CHAPTER II

LITERATURE REVIEW

2.1 Cassava Wastewater

Cassava starch is produced in Thailand about 18 million tons per year. One kilogram of fresh roots yields 0.2 kg of starch, 0.4-0.9 kg of cake, and about 5-7 L of wastewater (Reungsang *et al.*, 2004). Cassava wastewater is generated from washing and starch extraction processes. Sahamit Tapioca Chomburi Limited Part is a starch production plant in Thailand. The production capacity is about 110,000 kilogram per day. The process of native tapioca starch is shown in Figure 2.1.

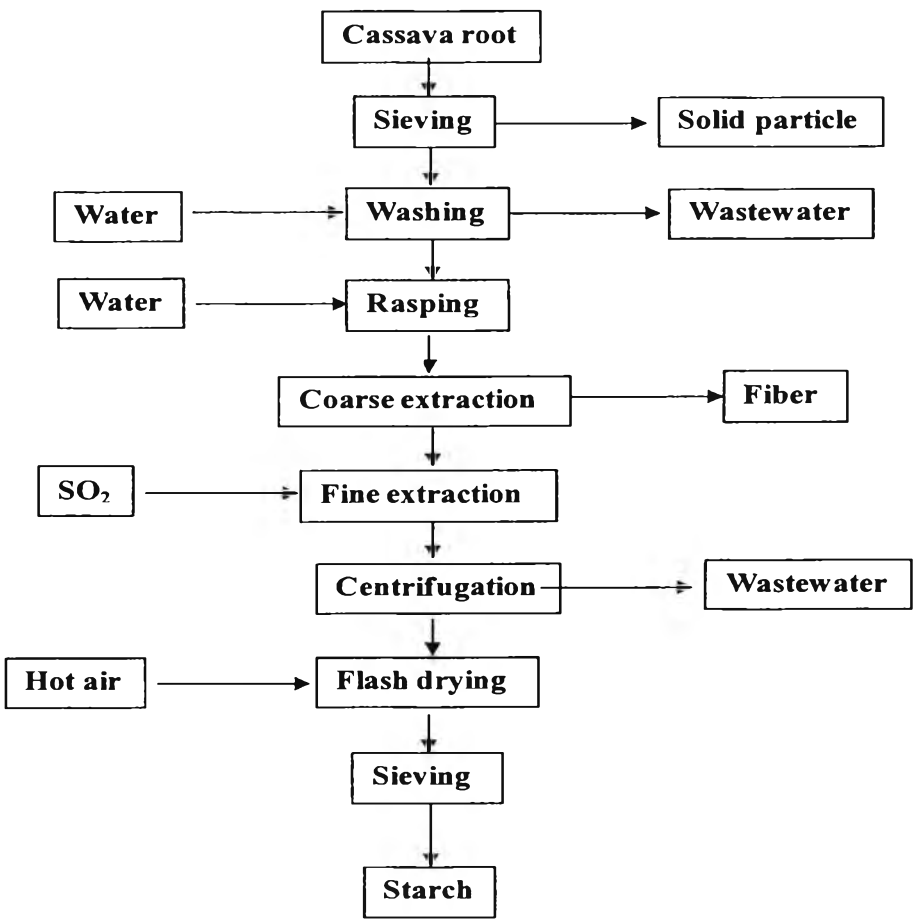

Figure 2.1 Flow diagram of tapioca starch process from cassava roots at Sahamit Tapioca Chomburi Limited Part.

Generally, the processes need to use much water, approximately 30 m^3 per ton of starch. The treatment of the cassava wastewater is a necessary step according to the environmental concern.

2.2 Wastewater Treatment

2.2.1 Fundamental of Wastewater Treatment

The goal of wastewater treatment is to reduce or remove organic matter, solids, nutrients, disease-causing organism, and other pollutants from wastewater. Wastewater treatment is a multi-stage processes to clean wastewater before discharged of the environment. A common set of process that might be used are as follows.

2.2.1.1 Preliminary Treatment

Preliminary treatment is the first step in the wastewater treatment to remove or separate large or hard solid. Grinners bar screens and grit channels are examples of medium in the entering of treatment plant as treatment equipment.

2.2.1.2 Primary Treatment

Primary treatment is the second step in the wastewater treatment to separate suspended materials. Most of the materials do not have a density much different from wastewater, thus they need to be held with enough time to separate. This step allows the suspended materials to settle out at the bottom and skim off at the surface. The clarified wastewater flows to the next stage of wastewater treatment.

2.2.1.3 Secondary Treatment

Secondary treatment is a biological treatment process to remove the dissolved organic matter from wastewater. Generally, the biodegradation of the pollutants can be supplied by microorganisms. The microorganisms are added to the wastewater and adsorb organic matter from wastewater supply. Three approaches are used to accomplish secondary treatment;

- Fixed Film Systems

Fixed film systems grow microorganism on substrate, such as rocks, sand, and plastic. The wastewater is spread over the substrate,

allowing the wastewater to flow past the film of microorganisms fixed onto the substrates. Organic matter and nutrients in wastewater are absorbed on the film, where microorganisms grow and thicken. Examples of fixed film systems are trickling filters, rotating biological contactors, and sand filters.

- Suspended Film Systems

Suspended film systems stir and suspend microorganism in wastewater. Organic matter and nutrients in the wastewater are absorbed by microorganisms, which will grow in size and number.

- Lagoon Systems

Lagoon systems are shallow basins, which hold the wastewater for several months to allow for the natural degradation of wastewater. These systems are slow, cheap, and relatively inefficient, but can be used for various types of wastewater.

2.2.1.4 Final Treatment

Final treatment focuses on removal of disease-causing organisms from wastewater. Treated wastewater can be disinfected by adding chlorine or by using ultraviolet light. High level of chlorine may be harmful to aquatic life in receiving streams. Treatment systems often add chlorine-neutralizing chemical to the treated wastewater before draining to environment.

2.2.1.5 Advanced Treatment

Advance treatment is necessary in some treatment systems to remove nutrients from wastewater. Chemicals are sometimes added during the treatment process to help settle out or skim off phosphorus or nitrogen. Some examples of nutrient removal systems include coagulant addition for phosphorus removal and air stripping for ammonia removal.

2.2.1.6 Sludge Treatment

Sludge is generated through the sewage treatment process. For primary sludge, material that settles out during primary treatment, it has a strong odor and requires treatment prior to disposal. Secondary sludge is the extra microorganisms from the biological treatment processes. The goals of sludge treatment are to stabilize the sludge and reduce odors, remove some of the water and

reduce volume, decompose some of the organic matter and reduce volume, kill disease causing organisms, and disinfect the sludge.

2.2.2 Wastewater Treatment to Remove Pollutants

Industrial wastewater usually contains pollutants both biodegradable and non-biodegradable materials. Thus, the industrial wastewater treatment can be divided into 2 major types.

2.2.2.1 Physical/Chemical Treatment

Physical/chemical treatment is used to treat the non-biodegradable wastewater. A physical process usually treats suspended, rather than dissolved pollutants. It can be dived into two processes, passive and mechanically aided. For the passive process, it allows suspended pollutants to settle down or float to the top depending on the density of suspended pollutants. On the other hand, the mechanically aided process may be used with flocculation, flotation, filtration techniques. A chemical process usually treats dissolvable metal or toxic pollutants into solid or harmless compounds. Dissolved metal pollutants can precipitate in settleable form by adding alkaline materials or organic coagulant aids, like electrolytes, to help flocculate and settle the precipitated metal. Highly toxic pollutants can be converted into harmless compounds by oxidizing them with chloride or using ozone to destroy organic chemicals.

2.2.2.2. Biological Treatment

Biological treatment is commonly used to treat domestic or combined domestic and industrial wastewater. The process keeps wastewater under controlled conditions, so that the cleansing reaction is completed before the water is discharged into the environment. Biological treatment can be divided into 2 types;

2.2.2.2.1. Aerobic Biological Treatment

Aerobic treatment systems require oxygen for microorganisms to be able to digest organic compounds in wastewater to harmless components. Sometimes, the wastewater receives pretreatment before it enters the aerobic unit. Treated wastewater leaving the unit requires additional treatment (passage through a soil absorption field) before being returned to the environment.

2.2.2.2.2. Anaerobic Biological Treatment

In anaerobic treatment, there is an absence of gaseous oxygen. The anaerobic treatment of wastewater has now emerged as an energy saving wastewater treatment technology. Organic compounds are degraded to produce biogas. Due to increasing energy cost in the aerobic treatment, the technique of anaerobic wastewater treatment has gained substantial importance.

The comparison of aerobic and anaerobic biological wastewater treatments is shown in Table 2.1

Table 2.1 Comparison of aerobic and anaerobic biological wastewater treatments

	Aerobic treatment	Anaerobic treatment
Start up	- Short start-up period.	- Long start-up period.
Process	- Integrated nitrogen and phosphorus removal possible. - Production of high excess sludge quantities. - Large reactor volume necessary. - High nutrient requirements.	- No significant nitrogen or phosphorus removal, nutrients removal done via post treatment. - Production of very little excess sludge (5-20%). - Small reactor volume can be used. - Low nutrient requirements
Carbon balance	- 50-60% incorporated into CO_2; 40-50% incorporated into biomass.	- 95% converted to biogas; 5% incorporated into microbial biomass.
Energy balance	- 60% of available energy is used in new biomass; 40% lost as process heat.	- 90% retained as CH_4, 3-5% is lost as heat, and 5-7% is used in new biomass formation.
Residuals	- Excess sludge production. - No need for post-treatment.	- Biogas, nitrogen mineralized to ammonia. - Post-treatment required for removal of remaining organic matter and malodorous compounds.
Costs	- Low investment costs. - High operating costs for aeration, additional nutrient and sludge removal, and maintenance.	- Often moderate investment costs. - Low operating costs due to low power consumption and additional nutrients hardly required.
State of development	- Established technology.	- Still under development for specific applications.

2.3 Anaerobic Biological Treatment

Anaerobic digestion or methane fermentation is a technologically simple process that is used to convert organic materials from a wide range of wastewater types, solid wastes, and biomass into methane. Anaerobes or microorganisms of anaerobic system access oxygen from other sources, which are not the surrounding air. The oxygen source for these microorganisms can be the organic material itself or alternatively may be supplied by inorganic oxides within the input material. When the oxygen source in an anaerobic system is derived from the organic material itself, the intermediate end products are primarily alcohols, organic acid, carbon dioxide, and hydrogen. The intermediates are converted to the final end products of methane and carbon dioxide. The anaerobic digestion process can be subdivided into 4 key biological and chemical stages of anaerobic digestion, depending on its own characteristic group of microorganism.

Stage 1: Hydrolysis

The hydrolysis process is the complex polymers such as carbohydrates, proteins and lipids are broken down into monomers such as sugars, fatty acid, and amino acid by the extracellular enzymes produced by microorganisms (e.g., cellulase, amylase, protease and lipase). Hydrolysis is a relatively slow process, and generally it limits the rate of the overall anaerobic digestion process.

Stage 2: Acidogenesis

The biological process of acidogenesis or acidification is used to further break down the remaining components by acidogenic (fermentative) bacteria, which ferment the monomer to a mixture of low molecular weight organic acid and alcohol. Volatile fatty acids are created along with ammonia, carbon dioxide, and hydrogen sulfide, as well as other by-products. Acidification is affected by a very diverse group of bacteria, the majority of which are strictly anaerobic, i.e. the presence of oxidants like oxygen or nitrate is toxic. Luckily for these strict anaerobes, there are always bacteria present that will use oxygen whenever it is available. The presence of these bacteria is important to remove all oxygen that might be introduced into the system, for instance together with the excess sludge. The acidogenic bacteria are able to metabolize organic material down to a very low pH of around 4.

Stage 3: Acetogenesis

The fermentation products are further oxidized to acetic acid, carbon dioxide, and hydrogen by obligatory hydrogen-producing acetogenic bacteria (acetogens). The first three stages of anaerobic digestion are often grouped together as acid fermentation. It is important to note that in the acid fermentation, no organic material is removed from the liquid phase: it is transformed into a form suitable as substrate for the subsequent process of methanogenesis.

Stage 4: Methanogenesis

Methanogens utilize the intermediate products of the proceeding stages and convert them into methane, carbon dioxide, and water. It is these components that make up the majority of the biogas emitted from the system. Methanogenesis is sensitive to both high and low pHs and occurs between pH 6.5 and pH 8.

2.3.1 Biological Hydrogen Production

The present hydrogen production process can be divided into physical/chemical or thermochemical and biological methods. At the present, hydrogen production is mainly produced from fossil fuels, particularly from natural gas by steam reforming. Hydrogen from thermochemical production usually cannot be regarded as an alternative, pollution free energy source. Regarding a sustainable energy production, the biological production of hydrogen represents a particularly pollution free and energy-saving process. Renewable energy resource, such as biomass, is very interesting to be used for hydrogen production by many routes, as shown in figure 2.2 (Manish *et al.*, 2008).

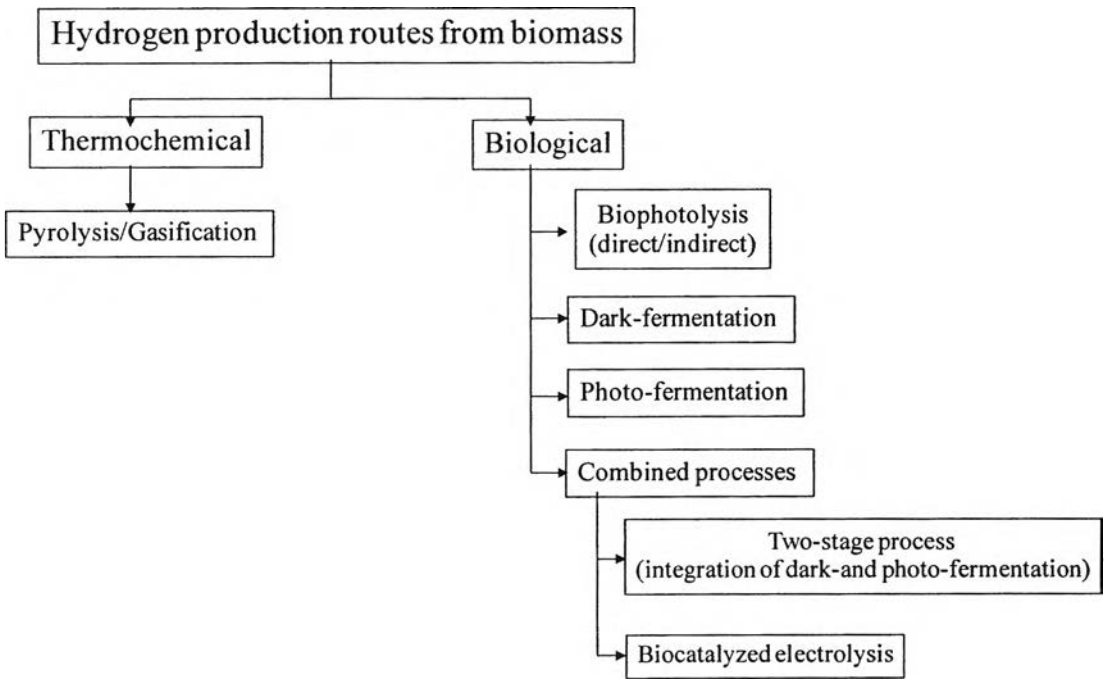

Figure 2.2 Hydrogen production routes from biomass.

2.3.1.1 Biological Hydrogen Processes

Biohydrogen or biological hydrogen production processes can be classified as follow (Manish *et al.*, 2008):

2.3.1.1.1 Biophotolysis of Water by Using Algae and Cyanobacteria

2.3.1.1.1.1 Direct Biophotolysis (Using Algae)

This method is the same process as found in plants and algae photosynthesis. The solar energy as the light source is directly converted to hydrogen in the photosynthesis reaction (Equation 2.1).

$$2H_2O + \text{light energy} \rightarrow 2H_2 + O_2 \qquad (2.1)$$

This is an attractive process because solar energy is used to convert a readily available substrate, water, to oxygen and hydrogen. However, only under this condition, hydrogen production is possible by

using Fe-hydrogenase as an enzyme since the activity of this enzyme is extremely oxygen-sensitive.

2.3.1.1.1.2 Indirect Biophotolysis (Using Cyanobacteria)

In indirect photolysis, the sensitivity of hydrogen processing problems is potentially circumvented by separating temporally oxygen and hydrogen evolution. Thus, indirect biophotolysis processes involve separation of the hydrogen and oxygen evolution reactions into separate stages, coupled through carbon dioxide fixation. Cyanobacteria or blue-green algae have the unique characteristics of using carbon dioxide in the air as a carbon source and solar energy as energy source (Equation 2.2). The cell takes up carbon dioxide first to produce cellular substrates, which are subsequently used for hydrogen production (Equation 2.3). The overall mechanism of hydrogen production in cyanobacteria can be represented as:

$$2H_2O + 6CO_2 + \text{light energy} \rightarrow C_6H_{12}O_6 + 6O_2 \quad (2.2)$$

$$C_6H_{12}O_6 + 12H_2O + \text{light energy} \rightarrow 12H_2 + 6CO_2 \quad (2.3)$$

2.3.1.1.2 Photo-Fermentation of Organic Compounds by Photosynthetic Bacteria

Photosynthetic bacteria evolve molecular hydrogen catalyzed by nitrogenase under a lack of nitrogen conditions using light energy and reduced organic acids. These bacteria themselves are not powerful enough to spilt water. However, under anaerobic conditions, these bacteria are able to use simple organic acids, like acetic acid, as electron donors. These electrons are transported to the nitrogenase by ferridoxin using energy in the form of ATP. When nitrogen is not present, this nitrogenase enzyme can reduce proton into hydrogen gas again using extra energy in the form of ATP (Equation 2.4).

$$\{CH_2O\}_2 \rightarrow \underset{\uparrow \text{ATP}}{\text{Ferredoxin}} \rightarrow \underset{\uparrow \text{ATP}}{\text{Nitrogenase}} \rightarrow H_2 \quad (2.4)$$

The overall reaction of hydrogen production can be given as Equation 2.5.

$$C_6H_{12}O_6 + 6H_2O + \text{light energy} \rightarrow 12H_2 + 6CO_2 \qquad (2.5)$$

2.3.1.1.3 Fermentative Hydrogen Production from Organic Compounds by Fermentative Bacteria

Hydrogen can be produced by anaerobic bacteria, grown in the dark on carbohydrate rich substrate. The majority of microbial hydrogen production is driven by the anaerobic metabolism of pyruvate, formed during the catabolism of various substrates. Carbohydrates are the preferred substrate for hydrogen producing fermentations. In strict anaerobic bacteria, a theoretical maximum of 4 moles of hydrogen per mole of glucose is obtained (Equation 2.6).

$$C_6H_{12}O_6 + 2H_2O \rightarrow 4H_2 + 2CO_2 + 2CH_3COOH \qquad (2.6)$$

The hydrogen productive fermentation has several advantages for industrial production, such as:

- Fermentative bacteria has very high rate of hydrogen evolution.
- Fermentative bacteria can produce hydrogen constantly from organic substrate.
- Fermentative bacteria can have growth rate good for supply of microorganism to the production system.

2.3.1.1.4 Hybrid Systems using Photosynthetic and Fermentative Bacteria

Hybrid systems can enhance the hydrogen production. Variety of carbohydrates as substrates can be digested by fermentative bacteria to produce hydrogen with degradation of substrates without using light (Equation 2.7).

$$C_6H_{12}O_6 + 2H_2O \rightarrow 4H_2 + 2CO_2 + 2CH_3COOH \qquad (2.7)$$

Resulting organic acids, like acetic acid, from dark-fermentation stage can be oxidized by photosynthetic bacteria with using light in the next stage to produce hydrogen (Equation 2.8).

$$CH_3COOH + 2H_2O + \text{light energy} \rightarrow 4H_2 + 2CO_2 \qquad (2.8)$$

Hence, hydrogen is produced continuously at the maximum yield with integrating dark- and photo-fermentation, respectively.

2.3.1.1.5 Biocatalyzed Electrolysis

Another way of oxidizing the acetic acid (or organic acid from the effluent of dark-fermentation stage) is the use of electrical energy instead of solar energy. In the bioreactor containing acetic acid forms, the anodic compartment of an electrolyzer cell produce photons and electrons by bacteria. Hydrogen are collected at cathode.

Anode and cathode reactions are as follows.

Anode: $CH_3COOH + 2H_2O \dashrightarrow 2CO_2 + 8H^+ + 8\ e^-$

Cathode: $8H^+ + 8e^- \rightarrow 4H_2$

The processes for the production of biohydrogen differ primarily concerning the involved microorganisms, the substrates, and the light dependence. Common division of the procedures for the biological production of hydrogen according to the light dependence into dark fermentation and photosynthetic fermentation processes is presented in Table 2.2. For the dark fermentative production of hydrogen, the microorganisms need only the chemical energy obtained from the substrate for their metabolism. In photosynthetic fermentation processes, the solar radiation is used as an additional energy source.

The production of hydrogen occurs in the second and third step of anaerobic degradation (acidogenesis and acetogenesis). Thereby, methanogenic bacteria must be inhibited to avoid the consumption of H_2 to produce CH_4 (Wang *et al.,* 2007). The dark fermentative production of hydrogen is characterized by a lower technical complexity compared to photosynthetic fermentation (Yu *et al.,* 2002). Light-independent hydrogen production anaerobic

microorganisms are always involved, converting the organic compounds to organic acids, hydrogen, and carbon dioxide.

Table 2.2 Processes of biological production of hydrogen classified by the light dependence

Dark fermentation	**Photo fermentation**
Fermentative H_2 production from biomass by fermentative bacteria $C_6H_{12}O_6 + 2H_2O \rightarrow 2CH_3COOH + 2CO_2 + 4H_2$	Biophotolytic H_2 production by green algae or cyanobacteria (water splitting) $12H_2O \rightarrow 12H_2 + 6O_2$
H_2 production from CO by photosynthetic bacteria $CO + H_2O \rightarrow H_2 + CO_2$	Photoproduction of H_2 from biomass by photosynthetic bacteria $C_6H_{12}O_6 + 6H_2O \rightarrow 6CO_2 + 12H_2$

Hydrogen gas production can be obtained by photo-fermentation and dark-fermentation by the pure or mixed culture at the ambient temperature and pressure. Dark-fermentation has more several advantages compared to photo-fermentation to produce hydrogen production, such as;

- availability of conventional bioreactor
- compactness of process
- availability of many kinds of sugar
- potentially high rate of production

However, the maximum theoretical yield by fermentative hydrogen production is much lower than photo-fermentative hydrogen production. And, the actual hydrogen yield is usually lower even than theoretical values. Furthermore, formation of by-products, such as fatty acids and alcohols, are inevitable, which can be used to produce methane in the second step.

Thus, in this work, dark fermentation was used to mainly produce hydrogen and methane by the two-step process.

2.3.2 Dark hydrogen fermentation

Under anaerobic or dark fermentation conditions, hydrogen is produced as a by-product during conversion of organic wastes into organic acids, which are then used as a feed substrate for methane production. Acetogenesis stage of anaerobic digestion of wastes can be manipulated to enhance hydrogen production. Methanogenesis is inhibited by operating at low pH and using heat treatment of sludge.

Dark fermentation of biohydrogen production is a process of applying biological fermentation method to produce hydrogen from organic wastes or wastewater. Normally, the dark fermentation process for biohydrogen production is operated under anaerobic conditions and does not require light. Under anaerobic condition at a very high organic loading, organics will be degraded anaerobically to produce hydrogen and organic acids as main products, as shown in Figure 2.3.

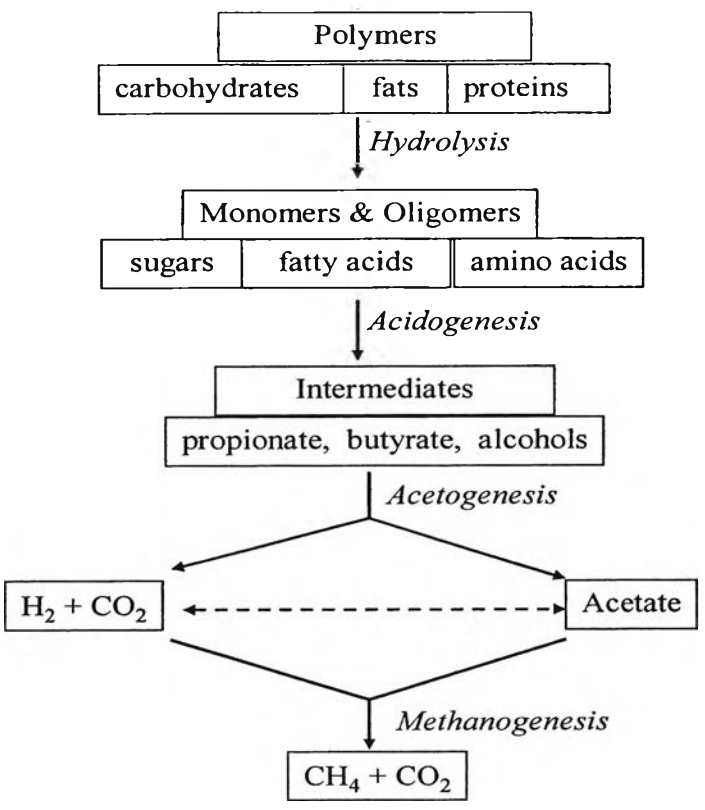

Figure 2.3 Flow diagram of anaerobic digestion (Largus *et al.*, 2004).

2.3.2.1 Physiology of Dark Hydrogen Fermentation

Dark hydrogen fermentation is operated under anoxic or anaerobic conditions, in the absence of oxygen as an electron accepter, to produce hydrogen. A wide variety of bacteria use the reduction of protons to hydrogen to dispose organic compounds into smaller compounds. Many organic compounds enable the production of hydrogen during dark fermentation; estimations of potential yields are mostly based on hexose conversions. The theoretical yield per mole of glucose is described in the following reaction:

$$C_6H_{12}O_6 + 4H_2O \rightarrow 2CH_3COOH + 2CO_2 + 4H_2$$

A maximum of 4 moles of hydrogen per mole of glucose can be produced concurrently with the production of energy (206 kJ per mole of glucose), which is sufficient to support microbial growth. The remainder of the hydrogen in the hexose is conserved in the by-product acetic acid, and under non-ideal circumstances, in more reduced products like ethanol, lactic acid. The complete oxidation of glucose to hydrogen and carbon dioxide yields a stoichiometry of 12 moles of hydrogen per mole of glucose, but in this case, no metabolic energy is obtained. The yield of hydrogen during dark fermentation is several affected by the partial pressure of the product. At high hydrogen pressures, a metabolic shifts to produce more reduced products, like lactic acid, thereby decreasing the hydrogen yield.

2.3.2.2 Feedstock for Dark Hydrogen Fermentation

Carbohydrates are suitable feedstock for dark hydrogen fermentation. Protein, peptides, and amino acids are probably less suitable for dark hydrogen production. Lipids are not suitable for dark hydrogen production.

2.3.2.3 Hydrogen Utilization

Today, environmental pollution is a great concern to the world, mainly due to rapid industrialization and urbanization. So, increasing focus is being placed on clean energy alternatives for satisfying growing energy demand. Hydrogen is considered as a clean energy because it has no emission, and moreover, it has much higher energy content per weight than the other fuels, as shown in Table

2.3. Hydrogen has various other uses (Das and Veziroglu, 2001), which can be broadly divided into the following categories:

1. As a reactant in hydrogenation processes: hydrogen is used to produce lower molecular weight compounds, saturate compounds, crack hydrocarbons, or to remove sulfur and nitrogen compounds.

2. As an O_2 scavenger: hydrogen is used to chemically remove trace amounts of O_2 to prevent oxidation and corrosion.

3. As a fuel in rocket engines.

4. As a coolant in electrical generators to take advantage of its unique physical properties.

Table 2.3 Energy content per weight of different fuels (Ni *et al.*, 2006)

Fuel	Energy content (MJ/kg)
Hydrogen	120
Liquified natural gas	54.4
Propane	49.6
Aviation gasoline	46.8
Automotive gasoline	46.4
Automotive diesel	45.6
Ethanol	29.6

2.3.3 Biological Methane Production

The final product for anaerobic wastewater treatment is biogas which is a mixture of methane and carbon dioxide. Methanogenic bacteria or methanogens decompose compounds with a simple molecule. For example, they utilize hydrogen and acetic acid to form methane and carbon dioxide, as shown in Equations 2.9 and 2.10.

$$CO_2 + 4H_2 \rightarrow CH_4 + 2H_2O \quad (2.9)$$

$$CH_3COOH \rightarrow CH_4 + CO_2 \quad (2.10)$$

Under natural conditions, methananogens are suitable to produce the maximum methane production, but they are sensitive to environmental change.

2.3.4 Parameter and Process Optimization

2.3.4.1 Temperature

Temperature range of anaerobic fermentation is in principle possible between 3°C and approximately 70°C. It is one of the important factors influencing the biological hydrogen fermentation process. The change of temperature has affected on substrate degradation, hydrogen production, product distribution, and bacterial growth, is dependent the end products that are needed. Anaerobic digestion being normally operated within 2 temperature ranges, as following classification:

2.3.4.1.1 Mesophilic Temperature

Mesophilic digestion is the most commonly used process for anaerobic digestion, in particular waste sludge treatment. Decomposition of the volatile suspended solids (VSS) is around 40% over a retention time of 15 to 40 days at a temperature of 30 to 40°C, which requires larger digestion tanks. It is usually more robust than the thermophilic process, but the biogas production tends to be less, and additional sanitization is usually required.

2.3.4.1.2 Thermophilic Temperature

Thermophilic digestion operates at a high temperature. The digester is heated to 55°C and held for a period of 12 to 14 days. The microorganisms rapidly break down organic matter and produce large volumes of biogas. The quick breakdown means that the digester volume can be smaller than That of other systems. Thermophilic digestion systems provide higher biogas production, but the technology is more expensive, more energy is needed, and it is necessary to have more sophisticated control and instrumentation. Greater insulation is necessary to maintain the optimum temperature range. These systems may be more sensitive to upset due to temperature variations. However, these systems are more effective in pathogen removal.

2.3.4.2 Available Nutrients

Microorganism requires mineral nutrients more than just a supply of organic substrates as a source of carbon and energy to grow of microorganisms. In addition to carbon, oxygen, and hydrogen, the generation of

biomass requires an adequate supply of nitrogen, sulfur, phosphorus, potassium, calcium, magnesium, and a number of trace elements, such as iron, manganese, molybdenum, zinc, cobalt, nickel, etc. Agricultural residues or municipal sewage usually contain adequate amounts of the mentioned elements. Higher concentration of any individual substrate usually has an inhibitory effect, so that analyses are recommended on a case to case basis to determine which amount of which nutrients, if any, still needs to be added.

2.3.4.3 Organic Loading Rate and Hydraulic Retention Time

The organic loading rate is the quality of organic matter fed per unit volume of digester per unit time. Organic loading rate plays an important role in anaerobic wastewater treatment in continuous systems and is a useful criterion for assessing performance of the reactors. Retention time is approximated by dividing the digester volume by the daily effluent rate and is one of the most important design parameters influencing the economic of digestion. Organic loading rate and hydraulic retention time depend on several factors, such as reactor type, process temperature, substrate quality, volumetric load determination, and the cost efficiency of the biological process.

2.3.4.4 pH Level

The anaerobic degradation process is highly pH dependent because each of the microbial groups involved in the reactions has a specific pH range for optimal growth. The optimal pH for methane-producing bacteria lives best under neutral to slightly alkaline conditions (6.8-7.2), while for acid-forming bacteria, it is around 5.5. The growth rate of methanogenic microbes decreases sharply below pH 6.6. If the pH value drops below 6.6, the medium will have a toxic effect on the methanogenic bacteria. Variations in pH levels from 6.0 to 8.0 have affected the dominant microbial population in the hydrogen-producing bacteria. In a one-step treatment process, the pH is typically maintained at conditions more optimal for methanogens to prevent the predominance of acid-forming bacteria, which may causes the accumulation of volatile fatty acids. An important factor to control pH in anaerobic digestion system is alkalinity, which needs to maintain pH for stable operation.

2.3.4.5 Nitrogen Inhibition and C/N Ratio

All substrates contain nitrogen. For high pH values, even a relatively low nitrogen concentration may inhibit the fermentation process. Microorganisms need both nitrogen and carbon for assimilation into their cell structure bacteria depending on the nature of the substrate.

2.3.4.6 Substrate Solid Content and Agitation

The mobility of the methanogens within the substrate is gradually impaired by an increasing solid content, and the biogas yield may suffer as a result. Many substrates and various modes of fermentation require some sort of substrate agitation or mixing in order to maintain process stability within the digester.

2.3.4.7 Inhibitory Factors

The presence of heavy metals, antibiotics, and detergents used in livestock husbandry can have an inhibitory effect on the process of methane production.

2.3.5 Microorganisms for Anaerobic Fermentation

2.3.5.1 Strict Anaerobes

2.3.5.1.1 Clostridia

Many anaerobes produce hydrogen from glucose in acetic acid, butyric acid, and ethanol-butanol fermentations. The highest maximum yield of 4 moles of hydrogen from 1 mole of glucose is produced in acetic acid fermentation. The production of other more reduced organic acids and/or alcohols lower the yield of hydrogen. For instance, the conversion of one mole of glucose into butyrate is accompanied by the production of only 2 mole of hydrogen. Usually a mixture of products is produced by *Clostridia*, and the available hydrogen from glucose is determined by the butyrate/acetate ratio.

2.3.5.1.2 Rumen Bacteria

Other strict anaerobic bacteria producing hydrogen are *Rumen bacteria. Ruminococcus lbus* has long been known to produce hydrogen together with other products like acetate, ethanol, formate, and CO_2 from carbohydrate.

2.3.5.1.3 Thermophiles

The hyperthermophile *Pyrococcus furiosus* produces hydrogen, organic acids, and CO_2 from carbohydrates. Extreme and hyper-thermophiles can provide higher hydrogen yield from glucose than mesophilic facultative and strict anaerobes.

2.3.5.1.4 Methanogens

Methanogens are characterized by the presence of hydrogenase, which is usually involved in the oxidation of hydrogen coupled to methane production and carbon dioxide reduction.

2.3.5.2 Facultative Anaerobes

Facultative anaerobes are resistant to oxygen. These bacteria have the advantage of rapidly consuming oxygen, so restoring anaerobic conditions immediately in reactors. Strict anaerobes are very sensitive to oxygen and often do not survive in the presence of low oxygen concentrations.

2.3.5.2.1 Enterobacter

Enterobacter can have several beneficial properties favorable for hydrogen production. In addition to high growth rates and utilization of a wide range of carbon sources, hydrogen production by *Enterbacter* is not inhibited by high hydrogen pressures. However, the hydrogen yield from glucose is normally lower compared to that of *Clostridi*a.

2.3.5.2.2 E. coli

E. coli has been shown to be capable of producing hydrogen and carbon dioxide from formate in the absence of oxygen.

2.3.5.2.3 Citrobacter

A *Citrobacter* species, *Citrobacter* sp. Y19 isolated from sludge digestion, has been shown to produce hydrogen from CO and H_2O by the water gas-shift reaction under anaerobic conditions.

2.3.5.3 Mixed Culture

Anaerobes for mixed culture have been isolated from various sources, such as fermented soybean meal or sludges from municipal sewage. These anaerobes often contain unwanted bacteria, in case of hydrogen production, such as methanogens, which consume the produced hydrogen and convert it to methane.

Enrichment culture of the anaerobes is prepared by heat treatment, which inhibits the activity of the hydrogen consumes while the spore-forming anaerobic bacteria survive. In industrial applications, the use of mixed cultures for hydrogen production from organic wastewater or waste might be more advantageous because pure cultures can easily become contaminated with hydrogen-consuming bacteria.

2.3.6 Bioreactors for Anaerobic Fermentation

Biohydrogen, or biological hydrogen, is a by-product in acetogenesis stage from the anaerobic fermentation process with organic compounds or organic wastewater as the substrate, such as glucose, municipal solid waste, starch effluent, food processing waste, rice winery, and biodiesel wastewater (Ito *et al.*, 2005). Metcalf and Eddy (2003) classified anaerobic bioreactor into three categories:

2.3.6.1 Anaerobic Suspended Growth Reactor

Suspended growth processes freely suspend microorganisms in water. In these processes, microorganisms convert the organic matter or other constituents in the wastewater into gases and cell tissue. Suspended growth technologies are conventional activated sludge treatment systems that use various process modes, ranging from conventional, extended aeration, contact stabilization, sequencing batch, and single sludge, which are available for polishing anaerobically treated effluents.

Under optimum conditions, the organisms break down material in the water and improve the water quality. Natural suspended growth treatment systems, such as wastewater biological treatment, can be used for organic wastewaters, such as municipal sewage, and tend to be lower in cost for operation and maintenance. The common suspended growth process consists of a batch reactor (Ting *et al.*, 2007, Zhu *et al.*, 2006), a continuously stirred tank reactor, or CSTR (Wang *et al.*, 2006, Zhang *et al.*, 2006, Lin *et al.*, 2006), an anaerobic contact filter reactor (Vijayaraghavan *et al.*, 2006), and anaerobic sequencing batch reactor, or ASBR (Arooj *et al.*, 2007).

2.3.6.2 Attached Growth Anaerobic Reactor

In these processes, microorganisms are held on a surface, the fixed film, which may be mobile or stationary with wastewater flowing past the surface. The packing can be submerged completely in liquid or not submerged, with

air or gas space above the biofilm liquid layer. These processes are designed to actively contact the biofilm with the wastewater.

Attached-growth anaerobic treatment reactors differ by the type of packing used and the degree of bed expansion. Packing materials used in attached growth processes include rock, gravel, slag, sand, and wide range of plastic and other synthetic materials. Attached-growth processes can also be operated as aerobic or anaerobic processes, such as an immobilized granular sludge bed reactor (Lee *et al.*, 2004), an anaerobic fluidized bed reactor or AFBR (Zhang *et al.*, 2007).

2.3.6.3 Anaerobic Sludge Blanket Reactor

This process uses an anaerobic process, while forming a blanket of granular sludge and being suspended in the tank. The key feature of this process that is the anaerobic sludge inherently has superior flocculation and settling characteristics, which favorably provide the physical and chemical conditions for sludge flocculation. The separation of the gas from the sludge in this process such as the up-flow anaerobic sludge blanket reactor or UASB (Han *et al.*, 2005, Wang et al., 2007) is most commonly used.

Due to the different types of substrate and bioreactor, the amount of hydrogen production is different. However, an anaerobic wastewater treatment is rapidly growing in the application for hydrogen production. The upflow anaerobic sludge blanket (UASB) reactor provides continuous and high rate of production, low energy requirement, and simple operation (Metcarf and Eddy, 2003).

2.4 Two-Step Hydrogen and Methane Production

One of the significant problems in the fermentative hydrogen production process is that most of the organic fraction of the feeding wastewater remains as soluble fermentation products. Thus, a complementary stage after fermentation would be necessary for COD elimination. It is well known that VFA formation during acidogenesis of the organic matter is actually the precursor to methanogenesis. Therefore, the hydrogen production process could be efficiently coupled with a subsequent anaerobic digestion step with the conversion of the remaining organic content to biogas (mainly methane and carbon dioxide). A two-stage anaerobic digestion process, in which acidogenesis and methanogenesis occur

in separate reactors, may offer several advantages as shown in Table 2.4 in order to enhance hydrogen and methane production and achieve stabilization of the treated wastewater prior to disposal.

Two-stage anaerobic digestion is a process configuration using two separate reactors. The first reactor is acidogenic-stage, which is maintained at a low pH or alkalinity and develops a high CO_2 and low CH_4 content in the gaseous products. Acidifying organisms dominate in the first reactor, and the major biochemical reaction is enzymatic hydrolysis and fermentation. Another one is methanogenic-stage, which is maintained at pH around 7 and high alkalinity, resulting in high specific methanogenic activity.

Table 2.4 The advantages of the two-stage system over the one-stage system when treating the same waste or wastewater

• Have short hydraulic retention time for rapidly degradable waste
• Higher COD removal efficiency
• Higher methane concentration in the gaseous products because the specific activity of methanogenic bacteria increases.
• Better process reliability, resilience, and stability, especially with variable waste conditions and readily degradable waste, which causes unstable performance in one-stage system.
• Physical separation of the acidogenic and methanogenic bacteria for maximum hydrogen and methane production rate.
• The acid phase and methane phase can be started much more easily and quickly than in conventional, single-stage digesters.