

# CHAPTER I

## INTRODUCTION

### 1.1 Rationale and Background

Depletion of fossil fuel has brought the attention of people into the findings of renewable energy resource. Biomass from crops and wastes are the most potential and promising ones due to the abundant and low/no cost. Renewable energy from biomass includes ethanol from corn (Chi and Chen, 2009), sugarcane juice (Cheng et al., 2008), sweet sorghum juice (Laopaiboon et al., 2009) and cassava starch (Christiana et al., 2010); biodiesel from palm oil (Yoojongdee et al., 2010) and used oil (Pratil et al., 2010); methane from food waste (Behera et al., 2010), chicken manure (Abouelenien et al., 2010); and hydrogen from sweet sorghum juice/syrup (Antonopoulou et al., 2008; Saraphirom and Reungsang, 2010), sugarcane juice (Pattra et al., 2010), sweet potato (Tatyana et al., 2010), cassava wastewater (Sangyoka et al., 2007), and sludge (Thungklin et al., 2010; Sittijunda et al., 2010). Methane and hydrogen are renewable energy but hydrogen has more advantages than methane due to its cleanness, efficient and non-polluting characteristics (Lin et al., 2008). When hydrogen is combusted with oxygen, water is the by-product so no greenhouse gas is produced unlike methane. Hydrogen has a high energy content per unit weight i.e.  $122 \text{ kJ g}^{-1}$  (Mizuno et al., 2000). Hydrogen can be produced by physiochemical and biological methods. Physicochemical method includes water electrolysis, steam reforming of methane and hydrocarbons. This method is energy intensive process requiring a high temperature ( $>850 \text{ }^\circ\text{C}$ ) (Kapdan and Kargi, 2006). Biological method is more attractive because it is energy saving process when compared to physiochemical process (Lin et al., 2008; Benemann, 1996; Lay et al., 1999). Biological hydrogen production by microorganisms falls into two main categories i.e., light fermentation process by photosynthetic bacteria and algae and dark fermentation by anaerobic bacteria (Ueno et al., 2001). Dark fermentation has shown to have a great potential for development as a practical bio-hydrogen production system (Hawkes et al., 2002).

Substrate for bio-hydrogen production is various. It ranges from simple sugars (sucrose, xylose, arabinose), sugars in the energy crops (sugarcane juice, sweet sorghum syrup) to complex substrate such as food waste, activated sludge, and industrial wastes. However, using crop such as sugarcane or cassava as the substrate for energy production has led to the conflicts of using crop for food or energy production. Therefore, the search for a new energy plant has been carried out. Sweet sorghum (*Sorghum bicolor* var. Keller) has been received the attention as a potential alternative energy plant due to its high biomass yield and high sugar content (Jaisil, 2007). In Thailand, sweet sorghum can grow in almost every geographic part. Sweet sorghum can be harvested within 100-120 days i.e. 3 crops per year while sugarcane takes at least one crop per year. Its production is not much different than sugarcane in which the average production of sweet sorghum is 31.25-37.5 tons/ha while production of sugarcane is 56.25-62.50 tons/ha (Sakellariou-Makrantonaki et al., 2007). Thus, when consider the number of 3 crops per year, sweet sorghum seems to have the advantage over sugarcane. Fresh biomass and fresh stalk of sweet sorghum yield 8-11.5% and 9-14.5% of total sugar content, respectively (Bulawayo et al., 1996). The main sugars in the sweet sorghum syrup are sucrose, fructose and glucose which are good substrates for ethanol and hydrogen productions. Despite the fact that the sweet sorghum syrup contains high sugar content, but the aconitic acid in the sweet sorghum juice prevent the crystallization of sugar (Coleman, 1983). Hence, sweet sorghum has been primarily used as the animal feed (Kongama and Rumei, 2005) which in turn makes it to be the new promising energy crop.

Various groups of bacteria such as *Enterobacter*, *Bacillus* and *Clostridium* (Li and Fang, 2007) are known hydrogen producers. Pure culture strain *Ruminococcus albus* can produce hydrogen from sugars and sweet sorghum biomass (Ntaikou et al., 2008). Not only pure culture but also mixed anaerobic microorganisms obtained from sewage or wastewater treatment plant sludge are able to produce hydrogen via the dark fermentation process after a proper pre-treatment (Fang and Liu, 2002). Mixed cultures can produce hydrogen from sweet sorghum extract with the maximum yield of 0.86 mol H<sub>2</sub>/mol glucose consumed (Antonopoulou et al., 2008). In this study, mixed cultures in anaerobic sludge are used as the hydrogen producer due to its advantages including capable of operating under non sterile condition which allow a

broader choice of feedstock selection and reducing overall cost (Li and Fang, 2007). In addition, the relationship of cooperative of microbial populations can facilitate the hydrogen production. For example, some of microorganisms in mixed cultures might consume volatile fatty acids (VFAs) produced during hydrogen production or dissolved oxygen in the liquid medium thus reducing the inhibitory effects of VFAs and oxygen resulting in a better production of hydrogen (Xing et al., 2008; Chong et al., 2009).

Biohydrogen production using dark fermentation is greatly influenced by environmental factors such as temperature, pH, nutrient addition, ferrous iron and substrate concentration (Wang and Lu, 2005). In order to efficiently produce hydrogen, there is a need to optimize these environmental factors. However, it is laborious and time consuming to perform the optimization by a conventional technique or known as “a one factor at a time” method (Stanbury et al., 1997). A statistical experimental design by the Plackett-Burman and response surface methodology (RSM) can eliminate this limitation (Abdel-Fattah and Olama, 2002). It is not only a time saving method but also can minimize the error in determining the effects of parameters as well as be able to demonstrate the interactive effects among the tested variables (Abdel-Fattah and Olama, 2002). Although many studies have been done on the effect of environmental factors on hydrogen production from various kinds of synthetic substrates and wastes but the information on the statistically optimization of environmental factors on biohydrogen production from sweet sorghum by mixed cultures are still lacking. Therefore, the first objective of this study was to optimize the environmental factors affecting hydrogen production from sweet sorghum syrup by anaerobic mixed cultures by using the Plackett-Burman design to screen the significant variables. Then the Box-Behnken design of RSM is used to optimize the levels of the screened variables. Results from this study will provide the optimum condition that can improve hydrogen yield from sweet sorghum syrup.

Numerous studies on continuously production of hydrogen have been conducted in the continuous stirred tank reactor (CSTR) (Li et al., 2008; Shen et al., 2009; Yuan et al., 2010; Antonopoulou et al., 2008; Kim et al., 2006; Kongjan et al., 2010). However, the drawbacks of CSTR have been reported including the difficulty to

maintain high levels of hydrogen-producing biomass at a short hydraulic retention time (HRT). This is due to its intrinsic structure (Chen and Lin, 2003; Yu et al., 2003) that causes operational instability and limits the hydrogen production rate (HPR) (Cavalcante de Amorim et al., 2009). In addition, CSTR system is time-consuming operation with continuous feeding by immobilized microorganisms (Chen and Lin, 2001). For that reason, anaerobic sequencing batch reactor (ASBR) process has become the reactor option for producing hydrogen. Distinct advantages of ASBR when compared to CSTR system including high biomass concentration, a high degree of process flexibility, no requirement to apply a separate clarifier (Dague et al., 1992). Moreover, ASBR system is efficient in operating control, without primary or secondary settling, with high organic removal efficiency and simple operation (Ratusznei et al., 2000). The ASBR cycle is composed of four steps which were filling, reacting, settling and decanting steps (Dague et al., 1992). ASBR has been used to produce hydrogen from various kinds of substrates such as sucrose, dairy wastewater, chemical wastewater and palm oil mill effluent (POME) (Lin and Jo, 2003; Venkata Mohan et al., 2007a,b; O-Thong et al., 2007). Investigations on important parameters for ASBR operation including solid retention time (SRT), organic loading rate (OLR), cyclic duration and HRT have been reported. Among these parameters, HRT is one of the important parameters that determine the economics of the hydrogen production process. The lower HRT attributed to a smaller reactor, cost reduction and therefore enhanced the productivity of the hydrogen production process (Kim et al., 2006). OLR is another important parameter for continuously producing hydrogen in the bioreactors. In order to optimize a system for hydrogen production, it is essential to define either a range of the OLR that the system can handle effectively, or optimal OLR for a maximum hydrogen yield. However, from the literature search, there is no clear relationship between the hydrogen yield and the OLR. In some cases high OLR decreased the hydrogen yield (Van Ginkel and Logan, 2005) whereas in some others high OLR increased the hydrogen yield (Zhang et al., 2004). For waste activated sludge as a seed material, it appears that increasing the OLR within the ranges of 40–160 g-COD/L-d increased hydrogen yield in which the optimum yield of 1.6 mol H<sub>2</sub>/mol glucose was obtained at an OLR of 120 g-COD/L-d (Wu et al., 2006). However, the hydrogen yield was

found to decrease with an increase in OLR when anaerobically digested sludge (Kyazze et al., 2006) and soil microorganisms (Van Ginkel and Logan, 2005) were used as the inoculums. Previous research indicated the important of the optimization of the HRT and OLR in order to efficiently operating the continuous hydrogen production process from sweet sorghum syrup in ASBR. Therefore, the second objective of this study was to investigate the effects and to optimize the HRT and OLR on hydrogen production from sweet sorghum syrup based on the information obtained from the earlier batch fermentation.

Although lower molar hydrogen yields at higher OLR have been attributed to the inhibitory effect of higher hydrogen partial pressure in the growth medium (Van Ginkel and Logan, 2005; Ruzicka, 1996), variations in the composition of bacterial communities that become established at different OLR (Luo et al., 2008) may be a major reason for lower yields. Thus, it is suggested that microbial community analysis, in addition to chemical analysis such as the volatile fatty acids (VFAs) e.g., butyric acid, acetic acid, etc., should be conducted especially when mixed cultures were used as the seed inoculums. In this study, the third objective, polymerase chain reaction-denaturing gradient gel electrophoresis (PCR-DGGE) was used as a tool to analyze the microbial community at both steady and unsteady states in order to link the evolution of the microbial community to the performance of the reactor. The information obtained would provide a better understanding and a successful operation of the continuously hydrogen production process in ASBR.

Low hydrogen yield and hydrogen production rate can be improved by nutrient supplementation such as the work conducted by O-Thong et al., (2007) and Liu et al., (2011). Thus, our forth study focused on the improvement of hydrogen production using nutrient and vitamin supplementations.

Throughout the successful process of hydrogen production from sweet sorghum syrup, large amounts of organic wastewater were generated. Wastewater contained residual VFAs such as butyric and acetic acids and residual sugars. These organic compositions had been reported as valuable substrates for producing renewable energy such as ethanol and methane by various types of mixed and pure cultures of fermentative bacteria. In addition, VFAs present in these effluents can be further transformed to polyhydroxyalkanoates (PHAs) by microorganisms such as

*Cupriavidus necator* (formerly named *Ralstonia eutropha*) (Hafuka et al, 2011). PHAs are a group of biologically derived biopolyester of hydroxyalkanoates that accumulate as carbon/energy or reducing-power storage materials in microbial cells (Salehizadeh and Van Loosdrecht, 2004). When a carbon source is available in excess and other nutrients are growth-limiting, biopolyesters are deposited as water-insoluble cytoplasmic non-sized inclusions by *Eubacteria* and *Archaea* (Rehm, 2007). VFAs i.e. acetic and butyric acids under low oxygen/anoxic microenvironment can be converted to poly(b-OH)butyrate (PHB) via various biochemical routes catalyzed by the action of different enzymes in which PHB synthase plays a major role in biosynthesis of PHB (Solaiman and Ashby, 2005). Compared to carbohydrates, lipids and amino acids, VFAs have simple structure with lower number of carbon atoms, which facilitates its easy synthesis to PHAs without the requirement of glycolysis and b-oxidation pathways by using less number of metabolic reactions and enzymes (Venkata Mohan et al., 2010). Therefore, the last objective of this study was to explore the possibility of using hydrogenogenic effluent, effluent from hydrogen production process to produce PHAs by *Cupriavidus* sp. KKU38.

## 1.2 Thesis Objectives

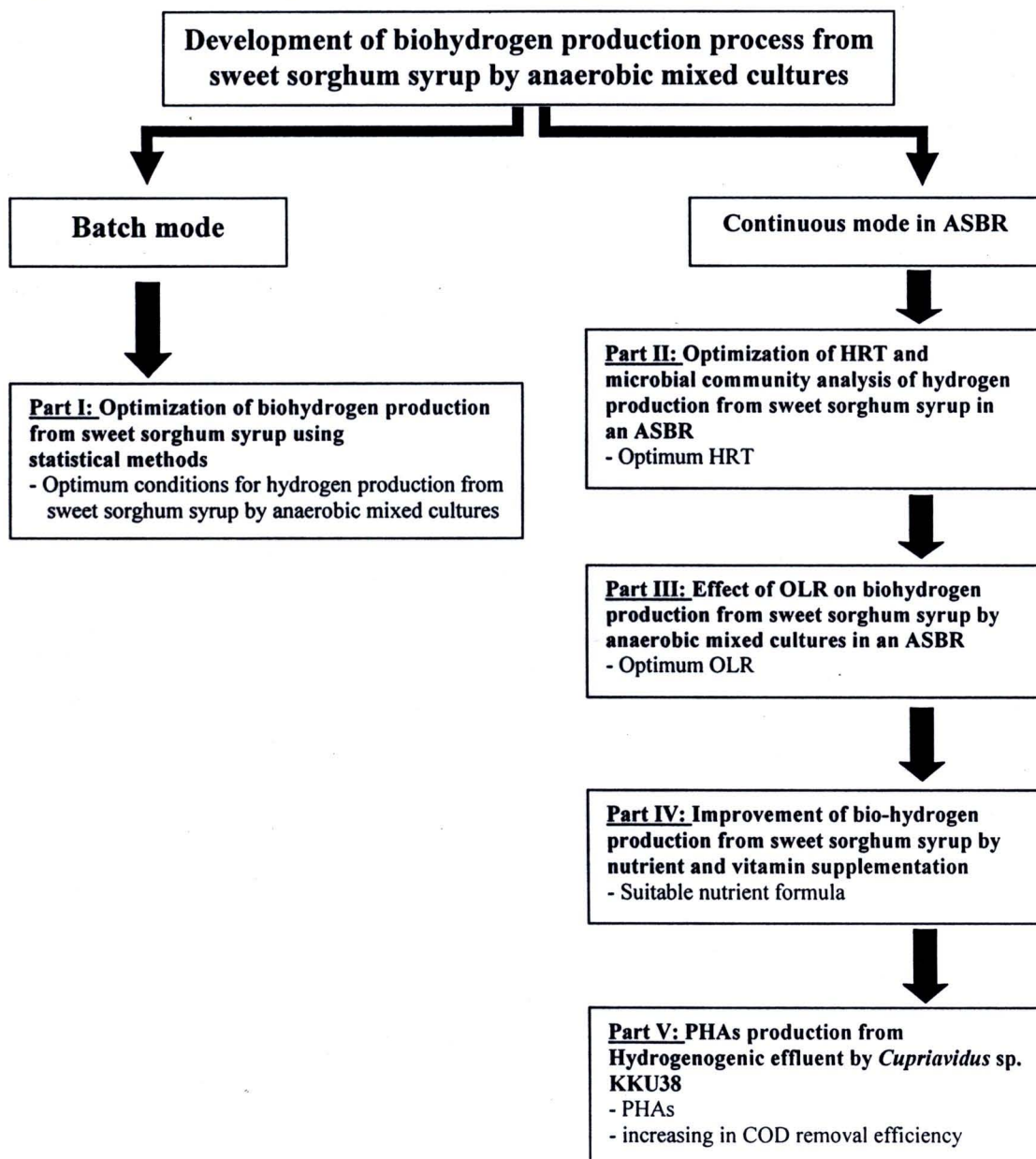
The ultimate goal of this study is to develop the bio-hydrogen production process from sweet sorghum syrup by anaerobic mixed cultures. In order to achieve the ultimate goal, we divided the goal into 5 sub-objectives as follows:

- 1) To optimize the environmental factors affecting batch fermentation of hydrogen from sweet sorghum syrup by anaerobic mixed cultures using statistical methods.
- 2) To investigate the effects of HRT and OLR on continuously hydrogen production from sweet sorghum syrup by anaerobic mixed cultures in an ASBR based on the information obtained from the batch fermentation.
- 3) To investigate the links between the microbial community and the reactor performance.
- 4) To improve the continuously hydrogen production from sweet sorghum syrup in ASBR by nutrient and vitamin supplementation.

5) To produce PHAs by *Cupriavidus* sp. KKU38 from the VFAs in the hydrogenogenic effluent.

### 1.3 Research Plan

Research plan of this study was divided into 5 parts according to the research objectives. The entire plan is summarized in Figure 1.1.



**Figure 1.1** Flow diagram of research planning for biohydrogen production from sweet sorghum syrup by anaerobic mixed culture

## 1.4 Thesis Structure

This thesis is organized into nine chapters. Chapter 1 is the introduction and overview of the research, the research rationale, objectives, research plan and organization of research and research sites. Chapter 2 is the literature review containing the literature search mainly in the areas of hydrogen and a touch in polyhydroxylalkanoates (PHAs) production. The third chapter presents the manuscript entitled “Optimization of biohydrogen production from sweet sorghum syrup using statistical methods”. This manuscript was published in International Journal of Hydrogen Energy (35), 2010 Page 134535-13444. The fourth chapter is the manuscript entitled “Optimization of hydraulic retention time and microbial community analysis of hydrogen production from sweet sorghum syrup in an anaerobic sequencing batch reactor”. This manuscript was prepared to submit to International Journal of Hydrogen Energy. Chapter 5 is the manuscript entitled “Biological hydrogen production from sweet sorghum syrup by mixed cultures using an anaerobic sequencing batch reactor (ASBR)”. This manuscript was published in International Journal of Hydrogen Energy doi:10.1016/j.ijhydene.2010.08.058. Chapter 6 is the manuscript entitled “Effect of organic loading rate on biohydrogen production from sweet sorghum syrup by anaerobic mixed cultures in an anaerobic sequencing batch reactor”. This manuscript was published in International Journal of Energy (4), 2010 Page 55-62. Chapter 7 is the manuscript entitled “Enhancement of biohydrogen production from sweet sorghum syrup in anaerobic sequencing batch reactor by nutrient and vitamin supplementations”. This manuscript was prepared to submit to International Journal of Hydrogen Energy. Chapter 8 is the manuscript entitled “Polyhydroxyalkanoates production from hydrogenogenic effluent by *Cupriavidus* sp. KKU38”. This manuscript was prepared to submit to International Journal of Hydrogen Energy. Chapter 9 is the general conclusions of this thesis.

## 1.5 Research Sites

This research has been conducted at the Department of Biotechnology, Faculty of Technology, Khon Kaen University, Khon Kaen, Thailand

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