OIL AND GREASE TREATMENT USING INDIGENOUS BACTERIAL BIOFILM SYSTEM

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Specially dedicated to my beloved

Family, Lecturers and Friends

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ABSTRACT

The Malaysian palm oil industry grows rapidly as the premier agriculture-based industry over the last two decades. However, the production of palm oil also resulted in the generation of large quantities of polluted wastewater commonly referred to as palm oil mill effluent (POME) as well as lignocellulosic biomass, especially from fronds, trunks, empty fruit bunch, palm kernel and mesocarp fibre. This study evaluates on the potential application of an immobilized bacterial-based system to reduce oil and grease (O&G) contamination in POME. From the 21 bacterial isolates, two isolates (i.e. isolate X7 and X10) were chosen for subsequent studies based on its high cell surface hydrophobicity, opaque halo formation and high culture turbidity. The bacterial isolates were evaluated for O&G degradation ability by varying contact time, initial POME concentration and initial pH. The isolated X10 showed the ability to completely degrade O&G from POME after 6 days of incubation at 200 rpm, 30^{o}C and pH 7 in the batch study. In the continuous flow-through column study, oil palm frond (OPF) - immobilized isolate X7 showed complete O&G degradation and 92.59% of chemical oxygen demand (COD) degradation from 100% (v/v) POME used after 8 days of contact time. Scanning Electron Microscope examination showed the presence of diverse morphology of microorganisms indicating the formation of mature biofilm after 200 days. One of the bacterial species isolated from the biofilm was identified as *Bacillus cereus* in whichmatch up to 99% of the query sequence. The finding of this study indicates the potential of using indigenous aerobic bacterial isolates as an alternative solution to degrade O&G in POME wastewaters.

ABSTRAK

Industri minyak sawit Malaysia berkembang dengan pesat sebagai industri berasaskan pertanian yang terulung sejak dua dekad yang lalu. Walau bagaimanapun, pemprosesan minyak kelapa sawit juga menyebabkan penghasilan kuantiti air sisa tercemar yang banyak, seringkali dirujuk sebagai buangan air sisa kilang minyak sawit (POME) atau dikenali sebagai biojisim lignoselulosik,khususnya pelepah, batang, buah tandan kosong, isirong sawit dan gentian mesokarp. Kajian ini memberi tumpuan terhadap penilaian potensi sistem bakteria tersekat gerak untuk mengurangkan pencemaran minyak dan gris (O&G) dalam POME. Berdasarkan 21 pencilan bakteria awal, dua pencilan bakteria (iaitu pencilan X7 dan X10) telah dipilih untuk kajian selanjutnya berdasarkan nilai yang tinggi bagi permukaan sel, formasi halo legap dan nilai kekeruhan kultur. Pencilan bakteria telah dinilai bagi keupayaan menguraikan O&G dengan mempelbagaikan masa tindakbalas, kepekatan asal POME dan pH asal. Pencilan X10 menunjukkan keupayaan untuk mendegradasi secara lengkap O&G dari POME selepas tempoh pengeraman selama 6 hari pada 200 rpm, 30^{o}C dan pH 7 menerusi kajian kelompok. Bagi kajian menggunakan aliran turus yang berterusan, pencilan X7 yang tersekat gerak pada pelepah kelapa sawit (OPF) menunjukkan keupayaan untuk mendegradasi secara lengkap O&G dan degradasi keperluan oksigen kimia (COD) pada tahap 92.59% daripada 100% (v/v) POME yang digunakan selepas 8 hari masa tindakbalas. Analisis mikroskop elektron pengimbas menunjukkan kehadiran morfologi pelbagai jenis mikroorganisma yang menandakan pembentukan biofilem matang selepas 200 hari. Salah satu daripada spesis bakteria yang diasingkan daripada biofilem telah dikenalpasti sebagai *Bacillus cereus* dengan nilai padanan setinggi 99%. Hasil daripada kajian ini menunjukkan potensi penggunaan pencilan bakteria indigenus aerobik sebagai penyelesaian alternatif untuk menguraikan O&G dalam air sisa buangan POME.

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LIST OF SYMBOLS

°C	-	Degree Celsius
%	-	Percentage
µg	-	Microgram
µL	-	Microliter
g	-	gram
d	-	Day
hr	-	Hour
kpa	-	kilopascal
kg	-	kilogram
K	-	Kelvin
rpm	-	revolutions per minute
ppm	-	parts per million
m	-	meter
mg	-	milligram
mM	-	millimolar
M	-	Molar
min	-	Minute
mL	-	Milliliter
mp	-	melting point
v/v	-	volume/volume

LIST OF ABBREVIATIONS

ADMI	-	American Dye Manufacturing Institute
HRT	-	Hydraulic retention time
NA	-	Nutrient Agar
NB	-	Nutrient Broth
LB	-	Luria Bertani
FT-IR	-	Fourier Transform Infrared
OPF	-	Oil Palm Frond
SEM	-	Scanning Electron Microscopy
COD	-	Chemical Oxygen Demand
BOD	-	Biological Oxygen Demand
TSS	-	Total Suspended Solid
VSS	-	Volatile Suspended Solid
TOC	-	Total Organic Carbon
POME	-	Palm Oil Mill Effluent
O&G	-	Oil and Grease
OD	-	Optical Density
BATH	-	Bacterial Adherence to Hydrocarbon
E_{24}	-	Emulsification Index
CFU	-	Colony Forming Unit
EPS	-	Exopolymeric substances
PCR	-	Polymerase Chain Reaction
DNA	-	Deoxyribonucleic acid
rRNA	-	Ribosomal Ribonucleic Acid
FASTA	-	DNA and protein sequence alignment
BLAST	-	Basic Local Alignment Search Tool
NCBI	-	National Centre for Biotechnology Information
IUPAC	-	International Union of Pure and Applied Chemistry

BJH	-	Barrett-Joyner-Halenda
SDS	-	Sodium Dodecyl Sulfate
PBS		Phosphate Buffer Saline
NaCl	-	Sodium Chloride
MeOH	-	methanol
HCl	-	Hydrochloric acid
H_2SO_4	-	Sulphuric acid
NaOH	-	Sodium hydroxide
CaCl	-	Calcium chloride
CH_2Cl_2	-	Dichloromethane

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CHAPTER 1

INTRODUCTION

1.1 Background of the Study

The Malaysian palm oil industry has grown rapidly over the past few years to become the world's second largest producer of palm oil, accounting for 46% of the world exports and 37% of world palm oil production in 2011 (according to the Malaysian Palm Oil Council, MPOC), (2011). The year 2011 was both remarkable and significant for the Malaysian palm oil industry which recorded its highest ever earnings of RM 80.4 billion, surpassing the RM 65.2 billion mark recorded in 2008 by 23.3% (MPOC, 2011; Wu *et al*., 2010). The palm oil industry also provides a source of livelihood to rural families in government land schemes and private small holders, as well as employment opportunities to agricultural workers in estates (Wu *et al*., 2010).

Malaysia has implemented a wet process for palm oil milling. Even though the process is effective, it is not suitable to be applied in large scale productions (Prasertsan and Prasertsan, 1996). This process involves sterilizing, stripping and threshing of bunches to free the palm fruit, in which huge amounts of water and steam is required for washing and sterilizing. Other operational processes in the palm oil mills produce waste load in the form of gaseous emissions from boilers and incinerators, solid wastes materials and by-products such as empty fruit bunch, potash ash, palm kernel, fiber, shells and liquid waste. During the process, more than 50% of the water will be discharged to the environment as palm oil mill effluent

(POME), while the rest are lost either as steam in the boilers blow-down, washing water or leakage (Ahmad *et al*., 2003a).

POME has always been regarded as the most significant pollutant from palm oil milling activities (Poh and Chong, 2009). POME is a viscous, brownish liquid containing about 95–96% water, 0.6–0.7% oil and 4–5% total solids that includes 2–4% total suspended solids (TSS) respectively (Salihu *et al*., 2011). It is acidic (pH 4–5), hot (80–90°C), non-toxic (as no chemicals added during oil extraction), high organic content (COD 15,000–100,000 mg/L, BOD 10,250–43,750 mg/L) and contains appreciable amounts of plant nutrients such as phosphorus, potassium, calcium, magnesium, zinc and sodium (Najafpour *et al.*, 2005). It was estimated that for every tonne of crude palm oil produced, about 5–7.5 tonnes of water is required with more than 50% (about 2.5–3.5 tonne) ends up as POME (Norli *et al.*, 2006). It was then estimated that more than 40 million tonnes of POME could be generated from 372 mills throughout Malaysia (Yacob *et al*., 2006). Most of the POME produced originates from different sources such as sterilizer condensate, hydrocyclone waste and clarifier sludge in the form of liquid effluent from the large amounts of steam or hot water used (Norli *et al.*, 2006).

In Malaysia, over 85% of the mills use the conventional anaerobic ponding system as the main treatment system for POME. The system usually based on the suspended growth of activated sludge with relatively low operation cost or something called as facultative pond. Other processes employed include aerobic and anaerobic digestions, physicochemical treatment and membrane filtration (Wu *et al*., 2010). In Malaysia, all discharged effluents must adhere to the regulations as outlined in the Environmental Quality Act, 1974. Anaerobic process or biological treatment has considerable advantages over other processes such as less energy demands, minimum sludge formation and production of methane due to efficient break down of organic substances by anaerobic bacteria (Rincón et al., 2006).

1.2 Problem Statement

Oil and grease (O&G) is one of the major organic-rich compounds that directly contribute to severe environmental pollution as well as affecting biological wastewater treatment processes where the high-organic load may lead to system shut-down due various number of reason such as toxicity to the microbial population in the activated sludge, incomplete degradation process and huge volumes of sludge being generated. O&G would form layers on water surface that may prevent the dissolution of oxygen and reduce the oxygen transfer rates into the aqueous environment (Prasad and Manjunath, 2011). Being the preferred method for the treatment of POME in Malaysian palm oil mills, the ponding system has received a lot of attention from researchers notably in Malaysia. However, amongst notable disadvantages in the ponding system includes the requirement for large land area (to accommodate a series of ponds and volume of wastewater) and long hydraulic retention time (HRT), up to 20 days for each pond. Therefore, there is need to have alternative treatment system that requires small land area and able to shorten the HRT. This approach is expected to substantially reduce the operating cost and also minimalize the impact to the environment.

1.3 Objectives of Study

i. To isolate, identify and characterize O&G degrading bacterial species from POME in batch and immobilized.
ii. To evaluate the feasibility of using the indigenous O&G-degrading bacteria in an immobilized continuous flow O&G removal system.
iii. To optimize indigenous O&G-bacteria degradation ability in batch and column study.

1.4 Scope of Study

This study focuses on the degradation of O&G in the mixing anaerobic pond from POME using biological treatment system and oil palm frond biomass as a support material. POME was characterized for biological and chemical parameters where the isolated indigenous O&G-degrading bacteria were determined for its O&G-degradation properties in POME. Oil palm frond was used as support material for the immobilization of bacteria for biofilm development. Factors that may affect optimum O&G degrading performance of the bacteria such as pH of the system, HRT, flowrate, influent and effluent COD concentrations and O&G contents were evaluated.

1.5 Significance of the Study

High concentration of O&G could cause severe environmental pollution and conflicts in water treatment process. Therefore, the estimated outcome of this study is initial indication on the feasibility of using an indigenous O&G-bacteria as alternative biological treatment system for the treatment of POME prior to entering the anaerobic pond (primary treatment system). It is expected that the retention time for the anaerobic system will be reduced significantly, hence reducing the overall treatment period for POME. Therefore, this alternative biological treatment may be suggested or implemented in the palm oil mills for its O&G degradation capability.

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