



UNIVERSITI PUTRA MALAYSIA

**OPTIMIZATION OF BIOHYDROGEN PRODUCTION FROM PALM OIL
MILL EFFLUENT BY NATURAL MICROFLORA**

**ZATILFARIHIAH BINTI RASDI
FBSB 2009 32**



**OPTIMIZATION OF BIOHYDROGEN PRODUCTION FROM PALM OIL
MILL EFFLUENT BY NATURAL MICROFLORA**

By

ZATILFARIHIAH BINTI RASDI

**Thesis submitted to the School of Graduate Studies, Universiti Putra Malaysia,
in Fulfilment of the Requirements for the Degree of Master Science
(Environmental Biotechnology)**

November 2009



Abstract of thesis presented to the Senate of Universiti Putra Malaysia in
fulfilment of the requirement for the degree of Masters of Science

**OPTIMIZATION OF BIOHYDROGEN PRODUCTION FROM PALM OIL
MILL EFFLUENT BY NATURAL MICROFLORA**

By

ZATILFARIHIAH BINTI RASDI

November 2009

Chairman : Dr. Nor'Aini Abdul Rahman

Faculty : Faculty Biotechnology and Biomolecular Sciences

Biohydrogen is a promising clean fuel as it is ultimately derived from renewable energy sources. It is environmental friendly since it burns to water, gives high energy yield, and can be produced by less energy-intensive processes. Anaerobic treatment of palm oil mill effluent (POME) was chosen to produce biohydrogen as POME is a commercially known waste that is such a burden to the industry and the environment.

In this study, POME sludge was used as an inoculum to produce biohydrogen from POME. Heat-treated POME sludge acclimatized with POME incubated at 37°C for 24 h was used as a seed culture. Preliminary screening on the effects of inocula size, heat treatment, substrate concentration and pH of incubation by using a factorial design (FD) were conducted under mesophilic condition (37°C) using a serum vial (160 mL). The



experimental results from two-level FD showed that pH and chemical oxygen demand (COD) of POME as substrate concentration significantly affected biohydrogen production.

Optimizations of the specific hydrogen production (P_s) and the hydrogen production rate (R_m) were carried out by using a central composite design (CCD). The maximum P_s of 270 mL H_2 /g carbohydrate and R_m of 98 mL H_2 /h were obtained under optimum conditions of pH 5.86 and substrate concentration of 80 g/L. The optimized conditions obtained were subjected to a confirmation run and it showed a reproducible data with P_s of 282 mL H_2 /g carbohydrate and R_m of 137 mL H_2 /h.

For the second part of experiment, 2-L of bioreactor was employed for the production of biohydrogen with and without pH control. The optimum conditions obtained in the serum vial were applied in the bioreactor. The results obtained for uncontrolled pH experiment generated 1.3 L biogas/L medium. Throughout the fermentation, no methane-gas was detected. The biohydrogen yield (P_s) was approximately 1 L H_2 /L medium, with hydrogen production rate (R_m) at 112 mL H_2 /h. For the controlled pH experiment, pH was controlled manually every 3 h at 5.86. The biogas generated from the fermentation was 2.5 L/L medium, which is almost 2-fold of biogas production from uncontrolled pH experiment. The P_s and R_m generated were 1.3 L H_2 /L medium and 144 mL H_2 /h, respectively.

Abstrak tesis yang dikemukakan kepada Senat Universiti Putra Malaysia
sebagai memenuhi keperluan untuk Ijazah Master Sains Bioteknologi
Alam Sekitar

**PENGOPTIMUMAN PENGHASILAN BIOHIDROGEN DARIPADA SISA
KILANG MINYAK SAWIT OLEH MIKROFLORA SEMULAJADI**

Oleh

ZATILFARIIHAH BINTI RASDI

November 2009

Pengerusi : Dr. Nor'Aini Abdul Rahman

Fakulti : Fakulti Bioteknologi and dan Sains Biomolekul

Biohidrogen adalah salah satu bahan api yang diterbitkan daripada sumber tenaga yang diperbaharui, di mana tidak mencemarkan alam sekitar memandangkan pembakarannya menghasilkan air serta memberikan kadar hasil yang bertenaga tinggi, dan boleh dihasilkan tanpa memerlukan tenaga tambahan. Rawatan anaerobik ke atas sisa kelapa sawit dipilih untuk menghasilkan biohidrogen kerana sisa tersebut secara komersialnya dikenali sebagai sisa yang memberi beban kepada industri. Dalam pembelajaran ini, 'sludge' digunakan sebagai inokulum untuk penghasilan biohidrogen daripada sisa minyak kelapa sawit. 'Sludge' yang telah dipanaskan, kemudian diberi penyesuaian bersama sisa minyak kelapa sawit dan disimpan dalam 37°C selama 24 jam, digunakan sebagai inokulum. Penyaringan awal terhadap kesan saiz inokulum, rawatan pemanasan,

kepekatan substrat dan pH menggunakan “factorial design” (FD) telah dilakukan di bawah keadaan mesofilik (37°C), di dalam botol serum (160 mL). keputusan eksperimen daripada FD menunjukkan kepekatan substrat dan pH memberikan kesan yang ketara terhadap penghasilan biohidrogen.

Pengoptimuman penghasilan biohidrogen dan kadar penghasilan telah dijalankan menggunakan “central composite design” (CCD). Penghasilan biohidrogen tertinggi dianggarkan 270 mL H₂/g karbohidrat dengan kadar 98 mL H₂/j, pada keadaan optimum iaitu pH 5.86 dan kepekatan substrat 80 g/L. Eksperimen penentuan dijalankan pada keadaan optimum untuk memastikan keputusan yang diperoleh boleh digunakan. Berdasarkan keputusan yang terhasil menunjukkan data ini boleh diterima apabila biohidrogen yang terhasil adalah 282 mL H₂/g karbohidrat pada kadar 137 mL H₂/j, lebih tinggi berbanding yang dianggarkan.

Bagi eksperimen kedua, bioreaktor 2-L digunakan dalam penghasilan biohidrogen menerusi pengawalan pH dan tanpa pH. Keadaan optimum yang diperoleh digunakan di dalam eksperimen ini. Sepanjang eksperimen, tiada penghasilan gas metana dikenalpasti. Berdasarkan kepada keputusan, biogas terhasil adalah 1.3 L/L media tanpa kawalan pH. Penghasilan biohidrogen menghampiri 1 L H₂/L media, dengan kadar penghasilan pada 112 mL H₂/j. bagi eksperimen dengan kawalan pH, pH dikawal secara manual setiap 3 j pada 5.86. Biogas yang terhasil adalah 2 kali ganda tinggi berbanding biogas daripada eksperimen tanpa kawalan pH. Penghasilan biohidrogen pula adalah 1.3 L H₂/L media pada kadar 144 mL H₂/j.

ACKNOWLEDGEMENTS

IN THE NAME OF ALLAH, MOST GRACIOUS AND MERCIFUL.

Alhamdulillah, thanks to Allah because I have finished my master project entitled “Optimization of biohydrogen production from Palm Oil Mill Effluent by natural microflora”. I would like to express my appreciation to my most respectful supervisory committee, Dr. Nor’Aini Abdul Rahman (chairman) and Prof. Dr. Mohd Ali Hassan for their guidance, encouragement, concern and support.

Special thanks to all members in bioprocess laboratory for their advice and criticism throughout this study especially to Environmental Biotechnology group. It has been wonderful experiences working with them.

To biohydrogen group, special thanks to all of their constructive advice and assistance throughout finishing this project. It has been a joyous, experience and wonderful experiences working together.

My heartfelt gratitude and love to my husband, Syazree Safwan for his support, encouragement and patience. For my children, Daniel Rasdi and Darwisy Ikhwan, who enlighten my life, thanks for understand me throughout the period. Most of all, I would like to acknowledge and thanks to my beloved parents, Mr. Rasdi and Mrs. Anis Zubaidah, and family members for their support, guidance and endless love. Also, special thanks to my mom-in-law, Mrs Noor Bishah and families for their understanding and encouragement.



I certify that a **Thesis Examination Committee** has met on 3rd November 2009 to conduct the final examination of Zatilfariyah binti Rasdi on her thesis entitled "**Optimization of Biohydrogen Production from Palm Oil Mill Effluent by Natural Microflora**" in accordance with **the Universities and University Colleges Act 1971 and the Constitution of the Universiti Putra Malaysia [P.U.(A) 106] 15 March 1998**. The Committee recommends that the student be awarded the Master Science (Environmental Biotechnology).

Members of the **Thesis** Examination Committee were as follows:

Mohd. Noor Abd. Wahab, PhD

Associate Professor
Faculty of Biotechnology and Biomolecular Sciences
Universiti Putra Malaysia
(Chairman)

Rosfarizan Mohamad, PhD

Associate Professor
Faculty of Biotechnology and Biomolecular Sciences
Universiti Putra Malaysia
(Internal Examiner)

Norhafizah Hj Abdullah, PhD

Associate Professor
Faculty of Engineering
Universiti Putra Malaysia
(Internal Examiner)

Jamaliah Md. Jahim, PhD

Associate Professor
Department of Chemical & Process Engineering
Faculty of Engineering & Built Environment
Universiti Kebangsaan Malaysia
Malaysia
(External Examiner)

BUJANG BIN KIM HUAT, PhD

Professor and Deputy Dean
School of Graduate Studies
Universiti Putra Malaysia

Date:



This thesis was submitted to the Senate of University Putra Malaysia and has been accepted as fulfillment of the requirement for the Degree of Master Science (Environmental Biotechnology). The members of the Supervisory Committee were as follows:

Nor ‘Aini Abd Rahman, PhD

Faculty of Biotechnology and Molecular Sciences
Universiti Putra Malaysia
(Chairman)

Ali Hassan, PhD

Professor
Faculty of Biotechnology and Molecular Sciences
Universiti Putra Malaysia
Malaysia
(Member)

HASANAH MOHD GHAZALI, PhD

Professor and Dean
School of Graduate Studies
Universiti Putra Malaysia

Date: 11 February 2010



DECLARATION

I hereby declare that the thesis is based on my original work except for quotations and citations, which I have been duly acknowledged. I also declare that it has not been previously or concurrently submitted for any other degree at UPM or other institutions.

ZATILFARIHIAH RASDI

Date:

TABLE OF CONTENTS

DEDICATION	
ABSTRACT	ii-iii
ABSTRAK	iv-v
ACKNOWLEDGEMENT	vi
APPROVAL SHEETS	vii-viii
DECLARATION	ix
LIST OF TABLES	xii
LIST OF FIGURES	xiii
ABBREVIATIONS	xiv
CHAPTER	
1 INTRODUCTION	1-5
2 LITERATURE REVIEW	
2.1 Hydrogen Energy System	
2.1.1 Definition of hydrogen	6
2.1.2 Hydrogen production methods	7
2.1.3 Biological hydrogen production methods	8-10
2.1.3.1 Fermentative Hydrogen Production	10-12
2.1.4 Advantages of hydrogen energy	12-13
2.1.5 Microorganisms involved in hydrogen production	14-16
2.1.6 Bioreactor related to biohydrogen production	17-18
2.1.7 Factors affecting hydrogen production	18-20
2.2 Anaerobic digestion process	
2.2.1 Theory	22-24
2.2.2 Advantages and disadvantages of anaerobic treatment	25-26
2.3 Palm Oil Mill Effluent (POME)	
2.3.1 Source of POME	26-27
2.3.2 Characteristics of POME	27-28
2.3.3 Treatment technology of POME	29-32
2.3.4 Factors influencing POME treatment	32-35
2.3.5 POME as reusable products	35-38
2.4 Kinetic analysis	
2.4.1 Modified Gompertz Equation	39-41
2.4.2 Hydrogen gas production	41
2.5 Response surface methodology (RSM)	
2.5.1 Theory	42-45
2.5.2 Previous work on optimization of hydrogen production	46-48
2.5.3 Advantages and disadvantages of RSM	48-49
2.6 Fermentation mode	50-52
2.6.1 Comparison between different fermentation mode	52-54



3	GENERAL MATERIALS AND METHODS	
3.1	POME	55
3.2	Inoculum preservation	55
3.3	Inoculum preparation	56
3.4	Analytical methods	
3.4.1	Organic acids determination	57
3.4.2	Chemical Oxygen Demand (COD)	58
3.4.3	Total solids (TS)	59
3.4.4	Suspended solids (SS)	59-60
3.4.5	Volatile suspended solids (VSS)	60-61
3.4.6	Gas analysis	61-62
3.4.7	Data analysis	62-63
3.4.8	Total sugar determination	63
3.4.9	Optimization on biohydrogen production	64-66
3.4.10	Reactor operation and monitoring	69-70
4	OPTIMIZATION AND PRODUCTION OF BIOHYDROGEN PRODUCTION USING POME	
4.1	Introduction	71-75
4.2	Results and discussion	
4.2.1	Evaluation of significant variables using two-level factorial design	75-79
4.2.2	Central composite design	80-102
4.2.3	Bioreactor performance	102-108
4.3	Conclusion	109-110
5	SUMMARY, CONCLUSION AND SUGGESTION FOR FUTURE WORK	111-114
6	PAPER PUBLICATION	115
7	BIODATA OF STUDENT	116-117
	REFERENCES	118-130
	APPENDICES	131-136



LIST OF TABLES

Table	Page
1: Comparison of energy and emissions of combustible fuels	13
2: Characteristics of POME and its concentration	28
3: Variables in actual values for factorial design	65
4: Two-level factorial design	66
5: Response of two-level factorial design	78
6: ANOVA for P_s for two-level factorial design	79
7: ANOVA for R_m for two-level factorial design	79
8: Coded and actual value for CCD	81
9: CCD with responses	82
10: ANOVA for P_s	88
11: ANOVA for R_m	89
12: Summary of optimum conditions	90
13: Comparison of optimal conditions with literature	95
14: Data of total volatile fatty acids production	101
15: Data of profile for biohydrogen production	102
16: Estimated parameters of modified Gompertz equation	104
17: Comparison of biohydrogen production with literature	109

LIST OF FIGURES

Figure	Page
1(a): Response surface plot and corresponding contour plot for P_s	91
1(b): Response surface plot and corresponding contour plot for R_m	92
2: Overlay plot of both contour for P_s and R_m	93
3: The time-course profile and characteristics of hydrogen production	99-100
4: The COD removal of hydrogen production under optimized conditions	101
5: The cumulative production of biohydrogen with and without pH controlled	105
6: Metabolism of biohydrogen production under pH controlled	107
7: Metabolism of biohydrogen production at uncontrolled pH	107

ABBREVIATIONS

AD	Anaerobic digestion
POME	Palm Oil Mill Effluent
CCD	Central Composite Design
COD	Chemical Oxygen Demand
CPO	Crude palm oil
FD	Factorial Design
HCl	Hydrochloric Acid
NaOH	Sodium Hydroxide
EGSB	Expanded granular sludge bed
UASFF	Up-flow anaerobic sludge fixed film
RSM	Response Surface Methodology
P_s	Specific hydrogen production potential (yield)
R_m	Hydrogen production rate
VFAs	Volatile fatty acids
P	Hydrogen production potential
g	Gram
g/L	Gram per liter
L	Liter
mL	mililiter
mg	miligram

CHAPTER 1

1.0 INTRODUCTION

In the tropical region, particularly in Malaysia and Indonesia, oil palm (*Elaeis guineensis*) is one of the most versatile crops. There are about 1.5 m³ water are used to process one tonne of fresh fruit bunches (FFB), and half of this quantity would end up as waste; palm oil mill effluent (POME). POME poses a great threat to the environment because of its highly biological and chemical oxygen demands (Zhang *et al.*, 2008).

The raw or partially treated POME has an extremely high content of degradable organic matter, which is due in part to the presence of unrecovered palm oil. This highly polluting wastewater can therefore cause severe pollution of waterways due to oxygen depletion and other related effects. Currently, there are about 265 active palm oil mills in Malaysia with a combined annual crude palm oil (CPO) production capacity of about 13 million tonnes (Zinatizadeh *et al.*, 2007). Thus, an efficient and practical approach is an urgent need to preserve the environment while maintaining the economy.

There are several techniques to treat POME. There are also many methods to control POME pollution including crop irrigation, flotation, adsorption, ultrafiltration and various biodegradation process (Zhang *et al.*, 2008). The most widely used in the



treatment of POME is biological methods which include anaerobic, facultative and aerobic degradation compared to physical and chemical treatments. But, because of too low nutrient content in POME, an anaerobic treatment process is sufficient rather than aerobic treatment process (Atif *et al.*, 2005).

As the reserves of oil and gas are being depleted, energy is one of the most important factors to global prosperity (Sivaramakrishna *et al.*, 2009). Therefore, the security of energy supply has raised the demand towards the establishment of hydrogen economy. Sustainable hydrogen energy seems to be a logical conclusion to numerous environmental problems like acid rain, green house gases and overcoming the local and transboundary pollutants (Maddy *et al.*, 2003). Logan (2005) also agreed with the above statement that hydrogen-based fuel cells offer great promise for non-polluting energy production. The technology to turn hydrogen into electricity already exists, and is forming the basis of a new, global shift to a “hydrogen-based” fuel economy. A move towards hydrogen is motivated by international environmental concerns and diminishing petroleum reserves.

To date, the majority of research on hydrogen production has focused on using organic wastes and wastewater as substrates (O-Thong *et al.*, 2007). POME is relatively resistant to biodegradation but clearly has a potential as a substrate for generation of hydrogen.

There are several studies have been conducted using POME to produce hydrogen (Atif *et al.*, 2005; O-Thong *et al.*, 2007; Zinatizadeh *et al.*, 2007; Chong *et al.*, 2009). Atif *et al.* (2005) produced hydrogen at 60°C, while Chong *et al.* (2009) produced hydrogen at 37°C using single strain, *Clostridium butyricum* EB6. For O-Thong *et al.* (2007), they studied the hydrogen production with nutrient supplementation at thermophilic condition. It is different for Zinatizadeh *et al.* (2007) when they did a comparative study for hydrogen production in an up-flow anaerobic sludge fixed film bioreactor (UASFF). The natural microflora are also used in various wastewater treatment process because they can adapt to various compounds in the wastewater and because no sterilization process is needed (Atif *et al.*, 2005).

However, there still have challenges towards production of hydrogen. Many factors that fall under the rubrique of bioprocess parameters have been studied and of course, particular experimental conditions are dictated by the goal of the study. Furthermore, the used of mixed culture, with unknown organism, have to manipulate the metabolic pathways through bioprocess parameters. Thus, it is difficult to compare one study with another because of mixed culture is very dependent upon inoculum source and history (Patrick, 2009).

The challenge towards bioreactor studies is when there is a gas over-saturation in liquid phase which induces formation of bubbles. Thus, this will lead to inhibition of hydrogen production. The important thing should be considered is to have a good control of the dissolved gas concentration and keep it as low as possible (Hussy *et al.*, 2005)

This project was grant by government where four institutions involved; UPM, UKM, UM and SIRIM. Our project leader was informed to conduct the research in mesophilic conditions. Currently, our research group has done and still pursuing research on anaerobic treatment of POME for biohydrogen production by a single culture namely *C. butyricum* EB6 (Chong *et al.*, 2009). They did optimization on the production of biohydrogen using isolated culture. The significant production was achieved with 3.2 L/L POME. Thus, this gave us the motivation to extent the research as we found that sludge can be used as seed culture for the production. This study is similar with Atif *et al.* (2005) in terms of substrate and inoculum used which were POME and POME sludge. The differences between these studies was temperature used for the fermentation, Atif *et al.* (2005) used 60°C while this study performed at 37°C.

For the present study, an experiment has been conducted to produce biohydrogen using natural microflora from POME sludge. An optimization experiment was designed based on the Response Surface Methodology (RSM). By optimization, the different parameters

such as substrate concentration, pH, inoculum size and different temperature for heat treatment can be tested and studied in order to obtain optimum operations. The justification of factors chosen for optimization based on current problems involved during the hydrogen production. The factors were discussed further in Chapter 2.

The objectives of this study are:

1. To optimize biohydrogen production from POME using RSM by natural microflora.
2. To produce biohydrogen in a bioreactor at pH 5.8 and COD of POME at 80 g/L.

CHAPTER 2

2.0 LITERATURE REVIEW

2.1 Hydrogen Energy System

2.1.1 Hydrogen

Hydrogen is an excellent energy option; a clean renewable energy alternative with no emissions of greenhouse gases, which helps in addressing the challenge of global climate change. Hydrogen is considered to be the most promising fuel for the future and its world consumption is rapidly growing year by year. Therefore, demand on hydrogen production has increased considerably in recent years. Ust'ak *et al.* (2007) have expected that in the coming years, the annual biohydrogen yearly increment should reach about 10%. This fuel is the only one that does not produce (during its combustion) any harmful substances or greenhouse gases- its only product of combustion is water (steam).



2.1.2 Hydrogen Production Methods

Production of hydrogen is one of the vital components in hydrogen energy platform (Wu and Chang, 2007). The production of hydrogen can be divided into physical/chemical methods and biological methods. Physical and chemical methods cannot be considered as an alternative, non-polluting energy source since the traditional non-renewable fossil fuels are used to produce the hydrogen gas.

Previous researchers stated that the hydrogen might be produced by a number of processes, including electrolysis of water, thermocatalytic reformation of hydrogen-rich organic compounds and biological processes (Levin *et al.* 2004; Atif *et al.*, 2005; Vijayaraghavan and Ahmad, 2006). Currently, hydrogen is produced, almost exclusively, by electrolysis of water or by steam reformation of methane. The production of hydrogen is highly depends on the process condition such as pH, hydraulic retention time (HRT) and gas partial pressure (Levin *et al.*, 2004).

2.1.3 Biological Hydrogen Production

Among the various technologies for hydrogen production, a biological approach has received special attention recently because organic waste, water and gases are relatively cheap and plentiful (Kapdan and Kargi, 2006). Biological hydrogen production may be either by fermentation or photosynthesis process (Sivamarakrishna *et al.*, 2009). It is the most challenging area of biotechnology with respect to environmental problems. It depends not only on research advances, which is only concentrate on the improvement in efficiency through genetically engineering microorganisms and/or the development of bioreactors, but also on economic considerations (the cost of fossil fuels), social acceptance, and the development of hydrogen energy systems (Vadakke, 2003).

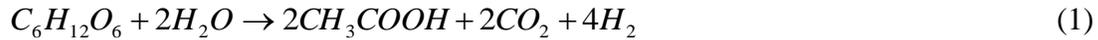
Biological production of hydrogen provides a feasible means for the sustainable supply of hydrogen with low pollution and high efficiency, thereby being considered a promising way of producing hydrogen (Das and Veziroglu, 2001; Levin *et al.*, 2004). Biological production of hydrogen (biohydrogen), using microorganisms is found to be an exciting new area of technology development that offers the potential production of usable hydrogen from a variety of renewable resources.

Wu and Chang (2007) have reviewed that there are various approaches of biological system that can be used to generate hydrogen. Direct photolysis, indirect biophotolysis, photo fermentation and dark fermentation are the example of biological hydrogen production processes.

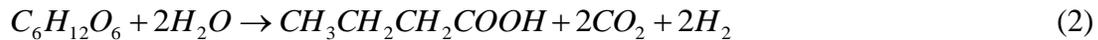
Hydrogen is a key compound in the metabolism of many anaerobic, as well as a few aerobic microorganisms (Patrick, 2009). Many organisms have the capacity to use this energy-rich molecule to drive energy generation.

Of the variety of possible substrates, practical hydrogen fermentations are restricted to carbohydrate-rich materials. There are two pathways to produce hydrogen which are butyrate pathway or acetate pathway. The cleavage of pyruvate to acetyl-CoA, CO₂ and H₂ is catalyzed by pyruvate: ferredoxin oxidoreductase (PFOR). Through this pathway, a part of the electrons is transferred to protons to produce H₂ and the other to NAD⁺ to generate NADH₂. NADH₂ is then used to produce H₂ in the second pathways which involve hydrogenase where electron will be transferred to ferredoxin then to H⁺.

Glucose (or in principle its isomer hexoses or its polymers starch and cellulose) in biomass gives a maximum yield of 4 mol H₂ per glucose when acetic acid is the by-product.



However, half of this yield per glucose is obtained with butyrate as the fermentation end product.



These equations (Eq. 1 and 2) give understanding of biochemical pathways of hydrogen production with different by-products. Usually mixtures of products are produced by Clostridia and the available hydrogen from glucose is determined by butyrate/acetate ratio. The process conditions have a significant effect on H₂ yield, as they influence the fermentation end products (Hawkes *et al.*, 2002). Fermentations of hexose to acetate or butyrate produce H₂ and CO₂. Fermentations to propionate or lactate produce no H₂. It is important to establish bacterial metabolism resulting in acetate and butyrate as end products.

2.1.3.1 Fermentative Hydrogen Production

Fermentative is one of biological methods. Fermentative hydrogen production can be achieved by dark fermentation (with obligate or facultative anaerobes) or by photo fermentation (with photoheterotrophic bacteria) (Das and Veziroglu, 2001; Levin *et al.*,

