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Comparative Studies of the Electricity Generation, Desalination and Wastewater Treatment Performance of the Three-Chamber and Newly Developed Five-Chamber Microbial Desalination Cells

BY

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DECLARATION

I hereby declare that, this submission is my own work towards the award of a PhD and that, to the best of my knowledge, it contains no material previously published by another person, nor material which has been accepted for the award of any other degree of the University, except where due acknowledgement has been made in the text.

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ABSTRACT

Poor wastewater treatment and limited potable water supply in the country are problems that require sustainable solutions. Available technologies to solve these problems are expensive especially to a developing country like Ghana. A recently developed technology, Microbial Desalination Cell (MDC) is considered a less expensive alternative to conventional wastewater treatment and desalination technologies. In MDCs, wastewater treatment occurs in the anode chamber and desalination in the desalination chamber. A third chamber, the cathode chamber is present to create a potential difference across the anode chamber and itself for electricity production. One setback of MDC systems however is that, most operate with chemical oxidants and buffers which can make this technology expensive. Thus, this research work sought to build MDCs which operate on alternative non-chemical oxidants sources and also, MDCs which are able to buffer electrolytes without the use of chemical buffers. Another objective of this study was to investigate the effects of rhamnolipid on MDCs electricity generation, desalination and wastewater treatment performances. The interactive effect of rhamnolipid and stirring on a five-chambered MDC was also investigated and ion exchange membranes of selected MDCs examined for the occurrence of fouled layers. A comparison between the electricity generation, desalination and wastewater treatment performances of three-chamber MDCs operating on different electron acceptor sources showed that, the threechamber chemical catholyte (3 C C) MDC performed better than the three-chamber water catholyte (3 W C) MDC and three-chamber plant-supported (3 P C) MDC. The 3 C C MDC produced the highest peak voltage of 282.91 ± 0.09 mV, coulombic efficiency of 78.61% and could desalinate 46.66% of saltwater. Its power $(0.35 \pm 0.10 \text{ W/m}^3)$ and current $(1.24 \pm 0.35 \text{ A/m}^3)$ densities were also the highest amongst the three-chambered MDCs. It produced a 42.81% COD reduction, 1.14% nitrate reduction and a 10.71% phosphorus reduction. On the other hand, the 3 P C MDC produced the least performance. The highest voltage recorded from it was 193.99 ± 0.80 mV. The 3 P C MDC could desalinate 23.34% of saltwater, produce $0.25 \pm 0.06 \text{ W/m}^3$ of power, $1.09 \pm 0.11 \text{ A/m}^3$ of current density with coulombic efficiency (CE) of 10.03%. It achieved a percentage COD reduction of 41.79%, highest percentage nitrate removal of 1.05% and phosphorus reduction of 9.97%. Apparently, the pH stabilization ability of neutralization chambers was lower than that of potassium phosphate buffer they were compared to. For instance, with the neutralization chamber, pH-change in the anolyte of the five-chamber chemical catholyte (5 C C) MDC was 1.66 ± 0.11 whiles pH change in the 3 C C MDC was 1.52 ± 0.15. Nonetheless, the 5 C C MDC produced a comparatively higher voltage of 343.57 ± 0.25 mV, percentage desalination of 50.01%, but a lower CE of 25.20%. Its power and current densities were $0.62\ 2 \pm 0.13$ W/m^3 and $1.61 \pm 0.21 \text{ A/m}^3$ respectively. It was able to reduce COD concentration by 63.42%, nitrate concentration by 2.12% and phosphorus by 3.85%. The supply of rhamnolipids to anolytes of the five-chamber MDCs increased their performances. Voltage production of the 5 C C MDC increased from 343.57 ± 0.25 mV to 630.60 ± 1.44 mV, percentage desalination from 50.01% to 67.63% and COD, from 63.42% to 72.78%. In the case of the five-chamber water catholyte (5 W C) MDC, its voltage increased from 164.5 ± 0.11 to 623.7 ± 1.32 mV,

percentage desalination from 43.74% to 63.21% and percentage COD reduction from 48.74% to 65.31%. Stirring (60 rpm) of water catholyte alone, could increase the percentage desalination of 5 W C MDC from 48.74% to 65.29% and voltage production from 164.5 ± 0.11 to 567.27 ± 18.06 mV. However, the interaction effect of rhamnolipid and stirring on the performance of the 5 W C MDC was insignificant. Analysis of membranes of the 5 W C MDC showed that, the membranes were fouled on both sides. And a comparison between the degree of fouling on cation exchange membranes (CEMs) of the 5 C C MDC and 5 W C MDCs revealed that, the CEM of the 5 C C MDC was more fouled though used for a lesser period of time. Having assessed the core performances and material requirements of MDCs investigated in this study, the 5 W C MDC was found to be the ideal MDC for possible future use in Ghana. Thus, the 5 W C MDC when integrated with conventional wastewater treatment and desalination technologies can aid these technologies achieve higher treatment qualities aside the additional benefit of ‗free' electricity the 5 W C MDC can provide.

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CHAPTER ONE

1.0 Introduction

1.1 Background to the Study

Microbial Desalination Cells (MDCs) fall under the broad category of technology referred to as BioElectrochemical Systems (BES). MDCs are able to treat wastewater, desalinate salt water and produce electricity (Cao et al., 2009). It is a cheaper alternative to conventional water and wastewater treatment technologies which are high energy consumers (Gude, 2012). For instance, in the USA, conventional wastewater and water treatment establishments consume about 4 - 5% of electrical energy generated (Gude, 2012). This can be computed to an approximated 116.07 – 145.08 out of the 2,901.67 USD/MWh costs of electricity production using a 10% discount on ‗levelised' cost of electricity generation (International Energy Agency et al., 2015).

The MDC technology‗s relative inexpensiveness and additional benefit of electricity generation makes it an ideal technology for a developing country like Ghana. MDCs can be integrated with conventional wastewater treatment technologies to support the treatment of the anticipated large volumes of wastewater to be generated in Ghana in the near future. That is, urban wastewater generation is expected to increase from an estimated 530,346 m^3/day to 1,452,383 m^3/day by 2020 (Agodzo et al., 2003). ‗Economic-wise', conventional wastewater treatment technologies will not be ideal for solving this problem because these technologies are high energy consumers (Gude, 2012), thus the need for cheaper alternatives like MDCs.

The integration of microbial desalination cells with wastewater treatment technologies will also help increase the wastewater treatment coverage in Ghana. For instance, The World Bank (2015) reported that, less than 8% of domestic wastewater generated in Ghana is treated (World Bank, 2015). This is an alarming fact which needs a sustainable solution.

MDCs also have the potential to support the production of potable water in the country through desalination. Electricity produced from MDCs can also add to the energy grid of the country thus supporting Ghana's drive to have some contribution of power from renewable energy sources (Renewable Energy Act, 2011).

The potentials and flexibility of designs of MDC technology have brought about several research works on it. Recent ones include: Treatment of two different water resources in desalination and microbial fuel cell processes by poly sulfone/sulfonated poly ether ketone hybrid membrane (Ghasemi et al., 2016); Energy-positive wastewater treatment and desalination in an integrated microbial desalination cell-microbial electrolysis cell (Li et al., 2017) and also research on super capacitive microbial desalination cells by Santoro et al. (2017).

In spite of the advantages of microbial desalination cell technology it still requires some improvements. That is, the chemical oxidant (potassium ferricyanides) and buffers (potassium phosphate buffers) used in it need to be replaced with relatively inexpensive alternatives. Moreover, efficient designs and improved operational conditions are required to improve MDCs electricity productions, desalination and wastewater treatment performances. Continuous research on membrane fouling is also needed for the

development of effective solutions to the membrane-fouling problem associated with this technology.

Thus in this study, the ability of alternative electron acceptor (oxygen) sources to support MDC operations were assessed and compared with the ability of potassium ferricyanide oxidant to do same. Also, a design modification (neutralization chamber) was operated as an alternative to chemical buffers for pH control. In addition, the effects of rhamnolipid and stirring on MDCs electricity generation, desalination and wastewater treatment performances were investigated. Ion exchange membranes were examined for fouled layers.

1.2 Problem Statement

Poor domestic and industrial wastewater treatment coverage has been the major cause of environmental pollution in most communities of the developing world (World Bank, 2015). In these communities, domestic wastewaters comprising both grey- and black sewage are often discharged untreated into surface water bodies *via* open drains. Unfortunately, some peasant vegetable farmers at times use water from these surface water bodies to irrigate their vegetable farms thereby contaminating them and creating conditions of public health concerns. Presently only about 8% of domestic wastewater is subject to some form of treatment in Ghana (World Bank, 2015). Most industries in Ghana also lack the infrastructure to largely treat their wastewater. Microbial desalination cells when integrated with conventional wastewater treatment technologies can help improve upon the poor wastewater treatment situation in Ghana.

Also, power supply in Ghana has been a major setback affecting the economy and the industrialisation drive of the country. Recent demonstrations and grievances expressed concerning the higher domestic and commercial electricity tariffs are testimonies of the crisis caused by the inadequate electricity generation in the country (Mohammed, 2015). This notwithstanding, a well-developed MDC can contribute some power to the 4,310 MW installed electricity energy generation capacity the country has (Energy Commission, 2018). This is necessary to prevent future erratic power supply to the growing population of Ghana.

In spite of the demonstrated capacity of the MDC technology, certain limitations have been identified with it. These include its reliance on mostly expensive chemical oxidants, example potassium ferricyanide and expensive buffers, example phosphate buffers. These problems make the technology unattractive to the developing world. Phosphate buffer saline powders for instance, costs between EUR 14.10 and 359.00 for just 10.0 g depending on specifications and a 50 g of potassium ferricyanide costs about EUR 43.00 (www.sigmaaldrich.com). Besides, chemical oxidants like ferricyanide are environmentally unfriendly due to their toxicities (Kokabien and Gude, 2015). Even where more environmentally friendly applications like the use of microalgae as source of terminal electron acceptors (Kokabien and Gude, 2013; Girme, 2014; Kokabien and Gude, 2015) were applied, the problem of high cost persisted. Also, when electrolyte circulation for pH control (Qu et al., 2012; Davies, Kim and Logan, 2013) was investigated, the problem of high cost could not be taken care of.

For instance, the maintenance of algae in biocathodes through supply of nutrients increases the cost of operations of this type of cathode. A 60 g of algae nutrient medium cost $23.95

(www.amazon.com) which translates to millions of dollars considering large scale applications. Circulation of electrolytes for pH control on the other hand increases cost through energy demand for pumping electrolytes between anode and cathode chambers.

Another problem with this technology is membrane fouling (Ping et al., 2013) and studies conducted to address this problem have been skewed towards MDCs working with chemical (potassium ferricyanide-based) catholytes. Aside the earlier discussed problems, the technology's current productions and desalination performances require some improvements too (Zhang et al., 2012; Ping et al., 2016). Also, how the simultaneous variations in conditions of the anolyte and catholyte affect MDCs wastewater treatment, electricity generation and desalination performances have not been adequately reported in available literature.

1.3 Justification of Study

Poor wastewater handling practices create favourable conditions for sanitation related diseases. However, the MDC technology has the potential to alleviate if not solve this problem through its capacity to improve wastewater quality before discharge. Thus the MDC technology can be an alternative or supporting technology to conventional wastewater treatment technologies given MDCs assembling and operational simplicity and additional benefit of electricity production. A fully operational MDC can thus be setup as a satellite treatment plant for treating domestic and industrial wastewaters where needed.

Also a well-developed MDC will be useful as an auxiliary technology to conventional desalination technologies. Energy produced from MDCs can reduce energy requirements of thermal and reverse osmosis systems thus reducing the cost of operations of these

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technologies. An economically viable MDC will therefore be beneficial to the Accra Desalination Plant which was shut-down in 2018 due to challenges associated with cost recovery (Gadugah, 2018). Electricity produced from MDCs can also add to the energy grid of the country thereby supporting Ghana's drive to have some contribution of power from renewable energy sources (Renewable Energy Act, 2011). Considering the ability of MDCs to provide clean water and some energy, the MDC technology can thus be put among technologies that have the potential to support the attainment of the sustainable development goals six (access to clean water and sanitation for all) and seven (access to affordable and sustainable energy for all).

1.3.1 Need for Research

Thorough studies of available literature have revealed the following knowledge gaps that this research seeks to address:

- The unavailability of information on design modification for pH control
- The unavailability of information on the enhancement of MDC performance through rhamnolipid addition
- The interaction effects of rhamnolipid and stirring on MDC electricity generation, desalination and wastewater treatment performances has been eluded in available literature
- Information on membrane fouling in MDCs operating with non-chemical basedcatholytes has not been found in available literature.

1.4 Objectives of Research

In view of the problem statement, justification and research needs outlined, the following objectives were set for this study.

Main Objective

The main objective of this study was to develop an economical and efficient MDC with reduced incidence of membrane fouling

Specific Objectives

The specific objectives were;

- Design and construct three-chamber and five-chamber MDCs to operate on a chemical catholyte, a plant-supported cathode and a water catholyte.

- Assess the electricity generation, desalination and wastewater treatment potentials of MDCs operating with different terminal electron acceptors.

- Compare and contrast the pH stabilization capacity and performance efficiencies of the five and three-chamber MDCs

- Investigate the effects of the addition of rhamnolipid on the best performing MDCs

- Investigate the interaction effects of rhamnolipid and stirring on the selected MDC's voltage production, desalination and COD reduction performances

- Examine the membranes of the selected MDCs for the occurrence of fouled layers.

CHAPTER TWO

2.0 Literature Review

2.1 Microbial Desalination Cell (MDC) Technology

Microbial Desalination Cells falls under the broad category of technology referred to as BioElectrochemical Systems (BES). MDC was developed by Cao et al. (2009) to

desalinate salt water and produce electricity. It evolved from the concept of Microbial Fuel Cells (MFC). Microbial fuel cells typically consist of two chambers, an anode chamber (wastewater treatment and electron release occur here) and cathode chamber (create potential difference/completes the circuit) (Offei, 2015). Unlike the two chambered MFCs, a typical MDC (Plate 2.1) has a third chamber called the Desalination Chamber which is created between the anode and cathode chambers with anion and cation exchange membranes positioned at specified distance(s).
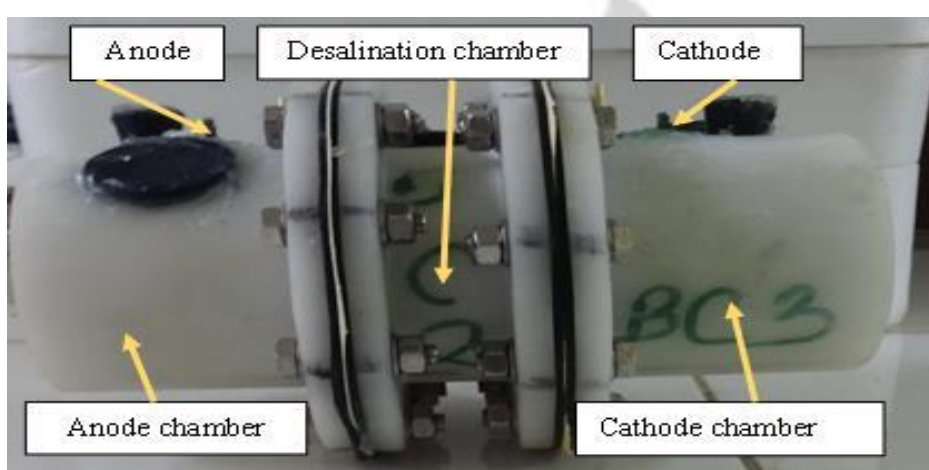
Plate 2.1 Conventional MDC

By principle, MDCs desalination mechanism is dependent on electricity produced by bacteria (exoelectrogens). The exoelectrogens breakdown organic matter in wastewater and transfer produced electrons to the anode to obtain energy. This causes the accumulation of protons in the anode chamber and consequently attracting negative ions

(Cl^-) from the desalination chamber into the anode chamber to balance charge (Cao et al., 2009). The transferred electrons at the anode surface travel through an external wire and across an external resistor to reduce oxidised species (electron acceptors) on the cathode causing the movement of cations (Na^+) from the desalination chamber into the cathode

chamber (Cao et al., 2009). By these processes, wastewater is treated, electricity is produced and salt water is desalinated.

2.2 Energy in Wastewater Recoverable by MDC Technology

Domestic wastewater is described to contain 120 – 380 mg/l BOD of recoverable organic energy (Tchobanoglous et al., 2003). Also, in terms of power, it (domestic wastewater) has been reported to possess potential energy between the range of 1.8 and 2.1 kWH/m^3 (Kim and Logan, 2013; Heidrich, Curtis and Dolfing, 2011; Shizas and Bagley, 2004). However, to guide the development of useful technologies that can utilise the energy resources of wastewater, there is a need for categorisation. Hence energies in wastewater have been put into the following categories:

i) Energy derived from organic matter ~1.79 KWh/m^3 ii) Energy derived from nutrients including nitrogen and phosphorous ~0.7

KWh/m^3 iii)   Energy   derived

from heat ~7 KWh/ m^3

(McCarty, Bae and Kim, 2011; Gude, Kokabien and Gadhamshetty, 2013).

Amongst the above listed categories, organic matter and nutrients are most useful in MDC technology.

It is important to note that, wastewater treatment facilities, which incorporate algae harvesting, add value to their operations. The U.S. Department of Energy National Algal Biofuels Technology Roadmap estimates that, the average gross energy content of algae biomass is 18 MJ/kg (Ferrell and Sarisky-Reed, 2010). Thus the successful cultivation of algae in wastewater treatment facilities can provide some useful energy which can reduce their high costs of operations.

2.3 MDC, an Ideal Technology for Producing Energy from Wastewater and for Desalination

The Microbial desalination cell is one of the technologies tipped for the optimum utilization of wastewater resources and this claim is supported by the following reported research works. According to Luo, Xu, Roane et al. (2012), the usage of domestic wastewater (pH, 6.9±0.1; conductivity, 1.3 ± 0.1) alone as sole substrate in an MDC could produce a power output of 8.01 W/m^3. Also, taking into consideration energy generated in MDC and energy saved when not employing conventional desalination technologies, a total 4 KWH/m^3 of energy output can be retained (Gude et al., 2013).

Moreover, on estimate, Jacobson, Drew and He (2011a) reported that MDCs at a litrescale could generate up to 58% of electricity needed by an attached reverse osmosis (RO) unit when using wastewater as anolyte. This finding is very important for RO systems especially because of their high energy requirements for optimum operations. In another study, Kim and Logan (2013) argued that, the salinity gradient created in MDCs between wastewater and seawater in itself was a valuable recoverable energy source which has so far not been well studied and quantified.

Based on the accounts of the above research works, envisaging microbial desalination cell technology as a possible contributor of power to Ghana's energy mix is not out of place. Besides electric energy that can be derived from MDCs, the MDC technology can help improve Ghana's domestic wastewater treatment coverage of just 8% (World Bank, 2015) and the 87% coverage of safe-water supply (WHO, 2015) through desalination. There are additional benefits with this technology such as microalgae (*Chlorella vulgaris*) cultivation and wastewater reuse.

2.4 MDC Architectures

Since the inception of the MDC technology, it has received a lot of design modifications and applications. The conventional three-chamber MDC type was first produced by Cao et al. (2009) whiles Ping and He (2013) constructed a spatially decoupling anode and cathode MDC to make the technology more easier to assemble. Ping and He (2013) constructed their cell such that a separated anode and cathode units were placed in a 4 L tank which served as a desalination chamber. This configuration could allow for additional anode and cathode units. With a parallel electric circuit connection, increasing the cathode unit by one could increase the MDC's current density from 72.3 to 116.0 A/m^3.

Another MDC design studied was the hydraulic coupled MDC constructed by Qu et al. (2013). The design allowed for the anolyte of the first MDC to flow into its cathode chamber, and subsequently to the anode chamber of the next cell in that order. Also, salt water moved through assembled desalination units in series. In all, four cells were coupled to achieve an improved MDC performance. With this MDC, Qu et al. (2013) were able to improve COD removal from 21% to $60 \pm 2\%$.

Ge, Dosoretz and He (2014) added a new design to the MDC design catalogue. They constructed the 10 cell pair MDC. A one centimetre diameter rubber spacer was used to enlarge the anode chamber of this MDC to acquire an anolyte volume of approximately 55 mL whiles the cathode chamber could contain liquids with volume of ~ 20 mL. Ge at al. (2014) were able to use this 10 cell pair (anion exchange membrane-cation exchange membrane pair) MDC to appreciably remove salt at a desalination rate of 90.8 ± 8.3 mg/h.

One other intriguing MDC design is the stack structure microbial electrolysis desalination and chemical-production cell constructed by Chen et al. (2012). This design didn't only have a desalination capacity but acid and alkali production capacity too. It consisted of four chambers, the anode, acid-production, desalination and the cathode chambers with two types of stack structures built in. The stack structures were; Anion Exchange Membrane-Cation Exchange Membrane stack structure and the Bipolar Membrane-Anion Exchange Membrane-Cation Exchange Membrane stack structure. With this design, a maximum salt removal rate of 33.9 ± 0.02 mg/h could be achieved (Chen et al., 2012).

Another unique MDC design is the tubular design and as the name implies, it is shaped like a tube. Jacobson, Drew and He (2011b) constructed one and named it, the Upflow tubular microbial desalination cells (UMDC). Their UMDC consisted of two chambers separated by ion-exchange membranes. The inner compartment was the anode, walled outwardly by an anion exchange membrane. The cathode consisted of a cation exchange membrane attached to an outer tube whiles the desalination chamber was the space between the anion-exchange and a cation-exchange membrane. With this setup, they produced currents of approximately 62 mA whiles treating salt solution of concentration

30 gTDS/L.

Zhang and He (2012) on the other hand, replaced the usual anion exchange membrane in a conventional MDC with a Forward Osmosis (FO) membrane to produce an Osmotic Microbial Desalination Cell (OsMDC). This configuration resulted in a FO membrane cation exchange membrane combination for desalination. The use of FO membrane provided an addition benefit for water abstraction from anolytes and facilitated electricity production through facilitated proton transfer across membranes (Zhang and He, 2012). With the OsMDC, Zhang and He (2012) proved that, microbial activities were largely

responsible for salt removal. Microbial degradation removed 57.8% salt whiles osmosis contributed only a 3.4% to salt reduction.

The above discussed designs were either constructed for improving electricity generation, salt reductions or resources recovery. However, none was designed for the purpose of pH control. To this, the novel five-chamber microbial desalination cell was constructed and assessed based on its ability to control pH, produce electricity, achieve appreciable desalination and treat wastewater. The five-chamber MDC is a modification of the threechamber MDC. By adding one chamber each to the anode and cathode sides of a threechamber MDC, the five-chamber MDC can control pH changes in its operations. This happens through two mechanisms; dilution of electrolytes (anolytes and catholytes) and migration of H^+ and OH^- into anolytes and catholytes respectively due to concentration differences.

2.5 Desalination Performances of MDCs

Desalinated seawater is an alternative source of potable water to our world (Shannon et al., 2008). A number of technologies are available for this purpose. MDC is an emerging technology in this regards and Cao et al. (2009) demonstrated for the first time the ability of MDCs to desalinate saltwater. They achieved a remarkable result of 93 ± 3% salt removal from 35 g/L salt solution. The very promising results obtained by Cao et al. (2009) caused the evolution of a vast number of researches on MDC technology primarily in efforts to improve percentage desalination to 100%. In this regard, Girme (2014) was able to achieve a 100% desalination using a ―photo MDC‖. Luo, Jenkins and Ren (2011) on the other hand achieved a relatively lower percentage desalination of 98.8% from a lower salt concentration of 10 g/L NaCl using a microbial electrolysis and desalination cell

(MEDC).

Various methods have been reported for improving the desalination efficiencies of MDCs. Amongst them include the use of membranes with improved ion exchange capacities and the use of ion exchange resins. For example, Mehanna et al. (2010) reported that they could increase the desalination efficiency of an MDC they studied from 50% to 63% by increasing the ion exchange capacities of the membranes used. Shehab et al. (2014) on the other hand used ion exchange resins in a Stacked Microbial Desalination Cell (SMDC) to improve the SMDC's desalination efficiency from 43% to 72% in reduced time of 80 hours (previous HRT of 110 hours)

Morel et al. (2012) also demonstrated the ability of ion exchange resins to increase the desalination efficiency of a MDC. The ion exchange resin supported MDC they investigated could produce a 58% conductivity reduction at a saltwater flow rate of 0.063 mL/min whiles a classical (three-chamber) MDC they compared it to recorded only a 45% conductivity reduction. With the application of multiple ions and ion-exchange resins, Zuo et al. (2013) achieved desalination efficiency of 99% from a solution with an initial electrical conductivity of 4.72 ms/cm (representing salt concentration) in their study of microbial desalination cells. An analysis of the reports above shows that, ion exchange resins have the capacity to increase percentage desalination but the use of them could increase the cost of constructing and maintaining MDCs.

A major factor found to affect the desalination efficiencies of MDCs is hydraulic retention time (HRT). To prove this, Morel et al. (2012) studied the effect of HRT on desalination and concluded that, a short HRT does not allow for effective desalination because microbial

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activities in the anode which drives desalination peaks gradually whiles longer HRT might lead to back diffusion of ions due to concentration gradient between the desalination chamber and adjacent chambers. An analysis of published literature on MDCs has shown that in most studies, HRT for desalination has been between a day and several months. Jacobson et al. (2011b), for example, worked with a four-day HRT and achieved an above 99% salt removal from a 30 g (TDS)/L salt solution using an Upflow MDC whiles Ping et al. (2016), achieved percentage desalinations of between 25.4 and 79.2%, and implicated hydraulic retention time as possible cause of the differences in performances (longer HRT improved desalination). Under any operational condition, a balance is required between short and long HRT for optimum desalination performance to be achieved (Morel et al., 2012).

Other investigators have focused on improving desalination performances of MDCs by modifying electrodes. These include the work of Forrestal, Xu and Ren (2012) who used a capacitive adsorption MDC in a batch cycle to achieve on average, a 69.4% salt removal from the desalination chamber through electrode adsorption. That is, the salt ions got adsorbed on electrode surfaces. This mode of operation prevents the accumulation of separated salt ions in receiving electrolytes.

Unlike most MDCs which rely on expensive chemical electron acceptors like potassium ferricyanide, Kokabien and Gude (2013) developed algae biocathodes with oxygen as alternative to chemical oxidant, potassium ferricyanide and achieved a desalination efficiency of 40%. This desalination efficiency was improved to 64.21% ± 0.5 for 500 mg/L COD anolyte solution and to 63.47% ± 0.1 for 1000 mg/L COD anolyte solution in a later study by same authors (Kokabien and Gude, 2015). Adding to research on biocathodes, Girme (2014) achieved a 100% salt removal from an initial salt concentration of 35 g/l NaCl

using the microalgae, *Nanochloropsis Salina* as source of terminal electron acceptor (oxygen). Such a high desalination performance and the comparative environmental friendliness of biacothodes support the call for the use of biocathodes in place of cathodes which depend on chemical oxidants.

Judging between desalination performances of MDCs is a problem due to the absence of standardized designs and operational conditions. Notwithstanding this, over 50% of research works on MDCs reviewed have reported percentage desalination of at least 50%. Table 2.1 presents information on percentage desalination recorded in some studies.

Table 2.1 Selected reports on cell types, salt concentrations and percentage salt removed from MDCs

Cell Type	Initial Salt Concentration	Percentage Salt Removed (%)	Citation
MDC	35 g/l	93 ± 30	Cao et al., 2009
MEDC	10 g/l	98.80	Luo et al., 2011
UMDC	30 g/l	99.00	Jacobson et al., 2011b
R-MDC	4.72 ms/cm	99.00	Zuo et al., 2013
MDC	35 g/l	100.00	Girme, 2014
FO-MDC	35 g/l	94.00	Yuan, Abu-Reesh and He, 2016
MDC-MEC	5 g/l	63.70	Li et al., 2017

MDC- Microbial Desalination Cell, MEDC- Microbial Electrolysis and Desalination Cell, UMDC- Upflow Microbial Desalination Cell, FO-MDC– Forward Osmosis Microbial Desalination Cell, R-MDC- Resins packed Microbial Desalination Cells

2.6 COD Reductions in Wastewater using MDCs

One objective of the MDC technology is wastewater treatment especially organic load reduction. Since organic matter reduction cannot be measured directly, COD reduction is an acceptable standard for its estimation. To this, several MDC types and operating conditions have been investigated in effort to reduce COD concentrations in wastewater. These include the Upflow MDC (UMDC) studied by Jacobson et al. (2011b). Jacobson et al. (2011b) successfully used the UMDC to remove a 92.0 ± 0.4% COD from anolytes receiving COD loading rate of 6.78 ± 0.36 g COD L^{-1} d^{-1}. Zhang and Angelidaki (2013) also used a submerged desalination-denitrification cell to cause an 87.7% COD reduction from an initial COD concentration of 800 mg-COD/L.

A microbial capacitive desalination cell (MCDC) built by Forrestal et al. (2014) could cause a COD reduction rate of 170 mg COD per liter per hour from an initial COD concentration of 800 mg L^{-1}. What was profound in the above study was the source of wastewater. That is, a natural gas produce-water and even with this type of wastewater an 85% COD was removed in just about 4 hours in the desalination chamber. In another study, Zhang and He (2015) used scaled up MDC to cause a high COD reduction of more than 96% from an initial COD concentration of 3000 mg L^{-1} COD. However, in Zhang and He (2015) study, COD removal was possibly facilitated by the post-aerobic run set-up in their experiments.

Some studies have shown that, COD reduction in MDCs is not to be influenced much by hydraulic retention time (HRT). Luo, Xu, Roane et al. (2012) and Luo, Xu and Ren (2012) studies provided some evidence to this claim. In Luo, Xu, Roane et al. (2012) study, an appreciable 52% COD removal from wastewater of initial COD concentration of 2744 ± 16 mg/L was achieved in about 8 days but in the latter study Luo et al. (2012) percentage COD removal increased by just 3% (from 52 to 55%) after 8 months of operation. Also, a later

study by Qu et al. (2013) supported this analogy. Qu et al. (2013) working on multiple MDC setups, recorded a very small difference (1%) in COD reductions between two different HRTs investigated. A 48 hours HRT produced a $60 \pm 2\%$ COD reduction whiles a 24 hours HRT produced a $59 \pm 2\%$ COD reduction. A difference of just 1%, thus other factors such initial COD concentration might be a more influencing factor on COD reductions as compared to HRT.

Investigations on the effect of initial COD concentration on percentage COD reduction showed a positive correlation between the two. In furtherance of this, Mehanna et al. (2010) reported that, at acetate concentration of 2 g L^{-1} a $82 \pm 6\%$ COD removal was achievable but when COD concentration was reduced to 1 g L^{-1} the percentage COD removal also reduced to $77 \pm 3\%$. This was not an isolated finding as Kokabien and Gude (2015) working on synthetic wastewater also demonstrated that, percentage COD removal correlates with initial COD strength of wastewater. With a synthetic wastewater of 500 mg/l COD concentration, Kokabien and Gude (2015) could achieve a $76.06 \pm 1.21\%$ COD reduction in a photosynthetic MDC whiles a 1000 mg/L COD concentration resulted in an $82.17 \pm 1.27\%$ COD removal.

Also, it was found that, complimenting MDCs with Forward Osmosis (FO) units improved MDCs COD reduction efficiencies. For example, using a coupled MDC – FO system Yuan, Abu-Reesh and He (2015) achieved an approximate total COD removal of 80% from wastewater with initial COD concentrations of 750 and 1000 mg L^{-1} COD. COD reductions in this study were attributed to the actions of both attached FO units and microbial degradation. A similar conclusion was drawn in their later study where a COD removal of 70.6% was achieved (Yuan et al., 2016).

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One other possible way to increase the rate and percentage COD reduction in MDCs is the use of rhamnolipid biosurfactants. Rhamnolipids are able to lower surface tensions thereby increasing the bioavailability of substrates to microbes for degradation (Whang et al., 2009). However, a search through available literature showed that, no work has been done in respect of the application of biosurfactants to improve COD reductions in MDCs. Biosurfactant addition from the understanding of its surfactant properties would increase COD reduction as it catalysis the breakdown of organic matter by microbes. Thus in this research, the effect of rhamnolipid on COD reductions was investigated.

2.7 Currents Generated in MDCs

An overview of published literature revealed that, differences in MDC architectures alongside differences in how currents are reported and differences in resistors used have made it difficult to compare current outputs from MDCs. However, MDCs have generally produced little current, which can be linked to a number of factors including low metabolic rates of microbes and potential losses at electrodes. Thus in MDC operations, currents as low as 3 mA have been recorded. For instance, in the work of Cao et al.

(2009), a maximum current of 3 mA at 200 Ω external resistor was produced. Whiles Zhu et al. (2013) working on acid and alkali production with an MDC generated a 3.5 mA of current.

However, Brastad and He (2013) in their work on water softening used an MDC with 1 Ω resistance to achieve a much appreciable current of 13.16 mA. Contrary to their work,

Girme (2014) achieved a very low current of 0.025 mA using a high external resistor of 2500 Ω. Zhang et al. (2012) also reported of low currents of 0.7 and 0.15 mA with an external resistor of 75 Ω. They reiterated that, even with the use of ion exchange resins they

could only increase currents to 0.65 and 1.4 mA respectively from the previous values. Ge et al (2014) did not achieve much current production either in their investigation of the effects of cell pair on the performance of MDCs. Low currents of 2.5 mA and below were reported. The applied external resistor in their work was just 10 Ω. Further investigations by Ge et al. (2014) however proved that, the supply of external voltage could increase electricity production by ~3 folds. Thus an external voltage of 0.8

V was connected to the MDC understudy and current production went above 8 A/m^2 (Ge et al., 2014).

Related studies that have reported current in densities have not produced much current either. This include the study by of Luo et al. (2012) who investigated the long-term performance and characterization of microbial desalination cells for the treatment of domestic wastewater. They achieved current densities of 190, 188 and 185 mA/m^2 respectively for three cycles of MDC operations. Also, Kokabian and Gude (2013) using an algal biocathode (100 ml) achieved a current density in mA/m^3 of value 2750 which when converted to the unit of measure, A/m^3 is small.

In spite of the several low current productions recorded from MDCs, some research works have shown that, much appreciable currents can be produced from MDCs. Amongst these are the 52.9 ± 2.1 mA, 35.9 ± 4.2 mA and 27.4 ± 1.3 mA currents produced from a hydraulically coupled forward osmosis microbial fuel cell – microbial desalination cell (Zhang and He, 2013). However, these currents were achieved under high current conditions (Connected external resistor was 0.1Ω). Comparatively higher currents have also been produced from brackish water. Ping et al. (2015) recorded a high current density of 12.3 ± 2.4 A/m^3 working on a brackish water of concentration, 5.9 g/l TDS. In Ping et al. (2015)

study, it was possible that, the transfer of ions along water flux between chambers was responsible for the high current generation aside electricity production by exoelectrogens. According to Zhang and He (2012), current production can be increased when water osmosis moves protons across membranes.

Demonstrating the flexibility of the MDC technology, Zhang and Angelidaki (2013) constructed the submersible MDC for underground water treatment (nitrate removal) but obtained a low current density of 3.4 A/m^2. In a later study, Zhang and Angelidaki, (2015) used a similar design to recover NH_3 from anaerobic reactors but this time round achieved a comparatively higher current density of 7.6 ± 0.3 A/m^2.

Based on a superficial analogy of previous research works, MDC designs could be argued to play a role in how much current is produced. For instance, Ping and He (2013) used a spatially decoupling anode and cathode design to obtain on the average a high current density of 68.3 ± 2.5 A/m^3 whiles Luo et al. (2011) obtained a maximum current density of 87.2 A/m^3 using microbial electrolysis and desalination cells. Ping et al. (2013) recorded a higher current density of 99 A/m^3 using multiple MDCs. However, as stated earlier, this ‗design type – current production' relationship only holds on a face value because, even when easily hydrolysable substrates are supplied in anolytes, unfavourable pH conditions and mass transport losses can lower current productions irrespective of the type of MDC used.

In the comprehensive study of Li et al. (2017), an effort was made to increase current production through catholyte modification. To this, ammonium (50 mg/l) was added in the cathode chamber and this increased voltage production from 0.32 to 0.37 V. This

observation was associated with the increase in electric conductivity of wastewater from 1.81 mS/cm to 2.50 mS/cm because of the added ammonium. However, current and power densities produced were low. That is; 2311.2 mA/m^2 and 293.7 mW/m^2 respectively. Such low current and power densities can be improved by lowering the internal resistance of MDCs concerned (Logan et al., 2006).

2.8 Varied Uses of Microbial Desalination Cell Technology

The MDC technology is gradually gaining importance among many water treatment and energy production technologies because of its flexibility. In this regard, it has been tried on diverse hypotheses including heavy metal removal, water softening, hydrogen gas production, acid and alkali production and algae cultivation.

According to Brastad and He (2013) MDCs can remove heavy metals such as arsenic, nickel, mercury and copper from a contaminated water. They reported an 89% arsenic (As) removal, 97% copper (Cu), 95% nickel (Ni), and 99% mercury (Hg) removal from initial concentrations of 13, 391, 357 and 11 mg/l of As, Cu, Ni and Hg respectively. The observed trend of metal removal was attributed to the relative affinities of the metals for ion exchange resin used. They also demonstrated the possibility of using MDC to soften hard water. In this, seven hard water sources in the USA were softened to percentages of between 84 and 95% CaCO$_3$ from the initial concentrations of between 220 and 2080 mg/L CaCO$_3$.

Another application of MDC technology is hydrogen gas production. Luo et al. (2011) used a Microbial Electrolysis and Desalination Cells to concurrently desalinate saltwater and produce hydrogen gas. Their work achieved a 48.7 mL H$_2$ gas production and 98.8% salt removal within 96 hours working period. What was found to be disadvantageous of using

MDC for hydrogen gas production was the requirement of the supply of external voltage which can increase energy requirements and therefore cost of operation. This requirement can make it prohibitively expensive for commercial operations.

MDCs have also been used for the production of acids and alkalis. Acid and alkali of quantities, 1.35 ± 0.13 mmol and 0.59 ± 0.14 mmol respectively were produced with a MDC in the work of Zhu et al. (2013). The acid production efficiency was $58 \pm 3\%$, while the alkali production efficiency was $25 \pm 3\%$. Also, Chen et al. (2012) achieved maximum acid and alkali production rates of 0.079 ± 0.006 and 0.13 ± 0.02 mmol/h, respectively using two-desalination-chamber microbial electrolysis desalination and chemicalproduction cell stack structure. Considering the quantities of acids and alkalis productions reported so far, more research is required to increase the production rates of these valuable

chemicals.

The ability of MDCs to remove nutrients from wastewater has also been investigated. Zhang and Angelidaki (2013) using submerged microbial desalination-denitrification cell removed 90.5% of NO_3^- from simulated groundwater whiles generating a 3.4 A/m^2 of current density in a 12 hour experiment. This work was a proof of concept that, nitrate- contaminated ground water could be treated using MDC technology. Zhang and Angelidaki (2015) in a later study also proved that a submersible MDC could be used to recover ammonia from anaerobic reactors. They achieved an 88% total ammonia

extraction with this MDC. The detection of nitrates in a ground water source is a threat to its dependents especially because of the association of nitrate with blue baby syndrome. Therefore, the development of a detachable-in-situ treatment technology as described above is welcoming as it can be used to make contaminated water safe for use.

2.9 Chemical, Air and Bio Cathodes

One of the most popularly used chemicals in cathodes of MDCs is potassium ferricyanide. It has a good electron acceptability and also offers high cathodic potential and faster reduction kinetics (Gude et al., 2013). However, it is not recommendable for large scale applications due to issues of chemical toxicity and cost. Oxygen is an alternative which is readily available in air (Gude et al., 2013). It is the foundation for air cathodes but suffer from slower oxidation-reduction kinetics under uncontrolled conditions consequently requiring expensive catalyst to work with (Saeed, et al., 2015).

When cathodes are built with living organisms capable of catalysing reduction reactions, they are referred to as biocathodes (Croese et al., 2011). Kokabian and Gude (2015) for example, investigated the important role photosynthetic activity of microalgae play in Photosynthetic Microbial Desalination Cells (PMDC) and concluded that the cathode and photosynthetic reactions are limiting factors in the operations of PMDCs. A maximum power density of 1.1 W/m^3, Net Cathode Compartment (NCC) and 0.77 W/m^3, Net Anode Compartment (NAC)) were realised from a COD concentration of 500 mg/L (Kokabien and Gude, 2015).

In reported works on biocathodes, microorganisms and not chemicals produce/serve as source of electron acceptors. This makes them an economic and environmentally friendly option. Microbial catalysed cathodes produce a stable voltage for longer times unlike the potential reduction that occurs when chemical oxidants like potassium ferricyanide is used as terminal electron acceptors (Wen et al., 2012; Kokabien and Gude, 2013). However, lack

of oxygen production during dark periods causes voltage drops in PMDCs due to the ceasing of photosynthetic activity in biocathodes. Notwithstanding this, continuous supply of light should not be advised because this will alter the natural growth pattern of algae as it alters oxygen production and use by algae (Kokabien and Gude, 2015). Poor algae growth will affect oxygen supply and algae biomass. Where algae harvesting is an integrated goal in MDC application, reduction in biomass will be a detrimental result.

Biocathode developments have largely been concentrated on bacteria and microalgae to the exclusion of plants. However, water plants could be used for this purpose too. Thus in this study, the potential of water plants for biocathode construction was investigated.

2.10 Operational Challenges in MDC Technology

Just as with most electrochemical technologies, MDC technology is faced with a number of challenges. These include concentration losses, increasing internal resistance, ohmic losses, and challenges with MDC architecture, electron acceptors, electrical conductivity changes and pH fluctuations.

2.10.1 Concentration Losses

Concentration losses directly affect current generation which in turn affects desalination. Concentration losses usually occur as mass transfer losses enhanced by shortage of nutrients/substrates in the anode chamber (Bower, 2013; Girme, 2014). It is high when the difference between oxidized species at the anode surface and that of un-oxidised species in solution is large. It depicts a state of high current density (Logan et al., 2006). In the cathode chamber, concentration losses come about as a result of large differences between reduced species at the cathode surface and oxidized species in solution (Logan et al.,

2006).

2.10.2 Internal Resistance

Internal resistance can be described as the resistance posed by an MDC (its components) to the flow of current excluding the connected external resistance. Internal resistance goes beyond just ohmic resistance though the two are used interchangeably (Logan et al., 2006). That is internal resistance unlike ohmic losses includes electrode over potentials. Even the substrate supplied to microbes can increase internal resistance. For example, Mehanna et al. (2010), studying the effect of substrate concentration on MDC performances in an air cathode MDC revealed that, 1 g L^{-1} acetate generated a lower internal resistance of $449 \pm 0.7 \ \Omega$ as compared to $809 \pm 0.6 \ \Omega$ recorded when 2 g L^{-1} acetate was used. Internal resistance can be reduced by the use of ion exchange resins. Zhang et al. (2012) successfully used ion exchange resins to reduce internal resistance of their setup from 7383 ± 69 to $1590 \pm 58 \ \Omega$ and from 641 ± 1 to $277 \pm 6 \ \Omega$ in a 50 mg/L and 700 mg/L anolyte COD concentrations respectively.

2.10.3 Ohmic Losses

Ohmic losses result from internal resistance of electrodes, membranes and electrolytes (Bower, 2013 in Girme, 2014). In other words, it is the resistance to the flow of ions and electrons (Logan et al., 2006). Kokabien and Gude (2015) reported that ohmic losses are dominant in over voltages and thus affects system performances. The effects of ohmic losses can be reduced by optimization of MDC designs. That is creating shorter electrode distances, using membranes with low resistance, ensuring proper electrode connection and improving the conductivity of anolytes (Logan et al., 2006).

Unlike internal resistance, a lot has been reported of ohmic losses (resistance). These include the first work done by Cao et al. in 2009. They reported an observed increase in ohmic resistance from 25 to 970 Ω after an $88 \pm 2\%$ desalination was achieved from a 5 g/L salt solution. Also, Chen et al. (2011) associated decreases in current density and desalination to an increase in ohmic resistance from 21 to 312 Ω during the desalination of 20 g/L NaCl in a stacked MDC.

Further, Luo et al. (2012) attributed the decline in power and desalination rate in their study to the rise in ohmic resistance especially the resistance of anode plus anion exchange membrane. Ohmic resistance can be reduced with ion exchange resins. For example, Morel et al. (2012) used ion exchange resins to reduce ohmic loses. The use of ionexchange resin stabilized ohmic resistance in the range of 3.0 to 4.7 Ω, irrespective of modifications in influent salt concentration and hydraulic retention time (HRT).

2.10.4 Challenges with MDC Architecture

The choice of MDC architecture affects its performance and this include the design, materials used in constructing the cell, membranes used, electrodes and electrode distances and in some cases HRT of operations. For instance, Girme (2014) found that, internal resistance of the MDC used in her work could rise to an estimated 2500 Ω and she attributed this observation to the architecture of the MDC. Considering the effect of electrode distance on internal resistance, Logan et al. (2006) reported that shorter distances between electrodes lowered the internal resistance of MFCs. The same phenomenon holds true for MDCs since they run on similar basic principles as MFCs.

Smaller surface area of anodes can increase activation losses in MDCs thus Girme (2014) recommended the use of anodes with larger surface areas to lower these losses and as well, increase fuel cell performance (Girme, 2014). Also, for optimal MDC performance the choice of HRT must be decided carefully. In this regard, Ping et al. (2015) stated that, for the achievement of more than 90% desalination in brackish water, a HRT of 7 days should be used.

2.10.5 Influence of Alternate Electron Acceptors

Alternate electron acceptors, such as NO_3^- and SO_4^{2-} in the anode have been implicated in the lowering of energy outputs in MDCs (Luo et al., 2012). That is, they short circuit reactions by accepting generated electrons in the anode chamber. Fortunately, this challenge is limited to the choice of anolyte conditions. Starting and maintaining anaerobic conditions in a MDC avoids the negative influence of NO_3^- and SO_4^{2-} on electricity productions. Also, careful studies have observed that complex substrates compete for electrons in the anode chamber thereby lowering columbic efficiencies and electricity productions (Ping et al., 2015). Using optimal concentrations of substrate should curb this

problem.

2.10.6 pH Challenges in MDCs
pH fluctuations in anodes and cathodes of MDCs is a serious limiting factor hindering the optimization and scale up of this technology. Typically, the anolyte pH drops to acidic conditions whiles the catholyte's pH increases to alkaline conditions. The slower movement of protons in relation to electrons is the cause of pH drops in MDC anode chambers (Luo et al., 2012) whiles pH increment in cathodic solutions is caused by consumption of protons and oxygen reduction (Qu et al., 2012). pH rises in cathodes cause especially potential losses (Zhang et al., 2010).

A number of researchers have associated the low performance of MDCs to pH changes. Amongst them is Luo et al. (2011). They reported a decrease in system performance and largely attributed it to drop in anolyte pH from 7.0 to between 5 and 6. For optimum MDC performances pH of electrolytes should be maintained at neutral or near neutral pH conditions.

2.10.6.1 Control of pH in MDCs

A number of investigators have applied different techniques to control or mediate the effects of pH on MDC performances and most of these applications have achieved appreciable results. Popular among these strategies are the use of anolyte replacement cycles, the use of potassium phosphate buffers, sodium hydrogen carbonate buffers and electrolyte recirculation.

Morel et al. (2012) for example used anolyte replacement cycles to control anode pH and recorded a slight decrease in pH from 6.43 ± 0.01 to 5.78 ± 0.09 over a batch cycle whiles Luo, Xu, Roane et al. (2012) used 50 mM potassium phosphate buffer solution with a pH of 7.0 to control catholyte pH. Also, Kokabien and Gude (2013) using the strong buffer, potassium phosphate were able to control anolyte pH between 6.4 and 6.8 and catholyte pH around pH 9.7. In their later work on sustainable photosynthetic biocathode, they reported of slight pH changes between 6.5 – 5.7 and linked it to the high buffer concentration in the anolyte (KH_2PO_4, 4.4 g/L and K_2HPO_4, 3.4 g/L). In Ping et al. (2016) work on bioelectricity inhibition of back diffusion in MDCs, they investigated the efficiency of $NaHCO_3$ in buffering electrolytes and realised that, it could appreciably stabilise pH.

Even though a number of researchers have used strong phosphate or carbon based buffers (50–200 mM) to maintain near neutral pH conditions, such chemical applications are not suitable given economic and environmental concerns (Nam et al., 2010). Consequently, approaches like electolyte recirculation are welcoming. This involves the circulation of solutions between the anode and cathode, which Qu et al. (2012) showed to effectively eliminate large pH imbalances. In Qu et al. (2012) study recirculation of electrolytes contributed to an improved COD removal from $38 \pm 5\%$ to $74 \pm 2\%$ and desalination efficiency from $39 \pm 1\%$ to $55 \pm 2\%$. They concluded that pH imbalances were responsible for the initial low performance of the MDC studied and not substrate limitation because control of pH improved performance.

With the several interventions applied to control pH, none has touched on design modification for this purpose. Consequently, in this study a design modification (neutralisation chambers units) was made to a conventional three-chamber MDC and the ability of the added on units to control pH investigated.

2.10.7 Scaling and Fouling

Scaling is caused mainly by the precipitation of ions especially cations. Cations of most interest include calcium and magnesium which are known to precipitate on Cation Exchange Membranes (CEM) (Zuo et al., 2013). One obvious effect of scaling is that, it limits ion transport and consequently increases ohmic resistance. There are very few studies on scaling of MDC membranes. This could be due to it being a secondary challenge which results when primary challenges like pH fluctuations haven't been resolved.

Popular among scaling studies is that of Zuo et al. (2013). In Zuo et al. (2013) study scaling was implicated in the reduction of MDC performances. They asserted that, the scaling observed on membranes of the MDC studied resulted from the presence of Ca^{2+} and Mg^{2+} in the desalination chamber and that, this scaling was facilitated by prolonged MDC operation. In another study, Luo et al. (2012) found that the cation exchange membrane from a disassembled MDC was covered with crystals on the side facing the cathode whiles the anion exchange membrane had crystals on the side facing the desalination chamber. The side of the anion exchange membrane in contact with the anode chamber was found to be colonized by a biofouling layer. Their investigation also showed that, the crystal-like structures were mostly constituted of calcium, magnesium and phosphate ions especially on the cation exchange membrane (CEM). They attributed the fouling of the CEM to pH rise in the cathode chamber.

A fouled membrane/membrane structure can allow the passage of macro molecules. Such a case was reported in the comprehensive work of Ping et al. (2013) who detected acetate in the desalination chamber and attributed it to long term operation of MDC. According to the authors, this possibly resulted from the fouling of anion exchange membranes (AEM). The detection of bacteria in the middle chamber in their study also confirmed the fouling or structural failure of the AEM used.

In another fouling study, Ping et al. (2016) found that a higher salinity gradient and shorter HRT (1.0 d) resulted in the most back diffusion of 7.1 ± 1.2% and 6.8 ± 3.1% phosphate and sulfate ions respectively from the anode. They indicated that, back diffusion of anions from the anode chamber to the desalination chamber against electricity generation should be expected to occur in MDC technologies. This claim is supported by the fact that,

industrialized AEMs do not usually have an ideal 100% perm-selectivity (Strathmann, 2004; Ping et al., 2016) thus even cations can pass through them.

Unlike inorganic fouling which is characterised mostly by scaling, biofouling is associated with the development of biofilm layers on membranes. Biofouling according to Luo et al. (2012) can increase the internal resistance of a MDC. They indicated that, biofouling on AEM caused by complex composition of anolyte and lengthy HRT of MDC was responsible for the increased internal resistance of the MDC they studied. This increase in internal resistance reported, could be linked to the increase in thickness of the AEM due to the formed biofilms on it.

Kokabien and Gude (2015) reported of fouled layers on membranes in their work too. In their study, a used anion exchange membrane had been bio-fouled and scaled and the scaling attributed to the deposition of inorganic compounds. Used cation exchange membranes also displayed evidence of inorganic fouling and biofouling caused by algal cells and rod shaped bacteria. Fouling generally compromises the performance of MDCs both in terms of current generation and desalination (Luo et al., 2012; Kokabien and Gude, 2015).

Ping et al. (2013) found a link between inorganic scaling and bacteria actions and stated that, scaling resulting from calcium carbonate depositions was facilitated by the presence of bacteria in the middle chamber. Therefore, it can be concluded that, fouling from cracks on AEM could cause the scaling of itself and other membranes because the cracks will allow bacteria passage which would then cause biofouling and as well, facilitate inorganic scaling on other membranes.

More studies on membrane fouling are needed for the development of sustainable solutions to this problem. However, a literature survey revealed that little work has been done especially on membranes of MDCs working on oxidants other than potassium ferricyanide. Thus in this study, used membranes of a MDC which operated on a water catholyte (oxygen as oxidant) were examined for fouled layers.

2.11 Microbes Involved in Electricity Generation

The most implicated microorganisms in electricity generation in bioelectochemical systems (MFCs and MDCs) are bacteria. These bacteria are generally referred to as exoelectrogens (Logan, 2008). Exoelectrogens because they are able to directly transfer electrons outside their cells to a compound or material and by so doing, respire. A vast number of bacteria have this ability but the most studied species are *Shewanella* and

Geobacter spp. The transfer of electrons outside the cell is by two main mechanisms. These are electron transport facilitated by mediators like pycocyanin produced by some bacteria including *Pseudomonas aeruginosa* (Rabaey et al., 2005; Logan, 2008) and transfer via nanowires associated with *Geobacter* and *Shewanella* species (Logan, 2008).

For most studies, a consortium of bacteria has been implicated in current generation. These consortia exist as biofilms which ensure their resilience to system disturbances. As a biofilm, they are able to metabolise complex organic compounds. The metabolic byproduct or intermediate products of one group of bacteria serve as source of carbon or energy to anothers. Exoelectogens that have been isolated from anodic biofilms include *Enterobacter cancerogenus*, *Thermanaerovibrio acidaminovorans* and *Desulfuromonas acetexigens* (Offei, 2015).

It is worth noting that, the successful proliferation of biofilms is necessary for the optimal performance of a MDC setup and to ensure this, suitable anode chamber conditions need be provided and maintained. These include suitable surfaces for attachment, optimum temperature and pH conditions (Saeed et al., 2015).

2.12. Biosurfactants

Biosurfactants are microbial products that have the ability to lower surface tension and facilitate microbial mineralisation of substrates (Pacwa-Plociniczak et al., 2011). They are friendly to the environment and possess less toxicity to humans (Das, Mukherjee and Sen, 2008). Because of the ability of biosurfactants to facilitate biodegradation, they are applied in contaminant clean up. For instance, Karami et al. (2016) used rhamnolipid biosurfactant to facilitate the degradation of pentaerythritoltetranitrate.

Rhamnolipid biosurfactant appears to be well-studied and applied. It is produced naturally by *Pseudomonas aeruginosa* (Sifour, Al-Jilawi and Aziz 2007; Zheng et al., 2015). Some researchers have demonstrated that, rhamnolipid improves MFC performances by lowering the resistance of bacteria cell membranes to electron transfer (Wen et al, 2010). With the addition of rhamnolipids, Wen et al. (2010) were able to increase the power density of a MFC from 22 to 275 Wm^{-3}.

Given the fact that, MDC is an extension of MFC, it is possible that rhamnolipid addition can also increase power productions from it. Thus, in this present research the effects of rhamnolipids on five-chambered MDCs were investigated.

2.13. Factorial Designs

Factorial designs create protocols that allow for a number of factors to be varied at different levels to produce measurable effects in responses (London and Wright, 2011).

With factorial designs, main effects and interaction effects of variables can be analysed. The presence or absence significant interaction effects is realised when factors are combined and examined together (London and Wright, 2011).

The general formula for a two level factorial design is 2^P (Ranjan, 2007). The number; 2 represents factor levels and P defines the number of factors. The levels usually considered are, maximum/high level coded as +1 and minimum/low level coded as -1. The simplest form of a factorial design is the 2x2 (2^2) design. The factors are the independent variables that can be manipulated to cause an effect in the response variable whiles the levels describe the values, degree, extent or categories at which the factors can be manipulated. Factorial designs have the advantage of creating fewer but efficient number of experimental runs to reach desired outcomes. Thus it promotes efficient use of resources (Pandis et al., 2014; Montgomery, Peters and Little, 2003).

2.14 Why MDC Technology will be Beneficial to Ghana

The following subsections present discussions on the wastewater situation in Ghana, discussions on why desalination is useful and how it can be made less costly. Power crisis in Ghana is also discussed.

2.14.1 Wastewater situation in Ghana

Urban Ghana generates an estimated annual wastewater volume of 280 million m^3

(Agodzo et al., 2003). To this, there is an estimated wastewater production rise from about 530, 346 m3/day to about 1,452,383 m3/day between the years of 2000 and 2020 (Agodzo et al., 2003). This estimated rise in wastewater generation is alarming because wastewater handling practices of most Ghanaians are inappropriate. According to Obuobie et al. (2006) about 38% of Ghanaians dispose of wastewater on streets, 21% in gutters, 35% in compounds and about 1% at undisclosed locations. This implies that, only about 5% dispose of wastewater responsibly. Unfortunately, though a reduction in unapproved methods of wastewater disposal is vital in wastewater management, wastewater management receives very little investment in developing countries (WHO, 2008).

The poor wastewater handling situation in Ghana has received international attention as The World Bank reported that, less than 8% of domestic wastewater in Ghana gets some form of treatment (World Bank, 2015). And even within this small percentage coverage, there are inequalities because about half of all treatment plants in Ghana are in the Greater Accra region (EPA, 2001), three in Tamale Metropolitan Assembly (UNICEF, 2016), whiles unfortunately the Brong Ahafo and Upper West regions have no treatment plants at all (Abuenyi, 2010; Adu-Ahyiah and Anku, 2003).

Recently, the Greater Accra region has received additional wastewater treatment plants. These are the Lavender Hill Faecal treatment plant and Mudor Wastewater treatment plant. With the large capacities of the two new plants in Accra, it is hoped that, most of the wastewaters generated in the region will receive some form of treatment. The Lavender Hill plant has a maximum treatment capacity of approximately 2,400 cu.m/day of sewage whiles the Mudor plant has a maximum of 18,000 cu.m/day for wastewater handling (Astrim, 2017). Treated wastewater from households, industries, and agriculture can be

reintroduced into the water cycle and accounted for in water budgets (Looker, 1998) when appropriate plans are executed.

On the average, conventional wastewater treatment plants require energy input in the range of 0.3 to 0.5 KWH/m^3 for their operations (Chen et al., 2016). Also, in a typical wastewater treatment plant, aeration alone can demand about 30 KWH/capita/annum of energy (Gude, 2012). These these energy demands make conventional wastewater treatment plants expensive to operate. Even in the USA the energy demand of water treatment establishments is felt as they consume about 4 - 5% of the U.S electrical energy grid (Gude, 2012). This is equivalent to 15.6 million household annual electricity consumptions (Luo, Xu, Roane et al., 2012). Therefore, conventional wastewater treatment technologies are not suitable for countries battling with energy crisis. However, the integration of a technology like the microbial desalination cell with conventional wastewater treatment plants will be beneficial because energy produced from MDCs can make up for the energy demand of the conventional facilities whiles also helping improve the effluent quality of these facilities.

2.14.2 Why Desalination is useful and how it can be made less costly

Fresh water sources span the length and breadth of Ghana (USAID, 2010). However, universal access to safe drinking water is yet to be met (WHO, 2015). That is, 87% of the Ghanaian population have access to safe water leaving 13% of the population to their faith (WHO, 2015).

Amongst areas most hit by limited access to safely managed drinking water are coastal communities like Teshie, Nungua and Cape coast. Cape coast especially has a long history of water supply challenges having suffered water shortages since the 1980s due to unreliable

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sources of raw water (Kumah, 2006). A readily available raw water source to coastal communities is the sea. Thus seawater can be desalinated for supply to coastal communities.

However, conventional desalination establishment like the Accra Desalination Plant are generally costly to operate. Even where renewable energies have been used, the cost of the technologies used have been estimated to be high (Karagiannis and Soldatos, 2008). For instance, the generalized cost of desalinating a meter cube of sea water ranges between $0.75 and $3 (Kumah, 2006) depending on the type of technology. Such high cost of production will make the price of desalinated water expensive, hence not suitable for a developing country like Ghana.

Nevertheless, the high energy demand of conventional desalination plants can be met even if not fully by a successfully developed MDC. Power generated from such a MDC can supply an attached desalination plant energy it requires whiles at the same time further support the conventional desalination plant by de-salting some seawater. This can also contribute to reductions in cost of desalinated water.

2.14.3 Power Crisis in Ghana

Ghana has an installed electricity generation capacity of about 4,310 MW (Energy Commission, 2018). This is insufficient and the insufficiency is evident in the power crisis the country is bedevilled with. Disturbingly, power crisis is not new in the country and is a challenge the country should have found sustainable solutions to long before now.

In the periods of 1982 – 1985 and 1998 – 2000, Ghana suffered major power deficits resulting in the importation of power from neighbouring La Cote d'Ivoire (Mohammed,

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2015). However, the country's power crisis has not necessarily always resulted from issues of installed capacity but also, the unavailability of fuel, faulty machines, poor rainfall and sometimes combination of factors. Poor rains caused the Akosombo and Kpong hydro-dams to underperform between the years 2006 and 2007 and this resulted in power generation deficits witnessed between these years (Mohammed, 2015). A cut-off of gas from the West African Gas Pipeline triggered the very troubling 2012 – 2015 power crisis which was further compounded by poor rains and inadequate financing for procuring other sources of fuel (Mohammed, 2015).

Inferring from the facts stated above, it is advised that, Ghana does more with regards to sustainable power production. For most parts of the world, renewable sources of energy are the way to go and for this, Ghana has not been left out. In 2011, the Renewable Energy Act, Act 832 was assented to with the main object of ensuring the sustainable provision and patronage of renewable energy sources in an environmentally responsible manner (Renewable Energy Act, 2011). Currently, the Volta River Authority has a solar farm in Navarongo which generates 2.5 MW of electricity for the community and its environs (Energy Commission, 2018). More solar projects and other renewable energy sources can be added on if some priority is given to cleaner technologies.

Microbial Desalination Cells are a source of renewable energy with a potential of adding some power to our country's energy mix. Thus its development and application should be welcoming as it is also in tune with the country's renewable energy goals. Also, its operation requirements allow for satellite electricity production with relative ease of supervision and maintenance.

CHAPTER THREE

3.0 Materials and Methods

3.1 Materials

3.1.1 List of Materials

Polyoxymethylene cylinders (Quadrant polypenco, Japan), Anion and Cation Exchange Membranes (Membrane International, NJ, USA), Carbon fibre fill material ((0.984‖ brush part, 400,000 tips per square inch) Mill-Rose, USA), Cloth Gas Diffusion Electrode - 2mg/cm² Pt - 5 x 5 (fuel cell store, USA), Vulcan XC-72R - 50 grams (fuel cell store, USA), Hach NO_3^-N (HR)-aCCUvAC Ampuls, Hach phosphorus (Total) USA, Hach COD HR and LR reagents (USA), Cow dung (KNUST cattle farm, Ghana), faecal sludge (Dompoase wastewater treatment plant, Ghana), rumen content (Kumasi abattoir, Ghana), *Ceratophyllum demersum* (Bari-Kesi, Kumasi, Ghana), *Lemna sp.* (KNUST, Ghana). Potassium ferricyanide - $K_3[Fe(CN)_6]$ (Sigma-Aldrich), biosurfactant (90% pure rhamnolipids, solid, AGAE, LLC), USA; sodium acetate anhydrous - CH_3COONa (Sigma-Aldrich, USA), M 9 minimal salts - Na_2HPO_4, KH_2PO_4, NaCl, NH_4Cl (SigmaAldrich, USA),

3.1.2 List of Equipment

Keithley multimeter (2700 module), Cyberscan Waterproof pH/conductivity/TDS/☐C/ ☐F PC 300 series multi-parameter, Hach HQ 30 d flexi DO/Temp- meter, Scanning Electron Microscope (Phenom ProX Generation), VWR CO310 Multi-parameter meter.

3.1.3 Parameters Investigated

Voltage, electrical conductivity changes, desalination, COD, nitrate and phosphorous reductions, pH, DO, internal resistance, power, current, power and current densities, coulombic efficiencies and voltage losses.

3.2 Methods

3.2.1 MDCs Design and Construction

Five different types of MDCs were designed and constructed in this study. They were the three-chamber chemical catholyte MDC, three-chamber water catholyte MDC, threechamber plant-supported MDC, five-chamber chemical catholyte MDC and five-chamber water catholyte MDC. Details of these MDCs are described in sections 3.2.1.1 and 3.2.1.2. Dimensions used in the construction of anode and cathode chambers were based on the sizes of electrode materials whiles those of the desalination chambers were based on the recommendation of Ping et al. (2014).

3.2.1.1 Chemical Catholyte Dependent MDCs

A. Three-chamber MDC Construction

This MDC was carved from polyoxymethylene cylinders and supported with cylindrical gaskets (Plate 3.1) to prevent leakages. Its compartments were held together with stainless steel bolts and nuts. The internal volumes of the anode and cathode of the MDC were 230 cm3 each while that of the desalination chamber was 77 cm3. The anode chamber was separated from the desalination chamber by Anion Exchange Membranes (AEM) and the cathode, by Cation Exchange Membranes (CEM). Electrode material used for both anode and cathode was carbon fibre fill material (0.984‖ brush part, 400,000 tips per square inch).

The inter-electrode distance was 10 cm and total internal volume considering all chambers was 537 cm^3.
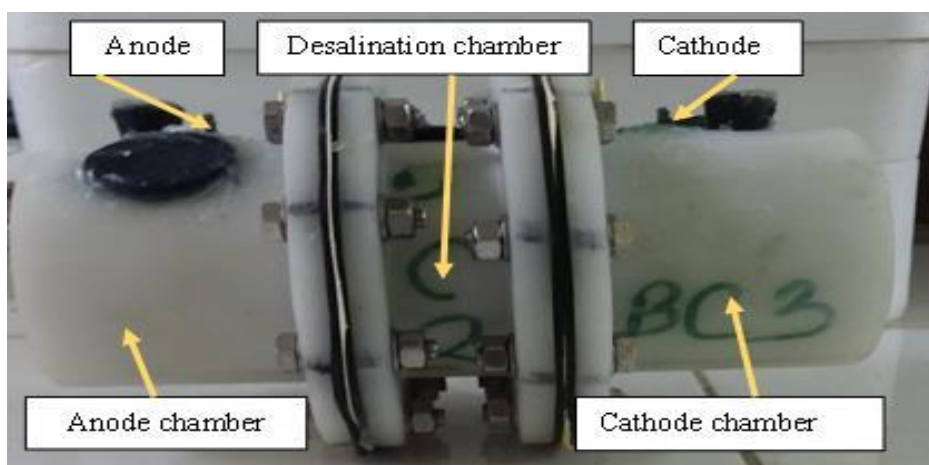
Plate 3.1: Three-chamber chemical catholyte MDC

B. Five-chamber MDC Construction

The five-chamber MDC was also made from polyoxymethylene cylinders (Plate 3.2). Its inter-electrode distance was 10 cm. It comprised of the; anode chamber, desalination chamber, cathode chamber and additional two chambers called the neutralization chambers. Its internal anode and cathode volumes were 230 cm^3 each, desalination chamber 77 cm^3 and neutralization chamber of volumes 150 cm^3 each. Its total internal volume considering all chambers was 837 cm^3. The electrodes used in it were carbon fibre fill material (0.984ǁ brush part, 400,000 tips per square inch) for both anode and cathode.
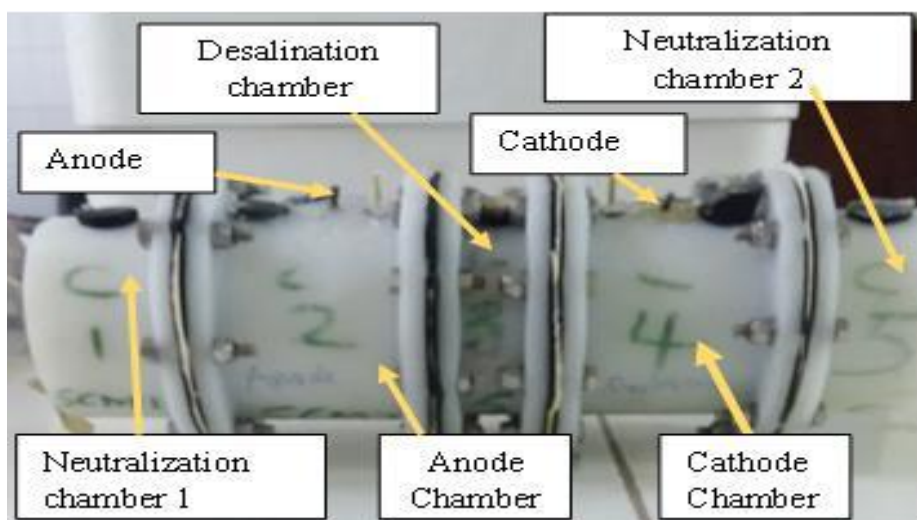
Plate 3.2: Five-chamber chemical catholyte MDC

This cell's anode chamber was separated from the desalination chambers by Anion Exchange Membranes (AEM) and the cathode, Cation Exchange Membranes (CEM). The neutralization chamber to the end of the anode (N_A) was separated from it by a CEM whiles that to the end of the cathode (N_C) an AEM. The constructed neutralization chambers were for pH control.

3.2.1.2 Non-Chemical Catholyte-Dependent MDCs

A. Three-chamber Plant-supported and Water catholyte MDC Construction

All dimensions of the three-chamber Plant-supported and water catholyte MDCs and their arrangements were same as with the three-chamber chemical catholtye MDC except that a 3.5 cm x 6 cm of their cathode roofings were cut opened (Plate 3.3) to make room for the growth of aquatic plants and also, for passive aeration. Their anodes were carbon fibre fill material (0.984‖ brush part, 400,000 tips per square inch) and cathode cloth gas diffusion electrode- 2mg/cm² Pt - 5 x 5 (fuel cell store) supported with 0.3 g Vulcan carbon black

(fuel cell store, USA). The platinum catalyst on the cathode was washed off before use.

This was done to mimic a no catalyst situation. The difference between the plantsupported MDC and water catolyte MDC was that, the Plant-supported MDC had an aquatic plant grown in it whiles the water catolyte MDC contained only tap water.
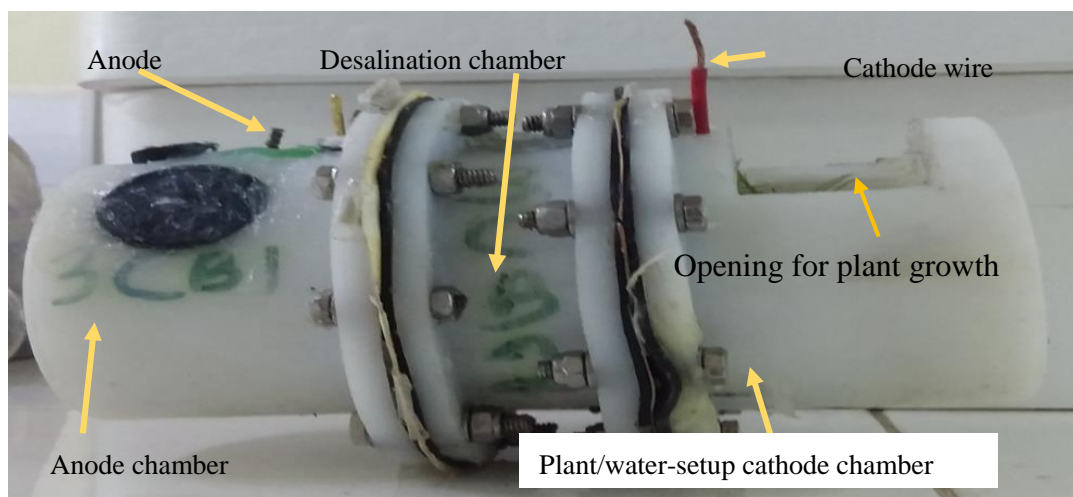
Plate 3.3: Three-chamber plant-supported/water catholyte MDC

B. Five-chamber water catholyte MDC Construction

The five-chamber water catholyte MDC was similar to the five-chamber chemical catholtye MDC in all aspects with the exception that, its cathode roof had a 3.5 cm x 6 cm opening to allow for passive aeration (Plate 3.4). Electrode materials used were carbon fibre fill material (0.984‖ brush part, 400,000 tips per square inch) for anode and cloth gas diffusion electrode- 2mg/cm² Pt - 5 x 5 (fuel cell store) supported with 0.3 g Vulcan carbon black for cathode. Platinum catalyst on cathode was washed off before use to present a condition of no catalyst.
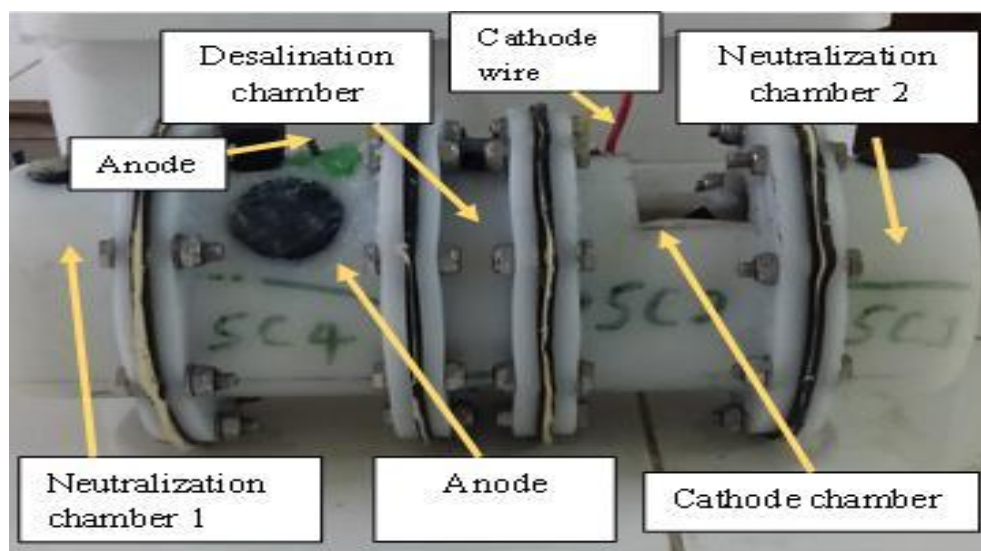
Plate 3.4: Five-Chamber water catholyte MDC (MDC)

3.3 Solutions and Materials Preparation

A. Simulated Seawater (NaCl solutions)

35 g of analytical grade NaCl was dissolved in 1 liter of tap water and used as simulated seawater. This concentration was used in all experiments.

B. Sodium Acetate Solution

This was prepared by dissolving 3 g of sodium acetate (analytical grade) in 1 liter distilled water

C. Buffer Solution

A buffer solution was prepared as follows; 1.07 g of K_2HPO_4 and 0.53 g of KH_2PO_4 were dissolved in 1 litre of di-ionized water as buffer (pH, 7.12) solution (Brastad and He, 2013).

D. Inoculum Source/Simulated Wastewater

Simulated wastewater used in the _actual experiments' in this study was prepared by mixing 10 g of cow dung and rumen contents in 100 ml of sterilized distilled water. This was topped up to 1000 ml with distilled water and filtered prior to use. Cow dung + rumen contents was

used for preparing wastewater because it proved to be the best inoculum source in a preliminary study (See section 3.4). Characteristics of wastewater were determined before use (Appendix 1).

E. Mineral solution

This was prepared by dissolving 5 g/l NH_4Cl and 2.5 g/l NaCl in 1 liter distilled water.

F. Chemical Catholyte

16.5 gL^{-1} of potassium ferricyanide ($K_3 [Fe(CN)_6$) solution was prepared. Depending on the type of MDC, a buffer solution was added to the catholytes.

G. Catholyte for Biocathode

Catholyte used in biocathode was water from Barekese dam. Chemical analysis of dam water is presented in appendix 1B

H. Water Catholyte

Tap water was used as water catholyte. Depending on the type of MDC, buffer solution was added to the catholyte

I. Membranes

Membranes were pre-conditioned in 5% salt solution for 24 hours before use according to manufacturer's (Membrane International, USA) recommendation.

J. Activation of Carbon Brush

Carbon brush electrodes were activated by heating to temperatures of 300 ⬚C for 30 minutes.

3.4 Preliminary Studies

The preliminary studies comprised of the following experiments:

- Selection of viable inoculum source,
- Selection of waterweed for biocathode construction

- Microbial acclimatization experiment.

Descriptions to the preliminary studies are presented below. All experiments were

triplicated.

3.4.1 Selection of Viable Inoculum Source

The following inoculum sources were investigated for their ability to evolve electricity

producing bacteria (exoelectrogens). Cow dung, faecal sludge, rumen contents, rumen

contents + cow dung. They were separately mixed (10 g) with tap water, filtered and used

as anolytes in a microbial fuel cell (MFC). The MFC setup for all _inoculum source'

experiments contained, inoculum source solution as wastewater (220 ml) dosed with 0.6 g/l

sodium acetate and buffered with 1.07 g/l of K_2HPO_4 and 0.53 g/l of KH_2PO_4. Catholytes

composed of 220 ml of 16.5 g/l potassium ferricyanide solution containing

1.07 g/l of K_2HPO_4 and 0.53 g/l of KH_2PO_4 buffer.

3.4.2 Selection of Waterweed for Biocathode Construction

Prior to the construction of the biocathode, an experiment was performed to choose the most

viable aquatic plant in terms of sustainable oxygen production. Thus, the ability of an

aquatic plant to maintain an approximate 8 mg/l oxygen concentration in water over at least

one batch cycle (ie 24 hours) of MDC operation was desired before it could qualify for

biocathode construction. Also the plant needed to be small enough to grow in

miniature cells.

The aquatic plants, *Lemna* spp and *Ceratophyllum demersum* were sampled and assessed

for biocathode construction based on the requirement of sustainable oxygen productions.

A control experiment (using only water) was run alongside the experiments to demonstrate that, the selected aquatic plants were responsible for increments in oxygen concentration in sampled water. Water used in this experiment were collected from the natural habitats of the aquatic plants concerned. Water from Barekese (for growth of *Ceratophyllum demersum*) contained 15.6 □ 0.62 mg/l nitrogen and 0.29 □ 1.00 mg/l phosphorus whiles that for the growth of *Lemna* spp was collected from small ponds behind KNUST, engineering laboratories. It contained 183.20 □ 0.33 mg/l nitrogen and 37.73 □ 01.05 mg/l phosphorus.

3.4.3 Microbial Acclimatization Experiment

To avoid a slow start in the operations of MDCs understudy, the selected inoculum source (Cow dung + rumen contents) was put to an acclimatization run in an MFC according to the protocol of Cao et al. (2009). This lasted eight (8) weeks with continuous change of electrolytes every week. In this experiment, the anolyte constituted of 3 g/l of sodium acetate in 220 ml of simulated wastewater and the cathode, 220 ml of 16.5 g/l potassium ferricyanide solution. Both anolyte and catholytes were buffered with 1.07 g/l of K_2HPO_4 and 0.53 g/l of KH_2PO_4. The experiment was monitored by recording the pH and voltage levels at the beginning and end of each batch test. Developed biofilms on anodes were transferred to MDCs for subsequent studies.

3.5 Performance Analysis of MDCs using different sources of Terminal Electron Acceptors (TEA)

This study was carried out on the three-chambered MDCs. The three-chamber chemical catholyte MDC operated with potassium ferricyanide as its terminal electron acceptor whiles the three-chamber water catholyte and plant-supported MDCs operated with dissolved oxygen as their electron acceptor. All three types of MDCs were run together in three batch cycles. Initial operating conditions were same for each cell except for differences in cathode setups (Table 3.1). The volumetric ratio between anolyte and saltwater was approximately 3:1. This was prepared based on the recommendation of Ping et al. (2014) who stated that, a range of 3-5 volumetric ratio of anolyte to saltwater is necessary for appreciable desalination to be achieved. Table 3.1 presents information on the constituents of each MDC.

A control experiment was conducted alongside the three experimental setups to demonstrate that electricity generation and desalination were the result of microbial metabolic activities. In this experiment, 220 ml autoclaved wastewater was used as the anolyte, salt water of concentration 35 g/l used as seawater and the catholyte comprised of 220 ml of 16.5 g/l potassium ferricyanide solution. Voltage produced and percentage salts removed were recorded to assess the performance of the control experiment.

Table 3.1: Constituents of anode, cathode and desalination chambers of threechamber MDCs

Type of MDC	Anolyte	Saltwater concentration	Catholyte
Threechamber chemical catholyte MDC	220 ml wastewater containing: 3 g/l sodium acetate, 1.07 g/l K_2HPO_4 and 0.53 g/l KH_2PO_4 buffer and 5 g/l NH_4Cl and 2.5 g/l NaCl mineral solution	75 ml of 35 g/l salt solution	220 ml solution of potassium ferricyanide containing: 16.5 g/l $K_3[Fe(CN)_6]$, 1.07 g/l K_2HPO_4 and 0.53 g/l KH_2PO_4 buffer solution

Threechamber plantsupported MDC	220 ml wastewater containing: 3 g/l sodium acetate, 1.07 g/l K_2HPO_4 and 0.53 g/l KH_2PO_4 buffer and 5 g/l NH_4Cl and 2.5 g/l NaCl mineral solution	75 ml of 35 g/l salt solution	220 ml Barekese water containing: 1.07 g/l K_2HPO_4 and 0.53 g/l KH_2PO_4 buffer
Threechamber water catholyte	220 ml wastewater containing: 3 g/l sodium acetate, 1.07 g/l K_2HPO_4 and 0.53 g/l KH_2PO_4 buffer and 5 g/l NH_4Cl and 2.5 g/l NaCl mineral solution	75 ml of 35 g/l salt solution	220 ml tap water containing: 1.07 g/l K_2HPO_4 and 0.53 g/l KH_2PO_4 buffer solution + 0.3 g Vulcan carbon

The circuits of all MDCs were closed individually by connecting the anodes and cathodes of each cell across a 1000 Ohm external resistor. The end of a desalination cycle was determined when voltage production dropped below 45 mV. This typically was approximately 72 hours unless otherwise stated.

The following parameters were monitored/measured/computed to assess the performances of MDCs understudy:

• **Electrical Conductivity (EC)**

This was monitored using Cyberscan Waterproof pH/conductivity/TDS/$\Box$C/ $\Box$F PC 300 series multi-parameter METER.

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☐

Percentage Desalination

Percentage desalination was computed as;

$$\% \text{ Desal.} = \frac{Initial\ salt\ solution\ conductivity - final\ salt\ solution\ conductivity}{Initial\ salt\ conductivity} \ x\ 100_\%$$

- **Voltage**:

  o Cell voltages were continuously measured every 5 minutes using Keithley (2700 module) digital multimeter

☐ **Current**

  o Currents were computed from voltage values as:

$$Current(I) = \frac{Voltage\ (V)}{Resistance(R)}$$

- **Power** was computed as;   P = V x I

  Where I = Current; V = Voltage; P = Power

- **Current and power densities were computed using the following formulae;**

  Current density $= \dfrac{Current\ (I)}{Anode\ chamber\ Volume\ (V)}$

  Power density $= \dfrac{Power\ (P)}{Anode\ chamber\ Volume\ (V)}$

- **pH**:

  o pH of solutions was measured using a Cyberscan Waterproof pH/conductivity/TDS/^{0}C/ ^{0}F PC 300 series multi-parameter meter

☐ **COD Analysis and Percentage COD Reduction** o Chemical Oxygen Demand (COD) was determined using reactor digestion method (Method 8000) o Percentage COD reduced was computed using the formula;

$$\% \text{ COD removed} = \frac{Initial\ COD - Final\ COD}{Initial\ COD} \ x\ 100\%$$

Coulombic Efficiency (CE): Computed as;

☐

$$(CE) = \frac{Ms \int Idt}{Fb\ V\ \Delta C} \times 100 \qquad \text{Logan et al. (2006)}$$

Where; Ms = molecular weight of oxygen

F = Faradays's constant

Δ C = Change in substrate concentration over the batch cycle (COD) b

= Number of electrons exchanged per mole of oxygen

V = volume of anodic solution

I = Average current produced within the operating time

☐ **Internal Resistance of MDCs**:

o This was estimated by Electrochemical Impedance Spectroscopy (EIS) using

Gamry PC 14 G750 potentiostat.

All EIS were performed at open circuit conditions over a frequency range of 20 x 10^3 to 0.1 Hz. Internal resistance was derived from Nyquist plots where the intercept of the curve with Z_{re} axis was defined as ohmic resistance (Cooper and Smith, 2006).

Polarization and power density curves were obtained by varying manually external resistances (8000-750 Ω) and recording the voltages across them. All MDCs were left in open circuit conditions for 24 hours before tests to obtain polarization curves were conducted. This is required for the establishment of stable open circuit voltages (Logan, 2008).

• **Dissolved Oxygen (DO)**

DO was monitored in biocathodes using a Hach HQ 30 d flexi DO/Temp- meter

• **Nitrate Analysis and Percentage Nitrate Reduction**

Nitrate analysis was carried out using the cadmium reduction method (Method 8039)
Percentage   nitrate reduction was computed as

$$\% \text{ Nitrate reduced} = \frac{Initial\ nitrate\ concentration - Final\ nitrate\ concentration}{Initial\ nitrate\ concentration}$$

- **Phosphorus Analysis and Percentage Phosphorus Reduction** ○

  Phosphorus analysis was carried out using the Molybdovanadate with

  acid persulfate method (Method 10127)

    ○ Percentage   (%)   phosphorus   reduced   was   computed   as

$$\frac{Initial\ phosphorus\ concentration - Final\ phosphorus\ concentration}{Initial\ phosphorus\ concentration}$$

3.6 pH Stabilization Effect of Neutralization Chambers and the Performances of three and five-chamber MDCs

In this study, the buffering capacities of neutralization chambers of the five-chamber MDCs were assessed. The electricity generation, desalination and wastewater treatment performances of the five-chamber MDCs were assessed and compared to that of the threechamber MDCs to evaluate how well neutralization chambers could support MDCs perform comparatively to or better than potassium phosphate-buffered MDCs (threechamber chemical and water catholyte MDCs). Table 3.2 presents information on the cell constituents of the five-MDCs and Table 3.1 presents that of the three-chamber MDCs.

Table 3.2: Constituents of anode, cathode and desalination chambers of five-chamber MDCs

Type of MDC	Anolyte	Saltwater Concentration	Catholyte
Five-chamber chemical catholyte MDC	220 ml wastewater containing: 3 g/l sodium acetate, 5 g/l NH_4Cl and 2.5 g/l NaCl mineral solution	75 ml of 35 g/l salt solution	220 ml solution of potassium ferricyanide containing: 16.5 g/l $K_3[Fe(CN)_6]$
Five-chamber water catholyte MDC	220 ml wastewater containing: 3 g/l sodium acetate, 5 g/l NH_4Cl and 2.5 g/l NaCl mineral solution	75 ml of 35 g/l salt solution	220 ml tap water + 0.3 g Vulcan carbon

*Neutralization chambers were supplied with 100 ml deionized water each

A drop in voltage production below 45 mV marked the end of a desalination cycle.

Parameters investigated in this study were same as previously described in section 3.5.

3.7 Effects of Rhamnolipid on the Performances of five-chamber MDCs

In this study, rhamnolipid (AGAE Technology, USA) was added to anolytes of the fivechambered MDCs to investigate its effects on the performances of these MDCs. An initial concentration of 80 mg/l (Wen et al., 2010) was used and then varied at an interval of 80 mg/l till 480 mg/l. Parameters investigated in the previous experiments were repeated in each experiment in this study. Each MDC was subjected to three batch runs. Table 3.3 below presents information on the constituents of each MDC in the rhamnolipid experiment.

Table 3.3: Constituents of anode, cathode and desalination chambers in rhamnolipid study

Type of MDC	Anolyte	Saltwater concentration	Catholyte

Five-chamber chemical catholyte MDC	220 ml wastewater containing: 3 g/l sodium acetate, 5 g/l NH$_4$Cl and 2.5 g/l NaCl mineral solution and varying concentrations of rhamnolipids in the order of: 80,160.240.320.400 and 480 mg/l	75 ml of 35 g/l salt solution	220 ml solution of potassium ferricyanide containing: 16.5 g/l K$_3$ [Fe(CN)$_6$]
Five-chamber water catholyte MDC	220 ml wastewater containing: 3 g/l sodium acetate, 5 g/l NH$_4$Cl and 2.5 g/l NaCl mineral solution and varying concentrations of rhamnolipids in the order of: 80, 160, 240, 320, 400, 480 mg/l	75 ml of 35 g/l salt solution	Tap water (220 ml) + 0.3 g Vulcan carbon

*Neutralization chambers of five-chamber MDCs contained 100 ml distilled water.

The circuits of all MDCs were closed individually by connecting anodes and cathodes of each MDC across a 1000 Ω external resistor. A drop in voltage production below 45 mV marked the end of a desalination cycle.

3.8 Factorial Study of the Combine effects of Rhamnolipid and Stirring on the Performances of the five-chamber water catholyte MDC

This study was carried out to find out if the application of both rhamnolipid and stirring could improve the core outputs (voltage production, desalination and COD removal) of the five-chamber water catholyte (5 W C) MDC. Preceding the factorial study was an investigation on the effects of stirring on the 5 W C MDC performances. Subsection 3.8.1

below provides details on the methodology of the ‗stirring experiment' and 3.8.2 provides details on the factorial study.

3. 8. 1 Effects of Stirring on the Performance of the Five-chamber Water Catholyte MDC

The five-chamber water catholyte MDC used in this experiment had similar composition as earlier described in Table 3.2. The only variation from the earlier experiment was stirring. Stirring experiments were preceded by an experiment to select an appropriate stirring speed. In this experiment, three stirring speed were studied for their influence on DO levels in water catholyte. These were 60, 80 and 100 rpms. The 60 rpm speed was the preferred speed because its effect on oxygen concentration was similar (produced comparative oxygen concentration) to those recorded from the higher stirring speeds (80 and 100 rpm) (Appendix 4 D).

Using the stirring regimes 1, 2 and 3, changes in voltage production and desalination were monitored to assess the effect of stirring on them. Stirring regime one (1) was defined as mechanical stirring at 60 rpm of water catholyte for 10 minutes once a day whiles stirring regime two (2) was defined as stirring at 60 rpm for 10 minutes, twice a day (12 hours' interval). Regime three (3) on the other hand, was 10 minutes of stirring at 60 rpm three times daily (8 hours' interval).

.8.2 Factorial Design

This study was design using DOE in Minitab14. Rhamnolipid concentration in anodes and stirring regimes in cathode chambers were the factors considered in the factorial study. Each factor was varied at two levels (low and high) with three replicates each of their corners

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points. This resulted in a total of 12 factorials runs. The factorial study was limited to one batch cycle of MDC operation. The outcomes measured were; desalination, voltage productions and COD removal.

Table 3.4: Factorial design for the study of effects of rhamnolipid and stirring on 5 W C MDC performances

Standard Order	Run Order	Center point	Blocks	Rhamnolipid	Stirring regime
7	1	1	1	-1	1
4	2	1	1	1	1
6	3	1	1	1	-1
11	4	1	1	-1	1
9	5	1	1	-1	-1
5	6	1	1	-1	-1
8	7	1	1	1	1
3	8	1	1	-1	1
12	9	1	1	1	1
10	10	1	1	1	-1
1	11	1	1	-1	-1
2	12	1	1	1	-1

Where:
- 1 represents rhamnolipid concentration of 240 mg/l and -1 represents 160 mg/l rhamnolipid
- 1 represents stirring regime of 3 times a day and -1 represents once a day

Residual plots were used in model diagnosis. Four in one residual plots were generated from minitab and analysed for conformation to assumptions of linear regression model.

3.8 Membrane Fouling Studies

The membrane fouling investigation was performed on all used membranes of the fivechamber water catholyte MDC and also, the used cation exchange membrane of the fivechamber chemical catholyte MDC. To appreciate the effect of catholyte on the integrity

of ion exchange membranes, a comparison (SEM images and EDS results) of the degree of fouling between the cation exchange membranes of the two types of five-chamber MDCs was made to ascertain which had more fouled layers.

The MDCs concerned were disassembled and their membranes analysed using Scanning Electron Microscope (SEM) with Energy Dispersive (X-ray) Spectroscopy (EDS) (Phenom ProX Generation 5, mag. 80 – 150,000x). A sterilized scissor was used to carefully cut middle portions of the membranes (AEM and CEM) for air drying overnight (Luo et al., 2012) and subsequent drying in a desiccator. The cut membranes were mounted on aluminum stubs with adhesive carbon papers then placed in the sample cupholder of the SEM for analysis. Optical and electron images of samples were obtained with the SEM and elemental compositions with EDS. With information from SEM and EDS, the nature and compositions of used and unused membranes were compared for the detection of possible fouled layers/structures.

CHAPTER FOUR

4.0 Results and Discussions

Microbial desalination cells (MDCs) used in this study were constructed using locally available materials. Details of each MDC are presented in chapter three under section 3.2.1 and the schematics of the cells in appendix 2. Amongst the MDCs constructed, the three-chamber plant-supported MDC and five-chamber MDCs were unique because these MDC designs were not found in available literature.

Also prior to the start of the main experiments, preliminary studies were conducted to select the best inoculum source, best aquatic plants for the building of the biocathode and an acclimatization experiment to develop electrogens on anodes. Results from these studies showed that, rumen contents and cow dung mixture was the best inoculum source (Appendix 5 A) and *Ceratophyllum demersum* was the most viable aquatic plant for the building of a biocathode (Based on oxygenation - Appendix 5 C). A stable reproducible voltage (Appendix 5 D) was obtainable in the third and fourth week of the acclimatization experiment after microbes had fully colonized anode surfaces.

4.1 Assessment of the Electricity Generation, Desalination and Wastewater Treatment Potential of three-chamber MDCs

Results of the three-chamber chemical catholyte MDC, three-chamber plant-supported and three-chamber water catholyte MDCs studied in this research are presented here. These MDCs performances were assessed and compared based on the following parameters; electricity production (voltage productions, volumetric power and current densities), desalination and wastewater treatment efficiencies (COD, nitrate and phosphorus reductions). Electrical conductivity and pH were also measured to support discussions on the performances of the MDCs

4.1.1 Electricity Production Performances of the three-chamber MDCs

Electricity production by the three-chamber chemical catholyte, water catholyte and plantsupported cathode MDCs was assessed and compared based on voltage and power productions. Also, polarization curves were used to describe voltages as a function of current densities.

4.1.1.1 Voltage Production by three-chamber MDCs

Voltage production in all MDCs immediately peaked upon start up but gradually declined towards the end of batch cycles. The highest voltage (282.91±0.09 mV) was produced by the three-chamber chemical catholyte MDC followed by the water catholyte MDC (193.99 ± 0.80 mV) and then the plant-supported MDC (153.96 ± 0.33 mV) (Fig. 4.1). The differences between their voltage productions were statistically significant (p = 0.000). A Tukey's test showed that, the difference principally resulted from the higher voltages of the three-chamber chemical catholyte MDC as compared to the smaller voltages of the three-chamber plant-supported MDC.
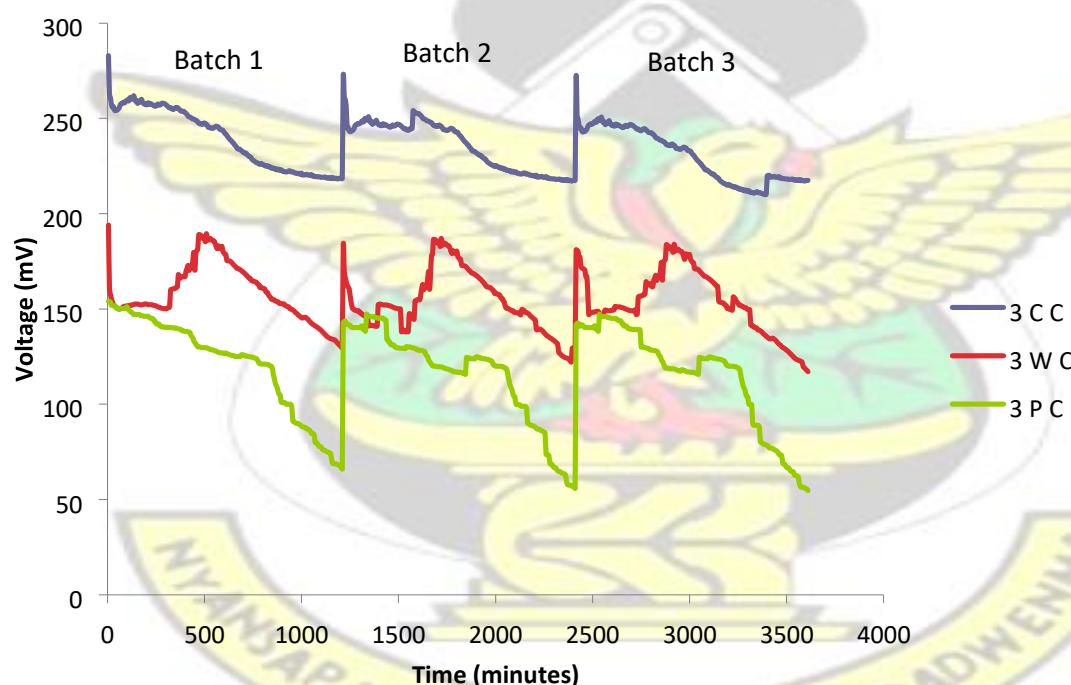
Figure 4.1: Voltage profiles of three-chamber MDCs
[3 C C - three-chamber chemical catholyte MDC; 3 W C - three-chamber water catholyte MDC; 3 P C - three-chamber Plant-supported MDC]

Monitoring voltages from control experiment showed negligible voltage productions (1.0 x 10^{-5} mV) for about 10 hours where it then produced an approximate 5 mV of electricity (Appendix 6). The realisation of minimal voltage production from the control experiment

supports the hypothesis that voltage productions in MDCs result principally from the metabolic activities of exoelectrogens and not from the mere presence of electrolytes. A similar conclusion was reached by Jharna, Asma and Kaur (2015) that electricity generation in MDCs is caused by _electric-active bacteria'. The control experiment from their study on an autoclaved sewage-anolyte could produce only an average of -1.37 ±

0.275 mV of electricity.

Additional peaks in voltages shown in the voltage profile of the three-chamber water catholyte MDC before the end of batch cycles (Figure 4.1) was due to stirring. When no stirring was applied, voltage production by the three-chamber water catholyte MDC dropped to the end of batch cycles without the intermittent _hikes' in voltage productions

(Fig. 4.2). Stirring at 60 rpm on the average increased the concentration of oxygen (electron acceptors) in the water catholyte from 4.37 ± 0.75 to 7.08 ± 2.9 mg/l. This increase in oxygen concentration and for that matter, increase in concentration of electron acceptors was implicated as responsible for the additional peak voltages recorded from the
3 W C MDC before the the end of batch cycles (Fig. 4.1). Amari, Vahdati and Ebadi (2015) also noticed this effect of changes in oxygen concentration on voltage production and reported that, supplying an oxygen-reliant MDC with adequate concentrations of oxygen supports higher current density productions.
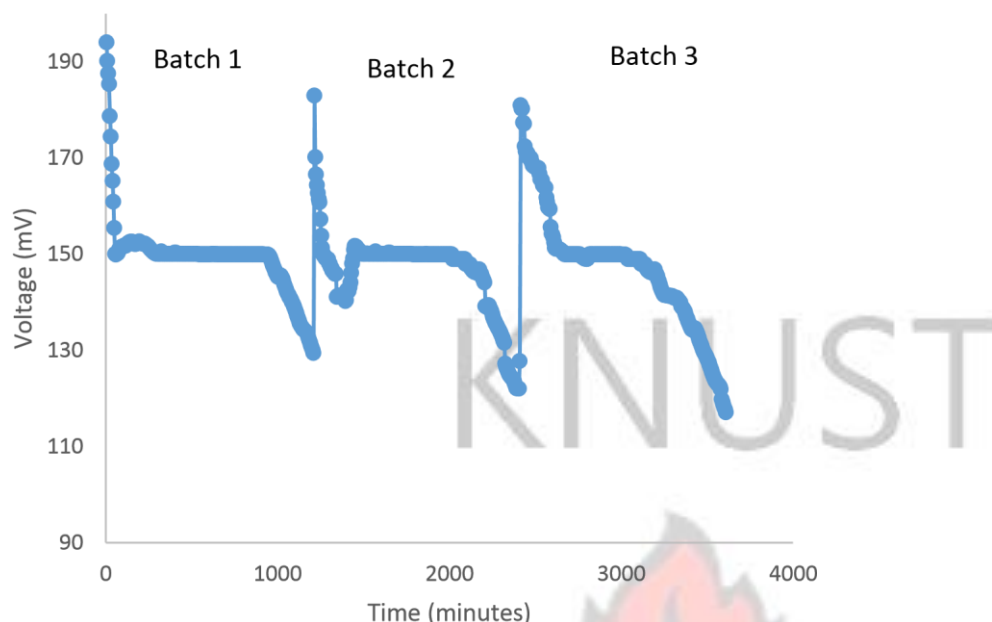
Figure 4.2: Voltage profiles of three-chamber water catholyte MDC in the absence of stirring

Generally, the declines in voltage production in the MDCs were attributed to pH fluctuations and changes in ohmic resistance. The pH of anolytes decreased to acidic conditions (Appendix 7a) while that of catholytes increased to alkaline conditions (Appendix 7b). Low pH conditions for instance affect microbial metabolic activities thus decrease electricity production (Qu et al., 2012). Internal resistance was also implicated in voltage drops because all MDCs recorded rise in internal resistance. The three-chamber chemical catholyte MDC's internal resistance increased from 41.11 to 47.78 Ω whiles that of the water catholyte MDC's increased from 127.90 to 146 Ω and the plant-supported MDC, from 138.20 to 441.00 Ω (Fig 4.3). Thygesen et al. (2009) also reported of voltage losses in their work and associated this with higher internal resistance as observed and reported in this work.
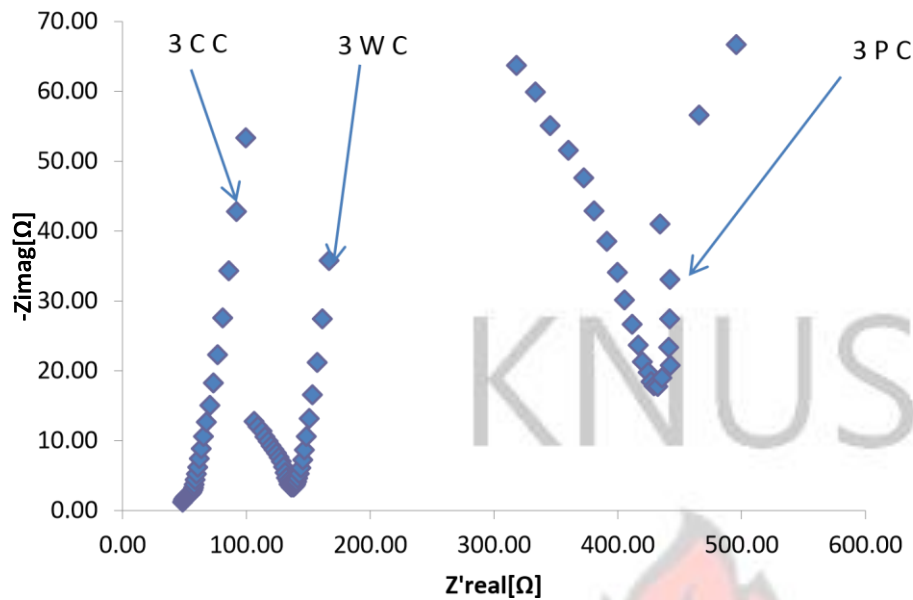
Figure 4.3 Final internal resistances of three-chamber MDCs (Nyquist plots)

Where; Zimag is imaginary impedance and Zreal is real impedance

4.1.1.2 Power Production and Polarization Curves of the three-chamber MDCs

Power density plots and polarization curves of the three-chamber MDCs are presented in Figures 4.4 – 4.6 below. The highest volumetric power density (0.35 ± 0.10 W/m^3; Fig. 4.4) was produced by the three-chamber chemical catholyte MDC and the least (0.25 ± 0.06 W/m3; Fig. 4.6) by the three-chamber plant-supported MDC. The power densities produced by these MDCs when compared to the 8.0 ± 0.4 W/m3 reported by Luo, Xu, Roane et al. (2012) were relatively small and this was possibly because of the large volume of the desalination chambers used in this study. According to Kim and Logan (2011), larger desalination chambers results in higher internal resistance which reduces power productions.

The polarization curve of the three-chamber chemical catholyte MDC (Fig 4.4) and that of the three-chamber plant-supported MDC (Fig 4.6) showed all three types of voltage losses. Rapid voltage losses at low current densities, constant voltage losses and then rapid voltage losses at higher current densities (Fig. 4.4).
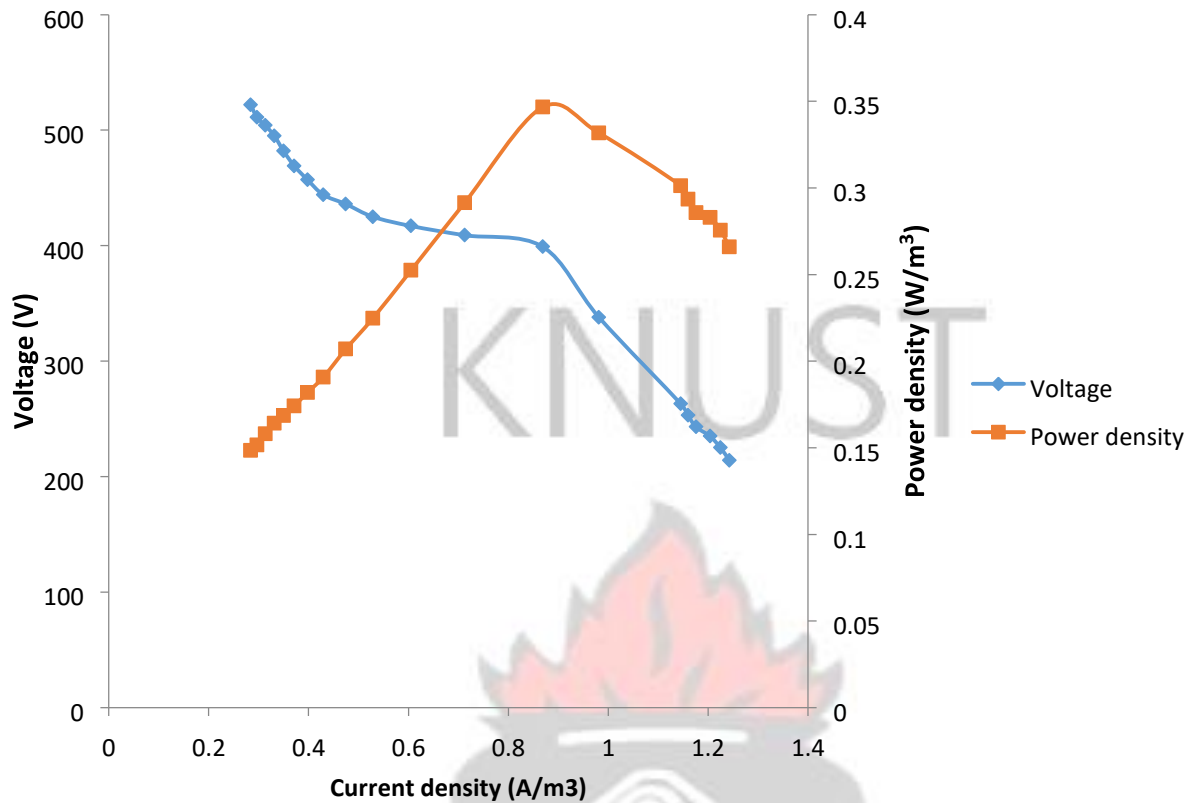
Figure 4.4: Power density and polarization curve of three-chamber chemical catholyte MDC

The water catholyte MDC on the other hand displayed rapid voltage losses right from low current densities through to high current densities (Fig. 4.5). Rismani-Yazdi et al. (2008) explained rapid voltage losses to be due to electrode overpotentials which can be reduced through the use of larger reaction surface areas. Constant drops in voltages generally reflect voltage losses due to internal resistances which largely come from resistance of electrolytes (Hayre et al., 2005). Rapid voltage losses at higher current densities on the other hand is caused by mass transport losses due to limitation of movement of reactants (for example, oxygen) or products (for example, water) especially in the cathode chamber (Rismani-Yazdi et al., 2008).
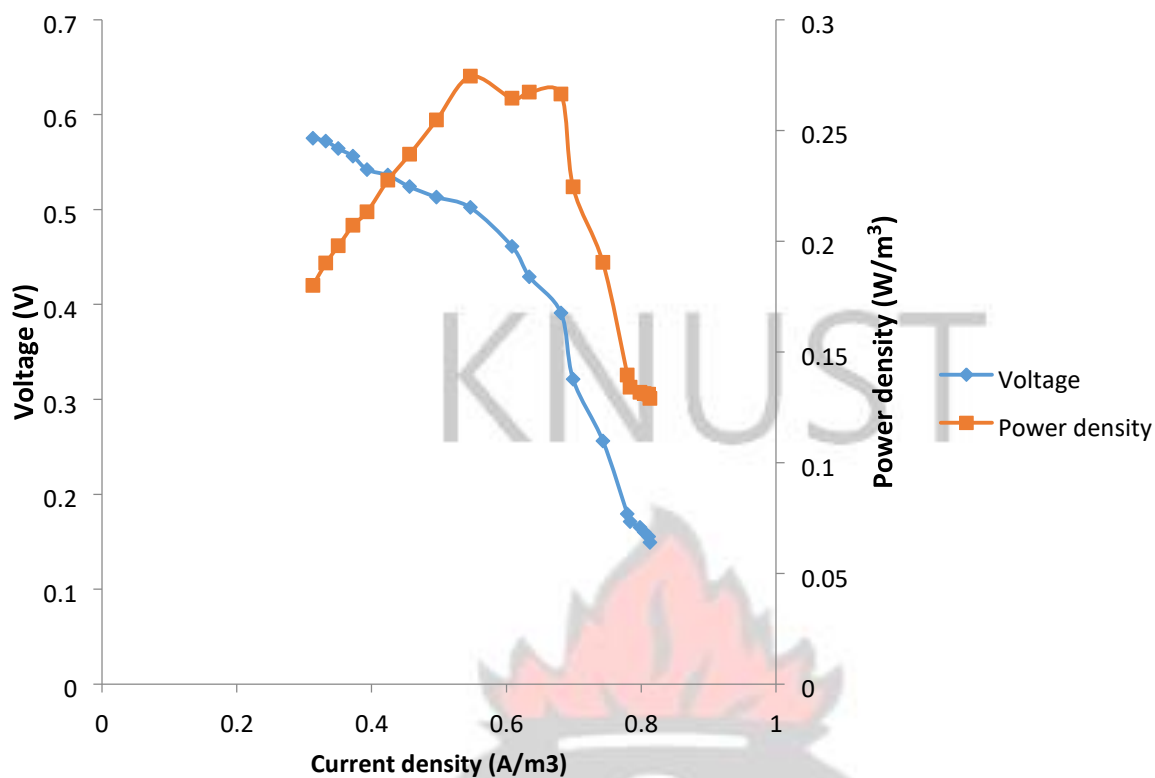
Figure 4.5: Power density and polarization curve of three-chamber water catholyte MDC
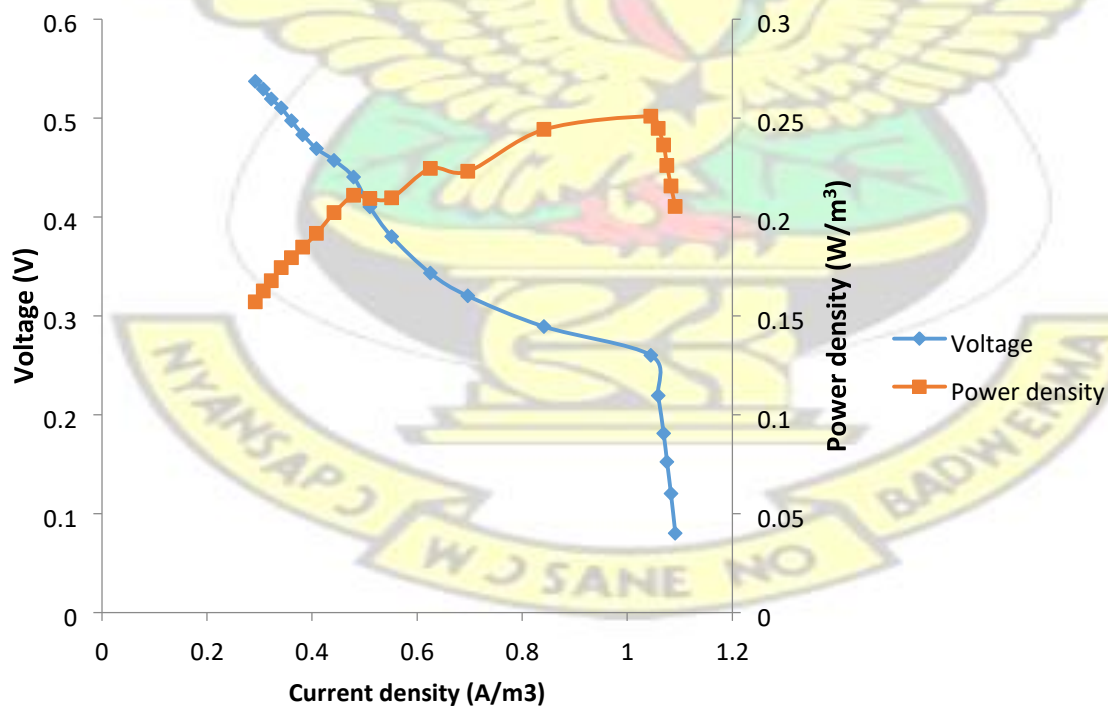
Figure 4.6: Power density and polarization curve of three-chamber plant-supported cathode MDC

4.1.2 Desalination Performances of the three-chamber MDCs

The three-chamber chemical catholyte MDC (3 C C MDC) produced the highest

percentage desalination of 22.06% in batch 2 and the least percentage salinity reduction of

4.65% recorded from the plant-supported MDC (3 P C MDC) (Fig 4.7).   A production of

1% salinity reduction in control experiment was attributed to dilution (water flux) due to

concentration gradient between the less concentrated anolyte and the concentrated solution

of the desalination chamber. A similar observation of dilution effect due to concentration

gradient was reported by Mehanna et al. (2010). On the other hand, desalination in 3 C C, 3

W C and 3 P C MDCs were attributed to electricity production by exoelectrogenic bacteria

because no obvious water flux was observed in these MDCs.

The comparatively higher desalination performance of the three-chamber chemical

catholyte MDC than the 3 W C and 3 P C MDCs could be attributed to its higher voltage

productions. Desalination in MDCs results from electricity production by exoelectrogens

(Cao et al., 2009; Yang et al., 2014). Meanwhile except for the three-chamber water

catholyte MDC, the other MDCs exhibited a non-uniform trend in desalination performance

across batches. The three-chamber chemical catholyte MDC's percentage

desalination increased in batch 2 and then declined in batch 3 (Fig 4.7). Its Batch 2 higher

percentage desalination could be attributed to the observation that; average voltage

productions recorded from it were higher than those recorded from the other batches (batch

1 and 3) (Fig 4.1).

On the other hand, the decline in percentage desalination of the plant-supported MDC in

batch two was attributed to the death of the aquatic plants. The death of the plants resulted

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in reductions in oxygen concentration from 6.80 ± 0.11 to 4.08 ± 0.22 mg/l and this

consequently resulted in the reductions in voltage production (Fig 4.1) and percentage

desalination (Fig 4.7) observed. However, when the dead aquatic plants were replaced with

fresher ones in batch 3, percentage desalination increased (Fig 4.7) as a result of an increase

in oxygen concentration (From 4.08 ± 0.22 to 6.10 ± 0.37) and voltage

productions (Fig 4.1).
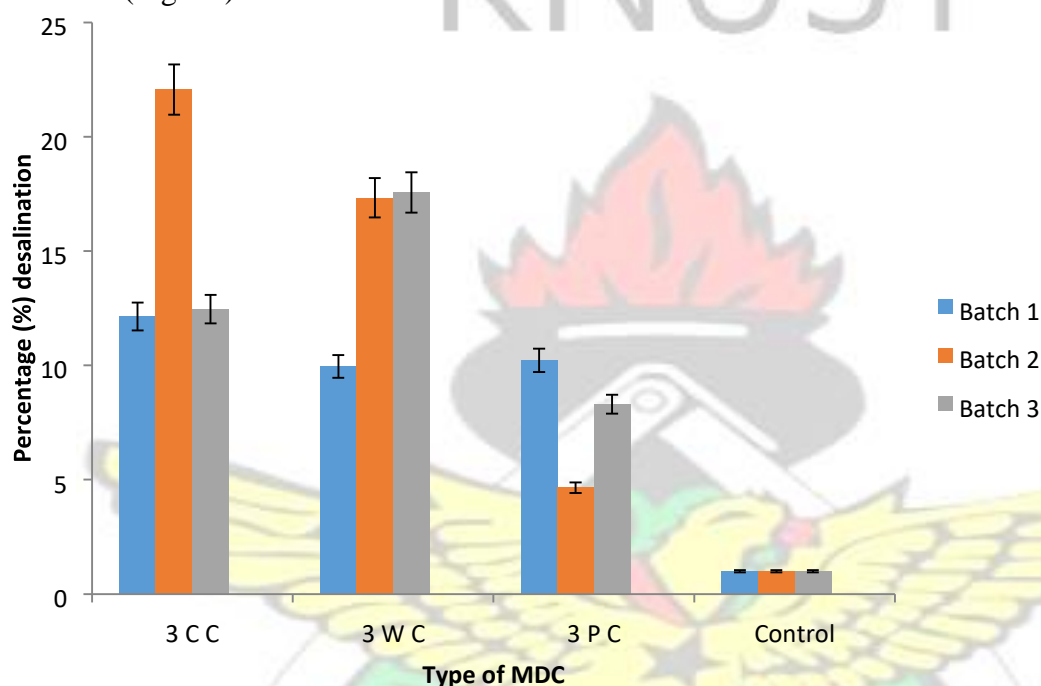
Figure 4.7: Percentage desalinations of the three-chamber MDCs

[3 C C - Three-chamber chemical catholyte 3 W C- Three-chamber Water catholyte, 3 P C - Three-chamber Plant-supported MDC]

Table 4.1: Final electrical conductivities of saline water in desalination chambers across batch cycles

MDC Type	Electrical Conductivity (mS/cm)		
	Batch 1	Batch 2	Batch 3
3 C C	47.00 ± 0.42	35.20 ± 4.08	28.54 ± 3.34
3 W C	48.18 ± 0.47	38.91 ± 0.28	29.51 ± 4.26
3 P C	48.03 ± 3.11	45. 59 ± 2.29	41.02 ± 2.86

Results from the measurement of changes in electrical conductivities (EC) of desalination chambers, anolytes and catholytes provided some proof of desalination due to ion migration from the middle chambers. The electrical conductivities of catholytes (Table 4.3) and anolytes (Table 4.2) increased whiles that of the salt solution in the middle chamber (Table 4.1) decreased. These EC changes resulted from the movement of Cl$^-$ and Na$^+$ ions out of the desalination chamber into anolytes and catholytes respectively in the desalination process. An earlier work by Zuo et al. (2013) on competitive migration of ions confirmed that, ions are able to move through selective membranes. Therefore, the association of changes in electrical conductivities of anolytes and catholytes to ion migration from the middle chamber was not an isolated conclusion.

Table 4.2: Anolyte electrical conductivities (EC) across batch cycles

MDC type	Initial EC (mS/cm)	Batch 1 Final(mS/cm)	Batch 2 Final(mS/cm)	Batch 3 Final(mS/cm)
3 C C	4.347 ± 0.07	1.86 ± 0.03	1.81 ± 0.02	1.18 ± 0.05
3 P C	4.347 ± 0.07	1.29 ± 0.06	1.36 ± 0.08	1.04 ± 0.06
3 W C	4.347 ± 0.07	1.55 ± 0.40	1.30 ± 0.37	1.16 ± 0.12

Where C C represents chemical catholyte; P C represents plant-supported catholyte; W C represents water catholyte. **Note: Fresh anolytes were used at the start of each batch cycle**

Table 4.3: Catholyte electrical conductivities across batch cycles

MDC type	Initial EC (mS/cm)	Batch 1 Final(mS/cm)	Batch 2 Final(mS/cm)	Batch 3 Final(mS/cm)
3 C C	6.95 ± 0.14	7.52 ± 0.25	7.23 ± 0.25	7.21 ± 0.12
3 P C	1.03 ± 0.06	1.60 ± 0.52	1.39 ± 0.03	1.57 ± 0.54

3 W C	1.01 ± 0.02	1.93 ± 0.07	1.24 ± 0.09	1.49 ± 0.54

Note: Fresh catholytes were used at the start of each batch cycle

4.1.3 Anolyte and Catholyte pH changes in three-chamber MDCs

The magnitude of anolyte pH change for all MDCs increased across batch cycles with the highest

pH change (2.45 ± 0.44) recorded in batch 3 of the water catholyte MDC (Fig 4.8).

The magnitude of catholyte pH change also increased across batch cycles with the highest pH change of 1.59 ± 0.35 produced in batch 3 by the three-chamber chemical catholyte

MDC (Fig 4.9).

The decreases in anolyte pH across batches were attributed to the increase in concentration

of H^+ ions within anode brush tips, biofilms and subsequently anolytes of the MDCs. According to Davis et al. (2013), the relative slow movement of buffers into biofilms compared to the faster proton production by bacteria causes the immediate environment of biofilms to be more acidic than the anolyte. Thus, the use of one anode brush for several batch cycles would likely result in situations where subsequent anolyte solutions become more acidic faster than previous ones irrespective of the type of buffer used. This explains the differences in magnitude of anolyte pH changes (Fig 4.8) across batch cycles observed in this study. Cathodic pH rise on the other hand was attributed to the build-up of hydroxyl ions in the cathode chamber (Qu et al. 2012).
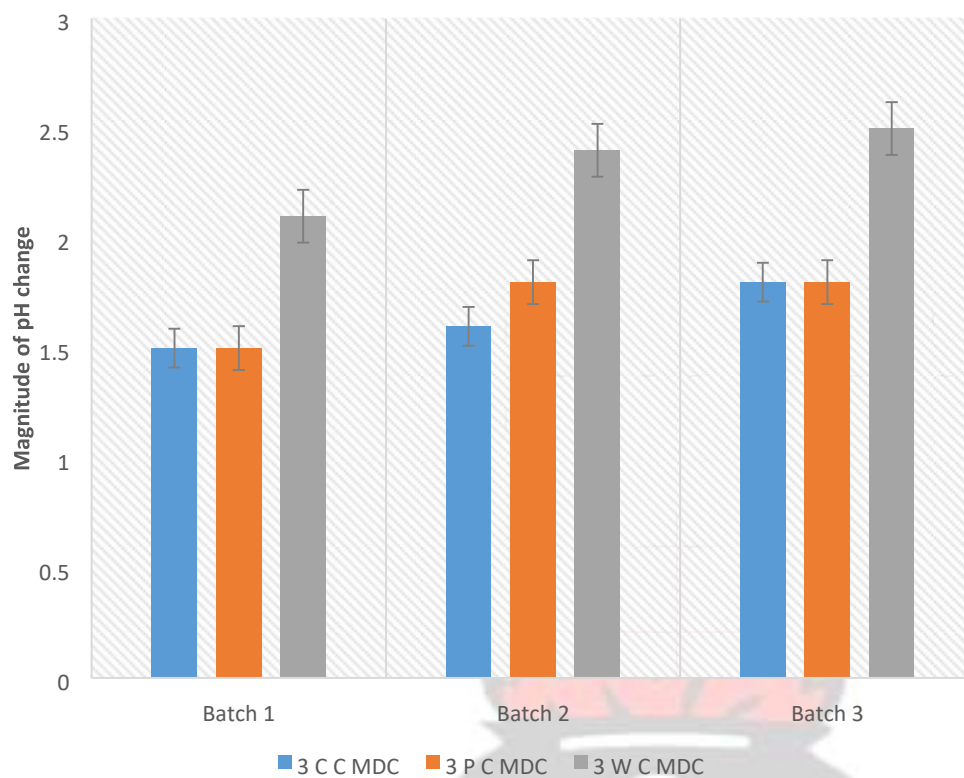
Figure 4.8: Magnitude of anolyte pH change across batch cycles
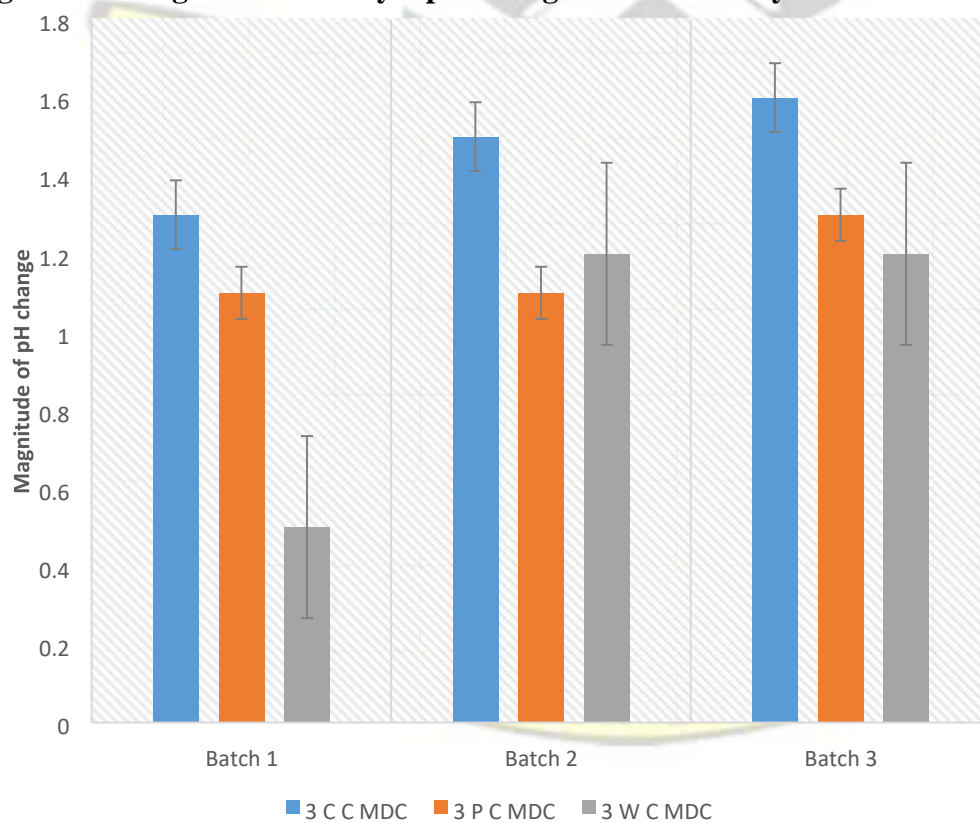
Figure 4.9: Magnitude of catholyte pH change across batch cycles

4.1.4 Wastewater Treatment Efficiencies of the three-chamber MDCs

The wastewater treatment performances of the MDCs were assessed based on their abilities to reduce COD, nitrate and phosphorus. Coulombic efficiency of each MDC was also assessed in relation to its COD reduction.

4.1.4.1 COD reduction and Coulombic efficiency (CE)

The highest percentage COD reduction (42.81%) from an initial of 645 ± 2.3 mg/l was achieved in batch 1 with the three-chamber chemical catholyte MDC whiles the least of 40.04% was achieved in batch 3 by the three-chamber Plant -supported MDC (Fig. 4.10). For the three-chamber chemical catholyte (3 C C) and plant-supported (3 P C) MDCs, COD reduction performance decreased from batch 1 through to 3 whiles that of the water catholyte (3 W C) MDC improved in batch 2 but declined in batch 3 (Fig. 4.10).

The decline in COD reduction efficiency observed in the 3 C C and 3 P C MDCs was attributed to the continuous decline in anolyte pH (Appendix 7a) across batches. Microbial metabolic activities (growth) lead to the release and accumulation of protons which then causes the reduction of pH to acidic conditions (Davis et al., 2013). Acidic conditions reduce the metabolic activities (can cause death) of microbes and thus will lower COD reductions as reported. An increase in COD reduction efficiency recorded for the threechamber water catholyte MDC in batch 2 was possibly due to the stirring effect (Stirring of its water catholyte). Stirring increased the concentration of dissolved oxygen in the water catholyte creating a concentration gradient between it and adjacent chambers. This possibly facilitated the diffusion of oxygen to the anolyte where it caused the oxidation of organic matter. Almatouq (2017) also reported of how the diffusion of oxygen to anolyte through aeration leads to COD reductions. Therefore, stirring or aeration of cathodes should be controlled to a point where diffusion of oxygen to anolytes would be impossible.

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On the average the three-chamber chemical catholyte (3 C C) MDC could produce a coulombic efficiency of 78.61% and the three-chamber water catholyte (3 W C) MDC, a coulombic efficiency of 19.52% whiles the three-chamber plant-supported (3 P C MDC) could produce a coulombic efficiency of 10.03%. Comparing the CEs of the 3 W C and 3 P C MDCs to the range of between 17.2 and 5.82% reported by Kokabien and Gude (2015) these values of 19.52% and 10.03% were not the worse in literature. The low coulombic efficiencies of the 3 W C and 3 P C MDCs may have resulted from the utilization of most of the organic substrates in their anolytes by fermentative bacteria instead of by exoelectrogens. The negative effect of fermenters can be reduced by sparging with nitrogen gas before start up (Zhang et al., 2012) however this was not carried out in this study.
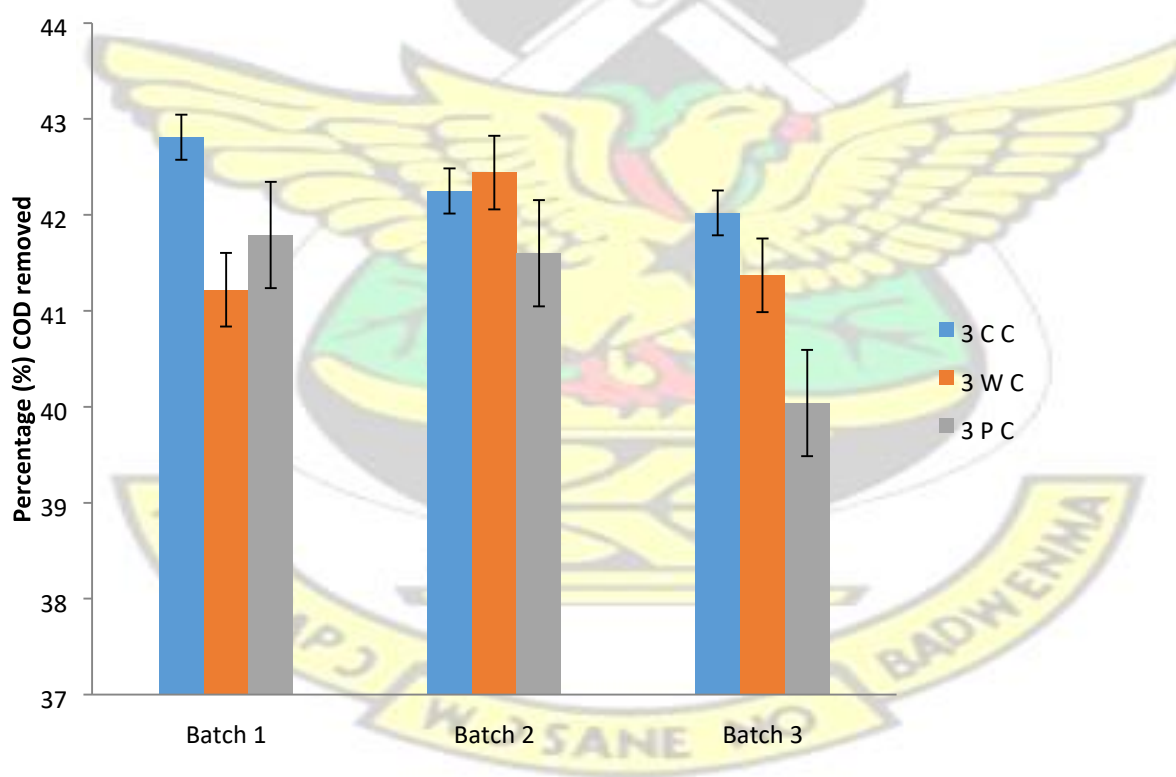
Figure 4.10: Percentage COD removed by the three-chamber MDCs
[3 C C - chamber chemical catholyte MDC, 3 W C – 3 chamber water catholyte MDC, 3 P C – 3 chamber plant supported-cathode MDC].

4.1.4.2 Nitrate and Phosphorus Reduction by the three-chamber MDCs

Nitrate removal efficiencies in all the types of MDCs showed a downward trend from batch 1 to batch 3 (Fig. 4.11). The highest of 1.41% from an initial of 18.54 ± 0.72 was achieved in batch 1 by the three-chamber chemical catholyte MDC whiles the least of 0.39 % was achieved in batch 3 by the three-chamber plant-supported MDC. The higher nitrate removal efficiency of the three-chamber chemical catholyte MDC was possibly due to its relatively favourable anolyte pH conditions (least anolyte pH drop to 5.32 ± 0.16; Appendix 7a). This possibly allowed for higher microbial survival and subsequent

utilization of the nutrient for growth.

Nitrate removal efficiencies achieved with the three-chamber MDCs were low when compared to the 90.5% removal (Initial of 20 mg/l) reported by Zhang and Angelidaki (2013). The better nitrate removal efficiency of the Submerged Microbial DesalinationDenitrification Cell (SMDDC) used by Zhang and Angelidaki (2013) than the MDCs used in this study was probably because of the use of pre-colonized denitrification bacteria cathodes in the SMDDC. Such pre-treatments facilitate reaction processes.
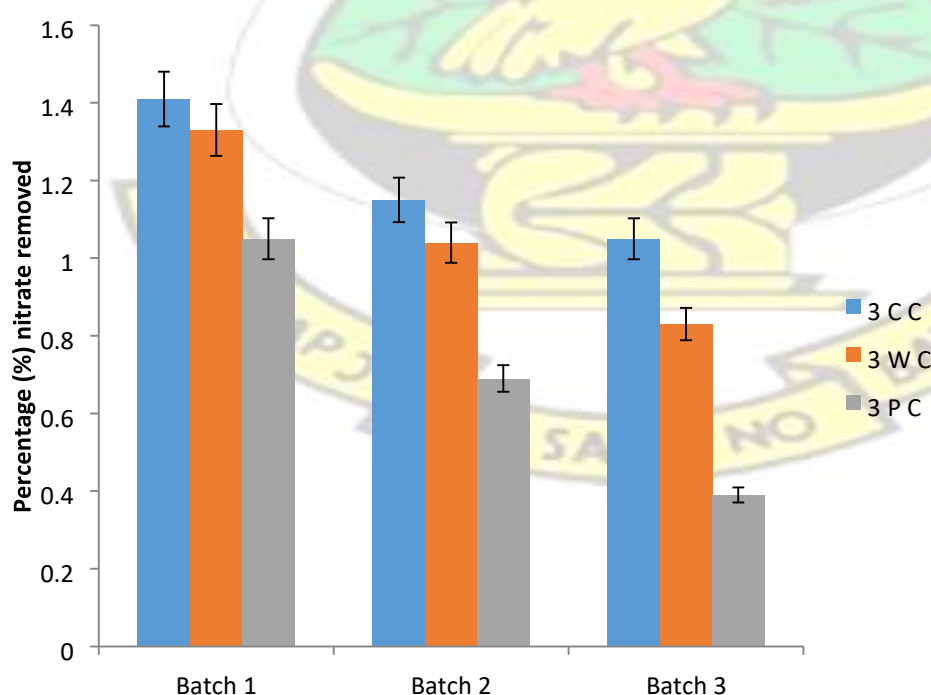
Figure 4.11: Percentage of nitrate removed by the three-chamber MDCs

[3 C C - 3 chamber chemical catholyte MDC, 3 W C – 3 chamber water catholyte MDC, 3 P C – 3 chamber plant supported-cathode MDC].

Similar to nitrate removal, phosphorus removal efficiencies decreased from batch 1 through to batch 3 (Fig 4.12). The highest percentage phosphorus reduction of 10.71% from an initial of 46.36 ± 0.6 was achieved by the three-chamber chemical catholyte MDC and the least of 5.28%, by the plant-supported MDC in batch 3. Phosphorus reduction in the MDCs was attributed to polyphosphate accumulating microorganisms (PAO) which can bring about the accumulation of phosphorus in the anode immediately after start up (before the anode chamber could became anaerobic). This was possibly the case because the anodes were not sparged with nitrogen gas to remove oxygen before start-ups. PAOs are known to accumulate phosphorus under aerobic (Tao et al., 2014) and anoxic (Zeng et al., 2003) conditions.
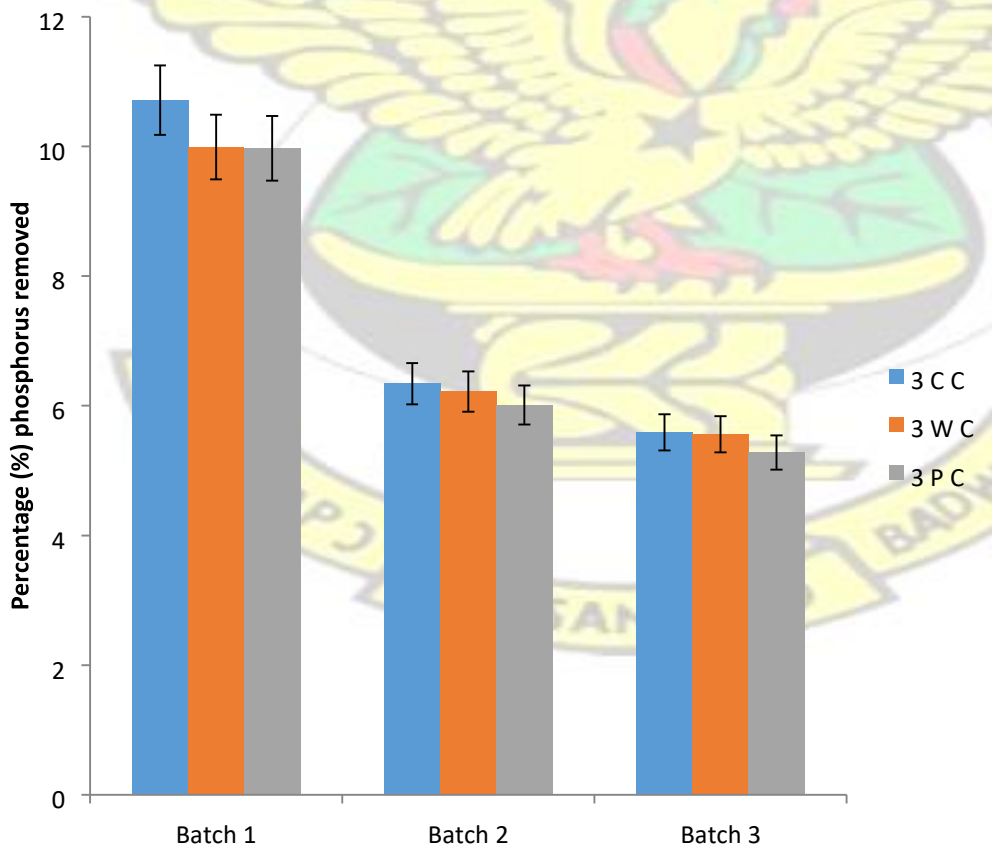
Figure 4.12: Percentage of phosphorus removed by three-chamber MDCs

[3 C C - chamber chemical catholyte MDC, 3 W C – 3 chamber water catholyte MDC, 3 P C – 3 chamber plant supported-cathode MDC].

4.2 Comparison between pH Stability and Performance Efficiencies of the fivechamber and three-chamber MDCs

Comparisons between the performances (pH control, electricity generation, desalination and wastewater treatment) of the five-chamber MDCs and the three-chamber MDCs are presented here. For pH control, comparisons were centred on the ability of neutralization chambers of the five-chambered MDCs to stabilize pH as compared to the ability of potassium phosphate buffer to do same in the three-chamber MDCs. The three-chamber MDCs selected for this study were the three-chamber chemical catholyte and water catholyte MDCs. This decision was based on their demonstrated potentials. MDCs compared were operated simultaneously.

4.2.1 Comparison between the pH Stabilization ability of Neutralization Chambers and Phosphate Buffers

The anolyte pH of the five-chamber chemical catholyte and five-chamber water catholyte MDCs decreased across batch cycles (Table 4.4) whiles their catholyte pH increased (Table 4.5). The pH of the neutralization chambers adjacent the anode side of both fivechamber MDCs decreased across batches whiles those on the cathode side increased (Table 4.4 and 4.5).

Table 4.4: pH changes in anolytes and neutralization chambers (N_A)

		Batch 1		Batch 2		Batch 3	
MDC Type	Initial pH	Final pH	pH change	Final	pH change	Final	pH change
5 C C	7.08 ± 0.02	5.42 ± 0.11	1.66 ± 0.09	5.29 ± 0.14	1.79 ± 0.11	5.10 ± 0.01	1.98 ± 0.01

MDC Type	Initial pH	Final pH	pH change	Final	pH change	Final	pH change
5 CC N_A	7.14 ± 0.06	7.03 ± 0.08	0.12±0.04	7.03 ± 0.02	0.12 ± 0.01	6.98±0.13	0.17 ± 0.02
5 W C	7.08 ± 0.02	5.00 ± 0.01	2.08 ± 0.01	4.94 ± 0.01	1.99 ± 0.01	4.90 ± 0.13	2.22 ± 0.10
5WC N_A	7.14 ± 0.06	7.01 ± 0.04	0.14 ± 0.02	6.99 ± 0.02	0.15 ± 0.01	6.99 ± 0.03	0.15 ± 0.01

Where:

5 C C - 5 chamber chemical catholyte MDC;

5 W C- 5 chamber water catholyte MDC

5 CC N_A- 5 chamber chemical catholyte MDC's Neutralization chamber, anode side;

5 WC N_A- 5 chamber water catholyte MDC's Neutralization chamber, anode side

5 CC N_C- 5 chamber chemical catholyte MDC's Neutralization chamber, cathode side;

5 WC N_C- 5 chamber water catholyte MDC's Neutralization chamber, cathode side

Between the five-chamber MDCs, the most stable anolyte pH conditions (batch 1) was produced by the five-chamber chemical catholyte (5 C C) MDC represented by anolyte pH change of 1.66 ± 0.11 (Table 4.4) and the most stable catholyte pH change of 0.52 ± 0.30 (Table 4.5) produced by the five-chamber water catholyte MDC. However, when these pH changes were compared to the most stable pH conditions produced by the three-chamber MDCs, those of the five-chamber MDCs were higher. The least anolyte pH change in the 3 C C MDC for instance was 1.52 ± 0.15 (Fig. 4.8, batch 1) whiles the least catholyte pH rise of 0.48 ± 0.54 (Fig 4.9) was recorded from the three-chamber water catholyte MDC. The pH stability in anolytes of the five-chamber MDCs were however appreciable than that of the three-chamber water catholyte MDC which produced a least pH change of 2.14 ± 0.58; Fig. 4.8, batch 1).

Table 4.5: pH changes in catholytes and neutralization chambers (N_C)

		Batch 1		Batch 2		Batch 3	
MDC Type	Initial pH	Final pH	pH change	Final	pH change	Final	pH change

5 C C	7.12 ± 0.11	8.65 ± 0.18	1.53 ± 0.11	8.78 ± 0.27	1.66 ± 0.12	8.90 ± 0.16	1.78 ± 0.11
5 CC N$_C$	7.14 ± 0.06	7.22 ± 0.08	0.08 ± 0.05	7.60±0.25	0.46 ± 0.20	7.69 ± 0.14	0.55 ± 0.09
5 W C	7.11 ± 0.09	7.63 ± 0.43	0.52 ± 0.30	7.97 ± 0.03	0.86 ± 0.01	8.08 ± 0.10	0.97 ± 0.09
5WC N$_C$	7.14 ± 0.06	7.17 ± 0.01	0.03 ± 0.01	7.19 ± 0.02	0.05 ± 0.01	7.21 ± 0.03	0.07 ± 0.01

Where:

5 C C - 5 chamber chemical catholyte MDC;

5 W C- 5 chamber water catholyte MDC

5 CC N$_A$- 5 chamber chemical catholyte MDC's Neutralization chamber, anode side;

5 WC N$_A$- 5 chamber water catholyte MDC's Neutralization chamber, anode side

5 CC N$_C$- 5 chamber chemical catholyte MDC's Neutralization chamber, cathode side; 5

WC N$_C$- 5 chamber water catholyte MDC's Neutralization chamber, cathode side

Unlike the three-chamber MDCs whose pH regulation was attributed to the buffer action of the phosphate buffer used, that of the five-chamber MDCs was attributed to two phenomena. That is, the movement of H^+ and OH^- ions from anolytes and catholytes respectively, into the adjacent neutralization chambers due to concentration gradient and the other, dilution of the concentrations of H^+ and OH^- ions in anolytes and catholytes respectively due to water flux from the neutralization chambers. The dilution effect was most obvious because, the volumes of the anolytes and catholytes of the 5 C C MDC for instance increased from 220 ml to approximately 222 and 224 ml respectively, with corresponding decrements in the volumes of water in the neutralization chambers (N$_A$, from 100 to 95 ml and N$_C$, 100 to 93 ml). This observation indicated the occurrence of water osmosis from the neutralization chambers into adjacent chambers.

Decrements in pH of the neutralization chambers (5CC N$_A$ and 5WC N$_A$) adjacent the anode chambers and increment in pH of the neutralization chambers (5 CC N$_C$ and 5 WC N$_C$) adjacent the cathode chambers were indications of movement of H^+ and OH^- ions into the

respective neutralization chambers. These ion migrations though very small as indicated in the small pH changes in the neutralization chambers (Table 4.4 and 4.5) were possibly facilitated by ionic gradients (H^+ and OH^- ions) between the electrolytes (anolytes and catholytes) and the tap water in the neutralization chambers.

4.2.2 Comparisons of the Electricity Generation Capacities of five-chamber and three-chamber MDCs

The electricity generation capacities of the five and three-chamber MDCs were assessed based on their voltage and power productions. Polarization curves were used to describe voltages as a function of current densities produced by the MDCs.

4.2.2.1 Voltage Produced by five-chamber MDCs and their Comparisons with three-chamber MDCs

Voltage productions of the five-chamber MDCs initially peaked but subsequently declined (Fig. 4.13). This declining trend was attributed to the increase in internal resistance as desalination progressed. The initial internal resistance of the MDCs was 48 Ω but this increased to 90 Ω for the five chamber chemical catholyte MDC and 185 Ω for the fivechamber water catholyte MDC (Fig 4.15). Cao et al. (2009) also reported of a similar observation of a decrease in voltage production due to increase in internal resistance resulting from the decreasing trend in electrical conductivity (desalination) in the desalination chamber.

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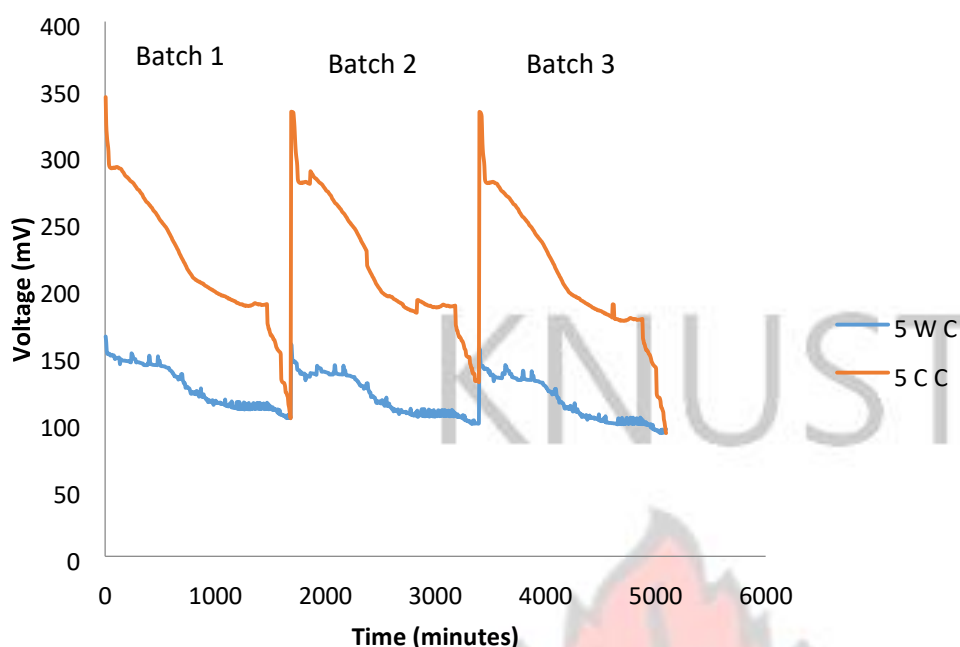
Figure 4.13: Voltage profiles of five-chamber chemical catholyte MDC (5 C C) and 5 chamber water catholyte MDC (5 W C)

The five-chamber chemical catholyte MDC produced the highest peak voltage of 343.57 ± 0.25 mV (Fig. 4.14) between the five-chamber MDCs. This was also higher than the peak voltages produced by the three-chamber chemical catholyte MDC (282.91 ± 0.09 mV; Fig. 4.14) and the three-chamber water catholyte MDC (193.99 ± 0.80 mV; Fig. 4.14). On the other hand, the five-chamber water catholyte MDC produced the least peak voltage of 164.46 ± 0.07 mV (Fig. 4.14) amongst all MDCs compared. Analysis of variance showed that the chemical catholyte MDCs (3 C C and 5 C C MDCs) produced significantly ($p = 0.000$) higher voltages than the water catholyte MDCs (3 W C and 5 W C). The lower voltage production of the water catholyte MDCs could be due to the slow oxygen reduction at the cathode surface under ambient conditions. Gude, et al. (2013) explained that, in the absence of a catalyst, reduction of oxygen is slow due high activation over potentials. The consequence of high activation over potentials, is low voltage production as observed in the water catholyte MDCs studied.
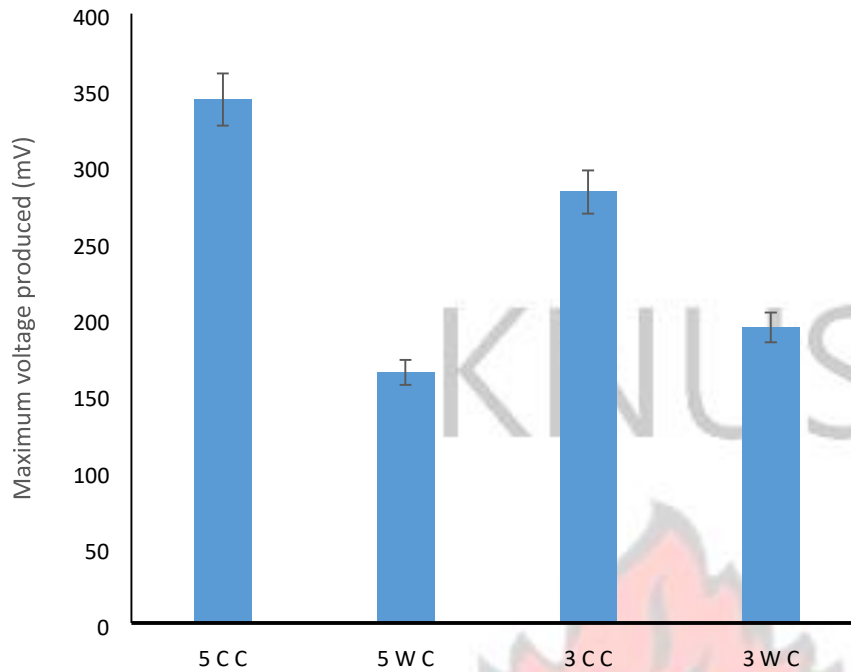
Figure 4.14: Peak voltages produced by the five and three-chamber MDCs

The high internal resistance (185 Ω, Fig. 4.15) of the five-chamber water catholyte MDC was implicated as a contributing factor to its low voltage production. This relation was drawn because MDCs in this research which produced higher voltages had relatively lower internal resistances. The internal resistance of the other (5 C C, 3 C C and 3 W C) MDCs were 90 Ω for 5 C C MDC (Fig. 4.15); 48 Ω for 3 C C MDC (Fig. 4.3) and 146 Ω for 3 W C MDC (Fig. 4.3).

Quite revealing, water osmosis was implicated as a contributor to voltage productions in the five-chamber MDCs. Water flux across membranes is able to move cations along thereby supporting current productions. This assertion was also made by Zhang and He (2012) who reported that, ion migration between anolytes and salt water in the desalination chamber was necessary for electricity production in the microbial desalination cell they studied. Though Zhang and He (2012) used a OsMDC unlike the five-chmaber MDC investigated in this study, the underlying fact for comparing inferences was the confirmed water flux. For

instance, the volume of salt water in the desalination chamber of the five-chamber water catholyte MDC increased from ~ 75 cm^3 to ~ 76.5 cm^3 providing evidence of water flux.
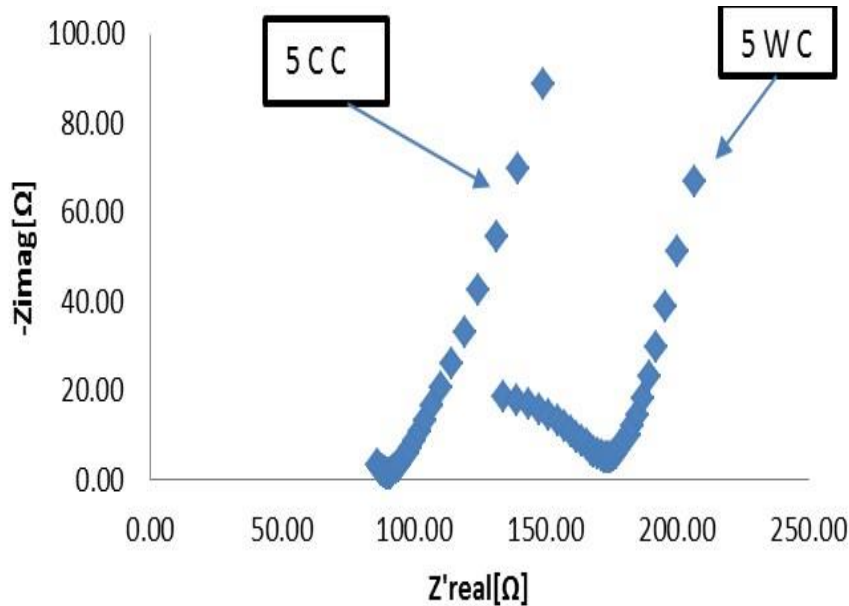
Figure 4.15: Internal resistance of five-chamber MDCs (Nyquist plots)
[zimag represents imaginary part of impedance and Z'real-represents real part of impedance]
4.2.2.2 Comparisons of Power Productions from the five and three-chamber MDCs

The five-chamber chemical catholyte MDC produced the highest power density of 0.622 ± 0.13 W/m^3 (Fig. 4.16) amongst the MDCs compared. The three-chamber water catholyte MDC on the other hand produced the least of 0.267 ± 0.06 W/m^3 (Fig. 4.5). Higher exoelectrogenic metabolic activities and electricity production due to ion transport along water osmosis were attributed to the higher power production of the five-chamber MDCs.
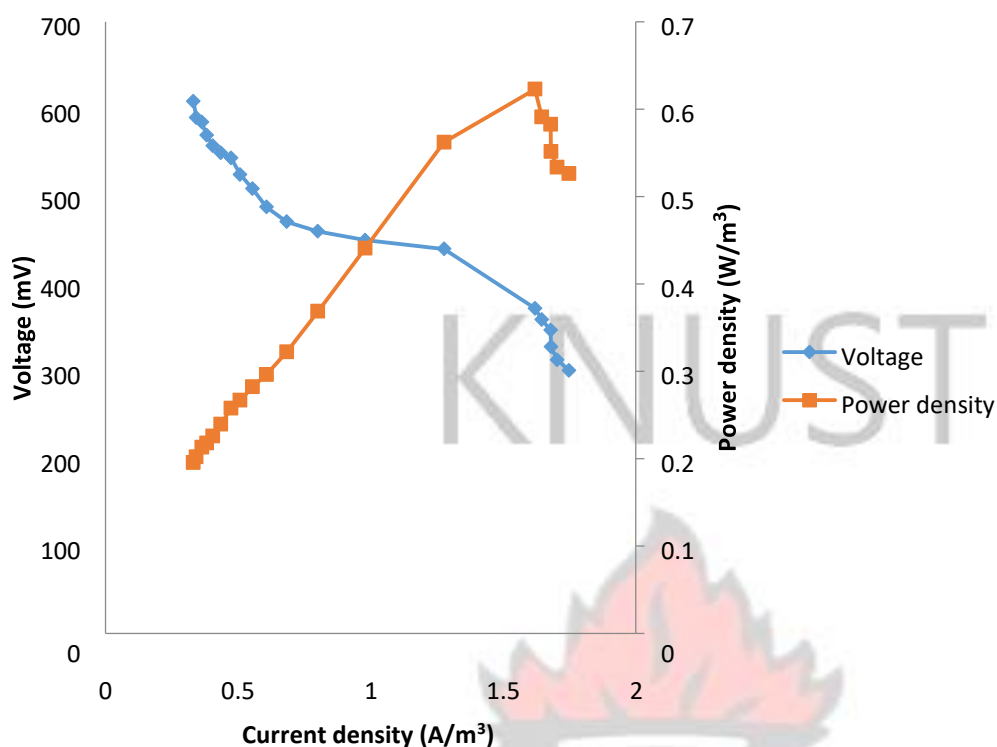
Figure 4.16: Power density and polarization curves of five-chamber chemical catholyte MDC

Polarization curves (Fig. 4.16 and 4.17) of the five-chamber MDCs showed that, the MDCs were affected by all three types of voltage losses associated with

bioelectrochemical systems. These voltage losses were rapid voltage loss at low current densities which is an indication of activation losses (Logan, 2008), constant voltage losses a reflection of ohmic losses (Logan, 2008) and then rapid voltage losses at higher current densities representing mass transport losses (Rismani-Yazdi et al., 2008). A similar trend of voltage losses was observed in the polarization curve of the three-chamber chemical catholyte MDC (Fig 4.4). Thus the three-chamber chemical catholyte MDC and the fivechamber MDCs were described as exhibiting similar voltage versus current density behaviours.
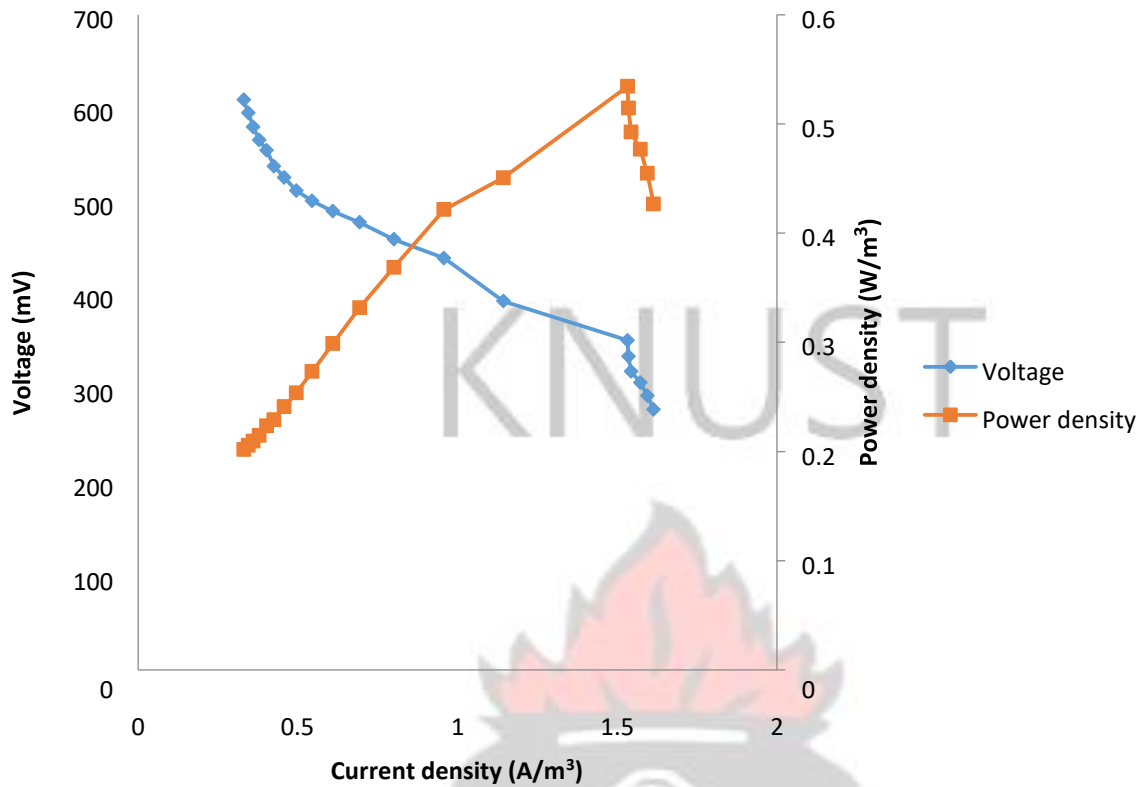
Figure 4.17: Volumetric power density and polarization curves of five-chamber water catholyte (5 W C) MDC

4.2.3 Desalination Efficiencies of five-chamber MDCs and their Comparisons with three-chamber MDCs

Percentage desalination in both five-chamber MDCs decreased from batch 1 through to 3 (Fig. 4.18). The highest total percentage desalination among the five-chamber MDCs after 3 batch runs was 50.01% achieved by the five-chamber chemical catholyte MDC (Fig 4.18). Amongst batch cycles, the least percentage desalination (8.11%) in a batch was produced by five-chamber water catholyte MDC (batch 3, fig 4.18) and the highest of 20.03% produced by the five-chamber water catholyte MDC (Fig. 4.18).

The 5 C C MDC recorded a higher total desalination (50.01%) performance than the threechamber chemical catholyte MDC which's total percentage desalination was 46.66% (Fig. 4.18). The three-chamber chemical catholyte and water catholyte MDCs recorded total

salinity reductions of 46.66% and 44.84% respectively (Fig. 4.18) which were in turn, higher

than the 43.74% (Fig. 4.18) of the five-chamber water catholyte MDC.
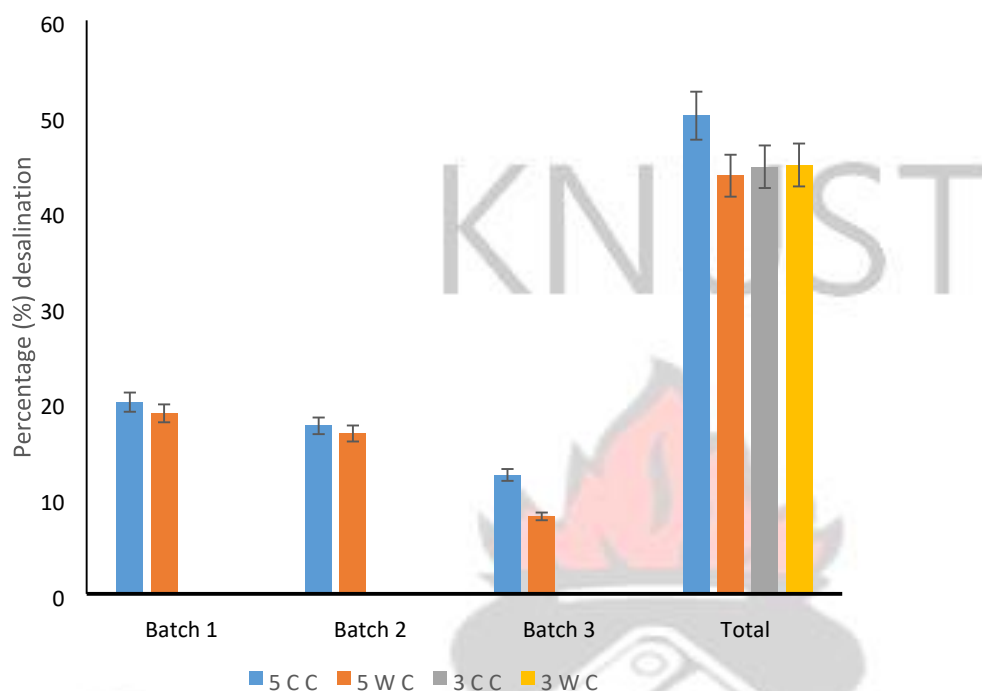
Figure 4.18: Percentage desalinations of the five and three-chamber MDCs

[5 C C-5 chamber chemical catholyte MDC; 5 W C- 5 chamber water catholyte MDC, 3 C C- 3 chamber chemical catholyte MDC, 3 W C- 3 chamber water catholyte MDC. NB: Batch cycle results for 3 C C and 3 W C are presented in figure 4.3]

Contrary to the three-chamber MDCs whose desalination capacities were associated mainly

with electricity production, those of the five-chamber MDCs were attributed to both

electricity production and dilution of the salt concentrations in the middle chamber.

The production of electricity in MDCs causes the movement of Cl^- and Na^+ ions out of the

desalination chamber into anolytes and catholytes respectively thereby leading to

desalination (Cao et al., 2009).

On the other hand, salinity reduction in the five-chamber MDCs due to dilution possibly

resulted from the movement of water from the neutralization chambers into anolytes and

catholytes further increasing the concentration gradient between these electrolytes and

saltwater in the desalination chambers. The resultant salinity gradient was possibly the cause

of water movement from the adjacent chambers into the desalination chambers thus diluting

the concentration of salt in them. Ping and He (2014) also reported of dilution through water

osmosis as a contributor to desalination. They also found that, a wider intermembrane

distance of 2.5 cm at higher salt concentration (30/L) facilitated water osmosis because of

the increase in concentration gradient (Ping and He, 2014).

The inter-membrane distance of the five-chamber MDC used in this study was 6 cm which

was large when compared to several studies including that of Ping and He (2014). Other

studies including that of Morel et al., (2012) and Zuo et al. (2013) used an inter-membrane

distance of 2 cm, whiles Qu et al. (2013) used 3 cm and Ge et al. (2014) used 2 mm spacer

between ion exchange membranes. Thus it was sound to state that, an inter-membrane

distance of 6 cm used in this study was large and facilitated water osmosis into the middle

chamber. This was evident in the increase in water volume of the desalination chamber from

an initial of 75 cm^3 to ~ 76.5 cm^3 at the end of a cycle.

The observation of a decreasing trend in desalination across batches was attributed to the decreasing

trend in electricity generation (Fig. 4.10) and also, the reduction in concentration gradient as

desalination progressed. Desalination due to electricity production is reduced when electricity

generation declines (Cao et al., 2009). Also, with the same salt solution used for all batch cycles, salt

reduction in a previous batch reduced the concentration gradient between the desalination chamber and

adjacent chambers in the subsequent batches thus reducing the contribution of dilution to desalination

in those batches.

4.2.4 Comparisons between Wastewater Treatment Efficiencies of five-chamber and three-chamber MDCs

Wastewater treatment efficiencies of the five and three-chamber MDCs were assessed based on COD, nitrate and phosphorus reductions. Also, the coulombic efficiency of each MDC was assessed in relation to its COD reduction.

4.2.4.1 Comparisons of COD reductions and Coulombic efficiencies of five and three-chamber MDCs

The five-chamber chemical catholyte (5 C C) MDC produced the highest percentage COD removal. It caused a maximum COD reduction of 63.42% (Fig. 4.19, batch 1) from an initial of 645 ± 2.3 mg/l. This was higher than the 48.74% (Fig. 4.19, batch 1) produced by the five-chamber water catholyte MDC, the 42.81% (Fig. 4.19) produced by threechamber chemical catholyte (3 C C) MDC and the 42.44% of the three-chamber water catholyte (3 W C) MDC (Fig. 4.19).

On the average each five-chamber MDCs could cause a COD reduction of 347.46 ± 47.61 mg/l whiles each three-chamber MDCs could produce an average of 271.02 ± 3.99 mg/l COD. This demonstrated that the five-chamber MDCs COD removal performances were 1.3 folds higher than those of the three-chamber MDCs. Aside bacteria reduction, oxidation of substrate by molecular oxygen in fluxed water from neutralization chambers possibly contributed to the high COD reductions of the five-chamber MDCs. This was much probable with the five-chamber water catholyte MDC as it had its catholyte exposed

to air.
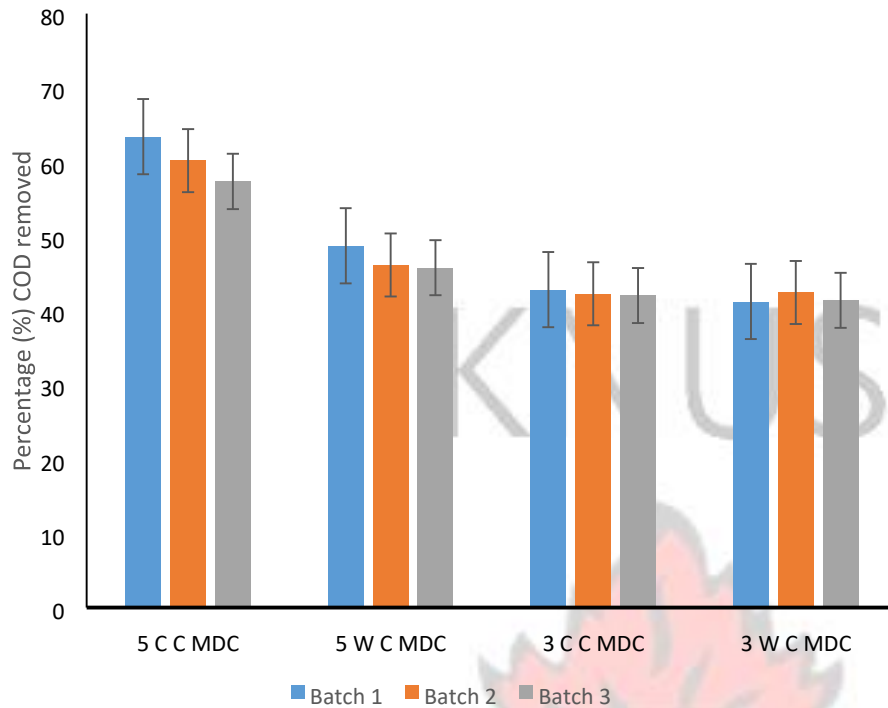
Figure 4.19: Percentage COD removed by the five and three-chamber MDCs

Reproducible coulombic efficiencies (CE) of the five-chamber MDCs were lower as compared to the 78.61% achieved with the three-chamber chemical catholyte MDC. The five-chamber water catholyte (5 W C) MDC produced a CE of 30.57% whiles the fivechamber chemical catholyte (5 C C) MDC produced a 25.20% coulombic efficiency. The low CEs of the five-chamber MDCs were associated with the oxidation of organic matter in anolytes by molecular oxygen in fluxed water from neutralization chambers. Previous studies by Davis et al. (2013) and Almatouq (2017) also linked low coulombic efficiencies to contribution of oxygen to the oxidation of organic substrates. Oxidation of organic matter by oxygen in the anode chamber reduces the concentration of substrates available to exoelectrogens for utilization in electricity production.

Another possible contributing factor to the low CEs in the five-chamber MDCs was the

‗high organic loading' of 3 g/l sodium acetate. High organic loading, according to Zhang and He (2013) supports the proliferation of other microbes (methanogens) to the detriment of exoelectrogens. Also, studies including those of Xiaoying, Yang and Sun (2018), Tom et al. (2016) and Darus (2011) which investigated the effect of substrate loading on CE found that, generally the use of lower organic loading resulted in higher coulombic efficiencies whiles higher organic loading supported other processing like methanogenesis. For instance, in the study by Xiaoying et al. (2018), substrate loading beyond 1 g/l glucose resulted in a decrease in CE by 7.6%.

It should be noted that, the use of a relatively larger MDC in this study informed the choice of 3 g/l acetate concentration but this concentration turned out to have some possible negative effects on CEs. Thus substrate loading in the operations of MDCs should be well controlled to support the production of higher coulombic efficiencies irrespective of the cell size used.

4.3.4.2 Comparisons of Nitrate and Phosphorus reductions of five and three-chamber MDCs

Nitrate reductions in the MDCs were attributed to denitrification by heterotrophic denitrifying bacteria. Tong and He (2013) made a similar assertion after recording a 208.2 $\pm$ 13.3 g NO$_3^-$-N m^{-3} d^{-1} nitrate reduction in their study. The 5 C C MDC yielded the highest nitrate reduction of 2.12% (Fig. 4.20, batch 1) from an initial of 18.54 $\pm$ 0.72 mg/l whiles the 5 W C MDC recorded the least nitrate reduction of 1.14 % (Fig. 4.20, batch 1). The 5 W C MDC nitrate reduction was not only lower than that of the 5 C C MDC but also lower than the highest nitrate reduction achieved with 3 C C (1.41 %; Fig. 4.20, batch 1) and 3 W C MDCs (1.33%; Fig. 4.20, batch 1). The higher nitrate reduction of the 5 C C MDC than the other MDCs was probably due to the contribution of dilution effect from

water osmosis resulting from the neutralization chamber adjacent the anode chamber of the 5 C C MDC.
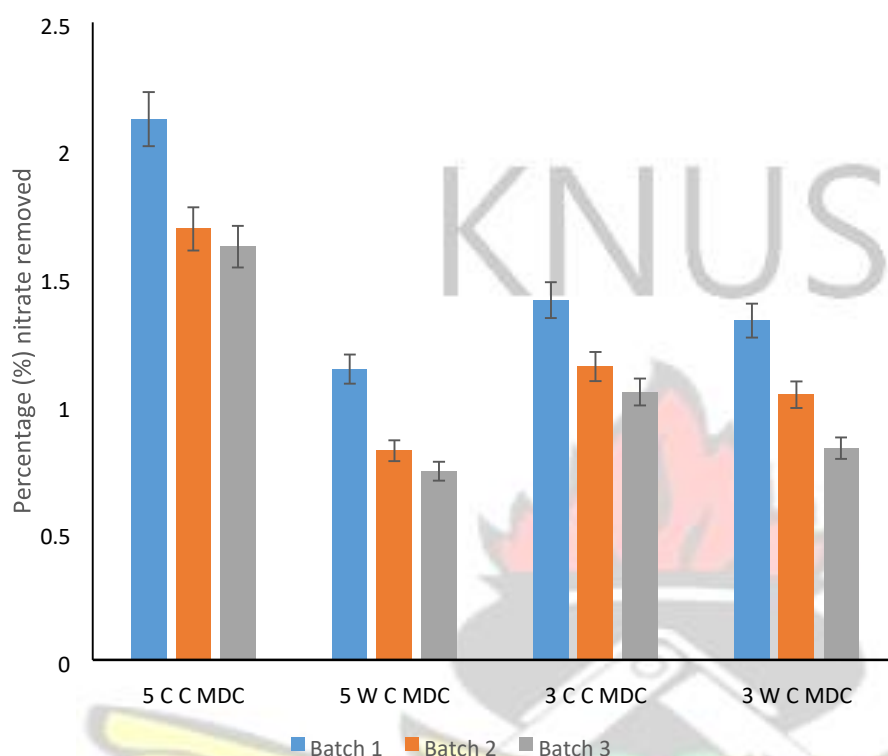
Figure 4.20 Percentage nitrate removed by the five and three-chamber MDCs

Phosphorus reduction in anolytes of MDCs studied was attributed to polyphosphate accumulating microorganisms (PAO) and dilution effect. POAs bio-accumulate phosphorus using oxygen (Tao et al., 2014) thereby reducing the concentration of phosphorus in a medium. Aerobic and anoxic bioaccumulation of phosphorus was possible in these MDCs because anolytes were not purged off oxygen gas before the start-up of experiments. This implied that, the establishment of anaerobic conditions delayed. Interestingly, under anaerobic conditions POAs release phosphorus rather than bioaccumulate them (Tarayre et al., 2016) thus strict anaerobic conditions will not favour phosphorus reduction in a MDC.

Reduction in phosphorus concentration due to dilution was possible especially in the fivechamber MDCs because of water osmosis from the neutralization chambers into anolytes of these MDCs. For instance, the volume of anolyte of the 5 C C MDC increased

from 220 ml to approximately 222 ml. This increase in volume provided evidence of water osmosis from the neutralization chamber and therefore could be responsible for the dilution effect stated.

Comparatively, the three-chamber MDCs recorded better phosphorus reductions than the five-chamber MDCs. The highest phosphorus reduction by the 3 C C MDC was 10.71% (Fig. 4.21) and that of the 3 W C MDC was 9.99% (Fig. 4.21) from an initial of 46.36 ± 0.6 mg/l. Whereas the highest phosphorus removal by the 5 C C MDC was 3.85% and that of the 5 W C MDC was 2.90% from an initial of 33.18 ± 4.7 mg/l (Fig. 4.21). The higher phosphorus reduction performance of the three-chamber MDCs was attributed to the possibility that, the established of anaerobic conditions in them delayed thereby allowing for a longer anoxic bioaccumulation of phosphorus by POAs.
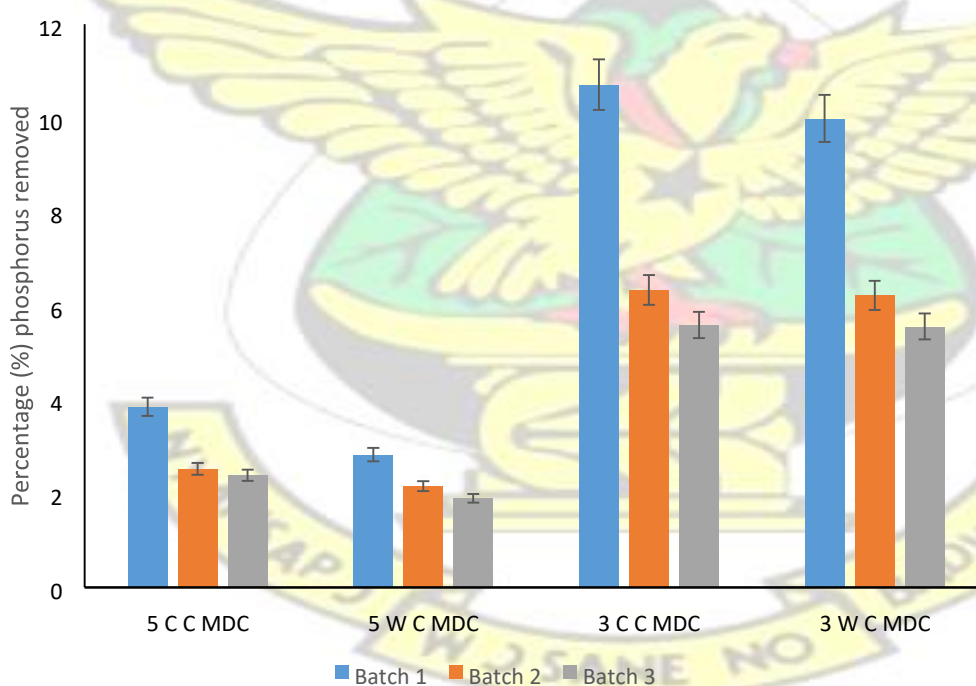
Figure 4.21 Percentage phosphorus removed by the five and three-chamber MDCs

4.3 Effect of Rhamnolipid on the Performances of Five-chamber MDCs

This section presents results and discussions on the effects of rhamnolipid biosurfactant on electricity generation, desalination, pH stabilization, internal resistance and wastewater treatment performances of the five-chamber (5 C C and 5 WC) MDCs.

4.3.1 Effect of Rhamnolipid on the Electricity Production Capacities of the fivechamber MDCs

The effect of rhamnolipid on the electricity production capacities of the five-chamber MDCs were investigated using their voltage, current and power density productions as

indicators.

4.3.1.1 Effect of Rhamnolipid Concentrations on Voltage Productions of the five-chamber MDCs

In this experiment, the highest voltage of 630.60 ± 1.44 mV was produced by the fivechamber chemical catholyte (5 C C) MDC and the least voltage of 164.5 ± 0.11 mV recorded in the experiment with the five-chamber water catholyte (5 W C) MDC (Fig. 4.22). Voltage productions initially increased as concentration of rhamnolipid increased till 240 mg/l of rhamnolipid addition where voltage then declined. The decline in voltages at higher concentrations of the rhanmnolipid was possibly due to the inhibition of bacteria metabolism at these concentrations. According to Nickzad and Deziel (2014) bacteria are intolerant to high concentrations of rhamnolipid.

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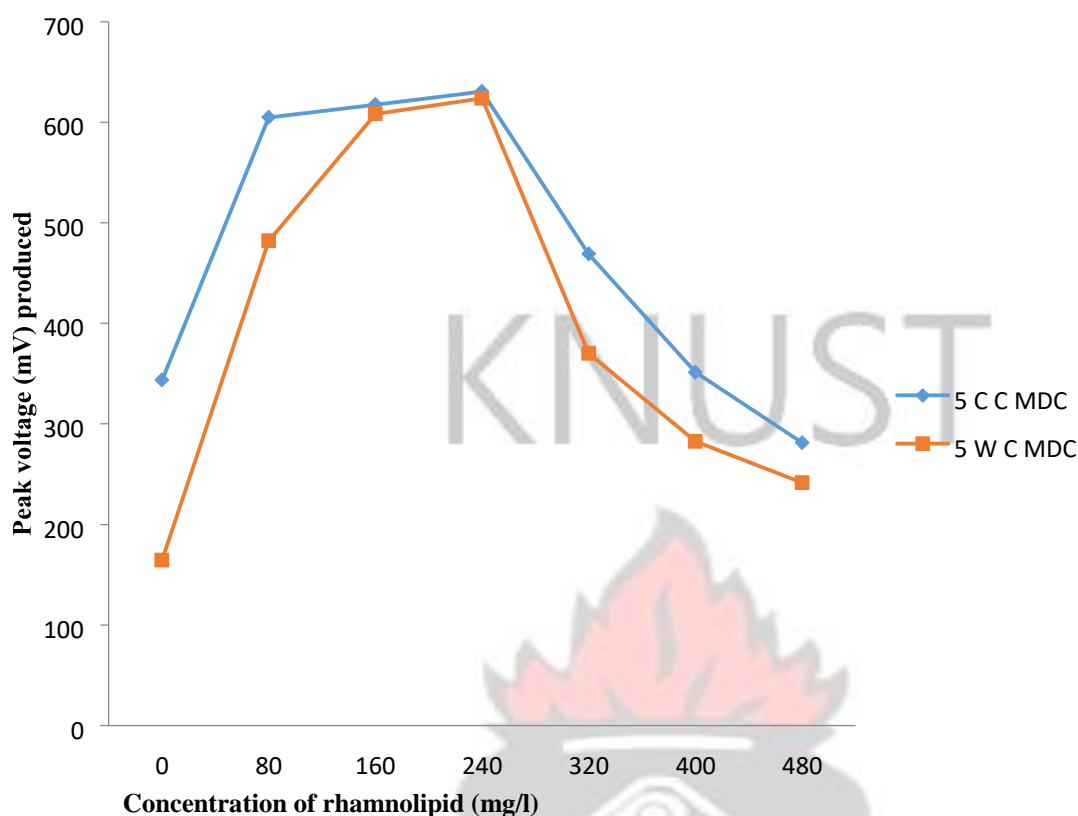
Figure 4.22: Peak voltage production in response to increasing rhamnolipid concentrations

The higher voltage production performance of the 5 C C MDC than the 5 W C MDC was probably because of the better electron acceptance of potassium ferricyanide than molecular oxygen. Kim and Logan (2013) reported of the superiority of potassium ferricyanide over molecular oxygen as terminal electron acceptor. Under ambient conditions, oxygen requires a catalyst to speed up its reduction reactions (Gude et al., 2013) but catalysts were not used in this study hence this could be the reason for the lower performance of the water catholyte MDC. There was however no statistical significant difference ($p = 0.412$) between the mean voltage productions of the five-chamber chemical catholyte MDC and the five-chamber water catholyte MDC.

A comparison between voltages produced at no rhamnolipid (0 mg/l) addition and when rhamnolipid concentration was 240 mg/l (Concentration which produced the highest voltage production, Fig. 4.22) showed that, the addition of 240 mg/l rhamnolipid resulted in a 4-fold increase in voltage production. Thus it was possible that, at the rhamnolipid concentration of 240 mg/l, the cell membranes of the exoelectrogens became much more permeable to electron transfer therefore facilitating higher voltage productions. An earlier study by Wen et al. (2010) also attributed enhanced electricity generation to reduction of bacteria cell membrane resistance to electron transfer due to rhamnolipid addition.

4.3.1.2 Effect of Rhamnolipid Concentrations on Current and Power Densities Current and power densities in both five-chamber MDCs (5 C C and 5 W C MDCs) peaked at 240 mg/l rhamnolipid concentration but declined with further increments in rhamnolipid concentrations (Fig. 4.23 and Fig. 4.24). The highest current density (3.93 ± 0.21 A/m3, Fig. 4.23) and power density (1.14 ± 0.27 W/m3, Fig. 4.24) were produced by the 5 C C MDC and the least current density (1.42 ± 0.05 A/m3, Fig. 4.23) and power density (0.53 ± 0.07 W/m3, Fig. 4.24), by the 5 W C MDC.
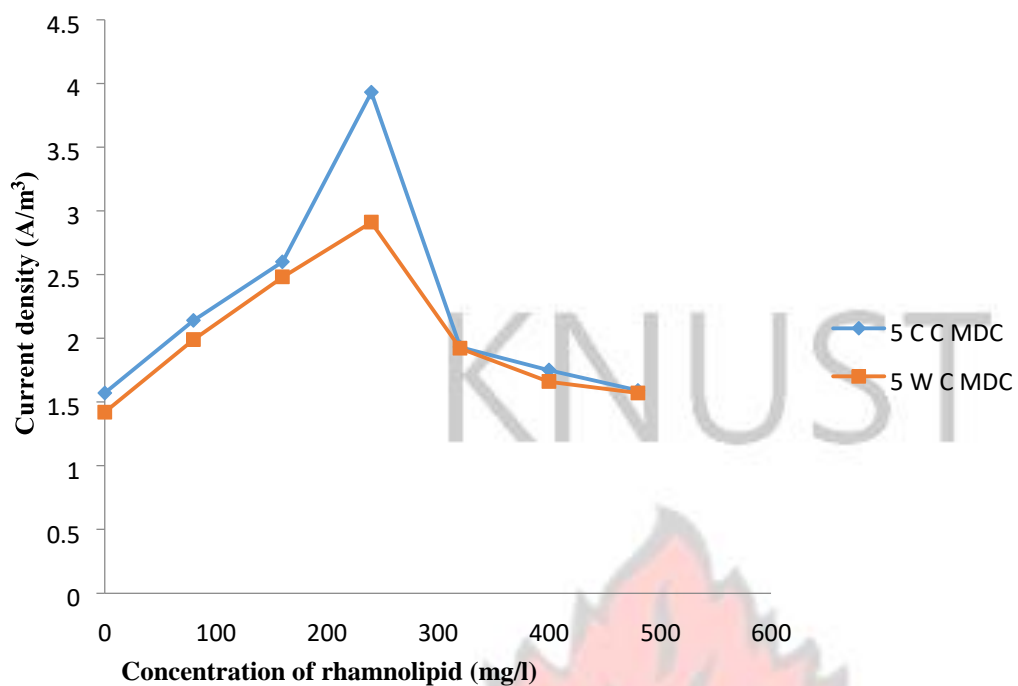
Figure 4.23: Changes in peak current density production in response to increasing concentrations of rhamnolipid
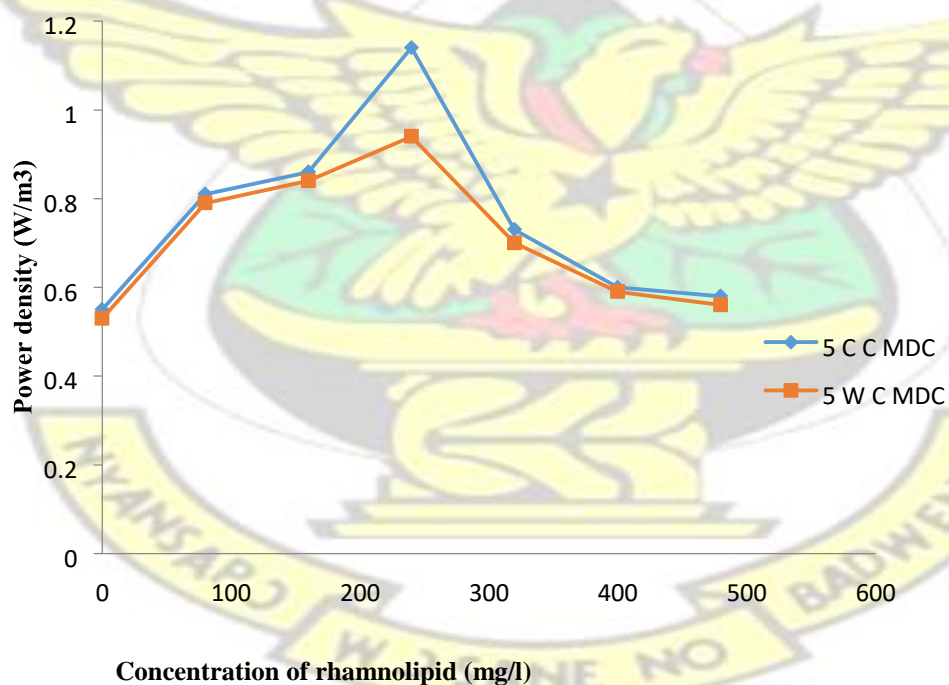
Figure 4.24: Changes in peak power density production in response to increasing concentrations of rhamnolipid

However, there was no statistical significant differences between the current ($p = 0.561$) and power ($p = 0.641$) density productions of both five-chamber MDCs when the MDCs were dosed with rhamnolipid. This implied that, the five-chamber water catholyte MDC could be substituted for the five-chamber chemical catholyte MDC for appreciable current and power density productions. The enhanced current and power densities recorded by the MDCs from 80 mg/l to 240 mg/l rhamnolipid concentration were attributed to the lowering of internal resistance by rhamnolipid (Fig. 4.28). However, the subsequent declines after 240 mg/l rhamnolipid concentration was attributed to the negative effect of higher rhamnolipid concentrations on microbes. According to Nickzad and Deziel (2014), bacteria are intolerant to higher concentrations of rhamnolipids.

Current densities produced by both MDCs (5 C C and 5 W C MDCs) at 240 mg/l rhamnolipid concentration (Concentration which produced the highest current density) were 2-folds higher than those produced when no rhamnolipid (0 mg/l) was supplied to anolytes (Fig. 4.23). Moreover, the power density of the 5 C C MDC at 240 mg/l rhamnolipid concentration was 3-folds higher than that produced at 0 mg/l whereas with the 5 W C MDC, the power density at 240 mg/l rhamnolipid was twice that produced when no rhamnolipid was supplied to its anolyte (Fig. 4.24). These observed improvements in current and power density productions at the 240 mg/l rhamnolipid concentration were associated with the increment in anolyte electrical conductivities upon the addition of the biosurfactant. Addition of rhamnolipid for example increased the initial electrical conductivities of anolytes from $2.37 \times 10^{-3} \pm 0.03$ to 5.33 ± 0.17 mS/cm (Initial EC for 0 and 240 mg/l respectively, Table 4.6) even before desalination started.

Rhamnolipid addition increases the electrical conductivities of anolytes (Wen et al., 2010) which results in reduction of internal resistance and an enhanced electricity generation (Liang et al., 2007).

4.3.2 Effect of Rhamnolipid on Percentage Desalination

Percentage desalination produced by both five-chamber chemical catholyte and fivechamber water catholyte MDCs increased with increasing concentration of rhamnolipid till 240 mg/l rhamnolipid concentration where it then decreased (Fig 4.25). In this experiment, the highest percentage desalination of 67.63% was produced by the 5 C C MDC whiles the least (43.74%) was produced by the 5 W C MDC (Fig 4.25). The better desalination performance of the 5 C C MDC than the 5 W C MDC was attributed to its' higher voltage production. However, a comparison between the average desalination performances of both types of MDCs showed no statistical significant difference (p =

0.355) between their performances.

A comparison of the desalination performances under the conditions of no rhamnolipid (0 mg/l) and when rhamnolipid concentration was 240 mg/l (concentration which produced the highest percentage desalination, Fig. 4.25) showed that, rhamnolipid addition could increase the percentage desalinations of the MDCs (5 C C and 5 W C) by a magnitude of

10%. Since desalination in MDC technology principally relies on electricity production (Yang et al., 2014), the enhanced desalination performance at 240 mg//l rhamnolipid concentration was attributed to the high electricity production at this concentration (Fig. 4.22).
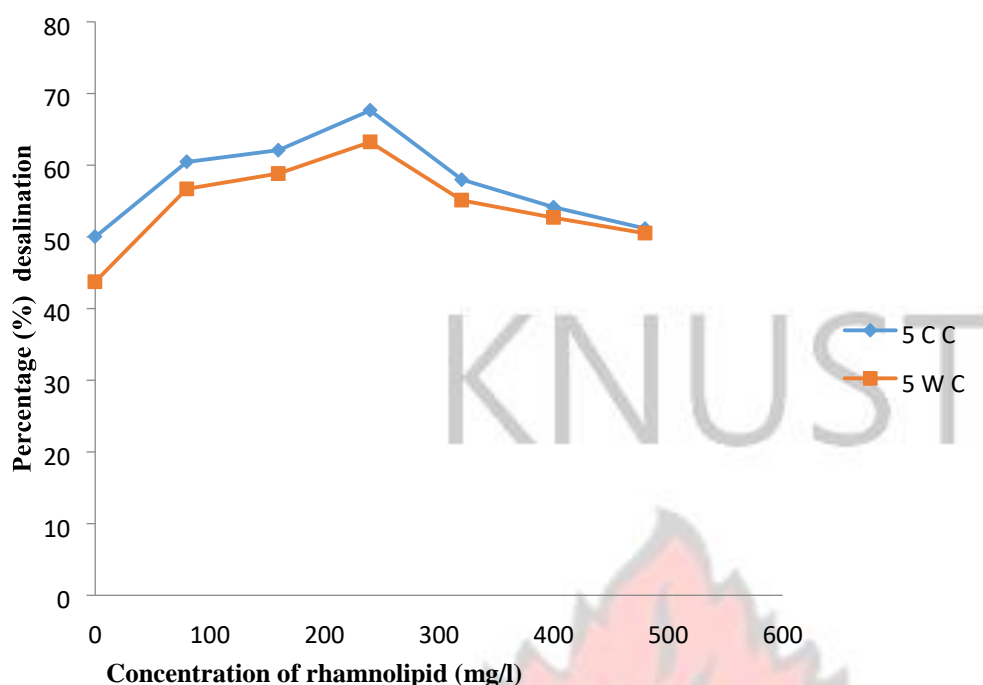
Figure 4.25: Changes in percentage desalination of five-chamber MDCs in response to increasing rhamnolipid concentrations

Also, changes in final anolyte electrical conductivities (EC) were measured to confirm if rhamnolipid addition increased ion migration from the desalination chamber into anolytes. This investigation showed that rhamnolipid addition produced higher final electrical conductivities in anolytes. For instance, rhamnolipid addition at 240 mg/l could produce an increment in electrical conductivity of the five-chamber MDCs from an initial of 2.365 x $10^{-3} \pm 0.01$ mS/cm to 9.48 ± 0.16 mS/cm for the five-chamber chemical catholyte MDC and to 9.12 ± 0.13 for the five-chamber water catholyte MDC (Table 4.6).

Table 4.6: Anolyte electrical conductivity changes with changing concentrations of rhamnolipid

Concentration of biosurfactant (mg/l)	Initial conductivity (mS/cm)	Final conductivity (mS/cm) of 5 C C MDC	Final conductivity (mS/cm) of 5 W C MDC
0	$2.37 \times 10^{-3} \pm 0.03$	2.55 ± 0.05	1.53 ± 0.09
80	1.42 ± 0.12	8.38 ± 0.19	7.94 ± 0.05

160	2.94 ± 0.08	8.66 ± 0.54	8.18 ± 0.24
240	5.33 ± 0.17	9.48 ± 0.16	9.12 ± 0.13
320	6.76 ± 0.03	8.35 ± 0.35	7.66 ± 0.37
400	7.93 ± 0.11	7.85 ± 0.81	7.57 ± 0.65
480	8.44 ± 0.06	7.24 ± 0.85	6.330.88

Where 5 C C; five-chamber chemical catholyte MDC and 5 W C; five-chamber water catholyte MDC

Aside the migration of chloride ions into anolytes, a possible increase in mobility of Na^+ and NH_4^+ ions in anolytes contributed to the rise in electrical conductivities observed. This was possible through the reduction of surface tensions in anolytes. According to Imoro (2012), biosurfactants reduce surface tension of water thereby increasing the mobility and detection of ions in solution.

4.3.3 Effect of Rhamnolipid Concentrations on pH Stability

Anolyte pH changes in both five-chamber chemical catholyte MDC (5 C C MDC) and five-chamber water catholyte MDC showed trends with peaks and troughs (Fig 4.26) as the concentration of rhamnolipid increased. Increments in magnitude of pH-change (peaks) observed at 80, 240 and 400 mg/l rhamnolipid concentrations were attributed to a possible weaker buffer action of neutralization chambers at these concentrations (Fig. 4.26).

The lower buffer actions (dilution effect) at these concentrations might have resulted from a low concentration gradient (of H^+ ions) between anolytes and the water in neutralization chambers. The low concentration of H^+ ions probably resulted from the consumption of H^+ ions in reactions with Cl^- ions to form HCl. The possible formation of HCl itself probably contributed to the increment in magnitude of pH-change observed.

Decrements in magnitude of pH-change (troughs) on the other hand reflected situations where the buffer actions of the neutralization chambers were considered adequate. Under the conditions of 0, 160, 320 and 480 mg/l rhamnolipid addition (Fig. 4.26), water flux from neutralization chambers probably facilitated by the concentration gradient (of H^+ ions) between anolytes and water in neutralization chambers caused the dilution of anolytes thereby reducing the effect of HCl and as well, the magnitude of pH-change.
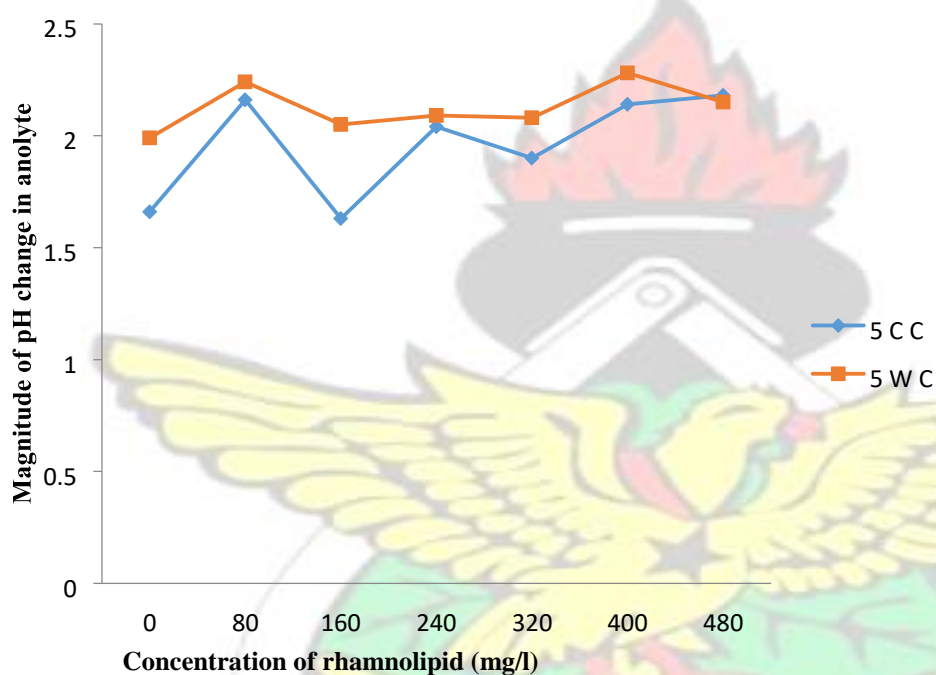
Figure 4.26: Magnitude of anolyte pH change in response to increasing concentrations of rhamnolipid. NB: Initial pH was ~7

In the cathodes however, the magnitude of pH-change of the 5 C C MDC initially decreased but subsequently increased after 160 mg/l rhamnolipid addition (Fig 4.27) whiles the catholyte pH of the 5 W C MDC showed no specific trend (Fig 4.27). Drops in the magnitude of catholyte pH-change (Fig. 4.27) indicated by troughs represented situations of good buffer actions of the neutralization chambers whiles increments in magnitude of pH-change (Fig. 4.27) represented situations of low buffer actions of the neutralization chambers. Low

buffer actions (dilution effect) possibly resulted from low concentration gradient of OH⁻ ions between catholytes and water in neutralization chambers due to the utilization of OH⁻ in the formation of NaOH.
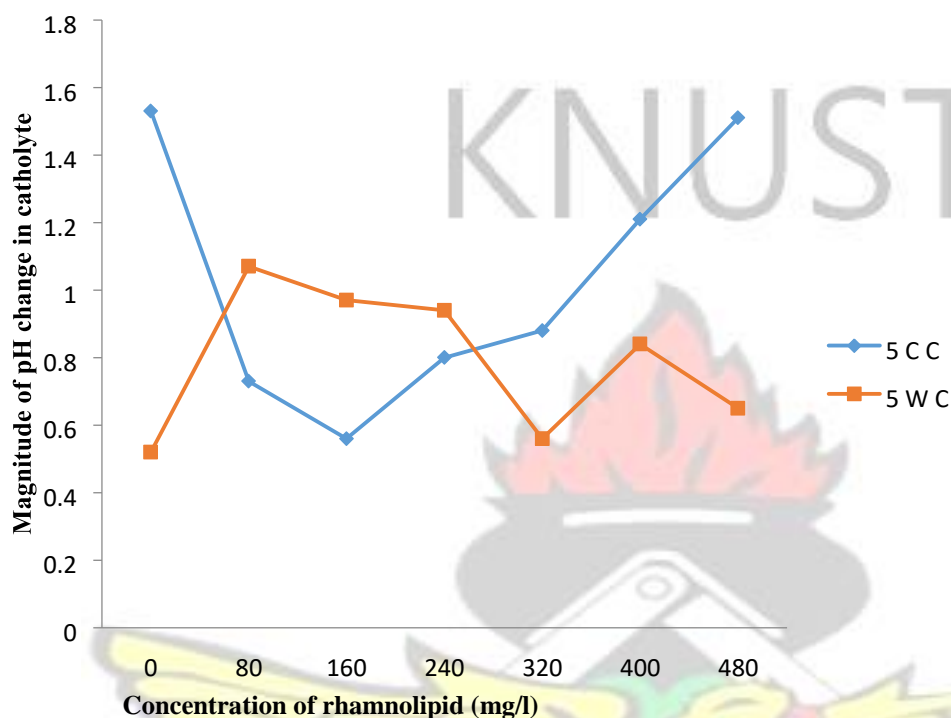
Figure 4.27: Magnitude of catholyte pH change in response to increasing concentrations of rhamnolipid. NB: Initial pH was ~7

On the average, the 5 C C MDC recorded smaller anolyte pH-changes as compared to the 5 W C MDC (Fig. 4.26). The 5 W C MDC on the other hand recorded smaller catholyte pH-changes as compared to the 5 C C MDC (Fig. 4.27). However, catholyte pH change is not much a concern as anolyte pH change in MDC operations (Zhang et al., 2010). This because, anolyte pH changes directly affects microbes involved in electricity generation. Thus it can be inferred that, the smaller changes in the anolyte pH of the 5 C C MDC possibly contributed to its better voltage production and desalination performance than the 5 W C MDC.

4.3.4 Effect of Rhamnolipid on Internal Resistance of the five-chamber MDCs

An internal resistance as high as 184.90 Ω was measured from the five-chamber water catholyte MDC and this was 4-fold higher than the least internal resistance of 4.25 Ω recorded from the five-chamber chemical catholyte MDC. Internal resistance generally, decreased with increasing concentration of rhamnolipid. Rhamnolipid's reduction of internal resistance was attributed to its contribution of ionic species to anolytes and also, its ability to increase the mobility of ions in solution or both. For example, the addition of 240 mg/l rhamnolipid resulted in an increase in EC of the MDCs from $2.37 \times 10^{-3} \pm 0.03$ to 5.33 ± 0.17 mS/cm (Table 4.6). In an earlier work by Wen et al. (2010), anolyte conductivities were also reported to increase with an increase in concentration (0 to 80 mg/l) of rhamnolipid. This supports the claim made in this study.

The higher internal resistances of the water catholyte MDC compared to the chemical catholyte MDC was due to its lower final electrical conductivity of 1.82 ± 0.13 mS/cm which was 9.7 folds lower than the 17.52 ± 1.39 mS/cm recorded from the five-chamber chemical catholyte MDC. Internal resistance and rhamnolipid concentration were negatively correlated (r = -0.868, p = 0.024).
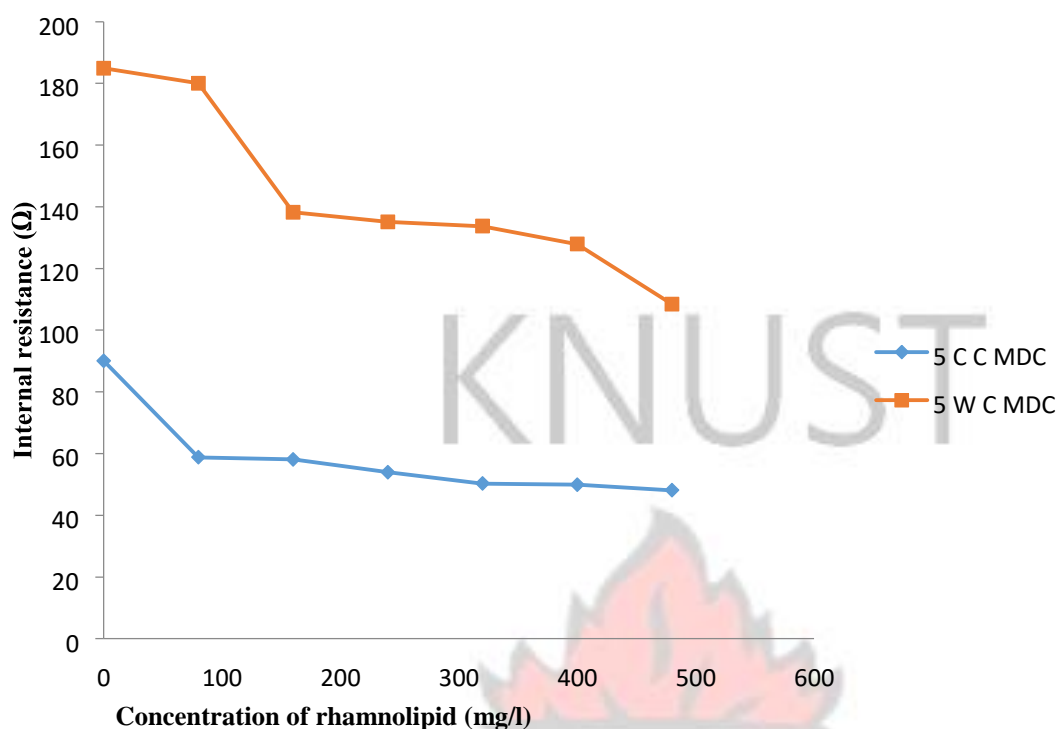
Figure 4.28: Changes in internal resistance in response to increasing concentrations of rhamnolipid

4.3.5. Effect of Rhamnolipid on Wastewater Treatment Performances of the fivechambered MDCs

The effect of rhamnolipid on the wastewater treatment performances of the fivechambered MDCs was investigated to ascertain the biosurfactant's influence on COD reduction, coulombic efficiency, nitrate and phosphorus reductions.

4.3.5.1 Effect of Rhamnolipid on COD reductions and Coulombic efficiencies of the five-chambered MDCs

Increasing the concentration of rhamnolipid increased the initial COD concentrations of anolytes through its contribution of chemical species to the anolytes. Interestingly this also caused increments in percentage COD reductions (Table 4.7). Thus, there was a positive and significant correlation (r = 0.957, p = 0.001) between rhamnolipid concentration and

percentage COD removed. This effect of rhamnolipid on COD reduction was probably due to the enhanced bioavailability of substrates to microbes for mineralization due to the increasingly lowering of surface tension by rhamnolipids. Whang et al. (2009) articulated this by reporting that; rhamnolipids lowers surface tension thereby increasing bioavailability of substrates to microbes for biodegradation.

Table 4.7: Effect of rhamnolipid concentration on COD reduction

Concentration of biosurfactant (mg/l)	Initial COD (mg/l)	% COD reduction in 5 C C MDC	% COD reduction in 5 W C MDC
0	645.00 ± 2.30	63.42	48.74
80	703.00 ± 1.20	67.78	54.48
160	751.60 ± 1.13	68.72	58.18
240	779.00 ± 2.00	69.57	61.26
320	800.20 ± 8.00	69.73	62.72
400	823.00 ± 0.91	70.11	64.17
480	841.14 ± 3.20	72.78	65.31

The realization of a positive correlation between increasing COD concentration and increasing percentage of COD removal was not an isolated finding. Yuan et al. (2015) also found that, increasing concentration of COD from 350 to 1000 mg/l increased COD reduction from 0.42 to 0.80 g/l/day and attributed this to microbial capabilities to degrade excess COD.

The five-chamber chemical catholyte MDC produced the highest COD reduction of 72.78% at a rhamnolipid concentration of 480 mg/l whiles the least COD reduction

(48.74%) was produced by the five-chamber water catholyte MDC at a rhamnolipid concentration of 0 mg/l (Table 4.7). The relatively higher COD reduction performance of the five-chamber chemical catholyte MDC than the five-chamber water catholyte MDC was attributed to the more favourable pH conditions (lesser pH change) of its anolytes (Fig. 4.26).

An analysis of results on coulombic efficiencies of the five chamber MDCs showed that, the five-chamber water catholyte (5 W C) MDC produced the highest coulombic efficiency of 98.75% at a rhamnolipid concentration of 240 mg/l and the least of 25.19%, produced by 5 C C MDC when no rhamnolipid (0 mg/l) was supplied to its anolyte (Fig 4.29). The relatively lower CE (96.66%) of the 5 C C MDC compared to the 98.75% of the 5 W C MDC at 240 mg/l (Fig 4.29) rhamnolipid concentration was not expected. This is because the 5 C C MDC's peak voltage of 630.60 ± 1.44 mV was higher than the 623.7 ± 1.22 mV of the 5 W C MDC (Fig 4.22) and therefore, its CE should have been higher. However, a further investigation on voltage productions showed that, at 240 mg/l rhamnolipid concentration the unit voltage productions of the 5 W C MDC were slightly higher than those of the 5 C C MDC, thus the higher CE of the 5 W C MDC's.

The initial increments in coulombic efficiencies with increasing rhamnolipid concentrations (Fig 4.29) were attributed to the possibility that, the preceding rhamnolipid concentrations (80 to 240 mg/l) favoured the proliferation of exoelectrogens against other members of the microbial consortium in anolytes. That is, exoelectrogens were probably more tolerant to lower concentrations of rhamnolipid than other microbes in the consortia. *Staphylococcus aureus* for instance have been reported to be intolerant to rhamnolipids (Silva et al., 2017). The subsequent declines in CE after 240 mg/l rhamnolipid concentration might have been due to the drop in pHs (increase in magnitude of pH change from ~pH 7,

Fig. 4.26) to acidic conditions and also, the possible oxidation of substrates by dissolved oxygen present in water fluxed into anolytes from neutralization chambers.
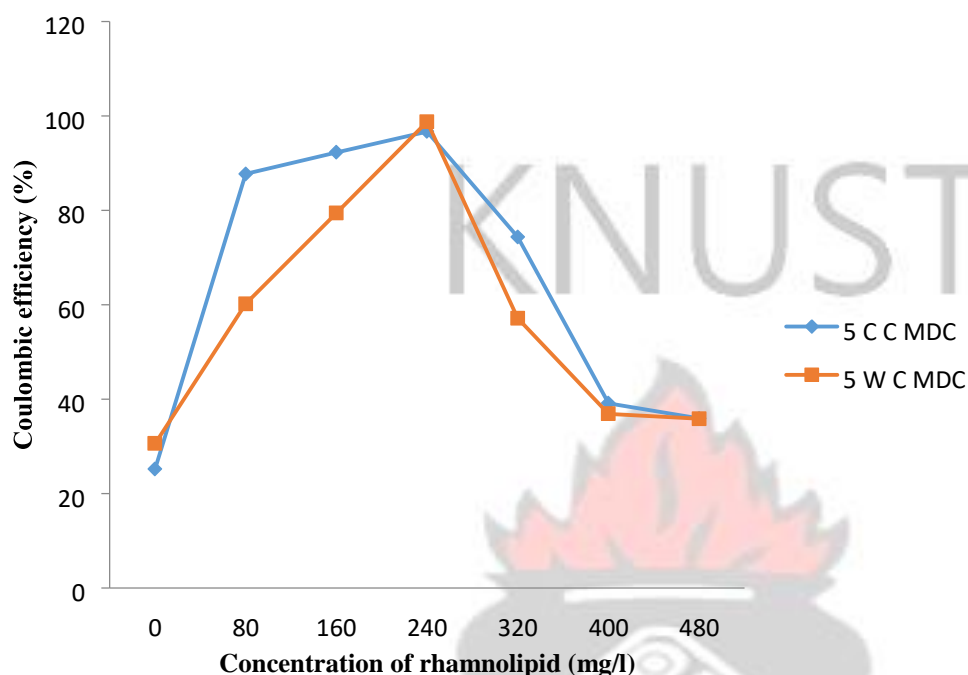
Figure 4.29: Changes in coulombic efficiencies in response to increasing concentrations of rhamnolipid

The general higher coulombic efficiencies recorded when rhamnolipid concentrations were above 0 mg/l (Fig. 4.26) was attributed to the ability of rhamnolipids to make substrate more bioavailable to exoelectrogens for mineralisation. A previous work by Whang et al. (2009) also reported that, addition of rhamnolipids increased the bioavailability of substrates to microbes and that, this was made possible through the lowering surface tensions.

4.3.5.2 Effect of Rhamnolipid on Nitrate and Phosphorus reductions in the five-chamber MDCs

The highest percentage nitrate removal of 2.52% was achieved with the 5 C C MDC and the least of 1.14 % with the 5 W C MDC (Figure 4.30). The higher nitrate removal efficiency of the 5 C C MDC compared to that of the 5 W C MDC was possibly due to it having a relatively more stable anolyte pH environment (Fig. 4.26). Similar to the percentage COD

reductions recorded by 5 C C and 5 W C MDCs, percentage nitrate reductions also increased with increasing concentration of rhamnolipid supplement (Fig. 4.30). This trend manifested as a strong positive correlation (r = 0.943), p = 0.003) between concentration of rhamnolipid and percentage nitrate removed. The explanation advanced for this observation was that, increasing the concentration of rhamnolipid continuously lowered surface tension thereby increasing the bioavailability of nitrates to microbes for utilization.
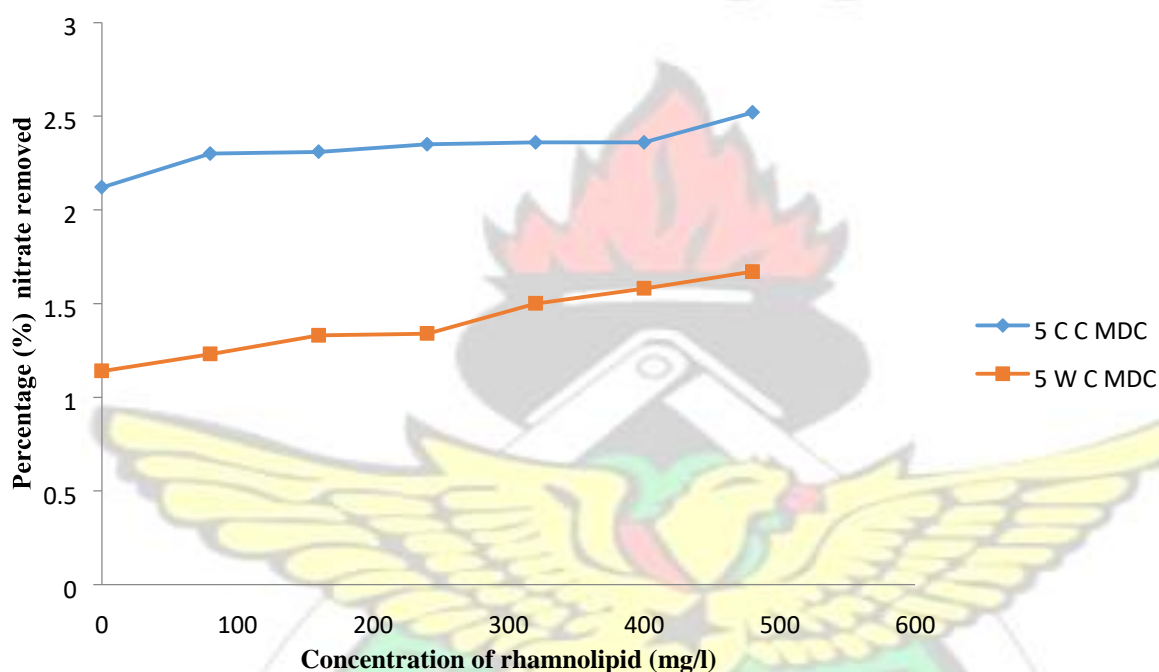
Figure 4.30: Changes in percentage nitrate removed in response to increasing concentrations of rhamnolipid

There was no phosphorus reduction in this experiment. The initial and final phosphorus concentrations were approximately 33.18 ± 4.7 mg/l. The maintenance of an approximately same phosphorus concentration throughout this experiment possibly resulted from a process which preceded with an initial bioaccumulation of phosphorus under anoxic conditions (Zeng et al., 2003) but subsequent release of phosphorus when anaerobic conditions were established. Under anaerobic conditions POAs release phosphorus instead of bio-accumulating them (Tarayre et al., 2016). Almatouq (2017) also realised a no phosphorus reduction in his study and attributed his findings to the anaerobic conditions in the anode.

4.3.6 Choice of MDC

Comparing the key performance indicators (peak voltage productions, total percentage desalinations and best COD reductions) of the five-chamber chemical catholyte (5 C C)

MDC and five-chamber water catholyte (5 C W) MDCs, it was realised that, the 5 W C

MDC performed fairly against the 5 C C MDC (Table 4.8).

Table 4.8: Summarised data on the performances and unit cost of producing the fivechamber MDCs

MDC Type	TEA	Highest % COD reduction	Peak voltage (mV)	Total% desalination	Cost of production (GHS)
5 C C	KFC	72.78	630.60±1.44	67.63	1,550.42
5W C	Oxygen	65.31	623.70±1.32	63.21	701.19

Where: TEA – Terminal Electron Acceptor   KFC – Potassium ferricyanide

However, the cost of producing the 5 C C MDC was higher than that of the 5 W C MDC.

This was principally because of the 5 C C MDC's requirement of an expensive potassium

ferricyanide as terminal electron acceptor. The cost of a 50 g potassium ferricyanide powder

for instance is about EUR 43.00 (GHS 248.54) (www.sigmaaldrich.com)

excluding shipment and duty whiles the industrialised cost of a meter cube of tap water in

Ghana is GHS 10.0702 (Ghana Water Company Limited, 2018). Thus, the cost of 50 g

potassium ferricyanide is about 25 times higher than a meter cube of tap water aside the fact

that, this quantity (50 g) will not be sufficient to produce a meter cube solution of potassium

ferricyanide considering 16.5 g/l concentration used in this study. Aside cost, potassium

ferricyanide is environmentally unfriendly due to its toxicity to living organisms (Kokabien and Gude, 2015).

Thus considering the appreciable performance (Table 4.8) of the 5 W C MDC against the 5 C C MDC and its use of a less costly source of electron acceptor (tap water), it was chosen as the MDC suitable for adoption in a developing country like Ghana. Following its selection, further experiments were conducted on the 5 W C MDC to improve its voltage production, desalination and COD reduction performances.

4.4 Factorial Study of the Effects of Rhamnolipid and Stirring on the Performances of five-chamber water catholyte MDC

Presented below in section 4.4.1 are results and discussions on the effects of stirring (60 rpm) on the voltage production and desalination performance of the five-chamber water catholyte MDC. This was required before the factorial study could proceed. Results and discussions on the factorial study are subsequently presented in section 4.4.2 as the effect of rhamnolipid and stirring on the performances of the 5 W C MDC.

4.4.1 Effects of Stirring on Voltage Production and Desalination Performance of the five-chamber Water Catholyte MDC

Peak voltage increased from stirring regime 1 through to 3 (Fig. 4.31). The highest peak voltage of 567.27 ± 18.06 mV was produced with stirring regime 3 and the least, 510.99 ± 15.54 mV with regime 1. The higher peak voltage production of the 5 W C MDC under regime 3 was attributed to regime three's (3) provision of higher oxygen concentrations in water catholyte (Table 4.9). Kokabien and Gude (2015) also observed that, voltage production was high when oxygen concentration of catholyte was high. The positive relationship between electricity production and oxygen concentration was also reported by

Rismani-Yazdi et al. (2008) and Oh, Min and Logan (2004). They concluded that, power

productions are proportional to dissolved oxygen concentrations in MFCs.
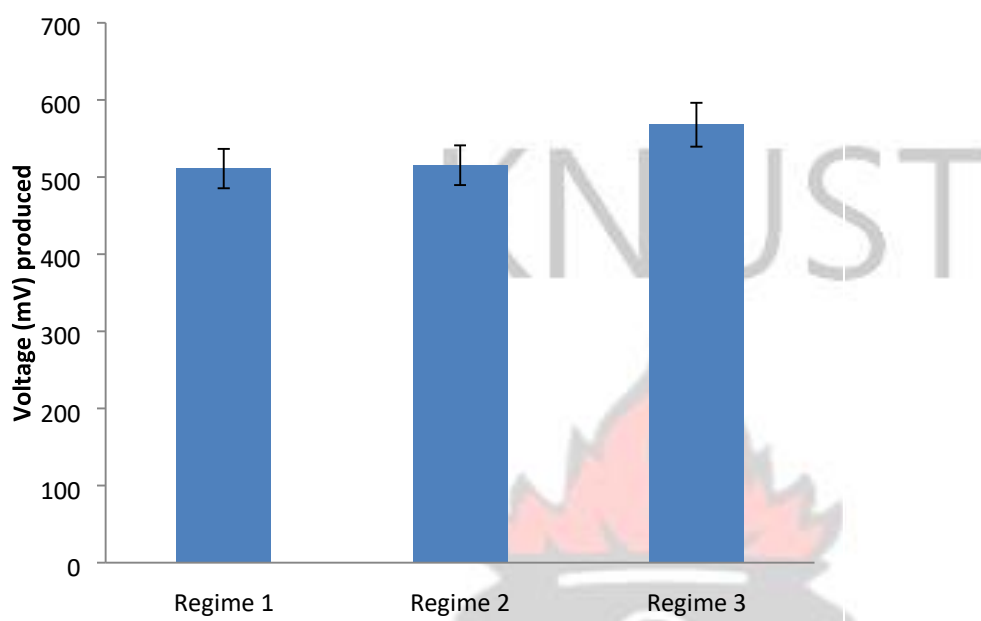
Figure 4.31: Effect of stirring regimes on peak voltage produced by the 5 W C MDC

Stirring generally created turbulence in exposed water catholyte thereby facilitating the

dissolution and the subsequent increments in oxygen concentrations in the water catholytes

(Table 4.9). Earlier studies have established the link between water turbulence and oxygen

concentrations. For instance, Atapaththu et al. (2017) found that, turbulence was positively

correlated with oxygen concentration in water.

Also, according to Rismani-Yazdi et al. (2008) stirring together with aeration is

recommendable for tackling the problem of mass transport losses in bioelectrochemical

systems which rely on water as catholyte. This notwithstanding, aeration through the use of

a pump together with stirring will increase the cost of operating a microbial desalination

cell.

Another possible positive effect of stirring on voltage production was its tendency to increase oxygen flux to the cathode surface for reduction reactions to take place and the displacement of reduced species from the cathode. In relation to this, Rismani-Yazdi et al., (2008) also reported that, stirring will reduce cathodic mass transport losses (voltage loss) through the increment in oxygen flux to cathode surfaces. In other words, stirring facilitates voltage productions as observed in this study.

Table 4.9: Dissolved oxygen (D O) concentrations in catholyte recorded in stirring experiments

Stirring Regime	Time intervals (Hours)	D O (mg/l) Before stirring	D O (mg/l) After stirring	Temperature (ºC)
1	24	4.08 □ 2.60	7.60 □ 1.00	28 ± 1.10
2	12	3.58 □ 3.40	7.40 □ 2.10	28 ± 2.00
	24	4.11 □ 1.30	7.70 □ 1.51	28 ± 1.19
3	8	5.08 □ 3.31	7.70 □ 2.39	28 ± 0.99
	16	5.16 □ 1.10	7.63 □ 3.09	28 ± 1.11
	24	5.30 □ 2.09	8.70 □ 2.22	28 ± 2.12

Percentage desalination increased in the same order as voltage production. The highest percentage desalination of 65.29% was achieved with stirring regime 3 whiles the least desalination efficiency of 59.43% was achieved with regime 1 (Fig. 4.32). The observation of similarity in performance trend between percentage desalination and voltage production was attributed to the fact that, desalination principally relies on electricity production. Electricity production causes the separation and movement of Cl^- and Na^+ ions out of the desalination chamber into the anode and cathode chambers respectively thereby causing desalination in the middle chamber (Cao et al., 2009).
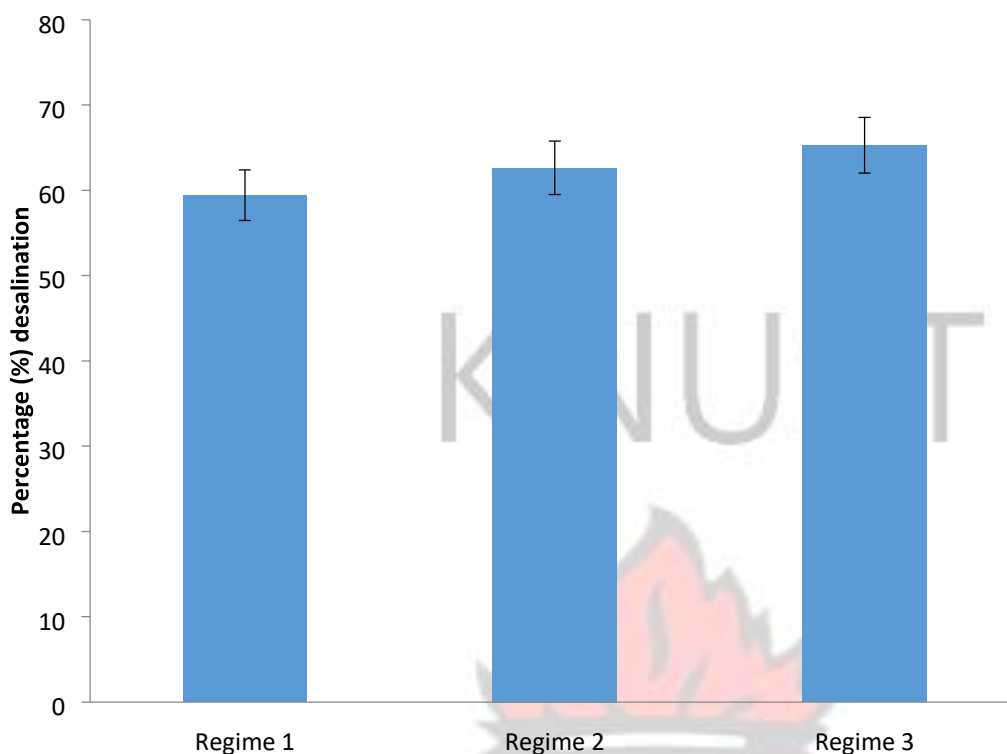
Figure 4.32: Effect of stirring regimes on percentage desalination produced by the 5 W C MDC

4.4.2 Effects of Rhamnolipid and Stirring on the Performance of the five-chamber water catholyte MDC

The parameters considered for assessing the effects of both rhamnolipid and stirring on the performance of the 5 W C MDC were; voltage production, desalination and COD reduction. These parameters were chosen because they represent the core functions of a microbial desalination cell.

4.4.2.1 Effect of Rhamnolipid and Stirring on Voltage Productions of the five-chambered water catholyte MDC

The highest voltage produced in the factorial study was 647.07 mV (Table 4.10). This was achieved at a rhamnolipid concentration of 240 mg/l and a high stirring regime of 3 stirring events a day. This voltage (647.07 mV) was higher than the highest voltage obtained (567.77

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mV; Fig. 4.31) when stirring was the only factor being manipulated and 623.70 mV (Fig. 4.22) obtained when rhamnolipid was the only factor varied.

The higher voltage production performance of the 5 W C MDC at high levels of both rhamnolipid and stirring was possibly due to an improved anolyte (accelerated electron transfer out of bacteria cells) and catholyte condition (adequate concentration of dissolved oxygen at cathode surface). An earlier study by Wen et al. (2010) also associated enhanced electricity generation to accelerated electrons transfer out of bacteria cells due to rhamnolipid addition.

The association of stirring to an improved cathode condition was because stirring increased the concentration of dissolved oxygen in the water catholyte (Table 4.9). Under adequate oxygen concentrations mass transport losses (voltage loss) can be reduced in an oxygen-depended cathode (Rismani-Yazdi et al., 2008) thereby improving voltage production.

Table 4.10: Performances of 5 W C MDC in factorial study

Standard Order	Run Order	Rhamnolipid concentration (Coded units)	Stirring regime (Coded units)	Responses		
				%Desalination	Peak voltage (mV)	%COD
7	1	-1	1	24.00	640.50	65.47
4	2	1	1	25.50	645.71	68.15
6	3	1	-1	24.50	642.53	67.43
11	4	-1	1	24.30	638.00	65.92
9	5	-1	-1	23.50	630.92	65.00

5	6	-1	-1	23.20	633.09	64.88
8	7	1	1	25.10	643.22	67.91
3	8	-1	1	24.60	635.87	66.24
12	9	1	1	25.00	647.07	67.6
10	10	1	-1	24.80	640.97	67.89
1	11	-1	-1	23.00	634.90	65.32
2	12	1	-1	25.20	644.00	67.31

Where; -1 represents rhamnolipid concentration at 160 mg/l (Low) and 1 represents 240 mg/l (High) -1 represents stirring regime 1 (Low) and 1 represents stirring regime 3 (High)

An ANOVA output (Appendix 3B-ii) showed that, the interactive effect of stirring and rhamnolipid though improved voltage production, had no significant (p = 0.337) effect on peak voltage productions. However, the main effects of stirring and rhamnolipid individually had significant impacts (F = 33.40, p = 0.000) on peak voltages produced (Appendix 3B-ii), and this was attributed to the ability of rhamnolipid to reduce internal resistance (Wen et al., 2010) and stirring (creation of turbulence) to increase oxygen (terminal electron acceptors) concentration in water (Atapaththu et al., 2017).

The insignificant effect of factor interaction on voltage production was attributed to the possibility that, the factor combinations at all levels whether high, low or both produced voltages near the optimum obtainable with the 5 W C MDC. Thus operating the MDC with any factor combination could not produce marked changes (Appendix 3B-ii) in peak voltages as observed.

The factorial analysis yielded the model:

Voltage = **639.732 + 4.185 (Rhamnolipid concentration) + 1.997 (Stirring regime)...(1)**

R^2 for the model was 89.45%.

4.4.2.2 Effect of Rhamnolipid and Stirring on Desalination Performance of the five-chamber water catholyte MDC

The highest percentage desalination recorded in the factorial study was 25.50%, produced when both rhamnolipid concentration and stirring were high (Table 4.10). This was higher than the highest desalination efficiency of 24.89 % achieved with the five-chamber water catholyte (5 W C) MDC under rhamnolipid conditions only (Appendix 4A) and the highest of 24.26% (Appendix 4B) produced under stirring only. The improved desalination performance of the 5 W C MDC under high stirring regime and high rhamnolipid concentration was attributed to the improved voltage production under these same conditions (240 mg/l rhamnolipid and regime 3) (Table 4.10). In MDCs, desalination results from electricity production (Yang et al., 2014) therefore improved voltage productions causes improvement in percentage desalination as observed in this factorial study.

The factorial analysis also showed that, the interactive effect of rhamnolipid and stirring did not produce a significant (p = 0.073, Appendix 3A-ii) effect on percentage desalination. This finding was attributed to the insignificant effects of the factor combinations of rhamnolipid and stirring on voltage production discussed earlier (section 4.4.2-1). An effect on voltage production affects desalination because of the dependence of desalination on voltage production.

However, ANOVA (Appendix 3A-ii) from the factorial study showed that, the main effects of rhamnolipid and stirring individually were significant (p = 0.000). The significance of the

main effects of these factors was exemplified in the percentage desalination produced by the 5 W C MDC when either rhamnolipid or stirring was high as compared to when both factors were high (Table 4.10). For instance, at high rhamnolipid concentration of 240 mg/l and low stirring regime (regime 1), a percentage desalination of 25.2% could be produced which was comparable to the 25.5% produced when both factors were high (Table 4.10).

The model produced from the factorial analysis had a high R^2 value of 90.49% and was defined as:

% *Desalination* = 24.3917 + 0.6250(Rhamnolipid concentration) + 0.3583(Stirring regime)…..…………………………………………………………………………….(2)

4.4.2.3 Effect of Rhamnolipid and Stirring on COD Reduction

At a rhamnolipid concentration of 240 mg/l and high stirring regime of 3 stirring events a day, a high percentage COD reduction of 68.15% was achieved. This was higher than the 61.26% produced when only rhamnolipid (240 mg/l) was applied (Table 4.7) and the 50.23% achieved when only stirring was the factor being varied (Appendix 4C). An analysis of variance (Appendix 3C-2) showed that, the interaction between rhamnolipid and stirring had no significant ($p = 0.221$) effect on COD reduction. This was possibly because COD reduction is primarily affected by anolyte conditions and not catholyte conditions, thus improvements in catholyte conditions through stirring would not have had any direct effect on COD reductions. However, the realization of a positive effect of stirring on COD reduction as shown in the model below was not expected. This unexpected positive effect of stirring on COD occurred possibly because of the contributions of dissolved oxygen (resulting from stirring) to oxidation of organic molecules.

The predictive model produced for % COD reduction was:

%COD = 66.5933 + 1.1217(Rhamnolipid concentration) + 0.02883(Stirring regime)

$$\dots\dots\dots\dots\dots\dots\dots\dots\dots\dots\dots\dots\dots\dots\dots\dots\dots\dots\dots\dots\dots\dots\dots\dots\dots\dots\dots\dots\dots\dots\dots\text{(3)}$$

R^2 value of the model was 95.63%

4.5 Examination of Membranes for Fouled Layers

Results from membrane analysis of both used and unused membranes of the five-chamber water catholyte MDC are presented in the following sections. The cation exchange membrane (CEM) of the five-chamber chemical catholyte MDC was also examined to find out the effect of potassium ferricyanide on its integrity.

4.5.1 Comparison between unused and used Anion Exchange Membranes (AEMs) of Five-Chamber Water Catholyte MDC

Used AEM of the anode chamber was fouled on both sides (Plate 4.2 and 4.3). The side of the AEM facing the anolyte was fouled predominantly by spherical shaped bacteria and this was attributed to the presence of bacteria (inoculum) in the anolyte. There was also a thin layer of debris (from wastewater) formed on the AEM with cracks on it. The cracks resulted from the subjection of the membranes to dryness (Plate 4.2). Inorganic scales were observed on the side of the AEM facing the desalination chamber and this primarily could be due the presence of salt (NaCl) in the desalination chamber.

Energy dispersive spectroscopy (EDS) revealed that, the side of the used AEM facing the anolyte contained C, O, Ca, P, F, Na, Cl whiles the side facing desalination chamber contained O, Na, Cl, K, Mg. The detection of C, O, F was expected because these elements are part of the composition of ion-exchange membranes (Zuo et al., 2013). The detection of especially Ca, C and O was possibly because of their increased concentration due to bacteria growth. According to Luo et al. (2012) bacteria growth increases the concentrations of C, O, Ca, K, thus making their detection on membranes easier (Luo et al., 2012).

The presence of Na and Cl on membranes was due to the use of NaCl solution in the desalination chamber whiles the precipitation of Mg on the membrane side facing desalination chamber was probably due to high pH (9.60 ± 0.7) in desalination chamber. According to Ping et al. (2013) a high pH of approximately 10.2 can facilitate the precipitation of Mg on cation exchange membranes.

Anion exchange membrane of the neutralization chamber facing the catholyte had numerous patches of inorganic scales (Plate 4.4) with the following elements; C, F, O, S, K, Na detected on it. The detection of S and K was not expected because the catholyte did not contain these elements. Thus, their presence on the AEM could be due to cross contamination from the anolyte. Analysis of the unused AEM (Plate 4.1) showed no obvious fouling layers/structures thus it provided the required contrast for assessing used membranes.
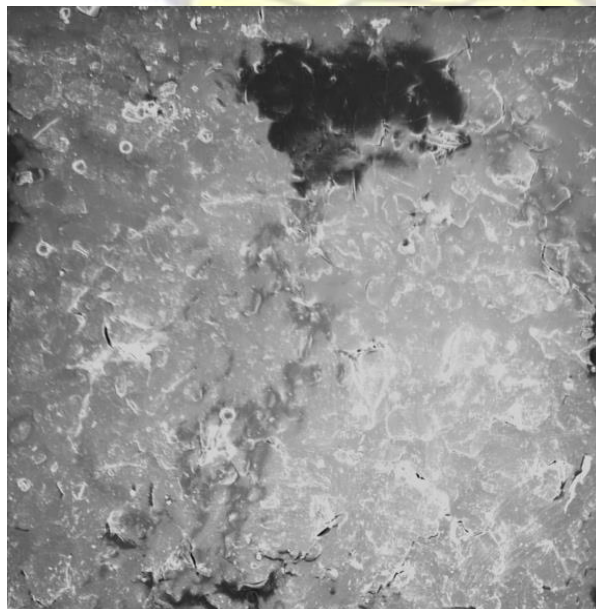
Plate 4.1 Unused AEM

Plate 4.2 Used AEM (Side facing anolyte),
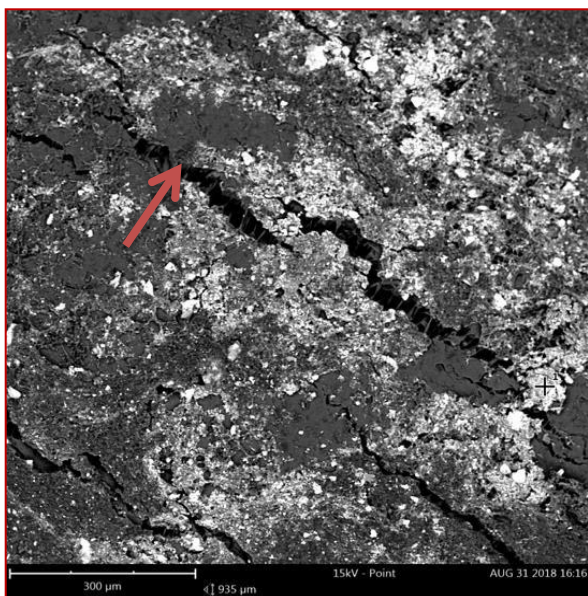
Plate 4.3 Used AEM (Side facing desalination chamber)
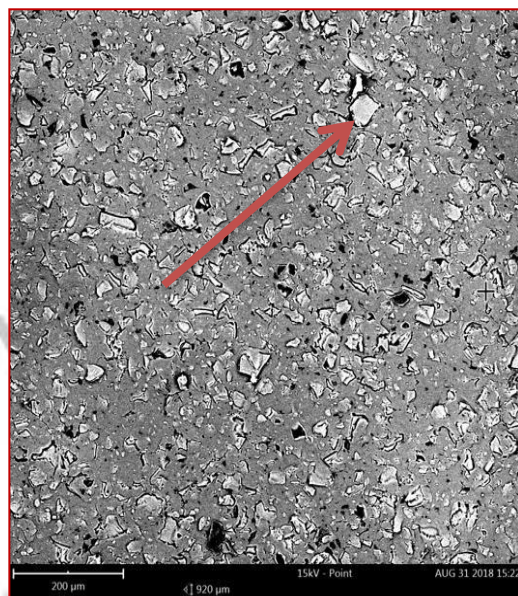
Plate 4.4 Used AEM of neutralization chamber (side facing catholyte)

4.5.2 Comparisons between unused and used Cation Exchange Membranes (CEMs)

The side of the cation exchange membrane facing the catholyte had dark patches all over its surface (Plate 4.5) and this was because of the use of Vulcan carbon in the cathode compartment. It contained the elements N, S, F, O and C (Plate 4.5). As indicated earlier, C, F, N and O form a part of the composition of ion exchange membranes so their detection was expected. Whiles S was from Vulcan carbon because Vulcan carbon has traces of S in it. On the other hand, the side of the cation exchange membrane facing the desalination chamber had patches of inorganic scales with the elements, C, F, O, Fe, Na, S, Ca and Cl on it (Plate 4.6).

The detection of Fe on the CEM was not expected and could represent a case of cross contamination from the tap water used as catholyte. The CEM (Plate 4.7) of the neutralization chamber (side facing anolyte) had a thin layer of debris on it with cracks.

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These cracks were as a result of dryness. The unused CEM on the other hand did not have obvious layers/structures of fouling (Plate 4.8).
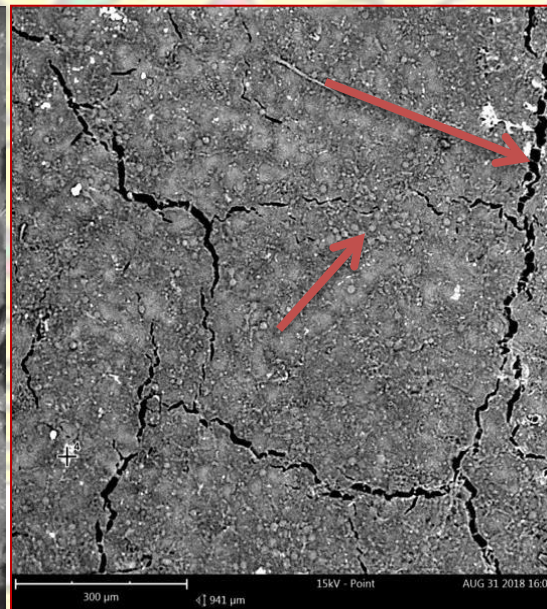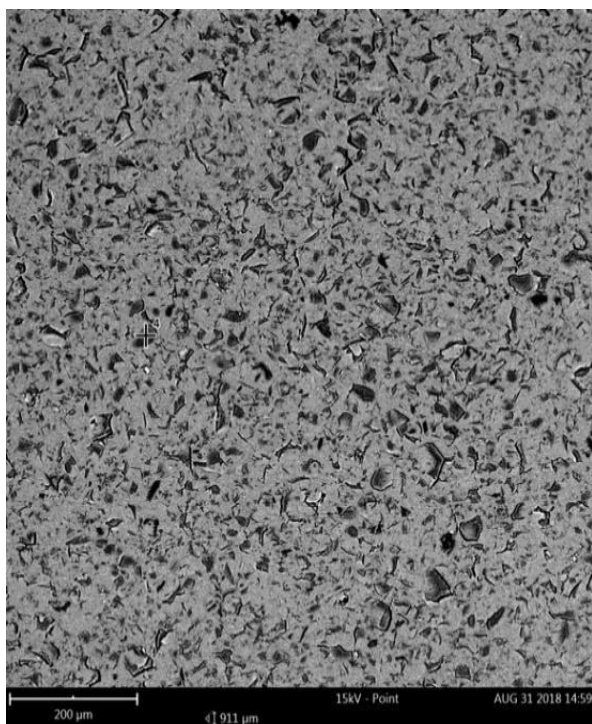
Plate 4.5 Used CEM (Side facing catholyte)
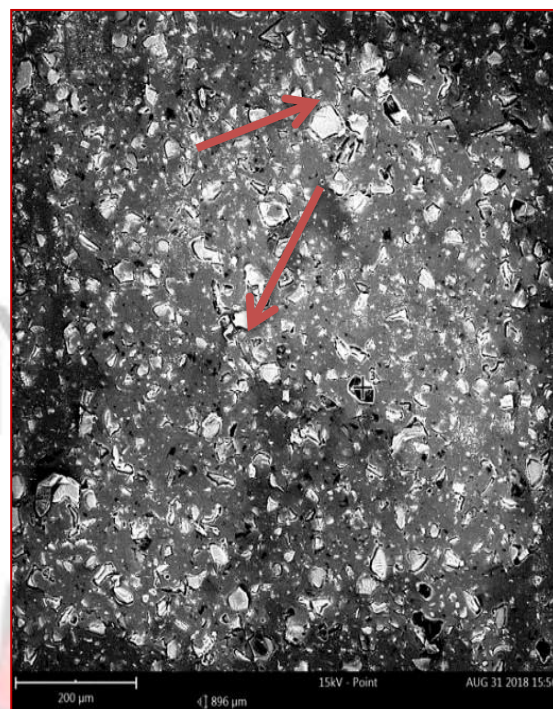
Plate 4.6 Used CEM (Side facing desalination chamber)
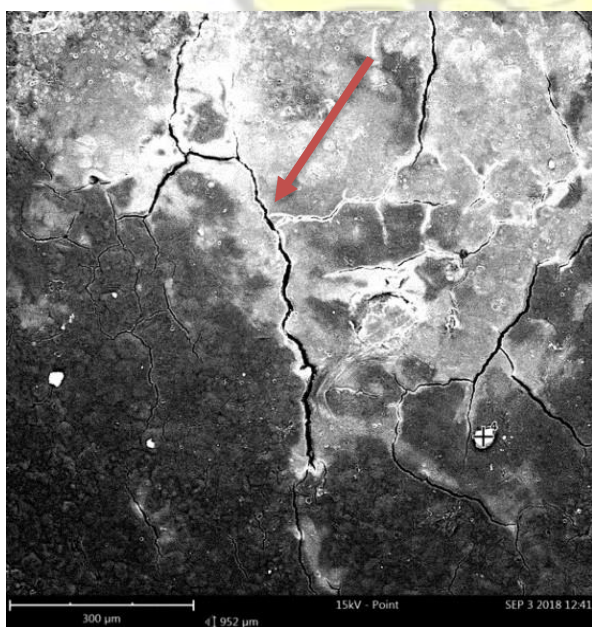
Plate 4.7 CEM of neutralization chamber (side facing anolyte),
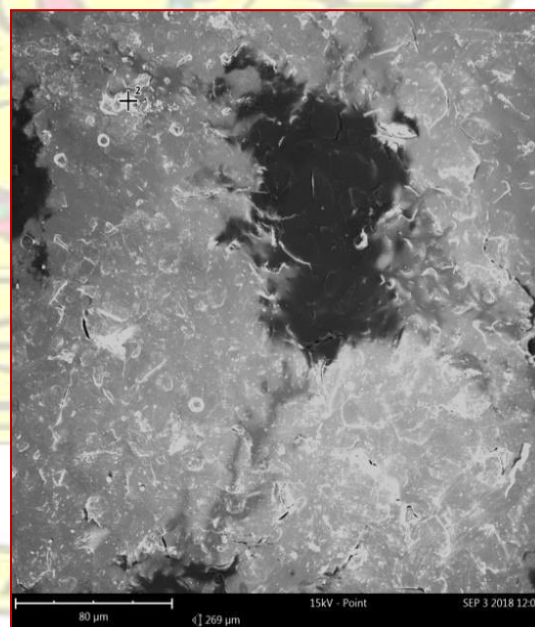
Plate 4.8 Unused CEM

4.5.3 Comparisons between Anion and Cation Exchange Membranes

The anion exchange membrane of the anode chamber showed more evidence of biofouling with the detection of spherical shaped bacteria and higher atomic concentrations of C, S, Ca and P on it (Table 4.11). Higher concentrations of these elements on membranes according to Luo et al. (2012) are as a result of bacteria metabolism, thus confirming biofouling.

Cation exchange membranes on the other hand were affected more by inorganic scaling. For instance, the side of cation exchange membrane facing the desalination chamber had the highest atomic concentrations of K, Na and Fe (Table 4.11). Layers of these elements could reduce the permeability of membranes to the migration of ions through them in a way similar to how biofouling reduces ion transport through ion exchange membranes.

Limited ion migration through membranes due to biofouling was previously confirmed by Choi et al. (2011) who indicated that the mechanism of reduction in ion migrations was either physical or chemical and that, biofilms can even limit the movement of liquids.

Table 4.11: Atomic concentrations of elements on ion exchange membranes

Elements	Atomic concentration (%) of CEM (Side facing desalination chamber) of 5 W C MDC	Atomic concentration (%) of AEM (Side facing anolyte) of 5 W C MDC	Atomic concentration (%) of CEM of 5 C C MDC
C	40.53	77.37	63.29
F	17.03	15.63	17.30
O	33.24	4.23	7.50
S	0.12	0.23	-
K	-	0.04	0.84
Na	8.06	0.34	31
Fe	0.08	-	0.22

Cl	0.06	5.80	0.06
Ca	0.04	0.11	0.04
P	-	0.04	-

4.5.4 Comparison between Cation Exchange Membranes of five-chamber Water Catholyte MDC and five-chamber Chemical Catholyte MDC

Though the cation exchange membrane (CEM) of the five-chamber water catholyte (5 W C) MDC was used for a much longer time (~150 days), it was less fouled compared to the CEM (Plate 4.9) of the five-chamber chemical catholyte (5 C C) MDC which was used for ~ 100 days. For instance, the used CEM of the 5 C C MDC had higher atomic concentrations of C, F, Fe, Na, and K compared to those on the CEM of the 5 W C MDC (Table 4.11). The higher concentration of Fe and K on the CEM of 5 C C MDC was possibly because of the potassium ferricyanide oxidant used in preparing its catholyte. Its higher carbon (C) concentration was associated with microbial growth on the CEM resulting from contamination from its anolyte. An earlier study by Luo et al. (2012) also attributed increased carbon (C) concentrations on membranes to biofouling resulting from bacteria growth.

The lower concentration of F on the CEM of the 5 W C MDC was attributed to the gradual release or loss of F from the CEM due to prolong use (~150 days). Zuo et al. (2013) reached a similar conclusion after observing a decrease in concentration of F on an AEM after a prolonged period of use (~64 days). This implies that, the prolonged use of membranes comprises membrane integrity and this should be factored in the optimization of MDC operations.
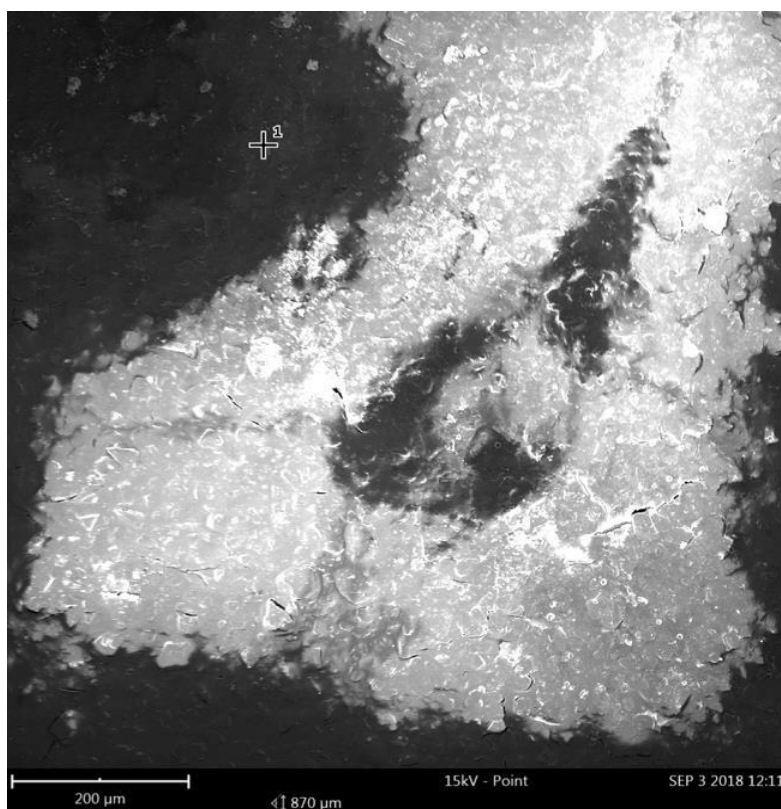
Plate 4.9: CEM of five-chamber chemical catholyte MDC (Side facing catholyte)

The CEM of the 5 W C MDC had a higher concentration of oxygen (O) in it as compared

to that in the CEM of the 5 C C MDC. This finding was attributed to the possibility that, the

microbial growth on the CEM of the 5 W C MDC was higher than that on the CEM of the

5 C C MDC. This was possibly the case because the cathode chamber of the 5 W C

MDC was opened to the atmosphere making it more prone to microbial contamination. The

association of higher oxygen concentration in the CEM of the 5 W C MDC to the higher

growth of microorganism on it is not an isolated claim. Earlier studies by Luo et al. (2012)

and Zuo et al. (2013) also drew similar conclusions about the effect of microbial growth on

oxygen concentration in membranes.

CHAPTER FIVE

5.0 General Discussion of Results

Results from this study showed that, generally, the chemical catholyte MDCs produced higher electricity, desalination and wastewater treatment performance than the water catholyte and plant-supported MDCs. The relatively lower performances of the ‗oxygendependent' MDCs were possibly due to the slow oxygen reduction reactions under ambient conditions. According to Gude et al. (2013), the slow reduction reaction of oxygen limits the performance of oxygen dependant cathodes and that catalysts are required to improve the performances of these types of cathodes.

An earlier study by Oh et al. (2004) demonstrated the superiority of the potassium ferricyanide catholyte over water catholyte. In their study, potassium ferricyanide catholyte could support power production up to 80% of the power (0.097 mW) a water catholyte could support/produce. Their work together with this present research thus provided evidences that, whether in an MFC or MDC, potassium ferricyanide would support bioelectrochemical systems operation better than water-based (oxygen dependent) catholytes.

Notwithstanding the above stated lower performances of water catholyte-MDCs compared to potassium ferricyanide-MDCs, when the water catholyte-MDC (5WC) investigated in this study was stirred, desalination (Fig. 4.32) and voltage productions (Fig. 4.31) of it improved. This improvement was attributed to increments in dissolved oxygen concentration near cathode surfaces due to stirring. The presence of adequate terminal electron acceptors (oxygen) at the cathode surface facilitates voltage production and hence desalination. In other words, keeping an optimum concentration of oxygen near the cathode

surface through effective mass transport of reactants (Oxygen) to the cathode surface and products (H_2O) away from cathode surface is essential for reducing voltage losses/cathodic concentration losses (Rimani-Yazdi et al., 2008) in a cell.

Gil et al. (2003) were able to link power production to oxygen concentration in catholyte under controlled temperature and stated that, dissolved oxygen concentration was proportional to power productions. Similarly, in this study, the temperature of water catholytes was about 28 ºC throughout experiments thus creating a basis for drawing justifiable relationships between oxygen concentration and electricity production. Jong et al. (2006) also found that the frequent change of air-saturated catholytes improved current production. Thus MDCs dependent on oxygen, should be operated at optimum oxygen concentrations if the best performances are desired.

The plant-supported MDC (PMDC) produced the least performance and this was attributed to the death of the aquatic plant as experiments progressed. The small volume (230 cm^3) of the cathode chamber of the PMDC and limited sunlight reaching the aquatic plant due to the translucent nature of the polyoxymethylene material used to build the PMDC were the possible causes of the deaths of the aquatic plants. Ejankowski and Solis (2015) reported that light limitation negatively affected *Ceratophyllum demersum* growth underneath water. Notwithstanding this challenge, as a proof of concept, *Ceratophyllum demersum* was able to support voltage production by increasing dissolve oxygen concentrations till when it started to die off. A search through available literature showed that, this the first work on plant-biocathode MDC (PMDC) thus the PMDC could serve as a baseline for further research on plant-biocathode developments in MDC technology.

The pH stabilization effects of the neutralization chambers were appreciable (could maintain anolyte pH change of 1.66 ± 0.11) and this contributed to the relatively higher electricity generation, desalination and COD reduction performances of the five-chamber chemical catholyte MDC as compared to the other MDCs studied. The introduction of neutralization chamber and its enhancement of the performances of MDCs thus positions the five-chamber MDCs as potential technology for scale-up because then, the expensive phosphate buffers and chemical oxidants usually employed in MDCs can be avoided. For instance, phosphate buffer saline powders costs between EUR 14 and EUR 359.00 per 10.0 g depending on specifications (www.sigmaaldrich.com). To use these buffers on large scale applications will mean spending millions of Euros on just chemical buffers aside other costs the technology might attract.

The improvement in voltage productions of MDCs in the presence of rhamnolipid supplement was anticipated as an earlier research by Wen et al. (2010) had found that, rhamnolipid could increase electricity production in a MFC. However, this current study according to available literature is the premier study so far as the application of rhamnolipid in a microbial desalination cell is concerned. According to Wen et al. (2010) rhamnolipid reduces the opposition of electron flow out of bacteria cell membranes by making the membranes more permeable. Increments in bacteria cell permeability in the presence of rhamnolipid has also been confirmed by Kim et al. (2015). However higher concentrations of rhamnolipid negatively affects MDC performance as demonstrated in this study. Nickzad and Deziel (2014) also reported that, high dosage of rhamnolipid impedes the initial formation of biofilms and this can result in a slow start up of electricity production.

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In Wen et al. (2010) work on improving the performance of MFC through rhamnolipid addition, they found that dosing a microbial fuel cell with rhamnolipid could increase power density from 22 to 275W m^{-3}, representing ~ 13-fold increment in power production (Wen et al., 2010). In this present study too, rhamnolipid addition could increase power density from 0.55 to 1.14 Wm^{-3}, representing ~2-fold increment in power density production. The comparative higher power densities recorded in Wen et al.

(2010)'s study was possibly due to the lower internal resistance (5 Ω) of the MFC they used. This internal resistance of 5 Ω was ~10-folds lower than for instance the 54 Ω of the 5 C C MDC at 240 mg/l rhamnolipid addition. The higher internal resistance of the 5 C C MDC than the MFC was expected because the MDC consisted of more compartments and components than the single chambered-MFC used by Wen et al. (2010).

The supply of rhamnolipid biosurfactants can increase the cost of MDC operations and thus can make it unattractive for practical applications. However, Zheng et al. (2015) found that, rhamnolipids could be produced in a MFC endogenously by *Pseudomonas aeruginosa*. Consequently, the exogenous supply of rhamnolipids might not be required in future MDC operations and thus, the associated cost of it can be avoided through the maintenance of endogenous production of the biosurfactant in MDCs.

One subtle but possible contributor to the appreciable performances of the water catholyte MDCs was the Vulcan carbon added to their cathodes. Vulcan carbon increased the conductivity of tap water and by so doing might have supported voltage productions. Vulcan carbon also possibly contributed to voltage productions in the MDCs by providing surfaces for oxygen reductions. Vulcan carbon has previously been used to support the performances of some bioelectrochemical systems. Duteanu et al. (2010) for example, used a chemically

modified Vulcan carbon to improve current density to 1115 mA/m^2 production above 849 mA/m^2 achieved with a platinum catalyst-supported MFC. Its good conductivity makes it useful for many applications including the support for catalysts.

Desalination performances of the MDCs studied were encouraging given the fact that, the MDCs worked with a high external resistor of 1000 Ω and each experiment was run under just three electrolyte replacement cycles. Though difficult to compare to other studies due to differences in operational conditions, the common initial salt concentration of 35 g/l allows for some comparison with results obtained in Cao et al. (2009) work. In their research, Cao et al. (2009) were able to achieve a 90% desalination but after several anolyte replacement cycles. So when their 90% desalination achieved with several anolyte replacement cycles was compared to for instance the 50.01% desalination of the 5 C C MDC achieved under three-anolyte replacement cycles, it was concluded that the 5 C C MDC desalination performance was appreciable.

Percentage COD reductions recorded in this study were satisfactory and compared favourably to those achieved in previous studies. For example, the COD reduction performance of 68.15% (Table 4.10) of the 5 W C MDC was comparable to the 68.9% COD removal achieved by Ping et al. (2015) who worked on wastewater with COD concentrations between 500 and 650 mg/l which were not too different from the 645 ± 2.3 mg/l used in this study.

Nutrient (nitrate and phosphorus) reductions recorded from the MDCs were low. Nitrate reductions were attributed to utilization by anaerobic microorganisms similar to the conclusion drawn by Luo, Xu, Roane et al. (2012) who reported a 38% ammonia removal

from wastewater. Phosphorus reduction was attributed to possible aerobic (Tao et al., 2014) and anoxic (Zeng et al., 2003) removal by polyphosphate accumulating microorganism before anaerobic conditions were established. Nonetheless, Almatouq (2017) demonstrated that, phosphorus could be better removed from the cathode of a microbial fuel cell by precipitation as struvite under high pH of pH > 8. This would be a more efficient way to remove phosphorus if phosphorus removal is considered the main goal of an MDC operation.

Improving the performance of the five-chamber water catholyte MDC was important as it was the preferred option for possible future application in Ghana. Thus the realization of an increased performance from it when either catholyte stirring or rhamnolipid addition or both were applied made its feasibility more promising. Results obtained with the 5 W C MDC were also comparable to those reported in previous studies (Cao et al., 2009; Brastad and He, 2013; Zhang et al., 2012).

Using external resistance as a basis for comparison, the 0.65 mA current produced in the factorial study by the 5 W C MDC compared favourably with the 3 mA achieved in the work of Coa et al. (2009) and 13.16 mA achieved by Brastad and He (2013). In the above referenced studies, lower external resistors were used as compared to the 1000 Ω used in this study. Cao et al. (2009) used a 200 Ω external resistor whiles Brastad and He (2013) used a 1 Ω resistor. Also, Zhang et al. (2012) even with the use of ion exchange resins and a comparatively lower external resistance of 75 Ω achieved a 0.65 mA current production. Thus it was sound to conclude that, the current (0.65 mA) produced by the 5 W C MDC was appreciable.

With the improved current productions from the 5 W C MDC, concerns of cost of power required for stirring catholytes is settled. In summary, the 5 W C MDC can therefore operate without chemical buffers, chemical catholytes and where *Pseudomonas aeruginosa* are seeded in anolytes, there would be no need for exogenous supply of rhamnolipids.

Membrane fouling studies are necessary for the evaluation of how long membranes can be efficient for the purposes they are meant for since prolonged use can affect membrane integrity. To obtain good quality desalinated water from MDCs, compromises on membrane efficiencies must be avoided. Thus the discovery of fouled layers on used membranes of the five-chambered MDCs demonstrated some possible setbacks with this technology. However, this not the first study to observe fouled layers on used membranes. Luo et al. (2012) working on long term performance of MDC reported of both biofouling and inorganic scaling. Further, Ping et al. (2013) also reported that, when anion and cation exchange membranes were used for a long time, they got fouled by bacteria and ion precipitates. For the optimal operation of MDCs more research works on membrane technology are required to solve the membrane fouling problems of this technology.

CHAPTER SIX

6.0 Conclusions and Recommendations

The following conclusions are made in view of the general outcome of the study against the set objectives

6.1 Conclusion

Microbial desalination cells were designed, locally constructed and successfully operated. A plant-supported MDC produced performed fairly against MDCs working with potassium ferricyanide-based catholytes. Amongst the three-chamber MDCs studied, the three-chamber chemical catholyte and three-chamber water catholyte MDCs demonstrated greater potentials for voltage production, desalination and wastewater treatment.

The ability of neutralization chambers to stabilize pH in MDCs was demonstrated in this research. Phosphate buffers however provided superior pH stabilization effects than the neutralization chambers. With the exception of coulombic efficiency and phosphorus reduction, the five-chamber chemical catholyte MDC produced higher voltage productions, desalination, power density, current density, COD and nitrate reductions performances than the three-chamber MDCs.

Adding rhamnolipid to the anolytes of both five-chamber chemical and water catholyte MDCs improved their electricity generation, desalination, COD and nitrate reduction performances. The five-chamber water catholyte MDC produced results comparable to those produced by the five-chamber chemical catholyte MDC.

Mechanical stirring improved the percentage desalination and voltage productions of the five-chamber water catholyte MDC. However, the interactive effect of stirring and rhamnolipid on voltage production, percentage desalination and percentage COD reductions was insignificant.

Used membranes of the five-chamber water catholyte MDC showed obvious fouled layers on both sides of all membranes. The cation exchange membrane of the five-chamber

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chemical catholyte MDC had more fouled layers than that of the five-chamber water catholyte MDC. The detection of fouled layers on membranes of the MDCs provided evidences that prolong use of membranes could limit the effectiveness of MDCs.

6.2 Recommendations

Presented below are recommendations for consideration

- A study on microbial succession in anolytes from the beginning of an experiment to the point of stable maximum voltage productions is recommended to provide comprehensive information on microbial evolution in MDCs.

- An optimization study can be conducted to determine the optimum concentration of rhamnolipid and stirring speed required to obtain the best electricity production, desalination and wastewater treatment efficiency from the five-chamber water catholyte MDC. This would help the development of a _global' model for predicting the performances of the 5 W C MDC.

- A pilot study on the performance of the five-chamber water catholyte MDC should be undertaken. The effects of different external resistors and COD concentrations can be investigated in this study to assess the MDCs performances under varied conditions. The pilot study can be accompanied with a business development plan.

- The effects of biosurfactants like sophorolipids on the performances of MDCs should be investigated too. It is possible that, sophorolipids might provide better positive effects on the performances of MDCs than rhamnolipid.

- It is recommended that, the government of Ghana provide some financial support for the development of bioelectrochemical systems technology in the country as this class of technology is in tandem with the country's renewable energy goal.

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Appendix 1.Results of Preliminary Studies

A. Table 4.3: Initial characteristics of simulated wastewaters

WASTEWATER SOURCE	PH	COD (MG/L)	NITRATE (MG/L)	PHOSPHORUS (MG/L)
COW DUNG	7.04 ± 0.20	730 ± 0.34	850 ± 0.78	220.00 ± 1.22
FAECAL SLUDGE	6.95 ± 0.11	730 ± 0.66	1124 ± 0.43	448.10 ± 0.87
RUMEN CONTENT	7.02 ± 0.23	730 ± 1.32	977 ± 0.19	320.07 ± 0.91
RUMEN CONTENT + COW DUNG	6.99 ± 0.25	730 ± 3.10	1854 ± 0.72	463.66 ± 0.60

B. Table 4.5: Characteristics of Barekese Dam water, water from farm dug outs and Tap water

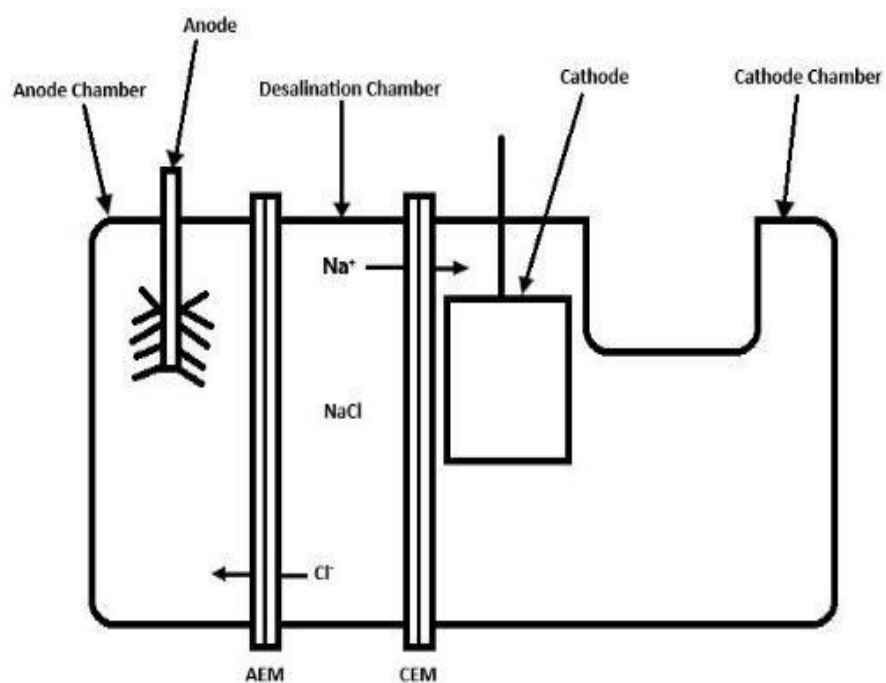| Water source	Electrical Conductivity (µS/cm)	pH	Nitrogen (mg/l)	Phosphorus (mg/l)
Barekese dam	983 ± 0.33	7.73 ± 0.05	15.6 ± 0.62	0.29 ± 1.00
Water from dug outs behind Engineering laboratories	844 ± 1.01	5.67 ± 0.36	23 ± 2.12	8.22 ± 0.23
Tap water	153.8 ± 0.14	6.57 ± 0.53	BDL	BDL

Appendix 2. Schematics of constructed MDCs

Figure A-2A. Schematic of three-chamber water catholyte MDC
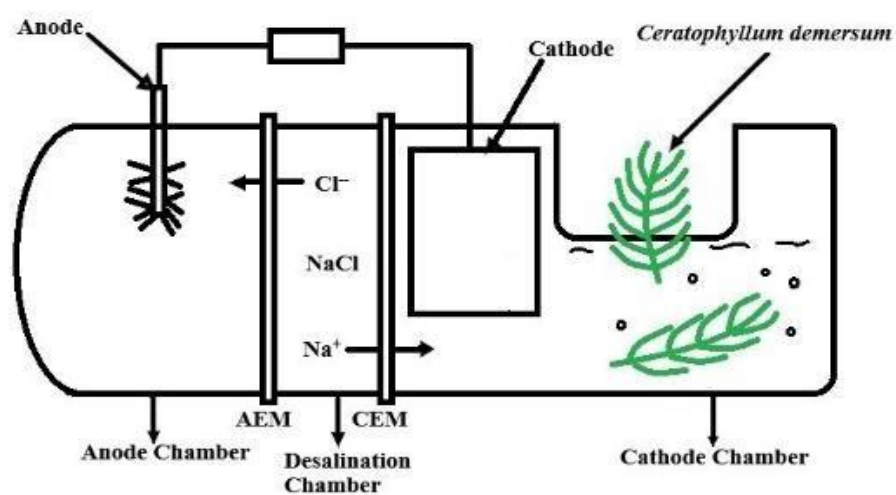

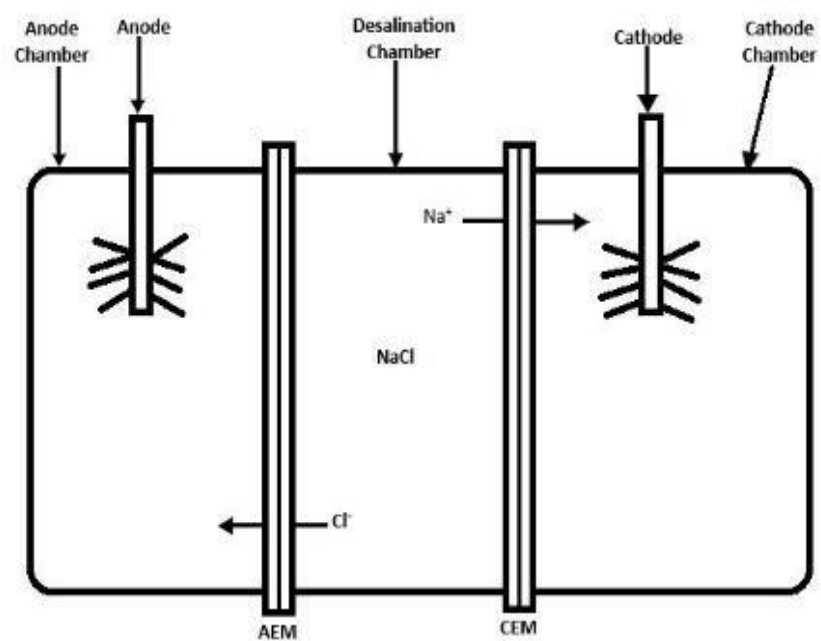**Figure A-2B. Schematic of three chamber plant supported cathode-setup MDC**

Figure A-2C. Schematic of three-chamber chemical catholyte MDC
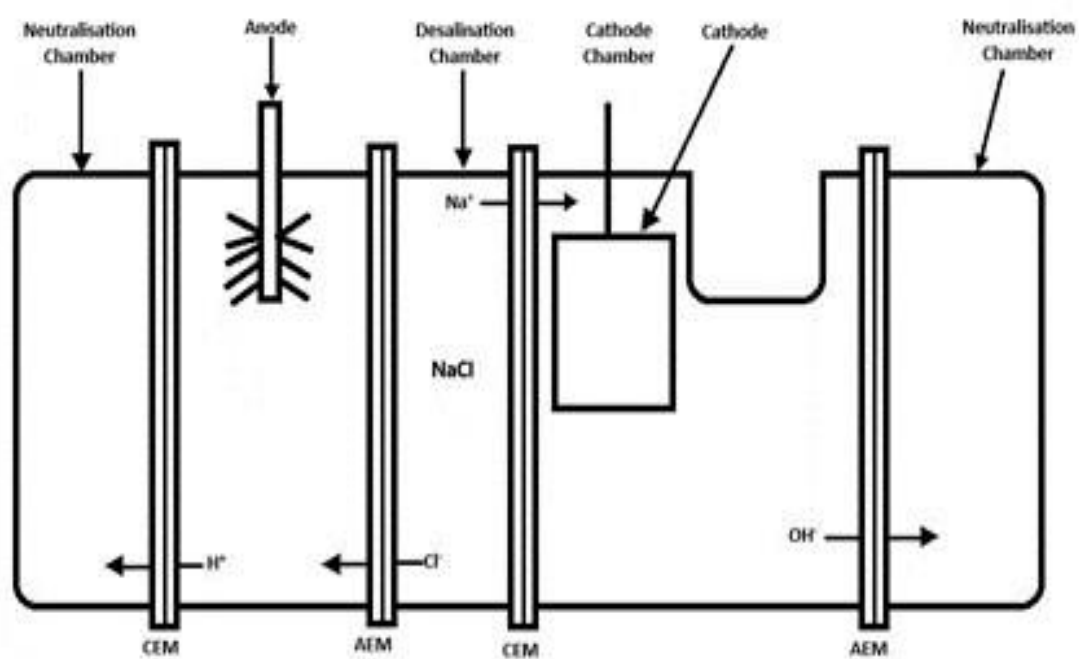
Figure A-2D. Schematic of five-chamber water catholyte MDC
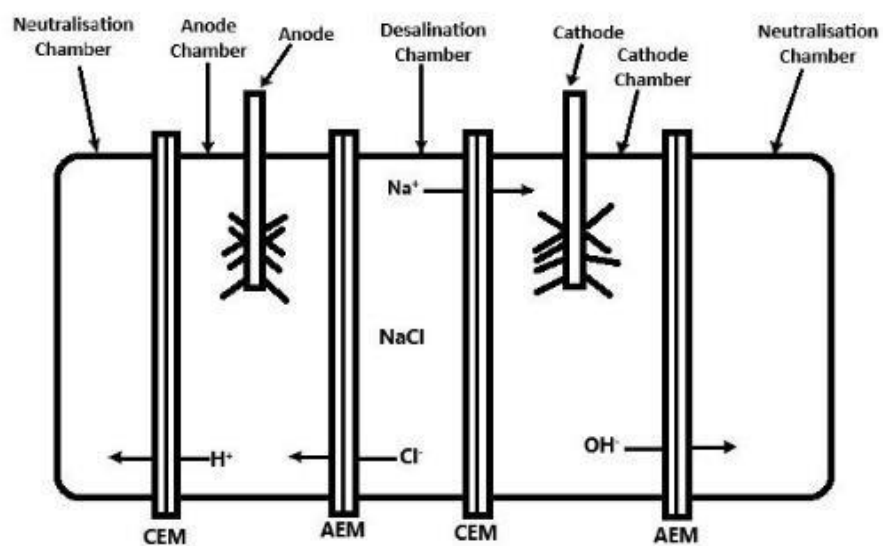
Figure A-2E. Schematic of five-chamber chemical catholyte MDC
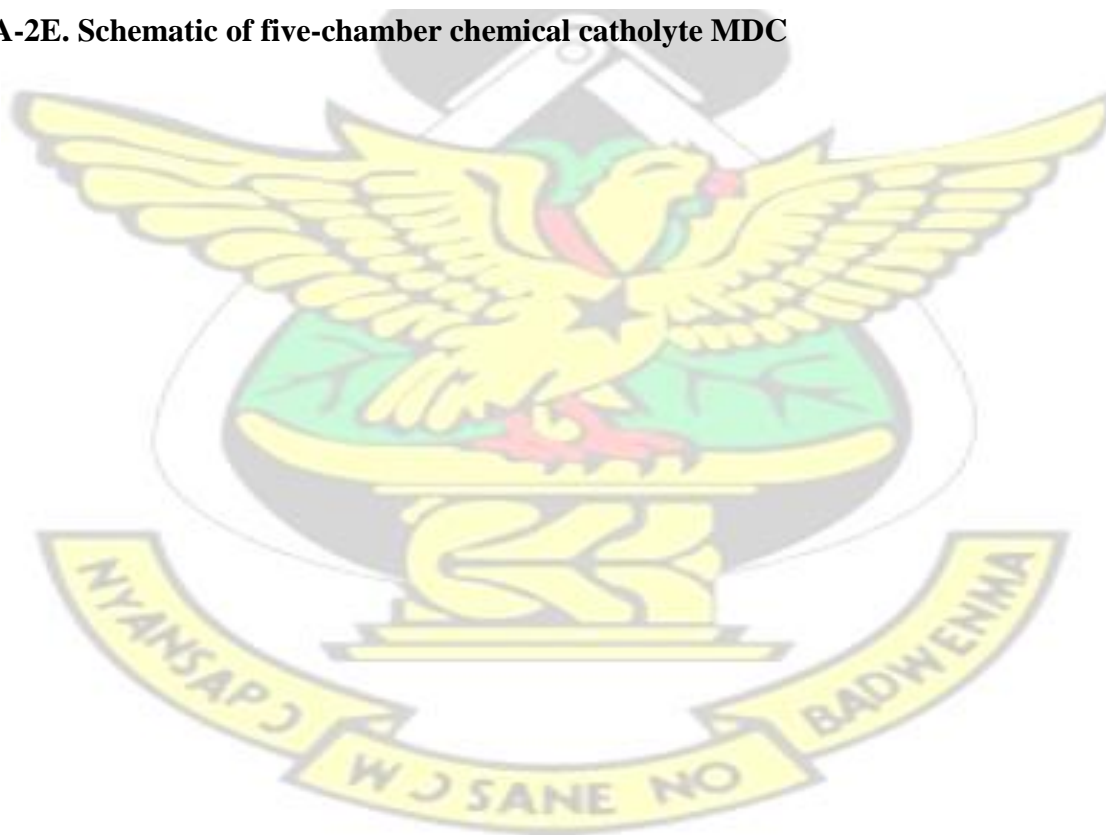
Appendix 3. Factorial Analysis

A. Factorial Analysis for Percentage Desalination

i. Estimated Effects and Coefficients for Desalination (coded units)

Term	Effect	Coef	SE Coef	T	P
Constant		24.3917	0.08498	287.02	0.000
Rhamnolipid	1.2500	0.6250	0.08498	7.35	0.000
Stirring	0.7167	0.3583	0.08498	4.22	0.003
Rhamnolipid*Stirring	-0.3500	-0.1750	0.08498	-2.06	0.073

S = 0.294392   R-Sq = 90.49%   R-Sq(adj) = 86.92%

ii. Analysis of Variance for Desalination (coded units)

Source	DF	Seq SS	Adj SS	Adj MS	F	P
Main Effects	2	6.2283	6.2283	3.11417	35.93	0.000
2-Way Interactions	1	0.3675	0.3675	0.36750	4.24	0.073
Residual Error	8	0.6933	0.6933	0.08667		
Pure Error	8	0.6933	0.6933	0.08667		
Total	11	7.2892				

Estimated Coefficients for Desalination using data in uncoded units
Term	Coef
Constant	18.8000
Rhamnolipid	0.0243750
Stirring	1.23333
Rhamnolipid*Stirring	- 0.00437500

B. Factorial analysis for Voltage productions

i. Factorial Fit: Voltages versus Rhamnolipid, Stirring

Estimated Effects and Coefficients for Voltages (coded units)

Term	Effect	Coef	SE Coef	T	P
Constant		639.732	0.5673	1127.62	0.000
Rhamnolipid	8.370	4.185	0.5673	7.38	0.000
Stirring	3.993	1.997	0.5673	3.52	0.008
Rhamnolipid*Stirring	-1.160	-0.580	0.5673	-1.02	0.337

S = 1.96529   R-Sq = 89.45%   R-Sq(adj) = 85.50%

ii.   Analysis of Variance for Voltages (coded units)

Source	DF	Seq SS	Adj SS	Adj MS	F	P
Main Effects	2	258.011	258.011	129.005	33.40	0.000
2-Way Interactions	1	4.037	4.037	4.037	1.05	0.337
Residual Error	8	30.899	30.899	3.862		
Pure Error	8	30.899	30.899	3.862		
Total	11	292.947				

Estimated Coefficients for Voltages using data in uncoded units
Term   Coef
Constant   609.013
Rhamnolipid   0.133625
Stirring   4.89667
Rhamnolipid*Stirring -0.0145000

   C. Factorial Analysis for COD Removal

   i.   Factorial Fit: COD versus Rhamnolipid, Stirring

Estimated Effects and Coefficients for COD (coded units)

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Term	Effect	Coef	SE Coef	T	P
Constant		66.5933	0.08793	757.33	0.000
Rhamnolipid	2.2433	1.1217	0.08793	12.76	0.000
Stirring	0.5767	0.2883	0.08793	3.28	0.011
Rhamnolipid*Stirring	-0.2333	-0.1167	0.08793	-1.33	0.221

S = 0.304604   R-Sq = 95.63%   R-Sq(adj) = 94.00%

ii.   Analysis of Variance for COD (coded units)

Source	DF	Seq SS	Adj SS	Adj MS	F	P
Main Effects	2	16.0953	16.0953	8.04763	86.74	0.000
2-Way Interactions	1	0.1633	0.1633	0.16333	1.76	0.221
Residual Error	8	0.7423	0.7423	0.09278		
Pure Error	8	0.7423	0.7423	0.09278		
Total	11	17.0009				

Estimated Coefficients for COD using data in uncoded units
Term   Coef
Constant   59.2417
Rhamnolipid   0.0338750
Stirring   0.871667
Rhamnolipid*Stirring -0.00291667

Appendix 4. Additional Experiments for 5 W C MDC

A. Percentage desalination by 5 W C MDC under rhamnolipid addition only

Concentration of rhamnolipid (mg/l)	Percentage desalination Cycle	Percentage desalination *Replicate (1)	Percentage desalination *Replicate (2)
0	18.86	16.75	8.10
80	19.69	18.90	18.07

160	20.21	20.60	18.00
240	24.89	22.18	16.03
420	21.34	20.00	13.73
400	20.03	18.27	14.36
480	20.00	17.35	13.14

B. Percentage desalination by 5 W C MDC under stirring (60 rpm) only

Stirring regime	Percentage desalination Cycle 1	Percentage desalination *Replicate (1)	Percentage desalination *Replicate (2)
1	12.15	16.61	17.46
2	16.40	23.44	22.73
3	23.42	17.61	24.26

C. Percentage COD removed under stirring (60 rpm) only

Stirring regime	% COD
1	49.11
2	49.57
3	50.23

D. Average concentration of DO under different stirring speeds

Stirring Speed (rpm)	Average DO (mg/l)
100	8.00 ± 2.11
80	7.98 ± 1.90
60	7.93 ± 1.05

Appendix 5. Results from Preliminary Studies 'B'

A.Voltage production by Inoculum sources

Residence Time (Hours)	Average Voltage (mV)			
	V1	**V2**	**V3**	**V4**
INITIAL	27.00 ◻ 2.33	46.10 ◻ 1.16	32.04 ◻ 0.22	52.00 ◻ 2.13
24	72.30 ◻ 3.11	69.30 ◻ 2.01	77.90 ◻ 2.98	106.80 ◻ 3.82
48	117.30 ◻ 3.01	134.10 ◻ 1.91	158.90 ◻ 1.77	190.62 ◻ 2.09
72	99.10 ◻ 1.89	72.10 ◻ 2.34	96.10 ◻ 2.43	119.90 ◻ 2.23
96	80.55◻2.15	53.30 ◻ 2.48	81.2 ◻ 3.10	87.90 ◻ 1.57
120	33.67◻ 3.24	40.11◻ 0.95	82.55 ◻ 1.27	81.08 ◻ 0.89

Where: V1= Voltages of cow dung inoculum V2 = Voltages of faecal sludge

V3 = Voltages of rumen contents V4 = Voltages of rumen contents + cow dung

B.pH changes in inoculum Sources

Residence Time (hours)	Average pH							
	pH of Cow dung		**pH of Faecal Sludge**		**pH of Rumen contents**		**pH of Cow dung + Rumen contents**	
	AD	**CD**	**AD**	**CD**	**AD**	**CD**	**AD**	**CD**
0	7.04 ◻ 0.20	7.12 ◻ 0.01	6.95 ◻ 0.11	7.12 ◻ 1.01	7.02 ◻ 0.23	7.12 ◻ 0.17	6.99 ◻ 0.25	7.12 ◻ 0.87
120	4.88 ◻ 0.09	11.14 ◻ 0.11	4.63 ◻ 0.16	11.20 ◻ 0.13	5.69 ◻ 0.27	10.88 ◻ 1.81	5.91 ◻ 0.93	11.91 ◻ 1.10

C.Oxygen production of selected waterweeds and algae

SPECIMEN	TIME (Hours)	DISSOLVED OXYGEN (mg/l)	TEMPERATURE ( ) °C
Control water) (Raw	24	8.29 ⬚ 3.41	29.20 ⬚ 1.01
	48	8.33 ⬚ 5.22	29.00 ⬚ 1.76
	72	7.30 ⬚ 2.87	29.47⬚1.22
	96	7.00 ⬚ 3.90	31.60 ⬚ 0.31
	120	7.22 ⬚ 1.13	31.98 ⬚ 1.54
Lemna sp.	24	7.82 ⬚ 4.01	29.40 ⬚ 5.50
	48	7.10 ⬚ 3.23	29.20 ⬚ 3.01
	72	6.93 ⬚ 5.11	30.60 ⬚ 4.90
	96	6.13 ⬚ 2.05	31.00 ⬚ 6.62
	120	4.83 ⬚ 2.89	31.32 ⬚ 1.88
Ceratophyllum demersum	24	9.08 ⬚ 4.11	29.20 ⬚ 4.39
	48	8.98 ⬚ 2.06	29.43 ⬚ 3.09
	72	8.30 ⬚ 1.39	30.70 ⬚ 2.16

	96	8.73 ☐ 4.01	31.00 ☐ 3.99
	120	8.21☐ 1.68	31.78 ☐ 2.05

Average voltages and pH changes in acclimatization experiment

Weeks	Average Max Voltage (Mv)	H	
		Anode	**Cathode**
1	183 ☐ 2.37	6.31 ☐ 1.93	8.12 ☐ 1.94
2	191 ☐ 4.21	5.83 ☐ 1.23	8.75 ☐ 2.03
3	300 ☐ 1.22	5.59 ☐ 3.01	8.94 ☐ 2.07
4	300 ☐ 5.29	5.22 ☐ 0.37	9.24 ☐ 2.33
5	297 ☐ 3.70	5.14 ☐ 2.10	9.41 ☐ 0.43
6	236 ☐ 0.74	5.09 ☐ 3.26	9.84 ☐ 3.17
7	221 ☐ 2.83	478 ☐ 1.04	10.07 ☐ 0.21
8	216 ☐ 5.42	4.33 ☐ 1.77	10.35 ☐ 1.44

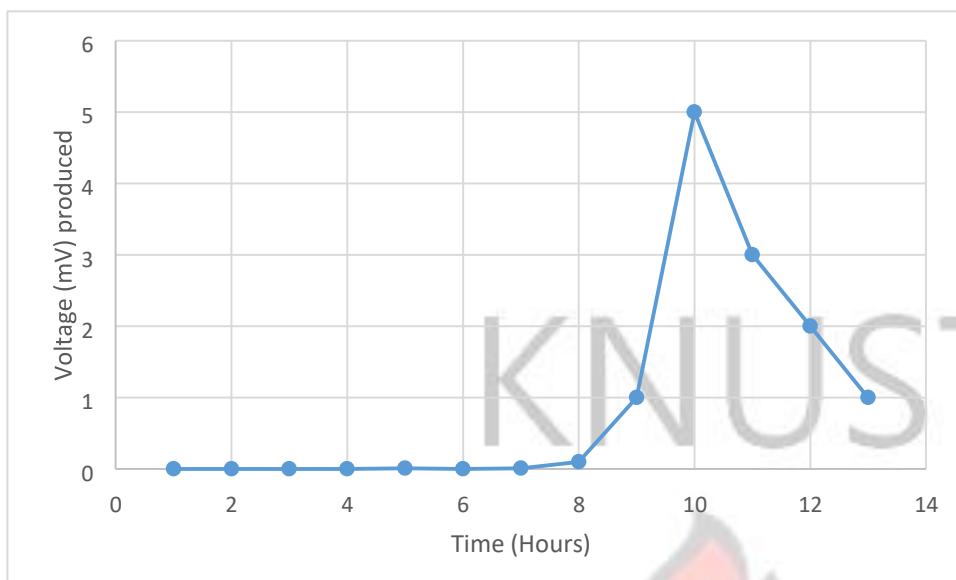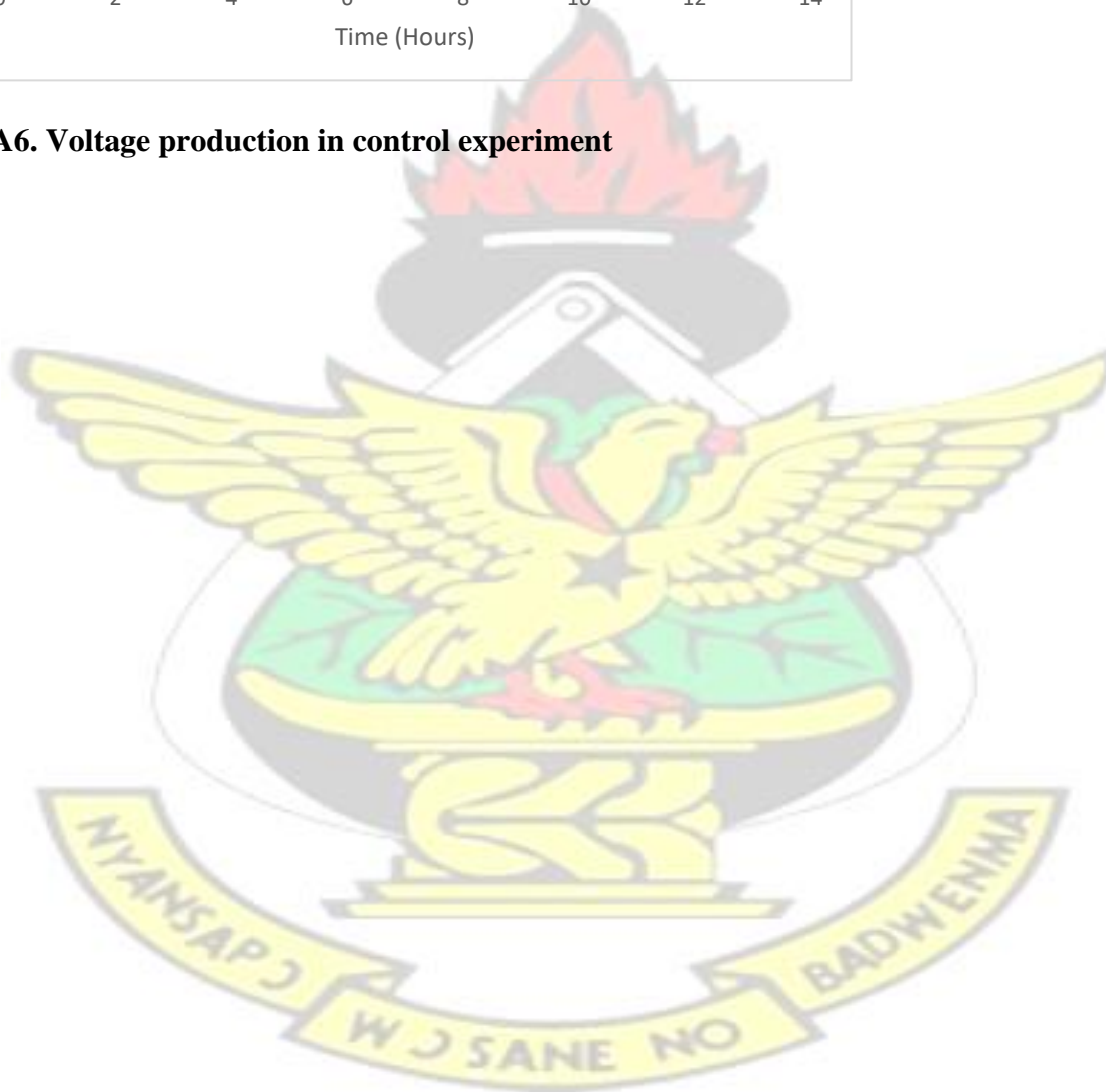**Appendix 6. Voltage production in control experiment**

Figure A6. Voltage production in control experiment

Appendix 7. pH changes in the anolytes and catholytes of the three-chamber MDCs.

    a. **Anode Chamber**

Table A: Anolyte pH across batch cycles

pH measurements		Batch 1	Batch 2	Batch 3
MDC type	Initials pH	Final pH	Final pH	Final pH
3 C C	7.08 ± 0.10	5.56 ± 0.25	5.48 ± 0.13	5.32 ± 0.16
3 P C	6.99 ± 0.01	5.54 ± 0.64	5.21 ± 0.46	5.17 ± 0.21
3 W C	7.04 ± 0.07	4.90 ± 0.58	4.62 ± 0.53	4.59 ± 0.44

    b. **Cathode Chamber**

Table B: Catholyte pH across batch cycles

pH measurements		Batch 1	Batch 2	Batch 3
MDC type	Initials pH	Final pH	Final pH	Final pH
3 C C	7.04 ± 0.40	8.34 ± 0.33	8.47 ± 0.54	8.63 ± 0.35
3 P C	7.10 ± 0.08	8.28 ± 0.12	8.23 ± 0.45	8.37 ± 0.52
3 W C	7.07 ± 1.02	7.55 ± 0.54	8.24 ± 0.07	8.31 ± 0.23