BIOHYDROGEN PRODUCTION FROM CASSAVA ETHANOL SPENT WASH (CESW) UNDER ANAEROBIC DIGESTION BY SEMI-COMPLETELY MIXED REACTOR

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ABSTRACT

Cassava ethanol spent wash (CESW) from a cassava ethanol plant was used as a substrate for hydrogen production under Anaerobic Digestion using a semi/completely Mixed Reactor with a 5 liter working volume of CESW. The initial MLSS and MLVSS concentrations were approximately 23,530, and 21,250 mg/L, respectively. Substrate concentration under all conditions at the organic loading rate was 30 kg COD/m^3-d. The F/M ratio for hydrogen production was 3.85 g COD/g VSS-d. The ratios of CESW and sugar at 1:2, 2:1, 1:1, and 3:1 were experimentally tested for 24 hr HRT. The results of the different ratios showed that the highest hydrogen production rates were 2.9, 0.70, 0.71, and 1.02 L H_2/d, respectively. The average hydrogen production rate was 1.02, 0.31, 0.24, and 0.21 L H_2/d, respectively. The maximum hydrogen production was attained with a ratio of 1:2. Statistical analysis at a confidence interval of 95% was done by SPSS 11.5 to determine the difference of hydrogen production for ratios of CESW and sugar. It was found that 1) the ratio of 1:2 was significantly different from all other ratios, 2) the ratio of 2:1 and 1:1 was not different, and 3) the ratio of 1:1 and 3:1 were not different. In addition, 12 hr HRT was experimentally tested at a ratio of 1:1 and the results showed that hydrogen production at 12 hr HRT (1.11 L H_2/d) was higher than that at 24 hr HRT (0.71 L H_2/d). The inflow pH, in the range of 5.0 – 5.5, and the effluent pH, in the range of 4.0 – 4.3, were controlled. The conditions led to production of hydrogen and volatile fatty acid (VFA). As a preliminary study, sludge was converted by the enzyme Cellulase to a reducing sugar as an initial substrate for hydrogen production. Results showed the average hydrogen production was 939 mmol H_2/mol hexose consumed, equivalent to 0.31 L H_2/d. Therefore the obtained hydrogen production rate was not high. Several indicators for the relatively low hydrogen production were considered. An effluent/influent VFA ratio of 1.25 was observed and the effluent/influent butyric acid ratio was 1.47. The effluent/influent propionic acid ratio was 0.96. The effluent/influent acetic acid ratio was 1.06. The effluent butyric/acetic ratio was 0.50, and the effluent propionic/acetic ratio was 0.34.

KEY WORDS: BIOHYDROGEN/ CASSAVA ETHANOL SPENT WASH/ ANAEROBIC DIGESTION/ SEMI-COMPLETELY MIXED REACTOR

105 pages

การผลิตไฮโดรเจนจากน้ำเสียที่ผลิตเอทานอลโดยใช้มันสำปะหลังเป็นวัตถุดิบภายใต้การหมักแบบไร้อากาศด้วยระบบกึ่งกวนสมบูรณ์

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บทคัดย่อ

น้ำเสียจากกระบวนการผลิตเอทานอลที่ใช้มันสำปะหลังเป็นวัตถุดิบนำมาเป็นสารตั้งต้นเพื่อผลิตไฮโดรเจนภายใต้การหมักแบบไร้อากาศด้วยระบบกึ่งกวนสมบูรณ์ในถังปฏิกรณ์ที่บรรจุน้ำเสียปริมาณ 5 ลิตร ความเข้มข้นของจุลินทรีย์ในรูปของเอ็มแอลเอสเอส และเอ็มเอลวีเอสเอสเท่ากับ 23,530 และ 21,250 มิลลิกรัมต่อลิตรตามลำดับ โดยมีอัตราภาระบรรทุกสารอินทรีย์เท่ากับ 30 กิโลกรัมซีโอดีต่อลูกบาศก์เมตรต่อวัน สำหรับอัตราส่วนของอาหารต่อจุลินทรีย์เท่ากับ 3.85 กรัมซีโอดี/กรัมวีเอสเอสต่อวัน การทดลองใช้อัตราส่วนของน้ำเสียต่อน้ำตาลเท่ากับ 1:2, 2:1, 1:1 และ 3:1 โดยมีระยะเก็บกักที่ 24 ชั่วโมง ผลพบว่าสามารถผลิตไฮโดรเจนที่อัตราสูงสุดเท่ากับ 2.9, 0.70, 0.71, และ 1.02 ลิตรไฮโดรเจนต่อวันตามลำดับ โดยที่อัตราค่าเฉลี่ยของไฮโดรเจนเท่ากับ 1.02, 0.31, 0.24, และ 0.21 ลิตรไฮโดรเจนต่อวันตามลำดับ นั่นคืออัตราการผลิตไฮโดรเจนสูงสุดนั้นอยู่ที่อัตราส่วนของน้ำเสียต่อน้ำตาลเท่ากับ 1:2 เมื่อทดสอบความแตกต่างทางสถิติที่ระดับความเชื่อมั่น 95% โดยใช้โปรแกรม SPSS 11.5 พบว่า 1) อัตราส่วนที่ 1:2 มีความแตกต่างอย่างมีนัยสำคัญจากอัตราส่วนอื่นๆ 2) อัตราส่วนที่ 2:1 และ 1:1ไม่แตกต่างกัน และ 3) อัตราส่วนที่ 1:1 และ 3:1ไม่แตกต่างกัน เมื่อนำอัตราส่วนน้ำเสียต่อน้ำตาลที่ 1:1 มาทดลองที่ระยะเก็บกัก 12 ชั่วโมง พบว่า อัตราสูงสุดของการผลิตไฮโดรเจนที่ระยะเก็บกัก 12 ชั่วโมงนั้น (1.11 ลิตรไฮโดรเจนต่อวัน) สูงกว่าระยะเก็บกักที่ 24 ชั่วโมง (0.71 ลิตรไฮโดรเจนต่อวัน) โดยการทดลองต้องควบคุม pH น้ำเข้าระบบในช่วง pH 5.0 – 5.5 และ pH น้ำออกระบบในช่วง pH 4.0 – 4.3 ซึ่งทำให้เกิดไฮโดรเจนและเกิดกรดไขมันระเหยง่าย นอกจากนี้ ได้ทำการศึกษาเบื้องต้นโดยนำกากตะกอนของน้ำเสียนี้มาย่อยด้วยเอนไซม์เซลลูเลสเพื่อเปลี่ยนเซลลูโลสเป็นน้ำตาลรีดิวซิ่งสำหรับเป็นสารตั้งต้นในการผลิตไฮโดรเจน พบว่าสามารถผลิตไฮโดรเจนที่อัตราค่าเฉลี่ยเท่ากับ 939 มิลลิโมลไฮโดรเจนต่อโมลเฮกโซสที่ถูกใช้ หรือเทียบเท่ากับ 0.31 ลิตรไฮโดรเจนต่อวัน ซึ่งนั่นคือมีปริมาณไฮโดรเจนไม่มาก โดยเมื่อพิจารณาตัวบ่งชี้การเกิดไฮโดรเจนปริมาณค่อนข้างต่ำ พบว่าอัตราส่วนของกรดอินทรีย์ในน้ำออกระบบต่อน้ำเข้าระบบมีค่าเท่ากับ 1.25 อัตราส่วนของกรดบิวทิริกในน้ำออกระบบต่อน้ำเข้าระบบมีค่าเท่ากับ 1.47 อัตราส่วนของกรดโพรพิโอนิกในน้ำออกระบบต่อน้ำเข้าระบบมีค่าเท่ากับ 0.96 อัตราส่วนของกรดแอซิติกในน้ำออกระบบต่อน้ำเข้าระบบมีค่าเท่ากับ 1.06 สำหรับอัตราส่วนน้ำออกระบบของกรดบิวทิริกต่อกรดแอซิติกมีค่าเท่ากับ 0.50 และอัตราส่วนน้ำออกระบบของของกรดโพรไพโอนิกต่อกรดแอซิติกมีค่าเท่ากับ 0.34

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LIST OF GLOSSARY

ASBR	Anaerobic sequencing batch reactor
BOD	Biochemical Oxygen Demand
BHP test	Biochemical hydrogen potential test
CESW	Cassava ethanol spent wash
Cl^-	Chloride
COD	Chemical Oxygen Demand
CSTR	Continuous Stirred Tank Reactor
FPU	Filter paper unit as unit of enzyme activity
HAc	Acetic acid as type of VFA
HBu	Butyric acid as type of VFA
HPr	Propionic acid as type of VFA
HRT	Hydraulic retention time
L H2/gCODr	The unit of hydrogen yield in form of liters hydrogen per gram COD removed
NH_4^+	Ammonia
OLR	Organic loading rate
PO_4^{3-}	Phosphate
SO_4^{2-}	Sulphate
TDS	Total Dissolved Solid
TKN	Total Kjeldahl Nitrogen
TS	Total Solid
TSS	Total Suspended Solid
TVS	Total Volatile Solid
VFA	Volatile Fatty Acid

CHAPTER I
INTRODUCTION

1.1 Statement of the Problem

Fuels have been one of products the major substances imported to Thailand for national economic development. The situation of high demand for energy is continuously increasing. The price of crude oil in the world market has been high and was increased steadily. Thailand, therefore, encounters economic disadvantages. According to the Energy Policy and Planning Office, Ministry of Energy, the imported 8,740,000 barrels per day of crude oil in the year 2009 (equivalent to 764,000,000 million baht) had increased by 7.6% approximately from the year 2008. Due to the worldwide increased economic recovery, the global need for crude oil has increased by 1.86% in the year 2010, which is from 84.6 – 86.2 million barrels per day in 2009.

The situation leads to a national energy crisis; therefore, the Thai government necessarily needs to find a domestic alternative energy source. Hence, the appropriate source of energy should be bio-energy such as biogas or hydrogen from fermentation. There are a large number of biomass for ethanol processing; for instance, cassava, sugar cane, molasses, sweet sorghum and sweet corn and so on (KAPI, 2006).

According to the Ministry of Energy's declaration on the national policy for supporting ethanol production from cassava in the years 2008 to 2022, cassava ethanol production in 2009 was equivalent to 2.15 million liters per day and has increased to 4.05 million liters per day in 2010 and 2011 (updated on 2010). The short term plan of the national policy for ethanol production supports the research and development onto value added wastes for ethanol production. Thailand is an agricultural country with a high volume of biomass such as cassava. Cassava has high carbohydrate content; approximately 65% is used primarily for fuel-energy (Srinorakutara, 2006). Since cassava can be easily grown in Thailand and is abundantly available in the market, it can be used in producing ethanol as an alternative source of bio-energy.

The availability of cassava ethanol spent wash is tremendous and deserves to be studied for its bio-energy production which is the generation of hydrogen. Hydrogen is a clean energy with low cost of production. The high energy hydrogen yield is 122 KJg^{-1}, which has an energy yield 2.75 times greater than hydrocarbon based fuels (Magnusson et al, 2008). It is a suitable alternative energy source biologically derived from anaerobic decomposition. The process of generating hydrogen from cassava ethanol waste is considered environmentally friendly because no external energy is produced and minimum amount of harmful gases are released into the atmosphere. Hence, this study aims to investigate the feasibility of bio-hydrogen production from cassava ethanol spent wash by semi-completely mixed reactor.

1.2 Research Objective:

1.2.1 Main Objective:

To investigate biohydrogen production through anaerobic digestion by Semi – Completely Mixed Reactor from cassava ethanol spent wash.

1.2.2 Specific Objectives:

- To study the possibility of applying anaerobic digestion from cassava ethanol spent wash to hydrogen production.
- To determine the optimum values of pH and HRT for hydrogen production.
- To investigate the yield of hydrogen related to pH level, HRT, and VFA (volatile fatty acid) concentrations.

1.3 Research Hypotheses:

- Production of biohydrogen is related to levels of pH.

- Production of biohydrogen is greater when HRT is at 12 hrs than at 24 hrs.
- Production of biohydrogen is related to VFA concentrations.

1.4 Research Variables:

1.4.1 Independent Variables

HRT, pH, ratio of substrate

1.4.2 Dependent Variables

Hydrogen yield

1.4.3 Control Variables

Effluent pH, Organic loading rate

1.5 Scope of the Study

1.5.1 The anaerobic granule sludge was collected from Serm Suk. Co., Ltd, Nonthaburi.

1.5.2 Cassava ethanol spent wash was collected from Sapthip Co., Ltd, Lopburi every month by grab sampling.

1.5.3 Reactor is made of acrylic with dimension of 14 centimeters (diameter) ×40 centimeters (height) and 5.0 liters of working volume, stirrer controlled with timer for semi – completely mixed reactor.

1.6 Limitation of the Research

The process of ethanol production in regards to volume may be uncontrolled from one month to the next or during the running process cycle. As a result, the characteristic of cassava ethanol spent wash may vary.

1.7 Definition of Key Words

1.71 Anaerobic Digestion (AD): the digestion of substances in wastewater by converting organic wastes into hydrogen, carbon dioxide, and other gases. The process occurs during anaerobic digestion and is responsible for digesting the sludge in wastewater. Anaerobic digestion is divided into hydrolysis, acidogenesis, and acetogenesis which is the final step for hydrogen production.

1.7.2 Semi-completely mixed reactor: it is the process of mixing CESW by agitation for 5 minutes, then stops for 2 hours throughout the course of operation.

1.7.3 Cassava ethanol spent wash (CESW): processing either with or without a decantor system usually produces a great deal of highly organic loading wastewater. These are by-product of the ethanol distillation process.

1.7.4 Hydrolyzed sludge: it is the sludge obtained from the same process as CESW, after being centrifuged. The sludge is dried in an oven, then hydrolyzed by adding cellulase enzyme.

1.7.5 Hydraulic retention time (HRT): the period of time that the wastewater is kept in the reactor which is equal to the volume of the tank divided by the flow rate.

1.7.6 Hydrogen production rate (HPR): the amount of hydrogen production in liters per day (L/d).

1.7.7 Specific hydrogen production rate (SHPR): the ratio of the hydrogen production rate (L/d) and working volume of reactor (Liters).

1.7.8 Hydrogen yield: the ratio of hydrogen production rate such as unit of liters per day and gram of COD removal per day.

1.7.9 Volatile fatty acid (VFAs): the hydrolysis of organics with high molecular weight to soluble organics, subsequently results in anaerobic organisms to produce volatile fatty acids, hydrogen, and carbon dioxide.

1.7.10 COD loading rate: the volumetric organic load is identified as the amount of organic matter utilized daily by the reactor per volume unit.

1.7.11 Biohydrogen: it is defined as hydrogen produced biologically mostly bacteria involving the conversion of organic wastes to hydrogen using a dark fermentative process.

1.8 Conceptual Framework

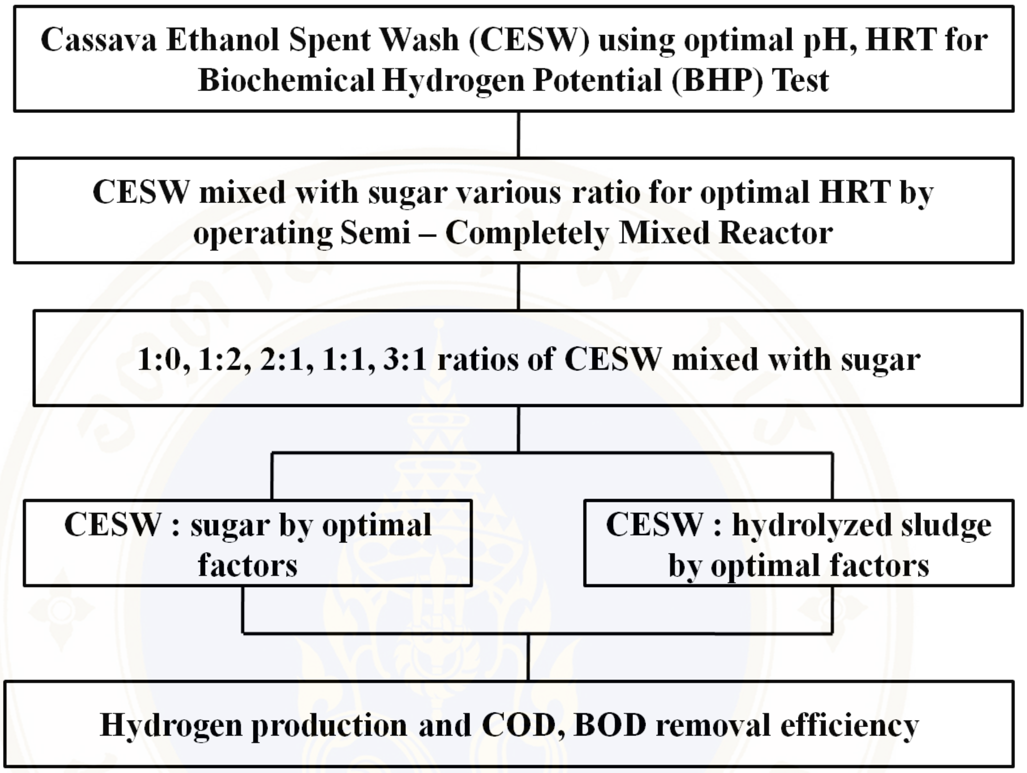

Figure 1.1 Conceptual Framework

CHAPTER II
LITERATURE REVIEWS

2.1 Anaerobic Digestion (AD)

The biological interaction of anaerobic digestion should be a primary step for pretreatment of BOD removal. Anaerobic digestion is divided into three steps: hydrolysis, acidogenesis, and methanogenesis (Tunthunwhet, 2009). However, hydrogen production just has only 2 steps: hydrolysis, acidogenesis.

In the first step, many kinds of wastes are hydrolyzed from polymeric plant structures or transformed from polysaccharides to monomers such as carbohydrate, protein, and lipid to convert to soluble substances: sugar, amino acid and a long chain lipid, respectively. The reaction is produced by extracellular enzymes of the microorganisms.

In the second step, acidogenesis, the soluble substances are absorbed into the bacteria cells and used as source of energy. They are then, transformed into VFAs in the form of acetic, butyric, propionic and others but in very small amount. It also produces hydrogen and carbon dioxide which are also produced through a biochemical process which is generated during the process of hydrolysis. Hydrogen yield depends on two factors: the type of organic waste and the partial pressure of hydrogen. For example, VFAs create butyric and propionic acids when it is under high hydrogen partial pressure. Starch from organic waste is converted into sugar and then into acetic acid, hydrogen, and carbon dioxide and passes internally through the Embden - Meyerhof’s cycle (low hydrogen’s partial pressure). However, at high partial pressure, the yield produces butyric and propionic acids.

Acetogenesis is the intermediate phase of methane production. Therefore, a volatile fatty acid that has more than two carbon atoms, will be decomposed to acetic acid. Subsequently, acetic acid links with methane gas in the methanogenesis pathway.

In the last step of the methanogenesis pathway, acetic acid and hydrogen are converted into methane by the action of obligate oxygen.

Consequently, hydrogen production influences the acidogenesis process. There are various microorganisms which participate in the acidogenesis process, such as Bacillus, Clostridium, Escherichea coli, etc. Theoretically, the organic compound is decomposed to volatile fatty acid by Clostridium spp. due to the presence of hydrogen and carbon dioxide.

Many researchers have studied under controlled conditions the production of hydrogen production from industrial wastewater especially agro-industrial wastes such as pH, partial pressure of hydrogen, nutrient, and types of volatile fatty acids and so on.

Schematic of drawing is illustrated below:

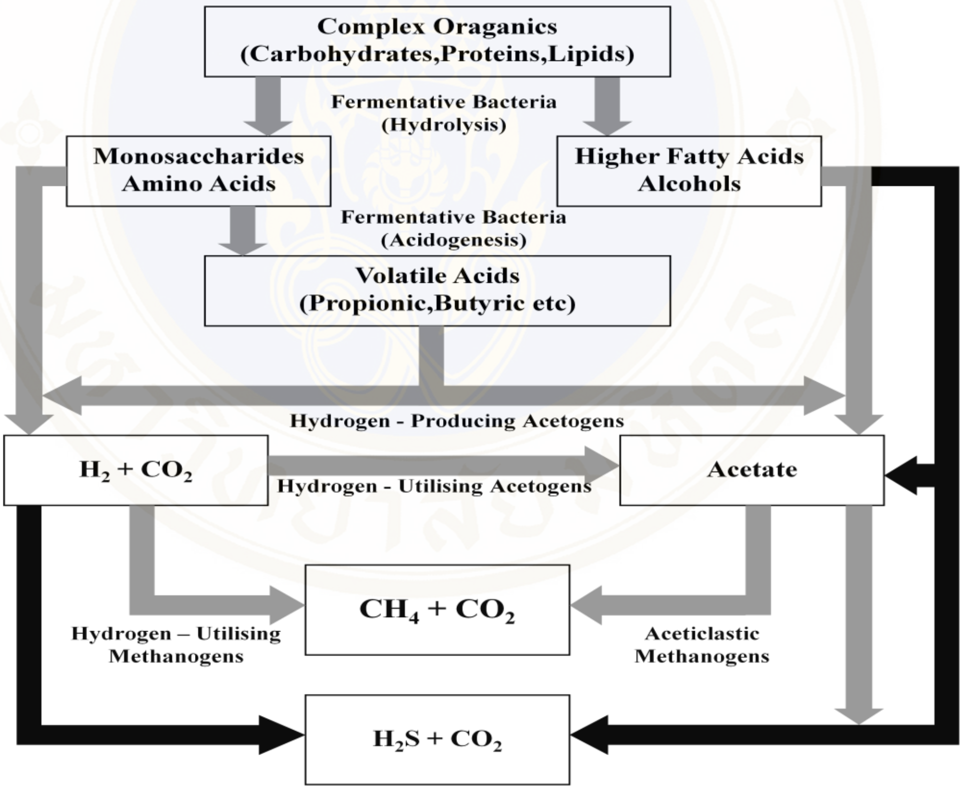

Figure 2.1: Metabolic pathways and microbial groups involved in anaerobic digestion [Adapted from Lettinga et al., (1996)]

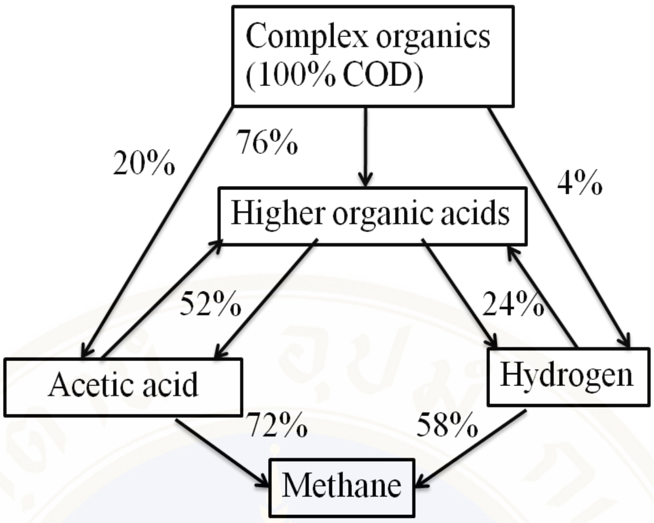

Figure 2.2 The formation from anaerobic fermentation of complex substrate (exemplified by McCarty, 1964 and 1981)

2.1.1 Microbiology and biochemistry of dark fermentative hydrogen production

Hawkes et al, (2007) restated that the maximum theoretical fermentation of glucose is 12 mole hydrogen per 1 mole of glucose as shown in equation (1):

$$C_6H_{12}O_6 + 6H_2O \rightarrow 12H_2 + 6CO_2 \qquad (1)$$

However, research results showed that 1 mole of glucose will be produced by 2 -3 mole of hydrogen. The production of each acid end-product produced various hydrogen yields. For example, the maximum theoretical yield of 4 mole of hydrogen per mole of hexose would be achieved from acetic acid production when as in Eq. (2) (Lens et al., 2007).

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

The pathway for hydrogen fermentation is related to the oxidative decarboxylation of pyruvate in regards to ferredoxin hydrogenase. The effect of hydrogen partial pressure on these reactions is summarized. If the hydrogen is at low partial pressure, NADH may be oxidized through hydrogenase, producing hydrogen up to the maximum theoretical yield of 4 mole hydrogen mol^{-1}hexose consumed and a maximal ATP yield. However, under normal reactor conditions, most of the NADH will be oxidized in reactions producing reduced fermentation end products, such as butyrate with a lower molar hydrogen yield and lower ATP yield as in Eq. (3) (Hakes et al., 2007).

$$C_6H_{12}O_6 \rightarrow CH_3CH_2CH_2COOH + 2CO_2 + 2H_2 \qquad (3)$$

However, in practice, the hydrogen yield produced by pure or mixed culture has been reported to range from 0.37 to 2.0 mole H_2/mole-glucose (Sung et al., 2002). Moreover, it results in a lower molar hydrogen yield from consumed hydrogen derived from the propionic acid and methane reaction as shown in equation (4) and (5) (Vavilin et al., 1995).

$$C_6H_{12}O_6 + 2H_2 \rightarrow 2CH_3CH_2COOH + 2H_2O \qquad (4)$$
$$4H_2O + 2CO_2 \rightarrow CH_4 + 2H_2O \qquad (5)$$

2.2 Factors in Hydrogen Production

2.2.1 Volatile fatty acids (VFA)

Volatile fatty acids are nutrients of bacteria in the anaerobic digestion process. There is a variety of volatile fatty acids which is produced during the hydrolysis step. Anaerobic digestion must involve acetogen bacteria in order to hydrolyze volatile fatty acid which has more than 2 carbon atoms. The most important volatile acids are acetic, butyric and propionic acid, which are precursors in hydrogen formation. The degradation of a complex substrate, namely the excess sludge from domestic sewage treatment plants or others of similar composition in the various metabolic steps was attained. VFA acid results mainly from the fermentation of carbohydrate and protein, while 30% of the organic compounds are also converted into this acid before finally being converted into methane. Acetic acid is the most substantial intermediate acid formed from all of the organic compounds. In regards to the degradation of complex substrate, acetic acid is the precursor of about 72% of the methane formed. When acetic acid is mixed with propionic acid, about 85% of the total methane is formed. A large part of the remaining 15% results from the degradation of other acids, namely formic and butyric acids as shown in Figure 2.3.

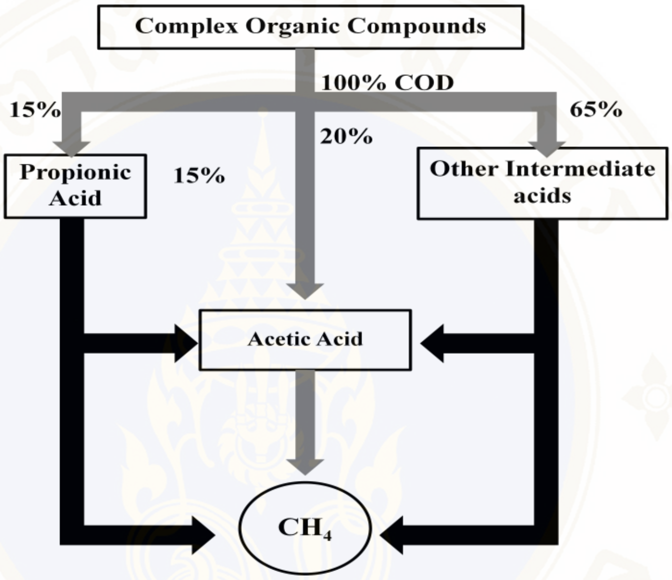

Figure 2.3 Routes for the fermentation of complex substrates (McCarty, 1964)

A preliminary evaluation will be conducted prior to the process. Wastewater concentration of biodegradable substrates can be reasonably estimated from the BOD and COD tests. In addition, other important chemical characteristics to be considered are the concentration of carbohydrates, protein, and lipids, pH, alkalinity, inorganic nutrients, temperature, and the potentially toxic compounds.

The volatile fatty acids (VFA) profile supported the fact that the butyrate to acetate (HBu/HAc) ratio was the most important parameter to justify hydrogen yield at various hydraulic retention times (HRT). Arooj et al, (2008) mentioned that hydrogen and carbon dioxide in the reactor might combine to form acetate under anaerobic conditions. Such acetate production is not favorable for hydrogen generation since hydrogen consumes itself to produce acetate. This acetate produced through this metabolic pathway is represented as HAc: $4H_2 + 2CO_2 \rightarrow CH_3COOH + 2H_2O$. In other words, acetate production through this pathway is represented by the equation: $C_6H_{12}O_6 + 2H_2O \rightarrow 2CH_3COOH + 2CO_2 + 4H_2$.

2.2.2 Temperature

Temperature is one of the most important factors in the selection of species. Microorganisms cannot control internal temperature. Therefore, the temperature inside the cell is determined by the external ambient temperature. The three temperature ranges associated with microbial growth in most of the biological process are: psycophilic range represents the temperature between 4 ˚C and approximately 15˚C, mesophilic range represents the temperature between 20 ˚C and approximately 40˚C, thermophilic range is the temperature between 45˚C and 70˚C, and above (Von Sperling and Chernicharo, 2005).

2.2.3 pH

The pH factor is a crucial factor for the proper operation of the anaerobic process. The pH affects the process in two main ways: directly and indirectly. The activity of the enzyme changes the proteic structure, and the toxicity of a number of compounds, respectively. The optimum pH depends on the type of microorganisms involved in the digestion process, as well as the type of substrate. The optimum pH range are based on the degradation of different substrates. The acid-producing bacteria are less sensitive to the pH than the methanogenic microorganisms. The acid-producing bacteria can still be very active, even if the pH value is below 4.5. However, the acid-producing bacteria have an optimum growth rate in the range of pH between 5 and 6. Hence, the pH factor is mainly achieved by the inhibition of methanogenic microorganisms.

2.2.4 Alkalinity and buffer capacity

The main substantial factors that affect the pH in anaerobic processes are carbonic acid and volatile acid. The buffer capacity of the anaerobic system depends almost completely on the carbon dioxide/alkalinity system. The amount of alkalinity is rarely controlled, because it is based on the characteristic and concentration of wastewater. However, if there is a high concentration of wastewater, the concentration of carbon dioxide produced will be high. Adding Na_2HCO_3 can increase the buffer effect in the water, which does not contribute to a change in carbon dioxide concentration. Additionally, adding NaOH can increase the buffer capacity, as well as decrease the concentration of carbon dioxide. Alternatively, the buffer capacity is based on the factor of chemical effect and its solubility in the chemical reaction. A factor of the chemical effect is the retention of carbon dioxide by the buffering capacity of the chemical. During the water solubility phase of the reaction if calcium is used it will produce carbonate. Therefore, sodium should be used since it can not precipitate. Since carbon dioxide is used during the precipitation phase, it does not increase the buffer capacity (Tunthunwhet, 1999).

Gang et al, (2010) stated that alkalinity was a crucial parameter influencing the fermentation process. When the influent was adjusted to pH of 6 by $NaHCO_3$ instead of NaOH, the hydrogen yield increased from about 40 to 52 mlH2/gVS in all experiments.

2.2.5 Hydraulic retention time (HRT)

Decreased HRT significantly enhances hydrogen measured during the period of time that a soluble compound remains in a reactor relative to the volume of the reactor tank or the influents flow rate as follows (Von Sperling and Chernicharo, 2005):

$$\mathrm{HRT} = \frac{V}{Q}$$

Where:

V = total volume of the reactor (m^3)

Q = flow rate (m^3/d)

2.2.6 Organic loading rate

The Organic loading rate: the volumetric organic load is identified as the amount of organic matter utilized daily in the reactor, per volume unit:

$$L_v = \frac{Q \times So}{V}$$

Where:

L_v = volumetric organic loading rate (kg COD/m^{3}·d)

Q = flow rate (m^3/d)

S_o = influent substrate concentration (kg COD/m^3)

V = total volume of the reactor (m^3)

2.2.7 Biological loading rate

The biological loading rate is the known sludge loading rate. The amount of organic matter utilized daily in the reactor, per unit of biomass present:

$$L_s = \frac{Q \times So}{M}$$

where:

L_s = biological or sludge loading rate (kg BOD/kg VS·d)

Q = average influent flow rate (m^3/d)

S_o = influent substrate concentration (kg BOD/m^3)

M = mass of microorganisms present in the reactor (kg VS/m^3)

2.2.8 Upflow velocity

The upflow velocity of the liquid is considered from the relation between the influent flowrate and the cross section of the reactor, as follows:

$$V = \frac{Q}{M}$$

Where:

V = upflow velocity (m/hour)

Q = flow (m^3/hour)

A = area of the cross section of the reactor, in this as the surface area (m^2)

2.2.9 Hydrogen production rate

It is the produced hydrogen volume ratio, in a given time:

$$\textbf{HPR = Q mixture gas / V}$$

where:

GPR = gas production rate (m^3 hydrogen / m^3 reactor day)
Q mixture gas = biogas flow rate, (m^3/day)
V = reactor volume, (m^3)

2.2.10 Nitrogen effect on hydrogen yield

Kalil et al, (2008) mentioned that a nitrogen source with proper C:N ratio enhanced hydrogen production. Using different nitrogen sources with different concentrations on bacterial productivity of hydrogen has to effect on both bacterial productivity of hydrogen and biomass concentration. The optimum organic nitrogen source at concentration of 13 g/l, hydrogen yield YP/S of 308 mL/g glucose utilized with biomass concentration of 1.1g/l.

2.2.11 Partial pressure hydrogen

Partial pressure has an effect on the process of hydrogen production. During the step of acidogenesis, for example, volatile fatty acid is degraded into acetic and hydrogen under low partial pressure, but it is degraded into butyric and propionic acid under high partial pressure (Tunthunwhet, 1999).

The fermentation reaction under low partial pressure as shown in equation (1) is the anaerobic digestion of 1 mole of glucose generates 2 mole of acetic acid, 2 mole of carbon dioxide, 4 mole of hydrogen and 4 mole of ATP.

$$C_6H_{12}O_6 + 2H_2O + 4ADP + 4P \rightarrow 2CH_3COOH + 2CO_2 + 4H_2 + 4ATP \qquad (1)$$

The fermentation reaction under high partial pressure as shown in equation (2) is the anaerobic digestion of 1 mole of glucose generating 1 mole of acetic acid, 1 mole of propionic acid, 1 mole of carbon dioxide, 1 mole of hydrogen and 3 mole of ATP.

$$C_6H_{12}O_6 + 3ADP + 3P \rightarrow CH_3COOH + CH_3CH_2COOH + CO_2 + H_2 + 3ATP \qquad (2)$$

The partial pressure is lower than 2×10^{-3} atm, causes butyric acid to be converted into acetic acid as the following equation (3) shows:

$$CH_3\ CH_2CH_2COOH + 2H_2O \rightarrow 2\ CH_3COOH + 2\ H_2 \qquad (3)$$

If the partial pressure is higher than 9×10^{-3} atm, propionic acid is generated during anaerobic digestion. Because it can not be converted into acetic acid as the following as equation (4) shows:

$$CH_3CH_2COOH + 2H_2O \rightarrow CH_3COOH + CO_2 + 2H_2 \qquad (4)$$

2.2.12 Type of feeding of hydrogen production

Sangyyoka et al, (2007) mentioned that repeated batch fermentation was more effective in producing hydrogen from cassava wastewater than batch fermentation. It resulted in the highest hydrogen yield, the highest potential maximal amount of hydrogen production rate and a relatively short lag time is 0.0094 mL mg-COD^{-1}, 12,532.80 mL, 540.46 mL h^{-1}, 3.5 mL g-VSS^{-1} and 5.31 hours, respectively.

2.3 Hydrogen Production by Biomass

It is the amount of alternative energy which can be produced from various sources to substitute for fossil fuel**,** it is divided into the followings:

- Thermo-chemical conversion processes
- Pyrolysis/charcoal production
- Gasification
- Liquid fuel production: Indirect liquefaction, direct liquefaction
- Ammonia production
- Biochemical conversion processes consist of: anaerobic digestion to methane and Ethanol fermentation

2.3.1 Principles of Electrolysis

Water substantially dissociates into hydrogen and hydroxyl ions (H^+ and OH^-), according to the reaction: $H_2O \rightarrow H_2 + ½\ O_2$.

An electrolysis cell for hydrogen production from water consists of an electrolyte usually in an aqueous basic solution revolving around two electrodes. The membrane is anticipated to generate hydrogen and oxygen gas from mixing, and separating the cell into anolyte and catholyte chambers, which allows transfer from one to the other. In a basic solution, the cathode reaction is

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

And oxygen is produced at the anode

$$2OH^- \rightarrow \tfrac{1}{2} O_2 + H_2O + 2e^-$$

The above description is the anode and cathode reactions combined to give a production of one mol of hydrogen plus one-half mol of oxygen from one mol of water. A general solution is used in preference to pure water so as to increase the conductivity of the solution. This could also be achieved using an acidic solution, however the corrosion problems would be more rigorous.

Utilizing pure-water in the process may appear to be expensive. But a great amount of water required to maintain the electrolysis is relatively small compared with a great deal of hydrogen produced. For instance, 1,000 gallons of water used in electrolysis will produce 166,000 scf of hydrogen. The latent heat of vaporization or the energy required for distillation is 9.8 Kcal/mol.

The electrolysis of water (H_2O) produces both hydrogen and oxygen improved energy efficiency at a lower cost. The result has been well known for many years of purity hydrogen. However, the approach has been improved energy efficiency cost. Energy efficiency has been considered because the low level of commercial interest did not provide motivating economic factors to start research and development. Therefore, commercial electrolysis has been small and relatively inefficient, and the technology has been rather inactive. It is generally reported that operating temperatures of 2000˚F and hydrogen and oxygen evolution pressure of 3,000 psi. The process involves an endothermic factor which is heat that must be added to the chemical reaction (Casper, 1978).

.2.3.2 Hydrogen from Coal

Hydrogen can be produced from coal by hydrogasification:

$$2H_2 + C \rightarrow CH_4$$

Steam-hydrocarbon reforming follows:

$$CH_4 + H_2O <--> CO + 3H_2$$

$$CO + H_2O <--> CO_2 + H_2$$

Giving the overall reaction $C + 2H_2O <--> 2H_2 + CO_2$

The above reaction uses coal solubilizing techniques to produce liquid coal which is hydrogasified to produce synthetic gas. Hydrogen is produced by steam reforming.

2.3.3 Thermo-chemical process

This process represents a complete oxidation to carbon dioxide and water to yield a variety of useful substrates which depends on a combination of temperature, pressure and various catalysts, limiting the oxygen supply, and the partial breakdown. The main thermo-chemical conversion approach consists of pyrolysis, gasification, and both direct and indirect biomass liquefaction.

2.3.4 Gasification

Gasification is a thermo-chemical conversion process. It is referred to as partial oxidation, which involves a limited amount of oxygen or air to the system under high temperature between 600 and 900 ˚C. This technique can convert a solid biomass to a component of hydrocarbon. The result of the output is to produce various gases such as 20 - 30% carbon monoxide, 50 – 60% nitrogen, 2-4% methane, 5-15% hydrogen, and the remaining 5-15% carbondioxide and 6-18% water. The process consists of 4 steps; drying zone, pyrolysis zone, combustion zone, and reduction zone; the end-products are oil and char.

2.3.5 Steam methane reforming process

Steam methane reforming process is utilized in many industries, such as the petrochemical industry, methanol industry, Ammonia industry and so on. This process converts liquid or gas fuel into synthesized gas, which is derived from the reaction of methane and carbon dioxide gas. Subsequently, synthesized gas is delivered to water-gas –shift. The catalyst such as carbon dioxide and water are added to form carbonmonoxide, and hydrogen gas as shown in the following reforming reaction equation:

$$CH_4 + H_2O <--> CO + 3H_2 \qquad \Delta H = +206.16\ kJ/mol\ CH_4$$

2.3.6 Pyrolysis/Charcoal production

Pyrolysis is the thermal decomposition of biomass under raised temperature in the absence of oxygen. When heating the biomass during the physical and chemical process it can be changed to yield a medium energy gas, a complex of pyrolysis both liquid and solid charcoal in proportion. These processes are influenced by several factors, such as temperature, moisture content, biomass composition, residence time and so on.

This process is usually achieved at atmospheric pressure and at a temperature between 200 and 600 ˚C. Under these conditions the typical product yields are 30 -35% of charcoal, 18 - 20% of organic liquids, and 20% of other gases.

The liquid products which compose an aqueous solution are commonly known as pyroligneous acids, namely methanol, acetone and acetic acid, and an insoluble fraction of tar and pitch. The end products are carbon dioxide, carbon monoxide, methane and hydrogen, which have heat content in the range of 10,500 – 15,000 kJ/m^3 as compared to natural gas which has 37,500 kJ/m^3 (Ferrero et al., 1984).

2.4 Usage of Hydrogen

2.4.1 Fuel cells

Fuel cells are similar to batteries which transform chemical energy into directly to electrical energy. Fuel cells can be continuously recharged, on the other hand, batteries have a limited supply which become exhausted.

Hydrogen flows to the anode where it disseminates into hydrogen ions and electrons according to the following equation:

$$\text{Anode} + H_2 + 2OH^- <=> (\text{Anode} + 2e^-) + 2H_2O$$

Oxygen, at the same time, flows to the cathode and reacts as illustrated by the following equation

$$\text{Cathode} + 2e^- + ½O_2 + H_2O <=> \text{Cathode} + 2OH^-$$

The net result of these two reactions on opposite sides of the cell is

$$H_2 + ½O_2 <=> H_2O$$

Hydroxyl ion is transferred from the cathode to the anode through an electrolyte and the electrons are transferred from the anode to the cathode by way of an external circuit. This electron flow is the valuable electrical output of the fuel cell. Electrolytes can be acids, bases, or molten salts. On the other hand, potassium hydroxide (KOH) is usually preferred to acid because it is less corrosive and has a higher potential of oxygen electrode.

The function of fuel cells is highly effective in some applications especially in speacecraft, advances in catalyst and electrode technology. However, fuel cells can be truly considered for general use in the residential, commercial, transportation or industrial market. The percentage of hydrogen gas needed must be considered.

2.4.2 Catalytic burners

The properties of hydrogen give it higher advantages than those of other fuels. Therefore, hydrogen production is able to fuel unique flameless heating devices. The burner operating temperature is under 1750˚K, which is the temperature for formation of significant quantities of nitrogen oxides. Besides, it is safe to operate. A device could be used for water heating, cooking, and for space heating purposes. However, it must also be inexpensive, and resistant to poison by fuel additives.

2.4.3 Utilizing other sources of energy

Digester can be used as fuel for automobile, hot water boilers, water pump engines, blowers, and electric generators. It can also be used to heat incinerators or burned to heat the influents sludge during pretreatment.

2.4.3.1 Advantages of hydrogen

- It can be manufactured from the molecular breakdown of water.
- Raw materials from agriculture can be the primary sources for hydrogen
- When combustion with air occurs hydrogen produce water vapor.
- The heat of combustion per unit weight is higher than that of other practical fuels.
- The biological process to generate hydrogen is more practical and efficient than that of other fuels.
- Spills of hydrogen disperse rapidly under conditions of good ventilation.
- Hydrogen can also be used in boilers for many processes (David and Mathis, 1976).

2.5 Ethanol Production

2.5.1 Raw materials for processing ethanol

Raw materials for ethanol production from various agricultural sources are divided into three categories:

- Starch – grains or cereals, e.g. rice, wheat, corn, barley and sorghum; and root vegetables, e.g. cassava, potato and sweet potato
- Sugar – e.g. sugarcane, molasses, beet-root, and sweet sorghum
- Fibers – e.g. rice, straw, dried sugarcane stems, corn cobs, rice bran, pieces of wood or paper, sawdust, weed, including industrial waste.

However, a wide variety of raw materials can be used to produce ethanol, depending on the various criteria such as prices, as well as yield of ethanol per unit of raw materials input and per area unit of cultivating agricultural products. It doesn’t affect human food production. Moreover, raw materials from cassava contribute to a positive net energy balance.

Based on these criteria, the choice of raw material for producing ethanol should be cassava (particularly fresh cassava). The production of ethanol yield is illustrated in Table 2.1.

Table2.1 Comparison of ethanol production from various raw materials

Raw material (ton)	**Biomass (ton/ha)**	**Ethanol (liter/ha)**	**Ethanol production (liters/ton biomass)**
molasses	-	-	270
Sugar cane	9	3600	70
Cassava	7	1800	180
Sorghum	5	1500	70
Potatoes	5	1800	-
Corn	3	1200	370
Coconut juice	-	-	83

Adapt from: Department of Alternative Energy Development and Efficiency, 2004; Martin, 1991.

2.5.1.1 Cassava

Cassava, also known as tapioca, is an important food product, and commercial plant. Cassava is mostly grown in northeastern, central and eastern Thailand, and the rest scattered in other regions. Cassava is widely cultivated, therefore there is an oversupply of cassava.

Table 2.2 Supply & Demand for Ethanol Production

Unit: Million tons/year

Raw Material Source	Description	2009	2010	2011
Cassava	Production	28.90	31.45	33.30
	Domestic Use (cassava, starch)	9.87	8.42	8.69
	Export	15.33	21.42	22.05
	The rest for Ethanol Production	3.70	1.61	2.56
	Ethanol from Cassava (Million Liters/day)	1.72	0.75	1.19
	Capacity of Ethanol Production from Cassava Plants (Million Liters/day)	2.15	4.05	4.05
Ethanol (excluding Consumption)		2.30	2.25	2.95
DEMAND of REDP*15 years		1.34	2.11	2.96

Source: Department of Alternative Energy Development and Efficiency, 2004.

Note: - Importing data 2010 during rectified.

- REDP: Renewable Energy Development Plan

2.6 Ethanol Production Process

There are several ethanol process technologies which are widely developed and utilized around the world such as Praj from India (www.praj.com), Katzen from United States (www.katzen.com), Maguin Interis from France (www.maguin.com), Alfa Laval from India (www.alfalaval.com), and Shandong from China (www. sdmeco.com). While each technology is similar in its process, it is different in the characteristics of the manufacturing process. General manufacturing process comprise raw material preparation, fermentation, separation and purification of ethanol as the following Figure 2.4 (In case of a raw material such as cassava, it has to be hydrolyzed in order to produce sugar).

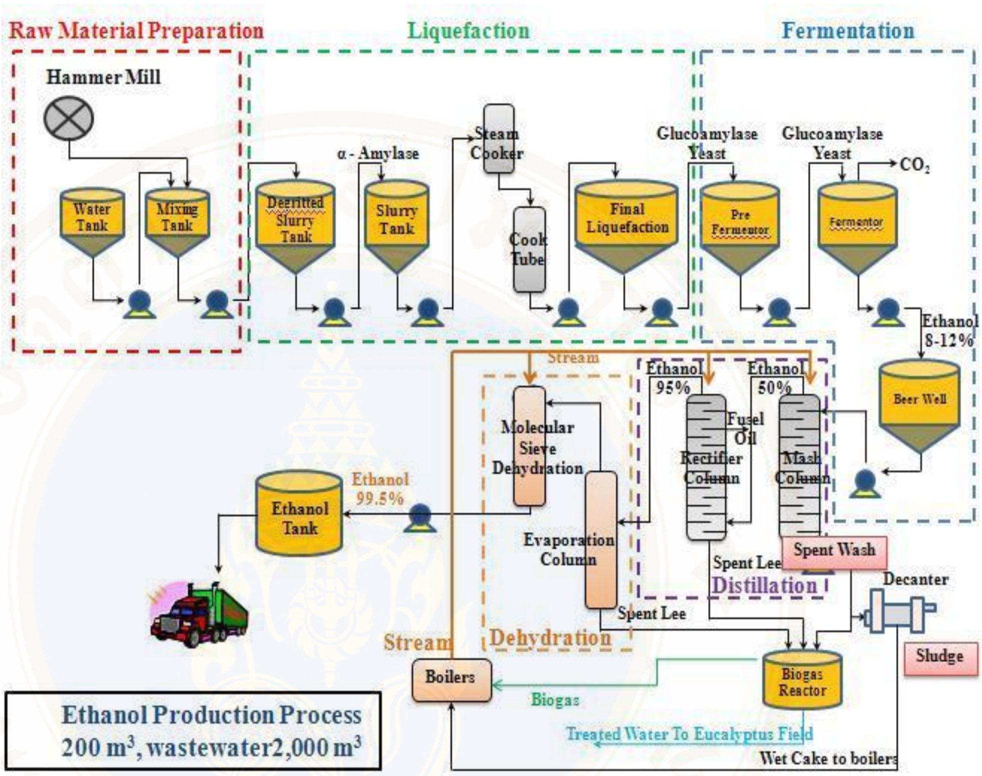

Figure 2.4 Flowchart of cassava ethanol plant

2.6.1 Raw materials preparation for ethanol production

The process of raw material preparation of cassava ethanol process for fermentation is complicated. The raw materials must be converted into sugar by using acids or enzyme hydrolysis until the raw materials are in an appropriate state before being transferred into the fermentation process. Most of the fermentation is adaptable because this type of hydrolysis is convenient, economical, and environmentally friendly.

2.6.2 Fermenting agent preparation and fermentation

2.6.2.1 Fermenting agent preparation

The subsequent step is the fermentation process to acquire sufficient quantities of strong microbes for fermenting without the contamination of the microbes. Raw material is poured into the fermentor. The condition of fermentation is controlled. For instance, the aeration rate, agitation rate, pH value, and temperature, are adjusted depending on the method of fermentation, type of product, and type of microbe used. Moreover, the type of yeast used in the fermentation process will be carefully selected to match the type of raw material. Yeast is a fermenting agent. However, the preparation of fermenting yeast will not be conducted if dry yeast is used.

2.6.2.2 Fermentation

Subsequently, the raw material is appropriately prepared, then placed in the fermentor. Fermentation is controlled at 30 - 35˚C for 2 – 3 days. An 8 - 12% concentration (by volume) of alcohol is acquired. After that, the dehydration of alcohol contributes to a 95% alcohol concentration (by volume) by molecular sieve or membrane filtration. The remaining 5% will partly become an energy source for the yeast and partly yield other by-products, such as acetaldehyde ranging from 0 to 0.03%, acetic acid ranging from 0.05 to 0.25%, and the rest is furfural.

2.6.3 Types of alcohol fermentation

Types of alcohol fermentation are classified below:

2.6.3.1) Batch fermentation

Raw materials, nutrients, and the fermenting agent are fed into the fermentor only once during the process to allow microbes to consume a greater amount of raw materials and fermenting agent.

2.6.3.2) Feed batch fermentation

Raw materials, nutrients, and fermenting agent are fed into the fermentor more than once during the process to permit the microorganisms to consume a great deal of raw materials and fermenting agent.

2.6.3.3) Continuous fermentation

Raw materials, nutrients, and the fermenting agent are continuously fed into and removed from the fermentor.

2.6.4 Separation and purification of ethanol

Ethanol concentration of 8 – 12% by volume is separated from the fermenting liquid, also known as spent wash, under a chemical process called partial transformation. The yield of ethanol is 95.6% by volume, otherwise it is known in the industry as 95% or hydrous ethanol. Transformation at atmospheric pressure cannot yield ethanol with higher concentrations. As a result of the process an azeotropic mixture is generated, which is a mixture containing substances with constant boiling points. The required fuel-grade ethanol is supposed to be 99.5% concentration by volume, called anhydrous ethanol or absolute ethanol.

The dehydration methods used in the process are as follows:

- Molecular sieve separation
- Extractive transformation using a third component, either an organic solvent or a strong salt solution to pause the azeotrope (The Department of Alternative Energy Development and Efficiency, 2004).

2.6.5 Utilization of By-product and waste from Ethanol Plant

In this last step, ethanol production yields the following by – products as shown below:

Carbon dioxide occurs during the fermentation of sugar to ethanol by using yeast. The estimated capacity of ethanol production is 150,000 liters/day which will create approximately 100 – 200 tons of carbon-dioxide per day. Utilization of carbon dioxide can be used in all states such as, gas, liquid, and solid. It is widely used in the food and the carbonated beverages industry. Moreover it is used in other industries such as the chemical industry and welding industry.

Fusel oil or sometimes called ‘Fusel alcohol’ is a by-product generated during the process of ethanol distillation. Fusel oil is composed of many different types of alcohol, the majority of which are 3, 4 or 5 atom carbons such as Isoamyl alcohol and Active amyl alcohol which is a main component that is highly valuable, other alcohols are butanol and propanol. Fusel oil is used to separate alcohol by distillation, chromatography or chemical process. After that, it passes through a purification process, then alcohol is taken and used as a solvent in industries.

DDGS, its full name is dry distillers grains with soluble. It is a by - product from the mixture of raw materials such as solid of stillage and soluble solids of stillage after separating the ethanol distillation for animal feeding. The production process of DDGS occurs in the separation of liquid and solid by centrifudge. The remaining liquid part after the evaporation of the ethanol distillation is mixed with solid part. Then the mixture is put through a drying and cooling process. DDGS production from cassava is lower than from corn; 10, and 25 percentage of protein by dry weight, respectively.

Biogas is a gas degraded by microorganisms naturally occuring under anaerobic conditions. It is used as an alternative fuel that can be produced from agricultural waste and wastewater. Biogas is composed of many types of gas; approximately 50% to 70% is methane, 30% to 50% is carbon dioxide, and the rest are hydrogen, oxygen, hydrogen sulfide, nitrogen, and vapor. Biogas mainly contains methane which makes it flammable, therefore it can be used in the steam boiler ethanol process. Heating value is approximately 22,400 KJ/m^3 for 65% methane.

Organic fertilizer wastewater stillage has humus and soluble salt which has many minerals such as potassium, nitrogen, and phosphorus. It can be used as a fertilizer (Department of Industrial Promotion, 2009).

2.7 Lignocellulose

Lignocellulose is a polysaccharide composed of a great deal of glucose (2,500 – 14,000 units). It is connected by β-1,4 glycosidic and hydrogen bond as shown in Figure 2.4. These make it a substantial structure of the primary cell wall of green plant, classified into 3 structures of fringe micelle, role or fold along the fiber of cellulose, ribbon and role into spiral. The different structures are a result of the space between the molecules which lead to discrete molecules. Also, it is comprised cellulose, hemicelluloses, and lignin. Lignocellulose is the largest source of hexose (C6) and pentose (C5) sugar. These are more complex substrate than starch, which is not easily hydrolyzed into fermentable sugar (Sara et al., 2010).

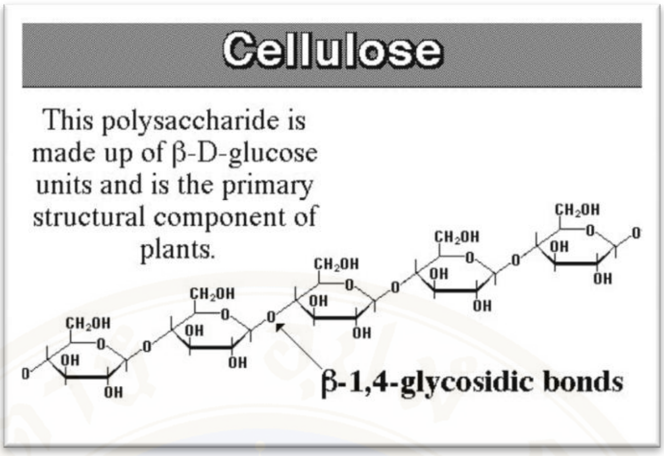

Figure 2.5 The structure of Cellulose

2.7.1 Pretreatment of lignocellulose

The purpose of the pretreatment is to remove the inhibitor of lignocellulosic such as lignin, hemicelluloses, and cellulose. Pretreatment enhances the ability to convert lignocelluloses to sugar by an enzyme. The main procedure for pretreatment consists of physical, chemical, biological, and physio-chemical. This study was done utilizing the psycho – chemical process to enhance pretreatment efficiency. Badal, (2005), compared the physio-chemical pretreatment by sodium hydroxide concentration, high temperature and pressure to wheat straw hydrolysis by enzyme. Physio-chemical pretreatment capability has the higher capability.

2.7.2 Hydrolysis of lignocellulosic material

Cellulase hydrolysis is comprised chemical and enzyme hydrolysis. Chemical hydrolysis, which is diluted acid, is commonly used to hydrolyze hemicellulose. Another alternative enzyme hydrolysis is considered in order to economically and environmentally produce hydrogen. Organic waste as biomass is broken down to sugar by enzyme, which is produced from bacteria and fungi. It produces a set of at least three different kinds of cellulose enzymes to effectively obstruct the complex cellulose.

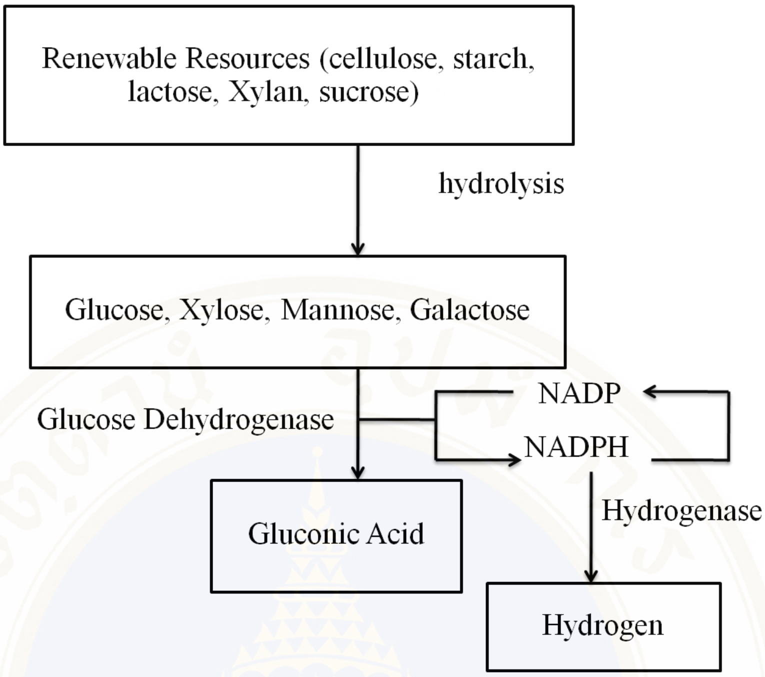

Figure 2.6 The pathway of enzymatic production of hydrogen (Woodward et al, 1999)

2.7.3 Mechanism for the enzymatic breakdown of cellulose

The enzyme used in enzyme reactions to break down cellulose, the cellulase, is an enzyme found in many organisms. Bacteria contained in the system of *Trichoderma sp*. are commonly used to produce the cellulose enzyme. The enzyme cellulase is classified in 3 steps which carries out the hydrolysis reaction. The function of the enzyme, which is called “Synergisticaction”, can be summarized as follows:

Endo-β-1,4-glucanase: Degradation of oligosaccharide and cellulose to cellobiose by degradating the reducing end of cellulose chains. One of these may be the enzyme that acts on crystalline cellulose; the main products are cellobiose and cellotriose.

Exo-β-1,4-glucanases: degrading cellulose substrates by successively removing cellobiose residues from the non-reducing end of the chain. This contains several components with varying degrees of randomness. Exo-β-l,4-Glucosidase removes a single glucose unit from the non-reducing end of the chain.

β-glucosidase: This hydrolyzes cellobiose and short chain cellooligosaccharides to glucose but has no effect on cellulose. This reaction supporting endoglucanase and exoglucanase enzyme are stimulated to enhance glucose. The amount of glucose converted is dependent on the ratio of β - glucosidase enzyme. Therefore, these are important keys for converting cellulose to glucose (Lee et al, 1980).

2.7.4 Reducing sugar and non-reducing sugar

Monosaccharide and disaccharide have mostly carbonyl group which is easily oxidized. All these groups of monosaccharide and maltose can be classified as reducing sugars. Their properties can reduce metal ions such as Cu^{2+} or Ag^{+}, which is an undissolved product. Reducing sugar has part of a hemiacetal, which can change to an open chain. On the other hand, Carbohydrates which are not oxidized capably are called non-reducing sugar. Since, these anomeric carbons are held on glycosidic bond (Horton et al., 1996).

2.8 Related Research

2.8.1 Effect of pH

The pH plays an important role in determining the type of anaerobic fermentation pathway in the anaerobic bio-hydrogen processes. Hydrogen production can be achieved at a weakly acidic pH. Chou et al. (2008) conducted an experiment with a sequencing batch operation at optimum conditions of pH 6.0., and Yang et al. (2006) used initial pH of 6.8–7.2 in continuous biological hydrogen production using wastewater from citric acid factory as raw material. But Wu et al. (2010) investigated the use of liquid swine manure supplemented with glucose (10 g/L) as substrate for hydrogen production using an anaerobic sequencing batch at pH of 5.0. Similarly, in the study by Hwang et al. (2004), hydrogen was produced by ethanol–acetate fermentation at pH of 5.0 ± 0.2. Shi et al. (2009) reported influent pH value of 5.0–6.0, effluent pH value of 4.0–4.5; and Lin et al. (2008) applied the optimal initial cultivation pH (tested range 5–7). All these values both in influent and effluent pH were similar to those in the control set of this study.

2.8.2 Effect of stirring Semi-Continuous system

Chou et al, (2008) mentioned that the pH level was a more important factor than the stirring on shortening hydrogen production lag and increasing hydrogen yield. Optimum condition of stirring speed was at 120 rpm using the 100 L reactor experiment with a sequencing batch mode operation confirmed that the hydrogen production rate obtained with laminar flow was significantly more stable and reproducible than with turbulent flow. However, Ding, et al. (2010) mentioned that impellers with different type and speed generated different flow patterns, which resulted in different efficiencies for biohydrogen production. The optimized impeller at speeds between 50 and 70 rev/min is the most suitable for economical biohydrogen production.

2.8.3 Effect of hydraulic retention time

Increasing hydrogen production rates were generally observed when the HRT was lower. Wu et al, (2009) investigated the use of liquid swine manure supplemented with glucose (10 g/L) as a substrate for hydrogen production, using an anaerobic sequencing batch reactor under different hydraulic retention times (HRTs). Decreasing HRT from 24 to 8 hours had some effects on the increase of hydrogen. The optimal HRT at12 hours could produce best hydrogen yield. In this regard, Yang et al, (2006) reported continuous biological hydrogen production using wastewater from a citric acid factory as raw material, while Zong et al, (2009) mentioned continuous biohydrogen production from biopolymers like starch, and Mitchell et al, (2009) applied an immobilized *Clostridium tyrobutyricum* culture, grown under continuous culturing conditions. However, Shi et al, (2009) revealed that the anaerobic fermentation to treat kitchen wastes for hydrogen production was achieved at 7 hr HRT.

2.8.4 Effect of feeding of process

Vazquez et al. (2004) mentioned that the moderate-to-high yields obtained with the semi-continuous process used in their work are in disagreement with previous reports which claimed that batch and semi continuous processes are less efficient than continuous ones.

2.8.5 Effect of volatile fatty acid

Chen et al. (2009) showed that using a ratio of butyrate to acetate to correlate the performance of hydrogen production is not appropriate due to the growth of homoacetogens, as a result the carbohydrate removal efficiency declined to 56% at pH of 4.9, which indirectly resulted in the reduction of total volatile fatty acid production. Acetate fermentation was the dominant metabolic pathway at pH 4.9.

2.8.6 Hydrogen yield

Anaerobic sludge, pretreated by chloroform, base, acid, heat and loading-shock, as well as untreated sludge were evaluated for their thermophilic fermentative hydrogen-producing characteristics from cassava stillage in both batch and continuous experiments. Hydrogen yields (varied from 32.9 to 65.3 mL H_2/g VS) among the tested pretreatment methods in batch experiments were reported (Luo et al., 2010**).** When the influent was adjusted to pH of 6 by $NaHCO_3$ instead of NaOH, the hydrogen yield increased from about 40 to 52 mL H_2/g VS in all experiments. Therefore, pretreatment of anaerobic sludge is unnecessary for practical thermophilic fermentative hydrogen production from cassava stillage.

Sreethawong et al, (2010) investigated hydrogen production from cassava wastewater using anaerobic sequencing batch reactors (ASBR). They found maximum hydrogen production performance in terms of specific hydrogen production rate (SHPR) (388 mL H_2/g VSS d or 3800 ml H_2/L- d) and hydrogen yield (186 ml H_2/g COD removed).

In the study by Argun et al, (2009) hydrogen formation performances of different anaerobic bacteria were investigated in batch dark fermentation of waste wheat powder solution (WP). The heat-treated anaerobic sludge was found to be the most effective culture with a cumulative hydrogen formation of 560 mL, hydrogen yield of 223 mL H_2 g^{-1} starch and a specific hydrogen production rate of 32.1 mL H_2 g^{-1} h^{-1}.

Shi et al, (2009) revealed that in the anaerobic fermentation of kitchen wastes for hydrogen production, the maximum efficiency of hydrogen bio-production in a continuous stirred tank reactor (CSTR) was 4.77 LH_2/ L reactor-d**.**

Wu et al, (2009) used liquid swine manure supplemented with glucose (10 g/L) was used as substrate for hydrogen production in an anaerobic sequencing batch reactor. They reported hydrogen yield ranging between 1.18 and 1.63 mol-H2/mol glucose at 12 hr HRT.

Zong et al, (2009) mentioned that in dark fermentation, the average yield of hydrogen was approximately 199 ml H_2 g-1 cassava and 220 ml H2 g-1food waste. Maximum hydrogen yield was 0.92 mol H_2/mol glucose added which was observed at 12 hr HRT with the highest HBu/HAc ratio of 4.3.

Dong et al, (2008) stated that the hydrogen production potentials of rice, potato and lettuce were 134 mL/g-VS, 106 mL/g-VS, and 50 mL/g-VS, respectively. In addition, the hydrogen percentages of the total gas produced from rice, potato and lettuce were 57–70%, 41–55% and 37–67%. Samples used in the study were from organic fraction of municipal solid waste (OFMSW) by anaerobic mixed culture fermentation using batch experiments at 37˚C.

Wang et al, (2007) found that direct starch fermentation attained the highest maximum H_2 production rate *(R*max*)*, overall Hydrogen production rate *(R*overall*)*, and H_2 yield *(Y*H_2 *)* were 25.6 mL/h, 88 mL/h/l, and 5.28 mmol H_2/g starch (4.64 mmol H_2/g COD), respectively. In contrast, using hydrolyzed starch as the substrate gave rise to a much higher hydrogen production performance, as the highest *R*max, *R*overall, and *Y*H_2 values increased to 43.1 mL/h, 210 mL/h/l, and 6.1 mmol H_2/g COD, respectively.

Fang et al, (2006) conducted an experiment on hydrogen production using rice slurry containing 5.5 g carbohydrate/L by using Clostridium sp. developed from acidophilic hydrogen producing sludge. The hydrogen yield of 346 mL H_2/g carbohydrate was obtained at a low pH of 4.5 which was 62.6% of the theoretical yield at 553 mL hydrogen per gram of polysaccharides.

Hwang et al, (2004) mentioned that hydrogen was produced by an ethanol–acetate fermentation, giving hydrogen yield 100–200 mL g Glu^{-1} with hydrogen content of 25–40%.

In the study by Chen et al, (2009), an anaerobic sequencing batch reactor (ASBR) was used to evaluate biological hydrogen production from carbohydrate-rich organic wastes. The maximum hydrogen yield of 2.53 mol H_2/mol sucrose consumed was achieved. Besides, pH and cyclic duration under the unique experimental state profoundly impacted fermentative hydrogen production.

Chong et al, (2009) referred that dark fermentative biohydrogen production can use biomass such as food and starch-based wastes, cellulosic materials, dairy wastes, palm oil mill effluent and glycerol. Several factors are affected during the biohydrogen production such as undissociated acid, hydrogen partial pressure and metal ions.

Lee et al, (2008) used cassava starch as the feedstock to produce hydrogen via dark fermentation. The effect of hydrogen production namely, on temperature, pH and starch concentration were intensively examined for their effects on Hydrogen production activity.

CHAPTER III

MATERIALS AND METHODS

This study was an experimental research done to determine bio - hydrogen production from cassava ethanol spent wash under anaerobic digestion by Semi - Complete Mixed Reactor. The experiment was divided into two phases. In phase I, the characteristic of cassava ethanol spent wash influent and researching the effects of hydraulic retention times (HRTs), and pH for biochemical hydrogen potential (BHP) test were studied. In phase II, the relationship effluent pH, VFA (volatile fatty acid) and hydrogen yield at various ratio of substrate for anaerobic digestion by Semi-Completely Mixed Reactor were determined.

3.1 Experimental Design

The bench-scale of Semi-Completely Mixed Reactor under anaerobic conditions was carried out in the laboratory at the Environmental and Resources Department, Thailand Institute of Scientific and Technological Research. The samples of cassava ethanol spent wash (liquid phase) and sludge (solid phase) after being centrifuged) from ethanol distillation process were used for this study. The sludge was dried in an oven, then hydrolyzed by adding enzyme Cellulase. In phase I, the experimental design was 7×2 factorial with various pH levels: 4.0, 4.5, 5.0, 5.5, 6.0, 6.5 and 7.0 (7 factors) observed at 24 and 48 hr HRT (2 factors). Operational condition for BHP test at ambient temperature is illustrated in Table 3.1. In phase II, the experiment was designed with 24 hr HRT at COD loading rate of 30 kg COD/m^3-d and various ratio of cassava ethanol spent wash (CESW) mixed with sugar at 1:0, 1:1, 2:1, 1:2, and 3:1 were operated. Subsequently, the optimal ratio condition (1:1) from 24 hr HRT was operated at 12 hr HRT. The same optimal ratio (1:1) was also operated for hydrolyzed sludge mixed with CESW, but at 24 hr HRT.

Table 3.1 The factor of hydraulic retention times (HRTs) and pH of Cassava ethanol spent wash in BHP test

Experimental condition	**HRT (hours)**	**Influent pH**
1	24	4.0
2	24	4.5
3	24	5.0
4	24	5.5
5	24	6.0
6	24	6.5
7	24	7.0
8	48	4.0
9	48	4.5
10	48	5.0
11	48	5.5
12	48	6.0
13	48	6.5
14	48	7.0

Phase I: The conditions of HRT and pH of Cassava Ethanol Spent Wash for biochemical hydrogen potential (BHP) test

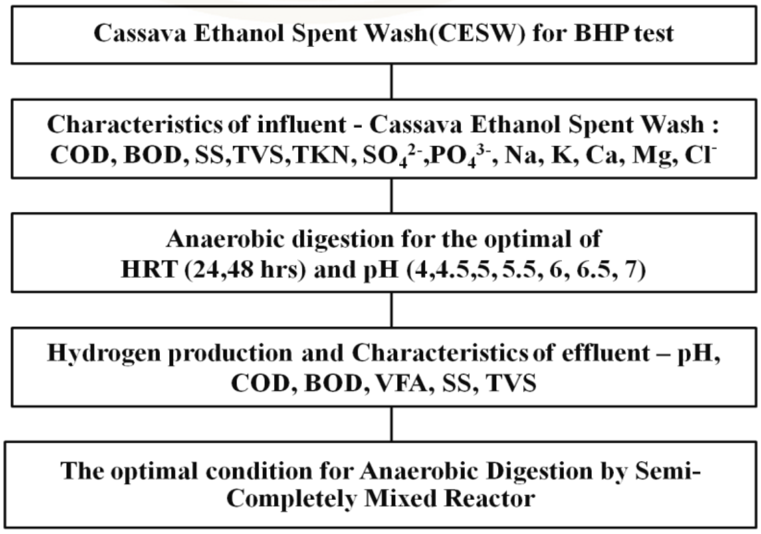

Figure 3.1 Experimental Design for Phase I

Table 3.2 The various ratio of substrate for biohydrogen production by operating Semi - Completely Mixed Reactor

Condition	**Organic Loading Rate (Kg COD/m^3-d)**	**Values of COD of CESW: COD of sugar (Kg/m^3)**	**Ratio of CESW: sugar**
1	30	30: 0	1:0 (no sugar added)
2	30	15: 15	1:1
3	30	20: 10	2:1
4	30	10: 20	1:2
5	30	22.5: 7.5	3:1
6	30	15: 15 (COD of CESW : COD of hydrolyzed sludge)	1:1

Note: Each condition was operated for 40 days, CESW: Cassava Ethanol Spent Wash

Phase II The various conditions of CESW mixed with sugar and CESW mixed with hydrolyzed sludge for anaerobic digester by Semi-Completely Mixed Reactor

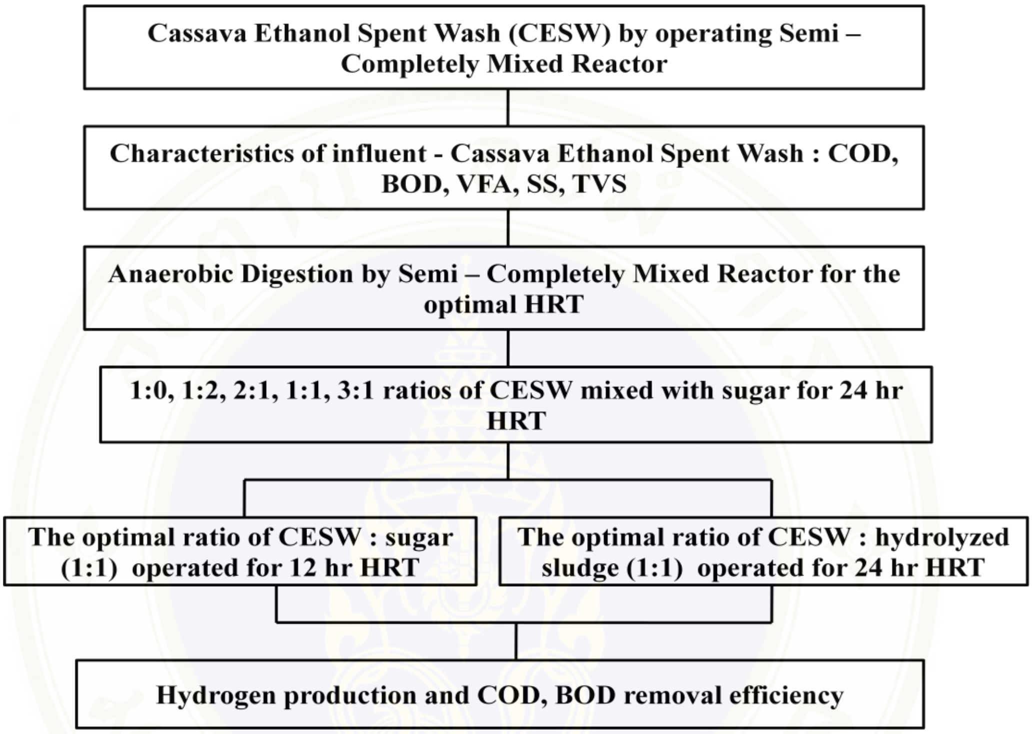

Figure 3.2 Experimental Design for Phase II

3.2 Research Model

3.2.1 Equipment and materials for the bench scale as Semi - Completely Mixed Reactor

3.2.1.1 Reactor is made of acrylic with dimensions of 14 centimeters (diameter) × 40 centimeters (height) as illustrated in Figure 3.1. There was 5.0 liters of working volume of CESW.

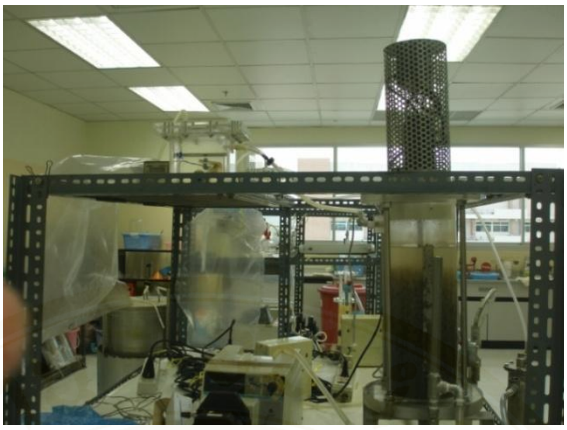

Figure 3.3 Reactor of experimental operating system

3.2.1.2 Stirrer of reactor is completely mixed by central stirrer shaft connected with speed motor.

3.2.1.3 Timer is used to set time for feeding and stirring.

3.3.1.4 Influent and effluent container is made of plastic with a volume of 15 liters.

3.2.1.5 The influent was fed by peristaltic pump.

3.2.1.6 The mixture of gas was measured by volume with the gas counter and collected in a gas bag for sampling and analysis with gas chromatography.

3.2.1.7 Sodium hydroxide was fed by stroke pump to adjust pH of the influent.

3.2.2 Equipment and materials for laboratory analysis

3.2.1.1 pH meter
3.2.2.2 Hot-air oven
3.2.2.3 Desiccators
3.2.2.4 Refrigerator
3.2.2.5 Muffle Furnace
3.2.2.6 Beaker size 50, 100, 500 and 1,000 mL
3.2.2.7 Cylinder size 50, 100 and 1,000 mL
3.2.2.8 Volumetric flask size 50, 100, 500 and 1,000 mL
3.2.2.9 Volumetric pipette size 1, 2, 3, 4, 5, 10, and 25 mL

3.2.2.10 Pipette size 1, 5 and 10 mL

3.2.2.11 Burette size 25 and 50 mL

3.2.2.12 The serum bottle 50, 120 mL

3.2.2.13 Aluminium cap, capper, syringe, and needle

3.2.14 Gas chromatography (Model 2014, Shimadzu, Japan)

3.2.15 High performance liquid chromatography

3.3 Procedures and Analytical Methods

3.3.1 Seeding and Acclimatization

The anaerobic granule sludge collected from Serm Suk. Co., Ltd, Nonthaburi was heated at 90˚C for 15 minutes to inhibit methane-producing bacteria activity (Arooj et al., 2008; Sung et al., 2002). The heated sludge was acclimatized with the cassava ethanol spent wash, collected from Sapthip Co., Ltd, Lopburi, for two weeks in the following Appendix A. The operating Semi – completely Mixed Reactor was activated by nutrient as shown in the Table 3.3.

Table 3.3 Nutrients for acclimatization of granule sludge in operating Semi – Completely Mixed Reactor

Nutrient	Ingredient	Unit (g/L)
Buffer solution	$Na_2HPO_4.7H_2O$	273
	K_2HPO_4	105
Nutrient (10mL/5 L wastewater)	NH_4HCO_3	12.5
	KH_2PO_4	0.875
	$MgSO_4.7H_2O$	0.075
	Na_2CO_3	0.0075
	$NaMoO_4.2H_2O$	0.0075
	$CaCl_2.2H_2O$	0.0075
	$MnSO_4.7H_2O$	0.0125
	$FeCl_2$	0.0025

Source: Lin, 2006

3.3.2 The biochemical hydrogen potential (BHP) test

The biochemical hydrogen potential (BHP) test was done in order to study the effect of HRT and pH to hydrogen yield. Moreover, the relationship factor to hydrogen yield was demonstrated after operating the bench-scale continuously. Each batch of the experimental condition batch test was done in triplicate. Starting-up each batch test, a mixture of 20 mL heat-pretreated and 80 mL of substrate was added in the serum bottle of 120 mL, then nitrogen gas was purge in the bottle replaced oxygen gas. The serum bottle was capped with a plastic and aluminum cap. The controlled temperature was 30 ± 1˚C in an incubator. An optimal primary pH value was determined from the range between 4.0 and 7.0 at an interval of 0.5 units. A stable cumulative hydrogen production was conducted for 24 and 48 hr HRT for each batch during the experimental phase as shown in Figure 3.4.

Figure 3.4 The biochemical hydrogen potential test

A mixture of gases produced during the BHP test was kept in the 50 milliliter serum bottles which were prepared by the following procedure. The serum bottles were filled with deionized water with an adjusted pH of 2 by sulfuric acid or hydrochloric acid. This pH 2 deionized water was then aerated by either helium or nitrogen gas to inhibit oxygen. The serum bottles were then capped by plastic and aluminum cap. Water displacement procedure was applied to replace water with mixed gases (Lin et al., 2008). A quantity of mixed gases was taken from a BHP test bottle using a 50 milliliter gas syringe, then injected into the prepared serum bottle. At the same time, a 5 milliliters syringe was used to remove deionized water out of the serum bottle (see Figure 3.5). The volume of water must be less than the quantity of gas replaced in order to prevent ambient air from getting into the serum bottle.

Figure 3.5 The water displacement to obtain mixture of gas

The mixture of gases was analyzed for the percentage of hydrogen gas by gas chromatography (model: GC2014 shimadzu). This percentage of hydrogen gas was multiplied by the volume of collected mixture of gases to derive the milliliters of hydrogen production from the BHP test.

3.3.3 Experimental Operation for Semi - Completely Mixed Reactor

A schematic drawing of the experimental unit, which was used in this study, was given in Figure 3.6. The system consists of reactor supported with stirrer, wastewater feeding pump, pH controller and NaOH feeding pump, influent and effluent containers, timers, gas counter, and gas bag as shown in Figure 3.6.

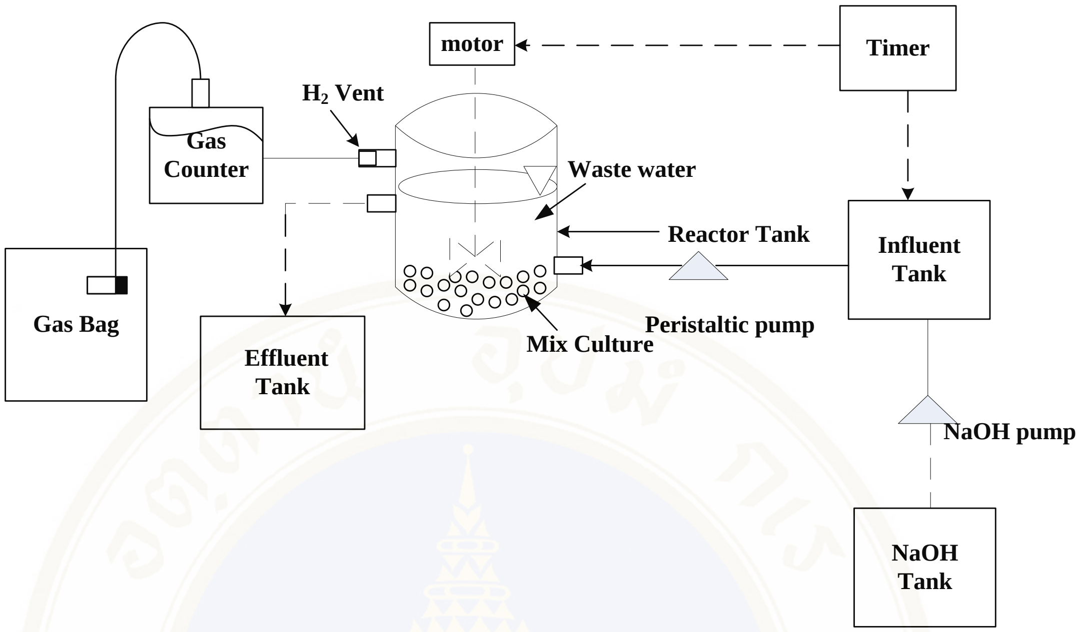

Figure3.6 A flowchart of Semi – Completely Mixed Reactor

The operation of Semi-Completely Mixed Reactor to produce hygrogen gas is demonstrated in Figure 3.6. The CESW was cleaned by passing through a screen to remove suspended contaminants before use. Dilutions were made according to the designed COD. Then, a large enough volume of this prepared CESW was contained in a tank to be delivered to the reactor by a peristaltic pump. The reactor consisted of a paddle shaft at the bottom which generated a horizontal circulation pattern. The influent entered the reactor in the bottom, while the effluent was discharged through the overflow line, in the upflow direction.

Mixed cultures of microorganisms fed on the influent as their substrate. As a result, mixed gases were produce, then delivered to the gas bag through gas counter. The mixed gases was analyzed for the percentage of hydrogen gas by gas chromatography (model: GC2014 shimadzu; Japan). This percentage of hydrogen gas was multiplied by the recorded volume of mixed gases from the counter to obtain the hydrogen production rate- milliliters of hydrogen per day.

Anaerobic microorganism fermentation in the reactor during operating system was controlled by influent pH value in the range of 5.0 – 5.5, and effluent pH value in the range of 4.0 – 4.3 by using sodium hydroxide and sulfuric acid in order to adjust pH. When influent pH dropped to lower than 5.0, the sodium hydroxide pump would automatically adjust the pH to the desired range. Flow rate of influent was controlled at 150 mL min^{-1} for 3 min, and stopped feeding for 2 hours. Subsequently, the stirrer would start operating at 55 rpm for 5 minutes. Therefore, the continuous operating system was 12 cycle per day under the Semi – Completely Mixed Reactor.

3.3.4 Experiment on hydrolyzed sludge by enzyme cellulase

Activity of cellulase enzyme was 367 ± 3.10 FPU/mL, which was purchased from East Asiatic Co., Ltd. The calculation that enzyme used 0.16 mL/ g substrate or 0.68 mL/g fiber shown in Appendix B.

3.3.4.1 Pretreatment and hydrolyzed sludge

The natural cellulose is an important characteristic in determining the effect of enzymatic hydrolysis. Sludge as biomass has an arrangement of fraction of lignin which is structure of crystallinity. It has an effect on the degradability of the substance. Therefore, pretreatment of sludge before being hydrolyzed was done as demonstrated in Figure 3.7.

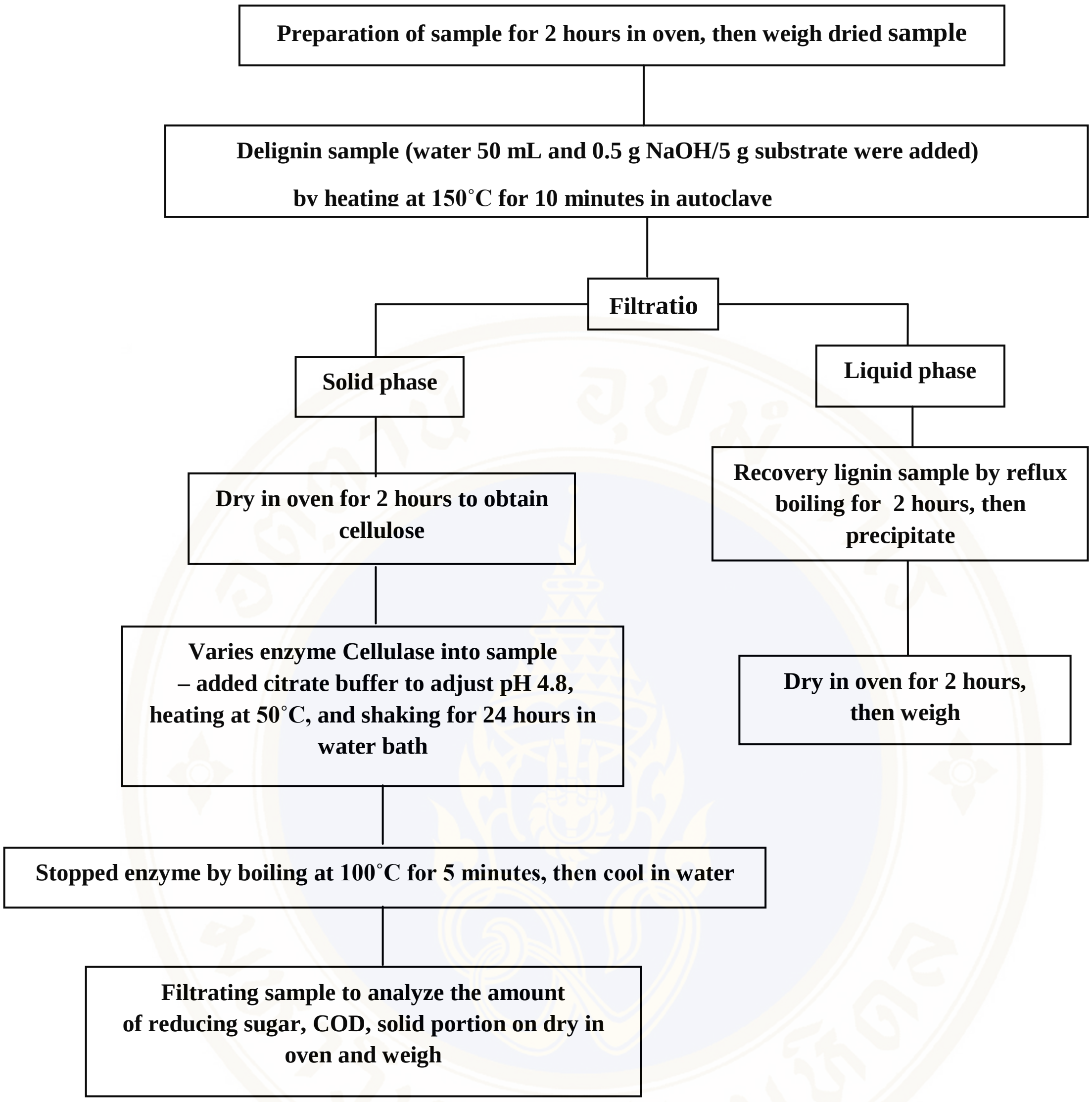

Figure 3.7 Procedure of pretreatment and hydrolyzed sludge (Klyosov et al, 1998)

3.3.4.2 Determination of reducing sugar

The titration method is done to determine the concentration of reducing sugar, for instance the Lane-Eynon is used as a titration method (Chapter 19 Determination of Reducing sugar by Lane-Eynon Method). For the analysis, it used a burette to add the carbohydrate solution to a flask containing an amount of boiling copper sulfate solution and a methylene blue indicator, to reduce sugar in the carbohydrate solution, which reacts with copper sulfate that is present in the flask. The copper sulfate in the solution reacts immediately, and there are no additional reducing sugar resulting which is indicated by the change from blue to white color and finally red-brick color. When the end point is reached, the volumes of sugar must be recorded.

3.3.4.2.1 Materials

- Hot plate
- Burette

3.3.4.2.2 Chemical

Felhing's A Solution: Dilute 69.278 g $CuSO_4 .5H_2O$ adjusted in 1 liter of distilled water. Preserve Cu is approximately 440.9 mg/25ml in refrigerator.

Felhing's B Solution: Dilute 346 g of potassium tartate and 100 g of sodium hydroxide adjusted in 1 liter of distilled water. Leave it set to 2 days and filtered through asbestos.

Dextrose solution 0.5%: Scale anhydrous dextrose 5 g, then adjusted in 1 liter of distilled water.

Indicator**:** Dilute methylene blue 1 g adjusted in 100 milliliter of distilled water.

3.3.4.2.3 Procedure of blank (control sample)

- Pipet 5 mL Fehling's A solution into Erlenmeyer flask 500 mL.
- Adding 5 mL Pipet Fehling's B into Fehling's A solution and shaked for mixing.
- Boil the solution on a hot plate with dextrose at 0.5 % by titration. Solution on the hot plate is heated until nearly boiling. The titration solution by 0.5% dextrose is approximately 18 ml.
- When close to boiling, add 5 drops of Methylene blue followed by titration until the blue solution disappears, and a heavy brick red color appears at the end. All the reactions should not take more than 3 minutes from the moment they start boiling to be completed, which in theory is without substance sample which should not exceed 0.5% dextrose and approximately 21.8 ml.
- The titration volume was assumed by the value of B in equation.

3.3.4.2.4 Procedure of sample

- Procedure is the same as control sample.
- Adding volume sample depends on sample characteristic. Before ending point add methylene blue.
- Titration solution by 0.5% dextrose, mixed with blue solution disappeared, and heavy brick red color is visible at the end.
- Recording volume titration was assumed by the value of A in the equation below (1).
- Calculating the amount of dextrose from

$$\text{R.S.} = \frac{\text{(B-A)(0.005g/L)}}{\text{Volume of sample}} \qquad (1)$$

B = Blank volume titration of 0.5 % dextrose solution

A = Sample volume titration of 0.5 % dextrose solution

R.S. = Reducing sugar

3.4 Analytical Methods

Gas analysis: the hydrogen gas percentage was calculated by taking an amount of sample mixing gas using a gas chromatograph (Model 2014, Shimadzu, Japan) equipped with a thermal conductivity detector (TCD). Helium was the carrier gas at a flow rate of 50 mL min $^{-1}$.

VFA analysis: the concentration of the volatile fatty acid (VFA) was analyzed using the same GC with a flame ionization (FID) after pretreatment with 0.45 µm membrane filter. The temperature of column, and the detector were 130, and 230 ºC, respectively. Helium was carrier gas at flow rate of approximately 15 mL min $^{-1}$.

Hydrolyzed hexose by enzyme analysis: the hexose concentration was analyzed using high performance liquid chromatograph (HPLC). The reflective index (RI) detector is the detector of choice to detect monosaccharide and disaccharide. The temperature of the column was 30 ºC. Mobile phase used 75% of acetonitrile at a flow rate 1 mL min $^{-1}$. Run time of each sample was injected into the HPLC at 20 µL.

The parameter and analytical methods of experimental cassava ethanol spent wash were illustrated in the table 3.4

Table 3.4 The parameter and analytical methods of experimental Cassava Ethanol Spent Wash

Parameter	**Analyzed**
pH	Electrometric Method
TS	heated at 105˚C for 2 hours
TSS	filtration and heated at 105˚C for 2 hours
TVS	heated at 550˚C for 15 minutes
BOD	Incubate and titration
COD	Close reflux and titration
TKN	Kjeldahl Method
TP	Spectrophotometric Molybdovanadophosphate Method
Ca, Mg, Na, K	Atomic Absorption with Air-acetylene
Cl^-	Argentometric Method
SO_4^{2-}	Turbidimetric Method

Source: (APHA, AWWA, WPCF, 2005).

3.5 Statistical Analysis

3.5.1 Descriptive Analysis: the VFA, BOD and COD removal efficiency, gas production rate (GPR), specific gas production (SGP) from anaerobic digestion were described as mean values, minimum values, maximum values and standard deviation (S.D.)

3.5.2 The One-Way ANOVA at 0.05 levels of significance was used to test for significant differences among the levels of dependent variables. All data were analyzed by using the package program of SPSS 11.5.

3.5.3 Regression analysis was performed to decide whether BOD, COD, pH and VFAs affecting the hydrogen production.

CHAPTER IV

RESULT AND DISCUSSION

This chapter is classified into 2 parts as follows: Part 1: The possibility of effective parameters on hydrogen production for biochemical hydrogen potential test. Part 2 is divided into 2.1) The effect of parameters on hydrogen production for operating the reactor at various ratio of cassava ethanol spent wash (CESW) mixed with sugar for 24 hr HRT, 2.2) The effect of the parameters on the hydrogen production for operating reactor at the optimal ratio of 1:1 of cassava ethanol spent wash (CESW) mixed with sugar for 12 hr HRT, and 2.3) The effect of parameters on hydrogen production for operating the reactor of hydrolyzed sludge mixed with CESW at the ratio 1:1 for 24 hr HRT.

Cassava ethanol spent wash (CESW) was used in this study. The main characteristic of CESW is as follows Table 4.1. It had a pH value of 4.0, the average value of Total Solid, Total Volatile Solid, Total COD, soluble COD, and soluble BOD were 59,400, 33,125, 103,810, 81,950, and 50,500 mg/L, respectively. These characteristics were similar to those from other study for the cassava ethanol plant in Taicang, Jiangsu province, PRC (Luo et al., 2010). It is highly likely that hydrogen is produced, because BOD is highly organic substrate. Besides, the controlled organic loading rate (OLR) was 30 kg COD/m^3-d or 30 gCOD L^{-1}-d. Kim et al, (2006), reported the continuous dark fermentative hydrogen production by mesophilic microflora of 35 g COD L^{-1}which was an overload, resulting in hydrogen inhibition. Moreover, they concluded that the maximum molar hydrogen yield of 1.22 mol mol^{-1} hexose consumed at sucrose concentration of 30 g COD L^{-1}. Besides, when COD loading rate was greater than 20 g COD L^{-1}, but not exceeding 30 g COD L^{-1}, methane was inhibited until it approached zero. Therefore, the upper limit of 30 g L^{-1} substrate concentration for sucrose or glucose was related to enhancing molar hydrogen. The mesophilic bacteria are not dependent on sunlight but require a supply of chemically bound energy in the form of wastes namely cassava ethanol spent wash, and hydrolyzed sludge (Baldursson, 2006). It contributed to an increased hydrogen yield. The results of BHP test, showed that hydrogen production for 24 hr HRT is better than that of 48 hr HRT. Therefore, 24 hr HRT was selected for the operation by a Semi – Completely Mixed Reactor which related to shorter HRT to enhance hydrogen yield (Hawkes et al, 2007), and because the Semi – Completely Mixed system is influent and effluent process which occurs simultaneously leading to more practical than the ASBR process (Gregor et al., 2010).

Table 4.1 Characteristics of Cassava Ethanol Spent Wash

Parameter	**Unit**	**Min - Max**	**Average**
pH	-	4.0 – 4.17	4.08
Total COD	mg/L	79,000 – 128,620	103,810
Soluble COD	mg/L	56,000 - 107,900	81,950
Soluble BOD	mg/L	17,000 – 84,000	50,500
Total Suspended Solid (TSS)	mg/L	11,000 – 22,800	16,900
Total Solid (TS)	mg/L	45,000 – 73,800	59,400
Total Volatile Solid (TVS)	mg/L CH_3COOH	30,250- 36,000	33,125
TKN	mg/L	900 - 1,064	982
PO_4^{3-}	mg/L	396 - 690	845
NH_4^+	mg/L	33 - 141	87
Cl^-	mg/L	26 - 60	43
SO_4^{2-}	mg/L	179 - 1507	912
Na	mg/L	10 - 34	23
Ca	mg/L	141-144	142
K	mg/L	358-585	466
Mg	mg/L	84-110	94

Source: APHA-AWA-WEF, 2005.

4.1 Optimal Parameters on Hydrogen Production in the Biochemical Hydrogen Potential (BHP) test

The important parameters of the biochemical hydrogen potential (BHP) test were pH, HRT and VFA, the indicators of hydrogen production (Shi et al., 2009 and Gang et al., 2010).

4.1.1 The effect of pH on hydrogen production in the BHP test

According to the results in Figure 4.1 and Table 4.2, the effluent pH of fermentation of both at 24 hr and 48 hr HRT were in the range of 3.95 to 4.80 which was similar to the study of Gang et al, (2010). The data demonstrated that the effluent pH at both 24 hr and 48 hr HRT was lower than the influent of each other. The influent pH of 5.5 leading to the effluent pH of 4.5 at 24 hr HRT could produce the maximal hydrogen yield similar to the research by Krupp et al, (2009).

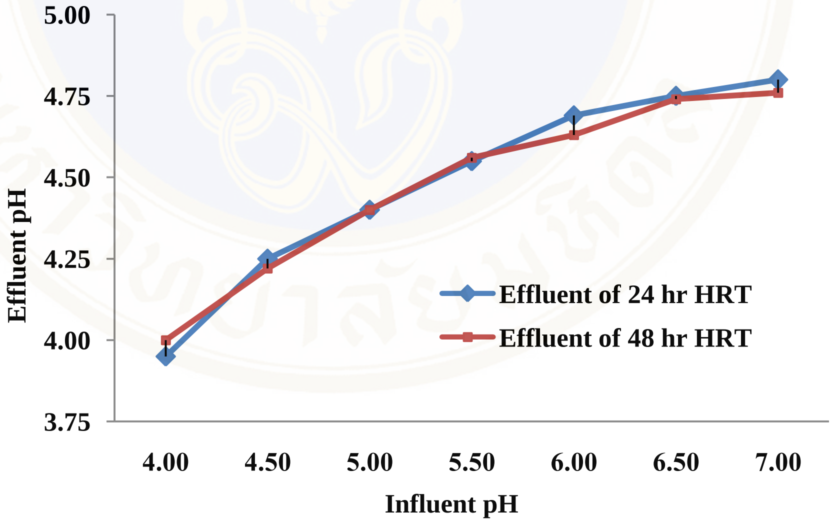

Figure 4.1 The influent pH and effluent pH for 24 and 48 hr HRT in BHP test

Table 4.2 The influent pH and effluent pH for 24 and 48 hr HRT in BHP test

Influent pH	Effluent pH (24 hr HRT)	Effluent pH (48 hr HRT)
4.00	3.95	4.00
4.50	4.25	4.22
5.00	4.40	4.40
5.50	4.55	4.56
6.00	4.69	4.63
6.50	4.75	4.74
7.00	4.80	4.76

4.1.2 The hydrogen production performance in the BHP test

From Figure 4.2 and Table 4.3, the graph shows hydrogen production rate and percentage of hydrogen. The maximal percentage of hydrogen was 20% at a pH value of 5.5 for 24 hr HRT and 5% at a pH value of 6.0 for 48 hrs HRT. As a result, the highest hydrogen production was 156 mL H_2 at a pH value of 5.5 for 24 hr HRT. Thus, the optimal condition of pH and HRT in the BHP test would be applied to the next phase using the Semi – Completely Mixed Reactor.

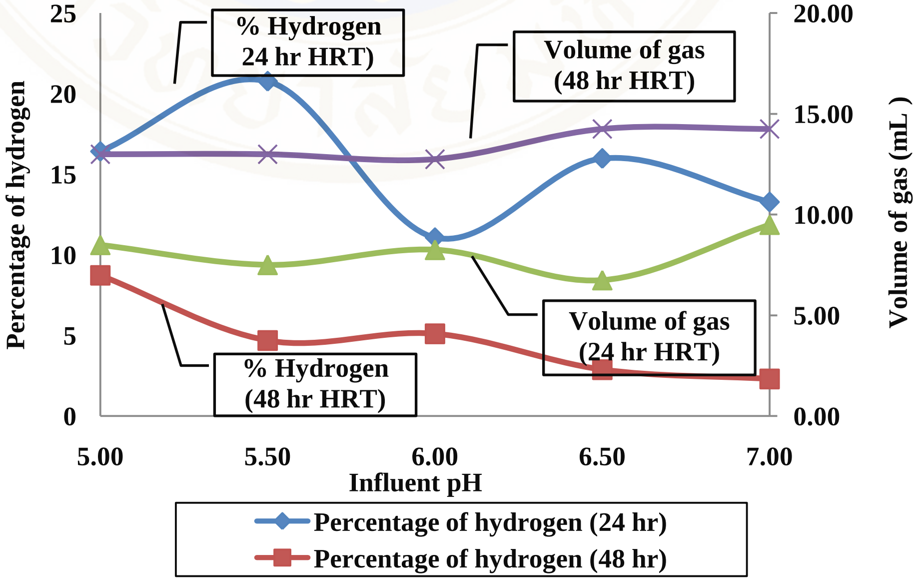

Figure 4.2 The pH, volume, and percentage of hydrogen gas for 24 hr and 48 hr HRTs in BHP test

Table 4.3 The pH, volume, and percentage of hydrogen gas for 24 hr and 48 hr HRT in BHP test

pH (Influent)	**%H_2 (24hr)**	**%H_2 (48hr)**	**volume gas (24hr)**	**volume gas (48hr)**	**Hydrogen production for 24 hr HRT (mL H_2)**	**Hydrogen production at 48 hr HRT (mL H_2)**
5.00	16.43	8.73	8.50	13.00	139.66	113.49
5.50	20.765	4.685	7.50	13.00	155.74	60.91
6.00	11.08	5.105	8.25	12.75	91.41	65.09
6.50	15.975	2.88	6.75	14.25	107.83	41.04
7.00	13.275	2.305	9.50	14.25	126.11	32.85

4.1.3 The effect of VFA on hydrogen in the BHP test

Figure 4.3 and Table 4.4 show that with effluent/influent VFA ratios at both 24 hr and 48 hr HRT the pH levels had the same characteristics that is the ratios were more than 1.00, excepting for pH values of 6.5 -7.0 which were less than 1.00. The higher ratio was linked to the higher hydrogen production. On the other hand, the lower ratio was related to the lower hydrogen production because the organic compound was converted to volatile fatty acids (VFAs) during the acidogenesis process. Subsequently, VFA could be decomposed to alcohol or acetone if the HRT is longer than those used in this study (Natpinit, 2010).

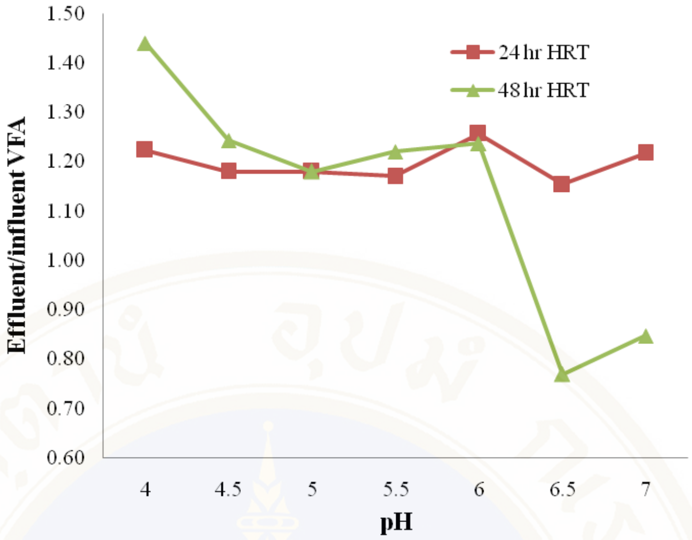

Figure 4.3 The effluent and influent VFA ratio in BHP test

Table 4.4 The effluent and influent VFA ratio in BHP test

pH (Influent)	24 hr HRT	48 hr HRT
4.0	1.22	1.44
4.5	1.18	1.24
5.0	1.18	1.18
5.5	1.17	1.22
6.0	1.26	1.24
6.5	1.15	0.77
7.0	1.22	0.85

4.1.4 The effect of each VFA on hydrogen in the BHP test

Figure 4.4 and Table 4.5 show the ratio of each VFA; the indicator of hydrogen production and yield depended on the type of VFA. That is, when the concentration of acetic and butyric acids were higher than the concentration of propionic acid, then it led to a greater hydrogen yield. (Natpinit et al., 2010). At optimal pH value of 6.5, HBu/HAc and HPr/HAc ratios were 0.47 and 0.21 for 24 hr HRT, while at pH value of 6.0, the ratios were 0.36, 0.38, respectively for 48 hr HRT. Therefore, the hydrogen production for 24 hr HRT was greater than that for 48 hr HRT, because, the HBu/HAc ratio (0.47) for 24 hr HRT was higher than that (0.36) for 48 hr HRT. Besides, the HPr/HAc ratio (0.21) for 24 hr HRT was lower than that (0.38) for 48 hr HRT. However, each VFA ratio was not associated with the level of pH in the BHP test, since was a batch operating system in order to possibly produce hydrogen. It was observed that CESW was decomposed to the initial acetic acid (6,700 mg/L) more than the initial butyric acid (113 mg/L) and propionic acid (675 mg/L). This could be due to its complex organic compound, because sugar from cassava was converted to ethanol and acetic acid. Hence, it is difficult to decompose CESW to hydrogen. Similarly, some researchers found that initial substrate with increasing acetic (HAc), butyric (HBu), and propionic (HPr) acid in the system tended to produce less hydrogen (Wang et al., 2008; Chatsiriwatana., 2009).

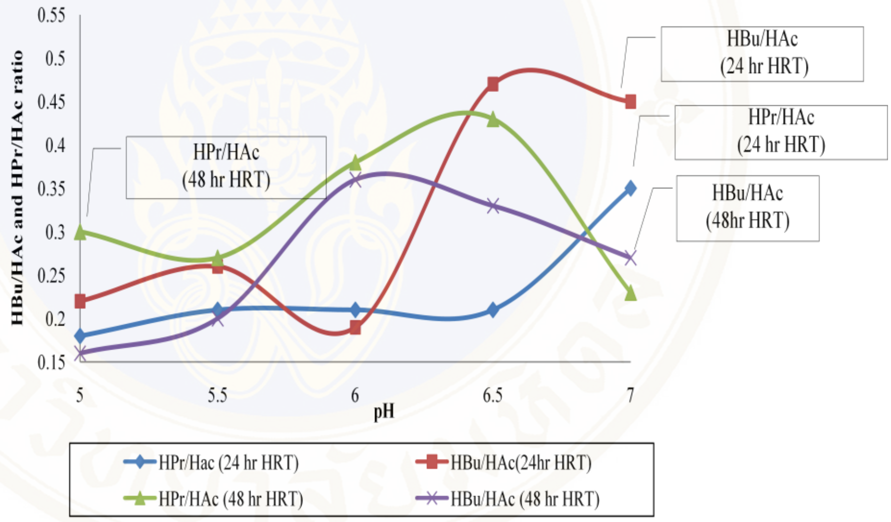

Figure 4.4 VFA ratio of effluent for BHP test

Table 4.5 VFA ratio of effluent for BHP test

Influent pH	**24hr HRT**		**48 hr HRT**	
	HPr/HAc	**HBu/HAc**	**HPr/HAc**	**HBu/HAc**
5.0	0.18	0.22	0.30	0.16
5.5	0.21	0.26	0.27	0.20
6.0	0.21	0.19	0.38	0.36
6.5	0.21	0.47	0.43	0.33
7.0	0.35	0.45	0.23	0.27

4.1.5 The ratio of CESW and sugar in BHP test

The biochemical hydrogen potential test to determine the feasibility of produce hydrogen for operating the reactor was performed. Table 4.6 in regards to hydrogen production, it was calculated as unit of milliliters hydrogen from the percentage of hydrogen measured by GC multiplied by volume of gas generated. It could be explained that the sample of CESW mixed with sugar at the ratio of 1:2 had highest hydrogen production (5.71 mL H_2) because, sugar was assumed to be converted to sucrose which could highly produce hydrogen. Regarding VFA influent, sample of CESW mixed with sugar at the ratio of 1:2 had the least amount. This means that the low VFA influent is suitable for producing hydrogen. Therefore, CESW dilution and sugar adding was conducted for continuous research by Semi-Completely Mixed Reactor in order to increase the hydrogen yield.

Table 4.6 The ratio of CESW and sugar in BHP test

The ratio of sample (g/L)	**Hydrogen production (mL H_2)**	**Effluent COD (mg/L)**	**Influent VFA (mg/L)**	**Effluent VFA (mg/L)**
CESW1:sugar0	0.94	27,648	6,700	7,429
CESW2:sugar1	0.98	29,952	4,400	6,381
CESW1:sugar1	1.73	25,344	3,350	6,000
CESW1:sugar2	5.71	36,096	2,200	6,095

Note: All ratio of CESW mixed with sugar based on COD concentration of 30 g/L

4.1.6 Use of hydrolyzed sludge in the BHP test

Table 4.7 shows hydrogen production from hydrolyzed sludge in the BHP test. It was found that using 0.8 mL enzyme Cellulase/ 5 g substrate gave the greatest hydrogen production of 6.95 mLH_2. Therefore, replacement of hydrolyzed sludge for sugar seems possible since it could yield high amount of hydrogen production.

Table 4.7 Application of hydrolyzed sludge in BHP test

Volume of enzyme used to hydrolyze sludge	%N_2	%CO_2	%H_2	Gas Volume (mL)	Hydrogen Production (mL H_2)
Enzyme Cellulase 0.8 mL	47.49	16.42	53.46	13.00	6.95
Enzyme Cellulase (0.6 mL)	41.66	18.23	40.10	11.00	4.41
Enzyme Cellulase (0.4 mL)	33.30	20.85	32.71	9.00	2.94
Enzyme Cellulase (0.2 mL)	10.30	28.04	42.12	6.00	2.53

Note: the quantity of enzyme per 5 grams of substrate (sludge)

4.2 Hydrogen Production Based on the Condition of CESW Mixed with Sugar at Different Ratios under Operating Semi – Completely Mixed Reactor

This experiments also had various ratio of CESW mixed with sugar (1:0, 1:2, 2:1, 1:1, 3:1 for 24 hr HRT; 1:1 for 12 hr HRT. The testing was done continuously for 40 days. The VFA of nondiluted CESW showed that the predominant acetic acid (6,700 mg/L), made it impossible for hydrogen to be produced. Thus, it should be diluted CESW before operating a Semi – Completely Mixed Reactor. The various ratios solutions of CESW mixed with sugar were used the purpose of which was to generate capable hydrogen-producing microorganism which could effectively degrade the organic substrate in the system to hydrogen gas.

4.2.1 The effect of VFA type on hydrogen production of CESW mixed with sugar at different ratios

Chen et al, (2009) and Kim et al, (2006) suggested the use of butyrate to acetate ratio (HBu/HAc) in relation to the performance of hydrogen production. From the result of this experiment at ratio 1:0, a sufficient volume of gas could not be produced since carbon source was insufficient to be converted to sugar. This contributed to a lack of the volume gas and then through a failing system. With the remaining ratios hydrogen could be produced.

AT the start – up, the system was still unstable. It was observed that the average effluent of HBu/HAc (0.63) was more dramatically decreased than the average influent of HBu/HAc (2.14) and effluent of HPr/HAc (0.98). Subsequently, the average of effluent HBu/HAc tended to increase at all ratios (Table 4.8 and Figure 4.5). The average of effluent HBu/HAc were 6.25, 5.46, 3.51, 8.50, and 6.16 at the ratio of 1:2, 2:1, 1:1, 3:1 for 24 hr HRT, and 1:1 for 12 hrs HRT respectively. The result of the average of effluent HBu/HAc at all ratios was higher than 1.0; this indicates that it enhance hydrogen production. However, the average of effluent HPr/HAc ratio should be equal to or less than 0.1 (Natpinit, 2010). The results showed that the average of effluent HPr/HAc were 1.83, 4.12, 1.37, 4.16, and 3.24 at the ratio of 1:2, 2:1, 1:1, 3:1 for 24 hr HRT, and 1:1 of 12 hr HRT, respectively. Although, the maximum of effluent HBu/HAc was at the ratio of 3:1 for 24 hr HRT (8.50), it was not the ratio for the maximum hydrogen production, resulted from greatest average effluent HPr/HAc of 4.16. Furthermore, it resulted in a high suspended CESW solid (8,320 mg/L).

In addition, the average effluent/influent VFA ratios (0.72) was less than 1, as shown in Table 4.9 and Figure 4.6. Therefore, this result was associated with the lower hydrogen production rate. Similarly, the average of effluent HPr/HAc (4.12) at the ratio of 2:1 was dramatically higher than those at the ratios of 1:2, and 1:1 for 24 hr HRT. Moreover, the high suspended CESW solid of 14,670 mg/L was found. Therefore, it was reasonable not to select these ratios for the next phase of experiment.

The average of effluent HPr/HAc at the ratio of 1:1 was lower than all of the rest. It meant that the lowest propionic acid was in accordance with increased hydrogen production. Moreover, the cost of commercial and technical term of sugar at the ratio of 1:1 was selected. Hence, the ratio of 1:1 was an optimal alternative through operating ratios of CESW mixed with sugar for 12 hr HRT (in order to compare with the capability of the ratio of 1:1 for 24 hr HRT). Another comparison was done by substitution of sugar with hydrolyzed sludge by enzyme Cellulase mixed with CESW for 24 hr HRT. This resulted in high effluent HBu/HAc values of greater than 1.5 reported by Kim et al, (2006) which operated on a continuous stirred tank reactor using sucrose at 12 hr HRT.

Table 4.8 The VFAs ratios of CESW mixed with sugar for 24 and 12 hr HRT

CESW: sugar ratio	Influent HBu/HAc	Influent HPr/HAc	Effluent HBu/HAc	Effluent HPr/HAc
1:2, 24 hr	4.08	1.66	6.25	1.83
2:1, 24 hr	2.48	2.58	5.46	4.12
1:1, 24 hr	2.98	1.34	3.51	1.37
3:1, 24 hr	5.02	2.80	8.50	4.16
1:1, 12hr	4.05	2.87	6.16	3.24
Min	2.48	1.34	3.51	1.37
Max	5.02	2.87	8.50	4.16
SD.	1.00	0.70	1.79	1.29

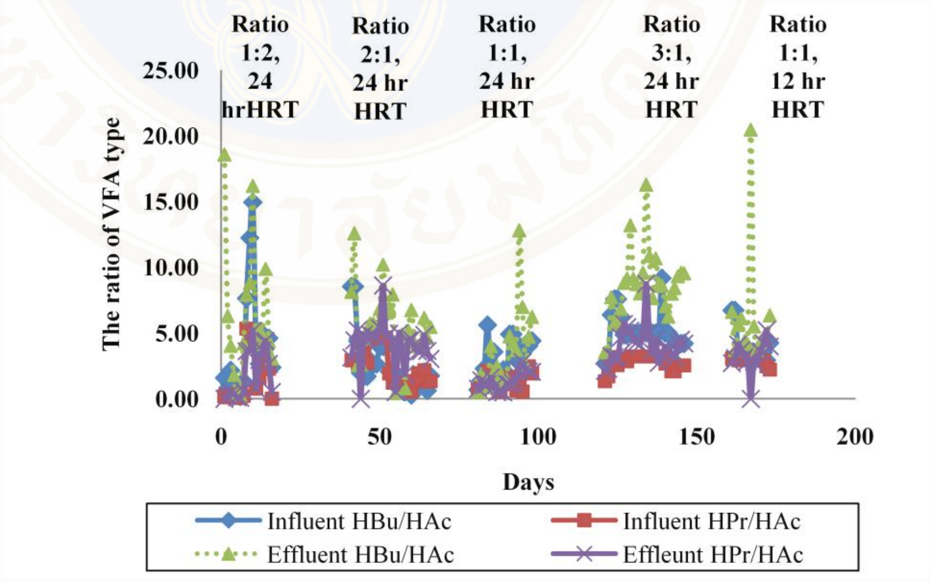

Figure 4.5 VFAs ratios of CESW mixed with sugar for 24 and 12 hr HRT

4.2.2 The effect of VFA concentration on hydrogen production of CESW mixed with sugar at different ratios

According to Table 4.9 and Figure 4.6, the average effluent/influent VFA ratio of 1:2, 2:1, 1:1, and 3:1 for 24 hr HRT were 1.67, 1.50, 0.63, and 0.72, respectively. The average effluent/influent VFA ratio of 1:1 for 12 hr HRT was 0.63. The results showed that at the ratios of 1:1 for 24 hr HRT and for 12 hr HRT were exactly the same. The highest value of 1.67 at the ratio 1:2 was associated with hydrogen production. It was involved in sufficiently low VFA concentration to prevent inhibition of hydrogen produced (Sung et al., 2002). It was similar to Wang et al, (2008) who restated that increasing added initial acetic acid, propionic acid, and butyric acid could be inhibited by the ability of mixed culture to degrade substrate during fermentative hydrogen production. Consequently, the main VFA types were butyric acid, acetic acid, and propionic acid.

Table 4.9 Effluent/influent VFA ratio of CESW mixed with sugar for 24 and 12 hr HRT

CESW: sugar ratio	average	Min	Max	S.D.
1:2, 24 hr	1.67	0.89	2.94	0.54
2:1, 24 hr	1.50	1.00	2.11	7:31
1:1, 24 hr	0.63	0.20	1.58	6:48
3:1, 24 hr	0.72	0.40	1.44	6:16
1:1, 12hr	0.63	0.49	0.96	3:29

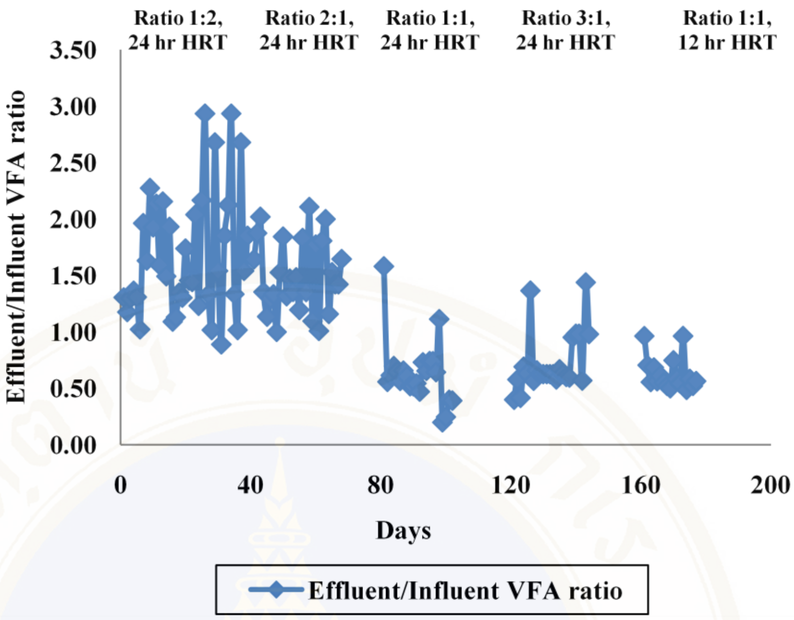

Figure 4.6 Effluent/influent VFA ratio of CESW mixed with sugar for 24 and 12 hr HRT

4.2.3 The effect of Butyric, Acetic, and Propionic acids on hydrogen production of CESW mixed with sugar at different ratios

According to Table 4.10 and Figure 4.7, the average of HBu effluent/influent at the ratio of 1:2, 2:1, 1:1, and 3:1 for 24 hr HRT were 2.12, 3.38, 1.78, and 2.45. Maintingker et al, (2008); Zhao and Yu, (2008) found that higher hydrogen production could be indicated by butyric acid generated in the reactor. This finding was similar to the result of this study which generated a higher concentration of butyric acid than acetic acid, and the least amount of propionic acid at the ratio of 1:2. On the other hand, the ratio of 2:1, 1:1, and 3:1 for 24 hr HRT, and also 1:1 for 12 hr HRT had a higher propionic acid concentration than acetic acid. As a consequence, hydrogen production decreased because it was consumed. Equation (1) shows propionic acid production (Antonopoulou et al., 2008).

Propionic acid fermentation is derived from 2 mole of hydrogen and 1 mole of glucose.

$$C_6H_{12}O_6 + 2H_2 \rightarrow 2CH_3CH_2COOH + 2H_2O \qquad (1)$$

At the ratio of 1:2, the highest hydrogen production was resulted from butyric and acetic acids producing hydrogen as illustrated in equations (2) and (3):

According to equation (2), 2 mole of hydrogen is produced from 1 mole of glucose in a butyrate ($C_4H_8O_2$) type of fermentation.

$$C_6H_{12}O_6 \rightarrow CH_3CH_2CH_2COOH + 2CO_2 + 2H_2 \qquad (2)$$

According to equation (3), 4 mole of hydrogen is produced from 1 mole of glucose in an acetate ($C_2H_4O_2$) type of fermentation.

$$C_6H_{12}O_6 + 2H_2O \rightarrow 2CH_3COOH + 2CO_2 + 4H_2 \qquad (3)$$

Table 4.10 The average value of effluent/influent butyric, propionic, and acetic acid ratio of the various ratios of CESW mixed with sugar for 24 and 12 hr HRT

CESW: sugar ratio	**HBu effluent/influent**	**HPr effluent/influent**	**HAc effluent/influent**
1:2, 24 hr	2.12	1.17	1.42
2:1, 24 hr	3.38	2.22	1.28
1:1, 24 hr	1.78	1.65	1.56
3:1, 24 hr	2.45	2.42	1.73
1:1, 12hr	1.31	1.52	1.41

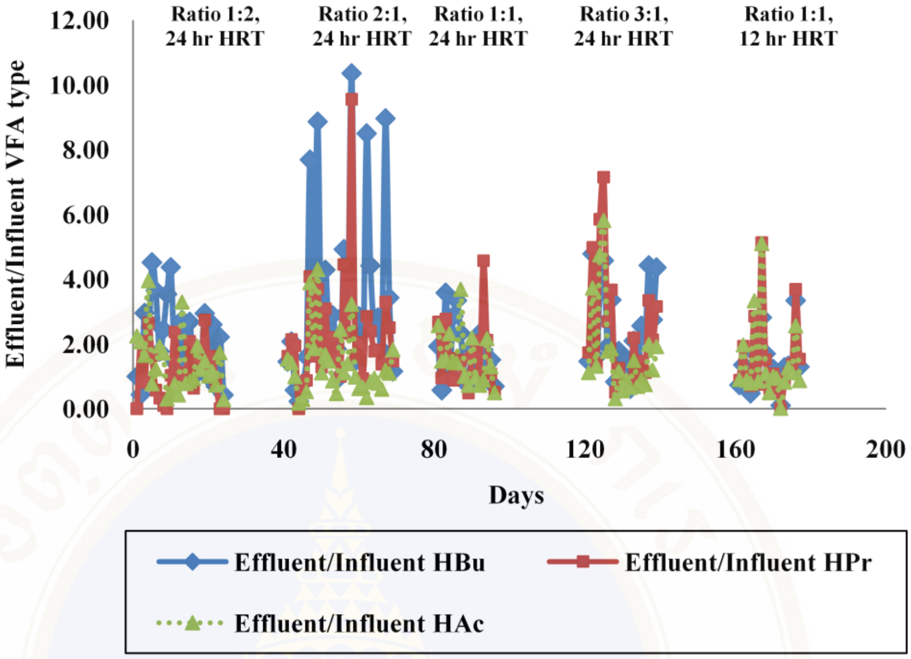

Figure 4.7 The effluent/influent butyric, propionic, and acetic acid ratio of CESW mixed with sugar for 24 and 12 hr HRT

4.2.4 The effect of pH on hydrogen production of CESW mixed with sugar at different ratios

The pH is a substantial parameter relative to hydrogen production, and VFA. This study had to maintain an influent pH value of 5.0 – 5.5, and effluent pH value of 4.0 – 4.3 all conditions of various ratios of CESW mixed with sugar and ratio of hydrolyzed sludge mixed with CESW as shown in Figure 4.8 (Natpinit et al, 2010). These were in the same trend as the results of Shi et al. (2009) and Gingkel et al. (2001) which found that the influent pH which occurred in the range of 5.0 – 6.0 was suitable for hydrogen production. Besides, the maximum hydrogen content in the biogas was 38% which was controlled to pH 5.5 (Kim et al., 2008; Gomez, et al., 2006). In terms of the effluent pH, a value of 4 was established (Lee et al., 2002), as well as effluent pH value of 4.3 (Mu and Yu, 2006). The influent pH was in the range of 5.0 – 6.0. After that, the stable operating pH was in the range of 5.0 – 5.5. It had to be maintained at this pH range which is sensitive to the amount of hydrogen. When organic acid accumulated, it led to a decreased level of pH. Then, it contributed to a production of toxic microorganisms and lower hydrogen efficiency. For instance, the lower pH of 4 was sensitive to acidic metabolites, which may have destabilized the cell's ability to maintain internal pH, resulting in less intracellular level ATP and inhibiting substrate uptake (O-Thong et al, 2008; Chen and Lin, 2003). On the other hand, an increased level of pH would result in a production of methane instead.

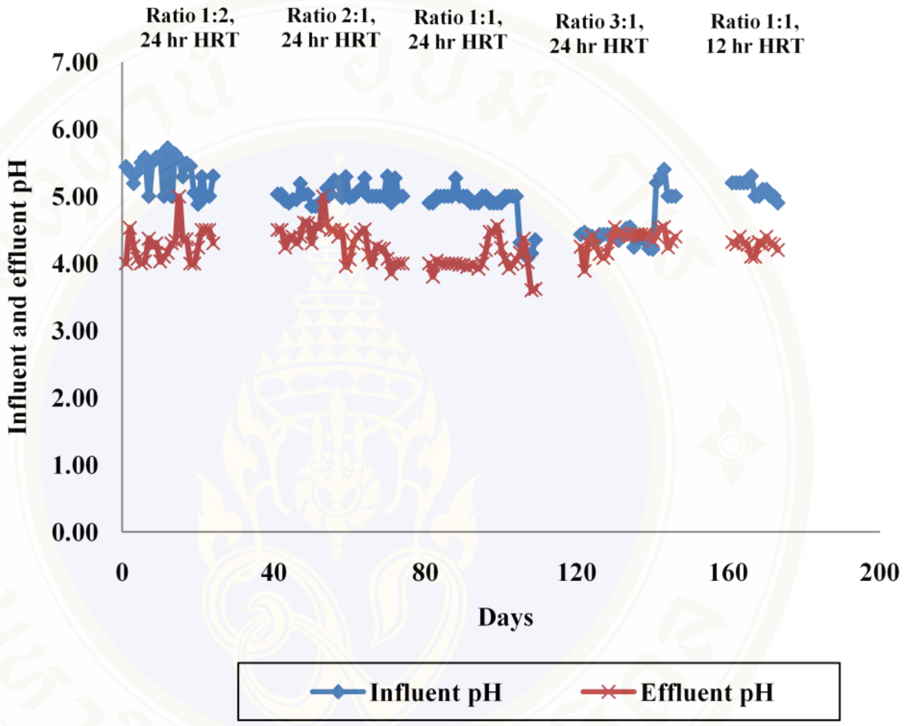

Figure 4.8 Influent and effluent pH of the various ratios of CESW mixed with sugar for 24 and 12 hr HRT

4.2.5 The hydrogen production performance of CESW mixed with sugar at different ratios

According to Table 4.11 and Figure 4.9, microorganisms could be generated for the hydrogen production rate if the following ratios of CESW and sugar: 1:2, 2:1, 1:1, and 3:1 were experimentally tested for 24 hr HRT. Results of the different ratios showed that the highest hydrogen production rate were 2.9, 0.70, 0.71, and 1.02 LH_2/d, respectively. The average hydrogen production rate was 1.02, 0.31, 0.24, and 0.21 LH_2/d, respectively. The maximum hydrogen production was attained with a ratio of 1:2 which was equivalent to 0.07 LH_2/g CODr-d or 580 mL H_2/L reactor-d in the following Table 4.12 and 4.13. It was similar to Kim et al, (2004) who found that the highest efficiency of hydrogen yield was 0.0892 L H_2/g COD at the optimal pH of 5.5, which corresponded to a theoretical maximum yield of 0.467 L H_2/g COD.

Statistical analysis at the confidence interval of 95% was done by SPSS 11.5 to determine the difference in hydrogen production for ratios of CESW and sugar as shown in Appendix B. It was found that 1) the ratio of 1:2 was significantly different from all other ratios, 2) the ratios of 2:1 and 1:1 were not different, and 3) the ratio of 1:1 and 3:1 were not different. In addition, 12 hr HRT was experimentally tested for the ratio of 1:1, and results showed that hydrogen production for 12 hr HRT (1.11 LH_2/d) was higher than that at 24 hr HRT (0.71 LH_2/d). However, the statistical analysis at the confidence interval of 95%, taking into account of different HRT, was not significant. Furthermore, the relationship between effluent BOD of removal, influent pH hydrogen yield was significant by statistical analysis at the confidence interval of 95% as well in the following Appendix C.

Table 4.11 The average hydrogen production rate (LH_2/d) of the various ratio of CESW mixed with sugar for 24 and 12 hr HRT

CESW: sugar ratio	average	Min	Max	S.D.
1:2, 24 hr	1.02	0.02	2.90	0.85
2:1, 24 hr	0.31	0.06	0.70	4.15
1:1, 24 hr	0.24	0.05	0.71	3.19
3:1, 24 hr	0.21	0.02	1.02	6.02
1:1, 12hr	0.32	0.00	1.11	7.53

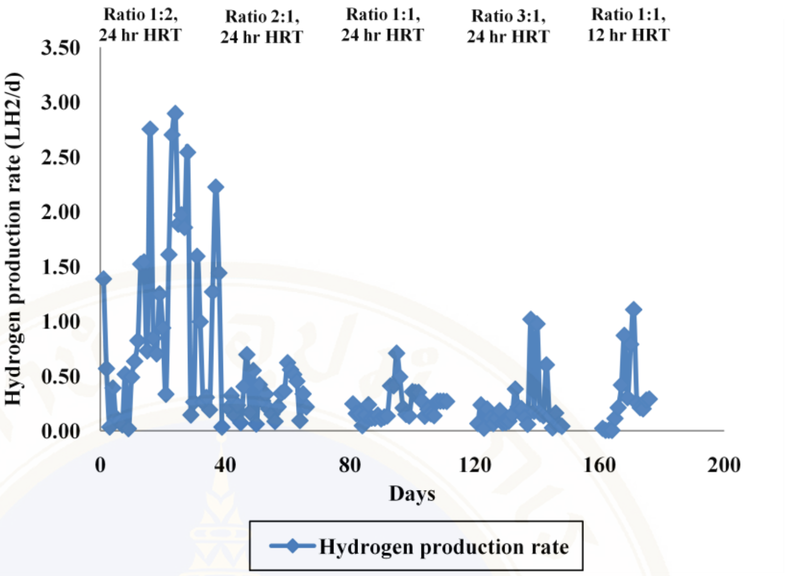

Figure 4.9 The hydrogen production rate of the various ratios of CESW mixed with sugar for 24 and 12 hr HRT

Table 4.12 The average hydrogen production rate (LH_2/g CODr-d) of the various ratio of CESW mixed with sugar for 24 and 12 hr HRT

CESW: sugar ratio	**average**	**Min**	**Max**	**S.D.**
1:2, 24 hr	0.03	0.01	0.07	0.02
2:1, 24 hr	0.01	0.01	0.02	0.00
1:1, 24 hr	0.01	0.01	0.02	0.00
3:1, 24 hr	0.01	0.01	0.03	0.01
1:1, 12hr	0.02	0.01	0.03	0.06

Table 4.13 The average hydrogen production rate (mL H_2/L reactor-d) of the various ratio of CESW mixed with sugar for 24 and 12 hr HRT

CESW: sugar ratio	average	Min	Max	S.D.
1:2, 24 hr	210.00	216.00	580.00	169.00
2:1, 24 hr	59.26	12.31	125.00	31.88
1:1, 24 hr	47.11	21.82	141.44	27.67
3:1, 24 hr	43.33	5.58	203.80	50.72
1:1, 12hr	84.21	40.24	221.50	63.63

4.2.6 The effect of COD and BOD concentration on hydrogen production of CESW mixed with sugar at different ratio

According to Figure 4.10, the average COD influent, effluent, and percentage of removal at the various ratio of CESW mixed with sugar were 28,475 mg/L, 22,059 mg/L, and 32 % COD removal, respectively. As well as shown in Figure 4.11, the average influent, effluent BOD, and percentage of BOD removal were 23,968 mg/L, 18,214 mg/L, and 24 % BOD removal, respectively.

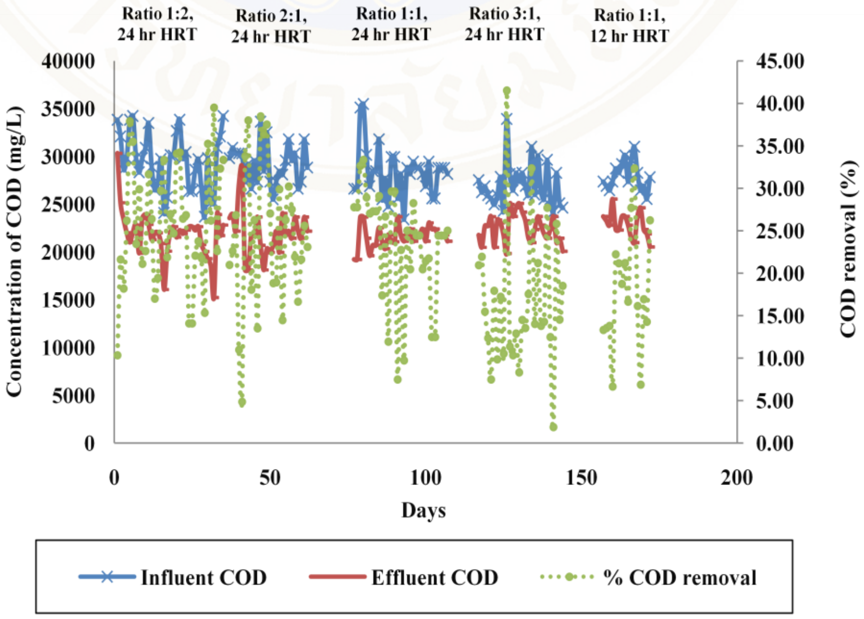

Figure 4.10 The influent COD, effluent COD and percentage of COD removal for various ratios of CESW mixed with sugar for 24 and 12 hr HRT

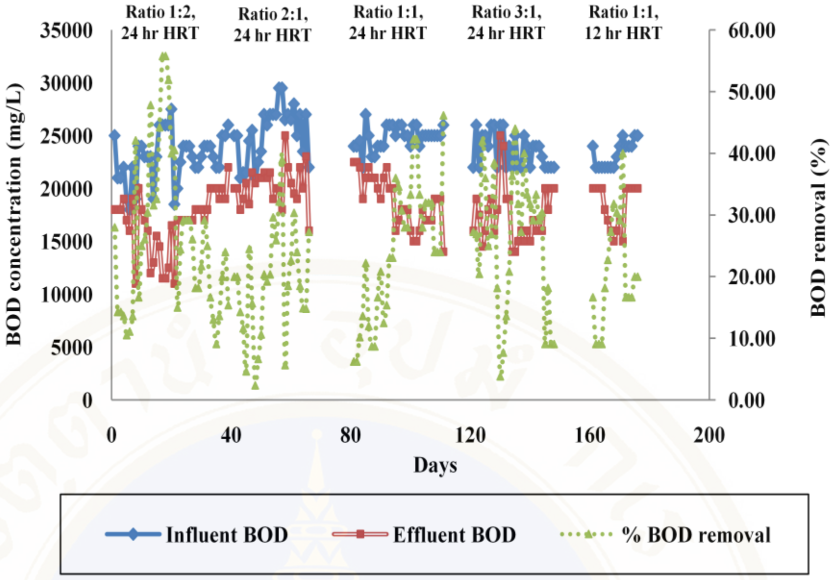

Figure 4.11 The influent BOD, effluent BOD and percentage of BOD removal for various ratios of CESW mixed with sugar for 24 and 12 hr HRT

4.3 Hydrolyzed sludge by enzyme Cellulase mixed with CESW condition for 24 hr HRT

Partly hydrolyzed sludge by enzyme Cellulase for 24 hr HRT was used during the experimental preliminary study in order to investigate the amount of reducing sugar in term of monosaccharide and disaccharide from sludge which were used as the carbon source for the mixed culture to produce hydrogen through dark fermentation. Because, no lactic acid concentration was found for 24 hr HRT (Antonopoulou et al., 2008). Besides, HRT has substantial influence on the composition of substrates that were more obstacle to biodegradation, resulting in lower hydrogen yield. As a result, decreased HRT could not always increase hydrogen production (Yu et al., 2002).

4.3.1 The possibility of hydrolyzed sludge by enzyme Cellulase

The capability of ethanol solid waste hydrolyzed by enzyme Cellulase was considered, it was mixed with cassava ethanol spent wash in order to add a carbon source. Consequently, 1:1 ratio as an alternative to the operating system followed the optimal ratio of CESW mixed with sugar and the capability of hydrogen production was determined. The concentration of enzyme Cellulase also affected the conversion efficiency. During this experiment which was done to determine the optimal enzyme Cellulase quantity of 0.16 mL/g substrate calculated as shown in Appendix C, which could be converted from a complex organic compound to disaccharide and monosaccharide as shown in Table 4.14.

Table 4.14 Concentration of reducing sugar from hydrolyzed sludge by enzyme Cellulase

Sample	**Composition of sample**	**Concentration (mg/L)**	
		Min - Max	**Average**
Hydrolyzed sludge by enzyme Cellulase	Glucose	21,250 - 46,020	33,635
	Fructose	0 –4,600	4,600
	(Reducing sugar	28,400 - 28,750	28,575)

The results showed that hydrolyzed sludge by enzyme Cellulase is converted to glucose and fructose in the range of 21,250 – 46,020, and 4,600 mg/L respectively. As well as, the average of reducing sugar from titration was 28,575 mg/L which was in the same trend. Hence, the reducing sugar could be assumed.

It was regarded as an alternative to utilized cellulose enzyme to convert cellulose so as to enhance monosaccharide as a substrate to produce hydrogen. Various factors were disputed, such as the capability of enzyme Cellulase in order to shift non-starch polysaccharides (cellulose, hemicelluloses, xylose, arabinose and pectin), and lignin. It was possible that not all of the cellulose in the substrate was available to the bacteria, considering the amount of reducing sugar concentration was linked to hydrogen yield. Besides, the indicator for marker organic substrate in order to convert to sugar considered the average of quantity of ash (1.54%), it is associated with the great deal of CESW as initial substrate (Gomez et al., 2006) as shown in Table 4.15.

Table 4.15 The data of percentage of ash to consider organic compound

Replicate of CESW	Percentage of ash
1	1.23
2	1.36
3	1.84
4	1.71
5	3.93
6	4.13
7	3.15
8	3.25
9	1.52
10	1.23
11	0.25
12	0.32
13	0.40
14	0.42
Average	1.52

4.3.2 The reducing sugar of hydrolyzed sludge

This graph shows that the amount of reducing sugar throughout operating Semi – Completely Mixed Reactor. The results of the average reducing sugar influent, effluent and percentage of removal were 1317 mg/L, 723 mg/L, and 45%, respectively as shown in Figure 4.12. It was observed that hydrolyzed sludge as carbon source could be converted to hydrogen gas.

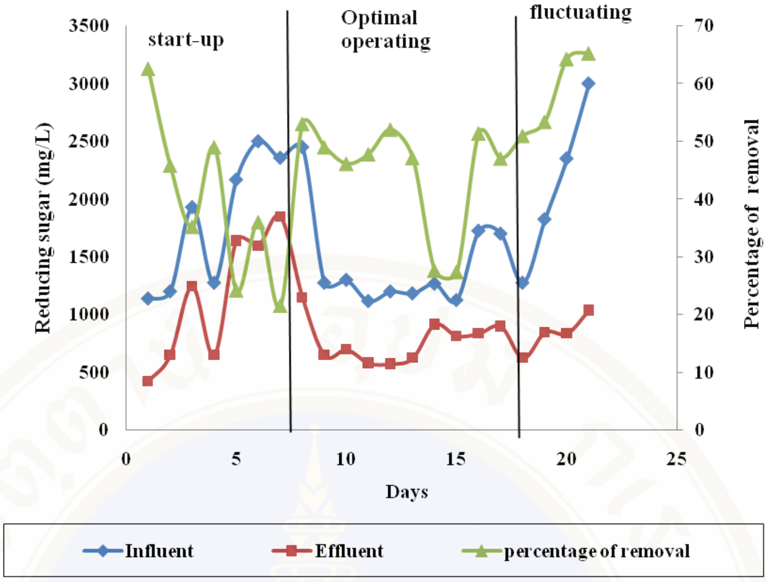

Figure 4.12 The effect of reducing sugar at (1:1) ratio of hydrolyzed sludge mixed with CESW ratio, 24 hr HRT

4.3.3 The hydrogen production at (1:1) ratio of hydrolyzed sludge mixed with CESW ratio for 24 hr HRT

The preliminary data in Table 4.16 show the optimal hydrogen yield as unit of mmolH_2/mol hexose, specific hydrogen production rate (SHPR) as unit of mL H_2/L reactor-d and hydrogen production rate as unit of mL H_2/d from the 6 replicates optimal data. As a preliminary result, the average hydrogen yield and SHPR at the 1:1 ratio of hydrolyzed sludge mixed with CESW was 939 mmol H_2/mol hexose or equivalent to 61 mL H_2/L reactor-d. In the other words, the highest and average hydrogen production rates were 427 and 308 mLH_2/d respectively for 24 hr HRT. The results of the study had similar results of hydrogen yield as those obtained during a previous study that was 0.9 mol H_2/mol hexose at pH 4.5 from the thermophilic acidogenesis of food waste (Shin et al., 2004). It was similar to the hydrogen yield increased from 1.74 mol H_2/mol hexose for 2 hr to 2.14 mol H_2/mol hexose for 24 hr HRT (Yu et al., 2002). It was in agreement with Wang et al, (2010), the average hydrogen yield of 288 mL/g TS-d was attained from the fermentative apple pomace by enzyme Cellulase at 45 ºC and initial pH 5.0 for 48 hr HRT. This study was similar to the hydrogen yield of 4.77 L H_2/L reactor-d treating kitchen waste by CSTR under

OLR of 53 kg COD/m^3-d at influent and effluent pH in the range of 5.0 – 6.0, and 4.0 – 4.5, respectively (Shi et al., 2009). This result concurred with Pattra et al, (2008) which stated that the optimal hydrogen yield of 1.73 mol H_2/mol total sugar or equivalent to 1.6 L H_2/L-d is obtained from fermentative sugarcane bagasse hydrolyzed by Clostridium butyricum at the influent pH 5.5 and influent sugar concentration of 20 g-COD/L. The result of hydrogen production rate acquired was only slightly higher than 160 mL H_2/d which was similar to Lo et al, (2009) which restated that using carboxymethyl cellulose hydrolysate as a carbon source to produce accumulative hydrogen resulted in a production and yield of 28.6 mL/L and 4.79 mmol H_2/g reducing sugar, respectively.

Table 4.16 The preliminary data of hydrogen production at (1:1) ratio of hydrolyzed sludge mixed with CESW ratio, 24 hr HRT

Replicate	**Hydrogen yield**		**Hydrogen production rate**
	mmolH_2/mol hexose	mL H_2/L reactor-d	mL H_2/d
1	605.15	50.08	250
2	1199.79	76.59	383
3	1059.21	83.47	427
4	878.41	61.17	306
5	926.01	57.39	287
6	964.38	39.17	196
Average	939.00	61.00	308
Min	605.15	39.17	196
Max	1199.79	83.47	427
S.D.	199.02	16.47	85.02

Note: Results from preliminary experiments.

4.3.4 The effect of VFA on hydrogen at (1:1) ratio of hydrolyzed sludge mixed with CESW ratio for 24 hr HRT

Several indicators for hydrogen production were evaluated. The influent and effluent VFA concentrations were 1912 and 2331 mg/L, respectively. An effluent/influent VFA ratio of 1.25 was found as shown in Figure 4.13. The higher value of effluent/influent VFA ratio led to increased hydrogen production. However, this result of lower hydrogen yield may have been a result of decreased COD removal, the hydrogen producing microorganism's limited capacity to degrade organics and inability to reduce carbon dioxide to acetate, and the sensitive level of pH.

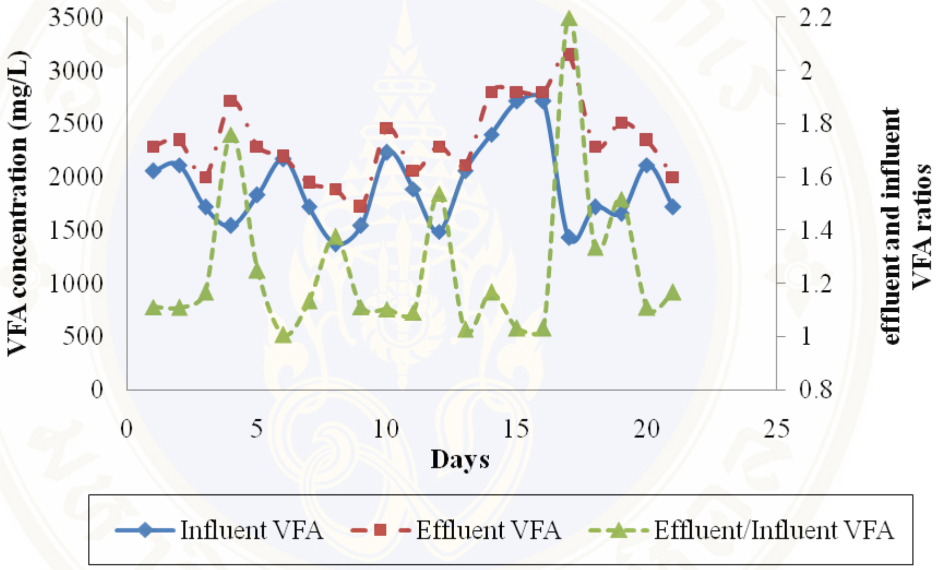

Figure 4.13 The VFA effect on hydrogen production at the (1:1) ratio of hydrolyzed sludge mixed with CESW ratio, 24 hr HRT

4.3.5 The effect of VFA type on hydrogen at (1:1) ratio of hydrolyzed sludge mixed with CESW ratio for 24 hr HRT

This result identified that there was hydrogen yield however, the effluent HBu/HAc was less than 1, which resulted in a decreased hydrogen yield. Besides, the effluent HPr/HAc was 0.34. It was observed that the high HPr/HAc consumed hydrogen. However, effluent HBu/HAc ratio was greater than HPr/HAc ratio as shown on Figure 4.14. The main by-products in hydrogen production by anaerobic dark fermentation were acetic, butyric, and propionic acid. It had an effect on the mixed cultures ability that can degrade substrate during hydrogen fermentation. Ethanol and methane were not detected in most of the hydrogen production. Because the elimination of methane was controlled by the heat digestion of sludge (Kapdan et al, 2006).

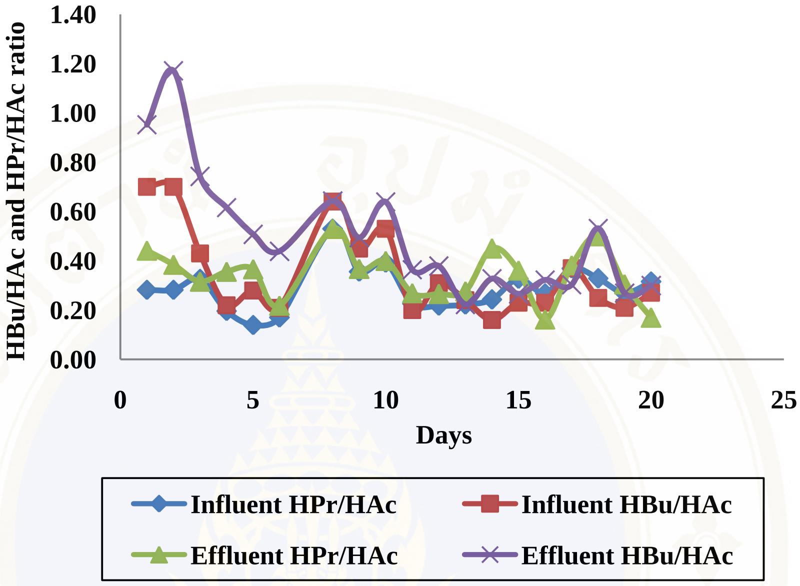

Figure 4.14 Each VFA at hydrolyzed sludge mixed with CESW ratio (1:1), 24 hr HRT

According to Figure 4.15, the average effluent/influent butyric acid ratio was 1.47. The effluent/influent propionic acid ratio was 0.96. The effluent/influent acetic acid ratio was 1.06. Butyrate - type fermentation was predominant and secondary abundant soluble metabolite which was acetate - type fermentation. It concurred with Pattra et al, (2008). Even if it was predominantly higher in butyric than in acetic acid, it resulted in lower in efficiency for hydrogen production by anaerobic hydrogen producing microorganisms.

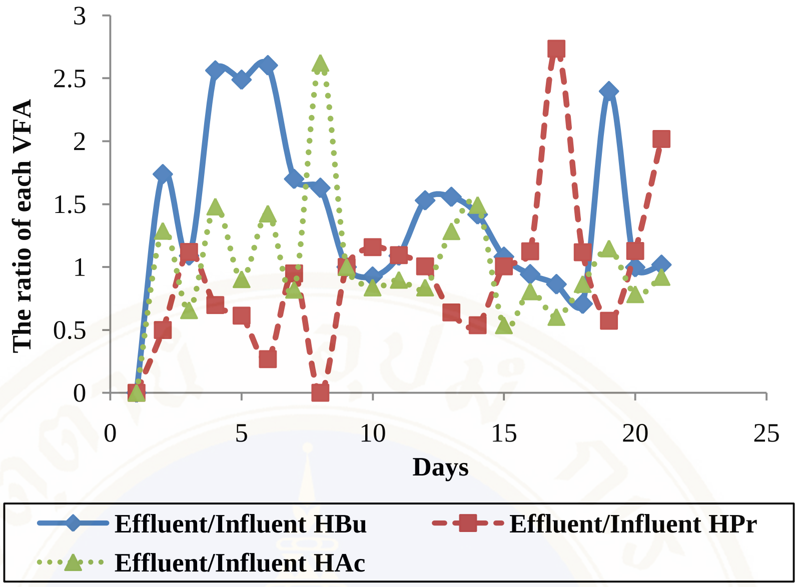

Figure 4.15 The ratio of effluent/influent butyric, propionic, and acetic acid at (1:1) ratio of hydrolyzed sludge mixed with CESW ratio, 24 hr HRT

4.3.6 The effect of pH on hydrogen at (1:1) ratio of hydrolyzed sludge mixed with CESW ratio for 24 hr HRT

This study had to maintain an influent pH value of 5.0 – 5.5, and effluent pH value of 4.0 – 4.3 at ratio of hydrolyzed sludge mixed with CESW as shown in Figure 4.16 (Natpinit et al, 2010). The pH level was slightly acidic which was in accordance with the results of several studies for instance; the optimal pH level was 5.5 for hydrogen yield of 1.74 mol H_2/mol hexose. Whereas, the hydrogen yield were 1.45 and 1.46 H_2/mol hexose at pH 5.0 and 6.0 from winery wastewater in an upflow anaerobic reactor by using mixed anaerobic cultures.

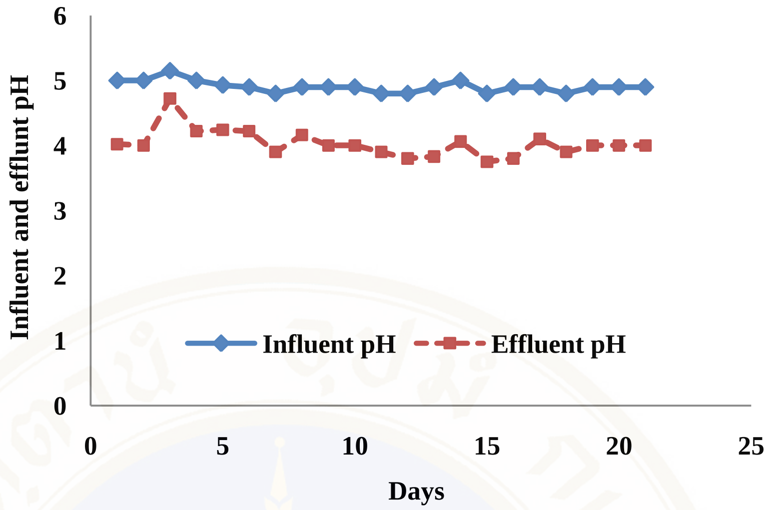

Figure 4.16 The effect of pH on hydrogen production at (1:1) ratio of hydrolyzed sludge with CESW ratio, 24 hr HRT

According to Figure 4.17, the influent, effluent of average COD and percentage of removal at the various ratios of hydrolyzed sludge mixed with CESW were 28,193 mg/L, 21271 mg/L, and 25% COD removal, respectively. As well as, the influent, effluent of average BOD and percentage of BOD removal were 18,300 mg/L, 14,000 mg/L, and 23% BOD removal, respectively as shown in Figure 4.18. The efficiency of COD and BOD removal of less than 30% was similar to Natpinit (2010).

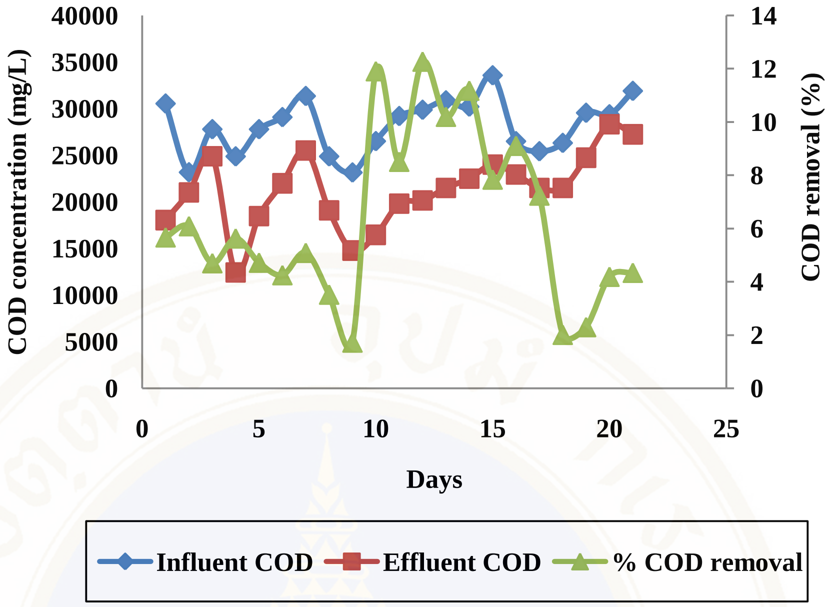

Figure 4.17 The influent COD, effluent COD and percentage of COD removal at (1:1) ratio of hydrolyzed sludge mixed with CESW ratio, 24 hr HRT

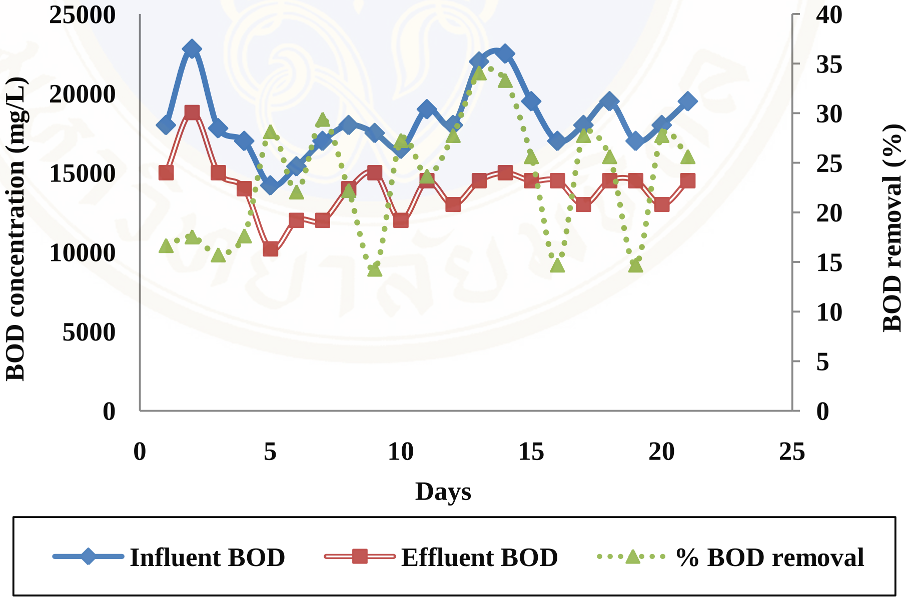

Figure 4.18 The influent BOD, effluent BOD and percentage of BOD removal at (1:1) ratio of hydrolyzed sludge mixed with CESW ratio, 24 hr HRT

This results concurred with the hypothesis of the study. It revealed that a decreased HRT, weakly acidic pH, and VFA concentration resulted in an increased hydrogen yield.

CHAPTER V

CONCLUSION AND RECOMMENDATION

5.1 Conclusion

For the biochemical hydrogen potential (BHP) test, the study condition of pH and HRT obtained the optimum pH of 5.5 for 24 hr HRT could produce maximum hydrogen of 20%. The ratio of VFA effluent and influent was an indicator of biochemical hydrogen potential testing that should be more than 1.0. Moreover, hydrogen yield of hydrolyzed sludge depended on the ratio of effluent butyric and acetic acid which should be more than 1. Whereas the ratio of effluent propionic acid and acetic acid that should be less than 1.0. CESW had a high initial VFA of 6,700 mg/l and less complex organic compound because sugar from cassava was converted to ethanol and acetic acid. So, it was difficult to decompose hydrolyzed sludge to hydrogen. CESW dilution and sugar adding was then conducted for continuous system by Semi-Completely Mixed Reactor in order to increase the hydrogen yield from CESW.

For the various ratios of CESW mixed with sugar operating Semi-Completely Mixed Reactor for 24 hr HRT, the results showed that CESW mixed with sugar at the ratio of 1:0 could not produce hydrogen since less carbon source (sugar) could be converted to hydrogen. The maximum hydrogen production rate at different ratios of 1:2, 2:1, 1:1, and 3:1 were 2.9, 0.70, 0.71, and 1.02 LH_2/d, respectively. As well as, the average hydrogen production rates were 1.02, 0.31, 0.24, and 0.21 LH_2/d, respectively. The maximum hydrogen production at ratio of 1:2 was 2.90 LH_2/d or equivalent to 0.07 L H_2/g CODr-d or 580 mL H_2/L reactor-d. The influent pH in the range of 5.0 – 5.5 and effluent pH in the range of 4.0 – 4.3 were controlled. The condition led to the production of hydrogen and volatile fatty acid (VFA). The average of effluent HPr/HAc at the ratio of 1:1 was lower than all other ratios at the 24 hr HRT. It meant that the lowest propionic acid was in accordance with increased hydrogen production. Moreover, the cost of commercial and technical term of sugar at the ratio of 1:1 was more feasible. Therefore, the ratio of 1:1 was selected as an optimal alternative through operating ratios of CESW mixed with sugar for 12 hr HRT (in order to compare with the capability of the ratio of 1:1 for 24 hr HRT). It was found that the operating 12 hr HRT had higher hydrogen production rate than at 24 hr HRT. However, the statistical analysis at the confidence interval of 95% was not significantly different to the two HRTs for hydrogen production.

As a preliminary study, hydrolyzed sludge was converted by enzyme Cellulase to a reducing sugar as an initial substrate for hydrogen production. Results showed the average hydrogen production of 939 mmolH_2/mol hexose consumed, or equivalent to 61 mL H_2/L reactor-d or equivalent to 0.380 L H_2/d. Relatively low hydrogen production was derived in this preliminary study of hydrolyzed sludge (in replacing sugar) mixed with CESW.

Several indicators for relatively low hydrogen production could be considered. An effluent/influent VFA ratio of 1.25 was found. The effluent/influent butyric acid ratio was 1.47. The effluent/influent propionic acid ratio was 0.96. The effluent/influent acetic acid ratio was 1.06. The effluent butyric/acetic ratio was 0.50, and the effluent propionic/acetic ratio was 0.34.

5.2 Recommendation

5.2.1 Further study on waste with high carbon source such as molasse and sugar cane in order to increase hydrogen production is recommended.

5.2.2 Further research on the methane phase using effluent from the hydrogen phase should be investigated.

5.2.3 Different organic loading rate to enhance hydrogen production should be studied.

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APPENDICES

APPENDIX A
PICTURES OF EXPERIMENTS

Cassava ethanol spent wash (CESW) as by – product derived after 50% of ethanol distillation from the cassava ethanol plant.

Condition of CESW mixed with sugar

Figure A-1. Cassava ethanol spent wash operated under dark fermentation by Semi – Completely Mixed Reactor

Condition of sludge derived from CESW hydrolyzed by Enzyme Cellulase

Figure A-2. Sludge derived from CESW as substrate

Figure A-3. Substrate after pretreatment

Figure A-4. Substrate after hydrolyzed by enzyme Cellulase

APPENDIX B

CALCULATED QUANTITY OF ENZYME CELLULASE

Table B-1. Cassava Ethanol Spent Wash using enzyme

Tradename of Enzyme	Fiber (g substrate)	Lignin (g)	Enzyme (ml)	Reducing Sugar (g product)	COD (mg/l)
Accellalase	1.5	3.5	0.8	0.2875	-
Celluclast	1.1769	3.8231	0.8	0.31	10066
Celluclast	1.379	3.621	0.6	0.11	9320
Celluclast	2.0695	2.9305	0.4	0.17	11557
Celluclast	1.0665	3.9335	0.2	0.23	10718
Celluclast	2.07	2.93	1	0.31	-

Note: Initial 5 g substrate

Capability of enzyme (g product/g substrate)

Accellalase (0.8 ml enzyme) = 0.2875×100/1.5 = 19.17%

Celluclast (1 ml enzyme) = 0.31×100/2.07 = 14.98%

Celluclast (0.8 ml enzyme) = 0.31×100/1.1769 = 26.34%

Celluclast (0.6 ml enzyme) = 0.11×100/1.379 = 7.98%

Celluclast (0.4 ml enzyme) = 0.17×100/2.0695 = 8.21%

Capability of enzyme (g product/ml enzyme)

Accellalase (0.8 ml enzyme) = 0.2875×100/0.8 = 35.94%

Celluclast (0.8 ml enzyme) = 0.31×100/0.8 = 38.75%

Celluclast (0.6 ml enzyme) = 0.11×100/0.6 = 18.33%

Celluclast (0.4 ml enzyme) = 0.17×100/0.4 = 8.21%

Celluclast (1 ml enzyme) = 0.31×100/1 = 31%

Quantity of enzyme (mL enzyme/g fiber)

Accellalase (0.8 ml enzyme) = 0.8/1.5 = 0.53 ml

Celluclast (0.8 ml enzyme) = 0.8/1.1769 = 0.68 ml/g fiber or (0.68/5g initial = 0.16 ml/g substrate)

Celluclast (0.6 ml enzyme) = 0.6/1.379 = 0.44 ml

Celluclast (0.4 ml enzyme) = 0.4/2.0695 = 0.19 ml

Celluclast (1 ml enzyme) = 1/2.07 = 0.48 ml

APPENDIX C

STATISTICAL ANALYSIS

Different various ratios of CESW mixed with sugar

Table C-1. One-way ANOVA Statistical Analysis between the ratios of CESW mixed with sugar

ANOVA

H_2

	Sum of Squares	df	Mean Square	F	Sig.
Between Groups	97425.852	3	32475.284	30.331	.000
Within Groups	127412.371	119	1070.692		
Total	224838.222	122			

Multiple Comparisons

Dependent Variable: H_2

LSD

OLR (I)	**OLR (J)**	**Mean Difference**	**Std. Error**	**Sig.**	**95% Confidence Interval**	
					Lower Bound	**Upper Bound**
1:2	2:1	55.7992(*)	8.70166	.000	38.5690	73.0293
	1:1	61.0831(*)	7.82980	.000	45.5794	76.5869
	3:1	74.0415(*)	8.70166	.000	56.8113	91.2716
2:1	1:2	-55.7992(*)	8.70166	.000	-73.0293	-38.5690
	1:1	5.2840	8.24304	.523	-11.0381	21.6060
	3:1	18.2423(*)	9.07529	.047	.2723	36.2123
1:1	1:2	-61.0831(*)	7.82980	.000	-76.5869	-45.5794
	2:1	-5.2840	8.24304	.523	-21.6060	11.0381
	3:1	12.9583	8.24304	.119	-3.3637	29.2804
3:1	1:2	-74.0415(*)	8.70166	.000	-91.2716	-56.8113
	2:1	-18.2423(*)	9.07529	.047	-36.2123	-.2723
	1:1	-12.9583	8.24304	.119	-29.2804	3.3637

* The mean difference is significant at the .05 level.

Comparison of various 12 hr and 24 hr HRT for CESW mixed with sugar

Table C-2. Paired samples t-test between 12 hr and 24 hr HRT for CESW mixed with sugar

Paired Samples Statistics

		Mean	N	Std. Deviation	Std. Error Mean
Pair 1	12 hr HRT	.2363	16	.17855	.04464
	24 hr HRT	.3175	16	.33040	.08260

Paired Samples Correlations

		N	Correlation	Sig.
Pair 1	12 hr HRT and 24 hr HRT	16	-.186	.491

Paired Samples Test

		Paired Differences					t	df	Sig. (2-tailed)
		Mean	Std. Deviation	Std. Error Mean	95% Confidence Interval of the Difference				
					Lower	Upper			
Pair 1	12 hr HRT and 24 hr HRT	-.0812	.40368	.10092	-.2964	.1339	-.805	15	.433

Various indicator of hydrogen production

HBu effluent/influent

Coefficients(a)

Model		Unstandardized Coefficients		Standardized Coefficients	t	Sig.
		B	Std. Error	Beta		
1	(Constant)	.553	.109		5.089	.000
	C4effluent/influent	-.008	.037	-.024	-.229	.819

a Dependent Variable: H_2

HPr effluent/influent

Coefficients(a)

Model		Unstandardized Coefficients		Standardized Coefficients	t	Sig.
		B	Std. Error	Beta		
1	(Constant)	.703	.103		6.819	.000
	C3effluent/influent	-.095	.044	-.223	-2.165	.033

a Dependent Variable: H_2

HAc effluent/influent

Coefficients(a)

Model		Unstandardized Coefficients		Standardized Coefficients	t	Sig.
		B	Std. Error	Beta		
1	(Constant)	.582	.112		5.171	.000
	HAc effluent/influent	-.034	.063	-.057	-.543	.588

a Dependent Variable: H_2

Effluent HBu/HAc

Coefficients(a)

Model		Unstandardized Coefficients		Standardized Coefficients	t	Sig.
		B	Std. Error	Beta		
1	(Constant)	.476	.122		3.904	.000
	Effluent HBu/HAc	.103	.183	.059	.564	.574

a Dependent Variable: H_2

Effluent HPr/HAc

Coefficients(a)

Model		Unstandardized Coefficients		Standardized Coefficients	t	Sig.
		B	Std. Error	Beta		
1	(Constant)	.691	.130		5.331	.000
	Effluent HPr/HAc	-.591	.414	-.149	-1.427	.157

a Dependent Variable: H_2

Influent pH

Coefficients(a)

Model		Unstandardized Coefficients		Standardized Coefficients	t	Sig.
		B	Std. Error	Beta		
1	(Constant)	-3.026	.884		-3.424	.001
	Influent pH	.717	.177	.392	4.038	.000

a Dependent Variable: H_2

Effluent pH

Coefficients(a)

Model		Unstandardized Coefficients		Standardized Coefficients	t	Sig.
		B	Std. Error	Beta		
1	(Constant)	-1.278	1.265		-1.010	.315
	Effluent pH	.423	.295	.149	1.433	.155

a Dependent Variable: H_2

APPENDIX D
HYDROGEN ALTERNATIVES

Table D-1. Comparative hydrogen production equivalent to other energy source

Type of fuel	Fuel alternative	Heating value (KJ/Kg) [1]	Fuel conversion (Kg/d)
Solid fuel	Bituminus	26,366.21	749.60
	Lignite	10,468.50	1,887.95
	Saw dust	10,878.42	1,816.81
	Rice Hask	14,397.92	1,372.70
	Bagasse	7,528.90	2,625.09
	Fire rubber wood	19,092.72	1,035.16
	Coal rubber wood	33,579.74	5,88.57
	Fire mangrove wood	18,548.41	1,065.54
	Coal mangrove wood	28,974.04	682.13
	Water hyacinth	13,398.40	1,475.10
Liquid fuel	Benzene fuel	34,525.00	572.45
	Diesel fuel	36,414.76	542.75
	Fuel oil A	41,274.02	478.85
	Fuel oil C	38,174.47	517.73
	Natural gas	36,694.47	538.61
	Liquefied petroleum gas	50,220.00	393.55
	Electricity	33,216.87 KJ/Kw-hr	595 Kw-hr/day

Source: [1] http://www.kmutt.ac.th/elearning/Energy Conservation in Industrial Plant/table/table5.html Retrieved 16 September, 2011

Note: Fuel conversion from solid and liquid fuel was calculated from heating value of hydrogen was 19764 MJ/d plus by heating value of other source.

Table D-2. Economic cost of alternative fuel conversion instead of biohydrogen production

Fuel alternative	**Fuel conversion (Kg/d)**	**Cost (Baht/ unit)**	**Total cost (Baht/day)**	**Total cost (Baht/years) (300day/ years)**	**Pay back period (years)**
Bituminus	749.60	3.50	2,623.59	78,7075.58	12 years 7months
Lignite	1,887.95	2.30	4,342.28	1,302,685.20	7years 7 months
Fire rubber wood	1,035.16	2.00	2,070.32	621,095.37	16years 1 months
Benzene fuel	572.45	24.48	14,013.69	4,204,107.63	2 years 4 months
Diesel fuel	542.75	23.67	12,846.82	3,854,046.11	2 years6 months
Fuel oil A	478.85	19.85	9,505.14	2,851,542.45	3years 5 months
Fuel oil C	517.73	16.00	8,283.65	2,485,095.41	4 years 1 months
Natural gas	538.61	8.50	4,578.18	1,373,454.91	7 years 3 months
Liquefied petroleum gas	393.55	20.00	7,870.97	2,361,290.32	4 years 2 months
Electricity		2.30	1,318	395,370	25 years3 months

Table D-1 and D-2 show the comparison in heating value of hydrogen and other alternative initial fuel sources both in terms of cost quantity and return on investment which are utilized in industry. The data from this study were based on soluble COD concentration of 81,950 mg/L, wastewater from ethanol production plant of 1000 m^3/d, hydrogen gas of 0.07 LH_2/g COD_r, and 32% of COD removal. Therefore, these calculation of hydrogen gas of 162 Kg/d in terms of heating value of hydrogen was 19,764 MJ/d, in order to substitute for other fuel source. Then, assumimg the capital cost and maintenance of hydrogen production plant for wastewater is approximately 10 million baht in order to calculate return on investment of payback period.

APPENDIX E

COST OF HYDROGEN GAS

Table E-1. Bulk hydrogen gas cost, USD in 2004

NG price,$/GJ	**Plant size, tons/day**	**Plant Cost,$**	**H_2 gas cost,$/kg**
3.5	22	23M	1.8
7.0	22	23M	2.4
15.0	22	23M	4.1
3.5	80	44M	1.2
7.0	80	44M	1.9
15.0	80	44M	3.0
3.5	600	210M	0.7
7.0	600	210M	1.0
15.0	600	210M	2.7

Source: Doty, 2004

Note: Natural gas (NG)

This part of the study was done to determine the feasibility of selling hydrogen gas. It was done using 99% hydrogen gas in reference to the cost of gas depending on size of the producing hydrogen capacity plant. For instance, the maximum hydrogen gas was 162 Kg/d which was based on soluble COD concentration of 81,950 mg/L, wastewater from ethanol production plant of 1000 m^3/d, hydrogen gas of 0.07 LH_2/g COD_r, and 32% of COD removal; assuming the following whole-sale prices of this study:

Capital cost	25,000	baht
Chemical and utility	1,320	baht
Maintenance 10%	2,500	baht
Net cost for 10 years	63,200	baht or $1,915.15
Produced gas from hydrogen production plant	48,600	Kg/years (300 days)

If the lowest and highest hydrogen gas cost followed \$0.7 and \$4.1/Kg for 10 years were \$340,200 and 680,400/Kg-10 years. Therefore, it would be feasible to invest in hydrogen gas. However, this study did not take into account the cost of having to purify the gas.

BIOGRAPHY

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