Enrichment and Electrochemical Performance of Electricigens from Different Environmental Niches using Microbial Fuel Cells

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IN

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بسم الله الرحمن الرحيم

This dissertation is dedicated to my Grand Mother who has loved and taken care of me for all my life. All I am I owe to my grandmother. I attribute all my success in life to the moral and intellectual education I received from her

DECLARATION

The research work presented in this thesis is my original work conducted at Quaid-I-Azam University and Newcastle University, Newcastle Upon tyne, United Kingdom. Additional support was provided by Higher Education commission of Pakistan (HEC) under IRSIP program. I have not previously presented any part of this work elsewhere for any other degree.

Zargona Zafar

Certificate

This thesis, submitted by **Miss. Zargona Zafar** is accepted in its present form by the Department of Microbiology, Faculty of Biological Sciences, Quaid-i-Azam University, Islamabad, Pakistan as satisfying the thesis requirement for the degree of Doctor of Philosophy in Microbiology.

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ARB	Anode respiring bacteria
AQDS	Anthraquinone-2,6-disulfonyl
ABTS	2,2^{0}-Azino-bis(3-ethylbenzthiazoline-6-sulfonic acid)
ANOVA	Analysis of variance
NH_4	Ammonium
NH_4Cl	Ammonium Chloride
BOD	Biochemical oxygen demand
H_3BO_3	Boric acid
BHM	Bushnell Hass media
BHIA	Brain heart infusion agar
BSA	Bovine serum albumin
CEM	Cationic exchange membrane
CE	Columbic efficiency
COD	Chemical Oxygen Demand
CV	Cyclic voltammetry
C-TAB method	Cetyl-trimethylammonium bromide
CSLM	Confocal scanning laser micrographs
$CaCl_2$	Calcium chloride
$CaCl_2$	Calcium chloride
$CoCl_2$	Cobalt chloride
$CuSO_4$	Copper sulphate
Id	Current density
CRA	Congo red agar assay
DMFC	Diesel inoculated MFCs
K_2HPO	Dipotassium phosphate
DCPIP	Dichlorophenol indophenol
EET	Extracellular electron transfer
EPS	Extracellular polymeric substances
EIS	Electrochemical impedance spectroscopy
EEC	Equivalent electric circuit
FISH	Fluorescent in situ hybridization
FTIR	Fourier Transform Infrared Spectroscopy
Fe^{3+}	Ferric ion
$FeSO_4$	Ferrous sulphate
HFC	Hydrogen fuel cell
HAS	Hot Spring Water
LSM	Laser scanning microscopy
MFCs	Microbial fuel cells
$MnSO_4$	Manganese sulphate
CH_4	Methane
MnO_2	Manganese oxide
MR-DNA	Molecular research DNA
MLAC-MFCs	Membrane-less air-cathode microbial fuel cells

NISO	National Information Standards Organization
NADH	Nicotinamide Adenine Dinucleotide
OCV	Open circuit voltage
OTUs	Operational taxonomic units
PEM	Proton exchange membrane
Pd	Power density
PCA	Principle component analysis
POME	Palm oil mill effluent
Pt	Platinum
PSA	Petroleum contaminated soil
PEI	Polyethyleneimine
PA	Pentanedioic acid
PCoA	Principal coordinate analysis
$KMnO_4$	Potassium permanganate
KCl	Potassium chloride
KH_2PO_4	Potassium dihydrogen phosphate
RVC	Reticulated vitreous carbon
RF	Riboflavin molecule
SMFCs	Sediment microbial fuel cell
SEA	Separator electrode assembly
Ag	Silver metal
AgCl	Silver chloride
NaH_2PO_4	Sodium dihydrogen phosphate
$NaHCO_3$	Sodium bi carbonate
SEM	Scanning electron microscopy
TSB	Tryptic soya broth
V	Voltage
VFA	Volatile fatty acid
$ZnSO_4$	Zinc sulphate

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Abstract

Renewable energy technologies (solar, wind, hydropower, 1^{st}, 2^{nd}, 3^{rd} generation biofuels etc) are under development that may replace conventional approaches which are adding more carbon footprints and causing environmental deterioration. In current scenario, among other renewable solutions, microbial fuel cells (MFCs) has been viewed as promising energy technology, besides it can help in treatment of waste and pollutants in more viable and cost-effective manner. This thesis focused on boosting electrochemical performance of MFCs by investigating key factors like enrichment of electricigens from different environmental niche, the electrogenic properties of electricigens under varying situation of anolytes and fuel cell configurations.

Experimental strategy include selection of (1) different environmental samples (petroleum contaminated soil, hot spring water, sludge, wastewater, sewer contaminated soil), (2) electrode materials (carbon cloth, graphite rod, carbon felt, graphite plates), (3) Fabrication of different MFCs [double chamber MFCs (PEM and salt bridge separators), air-cathode MFCs, parallel circuit air-cathode MFCs, membraneless air-cathode MFCs] (4) anolyte substrates (acetate, glucose, starch, bacterial growth medium) (5) catholytes (Bio-cathode (activated sludge), reducing compounds (K_2MnO_4), Air-cathode) under batch mode of operation.

In phase 1, bacterial consortia from two extreme environments i.e., petroleum contaminated soil (MFC-1) and hot spring water (MFC-2) has been investigated as a potential source of electricigens in bio-cathode MFCs. Maximum power density $2.9Wm^{-2}$ to $5.5Wm^{-2}$and $7.6Wm^{-2}$ to $1.3Wm^{-2}$ was recorded during enrichment stage 1 and 2 during operation of MFC-1 and MFC-2. The electrochemical performance of MFCs was significantly improved (1.8fold and 1fold) when applied in successive modes with enriched biofilms during enrichment stage 2 in two respective MFC setups. Additionally, biodegradation of petroleum contaminants and enhanced electrochemical performance of MFC-1 was further confirmed by the presence enriched bacterium *Stenotrophomonas maltophilia* on anode surface.

In phase 2, the succession and enrichment of bacterial communities from activated sludge in double chamber salt bridge MFCs was evaluated. During

enrichment stage 1, maximum voltage output of 136.2mV was recorded, that was increased by 3fold (418mV) during enrichment stage 2 with COD removal efficiency of 86.04% at ambient temperature. Molecular based phylogeny confirmed the enrichment of major contributing classes of α-proteobacteria 48.51%, β-proteobacteria 31.48% and γ-proteobacteria 16.16%. Several novel bacterial species i.e. *Massilia timonae, Duganella* sp. and *Paracoccus aestuarii* were identified to have bioelectrogeneic activity in MFC technology. Enrichment of anodic biofilms from already operating fuel cells, showed faster start-up and better in performance.

In phase 3, biocatalytic activity of sewer contaminated soil bacterial flora on two different anode materials (MFC1 = carbon cloth anode and MFC2 = graphite rod anode) using PEM-MFCs under batch mode with continuous anolyte mixing at 50 rpm at 35±2^{o}C. It has been observed that there is significant difference in power output between carbon cloth anode (27Wm^{-2}) and graphite rod (12Wm^{-2}). These results were further validated through ultra-micrographs of anodes using LSM and SEM. Biofilm developed on carbon cloth anode was much thicker (enriched) in terms of electricigens as compared to graphite anode. Maximum density of phylum Proteobacteria 99.1% including classes (Class: β-Proteobacteria>γ-Proteobacteria>α-Proteobacteria) was comparatively higher on graphite anode than carbon cloth anode [(94.5%) (Class: *γ*-Proteobacteria>β-Proteobacteria > Opitutae)]. Carbon cloth anode was enriched with *Pseudomonas* sp. (35.73%) followed by *Methyloversatilis universalis* (16.237%), *Pseudomonas plecoglossicida* (7.16%), *Pseudoxanthomonas Mexicana* (5.589%) etc, while, the graphite anode enriched with *Methyloversatilis universalis* (55.7) followed by *Nitrosomonas europaea* (13%), *Stenotrophomonas acidaminiphila* (11%) etc.

In phase 4, parallel circuit air-cathode MFCs were operated under different concentrations (0mlL^{-1} DMFC-1, 10mlL^{-1} DMFC-2, 50mlL^{-1} DMFC-3 and 100mlL^{-1} DMFC-4) of diesel taking bacteria from diesel oil contaminated soil. Maximum current (I_{max} = 43.11mA) under applied potential (-1V to 1V) was recorded using DMFC-3. Bioelectrochemical activity (13mA) of *Bacillus toyonensis* (MN173853) was monitored for the first time in MFC reactors. *Bacillus* sp. was found to have greatest electrochemical activity (22mA) and biodegradation capability (88%) in MFC. Up-scaling of MFCs at bench pilot scale, indicated that maximum current (Imax) of about 795mA in (2L) AC-MFC. Whereas, Imax (1098mA) was about 1.38 folds higher in 8L AC-MFC.

In phase 5, membrane less air-cathode MFCs (MLAC MFCs) were fabricated in order to optimize of anode to cathode spacing (between 20mm, 40mm, 60mm, 80mm) and associated biodegradation of simple (acetate, glucose) to complex (starch, wastewater) substrates which previously reported to have an influence on MFC performance was evaluated. Maximum potential output (Imax 1.8mA, PD 113.8±10.6 Wm^{-2} with COD removal efficiency of 95%) at minimum carbon felt anode distance (20mm) in MLAC-MFCs was recorded. Statistically, significant difference is observed between maximum current density generated (117mAm^{-2} ± 7.5) with carbon felt and graphite plate anode (94.31mAm^{-2}±5.7). Fermentation rate constant ($k=0.1523h^{-1}$) was much larger than hydrolysis and fermentation rate constant ($k = -0.0747h^{-1}$) which means hydrolysis is rate limiting step in performance of MLAC-MFCs when operated with simple (acetate, glucose) to complex (starch, wastewater) substrates.

Enrichment of electricigens from diesel contaminated soil under diesel influence (50mlL^{-1}) in parallel circuit air-cathode MFC was done. Efficiently enriched electricigens (*Bacillus* sp. (22mA) followed by *Bacillus lichenformis* (16mA), *Bacillus toyonensis* (13mA) etc) has significantly improved the MFC performance. Simple anolytes (acetate (137mAm^{-2}), glucose (135mAm^{-2})) has been proved to be better substrates for boosting MFC performance than complex substrates (starch, wastewater), showed faster start-up in less than 24hours and remained sustainable for 120hrs using acetate, 100hrs with glucose. Carbon felt anode (111.62Wm^{-2}) was shown to be better in performance and cost-effective material as compared to other electrode materials like graphite plate (106.62Wm^{-2}), carbon cloth anode (27Wm^{-2}) and graphite rod (12Wm^{-2}). The current study deciphered the relationships and profiles of enriched electricigens from different environmental niches on MFC performance that could be helpful guide for future up-scale MFC studies. Through our investigations we opened a plethora of possibilities to use MFC as cost-effective renewable energy technology in Pakistan in near future.