UNIVERSITI PUTRA MALAYSIA

BIOCONVERSION OF PALM OIL MILL EFFLUENT FOR THE PRODUCTION OF ORGANIC ACIDS AND POLYHYDROXYALKANOATES

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By

NOR 'AINI ABDUL RAHMAN

Thesis submitted in Fulfilment of the Requirements for the Degree of Master of Science in the Faculty of Food Science and Biotechnology Universiti Putra Malaysia.

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DEDICATION

GOD

You are my Supreme Love

"Mursyid"

Thanks for your dedication and leadership

Mum and family

Thanks for your love and care

Teachers

Thanks for your advise and guidance

Friends

Thanks for your help and support

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

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Chairman : Associate Professor Dr. Mohd. Ali Hassan

Faculty : Food Science and Biotechnology

In this study, palm oil mill effluent (POME) was utilized as a substrate for the production of polyhydroxyalkanoate (PHA) by *Alcaligenes eutrophus*. PHA production was achieved via a two-stage process; organic acids production from POME followed by PHA production from the organic acids. In the first stage, the study focused on optimisation of organic acids production as part of an integrated zero-emission system. Optimisation of organic acids production was performed by batch and continuous anaerobic treatments. In batch treatment, POME was mixed with different inoculum sizes (POME sludge) of 25%, 33%, 50% and 33% diluted sludge (two times) with tapwater in a 1 L conical flask. The result showed using inoculum size of 25%, 33% and 50% gave high organic acids production (7.0 g/L) after 4 days of treatment.

Continuous treatment was carried out at four different retention times (2.5, 5, 7.5 and 10 days). POME was mixed with an equal ratio of anaerobic POME sludge in a 12 L stirred-tank bioreactor. The pH was controlled at 7.0 to enhance organic acids production, particularly acetic and propionic. Total organic acids produced were 7.7 to 9.6 g/L for 2.5, 5 and 7.5 days retention times. More than 75% of the chemical oxygen demand (COD) conversion of POME was achieved during anaerobic treatment at 5, 7.5 and 10 days retention times. The best retention time was 5 days which produced 9.53±1.28 g/L of total organic acids, 11700 mg/L COD and 77% COD conversion of POME at steady state. After separation of the organic acids in the treated effluent by ion exchange chromatography, the residual COD of the effluent was less than 100 mg/L which met the effluent discharge standard set for the industry by the Department of Environment of Malaysia.

The effluent collected during continuous anaerobic treatment at 5 days retention time was concentrated by evaporation to be used in the second stage for polyhydroxyalkanoate production. The results showed that by evaporation, the organic acids could be concentrated ten-fold to about 100 g/L for use as substrates for the fed-batch fermentation. The concentrated organic acids were successfully converted to PHA by *Alcaligenes eutrophus* strain ATCC 17699 under a non-sterile fermentation system when the initial cell density was kept high at 4 g/L. After 150 hours, 20 g/L cells were obtained with more than 60% (g/g) PHA content being produced.

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

BIOPENUKARAN BAHAN BUANGAN KELAPA SAWIT UNTUK PENGHASILAN ASID ORGANIK DAN POLYHYDROXYALKANOATE

Oleh

NOR 'AINI ABDUL RAHMAN

Jun 2000

Pengerusi : **Profesor Madya Dr. Mohd. Ali Hassan**

Fakulti : **Sains Makanan dan Bioteknologi**

Di dalam kajian ini, bahan buangan kelapa sawit (POME) telah digunakan sebagai substrat untuk penghasilan polyhydroxyalkanoate (PHA) oleh *Alcaligenes eutrophus.* Tahap pertama kajian ini telah difokuskan terhadap pengoptimuman penghasilan asid organik yang merupakan sebahagian daripada keseluruhan sistem penyingkiran sifar. Pengoptimuman asid organik telah dijalankan secara rawatan anaerobik sesekelompok dan selanjar. Rawatan sesekelompok telah dijalankan dengan menggunakan saiz inokulum (enapcemar) yang berbeza iaitu 25%, 33%, 50% dan 33% sludge yang dicairkan dua kali dengan air paip. Keputusan menunjukkan pada saiz inokulum 25%, 33% dan 50% asid organik paling tinggi (7.0 g/L) dihasilkan selepas hari keempat rawatan.

Rawatan anaerobik selanjar telah dijalankan pada empat masa mastautin yang berbeza iaitu 2.5, 5, 7.5 dan 10 hari sehingga keadaan mantap tercapai.

POME dicampurkan dengan enapcemar dalam nisbah yang sama di dalam 12 L bioreaktor tangki berpengaduk. pH dikawal pada 7.0 untuk meningkatkan penghasilan asid organik terutamanya asetik dan propionik daripada POME. Lebih daripada 75% penukaran keperluan kimia oksigen (COD) telah dicapai dalam rawatan selanjar pada 5, 7.5 dan 10 hari masa tahanan. Masa mastautin terbaik ialah 5 hari dengan penghasilan 9.53±1.28 g/L asid organik, 11700 mg/L COD sisa cecair terawat dan 77% penukaran COD POME pada keadaan mantap. Selepas pemisahan asid organik pada sisa cecair terawat oleh penukar ion kromatografi, COD sisa cecair terawat adalah kurang dari 100 mg/L dan ini memenuhi piawaian bahan buangan industri oleh Jabatan Alam Sekitar Malaysia.

Sisa cecair yang dikumpul semasa rawatan selanjar anaerobik pada masa mastautin 5 hari telah dipekatkan menggunakan kaedah pemeluwapan untuk digunakan bagi tahap kedua iaitu penghasilan peolyhydroxyalkanoate (PHA). Hasil dari pemeluapan, organik asid telah dipekatkan sepuluh kali iaitu lebih kurang 100 g/L untuk digunakan sebagai substrat fermentasi suapan sesekelompok. Asid organik yang dipekatkan telah digunakan oleh *Alcaligenes eutrophus* strain ATCC 17699 menghasilkan PHA di dalam keadaan sistem fermentasi tak steril apabila ketumpatan sel awal 4 g/L digunakan. Setelah 150 jam fermentasi, 20 g/L sel dihasilkan mengandungi lebih 60% (g/g) PHA.

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TABLE OF CONTENTS

LIST OF TABLES

LIST OF FIGURES

CHAPTER I

INTRODUCTION

The issue of environmental pollution in relation to the disposal of nonbiodegradable plastic is a serious problem. The world production of plastics is over one hundred million tons per year (Chang, 1994). Plastics are non-biodegradable in the natural environment, thus they will remain indefinitely in landfill sites. During the combustion of plastic wastes, hydrogen cyanide can be formed from acrylonitrile-based plastics and hydrogen chloride is released from polyvinylchloride (PVC) (Brandl *et al*., 1990). Development and industrial production of degradable materials as replacement to non-biodegradable petrochemical-based plastic is one of the alternatives to solve the pollution problem.

Polyhydroxyalkanoic acid (PHA) produced by microorganisms has the potential to be used as a raw material for biodegradable plastics (Taga *et al*., 1996). Polyhydroxybutyrate (PHB) has received attention as a candidate for a novel biodegradable plastic material, since it has similar physico-chemical properties to polypropylene and polyethylene (Kim *et al*., 1996). The polymer is produced under unbalanced growth conditions in a nutrient limitation such as nitrogen, oxygen, or phosphate in the presence of excess carbon source (Martinez *et al*., 1995). Unfortunately, the widespread use of PHB has been hindered by the high production cost. Currently PHA in the form of poly(3-HB-co-HV) or PHB/v is being produced on a large scale by Monsanto Ltd. in USA under the trade name "Biopol" using *Alcaligenes eutrophus* H16. It cost US4.40/kg compared to $0.60/kg of petrochemical plastic (Hassan *et al*., 1997a). The cost of pure fermentation feedstocks, mainly glucose and propionic acid contributed significantly to the cost of the production.

The substrates usually used for the production of PHB are glucose, sucrose and fatty acids (Kim *et al*., 1996). However, for the practical application, cheaper carbon source needs to be utilised to reduce the production cost. PHB can be produced from relatively cheaper substrates such as methanol, carbon dioxide, beet molasses or ethanol. Wastewater can also be used as a cheaper carbon source in production of value-added products. In this study palm oil mill effluent (POME) was used as the raw material for PHA production. POME is a major contributor of industrial wastewater pollution in Malaysia. POME has BOD about 22 000 mg/L (Ho and Tan, 1985) and total nitrogen around 500 mg/L. The high carbon to nitrogen (C/N) ratio makes POME a suitable substrate for PHA production (Hassan *et a*l., 1997b, 1997c).

Previous studies carried out by Hassan *et al.* (1996) under batch anaerobic treatment of POME with sludge as inoculum for producing organic acids, mainly acetic and propionic acids, at pH 7.0. Further studies on anaerobic treatment of POME was performed in this project. Optimisation of organic acids production from POME was carried out mainly under continuous anaerobic treatment by using various retention times at pH 7.0.

A two steps process for wastewater treatment coupled with PHA production was proposed in this study. The first step involved the controlled anaerobic treatment of POME. During this process, complex organic compounds are broken down by bacteria into simple organic acids. The second step made use of the ability of a particular species of bacteria, *Alcaligenes eutrophus*, to consume the simple acids and convert them to intracellular biopolymers as energy and carbon reserve. However the amount of organic acids produced during anaerobic treatment was low and not enough to be used as raw material for PHA production. To solve this problem, the concentration of organic acids obtained was increased by evaporation. Then the concentrated acids were used as fermentation feedstock in fed-batch culture of *Alcaligenes eutrophus* for PHA production.

The objectives of this studies are :

1. To optimise organic acids production during partial anaerobic treatment of POME and to ascertain that the COD in the final effluent met of DOE's Standard B for the industry to be safely discharged
2. To produce polyhydroxyalkanoate (PHA) by *Alcaligenes eutrophus* using treated POME as fermentation feedstock.

This project uses the concept of coupling system, by combining the treatment of POME in a conventional wastewater treatment system with PHA production. This technique could significantly reduce the cost of PHA and, at the same time, reduce the cost of wastewater treatment.

CHAPTER II

LITERATURE REVIEW

Palm Oil Mill Effluent (POME)

Over the last three decades, the Malaysian palm oil industry has grown to become a very important agricultural-based industry (Ma, 1999). Palm oil contributes significantly in supplying the world's requirement for oils and fats. Total Malaysian crude palm oil production in 1997 is 9.03 million tonnes and 1.1 million tonnes of crude palm kernel oil (Ma, 1999). Yusof (1994) reported Malaysian crude palm oil contributed 16.4% of the world's total oils and fats output of 84.8 million tonnes.

A major trend that has evolved during the last two decades is the increasing contribution of palm oil in the international oils and fats market. With the increasing role of palm oil, there is also the corresponding increase in the participation of the palm oil producing countries in supplying oils and fats to the world trade. At present, only a few countries are major exporters of palm oil with Malaysia as the leading producer and exporter followed by Indonesia, Ivory Coast and Papua New Guinea (Yusof, 1994). About 90% of palm oil produced in Malaysia is exported to the world market and Malaysian palm oil accounted for about 52% of the world palm oil output (Ma, 1999). It is projected the oil palm planted area will increase to 2.9 million hectares by the year 2000 (Yusof, 1994).

Currently, there are more than 2.6 million hectares of land under oil palm and 290 palm oil mills to produce crude palm oil and palm kernel crushers and oleochemical companies (Ma, 1999). Whilst palm oil industry has contributed to the progress and development of the nation, its production of palm oil mill effluent (POME) has caused much environmental concern (Ma *et al*., 1982).

In a typical factory, the amount of POME generated is about double the tonnage of crude palm oil produced. POME is an organic wastewater from palm oil industry and has high carbon content (BOD in excess of 20,000 mg/L) with low nitrogen content (less than 200 mg/L ammoniacal nitrogen and less than 500 mg/L total nitrogen). The generally characteristics of POME is shown in Table 1. Fresh POME is a colloidal suspension of 95-96% water, 0.6%-0.7% oil and 4%-5% total solids including 2-4% suspended solids which are mainly debris from palm fruit mesocarp (Ma, 1999).

Table 1: Characteristics of Palm Oil Mill Effluent

Source: (Ma, 1999; Ma *et al*., 1997)

Parameters	Mean
pH	4.7
Oil & Grease	4000
BOD_3	25,000
COD	50,000
Total Solids	40,500
Ammoniacal Nitrogen	35
Total nitrogen	750
Phosphorous	180
Potassium	2270
Magnesium	615
Zinc	2.3
Calcium	439
Boron	7.6
Iron	46.5
Manganese	2.0
Copper	0.89

All parameters in mg/L except pH

POME has been singled out as one of the chief contributors to the nation's pollution problem. In 1997, Malaysian palm oil mills discharge 21 million tonnes of the effluent which in terms of biochemical oxygen demand (BOD) is about 100 times that of domestic sewage (Ma, 1999) and it is equivalent to that generated by about a population of 6.3 million (Ng *et al.,* 1987). Realising the seriousness of the problem, the government enacted the Environmental Quality Regulation (Prescribed Premises Crude Palm Oil) in 1977. The regulatory standards for watercourse discharge were made effective from 1st July 1978 (Ma *et al*, 1982) as shown in Table 2.

Table 2: Regulatory standards for palm oil mill effluent

Source: (Ma *et al*, 1982)

Parameters	Standard A 1.7.1978	Standard B 1.7.1979	Standard C 1.7.1980	Standard D 1.7.1981
Biological oxygen demand (BOD)	5000	2000	1000	500
Chemical oxygen demand (COD)	10000	4000	2000	1000
Total solids (T.S.)	4000	2500	2000	1500
Suspended solids (S.S.)	1200	800	600	400
Oil & grease (O & G)	150	100	75	50
Ammoniacal-nitrogen (NH_3-N)	25	15	15	10
Organic-nitrogen	200	100	75	50
pH	5.0–9.0	5.0–9.0	5.0–9.0	5.0–9.0
Temperature °C	45	45	45	45

- All except pH in mg/L

Treatment system of POME

In view of the high polluting strength and large volume (about 2.5 tonnes POME are produced per ton of oil extracted), there is an urgent need to treat this waste before permanent damage is done to the receiving waterways. Realising the seriousness of the situation, ways are actively being sought to reduce these organic loads and many treatments and disposal system have been proposed involving physical and biological methods.

Traditionally POME has been treated anaerobically in lagoons to produce biogas which is released into the atmosphere without any control. Anaerobic digestion of these wastes could indeed result in significant reduction in BOD and COD (Southwoth, 1979; Chin, 1981).

Over the last two decades, various treatment and disposal method have been successfully developed and employed by palm oil mills to treat their POME. Currently, most palm oil mill operators treat POME either aerobically using the activated sludge process or anaerobically in ponds and lagoons. Due to the high BOD strength of POME, high energy cost is associated with the activated sludge system. Conventional biological system consisting of a combination of anaerobic, aerobic and facultative processes are used instead. If well operated and maintained, these processes are able to treat POME to the discharge standards set by the Department of Environmental (DOE) (Table 3).

Biological treatment systems need proper maintenance and monitoring as the processes rely solely on microorganisms to breakdown the pollutants. The microorganisms are very sensitive to changes in the environment and thus great care has to be taken to ensure that a condusive environment is maintained for the microorganisms to thrive in (Ma, 1999).