CHAPTER 1 INTRODUCTION

1.1 Background

Combustion of fossil fuels has also caused global warming due to excessive emission of greenhouse gases. As a result, developing a new and more environmentally compatible alternative energy has become a hot issue in recent years [1]. The greenhouse effect caused by increasing amounts of carbon dioxide in the atmosphere and it brings a serious global environmental problem. Since large amounts of carbon dioxide are discharged by the combustion of fossil fuels, development of alternative energy resources to minimise the environmental impact is desired. Hydrogen is considered to be an ideal energy for the future because it is easily converted to electricity and is cleanly combustible. Among the processes of hydrogen production, microbial hydrogen production or 'biohydrogen' has been extensively investigated due to it being an energy-saving process [2].

Hydrogen is considered to be an ideal clean energy source, as vapour is only byproduct of combustion. Hydrogen is traditionally produced by hydrocarbon reformation or electrolysis of water. These processes require external energy sources for producing the hydrogen and therefore they are costly. Biohydrogen production is more economical and environmentally friendly than that of traditional methods since the organic waste residues are converted to hydrogen. The biohydrogen can be claimed as the waste utilisation [3]. Hydrogen is the highest massive energy, which is 122 KJ/g, the massive ratio of hydrogen energy is 2.75 times greater than the fossil fuels. Hydrogen can be produced biologically through microbes either by photosynthetic bacteria cultures under anaerobic light conditions or by fermentative bacteria [4]. Hydrogen producing inocula can consume water or other renewable resources as the substrate, enabling H_2 production at ambient temperature and pressure [1]. However, one of the challenges of biohydrogen processes is the feasibility of producing H_2 at a commercial scale with a low cost to meet the need of sufficient and cost-effective energy supply. Consequently, the substrate supplied to fermentative H_2 production must be abundant, easily available, and inexpensive [5].

Microbial hydrogen production by fermentative bacteria, photosynthetic bacteria and algae has been reported. Since sugars such as glucose and starch are suitable substrates for hydrogen-producing microorganisms, the organic residues enriched with the carbohydrate are desirable to be supplied to biohydrogen process. The sweet potato starch residue had the high contents of coarse starch, cellulose, hemicellulose, moisture and other organic substances. A mixed culture of *Clostridium butyricum* and *Enterobacteraerogenes* were employed to biohydrogen process, which was fed by the starch wastewater. The stoichiometry of starch biodegradation is 2 mole H_2/mole glucose. The hydrogen production was 6.6 mol H_2mole/ moleglucose could be obtained from the starch, when a mixed culture of *C. butyricum* and *Rhodobacter sp. M-19* were introduced [6]. As the suitable substrates for dark fermentative hydrogen production include carbohydrate-rich crops and food industry wastes. Starch manufacturing is one of the sources of low cost carbohydrate-rich waste. There are many starch factories in the north eastern region of Thailand. The starch manufacturing can contribute the significant amounts of organic wastewater and starch residues, which can be supplied to biohydrogen process. The dark fermentative hydrogen production processes may be employed to produce hydrogen gas from the starch waste residue. The specific inocula such as cellulosic enzyme producing microbes are required in order to convert the long chain cellulosic in starch to be a simple sugar, which can be consumed by hydrogen producing microbe. An non-sterile feedstock such as mixed microflora derived from natural sources, often contain the species of hydrogen-producing microbes that is *Clostridia*, these species can directly digest cellulose substrate in the hydrogen respiration pathway [7].

1.2 Statement of Problems

Hydrogen might be the alternative energy, which can reduce the impact global warming. The sustainable of hydrogen energy producing may be achieved whenever the technologies are proven in the aspects of environmentally safe and cost effectiveness. The biohydrogen can be recognised as the possible process, which can benefits these aspects. Many species of microbial inocula can biotransform organic waste to hydrogen. Instantly, algae and bacteria can produce H_2 via the photoreaction, some bacteria can produce H_2 through the dark fermentation. The bioreactors can be resembled as the anaerobic digestion process for dark fermentation or photobioreactor for light

fermentation [7]. Hydrogen production by anaerobic fermentation using organic waste as the substrate has drawn much attention, since this system requires low energy input and reasonable cost. The conventional anaerobic fermentation may generate both of H_2 CH_4 and CO_2. The dark fermentation biohydrogen process has similarly functioned to anaerobic fermentation process in term of biogas producing, but they can predominantly produce H_2 under acidic condition. The remaining carbon substrates are mainly transformed to organic acid and alcohols as the by-products or intermediate. These by-products may be utilised by the other microbes [8]. The efficiency of biohydrogen is related to the operating conditions and types of substrates and microbial seed. The rate of hydrogen evolution from an anaerobic fermentation depends on several parameters such as pH, substrate concentration and temperature. However, the available information is little, in the aspects of the relationship among hydrogen production, pH value, and substrate concentration in an anaerobic fermentation process. As a consequence, the pathways of hydrogen production are specified depending on the microbial species [8]. In order to promise the activities of hydrogen producing microbes, the mixed culture is always employed. The cost of H_2 can be reduced whenever low cost substrates are supplied to the biosystem. The possible sources of carbohydrate-rich waste are food processing. However the food preservative agent may inhibit the growth of microbial seed[9]. The starch waste is challenged to be the carbon source of hydrogen producing microbes. The limitation of starch utilisation is the low rate of biotransformation, which is the result of slow hydrolysis process. Therefore, the pre-fermentative microbes that can convert starch into simple sugar or directly digest the cellulosic molecules in the hydrogen respiration pathway are studied in this research. Since the H_2 producing microbes may associate with the simple sugar producing microbes, they can enhance the efficiency of bioprocess and bring the cost-effective H_2 production, as well as eliminating the limitation of starch hydrolysis reaction [10]. Starch is an important biopolymer in plants, so far the largest source of starch are corn (maize) wheat, potato, tapioca and rice [11]. The starch utilised in this research are govern from the tapioca flour manufacturer. The previous researches had investigated the key parameters for controlling the biohydrogen, including of identifying capable microorganisms and optimising systems to maximise hydrogen production potential[12]. The factors concerned in this study are set up as same as the previous research in order to understand the behaviours of pre-fermentative and

H_2producing microbes together, when starch residue is fed as the substrate in the biosystem.

1.3 Objectives

The aim of the research is to effectively produce H_2 from starch residue using the pre-fermentative coupled with dark fermentative processes. The optimum conditions of each process were separately examined and then the bench scale bioreactor was designed to be either single or two phase reactors. Therefore, the major objectives for this research are established as follows.

1. To evaluate the optimum condition for the pre-fermentative microbes to hydrolyse the starch residue and to determine the rate of glucose production.
2. To understand the role of H_2 producing microbes in the biosystem with starch residue feedstock.

1.4 Scopes

To completely achieve all objectives, the scopes of thesis were established as follows.

1. The microbial seeds were firstly prepared by either with or without heat shock treatment.
2. The treated and natural microbial seeds were acclimatised with glucose and starch until they were matured.
3. The synthesis wastewater was prepared by dissolving starch residue into clean water.
4. The pH of bioreactors was maintained at 5.5 and operated at room temperature.
5. The biotransformation activities were examined via the equations of Gompertz and Monod.
6. The bench scale bioreactor was designed based on the optimum conditions of both pre-fermentative and H_2 producing microbes.

1.5 Expected Outcomes

The findings of bioactivities of pre-fermentative and H_2 producing microbes are the useful information to design the demonstration unit of pre-fermentative coupled with biohydrogen processes. This research can provide the outcomes as:

1. The possible design of bioreactor for the biohydrogen with starch feedstock.

2. The optimum conditions for the biosystem containing pre-fermentative and H_2 producing microbes.

1.6 Research Development

The framework of this research is given in Figure 1.1, which can present the works conducted in this research. Based on the analytical thinking, the statement of problems is established in accordance with the reviewed literatures as given in Chapter 2. The hypothesis of research work is set up to define the parameters, which affect the performance of H_2 producing microbes and the alternative way to utilise the starch residue as carbon sources in biohydrogen process. The methods are required to prove the established hypothesis is illustrated in Chapter 3. The findings are critically discussed as provided in Chapter 4. The major results are summarised as given in Chapter 5.

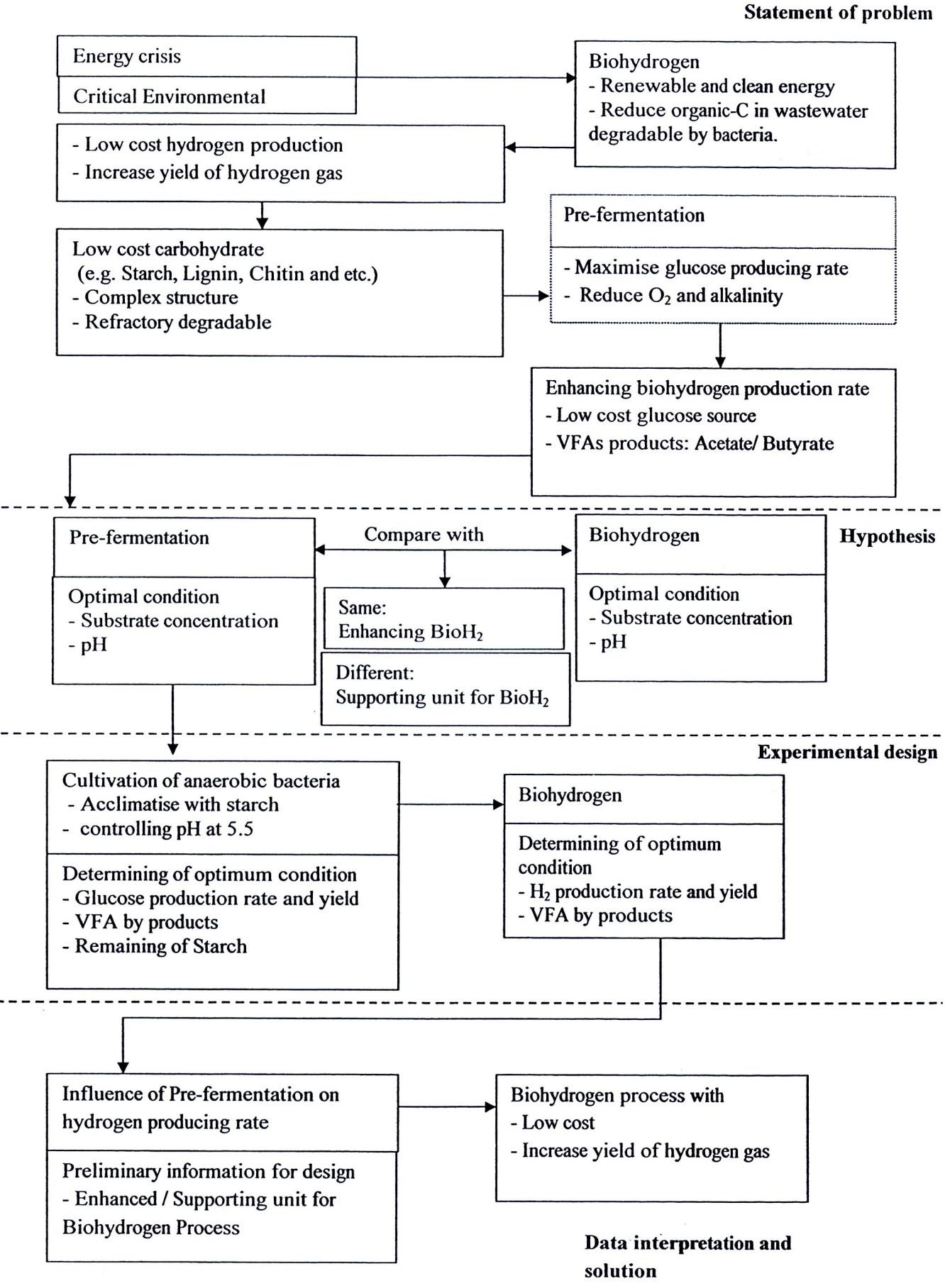

Figure 1.1 Research framework