UNIVERSITI PUTRA MALAYSIA

BIOGAS PRODUCTION AND DETERMINATION OF METHANOGENS FROM DIGESTER - TREATED PALM OIL MILL EFFLUENT

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By

MOHD RAFEIN BIN ZAKARIA @ MAMAT

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

December 2007

DEDICATION

This piece of work is dedicated to my lovely parent and my wife, who has always been by my side and given me the encouragement and support that carries me through my study period. Thanks for their undying love to me.

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

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Chairman: Professor Mohd Ali Bin Hassan, PhD

Faculty: Faculty of Biotechnology and Biomolecular Sciences

Due to increasing awareness of the risk of the environmental pollution and emission of green house gases (GHG) that caused global warming, a study of palm oil mill effluent (POME) treatment using biological processes in close digesters has been conducted. Potentially beneficial methane gas production from this treatment has driven the objectives of this study to explore the methanogens from POME sludge and their characteristic in order to improve the POME treatment efficiency. A pilot plant digester with 500 m^3 volume was operated for one year and biogas and methane production profiles were monitored daily. Parameters used for monitoring were pH, temperature, volatile fatty acids, chemical oxygen demand, biogas and methane concentration during the treatment. Structure of the flocs formation and methanogenic bacteria isolated from this digester was also carried out in order to understand the microbiological characteristics of the bioprocess involved.

Specific methanogenic activity test (SMA) and microscopic observation were carried out to support the existence of the methanogens that were able to produce methane gas. During POME treatment process, the highest methane concentration obtained was 55 % (v/v) and chemical oxygen demand (COD) removal efficiency was up to 95%. Anticipated active biomasses retained in the digester were determined as total solids (TS) and volatile suspended solids (VSS) at 2.5% and 1.5%, respectively. SMA test for acetolastic methanogens was determined in the range of 0.05 - 1.3 g COD/ g VSS/d. Microscopic observation of the sludge and isolated colonies have shown that the digester systems were dominated by microorganism resemble to *Methanosaeta* sp. and fluorescence microscope has proved that this microorganism exhibited autofluorescence green color.

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

PENGHASILAN BIOGAS DAN PENENTUAN METHANOGENS DARI TANGKI RAWATAN AIR PEMPROSESAN BUAH SAWIT

Oleh

MOHD RAFEIN BIN ZAKARIA @ MAMAT

Disember 2007

Pengerusi : Profesor Mohd Ali Bin Hassan, PhD

Fakulti : Fakulti Bioteknologi dan Sains Biomolekul.

Kesedaran yang tinggi tentang risiko pencemaran alam dari pelepasan gas kesan rumah hijau (GHG) yang menyebabkan pemanasan global telah mendorong kepada penyelidikan tentang rawatan air buangan dari pemprosesan kelapa sawit (POME) menggunakan kaedah proses biologi di dalam tangki tertutup. Secara potensinya, kelebihan penghasilan gas metana dari sistem rawatan ini telah mencetuskan matlamat kajian untuk mendalami ciri-ciri bakteria methanogens yang diperolehi dari mendakan POME bagi meningkatkan kecekapan rawatan. Loji pandu biogas yang berkapasiti 500 tan telah beroperasi selama satu tahun dan dipantau penghasilan biogas dan gas metana. Suhu, pH, kandungan asid lemak meruap, keperluan oksigen kimia (COD) juga adalah parameter yang dipantau semasa rawatan.

Struktur gumpalan yang terbentuk dan bakteria yang menghasilkan gas metana juga dikaji semasa proses rawatan POME dengan tujuan mengetahui dengan lebih mendalam tentang sifat-sifat mikrobiologi terhadap bioproses yang terlibat. Kajian SMA dan pengamatan menggunakan mikroskop juga dilakukan bagi mengesahkan kewujudan bakteria tersebut di dalam loji pandu biogas. Bacaan tertinggi gas metana yang diperolehi semasa kajian ini adalah sebanyak 55% dan kadar penurunan keperluan oksigen kimia (COD) adalah lebih dari 95%. Dianggarkan sebanyak 2.5% jumlah pepejal (TS) dan 1.5% pepejal terampai meruap (VSS) dapat dikekalkan di dalam sistem loji pandu. Kajian SMA bagi methanogens yang menggunakan asetik asid bagi penghasilan gas metana adalah dianggarkan sebanyak 0.05 – 1.3 gCOD/VSS/d. Pengamatan menggunakan mikroskop untuk mendakan POME menunjukkan loji pandu biogas didominasi oleh bakteria yang menyerupai spesis *Methanosaeta* dan ianya dibuktikan dengan pengamatan mikroskop fluoresen dimana bakteria tersebut menunjukkan warna hijau fluoresen.

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I certify that an Examination Committee met on 22 December 2007 to conduct the final examination of Mohd Rafein Bin Zakaria @ Mamat on his Master of Science thesis entitled "Methane Production and Determination of Methanogens from Digester Treating Palm Oil Mill Effluent" in accordance with Universiti Pertanian Malaysia (Higher Degree) Act 1980 and Universiti Pertanian Malaysia (Higher Degree) Regulations 1981. The committee recommends that the candidate be awarded the degree of Master of Science.

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This thesis was submitted to the Senate of Universiti Putra Malaysia and has been accepted as fulfilment of the requirement for the degree of Master of Science. The members of the Supervisory Committee were as follows:

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Date : 21st February 2008

DECLARATION

I declare that the thesis is my original work except for quotations and citations which have been duly acknowledged. I also declare that it has not been previously, and is not concurrently, submitted for any other degree at Universiti Putra Malaysia or at any other institution.

_____

MOHD RAFEIN ZAKARIA @ MAMAT

Date:

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LIST OF ABBREVIATIONS

AMA	Acetoclastic Methanogenic Activity
ATP	Adenine Triphosphate
BOD	Biological Oxygen Demand
CDM	Clean Development Mechanism
CDT	Closed Digester Tank
CER	Certified Emission Reduction
CFC	Chlorofluorocarbon
CH_4	Methane
CO	Carbon Monoxide
CO_2	Carbon Dioxide
CSTR	Continuous Stirred Tank Reactor
d	Day
DNA	Deoxyribonucleic Acid
COD	Chemical Oxygen Demand
CPO	Crude Palm Oil
DOE	Department of Environment
EFB	Empty Fruit Bunch
FFB	Fresh Fruit Bunch
g	Gram
GHG	Green House Gas
HOM	Hydrogen Oxidizing Methanogens
HRT	Hydraulic Retention Time

H_2	Hydrogen gas
L	Liter
MABR	Modified Anaerobic Baffled Reactor
NHOM	Non-Hydrogen Oxidizing Methanogens
ODT	Open Digester Tanks
OLR	Organic Loading Rate
PCR	Polymerase Chain Reaction
POME	Palm Oil Mill Effluent
RABR	Reversible Anaerobic Baffle Reactor
RNA	Ribonucleic Acid
SMA	Specific Methanogenic Activity
TMA	Total Methanogenic Activity
TS	Total Solid
UAF	Upflow Anaerobic Filter
UASB	Upflow Anaerobic Sludge Blanket reactor
UASFF	Upflow Anaerobic Sludge Fixed-Film
VFA	Volatile Fatty Acid
VS	Volatile Solids
VSS	Volatile Suspended Solid

CHAPTER 1

INTRODUCTION

1.0 Introduction

Since the global industrial revolution, environment has become polluted and unhealthy for living organisms. These are attributed to deforestation, discharge of pollutants into rivers, lands and air. Some hazardous compounds such as chlorofluorocarbon (CFC), nitrous oxide, carbon monoxide (CO), carbon dioxide (CO_2) and methane (CH_4) are green house gases (GHG) which have strong infrared absorption capacity and trap a large portion of the thermal radiation emitted from the earth's surface (Yang *et al*., 2003). Uncontrolled release of the pollutants into the air has led to an unstable environment threat. Some negative effects of these pollutants and massive concern causing worldwide are depletion of ozone layer, global warming and significant raise of ocean level. Malaysia, like other developing countries had achieved an outstanding performance of economic growth since implementing mass industrial revolution. One of big and outstanding industries in Malaysia is agriculture- based oil palm industry.

Oil palm industry was brought by Frenchman, Henri Fauconnier in the early 20th century (Teoh, 2002). Since then mass planting of oil palm has been promoted, the development and management of land has been expanded particularly in rural area, setting up research and development in recognized institutions have fuelled the

nation's economy. The oil palm planted area has expanded from 55,000 hectares in 1960 to 3.5 million hectares by 2001, occupying 60% of the agricultural land in the country with 380 palm oil mills of which about 70% are located in Peninsular Malaysia (Khoo, 2001). Malaysia is the largest palm oil producer, contributing approximately 11.8 million tonnes or 50.9% of total world production (Khoo, 2001). Despite being known as world's biggest palm oil producer, this industry also generates large amounts of wastes and is among the most polluting in the country.

The wastes or residues generated from palm oil industry are in two forms, solid wastes that consists of empty fruit bunch (EFB), shell, trunks, and fronds. Secondly is the liquid waste mainly palm oil mill effluent (POME), which is highly polluting at an average of 25,000 mg/L biochemical oxygen demand (BOD) and 50,000 mg/L chemical oxygen demand (COD) (Ma, 1999). Conventionally, POME is being treated using the cheapest technologies, open digester tanks and pond or lagoon systems. According to the strict regulations of wastewater disposal amendment by the Department of Environment (DOE) Malaysia, the POME should be treated first or converted into by-products with added value before it`s disposed to appropriate places. Generally, these systems need large land areas which release directly and uncontrolled GHG particularly CH_4 and CO_2 gas to the atmosphere. In 2005, the estimation methane of annual emission potential from POME was 5,000,000 tonnes (Tong and Jaafar, 2005).

Studies by Yacob and co-workers (2005a and 2005b) on the methane emission from lagoon and open digesting tanks treating POME have provided valuable information in establishing GHG emission particularly in palm oil industry. These studies were done in line with the initiatives laid out to reduce GHG emission under the Kyoto Protocol. Briefly, Kyoto Protocol encourages the involvement of the developing countries in reducing the climate problems on the way to development. Thus, there have been keen overwhelming interests in the palm oil industry due to the huge potential of untapped biogas and biomass as clean, green and renewable resources of energy during the mitigation of GHG emission. By ratifying the Kyoto Protocol, the implementation of the Clean Development Mechanisms (CDM) for palm oil industry is now possible. The industry could now contribute to global emissions reduction via CDM projects, in addition to earning economic benefits from Certified Emission Reduction (CER) credits. The palm oil industry can derive new economic, development and environment benefits through the implementation of CDM projects.

The increasing concerns for rapid depletion of the non-renewable fuel and the other world's environmental issues have resulted in searching for sustainable alternative fuels. Anaerobic degradation of organic residues for biogas production is the best option as a substitution for petrochemical fuel. Anaerobic degradation of organic waste/ residues in closed digesters is not a new technology. European countries have adopted and established this technology over than 50 years ago. However, this green and clean technology is not well appreciated in treating and handling organic residues in Malaysia. Quah and Gilles (1981) reported anaerobic degradation of POME in closed digester tanks produced approximately 28.3 m^3 gas per cubic meter

of POME digested where methane was found to be at the range of 54 -70% volume. Unfortunately, this valuable finding was not expanded and widely implemented because of several factors. The market demand for alternative fuels is insignificant and industries are pleased with the available resources. Most important factor is lack of enough motivation for improving and implementing of this technology as POME is only resources available in Malaysia.

Now the application of anaerobic digestion of POME using closed system has become attractive and expanded. There are a lot of researchers reported the potential application of the anaerobic digester in laboratory and pilot scale studies. There are varietion in the term of types of bioreactor design and system monitoring for treatment of POME such as the modified anaerobic baffled bioreactor (Faisal and Unno, 2001), anaerobic filter and anaerobic fluidized bed reactor (Borja and Banks, 1995), thermophilic upflow anaerobic filter (Mustapha, *et al*., 2003), continuous stir tank reactor (Tong and Jaafar, 2005), and reversible anaerobic baffled reactor (RABR) (Raof *et al*., 2005). However no study has been done on the microorganisms and their contribution to the successful anaerobic POME degradation process.

Previous studies have shown a lot of obstacles have been faced during anaerobic degradation of POME. High content of oils and fats that are not easy to hydrolyse, uncertainties about quantity and quality of fresh POME, bioreactor design, washout of active microorganisms especially methane producing bacteria are the factors that have to be taken into consideration. Specific methanogenic activity tests, diversity of characteristics and determination of methanogenic bacteria will help in understanding the characteristic of biomass sludge in anaerobic digesters thus

maintain the stability of POME digestion performance. Recently, similar efforts have been made by the largest palm oil company in the world, Federal Land and Development Authority (FELDA), through the R&D collaboration with Universiti Putra Malaysia (UPM) and Kyushu Institute of Technology (KIT), Japan to utilize the biogas and biomass for new bioproducts. Three projects have been conducted including; the generation of biogas for energy using 500 m^3 CDT (Yacob *et al*., 2006), production of organic acids and biodegradable plastics from POME (Nor `Aini *et al*., 1999) and saccharafication of EFB to produce sugar (Hassan *et al*., 2003). Those projects are examples for the mitigation methods to reduce GHG emitted from palm oil industries.

1.1 Objectives

The objectives of this study were;

1. To study the methane production in the closed digester tanks (CDT) based on organic loading rates (OLR) feeding pattern.

2. To study the microbial diversity of anaerobic fermentation in the CDT using microscopy observations and Specific Methanogenic Activity (SMA) test.

CHAPTER 2

LITERATURE REVIEWS

2.1 OIL PALM INDUSTRY

Oil palm (*Elaeis guineensis*) is vastly cultivated as a source of oil in West and central Africa where it is originated from, and in Malaysia, Indonesia and Thailand as well. In Malaysia, oil palm is one of the most important commercial items and accounts for 20% and 46% of the global oil and fat production and trade, respectively. Malaysia is the world's largest producer and exporter of palm oil with 11.80 million tonnes or 50% of the world palm oil production. The oil palm planted area increased to 3.5 million hectares by 2001, occupying 60% of the agricultural land in the country and the industry still expanding corresponding to the growing world population (Khoo, 2001).

Apart from edible oil, the palm oil industry also generates large amount of biomass. The biomass can be divided into two forms of renewable biomass, solid biomass and liquid biomass. Solid biomass comprises of empty fruit bunch (EFB), 53%, mesocarp fibre, 32%, and palm kernel shell, 15%. There are many practices of EFB utilization particularly, as it is rich in cellulose (50.4%), hemi-cellulose (21.9%), lignin (10%), and ash (17.7%), (Umikalsom, *et al*., 1997). Among the potential application of EFB are soil mulching (Weng, 1999), fiber board for the furniture industry (Tan and Kang, 1993), and as boiler fuel (Chua, 1991).