CHAPTER II

Literature review

2.1 Substrates used

2.1.1 Skim latex serum

Natural rubber latex (NRL) is a white and/or slightly yellowish opaque fluid with specific gravity range from 0.97-0.98 depending on rubber content, rubber tree species and terrain (Veerasamy and Ismail, 2012; Rippel *et al*., 2003). The natural latex as it comes from the rubber tree (*Hevea brasiliensis*) usually contains between 30-40% of dry rubber content and the remaining 60-70% being mainly non-rubber materials. Non-rubber material compositions of the natural latex rubber was containing two major compositions that consist of the first composition of serum, its density about 1.02 g/mL and contains the compositions of carbohydrates, proteins, sugars, carotenoids and amino acids, and the second composition of lutoids, its mostly bigger in size than the rubber particles (Sansatsadeekul *et al*., 2011; Santipanusopon and Riyajan, 2009).

2.1.1.1 Compositions of non-rubber materials in the serum

Non-rubber materials in the serum from concentrated latex production process by centrifugation method contain a wide variety of chemical species, including carbohydrates, proteins, sugars, carotenoids, fatty acids, amino acids and lutoids.

2.1.1.1.1 Carbohydrates

Mostly carbohydrates that found in natural rubber latex were 1-methylinositol or quebrachitol has been estimated about 1%. Moreover, minor carbohydrates were also found in the latex include glucose, sucrose, galactose and fructose.

2.1.1.1.2 Proteins

Natural rubber latex collected from the *Hevea* trees mostly contains about 1-1.5% of total proteins. However, these proteins can be separated in three parts that consist of 20-27% of the total proteins absorbed in rubber surface, 47-66% of the total proteins in aqueous serum, and 14-25% of the total proteins in lutoids (Archer, 1960).

2.1.1.1.3 Lipids and phospholipids

The stability and colloidal behavior of latex depending upon lipids and phospholipids associated with rubber and non-rubber particles in latex. Lipids and phospholipids associated in natural rubber latex can be separated in two parts, the first part is phospholipids of the rubber particles are α-lecithin and other lipids are a subgroup of lipids called triglycerides and waxes and the second part was presented in serum (Sansatsadeekul *et al*., 2011).

Some physical and chemical characteristics of skim latex serum are given in **Table 2.1**. The result shows that there are several limitations of using skim latex serum as substrate for generate biogas such as low C/N ratio around 7 as well as relatively high inhibitants content. These inhibitants that contains in the skim latex serum could potentially inhibit the microbial growth, resulting decrease in biodegradability. Thus, to enhance the biogas production potential as well as to prevent the risky of the process failure, the usages of anaerobic co-digestion and a two-stage process is a simply practical approach as previously mentioned. The process involved in concentration of natural rubber latex by using centrifugation method as shown in **Fig. 2.1**.

Table 2.1 Physical and chemical characteristics of skim latex serum (Kongjan *et al*., 2014; Sama *et al*., 2014).

Parameters	Unit	Concentrations
pH	-	4.8 – 5.0
Chemical oxygen demand; COD	g/L	29.2 – 35.8
Total nitrogen	g/L	5.1 – 5.2
Proteins	g/L	7.6 – 7.7
Carbohydrates	g/L	0.6 – 5.1
Total solid; TS	g/L	41.3 – 49.7
Total volatile solid; VS	g/L	38.0 – 43.5
Sulfate	g/L	0.3 – 3.6
Alkalinity	mg/L as $CaCO_3$	56 - 553

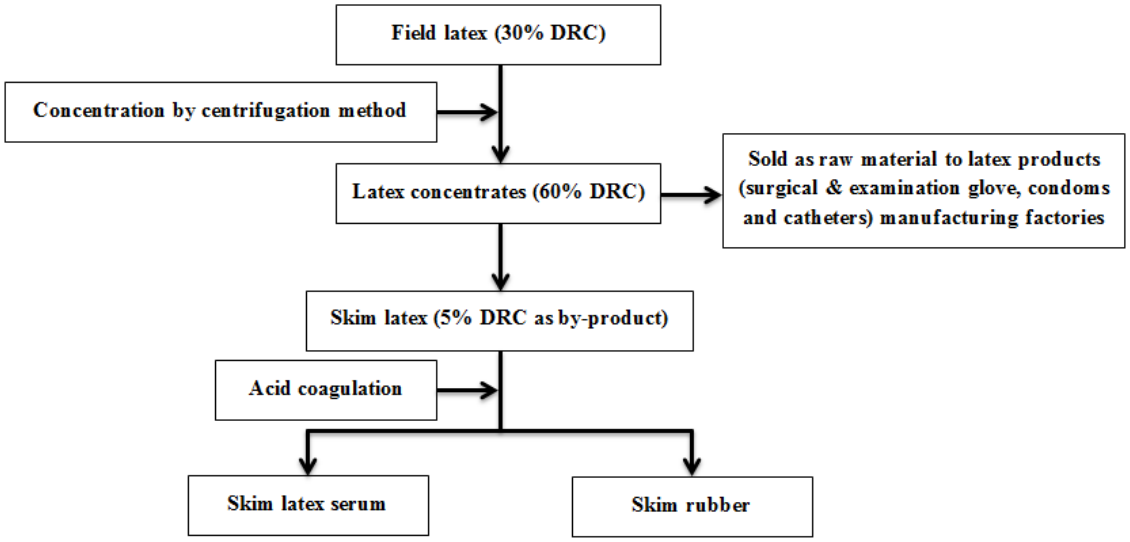

Fig. 2.1 Process involved in concentration of natural rubber latex using centrifugation method (Adapted from Taweepreda, 2013).

2.1.2 Palm oil mill effluent

There are several unit operations of milling of oil palm included sterilization, stripping or threshing, digestion, and extraction as shown in **Fig. 2.2**. Huge volumes and quantities of wastewater and solid wastes were produced in the palm oil mill industry. Generally, palm oil mill effluents (POME) were generated mainly from extraction, washing, and cleaning processes. The production of 1 tonnes crude palm oil is required approximately 5.0-7.5 tonnes of water and finally more than 50% of the water ends up as POME (Rupani *et al*., 2010). Every tonne of fresh fruit brunch, POME will be discharged from the mill approximately 0.50-0.75 tonnes, at the same time; large quantities of solids wastes is also generated such as 23% of empty fruit bunch (EFB), 12% of mesocarp fiber, and 5% of shell (Najafpour *et al*., 2005; Rupani *et al*., 2010). Raw palm oil mill effluent (POME) is a viscous acidic brownish fluid consisting of carbohydrate content ranged 8.3 – 24.7 g/L which is indicated that it could be used as a good potential substrate for generates hydrogen through dark fermentation process. Moreover, POME is also contains a relatively high lipid content which is indicated that it is an organic materials suitable and good substrate for generates methane through anaerobic dark fermentation process. Physical and chemical characteristics of POME obtained from several literature reviews are summarized in **Table 2.2**. There are several studies have been successfully of the hydrogen and methane production from POME as shown in **Table 2.3**.

Table 2.2 Physical and chemical characteristics of palm oil mill effluent (Yossan *et al*., 2012; Mamimin *et al*., 2012; O-Thong *et al*., 2008; Badiei *et al*., 2011; Fang *et al*., 2011; Zinatizadeh *et al*., 2007).

Parameters	Unit	Concentrations
pH	-	4.2 – 4.5
Chemical oxygen demand; COD	g/L	45.0 – 100.0
Total nitrogen	g/L	0.8 – 1.5
Total carbohydrate	g/L	8.3 – 24.7
Total phosphorus	mg/L	0.5 – 1.3
Oil	g/L	2.3 – 10.6
Total solid; TS	g/L	17.0 – 72.0
Total volatile solid; VS	g/L	13.2 – 48.6
Alkalinity	mg/L as $CaCO_3$	50.0 – 200.0
Total volatile fatty acid; VFA	g/L	0.8 – 3.3

Table 2.3 Hydrogen and methane production from anaerobic digestion of POME under mesophilic and thermophilic conditions.

Substrates	Source of inoculum	Reactor types	Temperature (°C)	H_2 yield (mL/g-COD)	CH_4 yield (mL/g-VS)
POME (O-Thong *et al*., 2008)	*Thermoanaerobactrium*-rich sludge	Batch	60	84.2	-
POME + EFB (O-Thong *et al*., 2012)	Digested manure	Batch	55	-	392
POME (Yossan *et al*., 2012)	Thermotolerant	Batch	55	17.5	-
POME (Fang *et al*., 2011)	Cow manure	UASB[1]	55	-	436
Deoiled POME (Fang *et al*., 2011)	Cow manure	UASB[1]	55	-	600
POME (Fang *et al*., 2011)	Cow manure	EGSB[2]	55	-	438
Deoiled POME (Fang *et al*., 2011)	Cow manure	EGSB[2]	55	-	555
POME (Mamimin *et al*., 2012)	*Thermoanaerobactrium*-rich sludge	CSTR[3]	60	92.0	-
POME (Khemkhao *et al*., 2014)	Mixed microflora	CSTR[3]	55	-	270
POME (Prasertsan *et al*., 2009)	*Thermoanaerobactrium*-rich sludge	ASBR[4]	60	270.0	-
POME (Badiei *et al*., 2011)	Mixed microflora	ASBR[4]	37	340.0	-

[1]UASB = Upflow anaerobic sludge blanket reactor

[2]EGSB = Expanded granular bed reactor

[3]CSTR = Continuously stirred tank reactor

[4]ASBR = Anaerobic sequencing batch reactor

Satisfactory hydrogen and methane production yield was obtained from batch assays was 3.5% (Yossan *et al*., 2012) and 16.9% (O-Thong *et al*., 2008) of hydrogen theoretical yield (498 mL H_2/g-COD) and 79.0% (O-Thong *et al*., 2012) of methane theoretical yield (496 mL CH_4/g-VS), respectively under thermophilic condition. These literature reviews shown in **Table 2.3** which could summarized are: (1) POME is a good potentially substrate used to generates both

hydrogen and methane which is satisfactory of hydrogen and methane production yield was achieved; (2) Source of inoculum is one of the important role of both hydrogen and methane formations, the highest hydrogen production yield was 270 mL H_2/gCOD using *Thermoanaerobacterium*-rich sludge which was 5 times greater than that achieved from the utilized of Thermotolerant as inoculum. Meanwhile, the utilization of cow manure as inoculum for generates methane is satisfied of methane production yield was achieved; (3) Reactor performance, methane production yield achieved from UASB and EGSB reactors are not different significant, despite of higher methane production yield achieved from EGSB reactor, in contrast the UASB reactor had more stable of methane production as well as lower of VFA accumulation; (4) Temperature, there are several advantages of thermophilic over mesophilic temperatures are including destruction of pathogens, higher both chemical and biological reaction rates, less variety of fermentation end-products and decreased contamination by hydrogen consumers.

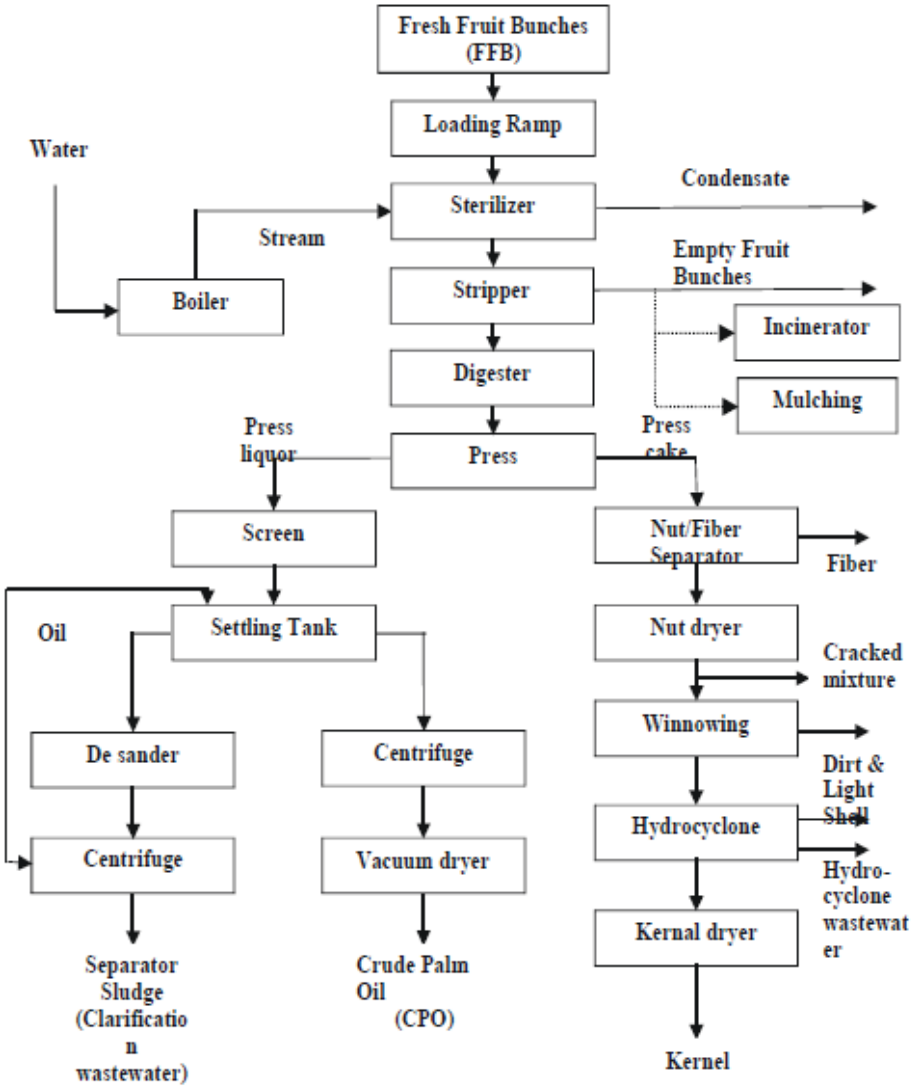

Fig. 2.2 Process involved in milling of oil palm (Rupani *et al*., 2010).

Recently, most of latex concentrates plants and palm oil mill plants, SLS and POME have been used as substrate to produce biogas in Malaysia and Thailand (Jawjit *et al*., 2010).

2.2 The biogas process

Anaerobic digestion process is a biological multi-step process with a specific groups of microorganisms involved in each individual step under the absence of oxygen to break down or

convert organic matter into the most reduced (CH_4) and most oxidized (CO_2) products respectively (Kongjan, 2010) as shown in **Fig. 2.3**.

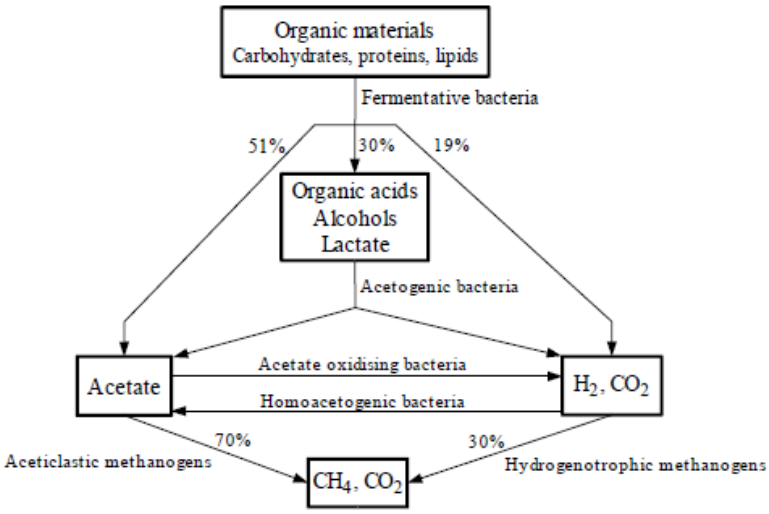

Fig. 2.3 Carbon flow diagram of the biogas process (Boe, 2006).

The four main stages of the important processes occurs in anaerobic digestion are comprised hydrolysis, acidogenesis, acetogenesis, and methanogenesis, respectively.

2.2.1 Hydrolysis

Hydrolysis is theoretically the first step in anaerobic digestion, in this stage the organic matters such as proteins, polysaccharides, and fats are broken down and/or converted to soluble oligomers and monomers. Hydrolytic enzymes are comprised cellulase, cellobiase, xylanase, amylase, protease, and lipase (Boe, 2006). Hydrolysis is an important step because the fermentative bacteria in the fermentation process cannot adsorb complex organic polymers directly into their cells. There are several step of the hydrolysis process itself such as enzyme production, adsorption, reaction, and enzyme deactivation step. Organic material size, surface area, biomass production, enzyme production, and adsorption play a vital role on the overall hydrolysis rate.

2.2.2 Acidogenesis

The second step of the anaerobic digestion process is acidogenesis or acidification; the products from hydrolysis step were converted into simple molecules with a low molecular weight such as volatile fatty acids (acetic, propionic, butyric, and lactic acids), alcohol (ethanol, and butanol), aldehydes, and gases (hydrogen, carbon dioxide, and traces of methane and hydrogen sulfide) by fermentative bacteria. Angelidaki *et al*. (2002) reported approximately 51% of acetate, 19% of hydrogen, and the rest are more products were comprised alcohols or aldehydes as the products from the acidogenesis step.

2.2.3 Acetogenesis

The products from acidogenesis such as fatty acids with longer than two carbon atoms, longer than one carbon atom of alcohols, and branched-chain and aromatic fatty acids, which cannot be converted to methane by methanogenic bacteria directly, were converted to methanogenic substrates (acetate, hydrogen, and carbon dioxide) in the acetogenesis stage. However, there are several factors affecting to hydrogen producing acetogens and homoacetogens comprehensive physical and chemical conditions (pH, temperature, acetate accumulation, and partial hydrogen pressure).

2.2.4 Methanogenesis

The final step of the anaerobic digestion process, the products achieved from the first three step of the anaerobic digestion mostly acetic acid, hydrogen, and carbon dioxide was further converted to methane and carbon dioxide by aceticlastic and hydrogenotrophic methanogens. However, 70% of methane was produced via the aceticlastic pathway by aceticlastic methanogens and the rest of 30% was produced via the hydrogenotrophic pathway by hydrogenotrophic methanogens. However, some of acetate-consuming methanogens are the slow-growing microbes with a generation time of 1 to 12 days; in contrast, the hydrogen-utilizing methanogens are the fastest-growing microbes with a generation time of 6 h. In addition, hydrogen-consuming methanogens are more tolerant to environmental changes than aceticlastic methanogens. Nevertheless, methanogenesis is affected by environmental changes and reactor operating conditions include pH, temperature, hydraulic loading rate, organic loading rate, substrate composition (Boe, 2006).

2.3 Two-stage anaerobic digestion process

Two-stage anaerobic fermentation process is often used for sequential hydrogen and methane production. The process contains two phases in two separate reactors comprehensive (i) the production of VFAs and hydrogen by acidogens and hydrogen producers, by the results of the hydrolysis and acidogenesis, occur in the first phase and (ii) the second phase is methanogenesis, in which the hydogenogenic effluent of the first stage is transferred to and used as a substrate to produce methane by methanogens (Cooney *et al*., 2007). Two-stage fermentation of hydrogen and methane is more stable and effective than the one-stage process since each stage can be optimized separately, leading to a greater overall reaction rate and biogas yield (Mataalvarez *et al*., 1993; Park *et al*., 2010; Liu *et al*., 2006).

Fig. 2.4 below shows the anaerobic process is categorized into two-stage based on the products of hydrogen and methane. Hydrogen released from the first stage is called dark hydrogen fermentation, while the soluble end products generated by this stage are fed into the second stage, a methane phase for further anaerobic methane production by sequential acetogenesis and methanogenesis steps (Liu *et al*., 2006).

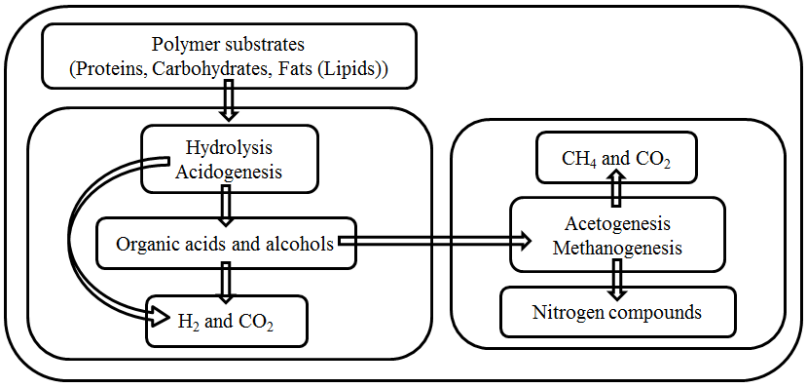

Fig. 2.4 Flow diagram of two-stage anaerobic process (Adapted from Kongjan, 2010).

2.4 Factors affecting the stability of the biogas production by dark fermentation

Apparently, there are several factors affecting the stability of the biogas production by dark fermentation are mainly comprehensive the operating conditions such as pH, temperature, buffer capacity, and mixing intensity, and feedstock including waste composition, waste concentration, toxic and inhibitory compounds; all of these factors are influence directly on the microorganisms are discussed below (Boe, 2006).

2.4.1 Substrate

Various types of substrate that can be used to produce biogas pass through dark fermentation are comprehensive pure sugars, such as sucrose, glucose, and lactose, and organic wastes from food factories such as apple processing wastewater, potato processing wastewater, starch wastewater, food waste, and palm oil mill effluent. However, the uses of pure sugars are only for trying to understand the microbial physiology of hydrogen production, it is not provided for hydrogen production in the industrial scale since it is too expensive. The selection of organic substrates to be used in dark fermentation for hydrogen production must consider including cost, availability, carbohydrate content, and also biodegradability (Kongjan, 2010). Moreover, the concentration of substrate also must consider because several researches reported about at high concentration of substrate could cause the substrate inhibition which could potentially inhibit or overload the process and lead to decrease in biodegradability.

2.4.2 Nutrients

The sufficient amounts of macro- and micro-nutrients are playing a vital role on microbial cell growth. Carbon, hydrogen, oxygen, and nitrogen are the major components of macro-nutrients in biomass cells. Moreover, sulphur, phosphorus, potassium, calcium, magnesium, and iron are required for specific proteins. Meanwhile, the micro-nutrients including nickel, cobalt, copper, manganese, molybdenum, zinc, selenium are also required in smaller amount. However, the present of these macro- and micro-nutrients in high concentration could be inhibitory.

Skim latex serum (SLS) is deficient in the macronutrients in carbon, while palm oil mill effluent (POME) is also deficient in nitrogen which is crucial for microbial growing. Thus, in

this work was carried out using anaerobic dark co-fermentation of SLS and POME under thermophilic temperature to enhance the efficiency of biogas formation.

2.4.3 Operating conditions

2.4.3.1 Temperature

A wide range of temperature is possible for anaerobic dark fermentation, divided into three temperature is comprehensive psychrophilic (< 20°C), mesophilic (25-40°C), thermophilic (45-60°C), and extreme-thermophilic conditions (> 60°C). Temperature is considered as one of the most important factors affecting on biogas production because it has direct effect on physical-chemical properties of all components in the digester and also affects thermodynamic and kinetic of the biological processes. There are several advantages of increasing temperature including increase chemical and biological reaction rate, shorter hydraulic retention time (HRT) in a continuous process, improve diffusivity of soluble substrate, decrease liquid viscosity, increase death rate of pathogenic bacteria. On the other hand, the increasing temperature in turn will also increase the concentration of free-ammonia (NH_3) which is inhibitory to microorganisms.

2.4.3.2 pH and buffers

pH value has play a vital role on enzyme activity in microorganisms because each group of microorganisms has different optimal pH range. Furthermore, enzyme is active in a specific pH range and also has maximum activity under the optimal pH. Several researchers reported most of the hydrogen producing bacteria is fast growing and prefers slightly acidic pH range from 5-6 with an optimal pH of 5.5 (O-Thong *et al*. 2008). On the other hand, methanogenic archae can function within a relatively narrow pH interval between 5.5-8.5 with an optimum pH interval between 7.0-8.0 (Boe, 2006). The pH level also affects acid-base equilibrium of different compounds in the digester. At high pH, the accumulation of free ammonia produced during degradation of proteins or by the present of ammonia in the feed can cause weak base, while low pH, the accumulation of free VFA can cause weak acid inhibition.

Bicarbonate (HCO_3^-, pKa 6.3) is often the main buffer in anaerobic digesters to resist the pH in the anaerobic digester change. Furthermore, the present at high concentration of ammonia (NH_4^+/NH_3, pKa 9.3), hydrogen sulfide ($H_2S/HS^-/S^{2-}$, pKa 7.1 and 13.3), and hydrogen

phosphate ($H_3PO_4/H_2PO_4^-/HPO_4^{2-}/PO_4^{3-}$, pKa 2.1, 7.2, and 12.3) are also influence the pH balance in the anaerobic digester (Boe, 2006).

2.4.4.3 Organic loading rate

The organic loading rate is an important operational parameter for continuously biogas production in continuous processes, to avoid overfeeding which could potentially inhibit or overload the process and lead to decrease in biodegradability and process failure. Organic loading rate is amount of organic matter fed per unit volume of digester capacity per unit time. A study from Saraphirom and Reungsang (2010) showed that the substrate removal efficiency was found to decrease when OLR was increased and also the microbial community composition in the digester was changed caused by the variation of OLRs.

2.4.4.4 Hydraulic retention time

Hydraulic retention time (HRT) is one of the most important operating parameter for dimensioning the biogas digester. The average time interval during the substrate is kept inside the digester is known as HRT. In fact, the retention time should be sufficiently long to ensure that the quantities of microorganisms removed in the effluent are not higher than the quantities of reproduced microorganisms. A study from Zhang *et al*. (2006) showed that there are some advantages and some disadvantages of using a short HRT are comprehensive reduction of diversity of microbial population associated with an elimination of propionate production without affecting the existence of dominant species, lower both substrate consumption and removal efficiency, and washout of microorganisms which was the presumably reason for the observed decrease in hydrogen yield. Moreover, the HRT could be used as a tool to select microbial populations whose growth rates are able to catch up with the mechanical dilution created by continuous volumetric flow.

2.4.3.5 Toxic/inhibiting compounds

VFA is the main intermediate compounds such as ethanol, butanol, acetate, propionate, butyrate, valerate, and lactate, produced during acidogenesis in anaerobic digestion process. Generally, the VFA inhibition is due to their accumulation inside the digester and consequently a

drop of pH-value. At lower pH values, much more of the VFAs exists in the undissociated form, which is much more toxic than ion form since its greater membrane permeability. A study from Amani *et al*. (2011) showed that under high mixing ratio of methanogens to acetogens (M/A = 3.1) the removal efficiency of propionic (HPr), butyric (HBu), and acetic acids (HAc) decreased because of the acetogens (propionate- and butyrate-oxidizing bacteria) were not sufficient to degrade high concentration of propionic (1543.5 mg/L) and butyric acids (2000.8 mg/L), they inhibited the acetogenic reactions and suppressed the growth of acetogens. At the same time, increasing M/A from 1.1 to 2.1, the removal efficiency of HPr, HBu, and HAc increased from 10, 18, and 20 to 41, 59, and 46%, respectively.

Ammonium ion (NH_4^+) and free ammonia (NH_3) are the two principal forms of inorganic ammonia nitrogen in aqueous solution come mainly from biodegradation of the nitrogenous compounds, mostly from proteins and urea. Free ammonia has been suggested to be the main cause of inhibition since it is freely membrane-permeable. The hydrophobic ammonia molecule may diffuse passively into the cell, causing proton imbalance, and/or potassium deficiency (Chen *et al*., 2008). The study from Cavinato *et al*. (2012) showed that the methane production rate decreased from 2.0 m^3/m^3_r d to 1.6 m^3/m^3_r d since the concentration of ammonia in the second stage increased to 2 g/L with free ammonia concentration of 916 mg/L. Under thermophilic conditions, 700 mg/L of free ammonia could be already toxic for methanogenic archae. Moreover, they also reported that 2 g-N/L of ammonia concentration was responsible of inhibition for biohydrogen production. Moreover, increasing of ammonia concentration from 960 mg/L to 1976 mg/L, the acetic and butyric acids decreased, while propionic acid increased from 696 mg-COD/L to 1904 mg-COD/L, which was the presumably reason for the observed decrease in hydrogen yield.

Proteins also had a constituent of both sulfate and sulfur compounds. In anaerobic condition, sulfate (SO_4^{2-}) is reduced to sulfide (S^{2-}) by responsible of two major groups of sulfate reducing bacteria are comprehensive complete and incomplete oxidizers. The reduce compounds such as lactate was converted to acetate and carbon dioxide by incomplete oxidizers and complete oxidizers, acetate was completely converted to carbon dioxide and bicarbonate (HCO_3^-) with reducing sulfate as electron acceptor. The competition of sulfate-reducing bacteria does not occur in the hydrolysis stage since it does not degrade natural biopolymers such as starch, proteins, and lipids. However, the competition of sulfate-reducing bacteria with methanogenic archaea for hydrogen and acetate is occurred at low concentrations of sulfate, while the competition of sulfate-reducing bacteria with acetogenic bacteria for propionate and butyrate is occurred under high concentration of sulfate.

Light metals ions including sodium (Na), potassium (K), calcium (Ca), magnesium (Mg), and aluminum required at low concentration as stimulate nutrients for microbial growing. Cabirol *et al*. (2003) reported the mechanism of aluminum inhibition due to its adhesion to the microbial cell membrane and microbial cell wall, which affect microbial growth. Both acetogenic and methanogenic microorganisms decreased by 50% and 72%, respectively after

exposed to 100 mg/L $Al(OH)_3$ for 59 days. The excessive amounts of calcium lead to precipitation of carbonate and phosphate, which affect biomass activity because of mass transfer limitations. A study from Yu *et al*. (2001) showed that the low Ca^{2+} concentration from 100-300 mg/L to be beneficial for sludge granulation in an UASB reactor. The optimal sodium concentration for mesophilic aceticlastic and hydrogenotrophic methanogens growth was 230 mg/L and 350 mg N^+/L, respectively (Kugelman and Chin, 1971; Patel and Roth, 1977). Moreover, a study the effect of potassium and magnesium on microorganism's growth shows that at high concentration of potassium (0.15 M K^+) caused 50% inhibition of acetate-utilizing methanogens, while under high magnesium ions concentration, stimulate the production of single cell, which causes loss aceticlastic activity in the digesters. Consequently, under high salt levels could cause bacterial cells to dehydrate due to osmotic pressure, and therefore suppress microbial growth, and consequently affect specific growth rate.

Several industrials and domestic wastewater was found a significant concentration of heavy metals such as zinc (Zn), copper (Cu), cadmium (Cd), nickel (Ni), iron (Fe), chromium (Cr), and etc. Heavy metals are bio-available and it's a necessary as nutrients at low concentration to microbial growth. Heavy metals are toxic when present in ionic form since it's attributed to disruption of enzyme function and structure by binding to ion-exchange site on the cell membrane and form a matrix with extracellular enzymes. Takashima and Speece (1989) reported the following order of heavy metal composition in the cell of ten methanogenic strains: Fe >> Zn > Ni > Co = Mo > Cu, and also acidogens are more resistant to heavy metal toxicity than methanogens. There are several forms of heavy metal such as precipitation as sulfide, carbonate, and hydroxides, and sorption to the solid fraction. There are, however, only metals in soluble, free form are toxic to the microorganisms (Oleszkiewicz and Sharma, 1990). The study from Lin (1993) showed that the relative toxicity of heavy metals in inhibition test on sensitivity of methanogens as Pb > Ni > Cd > Cu > Cr > Zn, while the relative toxicity of heavy metals on sensitivity of acidogens is Cu > Zn > Cr > Cd > Ni > Pb (Lin, 1992).