# CHAPTER I
# INTRODUCTION

## 1.1 Rationale and Background

Sugarcane is one of the important industrial crops in Thailand. It can be cultivated in all parts of Thailand, except in Southern Thailand (Office of the Cane and Sugar Board, 2006), with the cultivation area of more than 960,000 ha; approximately 48 million tons of sugarcane is produced per year (Office of the Cane and Sugar Board, 2006). An attempt to develop new breeds of sugarcane in order to increase its productivity has been experimented continuously. This may result in an excess of sugarcane product over the capacity of sugar factories which mainly use sugarcane as raw material to produce sugar. Therefore, this research was designed to investigate an alternative way to value-added sugarcane by producing a clean and renewable energy i.e. hydrogen. Hydrogen is alternative energy of our interest due to it is a clean and environmental friendly fuel, which produces water instead of greenhouse gases after combustion. Hydrogen can give high energy yield of 122 KJ/g which is 2.75 times greater than that of petroleum fuel (Han, Shin, 2004; Kim et al., 2004).

The use of renewable energy derived from the crop avoids many of the environmentally detrimental aspects of petroleum-based fossil fuels such as the greenhouse effect which led to a global warming. However, renewable energy has its own environmental costs including ecological damages caused by nitrogen and phosphorus fertilizers, pesticides, and soil erosion (Hill, 2007). In addition, as demand for both food and energy rise in the coming decades, the conflicts of using crop for food or energy production could occur. These reasons draw our interest of using lignocellulosic materials which are the most abundant and widely spread renewable biomass in the world as a feed stock for hydrogen production. Sugarcane bagasse (SCB), a waste left after sugarcane extraction process, is one of lignocellulosic materials. Since the bagasse accounted for approximately 25% of sugarcane mass, about 12 million tons of SCB is produced annually in Thailand (Neureiter et al., 2002). The most common use for SCB is the energy production by combustion which can cause environmental problem from the emissions of $CO_2$. In addition, SCB can be used also to produce chemical compounds such as furfural or hydroxymethylfurfural (Almazan et al., 2001), paper paste (Caraschi et al., 1996) or ethanol (Laser et al., 2002). However, the ratio of used sugarcane bagasse to sugarcane bagasse waste is still low. Therefore, we are interested in using SCB to produce hydrogen in which this approach will not only adding value to SCB, in a form of safe and clean energy, but also one of the solution approaches for making use of this abundant waste.

In the hydrogen production process using pure culture, the reactors are mostly started up and operated under sterile condition which requires high cost for hydrogen production in the industrial scale. To overcome this problem, the addition of pure culture to the reactor under non-sterile condition has drawn our attention. During hydrogen production under non-sterile condition, the relationship of competitive and cooperative of microbial populations could be found. For the competitive relationship, some isolates augmented into the reactors can not become dominant due to their inability to grow in the communities depending on the environmental condition. In contrast, for the cooperative relationship, the mixed indigenous microorganisms in the substrate could consume volatile fatty acids (VFAs) produced during hydrogen production, which in turn facilitate hydrogen production of bioaugmented producer by reducing the product inhibitory effect. According to these phenomena, the microbial community in the hydrogen production system under non sterile condition should be investigated in order to verify the relationship between the augmented microorganism and normal flora in the substrate.

Throughout the successful process of hydrogen production from sugarcane juice and SCB, large amounts of organic wastewater were generated. Wastewater contained residual organic matter such as butyric and acetic acids and residual sugars. These organic compositions had been reported as valuable substrates for ethanol and methane productions by various types of mixed and pure cultures of fermentative bacteria. Therefore, the possibility of using hydrogenogenic effluent, effluent from hydrogen production process, to produce ethanol and methane under non-sterile condition was also explored in this study.

The main objective of our studies is to explore the possibility of using sugarcane to produce multibiofuels (biohydrogen, methane and ethanol) by *Clostridium butyricum* and mixed cultures which including 4 sub-objectives as follows: (i) to investigate the bio-hydrogen production from sugarcane juice by the immobilized *C. butyricum* on sugarcane bagasse in batch and repeated batch fermentation systems, (ii) to investigate the bio-hydrogen and ethanol productions from sugarcane juice under non-sterile condition and determine the relationship between the augmented microorganism and normal flora in the substrate, (iii) to examine the bio-hydrogen production from the fermentation of sugarcane bagasse hydrolysate by *C. butyricum* and (iv) to recover the methane from hydrogenogenic effluent by anaerobic sludge using response surface methodology.

## 1.2 Thesis Objectives

### 1.2.1 Main objective

The research aims to explore the possibility of using sugarcane to produce multi-biofuels (bio-hydrogen, methane and ethanol) by *Clostridium butyricum* and mixed cultures.

### 1.2.2 Sub-objectives

1) To determine the bio-hydrogen production from sugarcane juice by the immobilized *C. butyricum* on sugarcane bagasse in batch and repeated batch fermentation processes.

2) To investigate the bio-hydrogen and ethanol productions from sugarcane juice by *C. butyricum* under non-sterile condition and determine the relationship between the augmented microorganism and normal flora in the substrate during hydrogen and ethanol fermentations.

3) To examine the bio-hydrogen production from the fermentation of sugarcane bagasse hydrolysate by *C. butyricum*.

4) To recover methane from hydrogenogenic effluent by anaerobic sludge using response surface methodology as the optimization tool.

## 1.3 Research Plan

Research plan of this study was divided into 4 parts according to the research objectives. The entire plan is summarized in Figure 1.1

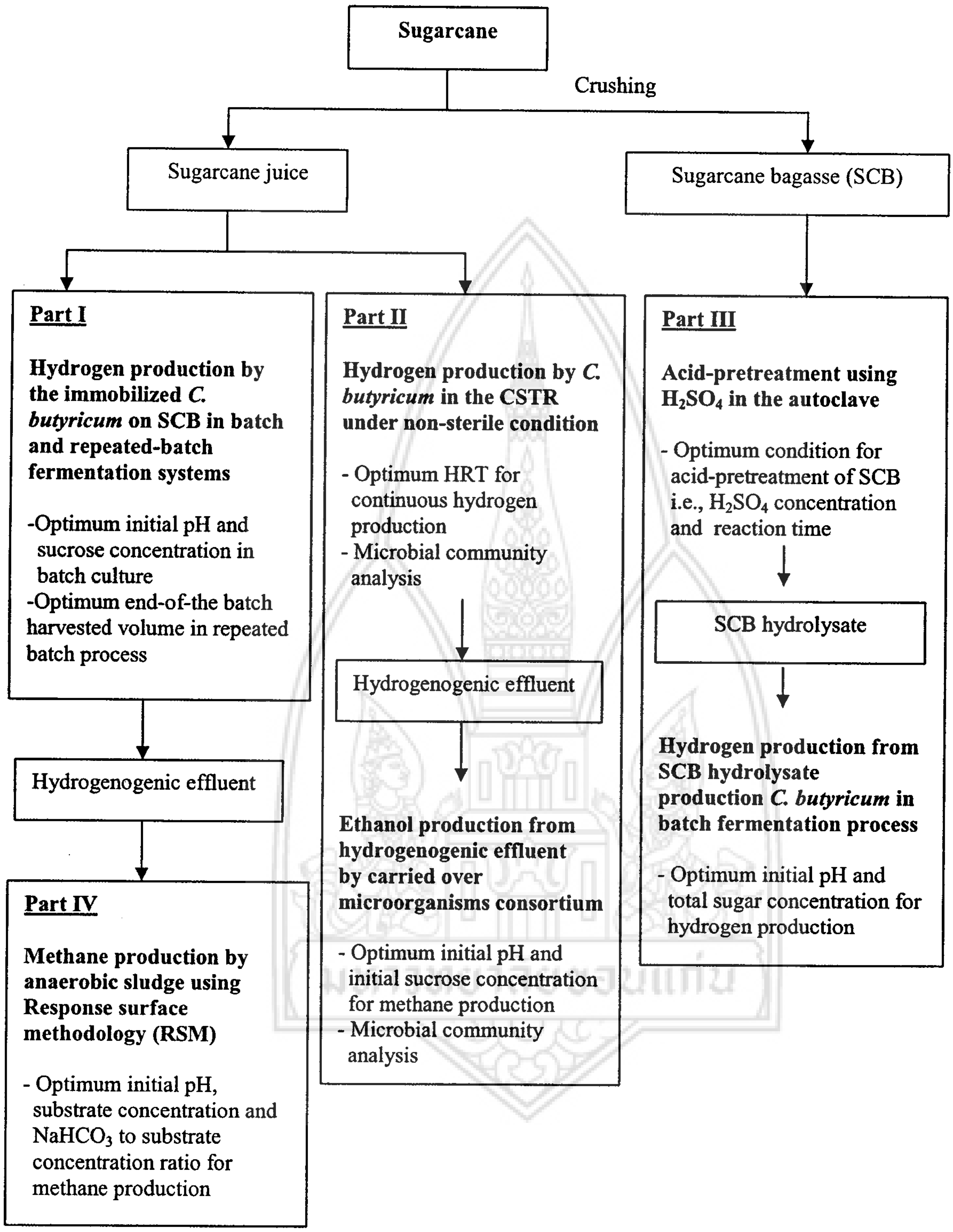


**Figure 1** Flow diagram of the experiments on multi-biofuels production from sugarcane by *C. butyricum* and anaerobic mixed-culture

## 1.4 Thesis Structure

This thesis is organized into seven 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 information on the previous research in the areas of bio-fuel production. The third chapter presents the manuscript entitled "Bio-hydrogen production from sugarcane juice by immobilized *Clostridium butyricum* on sugarcane bagasse". This manuscript was prepared to submit to World Journal of Microbiology and Biotechnology. The fourth chapter is the manuscript entitled "Two stages fermentation bio-hydrogen and ethanol by *Clostridium butyricum* from sugarcane juice". This manuscript was prepared to submit to International Journal of Hydrogen Energy. Chapter 5 is the manuscript entitled "Bio-hydrogen production from the fermentation of sugarcane bagasse hydrolysate by *Clostridium butyricum*". This manuscript was published in International Journal of Hydrogen Energy 33 (2008) 5256-5265. Chapter 6 is the manuscript entitled "Optimization of process parameters on methane production from hydrogenogenic effluent by Response Surface Methodology". This manuscript was submitted to Process Biochemistry. Lastly, the summarization, general conclusions of the thesis and recommendations for future research are presented in chapter 7.

## 1.5 Research Sites

This research has been conducted at the following laboratories:

1.5.1 Department of Biotechnology, Faculty of Technology, Khon Kaen University, Khon Kaen, Thailand

1.5.2 Department of Environmental Engineering, Faculty of Engineering, Khon Kaen University, Khon Kaen, Thailand

1.5.3 Department of Civil and Environmental Engineering, Faculty of Engineering, Yamaguchi University, Yamaguchi, Japan

1.5.4 Biohydrogen Laboratory, Department of Environmental Engineering and Science, Feng Chia University, Taichung, 40724, Taiwan

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1.6.2 Research Group for Development of Microbial Hydrogen Production Process from Biomass, the Commission on Higher Education, Thailand

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1.6.4 Feng Chia University (Contract No. 07G27501 and 08G27201) and Bureau of Energy, Taiwan (Grant No. 97-D0137-2)

1.6.5 Office of the Cane and Sugar Board, Thailand