OPTIMIZATION OF SOLID STATE FERMENTATION PARAMETERS FOR BIOETHANOL PRODUCTION FROM WASTE GLYCEROL USING IMMOBILIZED *ESCHERICHIA COLI*

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DOCTOR OF PHILOSOPHY

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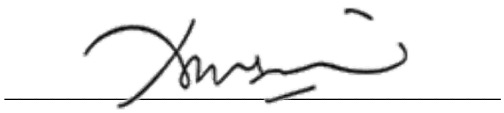

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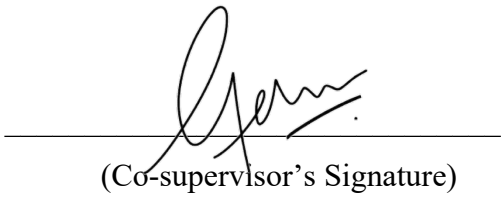

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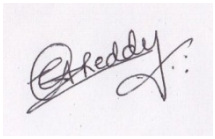

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ABSTRAK

Pembangunan perindustrian dan ekonomi yang pesat menggunakan bahan api fosil dan sumber tenaga lain. Rizab minyak yang terhad, isu alam sekitar dan kos pengangkutan yang tinggi membawa kepada penerokaan bahan api yang boleh diperbaharui dan mampan. Berbanding dengan bahan api berasaskan karbon, biodiesel menarik perhatian seluruh dunia sebagai biofuel untuk mengurangkan pergantungan global terhadap bahan api fosil dan kesan rumah hijau. Semasa pengeluaran biodiesel, kira-kira 10% gliserol terbentuk sebagai hasil sampingan. Malaysia adalah salah satu pengeluar minyak sawit terbesar di pasaran global; justeru, minyak sawit digunakan sebagai sumber utama pengeluaran biodiesel. Di Malaysia, 480 juta liter biodiesel dihasilkan pada 2017 dan dijangka mencecah 815 juta liter pada 2027, menunjukkan peningkatan sebanyak 66%. Selain itu, sisa industri minyak sawit adalah satu cabaran besar untuk memberikan kelebihan ekonomi yang ketara melalui pendekatan yang mampan. Oleh itu, terdapat keperluan mendesak untuk mencari cara berkompromi bagi mengimbangi perlindungan alam sekitar dan penggunaan semula mampan sisa gliserol daripada industri biodiesel. Dalam penyelidikan ini, matlamat kami adalah untuk menghasilkan bioetanol daripada sisa biodiesel menggunakan sel Escherichia coli yang tidak bergerak melalui penapaian keadaan pepejal. Sisa gliserol daripada industri biodiesel digunakan sebagai sumber karbon untuk penukaran kepada etanol tanpa rawatan primer menggunakan *Escherichia coli*. Sel *Escherichia coli* dipegunkan menggunakan Na alginat. Kestabilan, kebocoran dan kitar semula sel pegun telah dikaji untuk penapaian keadaan pepejal. Kromatografi cecair berprestasi tinggi digunakan untuk menganalisis kepekatan gliserol dan etanol. Spektroskopi inframerah transformasi Fourier digunakan untuk menganalisis kumpulan berfungsi gliserol dan etanol. Etanol yang telah dihasilkan telah dioptimumkan oleh Metodologi Permukaan Tindak Balas. Parameter bio-proses penting seperti inokulum (20%), jisim substrat (20 gram) dan masa (12 jam) dioptimumkan untuk mencapai pengeluaran etanol maksimum sebanyak 10.0 g/L oleh sel *E.coli* pegun. Kajian kinetik sel bebas dan sel pegun telah dikaji. Keputusan yang diperoleh untuk kadar pertumbuhan spesifik maksimum μmaks ialah 0.028 h^{-1} dan KS 6.23 g diperoleh untuk sel pegun, manakala bagi sel bebas, kadar pertumbuhan maksimum μmaks ialah 0.025 h^{-1} dan K_S sebanyak 5.11g. Kajian ini berjaya menggunakan sisa gliserol hasil sampingan biodiesel sebagai sumber karbon untuk penapaian, menggunakan sel *E.coli* pegun untuk menghasilkan etanol.

ABSTRACT

The rapid industrial and economic development runs on fossil fuels and other energy sources. Limited oil reserves, environmental issues, and high transportation costs lead towards carbon unbiased renewable and sustainable fuel. Compared to other carbon-based fuels, biodiesel is attracted worldwide as a biofuel to reduce global dependence on fossil fuels and the greenhouse effect. During biodiesel production, approximately 10% of glycerol is formed as byproduct. Malaysia is one of the largest producers of palm oil in the global market; thus, palm oil is used as the primary source for biodiesel production. In Malaysia, 480 million liters of biodiesel was produced in 2017 and is expected to reach 815 million liters in 2027, showing a 66% increase. Valorizing industrial waste is a big challenge to provide a significant economic advantage through the sustainable approach. Hence, there is an urgent need to find a compromising way to balance the environmental protection and sustainable reuse of the glycerol residue from the biodiesel industry. Our present work aims to produce bioethanol from biodiesel waste using immobilized *Escherichia coli* cells by solid-state fermentation. Hence, glycerol waste from the biodiesel industry is used as the carbon source for bioconversion to ethanol without primary treatment using *Escherichia coli*. *Escherichia coli* cells were immobilized using Na alginate. The stability, leakage and recycling of immobilized cells were studied for solid-state fermentation. High-performance liquid chromatography was used to analyze the concentration of glycerol and ethanol. Fourier transform infrared spectroscopy was used to analyze the functional group of glycerol and ethanol. Ethanol that has been produced was optimized by Response Surface Methodology. The important bio-process parameters such as inoculum (20%), mass substrate (20 g) and time (12 h) optimized to achieve maximal ethanol production of 10.0 g/L by immobilized *E.coli* cells. The kinetic study of free cells and immobilized cells were studied. The results obtained for maximum specific growth rate μ_{max} was 0.028 h^{-1} and K_S 6.23 g obtained for immobilized cells, while for the free cells, maximum growth rate μ_{max} was 0.025 h^{-1} and K_S of 5.11g. This study successfully used the biodiesel byproduct waste glycerol as the carbon source for the fermentation, using immobilized *E.coli* cells to produce ethanol.

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LIST OF SYMBOLS

°C	degree Celsius
Ca^{2+}	ion calcium
µm	micrometer
%	Percentage
3-D	Three- dimensional
g mol^{-1}	gram per mol
g/L	gram per liter
g	gram
kPa	kiloPascal
h	hour
L	liter
min	minute
ml	milliliter
n	Variable quantity
NAD^{+}	nicotinamide adenine dinucleotide
nm	nanometer
MT	metrik tons
OD	optical density
Pa.s	Pascal per second
rpm	rotation or revolution per minute
R_t	retention time
v/v	volume per volume
w/v	weight per volume
w/w	mass fraction (mass per mass)

LIST OF ABBREVIATIONS

FFV	flexible fuel vehicles
ETBE	ethyl tertiary butyl ether
DEFC	direct ethanol fuel cells
PEMFC	polymer electrolyte membrane fuel cells
ICE	internal combustion engine
ANOVA	analysis of variance
CFU	colony forming unit
DM	dried matter
DOE	design of experiment
FFD	full factorial design
FTIR	Fourier Transform Infrared Spectroscopy
DM	dried matter
DOE	design of experiment
FFD	full factorial design
FTIR	Fourier Transform Infrared Spectroscopy
H_2SO_4	Sulfuric Acid
HPLC	high performance liquid chromatography
MONG	Matter-non-glycerol
NaOH	Sodium Hydroxide
Na_2CO_3	Sodium Carbonate
$C_6H_{12}O_6$	Glucose
$NH_4H_2PO_4$	Ammonium phosphate monobasic
NaCl	Sodium chloride
K_2HPO_4	potassium phosphate dibasic
$MgSO_4$	magnesium sulfate
OD	optical density
OFAT	one-factor-at-a-time
LB	lysogeny broth
RID	refractive index detector
RSM	response surface methodology

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