CHAPTER 3 MATERIALS AND METHODS

3.1 Overview

This work was designed to achieve an efficient hydrogen production system from important biomasses of starch and glucose. This research was aimed at investigating the hydrogen-producing bioactivity of anaerobic at optimum pH and substrate concentration values for batch fermenting starch from synthetic wastewater. Specifically, the effects of these factors on hydrogen gas content, hydrogen yield (the ability converting starch into hydrogen), and the rate of hydrogen production from the reactor were determined. The conditions of anaerobic fermentation were studied in order to understand the effects of the operating conditions and process control on hydrogen production. Effect factors such as starch concentrations were investigated for determining the optimum operation conditions at hydrogen production. The whole framework of experiment is described in Figure 3.1.

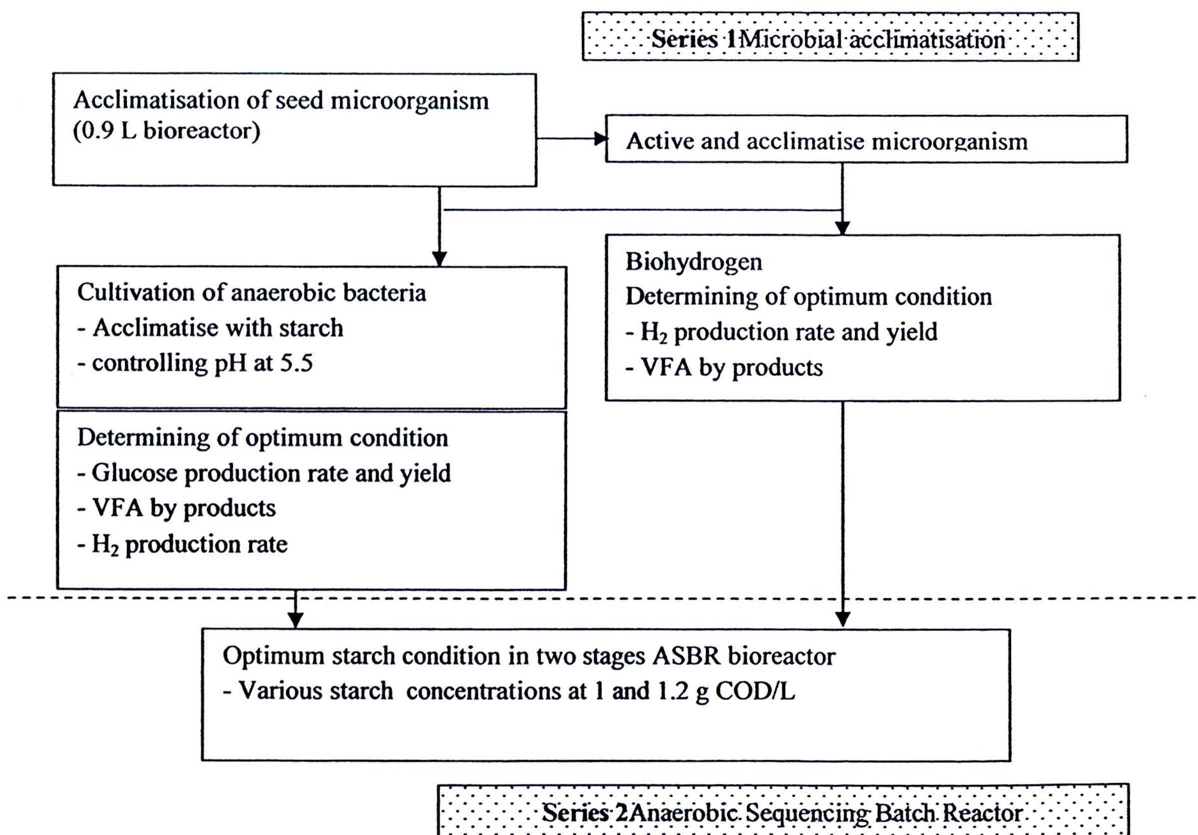

Figure 3.1 Overview of experimental setup

3.2 Seed Inoculums and Substrate Preparation

In fact, the anaerobic sludge samples were collected from many sources as same as the previous study of Surachote (2010) [114]. However, they were not able to digest the starch residues and turn the starch substrate to the H_2. Besides, another sludge sample collected from starch factory was also preliminarily tested. The sludge sample was familiar with thermophillic condition. The microbial seed could not adapt to survive in the mesophilic condition that was employed in this study. Therefore, anaerobic sludge samples from the Dindaeng (DD) and Nongkheam (NK) domestic wastewater treatment plants, Bangkok, Thailand were used as inocula in this study. These sludge samples were obtained from the wasted sludge chamber, before dewatering. Both of DD and NK inoculum were heat-shock treated by heating at 104 °C for 2 hours followed by the shocking in the ice box. These inoculums were pretreated using heat-shocked process to refine the hydrogen producing microbes. The synthesis wastewater samples were prepared by dissolving the starch residues 1.2 g and glucose 9.4 g into a clean water 1 L, controlling the COD concentration at 1 gCOD/L of starch and 10 gCOD/L of glucose, respectively. The starch solution was boiled for 15 minutes for dissolving the starch residues.

3.3 Experimental Setup

Two series of experiment investigated the performance of fermentative hydrogen production as follow:

Series 1: Cultivation of anaerobic bacteria

Eight batch experiments using either the incubated or the natural DD and NK sludges were performed as shown in Table 3.1. The experiments were set up by the fractional factorial design (FFD) to reduce the biased error. In the incubation process, 300 mL of dried inocula were added to 1 L borosilicate glass reactors. According to the prescription, the 600 mL of synthesis wastewater and 4 mL of nutrient solution were added to the reactors. Each litre of nutrient solution contains 2.0 g of NH_4HCO_3, 1.0 g of KH_2PO_4, 100 mg of $MgSO_4 \cdot 7H_2O$, 10 mg of NaCl, 10 mg of $Na_2MoO_4 \cdot 2H_2O$, 10 mg of $CaCl_2 \cdot 2H_2O$, 15 mg of $MnSO_4 \cdot 7H_2O$, 4 mg of $NiCl_2 \cdot 6H_2O$ and 2.78 mg of $FeCl_2$ [115]. The volumes of every bioreactor were totally at 900 mL and the reactors were immediately closed with the rubber plug connecting with the inlets and outlets of gas

and liquid tubes. The reactors were flushed with nitrogen gas for 15 s to eliminate oxygen in the system. The pH was adjusted to 5.5 using either 1 N of H_2SO_4 or 1 N of NaOH. The reactors were covered with aluminum foil to stimulate the dark fermentation and placed on the stirrer. The hydraulic retention time was 2.0 days. The experimental setup is presented in Figure 3.2.

Table 3.1 Batch experiment design

No.	Substrate type	[Substrate] (gCOD/L)	Matured inoculum	Initial VSS (g/L)
1A	Glucose	10	Treated DD	3.933
2A	Glucose	10	Treated NK	3.467
3A	Starch	1	Treated DD	3.700
4A	Starch	1	Treated NK	3.567
1B	Glucose	10	Natural DD	3.300
2B	Glucose	10	Natural NK	3.933
3B	Starch	1	Natural DD	3.500
4B	Starch	1	Natural NK	3.900
1AA	Glucose 80% + Starch 20%(80G+20S)	8.2	Treated DD	3.633
2AA	Glucose 80% + Starch 20%(80G+20S)	8.2	Treated NK	3.433
3AA	Starch 80% + Glucose 20%(80S+20G)	2.8	Treated DD	3.667
4AA	Starch 80% + Glucose 20%(80S+20G)	2.8	Treated NK	3.700
1BB	Glucose 80% + Starch 20%(80G+20S)	8.2	Natural DD	3.600
2BB	Glucose 80% + Starch 20%(80G+20S)	8.2	Natural NK	3.333
3BB	Starch 80% + Glucose 20%(80S+20G)	2.8	Natural DD	3.433
4BB	Starch 80% + Glucose 20%(80S+20G)	2.8	Natural NK	3.900

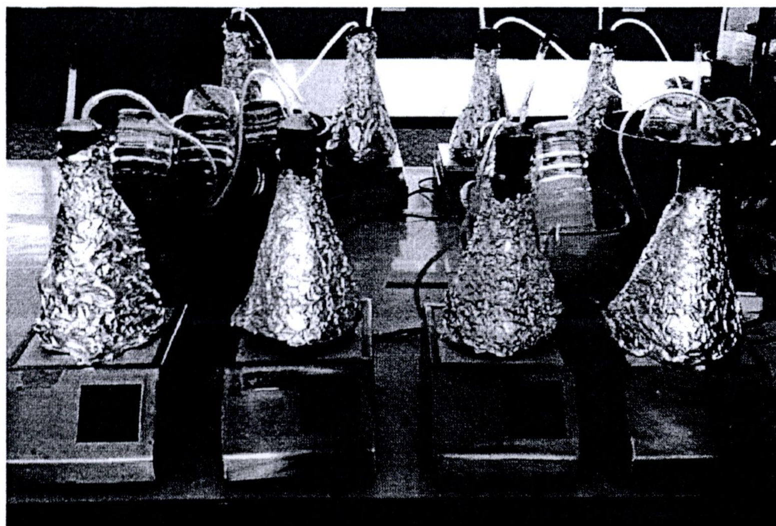

Figure 3.2 Experimental setup of dark fermentation for biohydrogen process

Series 2: Anaerobic Sequencing Batch Reactor

According to the previous experiment, the actively acclimatised hydrogen producing bacteria was selected. Four anaerobic sequencing batch experiments using either the incubated or the natural DD sludges were performed as shown in Table 3.2. In the incubation process, 1000 mL of dried inocula were added to 4 L bioreactors. The 3000 mL of synthesis wastewater and 16 mL of nutrient solution were added to the reactors. The same nutrient solution was prepared as Series 1.

Table 3.2 Anaerobic Sequencing Batch experiment design

No.	Substrate type	Matured inoculum	Initial VSS (g/L)
DD1	Starch	Natural DD	3.100
DD2	Starch residue from DD1	Treated DD	3.066
DD3	Starch	Natural DD	3.033
DD4	Starch residue from DD3	Treated DD	3.000

The total volume of bioreactor was 4000 mL. The inlets and outlets of gas and liquid tubes were connected at the reactor rid. The reactors were flushed with nitrogen gas for 15 s to eliminate oxygen in the system. The pH of the reactor was maintained at 5.5 by adding either 1 N of H_2SO_4 or 1 N of NaOH. The black plastic sheet was tried around the reactor to prevent the light. The paddle was investigated to agitate the wastewater. The reactor operated as an anaerobic sequencing batch reactor (AnSBR). The hydraulic

retention time was kept for 1.0 day for the whole cycle of each batch, which were 15 minutes for fill, 23 hours for anaerobic react, 30 minutes for settle and 15 minutes for decant phases. In all cases the exchange volume was 2 L or 50% of the total liquid volume per cycle. The systems were fed with starch solution at the concentrations of 1 and 1.2 mg COD/L. The anaerobic sequencing batch experiment is presented in Figure 3.3.

Figure 3.3 Anaerobic sequencing batch experiment

3.4 Analytical Methods

The influent and effluent samples, biosolids and biogas samples were collected and analysed in order to maintain as well as evaluate the performance of biohydrogen process. The concentrations of glucose and volatile fatty acids (VFAs), biogas compositions and volume, and H_2 production were examined weekly. As soon as the system achieved the stationary stage, the amount of biomass is indirectly determined by SS and VSS. Total gas volume was collected by acidic water (pH=3) displacement method. Biogas composition and H_2 yield were determined using a gas chromatography, model Shimadzu: GC-8A with thermal conductivity detector (TCD). The VFA was determined by Shimadzu GC-14B with flame ionisation detector (FID). Glucose concentration was analysed by Nelson-Somogyi Method [116, 117]. The key parameters required to be examined as follows.

3.4.1 Hydrogen Gas Collection Procedure and Gas Analysis

During batch operating, the quantity and compositions of gas products were monitored at every interval. Hydrogen gas was withdrawn from the head space of the bottle by using a gas-tight syringe and concentration of hydrogen gas was determined by using a gas chromatography (GC) (Model 8A, SHIMADZU, JAPAN) equipped with a thermal conductivity detector (TCD). The temperatures of injector, detector and column were 110, 60 and 60 °C, respectively. Argon gas was applied as carrier gas with a flow rate of 30 mL/min and the head pressure was 22 pound per square inch (psi). The amount of total gas produced was accounted by water displacement method and the gas outlet pipe was submerged into a gas collection bottle that contained one litre of mixture solution of 2% (v/v) sulfuric acid and 10% (v/v) NaCl [118].

3.4.2 Volatile Fatty Acid (VFAs) Analysis

The concentration of acetic, butyric, propionic, valeric, iso-valeric, capoice and heptanoice acids were determined by gas chromatography (GC) (Model 14B, SHIMADZU, JAPAN) equipped with a flame ionisation detector (FID). The temperatures of injector, detector and column were 130, 180 and 200 °C, respectively. Helium, nitrogen, hydrogen and pure air were used as carrier gases at flow rates of 30, 50, 60 and 500 mL/min, respectively.

3.4.3 Analytical Parameters

The influent and effluent characteristics were determined routinely. The influent sample was collected before feeding, while the effluent sample was collected at the outlet. The biosolids represented by SS and VSS were collected when the system achieved the declined condition. The analytical parameters and their methods are presented in Table 3.3.

Table 3.3 Analytical methods for routine examination

Parameter	Method	Reference
sCOD(mg/L)	Closed reflux, Titrimetric method	[119]
SS (mg/L)	Method for determination of mix liquor suspended solid dried at 103-105 °C for 2 h.	[119]
VSS (mg/L)	Method for determination of mix liquor volatile suspended solid incinerated at 550°C for 30 min	[119]
pH	pH meter method	[119]
VFAs (mmole/L)	Gas chromatography (GC) (GC-14B, Shimadzu) flame ionization detector (FID)	[119]
Hydrogen gas	Gas chromatography (GC) (GC-8A,Shimadzu) thermal conductivity detector (TCD)	[119]
Glucose concentration (mg/L)	Nelson-Somogyi Method	[116, 117]

3.5 Data Analysis

The data governed from the experiment were fitted with modified Gompertz equation in order to determine the yield of H_2 and VFAs in the bioreactor. Monod's equation was introduced to estimate the influence of substrates on to hydrogen respiration process. The results and discussions are expressed in the next chapter.