DEVELOPMENT OF INDIGENOUS BIOFILM FOR PRE-TREATMENT OF PALM OIL MILL EFFLUENT AND BIOGAS PRODUCTION

BY

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A thesis submitted in fulfilment of the requirement for the degree of Master of Science (Biotechnology Engineering)

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JUNE 2021

ABSTRACT

The process of producing crude palm oil required lots of water, creating a massive organic wastewater called palm oil mill effluent (POME). Current application favoured open ponding system which effectively treats POME to a safe level before release to the environment but have poor methane production. This research aims to develop indigenous biofilm for pre-treatment of POME in order to improve biogas production. Bacteria producing biofilm were isolated from local sources such as POME, palm kernel cake and food waste compost available in International Islamic University Malaysia (IIUM). Two bacterial isolates, *Bacillus cereus* and *Bacillus substilis* have been identified as biofilm producing bacteria with ability to excrete hydrolytic enzymes. Biofilm production was detected with crystal violet staining on the wall of the 96 well microplate containing bacterial cultures. Further study on biofilm screening showed better biofilm production for mixed bacterial cultures compared to the single culture. Addition of biofilm carrier, granular activated carbon (GAC) immobilized and mixture of identified *Bacillus* strains was found to 75% of removal of lipid content in POME compared to the control POME for hydrolysis analysis. Optimization of hydrolysis process of POME was done using Design Expert Software by Face Centered Central Composite Design (FCCCD) with two manipulated variables of total suspended solids (TSS) of POME and biofilm carrier (GAC) amount. Optimization of hydrolysis process indicated that the system was effective at 0.93% (volume of Total suspended solids/Total Volume) TSS value with no significant amount of biofilm needed. The analysis of variance (ANOVA) has showed value at $R^2 = 0.8968$ which was significant to the regression line of this model. Anaerobic digestion of biofilm mediated hydrolysed POME for biogas production was done suing One Factor at a Time (OFAT) method with two variables tested, inoculum ratio and hydraulic retention time (HRT). Anaerobic digestion of POME was found to give highest yield of gas produced for inoculum ratio of 10% (volume of activated sludge/total volume) and HRT of 25 days. Comparison study showed significant increase in volume of biogas yield by 81.92% and 52.67% to anaerobic digestion of raw POME and non-biofilm mediated POME respectively. The COD removal of the effluent was obtained within range of 85-92%. The methane content in the biogas was found to be at 79 – 84% (v/v). Positive achievement of this study will be helpful in managing the POME waste in this country by having more enhanced biogas production as a new source of energy.

خلاصة البحث

تتطلب عملية إنتاج زيت النخيل الخام الكثير من الماء ممّا يؤدّي إلى إنتاج كميات ضخمة من مياه الصرف العضوية تسمى نفايات مطحنة زيت النخيل (POME). يُفضِّل التطبيق الحالي نظام الحوض المفتوح والذي يعالج POME إلى مستوى آمن قبل إطلاقه إلى البيئة ولكنّه يتميز بإنتاج رديء لغاز الميثان. يهدف هذا البحث إلى تطوير أغشية حيوية فِطريّة لمعالجة POME من أجل تحسين إنتاج الغاز الحيوي. لذلك تمّ عزل البكتيريا المُنتِجة للغشاء الحيوي من مصادر محلية مثل POME وكعكة نواة النخيل وسماد نفايات الطعام المتوفر في الجامعة الإسلامية العالمية بماليزيا (IIUM). وتمّ التعرف على اثنتين من العزلات البكتيرية، *Bacillus cereus* و *Bacillus subilis* على أنها بكتيريا منتجة للغشاء الحيوي مع القدرة على إفراز الإنزيمات المحَلِّلة. وتمّ الكشف عن إنتاج الغشاء الحيوي (البيوفيلم) بواسطة الصبغ البنفسجي المتبلور على جدار صفيحة المعايرة الدقيقة (96 فراغاً) التي تحتوي على المزارع البكتيرية. وأظهرت دراسة لاحقة حول تحرِّي الأغشية الحيوية إنتاجًا أفضل للبيوفيلم للمزارع البكتيرية المختَلَطة مقارنةً بالمنفردة. وتمّ العثور على أنّ إضافة البيوفيلم والكربون المنشط الحبيبي (GAC) المثبّت ومزيج من سلالات البكتيريا العضوية المحددة *Bacillus* أدّى إلى إزالة 75% من محتوى الدهون في POME في مقابل عينة المقارنة المتحللة. وتمّ تحسين عملية تحلّل POME باستخدام برنامج Design Expert من خلال التصميم المركب المركزي (FCCCD) مع متغيرين معالجَين هما إجمالي المواد الصلبة العالقة (TSS) في عينة POME وكمية الناقل الحيوي (GAC). وأشار تحسين عملية التحلل إلى أن النظام كان فعالاً عند 0.93% من قيمة TSS (حجم إجمالي المواد الصلبة العالقة/ الحجم الإجمالي) دون الحاجة إلى كمية كبيرة من البيوفيلم. كما أعطى تحليل التباين (ANOVA) قيمة R^2 عند 0.8968 مما له كبير الأثر في خط الانحدار لهذا النموذج. وتمّ إجراء الهضم اللاهوائي لـعينة POME المتحللة بواسطة البيوفيلم لإنتاج الغاز الحيوي باستخدام طريقة عامل واحد في وقت (OFAT) مع متغيرين تم اختبارهما، وهما نسبة اللقاح و زمن المكوث الهيدروليكي (HRT). وأنتج الهضم اللاهوائي لعينة POME أعلى مردود للغاز عند نسبة لقاح 10% (حجم الحَمْأة المُنشَّطة/الحجم الكلي) و HRT لمدة 25 يومًا. كما أظهرت دراسة المقارنة زيادة كبيرة في حجم إنتاج الغاز الحيوي بنسبة 81.92% و 52.67% في الهضم اللاهوائي لعينة POME الخام وغير المنشّط بواسطة البيوفيلم على التوالي. وتمّ الحصول على إزالة COD من النفايات السائلة بنسبة 85-92%، بينما كان محتوى الميثان في الغاز الحيوي 79-84% (حجم/حجم). إنّ الإنجاز الإيجابي لهذه الدراسة يمكن أن يكون مفيدًا في إدارة نفايات POME في هذا البلد من خلال زيادة إنتاج الغاز الحيوي المحسّن كمصدر جديد للطاقة.

APPROVAL PAGE

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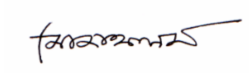

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This thesis is dedicated to my beloved family and colleagues for the tremendous support that has been bestowed upon me

ACKNOWLEDGEMENTS

All glory is due to Allah, the Almighty, whose Grace and Mercies have been with me throughout the duration of my programme. Although, it has been tasking, His Mercies and Blessings on me ease the herculean task of completing this thesis.

I am most indebted to by supervisor and co-supervisors, Prof. Dr Md Zahangir Alam, Dr Mariatul Fadzillah binti Mansor, and Dr Azlin Suhaida binti Azmi, whose enduring disposition, kindness, promptitude, thoroughness and friendship have facilitated the successful completion of my work. I put on record and appreciate their detailed comments, useful suggestions and inspiring queries which have considerably improved this thesis. Their brilliant grasp of the aim and content of this work led to his insightful comments, suggestions and queries which helped me a great deal. Despite their commitments, they took time to listen and attend to me whenever requested. The moral support they extended to me is in no doubt a boost that helped in building and writing the draft of this research work.

Lastly, my gratitude goes to my beloved family and lovely colleagues; for their prayers, understanding and endurance while away. Once again, we glorify Allah for His endless mercy on us one of which is enabling us to successfully round off the efforts of writing this thesis. Alhamdulillah.

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LIST OF ABBREVIATIONS

APHA	American Public Health Association
ANOVA	Analysis of Variance
AD	Anaerobic digestion
BCG	Bromocresol green
BOD	Biological Oxygen Demand
Bp	Base pair
CMC	Carboxymethyl cellulose
COD	Chemical Oxygen Demand
DoE	Department of Environment
EPS	Extracellular polymeric substance
FFA	Free fatty acids
gDNA	genomic deoxyribonucleic acid
GAC	Granular activated carbon
HRT	Hydraulic retention time
IIUM	International Islamic University Malaysia
LB	Luria Bertani
MW	molecular weight
m	mass
MPOB	Malaysian palm Oil Board
MPOC	Malaysian Palm Oil Council
N	Normality
OD	Optical density
OLR	Organic loading rate
PCR	Polymerase chain reaction
PKC	Palm kernel cake
POME	Palm oil mill effluent
PVA	Poly-vinyl alcohol
PEG	Polyethylene glycol

SS	Suspended solids
TFE	Tetrafluoroethylene
TPAD	Two phase anaerobic digestion
TSS	Total suspended solids
TS	Total solids
TVS	Total volatile solids
TN	Total nitrogen
V	Volume
VS	Volatile solids
VSS	Volatile suspended solids

LIST OF SYMBOLS

%	percentage
^{o}C	degree Celsius
µl	microliter
CO_2	Carbon dioxide
CH_4	Methane
g	gram
H_2O	water
kg	kilogram
m	mass
mg/l	milligram per liter
ml	milliliter
M	Molarity
MW	Molecular weight
N	Normality
nm	nanometer
V	Volume
v/v	volume per volume
w/v	weight per volume

CHAPTER ONE

INTRODUCTION

1.1 BACKGROUND

As of 2020, Malaysia Palm Oil Council (MPOC) has recorded about 25.8% and 34.3% of world's palm oil production and exports, respectively. According to Malaysia Palm Oil Board (MPOB), there are 4 sectors to palm oil mills processing (fresh fruit bunch mills, palm kernel crushers, oil refineries, and oleochemicals). In 2020, MPOB has recorded 569 working sectors with capacities over 100 million tonnes per year. As one of world's largest palm oil producer, the by-product, palm oil mill effluent (POME) is also largely generated. POME is slated to be treated prior to release to the environment due to its high organic content which is harmful to the nature, however, with recent technologies and various studies, the opportunity to utilize the high organic content to generate wealth also arises.

POME originates from oil palm processing, which requires the use of water which was estimated about 5 to 7.5 tonnes of water in order to process one tonne of crude palm oil (Wu et al, 2010a). About more than 50% of this water ends up as wastewater which is POME (Ahmad et al., 2010). This wastewater is brownish in colour, contains high amount of organic contents and solids which can harm the environment if released without prior treatment. In Malaysia, most POMEs were treated just to reduce the organic content until the acceptable level of several parameter outlined by the department of environmental (DoE) Malaysia (Chin et al., 2013a). In 2017, there were proposal to stricken the regulation of wastewater treatment due to its high toxicity (Bello & Abdul Raman, 2017).

There are several methods available for POME treatment. A review of available ongoing studies of general wastewater pretreatment/treatment technology has been compiled in 2015 by Ahmad et al (2015) which includes conventional open ponding system, upflow anaerobic sludge blanket, continuous stirred tank reactor, anaerobic filtration and others. These technologies come with their own advantages and disadvantages systematically, economically and environmentally. Malaysia commonly utilises conventional open ponding system due to its minimal setup and low operating cost. The biggest oil palm mill in Malaysia, Sime Darby Sdn. Bhd. reported the use of ponding system to treat POME in their 2019 sustainability report. However, open ponding system have many downsides such as vast land occupation and higher methane emission since the ponds are mostly open to the atmosphere. Remediation of this problem can be executed by reading, experimenting and exploring previous researches in order to obtain the benefit of POME. Biogas can be attained from POME with suitable method as POME have high organic content (Langer et al., 2014).

Several approaches have been made to enhance the production of biogas while also reducing the chemical oxygen content (COD) of any type of organic wastewater. These methods have their own pros and cons, and gaps in the studies becomes more specific and intriguing to be investigated (Ahmed et al., 2015). Improvement of biogas yield has been reported in many studies when their substrate or organic source were pre-treated prior to anaerobic digestion. Nonetheless, these studies has recorded unfavourable limiting factor in the pretreatment, such as biomass washout from a continuous stirring tank reactor resulting in low treatment efficiency (J. Wang et al., 2015). Biofilm mediated anaerobic digestion of organic wastewater is a new field of study that has yet to be explored deeply, particularly in POME wastewater.

Biofilm is a complex structure that is made of a group of bacteria that attaches themselves onto a surface and enveloped in a matrix called extracellular polymeric substances (EPS). Biofilm has been proven to improve biogas production in manure wastewater (Szentgyörgyi et al., 2010). Additionally, the size and type of biofilm carriers also plays role in both digestion of organic wastewater and biogas production (Dutta et al., 2014; Jamali et al., 2016). Introduction of biofilm in the system may potentially improve the treatment of POME, thus enhancing biogas production.

Enhanced biogas production may be a new source of energy and wealth to this country. With proper research approach, this study may yield as the booster of this country's economy.

1.2 PROBLEM STATEMENT AND SIGNIFICANCE OF STUDY

Malaysia is one of the biggest palm oil producers in the world. The process of extracting palm oil from palm oil fruit bunches produces by-product called palm oil mill effluent. Palm oil mill effluent contain very high organic content making it unsafe to undergo direct discharge to the environment. On the other hand, the high organic content is favourable to produce biogas. There are many ways to treat POME wastewater. About 85% of palm oil mills in Malaysia are adopting anaerobic pond system (Yacob et al., 2006) due to its low operational cost. However this system makes the use of vast land area and the methane produced are uncontrollable (Wu et al., 2010b). To overcome the disadvantages, anaerobic digester becomes an effective treatment technology which are more cost effective to treat POME. However, most application of anaerobic digester does not cater to the biogas yield (focused more on treatment) (Zhang et al., 2008). Biogas production can be increased with better pretreatment (digestion) (Sapci, 2013; Taherdanak & Zilouei, 2014; Tedesco et al., 2013). Biofilm based reactor to treat wastewater has been studied by other researchers and proven to be successful in reducing COD and increasing biogas production (Chai, Guoa, Chaib, Caia, & Gaoa, 2014; Dutta et al., 2014; Jamali et al., 2016; Szentgyörgyi et al., 2010). However, the system has yet to be studied on POME wastewater for enhanced biogas production. On the other hand, isolated bacteria from POME has been proven to be more effective in treating POME compared from microbes from foreign source (Bala, Lalung, & Ismail, 2015). This research will address the improvement of hydrolysis of POME with indigenous bacteria with biofilm producing ability for further enhancement. Improved pre-treatment will result in increased biogas production during anaerobic digestions of POME in a semi continuous system for better biogas entrapment.

1.3 RESEARCH OBJECTIVES

The main objective of this study is to isolate and screen locally available bacteria from organic sources with biofilm producing ability and hydrolytic enzymes secretion in order to pre-treat POME and enhancing biogas production by anaerobic digestion.

The specific objectives are as follows;

1. To evaluate potential microbes producing biofilm and hydrolytic enzymes through isolation, screening and characterization for enhanced biogas

production from palm oil mill effluent (POME) and later identification of selected and potential strains of bacteria.

2. To evaluate and optimize hydrolysis process of POME using selected characterized microbes producing biofilm.
3. To determine process parameters of anaerobic reactor with biofilm mediated pretreatment strategy in semi-continuous process for high rate of biogas production.

1.4 SCOPE OF STUDY

The scopes of this research are as follows;

a. Only bacteria producing biofilm isolated from locally available organic waste sources were used to maintain indigenous condition.
b. Hydrolysis and anaerobic digestion of POME were done in laboratory scale.
c. Biofilm carrier used was granular activated carbon (GAC) only (readily available in laboratory).
d. Bacterial isolates must produce biofilm and hydrolytic enzymes amylase, lipase, cellulase to pre-treat POME effectively.
e. Hydrolysis of POME for pre-treatment with bacterial mixture with biofilm and hydrolytic enzymes producing ability should have better performance compared to control POME and non-added biofilm carrier POME.
f. Hydrolysis progress will be measured by studying the lipid removal and carbohydrate removal.
g. Optimization of hydrolysis process will be by Design Expert software by FCCCD method with two variables manipulated which are TSS value for substrate (POME) concentration and amount of carrier (biofilm concentration).

h. Anaerobic digestion of biofilm mediated pre-treated POME will be studied by adjusting the inoculum of anaerobic bacteria (anaerobic sludge) ratio to the pre-treated POME and HRT.

i. Enhancement of biogas production from biofilm mediated pre-treated POME will be studied by comparing the volume of biogas produced with non-biofilm mediated pre-treated POME and untreated POME.

1.5 THESIS ORGANIZATION

This thesis consist of 5 chapters which includes chapter one that covers on the background information, problems statements, objectives, scope of studies, and the overall flow of this study. Chapter two includes the literatures on previous researches on biogas production from POME, pre-treatment applications, biofilms and process parameters of anaerobic digestion organic substance. Chapter three focuses on the detailed methodology of experiments applied in this study. Chapter four presents the results and discussion of each findings on biofilms application for pre-treatment and biogas production from POME and chapter five highlights the findings and the conclusions of this study.

CHAPTER TWO

LITERATURE REVIEW

2.1 INTRODUCTION

This chapter focuses on previous study on biogas production in order to provide detailed background for the nature of raw materials used in this research. This is followed by descriptions of existing pre-treatment processes for the raw materials. Latest findings on biogas production using POME were reviewed in order to elucidate the current status in this area of study. Literature review also entailed the sections that describes the operating parameters for anaerobic fermentation of POME. Finally, summary information was provided with respect to research gap available to be explored by researcher at the end of this chapter.

2.2 PALM OIL MILL EFFLUENT

Palm oil mill effluent (POME) is a waste by-product from palm oil industry. POME was generated from during the process of crude palm oil extraction that involves washing and cleaning process in the mill which incorporate massive water usage thus generating wastewater (Ahmed et al., 2015). According to Malaysian Palm Oil Council (MPOC), this country currently accounts for 28% of world palm oil production and 33% world exports, making Malaysia as one of the biggest producers and exporters of palm oil and palm oil products. This indirectly also contributes to large POME generation, which calls for studies to manage or waste utilization.

POME visually have thick brownish, viscous mixture of oil, grease and water with an unpleasant odour. The general characteristics of POME was summarized in Table 2.1, which shows that POME have high concentration of chemical oxygen demand (COD), biological oxygen demand (BOD) and total suspended solids (SS). The content of nitrogen in the form of ammoniacal nitrogen contains organic acids can be used as carbon sources (Md and Yunus, 2006).

Table 2.1 General characteristic of POME

Parameters	Concentration range
Chemical oxygen demand (COD)	15000 – 100000 mg/l
Biochemical oxygen demand (BOD_5, 30^{o}c)	10250 – 43750 mg/l
Total solid (SS)	11500 – 79000 mg/l
Total suspended solid (TSS)	2000 – 54000 mg/l
Total volatile solid (TVS)	9000 – 72000 mg/l
Total nitrogen (TN)	180 – 1400 mg/l
Ammoniacal nitrogen	4 – 80 mg/l
Oil and grease	130 – 18000 mg/l
Temperature	80 – 90^{o}c
pH	3.4 – 5.2
Colour (ADMI)	> 5000

Source: Ahmed et al. (2015)

The carbon content in POME calls describes it as a high organic waste which is desirable in the renewable energy generation, particularly biogas production. The opportunity to turns waste into wealth breaks into many researches done to have optimum biogas production.

2.3 BIOGAS

Biogas is a renewable source of energy that is obtained through digestion of organic content by microorganism. The organic feedstock can be obtained from various sources such as animal waste, plant waste, municipal wastewater, sewage sludge and