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Mulaudzi, Vusani

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UNIVERSITY OF THE
WITWATERSRAND,
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ANALYSIS OF MICROBIAL FUEL CELLS FOR THE TREATMENT OF SELECTED INDUSTRIAL WASTEWATER EFFLUENTS (DOMESTIC AND BREWERY) AND GENERATION OF ELECTRICITY

PhD

Vusani Mulaudzi (812626)

A thesis submitted to the Faculty of Engineering and the Built Environment, University of the Witwatersrand, Johannesburg, in fulfillment of the requirements for the Degree of Doctor of Philosophy.

Supervisor:
Prof. Jean Mulopo

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Keywords

Microbial fuel cells; wasterwater; electricity; external resistance, biofilm

Abstract

The goal of this study was to evaluate the technical potential of Microbial Fuel Cells (MFCs) for harvesting bio-electricity from municipal and hospital wastewaters, as well as the effects of key parameters like biofilm growth rate, substrate dilution, and catalyst addition on COD removal efficiency and electricity generation in both open and closed circuit Microbial Fuel Cells. The study looked at MFC performance for organic matter removal from brewery and domestic wastewater as a function of biofilm growth rate at MFC start-up for open circuit and closed circuit in a double chamber MFC to investigate biofilm growth rate and its effect on MFC performance. In the second experiment, the impact of biofilm formation and optimum acclimation time in an MFC fed with both diluted and undiluted domestic and brewery wastewaters was investigated. The third and fourth experimental works synthesized and examined the electrocatalytic behavior of MnO_2/rGO as an oxygen reduction reaction catalyst in MFC applications for power generation in the treatment of selected industrial wastewaters. The analysis of the biofilm growth rate and biofilm growth constant revealed new information about substrate degradation pathways and microbial interactions. The addition of sludge to beer brewery wastewater reduces electron transfer ability at low external resistance, and external resistance was found to be highly proportional to anode colonization during biofilm production, as well as other MFC parameters such as current generation, microbial activity, biofilm growth rate, and power generated. The COD of the effluent was also evaluated as a function of external resistance, and it was discovered that as external resistance was increased, the COD of the effluent decreased, meaning that the substrate consumed by microorganisms increased. When the external resistance was increased, the MFC's remedial capacity was also increased. Finally, the results indicate that the low activity of the MnO_2/rGO catalyst in domestic wastewater fed MFCs may be attributed to catalyst toxicity, which reduces catalyst activity. Variations in bacterial yield or the presence of electron acceptors may also explain the lower performance of the catalyst coated cathode MFC in domestic wastewater fed MFCs. The MnO_2/rGO catalyst demonstrated higher ORR activity than plain cathode MFCs in brewery wastewater, making it more appropriate for brewery wastewater treatment rather than domestic wastewater treatment.

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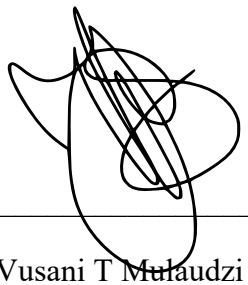
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List of Abbreviations

AC	Activated carbon
AEM	Anion-exchange membranes
ARB	Anode-respiring bacteria
BES	Bioelectrochemical system
BET	Brunauer-Emmett-Teller
BOD	Biochemical oxygen demand
CE	Coulombic efficiency
CEM	Cation exchange membranes
CLSM	Confocal laser scanning microscopy
COD	Chemical oxygen demand
CV	Cyclic voltammetry
DAMFC	Dual anode microbial fuel cell
DoE	Department of Energy
EAB	Electroactive bacteria
EIS	Electrochemical impedance spectroscopy
EPS	Extracellular polymeric substance
GCE	Glass carbon electrode
GNS	Graphene nanosheets
GO	Graphene oxide
MB	Methylene blue
MFC	Microbial fuel cell
MPPT	Maximum Power Point Tracking
ORR	Oxygen reduction reaction
PEM	Proton exchange membranes
PGM	Platinum group non-metals
PT	Polarization tests
RDDE	Ring disk electrode
SCFA	Short chain fatty acids
SCMFC	Single chamber microbial fuel cells
SEBS	Styrene-ethylene-butylene-styrene
SEM	Scanning electron microscopy
SHE	Standard hydrogen electrode
SMFC	Sediment microbial fuel cell
TOC	Total organic carbon
XRD	X-ray diffraction

Declaration

I declare that this thesis is my own, unaided except where otherwise acknowledged. It is being submitted for the Degree of Doctor of Philosophy to the University of the Witwatersrand, Johannesburg. It has not been submitted before for any degree or examination to any other University.



Vusani T Mulaudzi

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Chapter 1: Introduction

In today's world, economic and social sustainability is linked to transforming and adapting human behaviors and practices to future demands. Meanwhile, in South Africa, one of the most important economic and social driving factors, the water market, is currently at its most vulnerable due to increased water demand from growing human populations and industrial activities (Department of Water and Sanitation, South Africa, 2019). South Africa's water resource profile is currently under stress due to low water levels in major water supply sources, as previously stated (Strategic Overview of the Water Services Sector in South Africa, 2015). The study also describes South Africa's water supply profile as scarce, owing to erratic rainfall of 450 mm per year, compared to a global average of 860 mm per year. Inadequate infrastructure maintenance and investment in water systems, as well as recurrent drought and declining water quality, have been described as major contributors to South Africa's water crisis (Department of Water and Sanitation, South Africa, 2015). Since only 56% of wastewater treatment facilities are operating, 35% of households do not have access to clean drinking water, and only 63% have reliable water supply service. However, water security has remained steady at 98%, and annual water demand is expected to be 15 billion m^3, with 68% of the water supply coming from surface water, 13% from groundwater, 13% from return flows, and 6% from other sources including desalination.

Table 1.1 South Africa's annual water usage (*Source: Strategic overview of the water services sector in South Africa, DWA,2015*)

Sector	Usage (%)
Agriculture	62
Municipal	27
Energy and Afforestation	5
Mining and Industrial	6

There is a need to develop effective rainwater harvesting mechanisms while also intensifying wastewater recycling and remediation (e.g. effective sanitation and controlled effluent discharges). This would contribute to pollution control and increased water security.

1.1 WASTEWATER RECYCLING AND REMEDIATION

Some of the previous studies report exorbitant costs for pollutant removal in wastewater treatment as one of the major challenges in wastewater recycling and remediation, e.g. the USA alone needs 3-4% of its total annual electrical load each year to sustain its wastewater treatment capacity (McCarty et al., 2011; Wang et al., 2008). Some industrial operations have been documented to generate and discharge massive quantities of effluents on a yearly basis, for example, a typical beer brewery plant produces 3 to 5 litres of waste effluent per litre of beer generated, while requiring 15 litres of water to produce 1 litre of beer (Parawira et al., 2005; Braeken et al., 2004; Koroglu et al., 2014; Winkler, 2005). Other studies also shown that existing wastewater treatment costs are unsustainable due to units like the aeration unit, whose maintenance costs are over half of the plant's operating costs; for example, the United States processes over 126 billion litres of wastewater every day at a cost of over $25 billion a year (Liu et al., 2004). Energy is also a key driver in South Africa's wastewater treatment sector; for example, in the City of Johannesburg alone, electricity costs in wastewater treatment were projected to reach R300 million per year by 2020, up from R97 million per year, which if not met could have devastating consequences for the city's economy, environment, health services, and social activities (Water Research Commission, South Africa, 2018). Global energy security and environmental concerns are justifiable due to the dependence on limited sources such as fossils which face foresable depletion as well as their environmental unfriendly nature of CO_2 emission to the environment (Wen et al., 2009). A study findings report the global energy demand rising to 722 quadrillion BTUs by 2030 (Khanal, 2011) in addition to the 0.5-2 kWh/m^3 demand from the current typical wastewater treatment processes (Gude et al., 2015 (a)). Sludge treatment methods have been reported to use 21 billion kWh of electricity annually in the United States, with pumping and aeration units accounting for 21% and 30-55 percent of total treatment energy demand, respectively, while the United Kingdom has been reported to spend 3-5% of its annual national total electricity demand on wastewater treatment (Oh et al., 2010; DTI, 2005). The South African government recently released a survey outlines of the Department of Energy's (DoE) Integrated Resource Plan of 2010, which indicates that South Africa needs to increase

its capacity by 45000 MW by 2030 excluding Eskom's present capacity expansion projects (Department of Energy, South Africa 2018).

Previous research results have shown that energy can be found in wastewater in three forms: (i) organic matter (1.79kWh/m^3), (ii) nutritional elements such as nitrogen and phosphorus (0.7kWh/m^3), and (iii) thermal energy (7kWh/m^3) (McCarty et al., 2011). Furthermore, this energy in wastewater can be further classified as chemical energy (26%), available as carbon (measured as COD) and nutrients compounds (Nitrogen and Phosphorus) as well as thermal energy which holds approximately 74% of the energy potential (Gude, 2016). It was also discovered that, while chemical energy can be easily harvested, thermal energy can only be harvested using a heat pump and is subject to the temperature of the wastewater source (Gude, 2016). Burton et al., (2009) reported that the equivalent of 7% of Eskom's energy is available in South Africa's wastewaters (i.e. approximately 10 GWth of energy). A 2009 survey by the South African Water Research Commission on the quality and quantity of wastewaters listed the following three sectors as possible energy recovery sources: formal and informal animal husbandry (cows, pigs, and chickens); fruit and beverage industries (distillery, brewery, winery, fruit juicing and canning); and domestic backwater sewage (Water Research Commission, South Africa, 2018). The examples of South Africa's wastewaters and their future usable energy are shown in Table 1.2.

Table 1.2: Energy potential of various wastewaters produced in South Africa (Source: www.ee.co.za/wpcontent/uploads/legacy/Energize_2013/09_AT_03_Electricity.pdf)

Source		**Energy Potential (MWth)**
Domestic Wastewater		500-800
Animal Husbandry	Cattle	80-125
	Rural Cattle	1200-3500
	Piggeries	120
	Poultry Farms	20-70
	Abattoirs	1-55
Petrochemical		50
Fruit Production		70
Wineries and Distillers		73
Breweries		20
Textile Industries		25
Pulp and Paper		50-100

Furthermore, as a result of stringent environmental legislation and rising demand for water and sanitation, South Africa's energy consumption rises as a result of wastewater treatment. Recycling energy from usable energy in wastewater can be used to turn South Africa's water sector to low energy consumption. A wastewater treatment technology that is energy self-sufficient will provide a novel solution to the wastewater treatment and energy sectors. In this case, wastewater treatment plants are converted from energy consumers to power generators. Wastewaters are estimated to contain 3-10 times more energy than the energy needed to treat them (Gude, 2015(a); Logan, 2004). Wastewater is regarded as a valuable energy resource, with a capacity for energy harvesting of 3.8kWh/g COD (McCarty et al., 2011). However, potential methods for extracting energy from wastewater, such as methane recovery through anaerobic digestion, are said to be environmentally hazardous due to the possibility of inefficient methane gas collection, which could result in methane release into the atmosphere (Smith et al., 2013). Global climate change, as well as water and sanitation issues, have highlighted the urgent need to implement energy-efficient and environmentally sustainable wastewater treatment methods. Harvesting value-added products such as energy from wasteawater offers environmental as well as human

health and survival benefits as it contributes to water conservation, climate change mitigation, water pollution control and green energy production.

Even though investing in public wastewater treatment plants is important, it is difficult because they produce no revenue. According to reports, the United States spends about $2 billion a year on domestic wastewater treatment and needs another $300 billion to expand publicly owned treatment facilities (USEPA, 2008). A standard domestic wastewater treatment plant is said to use 0.6kWh of energy per m^3 of wastewater treated using aerobic activated sludge treatment and anaerobic sludge digestion technology (McCarty et al., 2011). Other systems, such as lagoons, use 0.09 to 0.05kWh of energy per m^3 of wastewater, trickling filters use 0.18 to 0.42kWh of energy per m^3, activated sludge uses 0.33 to 0.60kWh of energy per m^3, and advanced wastewater treatment uses 0.31 to 0.40kWh of energy per m^3 of treated wastewater (Logan, 2008). In comparison to traditional systems, other studies have shown that direct conversion of waste into renewable power, high value oil, or chemical products is a viable energy supply alternative because it avoids additional waste sources and energy challenges (Logan, 2008; Logan and Rabaey,2012). With global problems such as fossil-fuel depletion, deforestation, water scarcity, and other valuable resource shortages, practices such as ‘valuable resource recovery from pollutant removal processes,' which results in minimum energy usage and maximum energy recovery, are required (Gude, 2016).

Biological processes, such as the Bioelectrochemical method (BES), which can transform chemical energy (in the form of organic substances in wastewater) into electrical energy or other high-value products (Kumar et al., 2012), provide a novel and fascinating alternative. In microbial fuel cells (MFCs), BES uses indigenous exoelectrogenic bacteria to extract renewable energy from waste organic sources in the form of bio-electricity (Lovley, 2006). As a result, microbial fuel cells (MFCs) are seen as a promising renewable energy technology that can remove pollutants while still producing electricity in wastewater treatment processes (Qiao et al., 2010; Kim et al., 1999; Chaudhuri and Lovley, 2003; Rabaey et al., 2003; Liu et al., 2004; Min and Logan, 2004). And it's because of this one-of-a-kind capability that they continue to pique the attention of researchers working on self-sufficient energy systems for wastewater treatment (Feng et al., 2008).

1.2 PROBLEM STATEMENT

Though there have been numerous successful investigative study findings on microbial fuel cells (MFCs) for simultaneously treating wastewater effluents and generating electricity (Min and Angelidaki, 2008; Rozendal et al., 2006; You et al., 2008; Venkata-Mohan et al., 2008a, b; Kargi and Eker, 2007), there have been very few on harvesting electricity from real wastewater effluents. As a result, the majority of published studies (over 5000 scientific papers performed in the lab) used synthetic wastewaters based on glucose, acetate, sucrose composition, and other factors that do not reflect the composition or diversity of real wastewaters (WOS 2016; Menicucci et al., 2006; Freguia et al., 2007; Kargi and Eker, 2007; Liu and Li, 2007; Venkata-Mohan et al., 2008b; You et al., 2008; etc.). As a consequence, the restricted availability of qualitative data on wastewater remediation and electricity generation in microbial fuel cell (MFC) technology from real industrial effluents is of particular concern (WOS 2016; Menicucci et al.,2006; Freguia et al.,2007; Kargi and Eker,2007; Liu and Li, 2007; Venkata-Mohan et al.,2008b; You et al.,2008; etc.). Furthermore, little consideration has been given to using microbial fuel cells in their current state for other useful purposes, such as incorporation into existing wastewater treatment systems and the manufacture of high-value products.

1.3 RESEARCH QUESTIONS

Several key knowledge areas still need further interrogation and investigations in order to determine if microbial fuel cells can be economically and socially beneficial as well as environmentally sustainable. Some of these knowledge gaps will be addressed in this research, viz:

- Is it feasible to develop a MFC for South Africa's wastewater remediation in terms of treatment efficiency (COD removal) while harvesting sufficient energy?
- What are the areas of MFC optimization for effective real wastewater treatment purposes?

- What is the potential of MFC for other beneficial and value added purposes such as sanitation in remote areas?

1.4 RESEARCH AIM AND OBJECTIVES

The aim of this research is to look into harvesting electricity from actual industrial wastewater using microbial fuel cell technology. The efficiency of COD removal and electricity generation by an MFC from real wastewater are central to this goal (Domestic and Brewery wastewaters). As a result, the following objectives are crucial to this research:

- To assess the potential (techno-economic) of MFCs that can treat real wastewater (to minimize additional costs of diluents and to minimize risk of adding toxins).
- To identify and optimize the influential parameters in order to harvest sufficient electricity from real wastewaters (this would simplify the treatment process as real wastewaters are readily available).

1.5 THESIS STATEMENT

Earlier studies have successfully examined beer brewery and domestic wastewaters for electricity generation in MFCs using wastewaters diluted by glucose and sucrose with fixed pH of 6, 7 and 8. Research findings also show insignificant power output from MFCs and in this regard, this thesis will further investigate the feasibility of using MFCs primarily for COD removal. The success of such an investigation would reveal if MFCs can i) be cost-effective as compared to aeration procedures when treating wastewaters, ii), satisfy COD removal standards, and iii) offer solution to pollution solution in relation to sanitation problems.

1.6 REFERENCES

Strategic overview of the water services sector in South Africa, 2015. http://www.dwaf.gov.za/Downloads/WS/Macro_Planning_Products/STRATEGIC%20OVERVIEW%20OF%20WATER%20SERVICES/STRATEGIC%20OVERVIEW%20OF%20THE%20WATER%20SERVICES%202015%20(A6%20Booklet).pdf

McCarty, P.L., Bae, J. and Kim, J., 2011. Domestic wastewater treatment as a net energy producer–can this be achieved?

Winkler,H.,2005. Renewable energy policy in South Africa: policy options for renewable electricity. Energy policy,33(27-38).

Parawira, W., Kudita, I., Nyandoroh, M.G. and Zvauya, R., 2005. A study of industrial anaerobic treatment of opaque beer brewery wastewater in a tropical climate using a full-scale UASB reactor seeded with activated sludge. Process Biochemistry, 40(2), pp.593-599.

Braeken, L., Van der Bruggen, B. and Vandecasteele, C., 2004. Regeneration of brewery waste water using nanofiltration. Water research, 38(13), pp.3075-3082.

Köroğlu, E.O., Özkaya, B., Denktaş, C. and Çakmakci, M., 2014. Electricity generating capacity and performance deterioration of a microbial fuel cell fed with beer brewery wastewater. Journal of bioscience and bioengineering, 118(6), pp.672-678.

Liu, H., Ramnarayanan, R. and Logan, B.E., 2004. Production of electricity during wastewater treatment using a single chamber microbial fuel cell. Environmental science & technology, 38(7), pp.2281-2285.

Gude, V.G., 2016. Wastewater treatment in microbial fuel cells–an overview. Journal of Cleaner Production, 122, pp.287-307.

Chaudhuri, S.K. and Lovley, D.R., 2003. Electricity generation by direct oxidation of glucose in mediator-less microbial fuel cells. Nature biotechnology, 21(10), p.1229.

Kim, B.H., Kim, H.J., Hyun, M.S. and Park, D.H., 1999. Direct electrode reaction of Fe (III)-reducing bacterium, Shewanella putrefaciens. Journal of Microbiology and Biotechnology, 9, pp.127-131.

Qiao, Y., Bao, S.J. and Li, C.M., 2010. Electrocatalysis in microbial fuel cells—from electrode material to direct electrochemistry. Energy & Environmental Science, 3(5), pp.544-553.

Gude, V.G., 2015. Energy and water autarky of wastewater treatment and power generation systems. Renewable and Sustainable Energy Reviews, 45, pp.52-68.

Oh, S.T., Kim, J.R., Premier, G.C., Lee, T.H., Kim, C. and Sloan, W.T., 2010. Sustainable wastewater treatment: how might microbial fuel cells contribute. Biotechnology advances, 28(6), pp.871-881.

DTI, 2005. DTI Regional Electricity Consumption Statistics, UK

Khanal, S.K., 2011. Anaerobic biotechnology for bioenergy production: principles and applications. John Wiley & Sons.

Wang, S.G., Liu, X.W., Gong, W.X., Gao, B.Y., Zhang, D.H. and Yu, H.Q., 2007. Aerobic granulation with brewery wastewater in a sequencing batch reactor. Bioresource Technology, 98(11), pp.2142-2147.

Ince, B.K., Ince, O., Sallis, P.J. and Anderson, G.K., 2000. Inert COD production in a membrane anaerobic reactor treating brewery wastewater. Water research, 34(16), pp.3943-3948.

Kida, K., Tanemura, K., Sonoda, Y. and Hikami, S., 1994. Anaerobic treatment of distillery wastewater from barley-shochu making by UASB. Journal of fermentation and bioengineering, 77(1), pp.90-93.

Burton, S., Cohen, B., Harrison, S., Pather-Elias, S., Stafford, W., Van Hille, R. and Von Blottnitz, H., 2009. Energy from Wastewater-a Feasibility study. WRC, South Africa

USEPA, 2012 USEPA Inventory of US Greenhouse Gas Emissions and Sinks: 1990–2010

Visvanathan, C. and Abeynayaka, A., 2012. Developments and future potentials of anaerobic membrane bioreactors (AnMBRs). Membr. Water Treat, 3(1), pp.1-23.

Logan, B.E., 2004. Peer reviewed: extracting hydrogen and electricity from renewable resources.

Logan, B.E., 2008. Microbial fuel cells. John Wiley & Sons.

Logan, B.E. and Rabaey, K., 2012. Conversion of wastes into bio-electricity and chemicals by using microbial electrochemical technologies. Science, 337(6095), pp.686-690.

Kumar, A.K., Reddy, M.V., Chandrasekhar, K., Srikanth, S. and Mohan, S.V., 2012. Endocrine disruptive estrogens role in electron transfer: bioelectrochemical remediation with microbial mediated electrogenesis. Bioresource technology, 104, pp.547-556.

Min, B. and Angelidaki, I., 2008. Innovative microbial fuel cell for electricity production from anaerobic reactors. Journal of Power Sources, 180(1), pp.641-647.

Rozendal, R.A., Hamelers, H.V. and Buisman, C.J., 2006. Effects of membrane cation transport on pH and microbial fuel cell performance. Environmental science & technology, 40(17), pp.5206-5211.

Mohan, S.V., Saravanan, R., Raghavulu, S.V., Mohanakrishna, G. and Sarma, P.N., 2008. Bio-electricity production from wastewater treatment in dual chambered microbial fuel cell (MFC) using selectively enriched mixed microflora: effect of catholyte. Bioresource Technology, 99(3), pp.596-603.

Kargi, F. and Eker, S., 2007. Electricity generation with simultaneous wastewater treatment by a microbial fuel cell (MFC) with Cu and Cu–Au electrodes. Journal of Chemical Technology and Biotechnology, 82(7), pp.658-662.

Menicucci, J., Beyenal, H., Marsili, E., Veluchamy, R.A., Demir, G. and Lewandowski, Z., 2006. Procedure for determining maximum sustainable power generated by microbial fuel cells. Environmental science & technology, 40(3), pp.1062-1068.

Freguia, S., Rabaey, K., Yuan, Z. and Keller, J., 2007. Electron and carbon balances in microbial fuel cells reveal temporary bacterial storage behavior during electricity generation. Environmental science & technology, 41(8), pp.2915-2921.

Liu, Z.D. and Li, H.R., 2007. Effects of bio-and abio-factors on electricity production in a mediator-less microbial fuel cell. Biochemical Engineering Journal, 36(3), pp.209-214.

You, S., Zhao, Q., Zhang, J., Liu, H., Jiang, J. and Zhao, S., 2008. Increased sustainable electricity generation in up-flow air-cathode microbial fuel cells. Biosensors and Bioelectronics, 23(7), pp.1157-1160.

Liu, H. and Logan, B.E., 2004. Electricity generation using an air-cathode single chamber microbial fuel cell in the presence and absence of a proton exchange membrane. Environmental science & technology, 38(14), pp.4040-4046.

You, S.J., Zhao, Q.L., Jiang, J.Q., Zhang, J.N. and Zhao, S.Q., 2006. Sustainable approach for leachate treatment: electricity generation in microbial fuel cell. Journal of Environmental Science and Health Part A, 41(12), pp.2721-2734.

Smith, A.L., Skerlos, S.J. and Raskin, L., 2013. Psychrophilic anaerobic membrane bioreactor treatment of domestic wastewater. Water research, 47(4), pp.1655-1665.

www.ee.co.za/wpcontent/uploads/legacy/Energize_2013/09_AT_03_Electricity.pdf

Khanal, S.K., 2008. Anaerobic biotechnology for bioenergy production: principles and applications. John Wiley and Sons Inc, ISBN-978-0-813-82346-1[Chapter 10, microbial fuel cell: novel anaerobic biotechnology for energy generation from wastewater, Hong Liu, 221-246].

Feng, Y., Wang, X., Logan, B.E. and Lee, H., 2008. Brewery wastewater treatment using air-cathode microbial fuel cells. Applied microbiology and biotechnology, 78(5), pp.873-880.

Rabaey, K., Lissens, G., Siciliano, S.D. and Verstraete, W., 2003. A microbial fuel cell capable of converting glucose to electricity at high rate and efficiency. Biotechnology letters, 25(18), pp.1531-1535.

Min, B. and Logan, B.E., 2004. Continuous electricity generation from domestic wastewater and organic substrates in a flat plate microbial fuel cell. Environmental science & technology, 38(21), pp.5809-5814.

Wang, X., Feng, Y.J. and Lee, H., 2008. Electricity production from beer brewery wastewater using single chamber microbial fuel cell. Water Science and Technology, 57(7), pp.1117-1121.

http://www.wrc.org.za/News/Pages/EnergyEfficiencyintheSouthAfricanWaterIndustry.aspx

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Chapter 2: Literature Review

Over the last century, life dynamics have evolved and so has technology to a point where development has transformed to beyond boundaries of life. Evolved has life where humans sometimes exploit bacterial capabilities for innovative development. In this regard, the world has seen a multitude of future development approaches that rely on living microorganisms (Abrevaya et al., 2010). Microbiology and chemical engineering have recently put radical thinking on microorganisms' capacities, forcing a shift in the research world's attention to the potential of Microbial Fuel Cells technology (Loveley; 1988; Lovley, 1991; Nealson & Myers, 1992; Caccavo, 1994; Lovley, 1993). The remarkable microbial ability to harvest power during a reduction reaction in the anode of an MFC is fascinating. As a result, there is a move away from environmentally damaging combustion processes and towards biofriendly biocatalysts and fuel cell technology (Bullen et al., 2006; Picioreanu et al., 2010). As a result, using microbial fuel cells technology, it is now possible to directly extract renewable energy from waste remediation.

Studies have successfully shown that power can be harvested from diverse complex organics, e.g. Bond et al. (2002) reports 16mW/m^2 power density harvested from marine sediments, while others have reported power density in the range 216.4-971mW/m^2 harvested from swine wastewater, landfill leachate and corn stover hydrolysates using a single chamber MFC (Min et al., 2005; You et al., 2006; Zuo et al., 2006). MFC studies have also successfully generated power from pure compounds such as acetate (Bond and Lovley, 2003), glucose (Rabaey et al., 2003), sucrose (He et al., 2006), amino acid (cysteine; Logan et al., 2005), or a protein (bovine serum albumin; Heilmann and Logan, 2006). As shown in table 2.1, MFCs tests on these pure compounds yielded high power densities as compared to MFCs tests on actual wastewaters.

Table 2.1: Comparison of MFC power harvested from pure and mixed compounds (Fe et al., 2008).

MFC configuration	Substrate: single chemical	Power density (W/m^3)	Substrate: wastewater	Power density (W/m^3)	Reference
Single chamber	Glucose	12.4	Domestic	3.7	Liu and Logan (2004)
			Swine	6.5	Min et al. (2005)
			Corn stover hydrolysates	9.3	Zuo et al. (2006)
	Acetate	12.7	Biohydrogen reactor effluent	9.3	Liu et al. (2005b); Oh and Logan (2005b)
	Butyrate	7.6			
	Propionate	1.7			
	Bovine serum albumin	8.9	Meat packing	2.0	Heilmann and Logan (2006)
Two chamber MFC	Cysteine	0.35	Cereal	0.59	Logan et al. (2005); Oh and Logan (2005)
Baffled MFC	Glucose	37	Maple syrup with M9 medium	49	Rabaey et al. (2005a)
Tubular MFC	Acetate	58	Digester effluent	5	Rabaey et al. (2005b)

The innovative coupling of biological systems and electrical systems was discovered by Galvani in 1791 who applying electrical current to severed frogs' legs observed a twitch (Piccolino, 1998; Shukla et al., 2004; Bullen et al., 2006). And later on, inventions by Potter in 1911 also further revealed the potential to harvest power from the decomposition of organic matter during growth of species *Saccharomyces cerevise, Escherichia coli* and *Bacillus sp*. (Potter, 1911). Cohen published the first measurable MFC results in 1931, when he harvested 35V from a serial MFC using platinum electrodes (Aghababaie et al., 2015). Unfortunately, this ability of microorganisms to harvest energy in an MFC was not recognized until the 1970s, when the NASA research program investigated the possibility of simultaneous waste disposal and electricity production in an MFC (Rabaey and Verstraete, 2005; Bullen et al., 2006). And, as Rohrback et al. (1962) previously mentioned, NASA's research program looked into producing hydrogen from glucose fermentation in a fuel cell fed with *Clostridium butyricum biocatalyst* (Berk and Canfield, 1964; Shukla et al., 2004; Bullen et al., 2006). Berk and Canfield investigated the use of photosynthetic species in greater depth using microorganisms for bio-electricity generation from an MFC with

Ocillatoriaceae in the cathode chamber and *Rhodospirillum rubrum* in the anode chamber, and their findings reported insignificant power generation (Berk and Canfield, 1964). Davis and Yarborough's previous research looked at microbial fuel cells fed by *E. coli* and *Norcadia sp*, as well as glucose-fed enzymatic fuel cells (Davis and Yarborough, 1962).

Despite their potential, findings from earlier studies show that there was little commercial interest in biological fuel cells in the 1960s as compared to less expensive alternatives like photovoltaics (Shukla et al., 2004; Rabaey and Verstraete, 2005; Bullen et al., 2006; Lovley, 2006). Other report studies, however, show a resurgence of interest in biogological fuel cells in the 1970s and 1980s, as advocated by Bennetto and co. (Stirling et al., 1983; Bennetto et al., 1985; Bennetto, 1993; Shukla et al., 2004; Bullen et al., 2006). It was reported that a Tokyo Institute of Technology research group looked into immobilized *C.butyricum* cells as well as the use of microbial fuel cells as BOD sensors (Karube et al., 1977). During the 1970s and 1980s, one of the most troubling aspects of MFC research was the use of expensive mediators to allow electron transfer from microbes to the anode; however, later studies were able to produce MFCs that did not require mediators (Kim et al., 1999a; Kim et al., 1999b). Microbial fuel cells are not yet commercially feasible, but their research-based success demonstrates a desire and drive for clean green energy technology, which is also a climate change mitigation initiative (Katz et al., 2003; Prescott, 2009).

2.1 MICROBIAL FUEL CELLS

A microbial fuel cell (MFC) is a fuel cell that utilizes microorganisms as biocatalysts to directly convert chemical energy present in organic matter to energy in contrast to an enzymatic cell that utilizes enzyme and catalytic fuel cells that depend on inorganic molecules for energy generation (Rismani-Yazdi, 2008). Previous studies have found that electricity is generated in a microbial fuel cell as a result of electrons produced from the decomposition of organic matter by anaerobic microorganisms as depicted by equation 2.1 (Logan et al., 2006; Rabaey & Verstraete, 2005; Shukla et al, 2004; Yuan et al., 2017). Glucose is illustrated as an electron donor in the equation:

$$C_6H_{12}O_6 + 6H_2O \rightarrow 6CO_2 + 24H^+ + 24e^-(E^0 = 0.014V) \ldots\ldots\ldots\ldots..2.1$$

The electrons generated within the anode chamber are transferred to an insoluble electrode (i.e. anode electrode) instead of a dissolved terminal electron acceptor such as oxygen, nitrate or sulphate due to the high electrode potential (Logan et al., 2006; Rabaey & Verstraete, 2005). The transfer of electrons to the anode electrode may occur directly or indirectly by mediators to the anode and through an external circuit to the cathode (Bennetto et al., 1985; Allen & Bennetto, 1993; Shukla et al., 2004; Rabaey & Verstraete, 2005; Bullen et al., 2006; Lovley, 2006). During the catabolic process, protons are also generated which propagate to the cathode through a selective membrane to the cathode where they react with oxygen by the oxygen reduction reaction (ORR) to generate water as indicated by equation 2.2 (Yuan et al., 2017; Shukla et al, 2004):

$$6O_2 + 24H^+ + 24e^- \rightarrow 12H_2O \ (E^0 = 1.23V) \ldots\ldots\ldots\ldots\ldots\ldots\ldots 2.2$$

The overall reaction in a microbial reactor can be defined as the breakdown of the substrate to carbon dioxide and water while generating electricity in the process (equ. 2.3) (Yuan et al., 2017):

$$\text{Overall reaction: } C_6H_{12}O_6 + 6O_2 \rightarrow 6CO_2 + 6H_2O + electricity \ldots\ldots 2.3$$

In a microbial fuel cell, a proton exchange membrane or a salt bridge that is selective towards protons separates the anode and cathode chamber (Min et al., 2005). The anodic compartment contains substrate/fuel/wastewater while the cathode compartment employs oxidizing agent such as dissolved oxygen or ferricyanide (Yuan et al., 2017) (figure 2.1).

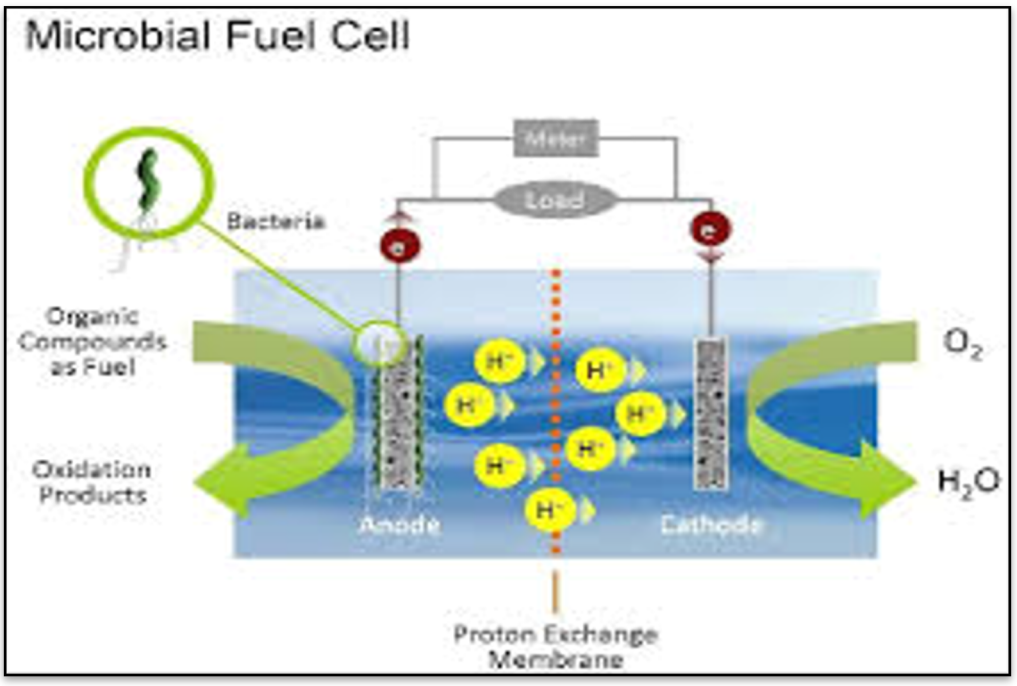


Fig 2.1: Schematic outline of a dual chamber microbial fuel cell and its components (Cameron, 2016).

Figure 2.2 further gives another illustration of an overall process occurring in a single chamber or Air-cathode MFC during power generation based on a carbon cloth anode and cathode electrodes where the cathode is coated with a manganese oxide-nanotubes (MnO_2-NTs) catalyst and the two-compartment separated by a Nafion 117 membrane (Khilari et al., 2013).

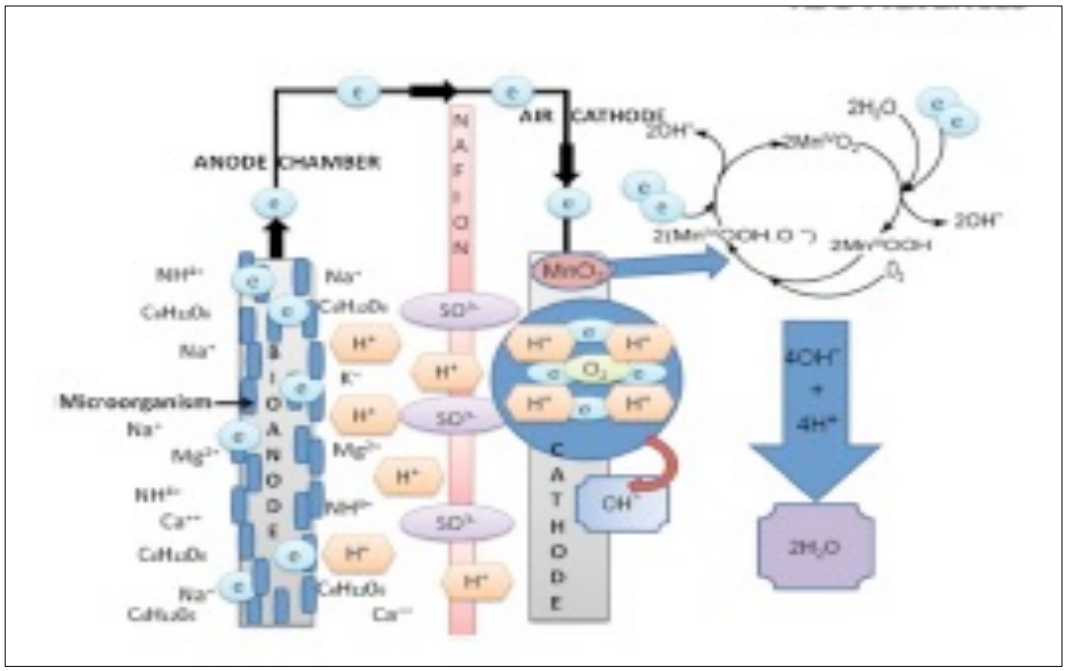


Fig. 2.2 A schematic single chamber MFC during the power generation process. (Khilari et al., 2013).

Research studies have also identified various factors that affect the efficiency of a MFC and these are summarized in figure 2.3.

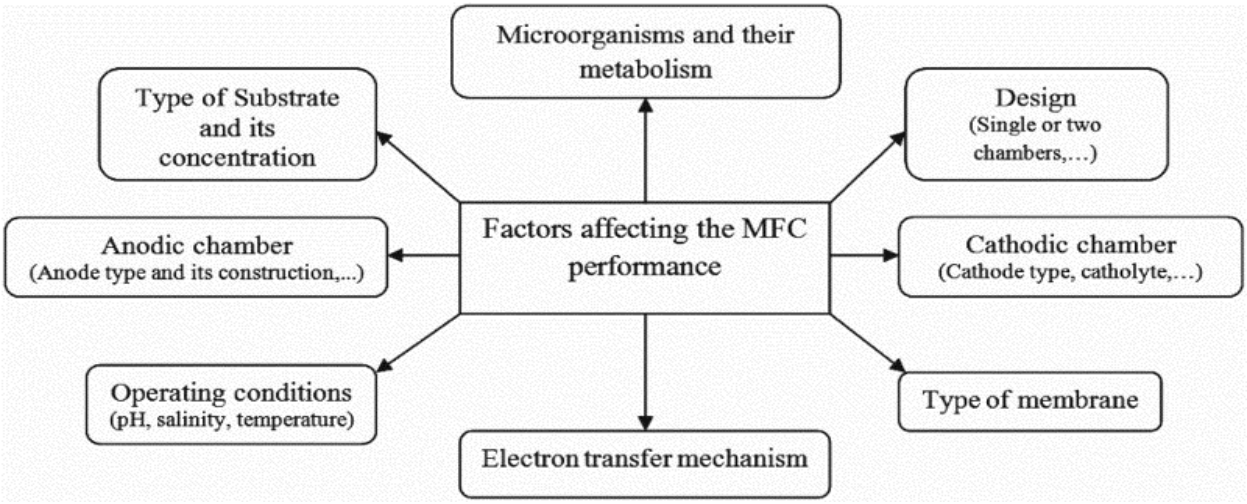


Fig 2.3. Summary of factors influencing the efficiency of a MFC (Aghababaie et al., 2015).

2.2 TYPES OF MFCS

Niessen et al., 2004; Schröder, 2007) investigated mediator-free MFCs in some studies. According to these studies' findings, electricity was produced when microbes (EABs) used the substrate during the inoculation phase (Niessen et al., 2004; Schröder, 2007). These microbes are said to pass electrons either directly or indirectly via membrane-bound proteins such as pili, c-type cytochromes, and filaments (Wang and Ren, 2013). It has been discovered that bacteria that generate electricity, such as Geobacter, form pili and c-type cytochromes Omcs (Bond and Lovley, 2003) and Shewanella, form bacterial nanowires (Gorby et al., 2006), allowing them to mediate electron transfer (Tharali et al., 2016). Moon et al. (2006) achieved 0.56W/m^2 power density in a mediator-less MFC using artificial wastewater, while Gil et al. (2003) found pH of 7, NaCl buffered solution, lower resistance, and improved aeration rate to be the best operating parameters for a mediator-less MFC (Moon et al., 2006; Gil et al., 2003).

The membrane-less MFC is another form of MFC that has been studied. Jang et al. (2004), Du et al. (2007), Ghangrekar and Shinde (2006), Jadhav et al. (2013), Tharali et al. (2016), and others have documented these forms of MFCs. Though Du et al.

(2007) reported a reduction in ohmic losses in electrolytes by reducing electrode spacing, Ghangrekar and Shinde (2006) claimed to have achieved 10.9 mW/m^2 and 10.16 mW/m^2 power density with a membrane-less MFC at 20cm electrode spacing, and Jadhav et al. (2013) claimed to have achieved 3.8 mA/m^2 current density and 0.52 mW/m^2 power density with a (Tharali et al., 2016).

Walter et al. (2019) explored the effects of various electrode immersion heights in a self-stratified membrane-less MFC that was fed with human urine. They used an advanced design known as a self-stratifying membrane-less microbial fuel cell (SSM-MFC) in their study, which is said to be versatile in size scalability, exhibit negligible power density losses, and have a configuration with a number of cathodes in the upper layers of the electrolyte and a number of anodes in the lower layers of the electrolyte (Walter et al., 2017; Walter et al., 2016; Walter et al., 2018; Jiang et al., 2011). The anolyte was human urine, and the cathode was made with an activated carbon-based substance as a catalyst. They looked into the effects of cathode immersion height on power production and catalyst durability in their research. At various catholyte heights of 1/4, 1/2, 3/4, and completely submerged, the cathode was immersed in the catholyte. When 34% submerged, the analysis achieved a maximum power output of 3.0mW, while stability tests for the 34% submersion yielded a maximum power output of 3.5mW.

2.3 EXOELECTROGENS IN MFCS

Exoelectrogens, electroactive bacteria (EAB), anode-respiring bacteria (ARB), or electricigens are anaerobic microorganisms that can produce electricity from waste matter decomposition, according to previous research findings (Varanasi and Das, 2018). The electrons produced in the anode chamber have been recorded to be transferred to the anode through shuttles or electron medators. *Pseudomonas, Enterobacter, Proteus spp.*, and *E. coli* (Kim et al., 2000; Park & Zeikus 2002; Ieropoulous et al., 2005; Rabaey et al., 2004; Rabaey & Verstraete, 2005; Kim et al., 2006) are just a few examples. Exoelectrogens include species such as *Shewanella putrefaciens, Geobacter sulfurreducens, Geobacter metallireducens*, and *Rhodoferax ferrireducens* (Liu et al., 2004; Bond & Lovley 2002; Holmes et al., 2006) and

Desulfuromonas acetoxidans (Bond et al., 2003), as well as *Shewanella species* such as *S.*

Table 2.2 summarizes the various electroactive bacteria identified as biocatalysts in various MFCs studies for power generation and they occur either as pure cultures or mixed cultures.

Table 2.2. Electroactive bacteria used in MFCs (Varanasi and Das, 2018)

Biocatalysts	**Electron donor**	**MFC type**	**Power density(mW/ m^2)**	**References**
Pure Cultures				
Escherichia coli	Complex medium	Single chamber	600	Zhang et al.(2006)
Shewanella oneidensis	Lactate	Miniature	3000	Ringeisen et al.(2006)
Geobacter sulfurreducens	Acetate	H-type	13	Bond and Lovley(2003)
Clostridial isolate	Complex medium	Two-chamber	n.a	Prasad et al.(2006)
Mixed cultures				
Activated sludge	Landfill leachate	H-type	n.a	You et al.(2006b)
-do-	Glucose	H-type	115.6	You et al.(2006a)
Domestic wastewater biofilm	Glucose	Single chamber	766	Cheng et al.(2006a)
-do-	Glucose	Single chamber	1540	Cheng et al.(2006b)
-do-	Domestic wastewater	Single chamber	464	-do-
Thermophilic effluent from anaerobic digestion of brewery wastewater	Acetate	Two-chamber	1030	Jong et al.(2006)
Activated sludge	Glucose, glutamate	Two-chamber	560	Moon et al.(2006)
Granular anaerobic sludge	Sucrose	Up-flow	n.a.	He et al.(2006)
Fly ash leachate	Fermentation effluent	Two-chamber	85.07	Varanasi et al.(2015)

2.4 ELECTRON TRANSFER MECHANISMS

Previous study findings indicate that electron transfer to the anode may occur either through exogenous mediators such a thionine or neutral red; mediators produced by bacteria; or by direct electron transfer from respiratory enzymes such as Cytochromes to the electrode (Liu et al., 2004; Niessen et al., 2004).

Mediated MFCs

According to studies, mediators trap electrons from the respiratory chain, which are then reduced for electron transfer to the electrode through the outer cell membrane (Reed and Nagodawithana, 1991). Kim et al. (2004) discovered a variety of inhibitors within the respiratory chain that can prevent electrical current from being produced in a microbial fuel cell (Kim et al., 2004). Other studies have identified processes that use oxidative phosphorylation as an electron transport mechanism, resulting in high energy efficiencies of up to 65 percent; for example, consortia containing Pseudomonas aeruginosa, Enterococcus faecium (Rabaey et al., 2004), Rhodoferax ferrireducens (Rabaey et al., 2004). (Chaudhuri and Lovley, 2003). Extracellular electron transfers to the electrode are often found to be mediated by a physical transport mechanism, which can take the form of (i) soluble electron shuttles (Roller et al.,1984 Delaney et al.,1984) or (ii) membrane-bound electron shuttling compounds (Roller et al1984). (Vandevivere and Verstraete, 2001; Kostka et al.,2002). Previous research has shown that compounds in the respiratory chain use the oxidative membrane associated electron transfer mechanism (Rabaey and Verstraete, 2005), which has been related to bacteria like Geobacter metallireducens (Vandevivere and Verstraete, 2001), Aeromonas hydrophila (Pham et al., 2003), and Rhodeferax ferriducens (Rabaey and Verstraete, 2005). (Chaudhuri and Lovley, 2003). Kim et al. (2004) discovered that electrons can be transported through NADH dehydrogenase, ubiquinone, coenzyme, or cytochrome (Champine et al., 2000; Beliaev, 2001; Nevin and Lovley, 2002) with NADH dehydrogenase, Fe/S (Iron/Sulphur) proteins, and quinones as electron carriers (Champine et al., 2000; (figure 2.4). Although some fermentative species have been confirmed to have hydrogenase, such as Clostridium butyricum (Park et al., 2001) and Enterococcus faecium (Rabaey et al., 2004).

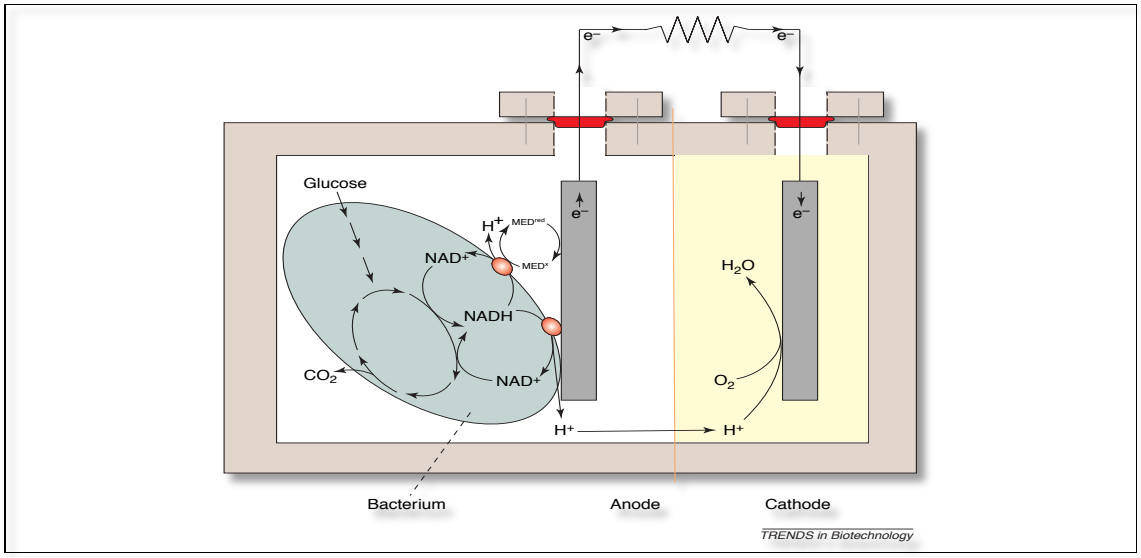


Fig 2.4: Electron transport with NADH dehydrogenase acting as electron carrier (Rabaey and Verstraete, 2005).

It is also stated that a good mediator must have the following characteristics: i) be able to easily cross the cell membrane, (ii) be able to grab electrons from the electron carriers of the electron transport chain, (iii) have a high electrode rate, (iv) have good solubility in the anolyte, (v) be non-degradable and non-toxic to microbes, and (vi) be of low toxicity. (Ieropoulos and colleagues, 2005a). Dyes and metallorganics such as neutral red (NR0), methylene blue (MB), thionine, meldola's blue (MelB), 2-hydroxy-1,4-naphthoquinone (HNQ), and Fe (III)EDTA are examples of artificial mediators. Park and Zeikus, 2000; Tokuji and Kenji, 2003; Veag and Fernandez, 1987; Allen and Bennetto, 1993; Ieropoulos et al., 2005a.The key issue with exogenous mediators (artificial/synthetic mediators) is their toxicity and volatility, which restricts their use in MFCs (Aghababaie et al., 2015).

Mediator-less MFCs

A mediator-less MFC is one that includes exoelectrogenic bacteria that transfer electrons using electrochemically active redox enzymes on their outer membranes rather than exogenous chemicals (Lithgow et al., 1986). Exoelectrogens with the ability to directly transfer electrons to the anode in an MFC should be anodophiles whose outer layers do not contain non-conductive lipid membranes, peptididoglycans, or lipopolysaccharides that obstruct direct electron transfer to the anode, according to

previous studies (Du et al., 2007; Davis and Higson, 2007). As a consequence, microorganisms that undergo electrogenesis can directly move electrons from the substrate to the anode using endogenous mediators or nanowires or pillis, as shown in figure 2.5 (Aghababaie et al., 2015), and thus can be used in mediator-free MFCs.

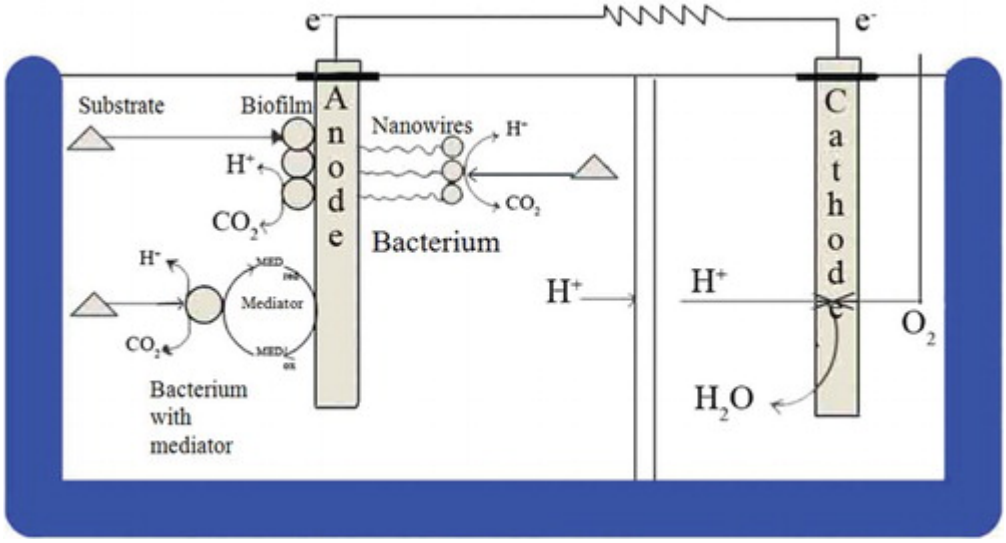


Fig 2.5 Electron transfer characteristics of electrogenesis bacteria (Aghababaie et al., 2015)

Electrogenesis bacteria are also electrochemically active and have the ability to reduce Fe (III) due to the presence of cytochromes in their outer membranes, according to studies (Kim et al., 2002; Gil et al., 2003). As a result, microorganisms like Shewanella and Geobacter have been discovered to produce highly conductive nanowires and form biofilms on the anode surface, as shown in figure 2.5. (Gorby et al., 2006). The MFC based on species that use their natural sulphate/sulphide mediating potential, i.e. MFC containing anodophilic species Geobacter sulfurreducens without a soluble mediator and the sulphate reducing species Desulfovibrio desulfuricans, performed better than the MFC based on synthetic mediators combined with E.coli (Ieropoulos et al.,2005).

Species that can directly transfer electrons to the anode, such as Shewanella putrefaciens (Kim et al., 2002), Geobacteraceae sulferreducens (Bond and Lovley,2003), Geobacter metallireducens (Min et al.,2005a), and Rhodoferax ferrireducens (Chaudhuri and Lovley,2003), have been recorded as stable (Kim et al., 1999a; Chaudhuri and Lovley, 2003).

2.5 MICROBIAL ACTIVITY

Exoelectrogens' growth capacity is influenced by essential factors such as substrate alkalinity and temperature, nutrient availability, type of electron acceptor, and so on, according to previous study findings (Varanasi and Das, 2018). Furthermore, each exoelectrogens bacterial species is said to have its own set of characteristics that are tailored to the environment. Various environmental/substrates conditions that are conducive to microbial growth are listed below.

2.5.1 pH of the substrate

The pH of the substrate is one of the environmental factors that influences exoelectrogen growth and substrate metabolism, and research has found that the pH impact on MFC output is dependent on microbial activity and substrate composition (Kaushik and Chetal, 2013). Studies have shown that the pH range of 6.5 to 7.5 is ideal for bacterial development (Patil et al., 2011). According to reports, this pH range maintains the intracellular pH of microbes and prevents the development of weak acid compounds as a result of pH drops during metabolism (He et al., 2008). Puig et al. (2010) discovered that raising the substrate/anolyte pH improves MFC efficiency, while Kaushik and Chetal (2013) discovered that the optimum pH for bacterial growth in an MFC is between 7 and 9. It's also been documented that the microbial community is highly sensitive to pH changes in the substrate (Varanasi and Das, 2018).

2.5.2 Temperature of the substrate

The kinetic and thermodynamic properties, as well as the nature of microbial communities, have been found to be influenced by changes in substrate temperature (Larrosa-Guerrero et al., 2010). Exoelectrogens were found to be less susceptible to temperature change than other bacteria, as they can respond to psychrophilic temperatures (20^{o}C) and thermophilic temperatures (>50^{o}C), whereas enzymes involved in biochemical reactions performed best at optimum pH and electrochemical

reaction rates were found to be directly proportional to temperature (Michie et al., 2011). Wastewater-fed MFCs and biosensors are examples of psychrophilic temperature MFCs, while thermophilic MFCs are said to have higher electrogenic activity, increased feed solubility, lower mass transfer limits, and a lower risk of contamination (Dopson et al., 2016). Wrighton et al. (2008) investigated a thermophilic MFC, studying the microbial culture of Firmicutes and found that 80% of the population was responsible for electricity generation (Wrighton et al., 2008). High temperature sludge has been identified as a possible source of thermophilic inocula in an MFC, according to Dopson et al. (2016). Thermophilic MFCs are being considered as possible solutions for handling high-temperature industrial wastewaters such as distillery effluents, petroleum refineries, pulp and paper mills, chemical plants, and so on (Varanasi and Das, 2018).

2.5.3 Type of substrate

Another factor that has been documented to affect exoelectrogen growth is the type of substrate used in an MFC. The form of medium used in an MFC has an effect on the dominant exoelectrogens species as well as the MFC's maximum power density and coulombic efficiency (Chae et al., 2009). Pant et al., 2010 discovered that the varying existence of substrates used in MFCs, such as simple carbohydrates and complex organic matters, at various operating conditions makes it difficult to make a definitive finding on which substrate yields the best MFC efficiency. Long-term MFC investigations are expected to fully comprehend microbial activity in various substrates.

2.5.4 Electrode material and membranes

Electrode material and membranes have been found to influence the electrochemical properties of an MFC, but there have been few studies on their impact on microbial activity (Varanasi and Das, 2018). Different electrode materials have different porosity and conductivity, which can affect bacterial adhesion on the surface, which is required for biofilm formation on the anode for electron transfer from bacteria to anode, according to Baranitharan et al. (2015). (Baranitharan et al., 2015; Varanasi and Das,

2018). Carbon-based materials such as graphite rod, carbon paper, carbon felt, carbon fabric, and other carbon-based materials are used as electrodes in MFC research (Mashkour and Rahimnejad, 2015).

2.5.5 Biofilms

Biofilms and biofilm formation mechanisms have piqued MFC researchers' interest. They were discovered to allow direct electron transfer between microorganisms (electron donor) and the anode (electron acceptor), resulting in increased current generation (Picioreanu et al., 2007). A biofilm's composition may also be made up of a single species or several microorganisms that use different metabolic pathways (Kim et al., 2006). Previous research has also suggested that investigations into microbial physiology be conducted in order to enhance MFC efficiency (Picioreanu et al., 2007).

Biofilms are said to develop on a surface via cell attachment, which is aided by the expression of exopolymers that bind to the surface, and cell growth, which is aided by the dispersion of microbial cells detached from the biofilm layer and attached to another part of the surface (Gottenbos et al., 1999; Davies et al., 1998). Biofilm growth is the result of detachment and attachment from and to the surface, which results in an increase in biofilm thickness. A growing biofilm in an MFC increases the anode surface area for electron transfer, increasing the MFC performance in terms of current generation (Picioreanu et al., 2007). A thicker biofilm that can preserve MFC effectiveness has been found to be efficient because it protects the dormant layer of bacteria by continuously restoring any dead or removed cells (Mah and O'Toole, 2001). Natural biofilms have been discovered to have a unique feature of intermediate group metabolism, in that they enable multiple microbes, such as Archaea and Bacteria, to form a microbial community in which one species' products serve as substrates for another (Hall-Stoodley et al., 2004). MFC biofilms, in this case, are bacteria that enable electron transfer to the anode and thus produce current. Geobacter species have also been found to be favored in previous studies due to their metabolic pathway, which lowers iron and metal oxides extracellularly as the terminal electron acceptor (Lovley, 2006; Logan, 2009). A mixed biofilm outperforms a single species biofilm in terms of MFC output (e.g., a mixed biofilm of *Pelotomaculum thermopropionicum, Methanothermobacter thermautotrophicus*, and *Geobacteraceae*

generates a substantial potential difference than a film of only *Geobacteraceae* colonies) (Kim et al., 2006); Logan, 2009; Gottenbos et al., 1999).

2.6 FACTORS INFLUENCING EXOELECTROGENS PERFORMANCE IN MFC

Mass transfer limitations, bacterial metabolism losses, activation losses, and other factors that have been found to increase the overall potential at bioanode have also been identified as factors that affect the performance of exoelectrogens in MFCs (Pham et al., 2009).

2.6.1 Mass transfer limitations

Mass transfer limitations are simply limitations in proton transfer caused by the accumulation of protons in the anodic chamber during the inoculation process, i.e. formation of biofilm at the anode and this affects the activity of exoelectrogens (Pham et al., 2009; Varanasi and Das, 2018). These limitations can also result in a pH gradient and as reviewed in the previous section, microbial community is very sensitive to pH change (Varanasi and Das, 2018). Furthermore, these limitations also promote concentration losses in MFC leading to limitations in the release of redox species to or from the electrode surface and there maybe an increased electrode potential caused by the increase in the ratio between the oxidized and the reduced species at the electrode surface.

2.6.2 Bacterial metabolism losses

Exoelectrogen output in MFCs has also been shown to be affected by bacterial metabolism in studies. Microorganisms have been discovered to obtain energy with the aid of the electron transport chain by transferring electrons from a low-potential substrate to a high-potential electron acceptor, such as oxygen (Varanasi and Das, 2018; Pham et al., 2009). As a result, the anode electrode is the terminal electron acceptor, and its potential is directly proportional to bacteria's energy gain. A large

difference between the redox potentials of the substrate and the anode results in a large bacterial metabolic energy gain. Low anode potentials are needed for high MFC performance; however, anode potentials should not be too low because this triggers fermentative pathways, which inhibit electron transfer due to high energy gained by fermentative pathways.

2.6.3 Activation losses

It has been stated that bacteria must overcome energy barriers in order to undergo redox reactions (Varanasi and Das, 2018), and that there are kinetic limitations in the transfer of electrons from the bacteria to the electrode surface during redox reactions, resulting in activation losses. Exoelectrogens also improve their metabolic energy gain by improving their electron transfer strategies, which contributes to a reduction in activation losses. Using biocompatible electrode surfaces to enhance bacterial-electrode interactions is said to improve electron transfer from the bacteria to the anode (Varanasi and Das, 2018).

2.6.4 Electron-quenching reactions

Studies have found that applications of MFCs using complex substrates such as real industrial or domestic wastewaters as compared to simple sugars such as glucose requires one to consider the mixed microbial communities present in the substrate and apart from exoelectrogens, there are other species of bacteria present competing for the substrate which may result in electrons being redirected to non-electrogenic pathways such as fermentation or methanogens (Varanasi and Das, 2018). Some of the electrons being transferred to the electrode surface are lost leading to low efficiency in the conversion of substrate to electrical current. It is further recommended that while operating MFCs, one should also consider the type of microbes, community composition, interactions between microbes to achieve high MFC performance (Varanasi and Das, 2018).

2.7 METHODS FOR STUDYING EXOELECTROGENS

The Zhi et al. (2014) devised microbiological and electrochemical methods to assist in the investigation of exoelectrogens (Zhi et al., 2014).

Electrochemical methods such as polarization techniques, cyclic voltammetry (CV), and electrochemical impedance spectroscopy (EIS) are used in studying the kinetics of single electrode reactions and can also be used to describe microbial reactions and electron transfer mechanisms in MFC studies (Zhi et al., 2014). Accordingly, these techniques are said to contribute to MFC research in the following ways: i) Polarization techniques provide insight into the various Overpotentials experienced by the MFC system; (ii) EIS, Nyquist, and Bode plots are used to determine the most influential resistance in an MFC system; (iii) and CV is a tool used in MFC investigations into redox processes, electron discharge patterns over time, metabolic changes, and the carriers involved during electron transport (Zhi et al., 2014).al.

2.8 MFC ELECTROCHEMISTRY

The main reaction in the cathodic chamber is the oxidation reduction reaction (ORR) which occurs via either a high energy conversion efficiency four-electron pathway to form water or via a two electron process to form hydrogen peroxide as a result of the absence of a platinum catalyst (equation 2.4) (Yuan et al., 2017). In a four-electron pathway the electron acceptor in the cathode, i.e. oxygen is converted to water in the presence of a platinum catalyst that eliminates overpotential during the reduction of oxygen (Oh et al., 2004).

$$\boldsymbol{O_2 + 4e^- + 4H^+ \rightarrow 2H_2O\ (E = +0.984\ V_{SCE})} \text{ ... ... .2.4.}$$

In a two electron pathway, oxygen is reduced on carbon-based electrodes producing hydrogen peroxide (H_2O_2) as a product (equation 2.5) (Fu et al., 2010).

$$\boldsymbol{O_2 + 2e^- + 2H^+ \rightarrow H_2O_2\ \ (E = +0.450 V_{SCE})} \text{ ..... 2.5.}$$

H_2O_2 cannot be further oxidized at a faster rate and therefore no further reduction reaction occurs and as a result H_2O_2 is the final product.

Previous research into the cathode kinetics of an MFC has shown that the cathode kinetics are guided by the oxidation reduction reaction, which occurs by either a four-electron pathway or a two-electron pathway depending on the type of catalyst and pH of the catholyte solution, as previously stated (Kinoshita, 1988; Kinoshita, 1992; Artyushkova et al., 2015; Shao et al., 2016). Other research findings have identified cathode kinetics as one of the output limiting factors in MFCs, owing to the low concentration of H^+ and OH^- in neutral solutions (Rismani-Yazdi et al., 2008; Madjarov et al., 2017; Erable et al., 2012; Kinoshita, 1988; Kinoshita, 1992; Artyushkova et al., 2015; Shao et al., 2016). It was also discovered that under acidic conditions, the ORR generates H_2O from O_2 reduction through the four-electron pathway (direct $4e^-$), as shown in equation 2.4. (Oh et al., 2004; Yuan et al., 2017; Kodali et al.,2018) As shown in equation 2.5, the ORR occurring through the two electron pathway (direct $2e^-$) has been found to produce H_2O_2 from O_2 reduction (Fu et al., 2010). It was also discovered that in alkaline medium, ORR occurring via a four-electron pathway (direct $4e^-$) generates OH^-, while ORR occurring via a two-electron pathway (direct $2e^-$) generates HO^{2-} and OH^- (Kinoshita, 1988; Kinoshita, 1992; Artyushkova et al.,2015; Shao et al.,2016; Kodali et al.,2018). One of the many limiting factors in the efficiency of microbial fuel cells has been stated to be cathodic limitations (Rismani-Yazdi, 2008). The continuous supply of substrate in an ideal MFC results in constant current generation while maintaining steady voltages (Rismani-Yazdi et al., 2008). Moreover, the Nernst equation in this case can be used to estimate the MFC theoretical ideal voltage, E_{thermo} (V) as follows:

$$E_{thermo} = E^0 - \frac{RT}{nF}\ln(\Pi) \quad \ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots 2.6.$$

where E^0 is the standard cell potential (V), R is the ideal gas constant (8.314J.mol-1K-1), T is the temperature (K), n is the number of electrons transferred in the reaction, F is the faraday's constant (96485 C mol-1) and Π is the chemical reactivity of products divided by the chemical reactivity of reactants (Rismani-Yazdi et al.,2008). However, it has also been found that due to irreversible losses (i.e. Overpotentials within the cell) such as activation losses, ohmic losses and mass transport losses, the actual cell voltage output is practically less than the predicted thermodynamic ideal voltage as illustrated by figure 2.6 (Rismani-Yazdi et al., 2008). These losses are defined as the voltage required compensating for the current loss due to electrochemical reactions, charge transport and mass transfer processes taking place in the anode as well as the cathode

compartments (O'Hayre et al., 2005). The actual cell voltage output (i.e. real operational voltage output, Vop) can be thermodynamically predicted by the following equation:

$$V_{op} = E_{thermo} - [(\eta_{act} + \eta_{ohmic} + \eta_{conc})_{cathode} + ((\eta_{act} + \eta_{ohmic} + \eta_{conc})_{anode}] \quad \text{.....2.7}$$

where E_{thermo} is the thermodynamically predicted voltage, ηact is the activation losses due to reaction kinetics, η_{ohmic} is the ohmic losses due to ionic and electronic resistances and ηconc is the concentration losses due to mass transport limitations (Rismani-Yazdi et al., 2008). According to equation 2.7, optimizing both the anode and cathode can reduce the irreversible losses and as a result improve the MFC performance.

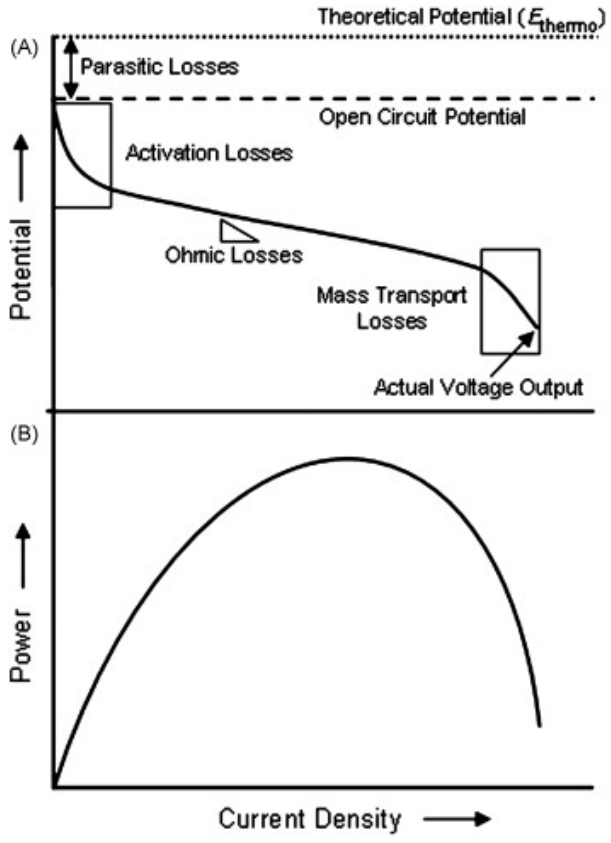


Fig. 2.6 (A) Schematic potential losses for a cathodic reaction displaying activation, ohmic, mass transport and parasitic regions. (B) A typical power–current curve (Rismani-Yazdi et al., 2008).

Studies on MFC performance investigations have found that the kinetics of the cathode oxidation reduction reaction (ORR) affects electricity generation and this is due to an activation energy barrier that hinders the conversion of the oxidant into a reduced form

(O'Hayre et al., 2005). And the activation energy barrier is overcome when some of the cathode potential is lost during current transfer from the cell and this cathode potential loss is what is termed cathodic activation loss (η_{act}) cathode. And in figure 2.6 above, a current-voltage curve illustrates how the activation losses cause characteristic and exponentially formed loss at low current densities. An increase in activation loss results in lower cell potential due to more current drawn from the MFC (Larminie and Dicks, 2003). The kinetic performance can be improved by reducing the activation energy barrier and increasing the reaction interface area, temperature or oxidant concentration (Rismani-Yazdi et al., 2008).

These are irreversible losses that are caused by internal resistance and they are defined as voltage lost to accomplish the electron and proton transfer processes and this loss follows the Ohm's law as described by equation 2.8 (Rismani-Yazdi et al., 2008):

$$\eta_{Ohmic} = iR_{Ohmic} \ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots 2.8.$$

where i is the current (A) and Rohmic is the Ohmic resistance (Ω) of the system.

The ohmic resistance is defined as a combination of both the ionic and electronic resistances as shown by equation 2.9.

$$R_{Ohmic} = R_{ionic} + R_{elec} \ldots\ldots\ldots\ldots\ldots\ldots 2.9.$$

It has also been reported that the internal resistance of the cell (R_{int}) is dominated by the electrolyte resistance due to electrical conductivity of the electrode being of higher magnitude than the ionic conductivity (O'Hayre et al., 2005). The ohmic resistance of the electrolyte is expresses by equation 2.10:

$$R_{ion} = \frac{l}{AK} \ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots 2.10.$$

where l is the distance (cm) and A is the cross-sectional area (cm^2) over which the ionic conduction occurs, and K is the specific conductivity (Ω.cm)-1 of the electrolyte (Larminie and Dicks, 2003). And according to the current-voltage curve in figure 2.6, the Ohmic losses are noticeable at medium current density and obeying Ohm's law (equation 2.8) which indicates that the voltage decreases linearly with current increase.

Studies have also found that at the cathodic chamber of a MFC, mass transport drives the oxidants supply and product removal and inadequate mass transport may result in

reactant depletion or product accumulation (Rismani-Yazdi et al., 2008). And as such the MFC performance is reduced as a result of the effects of reactant depletion on the Nernstian cell voltage and the reaction rates. The mass transport losses also known as cathodic concentration losses (η_{conc}) cathode are defined as the voltage required to drive mass transport process at the cathode and according to fig 2.6, they occur at high current density and they increase with increasing current density (O'Hayre et al., 2005; Rismani-Yazdi et al., 2008). It has also been stated that mass transport limitations in the anode compartment are marginal, and that only cathodic oxidant transport limitations should be considered in MFCs because they are more serious than those in the anode compartment (Rismani-Yazdi et al., 2008).

Cathodic limitations have been studied as a result of a slow oxygen reduction rate at the cathode electrode surface, especially on graphite/carbon electrodes, which stimulates a high reduction overpotential and thus affects MFC output (Gil et al., 2003). Different methods, such as the use of mediators, electrode modification with catalysts, and cathodic compartment operating conditions optimization, have been used in studies to increase cathodic reaction rates through reducing cathodic oxygen overpotentials (Zhao et al., 2006; Morris et al., 2007).

Cathodic overpotentials have been identified as one of the key limiting factors in microbial fuel cells, and as a result, some studies have looked at using mediators instead of oxygen because the reduction rate of mediators at the electrode surface has been found to be much faster than that of oxygen, potentially reducing cathodic overpotentials (Rismani-Yazdi et al., 2008). However, it has been reported that hiring mediators is still a problem because mediators need to be replaced on a regular basis and are thus impractical for long-term applications (Logan and Regan, 2006). Due to its high redox potential and faster reduction kinetics, ferricyanide is the most widely used mediator for cathodic reactions, and the ferricyanide solution concentration is stated to be unaffected by solubility (Rismani-Yazdi et al., 2008). Equations 2.11 and 2.12 illustrate the reversible redox reactions of ferricyanide in cathode kinetics.

$$[Fe(CN)_6]^{3-} + e^- \rightarrow [Fe(CN)_6]^{4-} \ (E'^0 = 0.358) \ldots\ldots\ldots\ldots 2.11.$$

$$O_2 + 4[Fe(CN)_6]^{4-} + 4H^+ \rightarrow [Fe(CN)_6]^{3-} + 2H_2O \ldots\ldots\ldots\ldots\ldots 2.12.$$

Jang et al. (2004) found that ferricyanide acts as an electron acceptor rather than as mediator due to the slow re-oxidation rate by oxygen (equ 2.11) (Jang et al., 2004). Oh et al. (2004) observed a 50-80% maximum power density increase when using ferricyanide as compared to that achieved when using oxygen or a platinum coated air-cathode MFC and this is due to the ferricyanide solution having a high open circuit potential as compared to that of dissolved oxygen (Oh et al., 2004). Investigations from Oh and Logan (2006), Ringeisen et al. (2006), Liu and Li (2007) all observed similar findings to Oh et al. (2004). Even though ferricyanide exhibits faster reduction kinetics than oxygen, its low regeneration rate by oxygen remains a challenge as it requires regular restoration which makes it unsustainable in long-term applications and it has also been found to be poisonous and can potentially diffuse through membranes in long-term applications (Logan and Regan, 2006).

Other studies have also explored the use of metal oxides as mediators in cathode kinetics and their findings revealed improved electron transfer kinetics resulting in improved MFC performance (Park and Zeikus, 2003). Alternatively, other investigative studies such as Heijne et al. (2006) explored the use of redox couple Fe^{3+}/Fe^{2+} as mediators due to its fast redox reaction rate and high standard potential (E^0=0.77V) and findings revealed a reduced cathode overpotential (Heijne et al., 2006). However, other studies have observed that applications of ferric ions in cathode kinetics is limited by their low solubility at high pH (>2.5) as many of the substrate have pH higher than 2.5 (Rismani-Yazdi et al., 2008). You et al. (2006) explored the use of permanganate as a cathodic oxidant under acidic conditions (equation. 2.13) and their findings revealed power density of 4.5 and 11 times higher than that of ferricyanide and oxygen respectively (You et al., 2006). However, other investigative study findings revealed that the application of permanganate as cathodic oxidant is hindered by its dependence on pH, low performance at alkaline conditions and the need to replenish it due to depletion (You et al., 2006).

$$MnO_4^- + 4H^+ + 3e^- \rightarrow MnO_2 + 2H_2O \ (E^0\text{=}1.70, \text{pH=}1.0) \ldots\ldots \ 2.13.$$

In order to speed up the overall reaction and boost the cathode kinetics of an MFC, research into platinum group metals (PGM) (e.g. platinum (Pt), ruthenium (Ru), palladium (Pd), rhodium (Rh), Osmium (Os), and iridium (Ir)) and platinum group non-metals (PGM-free) (e.g. carbon-based materials and transition metals) has (Yang et al.,2017; Antolini,2016; Antolini, 2015; Yuan et al., 2016; Wang et al., 2014;

Arbizzani et al.,2006). Researchers favor the use of PGM-free catalysts in MFC applications due to their low costs and resistance to poisoning from organics and pollutants in wastewater, despite their high costs and susceptibility to poisoning from organics and pollutants in wastewater (Santoro et al., 2015; Santoro et al., 2016, Minachev et al., 1952). Research studies have intensified efforts into investigating the applicability of PGM-free based catalysts as cathode catalysts in MFCs, with findings revealing that carbon-based materials such as activated carbon (AC), carbon nanotubes (CNT), carbon nanofibres, graphene nanosheets, and others perform poorly in MFCs when compared to transition metals such as Fe, Mn, Co, and Ni (Gratti). Burkitt et al., 2016 ; Jiang et al., 2017 ; Rojas-Carbonell et al., 2017 ; Kumar et al., 2016 ; Hou et al., 2016 ; Huang et al., 2015 ; Modi et al., 2016). Carbon-based materials have been found to be effective as a catalyst support material rather than as a catalyst.

Other studies have looked at using catalysts to minimize cathodic activation overpotential, and chemical and biological catalysts have been used in MFC systems (O'Hayre et al., 2005). Due to their low overpotential for oxygen reduction, precious metals such as Pt (Logan et al., 2005; Cheng et al., 2006) and Au (Kargi and Eker, 2007) have been tested as cathodic catalysts, with results showing improved MFC efficiency (Logan et al.,2005; Cheng et al., 2006; Kargi and Eker, 2007). Other studies have looked into the use of non-precious metals as cathode catalysts, such as the use of PbO_2-coated electrodes, and although the results showed improved power production as compared to Pt-coated electrodes, PbO_2 is regarded as a potential danger in the event of cathode leaching, so its use is limited (Zhao et al., 2006; Morris et al., 2007). Other studies have suggested using a binding material to boost catalyst stability, and Cheng et al. (2006) investigated the use of Nafion and polytetrafluoroethylene (PTFE) as a Pt binder, finding a 12 percent increase in cathode potential and a 14 percent increase in maximum power density with the Nafion binder (Cheng et al., 2006).

Zhao et al. (2006) and Oh et al. (2004) investigated the use of an iron-based material, pyrolyzed-Fe(II) phthalocyanine (Pyr-FePc), and a cobalt-based material, cobalt-tetramethylphenylporphyrin (CoTMPP), as cathode catalysts, and found that their findings were similar to that of a Pt-coated electrode (Zhao et al., 2006; Oh et al., 2004). Chen et al. (2006) investigated the use of CoTMPP coated electrodes in an air-cathode MFC and Zuo et al. (2007) investigated the use of CoTMPP coated electrodes

in a catalytic tubular membrane cathode, and these experiments yielded similar findings to Zhao et al. (2006) and Oh et al. (2004). Other research findings have shown that metal-based catalysts are susceptible to high cathodic pH, prompting Zhao et al. (2006) to investigate the effects of catholyte composition on iron and cobalt-based oxygen reduction catalysts. And their findings show that at pH 3.3, reducing the phosphate buffer (catholyte) concentration from 500 to 50 mM reduced pyr-FePc coated electrode output by 40%, while increasing pH from 2.4 to 7 at a 500mM phosphate buffer concentration resulted in an 80% reduction in oxygen reduction by a CoTMPP coated electrode. Zhao et al. (2006) stated that increasing catalyst loading can boost cathodic efficiency by compensating for the unfavorable low pH and low buffering ability (Zhao et al., 2006). Other research has looked into the possibility of cathode modification to reduce the cathodic activation overpotential by increasing the cathode surface region (Oh et al., 2004; Zuo et al., 2007; Freguia et al., 2007). More ORR reaction sites are given in this way, which improves cathode kinetics. Oh and Logan (2006) conducted this type of research, and their results showed that increasing the cathode surface area by 11-fold at a fixed anode surface area in a double chamber MFC increased maximum power density (Oh and Logan, 2006). Investigations by Oh et al., 2004; Kargi and Eker, 2007; Zuo et al., 2007; Fan et al., 2007) found similar results. However, since raising reactor volume to accommodate electrodes with large surface areas has proven difficult, studies have looked into the use of various electrode materials and designs to increase the number of available reaction sites while minimizing specific surface area (Logan and Regan, 2006; Rismani-Yazdi et al., 2008). The use of electrode materials such as woven graphite felt, woven graphite mat, granular graphite, and reticulated vitreous carbon, as well as tubular cathodes, was investigated, with results pointing to large-scale MFC applications (Gil et al., 2003; Park and Zeikus, 2003; Ringeisen et al., 2006; Zuo et al., 2007; Freguia et al., 2007; Rabaey et al., 2005; He et al., 2005).

Other research into cathode overpotential reduction has revealed how increasing the oxidant concentration affects MFC performance, as evidenced by the Nernst equation and reduction reaction kinetics (Srinivasan, 2006). The Nernst equation (equation 2.6) states that the optimal thermodynamic voltage is defined by the concentrations of reactants and products at the reaction site, but other experiments have found low thermodynamic gain as oxidant concentrations rise due to the logarithmic structure of

the Nernst equation, which compares with increased reaction rates, which improve cathode kinetics. Srinivasan (2006).

$$aO + e^{-} \rightarrow bR \quad \text{.....2.14.}$$

where O and R are the oxidant and reduced species respectively, while a and b are the corresponding stoichiometric coefficients and the reaction rate can be calculated by equation 2.15 below (O'Hayre et al., 2005):

$$r = k[O]^{a} \quad \text{..... 2.15.}$$

where [O] is the concentration of an oxidant and k is the rate constant.

The effect of operating temperature on oxidant reduction kinetics, as well as mass and proton transfer, has also been studied in MFC systems (Amirinejad et al., 2006). Further research from Liu et al. (2005) using a single chamber membrane-less MFC achieved a 9% increase in power density with an increase in operating temperature from 20^{0}C to 32^{0}C, due to increased cathodic potential (Rismani-Yazdi et al., 2008).

MFC efficiency is also harmed by cathodic ohmic losses, which can be minimized by increasing the conductivity of electrolyte materials used as catholyte and proton exchange membranes, as well as reducing electrode spacing (Rismani-Yazdi et al., 2008). Optimizing catholyte composition and concentration will increase MFC efficiency by reducing ohmic resistance caused by low proton concentration at neutral pH and catholyte's low ionic conductivity (Jang et al., 2004; Zhao et al., 2006). An improvement in current production can be achieved by improving cathodic proton transfer as a result of an increase in catholyte ionic power, according to reports (Jang et al., 2004; Gil et al., 2003). In the study Liu and Logan (2004), an improvement in electrolyte ionic strength from 100 to 400 mM with NaCl addition resulted in an 85 percent increase in power output in a single chamber MFC, and this increase in power output was due to a reduction in ohmic resistance (Liu and Logan, 2004).

Oh and Logan (2006) found that adding 0.4M KCl to a double chamber MFC reduced ohmic resistance by 42% (from 1087 Ω to 625 Ω), resulting in an increase in electrolyte conductivity from 10 to 60 mS/cm (Oh and Logan, 2006). Zhao et al. (2006) reported a 53 percent increase in power output at neutral pH double chamber MFC due to an increase in phosphate buffer (catholyte) ionic strength from 50 to 500mM,

resulting in decreased catholyte resistance to proton transfer (Zhao et al., 2006). It has also been stated that the catholyte's extremes of ionic strength and pH can negatively affect metal catalyst and biocatalyst activity, so research into catalyst tolerance to ionic strength and pH in MFC is critical (Rismani-Yazdi et al., 2008).

Physical properties and membrane type optimization, according to O'Hayre et al. (2005), will reduce the inherent resistance to proton transport and, as a result, reduce ohmic losses (O'Hayre et al., 2005). Oh and Logan (2006) investigated the effects of increasing membrane surface area from 3.5cm^2 to 30.6cm^2 in a double chamber MFC with fixed anode and cathode surface areas (22.5 cm^2), and their results revealed an improved power density output of 190 mW/m^2, up from 45 mW/m^2, owing to a reduction in internal resistance from 1110 Ω to 89 Ω. Other experiments have discovered that increasing the membrane surface area to the MFC's total volume increases proton transfer across the membrane (He et al., 2005; Rabaey et al., 2005).

Other research works have looked into electrode spacing to improve MFC efficiency, and Liu et al. (2005) found that reducing electrode spacing to 2cm from 4cm resulted in a 67 percent increase in power density in a membrane-less MFC, with the power increase due to the lower ohmic resistance (Liu et al., 2005). Others have looked into the effects of electrode spacing and found similar results to Liu et al (2005). However, in a membrane-less MFC, oxygen diffusion from the cathode to the anode has been recorded as a result of too small electrode spacing, which stimulates aerobic respiration rather than anaerobic respiration in the anode, resulting in a lower coulombic performance (Rismani-Yazdi et al., 2008). In an MFC with continuous advective flow from anode to cathode, Cheng et al. (2006) observed decreased oxygen diffusion and increased power production (Cheng et al., 2006). Fan et al. (2007) reported an increased Coulombic efficiency from 35 to 71 percent in a membrane-less MFC compared to an MFC without J-cloth at electrode spacing of 1.7cm (Fan et al. 2007). The increased power production in this case is due to oxygen diffusion.

Cathodic mass transport is one of the factors leading to lower MFC performance. It has been discovered that cathodic mass transport is influenced by convection and

diffusion, with convection influencing bulk catholyte mass transport and diffusive transport influencing mass transport at the cathode surface (Rismani-Yazdi et al., 2008). By maintaining high bulk concentrations and ensuring an even distribution of oxidant such as oxygen through the cathode compartment, as well as optimizing MFC parameters such as operating conditions, electrode material, and cathode compartment geometry, mass transport losses can be reduced.

Gil et al. (2003) discovered that the concentration of dissolved oxygen in the catholyte influences the power output of a double chamber MFC. However, the study discovered that increasing dissolved O_2 concentrations were limited by its solubility in water. As a result, increasing oxygen flux to the cathode can be accomplished by stirring and flushing the catholyte with air or pure O_2, as well as recirculating the catholyte. Jang et al. (2004) recorded a two-fold increase in current production after raising the cathode aeration rate by four times, and Jong et al. (2006) investigated the thermophilic MFC performance dependence on the retention time of air-saturated catholyte on a continuous flow between the anode and cathode compartments. Their findings indicated a two-fold increase in current output when retention time was reduced from 2.7min to 0.7min, although further decreases in retention time had little effect on current output, and MFC efficiency at retention times less than 0.7min was unaffected by oxygen availability, according to Jong et al. (2006). (Jong et al., 2006).

He et al. (2007) used an exogenous supply of sucrose as substrate to form a rotating cathode in a bench-scale sediment MFC in order to increase oxygen flux from the air to the underlying water column. They discovered that when half of the rotating cathode was submerged in water and the other half was exposed to air, the cathodic potential increased, which they attribute to O_2 limitation of the cathodic reaction. They also found an improvement in anodic potential as a result of O_2 diffusion in the anode (He et al., 2007).

Studies have shown that O_2 competing for electrons with the anode in the anolyte results in a decrease in current performance and low coulombic efficiency, which is due to an increase in oxygen diffusion to the anode as a result of the external supply of oxygen (Liu and Logan, 2004; Biffinger et al., 2007; He et al., 2007).

Cathode electrode design and material selection, according to Rismani-Yazdi et al. (2008), remain a mystery due to a lack of literature data. Cathode electrode design research should look into the effects of thickness, porosity, composition, structure, and the electrode's high specific area on reducing mass transport losses. It has been suggested that the hydrodynamic flow can be increased, allowing mass transport and thus preventing water accumulation in the cathode (Rismani-Yazdi et al., 2008).

Overpotential losses in the cathode compartment design are a source of concern, as they are said to reduce MFC efficiency. A double chamber MFC with aqueous catholyte, for example, is said to have mass transport limitations due to a lack of hydrodynamic flow and oxygen's low solubility in water (Rismani-Yazdi et al., 2008). Due to biofilm formation on the cathode, oxidant transfer to the cathode is limited in the double chamber MFC. Such limitations are said to become more widespread as the biofilm thickness thickens over time. Chemicals that provide catholyte buffering capacity and ionic conductivity, which reduces active reactive sites on the electrode and limits catalyst activity, are said to have an impact on cathode performance (Jang et al., 2005). Some research switched to air-cathode compartment design to avoid the problems associated with double chamber compartment MFCs, and investigations based on air-cathodes yielded lower mass transport losses (Park and Zeikus, 2003; Liu and Logan, 2004; Liu et al., 2004; Jang et al., 2005). Air-cathodes are said to have an advantage over double-chamber MFCs because they don't need any energy for oxygen supply and don't include catholyte, which removes all catholyte-related issues (Rismani-Yazdi et al., 2008). Jang et al. (2005) discussed issues with membrane-less air-cathodes, such as (i) decreased catalyst activity due to salt accumulation from cations and anions permeation through the membrane, and (ii) cathode flooding, which results in cathode mass transfer losses due to slow oxidation replenishment through diffusion (Jang et al., 2005). Cheng et al. (2006) proposed coating the air-side with polytetrafluoroethylene to reduce cathode flooding, but the study also suggested changing the coating material and thickness to control oxygen flux while reducing cathode flooding (Cheng et al., 2006). Parasitic losses are those that occur as a result

of substrate crossing and unintended reactions in the cathode (fig. 2.6). Zuo et al., 2007; Liu and Logan, 2004; Biffinger et al., 2007; Kim et al., 2007) These crossovers are said to occur as a result of molecular diffusion and electro-osmosis in membrane-less MFCs because there is no physical barrier separating the anode and cathode compartments (Jiang and Chu, 2004). Substrate crossover is said to have a number of negative effects on cathode production, including lowering cell potential below the thermodynamically expected value, decreasing coulombic efficiency due to substrate use away from the anode, and poisoning of the cathode catalyst (Rismani-Yazdi et al., 2008). Improvements in membrane materials, cathode catalysts, and electrode materials may be able to reduce substrate crossover and its negative effects on cathode performance.

The potential of MFCs to generate electricity in bioremediation processes provides a specific and systematic approach to waste management and energy generation. Large-scale applications of microbial fuel cells, however, remain a challenge due to the low power output offered by these devices. Researchers have increased their efforts in this area to improve microbial fuel production for large-scale applications. Because of their high power output, MFCs could be used in industries like manufacturing, municipal, and agricultural waste remediation.

Although the materials used to create a microbial fuel cell differ, some studies have used glass, polycarbonate, or plexiglass for MFC construction (Rhoads et al., 2005), and electrode materials such as carbon cloth, carbon paper, graphite rod, and graphite felt are commonly used (Zhang et al., 2011; Zhou et al., 2011 Wei et al., 2011; Sangeetha & Muthukumar, 2013). Others planned single-chamber MFCs with only the anodic chamber connected to an air-cathode, while others concentrated on two-chamber MFCs (Du et al., 2007). Table 2.3 lists some of the single chamber MFC designs that have been released.

Table 2.3: Examples of some of the single chamber MFC designs (Tharali et al., 2016).

Design	Anode	Cathode	Reference
Rectangular Cylindrical	Mn^{4+}graphite anode. Carbon paper without wet proofing.	Fe^{3+} graphite cathode. Carbon electrode/PEM assembly or rigid carbon paper without PEM.	Park and Zeikus (2003) Liu and Logan(2004); Cheng et al. (2005); Grot et al. (2005); Cheng et al. (2006)
Tubular Concentric design	Granular graphite matrix. Graphite	Ferricyanide solution. Air porous made up of carbon/Pt.	Rabaey et al. (2005) Liu et al.(2004)
Flate plate	Carbon paper	Carbon cloth	Min and Logan (2004)

Over the years' studies have utilized various complex organic substrates for electricity generation in microbial fuel cells. Some of the organic substrates utilized in microbial fuel cells include domestic wastewater (Choi & Ahn, 2013); swine wastewater (Min et al., 2005); Oil wastewater (Jiang et al., 2013; Choi & Liu, 2014) Waste sludge (Ge et al., 2013; Choi & Ahn, 2014); Fruit & vegetable wastes (Logrono et al., 2015); Although some studies have reported using basic substrates such as glucose, acetate, propionate, and butyrate, others have reported using food waste leachate (Ahn & Logan, 2010).

Although PEM contributes positively to the activity of an MFC, literature has also identified challenges associated with PEM, such as internal resistance, which limits power output due to a PEM's electrode surface area being smaller than the electrode surface area of an MFC (Oh and Logan, 2006; Tharali et al., 2016). Various PEMs used in MFCs have also been reported in the literature. Nafion (Bond & Lovley, 2003), Ultrex (Rabaey et al., 2005; Taskan et al., 2014), and salt-bridge are some of the most well-known (Nair et al., 2013). A sulfonated tetrafluroethylene copolymer with a hydrophobic fluorocarbon backbone ($-CF_2-$) bound to hydrophilic sulfonate groups (SO_3^-) is the chosen Nafion (Mauritz & Moore, 2004). Catholyte pH increase and decrease in anolyte pH effectively decreasing MFC output efficiency are some of the challenges faced when using Nafion membranes (Gil et al., 2003). The key cause of such pH variations is the accumulation of cations and increased conductivity in the cathodic chamber as a result of the membrane's preference for cationic species other

than protons (Rozendal et al., 2006). The thermally less stable chitosan membranes (Mukoma et al., 2004), sulfonated polyimides (Genies et al., 2001), BAM, and sulfonated styrene-ethylene-butylene-styrene (SEBS) membranes have all been documented in the literature (Hickner et al., 2004). Water absorption and conductivity are two recorded characteristics of a good membrane; however, water absorption has been found to influence the mechanical properties of a membrane, even though it facilitates proton transport through the membrane (Tharali et al., 2016; Hickner et al., 2004). Cation exchange membranes (CEM) and anion-exchange membranes have also been discussed in the literature (AEM). CEMs are separators with negatively charged groups such as $–PO_3^-$, COO^-, and $C_6H_4O^-$ that are selective towards positive ions and inhibit the flow of negative ions through them, according to Rahimnejad et al. (2015). (Rahimnejad et al., 2015). Peighambardoust et al. (2010) characterize AEMs as membranes with positively charged groups attached to the membrane matrix, such as NH_3^+, NHR_2^+, NR_2H^+, NR_3^+, PR_3^+, and SR_2^+, that are selective towards negatively charged ions while inhibiting the flow of positive ions through them (Rahimnejad et al., 2015). The research Kim et al. (2007) compared the efficiency of AEM, CEM, and three different ultrafiltration membranes with different molecular cut-offs, and found that AEMs were more efficient in terms of power density, which they attribute to the phosphate ions facilitating protons transport across the membrane and the low internal resistance (Kim et al., 2007). Other studies have used salt-bridges as separators in MFC studies, such as Sevda and Sreekrishnan (2012), who achieved a power density of 84.99mW/m^3 with a salt bridge containing 5% salt, and Nair et al. (2013), who researched the optimum concentration of agarose and found 10% agarose to be the optimum concentration for electricity generation (Tharali et al., 2016). As shown in the studies of Nair et al. (2013) and Sevda and Sreekrishnan, a salt-bridge can be used as an alternative to mediators in MFCs (2012).

2.9 RECENT DEVELOPMENTS IN MFC STUDIES

Recent research findings on MFC architecture (Logan 2010; Logan et al., 2006; Shimoyana et al., 2008), electron transfer mechanisms (Debabov, 2008; Reguera et al., 2005; Torres et al., 2010), and anode and cathode materials (Kang et al., 2003; Logan

et al., 2007; Rizzo et al., 2010) have increased (Jadhav and Ghangrekar, 2009). This section will go through some of the most recent developments in some of the parameters that are crucial to the MFC's performance.

Microbial fuel cells' low power output is a major barrier to their use in industry, prompting a number of research efforts to increase MFC performance (Pant et al., 2010). One of the parameters to consider is the impact of external resistance on MFC efficiency, and studies have looked into the role of external resistance in MFC for maximum power output. For example, Woodward et al. (2010) reports absolute control of external resistance (R_{ext}) as one of the ways to harness maximum power output from a microbial fuel cell (Woodward et al., 2010). (2005, Fuel Handbook) Pinto et al. (2011) state that R_{int} is dependent on parameters such as temperature, pH, influent strength, and influent composition, and that it is crucial to control and use the correct R_{ext} while running a microbial because the continuous metabolic method affects some of these parameters (Pinto et al., 2011).

Pasternak et al. (2018) investigated the impact of external resistance on the development of anode biofilms and their findings indicated a reliable MFC operation as well as an optimum start-up time for maximizing the power output of a microbial fuel cell (Pasternak et al., 2018). They (Pasternak et al. (2018)) tested a human-urine-fed microbial fuel under various external resistances ranging from 50Ω to 10kΩ and discovered that lower external resistance MFCs performed better for biofilm maturation than higher external resistance MFCs, which suffered from ohmic losses and poor performance. In their study, Pasternak et al. (2018) observed changes in biofilm behavior during maturation at various external resistances. The initial conditions of biofilm maturation, it has been noted, have a long-term effect on its structure, properties, and activity.

Studies into the effect of anode modification on microbial fuel cell production have been conducted, such as Hou et al. (2013), who investigated a carbon cloth anode coated with reduced graphene oxide and polyaniline and achieved a maximum power density of 1390mW/m^2, which is three times higher than an MFC with a plain carbon

cloth anode. Enhanced electron transfer efficiency as well as bacterial film loading are due to graphene oxide's high conductivity as well as the large surface area it offers for polyaniline (Hou et al., 2013). Hou et al. (2013) prepared and fabricated reduced graphene oxide on carbon cloth using an electrochemical synthesis method before coating the carbon cloth surface with polyaniline nano-fibres. Lin et al. (2019) investigated the effect of anode modification on biofilm formation and electrogenic activity using a carbon cloth fabricated with reduced graphene and polyaniline, and they discovered high power output with accelerated electrogenic biofilm formation and improved electrogenic bacterial colonization (Lin et al., 2019). The graphene and polyaniline modified anode was said to take 2.4 times less time to acclimate than the plain anode electrode when inoculated with wastewater (Lin et al., 2019).

Microbial fuel cell research is still looking into and exploring more critical factors that affect MFC performance, with improving Coulombic efficiency being one of the most important areas of study (Kim et al., 2008; Logan et al., 2006; Rabaey and Verstraete, 2005). In such investigations, researchers try to figure out what causes electron losses as measured by Coulombic efficiency. There are concerns about the amount of electrons released from substrate by microbes in the anode chamber that are efficiently used for electricity generation, and although electrons are transmitted through the circuit, other electron loss sources have been found, including microbial growth on the anode, gas production, and metabolite production (Kim et al., 2011). As a result, researchers have tried to classify electron pathways from subsequent microbial metabolism under different conditions, as well as using electron balances to assess electron losses. However, it's said to be difficult to generalize electron balances as a norm since different ecosystems have different microbial behaviors, resulting in different electron pathways (Freguia et al., 2007; Lee et al., 2008a). In addition to the electron balances method, other research identified methane production as a contributing factor to electron losses and proposed suppressing methane generation in the anode chamber to improve the Coulombic efficiency in microbial fuel cells (Chae et al.,2009a; Rabaey and Verstraete, 2009; Freguia et al.,2008; Wagner et al.,2009).

To investigate the Coulombic efficiency improvement, Kim et al. (2011) proposed and developed a dual anode electrode glucose-fed microbial. Initial tests on a single-anode glucose-fed MFC yielded a 15% Coulombic efficiency with major electron losses,

which were due to the formation of a glucose metabolite called residual propionate. As a result, Kim et al. (2011) carried out additional research on a dual anode microbial fuel cell (DAMFC) with two separate anodes, each filled with glucose and propionate, to improve Coulombic performance. The results showed that both propionate and glucose could be used effectively in a DAMFC, raising the Coulombic efficiency from around 33% to around 59%.

Despite recent developments in microbial fuel cell technology, one of the main focus areas for improving power output in microbial fuel cells has been research into cathode performance enhancement, due to the significance of scaling up for realistic applications (Logan,2010; Santoro et al.,2017; Rinaldi et al., 2018). As a result, research has focused on improving the cathode's various aspects, such as catalyst development, cathode material, and so on. Research efforts into improving cathode efficiency have increased in recent years as a result of low cathode performance caused by cathode overpotential and kinetic limitations of ORR. One of the most important research areas of MFC production has been the discovery of new ORR catalysts. Previously, platinum-group metal-based catalysts were the preferred ORR catalysts in MFCs, but their high costs and susceptibility to poisoning by various organics and pollutants in wastewaters necessitate the development of new catalysts that are functional, robust, reliable, and cost-effective. As a result, a wide range of materials are being investigated and tested as potential ORR catalysts, with many of them showing promise as oxygen reduction rection catalysts in MFCs.

Manganese dioxide (MnO_2), a possible cathode catalyst in MFCs, has recently been the subject of study. Zhang et al. (2009) developed three manganese dioxide catalysts, namely β-MnO_2, γ-MnO_2, and α-MnO_2, and tested them in an MFC as cathode catalysts (Zhang et al., 2009). These were created to replace Pt, as well as the highly toxic lead dioxide and the toxicity of transition metal macrocycles and pthalocyanines. As a result, in a neutral medium MFC, the performance of manganese oxide as a cathode catalyst was evaluated and compared to that of Pt. Zhang et al. (2009) found that all three manganese dioxide catalysts (β-MnO_2, γ-MnO_2, α-MnO_2) had strong ORR activity, with β-MnO_2 having the best catalytic activity in comparison to the others due to its large BET surface region. The study was able to achieve a maximum

power density of 373mW/m^2 with β-MnO_2, while also recommending that higher catalyst loading be used to increase power output.

In order to increase ORR activity in MFCs, Woon et al. (2015) investigated the use of MnO_2/CNT as a cathode catalyst. Carbon-based catalysts have been reported to have good conductivity, stability, and cost, while manganese oxide has been reported as a promising electrocatalyst with good ORR activity (Woon et al., 2015; Khan et al., 2015; Trogadas et al., 2014). To produce MnO_2/CNT, the researchers synthesized a MnO_2 catalyst and modified it by inducing it with carbon nanotubes (CNT) using a sonochemical-coprecipitation process. At 28.57mg/cm^2 catalyst loading, the analysis achieved a maximum power density of 215.57mW/m^3 and a COD removal efficiency of 22%.

Due to the high cost of Pt, Fe, and Co related macrocyclic complexes, Xia et al. (2018) developed cobalt oxides as an alternative. The ORR activity of a cobalt oxide cathode in an MFC was investigated because the other alternative manganese oxide produces unsatisfactory MFC ORR activity (Xia et al., 2018). With a cobalt oxide loading of 5mg/cm^2, Xia et al. (2018) obtained a maximum power density of approximately 1220mW/m^2, compared to 1360mW/m^2 with a Pt/C cathode. The study achieved a maximum power density of 1540mW/m^2 with a cobalt oxide loading of 30mg/cm^2, which was stated to be 13 percent higher than the power achieved by Pt/C. The research also discovered that the toughness of cobalt oxides is virtually equal to that of Pt/C, but the costs of Pt/C are considerably higher.

Other studies have looked at ferrous-based materials and found that they have strong catalytic activity (Xia et al., 2013; Birry et al., 2011; Zhao et al., 2005; Tang et al., 2010; Toda et al., 1999; Zhang et al., 2011; Lowy et al., 2006; Wu et al., 2012; Zhang et al., 2009a; Cheng and Wu, 2013). In a study by Fu et al. (2015), a modified activated carbon air-cathode coated with non-stoichiometric nano Fe_3O_4 was prepared and tested as a microbial fuel cell alternative. The study achieved a maximum power density of 1430mW/m^2, an improvement of 83.3 percent over a plain cathode, while also finding a decrease in external resistance. The research discovered non-stoichiometric nano

Fe_3O_4 as a promising cathode catalyst material in MFCs. Yuan et al. (2017) used a combination of a microbial fuel cell and a catalytic oxidation reactor to explore the feasibility of using iron phthalocyanine as a cathode catalyst for Congo red degradation. The study found that the overall power density was 808.3mW/m^3, and that Congo red was decomposed into less and more degradable organics including malonic acid and maleic acid, suggesting a promising alternative to pollutant removal.

Iron aminoantipyrine (Fe-AAPyr), graphene nanosheets (GNSs), and their physical mixture Fe-AAPyr-GNS, as well as activated carbon, were formed by Kodali et al. (2018), and their performances as MFCs cathode catalysts for oxidation reduction reactions were compared. The ORR activities of the catalysts were investigated using the rotating ring disk electrode (RDDE) at various loadings and in an air-cathode. According to the results, Fe-AAPyr had a maximum power density of around 218W/cm^2, 150W/cm^2 for GNS, and 235W/cm^2 for Fe-AAPyr-GNS, while activated carbon had a maximum power density of around 104W/cm^2. The amount of hydrogen peroxide found in AC and GNS suggested a possible two electron pathway (direct $2e^-$), while only a small amount of hydrogen peroxide was detected in Fe-AAPyr, suggesting a possible 2x$2e^-$ transfer mechanism (Kodali et al., 2018).

According to previous research, porous catalysts have strong ORR activity because they have additional active sites for oxygen, enhancing ORR activity (Ge et al., 2016). Kumar et al. (2016) investigated the potential of previously successfully tested cobalt oxide as a cathode catalyst to improve ORR kinetics in order to develop an effective low cost electrochemical catalyst that would result in a high ORR rate (Hou et al., 2016; Li et al., 2016; Liu et al., 2014; Song et al., 2015; Xu et al., 2012; Yang et al., 2016). Using a hydrothermal process and cobalt oxide as a possible ORR catalyst, the analysis synthesized varying concentrations of porous Co_3O_4 nanorods and investigated the possibility of using porous Co_3O_4 as cathode catalysts in a double chamber MFC. Researchers discovered improved cathode ORR kinetics, with a maximum power density of 503mW/m^2, owing to the porous property of the nanomaterial (Kumar et al., 2016). The study also suggested that porous catalysts be combined with other highly active ORR active catalysts as cathode catalysts in MFCs.

Recent interest in composite nanomaterials as potential ORR catalysts for improving MFC efficiency reflects recent developments in ORR catalysts in MFC. Jadhav et al. (2017), for example, investigated the possibility of using palladium-substituted-zirconium oxide and palladium-substituted-manganese oxide as cathode catalysts in a single chamber MFC. Electrochemical findings showed that these composite catalysts had higher ORR activity than single metal oxide cathode catalysts like MnO_2 and ZrO_2, indicating improved MFC power generation (Jadhav et al., 2017). Cao et al., 2016a evaluated a nitrogen/iron co-doped carbon (N/ Fe–C) composite cathode catalyst with a three-dimensional porous structure, which achieved a maximum power density of 745 mW/m^2 (11 percent higher than platinum) while also providing a lower charge transfer resistance (Cao et al., 2016a). Tian et al. (2017) investigated the use of a porous $Cu3(BTC)_2$ [BTC = 1, 3, 5-benzene tricarboxylate acid] metal framework as a possible novel type ORR catalyst in MFCs (Tian et al., 2017). The study achieved a power density of 1772 mW/m^2, nearly double that of a plain activated carbon cathode, while also revealing a higher BET surface area of 2159.7 m^2g^{-1} containing several pores that revealed divalent copper in the extended 3D frameworks and may have been the ORR redox centres (Tian et al., 2017).

There have also been studies that suggest creating mesoporous morphologies to improve ORR behavior (Gnana Kumar et al., 2014; Guerrini et al., 2015; Huang et al., 2015). The ORR activity is increased as a result of the availability of additional ORR active sites that enable electrolyte diffusion through the inner surface of the catalysts, as provided by the porous architecture of the catalysts (Ahn et al., 2014; Liu et al., 2015). Kumar et al. (2018) explored the use of novel mesoporous $MnCo_2O_4$ and Co_3O_4 nanorods as ORR catalysts in their research. In a neutral pH medium MFC, their output was investigated, with $MnCo_2O_4$ achieving a maximum power density of 587mW/m^2 (29 percent higher than Co_3O_4) and a current density of 6.01A/m^2 (12.4 percent higher than Co_3O_4) (Kumar et al., 2018). $MnCo_2O_4$'s improved MFC efficiency as an ORR catalyst is attributed to improved ORR kinetics and increased electro-conductivity as a result of its high BET surface area due to the presence of positively charged ions such as Co^{2+}/Co^{3+} and Mn^{3+}/Mn^{4+} on its surface. Low diffusion resistance (R_d), activation resistance (R_a), and ohmic resistance (R_{ohm}) were also observed in the study,

indicating that improved ORR kinetics resulted in improved MFC efficiency. As a result, the use of $MnCo_2O_4$ nanorods as cathode catalysts has potential in the production of MFCs.

Manganese and cobalt oxide are said to be more durable, cost-effective, and environmentally friendly than platinum metals (Wen et al., 2012; Zhang et al., 2015). When using MnO_2/CNT as an MFC cathode catalyst, Zhang et al. (2011) achieved 210mW/m^2 maximum power density, compared to 229mW/m^2 when using platinum as a cathode catalyst. In contrast, by using manganese-polypyrrole-carbon nanotube coated air-cathode in a single chamber MFC, Lu et al. (2013) achieved 213mW/m^2 maximum power density when compared to platinum catalyst. During MFC power generation, the manganese-polypyrrole-carbon catalyst exhibits long-term stability, according to the report (Lu et al., 2013). Similarly, Yu et al. (2015) studied the cobalt and nitrogen-doped carbon coated cathode MFC and found that the cathode had a higher electrocatalytic efficiency and power density than the platinum catalyst (Yu et al., 2015). In this case, combining highly electroactive cobalt oxide and manganese in a mesoporous structure improves cathode ORR operation.

Su et al. (2013) used a simple hydrothermal process to create Co_3O_4/N-G, a nanocomposite of cobaltosic oxide and nitrogen-doped graphene. The catalyst was also found to be highly active and stable in linear sweep voltammetry studies. MFC tests based on Co_3O_4/N-G coated cathodes achieved a maximum power density of approximately 1340mW/m^2, which was found to be four times higher than that of the plain cathode but lower than that of a Pt/C coated cathode (Su et al (2013). The study concluded that the catalyst could be used in future MFC applications based on their results.

Jiang et al. (2017) contrasted the efficiency of graphite/MnO_2 and MoS_2 nanocomposites as ORR catalysts in an MFC. Jiang et al. (2017) contrasted the efficiency of graphite, β-MnO_2, and MoS_2 mixtures with different weight proportions, such as 20:1:1, 30:1:2, and 30:2:1. A cathode catalyst made of graphite, β-MnO_2, and MoS_2 are measured at weight proportions of 20:1:1, as well as a cathode catalyst made of graphite,β-MnO_2 at weight proportions of 10:1. Without ultra-sonication, the analysis achieved a maximum power density of 120mW/m^2 with a mixture of graphite, β-MnO_2, and MoS_2 at weight proportions of 20:1:1, while ultra-sonication increased the maximum power density to 183mW/m^2.

However, the catalyst prepared with graphite, β-MnO_2, and MoS_2 at weight ratios of 20:1:1 obtained a maximum power density of 158mW/m^2, which is higher than the catalyst prepared with graphite, β-MnO_2, and MoS_2 at weight ratios of 20:1:1.

Overall, Jiang et al. (2017) discovered the following pattern in terms of catalyst efficiency in long-term operations, in decreasing order: (ß-MnO_2) > 20:1:1 (ultra-sonicated β-MnO_2) > 10:1 (ß-MnO_2) > 30:2:1 (β-MnO_2) > 30:1: According to the results, the best catalyst is one made with graphite, ß-MnO_2, and MoS_2 in a 20:1:1 ratio (Jiang et al). (2017).

Khilari et al. (2013) investigated MFC efficiency in a single chamber microbial fuel cell using graphene sponsored α-manganese dioxide nanotubes (α-MnO_2/CNTs) as a cathode catalyst. The researchers used a hydrothermal process to prepare a α-MnO_2/CNTs catalyst with graphene as a support (α-MnO_2/CNTs/graphene), -manganese dioxide nanotubes supported on multi-walled carbon nanotubes (α-MnO_2/CNTs/MWCNTs), and α-manganese dioxide nanotubes supported on Vulcan XC (α-MnO_2-NTs/Vulcan XC. The catalysts were tested on a single chamber MFC with a fixed catalyst loading of 0.3 mg/cm^2, and the effects of each carbon help on the α-MnO_2/CNTs were compared to that of Pt/C. The maximum power density for α-MnO_2/CNTs/graphene was 4.68 W/m^3, 3.94 W/m^3 for α-MnO_2/CNTs/MWCNTs, 2.2 W/m^3 for α-MnO_2/CNTs/Vulcan XC, and 5.67 W/m^3 for Pt/C catalyst in the analysis (Khilari et al., 2013). In addition, when compared to α-MnO_2/CNTs/MWCNTs and α-MnO_2/CNTs/Vulcan XC, the α-MnO_2/CNTs/graphene showed strong ORR catalytic activity and improved performance in terms of Coulombic efficiency (CE), COD elimination, and exhibited reduced resistance. It met the Pt/C benchmark, and as a result, it is considered a viable alternative candidate for large-scale MFC operations (table 2.4) (Khilari et al., 2013).

Table 2.4 Effect of different carbon supports on α-MnO_2/CNTs as compared to the Pt/C benchmark based on a single chamber MFC at fixed catalyst loading of 0.3mg/cm2 (Khilari et al., 2013).

sMFC with different cathode	Catalyst-free	α-MnO_2/CNTs /Vulcan XC	α-MnO_2/CNTs /MWCNTs	α-MnO_2/CNTs /graphene	Benchmark ptc
Maximum OCP(mV)	677	754	793	812	839
Max. Power density(W/m3)	0.57	2.2	3.94	4.68	5.67
Max. coulombic efficiency	5.0	8.4	11.0	11.5	12.6
COD removal efficiency (%)	69.23	78.7	82.9	83.7	84.37
Internal resistance(Ω)	172	108	97	85	75

Due to its specific structural, physicochemical, and mechanical properties, graphene-based materials have also been investigated as a catalyst support material in MFC applications (Ghorbani et al., 2015). (Zhang et al., 2017). Park and Ruoff (2009) published on some of graphene's properties, including high Young's modulus (1100GPa), strong fracture strength (125GPa), good thermal conductivity (5000W/m. K), good charge mobility (200, 000 cm^2/V.s), very high specific surface area (theoretical value of 2630 m^2/g), magnetism, and interesting transport phenomena (Park and Rouff, 2009). Rutter et al. (2007) discovered that carboxyl, hydroxyl, or epoxy groups bonded to graphene, as well as other atomic lattice defects that serve as strong scattering centers, can alter the graphene electronic structure and thus affect the electrical transport (Rutter et al., 2007). Li et al. (2007) investigated the possibility of reducing graphene oxide (GO) to obtain reduced graphene oxide (rGO) in order to restore the material's conjugated network and electrical conductivity (Li et al., 2007). Because of its simplicity, scalability, and low defect content, Li et al. (2007) used a hydrothermal conversion method to make reduced graphene oxide (rGO). Due to their remarkable properties, Interest in using graphene-based materials in mechanical, optoelectronic, capacitor, and sensing applications was stated by Avouris and Dimitrakopoulos (2012). Yang et al. (2011) looked into the electrochemical properties of graphene oxide-MnO_2 nanocomposites with graphene oxide aid. They used different graphite materials to make MnO_2 supported graphene oxide to use as a supercapacitor electrode material (Yang et al.,2 011).

2.10 AREAS OF APPLICATIONS OF MFCS

MFCs have been shown to have both economic and environmental advantages. This section outlines some of the areas of MFC applications, such as bio-electricity generation, bio-hydrogen generation, and wastewater management, among others, since the literature has recorded a wide range of MFC applications.

Despite the fact that the literature supports MFCs as a novel alternative to power generation, it is mentioned that implementing MFCs is difficult due to their low power output and high material costs, recent studies have shown improvements in power output, such as Logan (2004), who achieved maximum power outputs of up to 50mW/m^2 and 500mW/m^2 with domestic wastewater and gluconic acid, respectively (Logan, 2004). Rabaey et al. (2003) achieved a power density of 3.6W/m^2 using glucose as a substrate and a mixed consortium of microbial communities (Rabaey et al., 2003). Bio-electricity is a reality with MFCs, and efforts to make them operate on a large scale should be increased.

When an external potential is applied to the cathode of an MFC, however, it can be modified to generate hydrogen instead of electricity (Rabaey et al.,2004). Despite achieving hydrogen yields of 43%, Rabaey et al. (2004) discovered that electricity and hydrogen cannot be generated simultaneously in an MFC because hydrogen production falls below the detection limit as electron transfer increases (Rabaey et al., 2004).

The preceding sections addressed developments in research into the use of MFCs in industrial and urban wastewater treatment effluent for waste removal and electricity generation. In both energy production and wastewater treatment, MFCs have proved to be a novel alternative to existing approaches. Maximum power density, Coulombic efficiency, and COD have all been listed in the literature as measuring parameters for MFC efficiency (Tharali et al., 2016).

2.11 CHALLENGES AND PROSPECTS OF MFCS RESEARCH

The microbial populations and microbial behaviors are at the heart of microbial fuel cell research. Awareness and understanding of exoelectrogen characteristics (i.e. chemical, physiological, and electron transfer mechanism) are crucial to MFC research (Varanasi and Das, 2018). As a result, a breakthrough in exoelectrogens populations and operation will provide a novel and integrative solution to carbon-free wastewater

treatment and energy production processes that lead to environmental emission mitigation and waste reduction while still generating value-added goods.

Over the last decade, research into microbial fuel cells has accelerated, and progress has been made toward commercial application; however, large-scale implementations are still hindered by the low power production. Our research is also looking into the potential of microbial fuel cells in the sense of wastewater treatment in South Africa. The study is divided into four chapters: the first and second chapters look into the potential for electricity generation and pollutant removal from beer brewery and domestic wastewater as a measure of growth rate constant, while the third and fourth chapters look into MnO_2/rGO as an alternative catalyst for MFCs.

2.12 REFERENCES

Santoro, C., Serov, A., Villarrubia, C.W.N., Stariha, S., Babanova, S., Artyushkova, K., Schuler, A.J. and Atanassov, P., 2015. High catalytic activity and pollutants resistivity using Fe-AAPyr cathode catalyst for microbial fuel cell application. Scientific reports, 5, p.16596.

Shannon, M.A., Bohn, P.W., Elimelech, M., Georgiadis, J.G., Marinas, B.J. and Mayes, A.M., 2010. Science and technology for water purification in the coming decades. In Nanoscience and technology: a collection of reviews from nature Journals(pp. 337-346).

Aghababaie, M., Farhadian, M., Jeihanipour, A. and Biria, D., 2015. Effective factors on the performance of microbial fuel cells in wastewater treatment–a review. Environmental Technology Reviews, 4(1), pp.71-89.

Bresadola, M., 1998. Medicine and science in the life of Luigi Galvani (1737–1798). Brain Research Bulletin, 46(5), pp.367-380.

Caccavo, F., Lonergan, D.J., Lovley, D.R., Davis, M., Stolz, J.F. and McInerney, M.J., 1994. Geobacter sulfurreducens sp. nov., a hydrogen-and acetate-oxidizing dissimilatory metal-reducing microorganism. Appl. Environ. Microbiol., 60(10), pp.3752-3759.

Logan, B.E. and Elimelech, M., 2012. Membrane-based processes for sustainable power generation using water. Nature, 488(7411), p.313.

Shukla, A.K., Suresh, P., Berchmans, S. and Rajendran, A., 2004. Biological fuel cells and their applications. Curr. Sci, 87(4), pp.455-468.

Pant, D., Van Bogaert, G., Diels, L. and Vanbroekhoven, K., 2010. A review of the substrates used in microbial fuel cells (MFCs) for sustainable energy production. Bioresource technology, 101(6), pp.1533-1543.

Gorby, Y., Wanger, G., Yuzvinski, T., Fields, M. and El-Naggar, M., 2010, June. Electronic and biogeochemical properties of bacterial nanowires. In GEOCHIMICA ET COSMOCHIMICA ACTA (Vol. 74, No. 12, pp. A349-A349). THE BOULEVARD, LANGFORD LANE, KIDLINGTON, OXFORD OX5 1GB, ENGLAND: PERGAMON-ELSEVIER SCIENCE LTD.

Gorby, Y.A., Yanina, S., McLean, J.S., Rosso, K.M., Moyles, D., Dohnalkova, A., Beveridge, T.J., Chang, I.S., Kim, B.H., Kim, K.S. and Culley, D.E., 2006. Electrically conductive bacterial nanowires produced by Shewanella oneidensis strain MR-1 and other microorganisms. Proceedings of the National Academy of Sciences, 103(30), pp.11358-11363.

Holmes, D.E., Chaudhuri, S.K., Nevin, K.P., Mehta, T., Methé, B.A., Liu, A., Ward, J.E., Woodard, T.L., Webster, J. and Lovley, D.R., 2006. Microarray and genetic analysis of electron transfer to electrodes in Geobacter sulfurreducens. Environmental Microbiology, 8(10), pp.1805-1815.

Karube, I., Matsunaga, T., Mitsuda, S. and Suzuki, S., 1977. Microbial electrode BOD sensors. Biotechnology and bioengineering, 19(10), pp.1535-1547.

Davis, J.B. and Yarbrough, H.F., 1962. Preliminary experiments on a microbial fuel cell. Science, 137(3530), pp.615-616.

Qiao, Y., Bao, S.J. and Li, C.M., 2010. Electrocatalysis in microbial fuel cells—from electrode material to direct electrochemistry. Energy & Environmental Science, 3(5), pp.544-553.

Kumar, A.K., Reddy, M.V., Chandrasekhar, K., Srikanth, S. and Mohan, S.V., 2012. Endocrine disruptive estrogens role in electron transfer: bioelectrochemical remediation with microbial mediated electrogenesis. Bioresource technology, 104, pp.547-556.

Su, Y., Zhu, Y., Yang, X., Shen, J., Lu, J., Zhang, X., Chen, J. and Li, C., 2013. A highly efficient catalyst toward oxygen reduction reaction in neutral media for microbial fuel cells. Industrial & Engineering Chemistry Research, 52(18), pp.6076-6082.

O'hayre, R., Cha, S.W., Colella, W. and Prinz, F.B., 2016. Fuel cell fundamentals. John Wiley & Sons.

Du, Z., Li, H. and Gu, T., 2007. A state of the art review on microbial fuel cells: a promising technology for wastewater treatment and bioenergy. Biotechnology advances, 25(5), pp.464-482.

Allen, R.M. and Bennetto, H.P., 1993. Microbial fuel cells. Applied biochemistry and biotechnology, 39(1), pp.27-40.

Bennetto, H.P., 1985. Reduction of'Redox'mediators by NADH and electron transduction in bioelectrochemical systems. Chemistry and Industry, 21, p.695.

Berk, R. S., and Canfield, J. H., 1964. Bioelectrochemical Energy Conversion: Applied and Environment Microbiology, 12(1), pp. 10-12.

Piccolino, M., 1998. Animal electricity and the birth of electrophysiology: the legacy of Luigi Galvani. Brain research bulletin, 46(5), pp.381-407.

Bullen, R.A., Arnot, T.C., Lakeman, J.B. and Walsh, F.C., 2006. Biofuel cells and their development. Biosensors and Bioelectronics, 21(11), pp.2015-2045.

Abrevaya, X.C., Mauas, P.J. and Cortón, E., 2010. Microbial fuel cells applied to the metabolically based detection of extraterrestrial life. Astrobiology, 10(10), pp.965-971.

Gorby, Y.A., Yanina, S., McLean, J.S., Rosso, K.M., Moyles, D., Dohnalkova, A., Beveridge, T.J., Chang, I.S., Kim, B.H., Kim, K.S. and Culley, D.E., 2006. Electrically conductive bacterial nanowires produced by Shewanella oneidensis strain MR-1 and other microorganisms. Proceedings of the National Academy of Sciences, 103(30), pp.11358-11363.

Rohrback, G.H., Scott, W.R. and Canfield, J.H., 1962, May. Biochemical fuel cells. In Proceedings of the 16th Annual Power Sources Conference (Vol. 18).

Habermann, W. and Pommer, E.H., 1991. Biological fuel cells with sulphide storage capacity. Applied microbiology and biotechnology, 35(1), pp.128-133.

Zhang, H., Zhang, H., Aldalbahi, A., Zuo, X., Fan, C. and Mi, X., 2017. Fluorescent biosensors enabled by graphene and graphene oxide. Biosensors and Bioelectronics, 89, pp.96-106.

Khilari, S., Pandit, S., Ghangrekar, M.M., Das, D. and Pradhan, D., 2013. Graphene supported α-MnO 2 nanotubes as a cathode catalyst for improved power generation and wastewater treatment in single-chambered microbial fuel cells. Rsc Advances, 3(21), pp.7902-7911.

Katz, E., Shipway, A.N. and Willner, I., 2003. Handbook of fuel cells—fundamentals, technology and applications. Fundamentals and Survey of Systems, 1, p.355.

Mathuriya, A.S. and Sharma, V.N., 2010. Treatment of brewery wastewater and production of electricity through microbial fuel cell technology. International Journal of Biotechnology and Biochemistry, 6(1), pp.71-80.

Lovley, D.R., 1991. Dissimilatory Fe (III) and Mn (IV) reduction. Microbiology and Molecular Biology Reviews, 55(2), pp.259-287.

Prescott, C., 2009. Carbon accounting in the United Kingdom water sector: a review. Water Science and Technology, 60(10), pp.2721-2727.

Kim, B.H., Ikeda, T., Park, H.S., Kim, H.J., Hyun, M.S., Kano, K., Takagi, K. and Tatsumi, H., 1999. Electrochemical activity of an Fe (III)-reducing bacterium, Shewanella putrefaciens IR-1, in the presence of alternative electron acceptors. Biotechnology Techniques, 13(7), pp.475-478.

Lovley, D.R., 1993. Anaerobes into heavy metal: dissimilatory metal reduction in anoxic environments. Trends in ecology & evolution, 8(6), pp.213-217.

Reed, G. and Nagodawithana, T.W., 1991. Brewer's yeast. In Yeast technology (pp. 89-149). Springer, Dordrecht.

Kim, H.J., Hyun, M.S., Chang, I.S. and Kim, B.H., 1999. A microbial fuel cell type lactate biosensor using a metal-reducing bacterium, Shewanella putrefaciens. Journal of Microbiology and Biotechnology, 9(3), pp.365-367.

Niessen, J., Schröder, U., Harnisch, F. and Scholz, F., 2005. Gaining electricity from in situ oxidation of hydrogen produced by fermentative cellulose degradation. Letters in applied microbiology, 41(3), pp.286-290.

Bennetto, H.P., 1990. Electricity generation by microorganisms. Biotechnology education, 1(4), pp.163-168.

Potter, M.C., 1911. Electrical effects accompanying the decomposition of organic compounds. Proc. R. Soc. Lond. B, 84(571), pp.260-276.

Ieropoulos, I.A., Greenman, J., Melhuish, C. and Hart, J., 2005. Comparative study of three types of microbial fuel cell. Enzyme and microbial technology, 37(2), pp.238-245.

Suzuki, S., 1976. Fuel cells with hydrogen forming bacteria. Hosp. Hyg. Gesundheitswes. Desinfekt, 68, p.159.

Park, S. and Ruoff, R.S., 2009. Chemical methods for the production of graphenes. Nature nanotechnology, 4(4), p.217.

Ghorbani, M., Abdizadeh, H. and Golobostanfard, M.R., 2015. Reduction of graphene oxide via modified hydrothermal method. Procedia Materials Science, 11, pp.326-330.

Kargi, F. and Eker, S., 2007. Electricity generation with simultaneous wastewater treatment by a microbial fuel cell (MFC) with Cu and Cu–Au electrodes. Journal of Chemical Technology and Biotechnology, 82(7), pp.658-662.

Rutter, G.M., Crain, J.N., Guisinger, N.P., Li, T., First, P.N. and Stroscio, J.A., 2007. Scattering and interference in epitaxial graphene. Science, 317(5835), pp.219-222.

Xia, X., Li, M., Liu, T., Liang, P. and Huang, X., 2018. Facile synthesis of cobalt oxide as electrocatalyst for the oxygen reduction reaction in microbial fuel cells. Chemical Engineering Journal, 342, pp.395-400.

Biffinger, J.C., Ray, R., Little, B. and Ringeisen, B.R., 2007. Diversifying biological fuel cell designs by use of nanoporous filters. Environmental science & technology, 41(4), pp.1444-1449.

Srinivasan, S., 2006. Fuel cells: from fundamentals to applications. Springer Science & Business media.

Lithgow, A.M., Romero, L., Sanchez, I.C., Souto, F.A. and Vega, C.A., 1986. Interception of the electron transport chain in bacteria with hydrophilic redox mediators. I: Selective improvement of the performance of biofuel cells with 2, 6-disulphonated thionine as mediator. Journal of chemical research. Synopses, (5), pp.178-179.

Avouris, P. and Dimitrakopoulos, C., 2012. Graphene: synthesis and applications. Materials today, 15(3), pp.86-97.

Fu, Z., Yan, L., Li, K., Ge, B., Pu, L. and Zhang, X., 2015. The performance and mechanism of modified activated carbon air-cathode by non-stoichiometric nano Fe3O4 in the microbial fuel cell. Biosensors and Bioelectronics, 74, pp.989-995.

Fu, L., You, S.J., Yang, F.L., Gao, M.M., Fang, X.H. and Zhang, G.Q., 2010. Synthesis of hydrogen peroxide in microbial fuel cell. Journal of Chemical Technology & Biotechnology, 85(5), pp.715-719.

Roller, S.D., Bennetto, H.P., Delaney, G.M., Mason, J.R., Stirling, J.L. and Thurston, C.F., 1984. Electron-transfer coupling in microbial fuel cells: 1. comparison of redox-mediator reduction rates and respiratory rates of bacteria. Journal of Chemical Technology and Biotechnology, 34(1), pp.3-12.

Fan, Y., Hu, H. and Liu, H., 2007. Enhanced Coulombic efficiency and power density of air-cathode microbial fuel cells with an improved cell configuration. Journal of Power Sources, 171(2), pp.348-354.

He, Z., Minteer, S.D. and Angenent, L.T., 2005. Electricity generation from artificial wastewater using an upflow microbial fuel cell. Environmental science & technology, 39(14), pp.5262-5267.

He, Z., Shao, H. and Angenent, L.T., 2007. Increased power production from a sediment microbial fuel cell with a rotating cathode. Biosensors and Bioelectronics, 22(12), pp.3252-3255.

Yuan, G.E., Li, Y., Lv, J., Zhang, G. and Yang, F., 2017. Integration of microbial fuel cell and catalytic oxidation reactor with iron phthalocyanine catalyst for Congo red degradation. Biochemical engineering journal, 120, pp.118-124.

Woon, C.W., Ong, H.R., Chong, K.F., Chan, K.M. and Khan, M.M.R., 2015. MnO2/CNT as ORR electrocatalyst in air-cathode microbial fuel cells. Procedia Chemistry, 16, pp.640-647.

Min, C.K., 2014. Nanostructured Pt/MnO2 Catalysts and Their Performance for Oxygen Reduction Reaction in Air-Cathode Microbial Fuel Cell (Doctoral dissertation, Ph. D. Thesis, University Malaysia Pahang, Pekan, Pahang, Malaysia).

HAN, L., 2014. Simultaneous power generation and wastewater treatment by using air-cathode single chamber microbial fuel cell (Doctoral dissertation, UNIVERSITI MALAYSIA PAHANG).

Trogadas, P., Fuller, T.F. and Strasser, P., 2014. Carbon as catalyst and support for electrochemical energy conversion. Carbon, 75, pp.5-42.

Chaudhuri, S.K. and Lovley, D.R., 2003. Electricity generation by direct oxidation of glucose in mediator-less microbial fuel cells. Nature biotechnology, 21(10), p.1229.

Liu, H., Cheng, S. and Logan, B.E., 2005. Power generation in fed-batch microbial fuel cells as a function of ionic strength, temperature, and reactor configuration. Environmental science & technology, 39(14), pp.5488-5493.

Ahn, Y. and Logan, B.E., 2010. Effectiveness of domestic wastewater treatment using microbial fuel cells at ambient and mesophilic temperatures. Bioresource technology, 101(2), pp.469-475.

Jiang, J., Zhao, Q., Zhang, J., Zhang, G. and Lee, D.J., 2009. Electricity generation from bio-treatment of sewage sludge with microbial fuel cell. Bioresource Technology, 100(23), pp.5808-5812.

Wen, Q., Wu, Y., Cao, D., Zhao, L. and Sun, Q., 2009. Electricity generation and modeling of microbial fuel cell from continuous beer brewery wastewater. Bioresource Technology, 100(18), pp.4171-4175.

Jang, J.K., Moon, H.S. and Chang, I.S., 2005. Improved performance of microbial fuel cell using membrane-electrode assembly. Journal of microbiology and biotechnology, 15(2), pp.438-441.

Patil, S.A., Surakasi, V.P., Koul, S., Ijmulwar, S., Vivek, A., Shouche, Y.S. and Kapadnis, B.P., 2009. Electricity generation using chocolate industry wastewater and its treatment in activated sludge based microbial fuel cell and analysis of developed microbial community in the anode chamber. Bioresource technology, 100(21), pp.5132-5139.

Pant, D., Van Bogaert, G., Diels, L. and Vanbroekhoven, K., 2010. A review of the substrates used in microbial fuel cells (MFCs) for sustainable energy production. Bioresource technology, 101(6), pp.1533-1543.

Yasin, M., Jeong, Y., Park, S., Jeong, J., Lee, E.Y., Lovitt, R.W., Kim, B.H., Lee, J. and Chang, I.S., 2015. Microbial synthesis gas utilization and ways to resolve kinetic and mass-transfer limitations. Bioresource technology, 177, pp.361-374.

Zhi, W., Ge, Z., He, Z. and Zhang, H., 2014. Methods for understanding microbial community structures and functions in microbial fuel cells: a review. Bioresource technology, 171, pp.461-468.

Liu, H. and Logan, B.E., 2004. Electricity generation using an air-cathode single chamber microbial fuel cell in the presence and absence of a proton exchange membrane. Environmental science & technology, 38(14), pp.4040-4046.

You, S.J., Zhao, Q.L., Jiang, J.Q., Zhang, J.N. and Zhao, S.Q., 2006. Sustainable approach for leachate treatment: electricity generation in microbial fuel cell. Journal of Environmental Science and Health Part A, 41(12), pp.2721-2734.

Min, B. and Angelidaki, I., 2008. Innovative microbial fuel cell for electricity production from anaerobic reactors. Journal of Power Sources, 180(1), pp.641-647.

Zhan, Y.L., Zhang, P.P., Yan, G.X. and Guo, S.H., 2008. Constructing and operating of mediator and membrane-less microbial fuel cell. Journal of Chemical Engineering of Chinese Universities, 22(1), p.177.

Kim, H.J., Park, H.S., Hyun, M.S., Chang, I.S., Kim, M. and Kim, B.H., 2002. A mediator-less microbial fuel cell using a metal reducing bacterium, Shewanella putrefaciens. Enzyme and Microbial technology, 30(2), pp.145-152.

Picioreanu, C., van Loosdrecht, M.C., Curtis, T.P. and Scott, K., 2010. Model based evaluation of the effect of pH and electrode geometry on microbial fuel cell performance. Bioelectrochemistry, 78(1), pp.8-24.

Huang, L. and Logan, B.E., 2008. Electricity generation and treatment of paper recycling wastewater using a microbial fuel cell. Applied microbiology and biotechnology, 80(2), pp.349-355.

Mohan, S.V., Saravanan, R., Raghavulu, S.V., Mohanakrishna, G. and Sarma, P.N., 2008. Bio-electricity production from wastewater treatment in dual chambered microbial fuel cell (MFC) using selectively enriched mixed microflora: effect of catholyte. Bioresource Technology, 99(3), pp.596-603.

Feng, Y., Wang, X., Logan, B.E. and Lee, H., 2008. Brewery wastewater treatment using air-cathode microbial fuel cells. Applied microbiology and biotechnology, 78(5), pp.873-880.

Wang, X., Feng, Y.J. and Lee, H., 2008. Electricity production from beer brewery wastewater using single chamber microbial fuel cell. Water Science and Technology, 57(7), pp.1117-1121.

Logan, B.E. and Regan, J.M., 2006. Electricity producing bacterial communities in microbial fuel cells. TRENDS in Microbiology, 14(12), pp.512-518

Rabaey, K. and Verstraete, W., 2005. Microbial fuel cells: novel biotechnology for energy generation. TRENDS in Biotechnology, 23(6), pp.291-298.

Lovley, D.R., 2006. Microbial fuel cells: novel microbial physiologies and engineering approaches. Current opinion in biotechnology, 17(3), pp.327-332.

Pham, T.H., Rabaey, K., Aelterman, P., Clauwaert, P., De Schamphelaire, L., Boon, N. and Verstraete, W., 2006. Microbial fuel cells in relation to conventional anaerobic digestion technology. Engineering in Life Sciences, 6(3), pp.285-292.

Chang, I.S., Moon, H.S., Bretschger, O., Jang, J.K., Park, H.I., Nealson, K.H. and Kim, B.H., 2006. Electrochemically active bacteria (EAB) and mediator-less microbial fuel cells. Journal of Microbiology and Biotechnology, 16(2), pp.163-177.

Bullen, R.A., Arnot, T.C., Lakeman, J.B. and Walsh, F.C., 2006. Biofuel cells and their development. Biosensors and Bioelectronics, 21(11), pp.2015-2045.

Rout, S., Nayak, A.K., Varanasi, J.L., Pradhan, D. and Das, D., 2018. Enhanced energy recovery by manganese oxide/reduced graphene oxide nanocomposite as an air-cathode electrode in the single-chambered microbial fuel cell. Journal of Electroanalytical Chemistry, 815, pp.1-7.

Birry, L., Mehta, P., Jaouen, F., Dodelet, J.P., Guiot, S.R., Tartakovsky, B., 2011. Elec- trochim. Acta 56, 1505–1511.

Lowy, D.A., Tender, L.M., Zeikus, J.G., Park, D.H., Lovley, D.R., 2006. Biosens. Bioelectron. 21, 2058–2063.

Tang, Y.W., Cao, S., Chen, Y.F., Lu, T.H., Zhou, Y.M., Lu, L.D., Bao, J.C., 2010. Appl. Surf. Sci. 256, 4196–4200.

Toda, T., Igarashi, H., Watanabe, M., 1999. J. Electroanal. Chem. 460, 258–262.

Cheng, S., Wu, J., 2013. Bioelectrochemistry 92, 22–26.

Wu, Z.S., Yang, S.B., Sun, Y., Parvez, K., Feng, X.L., Müllen, K., 2012. J. Am. Chem. Soc.134, 9082–9085.

Xia, X., Zhang, F., Zhang, X.Y., Liang, P., Huang, X., Logan, B.E., 2013. Appl. Mater.Interfaces 5, 7862–7866.

Zhang, F., Cheng, S.A., Pant, D., Bogaert, G.V., Logan, B.E., 2009a. Electrochem.Commun. 11, 2177–2179.

Zhang, J.N., You, S.J., Yuan, Y.X., Zhao, Q.L., Zhang, G.D., 2011. Electrochem. Commun.13, 903–905.

Zhao, F., Harnisch, F., Schröder, U., Scholz, F., Bogdanoff, P., Herrmann, I., 2005. Electrochem. Commun. 7, 1405–1410.

Cameron, K.D., 2016. Microbial Fuel Cell Possibilities on American Indian Tribal Lands (No. SAND2016-7820PE). Sandia National Lab.(SNL-NM), Albuquerque, NM (United States).

Wang, H., Park, J.D. and Ren, Z.J., 2015. Practical Energy Harvesting for Microbial Fuel Cells: A Review. Environmental Science Technology, 49, pp.3267-3277.

Logan, B.E. and Regan, J.M., 2006. Electricity producing bacterial communities in microbial fuel cells. TRENDS in Microbiology, 14(12), pp.512-518.

Varanasi, J.L. and Das, D., 2018. Characteristics of Microbes Involved in Microbial Fuel Cell. In Microbial Fuel Cell (pp. 43-62). Springer, Cham.

Rismani-Yazdi, H., Carver, S.M., Christy, A.D. and Tuovinen, O.H., 2008. Cathodic limitations in microbial fuel cells: an overview. Journal of Power Sources, 180(2), pp.683-694.

Madjarov, J., Popat, S.C., Erben, J., Götze, A., Zengerle, R. and Kerzenmacher, S., 2017. Revisiting methods to characterize bioelectrochemical systems: the influence of uncompensated resistance (iRu-drop), double layer capacitance, and junction potential. Journal of Power Sources, 356, pp.408-418.

Erable, B., Féron, D. and Bergel, A., 2012. Microbial catalysis of the oxygen reduction reaction for microbial fuel cells: a review. ChemSusChem, 5(6), pp.975-987.

Kinoshita, K., 1988. Carbon: electrochemical and physicochemical properties.

Kinoshita, K., 1992. Electrochemical oxygen technology (Vol. 30). John Wiley & Sons.

Artyushkova, K., Serov, A., Rojas-Carbonell, S. and Atanassov, P., 2015. Chemistry of multitudinous active sites for oxygen reduction reaction in transition metal–nitrogen–carbon electrocatalysts. The Journal of Physical Chemistry C, 119(46), pp.25917-25928.

Shao, M., Chang, Q., Dodelet, J.P. and Chenitz, R., 2016. Recent advances in electrocatalysts for oxygen reduction reaction. Chemical reviews, 116(6), pp.3594-3657.

Yang, W., Kim, K.Y., Saikaly, P.E. and Logan, B.E., 2017. The impact of new cathode materials relative to baseline performance of microbial fuel cells all with the same architecture and solution chemistry. Energy & Environmental Science, 10(5), pp.1025-1033.

Antolini, E., 2016. Structural parameters of supported fuel cell catalysts: the effect of particle size, inter-particle distance and metal loading on catalytic activity and fuel cell performance. Applied Catalysis B: Environmental, 181, pp.298-313.

Picioreanu, C., Head, I.M., Katuri, K.P., van Loosdrecht, M.C. and Scott, K., 2007. A computational model for biofilm-based microbial fuel cells. Water research, 41(13), pp.2921-2940.

Kim, G.T., Webster, G., Wimpenny, J.W.T., Kim, B.H., Kim, H.J. and Weightman, A.J., 2006. Bacterial community structure, compartmentalization and activity in a microbial fuel cell. Journal of applied microbiology, 101(3), pp.698-710.

Gottenbos, B., Van der Mei, H.C. and Busscher, H.J., 1999. [38] Models for studying initial adhesion and surface growth in biofilm formation on surfaces. In Methods in Enzymology (Vol. 310, pp. 523-534). Academic Press.

Davies, D.G., Parsek, M.R., Pearson, J.P., Iglewski, B.H., Costerton, J.W. and Greenberg, E.P., 1998. The involvement of cell-to-cell signals in the development of a bacterial biofilm. Science, 280(5361), pp.295-298.

Mah, T.F.C. and O'Toole, G.A., 2001. Mechanisms of biofilm resistance to antimicrobial agents. Trends in microbiology, 9(1), pp.34-39.

Hall-Stoodley, L., Costerton, J.W. and Stoodley, P., 2004. Bacterial biofilms: from the natural environment to infectious diseases. Nature reviews microbiology, 2(2), p.95.

Lovley, D.R., 2006. Microbial energizers: fuel cells that keep on going. Microbe-American Society for Microbiology, 1(7), p.323.

Logan, B.E., 2009. Exoelectrogenic bacteria that power microbial fuel cells. Nature Reviews Microbiology, 7(5), p.375.

Antolini, E., 2015. Composite materials for polymer electrolyte membrane microbial fuel cells. Biosensors and Bioelectronics, 69, pp.54-70.

Yuan, H., Hou, Y., Abu-Reesh, I.M., Chen, J. and He, Z., 2016. Oxygen reduction reaction catalysts used in microbial fuel cells for energy-efficient wastewater treatment: a review. Materials Horizons, 3(5), pp.382-401.

Wang, Z., Cao, C., Zheng, Y., Chen, S. and Zhao, F., 2014. Abiotic oxygen reduction reaction catalysts used in microbial fuel cells. ChemElectroChem, 1(11), pp.1813-1821.

Arbizzani, C., Beninati, S., Manferrari, E., Soavi, F. and Mastragostino, M., 2006. Electrodeposited PtRu on cryogel carbon–Nafion supports for DMFC anodes. Journal of power sources, 161(2), pp.826-830.

Santoro, C., Serov, A., Villarrubia, C.W.N., Stariha, S., Babanova, S., Artyushkova, K., Schuler, A.J. and Atanassov, P., 2015. High catalytic activity and pollutants resistivity using Fe-AAPyr cathode catalyst for microbial fuel cell application. Scientific reports, 5, p.16596.

Santoro, C., Serov, A., Stariha, L., Kodali, M., Gordon, J., Babanova, S., Bretschger, O., Artyushkova, K. and Atanassov, P., 2016. Iron based catalysts from novel low-cost organic precursors for enhanced oxygen reduction reaction in neutral media microbial fuel cells. Energy & Environmental Science, 9(7), pp.2346-2353.

Li, D., Müller, M.B., Gilje, S., Kaner, R.B. and Wallace, G.G., 2008. Processable aqueous dispersions of graphene nanosheets. Nature nanotechnology, 3(2), p.101.

Minachev, K.M., Shuikin, N.I. and Rozhdestivenskaya, I.D., 1952. Poisoning of platinum catalysts with a low content of active metal on a carrier, under conditions of dehydrogenation catalysis. Russian Chemical Bulletin, 1(4), pp.567-575.

Grattieri, M., Suvira, M., Hasan, K. and Minteer, S.D., 2017. Halotolerant extremophile bacteria from the Great Salt Lake for recycling pollutants in microbial fuel cells. Journal of Power Sources, 356, pp.310-318.

Grattieri, M., Shivel, N.D., Sifat, I., Bestetti, M. and Minteer, S.D., 2017. Sustainable hypersaline microbial fuel cells: inexpensive recyclable polymer supports for carbon nanotube conductive paint anodes. ChemSusChem, 10(9), pp.2053-2058.

Karra, U., Muto, E., Umaz, R., Kölln, M., Santoro, C., Wang, L. and Li, B., 2014. Performance evaluation of activated carbon-based electrodes with novel power management system for long-term benthic microbial fuel cells. International Journal of Hydrogen Energy, 39(36), pp.21847-21856.

Watson, V.J., Nieto Delgado, C. and Logan, B.E., 2013. Influence of chemical and physical properties of activated carbon powders on oxygen reduction and microbial fuel cell performance. Environmental science & technology, 47(12), pp.6704-6710.

Watson, V.J., Delgado, C.N. and Logan, B.E., 2013. Improvement of activated carbons as oxygen reduction catalysts in neutral solutions by ammonia gas treatment and their performance in microbial fuel cells. Journal of Power Sources, 242, pp.756-761.

Guerrini, E., Grattieri, M., Faggianelli, A., Cristiani, P. and Trasatti, S., 2015. PTFE effect on the electrocatalysis of the oxygen reduction reaction in membrane-less microbial fuel cells. Bioelectrochemistry, 106, pp.240-247.

Wang, H., Wu, Z., Plaseied, A., Jenkins, P., Simpson, L., Engtrakul, C. and Ren, Z., 2011. Carbon nanotube modified air-cathodes for electricity production in microbial fuel cells. Journal of Power Sources, 196(18), pp.7465-7469.

Ghasemi, M., Shahgaldi, S., Ismail, M., Kim, B.H., Yaakob, Z. and Daud, W.R.W., 2011. Activated carbon nanofibers as an alternative cathode catalyst to platinum in a two-chamber microbial fuel cell. International Journal of Hydrogen Energy, 36(21), pp.13746-13752.

Santoro, C., Kodali, M., Kabir, S., Soavi, F., Serov, A. and Atanassov, P., 2017. Three-dimensional graphene nanosheets as cathode catalysts in standard and supercapacitive microbial fuel cell. Journal of power sources, 356, pp.371-380.

Sheikhyousefi, P.R., Esfahany, M.N., Colombo, A., Franzetti, A., Trasatti, S.P. and Cristiani, P., 2017. Investigation of different configurations of microbial fuel cells for the treatment of oilfield produced water. Applied energy, 192, pp.457-465.

Lu, G., Zhu, Y., Lu, L., Xu, K., Wang, H., Jin, Y., Ren, Z.J., Liu, Z. and Zhang, W., 2016. Iron-rich nanoparticle encapsulated, nitrogen doped porous carbon materials as efficient cathode electrocatalyst for microbial fuel cells. Journal of Power Sources, 315, pp.302-307.

Santoro, C., Gokhale, R., Mecheri, B., D'Epifanio, A., Licoccia, S., Serov, A., Artyushkova, K. and Atanassov, P., 2017. Design of Iron (II) Phthalocyanine-Derived Oxygen Reduction Electrocatalysts for High-Power-Density Microbial Fuel Cells. ChemSusChem, 10(16), pp.3243-3251.

Santoro, C., Serov, A., Gokhale, R., Rojas-Carbonell, S., Stariha, L., Gordon, J., Artyushkova, K. and Atanassov, P., 2017. A family of Fe-NC oxygen reduction electrocatalysts for microbial fuel cell (MFC) application: relationships between surface chemistry and performances. Applied Catalysis B: Environmental, 205, pp.24-33.

Birry, L., Mehta, P., Jaouen, F., Dodelet, J.P., Guiot, S.R. and Tartakovsky, B., 2011. Application of iron-based cathode catalysts in a microbial fuel cell. Electrochimica Acta, 56(3), pp.1505-1511.

Nguyen, M.T., Mecheri, B., Iannaci, A., D'Epifanio, A. and Licoccia, S., 2016. Iron/polyindole-based electrocatalysts to enhance oxygen reduction in microbial fuel cells. Electrochimica Acta, 190, pp.388-395.

Iannaci, A., Mecheri, B., D'Epifanio, A., Elorri, M.J.L. and Licoccia, S., 2016. Iron–nitrogen-functionalized carbon as efficient oxygen reduction reaction electrocatalyst in microbial fuel cells. International Journal of Hydrogen Energy, 41(43), pp.19637-19644.

de Oliveira, M.A.C., Mecheri, B., D'Epifanio, A., Placidi, E., Arciprete, F., Valentini, F., Perandini, A., Valentini, V. and Licoccia, S., 2017. Graphene oxide nanoplatforms to enhance catalytic performance of iron phthalocyanine for oxygen reduction reaction in bioelectrochemical systems. Journal of Power Sources, 356, pp.381-388.

Baranitharan, E., Khan, M.R., Yousuf, A., Teo, W.F.A., Tan, G.Y.A., Cheng, C.K., 2015. Enhanced power generation using controlled inoculum from palm oil mill effluent fed microbial fuel cell. Fuel 143, 72–79.

Ge, B., Li, K., Fu, Z., Pu, L., Zhang, X., Liu, Z., Huang, K., 2016. The performance of nano urchin-like NiCo2O4 modified activated carbon as air-cathode for microbial fuel cell. J. Power Sources 303, 325–332.

Ghasemi, M., Daud, W.R.W., Rahimnejad, M., Rezayi, M., Fatemi, A., Jafari, Y., Somalu, M.R., Manzour, A., 2013. Copper-phthalocyanine and nickel nanoparticles as novel cathode catalysts in microbial fuel cells. Int. J. Hydrogen Energ. 38, 9533–9540.

Gong, X.B., You, S.J., Wang, X.H., Zhang, J.N., Gan, Y., Ren, N.Q., 2014. A novel stainless steel mesh/cobalt oxide hybrid electrode for efficient catalysis of oxygen reduction in a microbial fuel cell. Biosens. Bioelectron. 55, 237–241.

Guo, K., Soeriyadi, A.H., Feng, H., Prévoteau, A., Patil, S.A., Gooding, J.J., Rabaey, K., 2015. Heat-treated stainless steel felt as scalable anode material for bioelectrochemical systems. Bioresource Technol. 195, 46–50.

He, Y.-R., Du, F., Huang, Y.-X., Dai, L.-M., Li, W.-W., Yu, H.-Q., 2016. Preparation of microvillus-like nitrogen-doped carbon nanotubes as the cathode of a microbial fuel cell. J. Mater. Chem. A 4, 1632–1636.

Hou, Y., Yuan, H., Wen, Z., Cui, S., Guo, X., He, Z., Chen, J., 2016. Nitrogen-doped graphene/CoNi alloy encased within bamboo-like carbon nanotube hybrids as cathode catalysts in microbial fuel cells. J. Power Sources 307, 561–568.

Hosono, E., Fujihara, S., Honma, I., Zhou, H., 2005. Fabrication of morphology and crystal structure controlled nanorod and nanosheet cobalt hydroxide based on the difference of oxygen-solubility between water and methanol, and conversion into Co3O4. J. Mater. Chem. 15, 1938–1945.

Huang, J., Zhu, N., Yang, T., Zhang, T., Wu, P., Dang, Z., 2015a. Nickel oxide and carbon nanotube composite (NiO/CNT) as a novel cathode non-precious metal catalyst in microbial fuel cells. Biosens. Bioelectron. 72, 332–339.

Huang, L., Liu, Y., Yu, L., Quan, X., Chen, G., 2015b. A new clean approach for production of cobalt dihydroxide from aqueous Co(II) using oxygen-reducing biocathode microbial fuel cells. J. Clean. Prod. 86, 441–446.

Huggins, T.M., Pietron, J.J., Wang, H., Ren, Z.J., Biffinger, J.C., 2015. Graphitic biochar as a cathode electrocatalyst support for microbial fuel cells. Bioresource Technol. 195, 147–153.

Kim, J.R., Kim, J.Y., Han, S.B., Park, K.W., Saratale, G.D., Oh, S.E., 2011. Application of Co naphthalocyanine (CoNPc) as alternative cathode catalyst and support structure for microbial fuel cells. Bioresource Technol. 102, 342–347.

Kumar, R., Singh, L., Zularisam, A.W., 2016. Exoelectrogens: recent advances in molecular drivers involved in extracellular electron transfer and strategies used to improve it for microbial fuel cell applications. Renew. Sustainable Energy Rev. 56, 1322–1336.

Kumar, R., Singh, L., Zularisam, A.W., Fadhil, M., 2015. Exoelectrogens in microbial fuel cells toward bio-electricity generation: a review. Int. J. Energy Res. 39, 1048– 1067.

Li, R., Dai, Y., Chen, B., Zou, J., Jiang, B., Fu, H., 2016. Nitrogen-doped Co/ Co9S8/partly-graphitized carbon as durable catalysts for oxygen reduction in microbial fuel cells. J. Power Sources 307, 1–10.

Liu, B., Brückner, C., Lei, Y., Cheng, Y., Santoro, C., Li, B., 2014. Cobalt porphyrin- based material as methanol tolerant cathode in single chamber microbial fuel cells (SCMFCs). J. Power Sources 257, 246–253.

Liu, Z., Ge, B., Li, K., Zhang, X., Huang, K., 2016. The excellent performance and mechanism of activated carbon air-cathode doped with different type of cobalt for microbial fuel cells. Fuel 176, 173–180.

Patil, S.A., Surakasi, V.P., Koul, S., Ijmulwar, S., Vivek, A., Shouche, Y.S., Kapadnis, B.P., 2009. Electricity generation using chocolate industry wastewater and its treatment in activated sludge based microbial fuel cell and analysis of developed microbial community in the anode chamber. Bioresource Technol. 100, 5132–5139.

Song, T.-S., Wang, D.-B., Wang, H., Li, X., Liang, Y., Xie, J., 2015. Cobalt oxide/nanocarbon hybrid materials as alternative cathode catalyst for oxygen reduction in microbial fuel cell. Int. J. Hydrogen Energ. 40, 3868–3874.

Wang, G., Shen, X., Horvat, J., Wang, B., Liu, H., Wexler, D., Yao, J., 2009. Hydrothermal synthesis and optical, magnetic, and supercapacitance properties of nanoporous cobalt oxide nanorods. J. Phys. Chem. C 113, 4357– 4361.

Winfield, J., Gajda, I., Greenman, J., Ieropoulos, I., 2016. A review into the use of ceramics in microbial fuel cells. Bioresource Technol. 215, 296–303.

Xu, J., Gao, P., Zhao, T.S., 2012. Non-precious Co3O4 nano-rod electrocatalyst for oxygen reduction reaction in anion-exchange membrane fuel cells. Energy Environ. Sci. 5, 5333–5339.

Yang, W., Kim, K.Y., Logan, B.E., 2015. Development of carbon free diffusion layer for activated carbon air-cathode of microbial fuel cells. Bioresource Technol. 197, 318–322.

Yang, T., Li, K., Pu, L., Liu, Z., Ge, B., Pan, Y., Liu, Y., 2016. Hollow-spherical Co/NC nanoparticle as an efficient electrocatalyst used in air-cathode microbial fuel cell. Biosens. Bioelectron. 86, 129–134.

Yazdi, A.A., D'Angelo, L., Omer, N., Windiasti, G., Lu, X., Xu, J., 2016. Carbon nanotube modification of microbial fuel cell electrodes. Biosens. Bioelectron. 85, 536–552.Zhang, X., Li, K., Yan, P., Liu, Z., Pu, L., 2015. N-type Cu2O doped activated carbon as catalyst for improving power generation of air-cathode microbial fuel cells. Bioresource Technol. 187, 299–304.

Nguyen, M.T., Mecheri, B., D'Epifanio, A., Sciarria, T.P., Adani, F. and Licoccia, S., 2014. Iron chelates as low-cost and effective electrocatalyst for oxygen

reduction reaction in microbial fuel cells. International Journal of Hydrogen Energy, 39(12), pp.6462-6469.

Kodali, M., Santoro, C., Serov, A., Kabir, S., Artyushkova, K., Matanovic, I. and Atanassov, P., 2017. Air breathing cathodes for microbial fuel cell using Mn-, Fe-, Co-and Ni-containing platinum group metal-free catalysts. Electrochimica acta, 231, pp.115-124.

Santoro, C., Artyushkova, K., Gajda, I., Babanova, S., Serov, A., Atanassov, P., Greenman, J., Colombo, A., Trasatti, S., Ieropoulos, I. and Cristiani, P., 2015. Cathode materials for ceramic based microbial fuel cells (MFCs). International Journal of Hydrogen Energy, 40(42), pp.14706-14715.

Santoro, C., Rojas-Carbonell, S., Awais, R., Gokhale, R., Kodali, M., Serov, A., Artyushkova, K. and Atanassov, P., 2018. Influence of platinum group metal-free catalyst synthesis on microbial fuel cell performance. Journal of power sources, 375, pp.11-20.

Burkitt, R., Whiffen, T.R. and Yu, E.H., 2016. Iron phthalocyanine and MnOx composite catalysts for microbial fuel cell applications. Applied Catalysis B: Environmental, 181, pp.279-288.

Jiang, B., Muddemann, T., Kunz, U., Bormann, H., Niedermeiser, M., Haupt, D., Schläfer, O. and Sievers, M., 2017. Evaluation of microbial fuel cells with graphite plus MnO2 and MoS2 paints as oxygen reduction cathode catalyst. Journal of The Electrochemical Society, 164(3), pp.H3083-H3090.

Rojas-Carbonell, S., Santoro, C., Serov, A. and Atanassov, P., 2017. Transition metal-nitrogen-carbon catalysts for oxygen reduction reaction in neutral electrolyte. Electrochemistry Communications, 75, pp.38-42.

Kumar, R., Singh, L., Zularisam, A.W. and Hai, F.I., 2016. Potential of porous Co3O4 nanorods as cathode catalyst for oxygen reduction reaction in microbial fuel cells. Bioresource technology, 220, pp.537-542.

Hou, Y., Yuan, H., Wen, Z., Cui, S., Guo, X., He, Z. and Chen, J., 2016. Nitrogen-doped graphene/CoNi alloy encased within bamboo-like carbon nanotube hybrids as cathode catalysts in microbial fuel cells. Journal of Power Sources, 307, pp.561-568.

Huang, J., Zhu, N., Yang, T., Zhang, T., Wu, P. and Dang, Z., 2015. Nickel oxide and carbon nanotube composite (NiO/CNT) as a novel cathode non-precious metal catalyst in microbial fuel cells. Biosensors and Bioelectronics, 72, pp.332-339.

Modi, A., Singh, S. and Verma, N., 2016. In situ nitrogen-doping of nickel nanoparticle-dispersed carbon nanofiber-based electrodes: Its positive effects on the performance of a microbial fuel cell. Electrochimica Acta, 190, pp.620-627.

Lee, J., Phung, N.T., Chang, I.S., Kim, B.H. and Sung, H.C., 2003. Use of acetate for enrichment of electrochemically active microorganisms and their 16S rDNA analyses. FEMS microbiology letters, 223(2), pp.185-191.

Kim, B.H., Park, H.S., Kim, H.J., Kim, G.T., Chang, I.S., Lee, J. and Phung, N.T., 2004. Enrichment of microbial community generating electricity using a fuel-cell-type electrochemical cell. Applied microbiology and biotechnology, 63(6), pp.672-681.

Champine, J.E., Underhill, B., Johnston, J.M., Lilly, W.W. and Goodwin, S., 2000. Electron transfer in the dissimilatory iron-reducing bacterium Geobacter metallireducens. Anaerobe, 6(3), pp.187-196.

Beliaev, A.S., Saffarini, D.A., McLaughlin, J.L. and Hunnicutt, D., 2001. MtrC, an outer membrane decahaem c cytochrome required for metal reduction in Shewanella putrefaciens MR-1. Molecular microbiology, 39(3), pp.722-730.

Nevin, K.P. and Lovley, D.R., 2002. Mechanisms for accessing insoluble Fe (III) oxide during dissimilatory Fe (III) reduction by Geothrix fermentans. Applied and environmental microbiology, 68(5), pp.2294-2299.

Rabaey, K., Boon, N., Siciliano, S.D., Verhaege, M. and Verstraete, W., 2004. Biofuel cells select for microbial consortia that self-mediate electron transfer. Applied and environmental microbiology, 70(9), pp.5373-5382.

Rabaey, K., Lissens, G., Siciliano, S.D. and Verstraete, W., 2003. A microbial fuel cell capable of converting glucose to electricity at high rate and efficiency. Biotechnology letters, 25(18), pp.1531-1535.

Delaney, G.M., Bennetto, H.P., Mason, J.R., Roller, S.D., Stirling, J.L. and Thurston, C.F., 1984. Electron-transfer coupling in microbial fuel cells. 2. performance of fuel cells containing selected microorganism—mediator—substrate combinations. Journal of Chemical Technology and Biotechnology, 34(1), pp.13-27.

Vandevivere, P. and Verstraete, W., 2001. Environmental applications. In Basic biotechnology (pp. 531-557). Cambridge University Press

Kostka, J.E., Dalton, D.D., Skelton, H., Dollhopf, S. and Stucki, J.W., 2002. Growth of iron (III)-reducing bacteria on clay minerals as the sole electron acceptor and comparison of growth yields on a variety of oxidized iron forms. Applied and Environmental Microbiology, 68(12), pp.6256-6262.

Pham, C.A., Jung, S.J., Phung, N.T., Lee, J., Chang, I.S., Kim, B.H., Yi, H. and Chun, J., 2003. A novel electrochemically active and Fe (III)-reducing bacterium phylogenetically related to Aeromonas hydrophila, isolated from a microbial fuel cell. FEMS Microbiology Letters, 223(1), pp.129-134.

Park, H.S., Kim, B.H., Kim, H.S., Kim, H.J., Kim, G.T., Kim, M., Chang, I.S., Park, Y.K. and Chang, H.I., 2001. A novel electrochemically active and Fe (III)-reducing bacterium phylogenetically related to Clostridium butyricum isolated from a microbial fuel cell. Anaerobe, 7(6), pp.297-306.

Davis, F. and Higson, S.P., 2007. Biofuel cells—recent advances and applications. Biosensors and Bioelectronics, 22(7), pp.1224-1235.

Park, D.H. and Zeikus, J.G., 2000. Electricity generation in microbial fuel cells using neutral red as an electronophore. Applied and environmental microbiology, 66(4), pp.1292-1297.

Tokuji, I. and Kenji, K., 2003. Vioelectrocatalyses-based application of quinoproteins and quinprotein-containing bacterial cells in biosensors and biofuel cells. Biochim Biophys Acta, 1647, pp.121-6.

Vega, C.A. and Fernández, I., 1987. Mediating effect of ferric chelate compounds in microbial fuel cells with Lactobacillus plantarum, Streptococcus lactis, and Erwinia dissolvens. Bioelectrochemistry and Bioenergetics, 17(2), pp.217-222.

Lovley, D.R., 1993. Dissimilatory metal reduction. Annual Reviews in Microbiology, 47(1), pp.263-290.

Kim, B.H., Kim, H.J., Hyun, M.S. and Park, D.H., 1999. Direct electrode reaction of Fe (III)-reducing bacterium, Shewanella putrefaciens. Journal of Microbiology and Biotechnology, 9, pp.127-131.

Kim, H.J., Park, H.S., Hyun, M.S., Chang, I.S., Kim, M. and Kim, B.H., 2002. A mediator-less microbial fuel cell using a metal reducing bacterium, Shewanella putrefaciens. Enzyme and Microbial technology, 30(2), pp.145-152.

Bond, D.R. and Lovley, D.R., 2003. Electricity production by Geobacter sulfurreducens attached to electrodes. Applied and environmental microbiology, 69(3), pp.1548-1555.

Min, B., Cheng, S. and Logan, B.E., 2005. Electricity generation using membrane and salt bridge microbial fuel cells. Water research, 39(9), pp.1675-1686.

Liu, H., Cheng, S. and Logan, B.E., 2005. Power generation in fed-batch microbial fuel cells as a function of ionic strength, temperature, and reactor configuration. Environmental science & technology, 39(14), pp.5488-5493.

Ahn, Y. and Logan, B.E., 2010. Effectiveness of domestic wastewater treatment using microbial fuel cells at ambient and mesophilic temperatures. Bioresource technology, 101(2), pp.469-475.

Wen, Q., Wu, Y., Cao, D., Zhao, L. and Sun, Q., 2009. Electricity generation and modeling of microbial fuel cell from continuous beer brewery wastewater. Bioresource Technology, 100(18), pp.4171-4175.

Patil, S.A., Surakasi, V.P., Koul, S., Ijmulwar, S., Vivek, A., Shouche, Y.S. and Kapadnis, B.P., 2009. Electricity generation using chocolate industry wastewater and its treatment in activated sludge based microbial fuel cell and analysis of developed microbial community in the anode chamber. Bioresource technology, 100(21), pp.5132-5139.

Pant, D., Van Bogaert, G., Diels, L. and Vanbroekhoven, K., 2010. A review of the substrates used in microbial fuel cells (MFCs) for sustainable energy production. Bioresource technology, 101(6), pp.1533-1543.

Yasin, M., Jeong, Y., Park, S., Jeong, J., Lee, E.Y., Lovitt, R.W., Kim, B.H., Lee, J. and Chang, I.S., 2015. Microbial synthesis gas utilization and ways to resolve kinetic and mass-transfer limitations. Bioresource technology, 177, pp.361-374.

Heilmann, J. and Logan, B.E., 2006. Production of electricity from proteins using a microbial fuel cell. Water Environment Research, 78(5), pp.531-537.

Logan, B.E., Hamelers, B., Rozendal, R., Schröder, U., Keller, J., Freguia, S., Aelterman, P., Verstraete, W. and Rabaey, K., 2006. Microbial fuel cells: methodology and technology. Environmental science & technology, 40(17), pp.5181-5192.

Zhi, W., Ge, Z., He, Z. and Zhang, H., 2014. Methods for understanding microbial community structures and functions in microbial fuel cells: a review. Bioresource technology, 171, pp.461-468.

Liu, H. and Logan, B.E., 2004. Electricity generation using an air-cathode single chamber microbial fuel cell in the presence and absence of a proton exchange membrane. Environmental science & technology, 38(14), pp.4040-4046.

You, S.J., Zhao, Q.L., Jiang, J.Q., Zhang, J.N. and Zhao, S.Q., 2006. Sustainable approach for leachate treatment: electricity generation in microbial fuel cell. Journal of Environmental Science and Health Part A, 41(12), pp.2721-2734.

Min, B. and Angelidaki, I., 2008. Innovative microbial fuel cell for electricity production from anaerobic reactors. Journal of Power Sources, 180(1), pp.641-647.

Zhan, Y.L., Zhang, P.P., Yan, G.X. and Guo, S.H., 2008. Constructing and operating of mediator and membrane-less microbial fuel cell. Journal of Chemical Engineering of Chinese Universities, 22(1), p.177.

Huang, L. and Logan, B.E., 2008. Electricity generation and treatment of paper recycling wastewater using a microbial fuel cell. Applied microbiology and biotechnology, 80(2), pp.349-355.

Mohan, S.V., Saravanan, R., Raghavulu, S.V., Mohanakrishna, G. and Sarma, P.N., 2008. Bio-electricity production from wastewater treatment in dual chambered microbial fuel cell (MFC) using selectively enriched mixed microflora: effect of catholyte. Bioresource Technology, 99(3), pp.596-603.

Feng, Y., Wang, X., Logan, B.E. and Lee, H., 2008. Brewery wastewater treatment using air-cathode microbial fuel cells. Applied microbiology and biotechnology, 78(5), pp.873-880.

Wang, X., Feng, Y.J. and Lee, H., 2008. Electricity production from beer brewery wastewater using single chamber microbial fuel cell. Water Science and Technology, 57(7), pp.1117-1121.

Logan, B.E. and Regan, J.M., 2006. Electricity producing bacterial communities in microbial fuel cells. TRENDS in Microbiology, 14(12), pp.512-518

Rabaey, K. and Verstraete, W., 2005. Microbial fuel cells: novel biotechnology for energy generation. TRENDS in Biotechnology, 23(6), pp.291-298.

Lovley, D.R., 2006. Microbial fuel cells: novel microbial physiologies and engineering approaches. Current opinion in biotechnology, 17(3), pp.327-332.

Pham, T.H., Rabaey, K., Aelterman, P., Clauwaert, P., De Schamphelaire, L., Boon, N. and Verstraete, W., 2006. Microbial fuel cells in relation to conventional anaerobic digestion technology. Engineering in Life Sciences, 6(3), pp.285-292.

Chang, I.S., Moon, H.S., Bretschger, O., Jang, J.K., Park, H.I., Nealson, K.H. and Kim, B.H., 2006. Electrochemically active bacteria (EAB) and mediator-less microbial fuel cells. Journal of Microbiology and Biotechnology, 16(2), pp.163-177.

Bullen, R.A., Arnot, T.C., Lakeman, J.B. and Walsh, F.C., 2006. Biofuel cells and their development. Biosensors and Bioelectronics, 21(11), pp.2015-2045.

Borole, A.P., O'Neill, H., Tsouris, C. and Cesar, S., 2008. A microbial fuel cell operating at low pH using the acidophile Acidiphilium cryptum. Biotechnology letters, 30(8), pp.1367-1372.

Chabert, N., Ali, O.A. and Achouak, W., 2015. All ecosystems potentially host electrogenic bacteria. Bioelectrochemistry, 106, pp.88-96.

Bond, D.R. and Lovley, D.R., 2003. Electricity production by Geobacter sulfurreducens attached to electrodes. Appl. Environ. Microbiol., 69(3), pp.1548-1555.

Cheng, S., Liu, H. and Logan, B.E., 2006a. Increased performance of single chamber microbial fuel cells using an improved cathode structure. Electrochemistry Communications, 8(3), pp.489-494.

Cheng, S., Liu, H. and Logan, B.E., 2006b. Increased power generation in a continuous flow MFC with advective flow through the porous anode and reduced electrode spacing. Environmental science & technology, 40(7), pp.2426-2432.

Dopson, M., Ni, G. and Sleutels, T.H., 2015. Possibilities for extremophilic microorganisms in microbial electrochemical systems. FEMS microbiology reviews, 40(2), pp.164-181.

He, Z., Wagner, N., Minteer, S.D. and Angenent, L.T., 2006. An upflow microbial fuel cell with an interior cathode: assessment of the internal resistance by impedance spectroscopy. Environmental science & technology, 40(17), pp.5212-5217.

Jong, B.C., Kim, B.H., Chang, I.S., Liew, P.W.Y., Choo, Y.F. and Kang, G.S., 2006. Enrichment, performance, and microbial diversity of a thermophilic mediator-less microbial fuel cell. Environmental science & technology, 40(20), pp.6449-6454.

Kaushik, A. and Chetal, A., 2013. Power generation in microbial fuel cell fed with post methanation distillery effluent as a function of pH microenvironment. Bioresource technology, 147, pp.77-83.

Moon, H., Chang, I.S. and Kim, B.H., 2006. Continuous electricity production from artificial wastewater using a mediator-less microbial fuel cell. Bioresource Technology, 97(4), pp.621-627.

Puig, S., Serra, M., Coma, M., Cabré, M., Balaguer, M.D. and Colprim, J., 2010. Effect of pH on nutrient dynamics and electricity production using microbial fuel cells. Bioresource technology, 101(24), pp.9594-9599.

Ringeisen, B.R., Henderson, E., Wu, P.K., Pietron, J., Ray, R., Little, B., Biffinger, J.C. and Jones-Meehan, J.M., 2006. High power density from a miniature microbial fuel cell using Shewanella oneidensis DSP10. Environmental science & technology, 40(8), pp.2629-2634.

Varanasi, J.L., Roy, S., Pandit, S. and Das, D., 2015. Improvement of energy recovery from cellobiose by thermophillic dark fermentative hydrogen production followed by microbial fuel cell. international journal of hydrogen energy, 40(26), pp.8311-8321.

You, S.J., Zhao, Q.L., Jiang, J.Q., Zhang, J.N. and Zhao, S.Q., 2006a. Sustainable approach for leachate treatment: electricity generation in microbial fuel cell. Journal of Environmental Science and Health Part A, 41(12), pp.2721-2734.

You, S., Zhao, Q., Zhang, J., Jiang, J. and Zhao, S., 2006b. A microbial fuel cell using permanganate as the cathodic electron acceptor. Journal of power sources, 162(2), pp.1409-1415.

Zhang, T., Cui, C., Chen, S., Ai, X., Yang, H., Shen, P. and Peng, Z., 2006. A novel mediator-less microbial fuel cell based on direct biocatalysis of Escherichia coli. Chemical communications, (21), pp.2257-2259.

Jung, S. and Regan, J.M., 2007. Comparison of anode bacterial communities and performance in microbial fuel cells with different electron donors. Applied microbiology and biotechnology, 77(2), pp.393-402.

Beecroft, N.J., Zhao, F., Varcoe, J.R., Slade, R.C., Thumser, A.E. and Avignone-Rossa, C., 2012. Dynamic changes in the microbial community composition in microbial fuel cells fed with sucrose. Applied microbiology and biotechnology, 93(1), pp.423-437.

Chabert, N., Ali, O.A. and Achouak, W., 2015. All ecosystems potentially host electrogenic bacteria. Bioelectrochemistry, 106, pp.88-96.

Patil, S.A., Harnisch, F., Koch, C., Hübschmann, T., Fetzer, I., Carmona-Martínez, A.A., Müller, S. and Schröder, U., 2011. Electroactive mixed culture derived biofilms in microbial bioelectrochemical systems: the role of pH on biofilm formation, performance and composition. Bioresource technology, 102(20), pp.9683-9690.

He, Z., Huang, Y., Manohar, A.K. and Mansfeld, F., 2008. Effect of electrolyte pH on the rate of the anodic and cathodic reactions in an air-cathode microbial fuel cell. Bioelectrochemistry, 74(1), pp.78-82.

Larrosa-Guerrero, A., Scott, K., Head, I.M., Mateo, F., Ginesta, A. and Godinez, C., 2010. Effect of temperature on the performance of microbial fuel cells. Fuel, 89(12), pp.3985-3994.

Mashkour, M. and Rahimnejad, M., 2015. Effect of various carbon-based cathode electrodes on the performance of microbial fuel cell. Biofuel Res. J, 2(4), pp.296-300.

Michie, I.S., Kim, J.R., Dinsdale, R.M., Guwy, A.J. and Premier, G.C., 2011. The influence of psychrophilic and mesophilic start-up temperature on microbial fuel cell system performance. Energy & Environmental Science, 4(3), pp.1011-1019.

Wrighton, K.C., Agbo, P., Warnecke, F., Weber, K.A., Brodie, E.L., DeSantis, T.Z., Hugenholtz, P., Andersen, G.L. and Coates, J.D., 2008. A novel ecological role of the Firmicutes identified in thermophilic microbial fuel cells. The ISME journal, 2(11), p.1146.

Dopson, M., Ni, G. and Sleutels, T.H., 2016. Possibilities for extremophilic microorganisms in microbial electrochemical systems. FEMS microbiology reviews, 40(2), pp.164-181.

Chae, K.J., Choi, M.J., Lee, J.W., Kim, K.Y. and Kim, I.S., 2009. Effect of different substrates on the performance, bacterial diversity, and bacterial viability in microbial fuel cells. Bioresource technology, 100(14), pp.3518-3525.

Baranitharan, E., Khan, M.R., Prasad, D.M.R., Teo, W.F.A., Tan, G.Y.A. and Jose, R., 2015. Effect of biofilm formation on the performance of microbial fuel cell for the treatment of palm oil mill effluent. Bioprocess and biosystems engineering, 38(1), pp.15-24.

Zhang, Y., Mo, G., Li, X., Zhang, W., Zhang, J., Ye, J., Huang, X. and Yu, C., 2011. A graphene modified anode to improve the performance of microbial fuel cells. Journal of Power Sources, 196(13), pp.5402-5407.

Zhou, M., Chi, M., Luo, J., He, H. and Jin, T., 2011. An overview of electrode materials in microbial fuel cells. Journal of Power Sources, 196(10), pp.4427-4435.

Wei, J., Liang, P., Cao, X. and Huang, X., 2011. Use of inexpensive semicoke and activated carbon as biocathode in microbial fuel cells. Bioresource technology, 102(22), pp.10431-10435.

Sangeetha, T. and Muthukumar, M., 2013. Influence of electrode material and electrode distance on bio-electricity production from sago-processing wastewater using microbial fuel cell. Environmental Progress & Sustainable Energy, 32(2), pp.390-395.

Park, D.H. and Zeikus, J.G., 2003. Improved fuel cell and electrode designs for producing electricity from microbial degradation. Biotechnology and bioengineering, 81(3), pp.348-355.

Liu, H., Cheng, S. and Logan, B.E., 2005. Production of electricity from acetate or butyrate using a single chamber microbial fuel cell. Environmental science & technology, 39(2), pp.658-662.

Liu, H., Grot, S. and Logan, B.E., 2005. Electrochemically assisted microbial production of hydrogen from acetate. Environmental science & technology, 39(11), pp.4317-4320.

Rabaey, K., Clauwaert, P., Aelterman, P. and Verstraete, W., 2005. Tubular microbial fuel cells for efficient electricity generation. Environmental science & technology, 39(20), pp.8077-8082.

Min, B. and Logan, B.E., 2004. Continuous electricity generation from domestic wastewater and organic substrates in a flat plate microbial fuel cell. Environmental science & technology, 38(21), pp.5809-5814.

Aelterman, P., Rabaey, K., Pham, H.T., Boon, N. and Verstraete, W., 2006. Continuous electricity generation at high voltages and currents using stacked microbial fuel cells. Environmental science & technology, 40(10), pp.3388-3394.

Choi, J. and Ahn, Y., 2013. Continuous electricity generation in stacked air-cathode microbial fuel cell treating domestic wastewater. Journal of environmental management, 130, pp.146-152.

Choi, J. and Ahn, Y., 2014. Increased power generation from primary sludge in microbial fuel cells coupled with prefermentation. Bioprocess and biosystems engineering, 37(12), pp.2549-2557.

Jiang, Y., Ulrich, A.C. and Liu, Y., 2013. Coupling bio-electricity generation and oil sands tailings treatment using microbial fuel cells. Bioresource technology, 139, pp.349-354.

Ge, Z., Zhang, F., Grimaud, J., Hurst, J. and He, Z., 2013. Long-term investigation of microbial fuel cells treating primary sludge or digested sludge. Bioresource technology, 136, pp.509-514.

Logroño, W., Ramírez, G., Recalde, C., Echeverría, M. and Cunachi, A., 2015. Bio-electricity generation from vegetables and fruits wastes by using single chamber microbial fuel cells with high Andean soils. Energy Procedia, 75, pp.2009-2014.

Ahn, Y. and Logan, B.E., 2010. Effectiveness of domestic wastewater treatment using microbial fuel cells at ambient and mesophilic temperatures. Bioresource technology, 101(2), pp.469-475.

Jiang, B., Muddemann, T., Kunz, U., e Silva, L.G.S., Bormann, H., Niedermeiser, M., Haupt, D., Schläfer, O. and Sievers, M., 2017. Graphite/MnO2 and MoS2 Composites Used as Catalysts in the Oxygen Reduction Cathode of Microbial Fuel Cells. Journal of The Electrochemical Society, 164(14), pp.E519-E524.

Oh, S.E. and Logan, B.E., 2006. Proton exchange membrane and electrode surface areas as factors that affect power generation in microbial fuel cells. Applied microbiology and biotechnology, 70(2), pp.162-169.

Tharali, A.D., Sain, N. and Osborne, W.J., 2016. Microbial fuel cells in bio-electricity production. Frontiers in life science, 9(4), pp.252-266.

Taskan, E., Özkaya, B. and Hasar, H., 2014. Effect of different mediator concentrations on power generation in MFC using Ti-TiO2 electrode. International Journal of Energy Science, 4(1), pp.9-11.

Nair, R., Renganathan, K., Barathi, S. and Venkatraman, K., 2013. Performance of salt-bridge microbial fuel cell at various agarose concentrations using hostel sewage waste as substrate. Int J Advance Res Technol, 2(5), pp.326-330.

Jana, P.S., Behera, M. and Ghangrekar, M.M., 2010. Performance comparison of up-flow microbial fuel cells fabricated using proton exchange membrane and earthen cylinder. International Journal of Hydrogen Energy, 35(11), pp.5681-5686.

Mauritz, K.A. and Moore, R.B., 2004. State of understanding of Nafion. Chemical reviews, 104(10), pp.4535-4586.

Gil, G.C., Chang, I.S., Kim, B.H., Kim, M., Jang, J.K., Park, H.S. and Kim, H.J., 2003. Operational parameters affecting the performannce of a mediator-less microbial fuel cell. Biosensors and Bioelectronics, 18(4), pp.327-334.

Rozendal, R.A., Hamelers, H.V. and Buisman, C.J., 2006. Effects of membrane cation transport on pH and microbial fuel cell performance. Environmental science & technology, 40(17), pp.5206-5211.

Mukoma, P., Jooste, B.R. and Vosloo, H.C.M., 2004. Synthesis and characterization of cross-linked chitosan membranes for application as alternative proton exchange membrane materials in fuel cells. Journal of Power Sources, 136(1), pp.16-23.

Genies, C., Mercier, R., Sillion, B., Cornet, N., Gebel, G. and Pineri, M., 2001. Soluble sulfonated naphthalenic polyimides as materials for proton exchange membranes. Polymer, 42(2), pp.359-373.

Hickner, M.A., Ghassemi, H., Kim, Y.S., Einsla, B.R. and McGrath, J.E., 2004. Alternative polymer systems for proton exchange membranes (PEMs). Chemical reviews, 104(10), pp.4587-4612.

Rahimnejad, M., Adhami, A., Darvari, S., Zirepour, A. and Oh, S.E., 2015. Microbial fuel cell as new technology for bio-electricity generation: a review. Alexandria Engineering Journal, 54(3), pp.745-756.

Peighambardoust, S.J., Rowshanzamir, S. and Amjadi, M., 2010. Review of the proton exchange membranes for fuel cell applications. International journal of hydrogen energy, 35(17), pp.9349-9384.

Kim, J.R., Cheng, S., Oh, S.E. and Logan, B.E., 2007. Power generation using different cation, anion, and ultrafiltration membranes in microbial fuel cells. Environmental science & technology, 41(3), pp.1004-1009.

Sevda, S. and Sreekrishnan, T.R., 2012. Effect of salt concentration and mediators in salt bridge microbial fuel cell for electricity generation from synthetic wastewater. Journal of Environmental Science and Health, Part A, 47(6), pp.878-886.

Fultz, M.L. and Durst, R.A., 1982. Mediator compounds for the electrochemical study of biological redox systems: a compilation. Analytica Chimica Acta, 140(1), pp.1-18.

Babanova, S., Hubenova, Y. and Mitov, M., 2011. Influence of artificial mediators on yeast-based fuel cell performance. Journal of bioscience and bioengineering, 112(4), pp.379-387.

Oh, S., Min, B. and Logan, B.E., 2004. Cathode performance as a factor in electricity generation in microbial fuel cells. Environmental science & technology, 38(18), pp.4900-4904.

Zuo, Y., Cheng, S., Call, D. and Logan, B.E., 2007. Tubular membrane cathodes for scalable power generation in microbial fuel cells. Environmental science & technology, 41(9), pp.3347-3353.

Zuo, Y., Xing, D., Regan, J.M. and Logan, B.E., 2008. Isolation of the exoelectrogenic bacterium Ochrobactrum anthropi YZ-1 by using a U-tube microbial fuel cell. Appl. Environ. Microbiol., 74(10), pp.3130-3137.

Niessen, J., Schröder, U. and Scholz, F., 2004. Exploiting complex carbohydrates for microbial electricity generation–a bacterial fuel cell operating on starch. Electrochemistry Communications, 6(9), pp.955-958.

Zuo, Y., Maness, P.C. and Logan, B.E., 2006. Electricity production from steam-exploded corn stover biomass. Energy & Fuels, 20(4), pp.1716-1721.

Schröder, U., 2007. Anodic electron transfer mechanisms in microbial fuel cells and their energy efficiency. Physical Chemistry Chemical Physics, 9(21), pp.2619-2629.

Wang, H. and Ren, Z.J., 2013. A comprehensive review of microbial electrochemical systems as a platform technology. Biotechnology advances, 31(8), pp.1796-1807.

Gorby, Y.A., Yanina, S., McLean, J.S., Rosso, K.M., Moyles, D., Dohnalkova, A., Beveridge, T.J., Chang, I.S., Kim, B.H., Kim, K.S. and Culley, D.E., 2006. Electrically conductive bacterial nanowires produced by Shewanella oneidensis strain MR-1 and other microorganisms. Proceedings of the National Academy of Sciences, 103(30), pp.11358-11363.

Moon, H., Chang, I.S. and Kim, B.H., 2006. Continuous electricity production from artificial wastewater using a mediator-less microbial fuel cell. Bioresource Technology, 97(4), pp.621-627.

Jang, J.K., Pham, T.H., Chang, I.S., Kang, K.H., Moon, H., Cho, K.S. and Kim, B.H., 2004. Construction and operation of a novel mediator and membrane-less microbial fuel cell. Process Biochemistry, 39(8), pp.1007-1012.

Ghangrekar, M.M. and Shinde, V.B., 2006, April. Wastewater treatment in microbial fuel cell and electricity generation: A sustainable approach. In 12th international sustainable development research conference (Vol. 8, p. 201).

Jadhav, G.S., Jagtap, Y.D. and Ghangrekar, M.M., 2013. Dual-chambered membrane microbial fuel cell: limitation on potential difference. International Journal of Engineering Research and Technology, 2(4).

Zhang, T., Zeng, Y., Chen, S., Ai, X. and Yang, H., 2007. Improved performances of E. coli-catalyzed microbial fuel cells with composite graphite/PTFE anodes. Electrochemistry Communications, 9(3), pp.349-353.

Cheng, S. and Logan, B.E., 2007. Ammonia treatment of carbon cloth anodes to enhance power generation of microbial fuel cells. Electrochemistry Communications, 9(3), pp.492-496.

Logan, B.E., 2004. Peer reviewed: extracting hydrogen and electricity from renewable resources.

Rabaey, K., Lissens, G., Siciliano, S.D. and Verstraete, W., 2003. A microbial fuel cell capable of converting glucose to electricity at high rate and efficiency. Biotechnology letters, 25(18), pp.1531-1535.

Rabaey, K., Boon, N., Siciliano, S.D., Verhaege, M. and Verstraete, W., 2004. Biofuel cells select for microbial consortia that self-mediate electron transfer. Appl. Environ. Microbiol., 70(9), pp.5373-5382.

Oh, S. and Logan, B.E., 2005. Hydrogen and electricity production from a food processing wastewater using fermentation and microbial fuel cell technologies. Water research, 39(19), pp.4673-4682.

Feng, Y., Wang, X., Logan, B.E. and Lee, H., 2008. Brewery wastewater treatment using air-cathode microbial fuel cells. Applied microbiology and biotechnology, 78(5), pp.873-880.

Yong, X.Y., Feng, J., Chen, Y.L., Shi, D.Y., Xu, Y.S., Zhou, J., Wang, S.Y., Xu, L., Yong, Y.C., Sun, Y.M. and Shi, C.L., 2014. Enhancement of bio-electricity generation by cofactor manipulation in microbial fuel cell. Biosensors and Bioelectronics, 56, pp.19-25.

Li, Z., Yao, L., Kong, L. and Liu, H., 2008. Electricity generation using a baffled microbial fuel cell convenient for stacking. Bioresource technology, 99(6), pp.1650-1655.

Di Lorenzo, M., Thomson, A.R., Schneider, K., Cameron, P.J. and Ieropoulos, I., 2014. A small-scale air-cathode microbial fuel cell for on-line monitoring of water quality. Biosensors and Bioelectronics, 62, pp.182-188.

Zhang, X., He, W., Yang, W., Liu, J., Wang, Q., Liang, P., Huang, X. and Logan, B.E., 2016. Diffusion layer characteristics for increasing the performance of activated carbon air-cathodes in microbial fuel cells. Environmental Science: Water Research & Technology, 2(2), pp.266-273.

Pinto, R.P., Srinivasan, B., Guiot, S.R. and Tartakovsky, B., 2011. The effect of real-time external resistance optimization on microbial fuel cell performance. Water research, 45(4), pp.1571-1578.

Aelterman, P., Rabaey, K., Pham, H.T., Boon, N., Verstraete, W., 2006. Continuous electricity generation at high voltages and currents using stacked microbial fuel cells. Environmental Science and Technology 40 (10), 3388e3394.

Aelterman, P., Versichele, M., Marzorati, M., Boon, N., Verstraete, W., 2008. Loading rate and external resistance control the electricity generation of

microbial fuel cells with different three-dimensional anodes. Bioresource Technology 99 (18), 8895e8902.

APHA, 1995. Standard Methods for the Examination of Water and Wastewater. American Public Health Association, Washington, DC.

Birry, L., Mehta, P., Jaouen, F., Dodelet, J.P., Guiot, S.R., Tartakovsky, B., 2010. Application of iron-based cathode catalysts in a microbial fuel cell. Electrochimica Acta, in press.

Chae, K.-J., Choi, M.-J., Kim, K.-Y., Ajayi, F.F., Park, W., Kim, C.-W., Kim, I.S., 2010. Methanogenesis control by employing various environmental stress conditions in two-chambered microbial fuel cells. Bioresource Technology 101 (14), 5350e5357.

Debabov, V., 2008. Electricity from microorganisms. Microbiology 77 (2), 123e131.

Fan, Y., Sharbrough, E., Liu, H., 2008. Quantification of the internal resistance distribution of microbial fuel cells. Environmental Science and Technology 42, 8101e8107.

Fuel Cell Handbook, 2005. National Energy Technology Laboratory U.S. Department of Energy. University Press of the Pacific.

Hua, C., Shen, C., 1998. Comparative Study of Peak Power Tracking Techniques for Solar Storage System. In: Anon (Ed.). IEEE, Anaheim, CA, USA, pp. 679e685.

Ieropoulos, I., Greenman, J., Melhuish, C., 2008. Microbial fuel cells based on carbon veil electrodes: stack configuration and scalability. International Journal of Energy Research 32, 1228e1240.

Jadhav, G.S., Ghangrekar, M.M., 2009. Performance of microbial fuel cell subjected to variation in pH, temperature, external load and substrate concentration. Bioresource Technology 100 (2), 717e723.

Kang, K.H., Jang, J.K., Pham, T.H., Moon, H., Chang, I.S., Kim, B.H., 2003. A microbial fuel cell with improved cathode reaction as a low biochemical oxygen demand sensor. Biotechnology Letters 25 (16), 1357e1361.

Logan, B., Cheng, S., Watson, V., Estadt, G., 2007. Graphite fiber brush anodes for increased power production in air-cathode microbial fuel cells. Environmental Science and Technology 41, 3341e3346.

Logan, B.E., 2008. Microbial Fuel Cells. John Wiley & Sons, Hobeken, New Jersey.

Logan, B.E., 2010. Scaling up microbial fuel cells and other bioelectrochemical systems. Applied Microbiology and Biotechnology 85 (6), 1665e1671.

Logan, B.E., Hamelers, B., Rozendal, R.A., Schröder, U., Keller, J., Freguia, S., Aelterman, P., Verstraete, W., Rabaey, K., 2006. Microbial fuel cells: methodology and Technology. Environmental Science and Technology 40 (17), 5181e5192.

Logan, B.E., Regan, J.M., 2006. Microbial fuel cells - Challenges and applications. Environmental Science and Technology 40 (17), 5172e5180.

Lovley, D.R., 2008. Themicrobe electric: conversion of organicmatter to electricity. Current Opinion in Biotechnology 19 (6), 564e571.

Lyon, D.Y., Buret, F., Vogel, T.M., Monier, J.-M., 2010. Is resistance futile? Changing external resistance does not improve microbial fuel cell performance. Bioelectrochemistry 78 (1), 2e7.

Martin, E., Savadogo, O., Guiot, S.R., Tartakovsky, B., 2010. The influence of operational conditions on the performance of a microbial fuel cell seeded with mesophilic anaerobic sludge. Biochemical Engineering Journal 51 (3), 132e139.

Oh, S.E., Logan, B.E., 2007. Voltage reversal during microbial fuel cell stack operation. Journal of Power Sources 167 (1), 11e17.

Pant, D., Van Bogaert, G., Diels, L., Vanbroekhoven, K., 2010. A review of the substrates used in microbial fuel cells (MFCs) for sustainable energy production. Bioresource Technology 101 (6), 1533e1543.

Pinto, R.P., Perrier, M., Tartakovsky, B. and Srinivasan, B., 2010a. Performance analyses of microbial fuel cells operated in series, Proceedings of 9th International Symposium on Dynamics and control of process systems, Leuven, Belgium.

Pinto, R.P., Srinivasan, B., Manuel, M.F., Tartakovsky, B., 2010b. A two-population bioelectrochemical model of a microbial fuel cell. Bioresource Technology 101 (14), 5256e5265.

Pinto, R.P., Tartakovsky, B., Perrier, M., Srinivasan, B., 2010c. Optimizing treatment performance of microbial fuel cells by reactor Staging. Industrial & Engineering Chemistry Research 49 (19), 9222e9229.

Reguera, G., McCarthy, K.D., Mehta, T., Nicoll, J.S., Tuominen, M.T., Lovley, D.R., 2005. Extracellular electron transfer via microbial nanowires. Nature Biotechnology 435, 1098e1101.

Rismani-Yazdi, H., Carver, S.M., Christy, A.D., Tuovinen, I.H., 2008. Cathodic limitations in microbial fuel cells: an overview. Journal of Power Sources 180, 683e694.

Shimoyama, T., Komukai, S., Yamazawa, A., Ueno, Y., Logan, B.E., Watanabe, K., 2008. Electricity generation from model organic wastewater in a cassette-electrode microbial fuel cell. Applied Microbiology and Biotechnology 80 (2), 325e330.

Tartakovsky, B., Manuel, M.F., Neburchilov, V.,Wang, H., Guiot, S.R., 2008. Biocatalyzed hydrogen production in a continuous flow microbial fuel cell with a gas phase cathode. Journal of Power Sources 182 (1), 291e297.

ter Heijne, A., Hamelers, H.V.M., Saakes, M., Buisman, C.J.N., 2008. Performance of non-porous graphite and titanium-based anodes in microbial fuel cells. Electrochemica Acta 53, 5697e5703.

Torres, C.I., Krajmalnik-Brown, R., Parameswaran, P., Marcus, A.K., Wanger, G., Gorby, Y.A., Rittmann, B.E., 2009. Selecting anode-respiring bacteria based on

anode potential: phylogenetic, electrochemical, and microscopic characterization. Environmental Science and Technology 43 (24), 9519e9524.

Torres, C.I., Marcus, A.K., Lee, H., Parameswaran, P., KrajmalnikBrown, R., Rittmann, B.E., 2010. A kinetic perspective on extracellular electron transfer by anode-respiring bacteria. FEMS Microbiology Reviews 34 (1), 3e17.

Woodward, L., Perrier, M., Srinivasan, B., Pinto, R.P., Tartakovsky, B., 2010. Comparison of real-time methods for maximizing power output in microbial fuel cells. AIChE Journal 56 (10), 2742e2750.

Lin, X.Q., Li, Z.L., Liang, B., Nan, J. and Wang, A.J., 2019. Identification of biofilm formation and exoelectrogenic population structure and function with graphene/polyanliline modified anode in microbial fuel cell. Chemosphere, 219, pp.358-364.

Chae, K.J., Choi, M.J., Lee, J.W., Ajayi, F.F., Kim, I.S., 2008a. Biohydrogen production via biocatalyzed electrolysis in acetate-fed bioelectrochemical cells and microbial community analysis. Int. J. Hydrogen Energy 33, 5184–5192.

Clauwaert, P., Verstraete, W., 2009. Methanogenesis in membrane-less microbial electrolysis cells. Appl. Microbiol. Biotechnol. 82, 829–836.

Freguia, S., Rabaey, K., Yuan, Z., Keller, J., 2007. Electron and carbon balances in microbial fuel cells reveal temporary bacterial storage behavior during electricity generation. Environ. Sci. Technol. 41, 2915–2921.

Freguia, S., Rabaey, K., Yuan, Z., Keller, J., 2008. Syntrophic processes drive the conversion of glucose in microbial fuel cell anodes. Environ. Sci. Technol. 42, 7937–7943.

Hallenbeck, P.C., Ghosh, D., 2009. Advances in fermentative biohydrogen production: the way forward? Trends Biotechnol. 27, 287–297.

Huang, L., Logan, B.E., 2008. Electricity production from xylose in fed-batch and continuous flow microbial fuel cells. Appl. Microbiol. Biotechnol. 80, 655–

Kim, I.S., Chae, K.J., Choi, M.J., Verstraete, W., 2008. Microbial Fuel Cells: Recent Advances, Bacterial Communities and Application Beyond Electricity Generation. Environ. Eng. Res. 13, 51–65.

Lee, H.S., Parameswaran, P., Kato-Marcus, A., Torres, C.I., Rittmann, B.E., 2008a. Evaluation of energy conversion efficiencies in microbial fuel cells (MFCs) utilizing fermentable and non-fermentable substrates. Water Res. 42, 1501–1510.

Lee, H.S., Salerno, M.B., Rittmann, B.E., 2008b. Thermodynamic evaluation on H2 production in glucose fermentation. Environ. Sci. Technol. 42, 2401– 2407.

Logan, B.E., Hamelers, B., Rozendal, R., Schr?der, U., Keller, J., Freguia, S., Aelterman, P., Verstraete, W., Rabaey, K., 2006. Microbial fuel cells: methodology and technology. Environ. Sci. Technol. 40, 5181–5192.

Rabaey, K., Verstraete, W., 2005. Microbial fuel cells: novel biotechnology for energy generation. Trends Biotechnol. 23, 291–298.

Torres, C.I., Kato Marcus, A., Rittmann, B.E., 2007. Kinetics of consumption of fermentation products by anode-respiring bacteria. Appl. Microbiol. Biotechnol. 77, 689–697.

Wagner, R.C., Regan, J.M., Oh, S.E., Zuo, Y., Logan, B.E., 2009. Hydrogen and methane production from swine wastewater using microbial electrolysis cells. Water Res. 43, 1480–1488.

Kim, K.Y., Chae, K.J., Choi, M.J., Ajayi, F.F., Jang, A., Kim, C.W. and Kim, I.S., 2011. Enhanced Coulombic efficiency in glucose-fed microbial fuel cells by reducing metabolite electron losses using dual anode electrodes. Bioresource technology, 102(5), pp.4144-4149.

Walter, X.A., Santoro, C., Greenman, J. and Ieropoulos, I., 2019. Self-stratifying microbial fuel cell: the importance of the cathode electrode immersion height. international journal of hydrogen energy, 44(9), pp.4524-4532.

Walter, X.A., Merino-Jiménez, I., Greenman, J. and Ieropoulos, I., 2018. PEE POWER® urinal II–Urinal scale-up with microbial fuel cell scale-down for improved lighting. Journal of power sources, 392, pp.150-158.

Walter, X.A., Gajda, I., Forbes, S., Winfield, J., Greenman, J. and Ieropoulos, I., 2016. Scaling-up of a novel, simplified MFC stack based on a self-stratifying urine column. Biotechnology for biofuels, 9(1), p.93.

Jiang, D., Curtis, M., Troop, E., Scheible, K., McGrath, J., Hu, B., Suib, S., Raymond, D. and Li, B., 2011. A pilot-scale study on utilizing multi-anode/cathode microbial fuel cells (MAC MFCs) to enhance the power production in wastewater treatment. international journal of hydrogen energy, 36(1), pp.876-884.

Santoro, C., Kodali, M., Kabir, S., Soavi, F., Serov, A. and Atanassov, P., 2017. Three-dimensional graphene nanosheets as cathode catalysts in standard and supercapacitive microbial fuel cell. Journal of power sources, 356, pp.371-380

Santoro, C., Arbizzani, C., Erable, B. and Ieropoulos, I., 2017. Microbial fuel cells: from fundamentals to applications. A review. Journal of power sources, 356, pp.225-244.

Rinaldi, A., Mecheri, B., Garavaglia, V., Licoccia, S., Di Nardo, P. and Traversa, E., 2008. Engineering materials and biology to boost performance of microbial fuel cells: a critical review. Energy & Environmental Science, 1(4), pp.417-429.

Abourached, C., English, M.J., Liu, H., 2016. Wastewater treatment by Microbial Fuel Cell (MFC) prior irrigation water reuse. J. Cleaner Prod. 137, 144–149.

Ahn, Y., Ivanov, I., Nagaiah, T.C., Bordoloi, A., Logan, B.E., 2014. Mesoporous nitrogen- rich carbon materials as cathode catalysts in microbial fuel cells. J. Power Sources 269, 212–215.

Bosch-Jimenez, P., Martinez-Crespiera, S., Amantia, D., Della Pirriera, M., Forns, I., Shechter, R., Borràs, E., 2017. Non-precious metal doped carbon nanofiber air-cathode for Microbial Fuel Cells application: Oxygen reduction reaction character- ization and long-term validation. Electrochimica Acta 228, 380–388.

Cao, C., Wei, L., Su, M., Wang, G., Shen, J., 2016a. Low-cost adsorbent derived and in situ nitrogen/iron co-doped carbon as efficient oxygen reduction catalyst in microbial fuel cells. Bioresour. Technol. 214, 348–354.

Cao, C., Wei, L., Su, M., Wang, G., Shen, J., 2016b. Enhanced power generation using nano cobalt oxide anchored nitrogen-decorated reduced graphene oxide as a high- performance air-cathode electrocatalyst in biofuel cells. RSC Adv. 6 (58), 52556–52563.

Gnana Kumar, G., Awan, Z., Suk Nahm, K., Xavier, J.S., 2014. Nanotubular MnO2/graphene oxide composites for the application of open air-breathing cathode microbial fuel cells. Biosens. Bioelectron. 53, 528–534.

Guerrini, E., Grattieri, M., Faggianelli, A., Cristiani, P., Trasatti, S., 2015. PTFE effect on the electrocatalysis of the oxygen reduction reaction in membrane-less microbial fuel cells. Bioelectrochemistry 106 (Pt A), 240–247.

Huang, J., Zhu, N., Yang, T., Zhang, T., Wu, P., Dang, Z., 2015. Nickel oxide and carbon nanotube composite (NiO/CNT) as a novel cathode non-precious metal catalyst in microbial fuel cells. Biosens. Bioelectron. 72, 332–339.

Hu, D., Zhang, G., Wang, J., Zhong, Q., 2015. Carbon-supported spinel nanoparticle MnCo2O4 as a cathode catalyst towards oxygen reduction reaction in dual-chamber microbial fuel cell. Aust. J. Chem. 68 (6), 987–994.

Jadhav, D.A., Deshpande, P.A., Ghangrekar, M.M., 2017. Enhancing the performance of single-chambered microbial fuel cell using manganese/palladium and zirconium/palladium composite cathode catalysts. Bioresour. Technol. 238, 568–574.

Kumar, R., Singh, L., Zularisam, A.W., 2017a. Mesoporous Co3O4 nanoflakes as an efficient and non-precious cathode catalyst for oxygen reduction reaction in air-cathode microbial fuel cells. J. Taiwan Inst. Chem. Eng. 78, 329–336.

Kumar, R., Singh, L., Zularisam, A.W., 2016a. Exoelectrogens: recent advances in molecular drivers involved in extracellular electron transfer and strategies used to im- prove it for microbial fuel cell applications. Renew. Sustain. Energy Rev. 56, 1322–1336.

Kumar, R., Singh, L., Zularisam, A.W., Hai, F.I., 2016b. Potential of porous Co3O4 na- norods as cathode catalyst for oxygen reduction reaction in microbial fuel cells. Bioresour. Technol. 220, 537–542.

Kumar, R., Singh, L., Zularisam, A.W., 2017b. Enhanced oxygen reduction reaction in air- cathode microbial fuel cells using flower-like Co3O4 as an efficient cathode catalyst. Int. J. Hydrogen Energy 42 (30), 19287–19295.

Li, B., Zhou, X., Wang, X., Liu, B., Li, B., 2014. Hybrid binuclear-cobalt-phthalocyanine as oxygen reduction reaction catalyst in single chamber microbial fuel cells. J. Power Sources 272, 320–327.

Liew, K.B., Daud, W.R.W., Ghasemi, M., Loh, K.S., Ismail, M., Lim, S.S., Leong, J.X., 2015. Manganese oxide/functionalised carbon nanotubes nanocomposite as

catalyst for oxygen reduction reaction in microbial fuel cell. Int. J. Hydrogen Energy 40 (35), 11625–11632.

Lima, F.H., Calegaro, M.L., Ticianelli, E.A., 2006. Investigations of the catalytic properties of manganese oxides for the oxygen reduction reaction in alkaline media. J. Electroanal. Chem. 590 (2), 152–160.

Liu, S., Wu, J., Zhou, J., Fang, G., Liang, S., 2015. Mesoporous NiCo2O4 nanoneedles grown on three dimensional graphene networks as binder-free electrode for high- performance lithium-ion batteries and supercapacitors. Electrochimica Acta 176, 1–9.

Lu, M., Guo, L., Kharkwal, S., Wu, H.N., Ng, H.Y., Li, S.F.Y., 2013. Manganese-poly- pyrrole-carbon nanotube, a new oxygen reduction catalyst for air-cathode microbial fuel cells. J. Power Sources 221, 381–386.

Mahmoud, M., Gad-Allah, T.A., El-Khatib, K.M., El-Gohary, F., 2011. Power generation using spinel manganese–cobalt oxide as a cathode catalyst for microbial fuel cell applications. Bioresour. Technol. 102 (22), 10459–10464.

Patil, S.A., Arends, J.B., Vanwonterghem, I., van Meerbergen, J., Guo, K., Tyson, G.W., Rabaey, K., 2015. Selective enrichment establishes a stable performing community for microbial electrosynthesis of acetate from CO(2). Environ. Sci. Technol. 49 (14), 8833–8843.

Tian, P., Liu, D., Li, K., Yang, T., Wang, J., Liu, Y., Zhang, S., 2017. Porous metal-organic framework Cu3 (BTC)2 as catalyst used in air-cathode for high performance of mi- crobial fuel cell. Bioresour. Technol. 244, 206–212.

Teichner, S.J., 1990. Recent studies in hydrogen and oxygen spillover and their impact on catalysis. Appl. Catal. 62 (1), 1–10.

Vidyadharan, B., Aziz, R.A., Misnon, I.I., Anil Kumar, G.M., Ismail, J., Yusoff, M.M., Jose, R., 2014. High energy and power density asymmetric supercapacitors using elec- trospun cobalt oxide nanowire anode. J. Power Sources 270, 526–535.

Wang, Y., Cui, X., Chen, L., Wei, C., Cui, F., Yao, H., Shi, J., Li, Y., 2014. One-step re- plication and enhanced catalytic activity for cathodic oxygen reduction of the me- sostructured Co3O4/carbon composites. Dalton Trans. 43 (10), 4163–4168.

Wen, Q., Wang, S., Yan, J., Cong, L., Pan, Z., Ren, Y., Fan, Z., 2012. MnO2-graphene hybrid as an alternative cathodic catalyst to platinum in microbial fuel cells. J. Power Sources 216, 187–191.

Xu, J., Gao, P., Zhao, T.S., 2012. Non-precious Co3O4 nano-rod electrocatalyst for oxygen reduction reaction in anion-exchange membrane fuel cells. Energy Environ. Sci. 5 (1), 5333–5339.

Xu, Y., Wang, X., An, C., Wang, Y., Jiao, L., Yuan, H., 2014. Facile synthesis route of porous MnCo2O4 and CoMn2O4 nanowires and their excellent electrochemical properties in supercapacitors. J. Mater. Chem. A 2 (39), 16480–16488.

Yu, Y.Y., Guo, C.X., Yong, Y.C., Li, C.M., Song, H., 2015. Nitrogen doped carbon nano- particles enhanced extracellular electron transfer for high-performance microbial fuel cells anode. Chemosphere 140, 26–33.

Zhang, X., Li, K., Yan, P., Liu, Z., Pu, L., 2015. N-type Cu2O doped activated carbon as catalyst for improving power generation of air-cathode microbial fuel cells. Bioresour. Technol. 187, 299–304.

Zhang, Y., Hu, Y., Li, S., Sun, J., Hou, B., 2011. Manganese dioxide-coated carbon na- notubes as an improved cathodic catalyst for oxygen reduction in a microbial fuel cell. J. Power Sources 196 (22), 9284–9289.

Zhao, F., Harnisch, F., Schröder, U., Scholz, F., Bogdanoff, P. and Herrmann, I., 2006. Challenges and constraints of using oxygen cathodes in microbial fuel cells. Environmental science & technology, 40(17), pp.5193-5199.

Morris, J.M., Jin, S., Wang, J., Zhu, C. and Urynowicz, M.A., 2007. Lead dioxide as an alternative catalyst to platinum in microbial fuel cells. Electrochemistry Communications, 9(7), pp.1730-1734.

Chapter 3: Research Methodology

This section outlines the methodologies employed throughout the experimental work of the research study. In this regard, a similar catalyst previously prepared, characterized and tested elsewhere (Nie et al., 2012; Wang et al.,2015) was also prepared for this study.

3.1 CATALYST PREPARATION

3.1.1 Catalyst synthesis

A non-metal-based catalyst MnO_2/rGO (Nie et al., 2012; Wang et al., 2015) was prepared using the Hummers and Offemann's method shown in figure 3.1.

3.1.1a. Materials/ Chemicals

The chemicals used in the experimental work for GO synthesis were graphite flakes, sulfuric acid (99%), hydrogen peroxide, potassium permanganate, sodium nitrate, hydrochloric acid and were supplied by Sigma Aldrich (South Africa). The chemicals used for MnO_2/rGO were also analytical grade and were used as obtained without further purification. Natural flake graphite with purity 99.95% and BS mesh 500, $KMnO_4$, concentrated sulphuric acid (purity of 98%) and $MnSO_4.H_2O$ (purity no less than 99%) were purchased from Sigma Aldrich (South Africa). Deionized water made by the reverse osmosis system was used throughout.

3.1.1. Methods

The method used to prepare GO was also used elsewhere (Nie et al.2012). GO was prepared by oxidation of graphite according to the Hummers and Offemann's method found elsewhere (Nie et al.2012). 10.0 g of graphite powder was mixed with to 5.0 g of sodium nitrate and added to 230 ml of concentrated sulfuric acid under constant stirring for 1hour at 0^0C, i.e. in an ice-bath. After an hour, 30.0 g of potassium

permanganate was slowly added to the above solution with vigorous stirring. After removing the ice-bath, the above solution was heated to a temperature of 35^0C, and the solution temperature was maintained for 30 minutes. 460ml of distilled water was then added slowly into the solution and the temperature was raised to 98^0C. After an hour, 1.4litres of warm water was added to the solution and also treated with 3 % hydrogen peroxide aqueous solution. Finally, the resulting suspension was filtered and washed with 5 % HCl aqueous solution.

The hydrothermal method was used for the preparation of the nanocomposite MnO_2/rGO as derived from a previous study (Wang et al.,2015). In this synthesis, 1.5mmol of manganese sulfate monohydrate ($MnSO_4.H_2O$) and 1mmol of potassium permanganate ($KMnO_4$) powders as well as GO with a mass ratio of $KMnO_4$ and GO equalling to 15:1 were dissolved in 40 ml deionized water successively under strong stirring at room temperature for about 30minutes to obtain purple aqueous solution. The resulting solution was then transferred into a 50ml Teflon lined stainless steel autoclave. The hydrothermal process was carried out in an oven at 90^0C for 12 h. After being cooled down to room temperature, the product samples were each rinsed 3 times with deionized water for 10 minutes via centrifuge at a rotation rate of 8000 rpm and the obtained powder was dried in a vacuum oven at 80^0C for 12 hours.

3.1.2 Characterization

The prepared samples, i.e., GO, and MnO_2/rGO characterized by the methods listed below. The morphology of each sample was studied using the FESEM (HITACHI, S-4800).



Fig 3.1 HITACHI, S-4800FESEM analyser:

The surface areas were measured by the BET method were N_2 absorption at 77K was applied and Carlo Erba Sorptometer was used.



Fig 3.2 Carlo Erba Sorptometer: BET analsyser.

FT-IR spectra of KBr powder pressed pellets were recorded on a Bruker VECTOR 22 spectrometer.


Fig 3.3 Bruker VECTOR 22 spectrometer: FT-IR analyser.

3.2 ELECTROCHEMISTRY

The cyclic voltammetric (CV) measurements were performed with an Auto-lab potentiostat (model PGSTAT 30) with a three-electrode system (Ecochemie, Netherlands). The glass carbon electrode (GCE, with a diameter 3.0mm) coated by catalyst severs as the working electrode, while a Pt wire and Ag/AgCl (sat. KCl, 222mV versus SHE) were used as the counter and reference electrodes, respectively.

The CV measurements were performed from -1.5V to 1.5V at scan rates of between 0.5 to 100mV/s in 0.1M KOH and electrolyte. The electrolyte solution is bubbled with O_2 or N_2 to establish aerobic or anaerobic environment, respectively, for 30min prior to each scan series and 3min between every two scans.

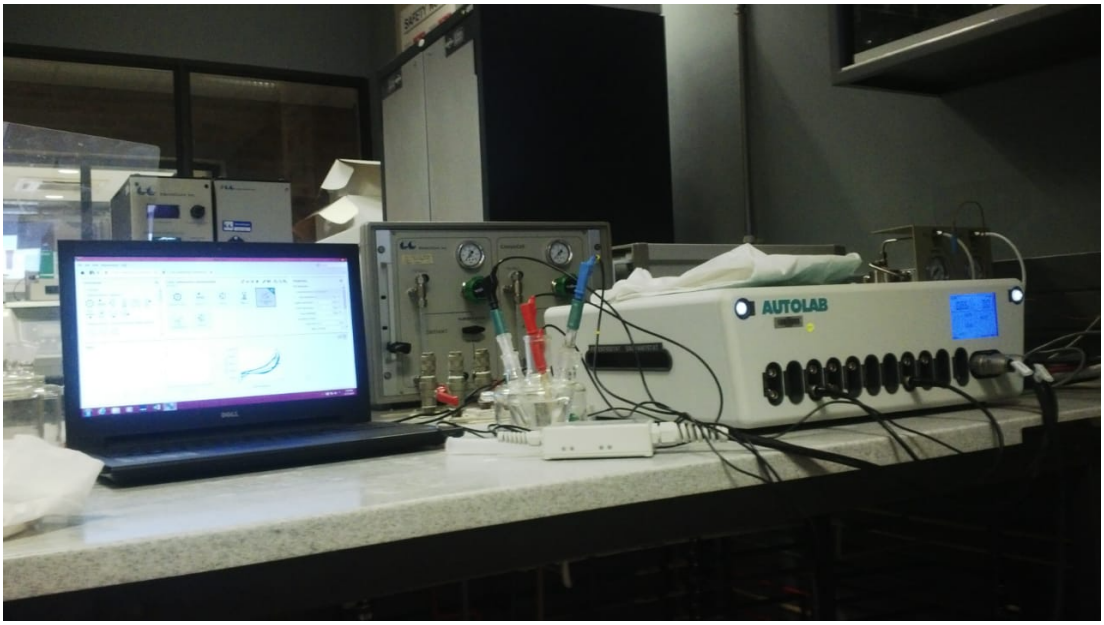


Fig 3.4 Auto-lab potentiostat (model PGSTAT 30): used for cyclic voltammetric (CV) measurements.

3.3 MICROBIAL FUEL CELL ANALYSIS

3.3.1 Wastewater samples

Beer brewery wastewater was collected from Inbev Inc Breweries in Alrode, Alberton, Johannesburg South Africa and stored in a refridgerator at 0^0C under anaerobic condition.

The domestic wastewater was collected from ERWAT wastewater treatment works in Eikenhof, Johannesburg, South Africa illustrated in figure 3.5. These samples were also stored in a refridgerator at 0^0C under anaerobic condition.

Raw wastewaster (brewey or domestic) samples (neither diluted nor treated) with a total volume of 350-400ml were randomly taken and fed to the anodic chamber of each MFC for each defined experiment.



Fig 3.5 ERWAT wastewater water treatment work, Ekurhuleni South Africa

Preparation of the cathode electrodes

The catalyst ink formulation method was utilised for the cathode electrode fabricated as follows: 1 mg of catalyst was dispersed in 1 mL of a solvent mixture of Nafion (5 wt %) and anhydrous ethanol (1:9, v:v) for 0.5−1 h under sonication. An ink dispersion method was used to fabricate the cathode electrodes tips with 10µl of the MnO_2/rGO catalyst solution.

3.3.2 Microbial Fuel Cell construction

Double chamber MFCs were constructed from plexiglass with a total volume of 500ml and working volume of 400ml. Both the anode and cathode chambers were separated by a Nafion 117 membrane, Merck. The electrodes for each MFC consisting of either plain graphite anode (L=75mm, d=3mm) and MnO_2/rGO coated graphite cathode (L=75mm, d=3mm) or plain graphite anode (L=75mm, d=3mm) and plain graphite cathode (L=75mm, d=3mm) were used in this study. Both electrodes (anode and cathode) were connected to a digital multimeter using a copper wire. The anodic chambers were fed with 400ml of sample from each wastewater while the cathodic compartment employed dionised water as catholyte utilising atmospheric oxygen as the electron acceptor for the electrode. The cathode chamber was operated at aerobic conditons while the anodic compartment was operated at anaerobic conditions as illustrated by figure 3.6.



Fig 3.6 A figure showing a double chambered microbial fuel cells reactors and a Fluke 17B+ digital multimeter.

The double chamber MFCs were operated at open and closed circuit. For each MFC unit, the anode chamber was fitted with a plain anode electrode made of graphite (Merck), and the cathode chamber fitted with a graphite cathode electrode containing a 20µg/cm2 MnO_2/rGO catalyst.

Before each experimental run, each anode chamber is filled and inoculated with a defined wastewater sample for a defined period of time. The composition for each influent sample is also measured and recorded.

3.4 MEASUREMENTS AND ANALYSIS

3.4.1 Current

A Fluke 17B+ digital multimeter was used to test the voltage on microbial fuel cells, with voltage readings taken at intervals at open and closed circuits.

The Current (I) was calculated using Ohm's law(equ.3.1):

$$I(mA) = \frac{V(mV)}{R(\Omega)} \dots\dots\dots\dots\dots\dots\dots 3.1$$

where V (V) is the measured-cell voltage and R is the external resistance (Ω) of the cell.

3.4.2 Current density

Current density was calculated as in equ. 3.2:

$$i(\frac{mA}{cm^2}) = \frac{E}{R.A} \dots\dots\dots\dots\dots\dots\dots\dots 3.2$$

where E (mV) is the cell-measured voltage, R (Ω) is the external resistance and A (cm^2) is the projected surface area of the cathode electrode.

3.4.3 Power

The overall performance of an MFC is evaluated in many ways, but principally through power output and Coulombic efficiency. Power is calculated as the voltage measured across a fixed external resistor (Rext), while the current is calculated from Ohm's law. Thus, power is usually calculated asin equ.3.3:

$$P(W) = \frac{E^2 cell}{R_{ext}} \dots\dots\dots\dots\dots\dots\dots\dots 3.3.$$

3.4.4 Power density

The power density was calculated according to equ. 3.4:

$$P\left(\frac{mW}{m^2}\right) = 10iE \dots\dots\dots\dots\dots\dots 3.4$$

where 10 is needed for the given units.

Power is often normalized to some characteristic of the reactor in order to make it possible to compare power output of different systems. The power density (P_{An}, W/m^2) is therefore calculated on the basis of the area of the anode (A_{An}) as in equ. 3.5:

$$P_{An}(\frac{W}{m^2}) = \frac{E^2 cell}{A_{An} R_{ext}} \ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots 3.5$$

Alternatively, the area of the cathode (A_{Cat}) can alternatively be used to obtain a power density (P_{Cat}). The power is normalized to the reactor volume as shown in equ.3.6:

$$P_V\left(\frac{W}{m^3}\right) = \frac{E^2 cell}{V R_{ext}} \ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots 3.6$$

where Pv is the volumetric power (W/m^3) and v is the total reactor volume (i.e., the empty bed volume). The use of the total bed reactor volume is consistent with a tradition in environmental engineering to use the total reactor size as a basis for the calculation. A comparison on the basis of total reactor volume, however, is not always level when comparing two- and single-chambered reactors because there is no "second chamber" for an open air-cathode. In such cases it is useful to compare reactors on the basis of the total anode compartment volume.

3.4.5 COD measurements

COD measurements were conducted according to the ALPHA standard protocol, 1998 and with a spectrophotometer (UV-VIS Spectrophotometer, Merck, Germany).



Fig 3.7 COD analysis using the UV-VIS Spectrophotometer-320.

The pH was measured using a benchscale 700 Eutech instrument and the experimental investigations were carried out at ambient temperature of 18^0C -25^0C.

3.4.6 Coulombic efficiency

The coulombic efficiency (CE) was calculated by the ratio of the total coulombs obtained in the experiment (Cp) to the theoretical amount (C_T) available from complete siubstrate oxidation (Liu et al, 2004) as in equ. 3.7 and 3.8:

$$CE = \frac{C_p}{C_T} \times 100\% \ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots 3.7$$

$$= \frac{I \times t}{F \times n \times \Delta S \times V} \times M \ldots\ldots\ldots\ldots\ldots 3.8$$

Where I represents the current (A); t is the time interval (s); F is the Faraday constant =96,485 C/mol; n is the number of moles of electrons produced per mol of substrate (n =4 for oxygen reduction on cathode); ΔS is the absolute removal amount of COD (mg/L); V is the volume of anolyte (L); M is the molecular weight of the substrate (32 g/mol for oxygen).

3.4.7 Treatment efficiency

MFCs have been proposed as a wastewater treatment system, so it's critical to assess their overall efficiency in terms of biochemical oxygen demand (BOD), chemical oxygen demand (COD), and total organic carbon (TOC) elimination. Other factors, such as soluble versus particulate removal and nutrient removal, may also be significant. We'll look at MFC output in terms of COD removal because it's a common way to evaluate wastewater treatment efficiency, and COD removal is necessary for Coulombic and energy calculations. The ratio between the removed and influent COD can be used to measure COD removal efficiency (COD). This parameter indicates how much of the available "fuel" in the MFC has been transformed, either into electrical current (via Coulombic efficiency) or biomass (via growth yield), or through competitive reactions with alternative electron acceptors (e.g., oxygen, nitrate, and sulfate). Since the MFC influent can contain both dissolved and particulate COD,

determining which fraction of the effluent particulate COD was due to biomass generated in the reactor versus untreated COD in the reactor influent can be difficult.

3.5 REFERENCES

Liu, H., Ramnarayanan, R. and Logan, B.E., 2004. Production of electricity during wastewater treatment using a single chamber microbial fuel cell. Environmental science & technology, 38(7), pp.2281-2285.

Nie, R., Shi, J., Xia, S., Shen, L., Chen, P., Hou, Z. and Xiao, F.S., 2012. MnO 2/graphene oxide: a highly active catalyst for amide synthesis from alcohols and ammonia in aqueous media. Journal of Materials Chemistry, 22(35), pp.18115-18118.

Wang, Y., Guan, H., Du, S. and Wang, Y., 2015. A facile hydrothermal synthesis of MnO 2 nanorod–reduced graphene oxide nanocomposites possessing excellent microwave absorption properties. Rsc Advances, 5(108), pp.88979-88988.

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Chapter 4: Microbial Fuel Cell Start-Up Performance at Open –Circuit and Closed Circuit: Effect of External Resistance

4.1 BACKGROUND

Controlling the critical factors that have the greatest effect on process efficiency is needed to achieve maximum results. In MFCs, the cell's external resistance has previously been identified as one of the parameters that influences the MFC's efficiency. Biofilm growth is also important for efficient MFC efficiency, and several studies have found that it is influenced by the MFC's external resistance, for example. The study Zhang et al. (2011) investigated biofilm formation in the ohmic range of 10Ω–1000Ω, documenting optimal biofilm formation conditions at 50Ω external resistance and maximum current generation at 10Ω external resistances (Zhang et al., 2011). Jung and Regan (2011) investigated methane production and biofilm activity after maturation phasing. According to their findings, operating the MFC at external resistance (R_{ext}) equal to internal resistance (R_{int}) results in maximum power efficiency and repression of methanogen formation (Jung and Regan, 2011). Aelterman et al. (2008) investigated fuel supply and biofilm activity after the maturation phase and found that operating the MFC at external resistance (R_{ext}) equal to internal resistance (R_{int}) results in optimum biofilm activity (Aelterman et al., 2008). Larrosa-Guerrero et al. (2010) investigated microbial diversity as a feature of current generation and anode content on a brewery wastewater-MFC, where biofilm activity was measured at open circuit and under external load. They discovered that the anode material had a greater influence on biofilm formation in open circuit than in closed circuit, and that the anode material had a greater influence on current generation than on biofilm formation in closed circuit (Larrosa-Guerrero et al., 2010). With an external resistance of 300 k, the analysis achieved an average voltage output of 0.6 V in closed circuit and 0.75 V in open circuit. The aforementioned studies, as well as others (Jung and Regan, 2011; Rismani-Yazdi et al., 2011; Katuri et al., 2011), have all found that an MFC's external resistance is an important factor in its success.

On the other hand, MFCs treatment of wastewaters containing sugars and carbohydrates has been found to generate many metabolites during the anaerobic digestion of glucose by microbes (Freguia et al., 2007; Lee et al., 2008a, b). And these metabolites are said to cause low coulombic efficiency (Huang and Logan, 2008; Torres et al., 2007). Some studies have shown that glucose-fed MFCs have lower coulombic efficiency than acetate-fed MFCs, as shown in table 4.1 derived from Kim et al. (2011).

Table 4.1: Coulombic efficiencies and power densities in MFCs with different electron donors (Kim et al., 2011)

Electron donor	Coulombic efficiency (%)	Power density (mW/m^2)	MFC type	References
Acetate	82	14	Two chamber	Bond et al.,2002
Acetate	61	1970	Single chamber	Cheng and Logan,2007
Butyrate	43	51.4	Two chamber	Chae et al.,2009b
Ethanol	10	488	Single chamber	Kim et al.,2007
Glucose	45	Not mentioned	Two chamber	Freguia et al.,2008
Glucose	15	156	Two chamber	Chae et al.,2009b
Wastewater	28	28	Single chamber	Liu and Logan,2004
Wastewater	8	182	Single chamber	Min et al.,2005

Hallenbeck and Ghosh (2009) established a two-stage method that can potentially harness the full amount of energy from the degradation of sugars and carbohydrates, as well as their metabolites, in order to remove the effects of metabolites on coulombic performance and they were able to get the most energy out of sugars and carbohydrates through dark fermentation and the use of fermentative metabolites. Kim et al. (2011) established a glucose-fed MFC and observed incomplete propionate degradation in their sample, which they attribute to hydrogen partial pressure inhibition, acetate inhibition, and pH due to the undissociated propionic acid (Boone and Xun, 1987; Fukuzaki et al., 1990). Kim et al. (2011) went on to construct glucose-fed dual-anode MFCs (DAMFCs), one open circuit and one closed circuit. Their Coulombic efficiency performances were compared by evaluating electron balances from studies of induced gas and metabolites. They also observed an improved coulombic efficiency from reduced electrons as a result of the effective use of glucose and propionate, and in this regard, they identified DAMFC as a potential alternative MFC configuration in MFCs

fed with hard-biodegradable organic compounds (Kim et al., 2011). The effects of external resistance on coulombic efficiency as a function of biofilm growth rate have rarely been documented. Analysis of the effects of external resistance on coulombic efficiency as a function of biofilm growth may reveal previously unknown microbial interactions as well as optimal operating conditions. By examining the effects of external resistance on MFC start-up output in both open and closed circuits, this chapter aims to analyze the biofilm growth rate as a useful technique for analyzing the coulombic quality. And, to date, systematic reporting on MFC success as a feature of biofilm growth rate is scarce. Expressing MFC start-up performance for wastewater treatment as a feature of biofilm growth rate can provide an important insight into MFC start-up performance when reporting on anodic biofilm enrichment. The ability to understand biofilm growth rate and its effect on MFC efficiency will be useful for the development of such systems.

4.2 MATERIALS AND METHIODS

The chemical oxygen demands (COD) measurements of the wastewater (beer brewery) were conducted with a spectrophotometer (UV-VIS Spectroquant 320, Merck) as reported in section 3.4.

6 double chambered MFCs with 400 ml total volume were used in this study. The chambers for each MFC were separated by a 25cm diameter Nafion membrane. The anode and cathode electrodes were each made of 3mm diameter and 75mm long graphite rods (Merck, South Africa). The MFCs were operated at ambient temperature, i.e., average of 24^0C. Brewery wastewater was collected from SAB/INBEV beer brewery and stored at 0^0C on the same day until use. All chemicals and reagents used were of analytical grade. The four MFCs (i.e. MFC-1, MFC-2, MFC-3 and MFC-4) were fed with pure beer brewery wastewater effluent while two more MFCs (MFC-5 and MFC-6) were fed with pure domestic wastewater. The initial composition of the wastewaters is represented by table 4.2.

Table 4.2. Initial wastewater composition

Wastewater	COD (mg/L)	pH
Beer brewery	3246	8.40
Domestic	825	8.05

The investigative study work was performed at open circuit and closed circuit. Each MFC operation was maintained long enough to ensure a steady state performance which was assessed based on manual measurements of the output current.

Steady state current(I_f) tests were performed for each MFC with random current measurements over a period of 7days. The resulting current and time values were used to construct current versus time curves where the MFC's steady state current can be estimated. The slope of the current versus time curve determines the biofilm growth rate constant by the exponential relationship,

$$ln\left(1-\frac{I_i}{I_f}\right)=\frac{1}{\tau}t_i \quad \text{.....4.1}$$

Where I_i (μA) is the current measured at a time ti(hrs), If (μA) is the steady state current achieved in a MFC and τ (hrs) is the biofilm growth rate constant for a given MFC. For each MFC, COD measurements were also performed.

The coulombic efficiency (CE) was calculated by the ratio of the total coulombs obtained in the experiment (Cp) to the theoretical amount (CT) available from complete substrate oxidation (Liu et al, 2004):

$$CE=\frac{C_p}{C_T}\times 100\%$$

$$=\frac{I\times t}{F\times n\times \Delta S\times V}\times M \text{ ... ...4.2}$$

Where I represents the current (A); t is the time interval (s); F is the Faraday constant =96,485C/mol; n is the number of moles of electrons produced per mol of substrate (n =4 for oxygen reduction on cathode); ΔS is the absolute removal amount of COD (mg/L); V is the volume of anolyte (L); M is the molecular weight of the substrate (32g/mol for oxygen).

4.3 RESULTS AND DISCUSSION

4.3.1 Comparative study analysis of MFC start-up performance at varying external resistance: current generation and COD removal

Previous studies have reported on limited current flow from anode to cathode as a result of high external resistance affecting anode colonization by microbes (Lyon et al., 2010; Ren et al., 2011). Figures 4.1 and 4.2 illustrate the results depicting current generation across the resistors of each MFC (i.e. MFC-1 to MFC-4). Figure 4.1 shows results for MFC-1 (operated at 33kΩ external resistor), MFC-2(operated at open circuit), MFC-3 (operated at 2.2 kΩ) and MFC-4 (operated at 51 kΩ) and all fed with beer brewery wastewater effluent. Figure 4.2 shows steady state current reached by MFC-1 as 6.7µA after 87hrs of start-up, 17.5 µA after 87hrs by MFC-2, 5.1 µA after 25hrs by MFC-3, and 9.2 µA after 25hrs by MFC-4. The trends in figure 4.1 show higher current generation for high external resistance MFC (i.e. MFC-4 operated at 51kΩ) and lowest current generation for MFC-1 operated at 33kΩ. The trends in figure 4.1 further indicate low external resistance (i.e. MFC-3 operated at 2.2kΩ) as favorable to higher current generation as compared to MFC-1 and MFC-2 operated at 33kΩ and open circuit respectively.

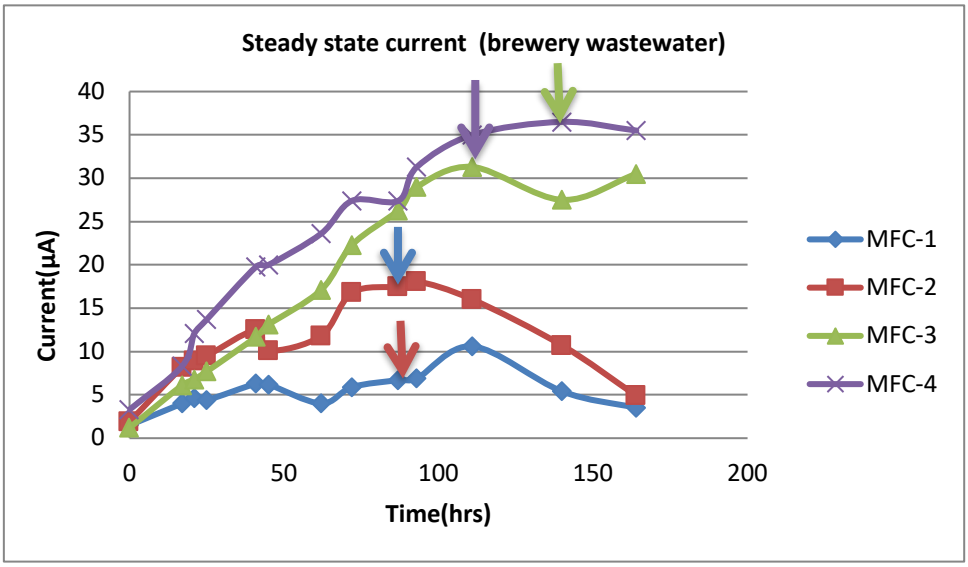


Figure 4.1 Steady state current during MFC start-up period for brewery wastewater at varying external resistance.

Figure 4.2 shows results for MFC-5 (operated at 2.2kΩ external resistor) and MFC-6 (operated at 51kΩ) fed with domestic wastewater. In the figure 4.2, steady state current is reached by MFC-5 at 31.3µA after 111hrs and by MFC-6 at 36.5 µA after 140hrs). The trends in figure 4.2 show higher current generation for MFC-5 operated at 2.2kΩ as compared to MFC-6 operated at 51kΩ.

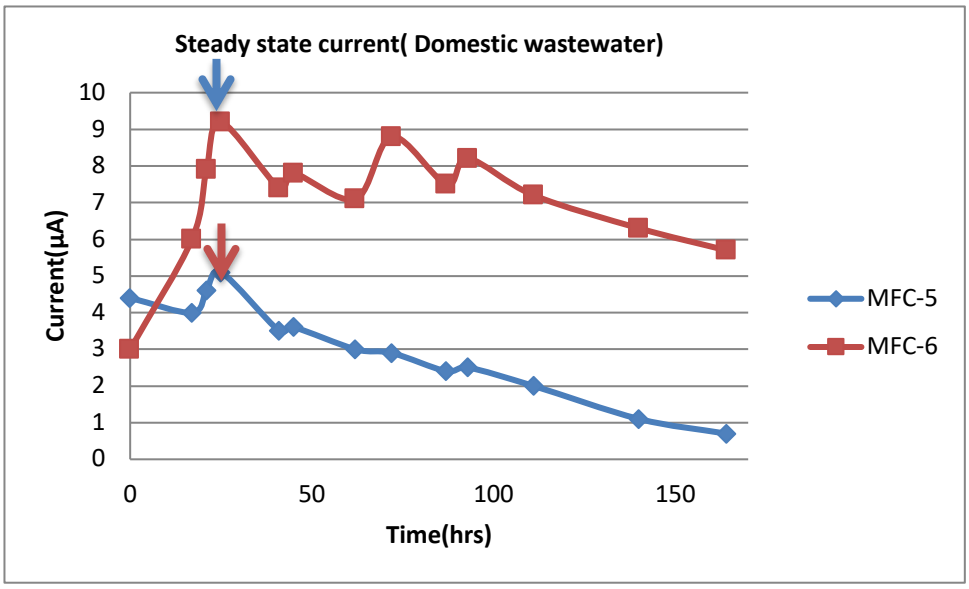


Figure 4.2 Steady state current during MFC start-up period for domestic wastewater at varying external resistance.

Figures 4.1 and 4.2 display low current in certain low external resistance systems (MFC-4 and MFC-5), contradicting previous findings (Freguia et al., 2008; Jung and Regan, 2011; Zhang et al., 2011). Figures 4.1 and 4.2 should illustrate indirect proportionality between current and external resistance, but only figure 4.1 reveals a marginally meaningful relationship between and external resistance. On the other side, the high external resistance MFC, i.e. MFC-4, and open circuit MFC, i.e. agree with the findings of Zhang et al. (2011), who discovered that high external resistance MFCs mimic an open circuit because microorganisms are unable to transfer electrons to an unfavorable electron acceptor (Zhang et al., 2011). Additionally, the high external resistance prevents current flow from anode to cathode, preventing microorganisms from colonizing the anode. Ghangrekar and Shinde (2007) published on the effects of external resistance on current generation and discovered results similar to Zhang et al (2011). As a result, the trends shown in Figures 4.1 and 4.2 indicate that one of the

factors restricting significant current generation in a microbial fuel cell as a function of biofilm growth rate is external resistance.

Previous research discovered that high external resistance has a substantial negative effect on anode colonization because it restricts current flow from the anode to the cathode (Lyon et al., 2010; Ren et al., 2011). The results of this study show that anode colonization is minimal, as seen in figures 4.1 and 4.2. Table 4.3 further indicate that for low external resistance MFC, steady-state conditions result in the fastest biofilm growth rate (i.e. brewery wastewater fed MFC-3). In terms of domestic wastewater fed MFCs, high external resistance MFCs exhibit the highest biofilm growth rate (i.e MFC-4). The biofilm growth rate method results from figures 4.3 and 4.4, as well as table 4.3, indicate no significant association between current generation and external resistance, which contradicts previous findings (Freguia et al., 2008; Jung and Regan, 2011; Zhang et al., 2011).

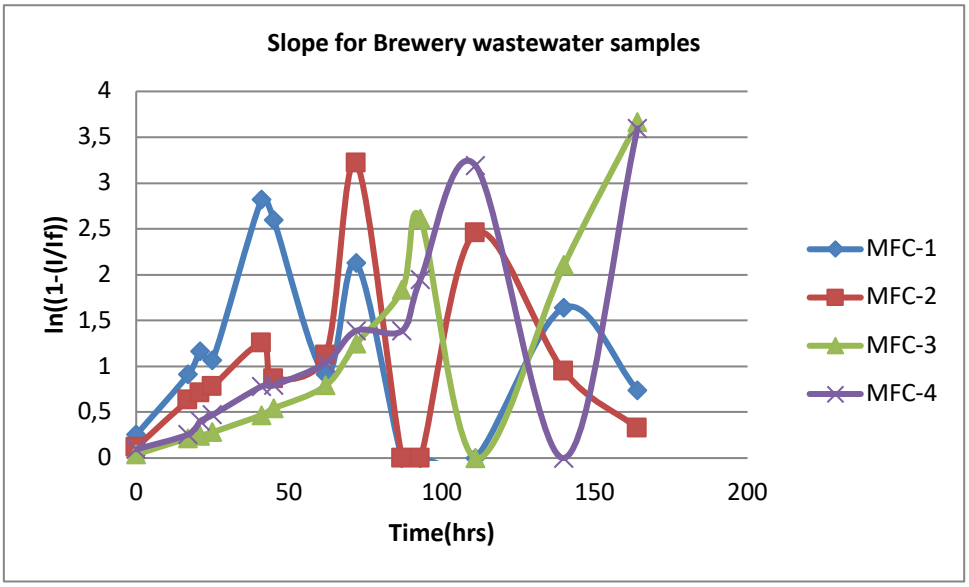


Figure 4.3 Slope determination for brewery wastewater samples.

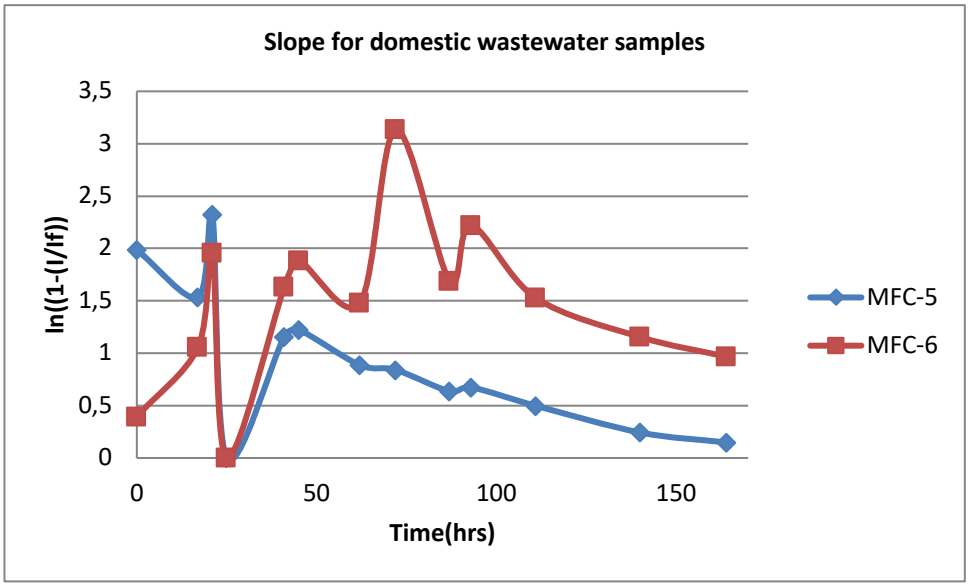


Figure 4.4 Slope determination for domestic wastewater samples.

Table 4.3 steady state current and biofilm growth rate constant for each MFC

	MFC-1	MFC-2	MFC-3	MFC-4	MFC-5	MFC-6
If(μA)	6.7	17.5	31.3	36.5	5.1	9.2
Time(hrs)	87	87	111	140	25	25
Biofilm growth rate $\frac{1}{\tau}(hr^{-1})$	0.003	0.0019	0.0184	0.0156	0.0027	0.0099
Biofilm growth rate constant τ (hrs)	333.3333	526.3158	54.34783	64.10256	370.3704	101.0101

Figures 4.5 and 4.6 show that high COD removal occurs at low external resistance and low COD removal occurs at high external resistance. Low anode potential results from high external resistance, resulting in altered metabolic activities of the anodic microbial population. MFCs with low external resistance have the potential to eliminate contaminants from wastewater treatment during and after MFC start-up, which is attributed to high MFC current generation, which encourages COD elimination. Sajana et al. (2014) discovered high COD removal when running a single chamber microbial fuel cell with low external resistance.

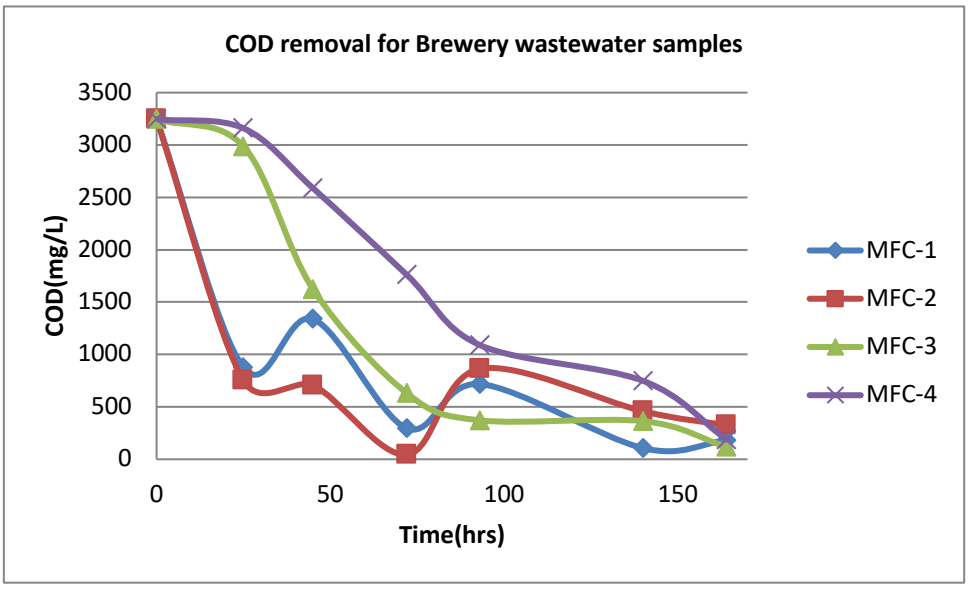


Figure 4.5 COD removal measurements for brewery wastewater-fed MFC at 2.2kΩ, 33kΩ, 51kΩ external resistance and open circuit.

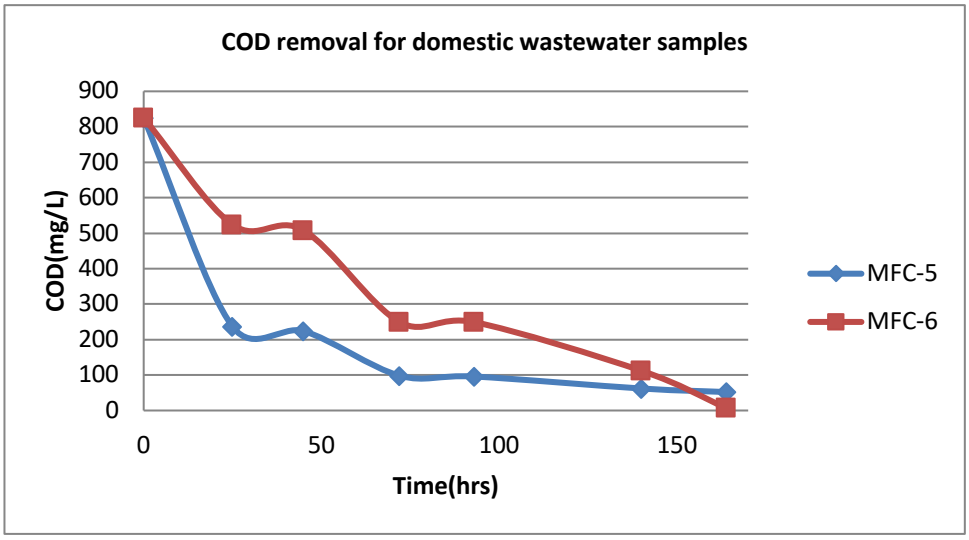


Figure 4.6 COD removal measurements for domestic wastewater-fed MFC at 2.2kΩ and 33kΩ external resistance

MFC performance, as calculated by coulombic efficiency, is a bottleneck in the use of MFCs in large-scale operations. Previous research discovered that the coulombic efficiency is highly dependent on the biocatalysts present in organic matter as well as the carbon content of organic matter (Franks and Nevin, 2010). Lanthier et al. (2008) obtained a coulombic efficiency of 56.2 percent due to the incomplete oxidation of the lactate (i.e. substrate) by the biocatalysts (i.e. Shewanella oneidensis) and losing unutilized electrons as waste products such as acetate during the oxidation of organic matter. Rabaey et al. (2003) recorded columbic efficiencies from wastewater ranging from 65 to 89 percent (Rabaey et al., 2003).

Other previous studies have discovered that low coulombic efficiencies suggest an unfavorable anodic reaction due to inadequate potential difference between the anode and cathode electrodes, resulting in insufficient assistance for electron transfer, which is consistent with the findings of this research as shown in figures 4.7 and 4.8.

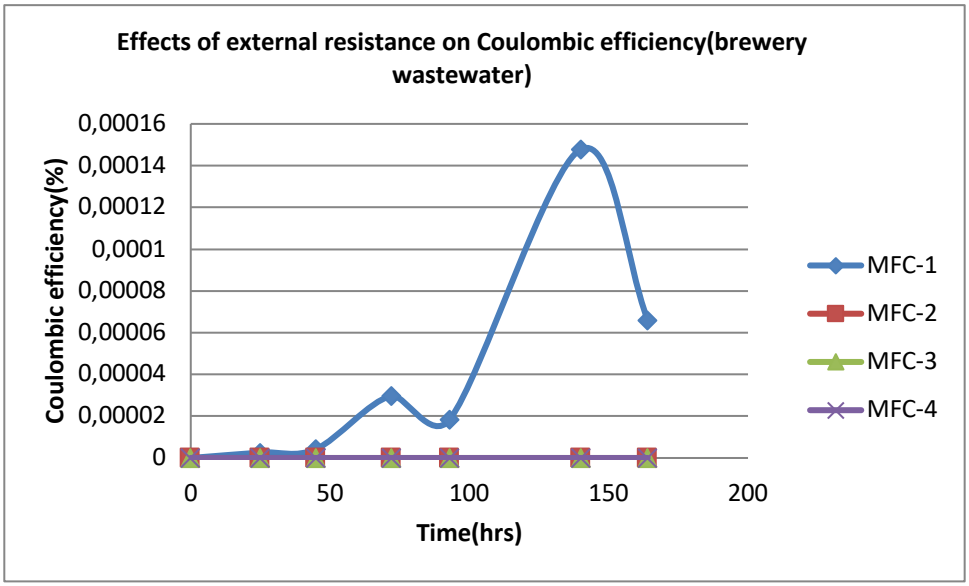


Figure 4.7 Effects of external resistance on coulombic efficiency as a function of time (Brewery wastewater).

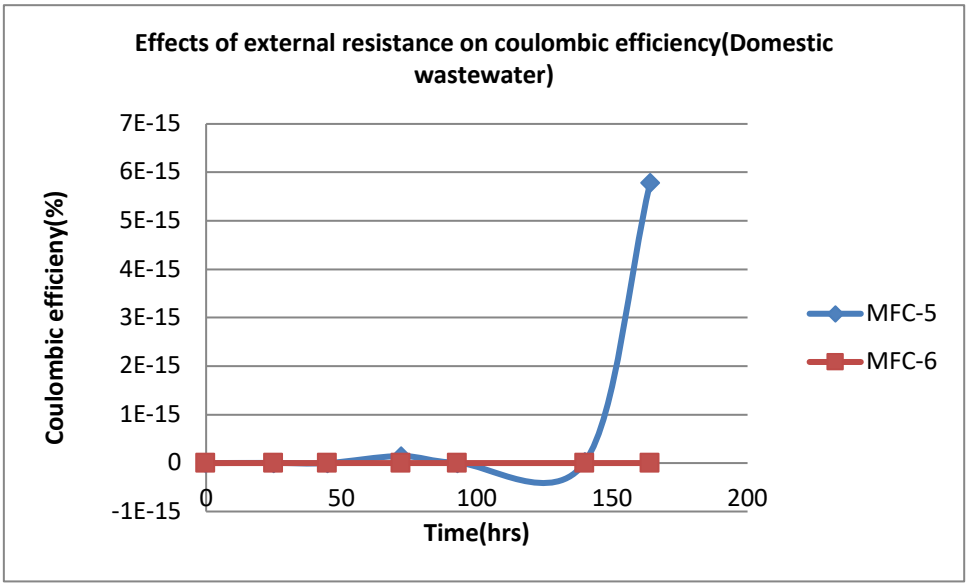


Figure 4.8 Effects of external resistance on coulombic efficiency as a function of time (Domestic wastewater)

Wei et al. (2010) stated that the upper potential of an organism's terminal electron donor limits the amount of energy an organism can obtain from a redox reaction (2010). Previous research has shown that high external resistance has a negative effect on coulombic efficiency because it reduces the election transfer rate to the anode, resulting in lower current generation (Freguia et al., 2008; Harnisch & Schroder,2010).

Tables 4.6 and 4.7 summarize the optimum COD removal and highest COD removal efficiency as determined by figures 4.9 and 4.10. They also show that the effect of external resistance on COD removal was positive. The biofilm growth rate and biofilm growth constant experiments contributed significantly to the investigation of microbial efficiency and yielded additional useful knowledge about substrate degradation pathways and microbial interactions. The experiment also showed evidence of correlating results close to global norm methods of investigating MFCs, as well as conflicting findings.

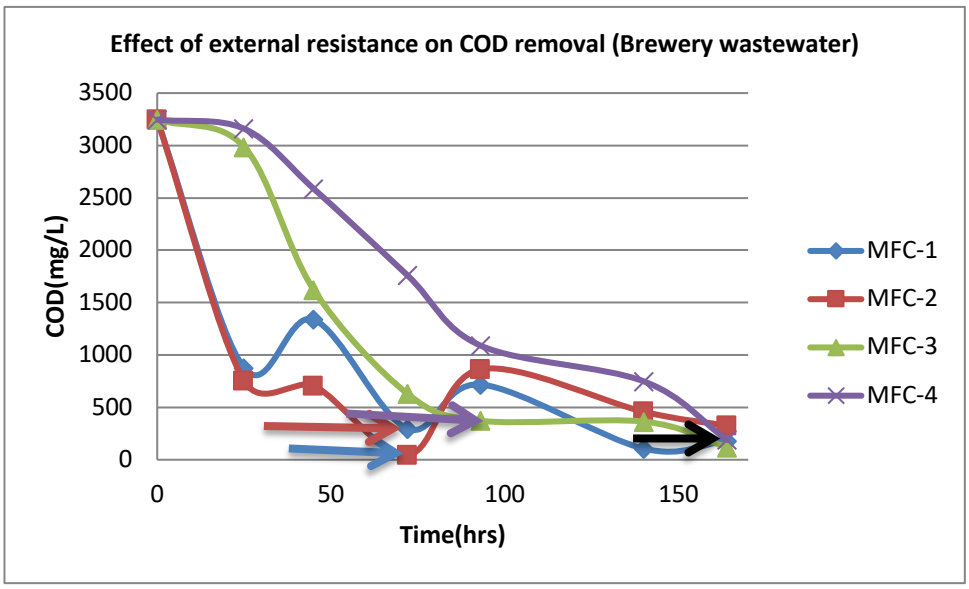


Figure 4.9 effect of external resistance on COD removal on brewery wastewater fed MFCs.

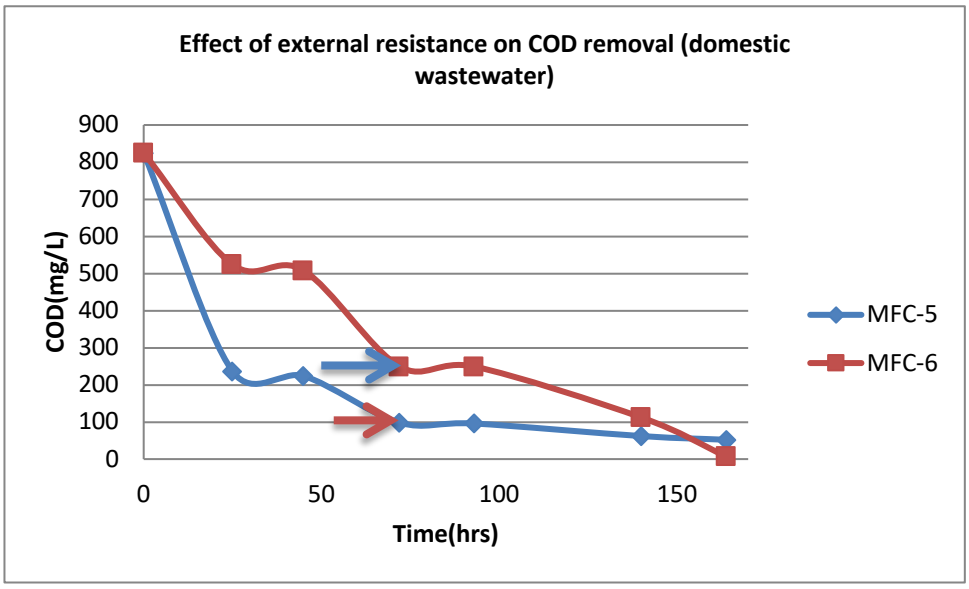


Figure 4.10 effect of external resistance on COD removal on domestic wastewater fed MFCs.

Table 4.6 Optimal COD removal for brewery wastewater-fed MFC at 2.2kΩ, 33kΩ, 51 kΩ external resistance and open circuit.

	MFC-1	MFC-2	MFC-3	MFC-4
Optimum I(μA)	6.7	17.5	31.3	36.5
Biofilm growth rate 1/τ(hr-1)	0.003	0.0019	0.0184	0.0156
τ (hrs)	333.3333	526.3158	54.34783	64.10256
Optimum COD(mg/L)	296.1	43.98	372	188
Highest COD removal(%)	96.73	98.65	96.36	94.21

Table 4.7 Optimal COD removal for domestic wastewater-fed MFC at 2.2 kΩ and 51kΩ external resistance.

	MFC-5	MFC-6
Optimum I(μA)	5.1	9.2
Biofilm growth rate 1/τ(hr-1)	0.0027	0.0099
τ (hrs)	370.3704	101.0101
Optimum COD(mg/L)	98	249
Highest COD removal (%)	93.70	99.23

4.4 CONCLUSIONS

Biofilm activity was measured in both open and closed circuits, as well as with and without an external resistance. The findings of this analysis show that high external resistance MFCs (operated at 51k) generate more current in closed circuit and low external resistance MFCs (operated at 2.2k) generate more current in open circuit. In this regard, our results contradict previous findings (Freguia et al., 2008; Jung and Regan, 2011; Zhang et al., 2011), since we predicted indirect proportionality between current and external resistance, but only figure 4.1 indicates a slightly relevant interaction between the two.

Figures 4.1 and 4.2, on the other hand, demonstrate that external resistance is one of the factors limiting major current generation in a microbial fuel cell as a function of biofilm growth rate. High external resistance, according to previous studies, has a

major negative influence on anode colonization because it limits current flow from the anode to the cathode (Lyon et al., 2010; Ren et al., 2011). Table 4.3 shows that steady-state conditions result in the highest biofilm growth rate for MFCs with low external resistance (i.e. brewery wastewater fed MFC-3). High external resistance MFCs have the fastest biofilm growth rate when fed domestic wastewater (i.e MFC-4). Figures 4.3 and 4.4, as well as table 4.3, reveal that there is no substantial connection between current generation and external resistance, which confirms previous results (Freguia et al., 2008; Jung and Regan, 2011; Zhang et al., 2011). Figures 4.9 and 4.10 from the report also indicate that external resistance has a beneficial impact on COD exclusion.

4.5 REFERENCES

Chae, K.J., Choi, M.J., Lee, J.W., Ajayi, F.F., Kim, I.S., 2008a. Biohydrogen production via biocatalyzed electrolysis in acetate-fed bioelectrochemical cells and microbial community analysis. Int. J. Hydrogen Energy 33, 5184–5192.

Chae, K.J., Choi, M.J., Lee, J.W., Kim, K.Y., Kim, I.S., 2009b. Effect of differentsubstrates on the performance, bacterial diversity, and bacterial viability inmicrobial fuel cells. Bioresour. Technol. 100, 3518–3525.

Clauwaert, P., Verstraete, W., 2009. Methanogenesis in membrane-less microbial electrolysis cells. Appl. Microbiol. Biotechnol. 82, 829–836.

Freguia, S., Rabaey, K., Yuan, Z., Keller, J., 2007. Electron and carbon balances in microbial fuel cells reveal temporary bacterial storage behavior during electricity generation. Environ. Sci. Technol. 41, 2915–2921.

Freguia, S., Rabaey, K., Yuan, Z., Keller, J., 2008. Syntrophic processes drive the conversion of glucose in microbial fuel cell anodes. Environ. Sci. Technol. 42, 7937–7943.

Hallenbeck, P.C., Ghosh, D., 2009. Advances in fermentative biohydrogen production: the way forward? Trends Biotechnol. 27, 287–297.

Huang, L., Logan, B.E., 2008. Electricity production from xylose in fed-batch and continuous flow microbial fuel cells. Appl. Microbiol. Biotechnol. 80, 655–

Kim, I.S., Chae, K.J., Choi, M.J., Verstraete, W., 2008. Microbial Fuel Cells: Recent Advances, Bacterial Communities and Application Beyond Electricity Generation. Environ. Eng. Res. 13, 51–65.

Lee, H.S., Parameswaran, P., Kato-Marcus, A., Torres, C.I., Rittmann, B.E., 2008a. Evaluation of energy conversion efficiencies in microbial fuel cells (MFCs) utilizing fermentable and non-fermentable substrates. Water Res. 42, 1501–1510.

Lee, H.S., Salerno, M.B., Rittmann, B.E., 2008b. Thermodynamic evaluation on H2 production in glucose fermentation. Environ. Sci. Technol. 42, 2401– 2407.

Logan, B.E., Hamelers, B., Rozendal, R., Schroder, U., Keller, J., Freguia, S., Aelterman, P., Verstraete, W., Rabaey, K., 2006. Microbial fuel cells: methodology and technology. Environ. Sci. Technol. 40, 5181–5192.

Rabaey, K., Verstraete, W., 2005. Microbial fuel cells: novel biotechnology for energy generation. Trends Biotechnol. 23, 291–298.

Torres, C.I., Kato Marcus, A., Rittmann, B.E., 2007. Kinetics of consumption of fermentation products by anode-respiring bacteria. Appl. Microbiol. Biotechnol. 77, 689–697.

Wagner, R.C., Regan, J.M., Oh, S.E., Zuo, Y., Logan, B.E., 2009. Hydrogen and methane production from swine wastewater using microbial electrolysis cells. Water Res. 43, 1480–1488.

Kim, K.Y., Chae, K.J., Choi, M.J., Ajayi, F.F., Jang, A., Kim, C.W. and Kim, I.S., 2011. Enhanced Coulombic efficiency in glucose-fed microbial fuel cells by reducing metabolite electron losses using dual anode electrodes. Bioresource technology, 102(5), pp.4144-4149.

Bond, D.R., Lovley, D.R., 2003. Electricity Production by Geobacter sulfurreducensAttached to Electrodes. Appl. Environ. Microbiol. 69, 1548–1555.

Boone, D.R., Xun, L., 1987. Effects of pH, temperature, and nutrients on propionatedegradation by a methanogenic enrichment culture. Appl. Environ. Microbiol.53, 1589–1592.

Fukuzaki, S., Nishio, N., Shobayashi, M., Nagai, S., 1990. Inhibition of thefermentation of propionate to methane by hydrogen, acetate, and propionate.Appl. Environ. Microbiol. 56, 719–723.

Cheng, S. and Logan, B.E., 2007. Ammonia treatment of carbon cloth anodes to enhance power generation of microbial fuel cells. Electrochemistry Communications, 9(3), pp.492-496.

Kim, J.R., Cheng, S., Oh, S.E. and Logan, B.E., 2007. Power generation using different cation, anion, and ultrafiltration membranes in microbial fuel cells. Environmental science & technology, 41(3), pp.1004-1009.

Liu, H. and Logan, B.E., 2004. Electricity generation using an air-cathode single chamber microbial fuel cell in the presence and absence of a proton exchange membrane. Environmental science & technology, 38(14), pp.4040-4046.

Min, B., Cheng, S. and Logan, B.E., 2005. Electricity generation using membrane and salt bridge microbial fuel cells. Water research, 39(9), pp.1675-1686.

Du, Z., Li, H. and Gu, T., 2007. A state of the art review on microbial fuel cells: a promising technology for wastewater treatment and bioenergy. Biotechnology advances, 25(5), pp.464-482.

Franks, A.E. and Nevin, K.P., 2010. Microbial fuel cells, a current review. Energies, 3(5), pp.899-919.

Freguia, S., Rabaey, K., Yuan, Z. and Keller, J., 2008. Syntrophic processes drive the conversion of glucose in microbial fuel cell anodes. Environmental science & technology, 42(21), pp.7937-7943.

Torres, C.I., Marcus, A.K. and Rittmann, B.E., 2007. Kinetics of consumption of fermentation products by anode-respiring bacteria. Applied microbiology and biotechnology, 77(3), pp.689-697.

Lee, H.S., Parameswaran, P., Kato-Marcus, A., Torres, C.I. and Rittmann, B.E., 2008. Evaluation of energy conversion efficiencies in microbial fuel cells (MFCs) utilizing fermentable and non-fermentable substrates. Water research, 42(6-7), pp.1501-1510.

Lanthier, M., Gregory, K.B. and Lovley, D.R., 2008. Growth with high planktonic biomass in Shewanella oneidensis fuel cells. FEMS microbiology letters, 278(1), pp.29-35.

Rabaey, K., Lissens, G., Siciliano, S.D. and Verstraete, W., 2003. A microbial fuel cell capable of converting glucose to electricity at high rate and efficiency. Biotechnology letters, 25(18), pp.1531-1535.

Song, T.S., Yan, Z.S., Zhao, Z.W. and Jiang, H.L., 2010. Removal of organic matter in freshwater sediment by microbial fuel cells at various external resistances. Journal of Chemical Technology & Biotechnology, 85(11), pp.1489-1493.

Aelterman, P., Versichele, M., Marzorati, M., Boon, N. and Verstraete, W., 2008. Loading rate and external resistance control the electricity generation of microbial fuel cells with different three-dimensional anodes. Bioresource technology, 99(18), pp.8895-8902.

Liu, H., Cheng, S. and Logan, B.E., 2005. Production of electricity from acetate or butyrate using a single chamber microbial fuel cell. Environmental science & technology, 39(2), pp.658-662.

Jadhav, G.S. and Ghangrekar, M.M., 2009. Performance of microbial fuel cell subjected to variation in pH, temperature, external load and substrate concentration. Bioresource Technology, 100(2), pp.717-723.

Jang JK, Pham TH, Chang IS, Kang KH, Moon H, Cho KS, Kim BH (2004) Construction and operation of a novel mediator and membrane-less microbial fuel cell. Process Biochem 39(8):1007–1012

Sajana, T.K., Ghangrekar, M.M. and Mitra, A., 2014. Effect of operating parameters on the performance of sediment microbial fuel cell treating aquaculture water. Aquacultural engineering, 61, pp.17-26.

del Campo, A.G., Cañizares, P., Lobato, J., Rodrigo, M. and Morales, F.F., 2014. Effects of external resistance on microbial fuel cell's performance. In Environment, energy and climate change II (pp. 175-197). Springer, Cham.

Rismani-Yazdi, H., Christy, A.D., Carver, S.M., Yu, Z., Dehority, B.A. and Tuovinen, O.H., 2011. Effect of external resistance on bacterial diversity and

metabolism in cellulose-fed microbial fuel cells. Bioresource technology, 102(1), pp.278-283.

Jung, S. and Regan, J.M., 2011. Influence of external resistance on electrogenesis, methanogenesis, and anode prokaryotic communities in microbial fuel cells. Appl. Environ. Microbiol., 77(2), pp.564-571.

Lee, C.Y., Chen, J.H. and Cai, Y.Y., 2010. Bio-electricity generation and organic removal in microbial fuel cells used for treatment of wastewater from fish-market. J Environ Eng Manage, 20(3), pp.173-180.

Katuri, K.P., Scott, K., Head, I.M., Picioreanu, C. and Curtis, T.P., 2011. Microbial fuel cells meet with external resistance. Bioresource technology, 102(3), pp.2758-2766.

Logan, B.E., 2009. Exoelectrogenic bacteria that power microbial fuel cells. Nature Reviews Microbiology, 7(5), p.375.

Pasupuleti, S.B., Srikanth, S., Dominguez-Benetton, X., Mohan, S.V. and Pant, D., 2016. Dual gas diffusion cathode design for microbial fuel cell (MFC): optimizing the suitable mode of operation in terms of bioelectrochemical and bioelectro-kinetic evaluation. Journal of Chemical Technology & Biotechnology, 91(3), pp.624-639.

Yong, Y.C., Dong, X.C., Chan-Park, M.B., Song, H. and Chen, P., 2012. Macroporous and monolithic anode based on polyaniline hybridized three-dimensional graphene for high-performance microbial fuel cells. ACS nano, 6(3), pp.2394-2400.

Aelterman, P., Freguia, S., Keller, J., Verstraete, W. and Rabaey, K., 2008. The anode potential regulates bacterial activity in microbial fuel cells. Applied microbiology and biotechnology, 78(3), pp.409-418.

Wei, J., Liang, P., Cao, X. and Huang, X., 2010. A new insight into potential regulation on growth and power generation of Geobacter sulfurreducens in microbial fuel cells based on energy viewpoint. Environmental science & technology, 44(8), pp.3187-3191.

Zhu, X., Tokash, J.C., Hong, Y. and Logan, B.E., 2013. Controlling the occurrence of power overshoot by adapting microbial fuel cells to high anode potentials. Bioelectrochemistry, 90, pp.30-35.

Zhang, L., Zhu, X., Li, J., Liao, Q. and Ye, D., 2011. Biofilm formation and electricity generation of a microbial fuel cell started up under different external resistances. Journal of Power Sources, 196(15), pp.6029-6035.

Jung, S. and Regan, J.M., 2011. Influence of external resistance on electrogenesis, methanogenesis, and anode prokaryotic communities in microbial fuel cells. Appl. Environ. Microbiol., 77(2), pp.564-571.

Aelterman, P., Versichele, M., Marzorati, M., Boon, N. and Verstraete, W., 2008. Loading rate and external resistance control the electricity generation of microbial fuel cells with different three-dimensional anodes. Bioresource technology, 99(18), pp.8895-8902.

Rismani-Yazdi, H., Christy, A.D., Carver, S.M., Yu, Z., Dehority, B.A. and Tuovinen, O.H., 2011. Effect of external resistance on bacterial diversity and metabolism in cellulose-fed microbial fuel cells. Bioresource technology, 102(1), pp.278-283.

Katuri, K.P., Scott, K., Head, I.M., Picioreanu, C. and Curtis, T.P., 2011. Microbial fuel cells meet with external resistance. Bioresource technology, 102(3), pp.2758-2766.

Larrosa-Guerrero, A., Scott, K., Katuri, K.P., Godinez, C., Head, I.M. and Curtis, T., 2010. Open circuit versus closed circuit enrichment of anodic biofilms in MFC: effect on performance and anodic communities. Applied microbiology and biotechnology, 87(5), pp.1699-1713.

del Campo, A.G., Cañizares, P., Lobato, J., Rodrigo, M. and Morales, F.F., 2014. Effects of external resistance on microbial fuel cell's performance. In Environment, energy and climate change II (pp. 175-197). Springer, Cham.

Lyon, D.Y., Buret, F., Vogel, T.M. and Monier, J.M., 2010. Is resistance futile? Changing external resistance does not improve microbial fuel cell performance. Bioelectrochemistry, 78(1), pp.2-7.

Ren, Z., Yan, H., Wang, W., Mench, M.M. and Regan, J.M., 2011. Characterization of microbial fuel cells at microbially and electrochemically meaningful time scales. Environmental science & technology, 45(6), pp.2435-2441.

Zhang, L., Zhu, X., Li, J., Liao, Q. and Ye, D., 2011. Biofilm formation and electricity generation of a microbial fuel cell started up under different external resistances. Journal of Power Sources, 196(15), pp.6029-6035.

Ghangrekar, M.M. and Shinde, V.B., 2007. Performance of membrane-less microbial fuel cell treating wastewater and effect of electrode distance and area on electricity production. Bioresource Technology, 98(15), pp.2879-2885.

Menicucci, J., Beyenal, H., Marsili, E., Veluchamy, R.A., Demir, G. and Lewandowski, Z., 2006. Procedure for determining maximum sustainable power generated by microbial fuel cells. Environmental science & technology, 40(3), pp.1062-1068.

Kamau, J.M., Mbui, D.N., Mwaniki, J.M., Mwaura, F.B. and Kamau, G.N., 2017. Microbial fuel cells: influence of external resistors on power, current and power density. J Thermodyn Catal, 8(1), pp.100-182.

Aelterman, P., Versichele, M., Marzorati, M., Boon, N. and Verstraete, W., 2008. Loading rate and external resistance control the electricity generation of microbial fuel cells with different three-dimensional anodes. Bioresource technology, 99(18), pp.8895-8902

Chapter 5: Analysis of Microbial Fuel Cell Start-Up: Biofilm formation

5.1 INTRODUCTION

Microbial fuel cell technology has received a lot of research attention in the last decade, and as a result, there have been a lot of studies looking at microbial activity factors including the formation of an efficient exoelectrogenic biofilm, anodic and cathodic surface property, electrogenic conductivity, and biocompatibility (Logan, 2009; Kumar et al., 2013; Yong et al., 2012; Pasupuleti et al., 2016). The study of biofilms and biofilm forming processes has piqued the attention of MFC researchers. They were discovered to allow direct electron transfer between microorganisms (electron donor) and anodes (electron acceptor), resulting in higher current generation (Picioreanu et al., 2007). Biofilms are formed on a surface through cell attachment, which is assisted by the expression of exopolymers that bind to the surface, and then by cell growth, which is caused by the dispersion of microbial cells detached from the biofilm layer and attached to another part of the surface, according to previous research findings (Gottenbos et al., 1999; Davies et al., 1998). Biofilm growth is the result of detachment and attachment from and to the surface, which results in an increase in biofilm thickness. A growing biofilm in an MFC increases the anode surface area for electron transfer, increasing the MFC performance in terms of current generation (Picioreanu et al., 2007). A thicker biofilm that can preserve MFC effectiveness has been found to be efficient because it protects the dormant layer of bacteria by continuously restoring any dead or removed cells (Mah and O'Toole, 2001). A natural biofilm has been discovered to have a special feature of intermediate population metabolism, which allows multiple microbes, such as Archaea and Bacteria, to form a microbial community in which one species' products are the substrates for another (Hall-Stoodley et al., 2004). MFC biofilms, in this case, are bacteria that enable electron transfer to the anode and thus produce current. Other studies have shown that a mixed biofilm of Pelotomaculum thermopropionicum, Methanothermobacter thermautotrophicus, and Geobacteraceae produces better MFC output than a single species biofilm (e.g., a mixed biofilm of Pelotomaculum thermopropionicum,

Methanothermobacter thermautotrophicus, and Geobacteraceae generates better potential difference than a film of only Geobacteraceae A biofilm's composition may also be made up of a single species or several microorganisms that use different metabolic pathways (Kim et al., 2006).

Also, biofilm formation is critical for microbial fuel cell electrogenic performance, prompting some researchers to investigate the impact of anode modification on MFC performance, due to poor MFC performance observed in carbon-based materials such as pain carbon cloth anode, which have a limited specific surface area and poor electrical conductivity (Yao et al., 2012). Due to their high surface area and electrical conductivity, some studies suggest using supported or improved conductive polymers and nanostructured materials to improve the surface characteristics, electrical conductivity, and biocompatibility of carbon-based anodes (Kumar et al.,2013; Zhou et al.,2011). Due to its high specific surface area and electrical conductivity, graphene is a material of interest for anode modification; however, its antibacterial effect has been found to have the potential to damage bacterial cell walls due to its anti-biofilm character (Zhu et al., 2010; Batzill, 2012; ElMekawy et al., 2017). While polyaniline was found to have good biocompatibility and be less costly by Yan et al. (2010), it does have limitations due to its limited specific surface area and electrical conductivity. Later studies proposed combining graphene and polyaniline for anode modification (Yong et al., 2012; Huang et al.,2016; Hou et al.,2013), but the effect of anode modification on the rate of biofilm formation, acclimation time, electrogenic bacterial enrichment, and performance remained a mystery (Lin et al.,2019). By inoculating carbon cloth anodes modified with graphene and polyaniline and plain carbon cloth anodes with varying percentages of domestic wastewater, Lin et al. (2019) investigated the effects of anode modification on rate of biofilm formation, acclimation time, electrogenic bacterial enrichment, and efficiency. Biofilm formation was continuously monitored on both modified and unmodified anodes, and scans from confocal laser scanning microscopy (CLSM) and scanning electron microscopy (SEM) were compared (SEM). As compared to unmodified electrodes, the modified anodes had a 1.9-fold higher maximum power output, a 2.4-fold shorter acclimation time, a 2.4-fold faster electrogenic biofilm growth, and a better electrogenic bacteria colonization.

Biofilm growth activity has been identified in other studies. According to the findings of Aelterman et al. (2008), optimum anodic biofilm growth and activity occur at potentials of 200 mV vs Ag/AgCl electrode (Aelterman et al., 2008). Wei et al. (2010) investigated biofilm formation at potentials ranging from 0 to 400 mV vs SHE (standard hydrogen electrode), and discovered that these conditions were optimal for biofilm development, resulting in maximum power density and reduced MFC start-up time, and were dominated by Geobacter sulfurreducens anaerobic exoelectrogens (Wei et al., 2010). Zhu et al. (2013) investigated the effects of biofilm formation with positive potentials and found that MFC power output improved, which they attributed to the decay of the power overshoot phenomenon (Zhu et al., 2013).

Others have looked into strategies for enriching electrochemically active bacteria on an electrode (Kim et al., 2005). By inoculating the anode with an inoculum (usually anaerobic sludge), these electrochemically active bacteria are enriched, resulting in biofilm maturation at the anode (Kim et al., 2005). Others have discovered that biofilm production enhances anode electrochemical activity (Wang et al., 2009; Ramasamy et al., 2008; Scully et al., 1993), while others have recorded increased power output with increased biofilm density (Wang et al., 2009; Ramasamy et al., 2008; Scully et al., 1993). (Rabaey et al., 2007). Larrosa-Guerrero et al. (2010) investigated anodic biofilm enrichment at open and closed circuits to report on MFC results. They looked at microbial diversity as a feature of current and anode content in their research. Their findings showed that the anode had an impact on bacterial community formation in open circuit, but that the anode content had a greater impact on current flow in closed circuit. The study also found that closed circuit with an average voltage output of 0.6V at an external resistance of 300k removed 95% of organic matter, while open circuit yielded 0.75V average voltage.

The aim of this research is to learn more about the impact of biofilm formation and the best acclimation time from a graphite rod electrode-based MFC that is fed with both diluted and undiluted domestic and brewery wastewaters. Ample research into the effect of external resistance on MFC efficiency using undiluted real wastewater and wastewater is still missing, resulting in a paucity of data in the literature.

5.2 MATERIALS AND METHODS

The chemical oxygen demands (COD) measurements of the wastewater (beer brewery) were with a spectrophotometer (UV-VIS Spectrophotometer, Merck). 8 double chamber MFCs were constructed, i.e. MFC-1, MFC-2, MFC-3 and MFC-4, MFC-5, MFC-6, MFC-7 and MFC-8. The two chambers for each MFC were separated by a 25cm diameter Nafion membrane. Each chamber had a total substrate volume of approximately 400ml. The anode electrodes were made of 3mm diameter and 75mm long graphite rods (Merck, South Africa). The cathode electrodes were made of 3mm diameter and 75mm long graphite rods (Merck, South Africa). The MFCs were operated at ambient temperature, i.e. average of 24^0C.

Brewery wastewater was collected from SAB/INBEV beer brewery and stored at -0^0C on the same day until use. The domestic wastewater sludge was collected from ERWAT wastewater treatment works plant and stored at 0^0C as well until use. All chemicals and reagents used were of analytical grade. 8 MFCs were fed with beer brewery wastewater samples. Four MFCs (i.e. MFC-1, MFC-2, MFC-3 and MFC-4) were fed with beer brewery wastewater diluted with 20% domestic wastewater sludge (COD-1 sample) and the other four MFCs (i.e. MFC-5, MFC-6, MFC-7 and MFC-8) were fed with pure beer brewery wastewater effluent (COD-2 sample). The wastewater was also further diluted with 20% deionized water in each MFC reactor.

The investigative study work was performed at open circuit and closed circuit. MFC-1 and MFC-8 were operated at 51kΩ external resistance, MFC-2 and MFC-7 were operated at 33kΩ external resistance, MFC-3 and MFC-6 were operated at 1.5kΩ external resistance and MFC-4 and MFC-5 were operated at open circuit voltage. Each MFC operation was maintained long enough to ensure a steady state performance which was assessed based on manual measurements of the output voltage.

Steady state voltage and current tests were performed for each MFC with random voltage and current measurements over a period of 7days. The resulting voltages, current and time values were used to construct voltage versus time and current vs time curves where the MFC's steady state conditions (i.e. Vf and If) can be estimated.

The slope of the voltage versus time curve can be used to estimate the biofilm growth rate constant by the linear relationship,

$$ln\left(1-\frac{V}{V_f}\right)=-\frac{1}{\tau}t \text{ .....5.1}$$

Where V (V) is the voltage measured at a time t(s), Vf (V) is the steady state voltage achieved in a MFC, $\frac{1}{\tau}$ is the biofilm growth rate (hr-1) and τ (hrs) is the biofilm growth rate constant.

The slope of the current versus time curve can also be used to estimate the biofilm growth rate constant by the linear relationship,

$$ln\left(1-\frac{I}{I_f}\right)=-\frac{1}{\tau}t \text{ .....5.2}$$

Where I (A) is the current measured at a time t(s), If (A) is the steady state voltage achieved in a MFC, $\frac{1}{\tau}$ is the biofilm growth rate (hr-1) and τ (hrs) is the biofilm growth rate constant. Voltage and current on the microbial fuel cells were measured at random time intervals by a Fluke 17B+ digital across open circuit cell, 2.2kΩ and 33kΩ external resistances.

Where Ecell (V) is the measured-cell voltage and Rext is the external resistance (Ω) of the cell.

Current density was calculated as:

$$i(\frac{mA}{cm^2})=\frac{E_{cell}}{R_{ext}.A} \text{ ..... 5.3}$$

Where Ecell (mV) is the cell-measured voltage, Rext (Ω) is the external resistance and A (cm2) is the projected surface area of the cathode electrode.

Power density was calculated as:

$$P(\frac{mA}{cm^2})=\frac{{E^2}_{cell}}{R_{ext}.A} \text{ ..... 5.4}$$

Where Ecell (mV) is the cell-measured voltage, Rext (Ω) is the external resistance and A (cm2) is the projected surface area of the cathode electrode.For each MFC, COD measurements were also performed.

5.3 RESULTS AND DISCUSSION

5.3.1 Evaluation of substrate dilution on microbial fuel cell start-up performance: analysis of biofilm growth rate

Previous studies have reported on limited current flow from anode to cathode as a result of high external resistance affecting anode colonization by microbes (Lyon et al., 2010; Ren et al., 2011). This subsection analyses steady state voltages during the

maturation phase of MFC at varying fixed resistance over diluted or undiluted substrate.

Figures 5.1 to 5.11 illustrate the results depicting voltage generation across the resistors of each MFC (i.e. MFC-1 to MFC-8). Fig 5.1 shows results for MFC-1 (operated at 51kΩ external resistor) fed with beer brewery wastewater diluted with 20% domestic wastewater sludge (COD-1 sample) and MFC-8 (operated at 51kΩ) fed with pure beer brewery wastewater effluent (COD-2 sample). Figure 5.1 shows steady state voltage (V_f) reached at voltage 144.9mV at time 99hrs by MFC-8 while steady state voltage (V_f) was observed at voltage 98.6mV at time 117hrs for MFC-1. Steady state voltages were observed earlier for MFC-8 than for MFC-1 as shown in figure 5.1

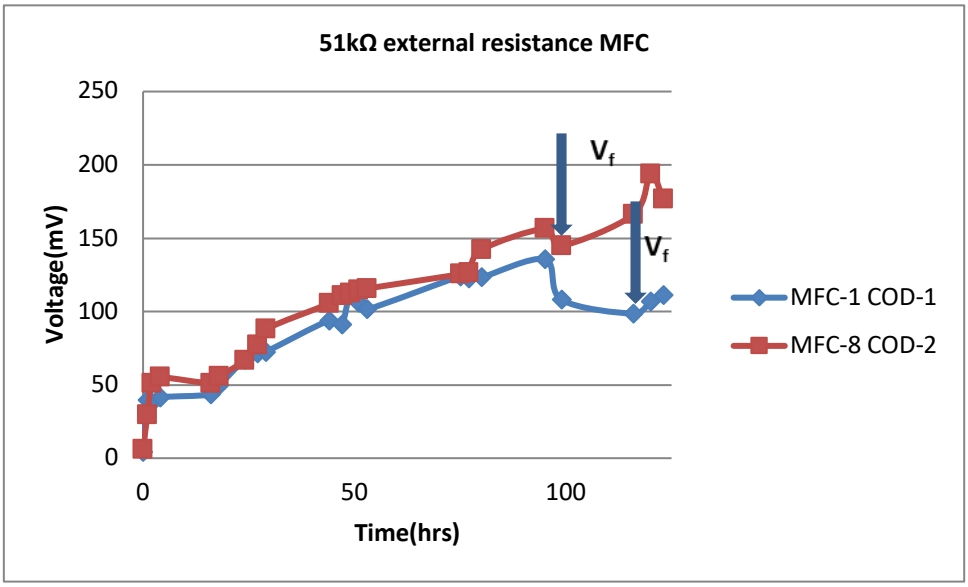


Figure 5.1 Steady state voltage (Vf) during MFC start-up period at 51kΩ external resistance.

Figs 5.2 shows results for MFC-2 (operated at 33kΩ external resistor) fed with beer brewery wastewater diluted with 20% domestic wastewater sludge (COD-1 sample) and MFC-7 (operated at 33kΩ) fed with pure beer brewery wastewater effluent (COD-2 sample). Figure 5.2 shows voltage steady state reached at voltage 83.8mV at time 120hrs by MFC-2 while steady state voltage was observed at voltage 168.1mV at time 116hrs for MFC-7. Steady state voltages were observed earlier for MFC-7 than for MFC-2 as shown in figure 5.2.

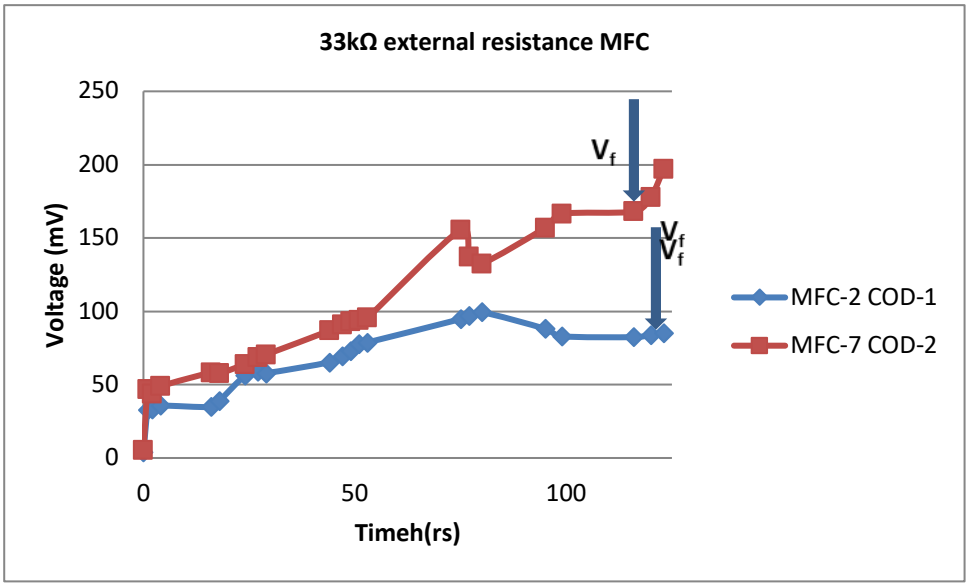


Figure 5.2 Steady state voltage during MFC start-up period at 33kΩ external resistance.

Likewise, Fig 5.3 shows results for MFC-3 (operated at 1.5kΩ external resistor) fed with beer brewery wastewater diluted with 20% domestic wastewater sludge (COD-1 sample) and MFC-6 (operated at 1.5kΩ) fed with pure beer brewery wastewater effluent (COD-2 sample). Figure 5.3 shows voltage steady state reached at voltage 3.7mV at time 95.9hrs by MFC-3 while steady state voltage was observed at voltage 9.3mV at time 116hrs for MFC-6. Steady state voltages were observed earlier for MFC-3 than for MFC-6 as shown in figure 5.3.

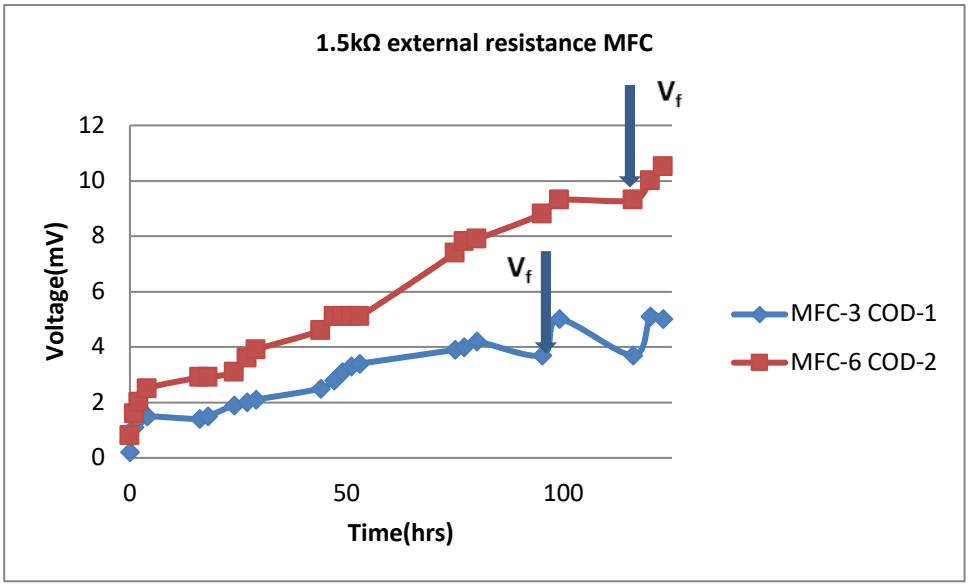


Figure 5.3 Steady state voltage during MFC start-up period at 1.5kΩ external resistance.

Fig 5.4 shows results for MFC-4 (operated as an open circuit cell) fed with beer brewery wastewater diluted with 20% domestic wastewater sludge (COD-1 sample) and MFC-5 (operated as an open circuit cell) fed with pure beer brewery wastewater effluent (COD-2 sample). Voltage steady state for figure 5.4 was observed at voltage 251.7mV at time 95.hrs by MFC-4 while steady state voltage was observed at voltage 413mV at time 123hrs for MFC-5. Steady state voltages were observed earlier for MFC-4 than for MFC-6 as shown in figure 5.4.

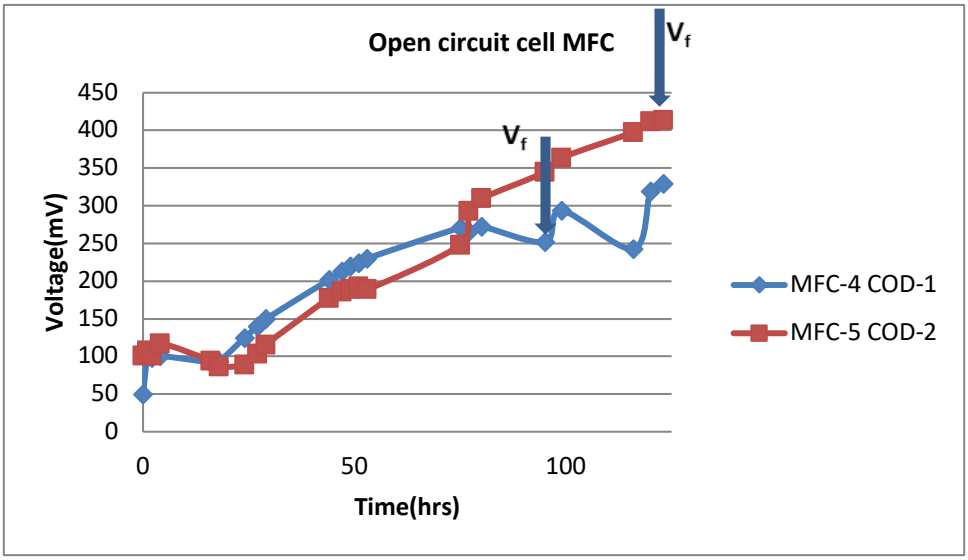


Figure 5.4 Steady state voltage during MFC start-up period for open circuit cells.

Figures 5.5 and 5.6 below are calculated from equation 4.1.1 wherein the slope of each curve, $\frac{1}{\tau}$ is the biofilm growth rate. The biofilm growth constant, τ relates to 63.3% biofilm having been formed on the anode electrode. The results are summarized in table 5.1

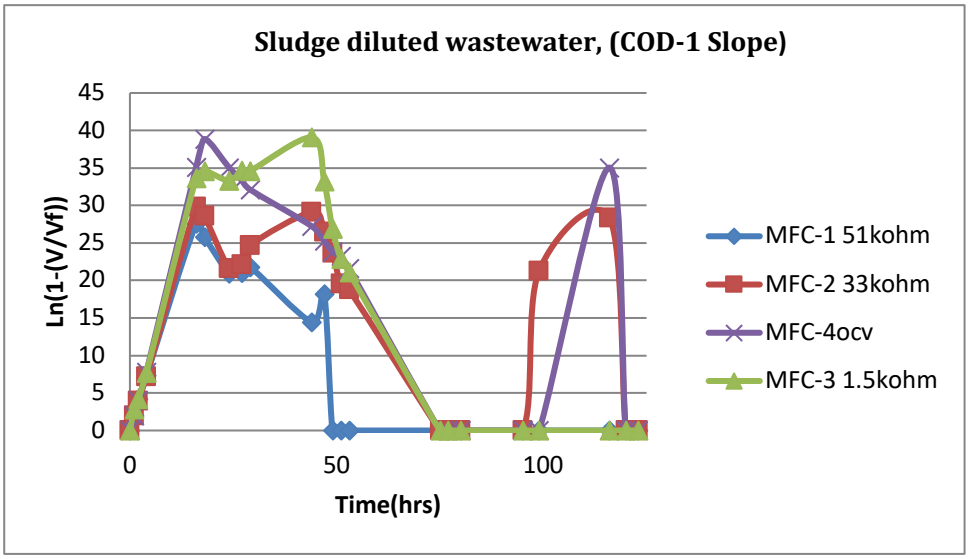


Figure 5.5 Slope determination for sludge diluted wastewater samples

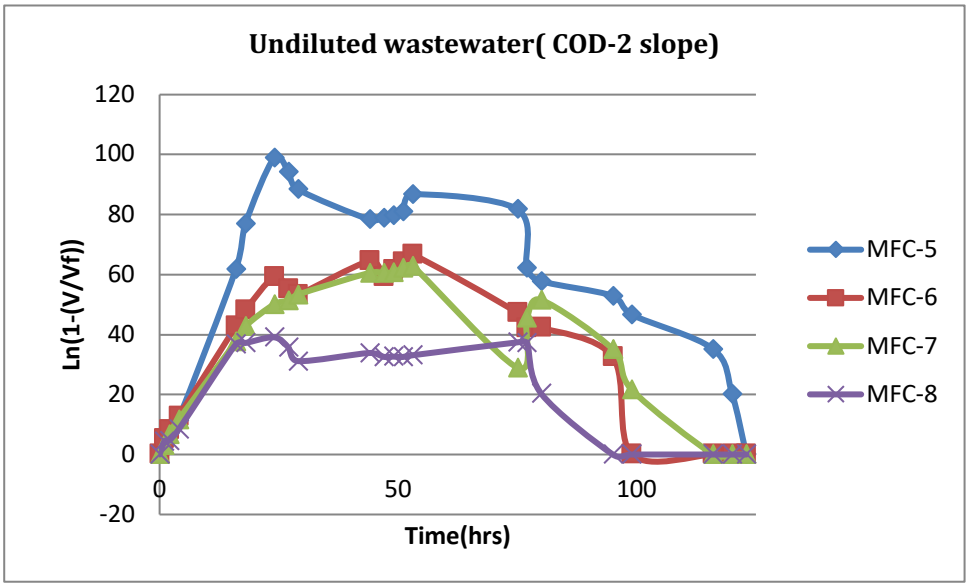


Figure 5.6 Slope determination for the undiluted wastewater samples

For the diluted wastewater, MFC-1 has a growth rate of 0.128hr^{-1} with growth rate constant of 7.83hrs, while MFC-2 shows a biofilm growth rate of 0.062hr^{-1} with a biofilm growth rate constant of 16.2hrs, MFC-3 shows the biofilm growth rate of 0.185hr^{-1} with a growth rate constant at 5.4hrs, MFC-4 shows a biofilm growth rate of 0.119hr-1 with a growth rate constant at 8.4hrs. The undiluted wastewater shows a growth rate of 0.044hr^{-1} with growth rate constant of 22.3hrs for MFC-5, while MFC-6 shows a biofilm growth rate of 0.152hr^{-1} with a biofilm growth rate constant of 6.6hrs, MFC-7 shows the biofilm growth rate of 0.093hr^{-1} with a growth rate constant at 10.8hrs, MFC-8 shows a biofilm growth rate of 0.130hr^{-1} with a growth rate constant at 7.7hrs.

Therefore, MFC-1 and MFC-8 which are both operated at 51kΩ and MFC-2 and MFC-7 which are operated at 33kΩ indicate higher value of biofilm formation rate and low values of biofilm growth constant for the undiluted wastewater-fed MFCs as compared to the diluted wastewater fed MFCs which indicate low value of biofilm formation rate and high values of biofilm growth constant. On the contrary, MFC-3 and MFC-6 which are both operated at 1.5kΩ and MFC-4 and MFC-5 which are both open circuit indicate higher value of biofilm formation rate and low values of biofilm growth constant for the diluted wastewater-fed MFCs as compared to the undiluted wastewater fed MFCs which indicate low value of biofilm formation rate and high values of biofilm growth constant.

Table 5.1 summary of steady state voltage conditions for each MFC

Parameter	MFC-1	MFC-2	MFC-3	MFC-4	MFC-5	MFC-6	MFC-7	MFC-8
Vf(V)	98.6	83.3	3.7	251.7	413	9.3	168.1	144.9
Time(hrs)	117	120	95	95	123	116	116	99
Biofilm growth rate,$\frac{1}{\tau}(\boldsymbol{hr^{-1}})$	0.127714	0.061728	0.185185	0.119048	0.044843	0.151515	0.092593	0.12987
Biofilm growth rate constant τ(hrs)	7.83	16.2	5.4	8.4	22.3	6.6	10.8	7.7

5.3.2 Steady state current

The steady state current (If) was also investigated as a function of biofilm formation rate. In this case, a method similar to that used to calculate steady state voltage was used to calculate steady state current. Figures 5.7 to 5.8 show the results depicting current generation across the resistors of MFC-2, MFC-4, MFC-5 and MFC-7.

Fig 5.7 shows results for MFC-2 (operated at 33kΩ external resistor) fed with beer brewery wastewater diluted with 20% domestic wastewater sludge (COD-1 sample) and MFC-7 (operated at 33kΩ) fed with pure beer brewery wastewater effluent (COD-2 sample). Figure 5.7 shows steady state current reached at current 35.4μA at time 111hrs by MFC-2 while steady state current was observed at current 6.7μA at time

87hrs for MFC-7. Steady state current was observed earlier for MFC-7 than for MFC-2 as shown in figure 5.7.

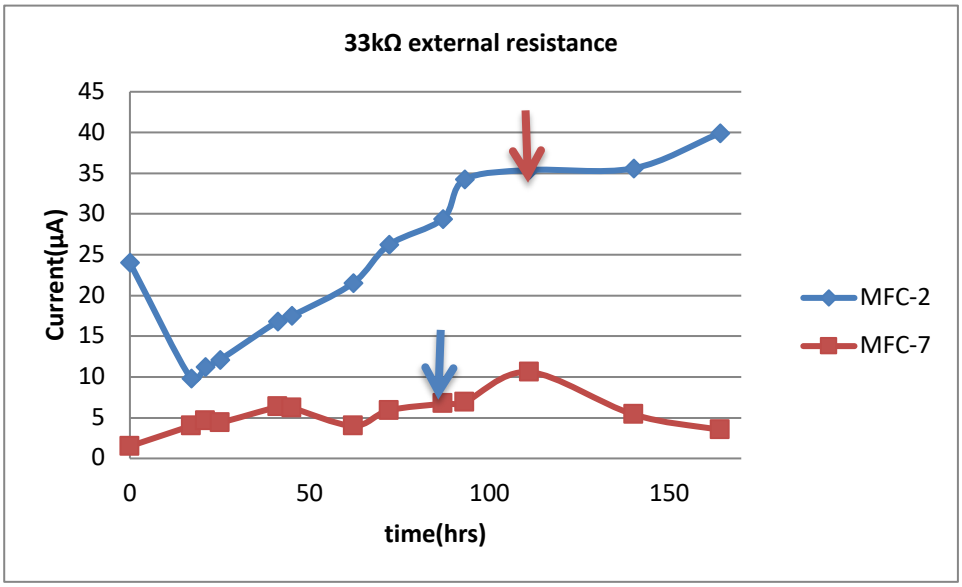


Figure 5.7 Steady state current during MFC start-up period at 33kΩ external resistance.

MFC-4 (operated at open circuit) fed with beer brewery wastewater diluted with 20% domestic wastewater sludge (COD-1 sample) and MFC-5 (operated at open circuit) fed with pure beer brewery wastewater effluent are shown in Fig 5.8 (COD-2 sample). Figure 5.8 indicates that MFC-4 reached steady state current of 35.5µA at time 140 hours, while MFC-5 reached steady state current of 17.5µA at time 87 hours. As shown in figure 5.7, steady state current was observed earlier for MFC-5 than for MFC-4.

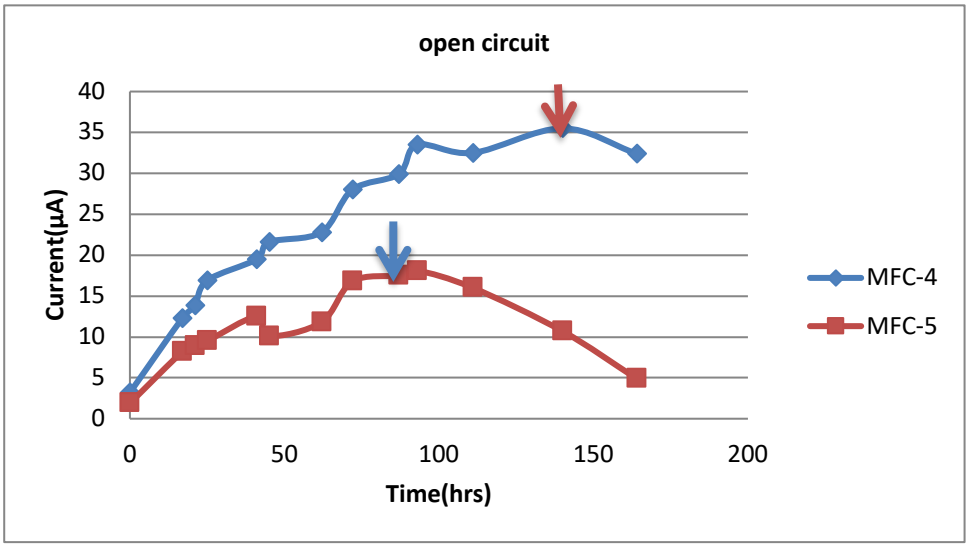


Figure 5.8 Steady state current during MFC start-up period at open circuit.

Similarly, equation 5.2 was used to estimate the biofilm growth rate and biofilm growth rate constant and table 5.2 below summaries the results of the calculations from equation 5.2.

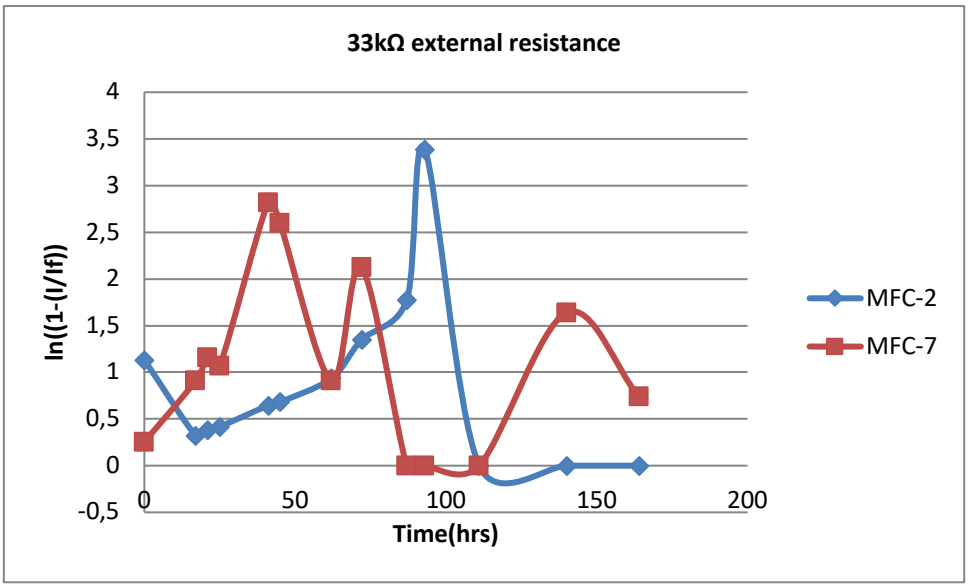


Figure 5.9 Slope determination for the 33kΩ external resistance MFCs

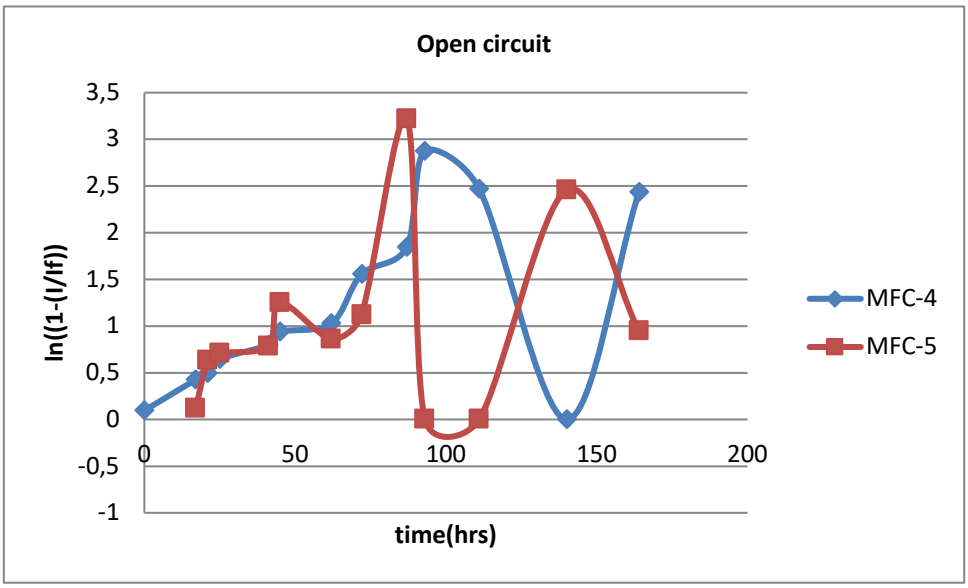


Figure 5.10 Slope determination for the open circuit MFCs

Table 5.2 summary of steady state current conditions for each MFC

Parameter	MFC-2	MFC-4	MFC-5	MFC-7
If(μA)	35.4	35.5	17.5	6.7
Time(hrs)	111	140	87	87
Biofilm growth rate,$\frac{1}{\tau}$ (hr^{-1})	0.0013	0.0034	0.0111	0.006
Biofilm growth rate constant τ(hrs)	769.2	294.1	90.1	166.7

The addition of sludge to the wastewater at low resistance indicates that voltage rises with time for open circuit cells, while voltage increases with an increase in external resistance for closed circuit cells. As demonstrated by the pure wastewater-fed MFCs' consistently higher current generation as compared to the sludge-diluted wastewater-fed MFCs. Sludge addition to beer brewery wastewater decreases the electron transfer power at low external resistance. Ghangrekar and Shinde (2007) reported similar results, observing an increase in cell voltage as external resistance increased from 0 to 4,000 Ω and they achieved a maximum voltage of 358 mV at an external resistance of 4,000 Ω while Menicucci et al. (2006) observed a decrease in cell voltage when external resistance decreased from 0.125 to 6KΩ.

5.3.3 Effect of external resistance on microbial fuel cell start-up performance: biofilm growth rate

Tables 5.1 and 5.2 show trends that are similar to those stated by Ghangrekar and Shinde (2007) and Rismani-Yazdi et al. (2011), who found that cell voltage is directly proportional to external resistance, i.e. the voltage generated decreases as the MFC's external resistance decreases. And the electrode reaction kinetics limitation, mass transfer losses, and charge transfer process limitations at one of the two electrodes that exhibits the slower charge transfer kinetics are reportedly all to blame for the voltage drop when external resistance drops (the current-limiting electrode).

5.4 CONCLUSION

MFCs run at 51k and 33k show a higher value of biofilm formation rate and a lower value of biofilm growth constant than diluted wastewater feeding MFCs, which show a low value of biofilm formation rate and a high value of biofilm growth constant. MFCs 1.5k and open circuit, on the other hand, show a higher value of biofilm formation rate and a lower value of biofilm growth constant for diluted wastewater-fed MFCs than undiluted wastewater-fed MFCs, which show a low value of biofilm formation rate and a high value of biofilm growth constant. In this respect, the addition of sludge to wastewater at low resistance means that voltage rises with time for open circuit cells and voltage rises with an increase in external resistance for closed circuit cells. As shown by the consistently higher current generation of pure wastewater-fed MFCs relative to sludge-diluted wastewater-fed MFCs. The addition of sludge to beer brewery wastewater reduces electron transfer capacity at low external resistance. External resistance is directly proportional to anode colonization during biofilm formation, and it affects other MFC parameters such as current generation, microbial activity, biofilm growth rate, and power produced. As a consequence, in this case, running an MFC at maximum external resistance for high MFC power output is critical. In contrast, steady-state current results from MFC-2 and MFC-7, both operated at 33k, and MFC-4 and MFC-5, both operated at open circuit, show higher values of biofilm formation rate and lower values of biofilm growth constant for the undiluted wastewater-fed MFCs than for the diluted wastewater-fed MFCs, which show low values of biofilm formation rate and high values of biofilm growth constant. Wastewater dilution tends to influence the rate of biofilm formation as well as the growth rate constant, and therefore the effects of steady state voltage and steady state current relations to biofilm formation show that sludge addition to beer brewery treatment in a microbial fuel cell can impact microbial activity.

5.5 REFERENCES

Logan, B.E., 2009. Exoelectrogenic bacteria that power microbial fuel cells. Nature Reviews Microbiology, 7(5), p.375.

Pasupuleti, S.B., Srikanth, S., Dominguez-Benetton, X., Mohan, S.V. and Pant, D., 2016. Dual gas diffusion cathode design for microbial fuel cell (MFC): optimizing the suitable mode of operation in terms of bioelectrochemical and bioelectro-kinetic evaluation. Journal of Chemical Technology & Biotechnology, 91(3), pp.624-639.

Yong, Y.C., Dong, X.C., Chan-Park, M.B., Song, H. and Chen, P., 2012. Macroporous and monolithic anode based on polyaniline hybridized three-dimensional graphene for high-performance microbial fuel cells. ACS nano, 6(3), pp.2394-2400.

Aelterman, P., Freguia, S., Keller, J., Verstraete, W. and Rabaey, K., 2008. The anode potential regulates bacterial activity in microbial fuel cells. Applied microbiology and biotechnology, 78(3), pp.409-418.

Wei, J., Liang, P., Cao, X. and Huang, X., 2010. A new insight into potential regulation on growth and power generation of Geobacter sulfurreducens in microbial fuel cells based on energy viewpoint. Environmental science & technology, 44(8), pp.3187-3191.

Zhu, X., Tokash, J.C., Hong, Y. and Logan, B.E., 2013. Controlling the occurrence of power overshoot by adapting microbial fuel cells to high anode potentials. Bioelectrochemistry, 90, pp.30-35.

Zhang, L., Zhu, X., Li, J., Liao, Q. and Ye, D., 2011. Biofilm formation and electricity generation of a microbial fuel cell started up under different external resistances. Journal of Power Sources, 196(15), pp.6029-6035.

Jung, S. and Regan, J.M., 2011. Influence of external resistance on electrogenesis, methanogenesis, and anode prokaryotic communities in microbial fuel cells. Appl. Environ. Microbiol., 77(2), pp.564-571.

Aelterman, P., Versichele, M., Marzorati, M., Boon, N. and Verstraete, W., 2008. Loading rate and external resistance control the electricity generation of microbial fuel cells with different three-dimensional anodes. Bioresource technology, 99(18), pp.8895-8902.

Rismani-Yazdi, H., Christy, A.D., Carver, S.M., Yu, Z., Dehority, B.A. and Tuovinen, O.H., 2011. Effect of external resistance on bacterial diversity and metabolism in cellulose-fed microbial fuel cells. Bioresource technology, 102(1), pp.278-283.

Katuri, K.P., Scott, K., Head, I.M., Picioreanu, C. and Curtis, T.P., 2011. Microbial fuel cells meet with external resistance. Bioresource technology, 102(3), pp.2758-2766.

Larrosa-Guerrero, A., Scott, K., Katuri, K.P., Godinez, C., Head, I.M. and Curtis, T., 2010. Open circuit versus closed circuit enrichment of anodic biofilms in MFC:

effect on performance and anodic communities. Applied microbiology and biotechnology, 87(5), pp.1699-1713.

del Campo, A.G., Cañizares, P., Lobato, J., Rodrigo, M. and Morales, F.F., 2014. Effects of external resistance on microbial fuel cell's performance. In Environment, energy and climate change II (pp. 175-197). Springer, Cham.

Lyon, D.Y., Buret, F., Vogel, T.M. and Monier, J.M., 2010. Is resistance futile? Changing external resistance does not improve microbial fuel cell performance. Bioelectrochemistry, 78(1), pp.2-7.

Ren, Z., Yan, H., Wang, W., Mench, M.M. and Regan, J.M., 2011. Characterization of microbial fuel cells at microbially and electrochemically meaningful time scales. Environmental science & technology, 45(6), pp.2435-2441.

Zhang, L., Zhu, X., Li, J., Liao, Q. and Ye, D., 2011. Biofilm formation and electricity generation of a microbial fuel cell started up under different external resistances. Journal of Power Sources, 196(15), pp.6029-6035.

Ghangrekar, M.M. and Shinde, V.B., 2007. Performance of membrane-less microbial fuel cell treating wastewater and effect of electrode distance and area on electricity production. Bioresource Technology, 98(15), pp.2879-2885.

Menicucci, J., Beyenal, H., Marsili, E., Veluchamy, R.A., Demir, G. and Lewandowski, Z., 2006. Procedure for determining maximum sustainable power generated by microbial fuel cells. Environmental science & technology, 40(3), pp.1062-1068.

Kamau, J.M., Mbui, D.N., Mwaniki, J.M., Mwaura, F.B. and Kamau, G.N., 2017. Microbial fuel cells: influence of external resistors on power, current and power density. J Thermodyn Catal, 8(1), pp.100-182.

Aelterman, P., Versichele, M., Marzorati, M., Boon, N. and Verstraete, W., 2008. Loading rate and external resistance control the electricity generation of microbial fuel cells with different three-dimensional anodes. Bioresource technology, 99(18), pp.8895-8902

Yu, J., Park, Y., Kim, B. and Lee, T., 2015. Power densities and microbial communities of brewery wastewater-fed microbial fuel cells according to the initial substrates. Bioprocess and biosystems engineering, 38(1), pp.85-92.

Wen, Q., Wu, Y., Zhao, L.X., Sun, Q. and Kong, F.Y., 2010. Electricity generation and brewery wastewater treatment from sequential anode-cathode microbial fuel cell. Journal of Zhejiang University SCIENCE B, 11(2), pp.87-93.

Parawira, W., Kudita, I., Nyandoroh, M.G. and Zvauya, R., 2005. A study of industrial anaerobic treatment of opaque beer brewery wastewater in a tropical climate using a full-scale UASB reactor seeded with activated sludge. Process Biochemistry, 40(2), pp.593-599.

Braeken, L., Van der Bruggen, B. and Vandecasteele, C., 2004. Regeneration of brewery waste water using nanofiltration. Water research, 38(13), pp.3075-3082.

Köroğlu, E.O., Özkaya, B., Denktaş, C. and Çakmakci, M., 2014. Electricity generating capacity and performance deterioration of a microbial fuel cell fed with beer brewery wastewater. Journal of bioscience and bioengineering, 118(6), pp.672-678.

Liu, H., Ramnarayanan, R. and Logan, B.E., 2004. Production of electricity during wastewater treatment using a single chamber microbial fuel cell. Environmental science & technology, 38(7), pp.2281-2285.

Feng, Y., Wang, X., Logan, B.E. and Lee, H., 2008. Brewery wastewater treatment using air-cathode microbial fuel cells. Applied microbiology and biotechnology, 78(5), pp.873-880.

Wen, Q., Wu, Y., Cao, D., Zhao, L. and Sun, Q., 2009. Electricity generation and modeling of microbial fuel cell from continuous beer brewery wastewater. Bioresource Technology, 100(18), pp.4171-4175.

Huang, L. and Logan, B.E., 2008. Electricity generation and treatment of paper recycling wastewater using a microbial fuel cell. Applied microbiology and biotechnology, 80(2), pp.349-355.

Wang, X., Feng, Y.J. and Lee, H., 2008. Electricity production from beer brewery wastewater using single chamber microbial fuel cell. Water Science and Technology, 57(7), pp.1117-1121.

Mathuriya, A.S. and Sharma, V.N., 2010. Treatment of brewery wastewater and production of electricity through microbial fuel cell technology. International Journal of Biotechnology and Biochemistry, 6(1), pp.71-80.

Larrosa-Guerrero, A., Scott, K., Katuri, K.P., Godinez, C., Head, I.M. and Curtis, T., 2010. Open circuit versus closed circuit enrichment of anodic biofilms in MFC: effect on performance and anodic communities. Applied microbiology and biotechnology, 87(5), pp.1699-1713.

Kim, J.R., Min, B. and Logan, B.E., 2005. Evaluation of procedures to acclimate a microbial fuel cell for electricity production. Applied microbiology and biotechnology, 68(1), pp.23-30.

Allen, M.J., 1972. Chapter IX cellular electrophysiology. In Methods in Microbiology (Vol. 6, pp. 247-283). Academic Press.

Allen, R.M. and Bennetto, H.P., 1993. Microbial fuel cells. Applied biochemistry and biotechnology, 39(1), pp.27-40.

Bennetto, H.P., Stirling, J.L., Tanaka, K. and Vega, C.A., 1983. Anodic reactions in microbial fuel cells. Biotechnology and bioengineering, 25(2), pp.559-568.

Wingard Jr, L.B., Shaw, C.H. and Castner, J.F., 1982. Bioelectrochemical fuel cells. Enzyme and Microbial Technology, 4(3), pp.137-142.

Bond, D.R., Holmes, D.E., Tender, L.M. and Lovley, D.R., 2002. Electrode-reducing microorganisms that harvest energy from marine sediments. Science, 295(5554), pp.483-485.

Bond, D.R. and Lovley, D.R., 2003. Electricity production by Geobacter sulfurreducens attached to electrodes. Appl. Environ. Microbiol., 69(3), pp.1548-1555.

Chaudhuri, S.K. and Lovley, D.R., 2003. Electricity generation by direct oxidation of glucose in mediator-less microbial fuel cells. Nature biotechnology, 21(10), p.1229.

Kim, B.H., Kim, H.J., Hyun, M.S. and Park, D.H., 1999a. Direct electrode reaction of Fe (III)-reducing bacterium, Shewanella putrefaciens. Journal of Microbiology and Biotechnology, 9, pp.127-131.

Kim, B.H., Park, H.S., Kim, H.J., Kim, G.T., Chang, I.S., Lee, J. and Phung, N.T., 2004. Enrichment of microbial community generating electricity using a fuel-cell-type electrochemical cell. Applied microbiology and biotechnology, 63(6), pp.672-681.

Kim, H.J., Hyun, M.S., Chang, I.S. and Kim, B.H., 1999b. A microbial fuel cell type lactate biosensor using a metal-reducing bacterium, Shewanella putrefaciens. Journal of Microbiology and Biotechnology, 9(3), pp.365-367.

Liu, H., Ramnarayanan, R. and Logan, B.E., 2004. Production of electricity during wastewater treatment using a single chamber microbial fuel cell. Environmental science & technology, 38(7), pp.2281-2285.

Wang, X., Feng, Y., Ren, N., Wang, H., Lee, H., Li, N. and Zhao, Q., 2009. Accelerated start-up of two-chambered microbial fuel cells: effect of anodic positive poised potential. Electrochimica Acta, 54(3), pp.1109-1114.

Scully, J.R., Silverman, D.C. and Kendig, M.W., 1993, March. Electrochemical impedance: analysis and interpretation. Philadelphia: ASTM.

Ramasamy, R.P., Ren, Z., Mench, M.M. and Regan, J.M., 2008. Impact of initial biofilm growth on the anode impedance of microbial fuel cells. Biotechnology and bioengineering, 101(1), pp.101-108.

Rabaey, K., Rodríguez, J., Blackall, L.L., Keller, J., Gross, P., Batstone, D., Verstraete, W. and Nealson, K.H., 2007. Microbial ecology meets electrochemistry: electricity-driven and driving communities. The ISME journal, 1(1), p.9.

Picioreanu, C., Head, I.M., Katuri, K.P., van Loosdrecht, M.C. and Scott, K., 2007. A computational model for biofilm-based microbial fuel cells. Water research, 41(13), pp.2921-2940.

Kim, G.T., Webster, G., Wimpenny, J.W.T., Kim, B.H., Kim, H.J. and Weightman, A.J., 2006. Bacterial community structure, compartmentalization and activity in a microbial fuel cell. Journal of applied microbiology, 101(3), pp.698-710.

Gottenbos, B., Van der Mei, H.C. and Busscher, H.J., 1999. [38] Models for studying initial adhesion and surface growth in biofilm formation on surfaces. In Methods in Enzymology (Vol. 310, pp. 523-534). Academic Press.

Davies, D.G., Parsek, M.R., Pearson, J.P., Iglewski, B.H., Costerton, J.W. and Greenberg, E.P., 1998. The involvement of cell-to-cell signals in the development of a bacterial biofilm. Science, 280(5361), pp.295-298.

Mah, T.F.C. and O'Toole, G.A., 2001. Mechanisms of biofilm resistance to antimicrobial agents. Trends in microbiology, 9(1), pp.34-39.

Hall-Stoodley, L., Costerton, J.W. and Stoodley, P., 2004. Bacterial biofilms: from the natural environment to infectious diseases. Nature reviews microbiology, 2(2), p.95.

Lovley, D.R., 2006. Microbial energizers: fuel cells that keep on going. Microbe-American Society for Microbiology, 1(7), p.323.

Logan, B.E., 2009. Exoelectrogenic bacteria that power microbial fuel cells. Nature Reviews Microbiology, 7(5), p.375.

Chapter 6: Impact of External Resistance on MnO_2/rGO Coated Cathode MFC Start-Up

6.1 INTRODUCTION

Some recent studies on the effect of external resistance on microbial fuel efficiency have demonstrated the importance of regulating an MFC's external resistance. Woodward et al. (2010) developed an online method known as Maximum Power Point Tracking (MPPT) to monitor the external resistance using an acetate-fed MFC, and in their investigations, they used various non-model real-time MPPT methods to trace an optimum R_{ext} (Woodward et al., 2010). Despite their performance, their investigations were focused on short-term MFC activity and thus do not expose MFC actions for long-term operation at optimal R_{ext}. Pinto et al. (2010a, b, c) used a model-based simulation of a microbial population to investigate the impact of external resistance on MFC performance, and their results revealed that MFC performance decreases when external resistance deviates significantly from optimum resistance. Pinto et al. (2011) studied the best external resistance for long-term MFCs using MPPT on acetate or synthetic wastewater. They were able to observe high power output and less methane production when R_{ext} was equal to R_{int} as compared to when $R_{ext}>>R_{int}$ or $R_{ext}<<R_{int}$. They also achieved average Coulombic efficiencies of 57% and 29% on acetate and synthetic wastewater, respectively at optimum resistance. They also suggested real-time optimal control as one of the crucial ways to achieve optimum power output, which should also be used in developing stacked MFCs for simultaneous wastewater treatment and electricity generation.

As a result, real wastewater (beer brewery and domestic) is used in this study to investigate the effect of external resistance on a MnO_2/rGO catalyst coated cathode electrode MFC during anode colonization.

6.2 MATERIALS AND METHODS

Experiments were perfomed over a MnO_2/rGO catalyst coated cathode electrode MFC as well as a plain cathode electrode MFC. Undiluted wastewaters were used in all tests. The raw domestic wastewater had 1710mg/l COD while raw brewery wastewater had

COD of 5789 mg/l. All chemicals and reagents used were of analytical grade. Wastewaters were collected on the same day and stored at 0^0C until use. Each anodic chamber was inoculated with 100ml of anaerobic sludge from a domestic wastewater treatment plant and 300ml of fuel, i.e. domestic or brewery wastewater.

8 double chamber MFCs were constructed, i.e. MFC-1, MFC-2, MFC-3 and MFC-4 were brewery wastewater fed and MFC-5, MFC-6, MFC-7 and MFC-8 were domestic wastewater fed and the two chambers for each MFC were separated by a 25cm diameter Nafion membrane. Each chamber had a total volume of approximately 400ml. The anode electrodes were made of 3mm diameter and 75mm long graphite rods (Merck, South Africa). The cathode electrodes were made of 3mm diameter and 75mm long graphite rods (Merck, South Africa). The cathode electrodes for MFC-3, MFC-4, MFC-5 and MFC-6 were coated with MnO_2/rGO catalyst with 0.2mg.cm^2 loading while MFC-1, MFC-2, MFC-7 and MFC-8 were operated with plain cathodes. The MFCS were operated at winter ambient temperature, i.e. average of 16^0C.

Each MFC's external resistance was individually connected and controlled by an external resistor which was manually controlled at resistor variation range of 33kΩ and 51kΩ. MFC-1, MFC-4, MFC-5 and MFC-8 were operated at a fixed external resistance of 51kΩ while MFC-2, MFC-3, MFC-6 and MFC-7 were operated at a fixed external resistance of 33kΩ. In order to account for process variability, each mode of operation was maintained long enough to ensure a steady state performance which was assessed based on manual measurements of the output voltage.

6.3 RESULTS AND DISCUSSION

6.3.1 Catalyst characterisation

A hydrothermal method was used to synthesize a nano-structured MnO_2 which was precipitated at room temperature. The results from the SEM images in figure 6.1 display a nano-structured MnO_2 with platelike morphology.

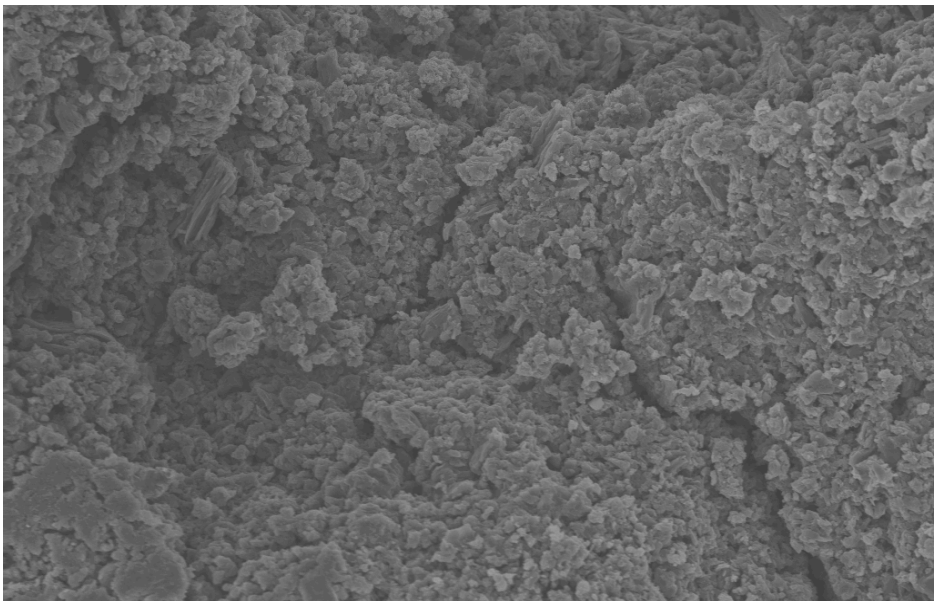


Figure 6.1 SEM image of nano-structured MnO_2

Figure 6.2 shows a SEM image of MnO_2/rGO catalyst depicting the surface morphology of MnO_2/rGO catalyst. Results from the image indicate consistent macropores indicating that the catalyst has good adsorption capacity.

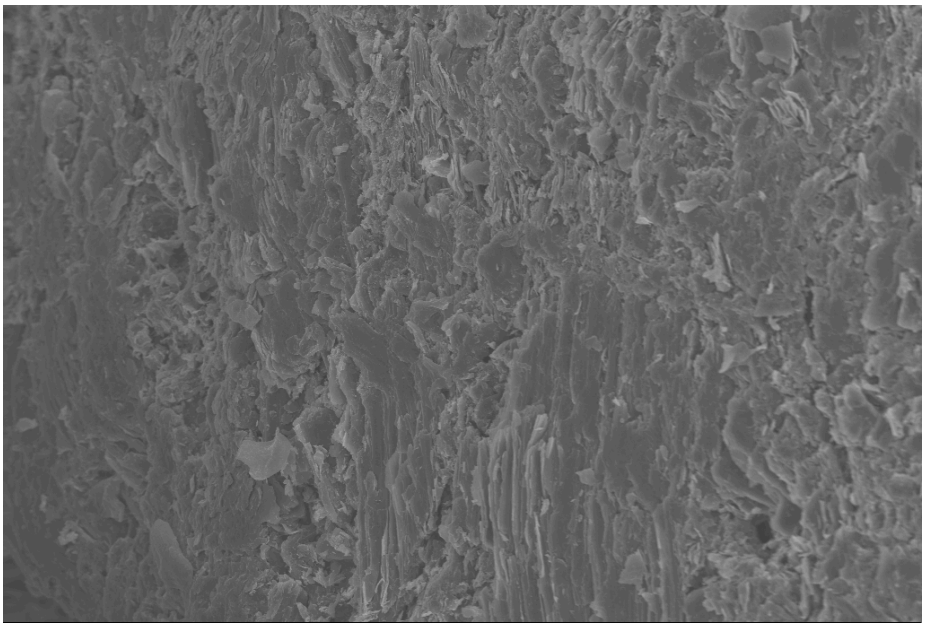


Figure 6.2 SEM image of MnO_2/rGO

The Brunauer-Emmett-Teller (BET) method was used to calculate the specific surface area obtained as 73.21 m^2/g with a total pore volume of 0.2570 cm3/g. The high surface area of the catalyst suggested the organic substrate's ability to adsorb on the cathode electrode, resulting in high oxygen absorption and electron acceptance on the catalyst's surface.

The functional group within the catalyst composite was investigated by the FT-IR spectrum as shown in figure 6.3. Results from the figure show a stretching frequency at 3205.3cm^{-1} indicating a broad band adsorption peaks for adsorbed water (H-O-H). It can also be seen that the angular deformation of the adsorbed water molecules band appeared at 1624.8cm^{-1} and 1126.13/cm^{-1} while the bands at 705cm^{-1} and 510 cm^{-1} correspond to the characteristic peaks of the metal oxide which shows the formation of MnO_2/rGO.

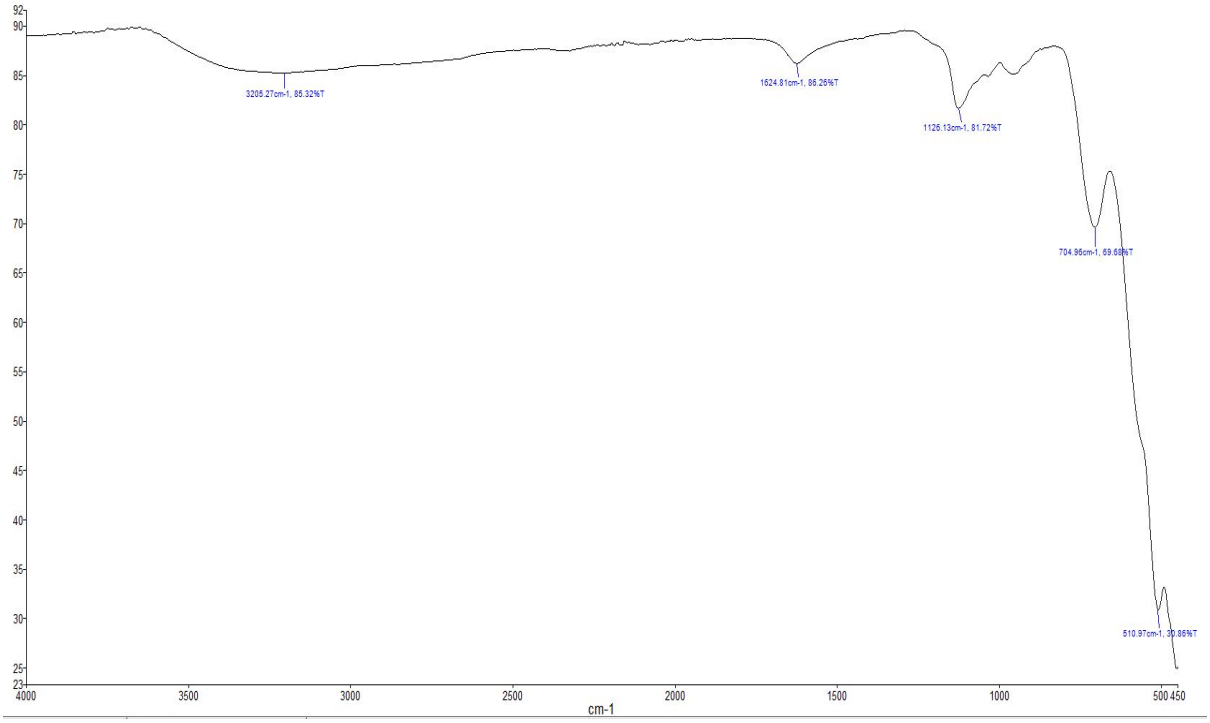


Figure 6.3 FT-IR spectrum of the MnO_2/rGO catalyst

6.3.2 MFC operation

The results of this research show that MFCs can produce electricity from both raw brewery wastewater and raw domestic wastewater. For four days, each MFC reactor was inoculated with anaerobic sludge and no voltage measurements were taken. The

experiemental tests entailed two varying external resistance (33kΩ and 51kΩ) in a series of MFC reactors fed with real wastewaters. During the first seven days (324hrs) of the experimental study after the inoculation period, a digital voltmeter was employed as a measuring instrument to monitor the voltage across the resistors. Figures 6.4 to 6.7 illustrate the results depicting measured voltage across the resistors of each MFC (i.e. MFC-1 to MFC-8). Fig 6.4 shows results for MFC-1 (operated at 51kΩ external resistor) and MFC-2 (operated at 33kΩ) and were fed with beer brewery wastewater and consisted of plain cathode electrodes. Fig 6.5 shows results for MFC-4 (operated at 51kΩ external resistor) and MFC-3 (operated at 33kΩ) and were fed with beer brewery wastewater however these reactors consisted of MnO_2/rGO catalyst coated cathode electrodes. Likewise, Fig 6.6 shows results for MFC-5 (operated at 51kΩ external resistor) and MFC-6 (operated at 33kΩ) however were fed with domestic wastewater and consisted of plain cathode electrodes. Fig 6.7 shows results for MFC-8 (operated at 51kΩ external resistor) and MFC-7 (operated at 33kΩ) and also were fed with domestic wastewater and however consisted of MnO_2/rGO catalyst coated cathode electrodes.

Fig 6.4 shows maximum voltage of 0.516V achieved by MFC-1 as compared to 0.338V achieved by MFC-2. MFC-1 shows more oscillatory microbial activity than MFC-2 during the operation period between 24hrs to 96hrs.

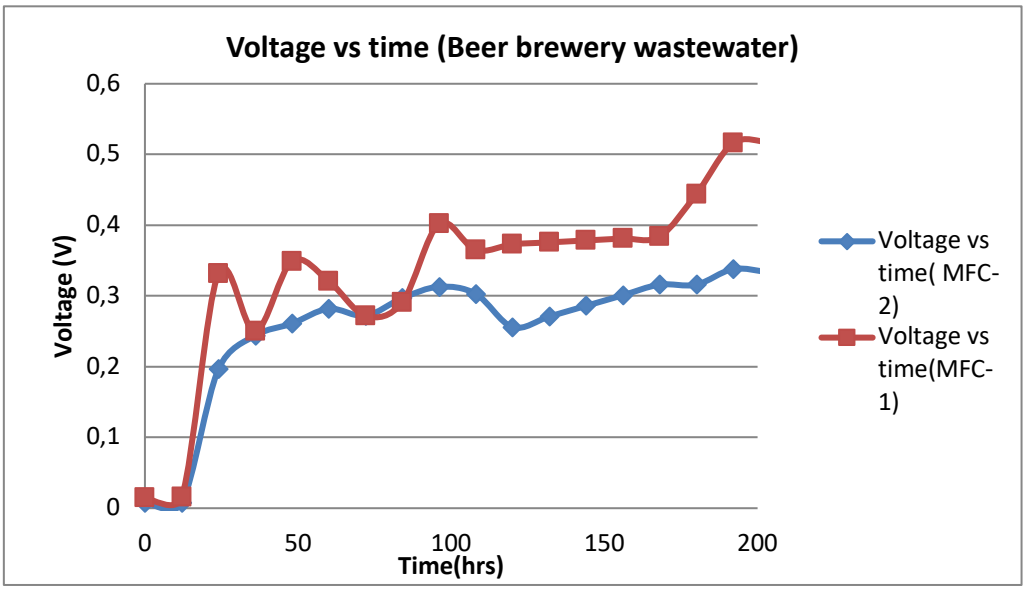


Figure 6.4 Effects of external resistance MFC start-up at external resistance of 33kΩ (MFC-2) and 51kΩ (MFC-1) (Plain cathode MFCs).

Fig 6.5 shows maximum voltage of 0.535V achieved by MFC-3 as compared to 0.505V achieved by MFC-4 however MFC-4 displays more oscillatory microbial activity than MFC-3 between period 24hrs and 84hrs.

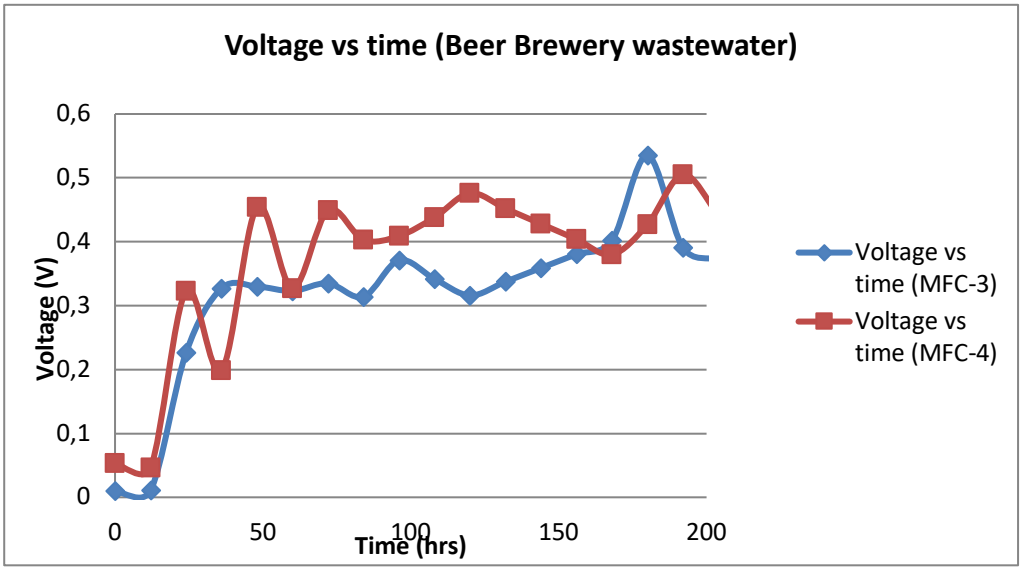


Figure 6.5. Effects of external resistance MFC start-up at external resistance of 33kΩ (MFC-3) and 51kΩ (MFC-4) (MnO2/rGO catalyst coated cathode MFCs).

The results of figure 6.6 indicate a maximum voltage of 0.445V achieved by MFC-8 as opposed to 0.363V achieved by MFC-7, but the two MFC exhibit identical oscillatory microbial behavior at operation periods ranging from 2hrs to 192hrs.

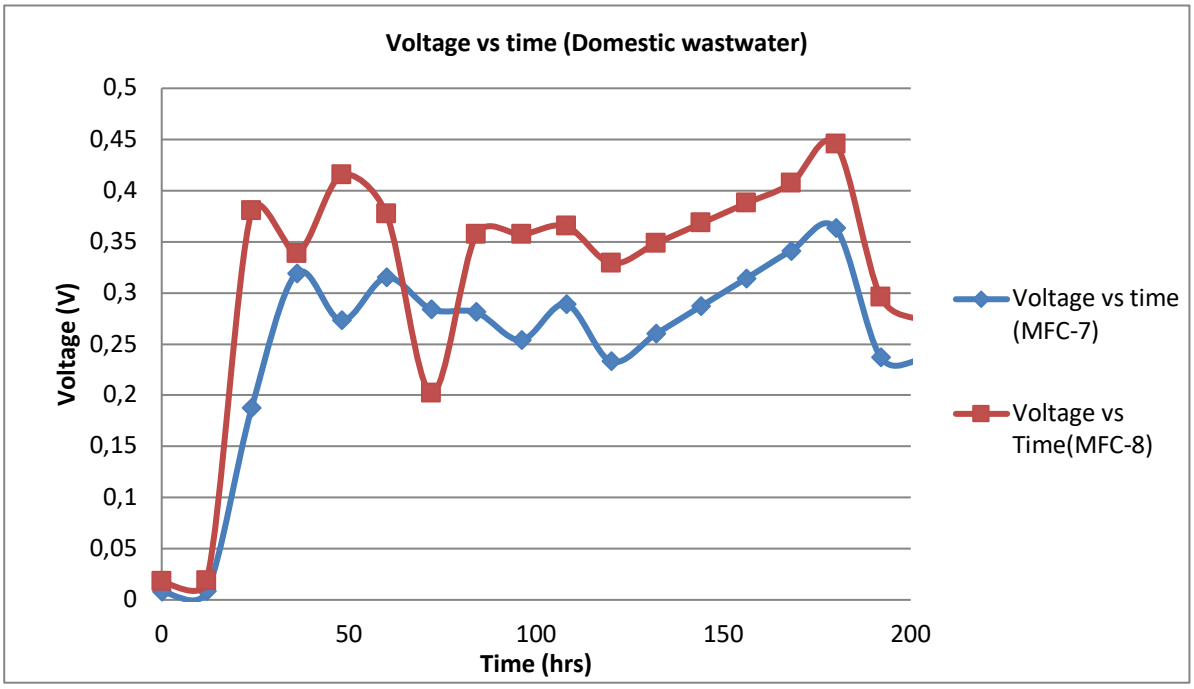


Figure 6.6. Effects of external resistance MFC start-up at external resistance of 33kΩ (MFC-7) and 51kΩ (MFC-8) (Plain cathode MFCs).

Figure 6.7 shows a maximum voltage of 0.327V achieved by MFC-5 as compared to 0.259V achieved by MFC-6 however MFC-6 displays more oscillatory microbial activity than MFC-5 at operation period between 24hr and 192hrs.

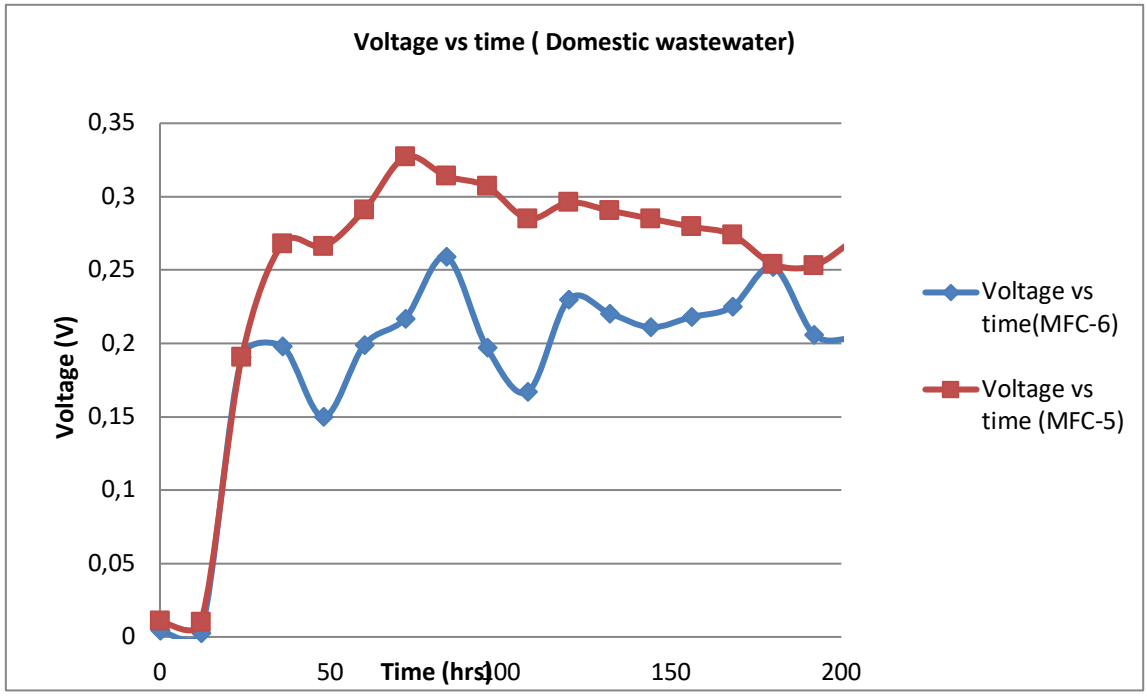


Figure 6.7. Effects of external resistance MFC start-up at external resistance of 33kΩ (MFC-6) and 51kΩ (MFC-5) (MnO2/rGO catalyst coated cathode MFCs).

Comparison of trends in figures 6.4 to 6.7 indicate that cell voltage is directly proportional to external resistance, i.e the voltage produced decreases as the external resistance of the MFC decreases. These findings also confirm the findings from a study Menicucci et al. (2006) which analysed the MFC performance at low external resistances between 6 to 0.125 kΩ. This chapter further evaluated the start-up MFC performance at high external resistance of 33 kΩ and 51 kΩ and observed high voltage yield at high external resistance MFCs. The study by Menicucci et al. (2006) attributed this behaviour to limitations imposed on electrode reaction kinetics, mass transfer, and charge transfer processes at one of the two electrodes that exhibits the slower charge transfer kinetics (the current-limiting electrode). Similar findings were also reported by Ghangrekar and Shinde (2007) and Rismani-Yazdi et al. (2011). In their study Ghangrekar and Shinde (2007) performed their investigation at external resistance from 0 to 4,000Ω and achieved a 358 mV maximum voltage at an external resistance of 4,000Ω.

6.3.3 COD removal at varying cell external resistances

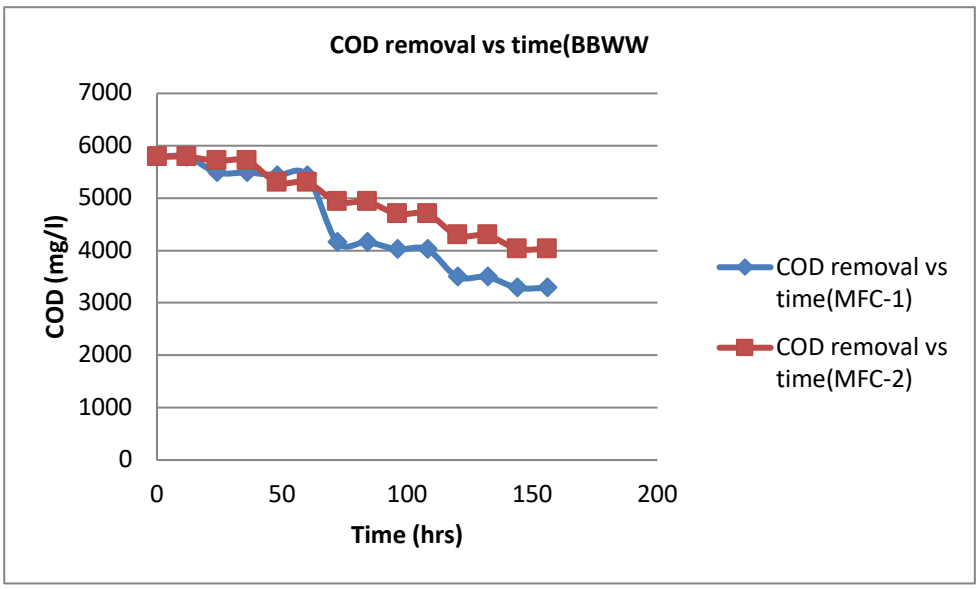


Figure 6.16. Effects of external resistance MFC start-up at external resistance of 33kΩ (MFC-2) and 51kΩ (MFC-1) (Plain cathode MFCs).

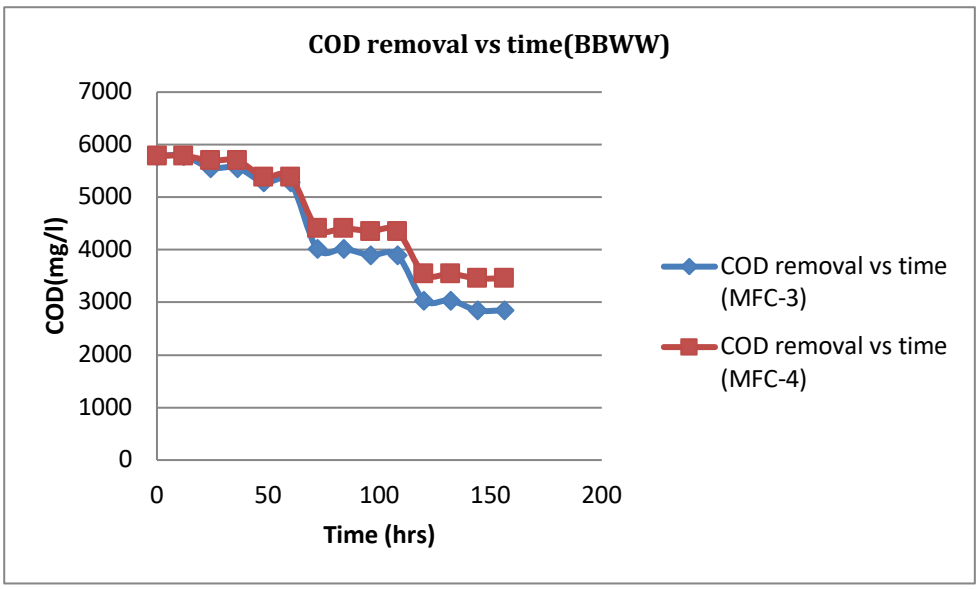


Figure 6.17. Effects of external resistance MFC start-up at external resistance of 33kΩ (MFC-2) and 51kΩ (MFC-1) (MnO2/rGO catalyst coated cathode MFCs).

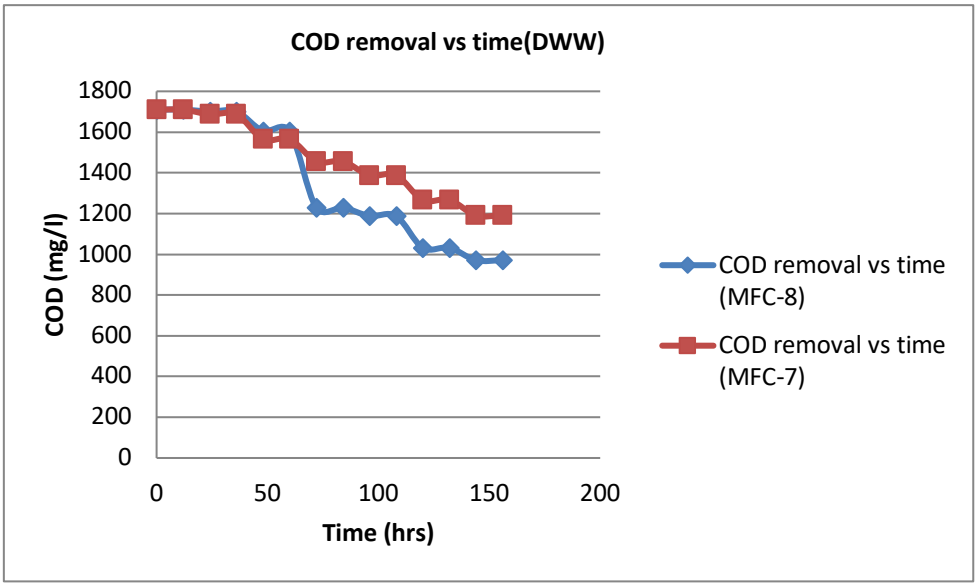


Figure 6.18. Effects of external resistance MFC start-up at external resistance of 33kΩ (MFC-2) and 51kΩ (MFC-1) (Plain cathode MFCs)

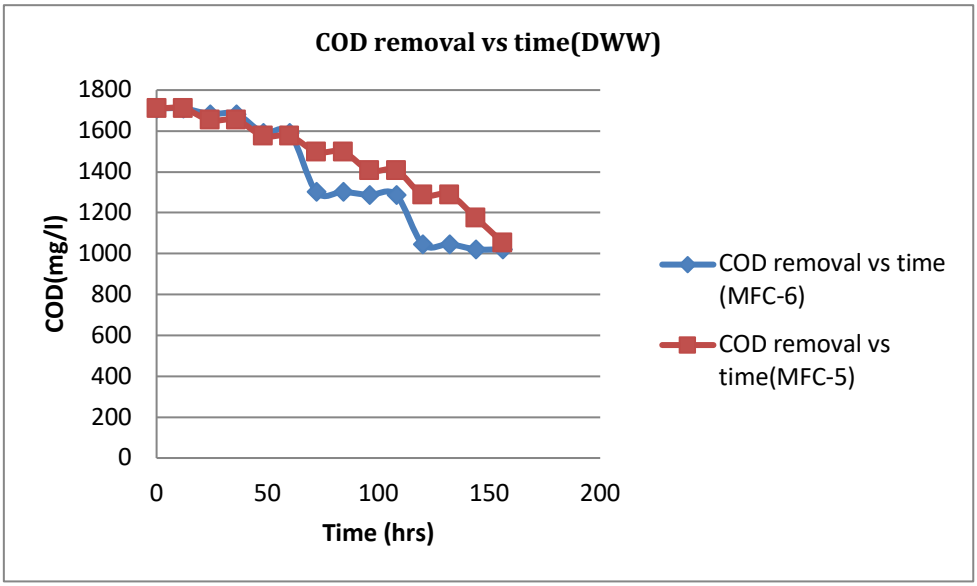


Figure 6.19. Effects of external resistance MFC start-up at external resistance of 33kΩ (MFC-2) and 51kΩ (MFC-1) (MnO2/rGO catalyst coated cathode MFCs).

In a MFC, the microorganisms of the anodic compartment oxidize the organic substrate to carry out its vital functions, thereby removing organic pollutants from wastewater. In this section, the effect of external resistance on the remedial capacity of MFC is studied. In Figure 6.16 to figure 6.19, the effluent COD as a function of external resistance is observed. As it can be seen, the COD of the effluent decreased when the external resistance was increased; therefore, the consumed substrate by microorganisms increased. In this way, the remedial capacity of the MFC enhanced when the external resistance was increased. However, taking into account that the

electricity production decreased, it is clear that greater consumption of organic matter was not caused by the improvement in the behaviour of electrogenic microorganisms. Thus, at the anodic compartment, there were other microorganisms (not electrogenics) that degraded organicmatter of wastewater

6.4 CONCLUSIONS

Findings from this study have shown that cell voltage is directly proportional to external resistance, i.e. the voltage generated decreases as the MFC's external resistance decreases. These results corroborate that of Menicucci et al. (2006), who investigated MFC efficiency at low external resistances ranging from 6 to 0.125 k. This chapter went on to examine the start-up MFC output at high external resistances of 33 k and 51 k, and it discovered high voltage yield at high external resistance MFCs. Menicucci et al. (2006) related this behavior to constraints placed on electrode reaction kinetics, mass transfer, and charge transfer processes at one of the two electrodes with slower charge transfer kinetics (the current-limiting electrode). External resistance influences anode potential and current, as well as other variables in an MFC such as microbial diversity, biofilm morphology, power produced, coulombic efficiency, and MFC stability. As a result, selecting the right external resistance to achieve maximum performance in the MFC is critical. It is vital to note that changes in operating conditions, such as electrode adjustment, will modify internal resistance and, as a result, MFC efficiency. Based on the findings, it is also possible to conclude that external resistance selects the microbial population that grows in the anodic chamber of the MFC. Comparing the current study results to other existing literature would be difficult because working temperature, configuration used, and substrate structure are all different and can influence MFC performance. More study is required to determine the effects of the MnO2/rGO catalyst on MFC production. The effect of various temperature levels on the action of the MnO2/rGO catalyst against ORR should be studied further. This is expanded upon in the study's investigations in Chapter 7.

6.5 REFERENCES

Woodward, L., Perrier, M., Srinivasan, B., Pinto, R.P., Tartakovsky, B., 2010. Comparison of real-time methods for maximizing power output in microbial fuel cells. AIChE Journal 56 (10), 2742e2750.

Pinto, R.P., Perrier, M., Tartakovsky, B. and Srinivasan, B., 2010a. Performance analyses of microbial fuel cells operated in series, Proceedings of 9th International Symposium on Dynamics and control of process systems, Leuven, Belgium.

Pinto, R.P., Srinivasan, B., Manuel, M.F., Tartakovsky, B., 2010b. A two-population bioelectrochemical model of a microbial fuel cell. Bioresource Technology 101 (14), 5256e5265.

Pinto, R.P., Tartakovsky, B., Perrier, M., Srinivasan, B., 2010c. Optimizing treatment performance of microbial fuel cells by reactor Staging. Industrial & Engineering Chemistry Research 49 (19), 9222e9229.

Menicucci, J., Beyenal, H., Marsili, E., Veluchamy, R.A., Demir, G. and Lewandowski, Z., 2006. Procedure for determining maximum sustainable power generated by microbial fuel cells. Environmental science & technology, 40(3), pp.1062-1068.

Ghangrekar, M.M. and Shinde, V.B., 2007. Performance of membrane-less microbial fuel cell treating wastewater and effect of electrode distance and area on electricity production. Bioresource Technology, 98(15), pp.2879-2885.

Rismani-Yazdi, H., Christy, A.D., Carver, S.M., Yu, Z., Dehority, B.A. and Tuovinen, O.H., 2011. Effect of external resistance on bacterial diversity and metabolism in cellulose-fed microbial fuel cells. Bioresource technology, 102(1), pp.278-283.

Chapter 7: Analysis of Microbial Fuel Cells for Treating Selected South African Industrial domestic and brewery wastewaters: MnO_2/rGO as an Alternative Catalyst

7.1 INTRODUCTION

Recent advances in microbial fuel cell science suggest an improvement in cathode electrochemical studies (Logan, 2010; Santoro et al., 2017; Rinaldi et al., 2008). The cathode has been documented to suffer from kinetic limitation of the oxidation reduction reaction (ORR), which impacts the efficiency of microbial fuel cells, and as a result, some studies have used catalysts in the cathode to accelerate the oxidation reduction reaction (Baudler et al.,2015; Erable,2017; Guerrini et al.,2014; Guo et al.,2015; Rismani et al., 2008; Madjarov et al.,2017; Kinoshita,1988; Kinoshita,1992; Erable,2012; Antolini,2015; Yuan et al.,2016; Wang et al.,2014). Recent research trends suggest a move toward the use of non-platinum group metal (PGM-free) based catalysts such as Fe, Mn, Co, and Ni, as well as carbon-based catalysts, due to lower costs, improved efficiency, and stability when compared to platinum (PGM) based catalysts, which are costly and prone to poisoning from contaminated environments (Antolini,2015; Yuan et al.,2016;Wang et al.,2014;Minachev et al.,1952; Santoro et al.,2013; de Oliveira et al.,2017; Kodali et al., 2017; Santoro et al.,2018; Santoro et al.,2017; Wang et al.,2017).

Activated carbon (AC) is currently the most favored carbon-based material due to its low costs and abundant availability, as well as its high strength in long-term operations and improved performance in neutral substrates (Merino-Jimenez et al.,2016; Zhang et al.,2014; Santini et al.,2017). Other research have used stainless steel mesh pasted with AC mixed with polytetrafluoroethylene (PTFE) as a cathode catalyst and discovered a low-cost stable catalyst with good results (Wang et al.,2017; Walter et al.,2018).

Manganese dioxide (MnO_2) has recently attracted research attention as a potential cathode catalyst in MFCs, and studies such as Zhang et al. (2009) examined manganese dioxide as a potential cathode catalyst in MFCs, e.g. β-MnO_2, γ-MnO_2, α-MnO_2 (Zhang et al., 2009). According to Zhang et al. (2009), these were developed as a replacement for Pt, as well as the highly toxic lead dioxide (PbO_2) and the instability of transition metal macrocycles and pthalocyanines. The catalytic activity of these manganese oxide catalysts was evaluated in a neutral medium MFC, and when compared to Pt as a cathode catalyst, their results yielded good ORR activity, with -MnO_2 exhibiting good catalytic activity in comparison to the others due to its high BET surface area. Zhang et al. (2009) also achieved a maximum power density of 1373mW/m2 with β-MnO2, while also suggesting higher catalyst loading to improve power output.

Studies have shown also that carbon-based catalysts have good conductivity, are highly stable, and are inexpensive, and manganese oxide is a promising electrocatalyst with ORR activity (Woon et al., 2015; Khan et al., 2015; Trogadas et al., 2014). Woon et al. (2015) investigated the use of MnO_2/CNT as a cathode catalyst in MFC to improve ORR activity. The researchers created a MnO_2 catalyst and changed it by inducing carbon nanotubes (CNT) using sonochemical-coprecipitation to produce MnO_2/CNT, achieving a maximum power density of 215.57mW/m^3 with a COD removal efficiency of 22 percent at 28.57mg/cm^2 catalyst loading (Woon et al., 2015).

Due to the high costs of Pt, Fe, and Co-based macrocyclic complexes, Xia et al. (2018) synthesized cobalt oxides as an alternative cathode catalyst. The other alternative manganese oxide cathode catalyst was stated to produce unsatisfactory MFC ORR activity (Xia et al., 2018), so the analysis looked into the ORR activity of a cobalt oxide cathode in an MFC. Xia et al. (2018) obtained a maximum power density of approximately 1220 mW/m^2 with a cobalt oxide loading of 5mg/cm^2 in their analysis, compared to 1360 mW/m^2 with a Pt/C cathode. However, at a cobalt oxide loading of 30 mg/cm^2, Xia et al. (2018) obtained an increased maximum power density of 1540 mW/m^2, which was 13% higher than the power achieved by Pt/C. The study discovered that the toughness of cobalt oxides is almost comparable to that of Pt/C, despite the fact that the costs of Pt/C are considerably higher.

Other studies examined ferrous-based materials as possible cathode catalysts in MFCs, and their results indicated that these materials had strong catalytic activities (Xia et al., 2013; Birry et al., 2011; Zhao et al., 2005; Tang et al., 2010; Toda et al., 1999; Zhang et al., 2011; Lowy et al., 2006; Wu et al., 2012; Zhang et al., 2009a; Cheng and Wu, 2013). Fu et al. (2015) developed and tested a modified activated carbon air-cathode coated with non-stoichiometric nano Fe_3O_4 as an alternative in microbial fuel cells in their research. Fu et al. (2015) found non-stoichiometric nano Fe_3O_4 to be a promising cathode catalyst material in MFCs, achieving 1430mW/m^2 maximum power density, an improvement of 83.3 percent over that achieved with a plain cathode. Yuan et al. (2017) explored the feasibility of using iron phthalocyanine as a cathode catalyst for Congo red degradation using a microbial fuel cell and a catalytic oxidation reactor. Yuan et al. (2017) demonstrated a maximum power density of 808.3mW/m^3 as well as the decomposition of Congo red into fewer and more degradable organics such as malonic acid and maleic acid, suggesting a promising alternative to pollutant removal. Kodali et al. (2018) created Iron aminoantipyrine (Fe-AAPyr), graphene nanosheets (GNSs), and their physical mixture Fe-AAPyr-GNS, as well as activated carbon, and contrasted their performances as MFC cathode catalysts for oxidation reduction reactions. The rotating ring disk electrode (RDDE) was used to examine the catalysts' ORR activities at various loadings as well as in an air-cathode, and results showed power densities of around 218W/cm^2 for Fe-AAPyr, 150W/cm^2 for GNS, and 235W/cm^2 for Fe-AAPyr-GNS, while activated carbon achieved a maximum power density of around 104W/cm^2 (Kodali et al., 2018). Due to the amount of hydrogen peroxide found, the study discovered a possible two electron pathway (direct $2e^-$) in AC and GNS, while only a small amount of hydrogen peroxide was detected in Fe-AAPyr, suggesting a possible 2x$2e^-$ transfer mechanism (Kodali et al., 2018).

A few studies have published on manganese oxide as possible cathode catalysts for MFCs (Clauwaert et al., 2007; Zhang et al., 2009). As a result, Clauwaert et al. (2007) compared the performances of a graphite felt cathode coated manganese oxide catalyst to a non-coated cathode and found that both cathodes performed similarly. Manganese oxides have been discovered to be strong cathode catalysts in MFCs due to their ability to inhibit peroxide formation and facilitate its decomposition (Klapste et al., 2002;

Mao et al., 2003). Therefore, this chapter aims to further investigate the performance of manganese oxide as a potential cathode catalyst for MFCs in real industrial wastewater effluents treatment. As a result, the aim of this chapter is to look into the performance of manganese oxide as a possible cathode catalyst for MFCs used to treat real industrial wastewater effluents. To the best of our knowledge, research into MnO_2/rGO as a cathode catalyst in psychrophilic MFCs is still lacking, so the aim of this study is to synthesize and further investigate the electrocatalytic activity of MnO_2/rGO as an ORR catalyst in MFC applications for power generation in industrial wastewater treatment.

7.2 MATERIALS AND METHODS

Eight double chambered MFCs (i.e. MFC-1, MFC-2, MFC-3, MFC-4, MFC-5, MFC-6, MFC-7 and MFC-8) with 400 ml volume each were used in the study. The two chambers for each MFC were separated by a 25cm diameter Nafion membrane. MFC-1, MFC-2, MFC-3 and MFC-4 contained MnO_2/rGO catalyst coated cathodes with catalyst loading of 0.25mg/cm^2 while MFC-5, -6, MFC7 and MFC-8 contained plain cathode electrodes. The anode electrodes were made of 3mm diameter and 75mm long graphite rods (Merck, South Africa). The cathode electrodes were made of 3mm diameter and 75mm long graphite rods (Merck, South Africa). The MFCs were operated at ambient temperature, i.e. average of 14^0C.

Brewery wastewater was collected from SAB/INBEV beer brewery and stored at 0^0C on the same day until use. MFCs (i.e. MFC-1, MFC-2, MFC-5 and MFC-6) were fed with pure beer brewery wastewater effluent. MFC-3, MFC-4, MFC-7 and MFC-8 were fed with raw domestic wastewater collected from ERWAT wastewater treatment works and which was stored at 0^oC on the same day until use. All chemicals and reagents used were of analytical grade. The initial composition of the wastewater is represented by table 7.1.

Table 7.1 Initial wastewater composition

Wastewater	COD (mg/L)
Beer brewery	3246
Domestic	825

The investigative study work was performed at open and closed circuit. In the first phase of the experiment, MFC-1, MFC-3, MFC-5 and MFC-7 were operated at 33kΩ external resistance while MFC-2, MFC-4, MFC-6 and MFC-8 were all operated at 51kΩ. And on the second phase of the experiment, similar experiments were repeated at open circuit.

Voltage outputs from microbial fuel cells were measured at random time intervals by a Fluke 17B+ digital multimeter across closed circuit cell, i.e. 33 kΩ and 51 kΩ resistances.

The Current (I) was calculated using Ohm's law as:

$$I(mA) = \frac{E_{cell}(mV)}{R_{ext}(\Omega)} \dots\dots\dots\dots\dots\dots\dots 7.1$$

where Ecell (V) is the measured-cell voltage and Rext is the external resistance (Ω) of the cell.

Current density was calculated as:

$$i(\frac{mA}{cm^2}) = \frac{E_{cell}}{R_{ext}.A} \dots\dots\dots\dots\dots\dots\dots\dots 7.2$$

Where Ecell (mV) is the cell-measured voltage, R_{ext} (Ω) is the external resistance and A (cm^2) is the projected surface area of the cathode electrode.

7.3 RESULTS AND DISCUSSIONS

7.3.1 Effects of MnO_2/rGO catalyst on microbial fuel performance at psychrophilic temperatures

Figure 7.1 shows voltage generation for two MFCs treating raw brewery wastewater one containing a cathode coated with a MnO_2/rGO catalyst and the other containing a plain cathode run at 33kΩ external resistance. The results show higher voltage generation for the microbial fuel cell containing a catalyst coated cathode.

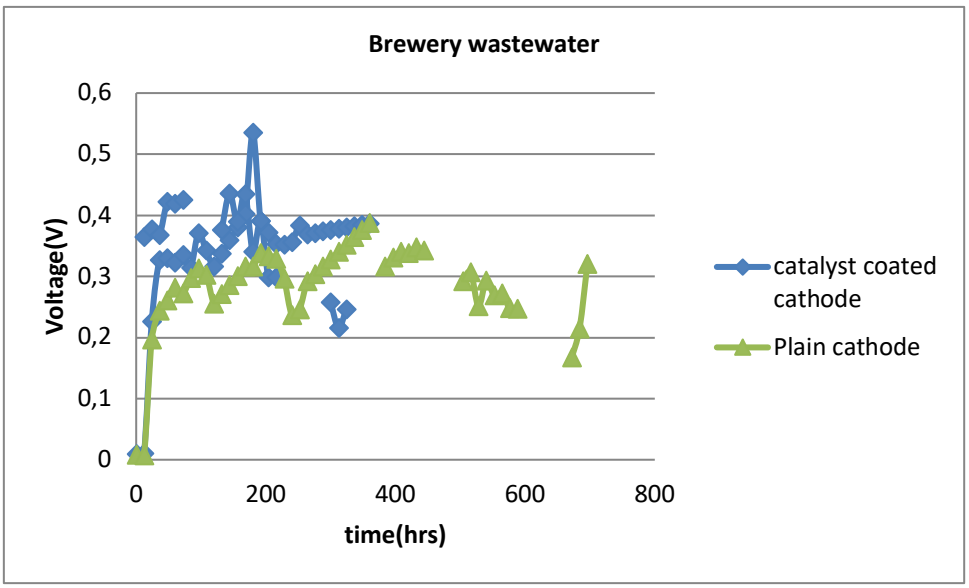


Figure 7.1Voltage generation for brewery wastewater-fed MFCs with catalyst coated and plain cathode electrodes at 33kΩ external resistance.

Figure 7.2 depicts the voltage generation for two MFCs treating raw domestic wastewater with one containing a cathode coated with a MnO_2/rGO catalyst and the other containing a plain cathode run at 33kΩ external resistance. These findings, however, are in contrast to those in figure 7.1, as they indicate that an MFC with a plain cathode generates higher voltage.

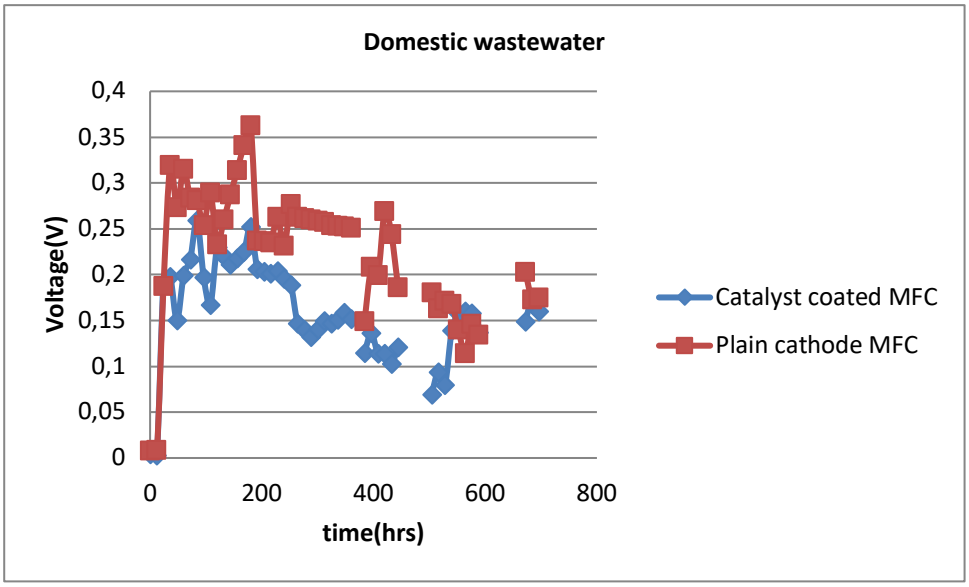


Figure 7.2 Voltage generation for domestic wastewater-fed MFCs with catalyst coated and plain cathode electrodes at 33kΩ external resistance.

Similar experimental runs were repeated for the above brewery and domestic wastewater fed MFCs at 51kΩ external resistance and their results are shown by figures 7.3 and 7.4.

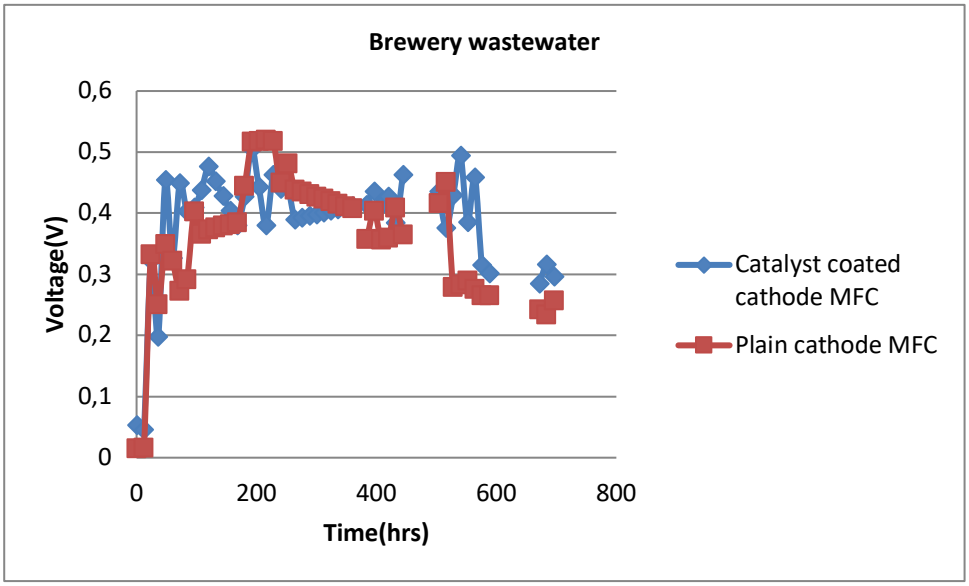


Figure 7.3 Voltage generation for brewery wastewater-fed MFCs with catalyst coated and plain cathode electrodes at 51kΩ external resistance.

Similar patterns can be seen here, where MFCs with a MnO_2/rGO catalyst have higher voltage generation for brewery wastewater fed MFCs than MFCs with plain cathode, whereas MFCs fed with domestic wastewater have higher voltage generation than MFCs with a MnO_2/rGO catalyst.

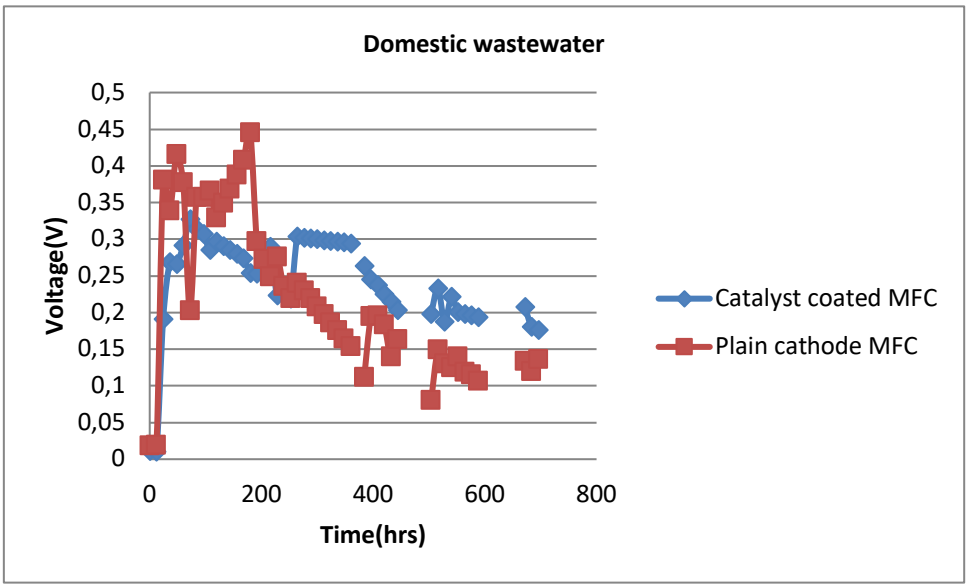


Figure 7.4 Voltage generation for brewery wastewater-fed MFCs with catalyst coated and plain cathode electrodes at 51kΩ external resistance.

In domestic wastewater fed MFCs for voltage generation, plain cathode MFCs outperformed catalyst coated MFCs, while catalyst coated MFCs outperformed plain cathode MFCs in brewery wastewater fed MFCs for voltage generation (figures 7.1 to 7.4). Similarly, in domestic wastewater fed MFCs, the power curves indicate higher power generation for plain cathode MFCs, suggesting low MFC output from catalyst coated graphite rod electrode MFCs as compared to brewery wastewater fed MFCs (figure 7.5 to 7.8).

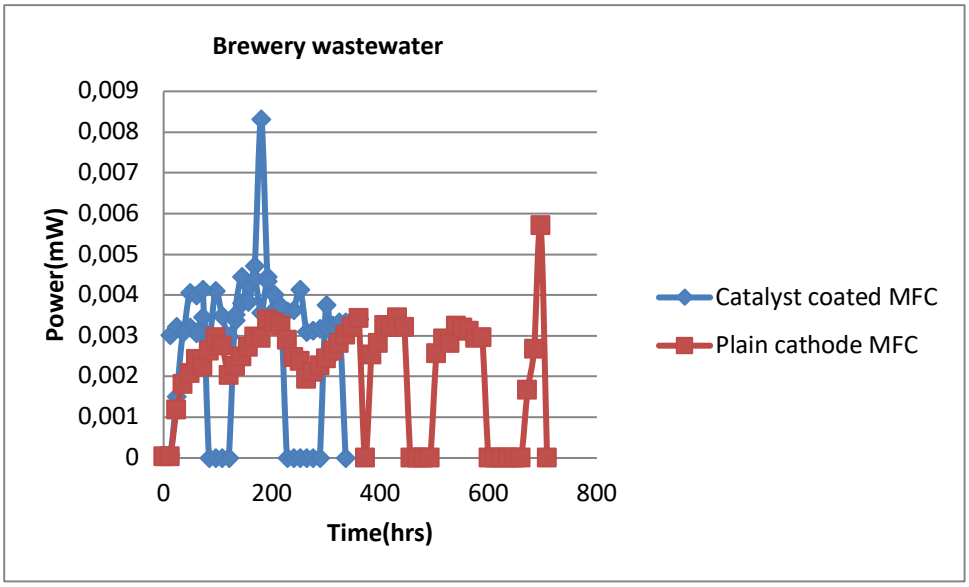


Figure 7.5 Power generation for brewery wastewater-fed MFCs with catalyst coated and plain cathode electrodes at 33kΩ external resistance.

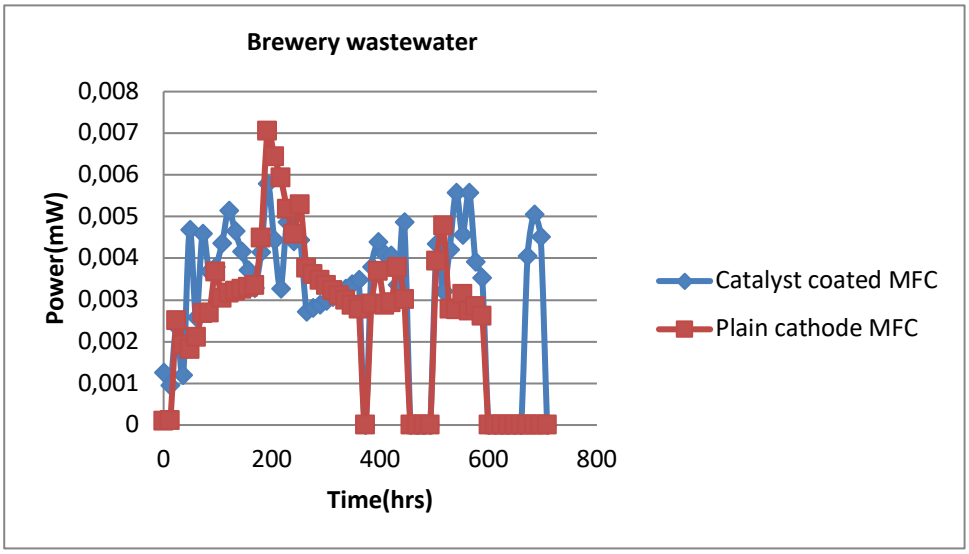


Figure 7.6 Power generation for brewery wastewater-fed MFCs with catalyst coated and plain cathode electrodes at 51kΩ external resistance.

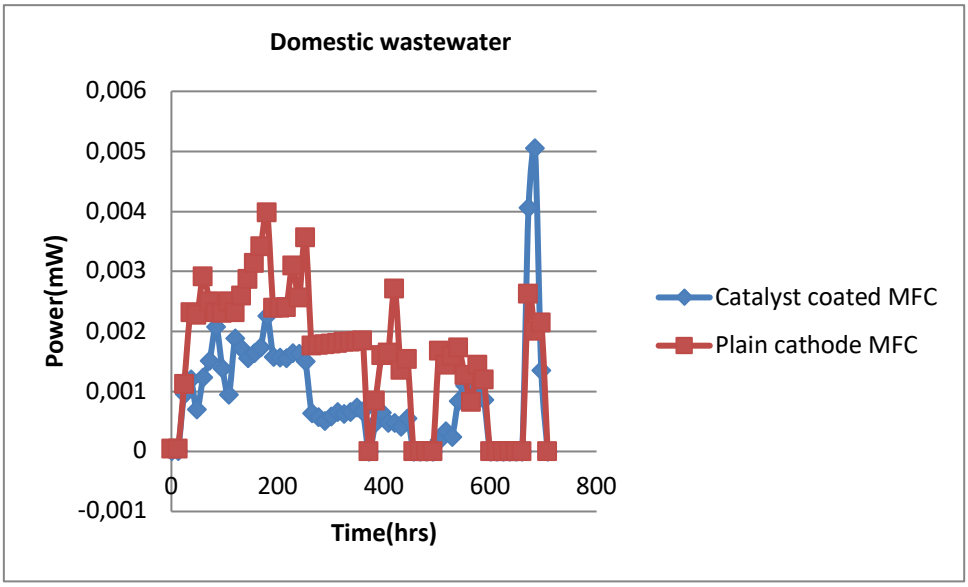


Figure 7.7 Power generation for domestic wastewater-fed MFCs with catalyst coated and plain cathode electrodes at 33kΩ external resistance.

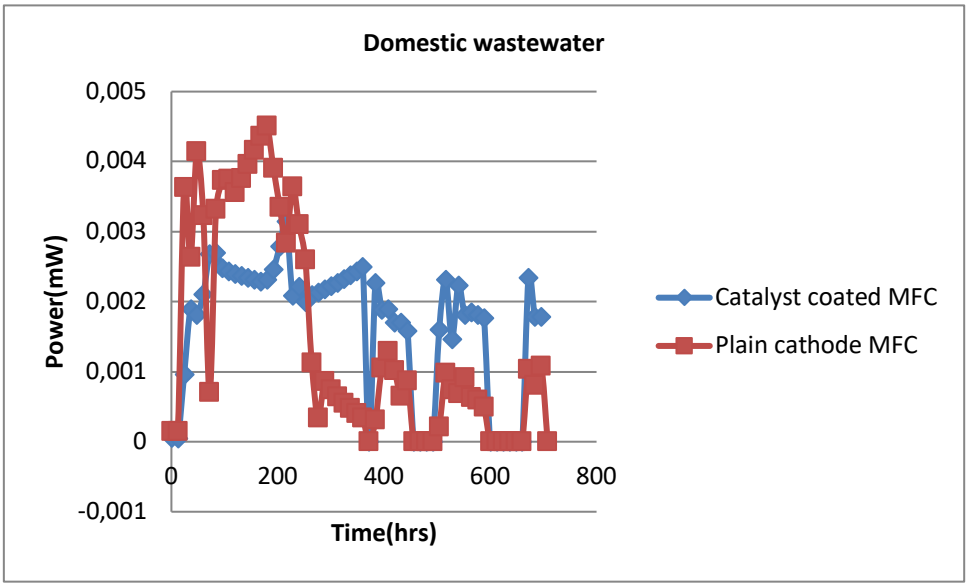


Figure 7.8 Power generation for brewery wastewater-fed MFCs with catalyst coated and plain cathode electrodes at 51kΩ external resistance

Every MFC's power peak decreases over time (figs. 7.5 to 7.8), likely due to biological growth on the cathode as well as a negative interaction between the catalyst and contaminants in the wastewater (Kodali et al., 2017). In this case, low MnO_2/rGO catalyst activity in domestic wastewater-fed MFCs may be due to catalyst poisoning, which decreases catalyst activity.

Another explanation for the catalyst coated cathode MFC's lower output in domestic wastewater fed MFCs (fig 7.2) may be variations in bacterial yield or the existence of electron acceptors such as such as NO_3^- and SO_4^{2-}. While the study by Zhang et al. (2009) achieved a maximum power density of 1373mW/m^2 with β-MnO_2 they also recommended a higher catalyst loading in order to increase the power output. Modifying the catalyst can improve MFC efficiency, as demonstrated by Woon et al. (2015), who synthesized a MnO_2 catalyst and modified it by inducing carbon nanotube (CNT) via a sonochemical-coprecipitation method to generate MnO_2/CNT and as such obtained an improved maximum power density (Woon et al., 2015). Cobalt-based catalysts can provide an alternative, as demonstrated by the study Xia et al. (2018), which synthesized cobalt oxides as an alternative cathode catalyst to boost ORR catalyst operation (Xia et al., 2018). Low temperatures could have harmed the microbial or ORR behavior of catalyst coated cathode MFCs in domestic wastewater fed MFCs more than plain cathode MFCs.

7.3.2 Effects of MnO_2/rGO catalyst on substrate pH at psychrophilic temperatures

The MnO_2/rGO catalyst was less active in domestic wastewater-fed MFCs than in brewery wastewater-fed MFCs, as previously discussed. As a result, the impact of the MnO2/rGO catalyst on the pH of each MFC fed with raw brewery and domestic wastewaters is examined in this subsection (figure 7.9 to 7.12). Figure 7.9 shows that catalyst coated MFCs have a higher pH increase over time than plain cathode MFCs, while figure 7.10 shows that catalyst coated MFCs have a marginally higher pH increase than plain cathode MFCs.

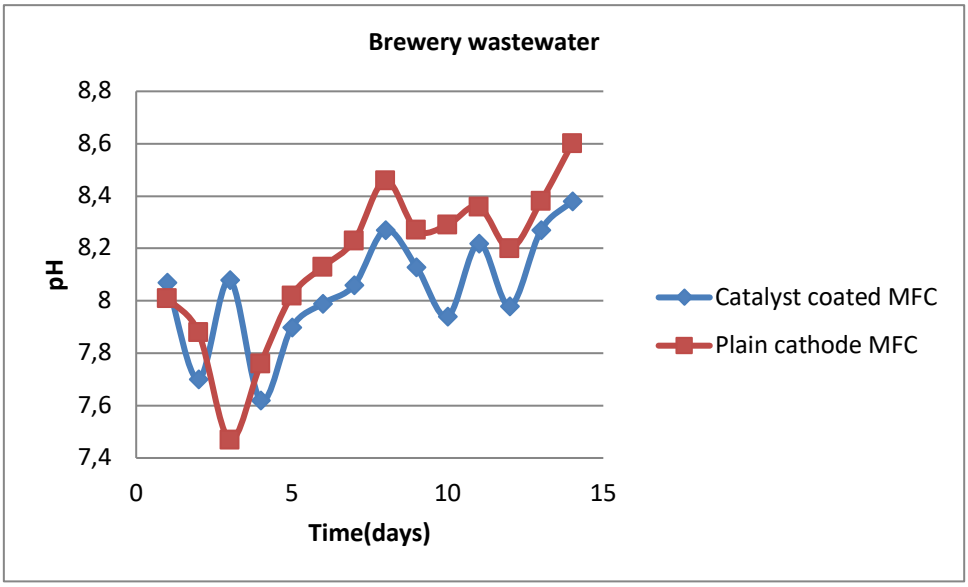


Figure 7.9 Effects of MnO2/rGO catalyst on substrate pH at 33kΩ external resistance (brewery and Domestic wastewater fed MFCs).

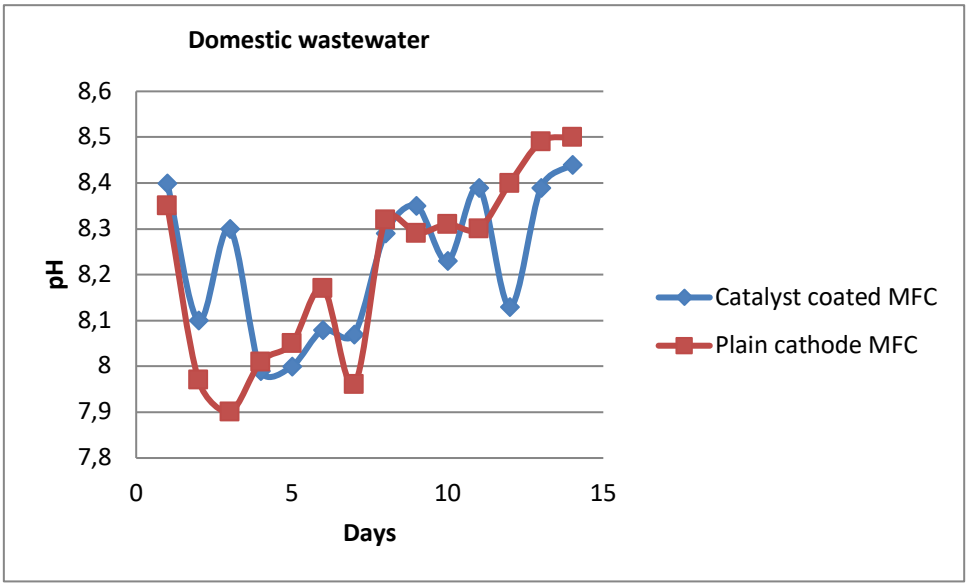


Figure 7.10 Effects of MnO_2/rGO catalyst on substrate pH at 33kΩ external resistance (Domestic wastewater fed MFCs).

In domestic wastewater fed MFCs, plain cathode MFCs display a slightly higher pH increase than catalyst coated MFCs (Figures 7.11 and 7.12). The results in figures 7.9 to 7.12 show that MFC performance improves as substrate pH increases, which is consistent with the findings of Puig et al. (2010), who found that a high substrate/anolyte pH results in improved MFC performance, and Kaushik and Chetal (2013), who recorded an optimum pH for bacterial growth in an MFC in the range of 7 to 9, and this review. The rise in pH had a positive effect on power production in this

case. The findings also show that the substrate composition may limit catalyst efficiency in MFC.

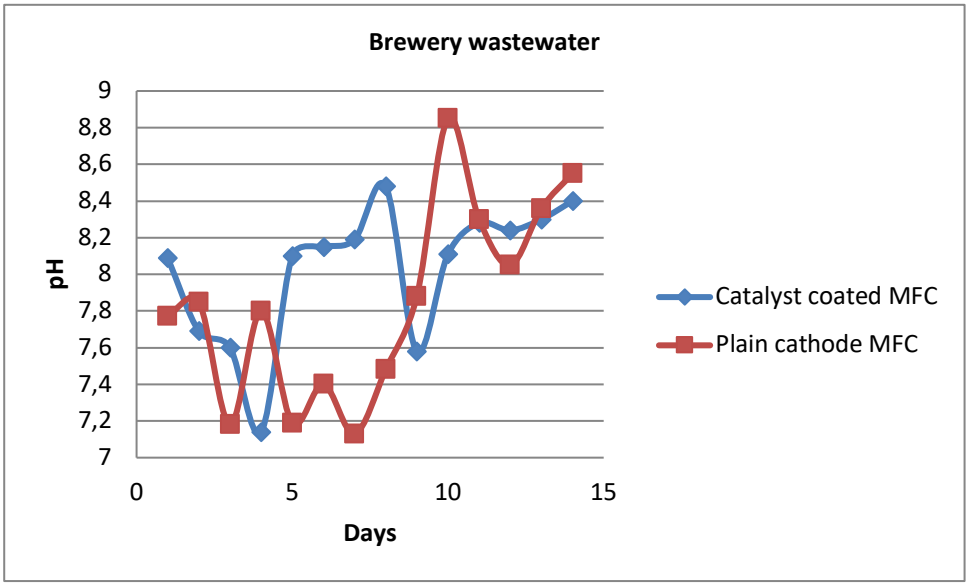


Figure 7.11 Effects of MnO2/rGO catalyst on substrate pH at 51kΩ external resistance (Domestic wastewater fed MFCs).

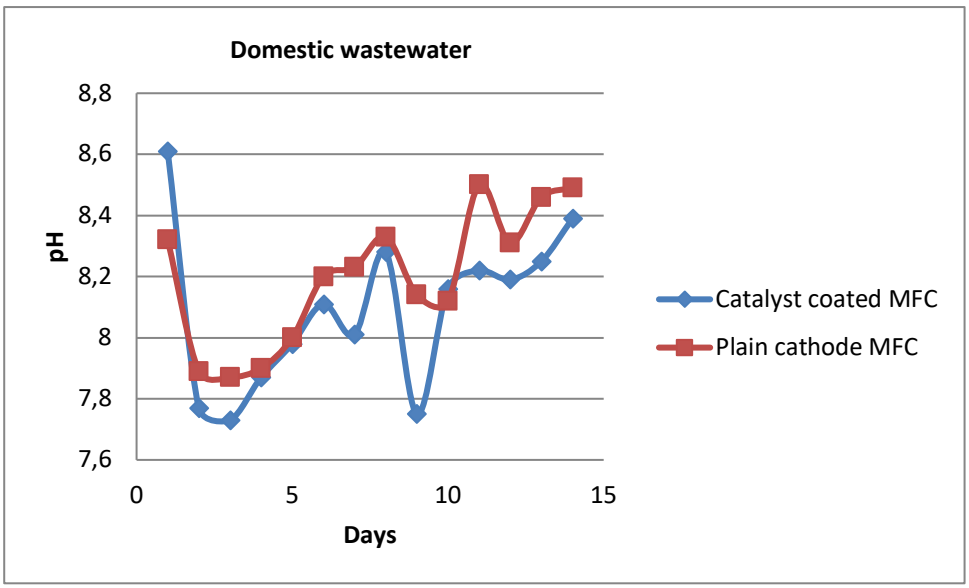


Figure 7.12 Effects of MnO2/rGO catalyst on substrate pH at 51kΩ external resistance (Domestic wastewater fed MFCs).

7.4 CONCLUSIONS

The performance of the MnO2/rGO catalyst in different mediums at different external resistances in a double chamber MFC under pyschrophilic conditions was assessed (i.e. operating temperature below 20ºC). The power peak of every MFC reduces with time, most likely due to biological development on the cathode and a detrimental association with the catalyst and toxins in the wastewater (Kodali et al., 2017). Low MnO_2/rGO catalyst activity in domestic wastewater-fed MFCs may be attributed to catalyst toxicity, which inhibits catalyst activity. Another possible reason for the lower performance of the catalyst coated cathode MFC in domestic wastewater fed MFCs may be differences in bacterial yield or the presence of electron acceptors such as NO_3^- and SO_4^{-2}. As shown by Woon et al. (2015), who synthesized a MnO_2 catalyst and modified it by inducing carbon nanotube (CNT) via a sonochemical-coprecipitation process to produce MnO_2/CNT and thus obtained an improved maximum power density, modifying the catalyst will boost MFC performance (Woon et al., 2015). As shown by the study Xia et al. (2018), which synthesized cobalt oxides as an alternate cathode catalyst to improve ORR catalyst activity, cobalt-based catalysts may provide an alternative (Xia et al., 2018). Low temperatures may have also had a greater impact on the microbial activity or ORR action of MnO_2/rGO catalyst coated cathode MFCs in domestic wastewater fed MFCs than plain cathode MFCs. Furthermore, the MnO_2/rGO catalyst was less involved in domestic wastewater-fed MFCs than in brewery wastewater-fed MFCs, and the effect of the MnO_2/rGO catalyst on the pH of each MFC fed with raw brewery and domestic wastewaters was investigated. The pH of each MFC increased as a result of the results, indicating that catalyst coated MFCs had a slightly higher pH increase than plain cathode MFCs. Data from domestic wastewater fed MFCs, on the other hand, show that simple cathode MFCs have a slightly higher pH rise than catalyst coated MFCs. In this regard, the results in indicate that MFC performance improves as substrate pH increases, which is consistent with the observations of Puig et al. (2010), who found that a high substrate/anolyte pH results in enhanced MFC performance, and Kaushik and Chetal (2013), who discovered an optimal pH for bacterial growth in an MFC in the range of 7–9, as well as this analysis. In this situation, the increase in pH has a positive impact on power generation. The findings also suggest that the substrate composition can restrict the efficiency of the catalyst in MFC. The MnO_2/rGO catalyst performed better in the

brewery wastewater fed MFCs than in the domestic wastewater fed MFCs, according to the findings. In addition, the results showed a proportional relationship between the pH of the substrate and the MFC production. In terms of ORR activity, the MnO_2/rGO catalyst outperformed plain cathode MFCs, rendering it more suited for brewery wastewater treatment than domestic wastewater treatment. Catalyst loading, catalyst alteration, and alternative catalysts can boost MFC efficiency under similar conditions.

7.5 REFERENCES

Walter, X.A., Santoro, C., Greenman, J. and Ieropoulos, I., 2019. Self-stratifying microbial fuel cell: the importance of the cathode electrode immersion height. international journal of hydrogen energy, 44(9), pp.4524-4532.

Logan, B.E., 2010. Scaling up microbial fuel cells and other bioelectrochemical systems. Applied microbiology and biotechnology, 85(6), pp.1665-1671.

Santoro, C., Arbizzani, C., Erable, B. and Ieropoulos, I., 2017. Microbial fuel cells: from fundamentals to applications. A review. Journal of power sources, 356, pp.225-244.

Rinaldi, A., Mecheri, B., Garavaglia, V., Licoccia, S., Di Nardo, P. and Traversa, E., 2008. Engineering materials and biology to boost performance of microbial fuel cells: a critical review. Energy & Environmental Science, 1(4), pp.417-429.

Baudler, A., Schmidt, I., Langner, M., Greiner, A. and Schröder, U., 2015. Does it have to be carbon? Metal anodes in microbial fuel cells and related bioelectrochemical systems. Energy & Environmental Science, 8(7), pp.2048-2055.

Erable, B., Byrne, N., Etcheverry, L., Achouak, W. and Bergel, A., 2017. Single medium microbial fuel cell: stainless steel and graphite electrode materials select bacterial communities resulting in opposite electrocatalytic activities. International Journal of Hydrogen Energy, 42(41), pp.26059-26067.

Guerrini, E., Cristiani, P., Grattieri, M., Santoro, C., Li, B. and Trasatti, S., 2014. Electrochemical behavior of stainless steel anodes in membraneless microbial fuel cells. Journal of The Electrochemical Society, 161(3), pp.H62-H67.

Guo, K., Prévoteau, A., Patil, S.A. and Rabaey, K., 2015. Engineering electrodes for microbial electrocatalysis. Current Opinion in Biotechnology, 33, pp.149-156.

Rismani-Yazdi, H., Carver, S.M., Christy, A.D. and Tuovinen, O.H., 2008. Cathodic limitations in microbial fuel cells: an overview. Journal of Power Sources, 180(2), pp.683-694.

Madjarov, J., Popat, S.C., Erben, J., Götze, A., Zengerle, R. and Kerzenmacher, S., 2017. Revisiting methods to characterize bioelectrochemical systems: the

influence of uncompensated resistance (iRu-drop), double layer capacitance, and junction potential. Journal of Power Sources, 356, pp.408-418.

Kinoshita, K., 1988. Carbon: electrochemical and physicochemical properties.

Kinoshita, K., 1992. Electrochemical oxygen technology (Vol. 30). John Wiley & Sons.

Erable, B., Féron, D. and Bergel, A., 2012. Microbial catalysis of the oxygen reduction reaction for microbial fuel cells: a review. ChemSusChem, 5(6), pp.975-987.

Antolini, E., 2015. Composite materials for polymer electrolyte membrane microbial fuel cells. Biosensors and Bioelectronics, 69, pp.54-70.

Yuan, H., Hou, Y., Abu-Reesh, I.M., Chen, J. and He, Z., 2016. Oxygen reduction reaction catalysts used in microbial fuel cells for energy-efficient wastewater treatment: a review. Materials Horizons, 3(5), pp.382-401.

Wang, Z., Cao, C., Zheng, Y., Chen, S. and Zhao, F., 2014. Abiotic oxygen reduction reaction catalysts used in microbial fuel cells. ChemElectroChem, 1(11), pp.1813-1821.

Minachev, K.M., Shuikin, N.I. and Rozhdestivenskaya, I.D., 1952. Poisoning of platinum catalysts with a low content of active metal on a carrier, under conditions of dehydrogenation catalysis. Russian Chemical Bulletin, 1(4), pp.567-575.

Santoro, C., Li, B., Cristiani, P. and Squadrito, G., 2013. Power generation of microbial fuel cells (MFCs) with low cathodic platinum loading. International Journal of Hydrogen Energy, 38(1), pp.692-700.

de Oliveira, M.A.C., Mecheri, B., D'Epifanio, A., Placidi, E., Arciprete, F., Valentini, F., Perandini, A., Valentini, V. and Licoccia, S., 2017. Graphene oxide nanoplatforms to enhance catalytic performance of iron phthalocyanine for oxygen reduction reaction in bioelectrochemical systems. Journal of Power Sources, 356, pp.381-388.

Kodali, M., Santoro, C., Serov, A., Kabir, S., Artyushkova, K., Matanovic, I. and Atanassov, P., 2017. Air breathing cathodes for microbial fuel cell using Mn-, Fe-, Co-and Ni-containing platinum group metal-free catalysts. Electrochimica acta, 231, pp.115-124.

Santoro, C., Kodali, M., Herrera, S., Serov, A., Ieropoulos, I. and Atanassov, P., 2018. Power generation in microbial fuel cells using platinum group metal-free cathode catalyst: effect of the catalyst loading on performance and costs. Journal of power sources, 378, pp.169-175.

Santoro, C., Kodali, M., Kabir, S., Soavi, F., Serov, A. and Atanassov, P., 2017. Three-dimensional graphene nanosheets as cathode catalysts in standard and supercapacitive microbial fuel cell. Journal of power sources, 356, pp.371-380.

Wang, Z., Mahadevan, G.D., Wu, Y. and Zhao, F., 2017. Progress of air-breathing cathode in microbial fuel cells. Journal of Power Sources, 356, pp.245-255.

Merino-Jimenez, I., Santoro, C., Rojas-Carbonell, S., Greenman, J., Ieropoulos, I. and Atanassov, P., 2016. Carbon-based air-breathing cathodes for microbial fuel cells. Catalysts, 6(9), p.127.

Zhang, X., Pant, D., Zhang, F., Liu, J., He, W. and Logan, B.E., 2014. Long-term performance of chemically and physically modified activated carbons in air cathodes of microbial fuel cells. ChemElectroChem, 1(11), pp.1859-1866.

Santini, M., Marzorati, S., Fest-Santini, S., Trasatti, S. and Cristiani, P., 2017. Carbonate scale deactivating the biocathode in a microbial fuel cell. Journal of Power Sources, 356, pp.400-407.

Walter, X.A., Greenman, J. and Ieropoulos, I., 2018. Binder materials for the cathodes applied to self-stratifying membraneless microbial fuel cell. Bioelectrochemistry, 123, pp.119-124.

Moon H, Chang IS, Kim BH (2006) Continuous electricity production from artificial wastewater using a mediator-less microbial fuel cell. Bioresource Technol 97: 621-627.

Logan BE (2008) Microbial fuel cells. Wiley, New Jersey, USA.

Debabov VG (2008) Electricity from microorganisms. Mikrobiologiia 77: 129-157.

Chaudhuri SK, Lovley DR (2003) Electricity generation by direct oxidation of glucose in mediatorless microbial fuel cells. Nat Biotechnol 21: 1229-1232.

Min B, Logan BE (2004) Continuous electricity generation from domestic wastewater and organic substrates in a flat plate microbial fuel cell. Environ Sci Technol 38: 5809-5814.

Liu H, Cheng S, Logan BE (2005) Production of electricity from acetate or butyrate using a single-chamber microbial fuel cell. Environ Sci Technol 39: 658-662.

Park DH, Zeikus JG (2003) Improved fuel cell and electrode designs for producing electricity from microbial degradation. Biotechnol Bioeng 81: 348-355.

Rabaey K, Lissens G, Siciliano SD, Verstraete W (2003) A microbial fuel cell capable of converting glucose to electricity at high rate and efficiency. Biotechnol Lett 25: 1531-1535.

Cheng S, Liu H (2006) Power densities using different cathode catalysts (Pt and CoTMPP) and polymer binders (Nafion and PTFE) in single chamber microbial fuel cells. Environmental Science & Technology 40: 364-369.

Lovley DR (2006) Bug juice: harvesting electricity with microorganisms. Nat Rev Microbiol 4: 497-508.

Fan YZ, Sharbrough E (2008) Quantification of the Internal Resistance Distribution of Microbial Fuel Cells. Environmental Science & Technology 42: 8101-8107.

Muralidharan VS (1997) Warburg impedance - Basics revisited. Anti-Corrosion Methods and Materials 44: 26-46.

Hoboken NJ (2005) Impedance spectroscopy: Theory, experiment, and applications. New York, USA.

Menicucci J, Beyenal H, Marsili E, Veluchamy RA, Demir G, et al. (2006) Procedure for determining maximum sustainable power generated by microbial fuel cells. Environ Sci Technol 40: 1062-1068.

Ghangrekar MM, Shinde VB (2007) Performance of membrane-less microbial fuel cell treating wastewater and effect of electrode distance and area on electricity production. Bioresour Technol 98: 2879-2885.

Rismani-Yazdi H, Christy AD, Carver SM, Yu Z, Dehority BA, et al. (2011) Effect of external resistance on bacterial diversity and metabolism in cellulose- fed microbial fuel cells. Bioresour Technol 102: 278-283.

Song TS, Yan ZS, Zhao ZW, Jiang HL (2010) Removal of organic matter in freshwater sediment by microbial fuel cells at various external resistances. J Chem Technol Biotechnol 85: 1489-1493.

Chae KJ, Choi MJ, Kim KY, Ajayi FF, Park W, et al. (2010) Methanogenesis control by employing various environmental stress conditions in two-chambered microbial fuel cells. Bioresour Technol 101: 5350-5357.

Jadhav GS, Ghangrekar MM (2009) Performance of microbial fuel cell subjected to variation in pH, temperature, external load and substrate concentration. Bioresour Technol 100: 717-723.

Jang JK, Pham TH, Chang IS, Kang KH, Moon H, et al. (2004) Construction and operation of a novel mediator and membrane-less microbial fuel cell. Process Biochem 39: 1007-1012.

Aelterman P, Versichele M, Marzorati M, Boon N, Verstraete W (2008) Loading rate and external resistance control the electricity generation of microbial fuel cells with different threedimensional anodes. Bioresour Technol 99: 8895-8902.

Zhang L, Zhu X, Li J, Qiang L, Ye D (2011) Biofilm formation and electricity generation of a microbial fuel cell started up under different external resistances. J Power Sources 196: 6029-6035.

Lyon DY, Buret F, Vogel TM, Monier JM (2010) Is resistance futile? Changing external resistance does not improve microbial fuel cell performance. Bioelectrochemistry 78: 2-7.

Ren Z, Yan H, Wang W, Mench MM, Regan JM (2011) Characterization of microbial fuel cells at microbially and electrochemically meaningful time scales. Environ Sci Technol 45: 2435-2441.

Del Campo AG, Cañizares P, Lobato J, Rodrigo M, Morales FF (2014) Effects of External Resistance on Microbial Fuel Cell's Performance. In: Environment, Energy and Climate Change, pp: 175-197.

Aelterman P, Versichele M, Marzorati M, Boon N, Verstraete W (2008) Loading rate and external resistance control the electricity generation of microbial fuel cells with different three dimensional anodes. Bioresour Technol 99: 8895- 8902.

Ren Z, Yan H, Wang W, Mench MM, Regan JM (2011) Characterization of microbial fuel cells at microbially and electrochemically meaningful time scales. Environ Sci Technol 45: 2435-2441.

Katuri KP, Scott K, Head IM, Picioreanu C, Curtis TP (2011) Microbial fuel cells meet with external resistance. Bioresour Technol 102: 2758-2766.

Logan BE, Regan JM (2006) Electricity-producing bacterial communities in microbial fuel cells. Trends Microbiol 14: 512-518.

Manohar AK, Bretschger O, Nealson KH, Mansfeld F (2008) The use of electrochemical impedance spectroscopy (EIS) in the evaluation of the electrochemical properties of a microbial fuel cell. Bioelectrochemistry 72: 149-154.

Jung S, Regan JM (2011) Influence of external resistance on electrogenesis, methanogenesis, and anode prokaryotic communities in microbial fuel cells. Appl Environ Microbiol 77: 564-571.

Lee CY, Chen JH, Cai YY (2010) Bioelectricity generation and organic removal in microbial fuel cells used for treatment of wastewater from fish-market. J Environ Eng Manage 20: 173-180.

Zhang Y, Mo G, Li X, Zhang W, Zhang J, et al. (2011) A graphene modified anode to improve the performance of microbial fuel cells. J Power Sources 196: 5402-5407.

Clauwaert, P., Van der Ha, D., Boon, N., Verbeken, K., Verhaege, M., Rabaey, K. and Verstraete, W., 2007. Open air biocathode enables effective electricity generation with microbial fuel cells. Environmental science & technology, 41(21), pp.7564-7569.

Zhang, L., Liu, C., Zhuang, L., Li, W., Zhou, S. and Zhang, J., 2009. Manganese dioxide as an alternative cathodic catalyst to platinum in microbial fuel cells. Biosensors and Bioelectronics, 24(9), pp.2825-2829.

Klápště, B., Vondrák, J. and Velicka, J., 2002. MnOx/C composites as electrode materials II. Reduction of oxygen on bifunctional catalysts based on manganese oxides. Electrochimica Acta, 47(15), pp.2365-2369.

Mao, L., Zhang, D., Sotomura, T., Nakatsu, K., Koshiba, N. and Ohsaka, T., 2003. Mechanistic study of the reduction of oxygen in air electrode with manganese oxides as electrocatalysts. Electrochimica Acta, 48(8), pp.1015-1021.

Chapter 8: Overall Conclusion

1. Dilution of wastewater has an effect on biofilm formation rate and growth rate constant, meaning that adding sludge to beer brewery treatment in a microbial fuel cell may affect microbial activity rate.
2. External resistance had a positive effect on COD elimination
3. At low external resistance, sludge addition to beer brewery wastewater reduces electron transfer capability.
4. External resistance was found to be highly proportional to anode colonization during biofilm production, as well as other MFC parameters like current generation, microbial activity, biofilm growth rate, and power produced.
5. Increase in external resistance resulted into decrease in the effluent COD due to absorption of more substrate by the microorganisms.
6. The MnO_2/rGO catalyst had a higher ORR activity than plain cathode MFCs, making it better suited to brewery wastewater treatment than domestic wastewater treatment.

Chapter 9: Recommendations

1. Further research into MnO_2/rGO catalyst tolerance to ionic strength is recommended. This is due the findings that indicated extremes of ionic strength and pH in the catholyte were shown to negatively affect metal catalyst and biocatalyst activity.
2. Further research into the effects of the MnO_2/rGO catalyst on pH, as well as the effects of the MnO_2/rGO catalyst on MFC efficiency and substrate pH.
3. Further research on the effects of temperature conditions on the action of the MnO_2/rGO catalyst against ORR .

Chapter 10: Appendices

Appendix A: Tables

Title: Microbial Fuel Cell Start-Up Performance at Open –Circuit and Closed Circuit: Effect of External Resistance

Table 4.4 Summary of Coulombic efficiencies at varying external resistance

	Coulombic efficiency					
	MFC-1	MFC-2	MFC-3	MFC-4	MFC-5	MFC-6
t(hrs)						
0	0	0	0	0	0	0
25	2.6029E-06	6.81608E-13	3.048E-19	1.1094E-25	5.9301E-33	5.3292E-40
45	4.3223E-06	1.28176E-12	4.27E-19	1.359E-25	2.2739E-32	3.643E-39
72	2.9748E-05	2.3652E-10	1.4508E-16	1.062E-22	7.7923E-29	2.5146E-35
93	1.8526E-05	8.04475E-12	4.3498E-18	2.967E-24	4.7945E-30	2.8539E-36
140	0.00014784	7.15937E-11	2.633E-17	3.07E-23	4.8077E-29	4.863E-35
164	6.6101E-05	2.0722E-11	5.7822E-18	1.0692E-22	5.7288E-28	2.2424E-33

Table 4.5 COD removal efficiency at varying external resistances as a function of time.

COD removal Efficiency						
	MFC-1	MFC-2	MFC-3	MFC-4	MFC-5	MFC-6
t(hrs)						
0	0	0	0	0	0	0
25	73.01	76.83	8.01	2.65	71.34	36.49
45	58.78	78.25	50.00	20.28	72.85	38.42
72	90.88	98.65	80.59	45.78	88.12	69.80
93	77.88	73.38	88.54	66.42	88.38	69.80
140	96.73	85.89	88.79	76.96	92.48	86.42
164	94.45	90.02	96.36	94.21	93.70	99.23

Appendix B: Graphs

Title: Analysis of Microbial Fuel Cell Start-Up: Biofilm formation

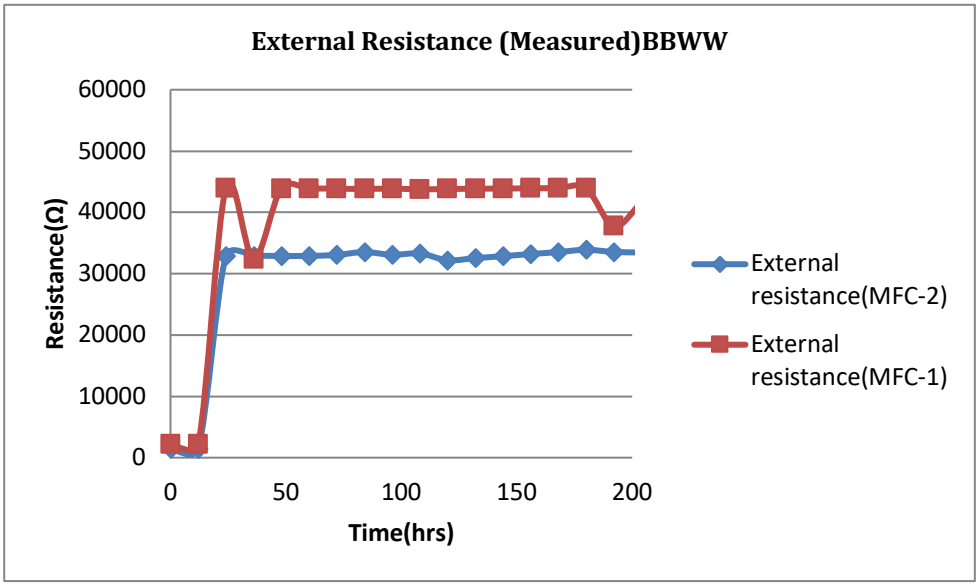


Fig 6.8 Effects of external resistance MFC start-up at external resistance of 33kΩ (MFC-2) and 51kΩ (MFC-1) (Plain cathode MFCs).

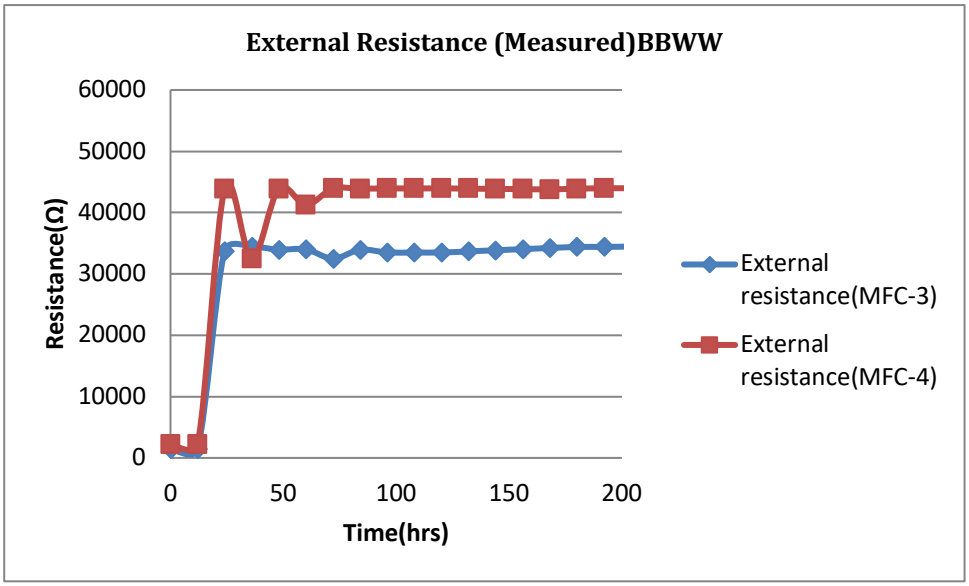


Fig 6.9 Effects of external resistance MFC start-up at external resistance of 33kΩ (MFC-2) and 51kΩ (MFC-1) (MnO2/rGO catalyst coated cathode MFCs).

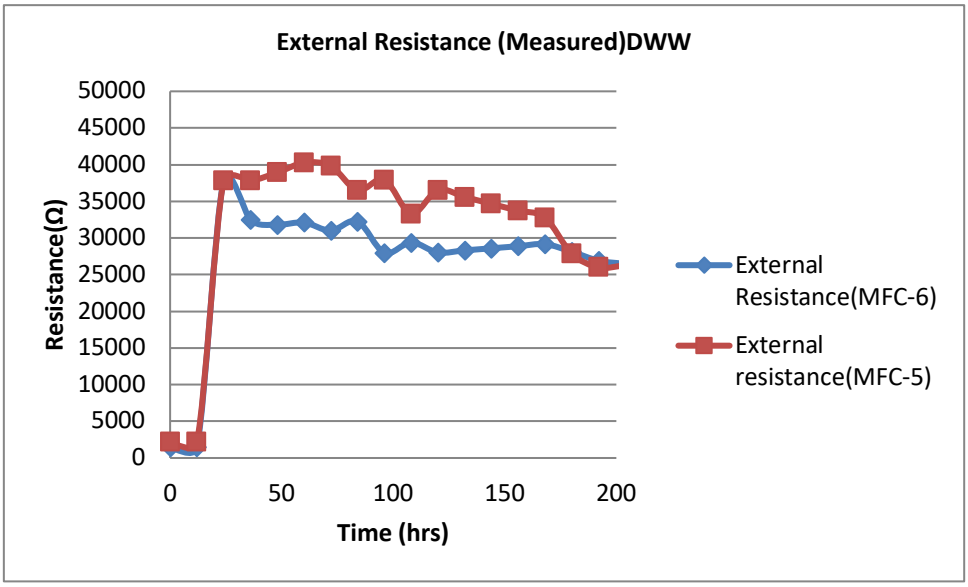


Fig 6.10 Effects of external resistance MFC start-up at external resistance of 33kΩ (MFC-2) and 51kΩ (MFC-1) (Plain cathode MFCs).

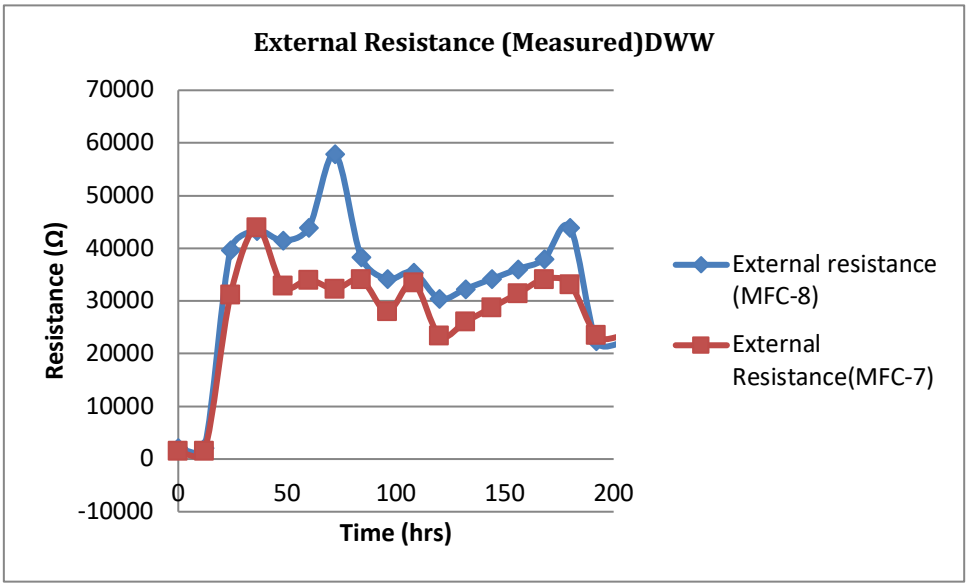


Fig 6.11 Effects of external resistance MFC start-up at external resistance of 33kΩ (MFC-2) and 51kΩ (MFC-1) (MnO2/rGO catalyst coated cathode MFCs).

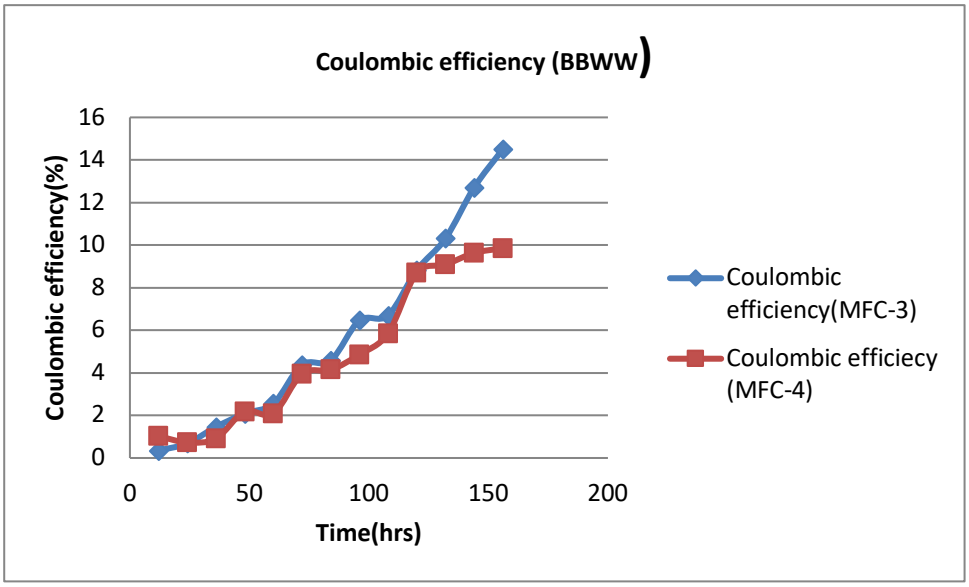


Fig 6.12 Effects of external resistance MFC start-up at external resistance of 33kΩ (MFC-2) and 51kΩ (MFC-1) (Plain cathode MFCs).

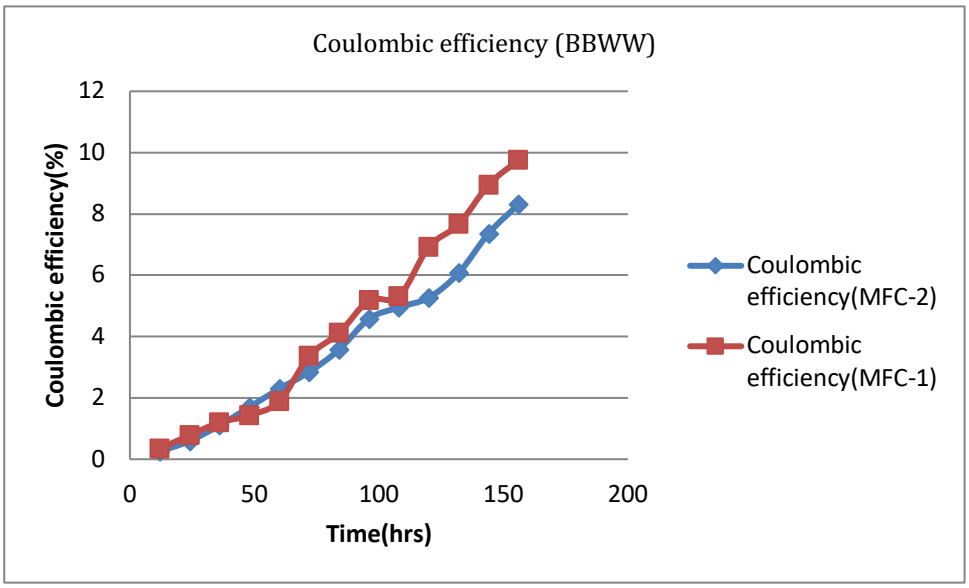


Fig 6.13 Effects of external resistance MFC start-up at external resistance of 33kΩ (MFC-2) and 51kΩ (MFC-1) (MnO2/rGO catalyst coated cathode MFCs).

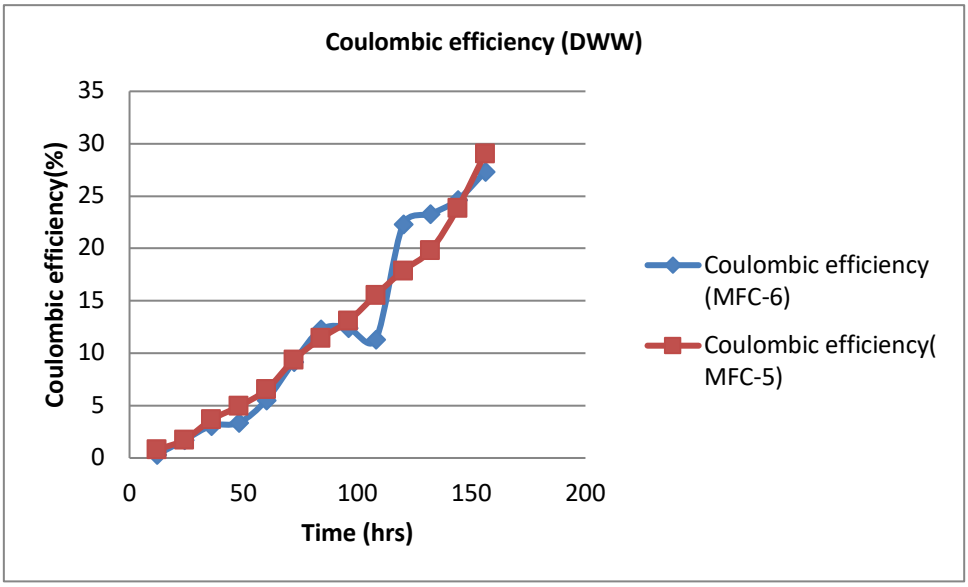


Fig 6.14 Effects of external resistance MFC start-up at external resistance of 33kΩ (MFC-2) and 51kΩ (MFC-1) (Plain cathode MFCs).

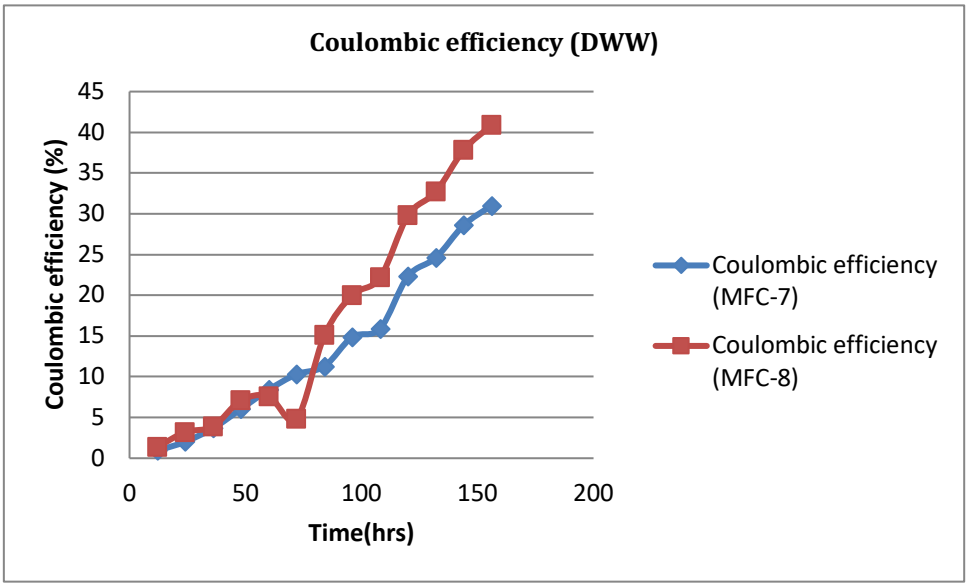


Fig 6.15 Effects of external resistance MFC start-up at external resistance of 33kΩ (MFC-2) and 51kΩ (MFC-1) (MnO2/rGO catalyst coated cathode MFCs).