UNIVERSITI PUTRA MALAYSIA

ENHANCEMENT OF METHANE PRODUCTION FROM PALM OIL MILL EFFLUENT THROUGH CO-DIGESTION AND BIO-AUGMENTATION TREATMENT

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By

NURLIYANA MOHD YAAKOB

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

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Degradation process of palm oil mill effluent (POME) via anaerobic digestion produced a valuable methane gas which has recently become a potential source of renewable energy in Malaysia. As to date, only a few palm oil mills in Malaysia are involved in recovering the biogas since current methane production by conventional technologies is below expectation. The composition of POME exhibits nutrient availability for the indigenous microorganisms and will affect the direction of the degradation process. Similarly, the performance of microbial community during the treatment process plays a significant role as they are primarily the agents that accomplish the desired treatment. This research aims at assessing the effects of POME characteristics on the methane production and subsequently investigating ways of enhancing the methane productivity by improving the feedstock characteristics and microbial consortia. This study was divided into three parts. The first part evaluates the POME physicochemical and biochemical characteristics. Total carbon of POME was found to be relatively low leading to low carbon to nitrogen (C/N) ratio value which affects the production of methane gas. In addition, a low pH value of POME was recorded, influencing the optimum condition of indigenous microbes specifically the methanogens during the digestion process. The second and third part of this study were conducted to improve the feedstock characteristics as well as to increase the methane productivity and substrate degradability by conducting the co-digestion and bioaugmentation treatment.

Co-digestion treatment of POME with natural carbon substrate in the palm oil mill, specifically empty fruit bunch (EFB), was investigated by varying the C/N ratio of the substrates loading. A significant effect of different C/N ratios towards methane production and methane yield were observed. The co-digestion treatment resulted in higher methane production where C/N ratio of 45 delivers the highest result with twelve times and 61.7% improvement in methane production and methane yield, respectively. While for substrate biodegradability,

a significant reduction of organic substances was identified with different C/N ratios except for degradation of cellulose content in EFB. However, co-digestion samples were found to be ineffective in terms of substrate degradability compared to mono-digestion of POME in total reduction of total solid (TS), biological oxygen demand (BOD) and chemical oxygen demand (COD) respectively for POME substrate. While in EFB substrate degradability, a low total reduction was observed for all co-digestion samples. Cellulose content particularly indicates an increase in percentage after the digestion process.

Bioaugmentation treatment towards POME and POME co-digested with EFB was investigated by addition of different microbial loadings of cellulolytic bacteria and methanogenic archaea by single or co-augmentation technique, to improve the microbial activity in the feedstock and to enhance the methane productivity and substrate biodegradability. Treatment of single augmentation by *Bacillus subtilis* in co-digestion sample at 10% microbial loading (C-B10) resulted in the highest methane production with 14.3% increment compared to non-augmented co-digestion sample. In the matter of substrate degradability, bioaugmentation treatment of C-B10 activated the cellulose reduction in EFB with 1.95% reduction as compared to non-augmented co-digestion sample which had an increased value of 4.4%. Besides, sample C-B10 recorded better TS, total volatile solid (TVS), COD, organic matter (OM) and lignin reduction with 27.2%, 22.3%, 90.9% 29.4% and 2.8 times higher degradation, respectively compared to the non-augmented co-digestion sample.

Treatment of co-augmentation between *B. subtilis* and mix methanogens was operated to maximize the methane productivity and substrate degradability. Sample of single digestion co-augmented at 5% microbial loading (S-B5-M5) gives a significant increase in methane productivity with 9.4-29.6% and relatively 2.1 times improvement compared to single augmented sample of similar microbial loading (S-B5 and S-M5) and the non-augmented single digestion sample respectively. In terms of substrate degradability, sample of S-B5-M5 showed a notable results in TVS and COD reduction where it attained the highest removal efficiencies among all co-augmented samples with 37.3% and 55.2% improvement measure up to non-augmented single digestion sample.

Co-digestion and bioaugmentation treatment were demonstrated as effective means of enhancing the methane productivity and substrate biodegradability of the feedstock. Optimum loading of the co-substrate and microbial cultures from this research can be implemented in the field to better develop the methane production system specifically in the closed anaerobic pond. A substantial number of palm oil millers will be able to apply the results of this study and broaden the chances to recover the biogas produced into a valuable renewable energy.

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

PENINGKATAN PENGHASILAN GAS METANA DARIPADA AIR PEMPROSESAN KILANG KELAPA SAWIT MELALUI RAWATAN DEGRADASI BERSAMA DAN PENAMBAHAN MIKROORGANISMA

Oleh

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Proses degradasi air pemprosesan kilang kelapa sawit (POME) melalui degradasi anaerobik menghasilkan gas metana berharga yang kebelakangan ini telah menjadi sumber potensi tenaga boleh diperbaharui di Malaysia. Sehingga kini, hanya beberapa kilang minyak sawit di Malaysia yang terlibat dalam mendapatkan biogas memandangkan pengeluaran semasa metana menggunakan teknologi konvensional adalah di bawah jangkaan. Komposisi POME menunjukkan ketersediaan nutrien untuk mikroorganisma sedia ada dan akan memberi kesan kepada arah tuju proses degradasi. Begitu juga prestasi komuniti mikrob semasa proses rawatan yang memainkan peranan penting, terutama kerana mereka adalah agen yang mencapai rawatan yang dikehendaki. Kajian ini bertujuan untuk menilai kesan ciri-ciri POME dalam pengeluaran metana dan seterusnya menyiasat cara untuk mempertingkatkan produktiviti metana dengan memperbaiki ciri-ciri bahan mentah dan konsortia mikrob. Kajian ini dibahagikan kepada tiga bahagian. Bahagian pertama adalah menilai ciri-ciri fisikokimia dan biokimia POME. Jumlah karbon POME secara relatif didapati rendah dan mengakibatkan nilai nisbah karbon kepada nitrogen (C/N) turut menjadi rendah dan akan menjejaskan pengeluaran gas metana. Di samping itu, POME didapati mencatatkan nilai pH yang rendah dan akan mempengaruhi keadaan optimum mikrob asli terutamanya metanogen ketika proses degradasi. Bahagian kedua dan ketiga kajian ini telah dijalankan untuk meningkatkan ciri-ciri bahan mentah serta untuk meningkatkan produktiviti metana dan keupayaan degradasi substrat dengan menjalankan rawatan untuk degradasi bersama dan penambahan mikroorganisma.

Rawatan degradasi bersama antara POME dengan bahan karbon asli daripada kilang minyak sawit, khususnya tandan kosong buah kelapa sawit (EFB), telah dikaji dengan mengubah nisbah C/N muatan substrat. Terdapat kesan yang ketara antara nisbah C/N yang berbeza ke arah pengeluaran metana dan hasil metana. Rawatan degradasi bersama telah menghasilkan peningkatan dalam pengeluaran metana di mana nisbah C/N 45 menghasilkan hasil tertinggi dengan

dua belas kali dan 61.7% peningkatan masing-masing dalam pengeluaran metana dan hasil metana. Manakala bagi keupayaan degradasi substrat, pengurangan ketara bahan organik dengan nisbah C/N yang berbeza telah dikenal pasti kecuali degradasi kandungan selulosa di dalam EFB. Walaubagaimanapun, sampel degradasi bersama didapati tidak berkesan dari segi keupayaan degradasi substrat berbanding degradasi POME secara tunggal dalam jumlah pengurangan jumlah pepejal (TS), keperluan oksigen secara biologi (BOD) dan keperluan oksigen secara kimia (COD) untuk substrat di dalam POME. Bagi keupayaan degradasi substrat di dalam EFB, jumlah pengurangan yang rendah telah dikenal pasti untuk semua sampel degradasi bersama. Kandungan selulosa khususnya menunjukkan peratusan peningkatan selepas proses degradasi.

Rawatan penambahan mikroorganisma ke dalam POME dan degradasi bersama antara POME dan EFB telah dikaji dengan penambahan muatan mikrob yang berbeza daripada bakteria selulosa dan arkea metanogen dengan teknik tunggal atau penambahan bersama, untuk meningkatkan aktiviti mikrob dalam bahan mentah dan meningkatkan produktiviti metana dan keupayaan degradasi substrat. Rawatan penambahan tunggal oleh *Bacillus subtilis* dalam sampel degradasi bersama pada 10% muatan mikrob (C-B10) menghasilkan pengeluaran metana tertinggi dengan 14.3% kenaikan berbanding sampel degradasi bersama yang tidak ditambah mikroorganisma. Dalam keupayaan degradasi substrat, rawatan penambahan mikroorganisma oleh C-B10 telah meningkatkan degradasi selulosa dalam EFB dengan jumlah pengurangan 1.95% berbanding dengan sampel degradasi bersama yang tidak ditambah mikroorganisma yang mempunyai peningkatan sebanyak 4.4%. Selain itu, sampel C-B10 mencatatkan pengurangan lebih baik dalam TS, jumlah pepejal mudah meruap (TVS), COD, bahan organik (OM) dan pengurangan lignin masing-masing dengan 27.2%, 22.3%, 90.9% 29.4% dan 2.8 kali lebih tinggi degradasi berbanding dengan sampel degradasi bersama yang tidak ditambah mikroorganisma.

Rawatan penambahan mikroorganisma bersama antara *B. subtilis* dan campuran methanogens dijalankan untuk memaksimumkan produktiviti metana dan keupayaan degradasi substrat. Sampel degradasi POME dengan penambahan mikroorganisma bersama pada 5% muatan mikrob (S-B5-M5) memberikan peningkatan yang ketara dalam produktiviti metana dengan 9.4-29.6% dan secara relatif 2.1 kali peningkatan masing-masing berbanding dengan sampel ditambah muatan mikrob yang sama secara tunggal (S-B5 dan S-M5) dan sampel degradasi POME yang tidak ditambah mikroorganisma. Dari segi keupayaan degradasi substrat, sampel S-B5-M5 menunjukkan keputusan yang ketara dalam pengurangan TVS dan COD di mana ia mencapai jumlah penyingkiran tertinggi dalam kalangan sampel yang ditambah mikroorganisma dengan 37.3% dan 55.2% peningkatan bebanding sampel degradasi POME secara tunggal yang tidak ditambah mikroorganisma.

Rawatan degradasi bersama dan rawatan penambahan mikroorganisma telah dikenal pasti sebagai cara yang berkesan untuk meningkatkan produktiviti metana dan keupayaan degradasi bahan mentah. Muatan optimum substrat degradasi bersama dan kultur mikrob daripada kajian ini dapat dilaksanakan di lapangan untuk membangunkan sistem pengeluaran metana yang lebih baik khususnya dari kolam anaerobik tertutup. Sebilangan besar daripada pengilang minyak kelapa sawit akan dapat menggunakan hasil kajian ini dan meluaskan peluang untuk menukar biogas yang dihasilkan menjadi tenaga boleh diperbaharui.

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I certify that a Thesis Examination Committee has met on 2016 to conduct the final examination of Nurliyana Mohd Yaakob on her thesis entitled Enhancement of Methane Production from Palm Oil Mill Effluent Through Co-digestion and Bio-augmentation Treatment in accordance with the Universities and University Colleges Act 1971 and the Constitution of the Universiti Putra Malaysia [P.U.(A) 106] 15 March 1998. The Committee recommends that the student be awarded the Doctor of Philosophy.

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TABLE OF CONTENTS

LIST OF TABLES

LIST OF FIGURES

LIST OF ABBREVIATIONS

ANOVA	Analysis of Varience
APHA	American Public Health Association
BOD	Biological Oxygen Demand
C/N	Carbon to Nitrogen
CFU	Colony Forming Unit
CMCA	Carboxymethylcellulose Agar
COD	Chemical Oxygen Demand
CRA	Congo Red Agar
DOE	Department of Environment
EFB	Empty Fruit Bunches
FFB	Fresh Fruit Bunches
GHG	Green House Gases
LEL	Lower Exposure Limit
LSD	Least Significant Difference
NA	Nutrient Agar
OM	Organic Matter
PCR	Polymerase Chain Reaction
PDA	Potato Dextrose Agar
POME	Palm Oil Mill Effluent
TA	Thioglycollate Agar
TS	Total Solids
TVS	Total Volatile Solids

CHAPTER 1

INTRODUCTION

1.1 General Background

Malaysia is a global leader in the palm oil industry that accounts for 33.1% of world palm oil production and 39.6% of world palm oil products exporter (MPOC, 2014). Currently, palm oil industry is one of the nation's important industries having the status as the largest contributor in the agricultural sector and the fourth largest contributor to the national economy as it covers the entire value chain from plantations to downstream activities. Despite a high economic return to the country, the industry is under incessant criticism for its performance from the environmental perception. The palm oil industry faces the challenge to balance between their economic and development sustainability as well as environmental preservation.

Oil palm processing generates a highly polluting wastewater commonly known as palm oil mill effluent (POME) that can cause a severe pollution to the waterways if discharged without proper treatment due to the oxygen depletion and other subsequent related effects. Likewise, a huge amount of solid wastes are produced from the oil palm extraction process predominantly empty fruit bunches (EFB), usually unsystematically dumped on the land causing pollution to the surrounding area. POME and EFB are the largest wastes produce in oil palm productions and these huge waste are nonetheless a good source of biomass due to their high organic contents and has recently become a potential source of renewable energy in Malaysia (Bakar, 2014).

Degradation process of POME via anaerobic digestion will emit greenhouse gases (GHG) primarily methane gas that is effective in trapping heat in the atmosphere, with a global warming potential over 20 times than carbon dioxide (Tabak, 2009). Wijbrans and Van Zutphen (2005) have reported that methane release from POME equivalents to 9kg/tonne fresh fruit bunch (FFB) (at 0.7 m^3 POME/tonne FFB, 28 m^3 biogas/m^3 POME and 65% methane in the biogas). Nevertheless, the methane gas produced can be a valuable source of bioenergy due to its high calorific value.

Methane collection can be carried out via various invented technologies either in a high efficiency closed-tank anaerobic digester system or covered anaerobic pond system. Harnessed methane gas is suitable not only to substitute non-renewable energy for multiple application but also can significantly reduce the GHG emission and dependence on fossil fuels to develop a more sustainable palm oil industry. Methane gas production by the conventional method of ponding system employed by the majority of the palm oil millers for POME treatment, however, are not fully recovered for utilization. As to date, only a few palm oil mills in Malaysia are involved in recovering the biogas to be utilized as additional sources of energy in the mills or supplying the energy to the national electricity grid. This is related to the current methane production by using the conventional technologies, which is below expectation (Chin et al., 2013).

The degradation process of POME into methane consists of four main stages which include hydrolysis to disintegrate the complex polymers, acidogenesis to further break down the soluble organic compounds, acetogenesis to degrade the resulting organic acids and lastly methanogenesis that produce methane and carbon dioxide gases (Gerardi, 2003; Poh and Chong, 2009). Energy is required for these cellular activities including growth and reproduction processes which is generally derived from the breakdown of the chemical bond mainly between carbon compounds. One of the reason for low methane production by POME might be due to its unstable nutrient availability which subsequently affected the nutrient requirements of the microorganisms. Besides, a deficiency in the desired microbial colonies to execute the degradation process could also influence the methane production (Cavaleiro et al., 2010).

Thus, effective methods should be implemented to counteract the inadequacies. EFB possessed a high carbon content that is suitable to be co-digested with POME to balance the nutrient availability and subsequently to assist in producing higher methane gas (Baharuddin et al., 2010). Since POME and EFB are comprised of cellulosic compounds, bio-augmentation by cellulolytic microorganisms might assist in the degradation of the complex compounds, as well as addition of methanogenic colony which might contribute to improve the methane production.

1.2 Problem Statement

Malaysian palm oil sector consistently seeks ways to further enhance its sustainability efforts. One of the effort implemented is via Entry Point Project No 5 (EPP 5) under National Key Economic Areas (NKEA) which has been inspired by the Government in the Tenth Malaysia Plan (2011 - 2015) that aims to develop the biogas facilities at palm oil mills to boost the renewable energy sector as well as reducing the environmental impacts. Besides, Malaysian Fifth Fuel Policy targeted to achieve 5% of national grid-connected electricity generation from renewable resources, particularly biomass.

With various biogas capturing technologies offered, covered lagoon was widely opted since ponding system was used by a majority of the palm oil millers besides it is cost-effective and easier to operate. However, low methane production was observed from the covered lagoon technologies as compared to the high-tech closed anaerobic digester tank leading the millers to merely flared the methane produced instead of utilizing it for peripheral use and lowering the chances to produce electricity for grid connection. One of the reasons might be due to the unbalanced nutrient in the feedstock since POME has been indicated to have a low carbon to nitrogen (C/N) ratio. Carbon act as the main energy sources for the indigenous microbes in the feedstock, while nitrogen support in structural material metabolism process. Hence, the unstable C/N ratio will subsequently affect their growth, methane productivity, and degradability rate.

Co-digestion of POME with a high carbon source will stabilize the nutrient availability in the feedstock. EFB is a lignocellulosic material with a high amount of carbon which makes it a potential natural co-substrate in the digestion process. Nonetheless, the loading of the co-substrate has to be specifically quantified to avoid unbalance nutrient availability during the digestion process. Loading proportion of POME as the primary substrate and EFB as the co-substrate in facultative digestion estimated by C/N ratio content on weight basis has yet to be reported.

The addition of prepared microbial cultures to a biomass treatment system or known as bio-augmentation can increase the density of desired microbes and their enzymes. The addition of cellulolytic bacteria and methanogens archaea as bio-augmentation treatment in facultative digestion can assist in improving the substrate degradability and methane productivity respectively. Thus far, limited studies had been reported towards bio-augmentation of *Bacillus subtilis* and mix methanogens in methane production from POME. Loading of the bacterium has to be quantified to avoid excess microbial colony and severe competition with the indigenous microorganisms during the digestion process.

Degradability of the substrates throughout the digestion process of POME was also in a limited search. Depletion of substrates in the feedstock in terms of organic substances after the digestion process signified total degradation and degradability of the materials. High degradability of biomass sample implies a high potential for methane productivity. Thus, the degradation rate of biomass feedstock can practically estimate the methane production and methane yield. Subsequently, methane production can be enhanced by improving the degradation rate of the substrates.

1.3 Justification

As mentioned earlier, methane production was affected by numerous factors from physical, chemical and biological properties of the feedstock as well as its operational parameters. The composition of the feedstock exhibits the nutrient availability for the indigenous microorganisms and will affect the direction of the degradation process. Similarly, the performance of microbial community during the treatment process plays a significant role in determining the production of methane gas, as they are primarily the agents that accomplish the desired treatment. Hence, it is essential to distinguish characteristics of the feedstock and to understand the microbial participating in metabolic reaction during the degradation process, in developing the digestion system operational efficiency.

Methane production from POME can be enhanced with numerous techniques and technologies available. In this study, two methods were evaluated which are 1) adding a co-substrate and 2) adding a commercialized microbes to investigate their effects toward the production of methane gas and substrates degradability. The addition of co-substrate can improve deficiency of the main substrates and subsequently enhance the methane production. Palm oil mills generate various types of cellulosic waste that can potentially be used as a co-substrate and EFB was selected for its high carbon content and high availability. In addition, microbes were added to increase the desired microbial colony in degrading the organic substrate. Cellulolytic bacteria were selected to assist in degrading the cellulosic material of POME and EFB while mix methanogens were selected to further enhance the methanogenesis process and subsequently the production of methane. Meanwhile, integration of both microorganisms was conducted to overcome the limitations of individual degradation potential and for further maximizing the yield of methane gas.

Sample of POME added with EFB at different loadings of C/N ratio was experimented in Part 1 of the study to observe the effect towards methane productivity. Eight loadings of C/N ratio were selected from 25, 30, 35, 40, 45, 50 and 55 including control where no EFB was added at all. These C/N ratios were selected in the range of original C/N ratio of both POME and EFB. Methane productivity and substrate degradability were observed throughout the digestion process and optimum C/N ratio was selected from the sample with the highest production of cumulative methane.

Meanwhile, bio-augmentation was done in Part 2 of the study where commercialized microbes of *B. subtilis* and mix methanogens were added at different concentrations of microbial loading to further observe the effect towards degradability of the substrate and methane productivity. Microbial loading was selected at 5% and 10% (v/v) for *B. subtilis* and mix methanogens at an average concentration of 10^6 colony forming unit (CFU). Both loading concentrations of singular cultures were further combined to observe the effects of their integration toward substrate degradation and methane productivity.

Optimized loading of the co-substrate and microbial colony from this research can be implemented in the field to better develop the methane production system. Since the majority of palm oil millers opted to apply ponding system for the POME treatment, a substantial number of palm oil millers will be able to apply the result of this study and broaden the chances to recover the biogas produced into valuable renewable energy. This thesis presents a research which aims at assessing the effects of palm oil mills bio-wastes characteristics on the methane production and subsequently investigating ways of enhancing the methane productivity by improving the feedstock characteristics and microbial consortia.

1.4 Objectives

The general objective of this research is to study the methane production from POME through co-digestion treatment with EFB and bio-augmentation treatment with *B. subtilis* and mix methanogens, via facultative digestion system. This research was carried out with the following specific objectives:

1) To determine the physicochemical, biochemical and microbial characteristics of raw POME and EFB sample.

2) To study the effect of different C/N ratios in co-digestion treatment of POME and EFB, on methane productivity and substrate degradability rate.

3) To study the effect of microbial types (*B. subtilis* and mix methanogens) and microbial loadings in bio-augmentation treatment of single and co-digestion sample (at selected C/N ratio), on methane productivity and substrate degradability rate.

4) To evaluate the co-augmentation treatment of single and co-digestion sample (at selected C/N ratio) towards methane productivity and substrate degradability rate, by incorporating *B. subtilis* and mix methanogens at different loadings.

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