



**UNIVERSITI PUTRA MALAYSIA**

**BIOHYDROGEN PRODUCTION FROM PALM OIL MILL EFFLUENT  
BY ANAEROBIC FERMENTATION**

**ATIF ABDELMONEIM AHMED YASSIN.**

**FK 2005 13**



**BIOHYDROGEN PRODUCTION FROM PALM OIL MILL EFFLUENT  
BY ANAEROBIC FERMENTATION**

**By**

**ATIF ABDELMONEIM AHMED YASSIN**

**Thesis Submitted to the School of Graduate Studies, Universiti Putra  
Malaysia, in Fulfilment of the Requirements for the Degree of Doctor of  
Philosophy**

**August 2005**



Dedicated to

My parents, wife, kids, brothers and sisters



Abstract of the thesis presented to the Senate of Universiti Putra Malaysia in fulfillment of the requirements for the degree of Doctor of Philosophy

**BIOHYDROGEN PRODUCTION FROM PALM OIL MILL EFFLUENT  
BY ANAEROBIC FERMENTATION**

By

**ATIF ABDELMONEIM AHMED YASSIN**

**August 2005**

**Chairman: Associate Professor Fakhru'l-Razi Ahmadun, PhD**

**Faculty: Engineering**

Biological hydrogen production was investigated using biomass in palm oil mill effluent (POME) and artificial wastewater containing 1% glucose, 0.2% yeast extract and 0.018% magnesium chloride hexahydrate under anaerobic fermentation in a batch process. Activated POME sludge and different types of composts were collected as sources of inocula for the study. The anaerobic microflora were found to produce significant amounts of hydrogen.

In the study with artificial media, 500 ml batch bioreactor was used. The experiments were carried out without pH control and at different temperatures. The maximum yield of 108.4 mmol-H<sub>2</sub>/L-med (2.01 mol-H<sub>2</sub>/mol-glucose) at the maximum evolution rate of 182 ml/(L-med hr) was obtained with Crest compost at 40°C.

Hydrogen production from POME was studied using a 5-L bioreactor optimal hydrogen production was observed at 60°C and a pH range of 5.5 to



6.0, the maximal hydrogen yields of 179 mmol/L-POME and 189 mmol/L-POME at evolution rates of 454 ml/(L-POME hr) and 421 ml/(L-POME hr) were obtained respectively.

Fed batch hydrogen production was conducted to study the reproducibility of microflora for hydrogen production from POME. Two liters of reaction medium was removed and 2 liters of fresh POME was added to the reaction medium every 24 hr (15 times) and the reproducibility of the fed batch process was checked by changing feeding time every 8 hr (10 times). A yield of 2382 ml-H<sub>2</sub>/ L-POME and 2419 ml-H<sub>2</sub>/ L-POME at maximum evolution rates of 313 ml-H<sub>2</sub>/(L-POME hr) and 436 ml-H<sub>2</sub>/(L-POME hr) were obtained respectively. Moreover, when the hydrogen production from POME using microflora was scaled-up to 10 L bioreactor, hydrogen yields of 140 mmol/L-POME and 96 mmol/L-POME at evolution rates of 361ml/(L-POME hr) and 188 ml/(L-POME hr) were obtained at pH of 5.5 and uncontrolled pH respectively.

Overall, hydrogen production was accompanied with the formation of acetate and butyrate. The experimental results showed that the gas composition contained hydrogen (66-68%) and carbon dioxide (32-34%). Throughout the study, methane gas was not observed in the evolved gas mixture. It was also found that the addition of nitrogen source in the medium caused a change in the hydrogen yield.

A simple model developed from Gompertz Equation was applied to estimate the hydrogen production potential ( $P$ ), hydrogen production rate ( $R_m$ ) and lag phase time ( $\lambda$ ), based on the cumulative hydrogen production curve. This study suggests that POME is suitable for biohydrogen synthesis without addition of any other nutrients. The finding of this study was highly reliable and showed that POME has potential for biological hydrogen production.

Abstrak tesis yang dikemukakan kepada Senat Universiti Putra Malaysia  
sebagai memenuhi syarat keperluan untuk ijazah Doktor Falsafah

**PENGHASILAN DARI EFFLUEN KILANG MINYAK KELAPA SAWIT  
MELALUI FERMENTASI ANAEROBIK**

Oleh

**ATIF ABDELMONEIM AHMED YASSIN**

Ogos 2005

**Pengerusi : Profesor Madya Fakru'l-Razi Ahmadun, PhD**

**Fakulti : Kejuruteraan**

Penghasilan hidrogen secara biologi dikaji dengan menggunakan biojisim dalam sisa kilang kelapa sawit (POME) dan airtsisa buatan yang mengandungi 1% glukosa, 0.2% pati yis dan 0.018% heksahidrat magnesium klorida di bawah penapaian anaerobik dalam proses berkelompok telah dikaji. Enapan POME yang diaktifkan dan berbagai-bagai jenis kompos dikumpul sebagai sumber inokula bagi kajian. Didapati mikroflora anaerobik tersebut mengeluarkan jumlah hidrogen yang banyak.

Dalam kajian dengan media tiruan, bioreaktor 500 ml telah digunakan. Eksperimen-eksperimen tersebut telah dijalankan tanpa mengawal pH dan pada suhu-suhu yang berbeza. Hasil maksimum 108.4 mmol-H<sub>2</sub>/L-med (2.01 mol-H<sub>2</sub>/mol-glukosa) pada kadar evolusi maksima 182 ml/(L-med jam) diperolehi dengan kompos Crest pada 40°C.



Penghasilan hidrogen dari POME dikaji mengguna bioreackor 5-L; penghasilan hidrogen optimum diperhati pada 60°C dan pH 5.5 hingga 6.0, penghasilan hidrogen maksimum sebanyak 179 mmol/L-POME dan 189 mmol/L-POME pada kadar evolusi 454 ml/(L-POME jam) dan 421 ml/(L-POME jam) masing-masing diperolehi.

Penghasilan hidrogen secara suapan berkelompok dijalankan untuk mengkaji penghasilan semula mikroflora bagi penghasilan hidrogen dari POME. Dua (2) L dari bahantara reaksi dikeluarkan dan 2 liter POME segar ditambah pada bahantara reaksi setiap 24 jam (15 kali) dan penghasilan semula proses suapan berkelompok tersebut diperiksa dengan mengubah masa menyuap setiap 8 jam (10 kali). Penghasilan 2382 ml-H<sub>2</sub>/L-POME dan 2419 ml-H<sub>2</sub>/L-POME pada kadar evolusi maksimum 313 ml-H<sub>2</sub>/(L-POME jam) dan 436 ml-H<sub>2</sub>/(L-POME jam) masing-masing didapati. Tambahan pula, setelah penghasilan hidrogen dari POME mengguna mikroflora dikembangkan ke bioreaktor 10 L, hasil hidrogen 140 mmol/L-POME dan 96 mmol/L-POME pada kadar evolusi 361 ml/(L-POME jam) dan 188 ml/(L-POME jam) didapati pada pH 5.5 dan pH tidak terkawal masing-masing.

Secara keseluruhan, penghasilan hidrogen diiringi dengan penghasilan asetat dan butirat. Keputusan eksperimen menunjukkan yang komposisi gas mengandungi hidrogen (66-68%) dan karbon dioksida (32-34%). Sepanjang kajian, gas metana tidak hadir dalam campuran gas yang terhasil.



Penambahan sumber nitrogen ke dalam bahantara juga menyebabkan perubahan dalam penghasilan hidrogen.

Sebuah model ringkas yang dikembangkan dari persamaan Gompertz diaplikasikan untuk menganggar potensi penghasilan hidrogen ( $P$ ), kadar penghasilan hidrogen ( $R_m$ ) dan masa bagi fasa ekor ( $\lambda$ ), berdasarkan kepada lengkung tokokan penghasilan hidrogen. Penyelidikan ini mencadangkan yang POME adalah sesuai bagi sintesis biohidrogen tanpa penambahan nutrien lain. Hasil pencarian kajian ini boleh dipercayai dan menunjukkan yang POME mempunyai potensi bagi penghasilan hidrogen secara biologi.

## ACKNOWLEDGEMENTS

First of all, I would like to thank our almighty God for shedding on me good health and keeping my brain working to the extent of completing this research, which I hope will contribute to the welfare of my nation.

Next, many thanks to my kind supervisor Associate Professor Dr. Fakhru'l-Razi Ahmadun, especially, I would like to sincerely thank him for his patience and understanding during the prolonged period of my study. What is indisputably true is that, it would have been quite impossible for me to have continued this study without his support, limitless assistance and beneficial advice. Thanks and appreciations are also extended to other members of the supervisory committee, Dr. Ir. Ma Ah Ngan and Associate Professor Dr. Sunny Iyuke.

My deep gratitude goes to the late Professor Masayoshi Morimoto, who initiated the idea of this project and whose moral support, and contributions remained immeasurable. I am also grateful to Professor Shigeharu Tanisho of the, Department of Environmental Science, Yokohama National University, for giving me continuous support and useful comments throughout the period of this study.

I wish to gratefully acknowledge Yang Berbahagia Tan Sri Datuk Dr. Yusof Basiron, the Director General of Malaysian Palm Oil Board (MPOB) for the



permission to conduct the research at the MPOB, and for his encouragement. My appreciation also goes to all the staff of the Chemistry and Technology Division in general, and the research staff in the effluent laboratory in particular, especially, Wan Fazlina Bint Zainal Alauddin and Lan Mee Ling. Working with them had been a great experience, despite the hectic hours I had to go through.

I would like to thank all individuals in the Department of Chemical and Environmental Engineering, Faculty of Engineering, Universiti Putra Malaysia.

My gratitude is also extended to my wife Gowaria Dafa Alla, my son Almontassir-Billah, and daughter Reem for their love, understanding, patience and support.

Last, but not the least, sincere appreciation goes to my parents, brothers and sisters, relatives and closed friends for their moral encouragement, patience and understanding throughout my study.

This work was conducted under the financial support of New Energy and Industrial Technology Development Organization (NEDO) of Japan, and Universiti Putra Malaysia.



## TABLE OF CONTENTS

	Page
DEDICATION	ii
ABSTRACT	iii
ABSTRAK	vi
ACKNOWLEDGEMENTS	ix
APPROVAL	xi
DECLARATION	xiii
LIST OF TABLES	xvi
LIST OF FIGURES	xviii
LIST OF ABBREVIATIONS	xxi
<b>CHAPTER</b>	
<b>1 INTRODUCTION</b>	<b>1.1</b>
1.1 Background	1.1
1.2 Problem statement	1.4
1.3 Objectives	1.6
1.4 Scope of the study	1.7
1.5 Significance of the study	1.8
<b>2 REVIEW OF LITERATURE</b>	<b>2.1</b>
2.1 Introduction	2.1
2.2 Anaerobic digestion process	2.1
2.3 Hydrogen energy system	2.4
2.3.1 Definition of Biohydrogen	2.7
2.3.2 Hydrogen production methods	2.7
2.3.3 Biological hydrogen production processes	2.9
2.3.4 Fermentative hydrogen production	2.10
2.3.5 Rate of biohydrogen synthesis	2.22
2.3.6 Hydrogen kinetics	2.22
2.3.7 Energy analysis	2.25
2.3.8 Purification of hydrogen	2.27
2.3.9 Hydrogen storage methods	2.28
2.3.10 Advantages and disadvantages of hydrogen energy	2.29
2.3.11 Biohydrogen: future directions	2.30
2.4 Palm oil mill effluent	2.33
2.4.1 Source of palm oil mill effluent	2.34
2.4.2 Characteristics of palm oil mill effluent	2.34
2.4.3 Microorganism in palm oil mill effluent	2.37
2.4.4 Treatment technology for palm oil mill effluent	2.39
2.5 Crest compost	2.42
2.6 Modified Gompertz Equation	2.43

3	MATERIALS AND METHODS	3.1
3.1	Introduction	3.1
3.2	Substrate	3.1
3.3	Seed microorganisms	3.2
3.4	Experimental design	3.2
3.5	Microflora and cultivation/hydrogen production	3.4
3.5.1	Screening of hydrogen-producing bacteria	3.4
3.5.2	Hydrogen production from palm oil mill effluent using 10 L fermentor	3.7
3.5.3	Fed batch hydrogen production from POME using 10 L bioreactor	3.8
3.6	Analytical Methods	3.9
3.6.1	Collection of evolved gas	3.9
3.6.2	Determination of gas composition	3.10
3.6.3	Determination of lower fatty acids	3.12
3.6.4	Parameters determination of cumulative hydrogen production curve	3.13
3.6.5	Determination of glucose concentration	3.14
3.6.6	Chemical oxygen demand (COD)	3.15
4	RESULTS AND DISCUSSIONS	4.1
4.1	Introduction	4.1
4.2	Experimental results	4.1
4.2.1	Screening of hydrogen-producing bacteria source	4.1
4.2.2	Hydrogen production from artificial media	4.10
4.2.3	Hydrogen Production from palm oil mill effluent using POME Sludge	4.27
4.3	Mathematical modeling and simulation results	4.48
4.3.1	Simulation results	4.48
4.3.2	Correlation of estimated parameters	4.52
5	CONCLUSIONS AND RECOMMENDATIONS	5.1
5.1	Introduction	5.1
5.2	Conclusions	5.1
5.3	Recommendations	5.2
	REFERENCES	R.1
	APPENDICES	A.1
	BIODATA OF THE AUTHOR	B.1

## LIST OF TABLES

Table	Page
2.1 Microorganisms used for hydrogen generation	2.11
2.2 Merits and demerits of different biological processes for hydrogen production	2.13
2.3 Comparison of the rate of hydrogen synthesis by different technologies	2.23
2.4 Comparison of energy and emissions of combustible fuels	2.26
2.5 Unit cost of energy obtained by different processes	2.27
2.6 Characteristics of palm oil mill effluent	2.36
2.7 Isolated aerotolerant bacteria from the digester	2.38
2.8 Bacteria in the aeration lagoon	2.39
2.9 Characteristics of palm oil mill effluent and its respective standard discharge limit set by the Malaysian Department of the Environment	2.40
2.10 Treatment technology for palm oil mill effluent	2.41
2.11 Some growth models used in the literature	2.44
4.1 Screening results of anaerobic microflora in the activated POME sludge for the production of hydrogen	4.4
4.2 Production of hydrogen by anaerobic microflora in the different types of compost	4.5
4.3 Production of hydrogen by different types of anaerobic microflora utilizing palm oil mill effluent	4.6
4.4 Production of biogas using disposable 50 ml syringe by anaerobic microflora in POME sludge at 60°C utilizing palm oil mill effluent	4.8
4.5 Production of biogas using disposable 50 ml syringe by anaerobic microflora in Crest compost at 60°C utilizing palm oil mill effluent	4.9



4.6	Fermentation products produced from glucose by anaerobic microflora in batch culture at 50°C and 60°C	4.12
4.7	Fermentation products produced from glucose using anaerobic microflora in batch culture at 40°C	4.18
4.8	Comparisons between hydrogen production at thermophilic and mesophilic temperature using microflora in Crest compost	4.23
4.9	Comparison of hydrogen yield with the literature	4.26
4.10	Effect of the culture condition on the hydrogen production from palm oil mill effluent using microflora in POME sludge	4.29
4.11	Hydrogen production from palm oil mill effluent by microflora in POME sludge using a 10-L bioreactor at 60°C	4.40
4.12	Amount of hydrogen produced from palm oil mill effluent at 60°C and pH 5.5	4.44
4.13	Kinetic parameter values of hydrogen production from palm oil mill effluent using POME sludge at 60°C and different pH values	4.49
4.14	Kinetic parameter values of hydrogen production from glucose	4.50
4.15	Comparison of the kinetics parameters of the hydrogen production obtained with those cited in the literature	4.51

## LIST OF FIGURES

Figure	Page
2.1 Process flow diagram of a conventional palm oil mill	2.35
3.1 The experimental design	3.3
3.2 Hydrogen production method using 500 ml fermentor. a) 500 ml fermentor, b) measuring cylinder, c) Acidic water, d) plastic container, e) wood box include gas collection bag, f) controller unit, g) acid and base	3.5
3.3 Syringe method for hydrogen production. a) Taking POME into The syringe, b) Inoculation, c) Attachment of silicone tube, d) Mixing of inoculum by pressing the tube and shaking, e) Pushing bubbles and air out of the syringe, f) Holding the tube with the syringe with a rubber band, g) Keeping at desired temperature, h) Fermentation and measurement of gas volume, i) Testing for the presence of hydrogen	3.6
3.4 Schematic diagram of 10 L bioreactor for batch hydrogen production. a) 10 L fermentor, b) sample tube, c) medium, d) stirrer, e) pH electrode, f) temperature sensor, g) condenser, h) silicone tube, i) glass tube, j) gas sampler, k) 20 L glass tank, l) rubber, m) evolved biogas, n) gas bubbles, o) acidic water, p) tape, q) displaced water, r) acid pump, s) base pump, t) acid container, u) base container, v) controller unit	3.8
3.5 Gas detector tube system (Kitagawa)	3.10
4.1 Time course of palm oil mill effluent fermentation pattern during cultivation of anaerobic microflora in Crest compost at 60°C using the syringe method	4.10
4.2 Time course of glucose fermentation pattern during cultivation of anaerobic microflora in: (A) sludge compost; (B) Crest compost at (□) 50°C and (O) 60°C	4.14
4.3 Cumulative hydrogen production from glucose by anaerobic microflora at 40°C and uncontrolled pH	4.17
4.4 The cumulative hydrogen production from glucose, during the cultivation of anaerobic microflora in Crest compost at 40°C and uncontrolled pH	4.19



4.5	Comparison of the fermentation pattern from glucose at $\square$ 50°C, $\circ$ 60°C and $\Delta$ 40°C	4.22
4.6	Effect of nitrogen source on hydrogen production by anaerobic microflora in Crest compost at 60°C	4.25
4.7	The fermentation pattern of palm oil mill effluent using microflora in POME sludge at 60°C and at different pH values	4.28
4.8	Comparison between mesophilic (40°C) and thermophilic (60°C) hydrogen production from palm oil mill effluent using microflora in POME sludge at pH 5.5	4.31
4.9	Comparisons between hydrogen yield and evolution rate from palm oil mill effluent using microflora in POME sludge at uncontrolled pH and controlled pH 5.5, at 60°C	4.33
4.10	Comparison between hydrogen production yields from palm oil mill effluent by microflora using 5 L and 10 L reactors at uncontrolled pH and 60°C	4.41
4.11	Comparison between hydrogen production yields from palm oil mill effluent by microflora using 5 L and 10 L reactors at pH 5.5 and 60°C	4.42
4.12	Cumulative hydrogen production from palm oil mill effluent at 60°C and pH 5.5	4.43
4.13	Typical fermentation pattern during the first fed batch process at 60°C and pH 5.5, feeding time of 24 hr	4.45
4.14	Typical fermentation pattern during the second fed batch process at 60°C and pH 5.5, feeding time of 8 hr	4.46
4.15	Comparison between the first and second fed batch process for hydrogen production, at 60°C and pH 5.5	4.47
4.16	Predicted vs. experimental hydrogen production from palm oil mill effluent using microflora in POME sludge at 60°C (predicted using Equation 2.27)	4.52
4.17	Predicted vs. experimental hydrogen production from glucose using microflora in POME sludge, sludge compost, and Crest compost at uncontrolled pH (predicted using Equation 2.27)	4.53

- 4.18 Residuals distribution with time, hydrogen production from palm oil mill effluent using microflora in POME sludge at 60°C (predicted using Equation 2.27) 4.54
- 4.19 Residuals versus predicted hydrogen production from palm oil mill effluent using microflora in POME sludge at 60°C (predicted using Equation 2.27) 4.54
- 4.20 Residuals distribution with time, hydrogen production from glucose using microflora in POME sludge, sludge compost, and Crest compost at uncontrolled pH (predicted using Equation 2.27) 4.55
- 4.21 Residuals versus predicted hydrogen production from glucose using microflora in POME sludge, sludge compost, and Crest compost at uncontrolled pH (predicted using Equation 2.27) 4.55
- 4.22 Cumulative hydrogen production curve from palm oil mill effluent using microflora in POME sludge at various pH and 60°C. Graph symbols show the experimental data. The lines are the nonlinear estimation results according to Equation 2.27 4.56

## ABBREVIATIONS

<i>A</i>	Asymptotic phase
COD	Chemical oxygen demand
cfu	Colony forming unit
BOD	Biological oxygen demand
<i>e</i>	2.718281828
FID	Flame ionization detector
GC	Gas chromatography
<i>H</i>	Cumulative biogas (hydrogen) production (ml)
HRT	Hydraulic retention time
$K_i$	Inhibition constant (g/l)
$k_s$	Saturation constant (g/l)
<i>N</i>	Number of organism
NADH	Nicotinamide adenine dinucleotide
<i>P</i>	Biogas (hydrogen) production potential (ml)
POME	Palm oil mill effluent
p- test	Probability distribution
$r_g$	Bacterial growth rate ( $h^{-1}$ )
$R_m$	Maximum biogas (hydrogen) production rate (ml/hr)
$r_m$	Biogas production rate (ml/hr)
$r_{su}$	Substrate utilization rate (mg COD/hr)
$R^2$	Correlation coefficient
<i>S</i>	substrate concentration (g/l)

$t$	Incubation time (hr)
$t$ -test	Student's test
TCD	Thermal conductivity detector
UV	Ultraviolet
$y$	Population size of bacteria at incubation time $t$
$Y_1$	Maximum yield coefficient (1/mg COD)
$Y_2$	<i>Maximum yield coefficient (mg COD/ml)</i>
$\lambda$	Lag phase time (hr)
$\mu$	Specific growth rate ( $\text{h}^{-1}$ )
$\mu_{max}$	Maximum growth rate (1/hr)
$\nu$	Specific hydrogen production rate
$\alpha$	Growth associated coefficient (dimensionless)
$\beta$	Non-growth associated coefficient ( $\text{h}^{-1}$ )

# CHAPTER 1

## INTRODUCTION

### 1.1 Background

The world population is expanding and so is the demand for non-renewable energy resources such as coal and oil. Ultimately this has been reflected in rising levels of energy consumption at both percapita and aggregate levels, at a global scale. However, most of energy consumption accrues to developed countries in North America, Europe and Japan (Pearce and Warford, 1993). Prospect of depletion of non-renewable resources has been a hot controversy since the early 1970s. The publication of the limits to growth, a highly celebrated contribution of its time marked the initiation. Hence after the debate over the limits to be imposed on economic growth as a result of ever dwindling stocks of non-renewable resources, such state of affairs has strongly brought into focus the issue of emphasizing the role of renewable resources as a principal pillar upon which sustainable development rests. This particularly applies to the crucial arena of energy production and consumption.

Within such circumstances, the interest in hydrogen production from biomass has been renewed and revitalized, particularly in Japan, Germany



and to some extent in the United States and Canada (Lay, 2001). Hydrogen is renewed as a clean, renewable, efficient energy source.

Hydrogen is mainly produced from fossil fuels, biomass and water. Currently, hydrogen is produced almost exclusively by electrolysis of water or by steam reformation of methane. Biological hydrogen production using wastewater and biomass as input has been gaining importance and attracting attention; the processes are mostly operated at ambient temperature and pressure (Das and Veziroglu, 2001). Thus, it is less energy intensive as compared to thermo-chemical and electrochemical process, and not only environmentally friendly (green house effect) but also leading to open a new avenue for the utilization of renewable energy resources, which are inexhaustible (Benemann, 1997; Greenbaum, 1990; Sasikala *et al.*, 1993; Miyamoto *et al.*, 1989; Tanisho *et al.*, 1983). The environmental friendliness of the process derived from its cleanness has been a major source for the increasing recognition for biomass-based production of hydrogen. On the other hand, its independence of fossil fuels has given a clear advantage both on cost effectiveness and environmental quality promotion grounds. In addition, the process can use various waste materials, which facilitates waste recycling.

Hydrogen production by microorganisms falls into two main categories: First, by means of photosynthetic processes involving organisms cultured under anaerobic light conditions. Second, *via* fermentation utilizing