UNIVERSITI PUTRA MALAYSIA

BIOHYDROGEN PRODUCTION DURING GROWTH OF AN INDIGENOUS BACTERIUM, OCHROBACTRUM PUTRANENSIS EB2, IN PALM OIL MILL EFFLUENT

CHEONG WENG CHUNG

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By

CHEONG WENG CHUNG

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

October 2006

Specially dedicated to,

My beloved parents who brought me to this world,

my brothers who gave me the encouragement and laughter,

and friends for their invaluable advice and morale support.

Sharon, thank you for being understanding throughout my study

and loving me……..

Abstract of thesis presented to the Senate of Universiti Putra Malaysia in fulfilment of the requirement for the Degree of Doctor of Philosophy

BIOHYDROGEN PRODUCTION DURING GROWTH OF AN INDIGENOUS BACTERIUM, *OCHROBACTRUM PUTRANENSIS* EB2, IN PALM OIL MILL EFFLUENT

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October 2006

Chairman: **Prof. Mohd. Ali Hassan, PhD**

Faculty: **Biotechnology and Biomolecular Sciences**

The study was on the production of hydrogen from palm oil mill effluent using indigenous bacteria. Three potential bacteria (WL1, EB2, and DT1) were isolated from wetland soil, termite's gut and anaerobic digester tank, respectively. All three isolates were Gram-positive, spore-forming, facultative anaerobe rods and exhibited motility by means of peritrichous flagella. All of the isolates were short straight rods, appeared in-pairs, clumped-together and formed biofilm on the agar surface. Using the 16s rRNA identification method, EB2 belonged to the *Brucellaceae* family and is closely related to *Ochrobactrum sp*., thus, designated as *Ochrobactrum putranensis* EB2; while WL1 and DT1 isolates were taxonomically positioned as *Bacillus cereus.* These bacteria differed by 30-35% in the 16s rRNA identification method from the existing bacteria recorded in the gene library. Since EB2 is different from other bacteria in the database, it is regarded as a new bacteria. In this study, the EB2 bacteria was used throughout the study to enhance the production of hydrogen.

The study on the optimization of hydrogen production was done on the *Ochrobactrum putranensis* EB2 and the parameters chosen were pH 4, 5 and 6; at

temperatures 30°C, 37°C and 45°C. Comparisons were done with VFA and hydrogen production as well as COD reduction. The *Ochrobactrum putranensis* EB2 was able to grow steadily in POME and produced hydrogen at the pH of 5.5 and temperature 37°C. The highest μ_{max} obtained was 0.384 h^{-1}. An average of 1300 mL of accumulated hydrogen volume was obtained within 72 hours of batch fermentation from 1 L of POME in the optimization study.

Production of hydrogen from palm oil mill effluent (POME) by *Ochrobactrum putranensis* EB2 was investigated in 2.0 L bioreactor with working volume of 1.0 L at 37°C and pH of 5.5 ± 0.3. The optimized conditions were applied in the hydrogen production study. The average hydrogen volume accumulated in the system was 1100 mL with 58.0% COD removal. Repeated experiments done on the batch fermentation confirmed the reproducibility of the results. Continuous fermentation were started after 72 hours of batch fermentation when there was no hydrogen production.

During continuous fermentation, hydrogen gas production was at 34.2 mL H_2/L per day for HRT 0.5 day, 13.0 mL H_2/L per day for HRT 1.0 day and 12.6 mL H_2/L per day for HRT 1.5 days and these show the HRT-dependent characteristics of hydrogen production. The efficiency of *Ochrobactrum putranensis* EB2 to convert the POME into hydrogen gas was about 31.67 mL H_2/g COD and was the highest recorded in batch fermentation reducing to 8 mL H_2/g COD at the end of the batch fermentation. The highest productivity obtained in the continuous fermentation was 1.07 mol H_2/mol glucose, was good in comparison with those reported earlier, 1.00-2.36 mol H_2/mol glucose. The high productivity was excellent as most of the COD

was converted to gas, based on the theoretical yield of 4 mol-H_2/mol-glucose. As such, HRT 0.5 day gave the best result compared to other HRT done in the study.

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

PENGHASILAN BIOHIDROGEN SEMASA PERTUMBUHAN BAKTERIA, *OCHROBACTRUM PUTRANENSIS* EB2, DALAM EFLUEN KILANG SAWIT

Oleh

CHEONG WENG CHUNG

Oktober 2006

Pengerusi: **Prof. Mohd. Ali Hassan, PhD**

Fakulti: **Bioteknologi dan Sains Biomolekul**

Kajian ini melibatkan penggunaan bakteria tempatan dalam penghasilan gas hidrogen menggunakan efluen kilang sawit (POME). Tiga bakteria yang berpotensi menghasilkan gas hidrogen, WL1, EB2 dan DT1, telah disaringkan daripada tanah di kawasan paya, perut anai-anai dan tangki anaerobik sistem rawatan efluen kilang sawit. Ketiga-tiganya merupakan bakteria Gram-positif, mempunyai keupayaan membentuk spora, berbentuk rod, fakultatif anaerobik, keupayaan bergerak dengan flagella dan membentuk biofilem di atas permukaan agar. Dengan menggunakan kaedah pengenalpastian 16s rRNA, EB2 digolongkan dalam keluarga *Brucellaceae* dan hampir serupa dengan spesis *Ochrobactrum*. Dengan itu, bakteria itu dinamakan *Ochrobactrum putranensis EB2*. Manakala, WL dan DT1 digolongkan secara taksonomi sebagai *Bacillus cereus*. Ketiga-tiga bakteria adalah berbeza daripada bakteria-bakteria yang dilaporkan dalam perpustakaan gen sebanyak 30–35% dalam proses pengenalpastian bakteria dengan kaedah 16s rRNA. Disebabkan kelainan bakteria EB2 daripada bakteria yang sedia ada,

ia dianggap sebagai bakteria yang baru untuk dikaji. Dengan itu, *Ochrobactrum putranensis* EB2 digunakan dalam kajian seterusnya.

Penyelidikan untuk mengoptimumkan penghasilan hidrogen dilakukan ke atas *Ochrobactrum putranensis* EB2 dengan menggunakan nilai-nilai parameter (pH 4, 5 and 6; suhu 30°C, 37°C and 45°C). Perbandingan dilakukan dengan penghasilan VFA dan hidrogen bersama penurunan COD dalam media. pH 5.5 dan suhu 37°C merupakan parameter yang optimum untuk penghasilan hidrogen dan *Ochrobactrum putranensis* EB2 dapat membiak di dalam POME. Nilai μ_{max} yang paling tinggi diperolehi dalam kajian ini adalah 0.384 h^{-1}. Secara purata sebanyak 1300 mL gas hidrogen telah dapat dikumpulkan dalam jangkamasa 72 jam selepas fermentasi dengan 1 L POME.

Penghasilan hidrogen daripada efluen kilang sawit (POME) menggunakan *Ochrobactrum putraensis* EB2 dalam 2.0 L bioreactor (1.0 L POME dengan suhu 37°C dan pH 5.5 ± 0.3) dikaji dengan cara fermentasi sesekelompok. Eksperimen fermentasi sesekelompok dijalankan dengan menggunakan parameter yang optimum untuk penghasilan hydrogen dan didapati tiada perubahan ketara. Sebanyak 1100 mL hidrogen dapat dikumpulkan dan penurunan kepekatan COD 58.0% dicatatkan. Eksperimen yang dibuat berulang kali menunjukkan keputusan yang serupa. Fermentasi selanjar dilakukan selepas 72 jam fermentasi sesekelompok setelah tiada penghasilan hidrogen dikesan.

Semasa fermentasi selanjar, penghasilan gas hidrogen ialah 34.2 mL H_2/L sehari untuk HRT 0.5 hari, 13.0 mL H_2/L sehari untuk HRT 1.0 hari dan 12.6 mL H_2/L sehari untuk HRT 1.5 hari. Ini menunjukkan penghasilan gas hidrogen menggunakan bakteria adalah bergantung kepada faktor HRT. Tahap kecekapan tertinggi dalam fermentasi sesekelompok *Ochrobactrum putranensis* EB2 untuk menukarkan POME kepada gas hidrogen ialah 67 mL H_2/g COD tersingkir, dan menurun kepada 8 mL H_2/g COD di akhir fermentasi dalam fermentasi selanjar. Produktiviti tertinggi ialah 1.07 mol H_2/mol glukos sehari adalah baik berbanding nilai yang dilaporkan oleh penyelidik yang lain pada 1.00-2.36 mol H_2/mol glukos. Produktiviti yang tinggi ini adalah nilai yang menunjukkan kebanyakan COD telah ditukarkan kepada gas. HRT 0.5 hari memberikan nilai yang terbaik berbanding keputusan HRT yang lain dalam kajian ini. HRT 0.5 hari memberikan nilai yang terbaik berbanding keputusan HRT yang lain dalam kajian ini.

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I certify that an Examination Committee met on 30th October 2006 to conduct the final examination of Cheong Weng Chung on his Doctor of Philosophy thesis entitled "Biohydrogen Production During Growth Of An Indigenous Bacterium, *Ochrobactrum Putranensis* Eb2, in Palm Oil Mill Effluent" in accordance with Universiti Pertanian Malaysia (Higher Degree) Act 1980 and Universiti Pertanian Malaysia (Higher Degree) Regulation 1981. The Committee recommends that the candidate be awarded the relevant degree. Members of the examination Committee are follows:

Arbakariya Ariff, PhD
Professor
Faculty of Graduate Studies
Universiti Putra Malaysia
(Chairman)

Mohd Arif Syed, PhD
Professor
Faculty of Biotechnology and Molecular Sciences
Universiti Putra Malaysia
(Internal Examiner)

Fakhrul Razi-Ahmadun, PhD
Assoc Prof,
Faculty of Engineering
Universiti Putra Malaysia
(Internal Examiner)

Abdul Jalil Abdul Kader, PhD
Professor
Faculty of Science and Technology
Universiti Kebangsaan Malaysia
Putra Malaysia
(External Examiner)

HASANAH MOHD. GHAZALI, PhD
Professor/Deputy Dean
School of Graduate Studies
Universiti Putra Malaysia

Date: 22 MARCH 2007

This thesis submitted to the Senate of Universiti Putra Malaysia and has been accepted as fulfilment of the requirement for the degree of Doctor of Philosophy. The members of the Supervisory Committee are as follows:

Mohd Ali Hassan, PhD
Professor
Faculty of Biotechnology and Biomolecular Sciences
Universiti Putra Malaysia
(Chairman)

Mohamed Ismail Abdul Karim, PhD
Professor
Faculty of Engineering
International Islamic University Malaysia (IIUM)
(Member)

Suraini Abdul Aziz, PhD
Associate Professor
Faculty of Biotechnology and Biomolecular Sciences
Universiti Putra Malaysia
(Member)

Vikineswary Sabaratnam, PhD
Professor
Faculty of Science
Universiti Malaya
(Member)

Yoshihito Shirai, PhD
Professor
Kyushu Institute of Technology (KIT)
Kitakyushu, Japan
(Member)

_____
AINI DERIS, PhD
Professor / Dean
School of Graduate Studies
Universiti Putra Malaysia

Date: 12 APRIL 2007

DECLARATION

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

_____

Name : **CHEONG WENG CHUNG**

Date : 27 FEBRUARY 2007

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