THE INFLUENCE OF MICROBIAL MUTUALISTIC INTERACTIONS AND BIOFILM FORMATION ON THE PERFORMANCE OF MICROBIAL FUEL CELL

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SUPERVISOR'S DECLARATION

We hereby declare that we have checked this thesis, and, in our opinion, this thesis is adequate in terms of scope and quality for the award of the degree of Doctor of Philosophy.

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I hereby declare that the work in this thesis is based on my original work except for quotations and citations which have been duly acknowledged. I also declare that it has not been previously or concurrently submitted for any other degree at Universiti Malaysia Pahang or any other institutions.

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LIST OF SYMBOLS

x_1	Inoculum composition
x_2	Substrate pH
x_3	Operational time
x_4	Initial chemical oxygen demand (COD) of substrate
y_1	Power density of microbial fuel cell performances (W/m^3)
y_2	COD removal efficiency
F	Faraday's constant
h	Hour
I	Current
P	Power
R_Ω	Ohmic resistance
R_{ct}	Charge transfer resistance
R_{dif}	Diffusion resistance
V	Voltage
W	Watt
E_e^{anode}	Anode potential
$E_e^{cathode}$	Cathode potential
Σ_η^{anode}	Anode overpotential
$\Sigma_\eta^{cathode}$	Cathode overpotential
ΔE_η	Overpotential difference between anode and cathode
ΔE_Ω	Ohmic voltage losses
ΔE	Real cell voltage
b_i	Linear coefficient
b_o	Constant coefficient
b_{ii}	Quadric coefficient
b_{ij}	Interaction of coefficient, x_i, x_j coded values
Σ	Summation

LIST OF ABBREVIATIONS

ANOVA	Analysis of variance
ATP	Adenosine triphosphate
AEM	Anion exchange membrane
APHA	American Public Health Association
BES	Bio electrochemical system
COD	Chemical oxygen demand
CE	Coulombic efficiency
CEM	Cation exchange membranes
CV	Cyclic voltammetry
DET	Direct electron transfer
DNA	Deoxyribonucleic acid
DGGE	Denaturing gradient gel electrophoresis
dNTP	Deoxynucleotide triphosphate
EAB	Electrochemically Active Bacteria
EET	Extracellular electron transfer
EIS	Electrochemical impedance spectroscopy
EPS	Extracellular polymeric substances
FAD	Flavin-adenine dinucleotide
PACF	Poly acrylonitrile carbon felt
g/L	Gram per liter
GC-MS	Gas chromatography mass spectrophotometry
kΩ	Kilo ohm
LB	Luria Bertani
µA	Micro ampere
µg	Micro gram
MFC	Microbial Fuel Cell
MEA	Membrane electrode assembly
mg/L	Milligram per litre
mM	Milli mole
mV	Millivolt
mW	Milliwatt

NAD	Nicotinamide-adenine dinucleotide
NADH	Nicotinamide adenine dinucleotide
NADPH	Nicotinamide adenine dinucleotide phosphate
NCBI	National center for biotechnology
OCV	Open circuit voltage
OD	Optical density
PEM	Proton exchange membrane
FESEM	Field emission scanning electron microscopy
RNA	Ribonucleic acid
RSM	Response surface methodology
rpm	Revolutions per minute
rRNA	Ribosomal ribonucleic acid
SD	Standard deviation
SHE	Standard hydrogen electrode
UV	Ultraviolet
VFA	Volatile Fatty Acid

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ABSTRAK

Sel bahan bakar mikrob (MFC) adalah peranti elektrokimia yang secara langsung menukarkan sisa tenaga kimia ke dalam elektrik melalui aktiviti metabolik mikroorganisma. Prestasi MFC boleh dipengaruhi oleh beberapa parameter utama seperti konfigurasi reaktor, bahan elektrod, kawasan permukaan elektrod, membran, ketebalan biofilm, dan inokulum. Di antaranya, komposisi komuniti mikrob dan biofilm anod sangat mempengaruhi prestasi MFC. Untuk menyediakan inokulum yang efektif, pilihan mikroorganisma harus berdasarkan kemampuan mereka untuk menggunakan substrat kompleks dan sifat elektrogenik. Dalam konteks ini, prestasi kultur tulen (*Klebsiella variicola*, *Klbesiella pneumonia*, *Bacillus cereus* dan *Pseudomonas aeruginosa*) telah disiasat dalam efluen minyak kelapa sawit (POME) yang dioperasikan oleh MFC. Bakteria yang digunakan telah diasingkan dan dicirikan menggunakan BIOLOG gen III, tindak balas rantai polimerase (PCR) dan analisis penjujukan. Kesan pembentukan biofilm berdasarkan masa oleh mikroorganisma pada prestasi MFC divisualisasikan dengan menggunakan mikroskop elektron pelepasan medan (FESEM) dan diklasifikasikan oleh voltammetry kitaran (CV) dan analisis spektroskopi impedans elektrokimia (EIS). Pengumpulan sel-sel mati dalam lapisan-lapisan biofilm di sekitar permukaan elektrod dari masa ke masa dalam anod biofilm didapati sangat memudaratkan generasi semasa atas peningkatan pemindahan caj dan penyebaran rintangan yang disahkan oleh EIS. Aliran tegasan geseran dan kaedah bantuan ultrasound telah digunakan untuk memulihkan semula biofilm dengan mengeluarkan biomass lengai untuk mengekalkan kuasa yang stabil dalam MFC. Tekanan ricih hidrodinamik 9.34 mPa dan 30 min rawatan ultrasound (20 kHz) telah berjaya mengurangkan ketebalan biofilm yang dipulihkan dalam waktu yang singkat dengan meningkatkan kadar pertumbuhan sel biofilm. Mekanisme pemindahan elektron dijelaskan dengan menggunakan analisis CV. Tambahan pula, inokulum kultur bersama dan campuran telah dihasilkan dengan menggunakan bakteria yang disasarkan (*Klebsiella variicola* dan *Bacillus cereus*, *Klebsiella variicola* dan *Pseudomonas aeruginosa*, *Bacillus cereus* dan *Pseudomonas aeruginosa*, *Klebsiella variicola* dan *Bacillus cereus* dan *Pseudomonas aeruginosa*). Ketumpatan kuasa tertinggi sebanyak 14.78 W/m3 dicapai oleh inokulum kultur bersama *Pseudomonas aeruginosa* dan *Klebsiella variicola* kerana hubungan sinergi mereka yang berkait menerusi metabolit berasaskan fermentasi. Selain itu, interaksi *Klebsiella variicola* dan *Bacillus cereus* telah mempengaruhi penjanaan kuasa dan inokulum kultur bersama secara positif dengan menghasilkan ketumpatan kuasa maksimum 11.78 W/m3 manakala hubungan yang sebaliknya telah ditunjukkan oleh *Bacillus cereus* dan *Pseudomonas aeruginosa*. Selain daripada itu, prestasi *Klebsiella variicola* dan *Pseudomonas aeruginosa* kultur bersama telah dioptimumkan melaui operasi parameter (kepekatan substrat, nisbah mikroorganisma yang berlainan, pH dan masa) dengan menggunakan metodologi tindak balas permukaan (RSM). Komposisi inokulum (perbezaan nisbah *Klebsiella variicola* dan *Pseudomonas aeruginosa*) memainkan peranan penting dalam penjanaan kuasa serentak dan penyingkiran chemical oxygen demand (COD) dari POME. Penemuan ini menunjukkan bahawa interaksi sinergistik mikroorganisma dalam inokulum dan pembentukan biofilm yang seterusnya seterusnya adalah penting untuk mencapai penjanaan kuasa yang dipertingkatkan dalam MFC yang berpotensi dilaksanakan untuk rawatan POME.

ABSTRACT

Microbial fuel cell (MFC) is an electrochemical device that directly converts chemical energy of wastes into electricity by the metabolic activity of microorganisms. The performance of MFC can be affected by several key parameters such as reactor configurations, electrode materials, electrode surface area, membrane, biofilm thickness, and inoculum. Among them, the microbial community composition and the anode biofilm severely influence the performance of MFC. To prepare effective inoculum, the choice of microorganisms should be based on their ability to utilize complex substrates and the electrogenic properties. In this context, the performance of targeted pure cultures (*Klebsiella variicola*, *Klbesiella pneumonia*, *Bacillus cereus* and *Pseudomonas aeruginosa*) were investigated in palm oil mill effluent (POME) driven MFC. The targeted bacteria were isolated and characterized using BIOLOG gene III, polymerase chain reaction (PCR) and sequencing analysis. The effect of time-course biofilm formation by the microorganisms on MFC performance was visualized using field emission electron microscopy (FESEM) and characterized by cyclic voltammetry (CV) and electrochemical impedance spectroscopy (EIS) analysis. The accumulation of dead cells in the multilayer biofilm at the vicinity of the electrode surface over time within the anode biofilm was found to be particularly detrimental to current generation that increased the charge transfer and diffusion resistances confirmed by EIS. Flow induced shear stresses and ultrasound-assisted methods were employed to revitalize the biofilm by removing inert biomass for the maintenance of stable power in MFCs. The hydrodynamic shear stress of 9.34 mPa and the 30 min of ultrasound treatment (20 kHz) successfully reduced the thickness of biofilm thus it revitalized within a short time by increasing the cell growth rate of the biofilm. The mechanism of electron transfer was elucidated using CV analysis. Furthermore, the co-culture and mixed cultures inoculum was developed using targeted bacteria (*Klebsiella variicola* and *Bacillus cereus*, *Klebsiella variicola* and *Pseudomonas aeruginosa*, *Bacillus cereus* and *Pseudomonas aeruginosa*, *Klebsiella variicola* and *Bacillus cereus* and *Pseudomonas aeruginosa*). The highest power density of 14.78 W/m3 was achieved by *Pseudomonas aeruginosa* and *Klebsiella variicola* co-culture inoculum due to their synergistic relationships which are inter-linked via fermentation-based metabolite. Besides, the interaction of *Klebsiella variicola* and *Bacillus cereus* positively influenced the power generation and the co-culture inoculum obtained maximum power density of 11.78 W/m3 whereas the antagonistic relationship was witnessed for *Bacillus cereus* and *Pseudomonas aeruginosa*. Apart from that the performance of *Klebsiella variicola* and *Pseudomonas aeruginosa* co-culture was optimized with respect of operational parameters (substrate concentration, different ratio of microorganisms, pH and time) by using response surface methodology (RSM). The inoculum composition (different ratios of *Klebsiella variicola* and *Pseudomonas aeruginosa*) played a crucial role in simultaneous power generation and chemical oxygen demand (COD) removal from POME. These findings demonstrate that the synergistic interaction of microorganisms in inoculum and their subsequent effective biofilm formation are crucial to achieve the enhanced power generation in MFCs that can potentially be implemented for POME treatment.

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