OPTIMAL CONDITION FOR BIOLOGICAL HYDROGEN PRODUCTION FROM FOOD WASTE

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OPTIMAL CONDITION FOR BIOLOGICAL HYDROGEN PRODUCTION FROM FOOD WASTE

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ABSTRACT

Hydrogen (H_2) production from food waste via anaerobic dark-fermentation was conducted using mixed culture under various environmental conditions (initial pH, initial F/M ratio, initial ferrous iron (Fe^{2+}), and temperature condition) in batch reactor. The optimal condition for maximizing H_2 production was initial pH 8, initial F/M ratio 4, initial Fe^{2+} 100 mg Fe^{2+}/l, and thermophilic condition (55°C). The maximum cumulative H_2 production and H_2 yield were obtained 543.97 ml H_2, and 44.83 ml H_2/g COD_{add}, respectively. Butyrate contained the main volatile fatty acids (VFAs). VFAs production including acetate, propionate, and butyrate were 324.69, 5.15, and 765.66 mg/l, respectively. Ratio of butyrate and acetate (B/A ratio) was 2.36. Pretreatment methods (Heat, repeated heat, ultrasonication, and chloroform) found that repeated heat improved H_2 production (656.57 ml H_2). The maximum H_2 yield of 46.19 ml H_2/g COD_{add} was achieved in the repeated heat method that found the VFAs production of acetate 324.25 mg/l, propionate 2.65 mg/l, and butyrate 838.38 mg/l. The ratio of butyrate and acetate was 2.59. Therefore, controls of optimum environmental and pretreatment conditions by the use of the repeated heat method supported the enhancement of fermentative hydrogen production of food waste.

KEY WORDS: BIO-HYDROGEN PRODUCTION/ FOOD WASTE/ OPTIMAL CONDITION/ PRETREATMENT

89 pages

สภาวะที่เหมาะสมต่อการผลิตก๊าซไฮโดรเจนทางชีวภาพจากเศษอาหาร
OPTIMAL CONDITION FOR BIOLOGICAL HYDROGEN PRODUCTION FROM FOOD WASTE

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วท.ม. (เทคโนโลยีที่เหมาะสมเพื่อการพัฒนาทรัพยากรและสิ่งแวดล้อม)

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บทคัดย่อ

การผลิตก๊าซไฮโดรเจนทางชีวภาพจากเศษอาหารโดยใช้เชื้อจุลินทรีย์ผสม (Mixed culture) ภายใต้สภาวะที่เหมาะสม (ค่าความเป็นกรด-ด่างเริ่มต้น อุณหภูมิ สัดส่วนของอาหารต่อเชื้อจุลินทรีย์ผสม และค่าเหล็กเริ่มต้นในรูปของเฟอร์รัส) พบว่าปริมาณและผลผลิตของก๊าซไฮโดรเจนคือ 543.97 มล. และ44.83 มล.ไฮโดรเจนต่อกรัมซีโอดี ตามลำดับ ที่ค่าความเป็นกรด-ด่างเริ่มต้น 8 ภายใต้สภาพเทอร์โมฟิลิก (55 องศาเซลเซียส) สัดส่วนของอาหารต่อเชื้อจุลินทรีย์ผสมที่ 4 และค่าเหล็กเริ่มต้น 100 มก. เฟอร์รัสซัลเฟต ต่อลิตร เกิดการผลิตของกรดไขมันที่ระเหยง่าย (VFAs) คือ กรดอะซีติก (324.69 มก./ล) กรดโพรพิโอนิก (5.15 มก./ล) และกรดบิวทิริก (765.66 มก./ล) ตามลำดับ ซึ่งสัดส่วนของกรดบิวทิริกต่อกรดอะซีติกเท่ากับ 2.36 สำหรับการศึกษาการบำบัดเบื้องต้น (ความร้อน ความร้อนซ้ำ คลื่นอัลตร้าโซนิก และสารคลอโรฟอร์ม) เพื่อยับยั้งจุลินทรีย์ที่ใช้ผลิตไฮโดรเจนพบว่า การใช้ความร้อนซ้ำทำให้เกิดปริมาณ และผลผลิตของก๊าซไฮโดรเจนสูงสุด คือ 656.57 มล. และ46.19 มล.ไฮโดรเจนต่อกรัมซีโอดี ตามลำดับ เกิดการผลิตของกรดไขมันที่ระเหยง่าย (VFAs) คือ กรดอะซีติก (324.25 มก./ล) กรดโพรพิโอนิก (2.65 มก./ล) และกรดบิวทิริก (838.38 มก./ล) ตามลำดับ ซึ่งสัดส่วนของกรดบิวทิริกต่อกรดอะซีติกเท่ากับ 2.59 ดังนั้นการควบคุมสภาวะที่เหมาะสมและการบำบัดเบื้องต้นของเศษอาหารจากการใช้ความร้อนซ้ำสนับสนุนการผลิตก๊าซไฮโดรเจนทางชีวภาพจากเศษอาหารได้สูงขึ้น

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CHAPTER I
INTRODUCTION

1.1 Background

At present, global warming is a major problem in the world that consensus greenhouse gas comes from combustion of fossil fuel use for develop industry and economic. In the world the major energy uses fossil fuel, which emits pollution such as CO_x, SO_x, C_xH_x, soot, ash, and organic compound has the component of greenhouse gas. Hydrogen is a promising energy alternative, because it is clean and renewable. Hydrogen gives the high energy yield (121 KJ/g), which is about 2.75 times higher than that of hydrocarbon fuel. Hydrogen can be directly used to an electricity by fuel cell. Hydrogen production can be generated in various methods, such as water electrolysis, thermochemical process, photolysis, and biological processes, etc (Lay et al., 1999; Mizuno et al., 2000 ; Das and Veziroğlu, 2001). Biological processes are the most environmental friendly and less energy, when compared to other processes. Biological hydrogen production processes can be classified into three major categories: biophotolysis of water using algae and cyanobacteria; photodecomposition of organic compounds by photosynthetic bacteria (Photo-fermentation); and fermentative hydrogen production from organic wastes (Dark-fermentation) (Hallenbeck and Ghosh, 2009). Dark-fermentation is the one method generating efficient H_2 gas production from a large carbohydrate obtained as refuse or organic waste (Kotay and Das, 2008). However it is difficult to establish a high hydrogen yield, because the amount of fermentative products is significantly influenced by various factors such as pH, temperature, and F/M ratio, etc. (Lay et al., 1999). Dark-fermentation producing hydrogen from low value biomass is also one advantages of biological hydrogen production (Bio-hydrogen production). In terms of substrates, food waste is one of current hydrogen studies, considering as a source of carbohydrate-rich for utilizing waste to energy (Han and Shin, 2002).

Thus, this research focuses on the study of bio-hydrogen production from food waste and the optimum of environmental condition under anaerobic fermentation process for enhancement of the maximum bio-hydrogen production.

1.2 Objectives of the research

1.2.1 To study the optimum of environmental conditions (Initial pH, temperature condition, initial iron concentration, and initial F/M ratio) on bio-hydrogen production from food waste using anaerobic fermentative process in order to maximize the bio-hydrogen production

1.2.2 To study the variation of pretreatment methods (Heat, repeated heat, chloroform and ultrasonication) of food waste and seed sludge in batch reactor for enhancing bio-hydrogen production from food waste.

1.3 Conceptual framework

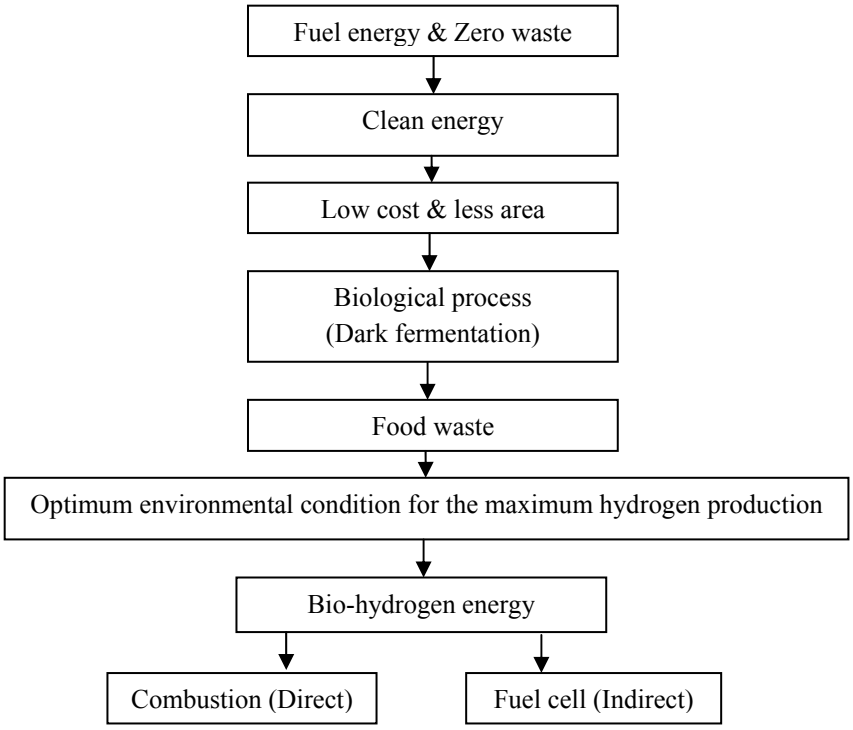

Figure 1.1 Conceptual framework

1.4 Hypothesis

1.4.1 Change in initial pH, temperature condition, initial F/M ratio and initial ferric concentration could affect on hydrogen production of food waste

1.4.2 Different of pretreatment methods could affect on an elimination of H_2 consuming bacteria and enhance the H_2 production.

1.5 Scopes of the research

1.5.1 Food waste was collected from central cafeteria of Mahidol University (Salaya campus), Thailand

1.5.2 Seed sludge was taken from anaerobic digestion of municipal excrement treatment plant in Nonthaburi, Thailand.

1.5.3 Study in a lab-scale. The experiments were carried on in lab-scale using batch system and anaerobic dark-fermentation process.

1.6 Variable

1.6.1 Independent variables

1) The initial pH of 4, 5, 6, 7, 8, 9, 10, 11 and 12 of the reactor under mesophilic (35±2°C) and thermophilic (55±2°C) condition.

2) The initial F/M ratios of 4, 6, 8, 10 and 12.

3) The pretreatment methods of heat, repeat heat, chloroform, and ultrasonication.

4) The initial ferrous ion concentrations of 0, 100, 200, 300, 400 mg Fe^{2+}/l.

1.6.2 Dependent variable

1) Bio-hydrogen yield
2) Volatile fatty acid
3) pH

1.6.3 Stable variable

1) Control the temperature and determine of optimal pH.
2) Control the optimal pH and temperature condition, and determine the proportion of F/M ratio.
3) Control the optimal pH, temperature condition and F/M ratio, and determine the proportion of iron concentration.
4) Control the optimum of pH, temperature condition and F/M ratio, and determine the variation of pretreatment methods.

1.7 Expected outcome

1.7.1 To obtain the optimum of environmental condition of anaerobic fermentation process for support the maximum bio-hydrogen production from food waste.

1.7.2 To select the pretreatment method of food waste, which can enhance the maximum bio-hydrogen production.

1.8 Definition

Bio-hydrogen production	Hydrogen gas production from fermentation process under anaerobic condition
Dark-fermentation	Fermentation process under environmental condition, which no light

Food waste	Food scraps from central cafeteria at Mahidol University (Salaya campus) in Thailand
Optimal factors	The best environmental condition can generate from food waste to maximum hydrogen yield production using fermentation process
F/M ratio	The proportion of food waste to microorganism
SRB	Sulfate-reducing bacteria
NRB	Nitrate-reducing bacteria

CHAPTER II
LITERATURE REVIEWS

2.1 Processing of hydrogen productions

2.1.1 Thermo chemical technology

There are commonly three techniques used to produce hydrogen from thermo chemical process: stream reforming, Gasification and pyrolysis.

1) Stream reforming generally, the steam re-forming process involves two reactions, namely, the splitting of hydrocarbons with steam (Eq 1) and the water gas shift (WGS) (Eq 2) (Navarro, 2007):

$C_nH_m \bullet nH_2O \longrightarrow nCO + (m/2)H_2$ $\Delta H°$ 298K 206.2 kJ/mol (1)

$CO + H_2O \longrightarrow CO_2 + H_2$ $\Delta H°$ 298K 41.2 kJ/mol (2)

For n = 1; m=2n+2

Almost the steam reforming is used in industry for produce hydrogen from raw materials such as natural gas, coal, methanol, C_2H_5OH, or even gasoline, where high temperature condition. Although, there many studies on catalysts, reactor materials, fluid dynamic, and heat transport continues, it emits large a mounts of carbon monoxide (Holladay et al., 2009).

2) Gasification and pyrolysis, gasification and pyrolysis processes are used produce hydrogen, when the feed stocks are solids (such as coal, wood, and other biomass) or semisolid (such as heavy or residual oils) (Haryanto et al., 2005). The pyrolysis process can be classified into slow pyrolysis and fast pyrolysis. In process biomass is heated at high temperature (600-800 K at 0.1-0.5 MPa) in the absence of air convert to liquid oils, solid charcoal and gaseous. Hydrogen gas can be found in fast pyrolysis process, where biomass is heated at high temperature in absence of air into a mixture of hydrogen, methane, carbon monoxide,

carbon dioxide and oil that remain liquid know as produce of fast pyrolysis (Jalan and Srivastava, 1999). The gasification can be converted biomass to gas at high temperature (1000 K), which the biomass particles undergo partial oxidation resulting in gas and charcoal production. However gasification aims to produce gaseous products, pyrolysis aims to produce bio-oils. The tar is major by-product that occurs in the process (Demirbas, 2002). One of the problems with technology is that a amount of resources must be used to the large amounts of biomass to the processing plant. So, the high logistics costs is limit of the gasification plants to be located (Holladay, 2009).

2.1.2 Electrochemical technology

Water is simply spitted by use electrical current pass through two electrodes to divide water into hydrogen and oxygen. It use electric current 53.4-70.1 kWh per 1 kg of hydrogen at 25°C, that electrical energy to chemical in the form of hydrogen and oxygen. The common electrolysis has three technologies include: alkaline base, proton exchange membrane electrolysis and solid oxide electrolysis cell. Although electrolysis can rapidly produce hydrogen, it more expensive than using large-scale fuel processing techniques. Further electricity are nonrenewable, that they have the highest electrical energy costs (Holladay et al., 2009).

2.1.3 Biological hydrogen production

Bio-hydrogen aspect, they all potentially offer the advantages of low cost and less energy intensive reactor operation than present industrial process for making hydrogen (Hallenbeck, 2005). Bio-hydrogen product process can be classified in to three major include: 1) biophotolytic of water (Direct biophotolysis) using algae or cyanobacteria, 2) photo-fermentation of organic compound using photosynthetic 3) dark-fermentative hydrogen production using anaerobic (Facultative anaerobic) bacteria (Hallenbech and Ghosh, 2009).

1) Biophotolytic (Direct biophotolysis) the concerted action of the two photosystems of plant-type photosynthesis to split water with absorbed photons and generate reduced ferredoxin to drive the reduction of protons to hydrogen, is carried out by both some green algae and some cyanobacteria (Figure 2.1). On the

other hand, its simultaneous production of oxygen and hydrogen poses a number of possibly severe problems; the generation of potentially explosive mixtures of these gases, and inhibition of hydrogenase (Green algae), highly sensitive to even moderately low concentrations of Oxygen. Hydrogen production by cyanobacteria, where hydrogen is usually produced by nitrogenase in heterocysts, is much less sensitive to oxygen. However, this comes at a metabolic cost, both due to heterocyst biosynthesis and maintenance, and to the burdensome adenosine triphosphate (ATP) requirement of nitrogenase (Hallenbeck et al., 2009).

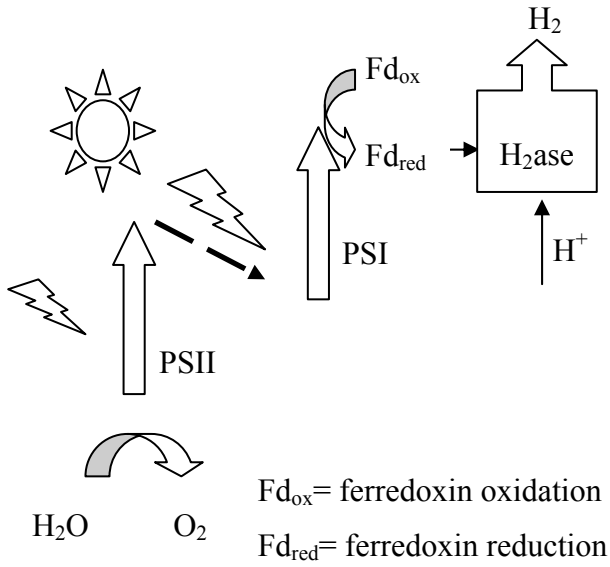

Figure 2.1 Biophotolysis (green algae-cyanobacteria) (Hallenbeck et al., 2009).

2) Photo-fermentative process is generated via photosynthetic degradation of organic compounds (Figure 2.2). Though the conversion of substrate is generally high, the production rate of hydrogen is slow and hydrogen yields are still far from the theoretical maximum. As with any other light-based production process, light diffusion and intensity play a key role in maximizing product (Hydrogen) yield. Increasing light intensity increases the hydrogen yield and production rate, but has a negative effect on light conversion efficiency. Expensive equipment and the requirement for large reactor surface areas remain serious drawbacks. Though cyclic light process operation (i.e. Light–dark cycles) has been shown to increase the amount

of hydrogen evolved when compared to continuous illumination (Koku et al., 2003) and a number of other improvements could possibly be made (Replace Nitrogenase with Hydrogenase, etc.), many questions remain about as to whether overall light conversion efficiencies could ever be high enough to warrant large-scale systems. Photosynthetic hydrogen production might have to be coupled with another process in order to make it economically viable (Hallenbeck et al., 2009).

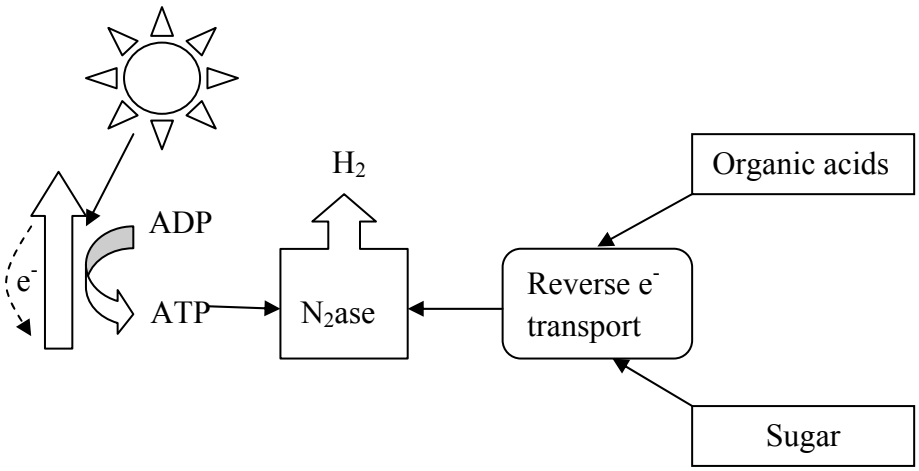

Figure 2.2 Photo-fermentation (Photosynthetic bacteria) (Hallenbeck et al., 2009).

3) Dark-fermentation biological production of hydrogen is dark fermentation, where hydrogen production is inherently more stable since it takes place in the absence of oxygen. Indeed, anaerobic systems have an advantage over their photosynthetic counterparts in that they are simpler, less expensive, and produce hydrogen at much higher rates (Hallenbeck et al., 2009). The degradation of organic matter in anaerobic environments by microbial consortia involves the cooperation of a population of microorganisms that generate a stable, self-regulating fermentation (Sterling et al., 2001). First, hydrolytic bacteria hydrolyze polymeric proteins and sugars. Then, fermentative bacteria form organic acids, hydrogen and carbondioxide from monomeric molecules (Figure 2.3). At that point, hydrogen and acetate can be utilized and produced by several microbial groups. Thus, acetate is generated during acetogenesis from carbon dioxide reduction and hydrogen gas by autotrophic acetogens via the Wood-Ljungdahl pathway, a process named homoacetogenesis

(Müller, 2003). Also, synthophic bacteria generate acetate along with additional H_2 from short-chain organic acids (Except acetate). Finally, for a complete degradation of organic matter, the consumption of organic acids and hydrogen by acetoclastic/hydrogenotrophic methanogens producing methane and carbon dioxide is essential (Garcia et al., 2000). In addition, when sulfates or nitrates are present, sulfate-reducing bacteria (SRB) and nitrate-reducing bacteria (NRB) are capable of using hydrogen as electron donors generating sulfides and ammonia, respectively (Figure 2.3). Thus, hydrogen is a key intermediate consumed mainly by methanogens, NRB, SRB and homoacetongens. The hydrogen consumption enables biochemical reactions carried out by syntrophic bacteria (Table 2.1) to become exergonic and syntrophs can produce additional hydrogen from organic acids (Thauer et al., 1977). This obligatory association between hydrogen producing and hydrogen utilizing microorganisms is called syntrophy. In consequence, hydrogen concentration and the activity of hydrogen utilizing microorganisms may regulate the fermentative pathways. Due to rapid hydrogen consumption, their concentration is usually extremely low and microorganisms have to compete for it. Therefore, establishment of one type of hydrogen consumer depends mainly on the type of inoculums, hydrogen concentration, carbon source, solubility of electron acceptor and capacity to utilize hydrogen traces.

Table 2.1 Hydrogen-producing and hydrogen-consuming reaction present in anaerobic processes (Vazquez and Varaldo, 2008).

Equation	Type of reaction	Reaction	Gibb free energy (Kj/reaction) $\Delta G^{\circ(a)}$	ΔG°(b)
1	Fermentation	$C_6H_{12}O_6 + 2H_2O \rightarrow 2H_2 + \text{butyrate} + 2HCO_3^- + 3H^+$	-135	-284
2	Fermentation	$C_6H_{12}O_6 + 4H_2O \rightarrow 4H_2 + 2\text{acetate} + 2HCO_3^- + 4H^+$	-207	-319
3	Anaerobic oxidation (syntrophy)	$\text{Butyrate} + 2H_2O \rightarrow 2\text{acetate} + H^+$	+48.2	-17.6
4	Anaerobic oxidation (syntrophy)	$\text{Propionate} + 3H_2O \rightarrow 3H_2 + \text{acetate} + HCO_3^- + H^+$	+76.2	-5.5
5	Hydrogenotrophic methanogenesis	$4H_2 + HCO_3^- + H^+ \rightarrow CH_4 + 3H_2O$	-136	-3.2
6	Acetogenesis from CO_2 and H_2	$4H_2 + 2HCO_3^- + H^+ \rightarrow \text{Acetate} + 4H_2O$	-105	-7.1
7	Sulfate reduction	$4H_2 + SO_4^{-2} \rightarrow HS^- + 3H_2O + OH^-$	NA	-165

NA= not analysis

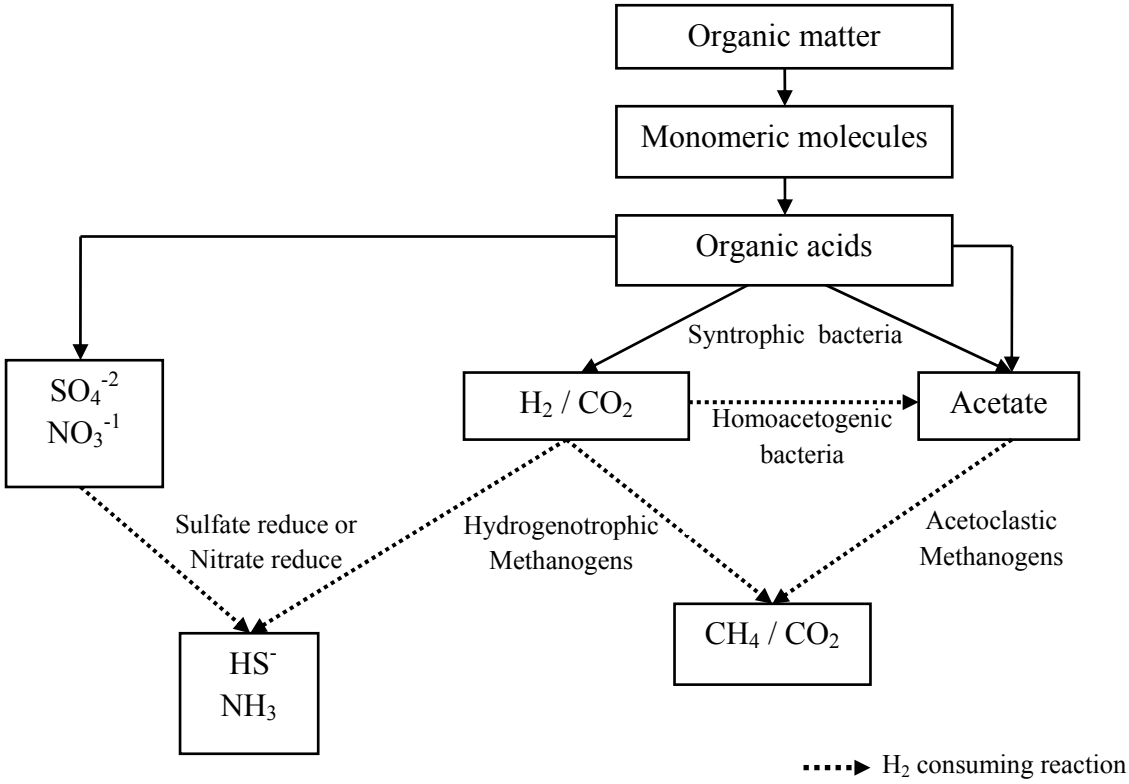

Figure 2.3 Pathway hydrogen role in anaerobic degradation of organic matter (Vazquez and Varaldo, 2008).

2.2 Food waste

Food waste is organic waste, which is residual food from cafeteria, market, shop and food industrial. Food wastes have component of rice, meat, fat, vegetable, and fruit etc. that can be easily generated to hydrogen, since microorganism degraded food waste for energy in metabolism. General food waste is discarded to a land field, then it is generated to methane, which released to environment arise climate change and liquid can contaminate to ground water that effect quality of water. Thus, value of organic waste can generate the energy and save the world from pollution.

2.3 Seed sludge

Nonthaburi city municipal has the eliminated excrement about 3,300 household and has the excrement about 9,145 cubic meters per year. Nonthaburi city municipal have eliminated excrement in anaerobic digestions operate for 28 days, and then release to sand trap, when sludge dries, it was utilized to fertilizer in soil (Bureau of Environment and Health Nonthaburi municipality, 2010). The excrement is the process of eliminating waste product of metabolism in mammals and other non-useful materials. There are many nature microorganisms, which can be degradable organic waste and can be transformed to gas and acid liquid in anaerobic process by metabolism of organisms, therefore the possibility to use sediment as seed sludge for hydrogen production.

2.4 Effect of environmental factors on anaerobic fermentative in dark-fermentation

2.4.1 Mixed culture

The hydrogen-producing had widely in the nature environment such as soil, wastewater and sludge. They can be used as inoculums for fermentative hydrogen production. Fermentative hydrogen production processes using mixed cultures are more operative than using pure cultures, because the mixed cultures are simpler to operate and easier to control, and can easily find source for feedstock (Li and Fang, 2007). In a fermentative hydrogen production process using mixed culture, the hydrogen gas may be consumed by hydrogen-consuming bacteria that can survive, when mix cultures are treated under crude condition. Thus, the mixed cultures can be pretreated by certain methods to suppress as hydrogen-consuming bacterial activity as possible still preserving the activity of the hydrogen-producing bacteria (Wang and Wan, 2008).

The pretreatment methods for elimination of hydrogen consuming bacteria were heat shock, acid, base, aeration, freezing, thawing, chloroform, sodium 2-bromoethansulfonate or 2-bromoethansulfonic acid (BESA), ultrasonic and

iodopropane treatment (Wang and Wan, 2008). The most widely used heat shock for pretreatment sludge, which applied at different temperatures (80-100°C) and different time periods (Haekes et al., 2007; Vazquez and Varaldo, 2008). Oh et al. (2003) reported that heat treatment can eliminate hydrogen-consuming bacteria. However, spore forming homoacetogenic bacteria may still remain in the culture for short heat treatment periods causing hydrogen consumption for acetic acid production. Hence, increasing the duration of heating may be useful in eliminating hydrogen consuming bacteria. Repeat heat pretreatment was reported to be an effective way of eliminating hydrogen consuming bacteria (Mohan et al., 2007; Arum and Kargi, 2009). Argun and Kargi (2009) reported that heat pretreatment was more effective than chloroform treatment alone. Hawkes et al. (2008) reported that some technical and economic difficulties associated with the heat treatment of anaerobic sludge at industrial scale as compared to acid or alkaline treatment. However, acid and alkaline pretreatments were not found to be as effective as 2-bromorthanesufonic acid and iodopropane used in chemical pre-treatment (Zhu and Béland, 2006). A combination of heat and chemical pretreatments may be more effective for elimination of hydrogen consumers. Utilization of 2-bromorthanesufonic acid or iodopropane was not recommended for large scale applications for economic reasons (Zhu and Béland, 2006).

2.4.2 pH

pH is an important factor that directly relatives with a metabolism pathway of hydrogen-producing bacteria. It has been demonstrated that in an appropriate range, increasing pH could increase the ability of hydrogen producing bacteria to capable hydrogen produce (Wang and Wan, 2009). Several reports observed that the hydrogen production initiation was carried out only after pH decreased to 5.5. Studies found that the hydrogenase activity measured in whole cells from acid-producing cultures maintained at pH 5.8 was about 2.2 times higher than that measured in cultures maintained at pH 4.5 (Andersch et al., 1983). In general, hydrogenase activity (Uptake and evolution) is low in cells maintained at a pH less than 5.2 (George and Chen, 1983). Also, Studies found that the activity of hydrogenase I increased steadily with decreasing pH with an optimum pH of 6.3 (Adams and Mortenson, 1984). Thus, these studies on hydrogenase activity are

directly correlated with those of hydrogen fermentation showing that pH affect to hydrogen production.

2.4.3 Temperature

Temperature is another important factor that influences the activities of hydrogen-producing bacteria in fermentative process. It has been demonstrated which in an appropriate range, optimum temperature could increase the ability of hydrogen-producing bacteria to hydrogen produce of metabolism in bacteria (Wang and Wan, 2009). Adams and Mortenson (1984) determined the effect of temperature on the rate of hydrogen catalysis by hydrogenases I and II from mesophilic *C. pasteurianum*. The authors obtained the Arrhenius plot for calculating the activation energy values and optimum temperatures for the reaction. With both enzymes in both assay systems, the plots were linear in the range of 15–50 °C. The rate of reaction decreased between 50 and 70 °C. These in vitro results, together with the in vivo observations, suggest that the optimum temperature is approximately 50 °C. In different systems (Reactors using soluble and solid substrates), it was observed that the specific hydrogen production rate and the hydrogen percentage increased with temperature. In both cases, an optimum hydrogen production rate was achieved at 55 °C and the maximum percentage of hydrogen was more than 60%. (Yu et al., 2002: Vazquez et al., 2005: Vazquez and Varaldo., 2008).

2.4.4 F/M ratio

The F/M ratio is ratio of the food concentration (F) was based on the chemical oxygen demand (COD) of substrate and the microorganism concentration (M) was estimated by the volatile suspended solid (VSS) concentration of the anaerobic digester sludge used as the inoculums. Foods to organisms have influence to fermentative hydrogen-producing process because of capable amount of organisms can rapidly degrade food. It has been demonstrated which in an appropriate range, optimum foods to organisms could increase the hydrogen produce (Wang and wan, 2009). Pan et al. (2008) studied the effect of different food waste to microorganism (F/M ratio) on the hydrogen production under anaerobic fermentation in a batch reactor at two temperature, 35±2 ^{o}C and 50±2 ^{o}C. They found the hydrogen

production mainly during the first 44 h of fermentation under the both condition. The optimal F/M ratio was 7 to 10 for hydrogen production via the thermophilic fermentation (50^{o}C) with the highest yield of 57 ml-H_2/g-VS. While the mesophilic condition, hydrogen was produced at a lower level and in a narrower range of F/M ratio, with the highest yield of 39 ml-H_2/g-VS at the F/M ratio of 6.

2.4.5 Iron concentration

As if at a higher concentration, metal ion may inhibit the activity hydrogen-producing bacteria, a trace level of metal ion is required for fermentative hydrogen production. Iron concentration is one of most important metal ion that seems be directly involve to hydrogenase activity since this enzyme consists of a binuclear iron site bound to a (4Fe-4S) cluster. Naturally iron concentration has two forms, which are ferric and ferrous ion. The most widely studies used ferrous iron concentration that effect on hydrogen production since have some reported effects of nitrate on hydrocarbon biodegradation may be indirect through the reoxidation of iron (Caldwell et al., 1999). Yan and shen, (2006) reported effect of ferrous iron concentration on anaerobic bio-hydrogen production from soluble starch in experiments were conducted to convert soluble starch to hydrogen at 35 °C. At pH 8, the hydrogen yield increased from 106.4 to 274 ml/g starch with iron concentration from 0 to 200 mg $FeSO_4$/l. When iron concentration continued to increase from 200 to 4000 mg $FeSO_4$/l, as iron concentration over 8000 mg $FeSO_4$/l inhibited hydrogen production. The optimal iron concentration at 150 mg $FeSO_4$/l and maximum cumulative hydrogen was 260.5 ml. Lay et al. (2005) found a much smaller optimal iron concentration (132 mg-Fe^{2+}/l) for hydrogen producing composts using solid food wastes as a substrate. Ding studied the effect of the ferrous concentrations ranging from 0 to 1473.7 mg/l on the fermentative hydrogen production from glucose (5 g/L) in batch tests by mixed cultures at 35°C and initial pH 4.7, obtaining the maximum hydrogen yield of 143.7 ml/g glucose at the ferrous concentration of 200 mg/l. Wang and wan (2008) studied the effect of the ferrous concentrations ranging from 0to1500mg/l on the fermentative hydrogen production from glucose was investigated in batch tests by mixed cultures at 35 °C and initial pH 7.0. The experimental results showed that in certain concentration range, ferrous was able to enhance the hydrogen

production rate, the cumulative hydrogen quantity, and the hydrogen yield by the mixed cultures. The maximum cumulative hydrogen quantity of 302.3 ml and the maximum hydrogen yield of 311.2 ml/g glucose were obtained at the ferrous concentration of 300 and 350 mg/l, respectively

2.5 Related researches

Sagnak et al. (2010) studied dark fermentation of acid hydrolyzed ground wheat starch for bio-hydrogen production. The highest hydrogen production rate (305 ml/d) was obtained at HRT = 6 h due to high total sugar loading rates at low HRTs. However, the yield was maximum (130 ml H_2/g total sugar) at HRT = 24 h due to presence of high hydrogen producing bacteria at this HRT. Specific and volumetric rates of hydrogen production were also the highest at HRT = 6 h. Hydrogen formation is strongly related with the type and concentration of VFAs produced by the dominant bacterial culture at every HRT. The effluent VFA composition also varied with HRT in parallel to variations in composition of mixed bacterial culture. High acetate/butyrate ratios obtained at low HRTs yielded high hydrogen formation rates.

Cakir et al. (2010) studied Hydrogen gas production potentials of acid-hydrolyzed and boiled ground wheat were compared in batch dark fermentations under mesophilic (37 °C) and thermophilic (55 °C) conditions. Heat-treated anaerobic sludge was used as the inoculum and the hydrolyzed ground wheat was supplemented by other nutrients. The highest cumulative hydrogen gas production (752 ml) was obtained from the acid-hydrolyzed ground wheat starch at 55 °C and the lowest (112 ml) was with the boiled wheat starch within 10 days. The highest rate of hydrogen gas formation (7.42 ml H_2/h) was obtained with the acid-hydrolyzed and the lowest (1.12 ml H_2/h) with the boiled wheat at 55 °C. The highest hydrogen gas yield (333 ml H_2/g total sugar or 2.40 mol H_2/mol glucose) and final total volatile fatty acid (TVFA) concentration (10.08 g/l) were also obtained with the acid-hydrolyzed wheat under thermophilic conditions (55 °C). Dark fermentation of acid-hydrolyzed ground wheat under thermophilic conditions (55 °C) was proven to be more beneficial as

compared to mesophilic or thermophilic fermentation of boiled (partially hydrolyzed) wheat starch.

Lee et al. (2009) studied the effect of iron concentration ($FeSO_4$) on continuous hydrogen production in a membrane bioreactor (MBR) was investigated using anaerobic mixed microflora under mesophilic condition. The hydrogen production of 41.6 l/day was obtained at 10.9 mg $FeSO_4$/l, which was 1.59 times higher than that at 2.7 mg $FeSO_4$/l. Between 2.7 and 13.7 mg $FeSO_4$/l, the hydrogen production rate increased in parallel with the hydrogen yield under high-cell-density. They reported that addition of iron and sulfur to an MBR is an important key factor in the enhancement of hydrogen production.

Argun and Kargi (2009) studied Hydrogen formation performances of the heat and chloroform pre-treated anaerobic sludges from different sources were compared by batch dark fermentation experiments using 20 g/l wheat powder as the substrate. Cumulative hydrogen formation (CHF), hydrogen yield (HY) and specific hydrogen production rate (SHPR) were used as the comparison criteria. Hydrogen consuming methanogens were eliminated and spore forming hydrogen producers were selected by different pre-treatment methods. Repeated heat treatment (2 × 5 h) was found to be more effective in selecting hydrogen producing bacteria compared to the other treatment methods tested on the basis of cumulative hydrogen production. The highest hydrogen formation (652 ml) and specific hydrogen production rate (SHPR = 25.7 ml H_2/g cells·h) were obtained with the anaerobic sludge pre-treated by repeated boiling. Both the type of anaerobic sludge and the pre-treatment method had considerable effects on bio-hydrogen production from wheat powder solution (WPS) by dark fermentation.

Kim and shin (2008) studied the continuous enriched culture for hydrogen production from food waste. The ground and diluted food waste (Volatile solids (VS) 4.4 ± 0.2% containing 27 g carbohydrate-chemical oxygen demand/l). The experiment was fed hydrogen production decreased below 7.1 ml H_2/g VS (0.10 mol H_2/mol hexose) within 20 days, because the substrate was consumed via non-H_2-producing acidogenesis. To suppress the unintended microbial reactions, three methods were examined: lowering hydrogen content by continuous CO_2 sparging, acid-pretreatment of food waste at pH 2.0 for 1 day, and base-pretreatment of food waste at pH 12.5 for

1 day. The base-pretreatment reduced indigenous anaerobic bacteria in food waste by 4.9 log and enabled a stable long-term operation over 90 days with the H_2 yields of 62.6 ml H_2/g VS (0.87 mol H_2/mol hexose).

Lee et al. (2008) studied the fermentation of vegetable kitchen wastes to produce hydrogen were conducted at pHs of 5.5, 6.0, 6.5, and 7.0 under a thermophilic (55°C) condition. The experiments were studied from initial substrate to microorganism ratio was kept at 10 g COD/g VSS. The hydrogen production contains 40, 43, and 73% at pHs 6.0, 6.5, and 7.0, respectively. A maximum specific hydrogen production rate of 0.48 mmol H_2/g VSS/h occurred at pH 6.0 and highest hydrogen yield of 0.57 mmol H_2/g COD at pH 7.0. The hydrogen production not found at pH 5.5. The major volatile acid produced was butyrate.

Zhu et al. (2008) studied bio-hydrogen production by anaerobic co-digestion of municipal food waste and sewage sludges. The result of these study show that All combinations of the feedstock such as food waste (FW) sludge (PS) and waste activated sludge (WAS) or mixture of PS and WAS produced the most hydrogen and a 1:1 v/v mix FW with a 1:1 v/v blend of PS and WAS was found to be optimal. The maximum hydrogen yield obtained from the co-digestion was 112 ml/g VS added when the ternary mixture of FW, PS and WAS was used. This yield was equivalent to 250 ml/g VS added if only FW contributed to hydrogen production. The reason for enhancement of hydrogen production was postulated to be multifold in which the increase in buffer capacity in the co-digestion mixture was verified.

Pan et al. (2008) studied The effect of different food to microorganism ratios (F/M) (1–10) on the hydrogen production from the anaerobic batch fermentation of mixed food waste was studied at two temperatures, 35 ± 2 °C and 50 ± 2 °C. Anaerobic sludge taken from anaerobic reactors was used as inoculums. It was found that hydrogen was produced mainly during the first 44 h of fermentation. The F/M between 7 and 10 was found to be appropriate for hydrogen production via thermophilic fermentation with the highest yield of 57 ml-H_2/g VS at an F/M of 7. Under mesophilic conditions, hydrogen was produced at a lower level and in a narrower ranges of F/Ms, with the highest yield of 39 ml-H_2/g VS at the F/M of 6. They reported that it provides a novel strategy for controlling the conditions for production of hydrogen from food waste via anaerobic fermentation.

Yang et al. (2007) studied Hydrogen (H_2) production from simulated cheese processing wastewater via anaerobic fermentation was conducted using mixed microbial communities under mesophilic conditions. In batch H_2 fermentation experiments H_2 yields of 8 and 10 mM/g COD fed were achieved at food-to-microorganism (F/M) ratios of 1.0 and 1.5, respectively. Butyric, acetic, propionic, and valeric acids were the major volatile fatty acids (VFA) produced in the fermentation process. Continuous H_2 fermentation experiments were also performed using a completely mixed reactor (CSTR). The pH of the bioreactor was controlled in a range of 4.0–5.0 by addition of carbonate in the feed material. Maximum H_2 yields were between 1.8 and 2.3 mM/g COD fed for the loading rates (LRs) tested with a hydraulic retention time (HRT) of 24 h. However, the microbial populations in the bioreactors were closely related to the conditions and performance of the bioreactors.

Van Ginkel et al. (2005) studied the bio-hydrogen gas production from food processing wastewater and domestic wastewater. The wastewaters were studied from four different food-processing industries that had chemical oxygen demand (COD) of 9 g/l (Apple processing), 21 g/l (Potato processing), 0.6 and 20 g/l (Confectioners A and B). The hydrogen yield conversion were 0.7-0.9 l-H_2/l-wastewater for apple waste, 0.1 l/l for confectioner-A, 0.4-2.0 l/l for confectioner-B, and 2.1-2.8 l/l for the potato wastewater. All of food processing wastewater contains 60% hydrogen gas.

Shin et al. (2004) studied the hydrogen production from food waste by use the mesophilic and thermorphilic acidogenic culture acclimated with food waste at 5 hydraulic retention time (HRT) for the effect of pH and volatile solid (VS) concentrations was evaluated. The biogas produced from the thermophilic acidogenic culture was free of methane at all tested pH and volatile solid concentrations, but methane was detected from the mesophilic acidogenic culture at all tested pH. The amount of hydrogen product from the thermophilic acidogenic culture was much higher than that from the mesophilic culture at all tested pH because of the methane free condition and negligible propionate production. Increasing VS concentrations from 3 to 10 g VS/l. resulted in the increase of quantity and quality of hydrogen production. The maximum hydrogen contains 69% (v/v) at 10 g VS/l. The hydrogen yield was 1.8 mol-H_2/mol-hexose at 6 g VS/l. For the volatile fatty acid mainly

produced was butyrate and the percentages of butyrate, acetate and propionate at tested VS concentrations were 54-60%, 22-31% and 22-31%, respectively.

Kim et al. (2004) studied feasibility of bio-hydrogen production by anaerobic co-digestion of food waste and sewage sludge that was performed in serum bottles under various volatile solids (VS) concentration (0.5-5.0%) and mixing ratios of two substrates (0:100-100:0, VS basic). However, the maximum specific hydrogen production potential of 122.9 ml/g carbohydrate-COD was found at the waste composition of 87:13 (food waste:sewage sludge) and VS concentration of 3.0%. The relationship between carbohydrate concentration, protein concentration, and hydrogen production potential indicated that enriched protein by adding sewage sludge might enhance hydrogen production potential. The maximum specific hydrogen production rate was 111.2 ml H_2/g VSS·h. Food waste and sewage sludge were, therefore, considered as a suitable main substrate and a useful auxiliary substrate, respectively, for hydrogen production. The metabolic results indicated that the fermentation of organic matters was successfully achieved and the characteristics of the heat-treated seed sludge were similar to those of anaerobic spore-forming bacteria, *Clostridium* sp.

Han and shin (2004) studied hydrogen fermentation of food waste in a leaching-bed reactor by heat-shocked anaerobic sludge, and also to investigate the effect of dilution rate (D) on the production hydrogen and metabolites in hydrogen fermentation. Among various reaction constraints affecting the fermentation of food waste, a key factor is the adjustment of environmental conditions during the fermentation because various components of food waste have different characteristics of degradation. D was used as a tool to keep the optimum conditions of hydrogen fermentation. The fermentation efficiency (58%) at initial D of 4.5/d was higher than those (51.4, 55.2, and 53.7%) at initial D of 2.1, 3.6, and 5.5/d. The chemical oxygen demand (COD) removed was converted to hydrogen (10.1%), volatile fatty acids (VFA) (30.9%), and ethanol (17.0%). The butyrate/acetate (B/A) ratios were maintained over 3.2 in the first 2 days. In addition, the fermentation efficiency improved from 58.0% to 70.8% by adjusting D from 4.5 to 2.3/d depending on the state of degradation. The COD removed was converted to hydrogen (19.3%), VFA (36.5%), and ethanol (15.0%). Compared to 0.7–2.2 with no D control, the B/A ratios were kept high (2.0–2.7) on days 3–7, accompanied by the second hydrogen peak. The

trend of B/A ratios was similar to the hydrogen production. D controlled environmental conditions to favor hydrogen production. This meant that the fermentation efficiency was improved by the enhanced degradation of slowly degradable matters. However, D control could delay the shift of predominant metabolic flow from hydrogen and acid-forming pathway to solvent-forming pathway.

CHAPTER III
MATERIALS AND METHODS

This study is an experiment at research using a batch reactor to investigate the optimal condition for the maximum bio-hydrogen production from food waste and sludge in laboratory of Faculty of Environment & Resource Studies at Mahidol University (Salaya campus), Nakhonpathom, Thailand. The diagram of experimental methodology is shown in Figure 3.1.

3.1 Experimental apparatus and procedure

3.1.1 Apparatus

1. Duran bottle 250 ml
2. Silicone rubber stopper
3. Screw caps
4. Syringe
5. Gas tight syringe
6. Air bag
7. Rotary evaporator
8. Tube
9. Beaker 50, 100, 250 and 1,000 ml
10. Centrifuge tube
11. Centrifuge
12. Pipette
13. pH Meter
14. Desiccators
15. Hot air oven
16. Vortex mixer
17. GC-MS
18. GC-TCD
19. Weighting machine
20. Ultrasonication

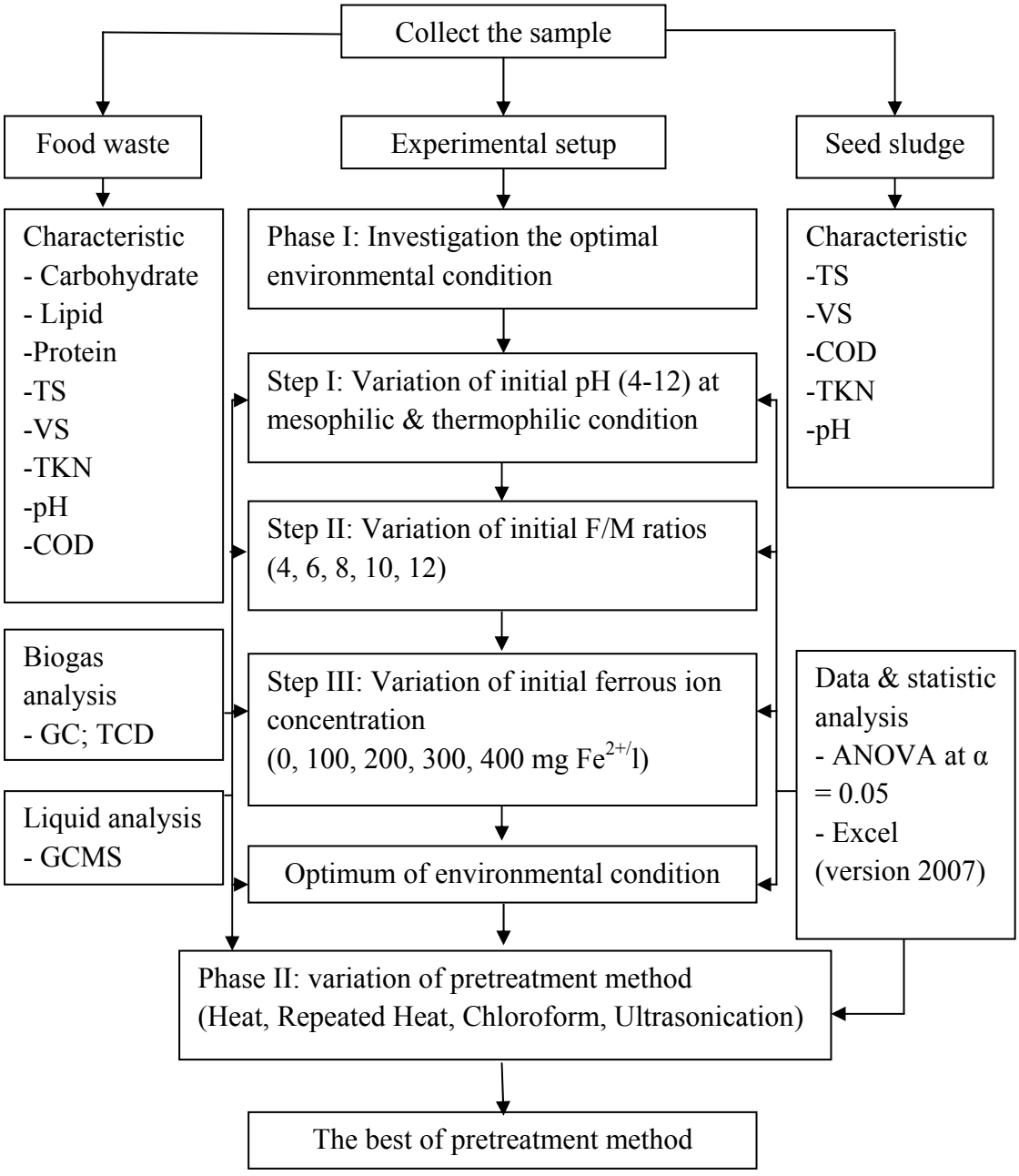

Figure 3.1 Diagram of experimental methodology

3.1.2 Chemicals

1. Acetic acid standard solution
2. Butyric acid standard solution
3. Propionic acid standard solution
4. Gas standard (60% H_2)
5. NH_4HCO_3
6. KH_2PO_4
7. $MgSO_4 \cdot 7H_2O$
8. NaCl
9. $Na_2MoO_4 \cdot 2H_2O$
10. $CaCl_2 \cdot 2H_2O$
11. $MnSO_4 \cdot 7H_2O$
12. $FeCl_2$
13. HCl
14. NaOH
15. Chloroform

3.1.3 Preparation of food waste

Food waste was collected from the central cafeteria at Mahidol University (Salaya campus) in Thailand. It was removed the difficult ground (e.g., animal bones and clamshells). The food waste was grinded using a blender and mixed in a container, then was sieved with a screen (Size 2 mm ID). The ground food waste was mixed with distilled water volume ratio of the ground food waste to distilled water 3:1. The ground food waste was stored in refrigeration at 4^{o}C and was thawed under ambient condition (35 ºC) before it was used in experiments. Characteristic of food waste was analyzed for carbohydrate (glucose equivalence), which was determined by the colorimetric method (Dubois et al., 1956). Chemical oxygen demand (COD) was determined by the closed reflux colorimetric method. Total solids (TS), volatile solids (VS), pH and Total Kjeldahl Nitrogen (TKN) were analyzed following standard methods (APHA/AWWA/WPCF, 2005). Total protein was calculated from organic nitrogen. Fat, oil and grease (FOG) were analyzed by the soxhlet method (APHA/AWWA/WPCF, 2005).

3.1.4 Preparation of seed sludge

The anaerobic sludge was taken from the anaerobic digestion excrement treatment plant of Bureau of Environment and Health, Nonthaburi municipality, Thailand. After that, the sludge was screened with a sieve (Size 2 mm ID) and added the distilled water volume, which was the ratio of seed sludge to distilled water 3:1. The seed sludge was stored in refrigeration at 4^{o}C and was thawed under ambient

condition (35 ºC) before use it in experiments. Total solids (TS), suspended solids (SS), volatile suspended solids (VSS), pH were measured the properties of sludge according to standard methods (APHA/AWWA/WPCF, 2005).

3.1.5 Experimental setup

All of batch experiments were set up in triplication and conducted using 250 ml serum bottles (Duran bottle) with a working volume of 200 ml. They were covered with black plastic to protect sunlight to inactivate bacteria that needed sunlight. Each reactor consists of the hydrogen producing seed which was obtained from the previous step, which was used as a microorganism for hydrogen production and food waste was used as a substrate. Add 10 ml of nutrient solution. The nutrient solution contains 200 g/l of NH_4HCO_3, 100 g/l of KH_2PO_4, 10 g/l of $MgSO_4 \cdot 7H_2O$, 1.0 g/l of NaCl, 1.0 g/l of $Na_2MoO_4 \cdot 2H_2O$, 1.0 g/l of $CaCl_2 \cdot 2H_2O$, 1.5 g/l of $MnSO_4 \cdot 7H_2O$ and 0.278 g/l of $FeCl_2$ (Lay et al., 1999). All bottles were purged with nitrogen in order to create an anaerobic condition and, then silicone rubber stoppers and screw caps were used to avoid gas leakage from the bottle, and then were connected with gas sampling bag. Diagram reactor is shown in figure 3.2.

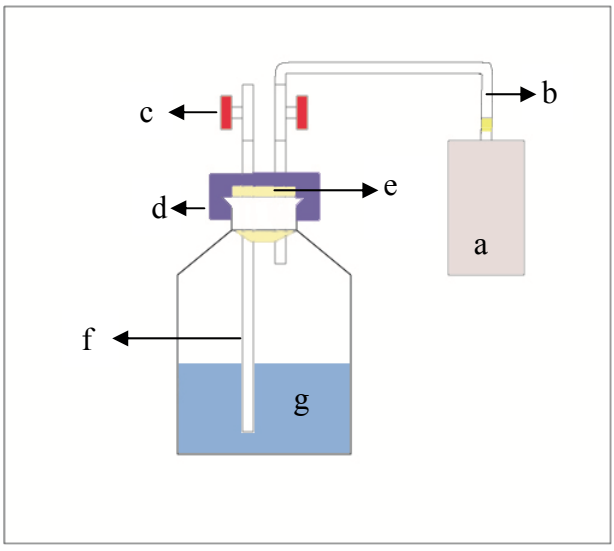

Figure 3.2 Diagram of batch reactor: a) Sampling bag, b) Rubber tube, c) Three-way valve, d) Screw cap, e) Rubber stopper, f) Needle for sampling collection, g) Mixed liquid of food waste, sludge and nutrient solution.

3.1.6 Operating procedure

The experiments in this study were divided into two phases according to Figure 3.1, so each phase was described below.

Phase I investigate the optimal environmental condition of anaerobic fermentative process (initial pH, temperature condition, initial ferric concentration, and initial F/M ratio) in this phase food waste and seed sludge were inactivated hydrogen-consuming bacteria by heat-shock (boiled at 90°C for 15 min). This phase consists of three steps for investigation of the optimum of environmental condition. In step I, pH 4, 5, 6, 7, 8, 9, 10, 11 and 12 were adjusted by either 2 N of HCl or 2 N of KOH. F/M ratio 10 was keep constantly in each bottle and the bottle reactors were placed in a water batch shaking with 100 rounds per minute (rpm) at 35±2 ºC and 55±2 ºC, for mesophilic and thermophilic conditions of test, respectively. In step II, the various initial F/M ratios at 4, 6, 8, 10, and 12 was investigated while the initial pH and temperature condition were keep constantly of the optimal value obtained from step I. In step III, the initial ferrous concentration various concentrations at 0, 100, 200, 300 and 400 mg Fe^{2+}/l, respectively. The initial pH, temperature condition and initial F/M ratio were keeping constantly of the optimal condition that obtained from step I and step II.

Phase II in order to investigate the variation of pretreatment methods to obtain the best pretreatment method for generating bio-hydrogen production. The pretreatment methods in this study were designed to evaluate the influence of mixing of food waste and seed sludge to enhance bio-hydrogen production. A total of four experimental sets (heat, repeat heat, chloroform, and ultrasonication) were designed and performed. In heat-shock pretreatment procedure, the mixed liquid was subjected to heating (maintained at 90 °C) for a period of 15 min. For repeated heat pretreatment procedure, the mix liquid (200 ml) was placed in 250 ml beaker, and was conducted by heating the mixed liquid for 15 min and rested at room temperature overnight, then It was repeated heat for 15 min and rest at room temperature until cool as room temperature. For Chemical inhibitor pretreatment procedure, the mix liquid (200 ml in 250 ml beaker) was exposed to 0.05% chloroform and kept for 1 day at room temperature. For Ultrasonic pretreatment procedure, the ultrasonic pretreatment (Branson) was performed with the help of a cell-breaker. The mixed liquid (200 ml)

was placed in 250 ml beaker and the ultrasonic probe positioned in 2 cm under the surface of sludge. The ultrasonic time is 2 min and the ultrasonic power density is 2 watt/ml. The initial pH, F/M ratios and temperature condition were used following previously phase.

3.2 Monitoring

Gas volume and gas composition were monitored initial at 4, 6, 8, 24 hours and daily day. The mixed liquor was collected from sampling ports in batch experiment determine between before and after experiment. pH liquor was analyzed daily by pH strip (Shleicher & schuell, Germany).

3.3 Analyses

3.3.1 Gas analysis

Biogas production from the reactor was collected by sampling bag. Volume gas was measured by a glass syringe with a capacity of 50 or 100 ml. Biogas was sampled using gas-tight syringe (500 µl injection volume), and injected to gas chromatography (GC, Varian Star 3400, USA) equipped with a thermal conductivity detector (TCD) and stainless-steel column packed (Alltech Molesieve 5A 80/100 10'x 1/8") with argon gas as carrier gas for hydrogen, nitrogen and methane analysis. Helium used as the carrier gas for carbon dioxide analysis (Selembo et al., 2009). The temperatures of injector, detector, and column were kept at 80 °C, 90 °C and 50 °C, respectively.

3.3.2 Liquid analysis

The liquid in batch reactor was analyzed for volatile fatty acid using a gas chromatography. It was collected about 5 ml by plastic syringe. The samples were centrifuged at 10,000 rpm for 2 minute through a membrane filter (0.45 µm) and then place in 5 ml vial bottle. All experiment was analyzed by a gas chromatography / mass

spectroscopy (AGILENT 5975C GC, China) equipped with headspace chromatographic analysis was performed using a MHS 02-00 B Volume 2.5 ml scale 60 mm ID 28 automatic headspace. The temperatures of the HS 40XL oven, needle and transfer line were set at 85. Injector, detector temperature was at 250 ºC, 250 ºC, respectively, with helium as a gas carrier at constant flow rates of 2 ml/min.

3.3.3 Data analysis

Hydrogen gas production was calculated from headspace measurements of gas composition and total volume of biogas produced at each time interval by using following equation (Eq 1) (Logan et al., 2002).

$$V_{H,\,i} = V_{H,\,i\text{-}1} + C_{H,i}(V_{G,1}\text{-}V_{G,i\text{-}1}) + V_H(C_{H,i} + C_{H,i\text{-}1}) \quad (1)$$

where $V_{H,i}$ and $V_{H,i-1}$ are cumulative hydrogen gas volumes at the current (i) and previous (i-1) time intervals, $V_{G,i}$ and $V_{G,i-1}$ are the total gas volumes in the current and previous time interval, $C_{H,i}$ and $C_{H,i-1}$ are the fraction of hydrogen gas in the headspace of the bottle measured using gas chromatography in the current and previous intervals, and V_H is total volume of headspace in the reactor.

A modified Gompertz equation (Zwietering et al., 1990; Lay et al., 1999) (Eq 2) was used to calculate cumulative hydrogen data depict.

$$H = H_{max} exp\left\{-exp\left[\frac{R_m e}{H_{max}}(\lambda - t) + 1\right]\right\} \quad (2)$$

where H (ml) is the cumulative hydrogen production H_{max} (ml) is the hydrogen production, R_m (ml/h) is the maximum hydrogen production rate, λ (h) is the lag phase time and e = 2.71828.

3.3.4 Statistic analysis

One factor analysis of variance (ANOVA) was used to determine the statistical significance of the differences in hydrogen production between the studies of pH, temperature, F/M ratio, ferrous ion concentration and variation of pretreatment method. The threshold level of statistical significance for this study was $\alpha = 0.05$. ANOVA was carried out using a Microsoft Excel Software 2007.

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.

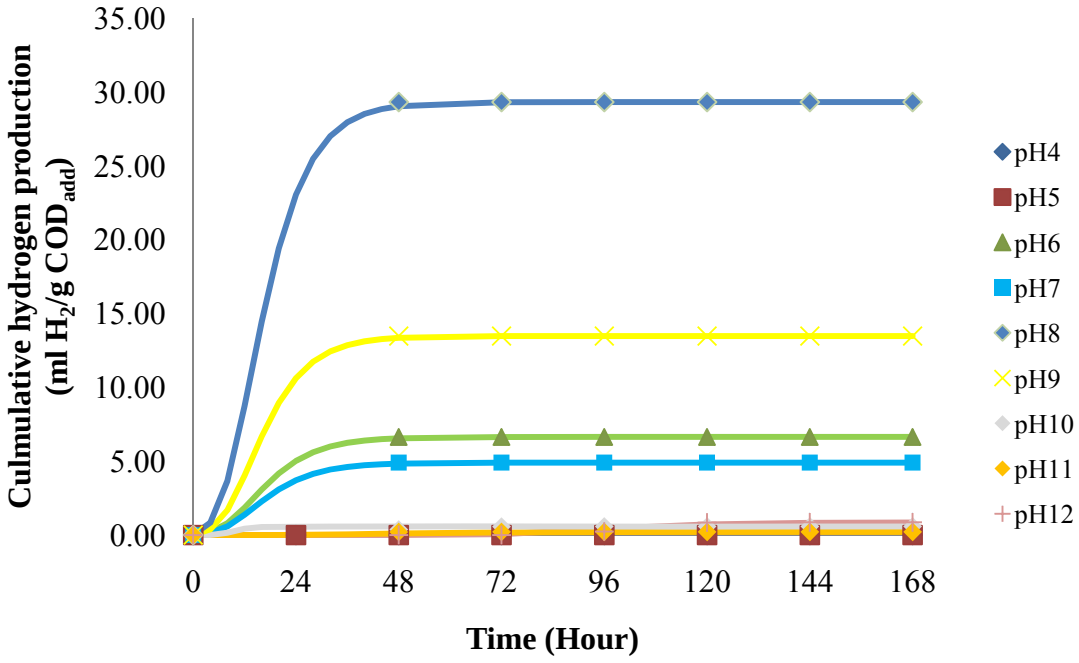

Figure 4.1 Cumulative hydrogen production at various initial pHs under mesophilic condition

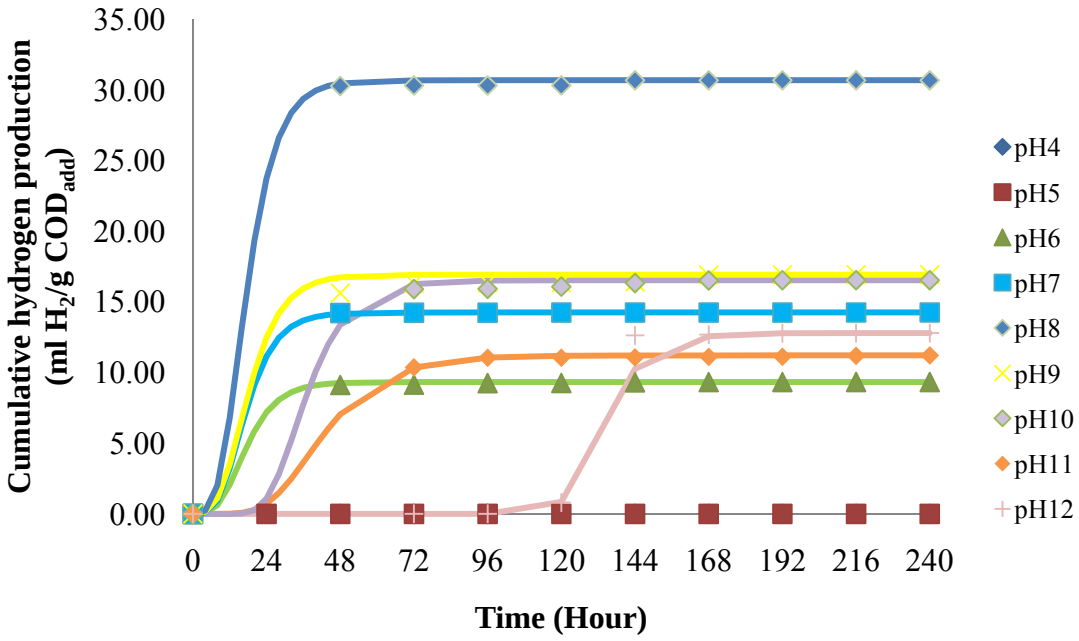

Figure 4.2 Cumulative hydrogen production at various of initial pHs under thermophilic condition

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.

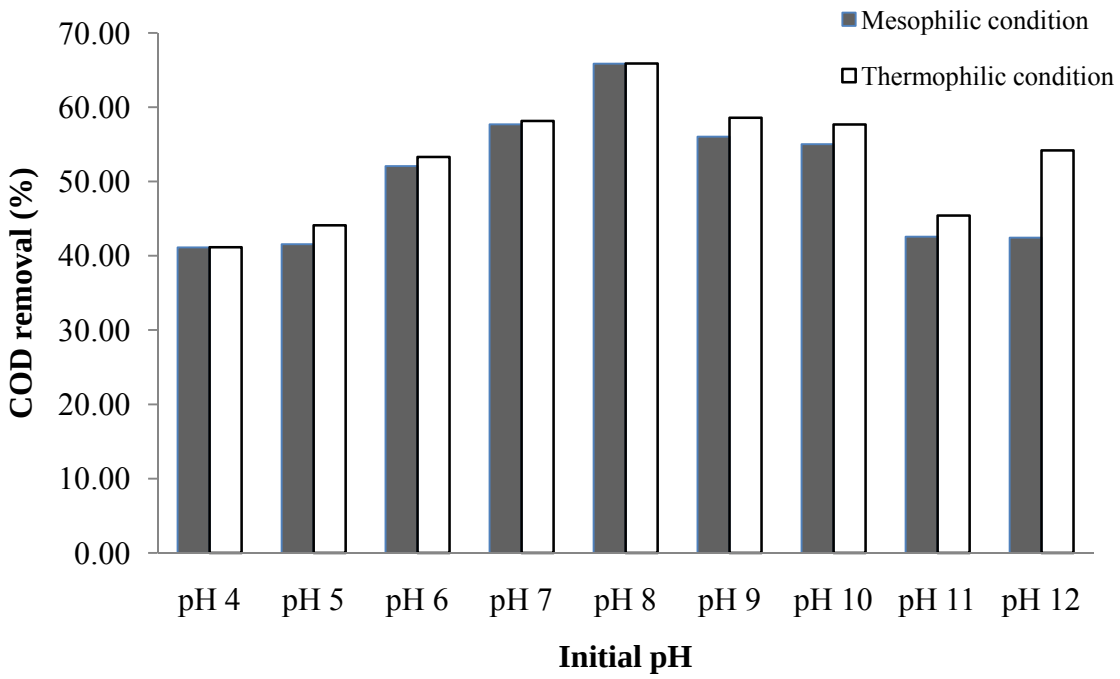

Figure 4.3 Percentage of COD removal under mesophilic and thermophilic conditions at various initial pHs

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}.

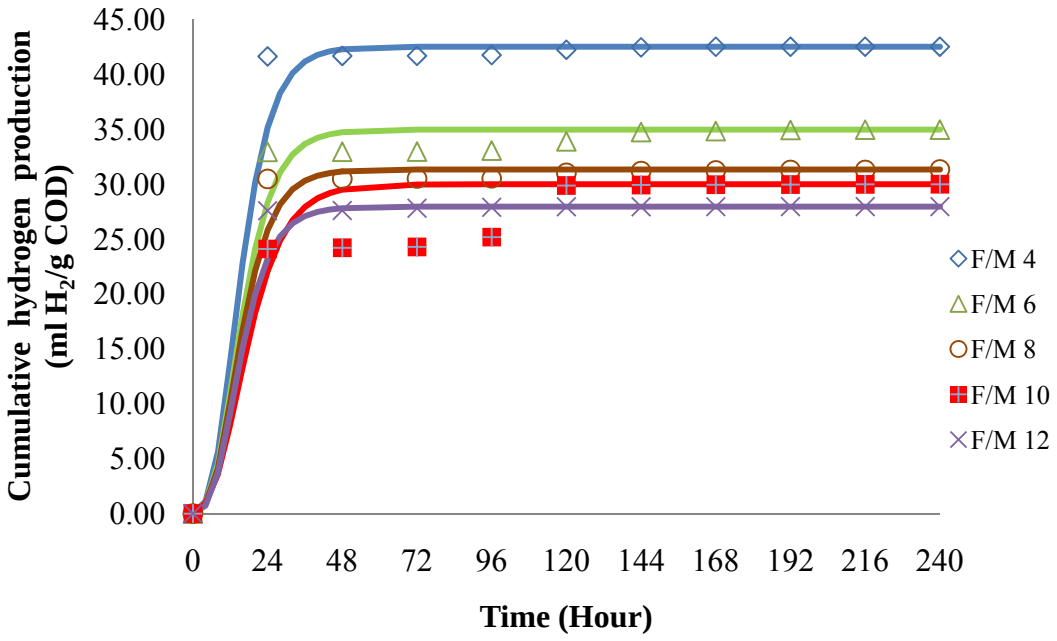

Figure 4.6 Cumulative hydrogen production at various initial F/M ratios

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.

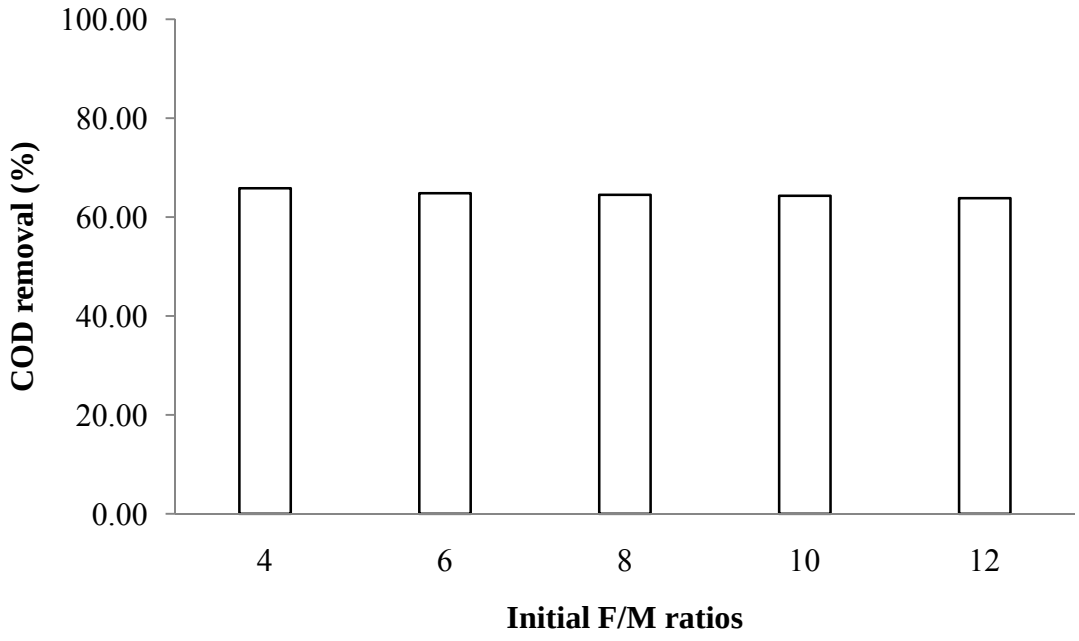

Figure 4.7 Percentage of COD removal of initial F/M ratios on bio-hydrogen production from food waste.

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.

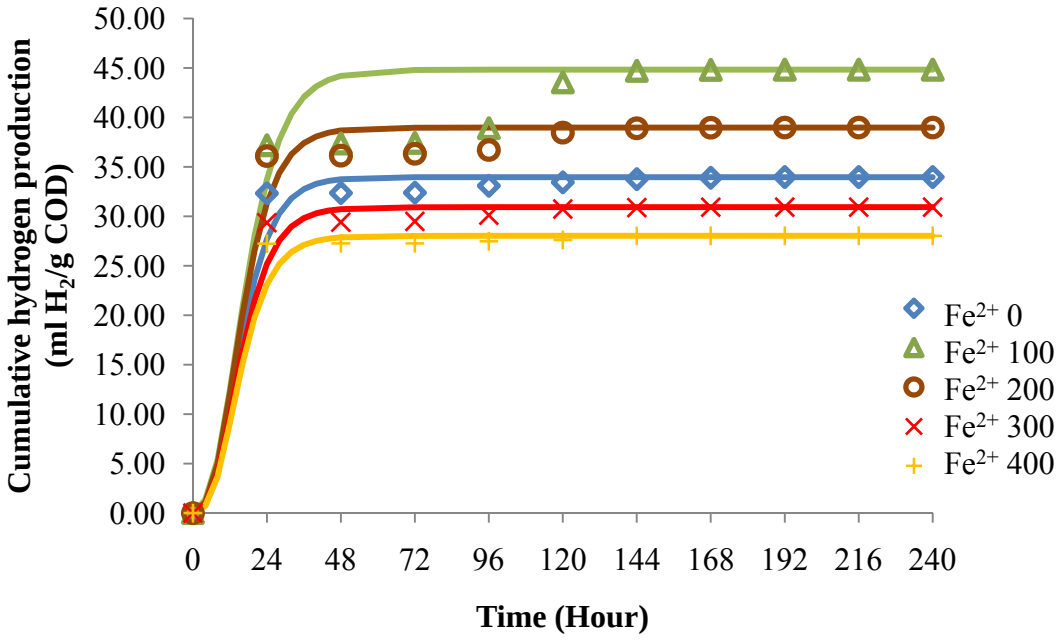

Figure 4.8 Cumulative hydrogen production at various initial ferrous ion concentration

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.

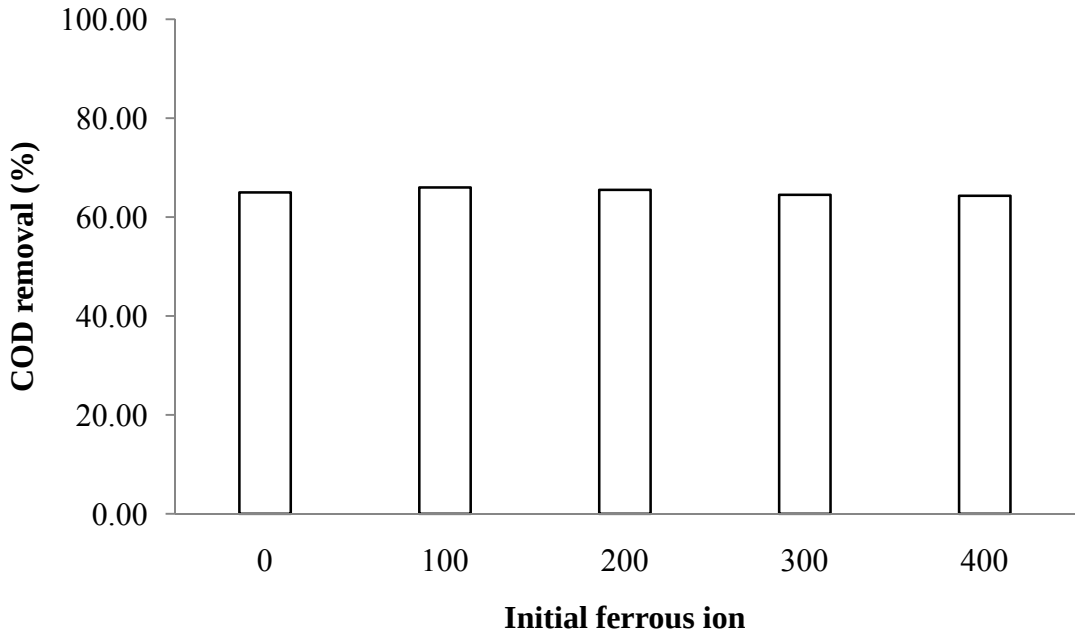

Figure 4.9 Percentage of COD removal at various initial ferrreous ion concentration on bio-hydrogen from food waste.

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.

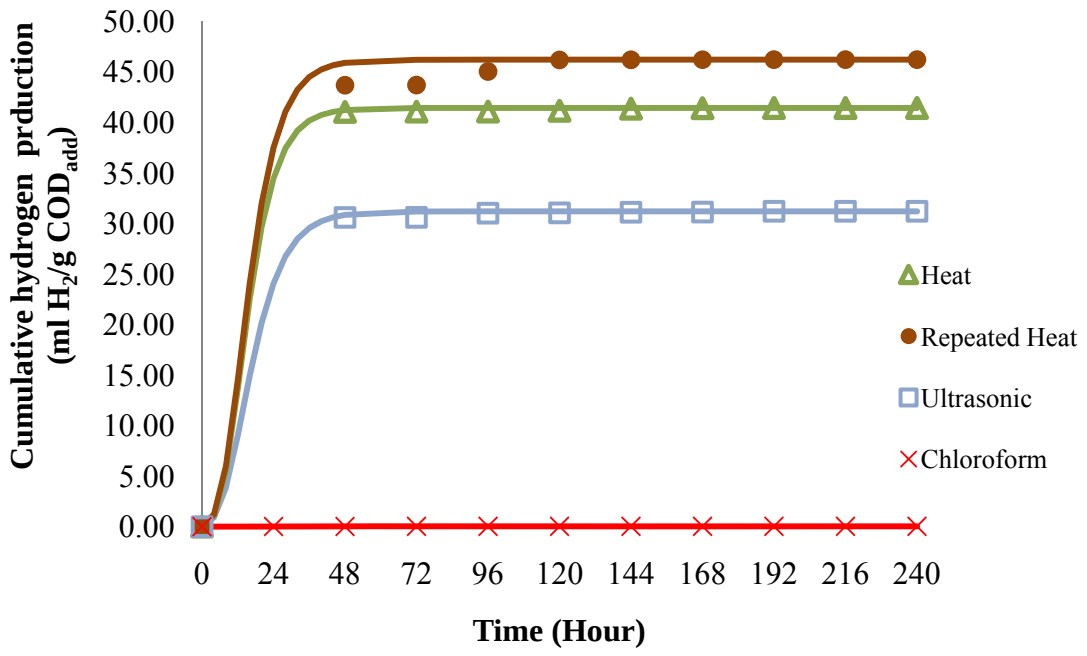

Figure 4.10 Cumulative hydrogen production of different pretreatment method

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.

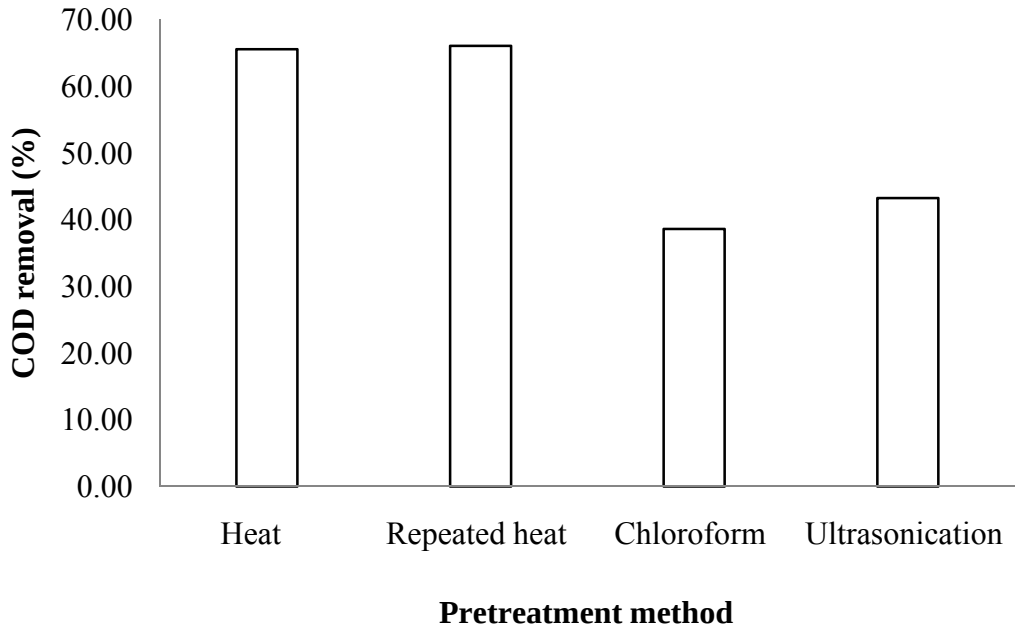

Figure 4.11 Percentage of COD removal of different pretreatment method

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}).

CHAPTER V
CONCLUSION AND RECOMMENDATION

5.1 Conclusion

All batch experiments were performed to evaluate the bio-hydrogen production from food waste and anaerobic sludge. The results could be concluded as follows:

5.1.1. In the experiment phase I, initial pH 4 to 12 (step I) were performed bio-hydrogen production under mesophilic (35±2°C) and thermophilic (55±2°C) conditions. Hydrogen production under initial pH 8 at 55°C was higher than it's under initial pH 8 at 35°C. The maximum cumulative hydrogen production and hydrogen yield were 767.64 ml H_2 and 30.69 ml H_2/g COD_{add}, respectively. Acetate, propionate and butyrate were mainly VFAs production which was 385.39, 5.67 and 758.56 mg/l, respectively and B/A ratio was 1.97. For initial F/M ratios (4, 6, 8, 10 and 12) (step II) were performed to enhance bio-hydrogen production under thermophilic condition that the optimal initial F/M ratio was 4. The maximum cumulative hydrogen production and hydrogen yield were 648.48 ml H_2 and 42.51 ml H_2/g COD_{add}, respectively. Acetate, propionate and butyrate were main VFAs production which was 402.97, 8.31 and 850.60 mg/l, respectively and B/A ratio was 2.11. For concentration ranges of initial $Fe2^+$ (100, 200, 300 and 400 mg Fe^{2+}/l) (step III) were effective on hydrogen production and the optimal Fe^{2+} concentration was achieved at 100 mg Fe^{2+}/l. The maximum cumulative hydrogen production and hydrogen yield were 543.97 ml H_2 and 44.83 ml H_2/g COD_{add}, respectively. Acetate, propionate and butyrate were main VFAs production which was 324.69, 5.15 and 765.66 mg/l, respectively and B/A ratio was 2.36.

The optimum of environmental condition was the initial pH 8, initial F/M ratio of 4 and initial Fe^{2+} concentration 100 mg Fe^{2+}/l under thermophilic (55±2°C) condition.

5.1.2. In the experimental phase II, it studied the effect of pretreatment methods (Heat, repeated heat, chloroform and ultrasonication) on bio-hydrogen production. The results showed that repeated heat method was the optimal method in this study. The maximum cumulative hydrogen production and hydrogen yield were 656.57 ml H_2 and 46.19 ml/g COD_{add}, respectively. The VFAs production (324.25, 2.65, 838.38 mg/l of acetate, propionate and butyrate, respectively) was observed in the batch reactor and the B/A ratio was 2.59.

5.2 Recommendation

The following aspects are recommended for further studies.

5.2.1. Optimal condition obtained from this study are helpful for design of bio-hydrogen production from food waste in a continuous fermentation process.

5.2.2. Pretreatment method shown the positive on H_2 production but it should consider the economic assessment of cost.

5.2.3. Bio-hydrogen production from other substrate (i.e., starch, organic waste, waste water etc.) should be more studied and applied for fuel cell as a clean energy.

5.2.4 Remaining COD (Effluent COD) of batch reactor should be analyzed total VFAs for conversion to percentage of COD removal.

5.2.5 Study more on the initial F/M ratio lower than 4 that may be the optimal condition.

5.2.6 Study more on the initial Fe^{2+} lower than 100 mg Fe^{2+}/l that may be the optimal condition.

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APPENDICES

APPENDIX A
METHODOLOGY OF ANALYSIS

Appendix A1 Chemical Oxygen Demand (COD) analysis (Closed reflux, Titration method)

Apparatus

- Digestion vessel 16 x 100 mm
- Hot air oven
- Heating block

Reagents

- Potassium standard solution. The following reagents were added into 500 ml distilled water. 4.913 g $K_2Cr_2O_7$ (Primary standard grade) previously dried at 103 ºC for 2 h, then added 167 ml conc. sulfuric and 33.3 g $HgSO_4$. The mixture was done until complete dissociation, thrown away to be cooled in room temperature, and finally diluted to 1000 ml.
- Sulfuric acid reagent. Added 1% W/V into conc. H_2SO_4. The mixture was let for 1-2 days for completely dissolved Ag_2SO_4.
- Ferroine Indicator
- 0.1 M Ferrous Ammonium Sulfate standard solution (FAS) 39.2 g $Fe(NH_4)_2(SO_4)_2$ dissolved into 500 ml distilled water, then added 20 ml conc. H_2SO_4, and finally diluted to 1000 ml.

Note: 0.1 M Ferrous Ammonium Sulfate standard solutions compare with Potassium Dichromate standard solution. Using 2.5 ml distilled water, 1.5 ml $K_2Cr_2O_7$, 3.5 ml H_2SO_4 and 1-2 drops Ferroine Indicator into flask titrate with 0.1 Fereous Ammonium Sulfate standard solution end point green blue to red brown.

$$\text{Concentration of FAS (M)} = \frac{\text{ml } K_2Cr_2O_7 \text{ x } 0.1}{\text{ml FAS}}$$

Procedure

- 2.5 ml of Sample was added to digestion vessel
- Add 1.5 ml digestion reagent, and then 3.5 ml sulfuric acid was slowly into the vessel.
- Seal was tightly with cork tube. Afterward, the vessel was gently inverted several times. (As mixing should put on grove and mask)**,** and the vessel was then placed in the hot air oven.
- The vessel was hearted at 150 ºC for 2 h, and then left to be cooled in room temperature.
- Afterward, Ferroine Indicator was dropped for 1-2 drop, and finally titrate with 0.1 M 0.1 M Ferrous Ammonium Sulfate standard solutions. (End point green blue change to red brown)

Calculation

$$\text{COD (mg/l)} = \frac{\text{(A-B) x M x 8000}}{\text{ml of Sample}}$$

where A = ml of FAS titrate with blank
B = ml of FAS titrate with sample
M = Molarity of FAS

Note: Distilled water was used to blank

Appendix A2 Total Suspended Solids (TSS) Analysis

Apparatus

- Desiccators
- Drying oven
- Glass-fiber filter disk (47 mm ID)
- buchner funnel
- Suction flask

Procedure

- The glass-filter disk was dried in a hot air oven at 105 ºC for 1 h. The disk was cooled in desiccators to balance temperature and weighed.
- The Sample was chosen to yield between 10 and 200 mg dried residue.
- The glass-filter was placed in buchner funnel which connect vacuum machine.
- The filter was wet with a bit volume of distilled water for fix in buchner funnel
- The sample volume was sieved in the Buchner funnel, after complete filtration also suction and wash filter for 3 min.
- The filter was carefully removed from Buchner funnel.
- The filter was dried at least 1 h at 105 ºC in an oven, afterward was cooled in desiccators in room temperature, and weight.

Calculation

$$\text{mg Total Suspended Solids/l} = \frac{(A-B)\times 1000\,(\text{mg})(\text{ml/l})}{\text{sample volume(ml)}}$$

A = Weight of filter + dried residue (mg)

B = Weight of filter (mg)

Appendix A3 Volatile Suspended Solids (VSS) Analysis

Procedure

- The residue produced by TSS method was ignited in a furnace at 550 ± 50 ºC.
- A furnace was heated up to temperature after inserting sample.
- Usually, 15 to 20 min ignition is required for 200 mg residue.
- The filter disk was left to partially cool in air until most of the heat was dissipated.
- The dist was transferred to desiccators, and weight as soon as if was cooled in room temperature.

Calculation

$$\text{mg Volatile Suspended solid/L} = \frac{(A-B)\times 1000}{\text{sample volume, ml}}$$

A = Weight of residue + disk before ignition (mg)

B = Weight of residue + disk after ignition (mg)

Appendix A4 Total Solid (TS) Analysis

Apparatus

- 50 ml crucible
- Water bath
- Analytical balance
- Muffle furnace
- Desicator
- Drying oven 103 to 105°C

Procedure

- Preparation of evaporation crucible : If volatile solids are to be measured ignite clean evaporaring crucible at 550°C for 1 h in a muffle furnace. Store and cool crucible in desiccator until needled. Weigh immediately before use.
- 25 ml sample , that homogeneous samples and then evaporate to dryness on water bath.
- Dry evaporate sample for 1 h in an oven at 103 to 105°C, and then cool crucible in desiccators for 30 min or until a constant weight.
- Determine weight in a weight balance.

Calculation

$$\text{mg Total solid/l} = \frac{(A-B)\times 1000}{\text{sample volume, ml}}$$

A = Weight of residue + crucible before ignition (mg)

B = Weight of residue + crucisle after ignition (mg)

Appendix A5 Volatile Solids Analysis

Apparatus

- The residue from Total Solids
- Muffle furnace
- Weight balance

Procedure

- The residue from Total Solids is ignited in the muffle furnace at 550°C for 1 h.
- Cool in desiccators to a constant temperature and weigh.
- Weigh Analysis in weigh balance.

Calculation

$$\text{mg Volatile solid/l} = \frac{(A-B)x1000}{\text{sample volume, ml}}$$

A = Weight of residue from total solids + crucible before ignition

B = Weight of residue + crucisle after ignition (ml)

Appendix A6 Total Nitrogen Analysis (Kjeldahl Methods)

Apparatus

- Weigh balance
- Hood
- Digestor
- Distilling unit
- 250 ml Digestor tube
- 250 ml Erlenmyer flask
- 100, 500 and 1000 ml of volumetric flasks
- 250 ml beaker
- 50 ml burette

- 5, 10, 15 and 20 ml of pipettes
- 25 and 100 ml of Cylinder
- Stir glass and rubber pump

Reagents

- conc.H_2SO_4
- 30% H_2O_2
- Sodium hydroxide Solution. The following reagents were added 1 kg sodium hydroxide (commercial grade NaOH) in to 1000 ml of distilled water
- Mixed catalyst, that consists of K_2SO_4 and $CuSO_4 \cdot 5H_2O$ in 100:10 ratio.
- Indicator (collected in a light brown bottle)

0.2 g of methyl red dissolve into 100 ml of 95% ethyl alcohol then collect in bottle

0.1 g of methyl blou dissolve into 100 ml of 95% ethyl alcohol and then collect in bottle

- 4% Boric indicator. Add 40 g of boric acid into 1 l of volumetric flak, that have a bit distilled water in it and add 10 ml of indicator (mixed 10 ml methyl red and 5 ml methyl blou) and then diluted to 1000 l.
- 0.1 N Sulfuric acid standard solution: pipette 1.5 ml conc.H_2SO_4 transfer to a 500 ml volumetric flask, fill flask to mark with distilled water. Titration certainly determine concentration of sulfuric acid standard solution with 40 ml of 0.05 N sodium carbonate. The following calculation express:

$$\text{Normality of sulfuric acid} = \frac{AxB}{53xC}$$

where: A = g Na_2CO_3 into 1 l flask

B = ml of Na_2CO_3 solution taken for titration,

C = ml acid used

Procedure

Digestor

- Add 100 ml saple in 250 ml digestor tube (blank use distilled water) and add 10.5 g mixed catalyst.
- Add 15 ml conc. Sulfuric acid and 2 ml hydrogen peroxide.
- Initial in digestor machine at temperature about 250°C, when temperature increase to 420°C count time for 2 hours.

Distillation

- Sample is distilled by auto Distillation unit (Kjeltec^{tm}2200). The program is fixed by Add 50 ml water and 30 ml sodium hydroxide distill for 4 min until get 150 ml and then was analyzed by titration.
- Add 25 ml boric indicator in 250 ml erlenmyer flask, which is supported from auto distillation.

Titration

- Titration with 0.1 sulfuric acid standard solution. The end-point change green to purple.

Calculation

$$\%N = \frac{(A-B) \times N \times 1400}{\text{ml sample}}$$

where: A = ml acid used titrate sample
B = ml acid used titrate Blank
N = Normality of standard acid

Appendix A7 Grease and oil Analysis via Soxhlet (Ruibal-mendieta et al., 2001)

Apparatus

- 150 ml beaker
- Soxhlet & distill bottle
- Extraction thimbles
- Glass beads

- Electric heating mantle
- Vacuum pump
- buchner funnel
- Water bath
- Clean cotton
- Desiccator
- Muslin remnant of cloth

Reagent

- Conc. HCl
- n-hexane

Procedure

- Add muslin remnant of cloth and filter paper, respectively in the Buchner funnel, that connect with vacuum pump and then add 100 mL water into funnel.
- Add 25 ml sample pass filter in funnel until dry.
- Collect muslin cloth and filter paper in extraction thimble. The residue of oil is wiped by n-hexane. Dry in oven for 30 min at 103 to 105°C.
- Extration thimble is add in soxhlet, that connect with distill bottle and soxhlet extraction for 4 h. n-hexane is Solvent in distill bottle. Extraction ratio 20 rounds per h.
- Afterward, distill extraction bottle until dry (n-hexane is distilled). Dry constant weight in oven at 103 to 105°C and place in desiccators until weigh constant.
- Determine weight by weigh blance.

Calculation

$$\text{mg/l oil and grease} = \frac{\text{mg increase in weight of flask x 1,000}}{\text{ml sample}}$$

Appendix A8 Carbohydrate Analysis (Colorimetric method)

Apparatus

- Tube
- Water bath
- Spectro photometer
- Micro pipette

Reagent

- 80% (w/w) phenol: 20 g phenol dissolve in 80 ml deionize water at 25°C.
- conc. Sulfuric acid
- 100 ppm of D-glucose standard solution:

Procedure

Standard curve

- Series of sugar containing 2, 4, 6, 8 and 10 µl of sugar solution is pipetted into colorimetric tube, 50 µl of 80% phenol is added. Then 5 ml of concentrated sulfuric acid is added rapidly.
- The tubes are allowed to stand 10 min, and then they are shaken and placed for 20 min in a water bath at 30°C.
- Characteristic of solution is yellow-orange color can be measured at 480, 484 and 490 nm with spectro photometer. Blank are prepared by substituting deionize water for the sugar solution

Sample Analysis

- Add 2 ml sample, that it is diluted 1000 times by deionize water in tube
- Add 50 µl of 80% phenol and then add 5 mL conc. Sulfuric acid is rapidly.
- The tubes are allowed to stand 10 min, then they are shaken and placed for 20 min in a water bath at 30°C. The same previously method.
- The sample is analyzed by spectro photometer at 480, 484 and 490 nm. Plot graph in standard curve and calculate concentration.

APPENDIX B
GAS CHROMATOGRAPH'S STANDARD GAS

Table B1 Gas chromatograph's calibration on standard curve (60% Hydrogen and 10% Methane)

Order	Hydrogen		Methane	
	% H_2	Area	% CH_4	Area
1	60	1810012	10	59149
2	60	1669967	10	61181
3	60	1568000	10	62757
4	60	1693790	10	61549
5	60	1894693	10	72672
6	60	1940139	10	73603
7	60	1912454	10	72642
8	60	1956328	10	72083
9	60	1874962	10	71123
10	60	1899974	10	70349
Average		1822031.90		67710.80

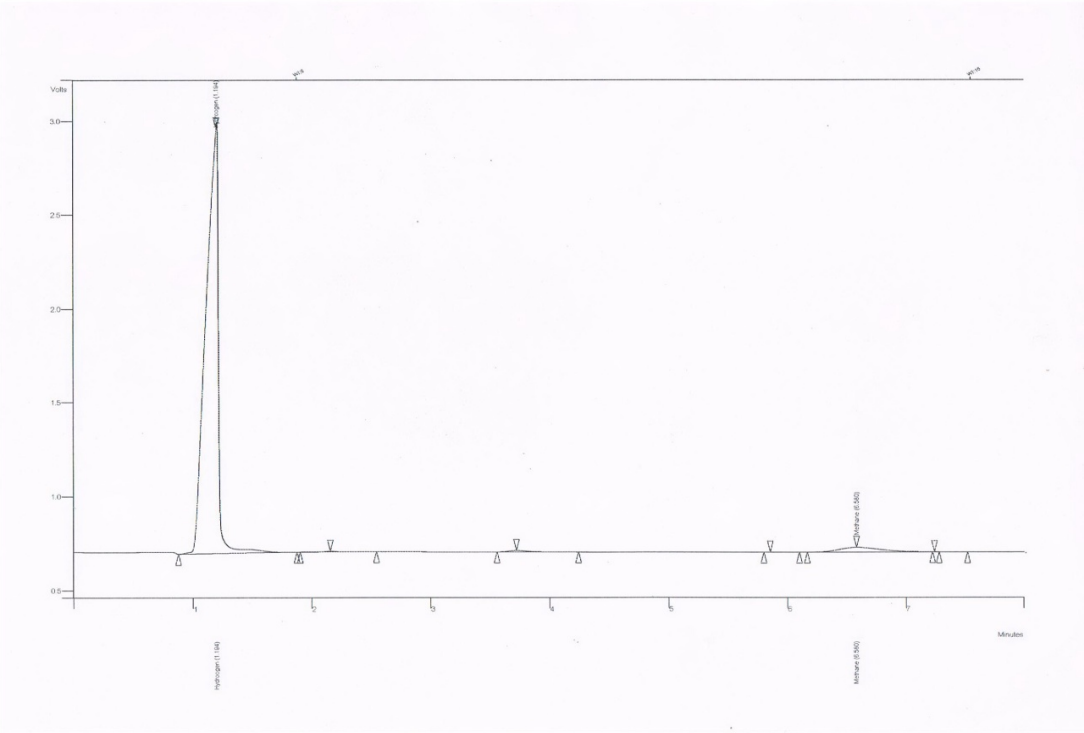

Figure B1 Graph of standard gas (Hydrogen and Methane)

Table B2 Gas chromatograph's calibration on standard curve (30% Carbon dioxide)

Order	% CO_2	Area
1	30	28325
2	30	27869
3	30	28738
4	30	28279
5	30	29111
6	30	29176
7	30	27006
8	30	31940
9	30	29337
10	30	27830
Average		28761.10

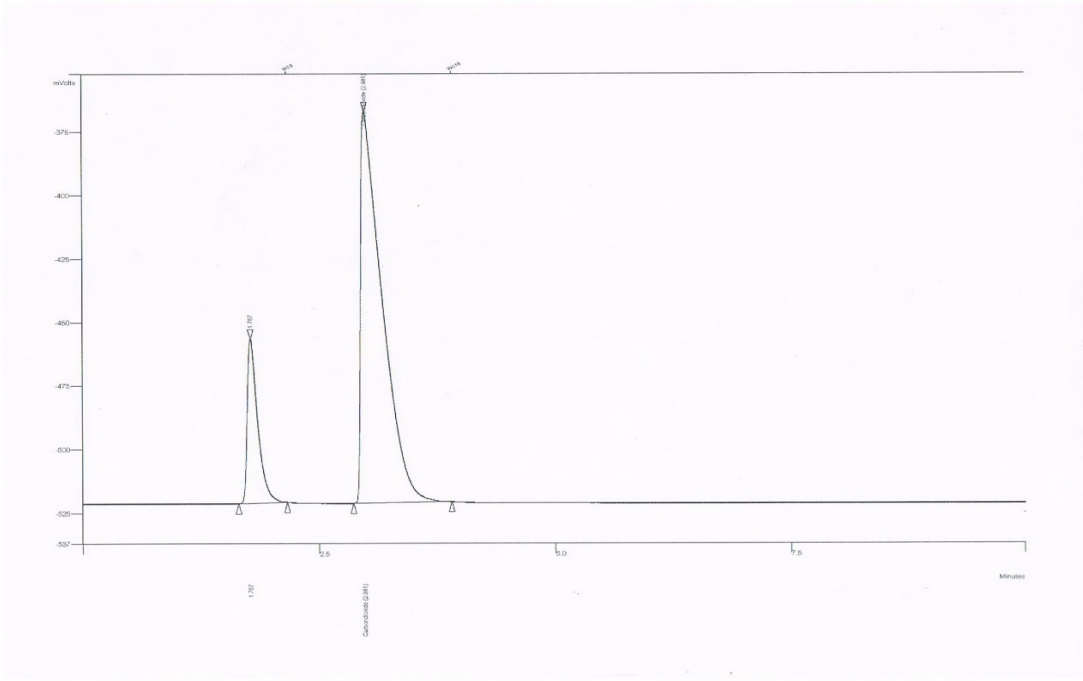

Figure B2 Graph of standard gas (Carbon dioxide)

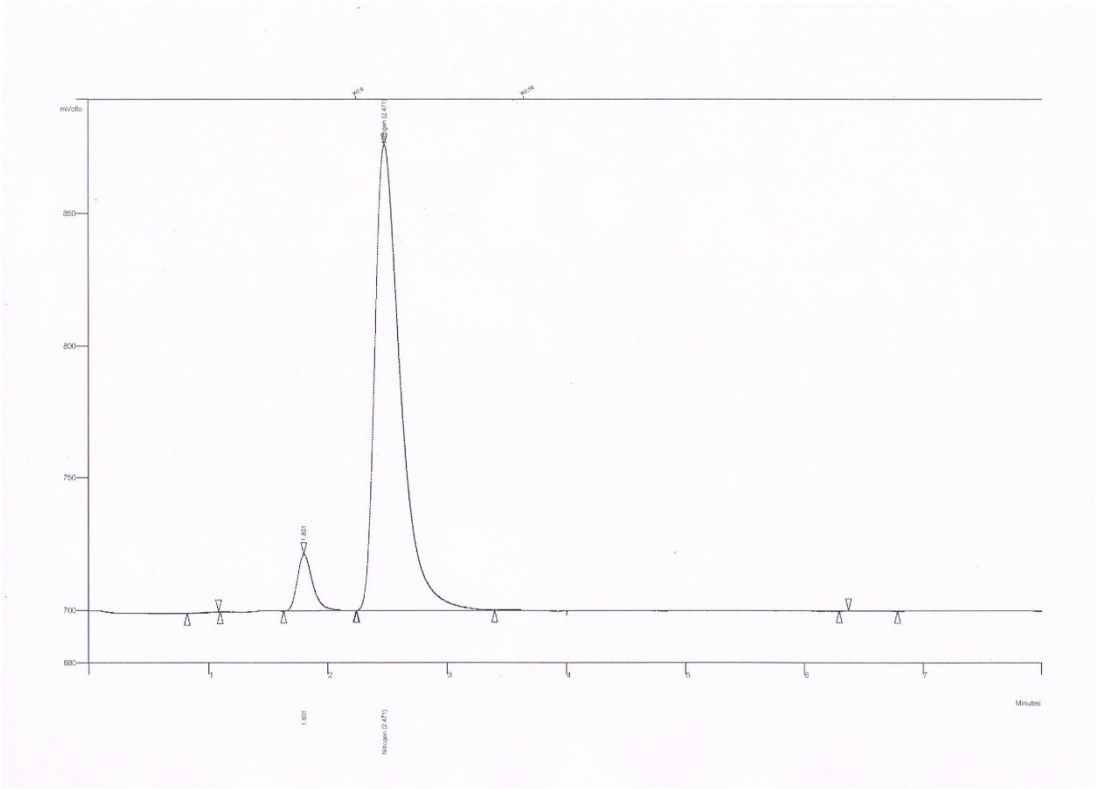

Figure B3 Graph of standard gas (Nitrogen)

Table B – 3 Gas chromatograph's calibration on standard curve (99.995% Nitrogen)

Order	% N_2	Area
1	99.995	317564
2	99.995	180162
3	99.995	196075
4	99.995	227666
5	99.995	260304
6	99.995	193392
7	99.995	221885
8	99.995	90977
9	99.995	84379
10	99.995	197031
Average		196,954.50

APPENDIX C
VOLATILE FATTY ACID (VFA)

Table C1 Gas chromatograph's calibration on standard curve for acetate

Concentration of acetate (mg/l)	Peak area
4	7004
6	7537
8	9071
10	9604
40	34604
60	45937
80	63847
100	78604

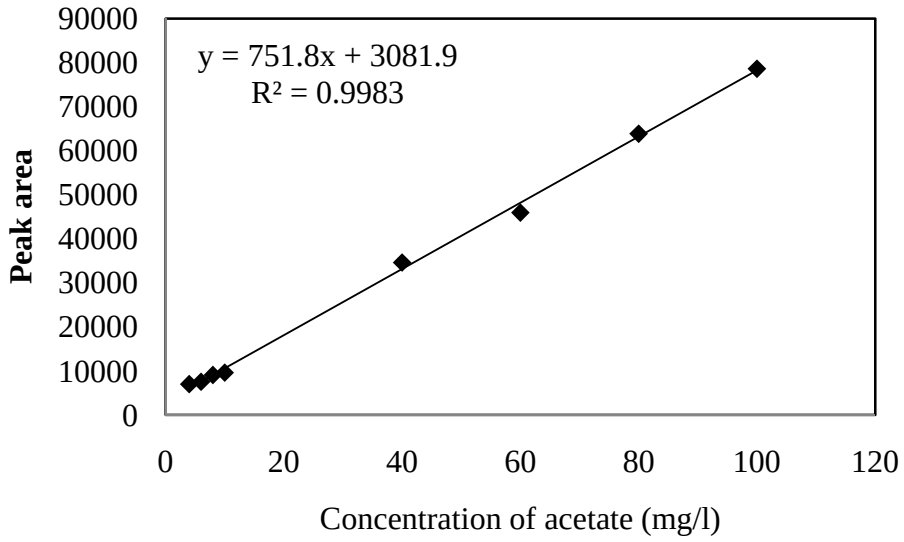

Figure C1 The relationship between peak area and different amount of standard acetic

Table C2 Gas chromatograph's calibration on standard curve for propionate

Concentration of propionate(mg/l)	Peak area
4	13853
6	16359
8	19780
10	20744
40	65198
60	94834
80	122836
100	145118

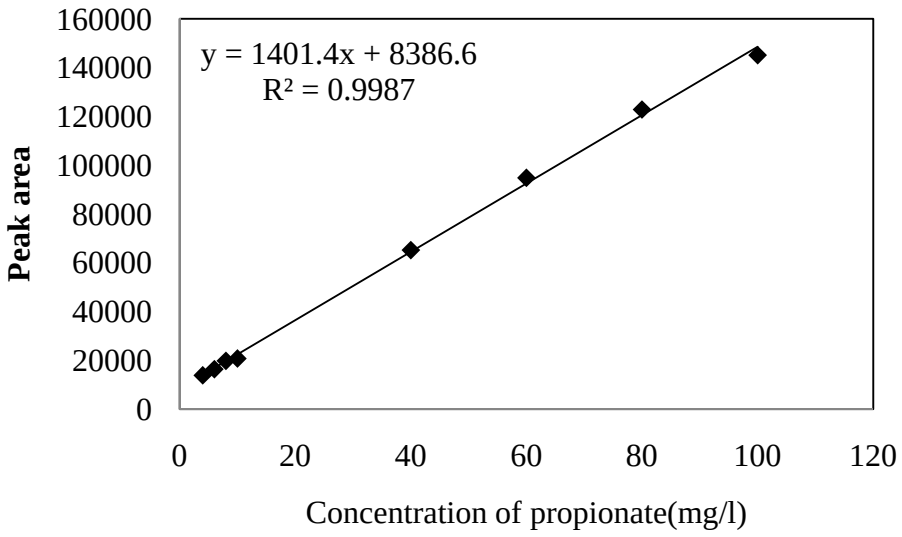

Figure C2 The relationship between peak area and different amount of standard propionate

Table C3 Gas chromatograph's calibration on standard curve for butyrate

Concentration of butyrate(mg/l)	Peak area
4	51890
6	62763
8	104789
10	122153
40	382619
60	576262
80	718848
100	893549

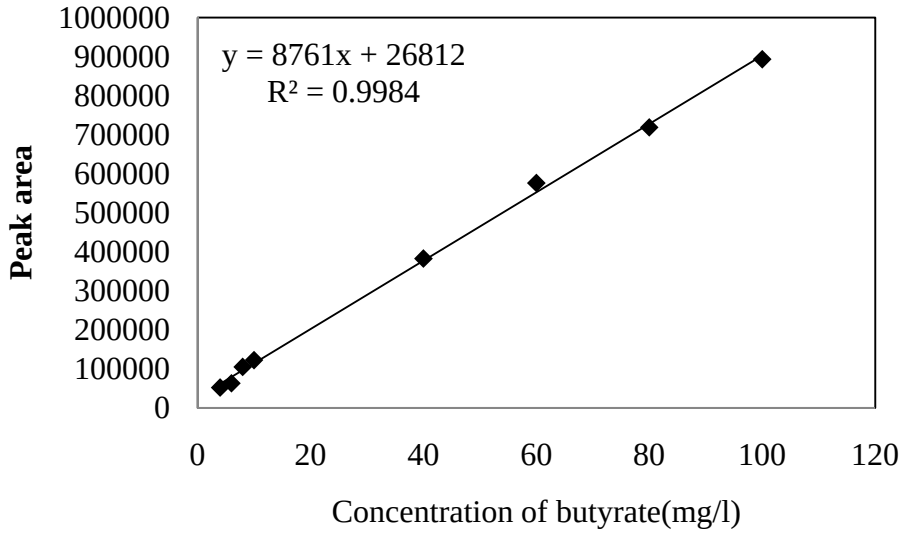

Figure C3 The relationship between peak area and different amount of standard butyrate

APPENDIX D
DATA OF BIOGAS

Appendix D 1 Raw data of hydrogen content in experiment batch reactor under mesophilic condition

Initial pH	Total biogas (ml)	H_2 (ml)	N_2 (ml)	CO_2 (ml)	% H_2	% N_2	% CO_2
pH4	116.83	0.00	49.72	67.11	0.00	42.56	57.44
PH5	167.83	0.10	53.79	113.94	0.06	32.05	67.89
pH6	561.33	166.22	107.16	287.95	29.61	19.09	51.30
pH7	357.42	122.68	76.34	158.39	34.32	21.36	44.32
pH8	1531.17	733.49	71.63	726.05	47.90	4.68	47.42
PH9	854.83	337.19	77.96	439.68	39.45	9.12	51.43
pH10	271.67	14.57	81.01	176.08	5.36	29.82	64.82
pH11	253.17	5.19	62.94	185.04	2.05	24.86	73.09
pH12	240.00	21.61	71.02	147.38	9.00	29.59	61.41

Appendix D2 Raw data of hydrogen content in experiment batch reactor under thermophilic condition

Initial pH	Total biogas (ml)	H_2 (ml)	N_2 (ml)	CO_2 (ml)	% H_2	% N_2	% CO_2
pH4	140.00	0.00	34.80	105.20	0.00	24.86	75.14
PH5	167.50	0.00	15.84	151.66	0.00	9.46	90.54
pH6	797.75	233.42	61.65	502.68	29.26	7.73	63.01
pH7	1118.00	356.58	61.34	700.08	31.89	5.49	62.62
pH8	1550.33	767.64	34.16	748.53	49.51	2.20	48.28
PH9	1339.67	423.55	36.98	879.14	31.62	2.76	65.62
pH10	1388.67	413.55	141.10	834.02	29.78	10.16	60.06
pH11	1832.17	280.62	163.12	1388.43	15.32	8.90	75.78
pH12	1079.00	320.23	112.56	646.21	29.68	10.43	59.89

Appendix D3 Raw data of hydrogen content in experiment batch reactor in different initial F/M ratios

F/M ratios	Total biogas (ml)	H_2 (ml)	N_2 (ml)	CO_2 (ml)	% H_2	% N_2	% CO_2
4	1571.67	648.43	87.54	835.69	41.26	5.57	53.17
6	1285.83	533.33	69.43	683.07	41.48	5.40	53.12
8	1164.50	477.94	60.79	625.77	41.04	5.22	53.74
10	1353.67	457.43	67.41	828.83	33.79	4.98	61.23
12	1272.00	426.30	68.43	777.27	33.51	5.38	61.11

Appendix D4 Raw data of hydrogen content in experiment batch reactor in different initial iron concentration

Initial Fe^{2+} concentration (mg $FeSO_4$/l)	**Total biogas (ml)**	**H_2 (ml)**	**N_2 (ml)**	**CO_2 (ml)**	**% H_2**	**% N_2**	**% CO_2**
0	1391.83	445.68	89.36	856.79	32.02	6.42	61.56
100	1532.67	588.25	89.66	854.76	38.38	5.85	55.77
200	1431.00	511.47	71.26	848.27	35.74	4.98	59.28
300	1313.00	405.90	75.23	831.87	30.91	5.73	63.36
400	861.00	367.88	44.86	448.26	42.73	5.21	52.06

Appendix D 5 Raw data of hydrogen content in experiment batch reactor in pretreatment method

Pretreatment methods	**Total biogas (ml)**	**H2 (ml)**	**N2 (ml)**	**CO2 (ml)**	**% H2**	**% N2**	**% CO2**
Heat	1435.50	588.65	588.65	103.93	41.01	7.24	51.75
Re Heat	1468.50	656.57	656.57	100.89	44.71	6.87	48.42
Chloroform	121.67	0.47	0.47	57.28	0.39	47.08	52.53
Ultrasonic	1537.50	443.36	443.36	153.44	28.84	9.98	61.18

APPENDIX E
ANOVA STATISTIC OF HYDROGEN ANALYSIS

Appendix E 1 Comparison of initial pH under mesophilic condition

	Sum of squares	df	Mean square	F	Sig.
Between groups	954955.034	8	119369.379	45727.510	.000
Within groups	23.494	9	2.610		
Total	954978.528	17			

(I) Initial pH	(J) Initial pH				95% Confidence interval	
		Mean difference (I-J)	Std. error	Sig.	Lower bound	Upper bound
4	5	.00000	1.61569	1.000	-3.6549	3.6549
	6	-166.22000*	1.61569	.000	-169.8749	-162.5651
	7	-122.68000*	1.61569	.000	-126.3349	-119.0251
	8	-733.89000*	1.61569	.000	-737.5449	-730.2351
	9	-337.18500*	1.61569	.000	-340.8399	-333.5301
	10	-14.57000*	1.61569	.000	-18.2249	-10.9151
	11	-5.19000*	1.61569	.011	-8.8449	-1.5351
	12	-21.61000*	1.61569	.000	-25.2649	-17.9551

(I) Initial pH	(J) Initial pH	Mean difference (I-J)	Std. error	Sig.	95% Confidence interval	
					Lower bound	Upper bound
5	4	.00000	1.61569	1.000	-3.6549	3.6549
	6	-166.22000*	1.61569	.000	-169.8749	-162.5651
	7	-122.68000*	1.61569	.000	-126.3349	-119.0251
	8	-733.89000*	1.61569	.000	-737.5449	-730.2351
	9	-337.18500*	1.61569	.000	-340.8399	-333.5301
	10	-14.57000*	1.61569	.000	-18.2249	-10.9151
	11	-5.19000*	1.61569	.011	-8.8449	-1.5351
	12	-21.61000*	1.61569	.000	-25.2649	-17.9551
6	4	166.22000*	1.61569	.000	162.5651	169.8749
	5	166.22000*	1.61569	.000	162.5651	169.8749
	7	43.54000*	1.61569	.000	39.8851	47.1949
	8	-567.67000*	1.61569	.000	-571.3249	-564.0151
	9	-170.96500*	1.61569	.000	-174.6199	-167.3101
	10	151.65000*	1.61569	.000	147.9951	155.3049
	11	161.03000*	1.61569	.000	157.3751	164.6849
	12	144.61000*	1.61569	.000	140.9551	148.2649
7	4	122.68000*	1.61569	.000	119.0251	126.3349
	5	122.68000*	1.61569	.000	119.0251	126.3349

(I) Initial pH	(J) Initial pH	Mean difference (I-J)	Std. error	Sig.	95% Confidence interval	
					Lower bound	Upper bound
7	6	-43.54000*	1.61569	.000	-47.1949	-39.8851
	8	-611.21000*	1.61569	.000	-614.8649	-607.5551
	9	-214.50500*	1.61569	.000	-218.1599	-210.8501
	10	108.11000*	1.61569	.000	104.4551	111.7649
	11	117.49000*	1.61569	.000	113.8351	121.1449
	12	101.07000*	1.61569	.000	97.4151	104.7249
8	4	733.89000*	1.61569	.000	730.2351	737.5449
	5	733.89000*	1.61569	.000	730.2351	737.5449
	6	567.67000*	1.61569	.000	564.0151	571.3249
	7	611.21000*	1.61569	.000	607.5551	614.8649
	9	396.70500*	1.61569	.000	393.0501	400.3599
	10	719.32000*	1.61569	.000	715.6651	722.9749
	11	728.70000*	1.61569	.000	725.0451	732.3549
	12	712.28000*	1.61569	.000	708.6251	715.9349
9	4	337.18500*	1.61569	.000	333.5301	340.8399
	5	337.18500*	1.61569	.000	333.5301	340.8399
	6	170.96500*	1.61569	.000	167.3101	174.6199
	7	214.50500*	1.61569	.000	210.8501	218.1599

(I) Initial pH	(J) Initial pH				95% Confidence interval	
		Mean difference (I-J)	Std. error	Sig.	Lower bound	Upper bound
9	8	-396.70500*	1.61569	.000	-400.3599	-393.0501
	10	322.61500*	1.61569	.000	318.9601	326.2699
	11	331.99500*	1.61569	.000	328.3401	335.6499
	12	315.57500*	1.61569	.000	311.9201	319.2299
10	4	14.57000*	1.61569	.000	10.9151	18.2249
	5	14.57000*	1.61569	.000	10.9151	18.2249
	6	-151.65000*	1.61569	.000	-155.3049	-147.9951
	7	-108.11000*	1.61569	.000	-111.7649	-104.4551
	8	-719.32000*	1.61569	.000	-722.9749	-715.6651
	9	-322.61500*	1.61569	.000	-326.2699	-318.9601
	11	9.38000*	1.61569	.000	5.7251	13.0349
	12	-7.04000*	1.61569	.002	-10.6949	-3.3851
11	4	5.19000*	1.61569	.011	1.5351	8.8449
	5	5.19000*	1.61569	.011	1.5351	8.8449
	6	-161.03000*	1.61569	.000	-164.6849	-157.3751
	7	-117.49000*	1.61569	.000	-121.1449	-113.8351
	8	-728.70000*	1.61569	.000	-732.3549	-725.0451
	9	-331.99500*	1.61569	.000	-335.6499	-328.3401
	10	-9.38000*	1.61569	.000	-13.0349	-5.7251

(I) Initial pH	(J) Initial pH	Mean difference (I-J)	Std. error	Sig.	95% Confidence Interval	
					Lower bound	Upper bound
11	12	-16.42000*	1.61569	.000	-20.0749	-12.7651
12	4	21.61000*	1.61569	.000	17.9551	25.2649
	5	21.61000*	1.61569	.000	17.9551	25.2649
	6	-144.61000*	1.61569	.000	-148.2649	-140.9551
	7	-101.07000*	1.61569	.000	-104.7249	-97.4151
	8	-712.28000*	1.61569	.000	-715.9349	-708.6251
	9	-315.57500*	1.61569	.000	-319.2299	-311.9201
	10	7.04000*	1.61569	.002	3.3851	10.6949
	11	16.42000*	1.61569	.000	12.7651	20.0749

*. The mean difference is significant at the 0.05 level.

Appendix E 2 Comparison of initial pH under thermophiclic condition

	Sum of squares	df	Mean square	F	Sig.
Between groups	868500.104	8	108562.513	14887.303	.000
Within groups	65.631	9	7.292		
Total	868565.735	17			

(I) Initial pH	(J) Initial pH				95% Confidence interval	
		Mean difference (I-J)	Std. error	Sig.	Lower bound	Upper bound
4	5	.00000	2.70042	1.000	-6.1088	6.1088
	6	-233.42000*	2.70042	.000	-239.5288	-227.3112
	7	-356.59000*	2.70042	.000	-362.6988	-350.4812
	8	-767.64000*	2.70042	.000	-773.7488	-761.5312
	9	-423.55000*	2.70042	.000	-429.6588	-417.4412
	10	-413.55000*	2.70042	.000	-419.6588	-407.4412
	11	-280.62000*	2.70042	.000	-286.7288	-274.5112
	12	-320.23000*	2.70042	.000	-326.3388	-314.1212
5	4	.00000	2.70042	1.000	-6.1088	6.1088
	6	-233.42000*	2.70042	.000	-239.5288	-227.3112
	7	-356.59000*	2.70042	.000	-362.6988	-350.4812
	8	-767.64000*	2.70042	.000	-773.7488	-761.5312
	9	-423.55000*	2.70042	.000	-429.6588	-417.4412
	10	-413.55000*	2.70042	.000	-419.6588	-407.4412
	11	-280.62000*	2.70042	.000	-286.7288	-274.5112
	12	-320.23000*	2.70042	.000	-326.3388	-314.1212
6	4	233.42000*	2.70042	.000	227.3112	239.5288
	5	233.42000*	2.70042	.000	227.3112	239.5288
	7	-123.17000*	2.70042	.000	-129.2788	-117.0612

(I) Initial pH	(J) Initial pH	Mean difference (I-J)	Std. error	Sig.	95% Confidence Interval	
					Lower bound	Upper bound
6	8	-534.22000*	2.70042	.000	-540.3288	-528.1112
	9	-190.13000*	2.70042	.000	-196.2388	-184.0212
	10	-180.13000*	2.70042	.000	-186.2388	-174.0212
	11	-47.20000*	2.70042	.000	-53.3088	-41.0912
	12	-86.81000*	2.70042	.000	-92.9188	-80.7012
7	4	356.59000*	2.70042	.000	350.4812	362.6988
	5	356.59000*	2.70042	.000	350.4812	362.6988
	6	123.17000*	2.70042	.000	117.0612	129.2788
	8	-411.05000*	2.70042	.000	-417.1588	-404.9412
	9	-66.96000*	2.70042	.000	-73.0688	-60.8512
	10	-56.96000*	2.70042	.000	-63.0688	-50.8512
	11	75.97000*	2.70042	.000	69.8612	82.0788
	12	36.36000*	2.70042	.000	30.2512	42.4688
8	4	767.64000*	2.70042	.000	761.5312	773.7488
	5	767.64000*	2.70042	.000	761.5312	773.7488
	6	534.22000*	2.70042	.000	528.1112	540.3288
	7	411.05000*	2.70042	.000	404.9412	417.1588
	9	344.09000*	2.70042	.000	337.9812	350.1988
	10	354.09000*	2.70042	.000	347.9812	360.1988

(I) Initial pH	(J) Initial pH	Mean difference (I-J)	Std. error	Sig.	95% Confidence interval	
					Lower bound	Upper bound
8	11	487.02000*	2.70042	.000	480.9112	493.1288
	12	447.41000*	2.70042	.000	441.3012	453.5188
9	4	423.55000*	2.70042	.000	417.4412	429.6588
	5	423.55000*	2.70042	.000	417.4412	429.6588
	6	190.13000*	2.70042	.000	184.0212	196.2388
	7	66.96000*	2.70042	.000	60.8512	73.0688
	8	-344.09000*	2.70042	.000	-350.1988	-337.9812
	10	10.00000*	2.70042	.005	3.8912	16.1088
	11	142.93000*	2.70042	.000	136.8212	149.0388
	12	103.32000*	2.70042	.000	97.2112	109.4288
10	4	413.55000*	2.70042	.000	407.4412	419.6588
	5	413.55000*	2.70042	.000	407.4412	419.6588
	6	180.13000*	2.70042	.000	174.0212	186.2388
	7	56.96000*	2.70042	.000	50.8512	63.0688
	8	-354.09000*	2.70042	.000	-360.1988	-347.9812
	9	-10.00000*	2.70042	.005	-16.1088	-3.8912
	11	132.93000*	2.70042	.000	126.8212	139.0388
	12	93.32000*	2.70042	.000	87.2112	99.4288
11	4	280.62000*	2.70042	.000	274.5112	286.7288

(I) Initial pH	(J) Initial pH	Mean difference (I-J)	Std. error	Sig.	95% Confidence interval	
					Lower bound	Upper bound
11	5	280.62000*	2.70042	.000	274.5112	286.7288
	6	47.20000*	2.70042	.000	41.0912	53.3088
	7	-75.97000*	2.70042	.000	-82.0788	-69.8612
	8	-487.02000*	2.70042	.000	-493.1288	-480.9112
	9	-142.93000*	2.70042	.000	-149.0388	-136.8212
	10	-132.93000*	2.70042	.000	-139.0388	-126.8212
	12	-39.61000*	2.70042	.000	-45.7188	-33.5012
12	4	320.23000*	2.70042	.000	314.1212	326.3388
	5	320.23000*	2.70042	.000	314.1212	326.3388
	6	86.81000*	2.70042	.000	80.7012	92.9188
	7	-36.36000*	2.70042	.000	-42.4688	-30.2512
	8	-447.41000*	2.70042	.000	-453.5188	-441.3012
	9	-103.32000*	2.70042	.000	-109.4288	-97.2112
	10	-93.32000*	2.70042	.000	-99.4288	-87.2112
	11	39.61000*	2.70042	.000	33.5012	45.7188

*. The mean difference is significant at the 0.05 level.

Appendix E 3 Comparison of initial pH 8 under between mesophilic with thermophilic condition

Independent Samples Test

		Levene's test for equality of variances		t-test for equality of means	
		F	Sig.	t	df
Hydrogen	Equal variances assumed	6.685E14	.000	-27.599	2
	Equal variances not assumed			-27.599	1.918

Appendix E 4 Comparison of initial F/M ratios

	Sum of squares	df	Mean square	F	Sig.
Between groups	61029.592	4	15257.398	452.040	.000
Within groups	168.761	5	33.752		
Total	61198.353	9			

(I) F/M ratio	(J) F/M ratio				95% Confidence interval	
		Mean difference (I-J)	Std. error	Sig.	Lower bound	Upper bound
4	6	115.09500*	5.80967	.000	100.1608	130.0292
	8	170.48500*	5.80967	.000	155.5508	185.4192
	10	191.19500*	5.80967	.000	176.2608	206.1292
	12	222.12500*	5.80967	.000	207.1908	237.0592
6	4	-115.09500*	5.80967	.000	-130.0292	-100.1608
	8	55.39000*	5.80967	.000	40.4558	70.3242
	10	76.10000*	5.80967	.000	61.1658	91.0342
	12	107.03000*	5.80967	.000	92.0958	121.9642

(I) F/M ratio	(J) F/M ratio	Mean difference (I-J)	Std. error	Sig.	95% Confidence interval	
					Lower bound	Upper bound
8	4	-170.48500*	5.80967	.000	-185.4192	-155.5508
	6	-55.39000*	5.80967	.000	-70.3242	-40.4558
	10	20.71000*	5.80967	.016	5.7758	35.6442
	12	51.64000*	5.80967	.000	36.7058	66.5742
10	4	-191.19500*	5.80967	.000	-206.1292	-176.2608
	6	-76.10000*	5.80967	.000	-91.0342	-61.1658
	8	-20.71000*	5.80967	.016	-35.6442	-5.7758
	12	30.93000*	5.80967	.003	15.9958	45.8642
12	4	-222.12500*	5.80967	.000	-237.0592	-207.1908
	6	-107.03000*	5.80967	.000	-121.9642	-92.0958
	8	-51.64000*	5.80967	.000	-66.5742	-36.7058
	10	-30.93000*	5.80967	.003	-45.8642	-15.9958

*. The mean difference is significant at the 0.05 level.

Appendix E 5 Comparison of initial iron concentration

	Sum of squares	df	Mean square	F	Sig.
Between groups	52404.908	4	13101.227	3262.445	.000
Within groups	20.079	5	4.016		
Total	52424.987	9			

(I) Iron concentration	(J) Iron concentration	Mean difference (I-J)	Std. error	Sig.	95% Confidence Interval	
					Lower bound	Upper bound
0	100	-131.84000*	2.00394	.000	0	100
	200	-60.84000*	2.00394	.000		200
	300	36.78000*	2.00394	.000		300
	400	71.94500*	2.00394	.000		400
100	0	131.84000*	2.00394	.000	100	0
	200	71.00000*	2.00394	.000		200
	300	168.62000*	2.00394	.000		300
	400	203.78500*	2.00394	.000		400
200	0	60.84000*	2.00394	.000	200	0
	100	-71.00000*	2.00394	.000		100
	300	97.62000*	2.00394	.000		300
	400	132.78500*	2.00394	.000		400
300	0	-36.78000*	2.00394	.000	300	0
	100	-168.62000*	2.00394	.000		100
	200	-97.62000*	2.00394	.000		200
	400	35.16500*	2.00394	.000		400
400	0	-71.94500*	2.00394	.000	400	0
	100	-203.78500*	2.00394	.000		100
	200	-132.78500*	2.00394	.000		200
	300	-35.16500*	2.00394	.000		300

*. The mean difference is significant at the 0.05 level.

Appendix E 6 Comparison of pre-treatment method

	Sum of Squares	df	Mean square	F	Sig.
Between groups	521872.958	3	173957.653	67527.196	.000
Within groups	10.304	4	2.576		
Total	521883.263	7			

(I) pretreatment method	(J) pretreatment method				95% Confidence Interval	
		Mean Difference (I-J)	Std. error	Sig.	Lower bound	Upper bound
Heat	Re-heat	-67.91500*	1.60503	.000	-72.3713	-63.4587
	Chloroform	588.18000*	1.60503	.000	583.7237	592.6363
	Ultrasonic	145.29000*	1.60503	.000	140.8337	149.7463
Re-heat	Heat	67.91500*	1.60503	.000	63.4587	72.3713
	Chloroform	656.09500*	1.60503	.000	651.6387	660.5513
	Ultrasonic	213.20500*	1.60503	.000	208.7487	217.6613
Chloroform	Heat	-588.18000*	1.60503	.000	-592.6363	-583.7237
	Re-heat	-656.09500*	1.60503	.000	-660.5513	-651.6387
	Ultrasonic	-442.89000*	1.60503	.000	-447.3463	-438.4337
Ultrasonic	Heat	-145.29000*	1.60503	.000	-149.7463	-140.8337
	Re-heat	-213.20500*	1.60503	.000	-217.6613	-208.7487
	Chloroform	442.89000*	1.60503	.000	438.4337	447.3463

*. The mean difference is significant at the 0.05 level.

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