# CHAPTER II
# LITERATURE REVIEW

## 2.1 Hydrogen production process

Nowadays, hydrogen is recognized as a renewable energy of the future and a clean fuel due to it does not emit a polluted gas such as carbon, nitrogen or sulfur during combustion. There are several methods for hydrogen production. This topic is summarized about technology of hydrogen production from fossil fuel and alternative resources (Holladay et al., 2009).

### 2.1.1 Hydrogen production from fuel

Fuel processing is converting of hydrocarbon fuels such as gasoline, methanol or methane into a hydrogen rich stream. The important fuel processing technologies are hydrocarbon reforming and pyrolysis.

1) Hydrocarbon reforming is producing of a gas stream that consists of hydrogen, carbon monoxide and carbon dioxide. There are three primary techniques such as steam reforming, partial oxidation (POX) and autothermal reforming (ATR) for hydrogen production from the reforming process. Generally, steam reforming is used in industry because it requires lower operating temperature than POX and ATR, and oxygen is unnecessary input to system. Moreover, it has high thermal efficiency (70 - 85% for methane reforming). However, air emission from steam reforming is the highest of all processes. Partial oxidation is hydrogen production that hydrocarbon was partially oxidized with oxygen. This process is more sulfur tolerant than the other process and it does not require a catalyst. The disadvantages of POX are high operating temperature and low thermal efficiency (60 - 75% for methane reforming). For Autothermal reforming, the heat is generated using the partial oxidation and hydrogen production is increased using steam reforming. The operating temperature of ATR is lower than POX. It does not require external heat source but it requires air or

oxygen. The thermal efficiency for methane reforming is 60 - 75%. Hydrocarbon reforming reactions can be generalized as follows:

Steam reforming

$C_mH_n + mH_2O \rightarrow mCO + (m + ½n)H_2$

$\Delta H$ = hydrocarbon dependent, endothermic

Partial oxidation

$C_mH_n + ½mO_2 \rightarrow mCO + ½H_2$

$\Delta H$ = hydrocarbon dependent, exothermic

Autothermal reforming

$C_mH_n + ½mH_2O + ¼mO_2 \rightarrow mCO + (½m + ½n)H_2$

$\Delta H$ = hydrocarbon dependent, thermally neutral

2) Pyrolysis is a hydrogen production process that hydrocarbon is decomposed into hydrogen and carbon using the high temperature. This process does not have carbon monoxide and carbon dioxide emission because it does not present water or oxygen in operating process. The reaction can be written as follows:

$C_mH_n \rightarrow mC + ½mH_2$

$\Delta H$ = hydrocarbon dependent

### 2.1.2 Hydrogen production from water

This hydrogen production process produces hydrogen and oxygen from water splitting. The water splitting can be divided into three categories that are electrolysis, thermolysis and photoelectrolysis.

1) Electrolysis is using two electrodes that are passed through with electrical current for separating of hydrogen and oxygen from water. It is a conversion of electrical energy to chemical energy. Alkaline electrolyzer is the most common electrolysis technology and lowest in capital cost. The efficiency of alkaline systems is

50 - 60% based on the lower heating value of hydrogen. The reactions at the anode and cathode for alkaline electrolyzer as follows:

Anode: $4OH^- \rightarrow O_2 + 2H_2O$

Cathode: $2H_2O + 2e^- \rightarrow H_2 + 2OH^-$

Overall: $H_2O \rightarrow H_2 + \frac{1}{2}O_2$ $\Delta H = -288$ kj/mol

2) Thermolysis or thermochemical water splitting decomposes water to hydrogen and oxygen using heat at high temperature and high pressure. It is known that water usually is separated at 2,500$^oC$. At this temperature and including of sustainable heat sources, it is not easily available for the materials stable.

3) Photoelectrolysis is directly separation of hydrogen and oxygen from water using sunlight. When a photoanode (n-type material with excess electrons) or a photocathode (p-type material with excess holes) is submerged in an aqueous electrolyte result in water is separated to hydrogen ions and gaseous oxygen.

### 2.1.3 Hydrogen production from biomass

Biomass is a renewable resources and it is easily available such as municipal solid wastes, agricultural waste, animal wastes, wastewater etc. There are many hydrogen production technologies from biomass such as gasification, pyrolysis, conversion to liquid fuels, liquefaction etc. and biological hydrogen production is included.

1) Gasification can be converts the biomass into a composition of hydrogen, carbon monoxide, carbon dioxide, nitrogen and methane at high temperature and large amount of tar are produced in the product gas at the temperature 800 - 1000$^oC$. Therefore, the product gas is cleaned and upgraded using a secondary reactor that applies nickel catalysts or calcined dolomite. Regularly, gasification require large amount of material to be continuously feed to system. Therefore, the high logistic cost is a limit for the gasification plants to be placed.

2) Biological hydrogen (biohydrogen) production produces hydrogen using organisms such as microorganisms, green algae or blue-green algae. There are three main types of biohydrogen production process as follows (Sinha and Pandey, 2011):

- Direct photolysis is generating of chemical energy in the form of hydrogen by using microalgaes photosynthetic system transform solar energy. The photosynthetic systems are photosystem I (PSI) producing reductant for carbon dioxide reduction and photosystem II (PSII) splitting water and developing oxygen. The microalgaes such as green algae and *Cyanobacteria* (blue-green algae) have ability to produce hydrogen because they contain of hydrogenase. In the process, when PSII absorbs solar energy result in the electrons are generated. Then, they are transferred to the ferredoxin (Fd) when PSI adsorbs solar energy. After that, they are accepted and produced to hydrogen by hydrogenase as shown in Figure 2.1 (Ni et al., 2006).

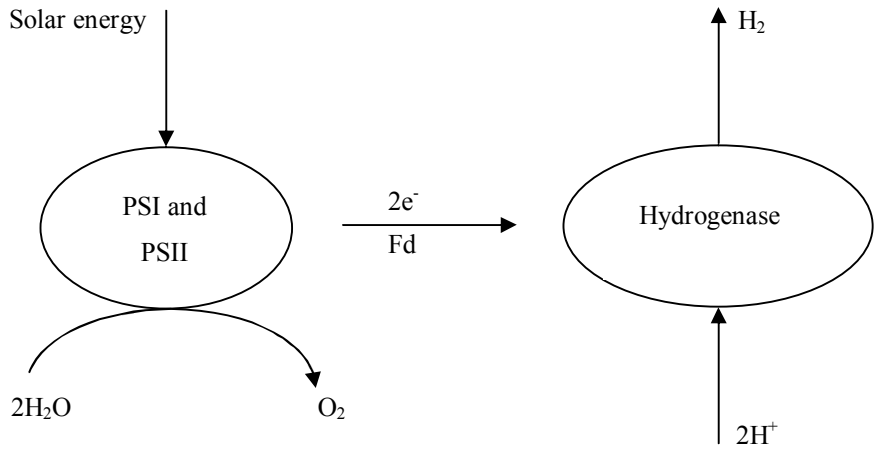


**Figure 2.1** Diagram of direct photolysis (Ni et al., 2006).

- Dark fermentation is hydrogen production at 30 - 80$^oC$ in dark condition using anaerobic bacteria on carbohydrate rich substrates. The products of this process are mostly hydrogen and carbon dioxide mixed with other gas such as methane or hydrogen sulfide depend on the reaction and the substrate that differ from the only hydrogen product of direct photolysis. The hydrogen 4 mole is produced per mole

glucose when the end product is acetic acid. If the end product is butyrate, hydrogen 2 mole is produced (Ni et al., 2006; Holladay et al., 2009).

- Photo fermentation, also called photosynthetic bacterial hydrogen production, is hydrogen production from solar energy and biomass or organic acids by nitrogenase. The advantage of this process is bacteria can be used under various conditions. However, this process has also the disadvantages are that the nitrogenase activity is not fast and amount of energy are greatly required (Ni et al., 2006; Holladay et al., 2009).

This research used dark fermentation for hydrogen production from food waste. Fermentative hydrogen production has main advantages of rapidly hydrogen production rate and easily operation in comparison to photosynthetic hydrogen production (Sinha and Pandey, 2011). The advantage and disadvantage of three types of biohydrogen production process are demonstrated in Table 2.1.

**Table 2.1** The comparison of three types of biohydrogen production process (Sinha and Pandey, 2011).

| **Biohydrogen production process** | **Example of organisms** | **Advantages** | **Disadvantages** |
|---|---|---|---|
| Direct photolysis | - Green algae<br>- Blue green algae | - Abundant substrate (water)<br>- Simple products | - Low light conversion efficiency<br>- Oxygen sensitive hydrogenase<br>- Expensive |
| Dark fermentation | - Enterobacteriaceae i.e. *Citrobacter Y-19* | - No require solar input<br>- Simple reactor<br>- Waste management | - Low hydrogen yield<br>- Large quantity of byproducts |
| Photo fermentation | - Purple non sulphur bacteria i.e. *R. sphaeroides* | - Complete conversion of organic waste to $H_2$ and $CO_2$ | - Low light conversion efficiency<br>- High energy demand by nitrogenase |

## 2.2 Fermentation process

Fermentation process involves digestion of organic matter in the nonappearance of oxygen or anaerobic condition. The complex organics are degraded in the four stages of fermentation process (Valdez-Vazquez and Poggi-Varaldo, 2009; Abbasi et al., 2012) (Figure 2.2).

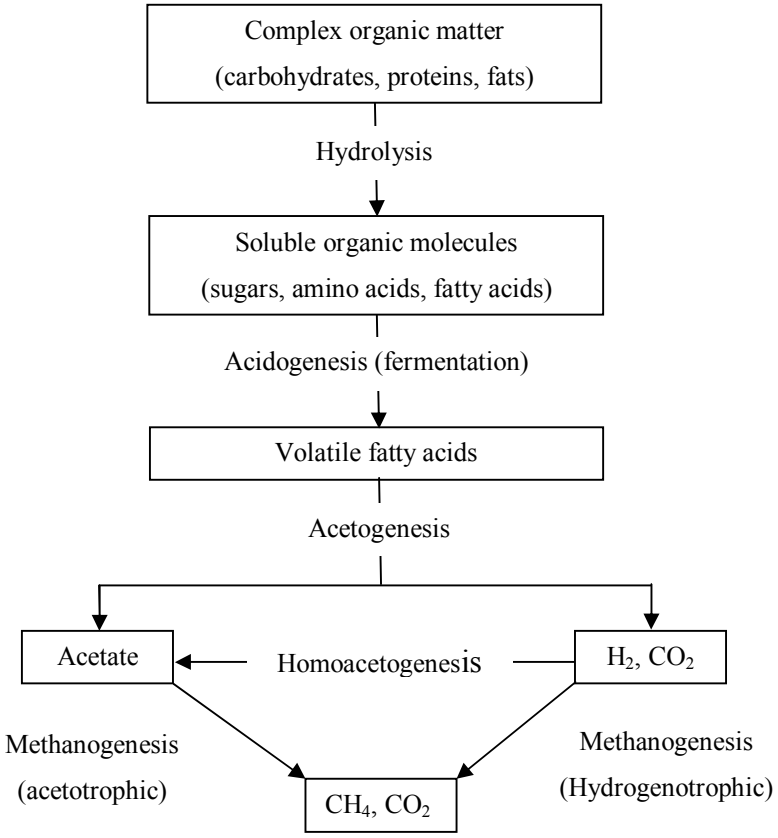


**Figure 2.2** Diagram of fermentation process
(Adapted from Abbasi et al., 2012).

**I Hydrolysis:** The polymer molecules (carbohydrates, proteins, fats) are broken into water soluble monomers (sugars, amino acids, long-chain fatty acids) by exoenzymes (hydrolase) of facultative and obligatorily anaerobic bacteria.

**II Acidogenesis (fermentation):** The monomers are degraded into short-chain ($C_1$-$C_5$) volatile fatty acids (VFAs e.g. acetate, butyrate, and propionate), and alcohols. Hydrogen and carbon dioxide are also produced in this process.

**III Acetogenesis:** The fermentation products are consumed by homoacetogenic microorganisms result in acetate, carbon dioxide and hydrogen are generated. Moreover, autotrophic acetogens generate acetate from carbon dioxide and hydrogen passing through the Wood-Ljungdahl pathway, this process called homoacetogenesis.

**IV Methanogenesis:** Acetate, hydrogen and some of carbon dioxide are consumed to producing methane and carbon dioxide by acetoclastic/hydrogenotrophic methanogens.

Methanogenesis can classified into three pathways depend on methanogenic bacteria (Chandra et al., 2012).

(i) Hydrogenotrophic methanogenesis

$$4H_2 + CO_2 \rightarrow CH_4 + 2H_2O$$

(ii) Acetoclastic methanogenesis

$$CH_3COOH \rightarrow CH_4 + CO_2$$

(iii) Methyltrophic methanogenesis

$$4CH_3OH \rightarrow 3CH_4 + CO_2 + 2H_2O$$

During fermentation process, if there are sulfate or nitrate in the system, hydrogen is used as electron donors to producing sulfide and ammonia by sulfate-reducing bacteria (SRB) and nitrate-reducing bacteria (NRB), respectively. In the fermentation process, hydrogen are both produced and consumed by hydrogen-producing and hydrogen-consuming microorganisms, respectively. Hydrogen is a main intermediate consumed by methanogens, homoacetogens, SRB and NRB. The reactions are shown in Table 2.2. Therefore, the process of fermentative hydrogen production is finished at acetogenesis.

**Table 2.2** Hydrogen-producing and hydrogen-consuming reactions present in fermentation process (Adapted from Valdez-Vazquez and Poggi-Varaldo, 2009).

| Equation | Type of reaction | Reaction |
|---|---|---|
| 1 | Fermentation | $C_6H_{12}O_6 + 2H_2O \rightarrow 2H_2 + \text{butyrate} + 2HCO_3^- + 3H^+$ |
| 2 | Fermentation | $C_6H_{12}O_6 + 4H_2O \rightarrow 4H_2 + 2\text{acetate} + 2HCO_3^- + 4H^+$ |
| 3 | Anaerobic oxidation | $\text{Butyrate} + 2H_2O \rightarrow 2H_2 + 2\text{acetate} + H^+$ |
| 4 | Anaerobic oxidation | $\text{Propionate} + 3H_2O \rightarrow 3H_2 + \text{acetate} + HCO_3^- + H^+$ |
| 5 | Hydrogenotrophic methanogenesis | $4H_2 + HCO_3^- + H^+ \rightarrow CH_4 + 3H_2O$ |
| 6 | Acetogenesis from $CO_2$ and $H_2$ | $4H_2 + 2HCO_3^- + H^+ \rightarrow \text{acetate} + 4H_2O$ |
| 7 | Sulfate reduction | $4H_2 + SO_4^{2-} \rightarrow HS^- + 3H_2O + OH^-$ |

## 2.3 Substrate

There are several substrates that have been applied for fermentative hydrogen production. Glucose, starch and sucrose are commonly used as substrate for study on hydrogen production. Moreover, organic waste such as food waste, agricultural waste, wastewater, etc. are being interested in currently (Wang and Wan, 2009b). Substrate can be divided into mainly four types as follows (Show et al., 2012):

### 2.3.1 Pure carbohydrates

The main source of hydrogen is carbohydrate. The extensive range of carbohydrates can produces hydrogen but pure monosaccharides (glucose), disaccharides (sucrose) and polysaccharides (starch, cellulose and hemicellulose) were used as substrate in most of studies. Simple sugars such as glucose, sucrose and lactose are easily degraded and have a short period of fermentation time, thus they were used as model substrate for biohydrogen production. However, pure carbohydrates might not appropriate to use as raw material for industrial biohydrogen production because they have the high cost. Pure carbohydrates provided the maximum hydrogen yield ranging from 2.40 mol $H_2$/mol hexose (cellulose) to 3.33 mol $H_2$/mol hexose (starch and glucose/or sucrose).

### 2.3.2 Food-based substrates

Food-related wastes such as food residue in municipal solid waste, food industry waste and food industry wastewater can be applied as substrate for biohydrogen production. The food manufacturing industry produces waste and wastewater that have the high carbohydrate in forms of starch, sugars and cellulose. There are various food-based substrates that have been investigated the feasibility of biohydrogen production such as rice and wheat bran, bean curd manufacturing waste, noodle manufacturing waste, sugars factory wastewater, rice winery wastewater, starch manufacturing waste, etc. The food processing waste or wastewater can provided the hydrogen yield ranging from 0.68 mol $H_2$/mol hexose (dehydrated brewery solid waste) to 2.70 mol $H_2$/mol hexose (sweet potato starch residue).

### 2.3.3 Wastewater sludge

Sludge regularly is produced in wastewater treatment process. It is different from carbohydrate-rich substrate because its mainly consists of microorganisms and organic matter. Wastewater sludge might be provided the low hydrogen yield due to a great of protein quantity. However, hydrogen yield can be enhanced by pretreatment of sludge. It has been reported that hydrogen yield of anaerobic activated sludge can be improved from 9.10 mL $H_2$/g dry solids into 16.60 mL $H_2$/g dry solids when substrate was pretreated by alkaline treatment.

### 2.3.4 Agricultural waste

In general, agricultural waste such as wheat straw waste, grass, woody waste, etc. consists of a complex polymer structure in terms of lignin, cellulose and hemicelluloses. In dark fermentation process, cellulose and hemicelluloses can be degraded into hydrogen, excluding lignin. Thus, agricultural waste has to pretreat to break bonding structure of lignin. The mostly pretreatment methods were used such as enzymatic treatment, biological treatment, physiochemical treatment (steaming explosion, acidification) and cooperation of several pretreatment, i.e. milling or extrusion and chemical pretreatment. It has been reported that wheat straw was pretreated with microwave heating and hydrochloric acid (HCl) can be provided the maximum hydrogen yield of 68.10 mL $H_2$/g VS.

This research used food waste from the central cafeteria in university as substrate. Food waste is a main component of municipal solid wastes because it is a major part of organic wastes that our community generates. It is a capability feedstock for biohydrogen production because food waste consists of the high carbohydrate that are the key source of hydrogen (Chu et al., 2008; Chu et al., 2012; Show et al., 2012). In addition to carbohydrate, food waste generally consists of fat and protein but their degradation to hydrogen is less than biohydrogen production from carbohydrate. The hydrogen production potential of carbohydrate-based wasted was reported as 20 times higher than that of fat-based wasted and protein-based wasted (Show et al., 2012).

## 2.4 Seed culture

Seed culture is also called inoculums. It is added in fermentation process to provide microorganisms (Liu et al., 2006). There are two kinds of inoculum that are pure cultures and mixed cultures. Many pure cultures of bacteria have been applied in fermentative hydrogen production especially *Clostridium* and *Enterobacter*. In the environmental such as compost, wastewater sludge, soil, etc., there are widely groups of bacteria that have capability to produce hydrogen. Therefore, these materials can be used as inoculum, especially the mixed cultures of bacteria from municipal sewage sludge, anaerobic sludge, soil and compost. Hydrogen production using the mixed cultures is easy to operate and have a broadly source of feedstock, is compared with that using the pure cultures. However, during biohydrogen production process using the mixed cultures, hydrogen is produced by hydrogen-producing bacteria maybe is used by hydrogen-consuming bacteria. Thus, the mixed cultures are necessary pretreated by some methods to restrain the activity of hydrogen-consuming bacteria whereas still conserving the hydrogen-producing bacteria (Wang and Wan, 2009b).

## 2.5 Pretreatment method

Hydrogen production can be enhanced by inhibition or control the growth of hydrogen-consuming bacteria with thermal and chemical methods (Chaganti et al., 2012). There are several pretreatment methods for restrain hydrogen-consuming bacteria from the mixed cultures such as heat-shock treatment, acid or alkali treatment, chemical treatment, etc. The first two methods were considered to be the effective pretreatment method and were widely used in the research (Wang et al., 2011).

### 2.5.1 Heat-shock treatment

Heat-shock treatment is the ultimate commonly used for inhibit hydrogen-consuming bacteria (Wang and Wan, 2009b). In anaerobic condition, the most important spore-forming bacteria are abundant genuses of acidogenic bacteria. Heat-shock treatment at temperature ~100$^{o}$C for 15 - 120 min has been used to remove non-spore-forming bacteria especially methanogens, while spores of acidogenic bacteria are selected that will germinate and produce hydrogen when the environmental conditions are suitable for growth (Valdez-Vazquez and Poggi-Varaldo, 2009).

### 2.5.2 Acid treatment and alkali treatment

Acid treatment and alkali treatment are pH adjustment of inoculum to acidity and alkalinity, respectively. These treatments are harvesting of the anaerobic consortia. Inoculum was adjusted extremely low or high pH for 24 h using HCl or NaOH, respectively that methanogens can be eliminated (Wang et al., 2011).

### 2.5.3 Chemical treatment

Chemical substances are used to inhibit methane formation of methanogens. There are two major groups of chemical substances that are nonspecific and specific inhibitors. Nonspecific inhibitors such as chloroform ($CHCl_3$), fluoroacetate ($FCH_2COO^-$) and acetylene ($C_2H_2$) inhibit methanogenesis through blocking the activity of some enzymes. Specific inhibitors such as BES (2-bromoethanesulfonate; $BrCH_2CH_2SO_3^-$) and lumazine (2,4-pteridinedione) that they are a structural analog of coenzyme M and some cofactors in methanogenesis, respectively (Valdez-Vazquez and Poggi-Varaldo, 2009).

## 2.6 A two-stage fermentation process

A two-stage process was studied to investigate the feasibility of using byproducts from the first stage as substrate in the second stage. The main product each stage might be same or different that depends on the process. For example, a two-stage fermentation process for hydrogen production by dark fermentation (first stage) and photo fermentation (second stage) that the product both of these processes is hydrogen. Another example, a two-stage process of anaerobic digestion system is often used for hydrogen fermentation (first stage) and methane fermentation (second stage) (Wang and Zhao, 2009).

In dark fermentation process for biohydrogen production, organic matters are converted into hydrogen which is energy carrier. Moreover, the products of biohydrogen production by dark fermentation are also volatile fatty acids and alcohols. As a result, hydrogen fermentation effluent has acidity. Therefore, it is necessary to treat the fermentation effluent that has three main methods for post treatment which are photo fermentation, anaerobic digestion and microbial fuel cells (Show et al., 2012) (Figure 2.3). The operation of anaerobic digestion is similar to a dark fermentation because it is an anaerobic process. Hence, it is an easy maintenance if anaerobic digestion is applied for a post treatment. Moreover, anaerobic digestion gives methane as a product that is a renewable energy and is similar to hydrogen. Hydrogen and methane production from a two-stage fermentation process have several advantages such as increase efficient of substrate digestion, high biogas product and stable system (Yang et al., 2011). Moreover, carbon dioxide and carbon monoxide emission from a two-stage process are less than a one-stage process (Luo et al., 2011).

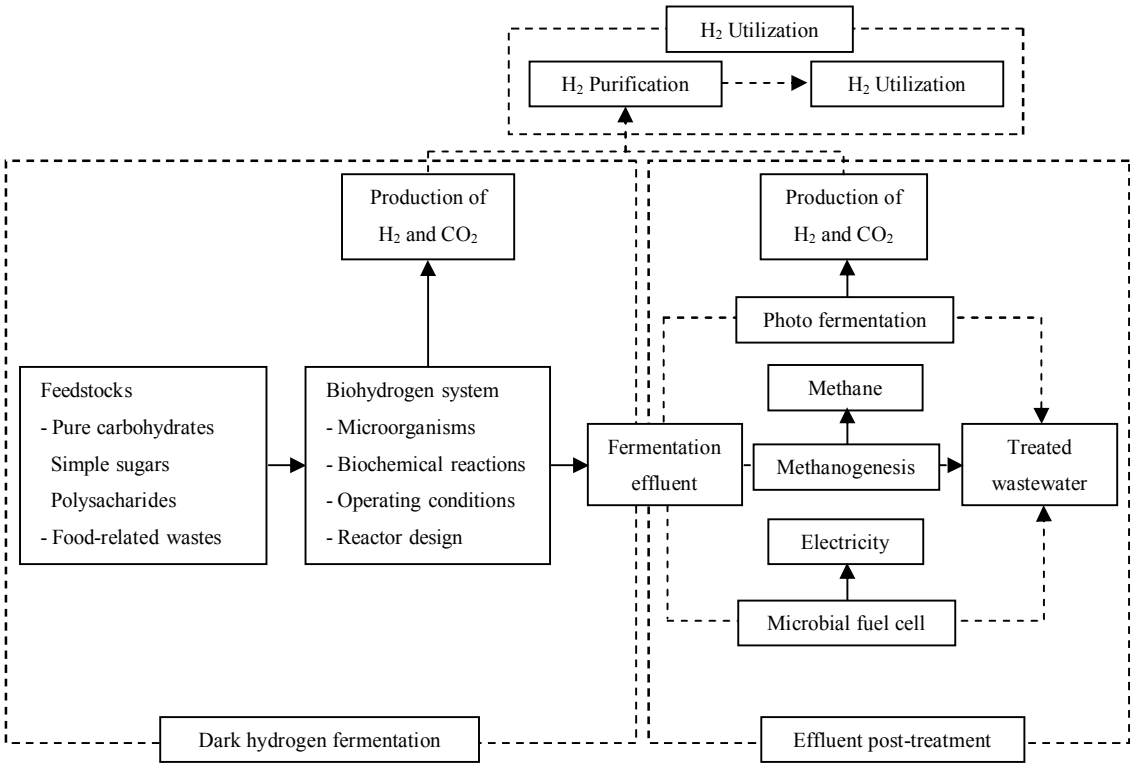


**Figure 2.3** Diagram of a two-stage process (Show et al., 2012).

## 2.7 Factors influencing biohydrogen production

Fermentative hydrogen production is a complicate process and encouraged by various factors such as substrate, inoculum, reactor type, temperature, pH, metal ion, phosphate and nitrogen. These factors at optimal conditions can promotes microorganisms activity and caused to achievement of the process (Wang and Wan, 2009b; Abbasi et al., 2012). The factors such as substrate concentration, initial pH, temperature and C/N ratio was described as follows:

### 2.7.1 Substrate concentration

The optimal substrate concentration can enhance the capability of hydrogen-producing bacteria to generate hydrogen during biohydrogen production. The increase of substrate concentration gives the hydrogen yield more than substrate concentration at low level. However, the high substrate concentration can reduce

hydrogen yield. Furthermore, the appropriate substrate concentration for biohydrogen production still remains confident difference (Table 2.3). The probable explanation for this difference was the variation of source and characteristics of substrate and inoculum, including substrate concentration range studied (Wang and Wan, 2009b).

**Table 2.3** The effect of substrate concentration on biohydrogen production in batch fermentation (Adapted from Wang and Wan, 2009b).

| Substrate | Inoculum | Substrate concentration (g COD/L) | | Maximum $H_2$ yield |
|---|---|---|---|---|
| | | Range studied | Optimal | |
| Sucrose | Mixed cultures | 1.50 - 44.80 | 7.50 | 38.90 mL/g COD culture |
| Sucrose | Municipal sewage sludge | 10.00 - 30.00 | 10.00 | 2.46 mol/mol sucrose |
| Starch | Anaerobic sludge | 9.80 - 39.00 | 9.80 | 67.00 mol/g starch |
| Starch | Anaerobic sludge | 5.00 - 60.00 | 20.00 | 2.20 mol/mol hexose |
| Food waste | Anaerobic digester sludge | 0 - 32.30 | 4.60 | 101.00 mL/g COD |
| Food waste | Anaerobic sludge | 3.20 - 10.70 | 6.40 | 1.80 mol/mol hexose |
| Food wastewater | Waste activated sludge | 10.00 - 160.00 | 40.00 | 47.10 mmol/g COD |

### 2.7.2 Initial pH

Initial pH is an essential factor that influences the activity of hydrogen-producing bacteria since pH affect to hydrogenase. Generally, the hydrogenase activity is low at the pH lower than 5.2 (Valdez-Vazquez and Poggi-Varaldo, 2009). The increase of initial pH can enhance the capability of hydrogen-producing bacteria to produce hydrogen. However, the high initial pH could decrease hydrogen production. Although, the initial pH on fermentative hydrogen production was studied in several researches, the disagreement of the optimal initial pH still exists (Wang and Wan, 2009b) (Table 2.4).

**Table 2.4** The effect of initial pH on biohydrogen production in batch fermentation (Adapted from Wang and Wan, 2009b).

| Substrate | Inoculum | Initial pH | | Maximum $H_2$ yield |
|---|---|---|---|---|
| | | Range studied | Optimal | |
| Food wastewater | Waste activated sludge | 4.0 - 8.0 | 6.0 | 47.10 mmol/g COD |
| Starch | Anaerobic sludge | 4.0 - 9.0 | 6.0 | 92.00 mL/g starch |
| Sucrose | Anaerobic digester sludge | 3.0 - 12.0 | 9.0 | 126.90 mL/g sucrose |
| Sucrose | Compost | 4.5 - 6.5 | 4.5 | 214.00 mL/g COD |
| Sucrose | Municipal sewage sludge | 5.5 - 8.5 | 7.5 | 2.46 mol/mol sucrose |
| Xylose | Municipal sewage sludge | 5.0 - 8.0 | 6.5 | 1.30 mol/mol xylose |

### 2.7.3 Temperature

Temperature is a significant factor because it influences on the activity of hydrogen-producing bacteria and the fermentation process of hydrogen production (Wang and Wan, 2008). The capability of hydrogen-producing bacteria to generate hydrogen can enhance by the increase of temperature. However, the high temperature could reduce hydrogen production. The effect of temperature on biohydrogen production that the mesophilic temperature (around 37$^{o}$C) and thermophilic temperature (around 55$^{o}$C) were studied in many researches (Wang and Wan, 2009b). As is shown in Table 2.5, there exists the difference of the optimal temperature on biohydrogen production.

**Table 2.5** The effect of temperature on biohydrogen production in batch fermentation (Adapted from Wang and Wan, 2009b).

| Substrate | Inoculum | Temperature ($^{o}$C) | | Maximum $H_2$ yield |
|---|---|---|---|---|
| | | Range studied | Optimal | |
| Glucose | Anaerobic sludge | 25 - 55 | 40 | 275.10 mL/g glucose |
| Glucose | Anaerobic sludge | 33 - 41 | 41 | 1.67 mol/mol glucose |
| Sucrose | Anaerobic sludge | 25 - 45 | 35 | 3.70 mol/mol sucrose |
| Sucrose | Anaerobic sludge | 25 - 45 | 35 | 252.00 mL/g sucrose |
| Starch | Municipal sewage sludge | 37 - 55 | 55 | 1.44 m mol/g starch |

### 2.7.4 C/N ratio

In general, the main composition of organic waste is carbon (C) while the nitrogen (N), phosphate (P) and other nutrients are deficient (Argun et al., 2008). Therefore, external addition of other nutrients could improve the activity of hydrogen-producing bacteria and biohydrogen production. Nitrogen is an essential nutrient for microorganisms because it is a component in enzymes, nucleic acids and proteins which are effect on the development of hydrogen-producing bacteria. In the mostly studies, ammonia nitrogen was used as nitrogen source to investigate the effect of nitrogen in term of carbon to nitrogen (C/N) ratio on fermentative hydrogen production (Wang and Wan, 2009b). In generally anaerobic digestion, microorganisms will rapidly consume nitrogen when C/N ratio is too high. On the other hand, when C/N ratio is too low, nitrogen in form of ammonia will be released and accumulated that as a result to the increase of pH in system (Abbasi et al., 2012).

Moreover, there are paltry other details but they are also important such as a specific-surface and dilution of the substrate. The substrate that has greatly specific surface can enhance efficiency of the surface contact between microorganisms and substrate. The small substrate is easily digested by microorganisms. The dilution of the substrate is also necessary. The substrate should be diluted by water to generate slurry. If the slurry is too thin, the solid particles possibly will settle in the bottom of the digester and might not get degraded as it should be. On the other hand, if the slurry is too thick, the blending and resistance the gas flow to the upper part of the digester might tough. Generally, the slurry density should be in the range of 10 - 25% of solids (Abbasi et al., 2012).

## 2.8 Factors influencing biomethane production

The optimal environmental factors for biomethane production and biohydrogen production are different since the difference of microorganisms each process. Methanogenic bacteria are important microorganisms to produce methane in anaerobic digestion process. The pH and temperature are the main factors that directly

influences on the activity of methanogenic bacteria. Furthermore, the other factors such as minerals and C/N ratio also affect on biomethane production.

### 2.8.1 pH

The mostly methanogens have the optimal pH in the range of 7.0 – 8.0, while the hydrogen-producing bacteria has the lower optimal pH (Raposo et al., 2011). However, the pH below 6.6 could inhibit the activity of methanogens and the pH of 6.2 is toxicity to methanogens (Chandra et al., 2012).

### 2.8.2 Temperature

Each species of methanogens has the most suitable condition for their activity and growth in three different temperature ranges that are psychrophilic (< 20$^{o}$C), mesophilic (20 - 40$^{o}$C), and thermophilic (50 - 65$^{o}$C). The temperature in the range of 40 - 50$^{o}$C could inhibit the activity of methanogens. The psychrophilic methanogens can produce the lowest of methane (Chandra et al., 2012). The most appropriate temperature for anaerobic digestion is the mesophilic temperature, while the thermophilic temperature is respected to be the ideal. However, fermentation process under thermophilic temperature is more proficient than that under mesophilic temperature to produce biogas (Abbasi et al., 2012).

### 2.8.3 Minerals

The minerals such as potassium, magnesium, calcium sodium, ammonium, and sulfur in small amount are necessary to microbial metabolism and growth. Moreover, heavy metals such as chromium, lead, zinc, nickel, and copper are also necessary for microbial growth in small amount. However, the high concentrations of minerals and heavy metals could become toxic and inhibits microbial growth (Abbasi et al., 2012).

### 2.8.4 C/N ratio

For anaerobic digestion process, the optimal C/N ratio is ranging from 20 to 30. If C/N ratio is too high, nitrogen will rapidly consumed and then will lack for microbial growth. As a result, biogas is low produced. If C/N ratio is too low, nitrogen

will be released and accumulated in ammonium ion ($NH_4$) form. The pH is increased due to the excess $NH_4$ and it could be toxic to methanogens at the pH higher than 8.5 (Chandra et al., 2012).

## 2.9 Related researches

Nathao et al. (2013) studied biohydrogen and biomethane production from food waste by a two-stage fermentation process. The experiment was carried out at mesophilic temperature (37$^{o}$C). The synthetic food waste was mixed with rice, vegetable and meat at a weight ratio 65, 17, and 18% (w/w), respectively. In biohydrogen production process (stage I), the anaerobic sludge was heated at 90$^{o}$C for 30 min and used as seed sludge. The batch reactor was varied food to microorganism (F/M) ratio in the range of 2.5 – 1.0 and adjusted initial pH at 6.0. Biomethane production process (stage II) was operated after hydrogen was not produced. The anaerobic sludge without heat treatment was added in the byproducts from stage I and then the initial pH was adjusted at 7.0. The result demonstrated that the lag phase time of biohydrogen and biomethane production were approximately 8 and 12 h, respectively. Hydrogen and methane yield increased with increasing F/M ratio from 2.5 to 7.5 and then they decreased at F/M ratio greater than 7.5. The optimal F/M ratio of 7.5 provided the maximum hydrogen yield of 55 mL $H_2$/g VS and the maximum methane yield of 94 mL $CH_4$/g VS.

Chu et al. (2012) studied the biological hydrogen and methane production from food waste materials by a two-stage fermentation process under thermophilic temperature. There are three types of food wastes were used as substrates: bean curd manufacturing waste (*okara*), kitchen garbage and potato. The anaerobic digester sludge was used as seed sludge. The substrates were added in biohydrogen production process (stage I). Then, the effluent from the biohydrogen production flooded to biomethane production process (stage II). The pH was controlled at 5.5 and the temperature was maintained at 55$^{o}$C. The hydrogen production rates of *okara*, kitchen garbage and potato were 0.4, 1.7 and 2.1 L/L/d, respectively. The methane production rates of *okara*, kitchen garbage and potato were 1.4, 1.5 and 1.2 L/L/d, respectively.

Hydrogen and methane yields were in the range of 20 - 85 mL $H_2$/g VS and 329 - 364 mL $CH_4$/g VS, respectively. Hydrogen yield increased in the sequence of potato, kitchen garbage and *okara*, but inversely, methane yield decreased. Hydrogen yield depended on the fraction of carbohydrate and the pH value. Hydrogen and methane yield were increased when the pH value in hydrolysis process was closed to the optimal pH for the activity of hydrogen-producing bacteria and methanogens, respectively.

Reungsang et al. (2012) studied the effect of key factors on biomethane production from hydrogen fermentation effluent. The effluent from biohydrogen production of sugarcane by *C. butyricum* was used as substrate. Hydrogen fermentation effluent contained the high concentrations of butyric acid, acetic acid and chemical oxygen demand. The key factors of substrate concentration (6,591 – 23,409 mg COD/L), $NaHCO_3$ to substrate concentration ratio (0.64 – 7.36) and initial pH (4.48 – 9.52) were investigated in the batch experiment under room temperature of 30$^{o}$C. The result showed that substrate concentration and initial pH was significantly individual influenced on methane yield but the interactive effect of these factors was not significantly affected on methane yield. The maximum methane yield of 367 mL $CH_4$/g VS was achieved at the optimal substrate concentration 13,823 mg COD/L, $NaHCO_3$ to substrate concentration ratio 3.09 and initial pH 7.07. In thus study, methane yield increased with increasing initial pH from 4.48 to 7.0 and then it decreased when the initial pH was increased from 7.0 to 9.52. It was not found methane production at initial pH 4.48 because the activity of methanogenic bacteria was inhibited at the pH value below 6.5.

Giordano et al. (2011) studied biohydrogen and biomethane production by a two-stage dark fermentation. The four kinds of the food industry wastes (common wheat, durum wheat, mashed potatoes and steam-peeling) were used as substrate for biohydrogen production and the byproducts of biohydrogen production was used as substrate for biomethane production. The pretreated anaerobic sludge was used as inoculum in biohydrogen production process and the anaerobic sludge without thermal treatment was used as inoculums in biomethane production process. All the batch experiments were operated at the initial pH 7.0 under mesophilic temperature (35 $\pm$ 1$^{o}$C). The hydrogen recovery and methane recovery of the food industry wastes were

in the range of 3.40 - 12.60% and 52.30 - 69.50%, respectively. The total gas recovery of the food industry wastes was in the range of 61.20 - 75.00%.

Sreela-or et al. (2011b) studied the optimization of key factors on biohydrogen production from co-digestion of food waste and sludge. The key factors such as C/N ratio, inoculum concentration, $Na_2HPO_4$ concentration and Endo nutrient addition were investigated in this study. The anaerobic seed sludge was heated at 105$^{o}$C for 3 h to deactivate hydrogen-consuming bacteria. The batch experiment was operated under room temperature (30 $\pm$ 2$^{o}$C). For the experiment of C/N ratio, total chemical oxygen demand and total nitrogen of food waste and sludge were calculated in C/N ratio. Food waste and sludge were mixed at various ratios for considering the final C/N ratio in the range of 10, 20, 30, 40 and 50. This study indicated that hydrogen yield significantly enhanced with increasing C/N ratio from 20 to 33.14 and then it reduced when C/N ratio greater than 33.14. Thus, the optimal C/N ratio of 33.14 provided the maximum hydrogen yield of 102.63 mL $H_2$/g VS and specific hydrogen production rate of 59.62 mL $H_2$/g VSS h.

Wang and Wan (2011) investigated the effect of temperature accompany with initial pH on biohydrogen production by mixed cultures. Glucose and anaerobic digested sludge were used as substrate and inoculum, respectively. The experiment was designed in the full factorial design. The temperature in the range of 30, 35, 40 and 45$^{o}$C, and the initial pH in the range of 6.0, 7.0, 8.0 and 9.0 were operated in the batch experiments. The result indicated that temperature and initial pH influenced on biohydrogen production. Generally, the optimal temperature and initial pH for biohydrogen production were about 37.8$^{o}$C and 7.1, respectively. In this study, temperature and initial pH which provided the maximum of hydrogen production (38.2$^{o}$C and 7.2), hydrogen production rate (37.4$^{o}$C and 6.9), and substrate degradation efficiency (37.8$^{o}$C and 7.1) were closed to the optimal temperature and initial pH.

Chen et al. (2010) studied biomethane production from various food wastes. The five kinds of food waste from a commercial kitchen, a cafeteria, a soup processing plant, a fish farm and a grease tap collection service were used as substrate. They had a highly different of C/N ratio. The fish waste had the minimum C/N ratio of 3 because of the high protein content, while the cafeteria food waste had the maximum C/N ratio of 23. The individually anaerobic digestion of these food wastes was varied

in food to microorganism (F/M) ratio of 0.5 and 1.0. Then, the batch reactor was conducted under mesophilic temperature (35 $\pm$ 2$^{o}$C) and thermophilic temperature (50 $\pm$ 2$^{o}$C). The result indicated that food wastes from the commercial kitchen, the cafeteria and the soup processing plant had similar biogas production because they contained of the high carbohydrate content. Moreover, the fish waste and grease trap waste had similar biogas production because of the high fat content. All kinds of food waste (except the grease trap waste), Biomethane production at F/M ratio 0.5 under thermophilic temperature provided higher methane yield (350 – 860 mL $CH_4$/g VS) than that under mesophilic temperature (250 – 510 mL $CH_4$/g VS). However, biomethane production at F/M ratio 1.0 under thermophilic temperature provided lower methane yield (250 – 380 mL $CH_4$/g VS) than that under mesophilic temperature (320 – 920 mL $CH_4$/g VS). Biomethane production from cafeteria food waste at F/M ratio of 0.5 under thermophilic temperature provided the maximum methane yield of 380 mL $CH_4$/g VS.

Nazlina et al. (2009) studied the effect of composition of substrate to sludge, initial pH, and temperature on biohydrogen production from food waste. Food waste was mixed with distilled water with a volume ratio of food waste to distilled water of 1:2. Anaerobic sludge was heated at 80$^{o}$C for 30 min to deactivate hydrogen-consuming bacteria and to harvest spore-forming hydrogen-producing bacteria. The batch experiment was carried out by addition various composition of substrate to sludge (90:10, 80:20, 70:30 and 60:40% (v/v)) with adjusted initial pH (5.0, 6.0, 7.0 and 8.0). Then, the batch reactor was operated at various temperatures (35, 40, 50, 55 and 60$^{o}$C). It was reported that hydrogen yield was low obtained at mesophilic temperature in the range of 35 – 40$^{o}$C, while it was higher obtained at thermophilic temperature in the range of 50 – 55$^{o}$C. However, the high temperature of 60$^{o}$C caused inhibition of hydrogen production. For the result of initial pH, it presented that the minimum hydrogen yield was obtained at initial pH 5.0. The maximum hydrogen yield was obtained at initial pH 7.0, followed by initial pH 8.0 and 6.0, respectively. Moreover, the composition of substrate to sludge significantly influenced on hydrogen yield. The composition of substrate to sludge of 90:10% (v/v) provided the minimum hydrogen yield because the high carbohydrate content of substrate might inhibit hydrogen production. The result demonstrated that the composition of substrate to

sludge of 70:30% (v/v), initial pH of 7.0 and temperature of 55$^{o}$C provided the maximum hydrogen yield of 593 mL $H_2$/g carbohydrate.

Argun et al. (2008) studied the effect of C/N and C/P ratio on biohydrogen production by dark fermentation of wheat powder solution. The wheat powder solution contained approximately 97% (w/w) starch, 3.40 mg/g total nitrogen and 1.72 mg/g phosphate. The nitrogen and phosphorus in the wheat composition were not contemplated in C/N and C/P ratio because the available of nitrogen and phosphorus were not examined. In this study, the external nitrogen and phosphorus source from urea ($CON_2H_4$) and $KH_2PO_4$ were considered in C/N and C/P ratio in the range of 20 - 200 and 50 – 1,000, respectively. The anaerobic sludge which was treated by heat-shock treatment was used as seed sludge. The experiment was operated under the initial pH at 7.0 and the temperature at 37$^{o}$C. The results of the Box-Wilson design experiment were hydrogen yield and formation rate. Hydrogen yield increased with increasing C/N and C/P ratio. At the maximum hydrogen yield and formation rate, low nitrogen contents required low phosphorus contents since prevailing stoichiometry of the anaerobic metabolism. Hydrogen formation was probably inhibited at the high concentrations of nitrogen and phosphorous since the metabolic pathway was changed. The maximum hydrogen yield (23 mg $H_2$/g starch) and formation rate (8 mg $H_2$/g biomass/h) were produced with the C/N/P ratio as 100/0.5/0.1. While hydrogen yield and formation rate of the control group were 6.09 mg $H_2$/g starch and 2.36 mg $H_2$/g biomass/h, respectively.

Forster-Carneiro et al. (2008) investigated the effect of different organic fraction of municipal solid wastes on anaerobic digestion. There are three different substrates were used in this study: food waste, organic fraction of municipal solid waste and shredded organic fraction of municipal solid waste. The digested sludge was used as inoculum. The batch experiment was operated under thermophilic temperature at 55$^{o}$C. The result from this study demonstrated that the characteristic of organic substrate influenced on methane production and degradation process. Food waste provided the maximum methane production of 0.18 L $CH_4$/g VS and the minimum degradation of 32.40% VS removal, whereas shredded organic fraction of municipal solid waste provided the lower methane production of 0.05 L $CH_4$/g VS and the higher degradation of 73.70% VS removal. For organic fraction of municipal solid waste,

methane production archived 0.08 L $CH_4$/g VS and it provided the maximum degradation of 79.50% VS removal.

Wang and Wan (2008) studied the effect of temperature on biohydrogen production by mixed cultures. Glucose and digested sludge were used as substrate and inoculum, respectively. The batch experiment was operated at the initial pH 7.0 accompany with the different temperature (20 - 50$^{o}$C). In this study, the potential of hydrogen production improved with increasing temperature from 20$^{o}$C to 40$^{o}$C, and then it reduced when temperature increased from 40$^{o}$C to 55$^{o}$C. In this study, the optimal temperature was 40$^{o}$C that the maximum hydrogen production (269.90 mL) and the maximum hydrogen yield (275.10 mL/g glucose) were achieved. The main soluble metabolites were ethanol, acetate, propionate and butyrate. The concentrations of ethanol and acetate enlarged with increasing temperature from 20$^{o}$C to 35$^{o}$C, and then they decreased with further increasing temperature from 35$^{o}$C to 55$^{o}$C. For each temperature, the hydrogen-producing bacteria have different capability of biodegradation and metabolic production. Moreover, the final pH reduced with increasing temperature from 20$^{o}$C to 35$^{o}$C although that increased with further increasing temperature from 35$^{o}$C to 55$^{o}$C. The final pH in the range of 3.34 - 5.11 was less than the initial pH of 7.0 in all the batch experiment. The possible reason for the value of final pH possibly that *Ethanoligenens harbinense,* the hydrogen-producing bacteria which responsible for hydrogen and metabolites production under the temperature at 35$^{o}$C thus the high concentration of metabolites leads to the low pH.

Lin and Lay (2004) studied the effect of C/N ratio on biohydrogen production from sucrose. The anaerobic sewage sludge was used as inoculum. The C/N ratio of 40 - 130 was varied in the batch experiment under mesophilic temperature (35 $\pm$ 1$^{o}$C). The C/N ratio of 47 provided the high of hydrogen production rate (270 mmol $H_2$/L-day) and hydrogen productivity (4.80 mol $H_2$/mol sucrose). Compared with the control group at the optimal C/N ratio, the hydrogen production rate and hydrogen productivity were increased by 80% and 500%, respectively. This study showed that biohydrogen production was enhanced with C/N ratio because the microorganism metabolic pathway was shifted.