, respectively. The component of biogas was hydrogen (0-47.9%), nitrogen (4.68-42.56%) and carbon dioxide (44.32-67.89%) under mesophilic condition. Otherwise, component of biogas was hydrogen (0-41.49%), nitrogen (2.20-39.46%) and carbon dioxide (56.31-75.78%) under thermophilic condition. All experiments were not found methane and hydrogen sulfide. The result showed that highest cumulative biogas production was found at the initial pH 8 under the both of mesophilic and thermophilic conditions. The percentage of hydrogen content under thermophilic condition was increased about 1.61% compared to the mesophilic condition. Vazquez, et al. (2005) reported that the percentage of hydrogen content under thermophilic condition increased 16% compared with mesophilic condition in semi-continuous reactor. Hydrogen content in this study was different to previous study due to different type of reactor. Gavala, et al. (2006) reported that the different percentage of hydrogen under thermophilic condition increased was 2% compared to mesophilic condition operating in a continuous stirred tank reactors at 12 hours of hydraulic retention times.

Table 4.2 Biogas production and biogas component at various initial pHs under mesophilic condition

Initial pH	Biogas production (ml)	H_2 (%)	N_2 (%)	CO_2 (%)
pH 4	116.25±37.83	0.00	42.56	57.44
pH 5	167.83±10.61	0.06	32.05	67.89
pH 6	561.33±53.54	29.61	19.09	51.30
pH 7	357.42±195.30	34.32	21.36	44.32
pH 8	1531.17±120.02	47.90	4.68	47.42
pH 9	854.83±25.84	39.45	9.12	51.43
pH 10	271.67±69.97	5.36	29.82	64.82
pH 11	253.17±28.81	2.05	24.86	73.09
pH 12	240.00±4.36	9.00	29.59	61.41

Table 4.3 Biogas production and biogas component at various initial pHs under thermophilic condition

Initial pH	Biogas production (ml)	H_2 (%)	N_2 (%)	CO_2 (%)
pH4	140.00±8.73	0.00	34.86	65.14
PH5	167.50±26.87	0.00	39.46	60.54
pH6	797.75±15.20	29.26	7.73	63.01
pH7	905.50±20.62	39.38	5.49	55.13
pH8	1550.33±51.44	49.51	2.20	48.28
PH9	1339.67±129.86	31.62	2.76	65.62
pH10	1388.67±123.74	29.78	10.16	60.06
pH11	1832.17±233.70	15.32	8.90	75.78
pH12	1079.00±42.18	29.68	10.43	59.89

2) Cumulative hydrogen production

Table 4.2 shows the correlation of coefficient parameter from Gompertz equation with parameter from reactor.

Table 4.4 Cumulative hydrogen production (H_{max}) under mesophilic condition by Gompertz equation

Initial pH	H_{max} (ml)	Lag time (Hour)	R_m (ml/hour)	R^2
pH4	0	0	0	-
pH5	0	0	0	-
pH6	166.22	6	7.72	0.9735
pH7	122.68	6	5.72	0.9794
pH8	733.49	6	36.35	0.9661
pH9	337.19	6	16.79	0.9654
pH10	14.57	6	1.94	0.8591
pH11	5.19	24	0.11	0.9039
pH12	21.61	72	0.45	0.9793

Table 4.5 Cumulative hydrogen production (H_{max}) under thermophilic condition by Gompertz equation

Initial pH	H_{max} (ml)	Lag time (Hour)	R_m (ml/hour)	R^2
pH4	0	0	0	-
pH5	0	0	0	-
pH6	233.42	8	12.61	0.9877
pH7	356.58	8	19.74	0.9868
pH8	767.64	8	41.69	0.9876
pH9	423.55	8	21.32	0.9884
pH10	413.55	24	16.22	0.9913
pH11	280.62	24	7.6	0.9703
pH12	320.23	120	12.32	0.9946

The cumulative hydrogen production was depicted from Gompertz equation. It was converted to ml H_2/g COD_{add} (Hydrogen yield) by ml of hydrogen production divided COD of food waste. The variation of initial pH at 4 to 12 under mesophilic condition is shown in tendency (Figure 4.1). No hydrogen production occurred in the initial pH 4 and 5. The initial pH was significantly ($P < 0.05$) affected on hydrogen production of the food waste under mesophilic condition (Appendix E, No.1). The cumulative hydrogen production increased with increasing initial pH in the range of 6 to 9. The maximum hydrogen yield of 29.32 ml H_2/g COD_{add} was obtained at initial pH 8 and the initial pH 10-12 was found the negligible cumulative hydrogen production. The cumulative hydrogen production at various initial pH 4-12 under thermophilic condition are plotted in Figure 4.2 and there was a significance ($P < 0.05$) of hydrogen production (Appendix E, No.2). The maximum hydrogen yield was found 30.69 ml H_2/g COD_{add} at initial pH 8. The cumulative hydrogen production occurred within 24 hours and increased at pH in the range of 6 to 9, whereas it was found after 24 hours at the initial pH range of 10, 11 and 12. The hydrogen production was not found at 4 and 5. The cumulative hydrogen production under thermophilic condition was higher H_2 production than mesophilic condition. The lag-phase time under mesophilic condition was shorter than thermophilic condition and was similar to the research by shin et al. (2004). The optimum of the initial pH may depend on pH of the seed sludge as microorganism that degraded organic waste to hydrogen production. By the result, the optimal condition for hydrogen production from food waste was suggested to be at initial pH 8 under thermophilic condition. This result was different from previous study (Shin et.al, 2004). It obtained the optimal pH at 4.5 under thermophilic condition, the reason might from variation of substrate and microorganism characteristics using in the studies. The microorganism in previous study used the mesophilic and thermophilic culture in a stirred acidogenic reactor. The pH of culture was about 5.5. It was reported that hydrogen production was 46.3 ml H_2, which it obtained at initial pH 4.5 and it was lower than in this study.

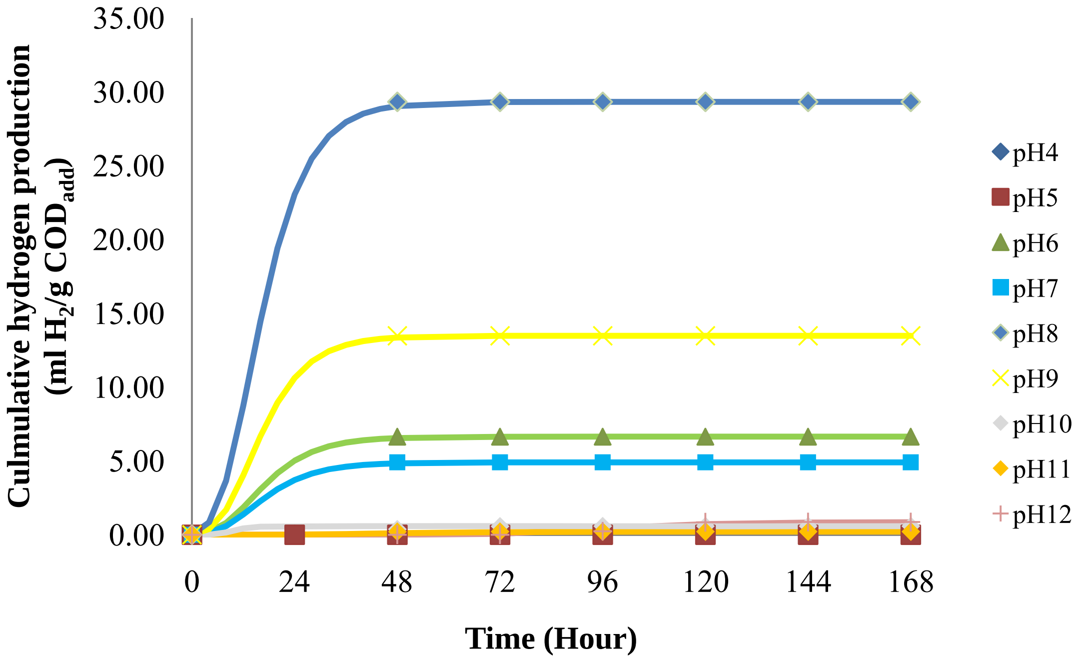

Figure 4.1 Cumulative hydrogen production at various initial pHs under mesophilic condition

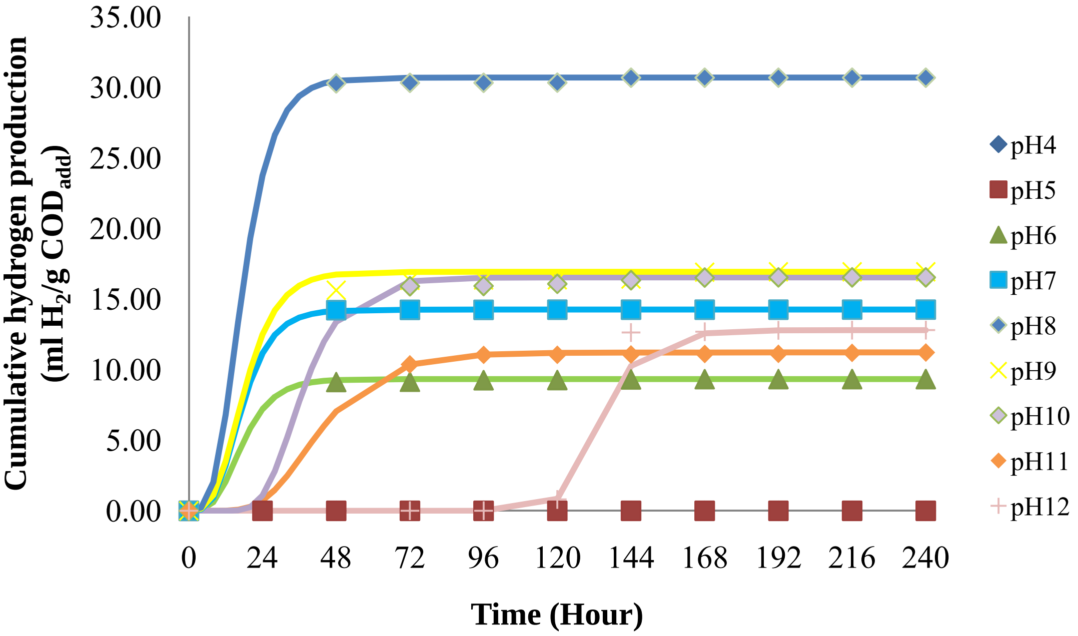

Figure 4.2 Cumulative hydrogen production at various of initial pHs under thermophilic condition

3) COD removal

In batch reactor, it used food waste as a substrate for bio-hydrogen production. Figure 4.5 shows that the efficiencies of COD removal under thermophilic condition were higher than their COD removal under mesophilic condition. The tendency of COD removal in percentage was according to cumulative hydrogen production. The highest percentage of COD removal was achieved at 65.89% at initial pH 8 under thermophilic condition.

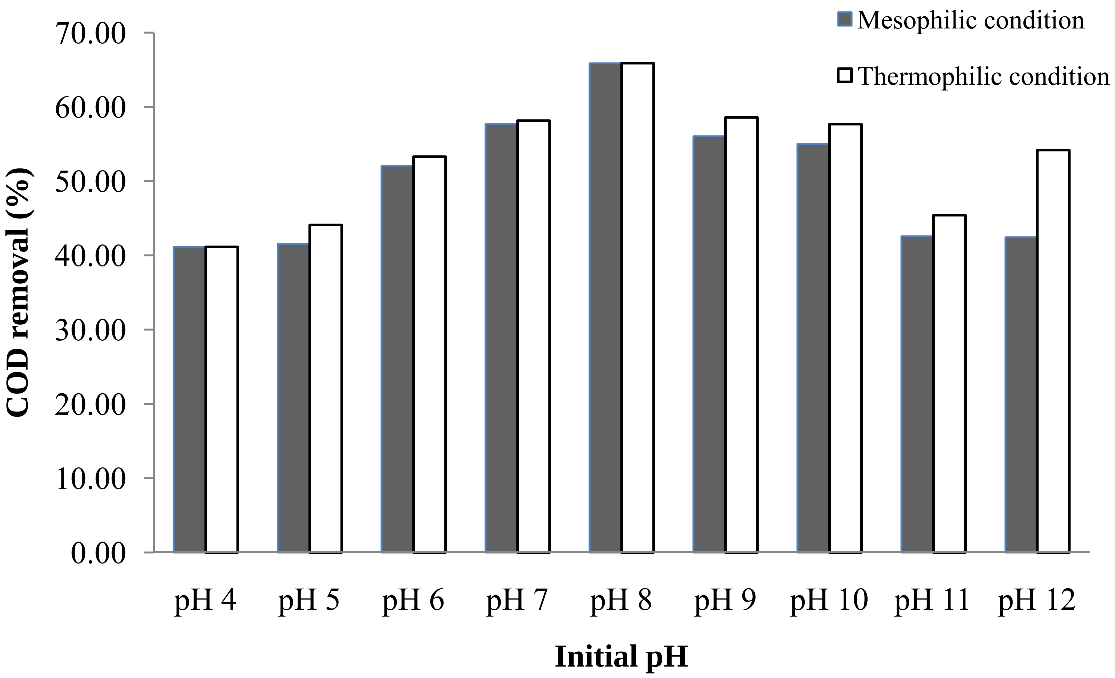

Figure 4.3 Percentage of COD removal under mesophilic and thermophilic conditions at various initial pHs

4) VFA production

Hydrogen production was accompanied with the formation of volatile fatty acids (VFAs) that was the intermediate anaerobic digestion products (acetate, propionate and butyrate). The acid products were mainly butyrate and acetate concentrations. These VFAs production might be a result from the pH decrease within a batch reactor. The ratio of butyrate/acetate (B/A) was always more than that of other acid in the late end time. The ratio has been frequently used as an indicator for evaluating the efficiency of hydrogen production (Yang and Shen, 2006; Annous et.al, 1995). High B/A ratio was favorable to hydrogen production. The variation of initial pHs under mesophilic condition was shown in Table 4.7. High B/A ratio was 1.88 at initial pH 8 that was according to hydrogen production. Otherwise, the variation of individual initial pH under thermophilic condition is shown in Table 4.8. High B/A ratio was 1.97 at initial pH 8 that was greater than mesophilic condition.

Table 4.6 VFA production at various initial pHs under mesophilic condition

Initial pH	Final pH	Acetate (mg/l)	Propionate (mg/l)	Butyrate (mg/l)	B/A ratio
4	4.0	3.73	8.17	4.43	1.19
5	4.0	8.86	10.10	0.41	0.05
6	4.5	429.87	14.48	645.76	1.50
7	4.5	456.70	22.82	659.86	1.44
8	4.5	374.43	10.84	702.56	1.88
9	4.5	464.42	13.58	701.40	1.51
10	4.5	90.25	6.10	110.69	1.23
11	4.5	23.60	6.04	14.72	0.62
12	4.5	18.91	37.01	10.31	0.54

Table 4.7 VFA production at various initial pHs under thermophilic condition

Initial pH	Final pH	Acetate (mg/l)	Propionate (mg/l)	Butyrate (mg/l)	B/A ratio
4	4.0	18.08	5.97	1.09	0.06
5	4.0	25.96	5.11	1.40	0.05
6	4.5	433.26	6.77	650.30	1.50
7	4.5	402.29	6.98	689.74	1.71
8	4.5	385.39	5.67	758.56	1.97
9	4.5	375.87	7.71	657.74	1.75
10	4.5	418.25	7.64	622.95	1.49
11	4.5	385.45	9.63	642.57	1.67
12	4.5	399.14	6.39	653.88	1.64

4.2.2 Step II: investigation of initial F/M ratios

The initial F/M ratios were set at 4, 6, 8, 10 and 12 and they were operated for 240 hours until out of biogas. All experiments were fixed at initial pH (8) under thermophilic condition (55°C) (results obtained from step I). The results of experiment are shown in below.

1) Biogas composition

Table 4.8 showed the cumulative biogas production in all experiments at different initial F/M ratios. The components of biogas were hydrogen (33.51-41.48%), nitrogen (4.98-5.57%) and carbon dioxide (53.12-61.23%). Methane and hydrogen sulfide were not found in all experiments. Highest biogas production was found at initial F/M ratio at 4. But the component of hydrogen concentration was lower than the experiment at initial F/M ratio of 6.

Table 4.8 Biogas production and biogas component at various initial F/M ratios

Initial F/M ratos	Biogas production (ml)	H_2 (%)	N_2 (%)	CO_2 (%)
4	1571.67±51.25	41.26	5.57	53.17
6	1285.83±47.44	41.48	5.40	53.12
8	1164.50±16.26	41.04	5.22	53.74
10	1353.67±188.73	33.79	4.98	61.23
12	1272.00±56.57	33.51	5.38	61.11

2) Cumulative hydrogen production

Table 4.9 showed the correlation coefficients parameter from Gompertz model. In this experiment, the hydrogen released after short lag time (8hours) and cumulative hydrogen production increased with time before reaching the maximum. The reason that hydrogen production decreased with increasing F/M ratios might be proportion microorganism higher than substrate (food waste). However, the objective in these studies was only F/M ratios of 4, 6, 8, 10 and 12.

Table 4.9 Cumulative hydrogen production at various initial F/M ratios by Gompertz equation

F/M ratios	H_{max} (ml)	R_m (ml/hour)	R^2
4	648.43	35.27	0.9871
6	533.33	27.91	0.9852
8	477.94	25.82	0.9868
10	457.43	20.42	0.9571
12	426.30	23.37	0.9874

The cumulative hydrogen productions at various initial F/M ratios are shown tendency in Figure 4.6. The initial F/M ratios were significantly ($P<0.05$) affected to hydrogen production of food waste (Appendix E, No.3). All experiment in this study was found lag time at 8 hours. The amount of cumulative hydrogen production decreased with increasing F/M ratios, which might be due to product inhibition of hydrogen and VFAs (Kim et al., 2004). Thus, the initial F/M ratio at 4 achieved the maximum hydrogen yield of 42.51 ml H_2/g COD_{add}.

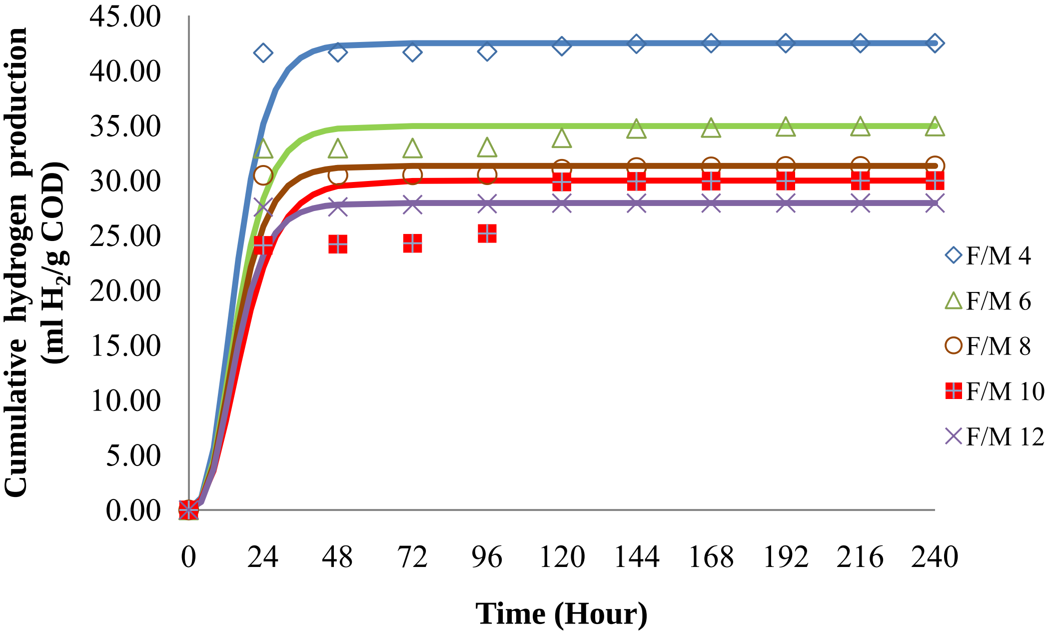

Figure 4.6 Cumulative hydrogen production at various initial F/M ratios

3) COD removal

Figure 4.7 shows the percentage efficiency of COD removal. The increased F/M ratios decreased efficiency favorable hydrogen production and percentage of COD removal have similarly trend that liquid in batch reactor may reside in any other form of VFAs (i.e. Lactate, ethanol, valerate, formate etc.). The maximum percentage of COD removal was about 65.83% at the initial F/M ratio of 4, while the percentage of COD removal of F/M ratios of 6, 8, 10 and 12 was 64.82, 64.50, 64.32 and 63.82 %, respectively.

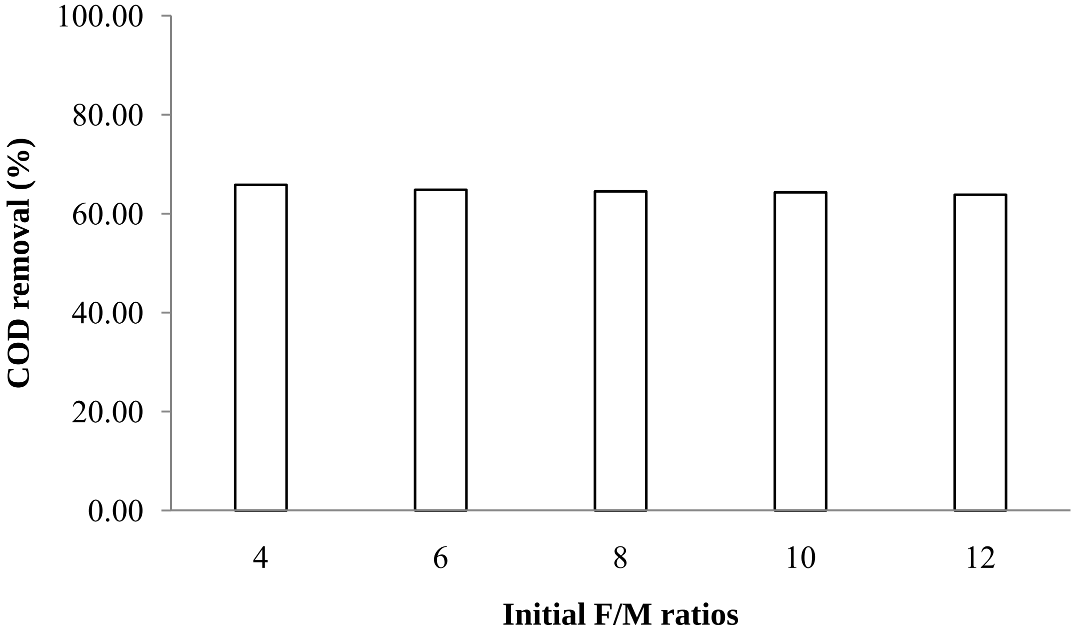

Figure 4.7 Percentage of COD removal of initial F/M ratios on bio-hydrogen production from food waste.

4) VFA

Table 4.10 presents the VFA production observed in the reactor. VFAs were the intermediate products. The highest of VFAs concentration (402.97, 8.31, 850.60 mg/l of acetate, propionate and butyrate, respectively) was observed at F/M ratio of 4. The B/A ratio was deceased with increasing F/M ratio. The high B/A ratio depended on the optimal proportion of substrate to microorganism.

Table 4.10 VFA production at various initial F/M ratios

F/M ratio	Final pH	Acetate (mg/l)	Propionate (mg/l)	Butyrate (mg/l)	B/A ratio
4	4.5	402.97	8.31	850.60	2.11
6	4.5	408.89	7.23	801.89	1.96
8	4.5	406.16	12.17	791.54	1.95
10	4.5	430.40	5.85	788.01	1.83
12	4.5	403.31	5.30	629.64	1.56

4.2.3 Step III: investigation of initial ferrous ion concentration

In batch reactor, the variation of initial ferrous ion concentration was set at 0, 100, 200, 300, and 400 mg Fe^{2+}/l as $FeSO_4$ and it was operated for 10 days until it was out of biogas production. All experiments were fixed at initial pH (8) under thermophilic condition (55±2°C) and initial F/M ratio of 4 (Results obtained from step II). The results are shown in below.

1) Biogas composition

The biogas comprised with hydrogen, nitrogen and carbon dioxide and no methane and hydrogen sulfide. Table 4.11 shows the results of biogas production and component of biogas at variation of initial Fe^{2+} concentrations. The components of biogas were hydrogen (32.02-42.73%), nitrogen (4.98-6.42%) and carbon dioxide (52.06-63.36%). The maximum of hydrogen gas was 42.73%. and hydrogen production was occurred about 340.19 ml H_2 at initial Fe^{2+} 400 mg $FeSO_4$/l that was lower than hydrogen gas (38.38%) and hydrogen production (543.97 ml) at the initial Fe^{2+} 100 mg Fe^{2+}/l.

Table 4.11 Biogas production and component at the variation initial ferrous ion concentrations

Initial Fe^{2+} (mg Fe^{2+}/l)	Biogas production (ml)	H_2 (%)	N_2 (%)	CO_2 (%)
0	1391.83±42.09	32.02	6.42	61.56
100	1532.67±221.87	38.38	5.85	55.77
200	1431.00±46.67	35.74	4.98	59.28
300	1313.00±29.70	30.91	5.73	63.36
400	861.00±82.02	42.73	5.21	52.06

2) Cumulative hydrogen production

Table 4.12 shows the correlation of coefficient parameter from Gompertz eqaution with parameter from reactor.

Table 4.12 Cumulative hydrogen production at initial ferrous ion (Fe^{2+}) concentration by Gompertz equation

Initial Fe^{2+} concentration (mg Fe^{2+}/l)	H_{max} (ml)	R_m (ml/hour)	R^2
0	412.13	21.79	0.9870
100	543.97	25.07	0.9668
200	472.97	24.33	0.9851
300	375.35	19.79	0.9872
400	340.19	18.36	0.9871

Figure 4.8 is depicted to show the result in experiment. The results showed hydrogen production was released after short lag time at 8 hours in all experiments and cumulative hydrogen production was increased with time before reaching the maximum. The initial ferrous iron concentration significantly ($P<0.05$) affected to the hydrogen production (Appendix E, No.4). The cumulative hydrogen production was increased with increasing of initial Fe^{2+} concentration from 0 to 200 mg Fe^{2+}/l, while it was decreased for initial Fe^{2+} concentrations of 300 and 400 mg Fe^{2+}/l. This result indicated that ferrous ion concentration more than 100 mg Fe^{2+}/l was harmful to the mixed microorganism and it was inhibition of hydrogen production in reactor. The highest hydrogen yield was 44.83 H_2/g COD_{add} at initial Fe^{2+} 100 mg Fe^{2+}/l. This result indicated that certain concentration Fe^{2+} was able to enhance the hydrogen yield by mixed cultures (Wang and Wan, 2008; Yang and Shen, 2006; Ding et al., 2004). The electron carrier ferredoxin in hydrogennase plays an important role in the fermentative hydrogen production. Iron is a fundamental component making up the ferredoxin (Wang and Wan, 2008). Wang and Wan (2008) reported that the optimal $FeSO_4$ concentration in hydrogen production form glucose was 350 mg $FeSO_4$/l (128 mg Fe^{2+}/l). Yang and Shen (2006) found the maximum hydrogen production from soluble starch was 150 mg $FeSO_4$/l (55 mg Fe^{2+}/l). Ding et al. (2004) found the optimal ferrous ion was 73 mg Fe^{2+}/l. and Lee et al. (2001) found the maximum hydrogen production from sucrose was 800 mg $FeSO_4$/l (352 mg Fe^{2+}/l). The results of previous studies shown the difference with this study may depend on the difference of the substrates, concentrations, initial pH values, and type of seed sludge, which were applied in the study. However, the optimal ferrous ion concentration also helps to improve hydrogen production from food waste.

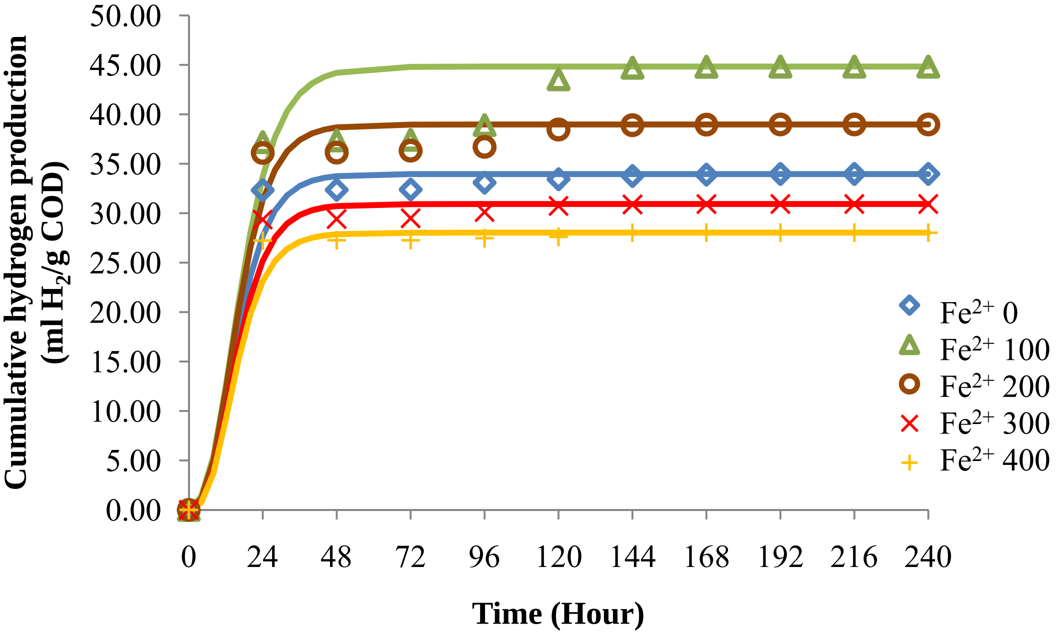

Figure 4.8 Cumulative hydrogen production at various initial ferrous ion concentration

3) COD removal

The trendency of COD removal value in all experiments was similarity that liquid in batch reactor may reside in any other forms of VFAs (i.e. Lactate, Ethanol, Valerate, Formate etc.). It was in accordance with hydrogen production. The percentage of COD removal was 66.00% at the optimal initial Fe^{2+} concentration (100 mg Fe^{2+}/l). The percentages of COD removal of the initail Fe^{2+} concentrations at 0, 200, 300 and 400 mg $FeSO_4$/l were 65.00, 65.50, 64.50 and 64.32 %, respectively. The result showed that the percentage of COD removal at the initial Fe^{2+} concentration (100 mg Fe^{2+}/l) was higher than the percentage of COD removal in the experiments of step I and II.

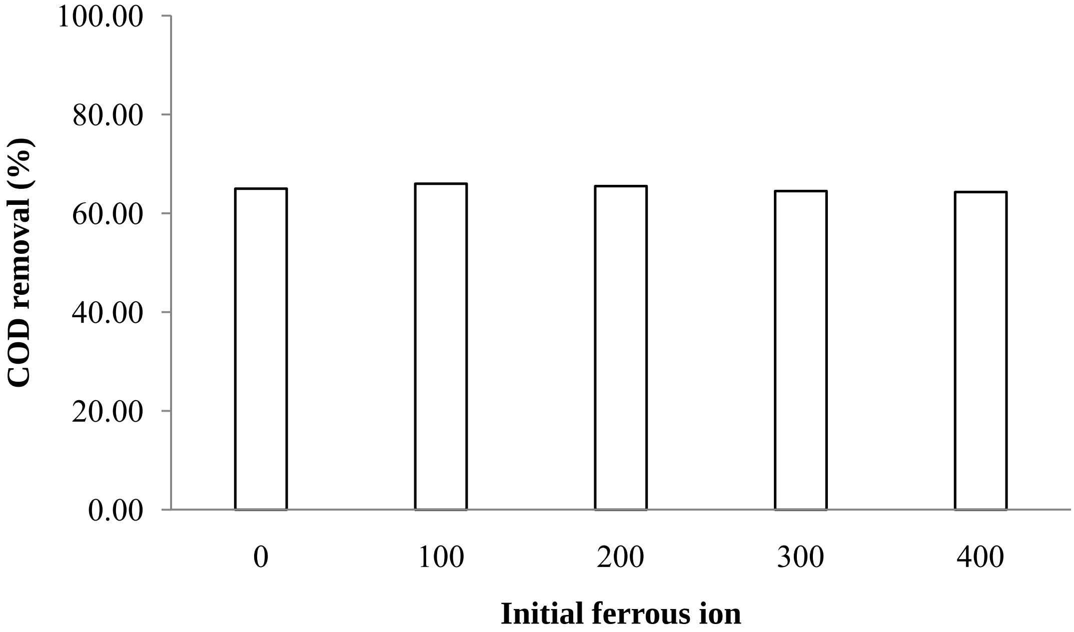

Figure 4.9 Percentage of COD removal at various initial ferrreous ion concentration on bio-hydrogen from food waste.

4) VFA production

Table 4.13 presents the VFA production in the batch reactor. VFA production was the intermediate product. The highest VFAs concentration (324.69, 5.15, 765.66 mg/l of acetate, propionate and butyrate, respectively) was observed at ferrous ion concentration 100 mgFe^{2+}/l that according to the hydrogen production (44.83 ml/g COD$_{add}$).

Table 4.13 VFA production at various ferrous ion concentrations

Fe^{2+} (mg Fe^{2+}/l)	Final pH	Individual VFA (mg/l)			B/A ratio
		Acetate	Propionate	Butyrate	
0	4.5	363.21	5.22	684.83	1.89
100	4.5	324.69	5.15	765.66	2.36
200	4.5	298.86	5.46	610.66	2.04
300	4.5	404.16	9.62	582.48	1.44
400	4.5	299.43	21.67	324.66	1.08

Phase I experiment, the optimum of environmental condition was obtained at initial pH 8, initial F/M ratio of 4 and initial ferrous ion concentration of 100 mg Fe^{2+}/l under thermophilic temperature (55±2°C) Table 4.14 showsthe result of hydrogen yeild production under the optimal condition. However, the results in this study was different from other reseaches (Table 4.15). The hydrogen yield production from starch was higher than the result of this study. it may be the starch primarily attributed to complex nature of substrate, which was less than food waste. From the result, it was suggested that the initial pH 8, initial F/M ratio 4 and initial $FeSO_4$ concentration under thermophilic condition (55±2°C) could enhance the hydrogen production from food waste.

Table 4.14 Hydrogen yield production under optimal condition

Experimental condition	Optimal condition				H_2 Yield (ml/g COD_{add})
	Initial pH	Temp	F/M ratio	$FeSO_4$(mg/l)	
Mesophilic	8	35	10	2.78	29.32
Thermophilic	8	55	10	2.78	30.69
F/M ratio	8	55	4	2.78	42.51
Fe^{2+}	8	55	4	100.00	44.83

Table 4.15 Hydrogen yield production from other reseaches

Feed stock	Micro organism	Optimal condition				H_2 Yield ml/g COD_{add}	Ref.
		Initial pH	Temp	F/M ratio	$FeSO_4$ (mg Fe^{2+}/l)		
Fw	MC	8.0	55	4	100	44.83	This study
Starch	MC	8.0	35	-	150	106.4	Yang&Shen
Fw	MC	5.5	36	-	20	15	Chen et al.
Garbage slurry	MC	6	60	-	-	46.3	Ueno et al.

Fw = Food waste, MC= Mixed culture

4.3 Phase II: Investigation of variation of pretreatment methods on bio-hydrogen production

In this phase, the experiment was investigated the effect of pre-treatment methods (Heat, repeated heat, chloroform and ultra-sonication) on hydrogen production. Experiment was operated for 10 days until out of biogas production. The operation was fixed for initial pH 8, initial F/M ratio of 4 and initial ferrous ion concentration 100 mg $FeSO_4$/l under thermophilic condition (55±2°C) (Obtained the results from phase I experiment). The results are shown in below.

1) Biogas composition

The biogas comprised hydrogen, nitrogen, carbon dioxide. All experiments were no found methane and hydrogen sulfide. Table 4.16 shows thethe result of biogas production. The biogas component was hydrogen (0.39-44.71%), nitrogen (6.87-47.08%) and carbon dioxide (48.42-61.18%). The repeated heat method was the maximum percentage of hydrogen in biogas production (44.71%).

Table 4.16 Biogas production and biogas component of different pretreatment method

Preteatment method	Biogas production (ml)	H_2 (%)	N_2 (%)	CO_2 (%)
Heat	1435.50±91.22	41.01	7.24	51.75
Repeated heat	1468.50±61.52	44.71	6.87	48.42
Chloroform	121.67±18.45	0.39	47.08	52.53
Ultrasonication	1537.50±286.38	28.84	9.98	61.18

2) Cumulative hydrogen production

Table 4.17 Cumulative hydrogen production by Gompertz equation

Pretreatment method	H_{max} (ml)	R_m (ml/hour)	R^2
Heat	588.65	32.38	0.99996
Repeated heat	656.57	34.46	0.99859
Chloroform	0.47	0.01	0.99863
Ultrasonication	443.36	21.29	0.95254

Figure 4.10 shows hydrogen production which is released after short lag time at 8 hours in all experiments and cumulative hydrogen production was increased with time before reaching the maximum. The pretreatment method shown the

significant ($P<0.05$) affected to hydrogen production (Appendix E, No.5). The maximum hydrogen yield was occurred in repeated heat method (46.19 ml/g COD_{add}). The result of this study was similarly with Sung et al. (2002) that they was found repeated heat was higher hydrogen production than heat treatment (100°C for 15 min) in batch experiment. Argun and kargi (2009) reported that repeated heat for 5 hours obtained the maximum hydrogen production. Chloroform (0.074% w/v) in this study was not found hydrogen production that was not similar to previous study. Chloroform pretreatment can suppress the activity of hydrogen-producing bacteria when chloroform concentration was 0.25-5 % in the medium (Hu & Chen, 2007). While Argun and kargi (2009) was also suggested that chloroform (0.05% w/v) was effective combination with other pretreatment methods (i.e. Heat, repeated heat) and was more efficient than its used alone.

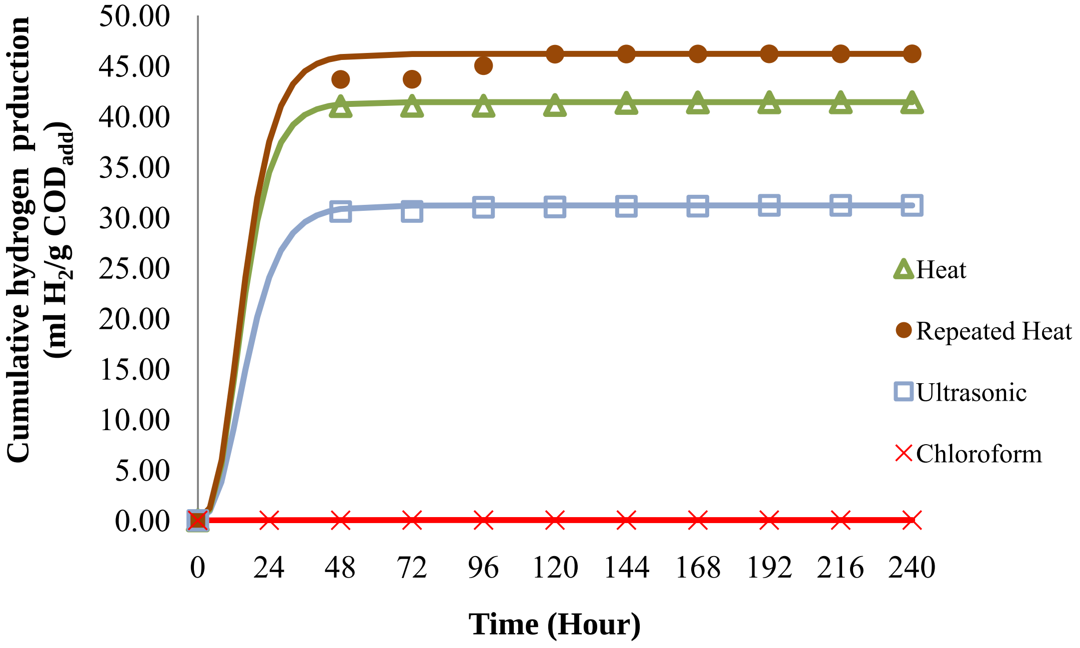

Figure 4.10 Cumulative hydrogen production of different pretreatment method

3) COD removal

The COD removal depend on hydrogen production. The percentages of COD removal of heat and repeated heat methods were nearly value (65.50 and 66.00 %, repectively). Although hydrogen production of chloroform method was neglilible, but the COD removal was 38.58 %. The hydrogen producing bacteria may be elimination, but other microorganism have alive in reactor.

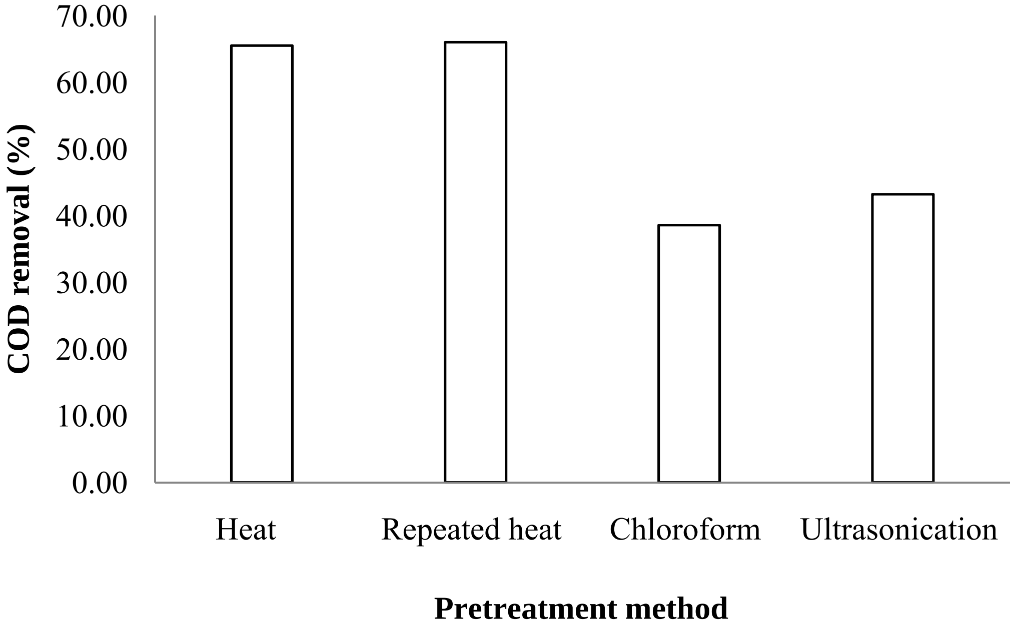

Figure 4.11 Percentage of COD removal of different pretreatment method

Table 4.18 VFAs production and butyrate/acetate ratio of different pretreatment method

Pretreatment method	Final pH	Individual VFA (mg/l)			B/A ratio
		Acetate	Propionate	Butyrate	
Heat	4.5	323.82	0.61	767.56	2.37
Repeated heat	4.5	324.25	2.65	838.38	2.59
Ultrasonication	4.4	423.41	6.73	503.69	1.19
Chloroform	4.8	52.79	1.36	37.05	0.70

4) VFA production

Tables 4.18 presents the VFAs production observed in the reactor. The VFA was the intermediate product in batch reactor after fermentation process. The highest VFAs concentration (324.25, 2.65, 838.38 mg/l of acetate, propionate and butyrate, respectively) was observed at repeated heat method pretreatment that was according to produce the hydrogen yield about (46.19 ml/g COD_{add}).