CHAPTER II
LITERATURE REVIEWS

2.1 Processing of hydrogen productions

2.1.1 Thermo chemical technology

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

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

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

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

For n = 1; m=2n+2

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

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

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

2.1.2 Electrochemical technology

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

2.1.3 Biological hydrogen production

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

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

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

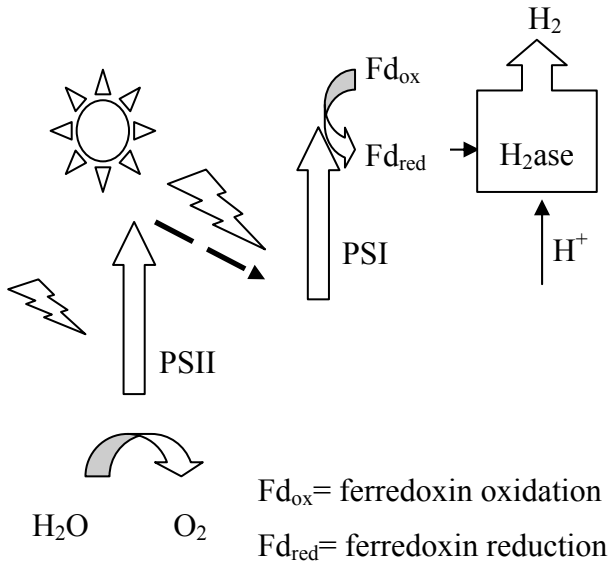

Figure 2.1 Biophotolysis (green algae-cyanobacteria) (Hallenbeck et al., 2009).

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

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

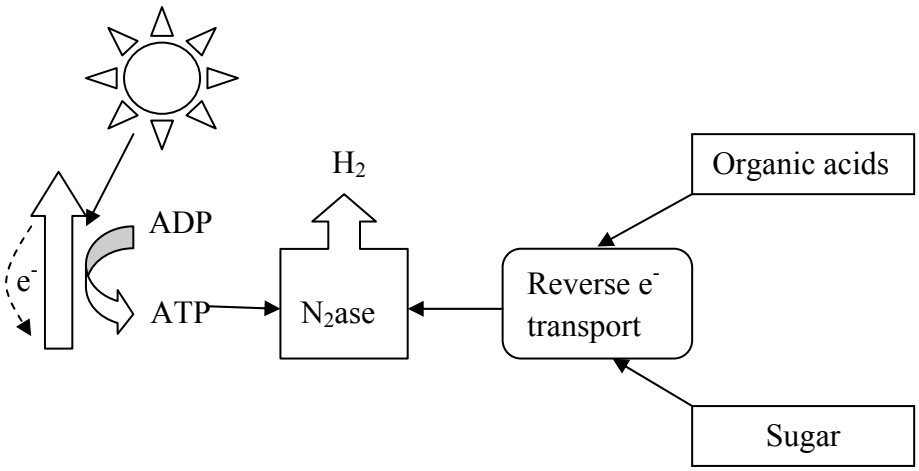

Figure 2.2 Photo-fermentation (Photosynthetic bacteria) (Hallenbeck et al., 2009).

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

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

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

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

NA= not analysis

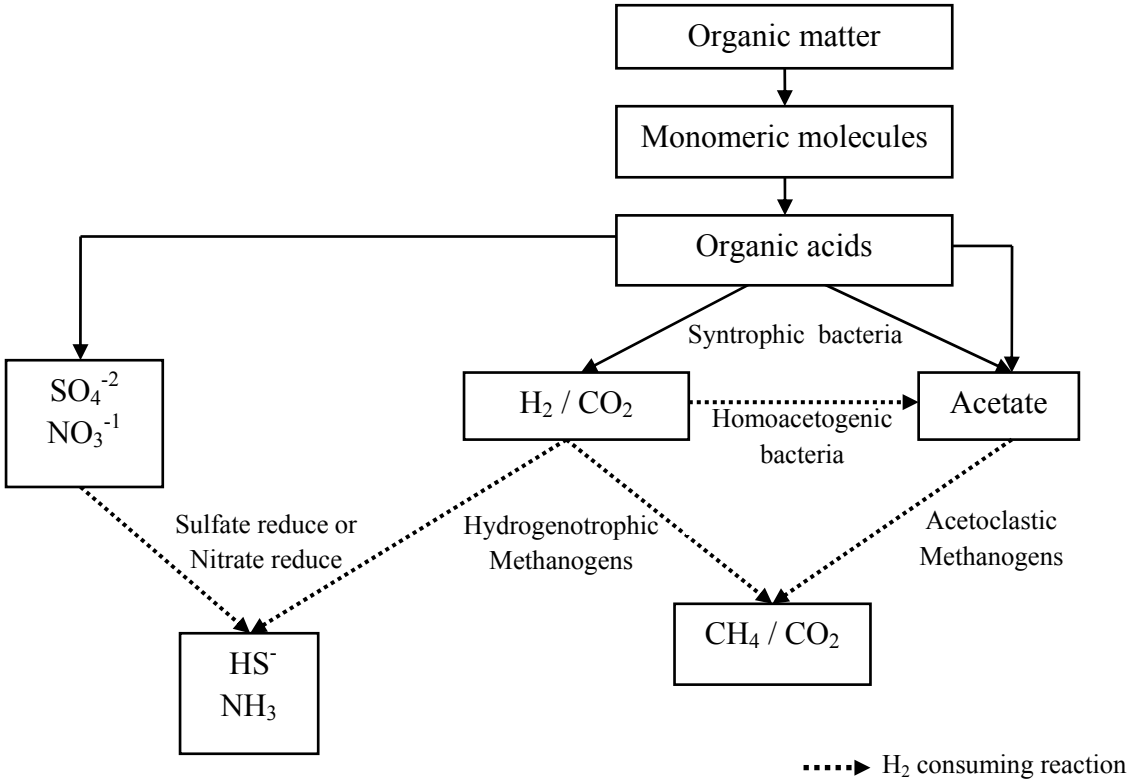

Figure 2.3 Pathway hydrogen role in anaerobic degradation of organic matter (Vazquez and Varaldo, 2008).

2.2 Food waste

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

2.3 Seed sludge

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

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

2.4.1 Mixed culture

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

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

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

2.4.2 pH

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

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

2.4.3 Temperature

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

2.4.4 F/M ratio

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

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

2.4.5 Iron concentration

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

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

2.5 Related researches

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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