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

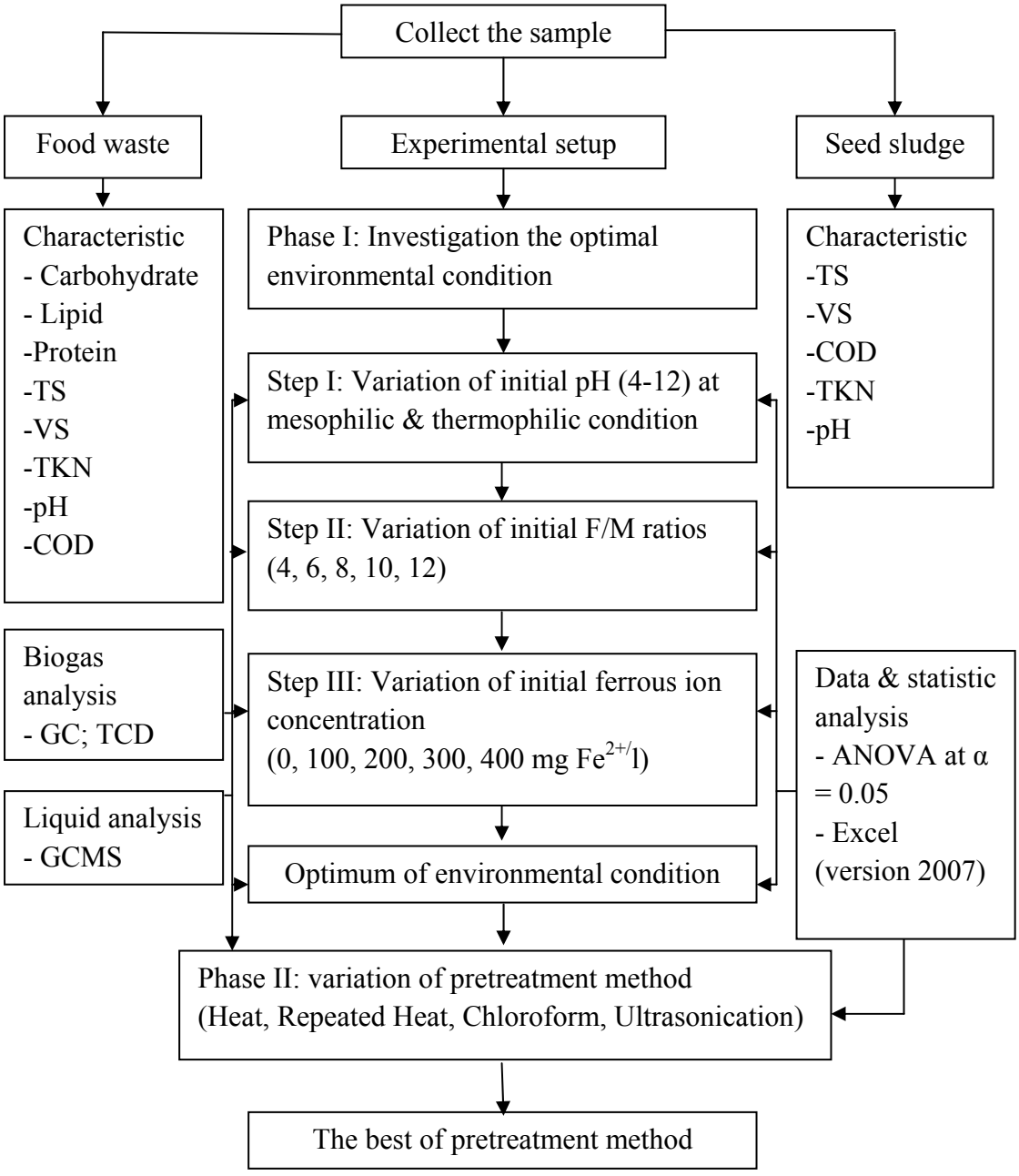

Figure 3.1 Diagram of experimental methodology

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.

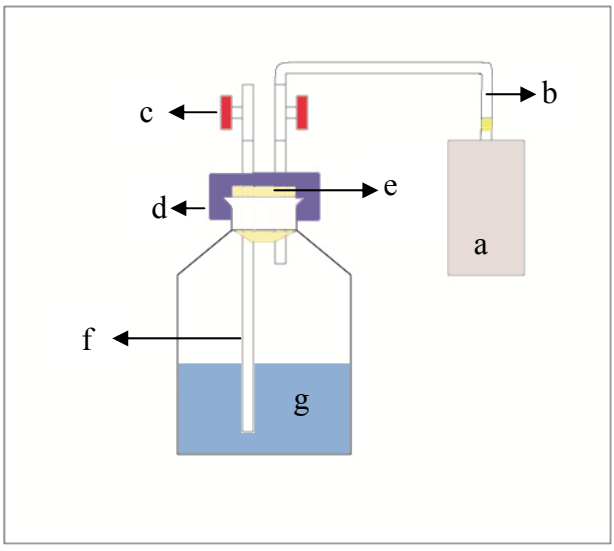

Figure 3.2 Diagram of batch reactor: a) Sampling bag, b) Rubber tube, c) Three-way valve, d) Screw cap, e) Rubber stopper, f) Needle for sampling collection, g) Mixed liquid of food waste, sludge and nutrient solution.

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.