

ISOLATION AND CHARACTERIZATION OF HYDROGEN PRODUCING  
BACTERIA FROM PALM OIL MILL EFFLUENT (POME)

MARYAM GHANBARIAN

A dissertation submitted in partial fulfillment of the  
requirements for the award of degree of  
Master of Science (Biotechnology)

Faculty of Biosciences and Medical Engineering  
Universiti Teknologi Malaysia

JANUARY 2013

## DEDICATION

*I would like to dedicate this thesis to special people in my life:*

*To my mother and father who always give me energy and without their encouragement and support I could not achieve this degree.*

*To Ali my amazing husband for her wonderful love, patience and sacrifice during this difficult stage of our life.*

*To my brother, Ali and my sister, Mahsa for their inspiration and love.*

*And finally, a special dedication goes to my in laws who have provided me all I need and have given me financial and moral support.*

## **ACKNOWLEDGEMENT**

Firstly, I would like to thank my supervisor, Dr. Mohd Firdaus Abdul Wahab, for his guidance and support throughout this study. Without his support, this dissertation would not have been possible. I appreciate all my lecturers and the staff of Faculty of Biosciences and Bioengineering, University Technology Malaysia.

I would like to thank all my classmates, who kindly helped me in my study. Especially Judit, Ali, Maryam, Arman, Karim, and Kiandokht who provided me with all information that I needed for my research. The learning experience with all of you will always be in my memory.

## ABSTRACT

Hydrogen gas and its usage in electricity generation and transportation is attracting wider interests nowadays. This is because it possesses a high-energy yields ( $122 \text{ kJ g}^{-1}$ ), it is a renewable energy source, and does not contribute to the greenhouse effect. In Malaysia, 15.2 million tons of wastewater is generated annually by the palm oil industry. The wastewater is known as Palm Oil Mill Effluent (POME). This study is focused on the isolation and characterization of hydrogen-producing bacteria from POME. Samples were taken from the sludge and raw waste of palm oil processing effluent. Bacterial isolation was performed to isolate facultative anaerobes and strict anaerobes. The isolated colonies of bacteria were identified and characterized by microbiological analysis and biochemical tests (catalase, indole, nitrate reduction, urea, TSI and citrate test). From TSI test, it was found that one facultative anaerobe (out of 13 colonies altogether) produced gas; and all strict anaerobes produced gas (8 colonies altogether). The gas-producing colonies were then cultivated at  $37^{\circ}\text{C}$  for 24 hours in a synthetic media simulating starch wastewater for screening of hydrogen production. Hydrogen gas production was then analyzed using RGA (Residual Gas Analyzer). Results show that the facultative anaerobe colony did not produce hydrogen gas by starch fermentation; and four colonies obtained via strict anaerobe isolation show hydrogen production. These colonies can be the subject of future studies to optimize hydrogen production from waste materials.

## ABSTRAK

Gas hidrogen dan penggunaannya dalam penjaanaan elektrik dan pengangkutan menarik lebih luas kepentingan pada masa kini. Ini adalah kerana ia mempunyai hasil yang tinggi tenaga ( $122 \text{ kJ g}^{-1}$ ), ia adalah satu sumber tenaga boleh diperbaharui, dan tidak menyumbang kepada kesan rumah hijau. Di Malaysia, 15200000 tan air sisa yang dijana setiap tahun oleh industri minyak sawit. Air sisa yang dikenali sebagai Kilang Minyak Sawit Efluen (POME). Kajian ini memberi tumpuan kepada pengasingan dan pencirian bakteria menghasilkan hidrogen dari POME. Sampel telah diambil daripada enapcemar dan sisa mentah pemprosesan minyak sawit efluen. Pengasingan bakteria telah dilakukan untuk mengasingkan fakultatif anaerobes dan anaerobes ketat. Jajahan terpencil bakteria telah dikenal pasti dan dicirikan oleh analisis mikrobiologi dan ujian biokimia (katalase, indole, pengurangan nitrat, urea, TSI dan ujian sitrat). Dari ujian TSI, ia telah mendapati bahawa satu fakultatif anaerobe (daripada 13 jajahan sama sekali) yang dihasilkan gas dan semua anaerobes ketat yang dihasilkan gas (8 jajahan sama sekali). Negara-negara jajahan gas menghasilkan kemudian ditanam pada  $37^\circ \text{C}$  selama 24 jam dalam media sintetik simulasi air sisa kanji untuk pemeriksaan pengeluaran hidrogen. Pengeluaran gas Hidrogen telah dianalisis menggunakan RGA (Gas Analyzer Residual). Keputusan menunjukkan bahawa koloni fakultatif anaerobe tidak menghasilkan gas hidrogen oleh penapaian kanji; dan empat jajahan yang diperolehi melalui pengasingan anaerobe ketat menunjukkan pengeluaran hidrogen. Tanah jajahan ini boleh menjadi subjek kajian masa depan untuk mengoptimumkan pengeluaran hidrogen daripada bahan-bahan buangan.