

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



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

A thesis submitted in partial fulfilment of the requirements for  
the degree of

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**IN**

**MICROBIOLOGY**



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*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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## List of Contents

List of Figures	i
List of Tables	v
List of Abbreviations	vi
Acknowledgment	viii
Abstract	x
<b>CHAPTER 1</b>	
<b>INTRODUCTION</b>	
1.1 Introduction	1
1.2 Aims and Objectives	5
<b>CHAPTER 2</b>	
<b>REVIEW OF LITERATURE</b>	7
2.1 Preface	7
2.2 Microbial Fuel Cells and Different Configurations	10
2.2.1. Two Component Microbial Fuel Cells	12
2.2.2 Single component Microbial Fuel Cells	12
2.2.3 Up-Flow mode MFC design	12
2.2.4 Stacked MFCs setups	13
2.3 Enrichment strategies and sources of electrochemically active bacteria	16
2.4 Electricigens: Molecular Machinery of MFC	18
2.5 Applications of MFCs	22
2.5.1 MFC Technology to Power up Electrical Machines	22
2.5.2 MFC Technology for Wastewater Treatment	24
2.5.3 MFCs as Biosensors	30
2.5.4 Challenges	30
2.5.5 Scope of MFC Technology for Future Use	31
<b>CHAPTER 3</b>	
<b>Enrichment and Electrochemical Performance of Electricigens from Different Environmental Samples in Bio-Cathode Microbial Fuel Cells</b>	
3.1 Introduction	34
3.2 Materials and Methods	36
3.2.1 Sample Collection	36

3.2.2 MFC Configuration	36
3.2.3 Electrochemical Analysis	38
3.2.4 Molecular Phylogeny and Bacterial Community Analysis	38
3.2.4.1 Viable bacterial isolation from anode surface	38
3.2.4.2 DNA Extraction	39
3.2.4.3 Ultra-Structure Analysis of Biofilms	39
3.3 Results and Discussion	40
3.3.1 Electrochemical Performance of Biocathode-MFCs	40
3.3.2 Effect of External Resistance on Power Output	44
3.3.3 Cyclic Voltammetry (CV) Analysis	46
3.3.4 COD Removal Efficiency	48
3.3.5 Biofilm Development and Sustainability	50
3.3.6 FTIR Analysis of Petroleum Degradation in MFCs	54
3.3.7 Molecular Based Bacterial Phylogenetic Analysis	56
3.3.8 Density and Diversity Profile Bacterial Community on Electrode Surfaces	58
3.4 Conclusions	61

## **CHAPTER 4**

### **Electrogenic Performance of Sludge Bacterial Flora Coupling Wastewater Treatment in Salt bridge Microbial Fuel Cells**

4.1 Introduction	63
4.2 Material and Methods	65
4.2.1 Sludge Sampling	65
4.2.2 MFC Fabrication and Operation	65
4.2.3 Power Calculation	68
4.2.4 Molecular Phylogeny of Anodic Biofilm	68
4.2.5 Heterotrophic Plate Count and Bacterial Isolation from Anode	68
4.2.6 Scanning electron microscopy	69
4.3 Results and Discussion	70
4.3.1 Electrochemical Power Performance in MFC	70
4.3.2 Effect of External Resistance	72
4.3.3 Cyclic Voltammetry	76
4.3.4 Treatment of Wastewater with Current Output	78
4.3.5 Culturable Electricigens in Anodic biofilms	79
4.3.6 Molecular Phylogeny of Electricigens in Anodic Biofilms	81



4.3.7 Principal Component analysis (PCA)	83
4.3.8 Anode Respiring Bacteria	85
4.3.9 Ultra Structure Analysis of Anodic Biofilms	89
4.4 Conclusions	90

## **CHAPTER 5**

### **Comparative Electrochemical Potentials of Biofilm Enriched Different Electrodes in PEM-Microbial Fuel Cells**

5.1 Introduction	92
5.2 Material and Method	94
5.2.1 Sampling	94
5.2.2 Configuration and Operation of MFCs	94
5.2.3 Electrochemical Monitoring	97
5.2.4 Isolation of ARB from Anode Surface	97
5.2.5 Biofilm Assay of ARB	97
5.2.6 Molecular based (pyrosequencing) Characterization of ARBs	98
5.2.6.1 DNA extraction	98
5.2.6.2 Ultra-Structure Analysis of Anodic Biofilms	99
5.3 Results and Discussion	100
5.3.1 Effect of Electrode Material on ARB Enrichment and Electrochemical Performance of MFCs	100
5.3.2 Power Density as a Function of Current	104
5.3.3 Cyclic Voltammetry	106
5.3.4 Density, diversity and biofilm forming ability of culturable bacteria in anodic chamber	108
5.3.5 Molecular Phylogeny of Electrochemically Active Bacteria	110
5.3.6 Similarity Analysis of Electroactive Biofilms in MFCs	118
5.3.7 Ultra-structure Analysis of Biofilms	121
5.4 Conclusions	125

## CHAPTER 6

### **Influence of Diesel Contaminants on Bioelectrogenic Performance of Electricigens in Air-cathode Microbial Fuel Cell**

6.1 Introduction	127
6.2 Material and Method	130
6.2.1 Diesel fed Air-cathode MFCs	130
6.2.2 Performance Evaluation and Analysis	132
6.2.3 Model Fitting of EIS	132
6.2.4 FT-IR Analysis of Diesel Degradation in DMFCs	133
6.2.5 Florescent in-situ hybridization (FISH), confocal laser scanning microscopy (CLSM), Scanning Electron microscopy (SEM)	133
6.2.6 Isolation of viable bacteria from anode surface	134
6.2.7 Microscopic Analysis of Isolated Electrochemically Active Bacteria	135
6.2.8 Biochemical Behaviour characterization of Hydrocarbon degrading EABs	136
6.2.9 Preliminary Screening and Selection of Efficient Hydrocarbon Degrading Bacteria	142
6.2.9.1 Biomass, Optical density and pH	142
6.2.10 Confirmatory Test for Hydrocarbon Degrading Bacteria	143
6.2.10.1 Dichlorophenol indophenols (DCPIP) Test	143
6.2.11 Qualitative and Quantitative Biofilm Detection Methods	144
6.2.11.1 Congo Red Agar Assay	144
6.2.11.2 Tube Assay	145
6.2.11.3 Quantitative; Microtiter Plate Assay	146
6.2.12 Capsule Staining of Biofilm Forming Bacteria	147
6.2.12.1 Maneval's Method	147
6.2.12.2 Positive Capsule Staining by Anthony's Method	147
6.2.13 Analysis of "Bacterial cell House in Biofilms"	148
6.2.14 EPS Component Characterization	149
6.2.14.1 Quantification of Polysaccharides in EPS	149
6.2.14.2 Quantification of Proteins in EPS	149

6.2.14.3 Fourier transformed infrared spectroscopy (FTIR) for estimation of Polysaccharides and Carbohydrates in EPS	150
6.2.15 DNA Extraction and Sequence Analysis	150
6.2.16 Bioelectrochemical Testing of Individual Bacterial isolates	151
6.2.17 Bioelectrochemical Performance of Bench Pilot Scale ACMFC	151
3.4 Results and Discussion	155
6.4.1 Effect of Diesel Oil Concentrations on Bioelectrogenesis of MFCs	155
6.4.2 Electrochemical Response of Anodic Biofilm	157
6.4.3 Electrochemical Impedance Spectroscopy	159
6.4.4 Biodegradation of Diesel Oil Contaminants in DMFC	163
6.4.5 Micrographic Analysis of Electroactive Biofilm	166
6.4.6 Characterization of Cultivable Electricigens from Anodic biofilm	173
6.4.7 Evolutionary Relationships of Hydrocarbon Degrading Bacteria	176
6.4.8 Screening of Diesel Degrading Bacteria	178
6.4.9 Capsule Screening of Electricigens	181
6.4.10 In-Vitro Quantification of Biofilm Formation by Bacterial Isolates	183
6.4.11 Evaluation of Amount of EPS Production and Composition	186
6.4.12 Investigation of EPS; FI-IR Spectrum	189
6.4.13 Electrochemical Response of Individual Bacterial Isolates	192
6.4.14 Electrochemical Performance of Large Scale MFCs	195
6.5 Conclusions	199

## **CHAPTER 7**

### **Effect of Anode to Cathode Spacing and Feeding of Different Organic Anolytes [simple (Glucose, Acetate), Complex (Starch, Wastewater)] on Electrochemical Performance of Membraneless Microbial Fuel Cell**

7.1 Background Study	201
7.2 Material and Methods	204
7.2.1 MLAC-MFCs Configuration and Operation	204
7.2.2 Electrochemical Measurements and Analysis	206

7.2.3 Chemical Characterization of Anolyte	206
7.2.4 Biofilm Imaging using Laser Scanning	207
7.4 Results and Discussion	209
7.4.1 Effect of Anode Spacing on Current Generation	209
7.4.2 Effect of Electrode Material on MFC Performance	213
7.4.3 Organic substrate Removal Efficiency and Change in pH	215
7.4.4 Effect of Different Substrates on MLAC-MFCs Performance	217
7.4.5 Cyclic Voltammetry	221
7.4.6 Volatile Fatty Acid Detection using Different Substrates using MLAC-MFCs	224
7.4.7 Micrographic Analysis of Electroactive Biofilms	228
7.4 Conclusions	230
<b>Chapter 8</b>	
<b>Discussion</b>	
8.1 General Discussion	232
<b>Chapter 9</b>	
<b>General Conclusions and Future Prospects</b>	
9.1 General Conclusions	246
9.2 Future Prospects	248
<b>References</b>	

## List of Figures

Sr. No.	Titles	Page No.
1.1	Working principle of microbial fuel cell (MFC) technology	2
1.2	Relevance of microbial fuel cells in different sector	4
2.1	Number of publications appeared in last five years (a) Publication's (review articles/ original research articles) on MFCs from 2011 to 2017 (b) Increase in MFC research interest imitated by a sudden rise in number of publications from 2011 to 2017 as compared to between 1980 and 2010	9
2.2	Different configurations of microbial fuel cells (A) Double chamber MFC (B) Air-cathode single chamber MFC (C) Up-flow MFC (D) Stacked MFC	14-15
2.3	Extracellular electron transfer mechanisms	21
2.4	(A) Gastrobots (B) Gastronome (C) EcoBot II operated with Microbial Fuel Cell	23
2.5	(A) Biosensor base microbial fuel cell (MFC) (B) MFC-based toxicity monitoring mechanism. Increased level of toxin fed in anode will inhibit the cell metabolic/viability, which reduces the output of current (C) Mechanism for biochemical oxygen demand (BOD) monitoring bases MFC biosensor	32
3.1	Basic bio-cathode double chamber microbial fuel cell fabrication	37
3.2	(A–B) Voltage (mV) output over period of time during operation of MFC-1, MFC1', MFC-2 and MFC-2' at 1 M $\Omega$ (C–D) Power density (mWm <sup>-2</sup> ) vs time at 550 $\Omega$ during operation of MFC-1 and MFC-2 in two enrichment phases	43
3.3	Polarization curves (A–B) Power density as a function of current density in MFC-1 and MFC-1' at 35 $\pm$ 2 $^{\circ}$ C (C–D) Power density as a function of current density in MFC-2 and MFC-2' during enrichment steps operated at 55 $\pm$ 2 $^{\circ}$ C	45
3.4	Cyclic voltammogram at – 1 to 1 V in (A) MFC-1 and MFC-1' (petroleum-contaminated soil inoculum) and (B) MFC-2 and MFC-2' (hot spring water) during enrichment stages	47
3.5	COD removal efficiency during 1st and 2nd enrichment stages in biocathode MFC fed with petroleum contaminated soil	49
3.6.1	Confocal laser scanning micrographs; (A) controlled electrode having no bacterial growth or deposits (B) Biofilm present on surface of anode (Petroleum contaminated soil fed MFC-1') (C) Biofilm on surface of cathode in MFC1' (D) Biofilm on surface of anode in MFC-2' (hot spring water) (E) Biofilm present on surface of cathode in MFC-2	51
3.6.2	Scanning electron micrographs of (A-B) Anodic biofilm in MFC-1' (C–D) MFC-2' (E) cathodic biofilm of MFC-1' (F) cathodic biofilm of MFC-2'	53

3.7	FTIR spectra of treated and untreated petroleum contaminants in soil (blue line represents untreated soil sample while red line represents treated sample in PEM -biocathode MFC-1)	55
3.8	(A) Rarefaction curve of phylogenetic assortment (B) Principal coordinate analysis (PCoA) based on weighted UniFrac distance matrix represented a visual demonstration of bacterial structural diversity among initial inoculum sample	57
3.9	(A)%age Relative Abundance at class level (B) species level	60
4.1	Fabrication of salt bridge microbial fuel cell for wastewater treatment	67
4.2	Effect of enrichment on voltage (mV) output during enrichment stage 1 and enrichment stage 2	71
4.3	Cyclic voltammogram of acetate containing synthetic wastewater inoculated in salt bridge MFC during 2 enrichment phases (1 and 2)	77
4.4	%age COD removal efficiency Vs time(days) during enrichment stage 1 and 2	79
4.5	Relative abundance (%) of biofilm (2) and sludge communities (2C) in acetate fed double chamber microbial fuel cell at (A) phylum level (B) Class level	82
4.6	Principle component analysis (PCA) of dominant bacterial classes in sludge Vs biofilm on anode surface in A-MFC (cut-off value was set at 0.1%)	84
4.7	Closely related species found on anode surface based on partial 16S rRNA gene sequences	86
4.8	Scanning electron micrographs of anodic biofilms (A-B) 1 <sup>st</sup> stage of enrichment (C-D) A-MFCs after 2 <sup>nd</sup> stage of enrichment	89
5.1	Basic materials for MFCs fabrication	96
5.2	Effect of electrodes on ARB enrichment and electrochemical performance of MFCs (A-B) Voltage (mV) output (3k $\Omega$ ) of microbial fuel cells (MFC1) and (MFC2) (C-D) Power density (57 $\Omega$ ) monitored over a period of time in MFC1 and MFC2 during two enrichment phases	103
5.3	Polarization curves as a function of current density and power density (A) MFC1 (B) MFC1* (C) MFC2 (D) MFC2	105
5.4	Cyclic voltammogram at 10mVs-1 using MFC1 (carbon cloth) and MFC2 (graphite rod) during two enrichment stages	107
5.5	(A) Heat map of relative abundance of different phyla present in MFC1 and MFC2 before and after enrichment. (B) Bar graph represents the relative abundance at class level in MFC1 and MFC2	112
5.6	%age relative abundance of species on anode surface (A) carbon cloth in MFC1* (B) graphite rod in MFC2*	114
5.7	(A) Rarefaction curve of phylogenetic bacterial assortment (B) Principal coordinate analysis (PCoA) based on weighted UniFrac distance matrix represented a visual demonstration	120

	of bacterial structural diversity in MFC1, MFC2 (stage 1) and MFC1* and MFC2* (stage 2)	
5.8	Scanning electron micrographs (A) Carbon cloth Anode after enrichment in MFC1* (B) Graphite rod anode after enrichment in MFC2*	123
5.9	Laser Scanning 3D biofilm micrographs on (A) Carbon cloth Anode after enrichment in MFC1* (B) Graphite rod anode after enrichment in MFC2	124
6.1	Biodegradation of diesel contaminants by electrochemically active bacteria in MFC	129
6.2	(A) Schematic representation of parallel circuit air-cathode microbial fuel cells (B) Air-cathode MFC in operation	131
6.3	EIS fitting model (EEC model) (Electric elements; C = Capacitors, R = Resistors)	133
6.4	Experimental strategy followed to screen ARB	137
6.5	Screening of isolated EAB on the basis of hydrocarbon degradation capabilities	142
6.6	Confirmatory test (DCPIP) for final selection of hydrocarbon degradation bacteria	144
6.7	Qualitative Congo red assay for confirmation of biofilm forming capabilities of EAB	145
6.8	Qualitative tube assay for biofilm formation estimation by electrochemically active bacteria	146
6.9	EPS extraction procedure	149
6.10	2L air-cathode microbial fuel cell fabrication and operation	153
6.11	8L air-cathode microbial fuel cells fabrication operation	154
6.12	Current generation profiles of DMFCs at different diesel concentrations in anode chamber	156
6.13	Cyclic voltammogram of DMFCs using potentiostat SP-150 (DMFC-1 0mL-1 diesel concentration, DMFC-2 10mL-1, DMFC-3 50mL-1, DMFC-4 100mL1 diesel fed in MFCs)	158
6.14	Nyquist plots (A) anode resistance and capacitance of DMFC-1 (B) DMFC-2 (C) DMFC-3 (D) DMFC-4	161-162
6.15	FT-IR spectra of diesel contaminated soil untreated and treated in microbial fuel cells at different concentrations	165
6.16	FISH-CLSM micrographs of DMFCs anode	168
6.17	CLSM micrographs of anode surface under different diesel concentrations	169-171
6.18	SEM micrographs of DMFC anode under different diesel concentrations	172
6.19	Phylogenetic tree based on 16S rRNA Analysis of six bacterial stains isolated from anode surface	179
6.20	(A) Biomass (B) OD (C) DCPIP quantification of diesel degrading bacteria	180
6.21	Polymeric capsular layer around bacterial cells by (A-B) Maneval's and (C-D) Anthony's staining	182
6.22	Screening of biofilm formers by (A) tube method (TM) (B) Congo Red Assay (CRA) (C) Microtiter plate assay (MTA)	185

6.23	(A) Dry weight of EPS extracted from bacterial species, (B) carbohydrate determination in EPS by UV-sulfuric Acid method, (C) Protein estimation by Lowery method (Carbohydrate amount compared to glucose standard solution, Protein amount compared to Bovine serum albumin standard in EPS of bacterial strains)	187-188
6.24	FT-IR spectrum of EPS extracted from six bacterial species compared with glucose standard for carbohydrates estimation in EPS	190
6.25	FT-IR spectrum of EPS extracted from six bacterial species compared with Bovine Serum Albumin (BSA) standard for protein estimation in EPS	191
6.26	(A) Linear sweep voltammogram (B) Cyclic voltammogram of six bacterial species isolated from mixed culture anode surface	194
6.27	Electrochemical performance of diesel (50mL-1) fed 2L AC-MFC and 8L AC-MFC (A) Current (mA) output Vs time (B) LSV in 8L AC-MFC (C) cyclic voltammogram (CV) at scan rate of 10mV/sec under applied potential of -1V to 1V	197-198
7.1	Hydrolysis and fermentation reaction mechanism in microbial fuel cell	203
7.2	Experimental set-up of Membrane-less Microbial Fuel Cells (A) Membrane-less Air-cathode MFCs (B) Picolog Multi-channel data logger (C) Picolog software (data storage in personal computer)	205
7.3	Procedure of laser scanning microscopy	208
7.4	Effect of anode distance from cathode on potential output in membraneless air-cathode microbial fuel cells	212
7.5	Effect of anode material and distance on voltage output (A) Continuous monitoring of voltage (mV) and current (mA) over different anode distance with passage of time (Hours) (B) Effect of anode material and distance on current density ( $\text{mA m}^{-2}$ )	214
7.6	Performance of MLAC-MFC with respect to wastewater treatment was evaluated by estimating the substrate (COD) removal	216
7.7	Effect of substrate type (simple to complex) on MLAC-MFC performance (A) effect of substrate (Acetate, glucose, wastewater and starch) on voltage output over time in membrane less air-cathode MFC with carbon felt anode (B) and with graphite plate anode	219
7.8	Cyclic voltammogram of different substrates in (A) MLAC-MFC1 (B) MLAC-MFC2	223
7.9	VFA peak analysis by ion-chromatography using (A) acetate (B) Glucose chromatogram (C) Starch (D) wastewater fed MFC (E) Control	226-227
7.10	LSM of anode biofilm using different substrates [simple (acetate, glucose) to complex (starch, wastewater)]	229



## List of Tables

Sr. No	Titles	Page. No
2.1	Summary of basic components required in MFCs	11
2.2	Microbial fuel cells (MFCs) performance for power production with pure cultures	26
2.3	Microbial fuel cell performance for bioelectrical performance with mixed cultures	28-29
4.1	Current outputs with diverse substrates in microbial fuel cells (MFCs)	74-75
4.2	Bacterial species identified at enrichment stage 2 on anodic biofilm	87-88
5.1	Biofilm formation assay of ARBs	98
5.2	Biochemical characterization of ARB isolated from anodic biofilm	109
5.3	Name of species identified on anodic surface, classification on class level, morphological description and %age relative abundance	115-117
6.1	Operating conditions for simultaneous power generation and diesel degradation in parallel circuit air-cathode microbial fuel cells	130
6.2	Biochemical characterization of isolated electrochemically active bacteria	138-141
6.3	Quantification of isolated electricigens on the basis of biochemical and molecular 16S rRNA	174-175
6.4	Biofilm forming ability of bacterial isolates by different method	184
7.1	Effect of anode spacing and electrode material on voltage and current output	211
7.2	Effect of different substrate on MFC performance	220

## List of Abbreviations

ARB	Anode respiring bacteria
AQDS	Anthraquinone-2,6-disulfonyl
ABTS	2,2 <sup>0</sup> -Azino-bis(3-ethylbenzthiazoline-6-sulfonic acid)
ANOVA	Analysis of variance
NH <sub>4</sub>	Ammonium
NH <sub>4</sub> Cl	Ammonium Chloride
BOD	Biochemical oxygen demand
H <sub>3</sub> BO <sub>3</sub>	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 <sub>2</sub>	Calcium chloride
CaCl <sub>2</sub>	Calcium chloride
CoCl <sub>2</sub>	Cobalt chloride
CuSO <sub>4</sub>	Copper sulphate
Id	Current density
CRA	Congo red agar assay
DMFC	Diesel inoculated MFCs
K <sub>2</sub> HPO	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 <sup>3+</sup>	Ferric ion
FeSO <sub>4</sub>	Ferrous sulphate
HFC	Hydrogen fuel cell
HAS	Hot Spring Water
LSM	Laser scanning microscopy
MFCs	Microbial fuel cells
MnSO <sub>4</sub>	Manganese sulphate
CH <sub>4</sub>	Methane
MnO <sub>2</sub>	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 <sub>4</sub>	Potassium permanganate
KCl	Potassium chloride
KH <sub>2</sub> PO <sub>4</sub>	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 <sub>2</sub> PO <sub>4</sub>	Sodium dihydrogen phosphate
NaHCO <sub>3</sub>	Sodium bi carbonate
SEM	Scanning electron microscopy
TSB	Tryptic soya broth
V	Voltage
VFA	Volatile fatty acid
ZnSO <sub>4</sub>	Zinc sulphate

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## Abstract

Renewable energy technologies (solar, wind, hydropower, 1<sup>st</sup>, 2<sup>nd</sup>, 3<sup>rd</sup> 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  $\alpha$ -proteobacteria 48.51%,  $\beta$ -proteobacteria 31.48% and  $\gamma$ -proteobacteria 16.16%. Several novel bacterial species i.e. *Massilia timonae*, *Duganella* sp. and *Paracoccus aestuarii* were identified to have bioelectrogenic 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  $35\pm 2^\circ\text{C}$ . It has been observed that there is significant difference in power output between carbon cloth anode ( $27\text{Wm}^{-2}$ ) and graphite rod ( $12\text{Wm}^{-2}$ ). 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:  $\beta$ -Proteobacteria >  $\gamma$ -Proteobacteria >  $\alpha$ -Proteobacteria) was comparatively higher on graphite anode than carbon cloth anode [(94.5%) (Class:  $\gamma$ -Proteobacteria >  $\beta$ -Proteobacteria > Opiritutae)]. 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 ( $0\text{mL}^{-1}$  DMFC-1,  $10\text{mL}^{-1}$  DMFC-2,  $50\text{mL}^{-1}$  DMFC-3 and  $100\text{mL}^{-1}$  DMFC-4) of diesel taking bacteria from diesel oil contaminated soil. Maximum current ( $I_{\text{max}} = 43.11\text{mA}$ ) 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 ( $I_{\text{max}}$ ) of about 795mA in (2L) AC-MFC. Whereas,  $I_{\text{max}}$  (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 ( $I_{max}$  1.8mA, PD  $113.8 \pm 10.6 \text{ Wm}^{-2}$  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 ( $117 \text{ mA m}^{-2} \pm 7.5$ ) with carbon felt and graphite plate anode ( $94.31 \text{ mA m}^{-2} \pm 5.7$ ). Fermentation rate constant ( $k=0.1523 \text{ h}^{-1}$ ) was much larger than hydrolysis and fermentation rate constant ( $k = -0.0747 \text{ h}^{-1}$ ) 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 ( $50 \text{ mL}^{-1}$ ) in parallel circuit air-cathode MFC was done. Efficiently enriched electricigens (*Bacillus* sp. (22mA) followed by *Bacillus licheniformis* (16mA), *Bacillus toyonensis* (13mA) etc) has significantly improved the MFC performance. Simple analytes (acetate ( $137 \text{ mA m}^{-2}$ ), glucose ( $135 \text{ mA m}^{-2}$ )) 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 ( $111.62 \text{ Wm}^{-2}$ ) was shown to be better in performance and cost-effective material as compared to other electrode materials like graphite plate ( $106.62 \text{ Wm}^{-2}$ ), carbon cloth anode ( $27 \text{ Wm}^{-2}$ ) and graphite rod ( $12 \text{ Wm}^{-2}$ ). 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.



