



UNIVERSITI PUTRA MALAYSIA

**UTILISATION OF ORGANIC ACIDS FROM ANAEROBICALLY
TREATED PALM OIL MILL EFFLUENT WITH AND WITHOUT SLUDGE
RECYCLE FOR POLYHYDROXYALKANOATE PRODUCTION**

SIM KEAN HONG

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By

SIM KEAN HONG

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

April 2003



SPECIALLY DEDICATED TO:

**MY BELOVED PARENTS, BROTHER, SISTER FOR THEIR
SACRIFICES AND INVALUABLE LOVE,**

**TO MY GRANDFATHER, GRANDMOTHER & RELATIVES
WHO ALWAYS SUPPORT ME,**

**TO MY MOM'S DAD WHO PASSED AWAY ON 11 JAN
2002, THANK YOU FOR EVERYTHING YOU GAVE TO ME,**

**AND FINALLY TO PUI LING WHO ALWAYS BE WITH
ME THROUGHOUT MY STUDY, I LOVE YOU ALWAYS.**



Abstract of thesis presented to the Senate of Universiti Putra Malaysia on fulfilment of the requirements for the degree of Master of Science

UTILISATION OF ORGANIC ACIDS FROM ANAEROBICALLY TREATED PALM OIL MILL EFFLUENT WITH AND WITHOUT SLUDGE RECYCLE FOR BIOPLASTIC PRODUCTION

By

SIM KEAN HONG

April 2003

Chairman: Professor Dr. Mohd. Ali Hassan

Faculty: Food Science And Biotechnology

Two-stage fermentation was carried out in this study where palm oil mill effluent (POME) was used as substrate for volatile fatty acids (VFA) production by continuous anaerobic treatment using a 50 L continuous stirred tank reactor (CSTR). The VFA obtained were then used for polyhydroxyalkanoate (PHA) production by *Ralstonia eutropha* ATCC 17699.

Three experiments were conducted in anaerobic treatment of POME until steady state was achieved, i.e. (1) pH 6.5, 30°C, 80 rpm, sludge/POME ratio 1:1, hydraulic retention time (HRT) 4 days, without sludge recycle and POME was fed continuously; (2) pH 6.5, 30°C, 100 rpm, sludge/POME ratio 1:1, HRT 4 days, without sludge recycle and POME was fed manually three times a day; (3) pH 5.5, 30°C, 150 rpm, sludge/POME ratio 3:7, HRT 4 days, SRT 8.2 days with partially sludge recycled (500 mL) and POME was fed manually three times a day. The major acids produced were acetic, propionic and butyric acids. The highest amount of VFA obtained were 15.36 g/L in 2nd experiment, followed by 1st experiment with 15.04

g/L and finally 10.23 g/L in 3rd experiment. For 2nd and 3rd experiments, COD removal was low which values at 12.5% and 31.1% while BOD yield were 58.3% and 43.4% respectively.

The treated POME obtained was mixed with concentrated H₂SO₄ and underwent recovery and purification process by evaporation. A clarified concentrated VFA comprised of 44.6 g/L acetic, 20.1 g/L propionic and 22.5 g/L butyric acids were obtained with recovery yield of 76%. The clarified concentrated VFA obtained were used for PHA accumulation by *Ralstonia eutropha* ATCC 17699 using pH-stat fed-batch fermentation under nitrogen limitation. PHA content over 90% with highest PHA concentration of 11.44 g/L and CDW of 12.5 g/L were able to be achieved using this system. In this study, PHA production was not inhibited by the high concentration of nitrogen when butyric acid was the preferred acid consumed by *Ralstonia eutropha*.

Abstrak thesis yang dikemukakan kepada Senat Universiti Putra Malaysia sebagai memenuhi keperluan untuk ijazah Master Sains

**PENGGUNAAN ASID ORGANIC PEROLEHI DARIPADA SISA KILANG
KELAPA SAWIT TERAWAT SECARA PENYAHUDARAAN DENGAN DAN
TANPA PENGEMBALIAN ENAPCEMAR UNTUK PENGHASILAN
PLASTIK BOLEH URAI**

Oleh

SIM KEAN HONG

April 2003

Pengerusi: Profesor Dr. Mohd. Ali Hassan

Fakulti: Sains Makanan Dan Bioteknologi

Dua peringkat fermentasi dijalankan dalam kajian ini di mana sisa kilang kelapa sawit (POME) digunakan sebagai substrak untuk penghasilan asid organik (VFA) melalui rawatan tanpa oksigen secara selanjut dengan menggunakan 50 liter bioreaktor (CSTR). VFA yang diperolehi kemudian digunakan untuk penghasilan polyhydroksialkanoate (PHA) oleh *Ralstonia eutropha* ATCC 17699.

Tiga eksperimen telah dijalankan dalam rawatan POME tanpa oksigen sehingga keadaan mantap tercapai, (1) pH 6.5, 30°C, 80 rpm, nisbah enapcemar/POME 1:1, hydraulic retention time (HRT) 4 hari, tanpa pengembalian enapcemar dan POME disuap secara berterusan; (2) pH 6.5, 30°C, 100 rpm, nisbah enapcemar/POME 1:1, HRT 4 hari, tanpa pengembalian enapcemar dan POME disuap secara manual tiga kali sehari; (3) pH 5.5, 30°C, 150 rpm, nisbah enapcemar/POME 3:7, HRT 4 hari, SRT 8.2 hari dengan pengembalian sebahagian enapcemar (500 mL) and POME disuap secara manual tiga kali sehari. Didapati asid

utama yang dihasilkan adalah asam asetat, propionat dan butirat. VFA tertinggi telah dihasilkan dalam eksperimen ke-2 dengan konsentrasi 15.36 g/L, diikuti dengan eksperimen pertama dengan konsentrasi VFA 15.04 g/L dan terakhir 10.23 g/L. VFA telah dihasilkan dalam eksperimen ke-3. Untuk eksperimen ke-2 dan ke-3, penolakan COD adalah rendah dengan hanya 12.5% dan 31.1% manakala untuk penghasilan BOD adalah 58.3% dan 43.4% masing-masing.

POME terawat yang diperolehi ditambah dengan asam sulfurik pekat dan menjalani proses pemulihan dan pembersihan melalui evaporasi. VFA yang telah dibersihkan dan dipekat terdiri daripada 44.6 g/L asam asetat, 20.1 g/L asam propionat dan 22.5 g/L asam butirat dengan pemulihan hasil sebanyak 76%. VFA yang sudah dibersihkan dan dipekat telah digunakan untuk pengumpulan PHA oleh *Ralstonia eutropha* ATCC 17699 dengan cara pH-stat suapan-sesekelompok dibawah keadaan kekurangan nitrogen. Kandungan PHA melebihi 90% dengan konsentrasi PHA tertinggi 11.44 g/L dan CDW 12.5 g/L telah diperolehi dengan menggunakan sistem ini. Dalam kajian ini, penghasilan PHA tidak dihalang oleh konsentrasi nitrogen yang tinggi apabila asam butirat adalah asam yang lebih disukai oleh *Ralstonia eutropha*.

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LIST OF ABBREVIATIONS

μ	–	Specific growth rate
μm	–	Micrometer
AN	–	Ammoniacal nitrogen
APB	–	Acids producing bacteria
BOD	–	Biological oxygen demand
C	–	Carbon
cm	–	Centimetre
CMC	–	Carboxymethylcellulose
COD	–	Chemical oxygen demand
CSTR	–	Continuous stirred tank reactor
D	–	Dilution rate
DCW	–	Dry Cell Weight
DOE	–	Department of Environmental
DS	–	Dissolved solids
EFB	–	Empty fruit bunches
FFB	–	Fresh fruit bunches
g/L	–	Gram per litre
GC	–	Gas chromatography
GCMS	–	Gas chromatography mass spectrometry
GM	–	Growth medium
HB	–	Hydroxybutyrate
Hg	–	Mercury
HPLC	–	High performance liquid chromatography
HRT	–	Hydraulic retention time
HV	–	Hydroxyvalerate
L	–	Litre
M	–	Molar
mL	–	Millilitre
mm	–	Millimetre
MRT	–	Microorganism retention time

°C	–	Celsius
OD	–	Optical density
P	–	Phosphorous
P(3HB-co-3HV)	–	Poly(3hydroxybutyrate-co-3hydroxyvalerate)
PHA	–	Polyhydroxyalkanoate
PHB	–	Polyhydroxybutyrate
PHV	–	Polyhydroxyvalerate
POME	–	Palm oil mill effluent
ppm	–	Part per million
rpm	–	Rotation per minute
rps	–	Rotation per second
RT	–	Retention time
S	–	Sulphur
SRT	–	Solid retention time
SS	–	Suspended solids
TKN	–	Total kjeldahl nitrogen
TS	–	Total solids
UASB	–	Upflow anaerobic sludge blanket
v/v	–	Volume per volume
VFA	–	Volatile fatty acids
vvm	–	Volumetric air flow rate
w/v	–	Weight per volume

CHAPTER 1

INTRODUCTION

In today's urbanized industrial society and the target of Vision 2020 set by Malaysia, it is becoming increasingly important to protect our vital and limited water resources from pollution by providing adequate treatment of liquid wastes emanating from domestic and industrial sources. The major pollutants constituents in these liquid wastes are suspended and dissolved solids and can be treated with biological processes. At the same time, desired products can be obtained by controlling the metabolic activity of microorganisms while treating these wastes.

In Malaysia, oil palm is the major commodity crop in world trade. The production of palm oil in the world is 10.8 million tones in 2000 (MPOB, 2001a) and about 61 per cent of this came from Malaysia (MPOB, 2001b).

There are currently about 300 palm oil mills operating with a capacity of more than 71 million tones FFB/year (MPOB, 2001c). With such a huge production of palm oil and its derivatives, a lot of wastes have been produced particularly in the form of palm oil mill effluent (POME). While such residues accumulate and methods of their disposal remain a problem, biological processes using microorganisms that economically convert lignocelluloses into products useful for man will be continually sought (Wood, 1985).

POME that has been known as the most polluting agro-waste in Malaysia with an average of 25000 ppm BOD needs to be treated before safe discharge into watercourse. With the consciousness of humanity toward the importance of environment getting higher and the strict standards set by Department of Environmental (DOE), Malaysia, various treatments have been proposed to treat POME in order to meet the discharge standard. The most commonly used systems are the ponding system, open tank digester (non-sterile), extended aeration system and land application system (Basiron and Darus, 1995).

Currently, anaerobic treatment of POME is widely used because of its low operation cost. However, this treatment process produced methane gas, which is harmful to the environment by contributing green house effect. Besides, most studies done by scientists were on the rate-limiting methanogenic phase and not many studies have been reported on acidogenic phase, which is more desirable, based on the economic values of the products. In acidogenic phase, organic particulates are transformed and fermented to volatile fatty acids (VFA) comprising mainly from acetic, propionic and butyric acids. These organic acids can be further transformed into methane gas under favourable conditions.

The conventional anaerobic ponding system requires huge area due to high retention time with more than 100 days for satisfactory treatment. Hassan *et al.* (2002) has studied the feasibility of integrating organic acids production from POME with conventional wastewater treatment as part of zero emission system for palm oil mill. In order to increase the rate of anaerobic digestion of POME, improvement have been carried out by retaining the sludge biomass inside the system, such as up-

flowed packed bed anaerobic reactor and UASB. Instead of using these bioreactors, Phang (2001) has tried on non-sterile anaerobic system (CSTR) by partially recycling the sludge back to the reactor to achieve higher solid retention time.

The VFA obtained could be subsequently used as alternative raw materials for polyhydroxyalkanoate (PHA) production under desirable conditions (Hassan *et al.*, 1996, 1997a, 1997b). The idea of using VFA obtained from anaerobic treatment of POME for the production of PHA is to reduce the production cost, which is many fold higher compared to petrochemical plastic. By using anaerobic treatment, a mixture of VFA can be obtained where acetic and butyric acids contribute to 3HB monomer while propionic acids contribute to 3HV monomer which become P3HB-co-3HV co-polymer by using *Ralstonia eutropha*.

Thermoplastic properties and biodegradability make poly-3-hydroxybutyrate-co-3-hydroxyvalerate (P3HB-co-3HV) a copolymer more preferable than polyhydroxybutyrate (PHB) (Steinbüchel, 1991). The range of applications of the copolymer is determined by properties that depend on the 3HV content (Holmes, 1985). PHA can be widely used in various areas such as packaging industry, agriculture, fishing industry, medicine, foodstuff industry, tobacco industry, chemical industry and others (Steinbüchel, 1996)