

MICROBIAL LIPID ACCUMULATION
THROUGH BIOREMEDIATION OF PALM OIL
MILL EFFLUENT BY CO-CULTURING YEAST
AND BACTERIA

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ABSTRAK

Pelepasan efluen kilang kelapa sawit (POME) ke tanah pertanian menyebabkan pencemaran alam sekitar kesan daripada ketinggian kepekatan komponen fenolik, keperluan oksigen kimia (COD) dan keperluan oksigen biokimia (BOD). Malah, penyusutan progresif bahan api fosil dan sumber mineral turut dikenal pasti sebagai cabaran pada masa hadapan. Kaedah penghasilan lipid mikrobial secara serentak melalui rawatan air sisa berpotensi menjadi penyelesaian terhadap kedua-dua isu tersebut. Kajian ini bertujuan untuk menghasilkan lipid mikrobial menggunakan bakteria dan yis oleaginous yang kuat iaitu *Bacillus cereus* (*B. cereus*) dan *Lipomyces starkeyi* (*L. starkeyi*) melalui bioremediasi POME dengan penapaian secara berkelompok. Kepekatan substrat POME yang berbeza (25%, 50%, 75%, dan 100%) digunakan sebagai nutrien untuk mengkaji tahap optimum kepekatan POME bagi pengeluaran hasil biojisim dan lipid paling maksimum. Hasil pemerhatian menunjukkan larutan POME yang mempunyai kepekatan sederhana (POME 50%) mempunyai kadar pertumbuhan mikrobial dan pengumpulan lipid yang lebih tinggi serta tahap bioremediasi yang signifikan. Tahap bioremediasi dinilai menggunakan beberapa parameter sisa air (BOD, COD, jumlah fenol, jumlah karbon organik dan lain-lain) dan indeks percambahan benih (GI) kacang hijau (*Vigna radiata*). POME yang dirawat menggunakan gabungan inokulum kultur (*B. cereus* dan *L. starkeyi*) menunjukkan penurunan ketara kadar pencemaran, khususnya COD bagi POME 50%, iaitu kecekapan penyingkiran sebanyak 83.66%. POME tersebut turut mencapai nilai GI yang lebih tinggi berbanding sampel yang lain (dirawat menggunakan kultur tulen dan tanpa rawatan) kesan daripada remediasi yang ketara terhadap kehadiran organik berbahaya di dalam POME seperti yang dibuktikan oleh analisis Gas Kromatografi-Spektometri Jisim (GC-MS). Gabungan inokulum kultur telah menyumbang kepada pertumbuhan biojisim tertinggi (9.16 g/L) dan penghasilan lipid (2.21 g/L), dengan kandungan lemak 24.12% (berdasarkan berat kering) dalam 50% (v/v) POME. Komposisi lipid dianalisa berdasarkan metil ester asid lemak menggunakan GC-MS. Kajian mendapati bahawa C16 dan C18 adalah asid lemak utama dalam lipid inokulum kultur yang membolehkan lipid mikrobial dapat digunakan sebagai bahan biodiesel. Satu kaedah baru pengekstrakan lipid, iaitu elektroporasi (EP) telah digunakan untuk mengekstrak lipid mikrobial dan kecekapan EP turut dibandingkan dengan beberapa kaedah konvensional yang lain. EP menunjukkan tahap kecekapan pengekstrakan lipid yang lebih tinggi sebanyak 31.88% (wt.%) berbanding dengan kaedah ultrabunyi (11.89%), reagen Fenton (16.80%), dan pengekstrakan pelarut (9.60%). Pengaruh parameter kajian seperti komposisi inokulum, pH, suhu, dan masa bagi penilaian kecekapan proses penyingkiran COD dan penghasilan lipid dioptimumkan menggunakan kaedah rangsangan permukaan. Pengoptimuman gabungan inokulum kultur menunjukkan bahawa komposisi inokulum, pH, suhu, dan masa mempunyai kesan yang signifikan terhadap prestasi penyingkiran COD dan pengumpulan lipid. Kecekapan maksimum penyingkiran COD sebanyak 86.54% dan pengumpulan lemak 2.95 g/L tercapai dengan komposisi inokulum, pH, suhu, dan masa inkubasi masing-masing adalah 50:50, 6.50, 32.5 °C dan 90 h. Oleh itu, hasil kajian ini menunjukkan bahawa gabungan kultur *B. cereus* dan *L. starkeyi* adalah inokulum yang berpotensi untuk mencapai pertumbuhan biojisim dan penghasilan lipid yang lebih tinggi dengan bioremediasi POME. Pendekatan kaedah gabungan bagi mencapai dwi-objektif kajian (bioremediasi POME dan penghasilan lipid mikrobial) memberikan sebuah strategi baru kepada pengilang minyak kelapa sawit.

ABSTRACT

The discharge of palm oil mill effluent (POME) on arable land causes large amounts of environmental distress due to its high concentration of phenolic compounds, chemical oxygen demand (COD), and biochemical oxygen demand (BOD). On the other hand, the progressive depletion of fossil fuels and mineral resources have also been identified as a future challenge. The approach of simultaneous microbial lipid production through the wastewater treatment could be a potential option to address both renewable energy production and environmental resilience. This study aims to produce microbial lipids using robust oleaginous bacteria and yeast of *Bacillus cereus* (*B. cereus*) and *Lipomyces starkeyi* (*L. starkeyi*) through the bioremediation of POME in batch mode fermentation. Different concentrations of POME substrates (25%, 50%, 75%, and 100%) were used as nutrients to determine the optimum POME concentration for achieving maximum yield of biomass as well as lipid production. It was observed that among the different dilutions, the moderately diluted solution of POME (50% POME) showed higher microbial growth and lipid accumulation and offered a significantly higher degree of bioremediation. The degree of bioremediation was assessed by evaluating several wastewater parameters (*i.e.*, BOD, COD, total phenol, total organic carbon, *etc.*) and determining the seed germination index (GI) of Mung bean (*Vigna radiata*). POME treated with a co-culture inoculum (*B. cereus* and *L. starkeyi*) substantially reduced the pollution load, particularly, in COD for 50% POME, thus demonstrating a removal efficiency of 83.66%. Furthermore, POME treated with co-culture inoculum obtained a higher GI value than the other samples (treated by pure cultures and untreated) due to the significant remediation of detrimental organics present in the POME as evidenced by Gas Chromatography-Mass Spectrometry (GC-MS) analysis. Nevertheless, the co-culture inoculum was found to have potential for the highest biomass growth (9.16 g/L) and lipid accumulation (2.21 g/L), with a lipid content of 24.12% (dry weight basis) in the 50% (v/v) POME. Lipid composition was analyzed in terms of fatty acid methyl esters using GC-MS. C16 and C18 were found to be the predominant fatty acids in the lipid of co-culture inoculum suggesting the potential of microbial lipid to be used as a biodiesel feedstock. A novel lipid extraction method, namely electroporation (EP) was used to extract microbial lipid and the efficiency of EP was compared with some other conventional methods. The EP demonstrated a higher lipid extraction efficiency of 31.88% (wt.%) compared to the ultrasound (11.89%), Fenton's reagent (16.80%), and solvent extraction (9.60%). Finally, the influence of several process parameters such as inoculum compositions, pH, temperature, and time on the performance of the COD removal efficiency and lipid accumulation were optimized using response surface methodology. Optimization of co-culture inoculum showed that the inoculum composition, pH, temperature, and time had a significant effect on the performance of the COD removal and lipid accumulation. The maximum COD removal efficiency of 86.54% and lipid accumulation of 2.95 g/L could be obtained while the inoculum composition, pH, temperature, and incubation time were 50:50, 6.50, 32.5 °C, and 90 h, respectively. Therefore, the results of this study suggest that the co-culture of *B. cereus* and *L. starkeyi* could be a promising inoculum for attaining higher biomass growth and lipid production in conjunction with the bioremediation of POME. This combined approach of achieving dual objectives (bioremediation of POME and microbial lipid production) that is utilized in the present study provides a novel strategy for palm oil millers.

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LIST OF SYMBOLS

V	Voltage
Hz	Hertz
SD	Standard Deviation
°C	Degree Celsius
Min	Minute
cm	Centimeter
D	Diameter
d	Days
g	Gram
s	Second
h	Hour
kg	Kilogram
L	Length
M	Micro Mole
m ³	Cubic Meter
V	Volume
μ	Micro (10 ⁻⁶)
C	Carbon
N	Normality
kV	Kilovolts (10 ³)
μs	Microsecond (10 ⁻⁶)
ms	Millisecond (10 ⁻³)
J	Joule
L	Liter
mL	Milliliter
π	Pi
σ	Sigma
atm	Standard atmosphere
Ω	Ohm
kJ	Kilojoule
W	Watt

kW	Kilowatt (10^3)
f	Frequency
T	Period
Σ	Summation
b_i	Linear coefficient
b_o	Constant coefficient
b_{ii}	Quadric coefficient
b_{ij}	Interaction of coefficient, x_i, x_j coded values
x_1	Concentration of inoculum A
x_2	Substrate pH
x_3	Temperature
x_4	Operational time
y_1	COD removal efficiency
y_2	Lipid accumulation

LIST OF ABBREVIATIONS

GHGs	Greenhouse Gases
AD	Anaerobic Digestion
VS	Volatile Solid
AS	Activated Sludge
BOD	Biochemical Oxygen Demand
TAGs	Triacylglycerols
COD	Chemical Oxygen Demand
PUFA	Polyunsaturated Fatty Acids
C/N	Carbon to Nitrogen Ratio
HRT	Hydraulic Retention Time
H ₂ O ₂	Hydrogen Peroxide
OLR	Organic Loading Rate
SCOs	Single-cell Oils
POME	Palm Oil Mill Effluent
TI	Treatment Intensity
DAGs	Diacylglycerols
SS	Suspended Solids
TN	Total Nitrogen
TOC	Total Organic Carbon
TPC	Total Phenolic Content
TSS	Total Suspended Solids
VFA	Volatile Fatty Acids
VSS	Volatile Suspended Solids
NN	Nitrate Nitrogen
AN	Ammoniacal Nitrogen
TS	Total Solids
TDS	Total Dissolved Solids
CH ₄	Methane
CO ₂	Carbon Dioxide
CO	Carbon Monoxide
DC	Direct Current

OD	Optical Density
EP	Electroporation
IRE	Irreversible Electroporation
RE	Reversible Electroporation
GC-MS	Gas Chromatography and Mass Spectrophotometry
FESEM	Field Emission Electron Microscopy
FAME	Fatty Acid Methyl Esters
CCD	Central Composite Design
SMR	Steam Methane Reforming
WGSR	Water Gas Shift Reaction
FFAs	Free Fatty Acids
FAME	Fatty Acid Methyl Ester
FAAE	Fatty Acid Alkyl Ester
DNA	Deoxyribonucleic Acid
PEF	Pulsed Electric Field
SCOD	Soluble Chemical Oxygen Demand
TCOD	Total Chemical Oxygen Demand
ECP	Exocellular Polymers
PHA	Polyhydroxyalkanoates
PHB	Polyhydroxybutyrate
FD	Factorial Design
ANOVA	Analysis of Variance
OFAT	One Factor at A Time
BBD	Box-Behnken Design
PBD	Plackett-Burman Design
DoE	Design of experiment
RSM	Response Surface Methodology
CFU	Colony Forming Units
DO	Dissolved Oxygen
GI	Germination Index
PMW	Pulse-Width Modulation
IDE	Arduino Software
DI	Deionization

LB	Luria Bertani
mV	Millivolt
rpm	Revolutions per Minute
mM	Milli Mole
mg/L	Milligram per liter
g/L	Gram per liter
APHA	American Public Health Association

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