UNIVERSITI PUTRA MALAYSIA

BIOHYDROGEN PRODUCTION FROM PALM OIL MILL EFFLUENT BY ANAEROBIC FERMENTATION

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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

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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_2/L-med (2.01 mol-H_2/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_2/ L-POME and 2419 ml-H_2/ L-POME at maximum evolution rates of 313 ml-H_2/(L-POME hr) and 436 ml-H_2/(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 (λ), 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 mengguna biojisim dalam sisa kilang kelapa sawit (POME) dan airsisa 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_2/L-med (2.01 mol-H_2/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_2/L-POME dan 2419 ml-H_2/L-POME pada kadar evolusi maksimum 313 ml-H_2/(L-POME jam) dan 436 ml-H_2/(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 ekoran (λ), 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.

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ABBREVIATIONS

A	Asymptotic phase
COD	Chemical oxygen demand
cfu	Colony forming unit
BOD	Biological oxygen demand
e	2.718281828
FID	Flame ionization detector
GC	Gas chromatography
H	Cumulative biogas (hydrogen) production (ml)
HRT	Hydraulic retention time
K_i	Inhibition constant (g/l)
$ks,$	Saturation constant (g/l)
N	Number of organism
NADH	Nicotinamide adenine dinucleotide
P	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
S	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	*Maximum yield coefficient (mg COD/ml)*
λ	Lag phase time (hr)
μ	Specific growth rate (h^{-1})
μ_{max}	Maximum growth rate (1/hr)
ν	Specific hydrogen production rate
α	Growth associated coefficient (dimensionless)
β	Non-growth associated coefficient (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