CHAPTER II
LITERATURE REVIEW

2.1 Background of Hydrogen

2.1.1 Advantages of Gaseous Hydrogen Utilization

2.1.1.1 Clean and Environmental Friendly Fuel

Hydrogen is considered as the cleanest fuel because it is a main content in the water. In a combustion engine, hydrogen is converted to heat, water and slight amounts of oxides of nitrogen. In a fuel cell, almost all products are water vapor. The reaction takes place at low temperature. Greenhouse gas, especially carbon dioxide, is considered as a main cause of climate change. Using of hydrogen is the benefit in long term to global environment. There are varieties of feedstock in the production of hydrogen, so the cleanest way to generate hydrogen can be chosen. The toxic pollutant from petroleum production is much higher than those of hydrogen production.

2.1.1.2 Harmless Fuel

Hydrogen is the safest fuel because of how light it is. Hydrogen is the lowest molar mass of all fuels. It is fourteen times lighter than the air and four times lighter than Helium. In the case of an accidental release, it disperses rapidly upward into the atmosphere while other fuels take longer to disperse or may spill in the ground.

2.1.1.3 Be Able to Use in Various Application

Hydrogen can be used in any applications, in which fossil fuels are being used, such as a fuel in furnaces, internal combustion engines, turbines, jet engines, automobiles, buses and airplanes. Nowadays, hydrogen can be directly applied to generate electricity through fuel cells, which are mostly utilized in transportation section. Moreover, hydrogen and fuel cell technology have the potential to strengthen our national energy security by reducing our dependence on foreign oil.

2.1.1.4 Be Produced from Various Sources

Hydrogen is also the most abundant element on earth. It is chemically attached to other atoms, such as carbon and oxygen. Additionally most of

hydrogen is bound as H_2O. The greatest advantage of hydrogen is that there are many ways to produce it, using both renewable and traditional energy sources. The hydrogen production concept is to detach the hydrogen atom from other components. The most common method of hydrogen production is by reforming fossil fuels, particularly natural gas. Electrolysis is another method of hydrogen production that uses electricity to split water into hydrogen and oxygen gases. One advantage of electrolysis is that one can perform electrolysis using renewable source so that the hydrogen produced as a renewable fuel. About 2.3 gallons of water and 45 kilowatts-hours (kWh) of electricity are needed to make enough hydrogen to generate energy content equivalent to a gallon of gasoline. Direct thermal dissociation of H_2O requires temperature more than 2,000 °C or temperature high than 900 °C with Pt/Ru catalyst.

2.1.2 Hydrogen Production Processes

Hydrogen production is the family of industrial methods for generating hydrogen. Currently the dominant technology for direct production is steam reforming from hydrocarbons. Many other methods are known including electrolysis and thermolysis.

There are many methods to generate hydrogen. Variety of feedstock, such as fossil fuels, water and biomass, can be converted to it.

2.1.2.1 Hydrogen Production from Fossil Fuels

Hydrogen is presently derived from natural gas, petroleum and coal by various ways:

2.1.2.1.1 Steam Reforming

Referring to steam methane reforming (SMR), converts steam and lighter hydrocarbons such as methane or refinery feedstock into hydrogen. There are two steps in this reaction. The first reaction is *the reforming of natural gas*. In this step, light gas is reformed to hydrogen and carbon monoxide called syngas under high temperature (750-800°C). This reaction is shown in Equation (2.1).

$$CH_4 + H_2O\ (+ \text{heat}) \rightarrow CO + 3\ H_2 \qquad (2.1)$$

The second reaction is *the water gas shift reaction.* In this step, carbon monoxide produced in the first reaction is reacted with high temperature steam to form hydrogen and carbon dioxide. This process occurs in two stages, consisting of a high temperature shift (HTS) at 350 °C (662 °F) and a low temperature shift (LTS) at 190-210 °C (374-410 °F). This reaction is shown in Equation (2.2).

$$CO + H_2O \rightarrow CO_2 + H_2 + \Delta H \qquad (2.2)$$

2.1.2.1.2 Thermal Cracking of Methane

Thermal cracking of natural gas, such as methane, is used for replacing steam reforming process because it does not release carbon dioxide. This reaction is carried out at high temperature. It requires temperature about 2,000 °C to produce 60 % of hydrogen and 40 % of carbon black that is a pigment used in many dying processes (Kothari *et al.*, 2004). The reaction is indicated in Equation (2.3).

$$CH_4 \rightarrow C + 2H_2 + 75.6\ kJ \qquad (2.3)$$

2.1.2.1.3 Partial Oxidation of Methane

Partial oxidation of methane is a reaction to convert natural gas into a synthesis gas (carbon dioxide and hydrogen), which can be converted to higher alkanes or methanol (Deutschmann and Schmidt, 1998). Because this process is the exothermic reaction, it can generate its own heat that is used as energy supply in the process itself. The reaction of this process is shown in Equation (2.4).

$$CH_4 + \tfrac{1}{2}\ O_2 \rightarrow CO + 2\ H_2, \quad H_R = -36\ kJ/mole \qquad (2.4)$$

2.1.2.1.4 Coal Gasification

Coal gasification is the oldest method to produce hydrogen. It is a process to convert the coal in solid state to gaseous state at 1,330 °C. The gaseous coal is treated with steam and controlled amount of oxygen to produce hydrogen, carbon monoxide and carbon dioxide. And then, carbon monoxide is reacted

with steam to produce more hydrogen and carbon dioxide in the water gas shift reaction. The reactions are shown in Equations (2.5-2.6).

$$\text{Coal} + O_2 + H_2O \rightarrow CO + CO_2 + H_2 + \text{Other species} \quad (2.5)$$

$$CO + H_2O \rightarrow CO_2 + H_2O \quad (2.6)$$

Coal gasification is expensive to produce hydrogen from coal as almost twice as from natural gas because of the low ratio of hydrogen to carbon; that is 4:1 in natural gas and 0.8:1 in carbon (or coal).

2.1.2.2 Hydrogen Production from Water

2.1.2.2.1 Electrolysis

It is the process to separate the water molecules into their basic elements of hydrogen and oxygen. Electricity is introduced to water through two electrodes, a cathode (negative plate) and an anode (positive plate), these ions are attracted to the opposite charged electrode. Therefore the positively charged hydrogen ions will collect on the cathode to form hydrogen gas and the negatively charged oxygen will collect on the anode to form oxygen gas. There is no carbon dioxide given off during the process and it can be results shown in Equation (2.7).

$$2H_2O + \text{energy} \rightarrow 2H_2 + O_2 \quad (2.7)$$

However, this method is not efficient when it comes to produce large amounts of hydrogen because it is energy-intensive and if using electricity generated from fossil fuels, carbon dioxide will be produced at an earlier stage in the process.

2.1.2.2.2 Thermal Decomposition

Thermal decomposition also called thermolysis, is defined as a reaction which a water breaks up into hydrogen and oxygen. When water is heated to a high temperature at 3,000 K (2,727 °C), it can be decomposed into hydrogen and oxygen. This process has high efficiency, but it is normally not applied to produce hydrogen. The chemical equation is shown in Equation (2.8)

$$H_2O \rightarrow H_2 + 0.5O_2 \quad (2.8)$$

2.1.2.2.3 Thermochemical Process

Thermochemical process is developed from thermolysis in order to decrease temperature required. The commercial process of thermochemical process is sulfur-iodine cycle (S-I cycle). The sulfur and iodine used in the process are recovered and reused. Concentrating solar power systems (CSP) is used as a heat source to drive an endothermic reversible reaction. Other commercial cycle is Copper–chlorine cycle.

2.1.2.2.4 Photolysis

Photolysis occurs when water molecules absorb the sunlight and use photons to separate water into hydrogen and oxygen in the presence of photocatalysts. This process can be divided into three kinds that are dependent on photocatalyst type.

(1) Biophotolysis Process

In biophotolysis process, hydrogen is produced from water by using sunlight and specialized microorganisms (photocatalyst), such as green algae and cyanobacteria. These microorganisms consume water and then produce hydrogen as a by-product of their natural metabolic processes, just like plants produce oxygen during photosynthesis.

(2) Photochemical Process

Photochemical process is similar to that of thermochemical cycles, which is to add a kind of photosensitive matter as an activator to increase the absorption of wave energy in sunlight. Hydrogen is then produced by photochemical reaction.

(3) Photoelectrochemical Process

Photoelectrochemical process uses sunlight and specialized semiconductors (photocatalyst) that are called photoelectrochemical materials to produce hydrogen from water. In the photoelectrochemical (PEC) system, the semiconductor can directly split water molecules into hydrogen and oxygen by using light energy. Different semiconductor materials work at particular wavelengths of light and energy.

2.1.2.3 Hydrogen Production from Biomass

Biomass is one of the most abundant renewable sources. It is formed by fixing and consuming carbon dioxide in the atmosphere during the process of plant photosynthesis (Ni *et al.*, 2006). It can also be used as a carbon source, which facilitates waste recycling (Manish and Banerjee, 2007). In hydrogen production processes, carbon dioxide is produced as a by-product, which means that they result in a near-zero net release of greenhouse gas. Moreover, biomass is carbon neutral in its life cycle. At present, about 12 % of today's world energy supply comes from biomass (Koutrouli *et al.*, 2006). A diverse array of biomass resources can be used to convert to energy (e.g. hydrogen, ethanol and methane/biogas). They can be divided into four general categories:

(1) Energy crops: agricultural crops, industrial crops, herbaceous energy crops, woody energy crops and aquatic crops.

(2) Forestry waste and residues: trees and shrub residues, logging residues and mill wood waste.

(3) Agricultural waste, wastewater and residues: crop waste, animal waste and wastewater from animal confinements.

(4) Industrial waste and wastewater, municipal waste and wastewater: municipal solid waste (MSW), sewage sludge and industry waste.

The available hydrogen production processes from biomass have two general categories: thermochemical and biological processes. Thermochemical processes can be divided into two types that are pyrolysis and gasification. Photo-fermentation and dark fermentation are the two types of biological processes.

2.1.2.3.1 Thermochemical Process

(1) Biomass Pyrolysis

In this process, biomass is heated at a temperature and pressure of 650-800 K (377-527 °C) and 0.1-0.5 MPa in the absence of oxygen (or air) to convert biomass into liquid, charcoal and non-condensable gases, acetic acid, acetone and methanol. The main gaseous products from pyrolysis are hydrogen, carbon dioxide, carbon monoxide and hydrocarbon gases. The reactions are shown in Equation (2.9).

$$\text{Biomass} + \text{heat} \rightarrow H_2 + CO + CO_2 + CH_4 + \text{Other products} \quad (2.9)$$

(2) Biomass Gasification

The basic process of biomass gasification is to gasify biomass at a high temperature (above 1,000 K). It is partially oxidized in the presence of oxygen (or air) to form gas and charcoal shown in Equation (2.10).

$$\text{Biomass} + \text{heat} + O_2 + H_2O \rightarrow H_2 + CO + CO_2 + CH_4 + \text{Light and heavy} + \text{Hydrocarbons} + \text{charcoal} \quad (2.10)$$

The gas and hydrocarbons products can be converted into more hydrogen by steam reforming and this process can be further improved by water gas shift reaction. Biomass gasification is available for biomass that has moisture content less than 35 % (Deutschmann and Schmidt, 1998).

As mentioned above, the products from biomass gasification are mainly gases while pyrolysis aims to produce bio-oils and charcoal. Also, biomass gasification is more favorable for hydrogen production than pyrolysis (Ni *et al.*, 2006).

2.1.2.3.2 Biological Process

Biological hydrogen production process is an alternative method for hydrogen gas production. This process uses microorganisms to decompose organic compounds in waste or wastewater, which are composed of carbohydrate-rich and non-toxic materials, to simple end-products, such as hydrogen, methane, carbon dioxide volatile fatty acids and alcohols. Most of biological processes are operated at an ambient temperature (30-40 °C) and normal pressure; hence, they are less energy-intensive and environmentally friendly. There are two types of biological processes: photo-fermentation and dark fermentation.

(1) Photo-fermentation

Photo-fermentation is the process to decompose organic compounds to hydrogen as the product by photosynthetic bacteria. They undergo an oxygenic photosynthesis with organic compounds or reduced sulfur

compounds as electron donors. Some non-sulfur photosynthetic bacteria are potent hydrogen producers, utilizing organic acids, such as lactic, succinic and butyric acids, or alcohols as electron donors. Hydrogen production by photosynthetic bacteria is mediated by nitrogenase activity, although hydrogenases may be active for both hydrogen production and hydrogen uptake under some conditions. Photosynthetic bacteria are the most promising microbial system for biohydrogen production because of their high theoretical conversion yields and lack of oxygen-evolving activity, which causes problem of oxygen inactivation of different biological systems. Moreover, they have the ability to use wide spectrum of light and consume organic substrates derived from wastes and wastewaters (Fascettiet *et al.*, 1998). If photosynthetic bacteria are combined with fermentative bacteria, the fermentative bacteria could produce the small organic acids, which the photosynthetic bacteria could then use.

(2) Dark Fermentation

Hydrogen production via dark fermentation is a special type of anaerobic digestion process comprising only hydrolysis and acidogenesis (Bartacek *et al.*, 2007). Fermentative bacteria producing hydrogen, carbon dioxide and some simple organic compounds, e.g. volatile fatty acid (VFA) and alcohols, in the dark may be cultivated in pure culture or occur in uncharacterized mixed cultures selected from natural sources, such as anaerobic digested sewage sludge and soil (Bartacek *et al.*, 2007; Vijayaraghavan *et al.*, 2005). The advantages of dark fermentation are that fermentative bacteria are capable of high hydrogen generation rate and hydrogen is produced throughout the day and night at a constant rate since it does not depend on energy provided by sunlight (Vijayaraghavan and Soom, 2006). This decreases the energy demand and the technology can be simpler (Bartacek *et al.*, 2007). In addition, fermentative bacteria can have good growth rate for supply of microorganisms to the production system. From these several advantages, hydrogen production by dark fermentation is feasible for industrial application (Das and Veziroglu, 2001).

2.2 Background of Methane

Methane is an important fuel nowadays. The consumption of natural gas for vehicle consisted of methane and ethane is increasing continuously. Methane is generated primary from petroleum, but its supply is going to disappear readily. There are many researcher find the solutions to solve this problem, especially by the best solution for methane production. This process consumes low energy and adds the value to wastewater.

2.2.1 Advantages of Methane

2.2.1.1 As an Abundant Fuel

The largest volume of methane in the world is generated from petroleum. There are not only from petroleum, but also from waste such as pig manure. In Thailand, pig farm has problems about infested smell pig manure. This problem is eliminated by fermentation of manure in anaerobic system. The direct result is eliminating the infested smell and methane as an indirect result.

2.2.1.2 Widely Application

There are widely applications for methane. It can be used as a fuel for driving engine, as a heat source for home, as a power source in factory as a feed stock for chemical production; such as ethanol, acetylene and methyl chloride. Natural gas cost, contain more than 90 percent of methane is cheaper and more stable than that of electricity for using as a power.

2.2.2 Methane Production

Methane is mainly obtained petroleum gas well. However, the impact of unlimited supply of methane is considered. There are many methods to produce methane which biological and chemical route is interesting.

2.2.2.1 Chemical Route

(1) The Sabatier Reaction

Sabatier reaction is the reaction of hydrogen with carbon dioxide at elevated temperatures (optimally 300–400 °C) and pressures in the presence of a nickel catalyst to produce methane and water. Optionally, ruthenium on alumina

(aluminum oxide) makes a more efficient catalyst. It is described by the following exothermic reaction. The chemical equation is shown in Equation (2.11).

$$CO_2 + 4\ H_2 + \text{energy} \rightarrow CH_4 + 2\ H_2O, \quad \Delta H = -165.0\ \text{kJ/mol} \qquad (2.11)$$

(2) Fischer–Tropsch Process

As Equation (2.12), converting of a mixture of H_2 and CO into aliphatic products. The Fischer–Tropsch process is the reaction converting a mixture of H_2 and CO into aliphatic products. This process is operated in the temperature range of 150–300 °C (302–572 °F). Higher temperatures lead to faster reactions and higher conversion rates but also tend to favor methane production. The reaction is shown in Equations (2.12)

$$3\ H_2 + CO \rightarrow CH_4 + H_2O \qquad (2.12)$$

Because of the cost of methane, it is unwanted product. This reaction is normally used to produce long chain aliphatic product.

2.2.2.2 Biological Routes

Methane is generated as a product of microorganism respiration in anaerobic system. The wastewater containing high organic matter is feed as a substrate for bacteria. There are abundant of bacteria for convert carbon dioxide to methane. Methane converted step is a step called methanogenesis step by methanogens in multistep process. The reaction of carbon dioxide converting to methane is shown in Equation (2.13).

$$CO_2 + 4\ H_2 \rightarrow CH_4 + 2H_2O \qquad (2.13)$$

The above equation is a generalized equation for the methane fermentations that carbon dioxide is an electron acceptor for the oxidation of the variety of alcohols and fatty acids known to be metabolized by these bacteria. This would explain why the hydrocarbon product is always methane no matter what organic substrate is fermented.

2.3 Wastewater Treatment

Wastewater which is the root cause for many problems including bad smell, decease, noisy, pest and contaminated in fresh water, is produced from many sources, such as household, industry, etc. Wastewater normally composes of water, pathogens (bacteria, virus and prion), non-pathogenic bacteria, organic particle (food hair, plant, NH_3, H_2S), animals (protozoa, insect and arthropods), toxin (pesticide, herbicide, poison), etc. So, the advantage of treated wastewater is not only supply water, but also reduce the chance to get decease from pathogen.

There are many ways of wastewater treatment, consisting of physical, chemical and biological method. In a conventional wastewater treatment process, there are four main steps, which is preliminary, primary, secondary, tertiary step, including with disinfection step as a disinfection step. The disinfection step is required in some countries. A conventional wastewater treatment diagram is shown in Figure 2.1.

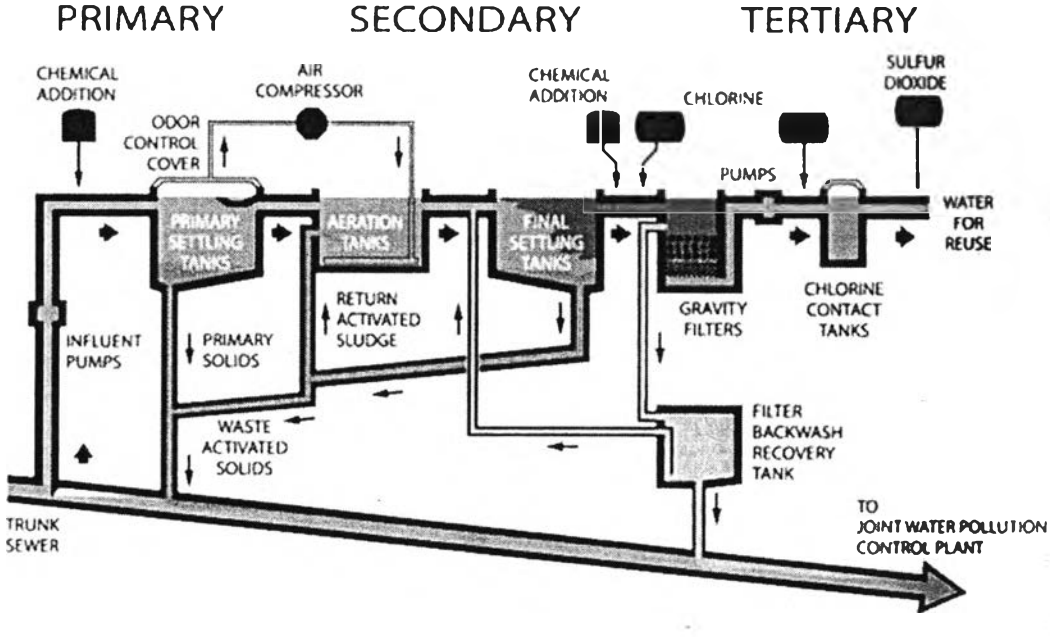

Figure 2.1 Schematics of wastewater treatment process (Sanitation Districts of Los Angeles County, 2015).

2.3.1 Steps in Wastewater Treatment

2.3.1.1 Preliminary Treatment

Preliminary treatment is the first unit for removing the large particle in wastewater for enhancing the efficiency of subsequent units. The preliminary part normally composes of molecular sieve or screening to strain the large particle out of the feed wastewater, grit chamber is used to settle the sand, grid and stone to bottom of the chamber while keeping the majority of suspended organic material in the water column. The removal grit is taken to the landfill and disposal. The objective of this unit is to long life the pump and other equipment.

2.3.1.2 Primary Unit

During the preliminary treatment, the sludge composed of settable organic and inorganic solid is precipitated to the bottom and floating materials, such as grease and oil, can be raised to skim on the surface. The sludge is sent to sludge treatment stage. Approximately 25 to 50 % of biochemical oxygen demand.

2.3.1.3 Secondary Treatment

The target of the secondary treatment is removal of the biodegradable dissolved organic matter and suspended solid from the effluent of primary treatment by biological method. The efficiency is up to 90 %. The biodegradable process can separate into 2 categories that it is aerobic and anaerobic biological treatment.

2.3.1.3.1 Aerobic Biological Treatment

Aerobic biological treatment is performed in the presence of oxygen. The organic matter including dissolved and suspended solid is digested by microorganism, algae fungi principally bacteria, for growing their cell. The end product from anaerobic process is normally operated in the high-rate biological process operated in small volume reactor and high concentration of microorganism. The most common method used to achieve secondary treatment is attached growth process and suspended growth process.

(1) Attach Growth Process

Attach growth process (also called fixed film process) is the process that any microorganisms is attached to the support (stone, wood, packing material) and consume the organic matter from the wastewater sprayed to the

basin. The growth microorganism form a layer called slime layer. The example of these processes is tricking filter, biotower and rotating biological contractor.

(2) Suspended Growth Solid

Suspended growth solid (also called activated sludge system) is the process that microorganism and sludge is mixed as liquor in the basin. In this basin, the mixed liquor is pumped vigorously by the air to serve oxygen sufficiently for growing the microorganism cell for several hours. The microorganism turns organic matter in wastewater to end gas and multiply themselves in the basin. Following this step, the effluent is sent to the tertiary treatment unit. The microorganism is clarified and separates into two parts. One is recycle to maintain the concentration of microorganism and another one is carried to solid disposal unit.

2.3.1.3.2 Anaerobic Biological Treatment

Anaerobic Biological Treatment is similar to aerobic treatment, but anaerobic treatment uses microorganisms that do not require the addition of oxygen. These microorganisms use the compounds other than oxygen to catalyze the oxidation of biodegradable organics and other contaminants, resulting in innocuous by-products

2.3.1.4 Tertiary Treatment

Tertiary treatment is employed to remove the specific constituent which cannot be removed by primary and secondary treatment. The specific constituent remained in the effluent from secondary treatment are dissolved solid, suspended solid, refractory organic, heavy treatment, principally nitrogen and phosphorus. Nitrogen and phosphorus is removed by an adaption of activated sludge process. Nitrogen in the influent is normally in the NH_3 form. Ammonia is converted to Nitrile and Nitrate form by nitrification process. Nitrate-rich mixed liquid is reduced by facultative bacteria to Nitrogen form in the anaerobic condition by denitrification reaction. Phosphorus removal is performed using addition of volatile fatty acid in the anaerobic fermentation tank.

2.3.1.5 Advanced Treatment Process

2.3.1.5.1 Disinfection

Before treated waste water is discharged to environment the waste water will be disinfected for reducing the number of microorganism. Common disinfection method is ozonation, chlorination and ultraviolet light.

2.3.1.5.2 Ozonation

Ozone is molecule which oxygen atom attached to oxygen molecule forming O_3. It's very unstable reactive and strong oxidizer for removing pathogen and bacteria. Ozone is generated by passing O_2 to high voltage electricity which storage is not important for it. Pathogen oxidized by ozone is produced fewer by product. The expensive equipment and complex operation are disadvantages of ozonation

2.3.1.5.3 Chlorination

The conventional method of disinfection waste water is chlorination. Low operating cost and long term history is effectiveness. However residue of chlorine can be generated toxic compound for aquatic life. Chlorinated organic compound is one of the toxic form chlorination process, it occurs between reaction of residue chlorine atom. This toxic compound is carcinogen and harmful to environment. Small amount of chlorine in the effluent of waste water processing be reacted with NH_3 to form chlorinated organic compound as well. Treatment unit after disinfection process is considered to be problems.

2.3.1.5.4 UV Light

Because of the toxicity of chlorinate compound. UV light is used instead of chlorine. This method doesn't use chemical s. Bacteria is depleted. It's genetic structure by UV radiation which make it incapable to production. UV light frequency have to specify with bacteria to make sure that it is not shield from UV radiation.

2.3.2 Important Terms in Wastewater Treatment

2.3.2.1 Total Solids (TS)

The total solid is a matter remained after wastewater sample is dried out at a specified temperature (103 to 105 °C). Total solids consist of suspended solids and dissolved solid shown Equation (2.14).

$$TS = TSS + TDS \tag{2.14}$$

2.3.2.2 Total Suspended Solids (TSS)

Total suspended solid is the nonfilterable residue remained on a filter, with a specific pore size after drying the sample at 105 °C.

2.3.2.3 Dissolved Solids (DS)

Dissolved solid is the solid that can pass through a filter with pore sizes of 2.0 microns or smaller. Then, the filtrate is dried out at 105 °C

2.3.2.4 Total Volatile Solids (TVS)

Total volatile solid is the volatilized solid after burning at 500 °C. The relation of volatile solid is shown in Equation (2.15).

$$TVS = TS - ash \tag{2.15}$$

2.3.2.5 Volatile Suspended Solids (VSS)

VSS is determined by igniting the TSS at 550±50 °C after placing the filter disk in a porcelain dish.

2.3.2.6 Biochemical Oxygen Demand (BOD)

BOD is a measuring parameter to determine the quantity of oxygen used by microorganisms in the aerobic stabilization of wastewaters and polluted waters. The limitations of the BOD test are as follows: (1) a high concentration of acclimated seed bacteria is required; (2) pretreatment is needed when dealing with toxic waste, (3) only biodegradable organics are measured.

2.3.2.7 Chemical Oxygen Demand (COD)

COD is a method to measure the concentration of organic compounds present in a wastewater sample using chemical oxidation reaction with

potassium dichromate under acidic condition. The value of COD is calculated in terms of oxygen required for this chemical oxidation reaction. It presents both biodegradable and non-biodegradable organics in the sample.

2.3.2.8 Hydraulic Retention Time

The hydraulic retention time (HRT) or τ (tau) is a measure of the average length of time that a soluble compound remains in a bioreactor. The theoretical hydraulic retention time is defined in Equation (2.16):

$$\tau = \frac{V}{Q} \tag{2.16}$$

Where τ = Hydraulic retention time, h

V = Volume of reactor, m^3

Q = Volumetric flow rate, m^3/h

2.4 Anaerobic Biological Treatment

Anaerobic biological treatment, also called anaerobic fermentation, is a digestion process made by microorganisms in absence of oxygen. The microorganisms multiple themselves in the metabolizing process by converting the dissolved substrates to hydrogen, carbon dioxide and methane.

2.4.1 Process Description

There are three steps involving in the process. It is consisted of hydrolysis, acidogenesis, acetogenesis and methanogenesis respectively:

2.4.1.1 Hydrolysis

Hydrolysis is the first step in fermentation process. The organic materials are converted to soluble compounds (normally in monomer forms), for example glucose, amino acid and fatty acid, preparing for digesting by microorganisms.

2.4.1.2 Acidogenesis

The second step is acidogenesis (referred to fermentation). In this step, organic matters, such as amino acid, sugars and some fatty acids, serve electron donors and electron acceptors. The principal products of this step are acetate, hydrogen, carbon dioxide, propionate and butyrate. The propionate and butyrate are further reacted to generate hydrogen, carbon dioxide and acetate. The final products (hydrogen, acetate, carbon dioxide) are precursors of methane formation at the next step.

2.4.1.3 Methanogenesis

The methanogenesis is a methane formation step. The methanogenesis involves microorganism group called methanogens. There are two groups of methanogens aceticlastic and hydrogen-utilizing methanogens groups. The first group, aceticlastic methanogen, is the microorganism that splits acetate to methane and carbon dioxide. The second group, hydrogen-utilizing methanogens, uses hydrogen as an electron donor and carbon dioxide as an electron acceptor in order to producing methane. The process diagram is shown in Figure (2.2).

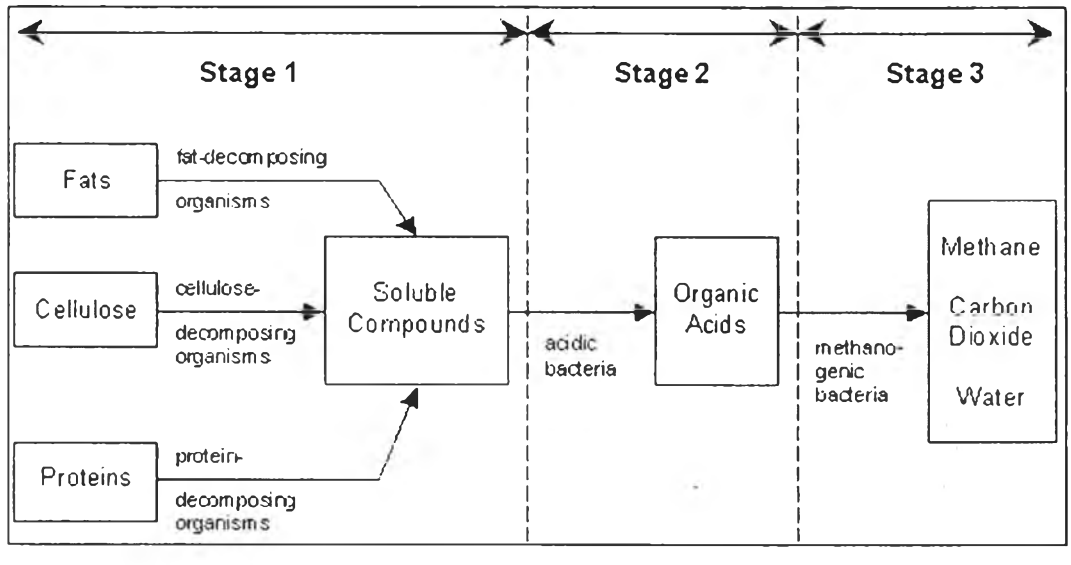

Figure 2.2 Schematic representation of anaerobic biological treatment (Das and Veziroglu, 2001).

2.4.2 Factors Affecting an Anaerobic Digestion

One of the important factors controlling the anaerobic biological treatment process is temperature. Substrate degradation, H_2 production, product distribution and bacteria growth are all affected by temperature.

2.4.2.1 Bacteria Culture

There are many species of bacteria used to produce hydrogen via anaerobic digestion such as *Escherichia coli, Clostridium, etc. Clostridium* found in soil (Van Ginkel *et al.*, 2001) had an endospore which make it survive in high temperature. From this advantage, the easy way to eliminate others bacteria population, such as methanogens, sulfate reducing bacteria, from *Clostridium* was boiling (Duangmanee *et al.*, 2002). In addition, Acid-based pretreatment was also developed to select the appropriate bacteria.

2.4.2.2 Substrate

There are many investigator reported the types of substrate conducted in anaerobic digester, such as glucose and sucrose (Majizat *et al.*, 1997; Mizuno *et al.*, 2000; Van Ginkel *et al.*, 2001; Duangmanee *et al.*, 2002; Lin and Jo, 2003; Khanal *et al.*, 2004), food wastes (Shin *et al.*, 2004; Van Ginkel et al., 2005; Wu and Lin, 2004), cellulose containing waste (Okamoto *et al.*, 2000), municipal wastes (Ueno *et al.*, 1996). Moreover, some researcher compared the performance of biogas gas production from different sources (Noike *et al.*, 2000).The bean curd manufacturing waste (protein-rich waste) gave higher hydrogen yield than that of rice and wheat bran waste (carbohydrate-rich waste). At the same time, the comparison of hydrogen yield from dark fermentation among carbohydrate-rich waste, protein-rich waste and fat-rich waste indicated that the highest hydrogen yield was obtained from carbohydrate-rich waste (Lay *et al.*, 2003).

2.4.2.3 Hydraulic Retention Time (HRT)

The hydraulic retention time (HRT) is defined as the average time that a substrate travel in a reactor. The most proper HRT was observed in many system. For continuous stirred tank reactor (CSTR), the maximum hydrogen yield (1.76 mole H_2/mole glucose) was obtained at HRT of 6 h via CSTR (Lin and Chang, 1999). The maximal hydrogen yield of 3.03 mole H_2/mole sucrose was found at a HRT of 0.5 h in a carried-induced granular sludge bed bioreactor (Lee *et al.*, 2004). In

addition, the best hydrogen yield of 2.67 mole H_2/mole sucrose was obtained under a three-phase fluidized-bed bioreactor with HRT of 2 h (Wu *et al.*, 2003). Moreover, some investigator specified that the HRT of 8 h can completely limit the methane production without any pretreatment in an anaerobic sequencing batch reactor (ASBR) (Lin *et al.*, 2003).

2.4.2.4 Product Inhibition

The accumulation of volatile fatty acid produced in acedogenesis step, such as butyric, propionic and valeric acid, was a toxic in hydrogen production. For butyric acid, the addition of butyric acid higher than 17.6 g/l began to inhibit the activity of *Clostridium butyricum* (Heyndrickx *et al.*, 1987)

2.4.2.5 pH

The pH was a parameter affected the physical properties of microbe which function to the metabolic pathway of dark fermentation. For batch reactor, the optimal initial pH for converting starch to hydrogen was found at 6.0 under thermal conditions (Zhang *et al.*, 2003), as well was hydrogen production from cheese whey, the maximum hydrogen yield was obtained at the initial pH of 6.0 (Ferchichi *et al.*, 2005). Moreover, the better performance of hydrogen production from rice slurry was obtained at an initial pH of 4.5 (Fang *et al.*, 2006). Based on these studies, it can be concluded that an optimal initial pH of hydrogen production was slightly acid. For continuous operation, a pH of 5.5 was found to be the optimum for hydrogen production from glucose (Fang and Liu, 2002). The optimum pH in hydrogen production from starch-synthetic wastewater was 5.2 (Lay *et al.*, 2005). The highest conversion efficiency from beer processing wastes to hydrogen was attained at pH of 5.8 (Lay *et al.*, 2005).

2.4.2.6 Macronutrients

The essential macronutrients in dark fermentation are carbon (C), nitrogen (N) and phosphorus (P) (Wong *et al.*, 2014), (Chairattanamanokorn *et al.*, 2009; Yossan *et al.*, 2012; Pakarinen *et al.*, 2008; Lin *et al.*, 2004). Carbon, obtained merely from various types of substrate, is utilized a food for bacteria. There are some investigator indicated nitrogen is affected to lag time of gas production, which is not help bacteria growth. There are many from of nitrogen, such as ammonium, nitrate nitrite and protein. Especially ammonium, not only to use as a

macronutrient but also provide the capacity of buffer against to organic acid production (Wang *et al.*, 2008; Xiao and Liu, 2009).Phosphorus generally appeared in phosphate form. The appropriate ratio of each nutrient was necessary for maximizing hydrogen production. The most suitable ratio of C/N/P, which should be 100/0.5/0.1 for mesophilic temperature (Argun *et al.*, 2008).

2.4.2.7 Micronutrients

Micronutrient are generally referred to heavy metal, such as nickel (Ni), iron (Fe), Chromium (Co), calcium (Ca), molybdenum (Mo), zinc (Zn) and cupper (Cu),). Ni and Fe are important nutrient to serve as a co-factor for hydrogenase (Oleszkiewicz *et al.*, 1990), which is an enzyme concerning with hydrogen production in Ni-Fe and Fe-Fe form (Frey, 2002; Shima *et al.*, 2004). The higher hydrogen yield by 1.5 fold was achieved by adding 18-55 mg/l Fe (Wang and Wan, 2008). In addition, 0.1 mg/l Ni supplementation in dark fermentation resulted in 2.4 fold increase in hydrogen yield compare to that of non-supplementation (Kim *et al.*, 2011). Calcium (Ca) concentration in the rage of 50-150 mg/l could enhance hydrogen yield (Kim *et al.*, 2011). Moreover, the concentration of 0.0042 mg/l of Molybdenum (Mo) increased the hydrogen yield by 29 % (Niu *et al.*, 2011). Zinc (Zn), copper (Cu) and chromium (Cr) are necessary to dehydrogenase, dismutase, methyltransferase enzyme, which involve in hydrogen production process (Chang and Lin, 2006). The threshold concentration of Zn, Cu and Cr was 0.24 mg/l, 30 mg/l, 15 mg/l, respectively. The higher supplementation than recommended value should be toxic to the system, leading to lower hydrogen production (Li *et al.*, 2007).

2.4.2.7 Temperature

There are two main temperatures for anaerobic biological treatment process.

2.4.2.7.1 Mesophilic Temperature (37 °C)

This is the most convenient temperature in anaerobic digestion because the system can be operated in an ambient temperature. Decomposition of the volatile suspended solids (VSS) is around 40 % over a retention time of 15 to 40 days. The high volume of reactor was required because of high retention time. This temperature cannot eliminate pathogens, which the disinfection system was required.

2.4.2.7.2 Thermophilic Temperature (55 °C)

At this temperature, the organic compound is rapidly broken down to soluble molecule which required a smaller size of reactor volume. The thermophilic temperature gives higher biogas yield and organic degradation, but this condition requires more energy to maintain the system temperature. However, an effective pathogen removal is one of important advantages.

2.4.3 Advantages and Disadvantages of Anaerobic Biological Treatment

Advantages and disadvantages of anaerobic biological treatment are shown in Table 2.1

Table 2.1 Comparison of advantages and disadvantages of anaerobic biological treatment

Advantages	Disadvantages
Consumes little energy (0.18-0.36 MJ/m^3) depending on the need for pumping and recycling effluent.	Longer start-up time to develop necessary biomass inventory
Less biological sludge production	May requires alkalinity addition
Fewer nutrients required	Much more sensitive to the adverse effect of lower temperatures on reaction rates
Provision of energy source through methane recovery	May requires further treatment with an aerobic treatment process to meet discharge requirements
Smaller reactor volume required	May be more susceptible to upset due to toxic substances
Elimination of off-gas air pollution	Potential to produce odors and corrosive gases
Rapid response to substrate addition after long periods without feed	Biological nitrogen and phosphorus removal is not possible.
Raw waste stabilization	
Relatively odor-free end-products	

Table 2.1 Comparison of advantages and disadvantages of anaerobic biological treatment (con't.)

Advantages	Disadvantages
Modern anaerobic treatment can handle very high loads, exceeding values of 30 g COD/L/day 30 °C and up to 50 g COD/L/d at 40 °C for medium strength, mainly soluble wastewater. The construction costs are relatively low.	
The space requirements of this process are lower than conventional systems.	
During anaerobic biological treatment, biodegradable compounds are effectively removed, leaving a number of reduced compounds in the effluents, as well as ammonium, organic N-compounds, sulfide, organic P-compounds and pathogens. Depending on the further use, a complementary treatment step is needed.	

2.4.4 Anaerobic Treatment Processes

Anaerobic treatment processes include anaerobic suspended growth, anaerobic sludge blanket and attached growth anaerobic processes:

2.4.4.1 Anaerobic Suspended Growth Processes

Anaerobic suspended growth processes are classified in three types:

(1) Complete-Mix Process

In the complete-mix anaerobic process, as shown in Figure 2.3(a), the hydraulic retention and solid retention times are equal. Generally, hydraulic retention time may be in the range of 15 to 30 d to provide sufficient safety

factor for operation and process stability. This process without sludge recycle is more suitable for waste that have high concentrations of solid or extremely high dissolved organic concentration, where thickening the effluent solids is difficult. Typical organic loading rates for this process are present in Table 2.1, are compared with anaerobic contact and anaerobic sequencing reactor processes.

(2) Anaerobic Contact Process

The anaerobic contact process, as shown in Figure 2.3(b), overcomes the disadvantages of a complete-mix process without recycle. Biomass is separated and returned to the complete-mix or contact reactor. So that the solid retention time (SRT) is longer than hydraulic retention time (τ). The anaerobic reactor volume can be reduced by increasing SRT values with a shortτ value. Gravitational separation is the most common approach for solid separation and thickening prior to sludge recycle. In some cases, gas flotation is used for solid separation by dissolving the process off-gas under pressure, which has been used in place of gravitational separation. Since the reactor sludge contains gas produced in the anaerobic process and gas production can be continue in the separation process. Solid-liquid separation can be inefficient and unpredictable.

(3) Anaerobic Sequencing Batch Reactor Process

The anaerobic sequencing batch reactor process, as shown in Figure 2.3(c), describes a suspended growth process with reaction and solid-liquid separation in the same vessel. The processes are much like that of aerobic sequencing batch reactor (SBR). The operation of ASBR consists of four steps: (1) feeding, (2) reacting, (3) settling and (4) decanting. During the reacting period, intermittent mixing for a few minutes each hour is introduced to provide uniform distribution of substrate and solid. The organic loading of the process can be changed by selecting hydraulic retention times from 6 to 24 h. At 25 °C, 92 to 98 % COD removal. This process can be achieved at volumetric organic loading of 1.2 to 2.4 kg COD/m^3d.

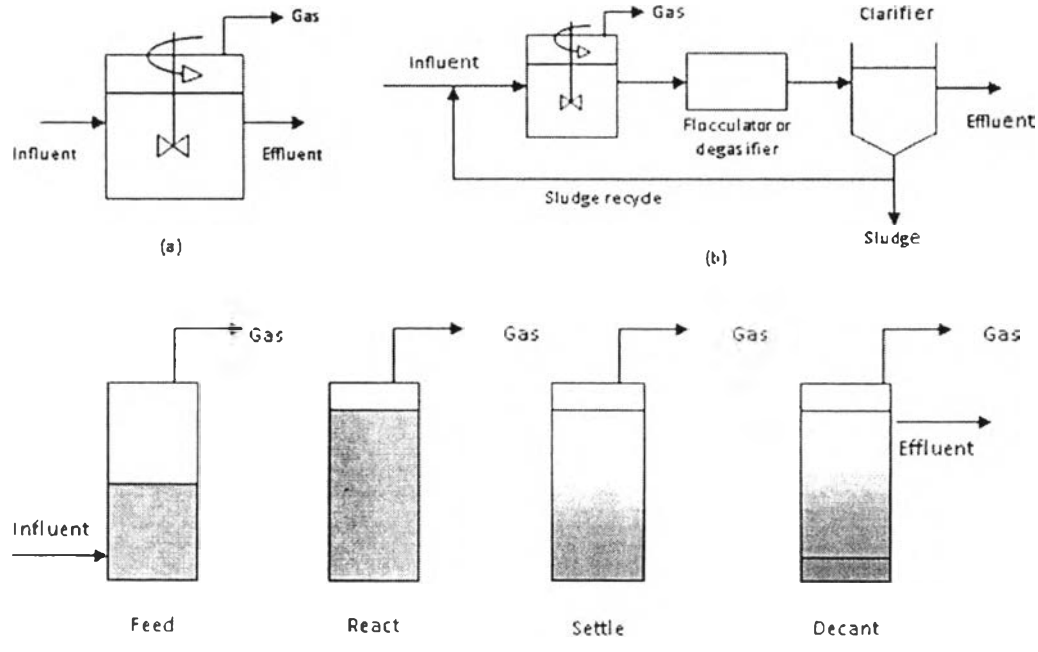

Figure 2.3 Anaerobic suspended growth processes: (a) complete-mix process, (b) anaerobic contact process and (c) anaerobic sequencing batch reactor process.

Table 2.2 Typical organic loading rates for anaerobic suspended growth processes at 30 °C

Process	Volumetric Organic Loading, kg COD/m^3 d	Hydraulic Retention Time τ, day
Complete-mix	1.0-5.0	15-30
Anaerobic contact	1.0-8.0	0.5-5
Anaerobic sequence batch reactor	1.2-2.4	0.25-0.50

2.4.4.2 Anaerobic Sludge Blanket Processes

This process uses an anaerobic process, whilst forming a blanket of granular sludge and being suspended in the tank. The key feature of this process is that the anaerobic sludge inherently has superior flocculation and settling characteristics, which favorably provide the physical and chemical conditions for sludge flocculation. When these conditions are met, a high solid retention time (at high HRT loadings) can be achieved, with separation of the gas from the sludge. One of the most notable developments in anaerobic treatment process technology is the upflow anaerobic sludge blanket (UASB) reactor. The principal types of anaerobic sludge blanket processes include (1) the original UASB process and modification of the original design, (2) the anaerobic baffled reactor (ABR) and (3) the anaerobic migrating blanket reactor (AMBR). Of these sludge blanket processes, the UASB is the most common used process for treating a wide range of industrial wastewater.

(1) Upflow Sludge Blanket Reactor Process

The basic UASB reactor is illustrated in Figure 2.4(a). The influent wastewater is distributed at bottom of the UASB reactor. Then it travels in an upflow mode through the sludge blanket. Critical elements of the UASB reactor design are the influent distribution system, the gas-solid separator and the effluent withdrawal design. Modifications to the basic UASB design include adding a settling tank, as shown in Figure 2.4(b), or the use of packing material at the top of the reactor, as shown in Figure 2.4(c). Both modifications intend to provide better solid capture in the system. Additionally they prevent loss of large amounts of the UASB reactor solid due to process upsets or changes in the UASB sludge blanket characteristics and density.

The key feature of the UASB process is that the development of a dense granulated sludge allows it to use high volumetric COD loadings compare to other anaerobic processes.

(2) Anaerobic Baffled Reactor Process

In the anaerobic baffled reactor process (ABR), as shown in Figure 2.5(a), baffles direct the flow of wastewater in an upflow mode through a series of sludge blanket reactors. The sludge in the reactor rises and falls with gas

production. However it flows through the reactor at a slow rate. Various modifications have been made to the ABR to improve its' performance. The modifications include: (1) changes of the baffle design, (2) hybrid reactors, where a settler is used to capture and return solids, or (3) packing is used in the upper portion of each chamber to capture solids.

(3) Anaerobic Migrating Blanket Reactor

The anaerobic migrating blanket reactor (AMBR) process is similar to the ABR with additional features of mechanical mixing in each stage and an operating approach to maintain the sludge in the system without resorting packing or settlers for additional solid capture, as shown in Figure 2.5(b). In the AMBR process, the influent feed point is changed periodically to the effluent side. And the effluent withdrawal point is also changed. By this way, the sludge blanket remains more uniform in the anaerobic reactor. The flow is reversed when a significant quantity of solids accumulates in the last stage.

2.4.4.3 Attached Growth Anaerobic Processes

Attached growth anaerobic treatment reactors are varied by the type of packing used and the degree of bed expansion. There are four types of attached growth processes:

(1) Upflow Packed-bed Reactor

In the upflow packed-bed reactor, as shown in Figure 2.6(a), the packing is fixed. The wastewater flows up through the interstitial spaces between the packing and biogrowth. Effluent recycle is generally not used for the packed-bed reactor, except for high-strength wastewaters. While the first upflow anaerobic packed-bed processes contained rock, a variety of design employing synthetic plastic packing is used currently. A large portion of the biomass is treated in the upflow attached growth anaerobic processes. It is loosely held in the packing void spaces and not just attached to the packing material. Low upflow velocity is generally kept to prevent the washout of the biomass. Over the time, solids and biomass will accumulate in the packing causing to plug and flow short-circuiting. At this point, solids must be removed by flushing and draining from the packing.

Advantages of upflow attached growth anaerobic reactors are: (1) high COD loading, (2) relatively small reactor volume and (3) operational simplicity. Major limitations are: (1) high cost of the packing material and (2) maintenance required associate with solid accumulation and possible packing plugging. The process is best suited for wastewater with low suspended solid concentration.

(2) Upflow Attached Growth Anaerobic Expanded-bed Reactor

The anaerobic expanded-bed reactor (AEBR), as shown in Figure 2.6(b), contains silica sand with diameters range from 0.2 to 0.5 mm and specific gravity of 2.65 as packing material which is support of biofilm growth. Recycle is employed to provide upflow velocity, resulting to 20 % bed expansion. The smaller packing size provides greater surface area per unit volume, theoretically fertilizes greater amount of biomass growth. The packing void fraction is about 50 % when expanded. With such a small packing and void volume, the expanded-bed operation is necessary to be prevented from plugging. Because the expanded-bed system is not fully fluidized, some solids can be trapped; as a result some degree of solid degradation occurs.

(3) Attached Growth Anaerobic Fluidized-bed Reactor

Anaerobic fluidized-bed reactor (AFBR) design, as shown in Figure 2.6(c), is physically similar to the upflow expanded-bed reactor. The packing size is more or less the same as the expanded-bed reactor, but the AFBR is operated at higher upflow liquid velocities of about 20 m/h which need to provide about 100 % bed expansion. Both fluidization and mixing of the packing material occurs in fluidized-bed system. Effluent recycle is required to provide sufficient upflow velocity.

The expanded and fluidized-bed reactors offer more surface area per reactor volume for biomass growth and better mass transfer than the upflow packed-bed reactor. In the other hand, it can capture lower solid.

(4) Downflow Attached Growth Process

The downflow attached growth anaerobic processes, as illustrated in Figure 2.7, have been applied for treatment of high-strength wastewater.

It is possible to employ varieties of packing materials including: cinder block, random plastic and tubular plastic. Systems are designed to allow recirculation of the reactor effluent.

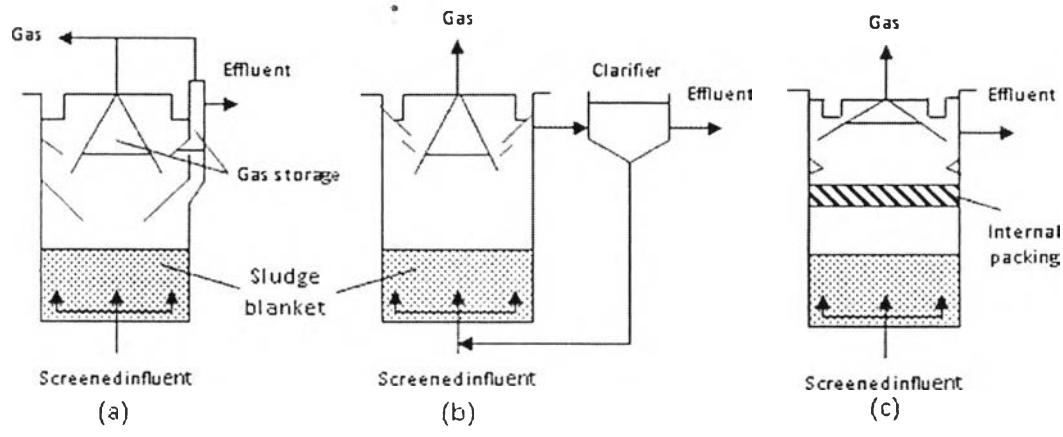

Figure 2.4 Schematic of the UASB process and some modifications: (a) original process, (b) UASB reactor with sedimentation tank and sludge recycle and (c) UASB reactor with internal packing for fixed-film attached growth.

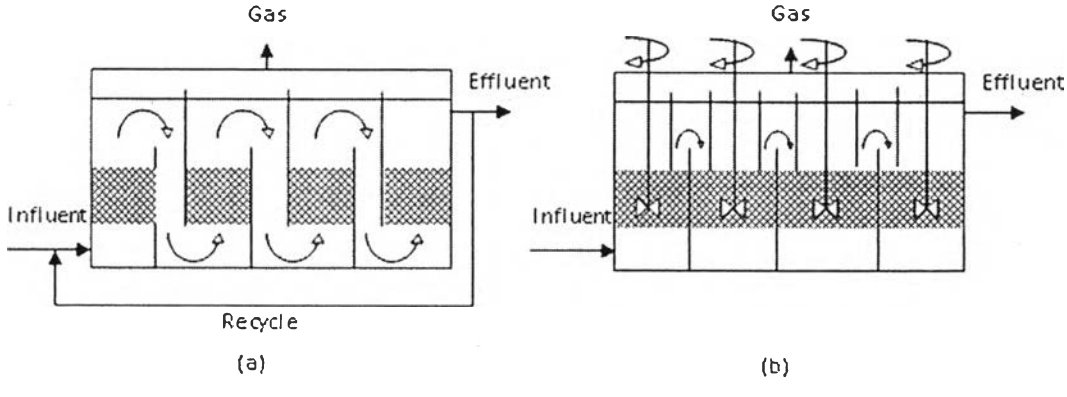

Figure 2.5 Schematic of alternative sludge blanket processes: (a) anaerobic baffled reactor (ABR) and (b) anaerobic migrating blanket reactor (AMBR).

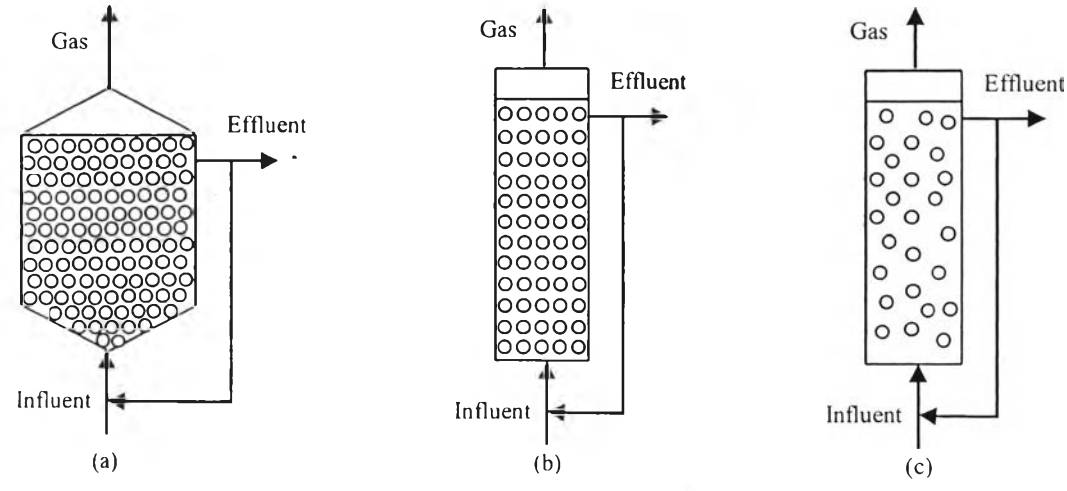

Figure 2.6 Upflow anaerobic attached growth treatment reactors: (a) anaerobic upflow packed-bed reactor, (b) anaerobic expanded-bed reactor and (c) anaerobic fluidized-bed reactor.

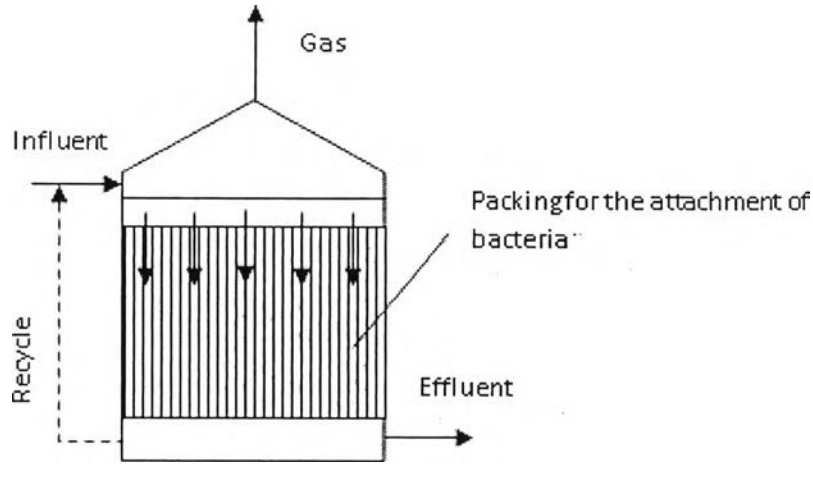

Figure 2.7 Downflow attached growth anaerobic treatment reactor.

2.5 Bio-Ethanol Production

Ethanol or ethyl alcohol, CH_3CH_2OH, is a volatile, clear, colorless and flammable liquid that is the intoxicating agent in liquors and is also used as a fuel or solvent. Ethanol is the most important member of a large group of organic compounds, which are called alcohol. The hydrogen atom of the hydroxyl group can be replaced by an active metal, such as sodium, potassium and calcium, to form a metal ethoxide (ethylate) with the evolution of hydrogen gas.

Ethanol is one of the renewable fuels used for reduction of negative environmental impact. Bio-ethanol, derived from biomass, can be used in various methods as a transportation fuel. It can be used directly in vehicles or blended with gasoline. Adding bio-ethanol in gasoline is an effective way to reduce the petroleum used and also to reduce the emission of greenhouse gas. However, the method for bio-ethanol production is relatively complicated.

2.5.1 Bio-ethanol Feedstock

Bio-ethanol can be produced from agricultural raw materials. It is necessary to find out the cheapest carbohydrate sources for bio-ethanol production. There are different raw materials that have been used in the manufacture of ethanol via fermentation processes, which can be divided into three major groups: (1) sucrose-containing feedstock, (2) starchy materials and (3) lignocellulosic biomass. Based on economical point view, the main feedstock for bio-ethanol production are currently sugars and starch. However, these feedstock are likely to be expensive and also can disturb the food prices. Lignocellulosic biomass is an interesting due to its low cost and high availability and believes to be an ideal raw material for bio-ethanol production in the future.

2.5.1.1 Sucrose-containing Feedstock

Sugar cane, sugar beet, sweet sorghum and fruits are the most important feedstock for production of bio-ethanol. The conversion of sucrose to ethanol is easier than starchy materials because disaccharide can be broken down into ethanol directly, so the hydrolysis of sugar is not required.

2.5.1.2 Starchy Materials

Corn, wheat, rice, potatoes, cassava, sweet potatoes and barley are the high yield feedstock for bio-ethanol production, especially corn and wheat. In order to produce ethanol, hydrolysis is required to break down carbohydrate chain into glucose syrup (simple fermentable sugars), which is then converted to ethanol by yeast with fermentation process.

2.5.1.3 Lignocellulosic Biomass

Biomasses, such as wood, straw and grasses, are attractive to produce bio-ethanol. Lignocellulosic biomass consists of cellulose, hemicellulose, lignin, extractives, ash and other components. Because the structure of lignocellulose is complex, pretreatment step is needed. In the hydrolysis step, cellulose is enzymatically degraded to obtain glucose that is fermented further by yeast to form ethanol. Because this method is more complex than using cane beet or corn as feedstock, it leads to a higher ethanol production cost. However, lignocellulosic materials come from agricultural or domestic wastes. These offer high possibility for bio-ethanol production in large scale. So, it is considered that lignocellulosic biomass will become a main bio-ethanol feedstock for ethanol production in the future.

2.5.2 Bio-ethanol Production

Ethanol is a product of fermentation process by yeast. Fermentation is a sequence of reactions, which release energy from organic molecules in the absence of oxygen. In this application of fermentation, energy is obtained when sugar is changed to ethanol and carbon dioxide. All beverage alcohols and more than half of industrial ethanol are made by this process.

2.5.2.1 Bio-chemical Production of Ethanol by Biomass

This bio-ethanol process consists of four major units: (1) pretreatment, (2) hydrolysis, (3) fermentation and (4) product separation or distillation (Balat and Balat, 2009), as shown in Figure 2.8.

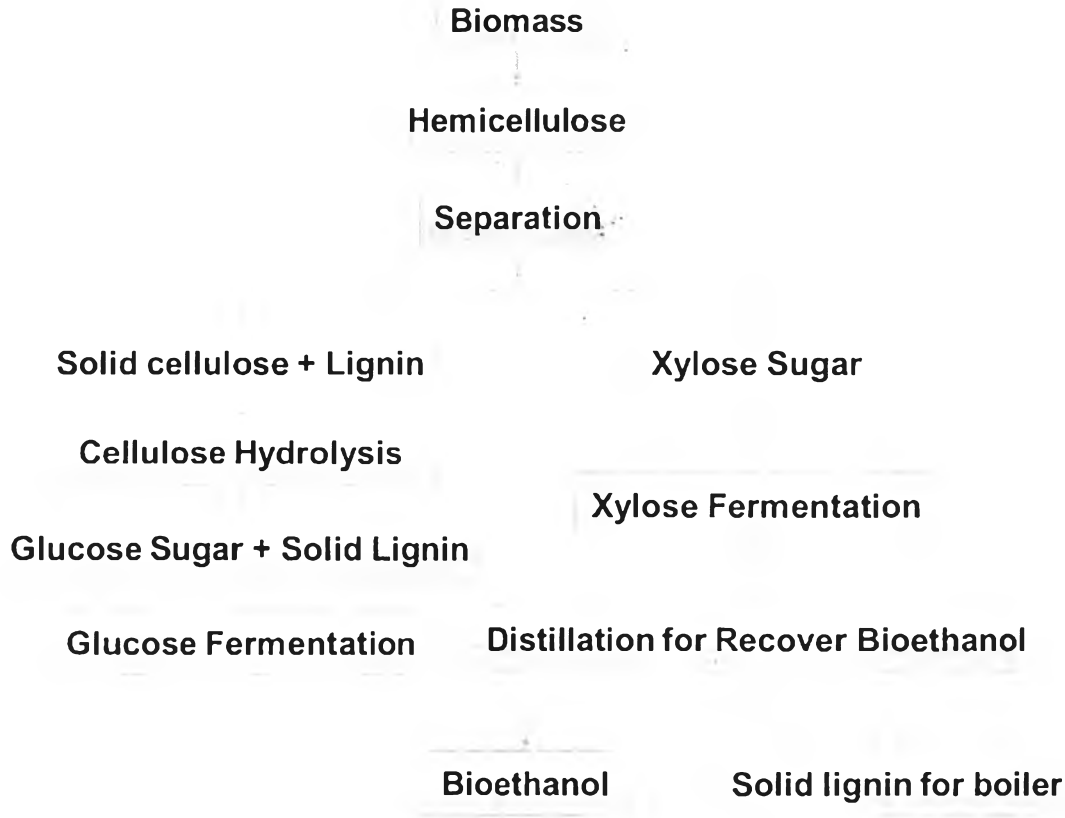

Figure 2.8 Flow chart for the production of bio-ethanol from lignocellulosic biomass (Balat and Balat, 2009).

The hydrolysis processes of lignocellulosic biomass can be classified into two groups:

(1) Enzymatic hydrolysis

(2) Acid hydrolysis, which is the old method for converting lignocellulosic biomass into sugars. Moreover, acid hydrolysis can be divided into two basic types: dilute and concentrated acid hydrolysis.

As shown in Figure 2.8, pre-hydrolysis is the first step for bio-ethanol production. The dilute sulfuric acid is used for hydrolyzing hemicellulose to cellulose, lignin and xylose sugar. After that, cellulose will be hydrolyzed by concentrated acid process to glucose and solid lignin. The obtained xylose sugar and glucose will be fermented in fermentation processes by microorganisms, such as yeast. The overall reaction in the fermentation process is shown in Equation (2.9).

$$C_6H_{12}O_6 \rightarrow 2C_2H_5OH + 2CO_2 \quad (2.9)$$

Theoretically, 1 kg of glucose will produce 0.51 kg of bio-ethanol and 0.49 kg of carbon dioxide. Practically, the actual yield is less than 100 % because the part of glucose is used by microorganisms for growth (Balat and Balat, 2009).

2.5.2.2 Ethanol Fermentation from Molasses

Molasses is an inexpensive raw material for ethanol production. It is a mixture of monosaccharides and disaccharides containing about 50 % of sugar, which can be classified into many types as follows:

- Cane molasses is a by-product of the manufacture or refining of sucrose from sugar cane.

- Beet molasses is a by-product of the sucrose production from sugar beets.

- Citrus molasses is the partially dehydrated juices obtained from the manufacture of dried citrus pulp.

- Starch molasses is a by-product of dextrose manufacture from starch derived from corn or grain sorghums, where the starch is hydrolyzed by enzymes and/or acid.

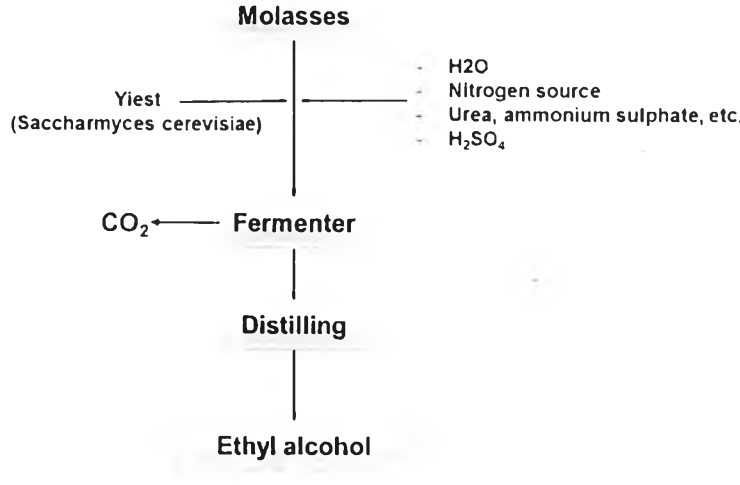

Figure 2.9 Ethanol production from molasses.

In the ethanol production process, as shown in Figure 2.9, molasses is diluted with water to reach a suitable sugar concentration around 15–16 %. A small quantity of nitrogen sources, such as urea, ammonium phosphate and ammonium sulphate, is added as the nutrient supplement for microorganism growth. It is concerned that microorganism used for ethanol production must have a high tolerance for produced alcohol and must produce high amount of ethanol. Yeasts, particularly *Saccharomyces cerevisiae*, which represent the best microorganism used in the production of ethanol, are then added. Yeast has two enzymes for converting the saccharides to ethanol. Firstly, invertase will convert dissacharides (sucrose) to monosaccharides by catalytic hydrolysis reaction and then glucose and fructose are converted to ethanol and carbon dioxide by the zymase enzyme. The pH is maintained at about 5.0. The fermentation starts and is allowed to proceed for about 24–40 h at about 25–30 °C.

2.5.3 Ethanol Wastewater in this Research

The ethanol wastewater in this research was obtained from Sapthip Co., Ltd, Lopburi, Thailand which has the capacity about 200,000 l/d. The ethanol production process is shown in Figure 2.1. First, cassava roots are ground before mixing with water. Then, the mixing solution is hydrolyzed enzymatically before fermentation. After that, the ethanol from fermentation was separated by distillation column. The wastewater coming out at the bottom of distillation column still contains high organic compound in both soluble and insoluble forms. The pretreatment process is required before discharging to the environment.

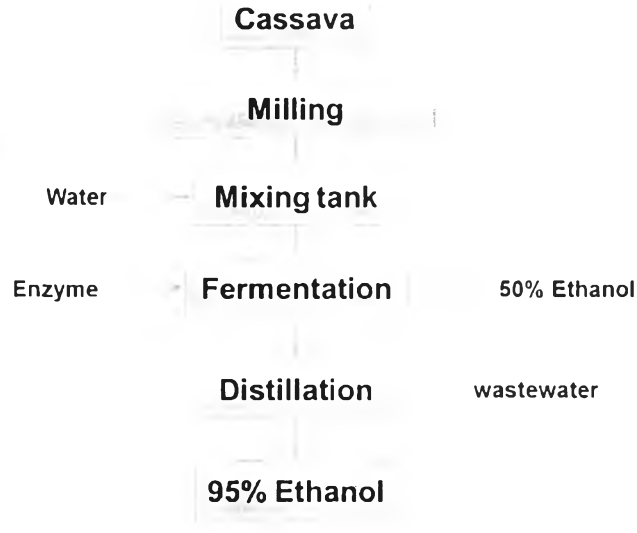

Figure 2.10 Flow diagram of ethanol production process from cassava at Sapthip Lopburi Co., Ltd.

2.6 Literature Review

Intanoo *et al.*, (2012) investigated the hydrogen production from alcohol wastewater using an anaerobic sequencing batch reactor (ASBR) under thermophilic temperature at pH of 5.5. The seed sludge was screened to remove inorganic particles and boiled for 15 min in order to remove methane producing bacteria. The COD:N:P which indicated the sufficiency of nutrient for microbial digestion in wastewater was 100:1:0.4, which higher than the theoretical ratio of 100:1:0.4 under thermophillic temperature (55 °C). The operation was performed until reach a steady state which the gas composition, gas production rate and COD removal is not varied with time. The result indicated that the maximum COD removal (32 %) was obtained at the optimal COD loading rate of 68 kg/m^3 d based on hydrogen UASB unit. The composition of volatile fatty acid (VFA) was propionic acid, valeric acid, acetic acid, butyric acid and ethanol.

Searmsirimongkol *et al.*, (2011) studied the hydrogen production from alcohol distillery wastewater in ASBR unit under mesophilic temperature (55 °C) and pH of 5.5 with 6 cycles per day. The seed sludge was pretreated by boiling for 15 minutes to remove the hydrogen-consuming bacterium. The alcohol distillery wastewater containing high potassium and sulfate. The result showed that the optimum

organic loading rate was 60 mg/m^3 d based on hydrogen UASB unit, hydraulic retention time (HRT) of 16 h which could yield hydrogen of 172 ml H_2/g COD removed, the specific hydrogen production rate (SHPR) of 270 ml H_2/g MLVSS d (or 3310 ml H_2/L_R d). However, beyond 40,000 mg/l of influent COD, the system was toxic by potassium and sulfate and lowered biohydrogen production performance.

Sreethawong *et al.*, (2010) studied the hydrogen production from cassava wastewater using anaerobic sequencing batch reactor (ASBR). The result indicated that the maximum specific hydrogen production rate (SHPR) of 388 ml H_2/g MLVSS d (or 3,800 ml H_2/L_R d), hydrogen yield of 186 ml H_2/g COD removed was at the COD loading rate of 30 kg/m^3 d and 6 cycles per day. After that, the NH_4HCO_3 was added into the system with the COD:N ratio of 100:2.2 under the optimum condition which maximized the SHPR and hydrogen yield to 524 ml H_2/g VSS d (or 5680 ml H_2/l d) and 438 ml H_2/ g COD removed, respectively. The excess nitrogen resulted in higher organic acids and ethanol production which decreased hydrogen production efficiency.

Jung *et al.*, (2013) investigated the production of hydrogen and methane from organic waste by a two-stage fermentation system. A two-stage fermentation system consisted of hydrogen ASBR unit following with comparison of methane production in ASBR and UASB unit. A two-stage system was operated at mesophilic condition (37 °C) with recycled of methane effluent as a diluting water in hydrogen fermentation. The ASBR was optimized at 6 cycles per days, with 0.05h filling, 18.9 h reaction and 5h decanting. The substrate was food waste taken from canteen and sewage sludge from wastewater treatment plant. The result showed that carbohydrate was degraded by 91 % in hydrogen fermenter and 90 % in the methane fermenter. A small amount of additional sewage sludge could enhance the hydrogen production performance. The total carbohydrate degradation is over 98 % after 15 days of hydraulic retention time (HRT) which was optimal condition. The alkalinity in hydrogen fermenter could be reduced 50 % when a use of methane ASBR effluent better than that of UASB effluent because the remaining organic compound contained in methane ASBR effluent was much higher than UASB unit.

Veeken *et al.*, (2000) studied the anaerobic hydrolysis rate of organic solid waste at fixed volatile fatty acid (VFA) concentrations ranging from 3 to 30 g COD/l and fixed pH values between 5 and 7. For separate control of both VFA and pH, a

special completely mixed reactor was designed. Results showed that hydrolysis of the organic solid waste followed first-order kinetics. Using a statistical analysis found that the hydrolysis rate constant was pH dependent but was not related to the total VFA and undissociated VFA concentrations.

Denise *et al.*, (2012) studied the effect of pH control and hydraulic flush on hydrolysis and VFA production and profile in anaerobic leach bed reactors digesting a high solids content substrate. The results showed that buffering at pH~6.5 improved hydrolysis (volatile solid (VS) degradation) and VFA production by ~50 %. Butyric and acetic acid were dominant, when reactors were buffered, while only butyric acid was produced at low pH. Hydraulic flush enhanced VS degradation and VFA production by ~15 % and ~32 %, respectively. Most Probable Number (MPN) of cellulolytic microorganisms indicated a wash out when hydraulic flush was applied, but pH control helped to counteract this. The highest VS degradation (~89 %), VFA yield (0.84 kg COD/kg VS added) and methane potential (0.37 m^3 CH_4/kg VS added) were obtained when pH control and hydraulic flush were applied.

Zitomer *et al.*, (1998) studied methanogenesis under oxygen-limited conditions and showed that the methanogenic activity can sometimes be even higher under microaerobic conditions in comparison with a purely anaerobic system.

Kato *et al.*, (1993) studied the effect of oxygen exposure on the methanogenic activity of anaerobic granular sludge. The results showed that the amount of oxygen that caused 50 % inhibition of the methanogenic activity after 3 days of exposure ranged from 7 % to 41 % oxygen. It can be indicated that methanogens located in granular sludge had a high tolerance for oxygen. The most important factor contributing to the tolerance was the oxygen consumption by facultative bacteria metabolizing biodegradable substrates. Uptake of oxygen by these bacteria creates anaerobic microenvironments, where the methanogenic bacteria are protected.

Diaz *et al.*, (2010) studied the removal performance of hydrogen sulphide in severely polluted biogas produced during the anaerobic digestion of sludge by employing pure oxygen. The results showed that the O_2 supply (0.25 Nm^3/m^3 feed) to the bioreactor successfully reduced the hydrogen sulphide content from 15,811 mg/Nm^3 to less than 400 mg/Nm^3.

Botheju *et al.*, (2010a) studied the impacts of limited aeration on an anaerobic bio-gasification process by anaerobic bioreactors operated at 35 °C in both semicontinuous and batch feed modes. The result showed that an increase in methane yield increased in the range of oxygenation loads of 0-16 % (% O_2 of COD input). The methane generation rates at the low oxygenation levels of 1.3 and 2.6% were higher than the strict anaerobic condition, while the prolonged higher oxygenation level of 4 % induced a slight negative impact on methane production. An accumulation of volatile fatty acids in aerated condition at the startup of the continuous feed reactors was lower than the strict anaerobic reactor. The positive effect of oxygen on methane production had much larger range in the batch feed mode compared to the semi continuous feed mode. Then, they studied the impacts of partial aeration in anaerobic bio-gasification by laboratory scale bioreactor (5.5 l working volume) operated for more than 120 days at 35 °C under the organic loading rate of 0.33 kg/m^3 d, hydraulic retention time of 33 days with the oxygenation loads of 0, 2.5, 5.0 and 10.1 % (% O_2 of COD input). The result showed that oxygenation under these operating conditions reduced the methane generation together with total biogas generation. The accumulation of volatile fatty acids was extensively reduced by oxygen introduction. Therefore, the authors suggested that methane production can be optimized by limited aeration in the first of two or more stages of anaerobic digestion. Partially aerated anaerobic digestion can be a useful and a stable process for enhanced waste treatment and resource recovery.

Jenicek *et al.*, (2008) studied effects of microaerobic conditions for anaerobic digestion of solid wastes containing slowly biodegradable compounds (high level of sulphur compounds) under mesophilic condition. The results showed that the hydrogen sulfide removal efficiency was very good and stable (average of 99.0 % is a realistic value at high initial concentration of 4,000-8,000 mg/m^3). The presence of the limited amount of oxygen in the digester does not destroy the digestion process, even though, oxygen is not consumed by sulphide oxidation in the systems. The VSS/TSS ratio of the digested sludge decreased due to the better efficiency in VSS degradation, including the decrease of the soluble COD concentration, ammonia, nitrogen and phosphate concentration in the sludge liquor. Besides, the decrease of the relative methane content in biogas was caused by the presence of nitrogen remaining in the

biogas from the dosed air. Therefore, microaerobic conditions (that means controlled dosing of small amount of air or oxygen into digester) was an efficient tool to increase the biodegradability of treated material and/or to increase the activity of methanogenic bacteria.

Jenicek *et al.*, (2010) studied a microaerobic experiments for anaerobic digestion at both high and low sulphide concentrations. The results showed that anaerobic bacteria including methanogens can be active also in microaerobic system. In a mixed culture, strict anaerobes can survive without inhibition as if the facultative microorganisms are able to consume the present oxygen quickly and fully. So, the microaerobic conditions were predominantly used for hydrogen sulphide removal from biogas.

Jenicek *et al.*, (2011) described that microaerobic condition was a condition between aerobic and anaerobic systems such as aerobic system with low oxygen concentration or anaerobic system with limited O_2 supply. Besides, they indicated the potential benefits and drawbacks of microaerobic conditions were as follows:

Potential Benefits of Microaerobic Conditions;

- Augmentation of microbial species diversity
- Desulphurization
- Improvement of organic compounds biodegradability

Potential Drawbacks of Microaerobic Conditions;

- Dilution of biogas by nitrogen if air is used
- Lower methane production (not in all cases)
- Lack of full scale experience
- Oxygenophobia of digester operators

Oxygen is considered as a potential toxic compound during anaerobic digestion, especially, for the end-of-food-chain microorgamisms, the acetogens and principally the methanogens, which are usually regarded as strict anaerobes. However, some previous studies showed that microaeration can be used in anaerobic digestion.

Diaz *et al.*, (2011) studied effect of the limited oxygen supply on the degradation kinetics of cellulose in batch-tests. The results showed that the performance of the digestion under anaerobic conditions operation (316±11 ml CH_4/g VS fed in 19 day) was lower value and longer period than that of the microaerobic

conditions (327±6 ml CH_4/g VS fed in 15 day). Besides, oxygen did not inhibit methanogenesis, or compete for the consumption of volatile fatty acids, as the methane yield was not reduced in the microaerobic assays.

Lim *et al.*, (2013) studied effect of microaeration pretreatment on the anaerobic co-digestion of brown water and food waste in batch-tests. After 4-day pretreatment with 37.5 ml O_2/L_R d added, the added oxygen was consumed fully by facultative microorganisms. At higher COD solubilization, microaeration pretreatment led to greater VFA accumulation and conversion of short chain fatty acids to acetate, which resulting in enhancement of hydrolytic and acidogenic activities. This study also found that 21 % and 10 % increase in methane yield was observed when microaeration pretreatment was applied to inoculated substrates and substrates without inoculum, respectively.