SELF SUSTAINABLE CATHODES FOR
MICROBIAL FUEL CELLS

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Declaration

I declare that the work in this dissertation was carried out in accordance with the requirements of the University's Regulations and Code of Practice for Research Degree Programmes and that it has not been submitted for any other academic award. Except where indicated by specific reference in the text, the work is the candidate's own work. Work done in collaboration with, or with the assistance of, others, is indicated as such. Any views expressed in the dissertation are those of the author.

Signed: Iwona Gajda

Date: 9/09/2015
Abstract

The ultimate goal of this thesis was to investigate and produce an MFC with self-sustainable cathode so it could be implemented in real world applications. Using methods previously employed [polarisation curve experiments, power output measurements, chemical assays for determining COD in wastewater and other elements present in anolyte or catholyte, biomass assessments] and with a focus on the cathode, experiments were conducted to compare and contrast different designs, materials and nutrient input to microbial fuel cells with appropriate experimental control systems. Results from these experiments show that:
Firstly, the choice of polymeric PEM membrane showed that the most effective materials in terms of power performance were cation exchange membranes. In terms of cost effectiveness the most promising was CM-I, which was the preferred separator for later experiments.
Secondly, a completely biotic MFC with the algal cathode was shown to produce higher power output (7.00 mW/m²) than the abiotic control (1.52 mW/m²). At the scale of the experimental system, the reservoir of algal culture produced sufficient dissolved oxygen to serve the MFCs in light or dark conditions. To demonstrate usable power, 16 algal cathode-designed MFCs were used to power a dc pump as a practical application.
It has been presented that the more power the MFC generates, the more algal biomass will be harvested in the connected photoreactor. The biomass grown was demonstrated to be a suitable carbon-energy resource for the same MFC units in a closed loop scenario, whereby the only energy into the system was light.
In the open to air cathode configuration various modifications to the carbon electrode materials including Microporous Layer (MPL) and Activated Carbon (AC) showed catholyte synthesis directly on the surface of the electrode and elemental extraction such as Na, K, Mg, from wastewater in a power dependent manner. Cathode flooding has been identified as an important and beneficial factor for the first time in MFCs, and has been demonstrated as a carbon capture system through wet scrubbing of carbon dioxide from the atmosphere. The captures carbon dioxide was mineralised into carbonate and bicarbonate of soda (trona).
The novel inverted, tubular MFC configuration integrates design and operational simplicity showing significantly improved performance rendering the MFC system feasible for electricity recovery from waste. The improved power (2.58 mW) from an individual MFC was increased by 5-fold compared to the control unit, and 2-fold to similar sized tubular systems reported in the literature; moreover it was able to continuously power a LED light, charge a mobile phone and run a windmill motor, which was not possible before.
Dedication

This work is dedicated to my parents, Witold and Janina Gajda
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# Table of Contents

Declaration ........................................................................................................................................... III
Abstract .............................................................................................................................................. IV
Acknowledgements .............................................................................................................................. VI
Table of Contents ............................................................................................................................... VIII
Figures ................................................................................................................................................. XIV
Tables .................................................................................................................................................. XXI
Glossary ................................................................................................................................................ XXII

1 Introduction and Background .......................................................................................................... 1
  1.1 OVERVIEW .................................................................................................................................. 1
  1.2 THESIS OUTLINE ....................................................................................................................... 3
  1.3 BACKGROUND ............................................................................................................................. 4
    1.3.1 Sustainability and self-sustainability ....................................................................................... 5
    1.3.2 Inspired by Nature. Energy sources in the natural environment .............................................. 7
    1.3.3 Energy and redox reactions .................................................................................................... 8
    1.3.4 Energy and metabolism .......................................................................................................... 9
    1.3.5 Bio-economy .......................................................................................................................... 10
    1.3.6 Energy and water nexus ......................................................................................................... 11
    1.3.7 Fuel Cell technology ............................................................................................................. 11
    1.3.8 Fuel cell operation principles ............................................................................................... 13
  1.4 BIOELECTROCHEMICAL SYSTEMS ............................................................................................. 14
    1.4.1 Microbial Electrolysis Cell (MEC) ......................................................................................... 15
    1.4.2 Microbial Electrosynthesis (MES) ......................................................................................... 16
    1.4.3 Microbial Desalination Cell (MDC) ...................................................................................... 16
  1.5 HISTORY OF THE MICROBIAL FUEL CELL .............................................................................. 17
  1.6 MICROBIAL FUEL CELL PRINCIPLES AND OPERATION ..................................................... 21
    1.6.1 Anode .................................................................................................................................... 23
    1.6.2 Proton exchange membrane .................................................................................................. 25
    1.6.3 Cathode system ...................................................................................................................... 26
      1.6.3.1 The oxygen reduction reaction (ORR) ......................................................................... 27
      1.6.3.2 Electroosmosis .............................................................................................................. 28
      1.6.3.3 Abiotic cathode ............................................................................................................. 29
      1.6.3.4 Catalyst free cathode .................................................................................................... 31
      1.6.3.5 Biotic cathode (Biocathode) ......................................................................................... 33
      1.6.3.6 Photosynthetic Biocathode ............................................................................................ 37
  1.7 AIMS AND OBJECTIVES OF THE CURRENT STUDY ................................................................... 41

2 Chapter 2. General Materials and Methods ................................................................................... 44
3.2.3 Activated Carbon (AC) on carbon cloth ......................................................... 46
2.2.2 Microporous Layer (MPL) on carbon cloth ....................................................... 45
1.1.1 Microporous Layer on carbon veil (MPL CV) ...................................................... 46
1.1.2 Activated Carbon (AC) on carbon cloth .............................................................. 47
2.2.3 Activated Carbon (AC) on carbon veil (in house preparation) ............................... 47
2.3 MEMBRANE MATERIAL ...................................................................................... 48
2.4 MFC DESIGNS .................................................................................................... 49
2.4.1 Standard, cubic shaped MFCs ............................................................................. 49
2.4.2 Biodegradable MFCs, Peanut-shell and Walnut-shell MFCs ................................. 49
2.4.3 Inverted tubular MFCs ...................................................................................... 50
2.5 INOCULATION .................................................................................................... 51
2.6 FEEDSTOCK AND MEDIA .................................................................................. 51
2.7 DATA CAPTURE .................................................................................................. 52
2.8 CALCULATIONS ................................................................................................... 52
2.9 POLARISATION EXPERIMENTS ......................................................................... 53
2.10 CONDUCTIVITY AND pH MEASUREMENTS ...................................................... 53
2.11 SCANNING ELECTRON MICROSCOPY (SEM) .................................................... 53
2.12 ENERGY-DISPERSE X-RAY (EDX) SPECTROSCOPY ........................................... 54
2.13 INDUCTIVELY COUPLED PLASMA—OPTICAL EMISSION SPECTROMETRY (ICP-OES) .................................................................... 54
2.14 X-RAY DIFFRACTION (XRD) .............................................................................. 54
2.15 BIOMASS ASSESSMENT ..................................................................................... 55
2.16 TOTAL VIABLE COUNT ..................................................................................... 55
2.17 CHEMICAL OXYGEN DEMAND (COD) ............................................................... 55

3 Chapter 3. Initial experiments .............................................................................. 57
3.1 MEMBRANE INVESTIGATION ............................................................................. 57
3.1.1 Introduction ...................................................................................................... 57
3.1.2 Polarisation experiments .................................................................................. 58
3.1.3 Endurance ....................................................................................................... 59
3.1.4 Maximum Power Density and Cost Effectiveness ............................................. 60
3.1.5 Conclusions .................................................................................................... 61
3.2 4th PIN ELECTRODE ............................................................................................ 62
3.2.1 Introduction ...................................................................................................... 62
3.2.1.1 Control Theory ........................................................................................... 63
3.2.2 Methods specific to the experiment ................................................................ 65
3.2.3 4th Pin connection to poise MFC voltage. Power “boost” ................................ 66
5 Open to air Cathode .................................................................................................................113

5.1 INTRODUCTION .................................................................................................................113

5.1.1 Methods specific to the experiment .................................................................................114

5.1.2 Results and discussion ......................................................................................................115

5.1.2.1 Direct contact of MPL based electrode and membrane .................................................115

5.1.2.2 MPL based electrode for improved power .................................................................118

5.1.3 Conclusions .......................................................................................................................120

5.2 WATER FORMATION AT THE CATHODE AND SODIUM RECOVERY ........................................121

5.2.1 Introduction .......................................................................................................................121

5.2.2 Methods specific to the experiment .................................................................................123

5.2.3 Results and discussion ......................................................................................................124

5.2.3.1 Polarisation curve experiment ......................................................................................124

5.2.3.2 Catholyte accumulation ...............................................................................................125

5.2.3.3 Relationship between catholyte accumulation and power generation .....................127

5.2.3.4 Analysis of the accumulated catholyte .........................................................................129

5.2.3.4.1 Conductivity and pH ...............................................................................................129

5.2.3.4.2 EDX and SEM .........................................................................................................130

5.2.3.4.3 GC MS and ICP OES ...............................................................................................130

5.2.3.4.4 XRD .......................................................................................................................131

5.2.3.5 Significance of catholyte accumulation to environmental cleanup ............................133

5.2.3.6 Sodium recovery through bioproduction .....................................................................133

5.2.4 Control of catholyte alkalinity .........................................................................................134

5.2.4.1 Carbon capture ..............................................................................................................134

5.2.5 Conclusions .......................................................................................................................135

5.3 ELECTRO-OSMOTIC-BASED CATHOLYTE PRODUCTION ..........................................................135

5.3.1 Introduction .......................................................................................................................136

5.3.2 Materials and Methods specific to the experiment .........................................................137

5.3.3 Results and discussion ......................................................................................................137

5.3.3.1 Power performance and catholyte generation .............................................................137

5.3.3.2 Catholyte properties ....................................................................................................141

5.3.4 Conclusions .......................................................................................................................144
Appendices .................................................................................................................. 216
A. H-TYPE MFC .............................................................................................................. 216
B. BIOMASS ASSESSMENT: ALGAL CELL COUNT CALIBRATION .......................... 219
C. POTASSIUM RECOVERY IN MICROBIAL FUEL CELL SYSTEM ....................... 221
D. FLAT PLATE MFC MADE OF TERRACOTTA FLOOR TILE .................................... 227
E. ALGAE MONOCULTURES IN CATHODE HALF-CELL ........................................ 229
Figures

Figure 1.1 Various modes of operation of Bioelectrochemical Systems ......................... 15
Figure 1.2 Scheme of the two-chamber Microbial Fuel Cell ........................................ 21
Figure 1.3 Microbial fuel cell with photo-cathode ......................................................... 37
Figure 2.1 Carbon veil electrode ...................................................................................... 45
Figure 2.2. MPL (Microporous layer) on carbon cloth used as folded and single layer electrode ...................................................................................................................... 46
Figure 2.3. MPL (Microporous layer) on carbon veil used as single layer electrode .... 46
Figure 2.4 Activated carbon on carbon veil electrode preparation procedure .......... 48
Figure 2.5 Single-chamber MFC with open to air cathode (left) and two-chamber MFC with aqueous cathode (right) ......................................................................................... 49
Figure 2.6 Novel design of inverted tubular MFC with anode wrapped around the outside of the terracotta tube ..................................................................................... 51
Figure 3.1 Power density vs current density values for each of the tested membranes. 58
Figure 3.2 Voltage vs current density performance for selected membranes during polarisation experiment ........................................................................................................... 59
Figure 3.3 AEM (MI A) and CEM (MI C) comparison of power curves after 3rd and 5th day of substrate supply .............................................................................................. 60
Figure 3.4 Representation of “value for money” of each tested membrane based on manufacturer’s pricing .............................................................................................................. 61
Figure 3.5 Control Theory adapted into the MFC system ................................................. 64
Figure 3.6 Working cell and the driver OFF mode (left), working cell and the driver in ON mode (poise) ................................................................................................................. 66
Figure 3.7 Average performance of tested triplicates in 10x poise manner ............ 67
Figure 3.8 Detailed voltage increase through 4th pin connection in all three working cells and their drivers (dotted lines as drivers) ................................................................. 68
Figure 3.9 Power increase through 4th pin connection in all three working cells. Error bars represent standard error of the triplicated data ................................................. 68
Figure 3.10 Voltage comparison through 4th pin connection between working cell and the driver against series and parallel configuration of the working cell and the driver. 69
Figure 3.11 Current comparison through 4th pin connection between working cell and the driver against series and parallel configuration of the working cell and the driver. 70
Figure 3.12 Power comparison through 4th pin connection between working cell and the driver against series and parallel configuration of the same working cell and the driver. .............................................................................................................. 71
Figure 3.13 Power comparison through poising working cell by the driver cell. ....... 72
Figure 3.14 Power improvement comparison through 4th pin connection between working cell and the driver. .............................................................................................................. 74
Figure 3.15 Power improvement through 4th pin connection between the driver and the working cell under various external resistance conditions. ......................................... 75
Figure 3.16 4th pin electrode performing open circuit potential “sensing” during a polarisation experiment. .............................................................................................................. 76
Figure 3.17 3rd and 4th pin electrodes poised by the Anode Driver and cathode driver cell in 10 sec and ten minutes (hold) cycles................................................................. 77
Figure 3.18 Power density curve (normalized per anode surface area) of the tested biodegradable MFCs in comparison to standard size MFC. ......................................................... 80
Figure 4.1 Experimental set-up; two-chamber microbial fuel cell with biotic cathode (left). Photoreactors and control bottles connected to the cathodic side of tested MFC (right)........................................................................................................................................... 82
Figure 4.2 Power and polarisation curves from 4 types of tested cathodes; the abiotic cathode is marked in blue.............................................................................................................. 83
Figure 4.3 Maximum power densities of biotic cathode (control) and abiotic cathodes based on different electrode material. Algae cellulose coated electrode polarization curve with a 200 x lower power output than algae wire electrode. ........................................... 84
Figure 4.4 Power curve of all tested MFCs after 120 days of operation. .................. 85
Figure 4.5 MFCs and bioreactors in temperature controlled light incubator........... 86
Figure 4.6 Power curves of tested MFCs. Right y-axis present the actual power generation and left y-axis represent power density (per total electrode surface area). Maximum power density values of each MFC are presented in an inset column graph........................................................................................................................................... 88
Figure 4.7 Voltage output under 8k Ohm resistor value during illumination and dark periods of algal based bio-cathodes. The white indicates the illuminated period and the shadow indicates the dark period................................................................. 89
Figure 4.8 Maximum power (μW) values of the polarisation curve experiments performed during the light and dark conditions on each of the experimental MFCs. The
red arrow highlights the difference between light and dark as a result of the previously
dabiotic becoming biotic MFC.............................................................. 90
Figure 4.9 Experimental set-up; two-chamber microbial fuel cell with biotic cathode
connected to the photoreactor. .................................................................. 95
Figure 4.10 Open circuit voltage (OCV) values of photosynthetic cathode MFCs. 14h
day/10night cycle. .................................................................................. 96
Figure 4.11 Open circuit voltage (OCV) values of photosynthetic cathode MFCs. 4h
day/20night cycle. .................................................................................. 97
Figure 4.12 Charging the capacitor to run an impeller pump in ~24h intervals. ..... 99
Figure 4.13 Demonstration of the practical implementation of the 16 MFC stack
connected in a series/parallel configuration (1- MFC stack, 2- super-capacitor, 3-DC
impeller pump). ...................................................................................... 103
Figure 4.14 Microbial Fuel Cell (MFC) with biomass harvesting photoreactor. The
source of fuel can be organic waste (sludge+20 mM sodium acetate) or biomass harvested
from the cathodic photoreactor. ................................................................. 104
Figure 4.15 Polarisation curve experiment of tested MFCs............................ 104
Figure 4.16 Calibration curves of optical density at $A_{678}$ to the cell density and
biomass.................................................................................................. 106
Figure 4.17 Harvested biomass from the photoreactors in relation to the MFC power
performance (right). ................................................................................. 107
Figure 4.18. SEM images of cathode electrodes with developed biofilm (b, d, e, and f)
and abiotic control electrode (a) which spontaneously turned biotic (c). .......... 108
Figure 4.19 EDX analysis on the biotic cathode crystallisation. ....................... 108
Figure 4.20 Algal biomass used as feedstock in comparison to acetate used at the
beginning of the test. .............................................................................. 110
Figure 4.21 Feedstock comparison; data are from day=1-8 and 15-23 from Fig.4.20
above. .................................................................................................. 111
Figure 5.1 Experimental set up. Left: control MFC with carbon veil electrode in the
cathode half-cell, right: single layer MPL coated carbon cloth pressed against the
membrane............................................................................................... 115
Figure 5.2 Polarisation curve experiment of the tested MPL material against the control
(CV). Normalised per identical anode surface area. ................................... 116
Figure 5.3 Maximum power density of the MPL material against the control (CV). Normalised per cathode surface area. ................................................................. 116
Figure 5.4 SEM image of carbon veil (left) and MPL (right). ............................... 117
Figure 5.5 Water formation on MPL surface. .......................................................... 118
Figure 5.6 Polarisation curve presenting average actual power of the fuel cells in triplicates of: single layer MPL based carbon cloth cathode MFCs and carbon veil (control). The inset represents the polarisation curves of tested triplicates. .......... 119
Figure 5.7 Polarisation data of all tested MFCs (mean value of triplicate MFCs). ..... 125
Figure 5.8 MFC with droplets of water forming on the cathode and collected liquid in the syringe (left), cathode chamber disassembled with salts precipitation (centre), catholyte liquid collected (right). ........................................................................ 126
Figure 5.9 Dry mass of salts evaporated from the catholyte formed in all 4 experimental MFC groups under various load conditions. ............................................... 126
Figure 5.10 Power average over 72 h period in relation to amount of catholyte collected after 72 h under various external resistances: 2.4 kΩ, 1.2 kΩ, 600 Ω and 300 Ω ....... 128
Figure 5.11 Conductivity and pH values of catholyte collected after 72 h under various load conditions: 2.4 kΩ, 1.2 kΩ, 600 Ω and 300 Ω. .......................................................... 129
Figure 5.12 EDX profile representing atomic content (%) of detected elements in precipitated salts from the electrode surface outside MFC and the evaporated liquid sample (left) and SEM image of the catholyte crystals (right). .................................................. 130
Figure 5.13 Detected trace elements in catholyte samples (g/L) by ICP OES. Sodium was off the measurable scale in all tested samples (*), as well as Potassium in sample AC (*). ........................................................................................................... 131
Figure 5.14 X-Ray Diffraction pattern comparison of Trona phase (grey line) with the experimental diffraction pattern (red line). ......................................................... 132
Figure 5.15 Power performance of all tested MFCs (mean values). .......................... 138
Figure 5.16 Catholyte formation in situ on the electrode surface as droplets on 2D electrode (left) and in 3D electrode sponge (right). Formed droplets are more visible on 2D structure. .............................................................. 138
Figure 5.17 Catholyte produced under the 300 Ω external resistor plotted against the current (µA) performance. .................................................................................. 139
Figure 5.18 A) Catholyte generated in open circuit conditions, B) Calculated catholyte volume transported via electroosmotic drag in relation to power generation shows linear regression. ................................................................. 141
Figure 5.19 pH of produced catholyte under load shows gradual increase with power performance. .............................................................................................................. 142
Figure 5.20 Conductivity of produced catholyte under load conditions shows linear increase with power .............................................................................................................. 143
Figure 5.21 Catholyte replacement and rapid pH change. ........................................ 146
Figure 5.22 Catholyte pH and MFC power change with MES (pH 5.5) buffer ......... 148
Figure 5.23 Power curve with catholyte controlled by buffer (acidic-MES, neutral-MOPS, alkaline-CAPS) under batch conditions when buffer was added fresh and after 24h ......................................................................................................................... 149
Figure 5.24 Power curve with catholyte controlled by buffers (acidic-MES, neutral-MOPS, alkaline-CAPS) under recirculation. ......................................................................................................................... 149
Figure 5.25 Maximum power levels of all tested conditions. ............................... 150
Figure 5.26 Immersed MFC with anode outside and cathode inside the ceramic tube (left) and MFC diagram (right). .............................................................................................................. 153
Figure 5.27 Experimental test from the start up- maturing. The peaks show the polarisation tests and the arrows external resistance. .............................................................................................................. 153
Figure 5.28 Catholyte formation inside the ceramic tube (left) and the recorded power output [μW] under 53 Ω resistor (right). .............................................................................................................. 154
Figure 5.29 MFC powering a red LED through the energy harvester system connected to the capacitor. .............................................................................................................. 155
Figure 5.30 Voltage and Current of the MFC and connected LED throughout the 7 day operation period. .............................................................................................................. 155
Figure 5.31 COD reduction after 7 days of continuous powering of the LED. ....... 156
Figure 5.32 Mobile phone voltage under charge/discharge connected to one MFC. .. 156
Figure 5.33 Continuous energizing a windmill by three MFCs connected in parallel. 157
Figure 5.34 Polarisation and Power Curves of all three tested MFCs. ................. 163
Figure 5.35 MFC performance during 13 day operation under external load (53 Ω). 164
Figure 5.36 MFC produced water in relation to current generation (left). The amount of water produced (under 53 Ω resistor) in working and OCP conditions (right). .......... 165
Figure 5.37 Catholyte formation (left) and the amount of water produced in working (under 53 Ω resistor) and OCP conditions (right).……………………………………………….. 166
Figure 5.38 pH and conductivity analysis of MFC anolyte and accumulated catholyte in working and open circuit conditions. …………………………………………………………….. 166
Figure 5.39 Power and COD analysis. ……………………………………………………………. 167
Figure 5.40 Cathode electrode on the air side. No visible biofilm growth was observed in working MFC (left) compared to visible biofilm formed in MFC left in open circuit (right)………………………………………………………………………………………….. 167
Figure 5.41 Three MFCs connected in series directly power single LED.……………….. 168
Figure 5.42 Urinal design in Reinvent the Toilet Fair in India (left, middle) with 40 ceramic MFCs placed inside the tank (right). ………………………………………………………… 171
Figure 6.1 MFCs as part of the floater design for practical implementation into the wastewater treatment tank.………………………………………………………………………………… 182
Figure 6.2 The proposed wet scrubbing MFC tower. ……………………………………….. 184
Figure 6.3 Catholyte samples from: Working and OCP conditions in serial dilutions cultivated on nutrient agar plates and using spread method. ……………………………. 186
Figure 6.4 Total Viable Count of colony forming units in 1mL of catholyte obtained from working tubular MFC (T working ) and MFC in open circuit (T OCP). ……. 186
Figure 9.1 H-type fuel cell (left), MPL as cathode/membrane assembly (centre), H-type fuel cell after 2 months of operation (right). …………………………………………………….. 217
Figure 9.2 Actual power [μW] and voltage [mV] of the large type fuel cell……………….. 218
Figure 9.3 Direct count using a calibration for the various experimental conditions used. ……………………………………………………………………………………………….. 219
Figure 9.4 Optical density calibration for the various experimental conditions used.……….. 219
Figure 9.5 Dry mass assessment calibration for the various experimental conditions used. ………………………………………………………………………………………….. 220
Figure 9.6. MFC operation and catholyte production using wastewater and potassium acetate…………………………………………………………………………………………… 221
Figure 9.7 Power performance over 18 day period of MFCs (A) and the amount of formed catholyte in the half-cell under various cathode materials (B) and the correlation of power to volume of formed liquid (C).…………………………………………………………………….. 222
Figure 9.8 EDX shows atomic content (%) in crystalized salt obtained from all samples (left) and the pH and conductivity analysis of the catholyte samples (right)…………… 223
Figure 9.9 Crystalline structure in catholyte sample observed under the digital microscope (inset), XRD profile showing a phase match to mineral pattern of kalicinite……………………………………………………………………………………………………………………………224

Figure 9.10 MFC made out of terracotta floor tile placed in the bucket filled with wastewater (left) and the same MFC directly energizing the windmill motor (right). 227

Figure 9.11 Scaled up MFC system containing six MFC plates immersed in the anolyte……………………………………………………………………………………………………………………………228

Figure 9.12 Power curves obtained from MFCs operated with selected monoculture of algae and cyanobacteria as the biocatalyst in the cathode. ......................................................231
Tables

Table 1-1 Oxygen reduction reaction (ORR) summary .................................................. 27
Table 2-1 Membrane material tested during the course of experimental work ............ 48
Table 3-1 Cost comparison of tested membranes ......................................................... 60
Table 3-2 Poising sequence description for Figure 3.17 .............................................. 77
Table 4-1 Cathode electrode modifications ................................................................. 82
Table 5-1 Types of electrodes in the open to air cathode half cells ................................. 124
Glossary

A – Ampere

AC – Activated Carbon

AEM – Anion Exchange Membrane

B-BiC – Bristol BioEnergy Centre

BES – Bioelectrochemical Systems

BRL – Bristol Robotics Laboratory

CCS – Carbon Capture and Storage

CEM – Cation Exchange Membrane

COD – Chemical Oxygen Demand

CV – Carbon Veil

DI – Deionised Water

DO – Dissolved Oxygen

ED – Electrodialysis

EET – Extracellular Electron Transfer

EPS – Extracellular Polymeric Substances

GDL – Gas Diffusion Layer

ICP-OES – Inductively Coupled Plasma – Optical Emission Spectrometry

MFC – Microbial Fuel Cell

mM – Millimolar

MPL – Microporous Layer
MPP – Maximum Power Point

OCV (OCP) – Open Circuit Voltage (Open Circuit Potential)

PEMFC – Proton Exchange Membrane Fuel Cell

$R_{int}$ – Internal Resistance

S – Siemens

SD – Standard Deviation

SEM – Scanning Electron Microscopy

UWE – University of the West of England

V – Voltage

W - Watts

XRD – X-Ray Diffraction
1 Introduction and Background

1.1 Overview

The current climate change threat caused by greenhouse gas emissions from the combustion of fossil fuels, in combination with the instabilities in fossil fuel markets and increasing energy prices, accelerates the urgency for development of alternative, renewable energy technologies. Alternative, carbon-neutral sources of energy such as solar, wind, biomass or nuclear energy are being explored and exploited to a considerable extent. Biomass has also been considered as a source of energy, but perhaps the potential of utilising wet biomass into direct electricity production has been largely overlooked. The rapidly evolving Microbial Fuel Cell (MFC) technology offers this distinct advantage. Microbial fuel cells are bioelectrochemical systems that extract energy from organic molecules via the metabolic activity of microbes. They promise not only energy regeneration out of biomass but also a waste (biomass) clean-up. Virtually any natural organic matter (if potentially biodegradable) could be used to feed the MFC, which makes the technology ideal for wastewater treatment, reuse and energy recovery. Microbial fuel cells are attracting more interest and development in terms of their practical applications, materials and design. The efforts in this field that contributed to expanding the knowledge about MFCs include UWE’s Bristol BioEnergy Centre and Bristol Robotics Laboratory. The MFC technology has already been implemented in small-scale practical applications although the possibility of large-scale implementation requires further research. It involves looking into many different materials, design and methodologies to improve the system’s performance and sustainability.

One of the major limiting factors of Microbial Fuel Cell technology has been found to be the rate of oxygen supply to and utilisation at the cathode electrode, which suggests
that the higher rates of oxygen or the appropriate modifications in the electro-catalytic properties would result in lower overall limitations to the MFC performance. Employing numerous catalysts both as electrolytes and as immobilised agents on the electrode surface have been shown to improve the overall MFC performance. However such materials require replenishment, which comes at a high economic cost, especially for noble metals. An alternative approach to the unsustainable manner of such catalysis is using photosynthetic organisms. Their property of oxygen generation optimising the reaction rate at the cathodic half-cell has an added advantage of self-maintenance as long as light radiation is provided; in addition excess biomass produced is removed and used as a biofuel source for the anodic reaction. These properties of photosynthetically active cathodes are representing the model of self-sustainability in the dual chamber MFC system. Another cathode modification involves catalyst-free carbon electrode material. The incorporation of a Microporous Layer on the surface of the open-to-air cathode electrode gives an advantage of higher specific surface area of highly porous carbon structures, which supports the oxygen diffusion and reaction rate in a similar manner as conventional would-be catalysts.

The contention is: **The cathode development of Microbial Fuel Cells improves the overall system performance and thus strengthens the case for self-sustainable MFCs that combine simultaneous electricity generation and product synthesis directly from waste.**
1.2 Thesis outline

The ultimate aim of this thesis was to produce a self-sustainable cathode for Microbial Fuel Cells. In the following sections of Chapter 1, a brief history of Microbial Fuel Cells is presented, followed by the principles of MFC operation and its main components focusing on the cathode half-cell. The Methodology has been described in Chapter 2 to list and outline the experimental procedures implemented in this work.

In Chapter 3, the initial experiments are presented to describe the evolution of the performed experimental work and progress in further exploration. These tests include the semi-permeable membrane investigation as a fundamental understanding of operational conditions in MFC system, power modulation through 3rd and 4th pin experiments and mini MFC based experiments on biodegradable materials. Chapter 4 focuses on one of the two main exploration routes, the aqueous cathode. In this mode of MFC operation photosynthetic organisms act as active oxygenators in situ on the cathode side. The work is described in three stages: photosynthetic cathode investigation, lagoon effect during the operation of the photosynthetic cathodes and the biomass growth analysis. The open to air cathode is the main focus in Chapter 5 as the other possible mode of operation. It includes the investigation into the cathode electrode materials and design as well as the incorporation of ceramic material such as terracotta as a separator. This exploration has met an outstanding MFC power performance and has been investigated further to achieve a scaled-up, simple to assemble and operate, MFC reactor stack. Chapter 6 provides a summary of work and outlines the possible directions for future research, in particular the possibilities of the improved design for practical implementation.

This work will begin with a brief introduction on the principles of MFC operation and focusing on one of its main components: the cathode, its latest developments, challenges and implications. The integrated work of optimising the cathode electrode,
the semi-permeable separating membrane, operational conditions and MFC configuration is crucial in improving cathode performance. The development of such biological fuel cell within various configurations is also discussed with regard to Bioelectrochemical Systems (BES) that do not focus on electricity production. The aims and objectives are followed by a description of work carried out to date, conclusions and future plans.

1.3 Background

Twice in human history the revolution led to the transformative changes in society. First, the agricultural revolution shifted human populations from hunting and gathering into agricultural cultivation of crops and domestication of animals. In the 18th century, the industrial revolution led to the utilisation of fossil fuels and technological growth. Nowadays we live in the era of the sustainability revolution (Edwards, 2005; Chen & Zhang, 2015) that needs to take place to maintain a good state of the planet for the future generations to come. Reinventing the energy economy is primarily driven by the population growth that accelerates the demand for food and energy production. At present, global energy requirements are mostly dependent on limited resources such as fossil fuels. Combustion of fossil fuels also has serious negative effect on the natural environment due to CO₂ emissions and consequent climate change.

A recent report from the U.S. Energy Information Administration (EIA) predicts that worldwide energy consumption will increase by 56 % between 2010 and 2040 (U.S. Energy Information Agency, 2013). With no action from humans, this will consequently result in energy-related carbon dioxide emissions increasing by 46 % as well. It is therefore vital to look for alternative, renewable and carbon neutral or carbon negative sources of energy (Canadell et al., 2007). The current climate change in
combination with the instabilities in fossil fuel markets and increasing energy prices, accelerate the urgency for the development of sustainable energy technologies.

1.3.1 Sustainability and self-sustainability

Sustainability has become a popular term in the research arena as well as everyday life from institutions and individuals concerned with the relationship between humans and the global environment. Delivering sustainability requires personal motivations and system change but also knowledge, behaviour and values needed to deliver a society that can live within the limits of our planet, both now and in the future. Given such a prominent and increasing level of attention directed towards this critical term, how exactly is sustainability being defined, or is it becoming a transcendent term that is difficult to measure and so becoming meaningless in its correct or incorrect usage?

The terms sustainable and sustainability are used to describe many different approaches towards improving our way of life. Sustainability does not have a rigid definition. The Oxford English Dictionary defines sustainable as:

Sustainable - (adjective) - “1. able to be maintained at a certain rate or level, conserving an ecological balance by avoiding depletion of natural resources, 2. able to be upheld or defended.”

to sustain - “to keep a person, community, etc. from failing or giving way; to keep in being, to maintain at the proper level: to support life, nature etc. with needs.”

The etymology of the terms originates in the Latin verb sustinere- “to hold up, support, endure”. Sustainability means taking the long-term view of how our actions affect future generations and making sure we do not deplete resources or cause pollution products at rates faster than the earth is able to re-cycle or renew them. Increased economic growth and social development are leading to a large gap between energy
demands and the availability of fossil fuels. Current methods to produce energy are not sustainable and hence the concerns about global warming and climate change require the development of new methods of energy production using renewable and carbon neutral sources.

With the depletion of carbon based fossil fuel resources, the cost of energy will undoubtedly increase suggesting that treating wastewater will likely become prohibitively expensive. In addition, the projected population with growing energy demands in the absence of technological alternatives requires a trade-off between keeping our aquatic systems clean and further warming the Earth’s climate. The Climate Change Act 2008 makes the UK the first country in the world to have legally binding long term framework to cut emissions by 80% by 2050 (DECC, 2008). Water utility companies are already addressing the problem, for example, in the UK water companies are committed to delivering carbon neutral wastewater treatment by 2020 to produce a quarter of energy from renewable sources (Howe, 2009). To address this it is required to combine aerobic wastewater treatment technologies with alternative, low energy consuming processes but also energy producing processes, if such technology is available. Before these concepts can be applied an extensive research addressing fundamental problems needs to be put in place to realise the technology’s full potential.

Self-sustainable - means able to continue in a healthy state without outside assistance. Self-sustainability is a quality of one’s independence, and self-sustainable living takes into account all interconnected elements of the natural world that influence one another to maintain a balance to survive. In the local scale for example is closely related to living “off the grid” and providing nutrition from the land rather than by buying or importing. Therefore the self-sustainability observed in nature and the cycle of energy in natural ecosystems would be the form of interactions that we could take the
inspiration from. For energy systems, self-sustainable would mean becoming an integral element of the environment that does not negatively influences the environment nor uses limited resources. It would imply continuous, long-term transformations with minimal maintenance with recyclable components that will show no environmental damage yet with many benefits.

1.3.2 Inspired by Nature. Energy sources in the natural environment

The primary production of biomass from atmospheric CO₂ is via the production of organic compounds by living organisms. Energy enters ecosystems in the form of sunlight, organic carbon or inorganic substances. Light is used by phototrophic organisms to synthesise new organic matter, which feeds chemoorganotrophs. Chemoorganotrophs use organic molecules as their source of energy and recycle the wastes of other organisms (Prescott, Harley & Klein, 2008). The energy used by animals is derived from carbohydrates, lipids and protein. Living organisms such as plants produce biomass that could be reused and recycled. This process is governed by the use of microorganisms that perform carbon and nitrogen cycles. The movement and exchange of organic matter is regulated by food chain pathways where matter is ultimately decomposed into mineral nutrients. Decay is an essential life process - which helps to digest food and recycle materials and is primarily governed by microorganisms such as bacteria and fungi that release enzymes to break down organic compounds. The most significant effect of microorganisms on Earth is their ability to recycle the primary elements that make up all living systems.

The dynamic transformation of energy in the natural world could be the inspiration required for building future energy systems for utilisation of organic waste, to mimic
the natural processes and ultimately (when deployed globally) help address climate change.

1.3.3 Energy and redox reactions

All living organisms are constrained by the laws of thermodynamics, they can only facilitate those reactions, which are thermodynamically possible. For example, biodegradation processes release energy, which is quantified by Gibbs’ free energy of reaction. It defines the maximum useful energy change for a chemical reaction at constant temperature and pressure. Negative values indicate that the reaction is exothermic (energy producing) and that the reactants will be transformed into the products and energy. Positive values indicate the endothermic reactions and, in order for the reaction to proceed, energy must be put into the system. Free energy changes are related to the equilibria of all chemical reactions including oxidation–reduction. In chemical terms, oxidation is the process where electrode accepts electrons (cathode), and reduction occurs at the electrode that donated electrons (the anode).

\[
\text{Acceptor (oxidant)} + n\text{e}^- \rightleftharpoons \text{Donor (reductant)}
\]

The acceptor (oxidant) and donor (reductant) pair is the redox couple. Oxidation-reduction (redox) reactions can be described as release of energy from an energy source when electrons are moving from electron donor to an electron acceptor. In biological systems the amount of free energy produced is determined mainly by the redox potential difference (ΔE) between electron donor and acceptor. These are listed in redox tower – summarising key biological half reactions that can be linked to find the redox potential change thus the free energy. The free energy change is the amount of energy in a system or a cell available to do work at constant temperature, pH and
pressure. The voltage \((E_0')\) is related to the free energy \((\Delta G^{\circ'})\) of the process that occurs in pH 7.0 by the equation:

\[
\Delta G^{\circ'} = - n F \cdot \Delta E_0'
\]

Where \(n\) is the number of electrons transferred per mole and \(F\) is Faraday constant. The equilibrium constant of a reaction is directly related to its change in free energy and it is standard reduction potential \((E_0')\) expressed in volts and it is a measure of the tendency of a donor to lose electrons. Volt is a unit of an electromotive force and it suggests that redox couples are the potential sources of energy while the overall voltage will be related to the free energy of the reaction (Hibbert, 1993).

### 1.3.4 Energy and metabolism

Energy may be defined as the capacity to do work as all physical and chemical processes are the result of movement of energy. Living cells carry out chemical, transport and mechanical work essential for life; metabolism is the collective of all these chemical reactions that occur in living cells. Considerable metabolic diversity exists in the natural world, however few biochemical processes are common to all types of metabolism. From the energy point of view they can be divided to energy conserving reactions or in general – catabolism which involves energy release from an energy source and anabolism which is a synthesis of complex molecules from simpler ones (Prescott, Klein & Harley, 2008). All these energy transformations in biological systems relate to the study of bioenergetics that centre on adenosine triphosphate (ATP) - the energy currency of living systems. ATP is formed from energy made available during aerobic respiration, anaerobic respiration, fermentation, chemolithotrophy and photosynthesis. Its breakdown to ADP and phosphate makes chemical, transport and mechanical work possible. Energy generation in the natural world is processed through the formation of ATP and reducing power of a co-enzyme
Nicotinamide Adenine Dinucleotide (NADH). It is influenced by the proton motive force (PMF) induced by the mobility of reducing equivalents in the reaction cascade towards a terminal electron acceptor. In the metabolic pathway, NADH plays a key role in the production of energy. Its interconversion between the reduced (NADH) and oxidised form NAD$^+$ forms a biological redox couple and it has negative standard redox potential (-0.32V) and can therefore give electrons to many acceptors including oxygen. The $E'_0$ of the reaction: $\frac{1}{2}O_2/H_2O$ is +0.82 V (Prescott, Harley & Klein, 2008) therefore the electrons move from a donor to an acceptor with more positive redox potential releasing energy. The difference in the standard redox potentials between the two couples discussed here is therefore: 1.14 V making free energy available (Ieropoulos, Greenman & Melhuish, 2008).

1.3.5 Bio-economy

The term ‘bio-economy’ represents a socio-ecological system and economic activity derived from utilising biological resources or bioprocesses to produce products such as food, energy and chemicals. According to the UK government, it represents a sustainable transition pathway towards a more circular economy, encouraging a more sustainable and efficient approach to resource use and management (HM Government, 2015). It is based on the renewable qualities of ecological systems that does not compromise the longevity of current ecosystem services. New technologies and new policies are desired to re-configure energy landscapes and make the bio-economy sustainable utilising organic waste in a similar manner nature does. In the UK, at least 100 million tonnes of carbon containing waste is being produced each year, therefore there is a significant waste feedstock availability (HM Government, 2015). Working towards bio-economy, producing high value products from waste resources is leading the way to a more sustainable approach to resource use and management through the development of innovative technologies.
1.3.6 Energy and water nexus

Water and energy are closely interlinked and interdependent. This creates energy-water nexus that has been recognised by governments and organisations across the world as one of the major factors to address. It is underlined especially in socio-political terms. There is a need for: “water for energy” for cooling, storage, growing biofuels, when the energy is being produced. Also, on the other hand, we need “energy for water” to recycle, pump, treat and desalinate water that has been used. Without energy and water we cannot satisfy basic human needs, produce food for a growing population and achieve economic growth. Rapidly growing cities depend on reliable energy and water supply; however, the sustainable development must reduce demands, and optimise resource utilisation by reuse, recycling and generation of energy from waste. This is where technologies aiming at the both: energy and water can truly become the viable option for the future.

To address these issues and focus on sustainable sources of water and energy for the future, in the recent report, biomass and household wastes – are identified as resources that demonstrate the greatest promise for the UK bioenergy sector, in terms of their availability, quantity and bioenergy potential (Welfle, Gilbert & Thornley, 2014). Substantial research has to be performed to reuse the available energy and water locked in wastewater to help secure the future and address the energy-water nexus.

1.3.7 Fuel Cell technology

Fuel cells are believed to be a modern technology; however their origins can be traced back to the beginning of 19th century. Fuel cells only developed in 1970s when the industrialised world experienced major oil shortages and realised that oil supplies were limited. During this time, more countries were also becoming concerned about air pollution and looking for technologies that would provide clean electricity generation.
The main advantage of fuel cell technology is that the production of greenhouse gases can be avoided, if they are running on hydrogen derived from renewable energy sources. Then it could be truly named as a clean energy source.

At the beginning of 19th century the discoveries such as the electrolysis of water by William Nicholson and Anthony Carlisle in 1800 producing hydrogen and oxygen, as well as the work of Humphrey Davy with the identification of 6 new elements (magnesium, potassium, sodium, calcium barium and boron) led to discovery of fuel cells. In 1802 Davy had reported the construction of a ‘galvanic combination’ a simple fuel cell based on reaction of carbon at one electrode and nitric acid at the other (Davy, 1802). However, the first positive identification of the fuel cell was in 1838 by a Swiss scientist Christian Friedrich Schoenbein, and the first fuel cell device was invented in 1845 by William Robert Grove. Grove's apparatus took in hydrogen and oxygen and produced electricity as well as water. It had a platinum electrode immersed in nitric acid and a zinc electrode immersed in zinc sulphate, and generated a current of about 12 amperes at about 1.8 volts (Hart & Womack, 1967).

In the late 1930s Francis Thomas Bacon began work on alkaline electrolyte fuel cells, and by 1939 he built a cell (Bacon cell) using nickel electrodes in a solution of potassium hydroxide, operating under high pressure. In 1958, Bacon demonstrated an alkaline cell using a stack of 10-inch diameter electrodes. Harry Karl Ihrig fitted a modified 15 kW Bacon cell to power an Allis-Chalmers agricultural tractor. This concept was later used by NASA where alkaline fuel cell used hydrogen and oxygen as fuel. Combining the two in electrochemical reactions it produced: electricity to power the spacecraft; water for drinking and for cooling equipment; and heat to keep the astronauts from freezing in space. This has been a great success in demonstration of fuel cell technology (Larminie & Dicks, 2003). Today, in light of severe
environmental damage, fuel cells are becoming a credible option to substitute other technologies in electricity production (Stambouli, 2011).

1.3.8 Fuel cell operation principles

The fuel cell is a device in which the energy produced by oxidation of fuel is converted directly into electrical energy. All fuel cells have the same basic configuration - an electrolyte and two electrodes, and the different types of fuel cells are based mainly on what kind of electrolyte they use. In the electrochemical cell, the two halves complete with the electrolyte are known as half-cells. The electrode that supplies electrons to the half-cell is the cathode and the one that removes electrons is called the anode. Thus, a reduction reaction occurs at the cathode and an oxidation reaction occurs at the anode half-cell (Hibbert, 1993).

Most fuel cells in use today, however, use hydrogen and oxygen as the chemical fuels. This technology has strong benefits over conventional combustion-based technologies currently used in many power plants and cars. They produce much smaller quantities of greenhouse gases and none of the air pollutants that create smog and cause health problems. If pure hydrogen is used as a fuel, fuel cells emit only heat and water as a by-product. Hydrogen-powered fuel cells are energy efficient, however, the hydrogen itself right now comes from reformed fossil fuels and the biggest hurdle for fuel cells today is cost. Fuel cells cannot yet compete economically with more traditional energy technologies, though rapid technical advances are being achieved. Although hydrogen is the most abundant element in the universe, it is difficult to generate, store and distribute.

A promising biological version of a fuel cell for producing electricity directly from biomass is the rapidly evolving Microbial Fuel Cell. Microbial fuel cells are Bio-Electrochemical Systems (BES) that extract energy from the metabolic activity of
microbes. The microbial ‘bio-engine’ within the fuel cell set up integrates the advantage of a self-replicating biological component with electrochemical techniques and engineering. They offer not only energy regeneration out of biomass but also a waste (biomass) cleanup. Virtually any organic matter that is biodegradable could be used to feed the fuel cell, which makes the technology ideal for wastewater treatment. The MFC links naturally occurring electrochemical processes of anaerobic bacteria breaking down organic compounds and fuel cell technology. It represents a true demonstration of technology that is aiming at the energy water nexus. It has the potential in the future to provide viable solutions to address environmental and energy concerns.

1.4 Bioelectrochemical Systems

In this section MFC technology will be discussed in detail since electricity generation is the primary aim of this research. However, the diverse application possibilities have been rapidly increasing in the past few years. Generally, research in the vast field of the Bioelectrochemical Systems (BES) has focused on converting compounds in wastewater to bioelectricity by Microbial Fuel Cell (MFC) or systems to produce energetically valuable products (Lovley & Nevin, 2011; Wang & Ren, 2013). However, many BES reactions require investment of electrical power into the system to drive the reactions (Fig. 1.1). The variety of configurations and extensive research has focused into this mode of BES operation neglecting the role of electricity production. Opposite to electricity production, the current input can be used to produce hydrogen or other valuable chemicals via Microbial Electrolysis Cell (MEC). Also, the electrons can be used in the cathodic chamber to synthesise organic compounds through microbial electrosynthesis (MES). The potential across the electrodes can also drive desalination in Microbial Desalination Cells (MDC).
Different research groups have used bioelectrochemical systems or as acronym MXCs as a platform of the technology (Harnisch & Schröder, 2010) where ‘X’ may present the main function of each specific cell. There are also many more sub divisions of each category, combining multiple functions and often involving complicated designs. Numerous publications have discovered dozens of functions. In this section the major types of BES are being discussed as the shared principle is the anodic chamber. It is then hypothesised that the compromise between the electricity and the product can be avoided and the MFC electricity generation can drive both product synthesis and water desalination simultaneously.

1.4.1 Microbial Electrolysis Cell (MEC)

If an external power source is added into the reactor the cell becomes a microbial electrolysis cell, this concept has been established in 2005 (Liu, Grot & Logan, 2005) and has been widely explored mainly for hydrogen production (Call & Logan, 2008; Boggs, King & Botte, 2009). Energy losses occurring in MEC add up to determine the applied voltage (Lee, Vermaas & Rittmann, 2010). If the voltage that needs to be applied becomes too high, the energy value of the produced hydrogen is lower than
the energy input (Lee & Rittmann, 2010). Moreover, the production of hydrogen may be contaminated by methanogenesis and severely affect large pilot scale reactors (Cusick et al., 2011). Methanogenesis is unfavourable because methanogens compete with exoelectrogenic bacteria and consume hydrogen (Clauwaert & Verstraete, 2009).

Various modifications to a MEC systems has been described and result in phosphate recovery to precipitate struvite (Cusick & Logan, 2012) or production of other chemicals such as hydrogen peroxide (Rozendal et al., 2009), caustic soda (Pikaar et al., 2013; Rabaey et al., 2010). In these processes, the microbial activity on the anode drive the chemical synthesis of the product on the cathode has been explored in recent years and defined as microbial assisted electrosynthesis. The consumption of protons at the cathode leads to increasing pH resulting in production of valuable oxidants and disinfectants (Rabaey & Rozendal, 2010). Therefore, through electricity input into the system the wastewater could be the source of valuable chemicals.

1.4.2 Microbial Electrosynthesis (MES)

Microbial electrosynthesis (MES) focuses on remediation and bioproduction in which electrons are supplied to living microorganisms via the cathode. Electrons derived from the cathode could be used for reduction of carbon dioxide into valuable products such as acetate, ethanol, and fatty acids (Nevin et al., 2010; Agler et al., 2011). This system only emerged recently and it is the fastest growing branch of BES technology (Wang & Ren, 2013).

1.4.3 Microbial Desalination Cell (MDC)

The electric potential difference between the two chambers has been proposed to drive desalination (Cao et al., 2009; Jacobson, Drew & He, 2011). This can be achieved by introduction of an additional chamber into the system and a pair of cation and anion exchange membranes. MDC designs accomplish desalination by transporting ions
from the middle chamber to the anode and the cathode chambers. This transport may affect a large decrease in pH of the anode and as a result, will inhibit microbial activity. Moreover, high pH at the cathode can result in potential losses reducing overall reactor performance (Luo, Jenkins & Ren, 2011).

### 1.5 History of the Microbial Fuel Cell

The microbial fuel cell is a unique technology linking biology, chemistry and physics. The fundamentals of electron transfer reactions originated in the 18th century when in the 1780s, a biologist at the University of Bologna, Luigi Galvani was the first to discover the physiological action of electricity and demonstrated the existence of bioelectric forces in animal tissue (Hart & Womack, 1967). The investigations of Luigi Galvani into the structure of animal organs established him as one of the founders of modern electro-technology alongside Benjamin Franklin and Alessandro Volta. His work was instrumental in leading Volta to the invention of the first electric battery and electrochemical series. Much of our current knowledge of chemical reactions can be traced almost directly to the experiments of Galvani and Volta. This then inspired the development of further electrolytic research by Humphry Davy and Michael Faraday. Historically, the biologists, physicists, and chemists were influencing each other’s research.

The earliest report about bacteria generating electricity was by a botanist from Durham University, Michael C. Potter, who demonstrated that *Escherichia coli* and *Saccharomyces cerevisiae* could generate electricity (Potter, 1911). Potter stated, “the disintegration of organic compounds by microorganisms is accompanied by the liberation of electrical energy” (Potter, 1911), observing a current flow between two electrodes emerged in a bacterial culture. In 1931 Barnett Cohen operated a potentiostat-poised half-cell and obtained a current of 0.2 mA and found that the
capacity of this device could be improved by introducing potassium ferricyanide or benzoquinone as artificial electron mediators in the anode (Cohen, 1931). During the 1960s, research on MFCs became popular due to the increased interest in converting organic waste into electric energy. Scientists were not able, however, to develop a MFC that could produce electricity at a consistent rate (Lewis, 1966). National Aeronautics and Space Administration (NASA) in the 1960s had a project to use microbial fuel cells as a waste disposal system which was prematurely terminated (Canfield, Goldner & Lutwack, 1963). In the 1980s, visionary work by H. Peter Bennetto started a new, very exciting period in MFC research that lasts until today. Bennetto proposed the first analytical prototype of the MFC and saw the fuel cell as a possible method for the generation of electricity for the developing countries and helped build an understanding of how fuel cells work (Allen & Bennetto, 1993). The first MFCs were successfully operated using artificial electron mediators to facilitate electron transfer in the anode. However, because of the cost and toxicity of these artificial mediators, commercial applications still remained out of reach, until it was found that species such as *Pseudomonas aeruginosa* produce metabolites that function as mediators transferring electrons (Habermann & Pommer, 1991). In the early 2000s, several research groups, including Derek Lovley’s at UMass Amherst, discovered that *Geobacter sulfurreducens* (Bond & Lovley, 2003) and *Rhodoferax ferrireducens* (Chaudhuri & Lovley, 2003), could transfer electrons directly to the anode of an MFC. Not only did this eliminate the need for mediators, but the biofilms that these bacteria formed were significantly more efficient at transferring electrons to the anode. This provided a major breakthrough in understanding electron transfer and proved that there is no need to use mediators. The exo-electrogenic mechanism nowadays being better understood is exactly what Potter has observed hundred years ago. The exocellular electron transfer (EET) is the process by which microorganisms transport electrons.
into and out of the cell towards or from an electron donor or acceptor. In *Shewanella putrefaciens* a direct electron transfer is facilitated by cytochromes located in the outer membrane (Kim *et al.*, 2002). At present, the same principle applies to the cathodic side and many workgroups focus on finding organisms as well as chemical reducing agents that would perform biocatalysis on the cathode. In the last decades MFC had gained profound interest due to its potential and sustainable nature.

Apart from electricity generation, MFCs became a platform for other applications generally named as Bioelectrochemical Systems (BES) (Wang & Ren, 2013a). Moreover, with the prediction of clean water shortages in the future, MFC reactors could be used mostly for water recovery and purification (Franks, Malvankar & Nevin, 2010).

The main focus of research within the MFC community is to improve the technology’s performance in terms of materials, design and methodology. The aim of the BioEnergy Centre research group is to find sustainable, cost effective materials and optimal design for use in stacks to power practical applications including autonomous robots.

The PhD thesis focuses on the improvement of the cathode chamber and this report will summarize previous abiotic cathode and biocathode studies and investigate the potential and feasibility of algal and photosynthetic bacterial cathodes in MFCs. With the development of micro-electronics and related disciplines the power requirement for electronic devices has drastically reduced. Typically, batteries are used to power chemical sensors and telemetry systems, but replacing batteries on a regular basis for some applications can be costly, time-consuming, and impractical. A possible solution to this problem is to use self-sustainable power supplies, such as MFCs, which can operate for prolonged periods using local resources. Extensive research towards developing reliable MFCs to this effect is focused mostly on selecting suitable organic
substances that could be used as sources of energy, but also improvement in the field of materials, MFC design and types of microbes being used. That may include development of autonomous robots and use for environmental measurement, data processing, information and locomotion (Ieropoulos & Melhuish, 2005). BRL’s development of the world’s first autonomous robot that integrates life and machine in a symbiotic manner as an exemplar of artificial life (Ieropoulos et al., 2010a) is a proof of concept and gives opportunities for the future as a basis for further research. This technology could possibly be scaled appropriately to be used both as an alternative power source and wastewater treatment in domestic and industrial environments.

The Microbial Fuel Cell (MFC) is a technology that is already 100 years old but only in the last decade has it become a point of great interest for many researchers around the world. Microbial fuel cells are seeing more and more development in terms of their practical applications, materials and design. The efforts in this field that contributed to expanding the knowledge about MFCs include UWE’s Bristol BioEnergy Centre, Bristol Robotics Laboratory. As the knowledge and the amount of research groups expand worldwide, MFCs have been used in a variety of applications such as:

- Gastrobot - (Chew-Chew) train moving on fixed train lines powered by MFCs fed on sugar cubes (Wilkinson, 2000)


- BOD monitoring (Kim et al., 2003)

- EcoBot-II - autonomous robot performing sensing, information processing, communication and actuation phototaxis, by utilising unrefined biomass (Ieropoulos & Melhuish, 2005)
- effective renewable power source for remote temperature monitoring (Donovan et al., 2008)

- meteorological buoy that measures air temperature, pressure, relative humidity, and water temperature (Tender et al., 2008)

- EcoBot-III - world's first robot to exhibit true self-sustainability (Ieropoulos et al., 2010a)

- development of a self-sustainable microbial fuel cell stack capable of self-maintenance powering single and multi-channel peristaltic pumps (Ledezma et al., 2013)

- a microbial fuel cell stack capable of charging a mobile phone (Ieropoulos et al., 2013)

1.6 Microbial Fuel Cell principles and operation

![Figure 1.2 Scheme of the two-chamber Microbial Fuel Cell](image-url)
MFC principle of operation is based on the metabolic activity of the microorganisms utilising organic fuel and the extraction of the electrical energy generated by the microbes. The MFC reactor consists of an anode, a cathode and a membrane that is located between the two half-cells (Fig 1.2). In the anode, microorganisms oxidise organic matter into CO$_2$ (ultimately), electrons and protons. Bacteria generally carry out their metabolic activities (anabolism and catabolism) in the presence of oxygen (aerobic) or in its absence (anaerobic) (Prescott, Klein & Harley, 2008).

They transform an electrochemically inactive substrate and its chemical energy into a form that is accessible for conversion into electric energy. This is thanks to the cell catabolic energy that liberates energy from the substrate. Microbes metabolise substrates and the abstracted electrons are transferred via redox carriers and finally transferred to an externally available terminal electron acceptor; the anode. The more positive the redox potential of a terminal electron acceptor, the higher the energy gain for the microbe. This is described as energy transformation (Schröder, 2007). The function of the MFC is harvesting the available electrons (e-) by introducing electrodes as terminal electron acceptors.

At the cathode, a chemical or microbiological assisted reduction takes place that, for example reduces oxygen to water, ferricyanide to ferrocyanide (Delaney et al., 2008; Roller et al., 2008; Thurston et al., 1985; Oh & Logan, 2006), nitrate to nitrogen gas (Clauwaert et al., 2007), ferric ion to ferrous ion (Ter Heijne et al., 2006). Electrons produced are collected on the electrode and travel along the circuit to the cathode, whilst protons migrate through the Proton Exchange Membrane (PEM). Proton exchange membranes physically separate two compartments, whilst allowing protons to transport to the cathode in order to sustain electrical current. The protons and electrons subsequently combine with oxygen in the cathode to form water. Electricity
in this case is generated through the metabolic activity of the microorganisms in the anode extracting energy from organic matter.

There are several important parameters that need to be summarised in the context of energy transfer in the MFC system.

*Redox potential difference* - is the difference between the two redox couples (two reactions in dissimilar conditions) and it is the driving force that governs the flow of electrons.

*Current [I]* - is the flow of electrical charge (electrons) from the anode to the cathode through the external circuit (wire). It can be calculated by Ohm’s law as the relationship of system’s voltage and applied resistance.

*Electrical Power [P]* - It is a rate of doing work, measured in Watts, it can be calculated as the product of system’s voltage [V] and current [I].

*Internal resistance* – is the ohmic, concentration and kinetic losses of the system where the internal resistance is the sum of the resistances of the anolyte, catholyte, a separator and also the electrode material and bacterial culture. For the MFC to achieve maximum power output, the internal resistance of the system must be as low as possible.

*Maximum Power Point (Maximum Power Transfer)* - in electrical systems it can be defined as the maximum level of power that a source with a fixed internal resistance can deliver across the external load. It can be achieved when the resistance of the applied load is equal to the systems internal impedance.

### 1.6.1 Anode

The anodic microenvironment influences the nature of metabolic activity and thereby affects the resulting redox reactions. The composition of microbial communities
located at the anodic biofilm affect the rate of electron transfer which determines the overall power output (Velasquez-Orta et al., 2011). Microbes with high electron discharge capabilities are considered to be electrochemically active. For bacteria to produce electricity in MFCs, the cells need to transfer electrons generated from metabolism to the outside of the cell via their lipid membranes. Several electron transfer mechanisms, including direct transfer of electrons via membrane bound c-type cytochromes or nanowires have been studied (Lovley, 2008; Reguera et al., 2005). This process of extracellular electron transfer (EET) also might involve solid components of the extracellular biofilm matrix (Marsili et al., 2008), or also through the redox mediators excreted by microbes showing a self-mediated electron transport (Rabaey et al., 2004; Rabaey & Verstraete, 2005).

Whilst anodes and cathodes can support and exploit bacterial respiration, most research has focused on understanding the microbial anodic electron transfer. This has revealed an extensive diversity of bacteria that transfer electrons onto external electron acceptors. Culture-independent studies of MFC anode biofilms indicate that the diversity of such microbial communities far exceeds that of the known available electricity-producing isolates, suggesting that many organisms with this capability have yet to be discovered (Ki et al., 2008). This knowledge has spurred interest in using a variety of alternative inoculum sources, operating conditions, and isolation methods to increase the known diversity of electrode-reducing organisms. Thus, the choice of the electrode material is crucial as it must be durable with high conductivity, high surface area for biofilm formation and relatively cheap when considering scale up.

What is most interesting in terms of waste treatment is that anodic heterotrophic bacteria are able to utilise a wide range of substrates (Franks & Nevin, 2010; Pant et al., 2010). This allows the treatment of many soluble and insoluble substrates and
macromolecules including animal and human urine (Ieropoulos et al., 2013; Kuntke et al., 2012) or uric salts (You et al., 2014) with an efficient nutrient removal (Gonzalez et al., 2008). One of the projects in B-BiC research group focuses on improving anode breakdown efficiency of organic waste and energy output levels.

1.6.2 Proton exchange membrane

Proton exchange membranes are one of the most important components in MFCs. Bioelectrochemical systems typically employ cation exchange membranes (CEMs) but it has been shown that cation species other than protons can diffuse through the membrane and their concentration is typically $10^5$ higher than protons (Rozendal, 2006). The MFC internal resistance (one of the most important performance characteristics which is described later) increase is mainly caused by the sulfonate functional groups of CEM being occupied with cations contained in the anolyte (Choi et al., 2011). Protons produced in the anode compartment of MFCs do not always bind with the PEM’s functional groups, and this causes a pH imbalance in the anode and the cathode chambers and reduces MFC performance (Chae et al., 2008; Rozendal, 2006). It should be noted however that several studies reported an improvement in MFC performance with increasing electrolyte strength due to the increased ion conductivity through a liquid medium (Gil et al., 2003; Liu, Grot & Logan, 2005) or movement of liquids. Anion exchange membranes (AEMs) have been reported to outperform CEMs (Kim et al., 2007; Mo et al., 2009) in the wastewater treatment application. Ceramic as porous, semi permeable membrane has also been recognised as a cost effective replacement for PEM (Behera, Jana & Ghangrekar, 2010; Ajayi & Weigele, 2012; Behera et al., 2010; Winfield et al., 2013a; Ieropoulos et al., 2013). From the wide variety of membranes it is important to choose low cost, durable and well performing materials and this was the main aim for the first experiments that were undertaken and is described in Chapter 2.
1.6.3 Cathode system

Oxidation processes on the anodic side have been extensively studied in MFCs, however reduction processes in the cathode half-cell have received less attention. However, in 2007 Rabaey et al, recognised that there may well be tremendous potential to develop novel biotechnological applications using the cathodic reduction (Rabaey et al., 2007). In order to operate the MFCs with a high energy output, the electric potential difference (voltage) must be as high as possible which means that the negative potential of the anode must be as high as possible versus a high positive potential in the cathode. In order to achieve this, the reduction and oxidation reactions must be driven at high rates. The cathode, where the oxidation occurs, must have an electron acceptor to be reduced. Generally, the cathodic reduction may be classified into aerobic and anaerobic depending on the final electron acceptor. In aerobic environments the oxygen reduction reaction will be the most dominant. Anaerobic respiration however is used to describe the process of energy conservation using electron acceptors other than oxygen. A range of molecules can act as electron acceptors for example nitrogen and sulphur compounds, perchlorate, phosphate etc. and the compound with a higher redox potential will be utilised over a compound with lower redox potential. One of the examples is the process of denitrification and if adopted in MFC it would represent the biocathode that will be discussed further in detail in this chapter. In abiotic environments the process of electron acceptance varies utilising various electron acceptors such as Fe$^{3+}$ or Mn$^{2+}$. For example reduction of ferric iron to ferrous iron in the cathodic compartment according to Fe$^{3+} + e^- \rightarrow$ Fe$^{2+}$ (Ter Heijne et al., 2006).

These reactions will be discussed below focusing on the environment of the cathode.
1.6.3.1 The oxygen reduction reaction (ORR)

Oxygen is the most readily available electron acceptor for biological systems having a reduction potential of +0.816 V (vs. SHE reference electrode) and it is widely used. The understanding of electrochemical reduction of oxygen is of prime importance in light of practical use in clean energy sources such as fuel cells and batteries (Kinoshita, 1992). The rate determining step is the initial oxygen adsorption on the surface of the electrode (Yeager, 1984) and it is dependent on the type of electrode material and the pH environment (Kinoshita, 1988). In general, 4-electron pathway is predominant on noble-metal catalysts such as platinum and metal oxides while 2-electron pathway occurs on carbon based electrodes (Kinoshita, 1988).

Table 1-1 Oxygen reduction reaction (ORR) summary.

<table>
<thead>
<tr>
<th>Conditions</th>
<th>Pathway</th>
<th>Reaction</th>
<th>EO (vs. SHE)[V]</th>
</tr>
</thead>
<tbody>
<tr>
<td>Acidic</td>
<td>4-electron pathway</td>
<td>O₂ + 4H⁺ + 4e⁻ → 2H₂O</td>
<td>1.229</td>
</tr>
<tr>
<td></td>
<td>2-electron (peroxide pathway)</td>
<td>O₂ + 2H⁺ + 2e⁻ → H₂O₂ Followed by reduction of peroxide: H₂O₂⁺ 2H⁺ + 2e⁻ → 2H₂O or decomposition : 2H₂O₂ → 2H₂O + O₂</td>
<td>0.67 1.77</td>
</tr>
<tr>
<td>Alkaline</td>
<td>4-electron pathway</td>
<td>O₂ + 2H₂O + 4e⁻ → 4OH⁻</td>
<td>0.403</td>
</tr>
<tr>
<td></td>
<td>2-electron (peroxide pathway)</td>
<td>O₂ + H₂O + 2e⁻ → HO₂⁻ + OH⁻ Followed by reduction of peroxide: HO₂⁻ + H₂O + 2e⁻ → 3OH⁻ or decomposition : 2HO₂⁻ → 2OH⁻ + O₂</td>
<td>-0.065 0.867</td>
</tr>
</tbody>
</table>

The oxygen reduction reaction (ORR) at the membrane/cathode interface consumes oxygen and generates water or hydrogen peroxide (Table 1-1). However, the peroxide pathway has not been fully explored in the MFCs primarily because of the instability of the molecule on carbon electrodes particularly in cathodic alkaline solutions (Kinoshita, 1988). Therefore, investigations should explore the possibility of any occurring peroxide pathway in more depth.
1.6.3.2 Electroosmosis

Electroosmotic flow was first reported in 1809 by F.F. Reuss who showed that water could be made to flow through a porous clay plug by the application of an electric field (Reuss, 1809). When an electric field acts on an electrolyte solution, cations move to the cathode and anions move to the anode. Transfer of momentum between moving ions and surrounding solvent molecules takes place. Electroosmosis can therefore be defined as the flow of a liquid induced by the potential across a microchannel. When a selective membrane is interposed, protons released upon the oxidation in the anodic half-cell move concertedly in the applied electric field at the ceramic-water interface and by dragging bulk water. The proton selective membrane properties and its configuration in dual-chamber MFCs give the opportunity to extract cations from the anolyte to the cathode (Rozendal, 2006). Therefore the cathode potential can be explored as a means for excluding specific contaminants, for example heavy metals (Rabaey & Keller, 2008). Application of an electric field to the system, where electrolyte solution is separated by the membrane to another electrolyte solution, causes not only transference of ions but also flow of liquid through the membrane. This leads to electroosmotic transport. As a result, recent studies have moved away from linking the cathodic processes to the oxidation of organic matter in wastewater at the anode as the source of electrons. Instead, the focus is on electricity consuming Microbial Electrolysis Cells where microbially assisted electrosynthesis can effectively be used for the production of oxidants or disinfectants (Rabaey & Rozendal, 2010). A similar phenomenon that occurs is water dissociation via electrodialysis where an external electrical energy source is used to provide the energy for separating the ionic species. However, it has been proposed that the same process of microbially driven electrosynthesis can be achieved with both energy production and simultaneous product extraction in a simple MFC design (Gajda et al., 2014a).
Moreover, it can generate highly saline catholyte that can additionally act as a drag solution which links the operational conditions of such system to Osmotic MFC. An osmotic Microbial Fuel Cell (OsMFC) incorporates forward osmosis membranes, catholyte NaCl solution and platinum electrodes. OsMFC represent a water extraction system from the anolyte by dragging water molecules through the membrane via osmotic pressure (Ge et al., 2013). The higher catholyte salinity increases current generation of the OsMFC (Zhang, Brastad & He, 2011). This relies on Forward Osmosis (FO), where the osmotic pressure gradient that exists between solutions of two different concentrations (thus different chemical properties) is driving the transport of water across the membrane. The driving force is created by high solute concentration solution and water transport occurs naturally via electro-passive transport. The disadvantage of FO reactors is the salt leakage such as NaCl across the membrane (Hancock & Cath, 2009). This leads to extracting water from anolyte simply by MFC operation without using external drag solution. To explore this path further it is important to look into the cost effective materials, design and methodology to showcase the technology to be ready for practical application in wastewater treatment plants.

1.6.3.3 Abiotic cathode

Conventional MFCs typically consist of a biological anode and an abiotic cathode. The abiotic cathode involves a terminal electron acceptor, which is typically oxygen. However, because of the slow reduction rates on the surface of carbon electrodes, a catalyst is normally incorporated to increase the rate of chemical reaction whereas the catalyst itself is not undergoing any physical change.

Metals such as iron and cobalt are also suitable electron mediators between the cathode electrode and oxygen, because of their high reaction rate between their changeable
redox states. Park and Zeikus have shown that Fe (III) –cathode improved the power output of the MFC compared to woven graphite cathode (Park & Zeikus, 2003). Fe (III) was reduced to Fe (II) by electrons generated from the anode reaction and Fe (II) was re-oxidised to Fe (III) by oxygen. In this way the iron compounds were acting as effective electron mediators. Platinum has been proven to have a high catalytic activity towards oxygen reduction (Liu & Logan, 2004; Logan et al., 2005) but because of it scarcity it comes at a high cost. The goal of many investigations is the development of new cathode materials efficient enough to replace the expensive Pt-catalysts. Other precious metals such as gold nanoparticles (El-Deab et al., 2005; Gotti et al., 2014) or gold-covered copper electrodes (Kargi & Eker, 2007) have shown to be suitable as catalysts. Many other oxidants have been used such as permanganate (You et al., 2006), persulfate (Pandit et al., 2011), lead dioxide (Morris et al., 2007). Another very popular catalyst is ferricyanide, which is used as an electron acceptor and its addition to carbon electrodes improves the MFC performance even in comparison with platinum (Oh & Logan, 2006). Ferricyanide is being reduced to ferrocyanide, thus, the chemical must be replenished after it is depleted, which is unsustainable and environmentally toxic (Liu et al., 2009). Independent of catalysts or artificial redox mediators, it is clear that none of them are suitable for use in field applications for the high cost and potential pollution, even though those modifications could greatly improve cathode performance.

Metal oxides are also suitable such as manganese oxide MnO₂ (Liu et al., 2010a; Zhang et al., 2009b; Rhoads, Beyenal & Lewandowski, 2005) and recent advances in nanotechnology are enabling fabrication of hybrid MnO₂ nanostructures as cathode catalysts (Haoran et al., 2014). Non noble metals such as pyrolised iron (II) phthalocyanine (FePc) and cobalt tetra methoxyphenylphorphirin (CoTMPP) had been reported to perform as well as a platinum cathode and slightly better than other iron
compounds, probably due to stronger back binding between oxygen and cobalt (Zhao et al., 2005), however, the long-term instability of the transition metal compounds makes them impractical. Although many studies have researched advanced nanomaterials and transition metal compounds they still remain costly when applied to MFC.

1.6.3.4 Catalyst free cathode

The use of ferricyanide in analytical laboratory conditions is highly efficient, used by researchers even in recent publications (Wei, Han & Shen, 2012), however due to the reduction of this oxidising agent by incoming electrons as well as taking up the hydrogen ions (H\(^+\)), replenishment is required. In contrast, oxygen for the gas diffusion cathode is available from the air and the only condition for successful operation is to keep the electrode moist in order for H\(^+\) ions to flow. The good example of such catalyst free MFC set up is the transition from ferricyanide based cathode half-cell in EcoBot-I (Ieropoulos, Greenman & Melhuish, 2003) to the open to air cathode developed for EcoBot-II (Ieropoulos & Melhuish, 2005) where it presents the practical use of non-catalyst based working carbon cathode.

The specific surface area of current popular air-cathode designs are orders of magnitude lower compared with for example the brush anode, making the cathode the limiting half-cell. The study of Freguia (Freguia et al., 2007) shows that a stable microbial fuel cell process can be obtained with a catalyst-free material as a cathode, which were plain graphite granules. It was shown that granular graphite is able to support cathodic oxygen reduction thanks to its very large specific surface area. Granular graphite is competitive with low surface catalysed cathodes in terms of cost and power production. Many researchers are looking into carbon materials with large specific surface area. There have been reports where further increases in the surface
area of the cathodes increased the total power output (Zuo et al., 2007). Carbon materials used as electrodes include carbon felt, carbon paper, carbon fibres (cloth, veil). Carbon veil has been used in multiple studies both as an anode and a cathode (Ieropoulos, Greenman & Melhuish, 2008; Zhao et al., 2008; Ieropoulos & Melhuish, 2005; Ieropoulos et al., 2010a; Ledezma et al., 2013) as carbon veil or carbon-paper based materials have a large microbially-accessible surface showing better platform for growing biofilm on its surface (Liu et al., 2010b) as well as in inner layers of the folded material (Ieropoulos et al., 2010b). As the cathode, carbon veil has been used successfully in MFC both in aqueous (Ieropoulos, Greenman & Melhuish, 2003) or open to air configurations (Ieropoulos & Melhuish, 2005) in order to drive robotic platforms Ecobot I, Ecobot II and EcoBot III. Activated carbon is one of the most widely used cathode materials at the moment (Santoro et al., 2013; Watson, Nieto Delgado & Logan, 2013; Zhang et al., 2014) due to its large specific surface area and physicochemical properties. It has been used widely in wastewater treatment as an odour adsorbent (Martin, 1980) or for the adsorption of heavy metals (Monser & Adhoum, 2002) or hydrogen sulphide (Le Leuch, Subrenat & Le Cloirec, 2003). Activated carbon as cathode material has gained a lot of interest in recent years, being an alternative material to platinum (Zhang et al., 2014; Ghasemi et al., 2011). Also carbon nanotubes showed potential, however, the accumulation of biofilm and chemical deposits decreased performance due to the blockage of active area and limited oxygen diffusion (Wang et al., 2011). The electrode material is another part of MFC architecture that plays an important role in performance as well as practical issues of cost, material preparation and operational conditions.

In a dual-chamber MFC with an aqueous cathode, oxygen must be continuously supplied for the reaction; continuous use of fresh and oxygenated water has nonetheless obvious limitations because of its economic and environmental cost.
Finding a solution that is analogous to the bio-catalytic properties of microbes and the role of biofilm at the anode has focused research in recent years on biotic cathodes (biocathodes).

1.6.3.5 Biotic cathode (Biocathode)

The output of such biocathodes can be much lower than using air cathode, however inexpensive non-catalytic electrode materials used as biocathodes may take part in secondary wastewater treatment. Recent reviews had been suggesting that an attractive alternative to abiotic catalyst is the biocathode where microorganisms are used as biocatalysts (Ben Liew et al., 2014; Huang, Regan & Quan, 2011). In the biocatalytic processes natural catalysts such as enzymes or excreted metabolites perform chemical transformations. The biocathode has the advantage of being sustainable since it utilises living organisms to produce oxygen or other compounds for the cathodic reduction reaction.

Biocathodes may give the same benefits as lowering the cost by eliminating precious metals for electrode material. Moreover, they give the advantage of sustainability as electron mediator replenishment occurs in situ. Finally, as in bioanodes, microbial metabolism may utilise unwanted compounds in the catholyte. For example, electrons extracted from oxidising nitrite compounds in the organic matter, result in denitrification. Applying a biocathode, Clauwaert et al. utilized nitrate as an electron acceptor to get an output of 8 W/m$^3$ (Clauwaert et al., 2007). A denitrifying MFC is not equivalent to oxygen when it comes to power production but it is compensated by the added value of removing nitrate. When the anode of an acetate oxidising tubular microbial fuel cell was combined with an open to air microbial cathode for energy production, the maximum power production was approximately 83 and 65 W/m$^3$ for batch fed and continuous systems, respectively (Clauwaert & Ha, 2007). Lefebvre
reported the use of denitrifying mixed culture bacteria in the cathode chamber. It was operated with 125 mL chambers and 25 cm² carbon paper electrodes using synthetic wastewater. The system was generating 9.4mW/m² and also removing 65% COD. This proves that the biocathode- in this case denitrifying- may be considered as an alternative to platinum (Lefebvre, Al-Mamun & Ng, 2008). Focussing on nitrogen removal, bioelectrochemical systems can reduce the carbon requirements needed for heterotrophic denitrification (Virdis et al., 2008). Also low performance levels were reported during the start-up period, so manganese oxides were used to improve the initial stages. Rhoads et al. described a biological manganese shuttling mechanism in the cathodic biofilm where *Leptothrix discophora* used Mn (²⁺) as the electron donor and oxygen as the electron acceptor (Rhoads, Beyenal & Lewandowski, 2005). The oxidised manganese was then chemically reduced at the electrode. A similar concept was also shown in benthic MFC (De Schamphelaire *et al.*, 2008).

However, increasing information about biocathodes is available and the mechanisms along with the microbial community working in a biocathode have been studied in various configurations (He & Angenent, 2006; Ben Liew *et al.*, 2014; Kelly & He, 2014) showing nutrient removal. The biocathode is a promising way to improve the cathode reaction, and it can be adopted into the MFC to improve the cathode performance, without the need for artificial mediators and catalysts (Zhang *et al.*, 2012b). For example growing *Acidithiobacillus ferroxidans* directly on the electrode suggested that Fe species excreted by the biofilm cells could be mediating electron transfer from the electrode to the cell (Carbajosa *et al.*, 2010). There are several research groups worldwide working with MFCs and significant effort has been made to use this technology in wastewater treatment. The wide known and proven thesis that microorganisms can use electrodes as electron acceptors on the anode raises the question of whether or not the reverse reaction can occur on the cathodic side whereby
electrodes could serve as electron donors for anaerobic respiration. Since a wide variety of species including *Geobacter, Shewanella, Arcobacter* and *Rhodoferax* appear to transfer electrons to the electrode surface (Bond & Lovley, 2003) it then becomes highly possible that electrons might also be transferred from the electrode to the microbes.

Cathodic biofilm growth gave a reported 4 fold increase in a fuel cell’s electric current in comparison to an abiotic oxygen cathode (Freguia et al., 2008). The system was reported to use anodic effluent as continuous feed for the cathode and proven to be stable for 9 months. However, the authors mention that oxygen consumption by the bacteria could compete with oxygen required for the cathodic operation and limit the electricity generation which implies limiting the organic loading to prevent overgrowth of heterotrophic bacteria.

Another publication reports *Geobacter sulphurreducens* biofilm activity on a stainless steel biocathode and concluded that current densities were 25 times higher than graphite electrodes in the same conditions (Dumas, Basseguy & Bergel, 2008). Removing the biofilm from the electrode surface resulted in a dramatic decrease of current and it was recorded that the *G. sulphurreducens* biofilm was catalysing the fumarate reduction to succinate. Not only the biofilm, but also the electrode material play an important role in the overall energy output. It is believed that stainless steel perform better because of its electrokinetic properties. Also, with regards to the electron acceptor, Dumas *et al.*, used fumarate which has a relatively low redox potential. However, the use of the acceptor with a higher redox potential, such as oxygen would theoretically perform better.

It has been demonstrated that electrodes can serve as electron donors for microbial reduction in *Geobacter* species where the electrode provides electrons for the
reduction of nitrate and fumarate. *Geobacter* can use electrodes as either an electron donor or an electron acceptor, depending upon the potential of the electrode (Gregory, Bond & Lovley, 2004). It was also presented that *Geobacter sulfurreducens* reduced U (VI) to U (IV) with an electrode serving as the electron donor (Gregory & Lovley, 2005). For example nitrate could not be reduced in the absence of *Geobacter metallireducens*; and the bio-reduction of nitrate provided energy for cell growth as there was a significant increase in microbial cells on the electrode surface when the electrode was donating electrons.

Looking at the electrode materials it has been reported that a seawater battery prototype is more effective using a carbon fibre cathode (Hasvold et al., 1997). The carbon based electrodes have the advantage over metal based ones that the process of corrosion is prevented. There has been a report (Dumas et al., 2007) describing current densities up to 140 mA/m² using stainless steel for both anode and cathode. It demonstrated that the biofilm-covered cathode in the marine MFC was working very efficiently and that the anode was limiting the performance. The reason why the biofilm-covered stainless steel cathode worked so efficiently seems to be due to the biocorrosion process, which occurred in the biofilm formed on the electrode in aerobic seawater and it induces catalysis of the oxygen reduction on stainless steel. Having the aforementioned reports in mind, and preparing the microbial fuel cell system based on similar principles but using mixed culture sludge microorganisms instead of marine sediment, stainless steel cathodes and carbon veil anodes could be an interesting combination to explore.

Corrosion of stainless steel in marine environments is known to be heavily affected by the biofilm and there have been many proposed explanations for this phenomenon. It is believed, that enzymes entrapped in the Extracellular Polymeric Substances (EPS) of the biofilm possess catalytic properties. It is now a question of how to utilise
corrosion on metal electrodes which is causing electrode fouling but at the same time corrosion is crucial to cathodic biofilm activity. The answer might be found in cathodic polarization. Inside the fuel cell electrons were provided through hydrogen oxidation on the anode, so that stainless steel was polarised at slightly cathodic potential values. It has been shown that weak cathodic polarization prevents corrosion. In other words - electrons provided by the anode are being consumed on the cathode so the stainless steel material is not affected and is not giving up its own electrons but instead it just acting as a conductor. This is common practice in steel ships, metal constructions and pipelines as a kind of cathodic protection. It has also been shown that seawater biofilms give stainless steel electrodes good catalytic properties for oxygen reduction (Bergel, Féron & Mollica, 2005). Low cost and easy to produce stainless steel cathodes have been previously reported as good alternatives to more expensive solutions (Zhang, Merrill & Logan, 2010).

1.6.3.6 Photosynthetic Biocathode

![Figure 1.3 Microbial fuel cell with photo-cathode.](image-url)
Considering that sunlight offers an unlimited source of energy, development of self-sustainable microbial fuel cells that rely on light instead of organics as the energy source has become a popular area of research in recent years. The research however has focused on photosynthetic anodes. In 1964 Berk and Cranfield successfully employed blue-green marine algae in a cathodic chamber and reported light dependent responses (Berk and Canfield, 1964). It was shown that Pt electrode in the presence of the photosynthetic microorganisms shifts the voltage upon illumination. *Rhodospirillum rubrum* in the cathode chamber exhibited a shift in the potential to a more negative one and marine algae in the cathode side shifted into more positive potentials when radiated with appropriate light. Previous solar powered fuel cells involved hydrogen production by photosynthetic microorganisms (Berk & Canfield, 1964; Kayano *et al*., 1981) or coupling conventional MFCs and photosynthetic bioreactors with anaerobic oxidation of organic substrates produced through photosynthesis (Strik, Hamelers & Buisman, 2010). Bio-hydrogen fuel cells involving an anode catalysed oxidation of hydrogen synthesised by *Chlamydomonas* or the purple bacterium *Rhodobacter* have also been demonstrated (Rosenbaum, Schröder & Scholz, 2005b, 2005a). The first illustration of positive light response (immediate increase in current upon illumination) was reported for aerobic photosynthetic microbial fuel cell that operated using cyanobacterial cultures (Tanaka, Tamamushi & Ogawa, 1985). A positive light response is consistent with the idea that electrons could be supplied directly by the photosynthetic electron transfer chain and not derived from the respiratory transfer chain or oxidation of hydrogen (Yagishita, Horigome & Tanaka, 2007). However, these studies were performed using two chamber MFCs with potassium ferricyanide in the cathode chamber, mediator and phosphate buffer in the anode chamber, which excludes it from implementation in practical applications.
In the past decade, one of the most promising attempts to improve the sustainability of the system was the integration of photosynthesis into the MFC (He & Angenent, 2006; He et al., 2009; Rosenbaum, He & Angenent, 2010), recognising that illumination - and as a consequence - the level of dissolved oxygen (DO), can effect polarity reversal (Strik, Hamelers & Buisman, 2010). Previous photo-cathode investigations (Fig. 1.3) showed that photosynthetic organisms can be used as active oxygenators in situ in the cathode (Ieropoulos, Greenman & Sauer, 2010), as a monoculture of Chlorella vulgaris (Powell et al., 2009; Powell & Hill, 2009a), or as a mixed culture cultivated on growth media (Walter, Greenman & Ieropoulos, 2013). Aiming towards practical applications, the use of mixed, wild algal cultures in the cathode chamber is more viable in comparison to monoculture, which requires aseptic conditions, pH control and continuous supply of sterile media. However, using active oxygenators such as photosynthetic organisms in situ leads to oxygen depletion during the dark phase, where photosynthesis stops and respiration occurs (Strik, Hamelers & Buisman, 2010; Wu et al., 2013; Walter, Greenman & Ieropoulos, 2013). A photoreactor or reservoir connected to the cathodic side of MFCs may theoretically produce more biomass as well as provide oxygen for the cathodic chamber (De Schamphelaire & Verstraete, 2009). The aspect of biomass growth in the photoreactor would be an additional function of nutrient recycling and carbon sequestration.

Algae use inorganic carbon in the form of CO2 as a carbon source and light as the energy source for growth (Prescott, Harley & Klein, 2008), and compared to terrestrial plants, algal species would be ideal for oxygenating the cathode chamber. Algae are responsible for producing up to 75% of the earth’s oxygen, which is continuously produced as long as there is access to light using carbon dioxide from the environment for growth during photosynthesis. The algal biomass production by fixing carbon dioxide during photosynthesis can be used for the production of biofuels (Rittmann,
2008), whilst microalgae also take part in wastewater treatment (Hammouda, Gaber & Abdelraouf, 1995) since they can assimilate a significant amount of nutrients such as nitrogen and phosphorus (Aslan & Kapdan, 2006). The main mechanisms in algal nutrient removal from waste streams include uptake into the cell and stripping ammonia through elevated pH (Hoffmann, 1998). Microalgae as a biofuel source offer several advantages over traditional agricultural crops (Pienkos & Darzins, 2009; McGinn et al., 2011) since many species of microalgae produce significant quantities of energy dense lipids and are grown in aquatic media therefore are not dependent on soil fertility. The growth of microalgae can be achieved in seawater or otherwise nonpotable water such as the effluent discharged from municipal wastewater treatment plants. Finally they have greater rates of aerial biomass productivity than plants. In raceway ponds, which are the mass culture systems most commonly used for commercial applications, the peak productivities can range from 70-90 dry t/ha per year (Zamalloa et al., 2011). However, the cultivation of microalgal biomass requires an expensive infrastructure and considerable energy for harvesting and processing the relatively diluted algal cultures.

Microalgal biofuels currently receiving much attention, however, in general, the cost of algal growth and harvesting of algal biomass is limiting the technology at large scale. Therefore, the combined use of High Rate Algal Ponds (HRAP) for both wastewater treatment and biofuel production is currently being recognised as an economically feasible option (Sutherland et al., 2015). It has been more apparent that HRAP microalgal biofilms (Craggs et al., 2011) are allowing recovery of nutrients such as nitrate and phosphate from municipal wastewater (Di Termini et al., 2011) as well as the removal of toxic pollutants (Muñoz & Guieysse, 2006). In this process, microalgae use the end products of bacterial metabolism (for example CO₂ and ammonia) and in turn, supply aerobic bacteria with the oxygen required for the
degradation of organic compounds. This process can increase resource efficiency turning eutrophication into an opportunity for biomass production. Wastewater treatment HRAPs could provide cost-effective and efficient tertiary-level wastewater treatment with the co-benefit of algal biomass production for biofuel use (Park, Craggs & Shilton, 2011). The high biomass productivity of wastewater-grown microalgae suggests that this cultivation method offers real potential as a viable means for sustainable energy (Pittman, Dean & Osundeko, 2011). The suitability of this biomass may not be recognised as being appropriate for the food industry however it would be suitable for energy conversion technologies including Anaerobic Digestion (AD) or a Microbial Fuel Cell system.

The novelty of this project involves further development of algal based biocathodes as shown previously by Ieropoulos et al., (Ieropoulos, Greenman & Sauer, 2010) incorporated in Microbial Fuel Cell stacks for sustainable and efficient electricity generation with an additional benefit of nutrient recycling and carbon fixing into new biomass.

1.7 Aims and objectives of the current study

This project involves experimentation with existing MFC designs and building knowledge that will aid in the completion of the ultimate project aim. That is: the optimisation of the running conditions for efficient oxygenation at the cathode electrode with minimum energy requirements, in order to produce self-sustainable cathodes for MFCs. This will be explored on algal cathodes in aqueous cathode scenarios and/or a self-sustainable cathode that will no longer require hydration in an open to air design. The exploration of such a system will require a trial and error testing of individual MFC designs, and different electrode materials, with the use of photosynthetic species. It will not only contribute to the knowledge of MFCs in terms
of recent attention on biocathodes but also to the development of more sustainable MFC units and as stack systems for the use in BRL’s autonomous robots and other practical demonstrations. It is aiming to demonstrate that a Microbial Fuel Cell is a viable and functional electricity generator whilst simultaneously driving the electrosynthesis of useful chemical products from treated waste. Essentially it aims to present MFC as an active wastewater electrolyser that generates rather than consumes electricity.

In order to achieve the goals of this study, the following objectives have been set in both aquatic and open to air cathode configurations depending on the environment the MFC set up may be used:

Biotic cathodes

- to develop a biotic cathode half-cell for active oxygenation and improved ORR as well as improved electricity level with a concomitant regeneration of algal biomass

- to demonstrate photosynthetic algal photoreactors as sustainable oxygen pools for prolonged MFC operation and biomass harvesting

- to present a sustainable operation of fully biotic MFC for biomass recovery in the cathode half-cell and its further utilisation as feedstock in the same MFC anode maximising energy efficiency

Abiotic, open to air cathodes

- to explore abiotic open to air cathode MFC systems based on low-cost carbon based materials for sustainable self-hydrating operation

- to present the open-to air cathode as water/catholyte formation unit

- to investigate the MFC unit as self-powered wastewater electrolyser for water and resource recovery through electro-osmosis and bioelectrosynthesis of caustic solution through ORR
The aim is also to improve electricity generation of a single MFC unit for a direct energising of practical applications to demonstrate useful power. Apart from cathode investigation this study also focused on a comprehensive approach towards MFC unit and investigates its elements such as a membrane, reactor design and sustainable materials useful for practical application of MFCs. It aims to demonstrate a simple, low cost and ready to implement MFC reactor that can be used to power real-life applications.
2  Chapter 2. General Materials and Methods

2.1  Introduction

This chapter describes the general materials and methods used during the course of the research period. Specific methods or adaptations of general methodology for specific experiments are described in the relevant chapter.

2.2  Electrode material

2.2.1  Carbon Veil standard folded electrode

All experiments were performed using carbon veil as the anode electrode (Fig 2.1) to keep the anode conditions equal and standard for all experiments performed. Carbon veil is a well-established electrode material for the microbial fuel cells both as anode and cathode, (see Introduction, p. 32-33). Also, carbon veil is both cheaper and easier to manipulate when constructing electrodes of different sizes. Anodes were made of carbon veil cloth with a density of 20 g/m$^2$ (PRF Composite Materials, Poole, Dorset, UK) and the total surface area of 270 cm$^2$. Electrode material was folded down so that it could fit into the chamber and be fully suspended in the anolyte fluid (Fig. 2.1). Also, the anode electrodes were matured during the course of the experiment or by keeping them suspended in sludge for 12 months prior to the experiment. Nickel- chromium (0.45 mm thickness) wire was used to connect the electrode. The electrodes and wire were applied without any chemical or physical pre-treatment. No mediators or chemical catalysts were used.

In terms of the cathode electrode the experiments were also based on carbon veil or carbon cloth as the basic current collector, substratum and a gas diffusion layer. Additional layers of carbon based coatings: Microporous layer (MPL) and Activated
Carbon (AC) were applied later onto the carbon veil substratum as the functional, catalytic surface where the ORR occurs.

Figure 2.1 Carbon veil electrode

2.2.2 Microporous Layer (MPL) on carbon cloth

This material has been prepared by Dr Carlo Santoro at the University of Connecticut (Santoro et al., 2012). Figure 2.2 shows carbon cloth (30% polytetrafluoroethylene (PTFE) wet proofed, E-Tek) with the geometric area of 150 cm² and 10 cm² used as the folded and single layer cathodes respectively. The material was composed by the gas diffusion layer (GDL, the hydrophobically treated carbon cloth) facing to air and the micro porous layer (MPL) facing to the PEM. The MPL was prepared by mixing carbon black particles, PTFE solution, distilled water and a Triton, non-ionic surfactant by following this procedure: firstly, 0.7 g of nano-size carbon black particles Vulcan XC-72R, 9.1 mL of distilled water and 21.5 mL of non-ionic surfactant (Triton X100, Sigma Aldrich), were added in a jar and mixed for 1 hour. Afterwards, 1 g of PTFE (60% emulsion, Sigma Aldrich) was added in the solution and mixed for 30 minutes. Then, the solution in the jar was put in an ultrasonic bath for 15 minutes followed by a mixing operation for 5 minutes, which was performed twice. The solution was then mixed for 1 hour after 2.75 g of carbon black particles was added to the jar. Once the
slurry (20% wt.) was formed, it was applied to the GDL using a silk screen technique. The product was then heated between two hot flat plates for 30 minutes with a temperature of 280°C in order to evaporate the water and non-ionic surfactant and then it was kept for 2.5 hours at the temperature close to the PTFE melting point (343°C).

![Figure 2.2. MPL (Microporous layer) on carbon cloth used as folded and single layer electrode](image)

1.1.1 Microporous Layer on carbon veil (MPL CV)

This material has been prepared by the author with help from the Engineering Department at UWE according to the MPL cathode preparation procedure described in section 2.2.2. MPL mixture has been applied directly onto the carbon veil fibre (20 g/m²) and used only as the single layer electrode (Fig. 2.3).

![Figure 2.3. MPL (Microporous layer) on carbon veil used as single layer electrode.](image)
1.1.2 **Activated Carbon (AC) on carbon cloth**

This material has been prepared by Dr Carlo Santoro at the University of Connecticut. Activated carbon (activated charcoal, BET area of 802 m$^2$g$^{-1}$, Calgon, Pittsburgh, PA) was prepared with a loading of 60±2 mg AC cm$^{-2}$ and PTFE (20% w/v) were mixed using a blender and pressed on a 30% wt PTFE treated carbon cloth (Fuel Cell Earth) that was used as the current collector in MFCs. The AC cathodes were prepared under pressure force (1400 psi) for 2 minutes and then heated at 200°C for 1 hour (Santoro *et al.*, 2014).

2.2.3 **Activated Carbon (AC) on carbon veil (in house preparation)**

To optimise the performance as well as the cost of the cathode electrode material, the expensive wet-proofed carbon cloth (Fuel Cell Earth) used as gas diffusion layer was replaced for carbon veil. To make carbon veil more robust and hydrophobic, it was treated with 30% of PTFE and left to dry. Activated carbon (activated charcoal, G. Baldwin and Co., London, UK) and PTFE (20% w/v) were mixed and pressed on a 30% wt PTFE treated carbon veil that was used as the current collector and gas diffusion layer in MFC cathode. The activated carbon loading was 60±2 mg cm$^{-2}$. The AC cathodes were prepared by applying activated carbon/PTFE paste onto the carbon veil with a spatula to obtain a flat, uniform surface and pressed under 150-200 °C with
a household iron (Fig. 2.4). This was a novel, cost effective approach, which was developed in-house by the author.

![Diagram of activated carbon on carbon veil electrode preparation procedure]

**Figure 2.4** Activated carbon on carbon veil electrode preparation procedure.

### 2.3 Membrane material

Membranes presented in Table 2-1, obtained from different manufactures were pre-treated and stored in line with the manufacturer’s guidelines. The total membrane area was 25 cm² and the membrane window was 18 cm².

**Table 2-1 Membrane material tested during the course of experimental work.**

<table>
<thead>
<tr>
<th>Abbreviation</th>
<th>Type</th>
<th>Model name</th>
<th>Manufacturer</th>
<th>Thickness (mm)</th>
</tr>
</thead>
<tbody>
<tr>
<td>VWR-C</td>
<td>CEM</td>
<td>551652U</td>
<td>BDH- Prolabo, VWR, Leicestershire, UK</td>
<td>Not Provided</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td>Measured: 0.10</td>
</tr>
<tr>
<td>VWR-A</td>
<td>AEM</td>
<td>5516425</td>
<td>BDH- Prolabo, VWR, Leicestershire, UK</td>
<td>Not Provided</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td>Measured: 0.10</td>
</tr>
<tr>
<td>MI-C</td>
<td>CEM</td>
<td>CMI-7000</td>
<td>Membranes International Inc., USA</td>
<td>0.45±0.025</td>
</tr>
<tr>
<td>MI-A</td>
<td>AEM</td>
<td>AMI-7001</td>
<td>Membranes International Inc., USA</td>
<td>0.45±0.025</td>
</tr>
<tr>
<td>FT-C</td>
<td>CEM</td>
<td>Fumasep FKE</td>
<td>FuMa- Tech GmbH</td>
<td>0.05-0.07</td>
</tr>
<tr>
<td>FT-A</td>
<td>AEM</td>
<td>Fumatech FAD</td>
<td>FuMa- Tech GmbH</td>
<td>0.08-0.10</td>
</tr>
<tr>
<td>AGC-C</td>
<td>CEM</td>
<td>Selelemion ™HSV</td>
<td>Ashahi Glass Co., Japan</td>
<td>0.15</td>
</tr>
<tr>
<td>AGC-A</td>
<td>AEM</td>
<td>Selelemion ™ASV</td>
<td>Ashahi Glass Co., Japan</td>
<td>0.13</td>
</tr>
<tr>
<td>MEGA-A</td>
<td>AEM</td>
<td>Ralex®AM-PP</td>
<td>MEGA a.s., Czech Republic</td>
<td>0.45</td>
</tr>
</tbody>
</table>
2.4 MFC designs

2.4.1 Standard, cubic shaped MFCs

The MFCs comprised 25 ml anode chambers and open to air cathodes or in an alternative dual-chamber configuration, both anode and cathode chambers were of 25 mL volume, separated by the subject membrane as shown in Fig.2.5. The chambers were made of Perspex ® material and the internal dimensions were: 53 mm (height), 43 mm (width), and 13 mm (depth). The chambers were assembled using rubber gaskets, 4 mm nylon washers, nuts and studding, and an aquarium sealant was used to prevent leakages (Wet Water Sticky Stuff, Acquatrix, Witham, Essex, UK). This sealant was selected due to its non-toxic properties unlike many of other aquarium sealants (Ieropoulos, Winfield & Greenman, 2010).

![Image of MFCs](image)

Figure 2.5 Single-chamber MFC with open to air cathode (left) and two-chamber MFC with aqueous cathode (right).

2.4.2 Biodegradable MFCs, Peanut-shell and Walnut-shell MFCs

Six peanut shells and two walnut shells were originally assembled as MFC chambers, where three of the peanut shells were used as single chamber MFCs with open to air cathodes and the other three were used as dual chamber MFCs. Walnut shells were used as dual chamber MFCs. In the dual chamber configuration, each of the matching
sides of the shell was an anode and a cathode chamber. The membrane used was made of terracotta plate (5.5 cm long and 3 cm wide). The anode electrodes, folded down carbon veil were: 40 cm² for walnut cells and 10 cm² for peanut cells fitted into the shells and assembled with 0.45 mm thick Ni-Cr wire. The dual chamber design cathode electrodes were again 40 cm² for walnut cells and 10 cm² made of carbon veil to mirror anodic side, whereas the peanut open to air was assembled with MPL coated carbon cloth (10 cm²) glued with the conductive, carbon based wire glue (Conrad, Germany) to the cathodic side of the terracotta plate. A non-toxic sealant was used to prevent leakages (Wet Water Sticky Stuff, Acquatrix, Witham, Essex, UK).

2.4.3 Inverted tubular MFCs

Terracotta caves (Orwell Aquatics, UK and Weston Mill Pottery, UK) of 10 cm length, 4.2 cm outside diameter, 3.6 inside diameter and 3 mm wall thickness, sealed at one end, were assembled with carbon veil anode and MPL cathode. The terracotta chassis was used both as a MFC structure and as a membrane/separator (Fig. 2.6). Initially the anode electrode was carbon veil (20 g/m²) size 810 cm² and the cathode was carbon cloth 72 cm² (30% wet proofed with PTFE-Fuel Cell Earth treated with Microporous Layer (MPL)). Subsequently the electrodes used were replaced for 2340 cm² anode and 90 cm² activated carbon cathode (prepared according to the section 2.2.3) to match the anode/cathode ratio from the cubic MFC design as indicated in description of the specific experiments. The anode was wrapped tightly around the ceramic tube while the cathode was inserted on the internal chamber. Ni-Cr wire was used to connect both electrodes to the logging device (Pico Technology, UK) and electrical load.
2.5 **Inoculation**

All experiments were inoculated with anaerobic activated sludge provided by the Wessex Water Scientific Laboratory (Saltford, UK). The sludge samples were added neat into each MFC, at pH 7, either fresh upon collection, or within 4 months from collection, after being stored under refrigeration at 4°C. No chemical pre-treatment or pH control was used for the MFC inoculation.

2.6 **Feedstock and Media**

To maintain the experimental anodes uniform in all experiments the feedstock used was anaerobic activated sludge provided by the Wessex Water Scientific Laboratory (Saltford, UK). Sludge was mixed with 20 mM or 0.1M sodium acetate prior to use, resulting in an initial pH of 7.2-7.6.

In terms of catholyte media for mixed algal communities, fresh pond water (Frenchay, Bristol) was used for culturing and further tap water and deionised water was used for the bioreactors.
Media used for both the culturing and performing monoculture experiments were: TAP medium for algae:

- *Chlorella pyrenoidosa*

- *Chlamydomonas reinhardii*

BG 11 for cyanobacteria:

- *Anabaena cylindrica*

- *Synechococcus leopoliensis*

Both TAP and BG 11 media composition is described in Appendix E.

### 2.7 Data capture

The output was recorded in volts (V) against time using an ADC-16 Channel Data Logger (Pico Technology LTD., Cambridgeshire, U.K.) and multi-channel Agilent 34972A, LXI Data Acquisition/ Switch Unit (Farnell, UK). The recorded data were processed using the GraphPad Prism® version 4 software package (GraphPad, San Diego, California, U.S.A.) and MS Office Excel.

### 2.8 Calculations

The current (I) in Amperes (A) was calculated using Ohm’s law, I=V/R, where V is the measured voltage in volts (V) and R is the known value of the external resistor expressed in ohms (Ω). Power (P) in watts (W) was calculated by multiplying voltage with current; P=I x V. Power density was calculated according to electrode total surface area; \( P_{\text{Density}} = \frac{P}{\alpha} \), where \( \alpha \) is the electrode surface area in square meters (m²).
The internal resistance was determined by recording the steady state open-circuit voltage \((V_{0-c})\) and the steady state current \((I_L)\) output was measured after a resistive load of known value \((R_L)\) was connected to the individual MFC. The equation is derived from applying Kirchhoff voltage law to a circuit where a power source is connected to a known load according to Ieropoulos et al., 2008 (Ieropoulos, Greenman & Melhuish, 2008).

Equation 1: \[ R_{int} = \frac{V_{0-c}}{I_L} - R_L \]

### 2.9 Polarisation experiments

Cell polarisations were obtained by connecting a DR07 decade resistor box (ELC, France) and also via automatic polarisation resistorstat tool device (Degrenne et al., 2012). Data were produced by varying the external resistance from 30KΩ to 10 Ω at time intervals of 3 minutes after the MFCs were allowed to reach their steady state open circuit voltage. All experiments were carried out at room temperature (22°C).

### 2.10 Conductivity and pH measurements

The pH was measured using a Hanna 8424 pH meter (Hanna, UK) with an operating range 0-14. The conductivity was monitored with a 470 Jenway multi range conductivity meter (Camlab, UK) with operating range 0-1999 mS.

### 2.11 Scanning electron microscopy (SEM)

Dry samples were mounted on aluminium mounts using contact adhesive. Samples were prepared for microscopy by sputter coating in gold using an Emscope SC500 sputter coating unit. Images were observed and captured using a Philips XL30 scanning electron microscope (SEM).
2.12 **Energy-dispersive x-ray (EDX) spectroscopy**

To determine elements present in solid samples, Energy-dispersive x-ray (EDX) was used in this study. EDX is a component of the aforementioned Philips XL30 SEM emitting an electron beam through a sample and producing x-rays. The x-rays emitted characterise the elements present in the upper layer of the sample and are measured by the EDX detector. Detection limits are typically 0.1 – 100 Wt%.

2.13 **Inductively coupled plasma – optical emission spectrometry (ICP-OES)**

The ICP-OES (Varian Inc. Vista-Pro ICP-OES using Axial Plasma) was used for elements detection in the produced catholyte. The equipment can determine the concentrations of metals and specific non-metals in liquid samples. The detection limits for these elements are generally in the μg per litre ranges (ppb). The high temperature (7,000 K to 14,000 K) emitted by a plasma bulb, breaks chemical bonds and transforms elements in the sample to a gaseous state. The light emitted by the excited atoms or ions in the plasma can determine the concentration of the metal present. The main advantage for using this technique is the ability to investigate more than one element at the same time and it is also very accurate and sensitive.

2.14 **X-Ray Diffraction (XRD)**

Dry samples obtained from catholyte were analysed further for phase identification of crystalline material. Powder X-ray Diffraction (XRD) analysis was determined using powder sample measurements, performed on a Bruker D8 Advance Diffractometer with the results being analysed using EVA software package (Bruker, UK). For the single crystal measurement, a unit cell check was performed on a Bruker Microstar
Cu-anode 4-circle diffractometer with the results being analysed with APEX II (Bruker, UK).

2.15 Biomass assessment

Algal biomass was determined using a direct cell count, optical density and dry weight assessment. All measurements were performed on samples calibrated against the conditions that algal sample is being taken for tests under dilution factor (DF): 1-0.2 for the regression analysis. Direct cell count was performed using a Neubauer bright line haemacytometer (Marienfeld, Germany) and a transmitted light microscope (AxioStar Plus, Carl Zeiss) 4 weeks after the start of the experiment. Optical density was measured using a 6300 spectrophotometer (Jenway, UK) at 678 nm (Chl a absorption peak). Microalgal dry weight (mg/L) was assessed using a vacuum filtration unit (Millipore, UK) to deposit cells on 47 mm (0.2 μm pore size) sterile membrane filters (Whatman, VWR, UK). Dry weight was determined by the analytical balance (HR120, Metler Toledo) after obtaining constant weight from drying filter papers for 24 h in room temperature and 1h under filament lamp (100W).

2.16 Total Viable Count

The total viable count (TVC) of organisms in catholyte samples have been performed by conventional serial dilution method with surface spreading of 0.1 mL dilution samples onto nutrient agar petri dishes which were incubated aerobically at room temperature 22 °C for 48 h. Microbial counts are expressed as log10 colony forming unit (CFU) per 0.1 mL sample of catholyte.

2.17 Chemical Oxygen Demand (COD)

COD was determined using the potassium dichromate oxidation method (COD MR, HR test vials, VWR, Lutterworth, England) and analysed using Lovibond MD 200
photometer (Lovibond, Amesbury, UK). For all laboratory tests, MR (medium range 0-1500 mg/L) samples (2 mL) or HR (high range 0-15000 mg/L) samples (0.2 mL) were taken before and after MFC treatment. They were filter sterilised to remove insoluble pollutants that cannot be treated by primary processes. Filtering the samples enabled the treatment efficiency to be described as a soluble form of COD. The samples were added to the COD test vials and heated for 2 hours in a heating block at 150 °C and after cooling the measurements were performed.
Chapter 3. Initial experiments

The initial experiments include the first experimental work performed during the first 1.5 year of studies. The experiments were carried out in collaboration with Jiseon You.

3.1 Membrane investigation

The results from these experiments have been reported as an oral presentation at the Electrochem 2011: Electrochemical Horizons: International Electrochemistry Conference in Bath on the 5th of September 2011.

3.1.1 Introduction

The aim of this study was to compare the performance of different ion exchange membranes in the air cathode Microbial Fuel Cells. Microbial fuel cell systems using wastewater as a substrate apply typically proton or cation exchange membranes to separate anode and cathode chamber and to transport protons. It is one of the most expensive components of the MFC therefore the appropriate choice of the type of membrane becomes very important both in terms of MFC performance and cost effectiveness. Several studies have shown that many cation species other than protons transport positive charge through the CEM membrane (Rozendal, 2006; Zhao et al., 2006) and the transfer of these species decreases the pH in the anode chamber, potentially impairing the microbial activity, whereas on the cathode side, the pH increase is reducing the cathode electrode potential (You et al., 2006). It should be noted that the MFC operated in a batch system will suffer from the pH imbalance as the energy intensive continuous flow or pH control of catholyte is being preferred to avoid this. AEM inhibits cation transfer, while allows transfer of negatively charged species including phosphate anions and it has been reported to increase power as well as enhancing buffering effect due to phosphate anion content (Kim et al., 2007). Our
study investigates this process using a range of anion and cation exchange membranes in the batch mode MFC operation.

### 3.1.2 Polarisation experiments

MFCs with CEMs produced higher values of maximum power density during the polarization experiment. Polarisation results presented in Figure 3.1 and 3.2 show that every CEM was performing better than AEMs in this configuration. The best performing cation exchange membrane was FT-C and its maximum power density was 4.53 mW/m², which was over 100% better than best performing MEGA A anion exchange membrane producing 2.04 mW/m².

![Figure 3.1 Power density vs current density values for each of the tested membranes.](image)
An overshoot phenomenon was observed during the polarisation experiments. Some studies suggested it is due to the immaturity of the biofilm and/or short time of sampling during a polarisation experiment, insufficient for allowing the anode microbial community to adjust to the applied resistance (Winfield et al., 2011) or due to feedstock composition.

### 3.1.3 Endurance

CEMs were found to be producing more constant levels of power than AEMs when the carbon source was consumed. Figure 3.3 illustrates power curves of AEM and CEM from the same supplier (Membranes International, U.S.A.) based on polarisation experiment repeated after the 3\textsuperscript{rd} and 5\textsuperscript{th} day following substrate feeding. Maximum power density of MI C decreased by only 4 \% from 3.95 mW/m\textsuperscript{2} on the 3\textsuperscript{rd} day of feeding to 3.39 mW/m\textsuperscript{2} on the 5\textsuperscript{th} day after adding carbon source whereas the maximum power of MI A decreased by 46 \% from 3.61 mW/m\textsuperscript{2} to 1.97 mW/m\textsuperscript{2} (Fig. 3.3).
3.1.4 Maximum Power Density and Cost Effectiveness

The re-calculation of cost effectiveness against cost per square meter of each purchased membrane (Table 3-1) gives a price comparison per square meter of each membrane against their performance value. As it is illustrated in Figure 3.4 in terms of cost effectiveness, the cation membrane MI C (Membranes International, U.S.A) appears to be the membrane that is most suitable for scaling up.

Table 3-1 Cost comparison of tested membranes.

<table>
<thead>
<tr>
<th>Membrane Make</th>
<th>Supplier</th>
<th>Cost (In British Pounds/m²)</th>
</tr>
</thead>
<tbody>
<tr>
<td>VWR C</td>
<td>VWR, Jencons</td>
<td>1896</td>
</tr>
<tr>
<td>FT C</td>
<td>Fumatech, Germany</td>
<td>279</td>
</tr>
<tr>
<td>AGC C</td>
<td>Asahi Glass Co., Japan</td>
<td>989</td>
</tr>
<tr>
<td>MI C</td>
<td>Membranes International, Inc. USA</td>
<td>100</td>
</tr>
<tr>
<td>VWR A</td>
<td>VWR, Jencons</td>
<td>1940</td>
</tr>
<tr>
<td>FT A</td>
<td>Fumatech, Germany</td>
<td>157</td>
</tr>
<tr>
<td>AGC A</td>
<td>Asahi Glass Co., Japan</td>
<td>755</td>
</tr>
<tr>
<td>MI A</td>
<td>Membranes International, Inc. USA</td>
<td>100</td>
</tr>
<tr>
<td>MEGA</td>
<td>Mega, Czech Republic</td>
<td>87</td>
</tr>
</tbody>
</table>
3.1.5 Conclusions

The investigation of the cost-effective membrane materials and the data from the experiments have resulted in the better choice of membrane material for future experiments. The findings and adoption of the more cost effective membrane had affected this project as well as have been widely implemented by other researchers in the Bioenergy Group.
3.2 4th Pin Electrode

The work presented in this section is stemming from previous B-BiC work and forms part of the original EPSRC Fellowship proposal EP/I004653/1, titled “Waste made useful by Microbial Fuel Cells for energy generation” submitted by I. Ieropoulos in 2010.

3.2.1 Introduction

Because the power output of single MFCs is low relative to other types of fuel cells, scaling up the power production for practical use is essential. It has already been suggested that MFC scale-up may be achieved by connecting multiple small units together rather than increasing the size of a single MFC unit (Ieropoulos, Greenman & Melhuish, 2008). Therefore, to increase the level of produced electricity, MFCs have to be connected together in a multiple units in a conventional manner: in a series, parallel configuration or the combination of the two. MFCs may be capable of powering sensors (Zhang, Tian & He, 2011) and biological microelectromechanical systems (Siu, 2008) to present self-maintenance (Li et al., 2011) and self-sustainability (Ledezma et al., 2013), when connected to peripheral systems. The possibility to control the MFC power output has been attempted by adjusting environmental conditions which affect physiological parameters of anodophilic organisms.

In a series circuit, the voltage across the circuit is the sum of the voltages across each component and the overall current is equal to that of an individual unit.

In a parallel circuit, the total current across the circuit is the sum of the current across each component and voltage is the same as that of an individual unit.
Therefore, it is required to apply the combination of the two conventional ways (series and parallel) of connections to achieve increase in both voltage and current that are required to drive the practical applications such as pumps, electronics, motors, mobile phones, or other electrical systems.

Additional to the above standard ways of connecting single units together is introducing an additional electrode in the MFC anodic and cathodic chamber. The additional electrode being inserted into the anode half-cell serves as a 3rd pin electrode and the additional electrode in the cathode half-cell, serves as 4th pin named for this purpose as 4th pin electrode. In this way, one MFC (Driver) may be connected to another MFC (Working Cell) via 3rd or 4th pin electrode. The purpose of this novel connection is the power “boost” of the “Working Cell” by connecting the “Driver” cell in such a way that is driving the cathodic potential, providing not only improved power but also power modulation and open circuit potential (OCP) sensing. The origin of this type of the connection lays in control theory and control signalling. For the purpose of clarity and to follow the main theme of this thesis the experimental work has focused on 4th pin electrode in the cathodic chamber.

3.2.1.1 Control Theory

Control Theory is certainly, at present, one of the most interdisciplinary areas of research, though the theory has a mathematical origin, the key ideas can be found in nature, evolution, sociology and psychology. The word control has a double meaning. First, controlling a system can be understood simply as testing or checking that its behaviour is satisfactory. In a deeper sense, to control is also to put things in order to guarantee that the system behaves as desired. In engineering and mathematics, control theory deals with the behaviour of dynamic systems. The usual objective of a control
theory is to calculate solutions for the corrective action from the controller that will result, if the system is to behave in the desired manner.

In Microbial Fuel Cell control can be performed through the control signal (voltage output) from one MFC to the other, as shown in Figure 3.5.

![Diagram of control theory](image)

**Figure 3.5 Control Theory adapted into the MFC system.**

In a similar manner as in electronics, a driver is an electronic component used to control another circuit or a component. Driver’s output provides the input of another circuit, for example a transistor. Optimal control theory has been applied to conventional fuel cell technologies already through algorithms (Najafizadegan & Zarabadiipour, 2012) or in hybrid vehicles where control computes the power splitting between two power sources which leads to a minimal hydrogen consumption (Bernard *et al.*, 2006).

In MFCs the control is used whenever voltage must be made to behave in desirable way over time and this may be achieved with the charge transfer from the driver cell to the working cell through a novel 4th pin electrode in the cathode chamber. This is the first time the control mechanism has been reported in the microbial fuel cell system.

The main objective of this study was to investigate the use of an additional electrode in the cathode chamber described as 4th pin to control and monitor the power generation of the MFC in order to:
1-investigate the voltage and power boost through a poising mechanism of connecting Driver cell to the Working cell through 4\(^{th}\) pin electrode.

2-improve the voltage, current and power performance in comparison to standard series parallel connection

3-investigate the mechanism of the charge transfer through different type of material used as 4\(^{th}\) pin and the suitability of the materials used as 4\(^{th}\) pin

4-monitor the open circuit potential with the use of 3\(^{rd}\) and 4\(^{th}\) pin electrodes

5-improve the performance with the use of 3rd and 4\(^{th}\) pin

3.2.2 Methods specific to the experiment

Three identical standard size MFCs were used as working cells (pin cells). The design was identical to the standard 25 mL MFCs and instead an open to air cathode, the 25 mL aqueous cathode chamber was used. The cathode consisted of a second “pin” electrode, it was 1 /16\(^{th}\) of a size of 270 cm\(^{2}\) standard electrodes inserted into the cathode chamber as it is shown on Figure 3.6, making sure that there is enough isolation between the main electrode and the “pin” to avoid short circuiting. The cells were loaded initially with 8 kΩ resistors and periodically fed with sludge + urine (1:1). Three identical driver cells but without pin electrode in the cathode side, were fed with the same mixture and left o/c.

Also, further testing other pin electrode materials included:

- carbon veil 20gsm (grams per square meter),
- carbon veil 30gsm,

- carbon cloth with microporous layer (MPL),

- stainless steel (SS)

3.2.3 4th Pin connection to poise MFC voltage. Power “boost”

To perform the pin connection test, all working cells were connected to a resistor value of 3 kΩ and the driver cells were kept o/c. The temporal connection of the driver to the working cell to poise the voltage of the working cell is shown in the Figure 3.7. Poising was repeated 10 times in on/off manner in order to investigate the MFC behaviour and to let the Driver cell to recover its o/c (open circuit) potential. Figure 3.8 shows the detailed behaviour of each of tested MFC in 5x poise manner and the connected driver cell o/c potential. The observed power increase of the Working cell when poised (ON mode) must be therefore related to the o/c potential decrease of the Driver and illustrates the charge transfer from one MFC to the other through the additional electrode in the same electrolyte (aqueous cathode). It is suggesting that the
4th pin electrode in this configuration is contributing to the power boost in the poising manner. It has been also observed that the strength of the poise becomes weaker in time. This is only preliminary and the more investigation is required, to for example, look at recovery time between each poise, to estimate how often poising could be performed to achieve the same amplitude of the poise signal.

Voltage boost increase varies thanks to the poising (temporal connection) and it might be dependent on the driver o/c value referred as driver strength, the higher the o/c potential of the driver connected to the working cell the higher poise as shown in Fig. 3.8, this requires further investigation.

Figure 3.7 Average performance of tested triplicates in 10x poise manner.
Figure 3.8 Detailed voltage increase through 4th pin connection in all three working cells and their drivers (dotted lines as drivers).

Power increase through power boost was calculated in % of improvement of power from before poising and the poising peak point and it is presented in Fig. 3.9. The improvement of power is shown to be up to 58 % (Poise 1, Working cell 3) and 36% on average.

Figure 3.9 Power increase through 4th pin connection in all three working cells. Error bars represent standard error of the triplicated data.
3.2.4 4th Pin connection vs serial and parallel type of circuit

To test the suitability of this type of connection for practical implementation the tested MFCs were connected in parallel and series configuration again in poised manner in order to compare voltage, power and current specifications to the output of the poised working cell. Here, 4th pin connection offers both voltage and current improvement in comparison to the control non-poised MFC as well as parallel and series connection (Fig. 3.10-3.12). The analysis focuses on the three aspects of the microbial fuel cell output: voltage (Fig. 3.10), current (Fig. 3.11) and power (Fig. 3.12). Voltage increases under a series connection, while current increases when units are in parallel, however as shown in Fig. 3.10 the 4th pin poising offers the voltage increase as well.

![Voltage comparison through 4th pin connection between working cell and the driver against series and parallel configuration of the working cell and the driver.](image)

*Figure 3.10 Voltage comparison through 4th pin connection between working cell and the driver against series and parallel configuration of the working cell and the driver.*
Current increase may be only possible when connecting single units in parallel configuration, while the connection made in series equals to the current of a single MFC, however as shown in Fig. 3.11, 4th pin poising offers the current increase against the series connection. Analysing both (voltage and current) graphs, the parallel and series configuration has to compromise current or voltage increase. Therefore the advantage of the 4th pin connection lays in immediate boost of both of these factors. Power output (Fig 3.12) as calculated on series or parallel configuration shows that the conventional ways of connecting two MFC units together gives more power than 4th pin connection. However, the magnitude of the poise signal and in result the voltage increase will be the subject of further study in next section of this chapter. This work shows only preliminary results of poising mechanism in the MFC system and points out the main advantages and room for improvement.
3.2.5 4th pin electrode material importance

This section focuses on 4th pin material comparison; it will be attempted to analyse and discuss the suitability of given electrode material for increasing the poise strength and its endurance.

3.2.5.1 Poise strength

The materials tested for 4th pin electrode included 20 gsm carbon veil as previously used in sections: 3.2.3-3.2.4 and other carbon based materials such as: carbon veil with higher carbon loading: 30 gsm carbon veil, and carbon based microporous layer (mpl). The only metal based 4th pin was assembled from stainless steel wire (ss). Again, the poising has been performed through ON/OFF manner for five repeats with the 6th poise leaving the Driver and the Working cell connected (“hold” position). The results show in Figure 3.13 that the worst performing 4th pin material was 20 gsm carbon veil. Higher increase of the poise signal may be achieved when using pin electrode made out of higher carbon loading carbon veil (30 gsm) or MPL. MPL was shown to be the most suitable from all chosen carbon materials. Every first poise gives the highest

Figure 3.12 Power comparison through 4th pin connection between working cell and the driver against series and parallel configuration of the same working cell and the driver.
power increase independently of the material used. In terms of the type of the material it was found that the biggest performance boost can be achieved with the use of MPL based pin. Carbon veil 30 gsm can perform better than 20 gsm, probably due to the higher carbon loading. Stainless steel pin appeared unsuitable as the additional electrode material as it negatively influenced the overall performance of the MFC therefore it has not included in further analysis.

![Graph showing power comparison through poising working cell by the driver cell.](image)

*Figure 3.13 Power comparison through poising working cell by the driver cell.*

This might be due to the dissimilar conductive materials used as electrodes in MFCs and their different electrode potentials. When two electrodes are placed in the same electrolyte, one acts as an anode and other as a cathode. The electropotential difference between dissimilar electrodes is the driving force for **Galvanic corrosion** where one electrode corrodes and the deposit is being collected on the cathodic electrode (Oldfield, 1988). This might be the explanation for the improved SS behaviour as the 4th pin electrode in terms of power increase (power boost) and the ability to “hold” the increase of power. In chemical fuel cells, carbon corrosion is also associated with carbon based catalyst support (Tang et al., 2006), therefore it is not impossible that carbon corrosion occurs in MFC systems between two dissimilar carbon-based
electrodes in the same electrolyte. The data suggested that the SS corrosion might the negative factor for the overall MFC performance (Fig. 3.13) and stainless steel might be unsuitable for use as pin electrode, however, it would require further investigation. The pin electrode signal transported to the main working electrode creates a feedback signal recorded as the working electrode output. This feedback refers to the process whereby the measured output may be processed and then used to adjust the signal of the control system (pin electrode material) in the feedback loop. It also helps to understand the mechanism of the charge transfer between the working and pin electrodes.

3.2.5.2 Poise endurance

Figure 3.14 presents the poise cycle comparison where the most suitable material in terms of power increase (in %) in repeated poise cycles, every first poise gives the highest power increase independently of the material used. However, the strength of every poise was maintained more efficiently by the pin electrode made of the MPL material. Figure 3.13 shows poise cycles and the 6th poise was in hold mode (continuously poised) when the Driver cell was kept connected to the Working cell. It showed that all Working cells drop the voltage after power boost point and only SS and MPL pin electrode materials are able to hold the improved power in continuous poise. This investigation shows that the most suitable material for the 4th pin would be MPL. This might be due to the MPL material characteristics and its microporous structure which large specific surface area. Such materials are used in the electrochemical double layer capacitors - the electrochemical storage systems that are able to store and deliver electric charge in instantaneous and reversible manner. Therefore, through the connection of an additional pin electrode, the MFC system
would also show a supercapacitive behaviour as the voltage increase through pin electrode (poise) is instantaneous and reversible.

![Graph showing power improvement comparison through 4th pin connection between working cell and the driver.](image)

*Figure 3.14 Power improvement comparison through 4th pin connection between working cell and the driver.*

Power increase (%) through 4th pin connection was also tested in two different conditions:

-when working cell is under 1 kΩ load

-when working cell is under 3 kΩ load

Fig.3.15 presents the % of increased power with the use of 4th pin. Again, MPL showed the biggest power increase during poising. In terms of chosen resistance 3 kΩ seems to be more suitable for the Working cell with 20 gsm and 30 gsm pin showing higher power increase during poising under 3 kΩ resistance. However, 1 kΩ seems more suitable choice for the use in a Working cell with MPL pin.

The magnitude of the power boost shows that under 1 kΩ resistance, the MPL based 4th pin is able to reach up to 100% power increase against single MFC output, therefore it shows to be a promising contender to traditional series and parallel connection types.
3.2.6 Monitoring the open circuit potential with the use of 3\textsuperscript{rd} and 4\textsuperscript{th} pin electrodes – \textit{Vo/c “sensing”}.

The appearance of the second electrode (4\textsuperscript{th} pin) in the cathode half-cell gives the opportunity look into the dynamics of the MFCs open circuit potential, using 4\textsuperscript{th} pin as a “sensing” electrode, however it is half-cell monitoring and it requires the mirrored configuration on the anodic side with an additional electrode in the anode chamber (3\textsuperscript{rd} pin). To perform sensing, a 3\textsuperscript{rd} pin electrode was inserted into the anode chamber and connected to a PC through differential mode logging device.

As shown in Figure 3.16, the \textit{Vo/c} values “measured” or “monitored” by the sensing pin electrodes sustain at almost the same level throughout the polarisation curve experiment. The additional electrode may act as an open circuit potential sensor while the working electrode is undergoing the polarisation experiment. This also shows a dynamic behaviour of \textit{Vo/c} as it is showing an initial drop and stabilisation of the level
throughout the experiment. These minor variations might be attributed to the change of external resistance during polarisation sweep that would further result in lower $R_{\text{INT}}$ values at those points as previously observed (Ieropoulos, Winfield & Greenman, 2010). Biological systems such as microbial consortia on the anode electrode evolve over time and the changes in $V_o/c$ are perhaps representative of the dynamic behaviour of the anodic ‘living engine’. It may indicating that there is a selection for organisms that colonise anodes under various external loads and under open circuit conditions as previously shown (Katuri et al., 2011). MFCs represent dynamic systems that are subject to change its electrochemical output as a response to the change of the environment which is similar to the balance in natural world (Ieropoulos, Winfield & Greenman, 2010).

![Graph](image-url)

*Figure 3.16 4th pin electrode performing open circuit potential “sensing” during a polarisation experiment.*

### 3.2.7 Improvement of the performance with the use of 3rd and 4th pin

This experiment was conducted with the use of both 3rd and 4th pin within the working MFC and two driver cells, one for the anode poise and another for the cathode poise.
Each poise is marked as “ON” mode with the procedure description and the corresponding data graph below.

Table 3-2 Poising sequence description for Figure 3.17

<table>
<thead>
<tr>
<th>Steps</th>
<th>Description</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>3rd pin ON only</td>
</tr>
<tr>
<td>2</td>
<td>4th pin ON only</td>
</tr>
<tr>
<td>3</td>
<td>Both 3rd and 4th pin ON</td>
</tr>
<tr>
<td>4</td>
<td>3rd pin ON only</td>
</tr>
<tr>
<td>5</td>
<td>4th pin ON only</td>
</tr>
<tr>
<td>6</td>
<td>Both 3rd and 4th pin ON</td>
</tr>
<tr>
<td>7</td>
<td>3rd pin ON only - hold the connection for 10 min.</td>
</tr>
<tr>
<td>8</td>
<td>4th pin ON only - hold the connection for 10 min.</td>
</tr>
<tr>
<td>9</td>
<td>Both 3rd and 4th pin ON - hold the connection for 10 min.</td>
</tr>
<tr>
<td>10</td>
<td>Both 3rd and 4th pin ON - hold the connection for 10 min.</td>
</tr>
<tr>
<td>11</td>
<td>Both 3rd and 4th pin ON - hold the connection for 10 min.</td>
</tr>
<tr>
<td>12</td>
<td>4th pin ON only - hold the connection for 10 min.</td>
</tr>
</tbody>
</table>

Figure 3.17 3rd and 4th pin electrodes poised by the Anode Driver and cathode driver cell in 10 sec and ten minutes (hold) cycles.
From the results shown in Fig.3.17 it might be concluded that the 4th rather than 3rd pin has got more influence on the power output. In terms of the carbon based material used for the pin, again, MPL seems to be more suitable as the more porous structure in power generation and poise endurance compared to carbon veil.

### 3.2.8 Conclusions

It is believed that through such an unconventional connection as a pin electrode, the power of the single unit such as individual MFCs can be improved by almost 100 % and controlled with the use of voltage potential of another MFC to improve and modulate the system performance. The transfer of electric potential from one MFC unit to the next one through an additional pin electrode also shows a capacitive behaviour of MFC electrodes. Furthermore, it may be used to monitor the dynamic change in the open circuit potential of a given MFC system thus it can be used as a diagnostic tool. Dynamic open circuit potential and its effect on internal resistance is an interesting area that requires further investigation.

### 3.3 Biodegradable MFCs based on peanut and walnut shells

#### 3.3.1 Introduction

Renewable energy generation and the treatment of waste are the two key challenges for the sustainability of future economies. Microbial Fuel Cells (MFCs) by converting biodegradable materials into electricity, present not only a technology for renewable energy production but also a wastewater treatment system as well, aiding bioremediation of elements. Virtually any organic matter may be converted to electricity, even substrates that are difficult to metabolise such as cellulose (Ren, Steinberg & Regan, 2008) or proteins (Heilmann & Logan, 2006). The efforts towards
low cost MFC materials has been made to show that using commercially available materials may be a good substitute to costly membranes such as Nafion. Despite excellent proton conductivity, Nafion has been reported to undergo operational problems such as: biofouling, oxygen leakage from cathode to the anode and cation accumulation in the cathodic half-cell (Chae et al., 2008). Therefore looking for alternative ion exchange membrane it has been shown that low cost and readily available materials are also suited for the task, for example ceramics (Behera, Jana & Ghangrekar, 2010), natural rubber (Winfield et al., 2013b) and gore-tex (Luo et al., 2013). Renewable sources such as peanut shells can serve as a source of activated carbon for electrode material. Its properties include removing various metal components such as Cadmium, Copper, Lead, Nickel and Zinc (Wilson et al., 2006) serving as an attractive low cost biosorbent for removal of heavy metal ions from aqueous media (Witek-Krowiak, Szafran & Modelski, 2011). Another study reporting activated carbon prepared from peanut shell (PAC) was used for the removal of Pb\(^{2+}\) from aqueous solution (Tao & Xiaojin, 2008). Also non processed peanut shells show similar effect, for example treatment with phosphoric or citric acid increased the amount of metal ions adsorbed by peanut shells for the metals such as Cd, Cu, Ni, Zn and Pb (Wafwoyo, Seo & Marshall, 1999). Walnut shells have similar properties when used as activated carbon (Orhan & Büyükgüngör, 2011; Gala & Sanak-Rydlewska, 2013), demonstrating excellent biosorption properties (Xie et al., 2013), also as non-processed substrate where hazelnut shell showed a good efficiency in removing toxic ions such as three- and hexavalent chromium, cadmium and zinc (Cimino et al., 2000) from aqueous solution.

This work aims to explore the suitability of low cost materials such as peanut and walnut shells as building blocks for MFC assembly with the ceramic based separator.
3.3.2 Materials specific to the experiment

The methodology section was described in Chapter 2.

3.3.3 Power density comparison with standard MFC

The performance of a small scale MFC based on a peanut shell (2 mL anodic volume) shows comparable results in comparison to large, standard size MFC (25 mL) in terms of power density, moreover, an open to air design in Peanut MPL cathode shows an even better performance of 2 fold (Fig 3.18).

![Power density curve](image)

*Figure 3.18 Power density curve (normalized per anode surface area) of the tested biodegradable MFCs in comparison to standard size MFC.*

3.3.4 Conclusions

The aim of presented work was to develop a MFC reactor constructed solely of biodegradable or waste recyclable materials, and the results indicated that almost any available material constructed accordingly may serve as the MFC bioreactor. To identify further novel materials that would serve as anode and cathode electrodes may to be carried out in the future work.
Chapter 4 Algal based cathodes

The Microbial Fuel Cell that is presented in this chapter is a complete biotic system based on aqueous cathode, which is able to degrade waste and produce electricity with the use of sunlight, and is aiming to demonstrate a way towards a more self-sustainable system. This line of work focuses on the effect of naturally occurring photosynthetic organisms that were used in the MFC as active oxygenators for the cathode. The research also investigated the transformation - and resultant performance improvement of abiotic cathodes into biotic half-cells.

This chapter describes the initial experiment and further development of photosynthetic cathodes, the algal “lagoon experiment” along with the biomass assessment and the closing off system by using harvested algae as fuel for the anode half cells.

4.1 Initial experiment

4.1.1 Introduction

The data from this experiment were presented (poster presentation) at the UCL Algal Biotechnology: Biofuels and Beyond Symposium in University College London, 13th February, 2012.

4.1.2 Methods specific to the experiment

The MFC design, materials, data capture and calculations were the same as described in Chapter 2. The anode and cathode electrodes were assembled of carbon veil 270m² and further modifications to the cathode were applied as presented in Table 4-1. The anodes were inoculated with sludge mixed with 0.1 M sodium acetate prior to use, pH 7.0. The pH was measured every time the anolyte was replaced and it varied from 6.4
to 7.2. The period of the experiments was 55 days. The cathodic side was inoculated with wild, mixed type algae obtained from the UWE pond, Frenchay Campus site, Bristol and grown in a well illuminated room for 2 months prior the experiment.

Table 4-1 Cathode electrode modifications.

<table>
<thead>
<tr>
<th>Name</th>
<th>Electrode type</th>
<th>Catholyte type</th>
<th>Cathode type</th>
</tr>
</thead>
<tbody>
<tr>
<td>water</td>
<td>Carbon veil (Control)</td>
<td>water</td>
<td>Abiotic</td>
</tr>
<tr>
<td>algae</td>
<td>Carbon veil</td>
<td>algae + pond water</td>
<td>Biotic</td>
</tr>
<tr>
<td>algae string</td>
<td>Carbon veil wrapped with cotton string</td>
<td>algae + pond water</td>
<td>Biotic</td>
</tr>
<tr>
<td>algae cellulose</td>
<td>Carbon veil with cellulose coating</td>
<td>algae + pond water</td>
<td>Biotic</td>
</tr>
<tr>
<td>algae wire</td>
<td>Carbon veil wrapped with stainless steel wire</td>
<td>algae + pond water</td>
<td>Biotic</td>
</tr>
</tbody>
</table>

First 40 days it was operated in the batch mode to allow cathode half-cell colonisation, afterwards, algae were re-suspended in fresh pond water (to make sure equal concentration) and transferred to the 0.5 L Schott bottles. Deionised water was autoclaved and used as controls. The set-up is presented in the pictures (Fig 4.1) below.

![Figure 4.1 Experimental set-up; two-chamber microbial fuel cell with biotic cathode (left). Photoreactors and control bottles connected to the cathodic side of tested MFC (right).](image)

Following 40 days of operation the experiment was changed to continuous flow mode using a 16-channel peristaltic pump (205U, Watson Marlow, Falmouth, UK) with a flow rate of 123 ml/h. Marprene tubing was used within the pump manifold connected to the inflow tubes of the MFC. The tubing was made of silicone, its length was 40 cm from the pump to the cell, 60 cm from the cell to the catholyte reservoir (0.5L Schott
bottle) and 30 cm from the reservoir to the pump. Only the catholyte was circulated, and the anode half-cell was operated in batch mode and periodically supplied with a fresh sludge mixed with 0.1 M sodium acetate as a source of carbon-energy substrate. Polarisation experiments were performed after 50 days and 120 days after the start of the experiment.

4.1.3 Results-Photosynthetic biofilm maturing

During batch mode operation and the first 2 weeks of continuous flow operation the algal cells performed unstably. After stabilising at the continuous flow mode the polarisation curve experiments were performed.

![Figure 4.2 Power and polarisation curves from 4 types of tested cathodes; the abiotic cathode is marked in blue.](image)

The performance of the abiotic cell, marked on the graph as water-abiotic with a maximum power of 1.55 mW/m² shows a 20% lower performance than algae cathode MFC with 1.93 mW/m² power density and 22% lower than the best performing, algae wire biocathode with 1.98 mW/m² (Fig. 4.2). The biocathode with string electrode although showing rich colonisation of the algae, gave only a maximum power density output of 0.94 mW/m². The maximum power output of the cellulose coated electrode
was 0.013 mW/m² which was 200 x lower than the best performing algae wire electrode (Figure 4.3). It is suspected that cellulose coating was limiting the oxygen flux while algae biofilm did not colonise the cellulose matrix sufficiently and requires further maturing.

Figure 4.3 Maximum power densities of biotic cathode (control) and abiotic cathodes based on different electrode material. Algae cellulose coated electrode polarization curve with a 200 x lower power output than algae wire electrode.

Figure 4.4 shows the power curves of 4 tested biotic and one abiotic MFC (water). The maximum power density generated by the best performing biotic MFC (algae) was 2.78 mW/m² which was 79% higher than the maximum performance of abiotic MFC that generated 1.52 mW/m².
Figure 4.4 Power curve of all tested MFCs after 120 days of operation.

4.1.4 Conclusions

In the initial experiments, algal based biocathode MFCs outperformed the microbial fuel cells with the abiotic cathode both in terms of power output and open circuit voltage and showed progression of maturity in terms of power output and produced biomass visible to the naked eye.

4.2 Photosynthetic Cathodes for Microbial Fuel Cells


4.2.1 Introduction

In a dual-chamber MFC with a non-catalyst-based cathode, oxygen must be continuously supplied for the reaction; continuous use of fresh and oxygenated water has nonetheless obvious limitations because of its economic and environmental cost. This work focuses on the effect of naturally occurring photosynthetic organisms that
were used in the MFC as active oxygenators for the cathode. The research also investigated the transformation - and resultant performance improvement - of abiotic cathodes into biotic half-cells.

4.2.2 Methods specific to the experiment

The design of the MFCs was as described in the section 4.1. Both photo-reactor bottles and MFCs were transferred to a temperature-controlled incubator (LMS Ltd., Kent, UK) using Cool White Daylight Tubes (3500 lux per tube) fitted in the cabinet (Fig 4.5). The temperature was set at 22°C and the illumination cycle was set at 14 hours day/10 hours dark periods.

Figure 4.5 MFCs and bioreactors in temperature controlled light incubator.
4.2.3 Polarisation curve experiment

The power curves obtained from the polarisation sweep experiment represent the maximum actual power generation and power density of individual MFCs (per total electrode surface area). The non-modified algal cathode MFC outperformed the other algal based cathodes, producing a maximum power density of 7.00 mW/m$^2$, compared to 2.92 mW/m$^2$ (algae/cellulose), 2.31 mW/m$^2$ (algae/wire) and the least performing algae/string with 1.74 mW/m$^2$. The reason for the latter might have been that the cotton string limiting the oxygen permeability to the electrode.

To explore the effect of oxygen saturation, the control MFCs were fed with fresh and oxygenated tap water. Two modes of operation were explored for the water based control MFCs: (i) following frequent replenishment of recirculated fresh tap water and (ii) no replenishment for a week (Figure 4.6). The fresh water based control (5.96 mW/m$^2$) was 75% higher than the non-replenished water-based control that produced only 1.52 mW/m$^2$ whereas the green algae based cathode MFC was outperforming the stagnated water cathode by 77% (6.8 mW/m$^2$) with a peak at 7.00 mW/m$^2$ and it was also higher than recirculated fresh water. The inset in Figure 4.6 summarizes the peak power output from the various cathodic configurations. The retracting part of the power curve observed in this experiment (Fig. 4.6) might be attributed to both electrical and ionic depletion effected primarily by the lower values of the resistance range and it was described previously in the literature as power overshoot (Winfield et al., 2011).
Figure 4.6 Power curves of tested MFCs. Right y-axis present the actual power generation and left y-axis represent power density (per total electrode surface area). Maximum power density values of each MFC are presented in an inset column graph.

4.2.4 Illumination

Preliminary results show an open circuit response to illumination of all MFC cells that included algae in the cathode chamber. The voltage output under a fixed external load of 8 kΩ is shown in Figure 4.7, which illustrates the positive response upon illumination and a subsequent decrease of output in the dark period. The MFCs with the highest increase in output appeared to have thicker algal growth, which was independent of temperature, since the light incubator temperature was thermostatically controlled. Figure 4.7 shows the behaviour cycle of the MFCs during one batch cycle; the overall decay in performance is probably due to the depletion of substrate in the anode, before the next feed.
4.2.5 Abiotic turned Biotic Cathode

The monthly replenishment of control catholyte was maintained throughout the experimental phase. After 2 months of operation it was observed that one of the abiotic cathode chambers (MFC Water 1) became densely colonised by algae whereas the other two remained abiotic. Polarisation curve experiments were performed to examine the effect this might have had on the MFC performance during the light and dark phases. Figure 4.8 presents the actual maximum power output (in μW) of each MFC. As can be seen, MFC Water 1 produced 48% more power during illumination than in the dark. MFCs Water 2 and 3 that appeared devoid of algal growth on the other hand produced a maximum power output percentage difference (day/night) of 6% and 5% respectively. During illumination, the maximum power produced by each of the biotic cells was much higher compared to the dark period. Although during the initial polarisation experiments, the MFC with the cotton string coated cathode was the least performing, in the endurance experiments below, this type of MFC showed
the highest difference between light and dark (42% average increase) compared to 30% with cellulose coating, 18% with stainless steel wire and 26% with plain carbon veil electrode. The highest performing MFC during illumination was the Algae 2, producing a maximum of 108 μW whereas the highest performance increase (between light and dark) was recorded from the cotton string MFCs. The reason for the latter might be due to the higher density of algal cells in a preferred environment for algal colonisation such as cotton fibres of the string.

![Figure 4.8 Maximum power (μW) values of the polarisation curve experiments performed during the light and dark conditions on each of the experimental MFCs. The red arrow highlights the difference between light and dark as a result of the previously abiotic becoming biotic MFC.](image)

4.2.6 Discussion

The development of photosynthetic cathodic half-cell has already been shown with a *Chlorella vulgaris* monoculture as the electron acceptor however, only with addition of chemical mediators (Powell & Hill, 2009b). The positive reaction of algae and cyanobacteria, as a result of illumination, has been previously reported with marine algae and platinum electrodes (Berk & Canfield, 1964). In the present study no
platinum material nor highly conductive sea water were used; instead naturally occurring pond water was further cultivated and adapted in the laboratory environment, and when it turned visibly “green”, it was then used as the inoculum and the source of phototrophic biofilm formation. The higher values of maximum power density obtained from the polarisation experiments of the control using fresh water were actually expected, due to the high oxygen content and presence of other electrolytes; however this quickly degraded as oxygen was depleted from the system.

In the natural environment algae are often found as a constituent part of mixed consortia composed of cyanobacteria and heterotrophic bacteria. In these communities heterotrophs utilise metabolites produced by their photosynthetic partners (Larsen et al., 2007). In a previous study it had already been shown that photosynthetic consortia displayed electroactive behaviour in the anode (Pisciotta, Zou & Baskakov, 2010). To investigate whether naturally occurring photosynthetic biofilms display any positive light responses in the cathode, a sample of fresh pond water was collected and cultivated under laboratory phototrophic conditions. Previous work have shown that the cathodic biofilm may facilitate the electron transport (Berk & Canfield, 1964) forming a completely biotic microbial fuel cell. It can thus be speculated that in present study the biocathode was also acting as a photosynthetic carbon dioxide harvester, since during the dark phase, electricity generation is suppressed and respiration occurs through the activity of photosynthetic organisms and heterotrophic bacteria producing more biomass. Such photosynthetic biofilms are completely self-sustainable communities that are able to grow in a variety of environments (Costerton et al., 1995; Stal, 2012). Another report showing photosynthetic oxygenation of the cathode led to the cell reversal upon illumination (Strik, Hamelers & Buisman, 2010), which has not been observed in our system, even though this would be expected under substrate
depleted conditions in the anode half-cell. Previous study reported an algal growth unit (with supplementation media to support algal growth) where harvested biomass was used as substrate for anaerobic digestion in a closed loop reactor, and found the algal culture can deliver the necessary electron acceptors for the cathode without mechanical aeration (De Schamphelaire & Verstraete, 2009). In the present study, no air sparging, no supplementation media or platinum as catalyst were used, keeping the MFC operational costs to a minimum and taking advantage of the bio-catalytic properties of natural photosynthetic consortia in the cathode. Also, in the current set-up the temperature was strictly controlled in an incubator and the algal growth was initiated from a natural source in the cathode chamber, not from a separate photoreactor unit. A simple MFC operation with minimal requirements has been demonstrated in order that it could serve in real field applications as well as in the laboratory set up.

4.2.7 Conclusions

Although the power produced by abiotic, water based MFCs might appear comparable with algal-based cathode systems, this is clearly limited and unsustainable in environments with no running water available. Biocathodes with active photosynthetic consortia provide sustainable aeration for continuous MFC activity. The positive response to illumination cycle of presented photosynthetic cathodes demonstrates that phototrophic organisms can actively oxygenate the cathode half-cell. Abiotic cathodes that had become contaminated by natural exposure turned into a biotic system that showed extended longevity and improved power. The photosynthetic biofilm appears to be a viable alternative biocatalyst for the real world implementation of MFCs as sustainable energy harvesters and it simultaneously presents the possibility for carbon fixation and/or re-cycling of carbon via cathodic biomass being re-utilised by the anodic side of the same system. This will be explored further in section 4.4.
4.3 **Algal lagoon**


4.3.1 **Introduction**

While the oxygen depletion during the dark phase, as shown in the previous section and in numerous studies (Jeon et al., 2012; Wu et al., 2013; Lobato et al., 2013), it may be resolved by an algal lagoon or photo-bioreactor connected to the cathodic side of MFCs that may theoretically produce more biomass and also provide oxygenation for the cathodic chamber. Whilst the electroactive bacteria biodegrade organic matter in the anode, cations diffuse through the cation selective membrane into the cathode (Rozendal, 2006; Kuntke et al., 2012) where they can be further assimilated by algal growth and locked into new biomass (Xiao et al., 2012). The biological reactions occurring in the algal ponds include carbon energy (C/E) utilisation by heterotrophic bacteria (a minor component) whilst the majority of the biological activity is CO₂ fixation with resultant increase in amount of mixed algal biomass produced from growth. The ideal attributes of algal species for use in wastewater treatment are: high growth rate when fed with predominantly N and P-rich wastewater effluents, and the formation of aggregates enabling simpler separation and harvesting of immobilized elements, leaving a lower concentration of soluble elements and general improvement to the quality of water.

Microalgal biomass obtained from photosynthetic systems compare favourably with conventional biofuel crops due to much higher growth rate, high CO₂ fixation and lack of requirement for fertile soil. Moreover, their life cycle may support the wastewater
treatment process (Oswald & Gotaas, 1957; Hoffmann, 1998) rather than add to the burden of fertilizer run off, a common problem for conventional biomass crops. In 1950s William J Oswald, the pioneer in the use of algae to treat wastewater, was one of the first researchers to study the symbiotic interactions between algae and bacteria in wastewater treatment ponds. He demonstrated a sustainable process that involves algae photosynthesis rather the electro-mechanical aeration devices. The study of such natural systems has developed in later decades into the field of ecological engineering. For treating high C/E wastewater, the algal system is termed as sewage lagoon (Oswald et al., 1953) or a photosynthetic pond and it is used in the wastewater treatment process. The development of new technologies could help utilise locked-in waste products for energy production in a carbon-neutral manner. This section is presenting the sustainable oxygenation of the MFC cathode by photosynthetic biocatalysts in a ‘lagoon’ kind of scenario and in addition the generated electricity by the stacked MFCs was used to power an impeller DC pump as a demonstration of an exemplar practical application.

4.3.2 Methods specific to the experiment

The experimental set-up is shown in Fig. 4.9. Similar to the previous experiment temperature was set at 22 °C and the illumination cycle at 14hours light/10hours dark (to aim at normal day/night setting) and 4h light/20h dark periods (short day), the recirculation was used optionally in OFF/ON mode. A DC impeller pump (M200-S, 3V DC, RS, UK) was powered continuously for a short period of time, by the energy produced from these same 16 MFCs (15 described above +added 1 MFC for balanced configuration). The energising of the pump was performed via a 5F super-capacitor (Figure 4.12).
Figure 4.9 Experimental set-up; two-chamber microbial fuel cell with biotic cathode connected to the photoreactor.

4.3.3 Results—Photocathode activity during illumination

Figure 4.10 presents the open circuit voltage (OCV) of algal-based cathode MFCs when the recirculating catholyte pump was switched OFF (batch mode). The voltage level exceeded 500 mV during the light phase, but decreased to 100 mV during the dark period. When the pump was switched ON (arrow on the graph) the open circuit voltage difference between light and dark rapidly decreased, suggesting that the algal growth in the photo-reactors (or reservoirs of high redox and/or oxygen concentrations) compensated for the lack of oxygen generation at the cathode during dark periods. This improved stability and performance over longer periods of time.
Figure 4.10 Open circuit voltage (OCV) values of photosynthetic cathode MFCs. 14h day/10night cycle.

In Figure 4.11 pumping was turned OFF once again, whilst the dark period was increased to 20 h night over 4 h day intervals. Voltage of the MFCs dropped into negative values and the short period of illumination was insufficient for the MFCs to fully recover. When the recirculation pump was switched ON, once again steady open circuit voltage levels were rapidly produced, with a lesser degree of fluctuation between the day/night cycles.
Figure 4.11 Open circuit voltage (OCV) values of photosynthetic cathode MFCs. 4h day/20night cycle.

Despite the fact that photosynthesis stops inside both the MFC cathode and photoreactor flasks, the amount of oxygen accumulated or pooled inside the bioreactor as a whole, compensates for the lack of oxygen production during the dark period inside the small-scale MFC cathode chamber. This can be termed as the algal lagoon effect. Furthermore, the fact that the amplitude of the OCV when the pump is switched ON is identical to the amplitude when operating in day/night batch mode (Fig 4.11) may suggest that the voltage output is a function of oxygen. Under open circuit conditions MFC would therefore behave as a sensor. Under load, the function of the MFC and its power output will be the dependent on the amount of oxygen in the reservoir (Fig. 4.12) therefore the ratio of cathode chamber volume to lagoon volume in the photoreactor is the key determinant.
Microscopic examination revealed cells of both cyanobacteria and photosynthetic eukaryotes as well as heterotrophic bacteria and protozoa are presenting a balanced phototrophic ecosystem. This synergistic interaction between photosynthetic organisms and heterotrophs exists in lagoons, microbial mat and coastal areas. It is unclear however whether cyanobacteria and algae have played an important role in electron transfer and this would need to be investigated further. However, the design of such system would work under water, providing there is light available, even if no exogenous oxygen is supplied, which goes along with sustainable wastewater treatment philosophy implemented first by Oswald in wastewater treatment plants.

A presented design of the phototrophic MFC systems will make it possible to convert solar energy into biomass and electricity and possibly power the remote sensoring systems for monitoring environmental conditions.

### 4.3.4 Results-Powering a real life application

In order to demonstrate that this pumping can be facilitated by the MFC stack itself, aiming towards self-sustainable applications, a dc impeller pump was powered continuously for a short period of time, by the energy produced from the same 15 MFCs with an additional control MFC to make n= 16 to give a final evenly balanced configuration of pairs of groups of units. The energising of the pump was performed via super-capacitor (Figure 4.13), when the MFC stack was connected in a series/parallel configuration. Under this condition, 2 MFC units were connected in parallel, and the resulting 8 pairs, were connected in series. The super-capacitor was charged to the stack’s maximum voltage for this configuration (3-3.5 V) for 24 h, which allowed the running of the impeller pump for 67 secs until the capacitor voltage decreased to 1.0 V, at which point the pump stopped the whole cycle was repeated in
3 cycles (Fig 4.12). Moreover, the charging was light dependent showing an algal cathode active demonstration as a biocathodes at work.

Figure 4.12 Charging the capacitor to run an impeller pump in ~24h intervals.

Figure 4.13 Demonstration of the practical implementation of the 16 MFC stack connected in a series/parallel configuration (1- MFC stack, 2- super-capacitor, 3- DC impeller pump).
4.3.5 Conclusions

This study presents the sustainable oxygenation of the MFC cathode by photosynthetic biocatalysts in a ‘lagoon’ type scenario. MFC operation in open circuit condition demonstrates also its possible use as a dissolved oxygen sensor. This is the first time that the stack of completely biotic MFCs incorporating photosynthetic organisms in the cathode has been shown to power a practical application such as the impeller pump. Furthermore, it is allowing a route for energy abstraction from the biomass grown in the photo-assisted MFC. Previous report documenting urine utilisation by Microbial Fuel Cell (Ieropoulos, Greenman, & Melhuish, 2012) suggests that the removal of N, P, K from urine is possible and that these elements (in the absence of carbon energy sources) stimulate algal growth. This could lead to locking these elements into algal biomass and stimulating algal growth in the cathode for oxygen production. This encourages the further investigation of solar powered microbial fuel cells to treat wastewater and harvest energy in aquatic systems.

4.4 Biomass assessment

This section has been presented at the International Biomass Conference in Manchester 11 March 2014 and has been published as:


4.4.1 Introduction

To limit the environmental impact of greenhouse gasses, the future substitute energy sources should be renewable and carbon neutral. This would include biomass products
for energy generation such as growth plants and crops, algae and organic waste. From the food security point of view, both micro and macro algae have attracted considerable interest globally as a potential feedstock for a bio based economy (Jones & Mayfield, 2012). Microalgae, are very efficient converters of solar power and have been used in mass culture for both biomass as well as high value product production. However, the cost of algal growth and biomass harvesting is limiting the technology at large scale. It has been more apparent that High Rate Algal Ponds (HRAP) and microalgal biofilms (Craggs et al., 2011) are allowing recovery of nutrients such as nitrate and phosphate from municipal wastewater (Di Termini et al., 2011) as well as toxic waste removal (Muñoz & Guieysse, 2006). In this process, microalgae use the end products of bacterial metabolism (for example CO₂ and ammonia) and, in turn, supply aerobic bacteria with the oxygen required for the degradation of organic compounds. This process can increase resource efficiency turning an eutrophication into an opportunity for biomass production. Wastewater treatment incorporating HRAPs could provide cost-effective and efficient tertiary-level wastewater treatment with the co-benefit of algal biomass production for biofuel use (Park, Craggs & Shilton, 2011). The high biomass productivity of wastewater-grown microalgae suggests that this cultivation method offers real potential as a viable means for sustainable energy (Pittman, Dean & Osundeko, 2011) being beneficial to the food chain in the local ecosystem. The suitability of this biomass may not being recognised as being appropriate for the food/feed applications, however it would be suited for energy conversion technologies including Anaerobic Digestion or Microbial Fuel Cell. The development of biocathodes is a viable alternative to chemical and noble metal cathode catalysts improving its sustainability and cost effectiveness (He & Angenent, 2006). Its operation has an important additional advantage of using both chambers for simultaneous treatment under different conditions, which helps in the removal
pollutants (Mohan & Srikanth, 2011) and photo cathode is the most promising option
for incorporation of photosynthesis into MFC systems (Xiao & He, 2014). The key
innovation on the large scale would lay in low cost biomass regeneration and nutrients
retrieval (such as nitrogen and phosphorus) for use as fertilisers. However, from the
practical point of view, most microalgal MFC studies contain specific, controlled
media and CO₂ supplementation to support algal growth (Powell et al., 2009; Lobato
et al., 2013). During MFC operation cations other than protons are being transported
from anode to the cathode through PEM (Rozendal, 2006; Xiao et al., 2012). Therefore,
the cationic transport may support algal micronutrient requirement to support and enhance algal growth. This experimental work is presenting further
development of photosynthetic cathodes presented in previous sections to access
biomass accumulation relation to MFC power performance. The harvested biomass
was subsequently used as the feedstock in the same MFCs, which demonstrated that
microalgae grown within the photo-reactors, can represent a sustainable feedstock for
MFCs.

4.4.2 Materials specific to the experiment

During long-term MFC operation, the control (abiotic) cathodes became
serendipitously biotic, showing growth of photosynthetic organisms visible to the
naked eye in the chambers as reported earlier. All MFCs have shown a well-developed
photosynthetic biofilm in the cathode side half-cell. This is when the photoreactors
were filled with fresh deionised water and the growth of photosynthetic organisms
were observed and accessed (Fig 4.14). Furthermore the newly formed biomass was
used as an anodic feedstock.
Figure 4.14 Microbial Fuel Cell (MFC) with biomass harvesting photoreactor. The source of fuel can be organic waste (sludge+20 mM sodium acetate) or biomass harvested from the cathodic photoreactor.

4.4.2.1 Biomass assessment methodology

A direct microscopic count was performed on the harvested samples of microalgal suspension using a Neubauer bright line haemocytometer (Marienfeld, Germany) and a transmitted light microscope (Axiostar Plus, Carl Zeiss) 4 weeks after the start of experiment. Optical density was measured using a 6300 spectrophotometer (Jenway, UK) at 678 nm (Chl a absorption peak). Microalgal dry weight (mg/L) was assessed using a vacuum filtration unit (Millipore, UK) and a 47 mm (0.2 μm pore size) sterile membrane filters (Whatman, VWR, UK). Dry weight was determined by the analytical balance (HR120, Metler Toledo) after obtaining constant weight after drying filter papers for 24 h in room temperature and 1h under florescent lamp (100W). Calibration was performed using dilutions in the range of dilution factor (DF) of 1-0.1.
Surface morphology image of cathode electrodes with the attached photosynthetic biofilms, were observed by scanning electron microscope (SEM) and analysed with Energy-dispersive x-ray (EDX) as described in Chapter 2.

4.4.3 Results

4.4.3.1 MFC power performance

The representation of the maximum actual power generation is being illustrated by the polarisation curve. Figure 4.15 presents the power curve where the actual maximum performance was obtained by the algae based cathode MFC 128 µW, algae water 81 µW, algae string 74 µW, algae wire 67 µW and algae cellulose 61 µW in comparison to abiotic MFC (control) with the lowest performance of 46 µW. The results support the previously reported data, that in the long term the algae were shown to improve the power performance and longevity.

![Figure 4.15 Polarisation curve experiment of tested MFCs.](image)

It has been observed, that the biomass accumulation in the connected to MFC cathode photoreactors shows dependence on the charge transfer of given MFC and can be
directly linked with the produced power. Therefore it is suspected that the more electricity the MFC generates, the more biomass may be obtained from its cathode photoreactor. The harvested biomass showed that the cell density for algal cathode was up to 31x 10^7/L. A platinum based cathode was previously shown to be able to maintain a monoculture of *Chlorella vulgaris* for CO₂ sequestration and oxygen generation (Wang *et al.*, 2010) or a mixed culture (Xiao *et al.*, 2012). Here it is further hypothesised that the cationic flux through the exchange membrane, which is dependent on MFC performance, can influence biomass growth (Xiao *et al.*, 2012) in a carbon based MFC system. This can significantly lower the cost of MFC technology making it feasible in the designs close to real applications. Power overshoot can be directly linked to the factors influencing internal resistance (Winfield *et al.*, 2011) linking it to oxygen starvation limiting cathode performance (Kulikovsky, Kucernak & Kornyshev, 2005).

### 4.4.3.2 Algal growth within the reactor

Cell density and biomass could be measured more easily by optical density rather than direct count or dry weight. Therefore, the relationships between optical density and cell density and dry weight were established by linear regression, as shown in Fig 4.16 and in Appendix B. As the samples were taken from the photoreactor bottles, it was observed that algae, algae wire and algae water showed uniform cell densities, the more aggregation was observed in string and cellulose. The aggregation in the MFC string and cellulose was limiting the optical density measurement as a reliable tool of biomass assessment. Therefore, the dry weight was chosen to correlate with the maximum power performance (Fig 4.17). Microalgal biomass growth at the cathode
was assessed to correlate with the power output. It shows that the most promising configuration for the algal cathode is the non-coated carbon veil matrix to allow better diffusion of dissolved oxygen to the electrode.

Figure 4.16 Calibration curves of optical density at A_{678} to the cell density and biomass.

The high cost of CO₂ as a feedstock for algal growth is the major obstacle turning into CO₂ regeneration techniques (Chi, O’Fallon & Chen, 2011). A previous study has already reported on algal growth correlated with power performance in wastewater supplemented cathode (Venkata Mohan et al., 2014). Here, it is suspected that the cathode acts more as the polishing chamber as no supplementing was supplied, only pond water as inoculum and deionised water in the test run.
Figure 4.17 Harvested biomass from the photoreactors in relation to the MFC power performance (right).

4.4.3.3 Photosynthetic Biofilm

The SEM images (Figure 4.18) show algal biofilm formed on all tested cathode electrodes in comparison to abiotic control without any biomass introduced into the cathode chamber. String and cellulose coating showed a layer consisted of microorganisms embedded in microbial extracellular polymeric substance (EPS) matrix formed on the electrode surface.
Oxygenic biofilm has been shown to enhance the MFC current production (Walter, Greenman & Ieropoulos, 2013). The charge-balancing cation transport from anode to cathode compartment (Harnisch, Schröder & Scholz, 2008; Xiao et al., 2012) might be responsible for increased growth in better performing MFCs. Also, looking at biocatalytic properties of algal colonies, the increased growth might influence the increased power generation. Previous research on cathodic biomass growth was studying light intensity (Juang, Lee & Hsueh, 2012), showing that intermittent illumination provides more efficient and prolonged operation of MFC (Wu et al., 2013). Natural biofilm communities include a number of microbes such as fungi, algae, protozoa and bacteria showing symbiotic interactions (Irving & Allen, 2011). It is showing the power correlation to the biomass produced and collected in the photoreactor. Therefore it might have been suspected that the level of charge transfer connected to the proton/cation transfer is influencing the biomass growth within given
reactor. The more power MFC generates, the more protons/cations is being transported from the anode to the cathode and accessible to support the photosynthetic organisms. The coexistence of both attached biofilm and suspended biomass in solution in a cathode compartment makes it challenging to accurately quantify the total biomass. It has also been observed that the biomass in the photoreactors forms aggregates making it difficult to perform reliable biomass measurement by optical density or haemocytometer. The test was performed on the least aggregated samples however the most aggregation was observed in the cellulose and string based cathodes. It might be due to the dense electrode colonisation as shown in Figure 4.18. Surface texture is one of the factors that influence microalgae attachment to different substrata. Generally, rough or porous surfaces are associated with larger surface area and better cell attachment (Christenson & Sims, 2012). It has been observed that the biomass growth was the lowest in algae wire MFCs, it might be related to stainless steel biocorrosion affecting the output and limit algal growth. Microscopic observation had shown a predominant colonisation by unicellular as well as colonial algae and cyanobacteria, bacterial species and protozoa. The development of biomass fixing cathode will help to make MFCs a carbon-neutral technology with enhanced efficiency and an element of self-sustainability.

The bright clusters observed on SEM images were investigated through EDX. Unlike the open to air cathode the sodium content was not that apparent, majority of the elements detected (except carbon) are presented in Fig. 4.19 show that the bright crystallization is predominantly calcium based salt. Other detected elements include: Na, K, Mg, Si, P, S and Cl.
4.4.3.4 Algae used as feedstock

The catholyte collected from photoreactors was harvested and separated into 4 feedstock solutions dependent on the type and the amount of biomass: non aggregated feedstock which biomass content was: a) 0.25 g/L, b) 0.39 g/L and aggregated feedstock: c) dry mass 0.56 g/L d) dry mass 0.71 g/L. The biomass was used as feedstock directly without any pre-treatment. The graph shows the average power of all five types of MFC when fed with sludge + 0.1 M sodium acetate in comparison to algal feedstock used as indicated. Figure 4.20 shows that algal feedstock produced by the MFC cathode may be successfully used as the anodic substrate. The performance increases with the amount of biomass fed to the anode half-cell. The endurance of this feedstock (Figure 4.21) in comparison to acetate is indicating that algal biomass seems to be a more complex substrate due to complex mineral composition. Algae have been used as feedstock for MFC, both microalgae (Kondaveeti et al., 2013) and macroalgae (Gadhamshetty et al., 2013) presenting a closed loop system with Anaerobic Digester (De Schamphelaire & Verstraete, 2009). Phototrophic biofilms grow in response to light, carbon dioxide and inorganic nutrients where the availability of nutrients
influences the type of biofilm formed (Hillebrand, Kahlert & Haglund, 2002). Moreover, biofuels from algal cells grown on non-arable land provides the solution in food-fuel dispute (Singh, Nigam & Murphy, 2011). Wastewater seem to be the best option for reducing the environmental burden from the cultivation of algal biomass, therefore current work aims to contribute to the development of algal biofuels towards self-sustainable MFC systems.

Figure 4.20 Algal biomass used as feedstock in comparison to acetate used at the beginning of the test.

Figure 4.21 Feedstock comparison; data are from day=1-8 and 15-23 from Fig. 4.20 above.
In the development of renewable energy sources and carbon sequestration technologies, one of the most popular methods of CO$_2$ reduction is the use of photosynthetic organisms such as algae and cyanobacteria that convert CO$_2$ into biomass. Incorporating such photo-assisted cathode for Microbial Fuel Cell (MFC) provides active oxygenation for oxygen reduction reaction with simultaneous carbon capture into the new algal biomass.

### 4.4.4 Conclusions

This study presents a complete microbiological system that is able to continuously cogenerate electrical current and biomass. While the algal activity supports the oxygen reduction reaction *in situ*, the MFC operation provides micronutrients for microalgal growth in the MFC cathode. Capturing the energy locked within the organic contaminants of wastewater to produce electric energy; and improving nutrient cycling could serve as a sustainable option increasing the energy recovery balance. Enclosed biofilm photo-bioreactors therefore offer a potentially more economical alternative to conventional tertiary treatments process for nutrient removal (Gonzalez *et al.*, 2008). It is promising to apply photosynthetic organisms in the cathode for oxygen supply, nutrient removal and biomass production (Xiao & He, 2014). It has been proposed that the separation of processes wastewater treatment in the anode and biomass production in the cathode can only be truly linked and combined when there is a current flow and electricity is generated, therefore the three valuable functions can be integrated.
5 Open to air Cathode

This Chapter focuses on the development of open-to-air cathodes in double-chamber configuration MFCs. Selected cathode materials (MPL and AC coatings on carbon cloth) were initially prepared by Dr Carlo Santoro at the University of Connecticut, U.S.A.

5.1 Introduction

Atmospheric or aqueous oxygen-based diffusion are the two most commonly used configurations for cathodes in MFCs. While the three dimensional electrodes would be ideal for the biofilm attachment in the anode, the air cathodes usually consist of a gas diffusion layer (GDL) which is exposed to air and a catalyst layer (CL) exposed to the electrolyte (anolyte or membrane). The catalyst layer is responsible for the Oxygen Reduction Reaction (ORR) therefore its optimisation with chemical or physical catalysts, determines the speed of ORR and overall performance. In terms of the electrode configuration, a single chamber, open to air MFCs have the greatest potential for practical applications due to the direct use of the oxygen from air and the simplicity of the design (Fan, Hu & Liu, 2007). There is usually a membrane between the chambers that physically separates the two dissimilar solutions within a reactor. With unmodified carbon based substrata, the ORR suffers at high overpotentials (Harnisch & Schröder, 2010) and it has been proposed to use carbon materials with high surface area to improve its catalytic activity (Freguia et al., 2007). The MPL (Microporous Layer) has been widely used in Polymer Electrolyte Fuel Cells (PEMFC) where it is usually placed between the GDL and the CL. It is made from a mixture of carbon powder such as Vulcan XC-72R and PTFE and its main function is to provide porous structure and hydrophobicity that will allow both oxygen transport and water removal from the reaction sites. MPL micro-structural and wetting characteristics affect the
The incorporation of MPL into Microbial Fuel Cells as a cathode electrode material has not been fully explored. Whilst researchers tested MPL with platinum catalyst layers in sediment MFC (Scott et al., 2008), it was concluded that the preparation of the electrode, where PTFE is a binder, is not suitable for a flooded cathode relying on dissolved oxygen for activity (Scott et al., 2008). It has been shown that chemical treatment with HNO₃ played a significant role in modifying the specific surface and improving the catalytic property of the Vulcan XC-72R carbon powder reaching power densities of 170 mW/m² (Duteanu et al., 2010). These novel cathode structures have already been successfully implemented in membrane-less single chamber MFCs (SCMFCs) demonstrating enhanced electricity production when MPL was inserted between the Catalyst Layer (CL) and the Gas Diffusion Layer (GDL) (Santoro et al., 2012); however, the catalyst used was nevertheless platinum. Various research groups have shown that the use of MPL results in improved performances (Santoro, Lei, Li, & Cristiani, 2012a; Wang et al., 2006). Here, we compare two catalyst-free and high surface area cathode materials and demonstrate that the MPL can act as a catalyst layer when in direct contact with the proton exchange membrane in MFC.

5.1.1 Methods specific to the experiment

The cathode electrode used as control was made with carbon veil (270 cm²) and folded down to fit inside the cathode chamber, as shown in Fig 5.1 (left) and was compared against: a single 10 cm² MPL (sMPL) and a folded 150 cm² MPL (fMPL). The study also investigated the use of MPL working as a 20 cm² Membrane-Electrode-Assembly (mMPL). In the second stage of experimentation, the triplicates of the same control
MFCs were as previously tested against the triplicates of single layer MPL based cathodes of total surface area of 17 cm$^2$ where 10 cm$^2$ was active cathode area and 7 cm$^2$ was used as a current collector (Fig 5.1. right) The direct contact between MPL and PEM was maintained by mechanically pressing the cathode electrode against the membrane. Electrodes and membrane were prepared without any chemical pre-treatment and without any mediators or chemical catalysts.

Cathode materials: MPL and carbon veil structures were observed fresh before inserting into the MFCs, using a SEM and it was done at the University of Connecticut thanks to Dr Carlo Santoro.

![Figure 5.1 Experimental set up. Left: control MFC with carbon veil electrode in the cathode half-cell, right: single layer MPL coated carbon cloth pressed against the membrane.](image)

### 5.1.2 Results and discussion

#### 5.1.2.1 Direct contact of MPL based electrode and membrane

Initially, fixed load (8200 Ω) data showed that the best performing fMPL produced 27 μW and sMPL 21 μW, which was 3-fold and 2.3-fold increase against the control (CV; 9 μW) respectively. Normalisation of data was performed using the anodic (Fig. 5.2) and cathodic electrode surface area (Fig 5.3).
Polarisation data showed that (Fig. 5.2 - anode normalisation) the maximum power density of the best performing fMPL MFC was 7.26 mW/m² (196 μW actual power), compared to sMPL 7.02 mW/m² (189 μW), mMPL 4.42 mW/m² (119 μW), CV1 4.22 mW/m² (114 μW) and CV2 4.34 mW/m² (117 μW). The increase in power generation (absolute power of MFC) as a result of the MPL was of the order of 40%. The mMPL underperformance was probably due to anolyte leakage through the material. Power density data (Fig. 5.3 – cathode normalisation) showed that the sMPL produced 189
mW/m² with electrode surface area 15 times smaller than fMPL that generated 13 mW/m². The mMPL performance was 59.75 mW/m² whilst the control CV1 and CV2 generated 4.22 mW/m² and 4.34 mW/m² respectively. These findings indicate that the reactive part of the cathode is the surface in direct contact with the electrolyte (membrane when PEM is being used or anolyte in membrane-less setup) and the 3 dimensional electrode in this case is not necessary. The SEM images of both carbon veil and micro-porous structure (Fig. 5.4) suggest that a higher active surface area per unit of the MPL may be responsible for the improved power generation.

Figure 5.4 SEM image of carbon veil (left) and MPL (right).

As previously described in the membrane investigation experiment, a 3-Dimensional carbon veil based air cathode requires periodic hydration to maintain a fluidic bridge between two chambers; however, after successfully operating the MPL based cathodes it was observed that these conditions do not require external hydration. Moreover, water formation was observed on the GDL surface in the form of droplets as shown in Figure 5.5. Therefore, the catholyte of the control MFCs was removed and electrodes were pressed against the PEM in order to maintain the same conditions as the MPL based cathodes. The transition from aqueous cathode to the open-to-air configuration would require periodic hydration of the 3D electrode structure. However, after 1 week
of operation in such conditions it was observed, that that external hydration is not necessary and after 2 weeks of operation, water formation on the cathode side of the MFC also occurred. The main difference between the open-to-air design (previous membrane experiment) to this one is that in previous conditions the cathode chamber was removed to ensure sufficient access to air. In the current conditions, the cathode chamber is closed with small air inlets at the top. It would explain the limited water loss through evaporation and improved humidity inside the chamber for sustaining liquid bridge and efficient ORR. The small air vents also provided enough air for the reaction to run. The water formation is one of the most important findings and it will be analysed in depth in the following sections of this chapter.

![Figure 5.5 Water formation on MPL surface.](image)

5.1.2.2 MPL based electrode for improved power

The above findings resulted in the second stage of experiment where the triplicates of control- carbon veil cathodes and single layer MPL were tested where the projected surface area of the electrode in direct contact with a membrane was 10 cm². Polarisation data (Figure 5.6) showed that the average actual maximum power of MPL was 168 μW, compared to control Carbon Veil that produced 68 μW. The average
power of these triplicates shows that the MPL cathodes outperform carbon veil cathodes by the factor of 2.5.

![Power curve](image)

**Figure 5.6** Power curve presenting average actual power of the fuel cells in triplicates of: single layer MPL based carbon cloth cathode MFCs and carbon veil (control). The error bars represent the standard deviation of the power curves of tested triplicates.

The positive effect of the increased cathodic surface area on MFC performance was previously reported on highly porous graphite granules (Freguia *et al.*, 2007) and nitric acid-activated graphite granules where an increase of 400 mV open circuit potential was recorded (Erable *et al.*, 2009). These results suggest that carbon can provide catalytic properties for the oxygen reduction reaction only when modified accordingly (pretreatment) or used in sufficient amounts (higher surface area).

Porous electrodes consist of an electrically conducting matrix that is interspersed with connected voids or pores. The electrochemical reactions in such electrodes occur predominantly in the pores, which represent the major fraction of the total surface area. Here, the external surface area of such electrodes is relatively small with respect to the structural area of pore walls. It is the high interfacial surface area available for electrochemical reactions that provides the major advantage of porous electrodes over smooth surface electrodes. The major driving force for the development of gas
diffusion electrodes lies in their attractive features for use in fuel cells. The gas diffusion electrode that is most commonly used is porous structure of catalysed carbon black and polytetrafluoroethylene (PTFE). Carbon provides the conductive substratum for the electrocatalyst, while PTFE provides the porous hydrophobic network for the diffusion of reactant and product gases through the electrode structure. This combination of two solid phases when fabricated into appropriate porous structure provides a stable three phase boundary for successful operation of gas diffusion electrodes. The early porous carbon electrodes were usually made of carbon or graphite plates. It was soon recognised that only a small fraction of available surface area is actively taking part in electrochemical reactions. In consequence the development of gas diffusion electrodes required thinner and more porous electrodes (Kordesch & Hacker, 2010). The common practice in the processing of PTFE bonded carbon electrodes is to sinter the composite mixture by heating at temperatures above the normal PTFE melting point (335-350°C). Thermal treatment of the PTFE and carbon black creates the structure consisting of PTFE films to cover the surface of carbon black particles. When PTFE and carbon black are heated at these temperatures, the PTFE flows slowly and penetrates into the porous carbon spreading as a very thin film. These films help to form the interpenetrating network of liquid filled and gas filled pores that are requirements for operation of gas-diffusion electrodes (Klinedinst, Vogel & Stonehart, 1976). The MPL coating on carbon based GDL cannot be uniform since it is done manually, therefore it can be speculated from the polarisation results of MPL that local non-uniformity affects the performance (Fig 5.6).

5.1.3 Conclusions

Microporous layer based cathodes can be used as a single layer electrode outperforming the carbon veil material used as cathode electrode in our set up, by a factor of 2.5. It appears this may be due to its micro-porous structure, which results in
a higher active surface area as well as higher carbon loading of the surface that is in
direct contact with the proton exchange membrane. Moreover the open-to-air cathodes
have been demonstrated to be self-hydrating which improves ionic transport and for
the first time, producing excess liquid in the form of droplets directly on the electrode
surface. This part of work will be investigated further.

5.2 Water formation at the cathode and sodium recovery

Two cathode materials used in this section: MPL and AC coatings on carbon cloth
were prepared by Dr Carlo Santoro at the University of Connecticut, U.S.A.

This section is based on:

Gajda, I., Greenman, J., Melhuish, C., Santoro, C., Li, B., Cristiani, P. and Ieropoulos,
I. (2014) "Water formation at the cathode and sodium recovery using Microbial Fuel
Cells (MFCs)" Sustainable Energy Technologies and Assessments. 7. 187–194.
Elsevier.

5.2.1 Introduction

There are very few previous studies reporting water production in MFCs via ORR or
through diffusion from the anode and its sufficiency for supporting cathode reaction;
thus, a water/ion bridge is necessary for MFC operation. Water production at the
cathode has been reported as an anolyte loss (Kim et al., 2009) or less frequent
hydration requirement (Ieropoulos & Melhuish, 2005) however, it has not been
visually observed in MFCs. Low water production was probably the result of lower
current generation by the MFC compared to a hydrogen fuel cell (Zhang et al., 2010)
and large evaporation losses. In addition, it has been found that in MFCs, cations, other
than protons, are more likely to complete the circuit via transportation from the anode
to the cathode (Rozendal, 2006). Therefore, by the active movement or synthesis of water it is possible to recover and remediate the cations from wastewater with the help of electro-osmotic drag (Chapter 1, page 29).

Air cathode MFCs are the most promising configuration for practical applications including wastewater treatment and power generation due to their improved electrical power output and operational simplicity of MFC reactor (Liu & Logan, 2004). However, the ORR of a working MFC in comparison to chemical fuel cells is limited due to the MFC neutral pH and ambient operating temperature. Mass transport limitations due to the diffusion of oxygen through the cathode structure results in the cathode being usually the limiting factor for power generation due to increased internal resistance, which hinders the overall system performance (Rismani-Yazdi et al., 2008).

Water is essential for the operation of MFC, providing an electrolyte bridge between anolyte, membrane and the cathode. In Proton Exchange Membrane Fuel Cells (PEMFCs) the water management is critical for achieving high performance. The polymer exchange membrane requires to be sufficiently hydrated to maintain high ionic conductivity, once there is too much water, the pores in gas diffusion layer and catalyst layer will be blocked, resulting in flooding problems and high mass transfer resistance (Hakenjos et al., 2004). During fuel cell operation, water molecules migrate through the membrane under electro-osmotic drag and molecular diffusion and water is being generated at the cathode/membrane interface due to the ORR. If the water generated is not removed, cathode flooding may hinder the oxygen transport (Yu et al., 2009; Holze & Vielstich, 1984) attributing it to hindered oxygen mass transfer by catalyst sites filled with water and pursuing a direction of development for anti-flooding binders (Zhang et al., 2012a). In PEMFCs, the liquid water transport mechanism is the most dominant parameter influencing the performance of the cathode
and current density-based loss distribution (Natarajan & Van Nguyen, 2003). Especially from the wastewater treatment point of view, the cathode flooding might serve as an advantage to the MFC technology. The generated or diffused water from the anolyte to the cathode chamber in the MFC system plays an important role in wastewater treatment as an additional method for waste clean-up, synthesis and bioremediation (recovery) of elements.

This section aims to present the MFC driven catholyte formation in situ on the range of low-cost carbon based cathode electrodes and explore the cathode flooding phenomenon in the dual-chamber MFC system in relation to power production. It also aims to understand the mechanism of biosynthesis, remediation and recovery of useful components from the wastewater; and analyse the newly formed catholyte solution in terms of its composition and a potential for carbon capture through the process of wet scrubbing.

5.2.2 Methods specific to the experiment

Twelve tested MFC reactors comprised of 25 mL anode and 25 mL cathode chambers as previously described in Chapter 2, separated by a cation exchange membrane (CMI Membranes International, U.S.A.). Already established and well matured anodes were used from previous MFC experiments, which have been running for at least 6 months under pseudo steady-state conditions. The cathode electrodes were used as shown in Table 5 and placed in transparent chambers with 2 vents (0.3 cm diameter) at the top. The anolyte was recirculated using a 16-channel peristaltic pump (205U, Watson Marlow, UK) with a flow rate of 47 mL/h. The tubing was made of silicone, its length was 40 cm from the pump to the MFC, 40 cm from the MFC to the 1L anolyte reservoir and 90 cm from the reservoir back to the pump, periodically supplemented with fresh
sludge mixed with 20 mM sodium acetate as a carbon-energy (C/E) source, in order to maintain C/E-replete conditions and avoid having the anode as the limiting half-cell.

The same folded carbon veil electrode used for the anode was used as the control for the cathode (CV) and was the only type of 3D cathode electrode used (Table 5-1). Microporous layer on carbon cloth and on carbon veil was prepared as described in Chapter 2. Activated carbon (Cabot) was prepared with a loading of $60 \pm 2 \text{ mg AC cm}^{-2}$ and PTFE (20% w/v) were mixed using a blender and pressed on a 30% wt. PTFE treated carbon cloth (Fuel Cell Earth) that was used as the current collector in MFCs. The AC cathodes were prepared under pressure force and heated for 1 hour as previously described (Santoro et al., 2014).

The cathode chamber had the additional hollow space at the bottom and included a syringe to allow the collection of the catholyte produced (see Fig.5.8). All 12 MFCs were divided in 4 experimental groups of identical triplicates (Table 5-1). No catalysts or buffers were used.

Table 5-1 Types of electrodes in the open to air cathode half cells.

<table>
<thead>
<tr>
<th>Electrode Name</th>
<th>Electrode Type</th>
<th>Cathode type, size</th>
</tr>
</thead>
<tbody>
<tr>
<td>CV</td>
<td>Carbon veil (Control) folded</td>
<td>3Dimensional (folded), 270cm$^2$, (projected surface area: 10cm$^2$)</td>
</tr>
<tr>
<td>MPL</td>
<td>Carbon cloth with Microporous layer</td>
<td>2D 10cm$^2$</td>
</tr>
<tr>
<td>CV MPL</td>
<td>Carbon veil with Microporous layer</td>
<td>2D 10cm$^2$</td>
</tr>
<tr>
<td>AC</td>
<td>Activated carbon on carbon cloth</td>
<td>2D 10cm$^2$</td>
</tr>
</tbody>
</table>

5.2.3 Results and discussion

5.2.3.1 Polarisation curve experiment

To assess the maximum power point (MPP), a polarisation experiment was performed; Fig.5.7 presents the polarisation data where the maximum power recorded for the
different electrodes was: AC 498 μW (SD±102 μW); MPL 419 μW (SD±90 μW); CVMPL 313 μW (SD±104 μW) and CV121 μW (SD±16 μW). From these it can be concluded that the 2D pressed cathode type materials such as AC, MPL, MPL CV are performing 4, 3.4, and 2.5-fold better, respectively compared to the control 3D cathode (CV). The improved performance might have been due to the higher specific surface area of the activated carbon in AC and MPL as discussed earlier and a better contact with PEM, which might have resulted in an improved ORR rate.

![Figure 5.7 Polarisation data of all tested MFCs (mean value of triplicate MFCs).](image)

5.2.3.2 Catholyte accumulation

Catholyte formation on the surface of the cathode electrode was first observed as droplets, visible to the naked eye (Fig. 5.8, left) and further on as accumulation of the catholyte from all the tested MFCs. The newly formed catholyte was clear (Fig.5.8, right) and when evaporated left a residue, which is soluble in water (Fig.5.8, centre). At the same time, anolyte loss was observed. The dry mass of collected precipitant was estimated on the basis of evaporation of collected catholyte samples of equal volumes.
The maximum salt concentration calculated for each cathode (see Figure 5.9) was shown to be: CV 40.1 g/L, AC 57.2 g/L, MPL 93.8 g/L, CV MPL 86.2 g/L.

Figure 5.8 MFC with droplets of water forming on the cathode and collected liquid in the syringe (left), cathode chamber disassembled with salts precipitation (centre), catholyte liquid collected (right).

Figure 5.9 Dry mass of salts evaporated from the catholyte formed in all 4 experimental MFC groups under various load conditions.
5.2.3.3 Relationship between catholyte accumulation and power generation

To explore the catholyte accumulation phenomenon, all MFCs were connected to 2.4 kΩ, 1.2 kΩ, 600 Ω and 300 Ω resistors. The 300 Ω resistor was chosen as the closest to the internal resistance for MPP, which was derived from the polarisation experiment. Voltage and produced catholyte “water” were monitored during a 72 h period at each resistor value. In terms of cathode material, the highest power was recorded for AC 262 μW, MPL 159 μW, MPL CV 152 μW at 300 Ω and CV 88 μW at 600 Ω (Figure 5.10). Catholyte accumulation was recorded after 72 h under each resistance value. The data show that water was produced from all cathode materials, at different levels, and was dictated by the level of power as well as by the cathode material. The maximum current was produced from AC 934 μA, MPL 728 μA, CV MPL 712 μA and CV 540 μA. In terms of power generation, the best performing cathode was the activated carbon (AC) with a maximum power density of 262 mW/m² (normalised to the total macro cathode surface area). Activated carbon was reported to produce power densities similar to those of Pt cathodes (Wei et al., 2012) also as AC was cold-pressed with PTFE binder to form the cathode around a nickel-mesh current collector, the performance of the AC cathodes was due to the high surface area of the material (Zhang et al., 2009a) and in long time run (1 year) performance dropped due to micropores clogging (Zhang, Pant & Logan, 2011). Here, the cathode electrode was purposefully enclosed within an empty chamber to prevent evaporation and salt precipitation. In this way, the formed droplets were dripping down and washing any deposits off, preventing clogging. The relationship between power generation under external resistance and the catholyte accumulation is shown in Figure 5.10.
Figure 5.10 Power average over 72 h period in relation to amount of catholyte collected after 72 h under various external resistances: 2.4 kΩ, 1.2 kΩ, 600 Ω and 300 Ω.

These findings seem to be similar to the previously reported water loss through the membrane, which was also dependent on the value of the external load resistance, suggesting that total charge transfer in the MFC is related to electro-osmotic drag of water (Kim et al., 2009; Zhuang et al., 2009). However, this has only been reported as a loss of anolyte volume and furthermore this is the first time that newly synthesised catholyte of such volumes, is actually collected from MFCs. This has also been reported for chemical PEM fuel cells (Park & Caton, 2008), which traditionally employ water management techniques to avoid catalyst flooding and improve performance.
5.2.3.4 Analysis of the accumulated catholyte

5.2.3.4.1 Conductivity and pH

Figure 5.11 Conductivity and pH values of catholyte collected after 72h under various load conditions: 2.4kΩ, 1.2kΩ, 600Ω and 300Ω.

The conductivity and pH behaved in a similar manner, with the highest conductivity and pH recorded for the highest power levels and water volumes. The pH of the collected samples in the range of 10.5-13.6 suggests high caustic content; Figure 5.11 shows the measured pH and conductivity values of the collected catholyte from MFCs under the different resistance loads. The data suggest that improved power generation and subsequent higher ion exchange rate between the anode and the cathode drives the OH⁻ accumulation on the cathode surface, therefore the more power produced by the MFC, the more caustic catholyte is collected. The higher reaction rates at MPP (300 Ω) would suggest that CO₂ buffering (reacting with OH⁻) would be suppressed compared with the lower power generation (i.e. slower reaction rates) recorded under 2.4 kΩ or 1.2 kΩ. As previously reported, in the aqueous cathode half cells, high pH of the catholyte is generating a large membrane pH gradient between the anolyte and the catholyte, causing a significant potential loss (Rozendal et al., 2007), which could be compensated by the increase in cathodic conductivity (Rozendal et al., 2008). In the present study the catholyte is constantly removed from the cathode surface by
gravity preventing cathode flooding, salt precipitation and high pH gradient build-up, therefore not affecting the MFC performance.

5.2.3.4.2 EDX and SEM

![Graph showing EDX analysis results]

**Figure 5.12** EDX profile representing atomic content (%) of detected elements in precipitated salts from the electrode surface outside MFC and the evaporated liquid sample (left) and SEM image of the catholyte crystals (right).

Given that the anolyte contained 20 mM sodium acetate, it would be expected to find sodium in the precipitated salts. The precipitated salts were crystalline (Fig. 5.12, right), transparent or white and soluble in water. The EDX analysis (Fig. 5.12, left) of the salts that precipitated on the surface of the part of electrode outside the MFC and from the evaporated catholyte solution, show 96.7% and 93.6% sodium content, respectively. This suggests that cations such as sodium (from sludge and sodium acetate feedstock) are being recovered from the anode to the cathode.

5.2.3.4.3 GC MS and ICP OES

The GC-MS analysis detected no organic content in the collected liquid (data not shown). With the exception of Na, which was off-scale for all the samples, and K which was off-scale for AC, the ICP OES profile is shown in Figure 5.13. The analysis showed varying amounts of potassium, magnesium and calcium primarily, depending on cathode material, and very small amounts of aluminium and zinc. Although the data
were not directly proportional with the level of power produced by each material, the highest peaks were recorded from the most powerful AC cathode.

Figure 5.13 Detected trace elements in catholyte samples (g/L) by ICP OES. Sodium was off the measurable scale in all tested samples (*), as well as Potassium in sample AC (*).

5.2.3.4.4 XRD

X-ray diffraction pattern analysis of crystallised catholyte samples from all tested cathode types, appear to be made up from very similar materials. Analysis using single crystal X-ray diffraction suggests that the primary component of the material has a monoclinic cell of \( a = 21.0 \), \( b = 3.5 \), \( c = 10.7 \) \( \beta = 106.41 \). A database search of materials with a known unit cell of this value with a Na content, suggests a mineral phase known as Trona or \( \text{Na}_3\text{H(CO}_3\text{)}_2\cdot\text{H}_2\text{O} \) (Figure 5.14). This matching leaves some peaks unaccounted for. Therefore a further search of the data suggests that the other possible phase present is \( \text{Na}_2\text{CO}_3\cdot\text{H}_2\text{O} \) phase with the parameters: \( a = 6.472 \), \( b = 10.724 \), \( c = 5.259 \beta = 90 \).
Recent developments in the field of BES research has shifted from power production to chemical synthesis, where the energy is stored as chemicals in the cathode. Here, it is suggested that it may be possible to integrate both energy generation and chemical synthesis as it appears that both processes are inherently linked. The recovery of caustic soda from wastewater and acetate solution used as anolyte has been previously reported (Rabaey et al., 2010), by using an energy-consuming MEC and a catholyte containing NaCl. Here, as no catholyte solution was used, the electricity produced by the MFCs is directly linked to the synthesis of caustic catholyte in situ. Similar carbonate salt deposits were previously reported on the electrode-membrane assembly however this accumulation was hindering the overall MFC performance (Pham et al., 2005). Similarly, in cases of membrane-less MFCs, the biocathode, free of platinum, seemed to be limited by carbonate deposits (Cristiani et al., 2013; Santoro et al., 2014). Here, it is suspected that the salt accumulation is prevented due to the effective electro-osmotic transport of water, and active ORR which removes salts by hydrodynamic flow away from the membrane and washing away potentially accumulating salts off from the cathode surface. Therefore, the cathode ‘flooding’ and draining away bulk water in the presented MFC system may be of benefit rather than of hindrance.

Figure 5.14 X-Ray Diffraction pattern comparison of Trona phase (grey line) with the experimental diffraction pattern (red line).
5.2.3.5 Significance of catholyte accumulation to environmental cleanup

In principle, the catholyte production is mainly due to the electro-osmotic drag and ORR, thus the volume of catholyte produced should be equal to the volume of anolyte lost (minus evaporative losses) and considering negligible the contribution of ORR-produced H₂O due to low current generation. However, high salt concentration in the formed liquid suggests that the water transport might be also supported by the passive osmosis, which requires further investigation. If 2 mL of catholyte can be produced over 3 days of operation as shown in Figure 5.10, from a 25 mL anode, which theoretically suggests that it would take (for example) 16 days to produce 10.6 mL of clear catholyte. If by the end of this period the anodic feedstock is completely depleted (which we expect to be the case), this would suggest a 40% recovery rate with a 60% evaporation rate. This is only a prediction based on visual inspection of system’s evaporative losses, and further work is required to validate this. Apart from sodium which was added to the feedstock as sodium acetate, the other detected elements through the ICP OES analysis included potassium, magnesium and calcium (Fig. 5.12). It is therefore suspected that these cations must have come from the wastewater through the PEM, and in effect this demonstrates a MFC-driven recovery. This would be valuable in extracting useful elements from highly polluted/toxic waste streams.

5.2.3.6 Sodium recovery through bioproduction

It has already been hypothesised that a 25 mL anolyte could produce 10.6 mL of catholyte. This would be the equivalent of 426 L of catholyte recovered from 1 m³ of anolyte. The concentration (5-9 %) and composition (90 % sodium) of salts in the obtained catholyte, may suggest that in theory, 45-81 g of sodium can be recovered per L of catholyte, which at a large scale would be the equivalent of 19.1-34.5 kg of salts from 1 m³ of anolyte. The analysis of the salt deposits suggests the mineral known
as trona Na₃(CO₃)(HCO₃)•2H₂O or Na₂CO₃-NaHCO₃•2H₂O which is one of the natural forms of sodium carbonate and it is a salt of sodium carbonate and sodium bicarbonate. Its potential could be quite high, as sodium-precursors are normally used in the production of sodium battery components (Slater et al., 2013).

5.2.4 Control of catholyte alkalinity

The typical pH of the collected catholyte throughout the experiment was 10.5-13.6. During the experiment the cathode chamber was closed, with only two open-to-air vents at the top of the reactor. As it was observed by obstructing the vents and preventing the ambient air to flow through the chamber, the pH of the catholyte collected subsequently increased to >13 (data not shown). This may suggest that by limiting the air flow and consequently the CO₂ flux and its buffering properties, it is possible to control the alkalinity of the catholyte. Moreover, elevating the already high pH of the catholyte simply by limiting the air flow indicates the possible use of the synthesised caustic solution as disinfectant and may prevent biofouling of the membrane by limiting the growth of biofilm on the cathode side (Xu et al., 2012).

5.2.4.1 Carbon capture

Removal of gaseous components through contact with a caustic solution is known as “wet scrubbing”. Carbon dioxide absorption from atmospheric air using alkaline solution has been explored in the 1940s (Spector & Dodge, 1942) and used at large/industrial scales (Lackner, 2009; Zeman & Lackner, 2004). In the air extraction process, alkaline solvent (typically NaOH) reacts chemically with the entrapped CO₂. Therefore, it is suspected that in the presented MFC cathode, an air flow (and consequently its CO₂ content) played an important role in controlling alkalisation of the caustic catholyte formed in the porous open-to-air cathode, and the further formation of sodium salts such as: trona - a mixture of carbonate and bicarbonate of
sodium. Furthermore, it has already been proposed that an addition of CO₂ is an important pre-condition for the formation of trona deposits (Earman, Phillips & McPherson, 2005). With the proposed MFC set up, caustic solution (and its carbonate content) may be directly synthesised on the MFC cathode to capture CO₂ from the anode and from the air, and allow sodium recovery from the anolyte. This would allow an MFC being truly carbon negative technology.

5.2.5 Conclusions

It has been shown for the first time that Microbial Fuel Cells have the ability to synthesise and extract useful elements on different carbon-based cathodes with the important advantage of electricity generation during this process. The in situ synthesis of catholyte appears to be independent of the electrode material used and it shows a significant correlation with MFC electrical performance. This is the first time that carbon capture through chemical synthesis of liquid catholyte has been recorded whilst generating (as opposed to consuming) electric current, which emphasises the benevolent potential of the MFC technology.

The formation and extraction of water in Microbial Fuel Cell might be extremely beneficial as an additional form of wastewater treatment and bioremediation of elements. It is one of the key findings. The reaction kinetics for oxygen reduction on carbon appears to be influenced by many factors: surface structure, chemical treatment, electrochemical treatment and humidity therefore it needs to be explored further.

5.3 Electro-osmotic-based catholyte production

This section has been presented at 225 ECS conference in Orlando, U.S.A. at 13 May 2014.
5.3.1 Introduction

As it was previously shown in section 5.2, the current-dependent transport results in electro-osmotically dragged water molecules reaching the cathode as well as osmotically dragged from the anode to the high concentration of salts on the cathode. However, to explore further the role of these two processes separately, it requires distinguishing between the two. The transfer of ions through the proton exchange membrane plays a major role in the charge-balancing ion flux from the anodic into the cathodic compartment. This leads to the formation of pH gradients between the anode and the cathode compartment (Zhao et al., 2006). Electroosmosis is observed with all types of membranes and the electrokinetic phenomena have been attributed to interactions between flow of electricity and flow of liquid in the electric double layer. In fuel cells, the transport is current dependent especially with the use of high water content membranes (Lakshminarayanaiah, 1969). It is representing an active transport in opposition to the passive flux through forward osmosis. In forward osmosis the use of catholyte of high salt concentration is acting as drag solution passively extracting water from the lower concentration to high (Zhang, Brastad & He, 2011).

This work aims to truly understand ’flooding’ in a Microbial Fuel Cell system and show as non-limiting way of bioelectrosynthesis of alkaline compounds directly onto the cathode surface. It is investigating the effect of electroosmotic drag and osmotic pressure in the MFC water transport. Moreover it is aiming to demonstrate the feasibility of MFC electricity production with simultaneous bioelectrosynthesis of caustic compound. This is particularly important for carbon capture and driving...
5.3.2 Materials and Methods specific to the experiment

The design and operating conditions were kept the same as in previous section 5.2, however the experimental test run lasted 7 days under: open circuit conditions and 7 days under external resistance 300 Ω. This is to allow the accumulation of larger volumes of catholyte under steady-state MFC operation. Also, to prevent the evaporation losses the cathode chamber vents were completely closed with silicon sealant. The amount of collected catholyte under those two specific conditions was analysed against the produced power by all MFCs.

5.3.3 Results and discussion

5.3.3.1 Power performance and catholyte generation

MFCs have been tested under external load conditions 300 Ω and the performance has been presented in Figure 5.15. The stability over 7 day period was achieved with the average output for AC 309 µW, CV MPL161 µW, MPL 91 µW, CV 81 µW. The improved performance of activated carbon has been reported earlier (Gajda et al., 2014). During this stable operation the accumulation of water has been observed (see Figure 5.16).
The formation of droplets was observed on the surface of carbon cathodes and further accumulation of the catholyte was collected and analysed. Previous study suggested that the catholyte accumulation is a function of power performance, therefore it was attempted to plot the current level vs volume of accumulated liquid (Figure 5.17) as the power performance was stable (Fig 5.15). The catholyte has been removed by gravity and collected in external vessel (syringe), therefore flooding (cathode chamber...
being filled with liquid) has been avoided as well as accumulation of salt deposit was sufficiently rinsed.

Figure 5.17 does provide a significant correlation of power performance to the volume of catholyte generated however only in case of 2D electrodes. It has been observed that 3D electrodes (CV) were outliers and producing relatively more catholyte in comparison to other 2-dimensional cathodes. The 3D structure was discovered to act as a sponge and when pressed, it released excess catholyte. Therefore it was suspected that the ability of folded CV “sponge” to absorb the catholyte is causing the osmotic pressure to drive the water transport from the anode (lower salt concentration) to the cathode (higher salt concentration). In order to understand the mechanism better, the total volume of catholyte has been divided by the two main contributions due to: i) current dependent electro-osmotic drag and ORR \( (V_{\text{osm}}) \) and ii) osmotic pressure
\( V_{\text{oosm}} \). Natural evaporation has been neglected as the cathode chamber has been sealed.

\[ V_{\text{cath}} = V_{\text{eosm}} + V_{\text{osm}} \]

Where:

- \( V_{\text{cath}} \) - The total catholyte produced (under external load)
- \( V_{\text{eosm}} \) - Catholyte actively transported by electroosmotic drag and synthesised via ORR
- \( V_{\text{osm}} \) - Catholyte transported via osmotic pressure (under open circuit)

To evaluate this assumption, MFCs have been left in open circuit (OCP) for another 7 days and the relative amount of catholyte was collected. This passive movement of electrolyte has been governed by the local water concentration gradient and osmotic pressure. The results showed that 3-Dimensional CV sponge electrodes produced relatively the most catholyte (Figure 5.18A). Values for the volumes of the collected catholyte in OCP were inserted into the rearranged equation 1:

\[ V_{\text{eosm}} = V_{\text{cath}} - V_{\text{osm}} \]

Figure 5.18B presents that the volume of electroosmotically transported catholyte is directly correlated with the electrical current generation.
Figure 5.18 A) Catholyte generated in open circuit conditions, B) Calculated catholyte volume transported via electroosmotic drag in relation to current generation shows linear regression.

The electrolyte in the anode makes contact with the cathode electrode through the membrane transferring a mixture of ions and water. During MFC operation, ions are being actively transported dragging water molecules with them. Thanks to the produced catholyte it is now possible to determine this transport as it only has been reported previously as net water loss (Kim et al., 2009; Zhuang et al., 2009) linking the water loss in open circuit potential to natural evaporation (Kim et al., 2009).

5.3.3.2 Catholyte properties

The quality of the formed catholyte has been investigated by pH and conductivity measurements. As suspected, pH values show linear correlation with power performance as pH range was 10.61-13.23 (Figure 5.19). These alkaline properties have been already investigated before in similar cathode half-cell configuration however with the air inlets open to atmospheric air (Gajda et al., 2014). Here, as the cathode half-cell was completely closed but not air tight, the pH values were significantly higher. The cationic flux from the anolyte to the cathode has been related to the catholyte pH increase. Rather than battling this pH imbalance, it could be used
as an advantage to produce the alkaline solution containing mainly hydroxides in BES systems that consume electricity (Rabaey et al., 2010). Nowadays with results from the current study this may be achieved with the net energy generation where pH splitting (Harnisch, Schröder & Scholz, 2008) does not affect the performance but it is directly related to the output.

![Figure 5.19 pH of produced catholyte under load shows gradual increase with power performance.](image)

Another aspect of active synthesis of hydrogen peroxide was shown in MFC previously and it was dependent on external resistor used (Fu et al., 2010) however, in alkaline conditions hydrogen peroxide undergoes decomposition (Navarro et al., 1984; Venkatachalapathy, Davila & Prakash, 1999) resulting in formation of OH-(Kinoshita, 1988). Alkaline sorbents are used in chemical carbon capture through wet scrubbing (Zeman & Lackner, 2004) and applied in industrial scale. The method involves use of an alkaline liquid sorbent such as NaOH and KOH to remove CO₂ from ambient air and produce carbonate salt. It also has been included in innovative concept idea of artificial trees (Lackner, 2009). The MFC based extraction of sorbents shows to be particularly attractive. Here, it is suspected that thanks to the peroxide
pathway resulting in OH- formation as well as the cationic Na⁺ transport, the sorbent is directly synthesised onto the cathode electrode. The cathode shown here is serving as a sorbent-based air scrubber.

Figure 5.20 Conductivity of produced catholyte under load conditions shows linear increase with power.

Increase in catholyte conductivity with MFC current performance (Fig. 5.20) indicates that the quality of the catholyte is dependent on current and does not affect the power output. This suggests that the catholyte solution washes the salt deposits off the electrode surface. Increase in pH can be important in terms of the possible use of it as disinfectant. Use of cathodic chamber for disinfection process in MFC has been reported so far with an external supply of disinfectant (Jadhav, Ghadge & Ghangrekar, 2014). The use of a saline solution MFC increases solution conductivity, resulting in decreased internal resistance and higher power densities without pH control (Ahn & Logan, 2013). In this way, this has the chance to become a technology suitable for practical implementation as a carbon capture and energy regeneration system from waste. It is important to further explore the cathode capabilities depending on application (Harnisch & Schröder, 2010) and use it as the platform for carbon
scrubbing (Gajda et al., 2014) or ammonia stripping (Kuntke et al., 2012) with simultaneous energy generation instead energy consumption.

5.3.4 Conclusions

MFCs were shown to produce useful catholyte whilst generating electricity, with plain Pt-free electrodes, thereby representing a promising route for sustainable electricity production and water recycling. Catholyte pH (up to 13.23 suggesting caustic content) and conductivity, showed gradual increase with power. Cationic transport might present a mechanism of water extraction from the anolyte via electro-osmotic drag (under load) and osmotic pressure (under open-circuit), where no electron transfer occurs. The total charge transfer in the MFC is related to the electro-osmotic drag of water through the membrane representing the active transport, whereas osmotic pressure gradient between dissimilar solutions is passive and dominant under open-circuit conditions.

5.4 Catholyte pH observation

5.4.1 Introduction

The detrimental effect of high pH in the cathode chamber has been reported by other researchers (Rozendal, 2006) and this has been the subject of this chapter’s investigation. In the cathode chamber, protons react with oxygen as corresponding electrons originating from the anodic electrode, result in a gradual increase in catholyte pH. Moreover, the passive electrolysis of water rather then transported from the anode is another factor increasing the pH. Accumulation of hydroxide ions (OH-) produced via water electrolysis creates a pH imbalance in membrane MFCs (You et al., 2009) with a consequent pH splitting. For that reason the pH control has been used in MFC
cathode studies (for example phosphate buffer) and when removed, the MFC performance decreases (Gil et al., 2003). This study is aiming to investigate the influence of pH control in the MFC cathode chamber with the use of biological buffers.

5.4.2 Methods specific to the experiment

Six standard dual chamber MFCs were used with MI cation exchange membrane and standard, folded carbon veil cathode. 3 of the MFC cathodes were kept in batch mode kept half full with tap water (10 mL) and the other 3 have the cathodes filled with catholyte and operated in recirculation mode from the catholyte bottles of the 400 mL volume. The batch MFC cathode chambers were kept half full to allow both sufficient hydration and oxygen flow. 1000 Ω resistor was connected as the external load. Preliminary test included only batch mode MFCs.

To maintain pH control in this experiment, the biological buffers (Good’s buffers) were used. These non toxic buffers were chosen to minimise the influence of the introduced buffer solution on the anodic community.

Biological buffers used:

MES-to maintain acidic conditions pH 5.5

MOPS- to maintain neutral conditions pH 7.0

CAPS- to maintain basic conditions pH 9.2

5.4.3 pH influence on power generation

The preliminary study showed that pH influences power generation, which was of course expected. The data illustrate power production during catholyte change in Veil 1, Veil 2 and Veil 3 MFCs, which were used as control MFCs in the previous experiment. The experiment was performed in steps as shown in Figure 5.21.
The step 1 demonstrates a whole catholyte (10 mL) removal from the cathode chamber and pH measurement of catholyte and as a result the power generation improvement. It demonstrates that the cathode chamber does not contain catholyte but the folded electrode (carbon veil) is still hydrated. High pH of the catholyte removed (high concentration of OH-) ranged from 10.28-10.35.

In the step 2, new catholyte, 10 mL of stagnated tap water (pH 7.5) was added and the power improvement was observed again, however diminishing in time. After 15 minutes the catholyte was removed and pH measurement indicated high pH values ranged from 10.26-10.34.

Again in the step 3, new catholyte was added (pH 7.5) with pH measurements performed after 15 minutes interval. Steps 4, 5 and 6 were performed by replacing the catholyte with fresh tap water (pH 6.95) in time intervals of 15 minutes. It was observed that the power generation improvement after the addition of fresh tap water
results in rapid but temporary power peak, which diminishes in time with the simultaneous pH rise of the freshly replaced catholyte. After step 6, and 30 minutes interval the catholyte was replaced one last time and the pH was measured. It has been observed, that with 1 unit of pH change, the voltage output may improve up to 100 %.

The conclusion of this test is that the pH of the freshly replaced catholyte rises very quickly, thus diminishing the performance. Although the performance improves greatly with catholyte change, this is not a long-term effect.

The high pH results in protons being removed from the solution through the oxygen reduction reaction (ORR). MFCs, unlike chemical fuel cells, must operate at neutral pH in the anode chamber to ensure optimum growth and activity of the microorganisms catalysing the reactions. At the cathode, OH⁻ ions cause a local increase in pH, due to a limiting rate of their transport. However, transfer of alkali cations such as Na⁺ is caused by the insufficient concentration of protons in microbial fuel cells, which is significantly lower than the concentration of alkali cations. The flux of alkali cations in combination with the proton consumption during the ORR leads to the accumulation of hydroxide and a pH increase. A rapid pH increase and its negative effect on the MFC performance would require an active pH control or the implementation of an open-to-air cathode with a catholyte-collection system. Active pH control with a use of chemical buffering agents (for example phosphate buffer) is not a sustainable approach as it is introducing more elements (phosphate) to the system. One of possible solutions used as active buffers would be the implementation of living organisms in the cathode chamber such as phototrophs and an active in situ regulation of the increasing pH alongside with oxygen production, which forms Chapter 4 of this thesis.
5.4.4 pH Control

Temporal data show that as in the preliminary experiment, the replenishment of the catholyte in the batch MFCs with MES buffer (pH 5.5) improves power generation. pH control performed by the buffer is showing power stability rather than quick decline.

![Graph showing pH and MFC power change with MES (pH 5.5) buffer.](image)

*Figure 5.22 Catholyte pH and MFC power change with MES (pH 5.5) buffer.*

However, the power curves of the MFCs under batch conditions performed after buffer has been replaced and after a 24 h period (Fig 5.23) show a significant decay in power. It has been also shown that the preferred pH for improved power generation is acidic or neutral.
Under catholyte recirculation conditions the power levels are higher in comparison to the batch mode test, as seen in Figure 5.24 and 5.25. This shows that constant replenishment through recirculation is required if pH control is chosen for the MFC operation. This also suggests that in the cathode half-cell acidic and neutral pH conditions are favourable for improved power production.
5.4.5 Conclusions

With the active pH control it is possible to improve the power generation in MFC and prevent the caustic formation, however such control would require external supply of a buffer and its frequent replenishment in the cathodic system. The energy consuming recirculation of the pH controlled catholyte results in improved performance; however it is less viable in real world conditions.

5.5 Inverted Tubular MFC-ceramic membrane

Parts of this study has been published as:

Also, parts of this section were presented at the Fourteenth International Conference on the Synthesis and Simulation of Living Systems, 2014 and have been published as:


5.5.1 Initial experiments

5.5.1.1 Introduction

The observation of catholyte generation and the microbially driven electrosynthesis of alkaline sorbents presented in previous sections of this thesis has been pushing forward this investigation into scaled-up designs. This is to obtain possibly larger volumes of catholyte, investigate cathode flooding and test if the catholyte would be generated in the absence of a cation-specific membrane. This investigation was inspired by the need for simple and scalable designs that might be put into a wastewater tank or a container and work without the need for continuous flow or recirculation, tubing connections and complicated, energy-intensive operation. Moreover, the thought behind the MFC development also focused on the cost effectiveness of the materials used and the method of preparation (i.e. electrode). This approach was centred on choosing the ceramic material (terracotta) as the key element and building block of a MFC structure. Ceramic, as a porous material, has shown great potential in MFCs as a membrane separator (Winfield et al., 2013a) or as an entire MFC structure in itself (Behera, Jana & Ghangrekar, 2010). So far, it has been used as a standard ‘pot’-configuration, in which the anode compartment is placed inside the ceramic container and the cathode outside. This requires connecting MFC units together via tubing and an external power
input to distribute the anolyte between MFCs (Ieropoulos et al., 2013). Were this configuration to be inverted (with an inner cathode and an outer anode), the MFC could be immersed in a wastewater tank where, in theory, the movement of the anolyte and recirculation of nutrients to the anodic biofilm could be achieved passively. This inverted construction was previously shown in an upflow MFC design (He et al., 2006) or as a bio-cathode (Liang et al., 2009) however, it was never developed further. In this section, the potential of a simple and cost-effective design was studied as a means to improve power and to encourage catholyte generation.

5.5.1.2 Methods specific to the experiment

Terracotta cave was initially assembled with carbon veil anode and MPL on carbon cloth cathode placed inside the ceramic cylinder as previously described in section 2.4.3. The MFCs has been placed inside the 1 L bucket (Fig. 5.26) which was filled with 500 mL of activated sludge and supplemented with 20 mM sodium acetate of pH 6.6-7.8 and periodically fed. In order to demonstrate the viability of a single ceramic MFC as a single and only power source a single red LED was connected via TI energy harvester (Texas Instruments, Farnell, UK) and a 6800 μF super-capacitor. The output voltage from the single ceramic MFC was lower than the input requirement of the LED and thus the TI harvester was used to continuously charge the capacitor and allow constant energising of the LED. Data were logged using a multichannel Agilent 34972A, LXI Data Acquisition/Switch Unit (Farnell, UK)
5.5.1.3 Results-Maturing and catholyte formation

Fig 5.27 shows the maturing of the single MFC submerged in the anolyte. It shows the improvement in power generation over the 20 day period under various external load conditions.

Figure 5.27 Experimental test from the start up- maturing. The peaks show the polarisation tests and the arrows indicate external resistance.
The catholyte collected was again of high pH and much higher conductivity than the anolyte. In terms of power stability represented in Fig 5.28 (right) shows the removal of the formed catholyte on day 1 of the test and the improvement of power performance during the 14 days of operation with the peak power of 1.3 mW. The catholyte was collected inside the cylinder and anolyte loss from the anode was observed. The catholyte properties and its relation to power performance will be investigated in detail in section 5.6.2.

5.5.1.4 Results-Powering a LED by a single MFC

To show the potential of a single MFC to power real applications, the described ceramic MFC was used to operate a red LED, which was successfully performed at a constant voltage of ~1.7 V through an energy harvester system (Figure 5.29). During the experiment, the MFC performance showed stable current generation (3.2 mA) at the constant voltage of 250 mV (Fig 5.30). The LED operated continuously, for as long as the MFC was fed, during the 7-day period. The LED was shown to be operated continuously as long as the substrate was provided for the MFC operation. During the continuous powering of the LED light, the formation of transparent liquid was observed on the cathode electrode surface (Fig 5.28), and further accumulation of the
liquid. The catholyte was allowed to evaporate, and the crystallised salt was analysed further.

Figure 5.29 MFC powering a red LED through the energy harvester system connected to the capacitor.

![Image of MFC powering a red LED](image.png)

Figure 5.30 Voltage and Current of the MFC and connected LED throughout the 7day operation period.

To show the real benefit of this application, the COD was measured before and after the LED operation for 7 days. Fig 5.31 shows the COD reduction sampled from the anolyte that the MFC was submerged in. The measurement was taken from the bottom, top and mixed anolyte tank and compared to the control COD measurement in the similar tank but not connected to the MFC. Real life application of the microbial fuel cell that does not require strictly controlled conditions has the potential to play a major role in developing sustainable urban wastewater systems.
5.5.1.5 Powering a mobile phone by a single MFC

In the same configuration the Samsung mobile phone was connected to the single MFC, Fig.5.32 demonstrates that the charging from 3.3V to 3.7 V was performed within 36 hours and after discharging when phone was in operation (standby) the charge was improved to 33 hours. This is the first time that a mobile phone was charged by an individual MFC.

Figure 5.32 Mobile phone voltage under charge/discharge connected to one MFC.
5.5.1.6 Powering a DC motor

While both of the above demonstrations were performed using a smart energy harvester system by Texas Instruments, to show ability of a MFC to directly energize a practical application, three similar MFCs (described in the section 5.5.2) were connected in parallel. Figure 5.33 shows the continuous voltage and current output of the stack while the windmill motor was running. At the third day of the windmill steady run, the capacitor was connected and charged to show that the electricity can be stored. On the sixth day of the test the windmill stopped running as the feedstock was depleted.

![Continuous powering a windmill](image)

*Figure 5.33 Continuous energizing a windmill by three MFCs connected in parallel.*

5.5.1.7 Discussion

To meet the global goal of carbon reduction process there is a need to develop an artificial system that may act as an autonomous CO\textsubscript{2} scrubber. This section describes the direct electricity generation by the Microbial Fuel Cell with the simultaneous bioelectrosynthesis of active solution resulting in further carbon capture and storage. The microbial biotransformation of organic waste results in direct current that may be
used to drive peripheral circuitry of the system such as LED lights, DC motors or a mobile phone, whilst the synthesised product *in situ* recreate some aspect of chemically driven carbon capture. While the biotic biofilm anode portraits the ‘living engine’, the abiotic cathode simultaneously produces a caustic agent that is able to fix CO₂. In the context of artificial life, this system mimics photosynthesis and recycles CO₂ into useful chemicals via the process of electrosynthesis. In a way it behaves like an artificial tree and if implemented in practice, it would be performing a similar function.

While the photosynthesis is a natural way to remove carbon dioxide, it is very challenging to reproduce artificially in any practical way. However, the carbon fixation can be performed through Carbon Capture and Storage (CCS- also known as Carbon Sequestration) process, which is a method of mitigating the CO₂ emitted from the combustion of fossil fuels. Currently, CO₂ removal involves exposing air containing carbon dioxide to a fluid with alkaline properties, which absorbs carbon dioxide and produces carbonate/bicarbonate salts. Air capture technology provides an important tool for carbon management through the development of the innovative concept of artificial trees (Lackner, 2009). The same function can be performed and driven by the Microbial Fuel Cell, where CO₂ is removed by the microbial utilisation of organic waste, to generate electrical current directly. The concept of self-powered scrubbing with additional energy generation is novel and may present a new opportunity for artificial life, in terms of tree-like systems that capture carbon dioxide and generate electricity. As a concept, it may contribute to the energy balance and economics of CO₂ recycling in the future.

During the MFC operation, electric current is generated when for every electron donated by microbes to the electrode surface, a proton is transferred from the anode to the cathode. The cathodic oxygen reduction reaction (ORR) results in the formation of alkaline liquid sorbent that naturally removes CO₂ from the ambient air by producing
dissolved carbonate ions. The caustic conditions driven by the MFC electron flow may result in further removal of carbon dioxide through wet scrubbing. Improved performance in terms of power production might also be due to the reducing the distance between the electrodes (Ahn et al., 2014) using cylindrical design.

5.5.1.8 Conclusions and outlook

The MFC design described in the present study represents a true integration of two bio-inspired technologies such as microbial oxidation and artificial photosynthesis. The proposed MFC with open to air cathode coupled with ceramic membranes could be used both for electricity generation, wastewater treatment, filtration and production of absorbent to demonstrate carbon sequestration through wet scrubbing. A similar activity has been reported using amine-based resins (Gray et al., 2008), however the novelty of the presented system herein, lies with the inherent generation by the MFC \textit{in situ} on the cathodic electrode surface.

The flux density of the solute (the formed salt) is proportional to the electric current, which is akin to the ion transport processes within living plant cells, where the metabolic energy is driving the system. There are many examples of biological membranes within plant cells that function on electro-potential gradients, which are regulating the metabolic processes. Ions can be actively transported across membranes in which case metabolic energy is involved.

The inspiration for the process herein, comes from the anatomy of the leaf where CO$_2$ enters the epidermal layer through stomata (pores), which are regulated by the guard cells into the intercellular air space. This is where the gases enter the mesophyll cells through their cell wall and plasma membranes. The anolyte would therefore be analogous to the engine of the process located in the mesophyll cells of the leaf, the ceramic separator is likened to the plasma membrane and finally the cathode chamber
functions as the intercellular air space where the air flow can be further regulated. It is now possible to illustrate a process where the CO$_2$ is absorbed into the catholyte, as the sodium carbonate/bicarbonate salt washed off the porous electrode by moisture and gravity. This is allowing the electrode to continue operating without large salt deposits and subsequent losses.

The presented “artificial leaf” scrubber in the form of the porous cathode electrode has a high specific surface area increasing the gas diffusion rate of carbon dioxide, as well as allowing the transport of water to rinse the carbonic salt in the chamber. The capture must not only work chemically, but it must also be practical, cost-effective and energy-efficient. The MFC system is driving the process through the microbially assisted electrosynthesis of chemical sorbent on the cathodic electrode leaf. Considering advances in material science of carbon-based materials (graphene for example) in the future, the MFC driven CO$_2$ capture seems feasible and very attractive.

Real autonomy, especially in the context of artificial photosynthesis is presented here by the simple ceramic-based MFC design that does not involve expensive catalysts. This is particularly important to exploit in real-life applications independent of the main energy grid, operating offline and utilising the energy-rich waste. It is envisaged that such systems could be deployed in various natural environments, where they could be performing the triple action of carbon dioxide removal, electricity generation and green chemistry. Depending on the architecture, these can be mobile (autonomous robots) or stationary (artificial trees/plants).
5.5.2 Microbially-Assisted Electrosynthesis in ceramic MFCs with electricity generation

5.5.2.1 Introduction

Water recovery and desalination is widely used in industry through the electrodialysis system (ED). Electrodialysis is an electrically driven membrane technique used for removing charged species from saline water through a series of alternating cation and anion exchange membranes (Xu & Huang, 2008), thus the capital cost of the technology is high. In this process the water dissociation and subsequent emergence of H+ and OH⁻ ions reduces power and causes pH splitting leading to extreme pH variations. This occurs where an external electrical energy source is used to provide the energy for separating the ionic species. This loss of ionic species and the separation occurs. The attempts into incorporation of the reverse electrodialysis process into platinum based MFC has shown that the process might avoid external power supply and be driven on salinity gradients (Zhu et al., 2013) and resulting in production of acid and alkaline solutions. As previously described, a similar process of alkaline generation in the cathode has been proposed as microbially assisted electrosynthesis (Rabaey & Rozendal, 2010). However, the same process of microbially assisted electrosynthesis can be achieved with both energy production and simultaneous product extraction shown in previous sections of this chapter. Moreover, it is generating high salt catholyte that can additionally act as a drag solution in Osmotic MFC (Zhang, Brastad & He, 2011) dragging water molecules through the membrane via osmotic pressure (Ge et al., 2013).

This leads to extracting water from anolyte simply by MFC operation without using an external drag solution. To explore this path further it is important to look into the cost effective materials, design and methodology to showcase the technology to be ready for practical application in wastewater treatment plants. Ceramic as porous, semi
permeable membrane has been recognised as cost effective replacement for PEM. The use of ceramic materials in MFCs has been reported before as septum/separator (Park & Zeikus, 2003) or as a whole MFC reactor (Behera, Jana & Ghangrekar, 2010; Ajayi & Weigele, 2012; Behera et al., 2010; Winfield et al., 2013a). Internal cathode design has been investigated before (Liu, Ramnarayanan & Logan, 2004; He et al., 2006) however its real potential never has been explored further. This section is aiming to present a simple, ceramic based MFC design for both energy recovery and microbially driven electrosynthesis of newly formed catholyte, and to demonstrate the catholyte generation in situ within the catholyte chamber as a platform for water recovery and carbon capture. It also aims to present a cost effective cathode electrode based on activated carbon layer applied on carbon veil fibre substratum.

5.5.2.2 Materials specific to the experiment

The total of six terracotta caves (Orwell Aquatics, UK) of the dimensions 10 cm length 4.2 cm outside diameter, 3.6 cm inside diameter, wall thickness 3 mm, were assembled with carbon veil anodes and AC cathodes. The activated carbon cathode based on carbon veil was prepared in the laboratory by the method described in section 2.2.5. The anode electrode was carbon veil (20 g/m²) size 2430 cm² folded down and wrapped around the terracotta cave, held together with Nickel Chromium (0.45 cm diameter) wire. The cathode was 90 cm² placed inside the cylinder. The ratio of anode to cathode was 27, the same as in the previous, standard size MFC designs in section 5.2 where the anode size was 270 cm² and the cathode 10 cm². In order to demonstrate the viability of the tested MFCs as a single and only power source for applications, single red LED (RS, UK) was connected via series connection and continuously powered without the use of energy harvesting systems.
5.5.2.3 Results and discussion

5.5.2.3.1 Power behaviour

The two sets of MFCs were operated under two conditions. The working MFCs (T1, T2, T3) was left to mature under external load conditions (53 Ω), set 2 (T4, T5, T6) was left to mature under open circuit conditions. To evaluate the electrical performance of this system the polarisations were performed only on working MFCs and shown in Fig.5.34. The maximum performance was achieved by T1 2.58 mW (286 mW/m²), T2 2.12 mW (235 mW/m²) and T3 1.16 mW (128 mW/m²). This is a 5–fold increase to control MFC (0.49 mW) presented earlier in the section 5.2.3 using the same anode to cathode ratio (27:1) and similar activated carbon cathode. Moreover, comparing to other tubular designs of similar volume, the best performing T1 is producing up to 12.3 W/m³ (calculated per anodic volume) and shows a 2-fold increase in power density to previously reported platinum based reactors producing 6.1 W/m³ (Kim et al., 2009) and 6.8 W/m³ (Zhang et al., 2013).

Figure 5.34 Polarisation and Power Curves of all three tested MFCs.
Calculating power production from the regular MFC operation under load shows an average of 2.70 mW, 1.08 mW and 1.98 mW for T1, T2 and T3 respectively corresponding to the data obtained from the polarisation experiment. The fact that all three MFC replicates produce different levels of power might be due to non-homogenous contact between the cathode electrode and the ceramic membrane inside the cylinder and lack of fixed pressing mechanism which requires further development.

![Figure 5.35 MFC performance during 13 day operation under external load (53 Ω).](image)

The performance under external load conditions (53 Ω) showed that all MFCs exhibit stable performance over 13 day period (Fig. 5.35), during which, the catholyte was generated on the surface of the cathode (Figure 5.37). The performance was T1 7.12 mA, T2 4.50 mA and T3 6.09 mA. Produced current was proportional to the amount of generated catholyte (Fig 5.36, left). T1 produced 68 mL, T2: 45 mL, T3: 55 mL of clear catholyte. Open circuit operations under the same conditions were carried out to measure the passive dialysis effects. MFCs in open circuit mode shown catholyte accumulation, however in significantly smaller volumes (Fig. 5.36, right) where T4 produced 15 mL, T5: 17 mL, T6 10 mL. Under the OCV conditions, small amount of
catholyte is suspected to diffuse passively from the anode compartment to the cathode through terracotta separator. During this time the anolyte loss was observed proportionally to the accumulated catholyte, thus the MFC performance as previously presented in the previous sections of this chapter.

![Graph showing catholyte produced [mL] vs. Current [mA]](image1)

**Figure 5.36** MFC produced water in relation to current generation (left). The amount of water produced (under 53 Ω resistor) in working and OCP conditions (right).

5.5.2.3.2 **Catholyte analysis**

Only the cathode electrode in working MFCs showed the production of droplets directly on the surface of the electrode and further accumulation of catholyte liquid. The catholyte generated was transparent and uniform, while the catholyte in OCP showed similar properties to anolyte. The analysis of the pH and conductivity was based on the properties of the anolyte and the catholyte in relation of presence or absence of external load. Figure 5.38 shows that both pH and conductivity of the catholyte in working MFCs is significantly higher to the MFC catholyte in OCP. Moreover, the difference between the anolyte and catholyte pH value is also significant while the OCP conditions show no significant change.
Figure 5.37 Catholyte formation (left) and the amount of water produced in working (under 53 Ω resistor) and OCP conditions (right).

![Catholyte formation and water production](image)

Figure 5.38 pH and conductivity analysis of MFC anolyte and accumulated catholyte in working and open circuit conditions.

![pH and conductivity analysis](image)

The investigation into the water treatment and COD analysis shows the significant COD reduction in working MFCs in comparison to the MFCs that do not produce any power (Fig 5.39).
5.5.2.3.3  **Powering the LED without harvesting systems**

To show the potential of a single MFC to power real applications, the described ceramic MFC was used to operate a red LED which was successfully performed at constant voltage of ~1.7 V directly and without an energy harvester system (Fig. 5.41). The LED was shown to be operated continuously for 7 day period showing constant current level of 4.7 mA until day 5 and slow decline in current until day 7. The lower
current level due to feedstock depletion allowed LED to still operate but with lower light output level.

![Figure 5.41 Three MFCs connected in series directly power single LED.](image)

5.5.2.3.4 **Carbon Capture via electrodialysis**

The key issue for the implementation of the Carbon Capture and Storage (CCS) is cost reduction of the process. Electrodialysis is a process that depends on the principal that most dissolved salts are positively or negatively charged and they will migrate to electrodes with an opposite charge. Electrodialysis systems use the set of membranes increasing the technology cost. In wastewater which is supplemented with sodium acetate, the mix of mineral composition and sodium dissociates while acetate is being used as a feedstock for the microorganisms. Sodium ions are attracted by the cathode and the electricity driven process is resulting in electrodialysis and ion separation.

With an electric field in the system, the process of anolyte dissociation takes place with the use of membrane systems. Electrical potential applied to the ion exchange separators will involve the ion movement within the reactor. This has been a basis for electrodialysis studies where the potential is set externally to drive the dialytic process
or desalination. Recently, it was proposed that alkali production in cathodic chamber of microbial electrolysis cell with external power source and a supply of highly concentrated salt solutions as catholyte (Rabaey et al., 2010) showing the potential of alkali production in situ in electrodialysis system (Chen et al., 2013) resulting in rather complicated designs (Zhu et al., 2013). This study is aiming to demonstrate the advantages of simple design to make MFC technology more applicable. The novelty of the use of ceramic to perform the co-generation of electricity and electrodialytic functions, extraction of water, production of alkaline catholyte is bringing this design closer to real applications that could be implemented in real wastewater treatment plants. It is shown here that water desalination can be accomplished without electrical energy input as a result of electricity generation. The fact that the power does not decrease during this trial while the cathode electrode has been flooded might be attributed to the solution concentration. Electrical resistance of ion exchange membranes strongly increases with the decrease of solution concentration especially in low strength solutions (Długolęcki et al., 2010; Harnisch, Schröder & Scholz, 2008). Moreover the electrical power obtained from mixing saline solutions is based on the membrane-based energy conversion techniques such as electrodialysis (Post et al., 2007; Pattle, 1954). The salinity gradient energy is a very promising energy source for generation electricity and in this design might be an additional driving element.

Ceramic based MFC cylinders were previously shown, however in standard anode-cathode configuration (Winfield et al., 2013a) showing the ability to power practical applications (Ieropoulos et al., 2013). The submerged MFC design described in the present study represents a true integration of two BES reactors (MFC and MEC) into one simple design that could be used in wastewater treatment systems. The calculated cost of in house prepared activated carbon cathodes is 120 £/m² which would be 7 times cheaper in comparison to the same coating on carbon cloth (Fuel Cell Earth).
This would allow a real life application of presented system that does not require strictly controlled conditions and has the potential to play a major role in developing sustainable urban wastewater systems. A novel, open-to-air cathode coupled with ceramic membrane could be used both for electricity generation and filtration of wastewater to regenerate water.

The observation of the cathode electrode shows biofilm growth only on MFCs that are in open circuit suggests that the catholyte of high pH and salinity generated by loaded MFCs might have antimicrobial properties or at least it is limiting biofilm formation on the cathode.

5.5.2.4 Conclusions

Considering the amount of wastewater produced globally and the potential chemical energy stored within it, it is important that the development of MFC technology could be pursued close to real applications. This work is aiming at efficient industrial-scale development that would recover energy from wastewater using a terracotta tubular design which is enhancing functionality of the MFC. This work is presenting simultaneous extraction of clean water from wastewater, element recovery and power production from catholyte free Microbial Fuel Cell system. A system of simple, cost effective design and operational conditions, without the need for platinum catalysts, pH control or saline catholyte. It is believed that the cathode potential can be explored further, looking into other functions such as in exclusion of specific contaminants, for example heavy metals (Abourached, Catal & Liu, 2014) and looking into its antimicrobial properties. This will be discussed in the future work section in Chapter 6 of this thesis. This ceramic design as well as the activated carbon cathode developed by the author have been used in the urinal design for the Reinvent the Toilet Fair
exhibit in India (Fig. 5.42), it is currently been used by researchers at Caltech and Durban, and it in PeePower urinal development at the UWE campus funded by Oxfam.

Figure 5.42 Urinal design in Reinvent the Toilet Fair in India (left, middle) with 40 ceramic MFCs placed inside the tank (right).
6 Chapter 6. Overall Conclusions and Future Work

6.1 Executive summary

This work has demonstrated the development of self-sustainable cathode half cells both in aqueous and open to air configurations, which would aid the development of MFC technology depending on the environment and required functional operation.

This work is presenting a simultaneous performance and sustainability improvement of the cathode half-cell. It is aiding an efficient MFC development that would recover energy and valuable products from wastewater to maximize the energy turnover in the route towards better self-sustainable society.

6.2 Conclusions and Summary of work

The ultimate goal of this thesis was to investigate and produce an MFC with self-sustainable cathode so it could be implemented in the real world applications. This challenge requires a study of the cathode element as an independent unit (an electrode) and its configuration within the Microbial Fuel Cell system.

As part of the initial work was the investigation of the MFC membrane as one of the most expensive parts, primarily, to understand the mechanism of ionic transport and to evaluate the alternative, more cost effective PEM. It was demonstrated that in general, cation exchange membranes outperformed anion exchange membranes and the most cost effective PEM out of the range of tested materials was shown to be the MI-C (Membranes International Inc.). These findings resulted in wider implementation of this membrane by other researchers in Bioenergy Centre. Another aspect in the initial experiments that also investigated was the adoption and influence of a second electrode inserted in the cathode chamber to explore the unconventional connection alternative to the parallel and series connection. The second electrode in
the cathode chamber known as a 4th pin showed a potential to control and modulate the MFC performance. It also shows an interesting path towards the control mechanism and galvanic corrosion within the cathode half-cell when the electrode used are made of dissimilar conducting materials. Also, in the attempt to examine the alternative MFC designs, biodegradable materials were used to develop a MFC reactor constructed solely of biodegradable or waste products, and show that almost any available material constructed accordingly may serve as the MFC bioreactor. This also identified the terracotta as a potentially valid membrane/separator for use in both open to air and aqueous MFC configurations.

The core of this work was the testing and improvement of the cathode with the self-sustainability being the main theme and the prime goal. Work was separated into two exploration routes according to the two well described in literature cathode configurations: aqueous and open to air.

The exploration of aqueous cathode was inspired by the natural ecosystems to create a biocathode based on self-sustainable phototrophic organisms. The use of biotic cathode of mixed phototrophic origin provides an exciting path towards enhancement of natural processes and environmental sustainability. Phototrophic organisms can perform the role of active oxygenators and therefore become vital biocatalysts. The photosynthetic biofilm appears to be a good biocatalyst for the real world implementation of MFCs as sustainable energy harvesters for carbon fixation. Moreover, the positive response to illumination of photosynthetic cathodes provides the evidence that microalgal organisms can actively influence the MFC performance.

To demonstrate the sustainable oxygenation of the MFC cathode by photosynthetic biocatalysts and avoid the diminishing performance during dark phase, photoreactor tanks were connected to the MFCs. The photoreactors were used as the oxygenation
ponds in a similar manner as in a ‘lagoon’ type of tanks used in wastewater treatment, where natural, sustainable oxygenation by photosynthetic organisms has been widely implemented instead of the mechanical aeration. The recirculation of the catholyte from the photoreactor to the MFC has to be done mechanically and through this investigation it has been shown that a stack of completely biotic MFCs can power a practical application such as the impeller pump to perform recirculation cycle. The practical use of generated electricity presents the self-sustainable configuration and biomass growth in the photo-assisted MFC system. The practical implementation of MFC technology into the wastewater treatment plants and sewage disposal systems is currently limited by the low concentration of available organic substrate in wastewater and relatively low power output from single MFC. However with the advances in cost effective multiplication of MFC systems to improve the level of usable power and thanks to the development of low power and smart-energy harvesting devices the real-world applications could be demonstrated in the near future.

This important second outcome of presented photo-cathode required further exploration into the newly formed biomass and its assessment against the MFC performance. It has been presented that the more power MFC generates, the more biomass will be harvested in the connected photoreactor. It is suspected that this can be linked to the total charge transfer, therefore the total protons and cation transported from the anode to the cathode. The cations recovered from the wastewater would become the supporting nutrition for the new algal cells which gives an opportunity to recover energy valuable biomass through the natural photosynthetic activity. Phototrophic biofilm formed on the surface of cathode electrodes demonstrates that balanced algal growth might be linked to good electricity generation, where either scarce or overpopulated cathode electrodes might be limiting power generation and in consequence the re-generation of biomass.
To further investigate the self-sustainability of this photo assisted MFC, the harvested biomass was demonstrated to be a suitable energy resource for the same MFC units. This “closed loop” is presenting MFCs working in a low resource setting, as an energy generation system utilising waste products and maximising energy turnover through biomass recovery. Essentially, it has the ability of turning light energy into electrical energy.

In the open-to-air design, the challenge lays in making modifications to the cathode electrode that will improve the performance without the implementation of expensive and non-sustainable catalysts. The various modifications to the carbon electrode materials include the incorporation of Microporous Layer (MPL) used as a coating applied to carbon cloth based cathode and compared to widely used carbon veil fibre electrode as the control. It has been demonstrated, that MPL can be used as a single layer electrode outperforming the multi-layer carbon veil material (used as control) by a factor of 2.5. This may be due to MPL micro-porous structure, and higher active surface area that is in direct contact with the membrane. Through this investigation, it becomes apparent, that direct, mechanical contact between the electrode and the membrane seems to be the most important feature of the open-to-air cathode design.

As experiments were carried out further, it has been observed for the first time that Microbial Fuel Cells have the ability to synthesize catholyte directly on different catalyst-free carbon-based cathodes. The best performance was achieved with the use of activated carbon (AC) based cathodes however, the in situ synthesis of transparent catholyte appears on the range of carbon based electrodes and it shows a significant correlation between volume generated with its electrical performance. This accumulation of water known as the cathode flooding in Microbial Fuel Cell might be extremely beneficial as an additional form of wastewater treatment and bioremediation of elements extracted from the treated waste. It is one of the key findings, which may
be attributed to the oxygen reduction reaction, electroosmotic drag and osmotic diffusion. The newly formed catholyte appeared to be consisted primarily of sodium salts (9% concentration), which was recovered from the anolyte feedstock containing sludge and sodium acetate. The recovered elements including cations such as potassium, magnesium and calcium seems more valuable in the light of future potential treatment of highly polluted/toxic waste streams. The further analysis of the newly formed catholyte is characterised by elevated pH levels and high sodium content which suggests the formation of caustic solution, which would act as wet scrubbing mechanism to entrap carbon dioxide from atmosphere. The proposed carbon capture in the porous open to air cathodes filled with liquid catholyte containing caustic soda has been recorded whilst generating (as opposed to consuming) useful electricity, which emphasizes the potential of the MFC as a true carbon–negative technology.

The importance of this phenomenon (water recovery, pH increase, CO$_2$- absorption along with electricity generation) is also representing a promising route for sustainable electricity production with water recycling. However, to fully understand the process, the further investigation of the water transport within the MFC has been developed under external load and open circuit conditions. It was shown that the total charge transfer in the closed-circuit MFC is related to the electro-osmotic drag of water through the membrane, whereas osmotic pressure gradient that exists between dissimilar solutions is dominant under open-circuit conditions, where no electron transfer occurs. The total water transport normalised per osmotic loss in open circuit conditions gives the value of the electroosmotically dragged water that linearly corresponds with the produced power. Moreover, the pH of produced catholyte showed gradual increase with power suggesting that the cationic transport relates to mechanism of water extraction from the anolyte via electro-osmotic drag.
To further explore the true potential of above findings utilized within laboratory conditions and standard, analytical MFC designs, it was attempted to develop an open-to-air tubular MFC where the cation exchange membrane was substituted by terracotta. The tubular design was kept close to the operational requirements of real world application and its novelty lies in its simplicity and inversion of the cathode/anode configuration. MFC design described in the final part of this thesis represents a true integration of findings presented earlier into one simple design that could be used in a wastewater treatment tank. Open-to-air cathode coupled with ceramic membranes could be used both for electricity generation and wastewater filtration to regenerate water and recover useful compounds. The improved power is showing a 4 fold increase in actual power comparison to the standard MFC performance and in terms of a similar size tubular MFC it was able to continuously power a LED light, mobile phone and windmill motor which was not possible before.

6.3 **Original contributions**

This study has demonstrated that it is possible to develop a sustainable cathode for the Microbial Fuel Cells both in aqueous and open to air configurations. During the course of this PhD study, the most significant findings resulted in novel contributions. This work contains seven original contributions in the field of cathode development for Microbial Fuel Cells.

Firstly, the membrane investigation contributed to the choice of more cost effective PEM for the experimental work in this project and the work performed by all members within the Bristol BioEnergy Centre research group.

Secondly, a completely biotic MFC with the algal cathode has been demonstrated to power a practical application such as a DC pump representing a photo-dependent system.
Thirdly, the biomass grown within the MFC cathodes shows correlation with MFC performance showing a new route into the biomass recovery and enhanced carbon capture.

Fourthly, the open to air cathode configuration shows a catholyte synthesis directly on the surface of the electrode and elements extraction such as Na, K, Mg from wastewater in a power dependent manner as a function of MFC operation. Cathode flooding has been identified as an important and beneficial factor that does not hinder the MFC performance.

Fifthly, for the first time, the cathodic formation and extraction of salts has been identified as a carbon capture system through wet scrubbing of carbon dioxide from atmosphere and locking it out with the use of caustic solution formed by the MFC itself. This reaction formed carbonate and bicarbonate of soda known as mineral form Trona.

Sixthly, the novel design of inverted, tubular MFC configuration integrates simplicity of design and operational conditions to show significantly improved performance to show real feasibility of MFC system to regenerate electricity from waste. Real life implementation includes for example: continuously powering a small windmill motor by the stack of three MFCs to powering a LED without any energy harvesting system or powering a mobile phone by a single MFC with the use of smart electronic harvester. This design has been implemented as part of the urinal presented at the Reinvent the Toilet Fair in India, it is currently being used by the researchers in Caltech and Durban and it is going to be the basis of the PeePower urinal development at the UWE campus funded by Oxfam.
Seventhly, the development of a novel, cost effective and simple in preparation cathode electrode material based on activated carbon powder has been developed and used in the inverted tubular MFCs. It has been used in all practical developments in previous point as well it has been widely used by researchers in Bristol BioEnergy Centre in BRL.
6.4 **Future work**

6.4.1 **Design of ammonia stripping MFC**

The removal of organic pollutants from industrial and municipal wastewater focuses mainly on nitrogen compounds which come from human and animal waste (mainly urine). Ammonia stripping is a simple desorption process used to lower the ammonia/nitrogen content of a wastewater stream. The process is based on adding caustic additive to increase wastewater pH to 11.5. In this process ammonium hydroxide ions are being dissociated to form gaseous NH$_3$. In a waste stream, ammonium ions exist in equilibrium with ammonia. Below pH 7, all the ammonia will be present in the form of soluble ammonia ions. Above pH 12, virtually all the ammonia will be present as a dissolved gas. The range between 7 and 12, both ammonium ions and dissolve gas exist together whereas temperature and pH increase favour removal of ammonia from solution. This process has been successfully implemented in the stripping towers where the ammonia rich waters enters the tower at the top, counterflow gasses are being introduced in the bottom with the addition of NaOH allows ammonia to be stripped from the water and leave in the gaseous form at the top of the stripper, where the ammonia gas is treated with sulphuric gasses to absorb the ammonia (ammonia sulfate solution may be used as fertilizer). Ammonia has been demonstrated to shuttle protons from the anolyte to the catholyte becoming a dominant cation to transfer positive charges across the CEM to the cathode (Cord-Ruwisch, Law & Cheng, 2011). Ammonia stripping has been already investigated in Pt -based MFC (Kuntke *et al.*, 2012), however it was using an active oxygenation system which is energy intensive. Preliminary test involved ammonium acetate as feedstock used in MFCs. The output catholyte was clear in color and with very strong
ammonium odour suggesting an active ammonium stripping mechanism with an important advantage of continuous electricity generation during this process.

6.4.2 Scale-up-Towards Floating MFC digester

The development of the single MFC as well as the collective of MFCs connected together have been shown in this thesis to show the importance of the cathode development and the MFC design. It has already been successfully implemented in urinal presented at the Reinvent the Toilet Fair in India, it is currently being used by the researchers in Caltech and Durban and it is was implemented at the UWE PeePower urinal funded by Oxfam. Further demonstrations of the power performance should include the scale-up of power production as well as implementation in real wastewater treatment plant. This design gives the advantage that it may be used directly dipped into the pool of waste and possibly in a floating device. This have been investigated before with regards of floating cathode (Zhang, Tian, & He, 2011) or the whole MFC reactor unit for the purpose of use in contaminated water (An et al., 2009). There also has been demonstrated that a floating MFC may be used in marine environment (Huang et al., 2012) showing a potential to power meteorological buoy (Tender et al., 2008). The continuous mode of harvesting energy from the MFC has limited practical applications because the low energy level from the MFC harvested at a slow rate suggests that only devices consuming low power can be operated. To power real life devices that require higher energy to run the electrical energy has to be collected in a capacitor and then dispensed intermittently, in bursts of high power. This has been presented in MFC powered robots (Ieropoulos, Melhuish, & Greenman, 2003). It has been shown that if the size of the capacitor is selected properly, as well as the time of each cycle, harvesting energy through intermittent mode of operation is more efficient than continuous MFC energy harvesting under constant external load.
The proposed dynamic reconfiguration reducing the charging times by allowing storage of the energy in a shorter period of time (Papaharalabos et al., 2014).

Microbial Fuel Cell (MFC) stack has been designed to power a real application (floater-boat device) to demonstrate via MFCs the electricity harvesting directly from wastewater in working wastewater treatment plant (Fig.6.1). It has been assembled and launched in wastewater treatment plant in Nosedo, Milan on 23-27 September 2014. This work is performed in collaboration with Prof. Pierangela Cristiani (RSE- Ricerca Sistema Energetico-Sustainable Development and Energy Sources) and focuses on data monitoring (power output) and knowledge exchange between UWE and RSE.

![MFCs as part of the floater design for practical implementation into the wastewater treatment tank.](image)

6.4.3 Self-powered, artificial tree: chemical carbon capture MFC

Removing carbon dioxide directly from the atmosphere could help combat climate change. Carbon capture is one way of removal and it is performed by wet scrubbing. Scrubbing is a common name given to the unit operation known as gas absorption and plays an important role in pollution control. In this process, mass (for example: carbon
dioxide) is being transferred from the gas phase into a liquid phase for the purpose of material removal from the gas stream. The implementation of the process uses sodium hydroxide (NaOH) based, alkaline liquid sorbent to remove the CO₂ from the ambient air by producing dissolved carbonate ions.

A packed tower air scrubber, or trickling filter, is a reactor that has been filled with an inert or inorganic packing material. The packing material usually has a large porosity, or void volume, and a large specific area. Within a proposed MFC-based reactor the porous and large specific surface area is the cathode electrode that is directly exposed to ambient air (Fig 6.2). Moreover, the utilization of porous terracotta as a MFC casing and a membrane allows more efficient air permeation into the system and improved scrubbing efficiency.

Experimental testing would include the quantitative determination of components in solution containing Sodium Hydroxide, Sodium Carbonate and Sodium Hydrogen Carbonate by Warder Method where the solution has 3 bases: OH⁻, CO₃²⁻ and HCO₃⁻. Differences between their strengths are large enough so that there are three inflection points on the titration curve. These could help determine the concentration of NaOH in the solution as well as carbonates.
6.4.4 Electrochemically active solution produced by MFC/Catholyte as disinfectant

Electrochemically activated solutions (ECAS) have been shown to have a significant antimicrobial activity (Thorn et al., 2012). This is particularly important in the healthcare institutions, especially from the acquired resistance to antibiotics perspective. ECAS is produced via the electrolysis (electrochemical activation) of 10% sodium chloride aqueous solution (the electrolyte) in the electrochemical cell. As a result of applied current electrolyte is being transformed into highly reactive solutions of acids and oxidants (anolyte) as well as alkali and reductants (catholyte) are produced. The catholyte has the properties that may be used for flocculation and transformation of heavy metal ions into colloid substances of hydroxides.
The killing power of catholyte is best studied using a bioassay that uses bioluminescent reporters (E. coli - lux) to calculate kill rate of neat or diluted and/or pH-neutralised collection samples from cathode. Methods to look for the generation of hydroxyl radical, singlet-oxygen or superoxide can be performed by spin-trap chemistry and detection of measurable end products.

Preliminary test included the experiment where the catholyte produced by the energy producing MFCs has been analysed against the catholyte passively diffused in the open circuit conditions. The total viable count (TVC) of organisms in catholyte samples have been performed by conventional serial dilution method with surface spreading of 0.1 mL dilution samples on to nutrient agar petri dishes which were incubated aerobically in room temperature at 22 °C for 48 h. The observation of colonies shown the growth of far more variety of growth of specimens on samples obtained from open circuit conditions in comparison to current generating MFCs.

6.4.4.1 Preliminary results

Cathode electrode observation showed that in all MFCs that were electricity generating (working MFCs), the electrodes were visually clean of the biofilm, or the cathodic biofilm growth was limited. In the same time, the open circuit electrodes have shown the thick biofilm growth as presented earlier in Fig 5.40, section 5.5.2.3.2. Synthesised caustic solution can be used as microbial growth limiting factor or even a disinfectant therefore it may prevent biofouling of the membrane.

Total Viable Count has shown that the catholyte obtained from the working cells had the much lower colony numbers grown on the agar plates (Fig 6.4). The comparative growth shows visible microbial growth inhibition on catholyte from working MFC (Fig.6.3) in comparison to the catholyte obtained from open circuit conditions.
Figure 6.3 Catholyte samples from: Working and OCP conditions in serial dilutions cultivated on nutrient agar plates and using spread method.

<table>
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<tr>
<th>Sample Type</th>
<th>Sample</th>
<th>$1 \times 10^1$</th>
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<th>$1 \times 10^4$</th>
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<td>Working MFCs</td>
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<td>Open Circuit MFCs</td>
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Figure 6.4 Total Viable Count of colony forming units in 1mL of catholyte obtained from working tubular MFC (T working) and MFC in open circuit (T OCP).

Disinfecting catholyte has been previously reported in MFC fabricated using clayware separator (Jadhav, Ghadge & Ghangrekar, 2014) however with externally introduced
hyperchlorite. The further investigation might look into the ORR reaction. A significant number of electrons oxygen atoms and protons react with water to produce hydroxyl radical via the $\text{H}_2\text{O}_2$ production and pH increases. This process is induced by the current generation that starts off electro-osmosis and maintains the pH splitting function to provide alkaline conditions at the cathode for hydroxyl radical production. This will be a very attractive investigation into electrochemically produced disinfectant (ECAS) from wastewater.
6.4.5 Removal of pollutants from industrial wastewater, Fenton reaction

Heavy metal pollution is one of the biggest environmental challenges. They are a serious danger to natural world, the environmental diversity and public health because of their toxicity, non-biodegradability and bio-accumulation. MFC as relatively novel wastewater treatment method has been utilised to study the removal of chromium, copper and mercury (Wang, Huang & Zhang, 2008; Heijne et al., 2010; Wang, Lim & Choi, 2011). The removal is occurring in the anaerobic cathode chamber through cathode metal reduction, this is useful for their removal and recovery. This short section is aiming to propose the use of the Microbial fuel Cell that has the ability to extract catholyte from wastewater and use it in the purpose of toxic, heavy metal removal from wastewaters such as textile industry waste streams.

For example, metal ions such as \( \text{Fe}^{2+} \) may be used in electro-Fenton reactions (Agladze et al., 2007) in which oxygen accepts two electrons produced by microorganisms to form hydrogen peroxide together with \( \text{Fe}^{2+} \) present in the cathodic chamber. The reaction below, adopted from Agladze et al, 2007 show cathodic reduction of oxygen at gas-diffusion electrodes:

\[
\begin{align*}
\text{O}_2 + 2\text{H}_2\text{O} + 2\text{e}^- & \rightarrow \text{HO}^- + \text{OH}^- & \text{(alkaline solutions)} \\
\text{FeOOH} + 3\text{H}^+ & \rightarrow \text{Fe}^{3+} + 3\text{H}_2\text{O} \\
\text{Fe}^{3+} + \text{e}^- & \rightarrow \text{Fe}^{2+} \\
\text{Fe}^{2+} + \text{H}_2\text{O}_2 & \rightarrow \text{Fe}^{3+} + \text{OH}^- + \cdot\text{OH}
\end{align*}
\]

This may produce OH radicals in cathodic chamber to perform disinfection, remove contaminants but also generate electricity (Lu et al., 2015).
7 **List of Publications**

Some of the most significant findings from this project have been submitted to various conference events and scientific journals. A list of published articles as well as the ones submitted for review is shown below.

2011


2012


2013


2014


2015


8 References


9 **Appendices**

A. **H-type MFC**

The H type is the analytical type of the MFC design where large chamber volume proved useful for exploring the potential for degrading capabilities of MFCs. Usually used as a two chamber design, here it was used to explore the MPL as a membrane. The H-type MFC was constructed according to the following protocol:

**Introduction**

It has been reported by researchers that by removing the membrane in a fuel cell improved outputs can be reached. By removing the proton exchange membrane (PEM) and integrating anode and the cathode in the same chamber the internal resistance can be reduced and the MFC design simplified (Liu and Logan, 2004). To address this investigation and to compare the results, a large H-type of a fuel cell was assembled as shown on Figure 9.1.

**Methods specific to the experiment**

The anodic chamber was containing anolyte- sludge and acetate, the electrode used was carbon veil of the total surface area 3000 cm² and the chamber was filled with 200 mL of anolyte. The cathode was sealed on the lower part of the MFC with an inner side (MPL) facing the solution and the outer side (GDL) facing to the air.

Before use the MFCs were autoclaved and stoppers rinsed in ethanol. A MFC consisted of media bottle connected with glass tube. Clamped between the flattened tube ends was a circular piece of MPL which was sandwiched between two neoprene-rubber gaskets. MPL was here tested as a Membrane-Electrode Assembly (MEA). Anode was constructed using carbon veil folded to fit into each glass tube and pierced with a piece
of nickel-chrome wire approximately 15 cm in length. The wire was fed out through an opening at the top of the chamber and then sealed with silicon sealant.

Figure 9.1 H-type fuel cell (left), MPL as cathode/membrane assembly (centre), H-type fuel cell after 2 months of operation (right).

After 2 months of operation the anolyte turned visibly green and the performance of the H type MFC did not improve even after additions of 20 mL of sludge +0.1M acetate as feed. The power curve can be compared with the actual power production of the standard two chamber fuel cell and the maximum power point is in the range of 10-5 times lower than our tested design (Figure 9.2).
This experiment was set up in order to compare to previous membrane-less, standard MFC where in the same manner MPL was used as membrane electrode assembly (as shown in Chapter 5.1) which suffered from anolyte leakage. The H type fuel cell did not leak and it is probably due to the much smaller MPL membrane used. Performance of H-type MFC has been at least 10 fold lower than standard rectangular MFC design.

**Conclusions**

The conclusion from this experiment is that despite the larger anode surface area and bigger volume of the MFC the lack of membrane between the anode and cathode did not improve the performance of the H-type MFC. It might be due to the specific surface area as well as the local properties of the handmade MPL material which would be responsible for leakages in the previous membrane electrode assembly in standard MFC.

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*Figure 9.2 Actual power [µW] and voltage [mV] of the large type fuel cell*
B. **Biomass Assessment: Algal cell count calibration**

![Figure 9.3 Direct count using a calibration for the various experimental conditions used.](image)

**Figure 9.3** Direct count using a calibration for the various experimental conditions used.

![Figure 9.4 Optical density calibration for the various experimental conditions used.](image)

**Figure 9.4** Optical density calibration for the various experimental conditions used.
Figure 9.5 Dry mass assessment calibration for the various experimental conditions used.
C. **Potassium recovery in Microbial Fuel Cell system**

This section has been a part of presented paper at the 226 ECS Meeting in Cancun, Mexico on 7th October 2014.


Analogous to the sodium acetate consumption resulting in driving energy to transport \( \text{Na}^+ \) to the cathode, the feedstock has been changed to potassium acetate. It is therefore suspected that in the same MFC salts formed on the cathode will be potassium carbonates/bicarbonates (Figure 9.6A).

![Image of MFC operation and catholyte production using wastewater and potassium acetate](image)

*Figure 9.6. MFC operation and catholyte production using wastewater and potassium acetate.*
The power performance has been monitored and the synthesis of clear catholyte has been detected (Figure 9.6 B). Figure 9.7A shows the power performance data where each MFC has been connected to 300 Ω external resistor. The average data show that the best performing AC was producing 220 µW, MPL 144 µW Veil MPL 152 µW and Veil 105 µW on average, during 18 day operation (Figure 9.7A). All MFCs have produced catholyte that has been collected in the syringe attached to the cathode half cell (Figure 9.6) and shown that the most catholyte has been collected from AC 20.85 mL, MPL 16.7, Veil MPL 15.5mL and Veil 14.6. The amount of the collected liquid is corresponding to the produced power as shown in Figure 9.7B. Plotting the catholyte against the power generation shows the linear relation of the two factors as shown in Figure 9.7C. As suggested previously, catholyte formed is due to the electrosmosis where cations are dragging water molecules across the CEM membrane.

![Image](image.png)

**Figure 9.7** Power performance over 18 day period of MFCs (A) and the amount of formed catholyte in the half-cell under various cathode materials (B) and the correlation of power to volume of formed liquid (C).
The dripping design allows the cathode to avoid flooding and collect the catholyte so the diffusion of hydroxide ions back from cathode to the anode at such high caustic strength may be prevented. High strength salt solutions (externally supplied) are used in Forward Osmosis (FO) systems (McCutcheon, McGinnis & Elimelech, 2005) where FO may be incorporated in Osmotic MFCs (OsMFCs) to draw and recover water from the anode (Zhang, Brastad & He, 2011). Here, the MFCs had not been supplied externally by adding draw solution in the cathode and water has been successfully recovered as the catholyte. It has been demonstrated that the carbonates may be successfully used as draw solutions in OsMFC therefore an MFC that is able to synthesize its own draw solution is particularly attractive.

*Figure 9.8 EDX shows atomic content (%) in crystalized salt obtained from all samples (left) and the pH and conductivity analysis of the catholyte samples (right).*
Results showed that all Pt-free cathodes used in this study including plain carbon veil, activated carbon, and microporous layer (MPL) applied on both carbon cloth and carbon veil generated electric current with simultaneous catholyte generation in the cathode chamber. During MFC operation, the production of catholyte on the surface of the cathode electrode was a direct result of electricity generation, and power output has been correlated with catholyte volume. Moreover, catholyte showed the pH values of 13.20 to 13.78 and increased conductivity, showed the catholyte to be strongly basic with a high salt concentration (Figure 9.8, right). Previously, the same MFC system has been supplemented with real wastewater with sodium acetate and shown sodium recovery on the cathode in the form of sodium carbonate/bicarbonate salts (Gajda et al., 2014). Here, when the anode feedstock was changed to potassium acetate, KOH was formed on the cathode half-cell with further crystallization of potassium carbonate salts. To evaluate this liquid samples were left to evaporate showing formed crystals of the solid salts which shown to have hygroscopic properties. It shown to be soluble.
in water and insoluble in ethanol. Phase composition of the salt was studied by through EDX showed the dominant atomic content of potassium and trace elements such as sodium and silicon in all tested samples as seen in Figure 9.8. XRD analysis shown that the salt precipitants shown crystalline structure (Figure 9.9) and the pH of the formed catholyte (>13) and conductivity, showed gradual increase with current generation. It was then suspected that the MFC system fed with real wastewater supplemented with potassium acetate, KOH was formed on the cathode half-cell with additional crystallization of potassium carbonate salts. Crystals of the solid salts shown to be hygroscopic and further XRD powder diffraction pattern analysis showed (Fig 9.9) this salt shown good comparison of the diffraction pattern mineral phase kalicinite (potassium bicarbonate). Potassium carbonate has an excellent CO₂ removal properties (Kothandaraman et al., 2009) to form potassium bicarbonate (kalicinite) that precipitated from the solution (Fig 9.9 inset). Catholyte conductivity values suggest high concentration of salts in the solution and its possible use as a draw solution for OsMFCs. Preparing the draw solution and generating the feed solution were examined previously in the MEC (Qin & He, 2014) showing the possibility of using self-synthesized draw solutions in bioelectrical systems (Gajda et al., 2015).

Hydroxide ions, which are known as one of the most reactive aqueous radical species, have strong attractions towards dispersed particles as well as counter ions to cause coagulation. OH- can also be used in electrocoagulation which is a rapidly developing method for contaminant removal in waste streams (Mollah et al., 2004). Electrochemical activation process of passing a diluted saline solution through the electrochemical reactors showing antimicrobial properties of acidic Electrochemically Active Solution (ECAS) have been widely reported (Robinson et al., 2010); alkaline catholyte with a pH between 11 and 13 and can be used for flocculation (e.g. of heavy
metals) and precipitation or coagulation or mineral-crystal formation (e.g. struvite, trona, kalicinate).

Potassium alkaline sorbent supported on the activated carbon (K$_2$CO$_3$/AC) has been shown more effective than sodium carbonate sorbents (Lee et al., 2006). This proof-of-concept system encourages further studies of system optimization with examination of actual wastewaters, including urine. Urine contains around 80% of the total nitrogen (N), 70% of the potassium (K) and up to 50% of the total phosphate (P) loads in municipal wastewater (Larsen & Gujer, 1996) and has been demonstrated as a good substrate for MFC operation and recovery (Ieropoulos, Greenman & Melhuish, 2012; Kuntke et al., 2012). These results have successfully shown the feasibility of coupling an electricity producing MFC with potassium-recovery in a form of caustic potash as a sorbent for carbon capture. This provides a sustainable source of alkaline draw solute in form of kalicinite that would be extracted from wastewater directly by MFC operation, without need for any electrical input, nor salt in the system whilst still generating electricity rather than requiring an exogenous source. Caustic potash (KOH) can be generated in situ from sewage using a divided MFC electrochemical cell, which avoids the need for transport, handling and storage of concentrated caustic solutions.

**Conclusions**

This approach leads to carbon capture through wet caustic scrubbing on the cathode, which locks the carbon dioxide into carbonate salts. Electricity is generated throughout.
D. Flat Plate MFC made of terracotta floor tile

High cost and low power performance are limiting MFCs from becoming economically viable. To address the high cost and to improve the performance, a flat plate MFC configuration using passive air-breathing cathode and a three dimensional anode was introduced. The design was inspired by the chemical fuel cells that operate as flat plates. The development of in house activated carbon cathodes made this method advantageous for another reason, it may be made to size and applied to larger membranes of the microbial fuel cell area. This investigation was to explore the use of larger membrane area materials for the utilisation into the MFC system. A terracotta floor tile (Topps Tiles, UK) of the dimensions 30 cm (W) by 30 cm (L) and thickness 3 cm was assembled with 1000 cm$^2$ carbon veil anode that was folded and pierced with Ni-Cr wire. The activated carbon cathode (176 cm$^2$) was prepared as previously described in section 2.2.5. and attached to the opposite side of the tile.

Figure 9.10 MFC made out of terracotta floor tile placed in the bucket filled with wastewater (left) and the same MFC directly energizing the windmill motor (right).

Figure 9.10 shows a MFC set up wit a single flat plate tile producing enough electrical power to operate small DC motor (windmill). This area is very exciting as it presents a very simple and low maintenance system where the anode and the cathode share the
same electrolyte taking the advantage of their different characteristics. The anode is submerged in the liquid while the cathode is partially exposed to air. It shows a promising new route to scaling up through multiplication (Figure 9.11) by connecting multiple plate MFCs in parallel configuration.

Figure 9.11 Scaled up MFC system containing six MFC plates immersed in the anolyte.
E. **Algae monocultures in cathode half-cell**

The monocultures of algae and cyanobacteria have been chosen to explore the activity of the single culture on the rate of ORR in the cathodic environment and its suitability for MFC operation. MFCs were assembled as shown previously in Chapter 4 however instead of mixed culture, the monocultures were used in 4 different experimental groups and an aseptic environment was maintained.

- TAP medium for green algae cultures such as *Chlamydomonas reinhardii* and *Chlorella pyrenoidosa* was prepared by making the following stock solutions (Andersen, 2005):

1. TAP salts (in 1000mL)
   - NH4Cl 15.0 g
   - MgSO4 . 7H2O 4.0 g
   - CaCl2 . 2H2O 2.0 g

2. Phosphate solution (in 100mL)
   - K2HPO4 28.8 g
   - KH2PO4 14.4 g

3. Hutner's trace elements (in 1000mL)
   - EDTA-Na2 50.0 g
   - H3BO3 11.14 g
   - ZnSO4 • 7H2O 22.0 g
   - MnCl2 • 4H2O 5.1 g
   - FeSO4 • 7H2O 5.0 g
   - CoCl2 • 6H2O 1.6 g
   - CuSO4 • 5H2O 1.6 g
   - (NH4)6Mo7O24 • 4H2O 1.1 g

To make the final medium the following: 2.42 g Tris, 25 ml solution no.1 (salts) 0.375 ml solution no. 2 (phosphate) and 1.0 ml solution no. 3 (trace elements) have been mixed, adjusting the pH 7.2 with glacial acetic acid, and adding water to 1 litre.

- BG 11 Medium has been used for cultivation of cyanobacteria (Barsanti & Gualtieri, 2005): *Anabaena cylindrica* and *Synechococcus leopoliensis* and prepared as follows:
Stock solutions prepared dissolving given component in 1000 mL of deionised water:

1. NaNO$_3$ 1.5 g
2. K$_2$HPO$_4$ 0.04 g
3. MgSO$_4$ 7H$_2$O 0.075 g
4. CaCl$_2$ 2H$_2$O 0.036 g
5. Citric Acid 6.0 mg
6. Ferric ammonium citrate 6.0 mg
7. EDTA 1.0 mg
8. Na$_2$CO$_3$ 0.2 g
9. Microelement Stock solution 1 mL

Microelement Stock solution:

- H$_3$BO$_3$ 2.86 g
- MnCl$_2$ 4H$_2$O 1.81 g
- ZnSO$_4$ 7H$_2$O 0.222 g
- Na$_2$MoO$_4$ 2H$_2$O 0.39 g
- CuSO$_4$ 5H$_2$O 0.079 g
- Co(NO$_3$)$_2$ 6H$_2$O 49.4 mg

- Distilled Water 1.0 L

100 mL of stock solution 1 have been added to 10 mL each of stock solutions 2 - 8 and 1 mL of stock solution 9. The final solution has been made up to 1 litre with deionized water and pH was adjusted to 7.1 with glacial acetic acid or NaOH.

Results

The monocultures of 4 different photosynthetic species were left to establish in the cathodic half-cell under 1 kΩ external load, and only when the chambers were visibly green the polarisation curve experiments were performed. Figure 9.12 shows power curves performed under 4 different photo-cathode conditions. These results suggest that the most suitable biocatalytic activity is the monoculture of cyanobactrium *Anabaena cylindrica*. It might be due to the filamentous structure of the culture that may have anchored to the matrix of the electrode as previously suggested (Walter, Greenman & Ieropoulos, 2013). The high maintenance of a cathode with single species requires constant supply of feedstock media and maintaining sterile conditions therefore it is not suitable for use in practical demonstrations. The results however bring new information about the development of cyanobacteria as active oxygenators in the cathode and require further investigation in the future.
Figure 9.12 Power curves obtained from MFCs operated with selected monoculture of algae and cyanobacteria as the biocatalyst in the cathode.