# OPTIMISATION OF PHYSICAL PARAMETERS AND MICROBIAL COMMUNITY ANALYSIS OF BIO-HYDROGEN PRODUCTION FROM FOOD WASTE

By

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**Thesis Submitted to the School of Graduate Studies, Universiti Putra Malaysia, in Fulfillment of the Requirements for the Degree of Master of Science**

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**October 2011**

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The demand for clean energy from renewable resources stimulates biohydrogen production from biomass as an alternative fuel to replace fossil fuel. Biohydrogen from food waste fermentation initiates clean technologies for energy generation thus provide the solution for waste treatment. However, the production of biohydrogen is inhibited by hydrogen consuming bacteria and soluble metabolites. This inhibition effects can be overcome by optimizing the physical parameters during biohydrogen production. The objectives of this study were to establish the optimum operating parameters for biohydrogen production from food waste in batch fermentation and to identify the main hydrogen-producing bacteria at different controlled pH values. The batch fermentation was conducted using 150 mL serum bottles incubated in facultative anaerobic condition. Cooked and uncooked food waste taken from cafeterias with composition ratios of 2:1:1

carbohydrate, protein and fiber were used as a substrate in this study. The concentration of food waste was standardized at 25 g/L carbohydrate before all the experiments was conducted. Palm oil mill effluent (POME) sludge was used as a seed culture. Heat treatment was carried out to POME sludge at 80°C for 30 minutes to eliminate hydrogen consuming bacteria. Biohydrogen production was performed at different temperatures (35°C, 40°C, 50°C, 55°C and 60°C), initial pH values (5, 6, 7 and 8) and various ratios of sludge to substrate (10:90, 20:80, 30:70 and 40:60 % (v/v)). Biogas was collected every 2 h and the composition of hydrogen and carbon dioxide in biogas was analyzed by gas chromatography with no methane gas detected in all experiments. The highest biohydrogen yield obtained was 83 mmol $H_2$/L-medium/d for the experiment conducted at a temperature of 55°C, initial pH 7 and sludge to substrate ratio at 30:70 % (v/v). The experiment was then studied using different controlled pH values of 5.0, 5.5 and 6.0 at temperature of 55°C in 500 mL bioreactor. The results showed that pH 5.5 gave the highest biohydrogen production yield (79 mmol $H_2$/L-medium/d). Microbial cells number was determined by using fluorescent *in situ* hybridization (*FISH*) technique. The quantification analysis showed that the number of *Clostridium* sp. from cluster I and XI from samples after acclimatization was 2.9 x $10^8$ cells/mL while the number of *Clostridium* sp. from fermentation medium at pH 5.0, 5.5 and 6.0 were 3.6 x $10^8$, 7.8 x $10^8$ and 5.4 x $10^8$ cells/mL, respectively. *Clostridium* sp. from cluster I and XI were found to be dominant at pH 5.5 (92% out of the total bacteria) which corresponded to the highest biohydrogen yield compared to the other pH values. *Clostridium* sp. cluster I produce butyrate as the main metabolites while cluster XI criteria is heterogenous includes non-spore forming and thermotolerance alkaliphiles species. Methanogens were

not detected in the culture broth due to the heat treatment. Microbial profile at different pH was also investigated using denaturing gradient gel electrophoresis (DGGE). It was revealed that the DGGE bands belonged to uncultured *Bacteroidetes*, uncultured bacterium, *Caloramator australicus* sp. and *Clostridium* sp. Thus, controlled operating conditions were important to enhance hydrogen-producing bacterial growth for optimum biohydrogen production.

Abstrak tesis yang dikemukakan kepada Senat Unversiti Putra Malaysia sebagai memenuhi keperluan untuk Ijazah Master Sains

# PENGENALPASTIAN PARAMETER FIZIKAL OPTIMUM DAN MIKROORGANISMA DALAM PENGHASILAN BIO-HIDROGEN DARIPADA SISA MAKANAN


Oleh

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Permintaan yang banyak terhadap tenaga bersih daripada sumber boleh diperbaharui telah menggalakkan penghasilan gas biohidrogen sebagai alternatif kepada bahan bakar daripada bahan api fosil. Biohidrogen daripada fermentasi sisa makanan merupakan teknologi bersih untuk penjanaan tenaga seterusnya adalah penyelesaian untuk rawatan sisa. Walaubagaimanapun, penghasilan biohidrogen boleh terjejas dengan kehadiran bakteria yang tidak menghasilkan hidrogen dan metabolit. Efek penjejasasan ini boleh ditangani dengan mengoptimakan parameter fizikal semasa proses penghasilan biohidrogen. Tujuan kajian ini dijalankan adalah untuk menentukan parameter operasi optima untuk penghasilan biohidrogen daripada sisa makanan melalui fermentasi kelompok dan untuk mengenalpasti bakteria penghasil hidrogen yang utama pada pH

yang berbeza. Fermentasi kelompok telah dijalankan menggunakan 150 mL botol serum yang dieram dalam keadaan anaerobik. Sisa makanan yang telah dimasak dan tidak dimasak yang diambil dari kafeteria dengan nisbah komposisi 2:1:1 (karbohidrat, protein dan serat) digunakan sebagai substrat dalam kajian ini. Kepekatan sisa makanan dikawal pada 25 g/L karbohidrat sebelum digunakan dalam semua eksperimen. Enapcemar daripada efluen kilang kelapa sawit (POME) digunakan sebagai kultur benih. Enapcemar diberikan rawatan panas pada suhu 80°C selama 30 minit untuk membunuh bakteria yang mengganggu proses penghasilan hidrogen. Penghasilan biohidrogen dijalankan pada suhu (35°C, 40°C, 50°C, 55°C dan 60°C), pH permulaan (5, 6, 7 and 8) dan pelbagai nisbah enapcemar terhadap substrat (10:90, 20:80, 30:70 dan 40:60 % (v/v)) yang berbeza. Biogas dikumpulkan setiap 2 jam dan komposisi hidrogen dan karbon dioksida di dalam biogas dianalisis menggunakan kromatografi gas dengan tiada gas metana dikenalpasti di dalam semua eksperimen. Penghasilan biohidrogen tertinggi ialah 83 mmol $H_2$/L-media/hari untuk eksperimen yang dijalankan pada suhu 55°C, pH permulaan 7 dan nisbah enapcemar terhadap substrat 30:70% (v/v). Kajian seterusnya dijalankan di dalam 500 mL bioreaktor yang dikawal pada pH 5.0, 5.5 dan 6.0 dan suhu 55°C. Hasil kajian menunjukkan pH 5.5 memberi hasil biohidrogen terbanyak (79 mmol $H_2$/L-media/hari). Jumlah sel mikrob dikaji menggunakan hibridisasi *in situ* fluorescent (FISH). Analisis kuantitatif menunjukkan jumlah *Clostridium* sp. dari kluster I dan XI dari sampel selepas diaklimitasi ialah $2.9x10^8$ sel/mL manakala jumlah *Clostridium* sp. di dalam medium pada pH 5.0, 5.5 dan 6.0 masing-masing adalah $3.6x10^8$, $7.8x10^8$ and $5.4x10^8$ sel/mL. *Clostridium* sp. dari kluster I meghasilkan butirat sebagai metabolik utama sementara kriteria kluster XI adalah bercampur-campur termasuk bukan bakteria penghasil spora dan boleh hidup dalam suhu yang panas dan beralkali. *Clostridium* sp.

dari kluster I dan XI didapati dominan pada pH 5.5 (92% daripada jumlah keseluruhan bakteria) di mana berkadaran terus dengan penghasilan biohidrogen tertinggi jika dibandingkan dengan pH lain. Bakteria penghasil gas metana tidak didapati didalam kultur disebabkan oleh rawatan haba. Profil mikrob pada pH berbeza juga dikaji menggunakan elektroforesis gel kecerunan denaturasi (DGGE). Keputusan daripada analisis DGGE menunjukkan bakteria yang terdapat di dalam sampel adalah daripada jenis *Bacteroidetes* yang tidak boleh dikultur, *Calaromator australicus* sp. dan *Clostridium* sp. Dengan itu, kawalan keadaan semasa penghasilan biohidrogen sangat penting untuk membantu perkembangan bakteria penghasil hidrogen untuk penghasilan biohidrogen yang optima.

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## LIST OF ABBREVIATIONS

| | |
|---|---|
| ATP | Adenosine triphosphate |
| Biohydrogen | Biological hydrogen |
| BLAST | Basic local alignment search tool |
| BOD | Biological oxygen demand |
| $C_6H_{12}O_6$ | Glucose |
| $CH_4$ | Methane |
| CLSM | Confocal laser scanning microscope |
| $CO_2$ | Carbon dioxide |
| COD | Chemical oxygen demand |
| DGGE | Denaturing gradient gel electrophoresis |
| DNA | Deoxyribonucleic acid |
| EDTA | Ethylenediaminetetraacetic acid |
| EtOH | Ethanol |
| Fd | Oxidized ferrodoxin |
| $FdH_2$ | Reduced ferrodoxin |
| Fe-hydrogenase | Iron-hydrogenase enzyme |
| FISH | Fluorescent *in situ* hybridization |
| F/M | Food to microorganism |
| GC | Gas chromatography |
| $H_2$ | Hydrogen |
| $H_2SO_4$ | Sulphuric acid |
| HCl | Hydrochloric acid |

| | |
|---|---|
| $HNO_3$ | Nitric acid |
| HPLC | High performance liquid chromatography |
| MSW | Municipal solid waste |
| NmL | Normalize volume in mL |
| NADH | Nicotinamide adenine dinucleotide |
| $NaHCO_3$ | Sodium bicarbonate |
| NaOH | Sodium hydroxide |
| NCBI | National Center for Biotechnology Information |
| NmL | Normalized volume (mL) |
| $O_2$ | Oxygen |
| PCR | Polymerase chain reaction |
| POME | Palm oil mill effluent |
| RCM | Reinforced Clostridial Media |
| rRNA | Ribosomal ribonucleic acid |
| SDS | Sodium dodecyl sulfate |
| SD | Standard deviation |
| SE | Standard error |
| TC | Total carbohydrate |
| TS | Total solid |
| TSS | Total suspended solid |
| TVS | Total volatile solid |
| VSS | Volatile suspended solid |
| $CH_3CH_2OH$ | Ethanol |
| $CH_3COOH$ | Acetic acid |

| | |
|---|---|
| $CH_3CHOCOOH$ | Lactic acid |
| $CH_3(CH_2)_2COOH$ | Butyric acid |
| $CH_3CH_2COOH$ | Propionic acid |
| $CH_3COSCoA$ | Acetyl-CoA |
| $CH_3COCOOH$ | Pyruvate |

## LIST OF NOMENCLATURE

| | |
|---|---|
| $C_{H,i}$ | Fraction of hydrogen gas in the headspace of the bottle measured using gas chromatograph in the current (%/100) |
| $C_{H,i-1}$ | Fraction of hydrogen gas in the headspace of the bottle measured using gas chromatograph in the previous time interval (%/100) |
| $e$ | 2.718281828 |
| $H$ | Cumulative biohydrogen produced (mL) |
| $P$ | Biohydrogen production potential (mL) |
| $Rm$ | Rate of biohydrogen production (mL/h) |
| $V_{G,I}$ | Total biogas volume at current (mL) |
| $V_{G,i-1}$ | Total biogas volume at previous time interval (mL) |
| $V_H$ | The total volume of gas in the head space of reactor (mL) |
| $V_{H,i}$ | Cumulative biohydrogen production at current (mL) |
| $V_{H,i-1}$ | Cumulative biohydrogen production at previous time intervals (mL) |
| $\lambda$ | Lag phase (h) |
| $t$ | Fermentation time (h) |

# CHAPTER 1

# INTRODUCTION

## 1.1 Introduction

Biological hydrogen production (Biohydrogen) can be the alternative fuel of future energy system. Hydrogen was recognized as clean energy as the product of hydrogen combustion is only water instead of greenhouse gases (Zhang *et al.*, 2007). Various attempts have been carried out in order to use cheap and renewable carbon sources. Substrate such as food waste (Kim *et al.*, 2008; Pan *et al.*, 2008), palm oil mill effluent (Ismail *et al.*, 2010), wastewater (Yang *et al.*, 2007), rice slurry (Fang *et al.*, 2005) and tofu processing waste (Zheng *et al.*, 2010) have been studied to produce biohydrogen. The usage of carbon sources readily available in biomass for biohydrogen production will generate less economic burden for the future by turning food waste into energy source. At the same time, the attempt was to overcome the pollution problems created by food waste.

Biohydrogen can be produced through anaerobic degradation of complex substrate. Fermentative biohydrogen production is very complex process (Wang and Wan, 2009). Thus, controlled environmental conditions such as pH, temperature, low hydrogen partial pressure, substrate concentration, metal ions concentration (ferum and magnesium) and nutrient supply (nitrogen and phosphate) should be taken into

consideration to achieve high biohydrogen yield (Wang and Wan, 2009). The controlled conditions can affect rates of biohydrogen production, product formation (organic acids, solvent and biohydrogen), bacterial activities and growth rates, also may change metabolic pathways of hydrogenase enzyme (Pan *et al.*, 2008). Anaerobic fermentation for biohydrogen production has been studied by many researchers using single or mixed culture. Single culture such as *Clostridium* sp. (Chong *et al.*, 2009a), *Escherichia coli* (Bisaillon *et al.*, 2006), *Enterobacter auerogenes* (Jo *et al.*, 2008), and *Rhodobacter sphaeroidetes* (Zheng *et al.*, 2010) were proved for biohydrogen production. Biohydrogen production using single culture cause high risk of contamination, require sterile substrate, difficult to cultivate, and not applicable for large-scale operation system (Pan *et al.*, 2008). Fermentative biohydrogen production from mixed culture was simple and less tendency of contamination when compared to single culture. The effectiveness of biohydrogen production using mixed culture as a inoculum has been verified by several researches from anaerobic sludge (Pan *et al.*, 2008; Yusoff *et al.*, 2009), compost (Akutsu *et al.*, 2008; Lee *et al.*, 2008), sewage sludge (Kim *et al.*, 2008) and cattle manure sludge (Cheong *et al.*, 2006). Anaerobic fermentation using mixed culture was suitable for biohydrogen production as the substrate was utilized by microorganism with fast rate, technically simple to operate without requirement of light and oxygen supply (Chong *et al.*, 2009b; Valdez-Vazquez and Pooggi-Varaldo, 2009).

Different microorganism serves to produce biohydrogen during their metabolism for biohydrogen production from non sterile fermentation (Kim *et al.*, 2009). In this study, denaturing gradient gel electrophoresis (DGGE) was useful to explain the pattern of

microbial communities during biohydrogen production. DGGE results provide microbial communities information to be a useful indicator for biohydrogen production performance, also explain the degree of correlation between cooperation and competition of microorganism in certain niche (Muyzer *et al.*, 2008; Wu *et al.*, 2008). The quantification, enumeration, visualization and localization of microbial cells can be identified through fluorescent *in-situ* hybridization (FISH) technique (Davenport and Curtis, 2008; Tabatabaei *et al.*, 2009). FISH provides adequate quantification analysis from specific probes labeled with fluorescent dye targeting specific sequence under optimal hybridization condition. Both DGGE and FISH are becoming important for characterization of mixed microbial culture analysis.

## 1.2 Objectives

To date, information for biohydrogen production has been studied from different kind of substrate. In this study, food waste was used as a substrate for biohydrogen production. However, biohydrogen production is inhibited by hydrogen consuming bacteria and soluble metabolites. Thus, optimized physical parameters are critical to eliminate the inhibition effects to enhance high yield of biohydrogen. The characterization of microorganism present in mixed culture also important for better understanding of process performance during biohydrogen production. The objectives of this study are:

1. To study the effect of different temperature, initial pH and substrate composition on biohydrogen production from food waste in batch fermentation.

2. To analyzed microbial morphology and the number of bacterial count by fluorescent *in-situ* hybridization (FISH) and to understand microbial profile in the different stages during biohydrogen production by denaturing gradient gel electrophoresis (DGGE) technique at different controlled pH values.

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