CHAPTER I

Introduction

1.1 Rationale and background

Currently, the worldwide awareness of global climate change, it was being caused by the extensive use of fossil fuel wherewith carbon dioxide (CO_2) emissions from fossil fuels containing carbon combustion. To reduce the emission of CO_2 from fossil fuels to the atmosphere, researchers are looking at alternative fuels that combat the mentioned problem. Among the alternative fuels, biogas including biohydrogen and biomethane is a promising one to in place of fossil fuels due to biohydrogen is clean energy with no CO_x, SO_x and NO_x emissions, high energy content which greater than other hydrocarbon fuel about 2.75 times, rapid burning speed and high-octane number (Hawkes *et al*., 2002). Additionally, gas mixture consisting of biohydrogen (10-60%) and biomethane were referred to the enhance performance (Kongjan, 2010). There are several biological ways to produce biogas from organic wastes and residues, among that anaerobic dark fermentation is more popular because environmentally friendly, renewable, sustainable, cheap energy and capable to utilize the organic wastes and residues to eliminate the pollution.

Biogas production by microbial fermentation pass through anaerobic digestion using organic wastes and residues is one of the oldest technologies for bioenergy formation as well as wastewater treatment. The process is a multi-step involving large groups of microbial populations, which are linked process steps, in which complex organic compound such as carbohydrates, proteins and lipids are degraded into single molecules, including mono sugar, amino acid and long chain fatty acid by hydrolytic bacteria. Acidogenesis, fermentative products of hydrolysis are further broken down into acetic acid (51%), hydrogen (19%) and the remaining are alcohols, lactic acid and higher VFA by fermentative and acidogenic bacteria. Acetogenesis, fermentation products with longer than two carbon atoms for volatile fatty acids (VFAs), longer than one carbon atom for alcohols are oxidized to acetate and hydrogen by obligate proton reducing bacteria in syntrophic relationship with methanogenic archaea (Boe, 2006). Methanogenesis, the intermediate products of the preceding stage are utilized by methanogenic archaea to further convert these products to methane and carbon dioxide. Mostly of methane formation of 70% is originated pass through acetate pathway by aceticlastic methanogens and the rest of 30% is generated from conversion of hydrogen and carbon dioxide by hydrogenotrophic methanogens.

There are various kinds of substrate can be used to produce biohydrogen and biomethane pass through anaerobic dark fermentation process, for example, monomeric sugars (Ren *et al*., 2009; Mangayil *et al*., 2011), sucrose (Hussy *et al*., 2005; Lin *et al*., 2006; Perera and Nirmalakhandan, 2010; Perera and Nirmalakhandan, 2011), Lactose (Calli *et al*., 2008; Davila-Vazquez *et al*., 2008; Rosales-Colunga *et al*., 2012), cheese whey (Kargi *et al*., 2012; Rosales-Colunga *et al*., 2012; Perna *et al.*, 2013; Rosa *et al*., 2014), starch (Argun and Kargi, 2010; Cakır *et al*., 2010; Xia *et al*., 2014). However, the usage of pure sugars is only for trying to understand the microbial physiology of hydrogen production since it's too expensive for use as substrate in an industrial scale. Thus, researchers are interested to produce biogas from wastewater generated from several industries due to their low costs and direct treatment of wastewater with bioenergy is profitable. Resulting, extensive research has been used wastewater generated from several industries including wastewater generated from food and beverage industries, waste sludge from wastewater treatment plants, mostly include slaughter house and meat-processing, daily industries, fish processing, starch processing, edible oil, olive mill, beverage and distilleries, fruit and vegetable processing. Moreover, wastewater generated from concentrated latex (Perrella and Gaspari, 2002; Abraham *et al*., 2009; Santipanusopon and Riyajan, 2009; Kongkaew *et al*., 2012) and wastewater generated from palm oil mill factories (O-Thong *et al*., 2008; Ismail *et al*., 2010; Alrawi *et al*., 2011; Fang *et al*., 2011; Khemkhao *et al*., 2012) have been successfully used to produce biohydrogen and biomethane by anaerobic dark fermentation.

Skim latex serum (SLS) is a by-product generated from concentrated latex production process by centrifugation method. This process about 10% of rubber materials lost as a by-product, however, it able to recover rubber by using 98% sulfuric acid. Nevertheless, rubber materials are recovered using 98% sulfuric acid, but skim latex serum is still remaining mainly nutrients, including carbohydrates, proteins, lipids, sugars and carotenoids. However, the microorganisms can be used these nutrients to growing (Santipanusopon and Riyajan, 2009; Abraham *et al*., 2009). Normally, the concentrated latex production process, ammonia (NH_3), zinc oxide and tetramethylthiuram disulfide (ZnO/TMTD) and sulfuric acid (H_2SO_4) was added to preservation and coagulation of latex (Perrella and Gaspari, 2002; Rippel *et al*., 2003; Santipanusopon and Riyajan, 2009). However, some of these chemical reagents are remaining in the skim latex serum which important to the microorganisms growing and also affecting to the efficiency of biogas production. Raw skim latex serum is acidic yellowish fluid consisting of chemical oxygen demand (COD) ranged 29.2 – 35.8 g/L, total Kjeldahl nitrogen (TKN) ranged 4.9 – 5.1 g/L, carbohydrate content ranged 379 – 603 mg/L and protein content ranged 6.4 – 7.6 g/L (Kongjan *et al*., 2014; Sama *et al*., 2014). Skim latex serum is a concentrated substrate with high concentrations of COD and TKN, resulting in low C/N ratio around 7 which are still limitations substrate used for biogas fermentation. Corresponding with a study from Kongjan *et al*. (2014) found that a relatively low of hydrogen production yield achieved from sole fermentation of skim latex serum was 59.2±2.4 mL H_2/g-VS, which is just only 11% of hydrogen theoretical yield (498 mL H_2/g-VS). They were reported the possible reason for having low hydrogen formation because the competition of hydrogen producing bacteria and sulfate reducing bacteria to produce either hydrogen or hydrogen sulfide. Hydrogen sulfide (H_2S) has inhibitory effect on methanogenic archaea at even low concentration of 20 – 30 mM (Boe, 2006). Thus, to enhance the biogas production potential, there are several methods such as using various biological and chemical additives under different operating condition, pretreatment of both substrate and seed sludge, co-digestion of organic substrates and two-stage process (Liu *et al*., 2006; O-Thong *et al*., 2008; Luo *et al*., 2010; Alrawi *et al*., 2011; O-Thong *et al*., 2012). Among various options, in this research was focused on two methods to enhance the biohydrogen production, including co-digestion of organic substrates and two-stage anaerobic process.

Anaerobic co-digestion of organic wastes has been extensively researched due to this method has several advantages compared to single substrate digestion such as increased process stability, improves the biogas production yield, synergistic effect and easier handling of mixed waste steams (Mata-Alvarez *et al*., 2000; Nayono *et al*., 2010; O-Thong *et al*., 2012). This research was carried out by using anaerobic co-digestion of skim latex serum (SLS); wastewater generated from concentrated latex plant and palm oil mill effluent (POME); wastewater generated from palm oil mill plant as substrate to produce biogas. POME was used as co-substrate due to it contains large quantities of nutrients, including carbohydrates ranged 8.3 – 24.7 g/L, total phosphorus ranged 0.47 – 1.25 mg/L and oil content ranged 2.3 – 10.6 g/L (Badiei *et al*., 2011; Fang *et al*., 2011; Khemkhao *et al*., 2012). POME has been widely used as substrate to produce biogas. Mamimin *et al*. (2012) used *thermoanaerobacterium*-rich sludge as inoculum to produce biohydrogen in a continuously stirred tank reactor (CSTR) with the highest hydrogen production yield of 4.2 L H_2/L POME was obtained under hydraulic retention time (HRT) of 2 days. Badiei *et al*. (2011) used anaerobic sequencing batch reactor (ASBR), the maximum hydrogen production yield was 0.34 L H_2/g-$COD_{feeding}$ achieved under the HRT of 72 h. At the same time, methane potential achieved from batch sole fermentation of POME and deoiled POME was 503 and 610 mL CH_4/g-COD_{added}, respectively. Meanwhile, biomethane production potential in continuously fed reactor was 436 and 438 mL CH_4/g-VS_{added} achieved from POME, while 600 and 555 mL CH_4/g-VS_{added} achieved from deoiled POME which was operated in upflow anaerobic sludge blanket (UASB) and expanded granular sludge bed (EGSB) reactors (Fang *et al*., 2011). Although satisfactory of biohydrogen and biomethane production from individual fermentation of POME was observed. There are, however, several researches was carried out by using co-digestion of POME such as co-digestion with rumen fluid take from the first compartment of cow's stomach (Alrawi *et al*., 2008), oil palm empty fruit bunches (O-Thong *et al*., 2012) and skim latex serum (Sama *et al*., 2014). Our previous study used anaerobic co-digestion of SLS and POME, the result shows that the optimal mixing ratio of SLS and POME was 75:25 (%v/v) with the highest hydrogen production yield was 35.0±1.2 mL H_2/g-COD which was 1 and 5 times greater than that achieved from sole fermentation of SLS and POME, respectively. One of the benefits of using POME as co-substrate is that to adjust C/N ratio in the mixture as it contains high C/N ratio ranged 37 – 68. Moreover, POME was also used to dilute several inhibitants in the SLS to enhance the efficiency of biogas production.

The usage of two-stage anaerobic digestion process is one of an approach used to reduce sulfate containing in the SLS in formed H_2S, which is easily released from the fermentation broth in the acidogenic stage (Kongjan *et al*., 2014). Furthermore, the reason for using two-stage process was to separate hydrolysis/acidogenesis and acetogenesis/methanogenesis microorganisms groups in separated reactor, due to there are several differences in term of nutritional need, growth kinetics and environmental conditions, leading to a large overall reaction rate and biogas production yield (Liu *et al*., 2006; Luo *et al*., 2010; Kongjan, 2010; Zhu *et al*., 2011; Nkemka, 2012). Under the optimal environmental conditions of acidogenic and methanogenic stages in two-stage process, there are several advantages over the single stage process such as more process stability, higher digestion efficiency and higher methane content in biogas. The first stage of two-stage anaerobic digestion process is acidogenic stage; the biopolymers are broken down to soluble oligomers and monomers and are then further degraded to organic acids, alcohols, hydrogen and carbon dioxide (Özmen and Aslanzadeh, 2009; Kongjan, 2010; Nkemka, 2012). The second stage is methanogenic stage; end products from the first stage are further converted to methanogenic substrates such as acetate, hydrogen and carbon dioxide and are then further converted to methane and carbon dioxide. However, various researches are reported about 70% of methane is originated from acetate via the aceticlastic pathway by methanogen groups and the remaining from conversion of hydrogen and carbon dioxide. Nevertheless, these microorganisms are more sensitive to environmental changes i.e. composition of feedstock, feeding rate, temperature, pH, presence of oxygen as well as presence and amount of inhibitors such as ammonia, sulfate and sulfur compounds, long-chain fatty acids, heavy metals and other aliphatic and aromatic compounds (Boe, 2006; Özmen and Aslanzadeh, 2009; Nkemka, 2012).

In this research was carried out by using two-stage anaerobic co-digestion of skim latex serum and palm oil mill effluent under thermophilic condition at the temperature of 55°C to produce biogas.

1.2 Thesis objectives

The main objective of this study is to utilize skim latex serum; wastewater generated from concentrated latex plant and palm oil mill effluent; wastewater generated from palm oil mill plant for the purpose of anaerobic co-fermentation to produce biogas using thermophilic mixed cultures fermentation under thermophilic temperature (55°C). In order to able to the main objective, the experiments are constructed into 4 parts as follows:

1) To determine the optimum mixing ratio of skim latex serum and palm oil mill effluent in order that hydrogen production.
2) To optimize nutrients and buffer concentrations for hydrogen production from the optimum mixing ratio of skim latex serum and palm oil mill effluent was using response surface methodology (RSM) with a central composite design (CCD).

3) To investigate the effect of hydraulic retention time (HRT) and organic loading rate (OLR) on hydrogen production from the optimum conditions in continuously stirred tank (CSTR) reactor.
4) To investigate the effect of organic loading rate (OLR) on methane production in upflow anaerobic sludge blanket (UASB) reactor.

1.3 Research scope

Scope of this study was divided into 2 parts as shown in **Fig. 1.1**

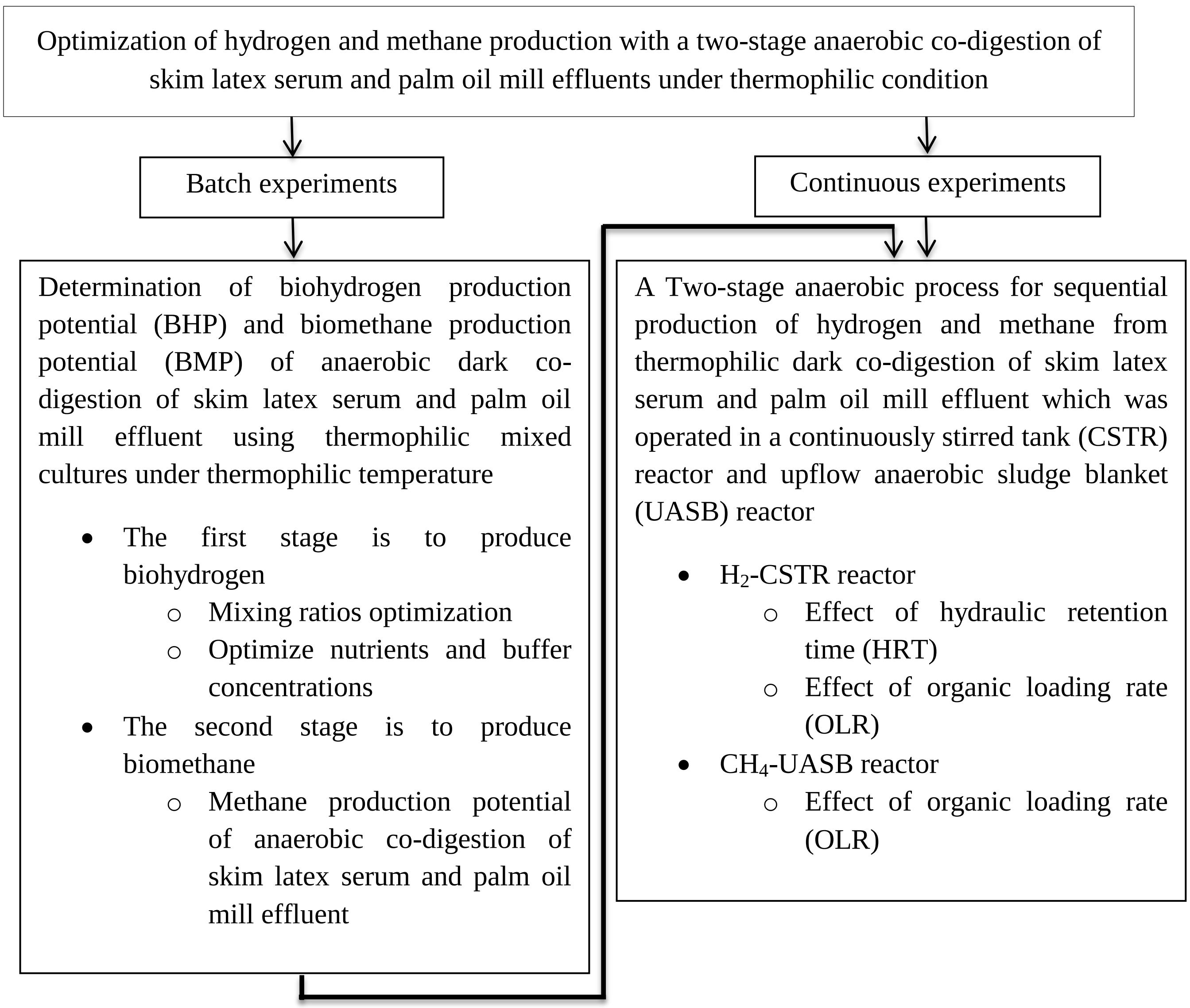

Fig. 1.1 Schematic diagram of research plan for hydrogen and methane production from anaerobic dark co-digestion of skim latex serum and palm oil mill effluent under thermophilic condition.